

MAURICIO FERNÁNDEZ OTÁROLA

**“FENOLOGIA REPRODUTIVA, ALOMETRIA E
ORGANIZAÇÃO ESPACIAL DE ESPÉCIES DIÓICAS DO
GÊNERO *VIROLA* (MYRISTICACEAE)”**

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UNIVERSIDADE ESTADUAL DE CAMPINAS
INSTITUTO DE BIOLOGIA

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ORGANIZAÇÃO ESPACIAL DE ESPÉCIES DIÓICAS DO
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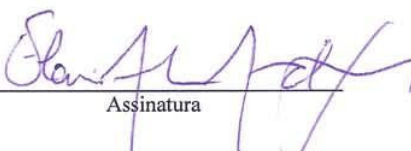
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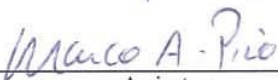
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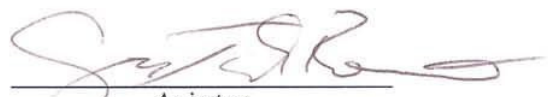
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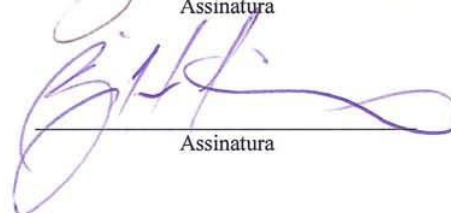
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A mi mamá, mi papá y mi hermano

He aprendido a mirar de una manera más viva:
como si mis abuelos por mi sangre miraran;
como si los futuros habitantes
alzaran mis pestañas.

Yo no miro la piel sino lo que en la piel
es fuego y esperanza.
Lo que aún en los muertos
sigue nutriendo razas.
Lo que es vida y es sangre
tras la inmovilidad de las estatuas.

Profundidad.
Jorge Debravo

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Resumo

Espécies filogeneticamente próximas ocorrendo em simpatria podem variar em diversas características relacionadas à redução de interferências negativas. Em espécies arbóreas podem ocorrer padrões distintos de distribuição espacial, de uso do estrato vertical, de organização e tamanho da copa, que podem ser relacionadas a diferentes tolerâncias ambientais e competição por espaço e nutrientes. Entretanto, interações negativas podem também ocorrer nos eventos reprodutivos, quando as espécies apresentam estruturas reprodutivas similares e são polinizadas pelos mesmos organismos. O gênero *Virola* (Myristicaceae) está composto por espécies arbóreas distribuídas na região neotropical. Estas espécies são dióicas, atingem porte médio a grande, e apresentam semelhanças em suas flores, frutos, polinizadores e dispersores. O objetivo deste trabalho foi estudar os padrões fenológicos, morfológicos (alométricos) e a distribuição espacial de espécies simpátricas do gênero *Virola* para contribuir ao entendimento dos mecanismos que permitem sua coexistência. Foram estudadas *V. bicuhyba* e *V. gardneri* no Brasil (São Paulo) e *V. koschnyi* e *V. surinamensis* na Costa Rica (Península de Osa). Os estudos alométricos foram realizados nos dois pares de espécies, medindo as relações entre o diâmetro e altura do caule e da copa. Os estudos de fenologia e distribuição espacial foram realizados apenas nas espécies brasileiras, observando-se mensalmente a cobertura da copa por flores e frutos e mapeando cada indivíduo e utilizando análises espaciais de padrões de pontos. Os padrões fenológicos, morfológicos e espaciais foram analisados populacionalmente e comparados entre os sexos e as espécies. A cobertura floral e sincronia reprodutiva de cada espécie foram analisadas em relação com o tamanho da árvore. Nas espécies brasileiras a floração inicia simultaneamente. Apenas em *V. bicuhyba* a intensidade e duração da floração são relacionadas com o tamanho da árvore e podem estar relacionadas com a incidência lumínica que varia segundo o tamanho desta.

Diferenças entre os sexos podem estar relacionadas com diferentes pressões fisiológicas e seletivas. *Virola gardneri* apresenta um episódio curto e sincronizado de floração e com um padrão individual bienal. Os frutos maduros nesta espécie são disponibilizados por um período de dois meses, enquanto em *V. bicuhyba* por até sete meses. As relações alométricas do tronco das quatro espécies foram similares, mas o tamanho da copa apresentou variação entre elas e esteve fortemente relacionado com o diâmetro do tronco. Não foi encontrado dimorfismo sexual nas relações alométricas. Finalmente, quanto à organização espacial das espécies brasileiras, apenas *V. gardneri* apresenta preferência por áreas particulares (mais elevadas) com maior iluminação na floresta que favorecem seu estabelecimento. *Virola gardneri* apresenta maior agregação dos indivíduos e uma maior estruturação genética espacial, o que pode estar relacionado com o curto período de frutificação gerando menor taxa de dispersão, mas também com o tamanho das sementes, que por serem maiores que as de *V. bicuhyba* são dispersas por menos espécies. Não foram observadas interações entre as espécies (agregação ou repulsão), com exceção de agrupamento de juvenis pequenos em distâncias curtas. Diferentes estratégias fenológicas, variação na alometria, especialmente da copa e a distribuição espacial estão relacionadas e explicam a coexistência destas espécies.

Abstract

Phylogenetically related species occurring in sympatry may vary in many characteristics related to the reduction of harmful interference. Tree species may present distinct patterns of spatial distribution, use of vertical strata, crown size and organization, which may be related to different environmental tolerances and competition for space and nutrients. However, negative interactions can also occur in reproductive events, when species have similar reproductive structures and are pollinated by the same organisms. The genus *Virola* (Myristicaceae) is composed of tree species distributed in the Neotropical region. These species are dioecious, achieve medium to large size, and have similarities in their flowers, fruits, pollinators, and seed dispersers.

The aim of this work was to study the phenological and morphological (allometric) patterns, and spatial distribution of sympatric species of the genus *Virola* to contribute to the understanding of the mechanisms that enable their coexistence. We studied *V. bicuhyba* and *V. gardneri* in Brazil (São Paulo) and *V. koschnyi* and *V. surinamensis* in Costa Rica (Osa Peninsula). The allometric studies were performed in both pairs of species, measuring the relationship between the diameter and height of the stem and crown size. Studies on phenology and spatial distribution were performed only in the Brazilian species, observing monthly canopy cover by flowers and fruits and mapping each individual and using point pattern spatial analysis. The phenology, morphology and spatial patterns were analyzed at the population level, and compared between individuals, sexes, and species. The relationship between floral coverage and reproductive synchrony to tree size was also analyzed. Flowering starts simultaneously for the Brazilian species. Only in *V. bicuhyba* the intensity and duration of flowering are related to the size of the tree and may be related to resource availability, which varies according to tree size. Gender differences may be related to

different physiological and selective pressures. *Viola gardneri* features a short and synchronized flowering episode, with a biennial pattern at the individual level. Ripe fruits are available for a period of two months, but for up to seven months in *V. bicuhyba*. Trunk allometric relationships were similar among the four species, but crown size was highly variable and was strongly related to the diameter of the trunk. No sexual dimorphism was found in the allometric relationships. Finally, regarding the spatial organization of the Brazilian species, only *V. gardneri* have preferences for particular areas in the forest, possibly related with light levels that favor its establishment. *Viola gardneri* showed the strongest aggregation of individuals and strong spatial genetic structure, which may be related to the short ripe fruiting time and a low dispersal rate, but also the size of the seeds, which are larger than those of *V. bicuhyba* are dispersed by fewer species. No interactions were observed between species (aggregation or dispersion), except for grouping of small juveniles over short distances. Different phenological strategies, variation in allometry (especially for the crown) and spatial distribution are related and explain the coexistence of these species.

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INTRODUÇÃO GERAL

Reduções no valor adaptativo dos indivíduos podem ocorrer quando coexistem com espécies similares, produto de interações que diminuem sua aquisição de recursos ou dificultam seus processos reprodutivos (Pfennig & Pfennig 2009). Nessas situações a coexistência de espécies é favorecida pela divergência fenotípica que permite o uso de nichos ecológicos diferentes (Silvertown 2004). Interferência e competição entre espécies são especialmente prováveis em espécies aparentadas cuja base filogenética determina características ecológicas e morfológicas semelhantes entre elas.

O processo pelo qual os indivíduos mantêm seu valor adaptativo em resposta aos heterospecíficos é chamado deslocamento de caracteres e é esperado em casos nos quais espécies previamente isoladas entram em contato (Pfennig & Pfennig 2009). Nos casos em que as espécies mantenham essa interferência pelo uso de recursos semelhantes teoricamente acontecerá exclusão competitiva e uma das espécies será eliminada da comunidade (Hardin 1960). Modelos populacionais iniciando com o modelo de Lotka-Volterra coincidem neste princípio de exclusão competitiva, concluindo que apenas quando a competição intraspecífica é maior que a competição interespecífica as espécies poderão coexistir (Chesson 2000). Caso contrário a espécie com a maior tolerância à diminuição de um recurso limitante será mantida na comunidade. Assim, espécies semelhantes podem diferir marcadamente em suas características ecológicas se os fatores seletivos determinam diferentes pressões evolutivas (Schaffer 1974).

Outro fator que potencialmente pode aumentar a complexidade no uso do ambiente por uma espécie é a variação fisiológica entre os indivíduos quando categorias bem definidas podem ser identificadas nas populações. Um exemplo disto são os dois sexos em plantas dióicas. Aproximadamente 6% das espécies de angiospermas são dióicas (Vamosi

& Vamosi 2004), mas em comunidades tropicais esse sistema sexual é muito mais comum alcançando valores ao redor de 15% (Bawa *et al.* 1985a; Ibarra-Manriquez & Oyama 1992; Oliveira 1996; Matallana *et al.* 2005; Vary *et al.* 2011).

As variações que favorecem a coexistência das espécies podem se refletir em diferentes estratégias reprodutivas (ver Pauw 2012). A reprodução das plantas dióicas é interessante pelas possíveis limitações que esse sistema reprodutivo pode gerar. Entre estas limitações estão a dependência dos polinizadores para a reprodução, a necessidade de sincronização entre os dois sexos para a produção de sementes e a restrição do fluxo gênico quando apenas alguns indivíduos da população possuem sincronismo na produção de flores (Lenza & Oliveira 2006). Muitas espécies de árvores dióicas apresentam uma série de atributos semelhantes nas suas características reprodutivas, como flores pequenas pouco especializadas e de cores pálidas, assim como frutos com uma ou poucas sementes grandes, dispersadas por vertebrados (Renner & Ricklefs 1995). No entanto, mesmo em sistemas reprodutivos como a dioicia é esperada variação em certos caracteres reprodutivos, especialmente quando espécies similares coexistem. Variação fenológica pode ser uma alternativa para evitar interferências e favorecer a coexistência de espécies simpátricas morfológicamente semelhantes. Exemplos de fenologia escalonada têm sido mencionados em estudos de comunidades ao considerar espécies polinizadas por grupos particulares como beija-flores (Stiles 1977; Buzato *et al.* 2000). Assim, em espécies muito similares, relacionadas filogeneticamente a variação fenológica seria uma alternativa à variação morfológica e a interferência reprodutiva seria evitada temporalmente e não mecânica ou espacialmente (Stiles 1975). Bawa *et al.* (1985b) mencionaram que árvores dióicas são polinizadas principalmente por diversos pequenos insetos e argumentaram que isto poderia restringir a dispersão do pólen devido a supostas limitações no deslocamento e ao hábito

generalista destes organismos (Renner & Feil 1993; Matsuyama *et al.* 2009). Entretanto, Bawa *et al.* (1985b) também mencionaram que uma estratégia dióica pode favorecer o deslocamento dos polinizadores pelos diferentes elementos de atração oferecidos pelas flores dos dois sexos.

Outro fator que pode favorecer a coexistência de espécies é o uso diferencial do espaço vertical e horizontal (Silvertown 2004). Quanto ao uso do espaço vertical, diferentes relações alométricas no tronco e copa entre as espécies de árvores tropicais podem refletir diferentes estratégias ecológicas e o uso diferencial do espaço vertical e aquisição de luz (e.g. Yamada 2005). Porém, restrições filogenéticas podem existir em espécies relacionadas limitando essa variabilidade (Niklas 1994). Variações no uso do ambiente também podem ocorrer em escala horizontal. Samambaias do gênero *Polybotrya* têm preferências particulares por certas condições edáficas na Amazônia ocidental (Tuomisto 2006). Resultados similares foram registrados por Yamada *et al.* (2000) para árvores do gênero *Scaphium* na Malásia. Alguns estudos com plantas dióicas têm encontrado uso diferencial do espaço entre os sexos, possivelmente relacionado com disponibilidade de nutrientes no solo (Cox 1981), entretanto outros têm mencionado que indivíduos de sexos diferentes na mesma espécie influem diferencialmente nas características do solo, modificando sua composição (ver Rhoades *et al.* 1994).

Neste trabalho formulamos a hipótese de que o uso do espaço e os processos reprodutivos podem estar relacionados em plantas dióicas, caso exista esforço reprodutivo diferencial entre os sexos. O esforço reprodutivo é dependente da aquisição de recursos, que está relacionado com a estrutura e a forma do indivíduo e seu uso do ambiente. Isso poderia se refletir em diferentes estratégias para a ocupação do espaço e interceptação de luz e poderia gerar diferentes relações alométricas do tronco e da copa, como resposta ao uso

de diferentes microambientes. Assim, variações no uso do espaço entre os sexos permitiriam aquisição diferencial de recursos entre eles. Propomos testar também a hipótese que espécies simpátricas semelhantes diferem nas suas características ecológicas (reprodução e uso do espaço) permitindo sua coexistência.

Testamos estas hipóteses principais usando árvores dióicas da família Myristicaceae como modelo. Esforço reprodutivo diferencial entre os sexos tem sido proposto para algumas espécies de Myristicaceae: *Myristica insipida* (Armstrong & Irvine 1989); espécies de *Iryanthera*, *Otoba* e *Viola* (Queenborough *et al.* 2007).

O maior dos gêneros da família Myristicaceae é *Viola*, que apresenta mais de 46 espécies. Desenvolvemos esse trabalho na mata atlântica do estado de São Paulo e na floresta chuvosa da Costa Rica. Na Mata Atlântica ocorrem duas espécies de *Viola*: *V. bicuhyba* conhecida popularmente como “bicuíba” e *V. gardneri* conhecida como “bucuibuçu”, as quais são simpátricas em boa parte da sua distribuição. Na Costa Rica estudamos adicionalmente a alometria de *V. koschnyi* e *V. surinamensis* chamadas localmente de “fruta dorada”.

Este trabalho tem por objetivos principais: (1) Determinar como a fenologia reprodutiva, as características morfológicas (alométricas) e o uso do espaço (vertical e horizontal) se relacionam entre os sexos e entre as espécies *V. bicuhyba* e *V. gardneri*. (2) Esclarecer como as estratégias reprodutivas e ecológicas diferem permitindo a coexistência dessas espécies.

O presente trabalho está dividido em três capítulos que analisam diferentes fatores da ecologia dessas espécies em populações simpátricas. O Capítulo 1 foca na fenologia reprodutiva de *V. bicuhyba* e *V. gardneri*, sendo descrita no nível da espécie (população), sexo e finalmente no nível do indivíduo. O comportamento em cada um desses níveis foi

comparado entre as espécies. No nível individual foi determinada a relação entre o tamanho, o esforço reprodutivo e o período de reprodução e suas possíveis implicações no fluxo de pólen nas populações. No Capítulo 2 foi verificada a existência de semelhanças nas relações alométricas entre dois pares de espécies simpátricas, congênericas, localizadas em florestas chuvosas em ambientes distantes e com características ambientais diferentes. Assim, além de *V. bicuhyba* e *V. gardneri*, foram estudadas *V. koschnyi* e *V. surinamensis* na floresta chuvosa do sul da Costa Rica. Isto permite determinar o grau de conservação das características alométricas dentro do gênero e como cada espécie aloca os recursos e faz uso do espaço. Foi analisada a morfologia do tronco e copa, assim como a presença de dimorfismo sexual nessas características. Finalmente, o Capítulo 3 descreve o uso do espaço horizontal pelas espécies brasileiras e a estrutura genética espacial dentro das populações, dividindo as populações em categorias de tamanho. Essa metodologia permite determinar os padrões de dispersão de sementes que determinam a organização inicial na população, assim como inferir parte dos processos que geram os padrões de distribuição observados ao longo da ontogenia.

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CHAPTER 1

Reproductive strategies of two dioecious tropical trees: The case of *Virola bicuhyba* and *V. gardneri* (Myristicaceae) in the Brazilian Atlantic Forest

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Abstract

Closely related and sympatric plant species may present variability in reproductive traits as different reproductive strategies assure coexistence by reducing negative interferences. An alternative to mechanistic/structural differences is the phenological variation in reproductive episodes. We quantified the reproductive effort and reproductive synchronization of two sympatric dioecious Wild Nutmeg species (*Virola*, Myristicaceae), and analyzed its relationships with tree size between and within species and sexes. Different flowering strategies were found between the species and sexes in timing, intensity, and synchronization. Two extreme strategies in flowering timing and intensity were found among individuals in *V. bicuhyba* (extended vs. peak flowering), but individuals vary between these strategies in relation to their size. Fruiting strategy also differed between the species in fruit size and timing of ripe fruits availability. Different strategies between the sexes can be related with different physiology and selective pressures. At the individual level the phenological strategies can be related to resource availability, which varies with tree size along the ontogeny. This has implications for sexual selection affecting the pollinators' attraction capacity, pollen dispersal, and individual reproductive success. Different reproductive strategies should be recurrent among highly related sympatric species helping to explain the high tree diversity and coexistence in tropical forests.

INTRODUCTION

The maintenance of species diversity in biological communities is a central issue in ecological studies. Of particular interest is the coexistence of closely related species, as natural selection may favor the evolution of phenotypic divergence and thereby reduce niche overlap. According to Pfennig & Pfennig (2009) character displacement refers to the evolutionary accentuation of phenotypic differences between species, stemming from selection to lessen resource competition or reproductive interactions between them.

Reproductive character displacement, together with the characterization of geographic variability, has been the focus of studies in sympatric and allopatric populations of congeneric species (e.g. Ashton *et al.* 1988; Lobo *et al.* 2003). However, when species share an essential resource with no alternative, as the pollinators, models predict that they will converge to similar resource uptake phenotypes, being more similar in sympatry than in allopatry (Fox & Vasseur 2008).

There is many evidence of character displacement in vertebrates, invertebrates, and plants (see Dayan & Simberloff 2005), but sympatric tropical trees have received relatively little attention considering the diversity of tropical forests and that congeneric species are frequently reported in the same community. The reproductive phenology of plant species can have important implications in their coexistence as species can interfere in the others' reproduction. Several studies analyzed the phenological patterns of tropical trees at the community level (e.g. Borchert 1983; Wright & Schaik 1994); however, the strategies of particular species, as well as intraspecific variation, are usually overlooked (Ollerton & Lack 1992; Newstrom *et al.* 1994). Coexisting tree species with similar reproductive characteristics (especially phylogenetically related ones) are common in tropical forest, but

how their specific reproductive niche differ, and how their reproductive phenology is modified in order to favor coexistence still need to be studied in detail.

For such studies, dioecious plants are particularly interesting as each sex may be subject to different reproductive pressures which can reflect in their reproductive phenology together with strong constraints to divergence, such as the need of flowering synchronization between staminate and pistillate plants (hereafter males and females). Dioecy occurs in approximately 6% of the angiosperm species (Vamosi & Vamosi 2004), but they represent an important component of tropical forest diversity (e.g. (Bawa *et al.* 1985; Ibarra-Manriquez & Oyama 1992; Oliveira 1996; Matallana *et al.* 2005; Vary *et al.* 2011)). In dioecious trees it is well known that males and females can have differences in reproductive characteristics, as they have different physiological requirements and reproductive strategies (e.g. Cox 1981; Wheelwright & Logan 2004; Rocca & Sazima 2006; Queenborough *et al.* 2007). But how the variability in reproductive characteristics is distributed among the individuals and which factors define it is still poorly understood. This is probably due to the difficulty to directly quantifying plant flowering intensity especially for trees.

The size of individual plants has been found to influence their flowering time and intensity (Schmitt 1983; Ollerton & Lack 1998), mediating the attraction of animal vectors dispersing the pollen (Klinkhamer *et al.* 1989). Plant size can also influence the resource acquisition, especially in forest ecosystems where light incidence decreases considerably from canopy to understory strata. The combined information about individual plant flowering characteristics and plant size will allow the study of plant reproductive strategies. Especially for tree species, the great variation in size during their life time can allow the

development of distinct reproductive strategies among individuals favorable for the particular conditions experienced during the ontogeny.

We studied two sympatric dioecious species of genus *Virola* (Myristicaceae) in the Brazilian Atlantic rain forest and compare in detail the reproductive phenology between and within species, between the sexes, and among individuals. We hypothesize that the reproductive phenology should vary at different levels, and predict that: -reproductive individuals should present different phenological strategies related to their size increasing the reproductive investment on larger individuals; - the sexes should present different reproductive phenology related to their different physiological characteristics, but restricted by their necessity of synchronization. Finally, species coexistence is influenced by their reproductive phenology which would be affected by their phylogenetic relationship. Similar reproductive phenology could be an advantage in cases of facilitation in the attraction of pollinators or dispersers among species, but some character displacement in reproductive time is expected in the case of negative interference in their reproduction. Variation in the reproductive strategies of tropical trees at species, sex, and individual levels is important to understand populations' performance and evolution, as well as species coexistence mediated by reproductive phenology divergence.

MATERIALS AND METHODS

Studied species

The neotropical genus *Virola* (Nutmeg trees) is comprised by approximately 46 dioecious species, and is one of the most diverse and abundant of the family Myristicaceae. *Virola bicuhyba* Schott ex Spreng. and *V. gardneri* (A. DC.) Warb. are endemic to Brazil and are the only two species of the genus found in the Atlantic rain forest biome. They overlap over

a broad geographical range, comprising the Atlantic rain forest between the states of Bahia and São Paulo (11°30'S to 25°14'S approximately; Rodrigues 2011).

Both sexes of both species produce inflorescences at the terminal parts of their small branches, having similar yellowish small flowers. Fruits are dehiscent and open two green valves to expose a single seed covered by a bright red aril. Seeds are conspicuous and dispersed by vertebrates, mainly large birds such as toucans (Galetti *et al.* 2000). *Virola bicuhyba* and *V. gardneri* can reach up to 44 and 35 m respectively in the studied area and they both start reproduction when individuals reach approximately 18 m in height.

Study Site

This study was conducted in the Serra do Mar State Park, Núcleo Picinguaba (47500 ha), São Paulo, Brazil in the Fazenda Capricórnio, near the city of Ubatuba (23°22'S, 45°05'W). The sampled area has 10 ha of continuous primary forest, classified as tropical moist evergreen according to Holdridge (1947), or submontane rain forest according to Oliveira-Filho & Fontes (2000). Average annual precipitation for the period 2008-2010 was 2918 mm, and average temperature varies between 18 and 27 °C along the year (Ubatuba climatic station, Instituto Agronômico de Campinas). Our study was conducted with populations located at middle elevations (vertical distribution 250-450 m a.s.l.).

Phenological evaluations

Within a permanent monitoring plot of 10 ha, 47 reproductive individuals of *V. bicuhyba* (20 females, 27 males), and 31 *V. gardneri* (14 females, 17 males) were marked and followed during the study period. The sex of each tree was determined by the analysis of

floral structures and/or fruit presence along the study period. Phenological observations were made every month, from May 2009 to April 2011. Individuals of *V. gardneri* were observed also during the reproductive seasons of 2008 (10 individuals), 2011 (31 individuals) to check for the presence of reproductive structures. As a direct count of flowers, inflorescences or fruits was not possible (the trees are tall and large and their production is massive), we estimated the proportion of the crown covered by inflorescences or fruits, as only the terminal part of the branches produce flowers and fruits (Fig. 1). To do this, each crown was divided into four equal parts that were carefully observed with binoculars to provide a flower or fruit coverage. Coverage estimate error was calculated to be 5% during repeated measurements of the same individuals in successive days. These monthly coverage values were summed to obtain the total flower coverage per individual for each year (annual coverage) and during the full study period (total coverage); for example: total coverage = 6.5 means the tree produced the equivalent of 6.5 crowns full of flowers during the study period. Some individuals were included in the quantification after the beginning of the study and followed from then on, having their reproductive efforts standardized to two years in order to have a common period for comparison. This was done only for males in *V. bicuhyba* as the full reproductive episode for females was observed, and no individual with less than 19 months of observations were included in the analyses.

Measurements

As seed size is an important component of the reproductive effort, the diameter, length and mass of a sample of eight fresh seeds from each species were measured. As our seed sample size was small we used an average between our values and the data found in Zipparro & Morellato (2005) and Galetti *et al.* (2011).

A crown illumination index, modified from (Clark & Clark 1992), was used to measure the light incidence on each tree. The scores were: 1 (no direct lateral or overhead light), 1.5 (little direct lateral light, no overhead light), 2 (some direct lateral light, no overhead light), 2.5 (substantial direct lateral light, no overhead light), 3 (some overhead direct light), 4 (full overhead direct light; in canopy), 4.5 (full overhead direct light; substantial direct lateral light), and 5 (full overhead and lateral direct light; emergent). An index value was given monthly at the same time as with the phenological observations; the average of the values for each tree was used in the analyses.

Data Analysis

In *V. bicuhyba*, we used a simple correlation to compare the total flower coverage of the individuals between the two years of study. The flowering synchrony of each individual was evaluated with the Augspurger index (Augspurger 1983), which compares the overlap of the flowering time of each individual to the rest of the population. This index ranges from zero (individual flowering alone) to one (individual flowering overlaps with all others); month was used as the unit for comparison. The first six months of study were excluded from these calculations to standardized data to a common period of observation for all the individuals. The index for three individuals is based in one year of observations. The flower coverage during the two months of flowering overlap per year was compared by a nested ANOVA (year was nested within species to control for the difference in the phenological pattern -annual vs. biennial- of the individuals). Tree sizes were estimated by the diameter at breast height (DBH) which is strongly correlated with the crown dimensions (see Chapter 2). The relationships of total flower coverage, synchrony and flowering time with sex and size were analyzed with ANCOVA using DBH as a covariate. The Box-Cox

transformation was used for the time of flowering, total flower coverage in *V. bicuhyba*, and flower coverage during the flowering overlap for both species, because of their better adjustment to the fitted models in comparison to the non-transformed variables. Analyses were performed in JMP 7.0 (SAS Institute Inc.).

RESULTS

Phenological strategies

Both species started flowering together the two years (Fig. 2). Precipitation and mean temperatures were correlated ($r = 0.50$, $p = 0.01$), but there was less variation in mean temperature than in precipitation.

Viola bicuhyba

Flowering in this species is annual; 88% of the individuals flowered in both years with a peak of three months during the spring. All individuals started flowering together; males and females were synchronized, but 9.1% of the females and 48% of the males continued flowering after peak population-wide flowering (Figs. 2 and 3).

Annual flower coverage presented great variation and was higher for males than females in both years (Table 1, Fig. 4). The annual flower coverage of each male was highly correlated between the two years ($r = 0.93$, $p < 0.0001$, $n = 24$), while females showed not such correlation ($r \approx 0$, ns, $n = 18$). A tendency to higher total flower coverage was found on larger individuals, but there is great variability, mainly in males (Fig. 5A, Table 2). Total flower coverage was higher for males than for females (Fig. 5A, Table 2).

The relationship of the crown illumination and the total flower coverage was significant only for males (males: $r^2=0.25$, $p=0.012$; females: $r^2=0.07$, $p=0.29$; Fig. 5B). Flowering in the biggest trees tended to be less synchronized with the population (ANCOVA $F_{(1,42)}=5.66$, $p=0.022$; Fig. 6A) and females are more synchronized than males (average Augspurger index values \pm SD: 0.74 ± 0.14 and 0.55 ± 0.16 respectively; ANCOVA $F_{(1,42)}=21.33$, $p<0.0001$). The variation in synchrony with tree size did not differ between the sexes (ANCOVA $F_{(1,42)}=0.034$, $p=0.85$).

Big trees flowered for longer periods than smaller individuals (ANCOVA $F_{(1,46)}=10.34$, $p=0.002$; Fig. 6B) and flower production in males extended longer than females (ANCOVA $F_{(1,46)}=22.99$, $p<0.0001$). The relationship between flowering time and tree size (DBH) did not differ between the sexes (ANCOVA $F_{(1,46)}=0.005$, $p=0.94$).

Fruit ripening began in June and lasted up to seven months (Fig. 2), with no clear peak; at every observation each female had just a few mature fruits with exposed seeds. Seed shape is elliptical, measuring on average 23.7×15 mm and weighing 2.7 g. Large females presented a higher fruit coverage than smaller ones ($r^2=0.30$, $p=0.012$).

Viola gardneri

This species showed contrasting reproductive patterns in comparison with the sympatric *V. bichuba* at the three levels analyzed (species, sexes, and individuals). Flower production in *V. gardneri* species is highly synchronized and lasted for two months (Fig. 2). While at the population level flowering individuals were observed each year, we detected that individuals (males and females) flower every other year, a biennial pattern that persisted

over two additional years of observations (Fig. 3). Among the reproductive trees, 53% flowered in 2009 and the remaining 47% the following year.

Average flower coverage was higher for males in *V. gardneri*, and also higher than the average in *V. bicuhyba* (Tables 1 and 2; Fig. 4). No significant relationship between tree size (DBH), crown illumination, and flower or fruit coverage was found in *V. gardneri*, for males or females (Table 2; Fig. 5C,D). Average Augspurger index values did not differ between males and females (0.87 ± 0.13 and 0.92 ± 0.1 respectively; $t=1.44$, $df=22$, $p=0.16$).

Fruit development takes a full year to ripening. The short ripening peak of two months overlapped the species flowering, as each tree flowers every other year; at the population level some trees had flowers while other trees had ripe fruits at the same time. Seed shape is slightly elliptical, with an average size of 24.9 x 20.6 mm and weighing 5.3 g (27% wider and 50% heavier than those of *V. bicuhyba*).

DISCUSSION

The two sympatric species of *Virola* here studied have a very similar floral and fruit morphology, attract the same pollinators, mainly flies (M. Fernández Otárola pers. obs.) as has been observed in other species in the genus (Jardim & Mota 2007), and share part of their seed dispersers, as toucans (Galetti *et al.* 2000; Pizo *et al.* 2002; M. Fernández Otárola unpublished data), and flower at about the same time. The need of synchronized flowering can produce stabilizing selection, where the availability of mates limits reproductive success as is the case in dioecious species (O’Neil 1997; Ollerton & Diaz 1999). Also the phylogenetic relationship between the species can impose restrictions in their phenology (Kochmer & Handel 1986; Wright & Calderon 1995).

Plant reproductive activities are related to environmental conditions that trigger the development of specialized structures. Day length has been reported as an explanation for phenological patterns in species of the Atlantic forest (Morellato *et al.* 2000), but there was a one month difference in the beginning of the flowering between 2008 and 2009 for both species, although day length remained constant. As environmental variables are markedly seasonal and correlated in the area (e.g. temperature and precipitation) it is very difficult to detect the most important one and probably the combination of these environmental variables determines the start of the reproductive cycle. *Virola bicuhyba* and *V. gardneri* are sympatric over a great range and are the only two species in the genus found in the Brazilian Atlantic rain forests. We are assuming that both species have an evolutionary history of coexistence and that some of their differences can be discussed as strategies related to patterns of resource partitioning. No phylogeny has been developed for the genus, and the exact relationship among the species is unknown.

Individual strategies (intrapopulational diversity)

According to the temporal distribution and flower coverage of each individual we identified two main patterns: the “peak strategists” that flowered only during the population peak and the “extended strategists” that presented large flower coverage during the peak and kept flowering for several months. *Virola gardneri* males and females are peak strategists, whereas *V. bicuhyba* exhibits the full range of variation between the two extremes, the males being more extended than females. Flowering during the population peak and its maintenance thereafter was described for *V. surinamensis* (Piña-Rodrigues 1999) and for the neotropical shrub *Piper arieianum* by Marquis (1988), and a high variation in flowering time among individuals has been observed for *Jacaratia dolichaula*, a dioecious tropical

tree (Bullock & Bawa 1981). These works show that reproductive phenological variation is common in tropical trees, although usually overlooked. When pollinators are scarce or respond differently to flower display, variation in flowering time and intensity can result in different reproductive success, especially if the amount of pollen transferred is correlated with the length of the flowering period (Willson 1994).

Synchronization is an important trait in sexual organisms enhancing the chance of mating. Synchronized flower production is an attractive resource for pollinators that, as a result, repeatedly visit plants of the same species. Although efficient in ensuring suitable levels of pollinated flowers, synchronization can also lead to intrapopulation competition for pollination agents. In *V. bicuhyba*, the synchronized males can pollinate the majority of females, as most of them flower during the peak; on the other hand, the ones with low synchronization can pollinate the synchronous females as well as those extending their flowering outside the peak population-wide flowering.

Variation in the flowering phenology according to tree size reflects different reproductive strategies at different stages of a plant reproductive life. When the tree size is about its maximum and vegetative growth slows down, resources can be mostly allocated for reproduction; by this time, when large light incidence is available at the canopy an extended strategy can increase the number of mates among the population of females. Populations of *Tibouchina pulchra* studied along an altitudinal gradient of the Atlantic rainforest showed temporal variation in flowering episodes, beginning its flowering in lowlands and gradually expanding it to higher elevations (Brito & Sazima 2012). If a similar pattern occurs in *V. bicuhyba* populations, extended flowering males may provide a means of interpopulation gene flow a hypothesis that can be tested in future studies with molecular markers. As bigger trees, both males and females extended their flowering, have

relatively higher flower coverage, and produce more fruits, we can propose a hypothesis of selection on this tendency, allowing the successful (biggest) individuals to reproduce longer and to disperse pollen and seeds further. On the other hand, the biennial reproductive behavior of *V. gardneri* could allow resource storage and massive flowering episodes independent of tree size.

Both species presented great variability in flower coverage, but for the males of *V. bicuhyba*, the similar crown coverage of each individual between the two years suggests non random differences in reproductive potential. In this species, individuals with similar sizes can present great variation in the crown coverage and time of flowering, showing that the strategies are not fully dependent on tree size. This variability in flower production among similar size individuals can allow the differential attraction of pollinators and be an ideal scenario for the occurrence of sexual selection in this species.

Sex specific strategies

Males and females of both species started flowering together, differing from *V. sebifera* (Lenza & Oliveira 2006) and other dioecious species in which males flower earlier than females, having longer and more intense flowering episodes (e.g. (Bullock & Bawa 1981; Rocca & Sazima 2006). A higher energetic cost of reproduction for females compared to males has been reported for Myristicaceae species. Armstrong and Irvine (1989) reported that females in *Myristica insipida* expended 4.2 times more energy than males per reproductive twig; Queenborough *et al.* (2007) discussed that females of five species in the genera *Virola* and *Otoba* used 8 to 30 times more energy for reproduction than males. Nevertheless, these studies considered only the dry mass of reproductive structures, ignoring either the temporal distribution of flowers, or the crown coverage per individual.

Our data show that flowering traits are different for each sex. In both species the flower coverage was higher in males; in *V. bicusbya* this difference is even greater, as is the number of flowers per inflorescence (preliminary data indicate that male inflorescences have around 16 times more flowers and are 3 times heavier). This, in addition to the longer flowering time, implies that males also have a very high resource use during reproduction. If only the fruiting episode is taken into account, females will appear to use a lot more energy than males, but when flowering and fruiting distribution along the year is considered a more comprehensive evaluation of the reproductive effort of the sexes can be done, and in this way a similar effort may be observed. Although fruits are heavy, their development occurs along many months (one year in *V. gardneri*), and as photosynthetic structures they contribute to their own development (Bazzaz *et al.* 1979; Cipollini & Levey 1991). The males' longer flowering episodes and higher flower coverage indicate that their reproductive effort may not be much lower than the females' as previously reported, and that traits other than flower and fruit average mass have to be used to compare sex related reproductive effort. Dry mass measures only carbon allocation and does not reflect the real limiting resources as nitrogen or phosphorus. Reproductive effort is highly dependant on the fruit size and nutrient composition, development time, flowering time, flower number, and flower crown coverage, all these factors being variable among the species. We demonstrate that flowering time, crown coverage per inflorescences, and flower number per inflorescence is much higher in males than females in these species and may be comparable to the investment in the nutritious aril in the fruits. We propose that the total reproductive effort has to be evaluated along the reproductive season, considering together with the fruits also the crown coverage and flowering time span in order to determine the actual differences between the sexes.

Species specific strategies

Lankau (2011), reviewing the theoretical framework for species coexistence, organized two general classes of mechanisms: those that promote diversity by creating differences between species and those that allow diversity owing to ecological similarities. *Virola bicuhyba* and *V. gardneri*, as sharing pollinators and seed dispersers, are expected to have synchronous seasons of flowers and mature fruits, and indeed there is a conspicuous overlap, mainly in flower peaks. However, they clearly have distinct strategies, distributing their resource demands differently over time.

As a general pattern, both species have concomitant flower peaks, and initiate the massive flowering episode together, in similar response either to the same environmental conditions or to the peak activities of pollinators (or both). Following the model of Fox & Vasseur (2008), these resemblances should be related to scarcity of pollinators, leading the coexisting species to be so similar in the traits related this resource need. By the other hand, there is a clear temporal character displacement in maturing the fruits: *V. gardneri* presents a peak of ripening fruits that starts when *V. bicuhyba* has lessen mature fruits, in what can be interpreted as resource partitioning.

We report, a very interesting reproductive phenological pattern: *V. gardneri*, though an annual species is composed by biennial individuals, half of them flowering each year. Only when the onset of flowering is heritable such a pattern might lead to sympatric isolation between two reproductive populations; if not so, if the entrance of a new individual to a group is triggered by physiological conditions, the genetic cohesion of the species is kept. This biennial reproduction may also contribute to coexistence with other species via stabilizing mechanisms that increase intraspecific relative to interspecific

competition (Chesson 2000; Lankau 2011). In this way, resource partitioning occurs among conspecifics, while different species will show overlap in resource use. The extended strategy described for *V. bicuhyba* may also fit this view of intraspecific competition, as those trees flowering for a longer time can potentially produce more offspring having reproductive access to peak flowering individuals, as well as extended ones.

The fruit ripening follows the flowering strategy, with an extended fruiting time in *V. bicuhyba* and a short pulse in *V. gardneri*. Mature fruits of *V. bicuhyba*, available over most part of the year, will keep seed dispersers around, but as these fruits are not an abundant resource, the seeds of this species might be dispersed further than those of *V. gardneri* that produces enough fruits to keep birds in a close neighborhood during its short fruiting peak. The seeds of *V. gardneri* are larger than those of *V. bicuhyba*, in accordance to a longer time for fruit maturation. The larger seeds of *V. gardneri* cannot be ingested by some bird species that would use *V. bicuhyba* seeds (Pizo *et al.* 2002), what is suggestive of a broader dispersal range for *V. bicuhyba*.

Concluding remarks

As far as we know, this is the first field study to evaluate the reproductive phenology of populations by taking detailed individual evaluations of crown coverage and relate it with tree size. Our work highlights the importance of this approach in the analysis of the reproductive strategies of tropical trees. Phenology is usually studied at species or population level and few studies have focused in the variation among individual within populations. Here we found different strategies between a pair of closely related sympatric species, at the species, sex, population, and individual levels. This has several implications

on the understanding of the mechanisms related to the great diversity of tropical forests, sexual selection in plants, and the evolution of reproductive strategies in plants.

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Table 1. Nested ANOVA comparing the crown coverage by flowers during the two months of flowering overlap, between species, sexes, and years of study (data of both years were pooled for *V. gardneri*).

Factor	df	F	p
Species	1	8.37	0.0048
Sex	1	19.29	<0.0001
Species*Sex	1	1.83	0.18
Year[Species]	1	1.43	0.23

Table 2. ANCOVA comparing the flower coverage and its relation with tree sex and size (DBH).

Factor	<i>V. bicuhyba</i>			<i>V. gardneri</i>		
	df	F	p	df	F	p
Sex	1;42	29.71	<0.0001	1;27	13.66	0.001
DBH	1;42	14.3	0.0005	1;27	0.14	0.71
Sex*DBH	1;42	1.83	0.18	1;27	0.92	0.34



Figure 1. A) Section of the crown of a male *Virola gardneri* with inflorescences on all terminal branches (coverage = 1). B) Detail of reproductive flowering twigs of *V. gardneri*. C) Terminal branch of *V. bicuhyba* with developing inflorescences. D) Male flowers of *V. bicuhyba*.

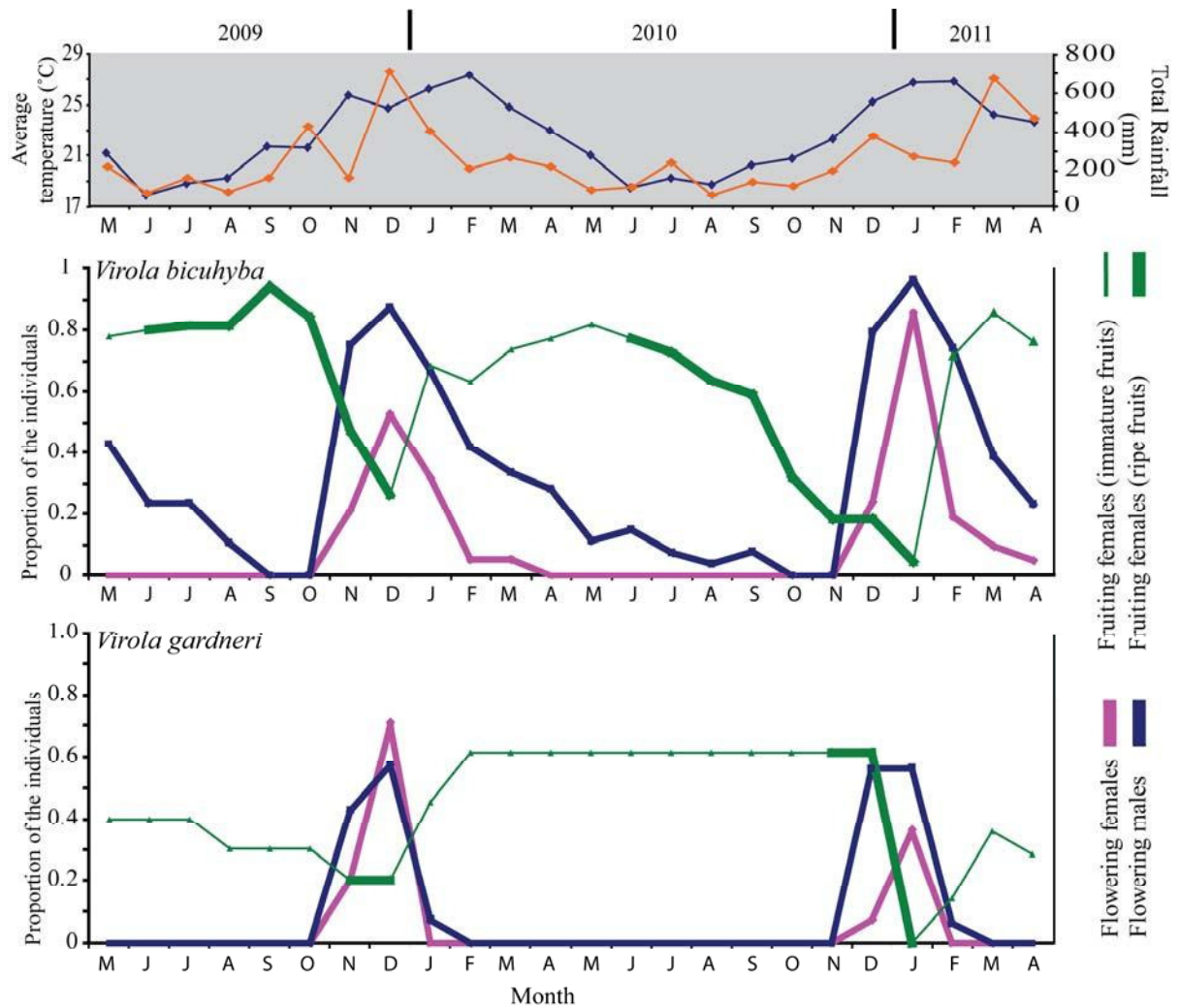


Figure 2. Average populational reproductive phenology for *Virola bicuhyba* and *V. gardneri* (dark graph: orange line – precipitation; blue line - temperature).

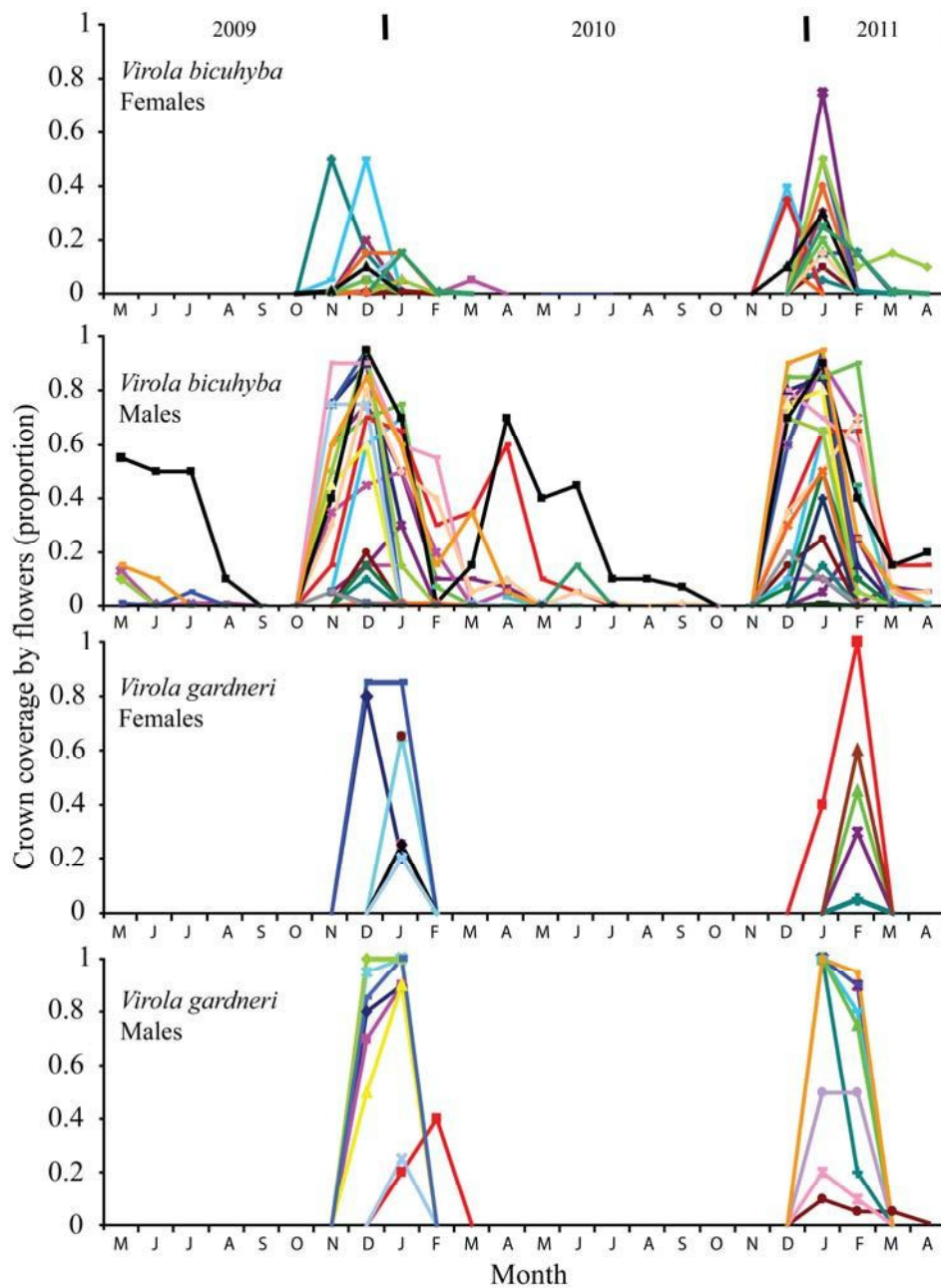


Figure 3. Monthly proportion of tree crown covered by flowers according to species and sex. Each line/color represents an individual.

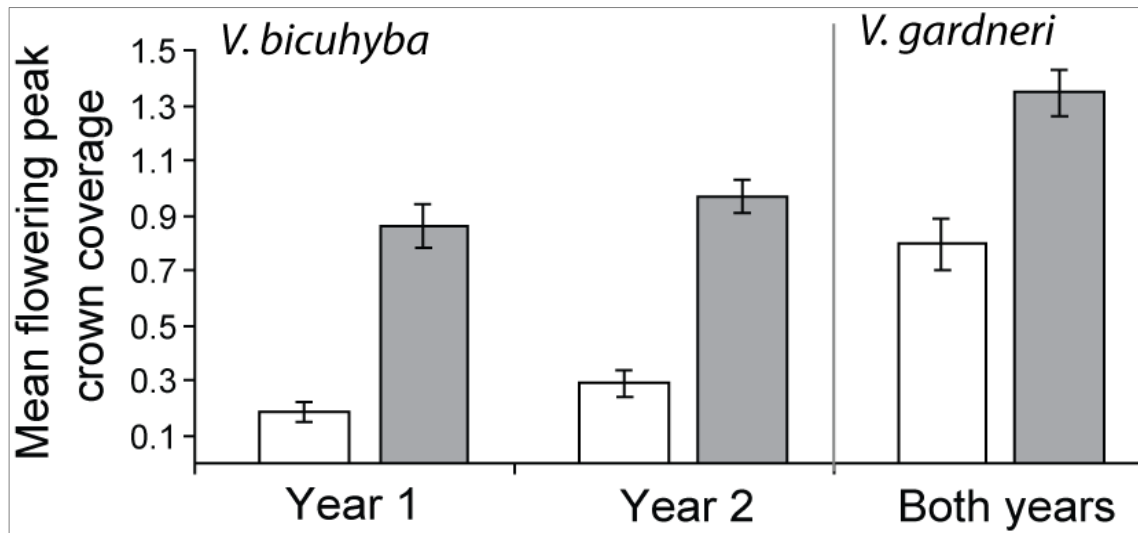


Figure 4. Average proportion of the crown area covered by flowers (\pm SE) during the first two months of the reproductive season (flowering overlap between the species) according to species and sex. As *V. gardneri* is biennial, each individual reproduces once during the study period and data for the two years was combined. Males are gray bars, and females are white bars.

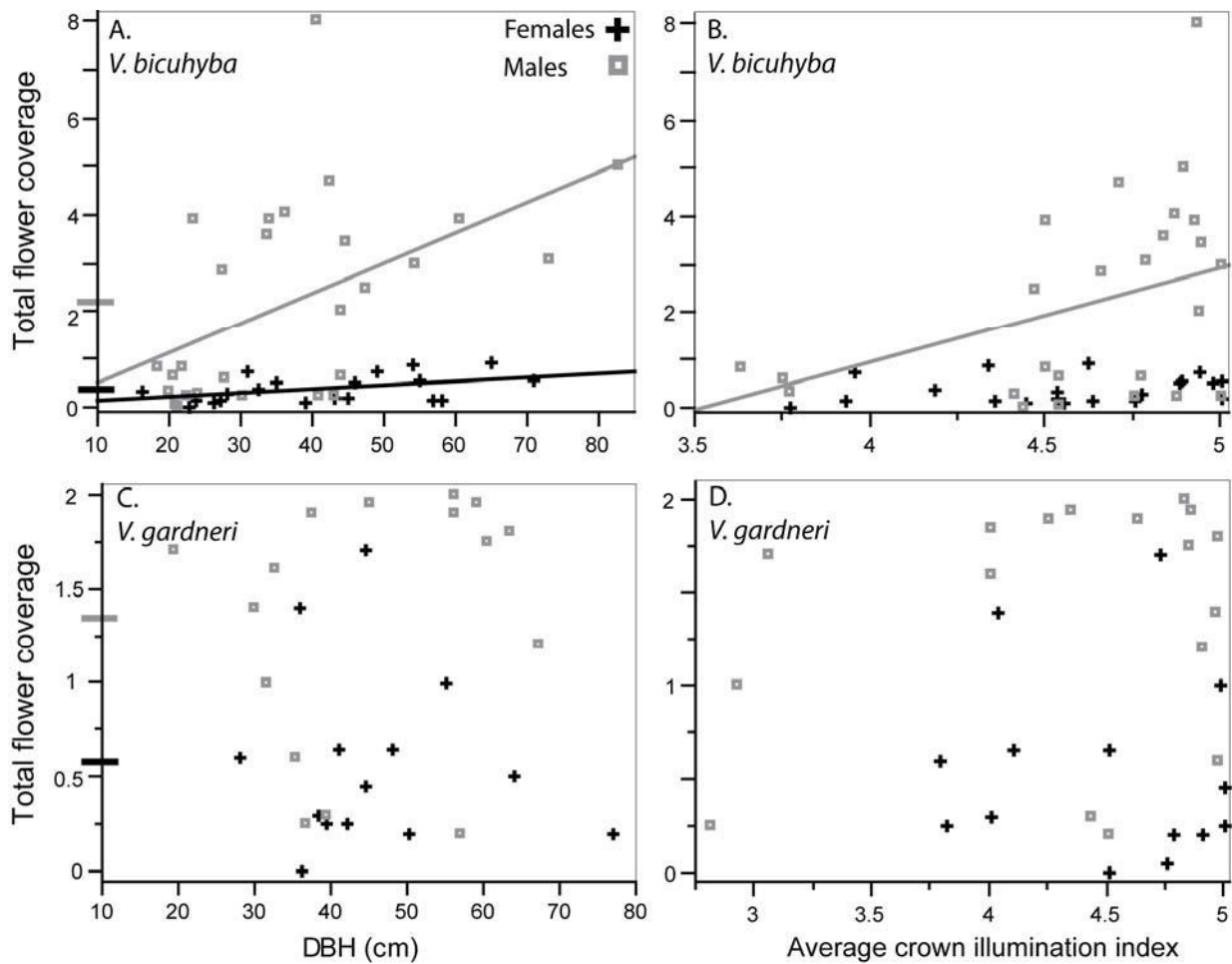


Figure 5. Relationship between total flower production, tree size (DBH), and average crown illumination. Fitted lines are shown only when significant relationships between the variables were found; A,C are for ANCOVAs, and B,D for simple regressions for each sex. Thick lines on the dependent axis are the mean coverage value for each sex.

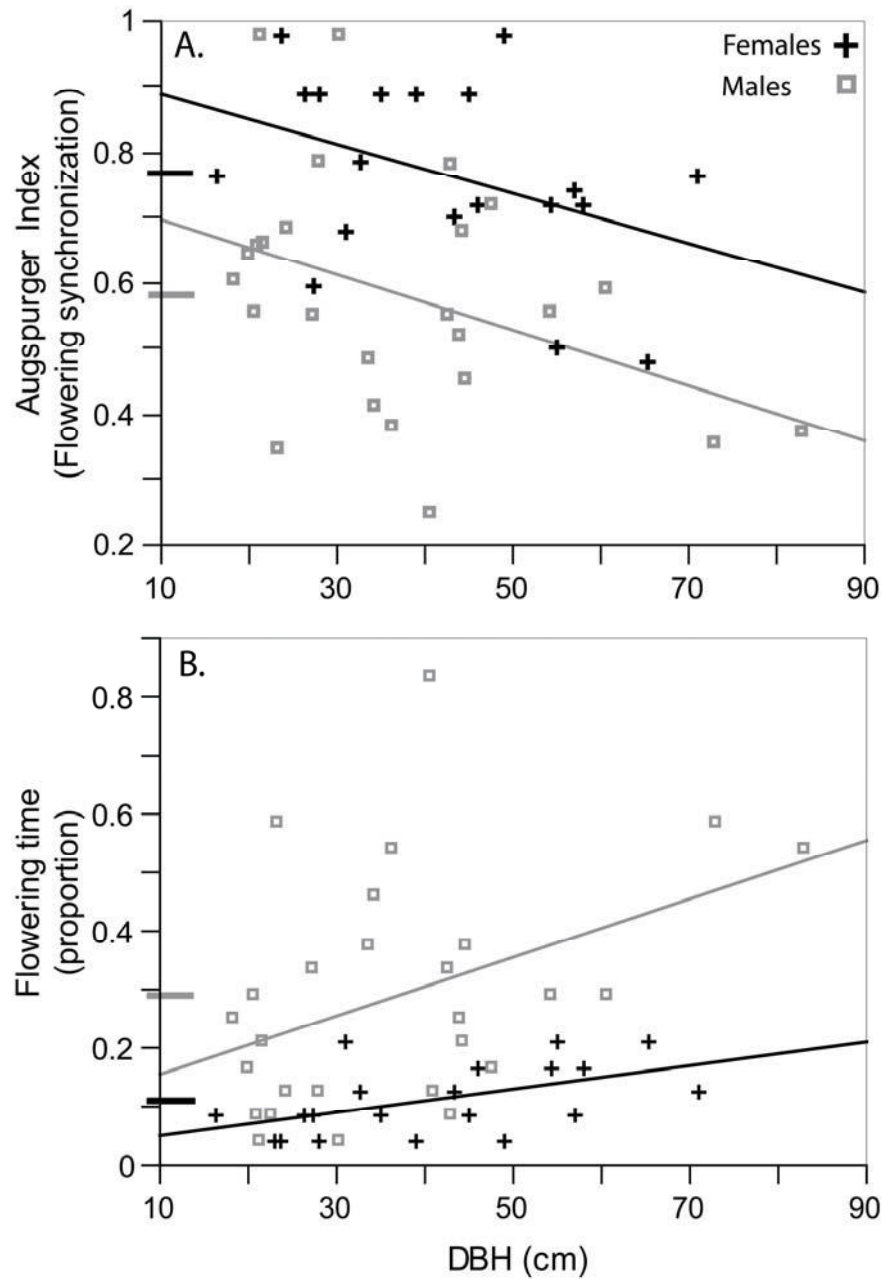


Figure 6. Relationship between tree size (DBH) and flowering temporal distribution according to the sex of the individuals for *Virola bicuhyba*. A) Flowering synchronization (Augspurger index); B) Proportion of the time producing flowers (in two years). Thick lines on the dependent axis are the mean value for each sex.

CHAPTER 2

Ecological significance of allometric relationships in dioecious tropical trees of the genus *Virola* (Myristicaceae) from Brazil and Costa Rica

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Abstract

Morphological adaptations can favor differential use of vertical space and resources in tropical forest. However, how tree morphology and allometry change among structurally different environments and related sympatric species is poorly understood. We compared the allometric characteristics of two pairs of dioecious species of the genus *Virola* (Myristicaceae) in two distant tropical forests in Brazil and Costa Rica. Our goal is to determine the way trunk and crown allometry vary in these related species in different environments, and how this variability can favor different use of the vertical space. We also tested for the effect of the sexual dimorphism in trunk and crown allometry. Tree height was highly different between the areas and trunk allometric characteristics were very similar among species, but initial height of reproduction was related with the canopy height. No sexual dimorphism in allometric characteristics was found. Crown size was highly correlated to trunk diameter and the scaling between crown size and trunk diameter markedly differed among species with implications for resource acquisition and structural stability. Our data shows great variation in crown size and strong conservatism in trunk structure at the genus level among sympatric pairs of species in structurally different and distant tropical rain forest. Crown variability can allow different use of the vertical space and light exposure having implications in the functional characteristics of these species.

INTRODUCTION

Resource allocation in plants imposes major tradeoffs on growth and habitat use strategies, integrating different selective pressures on morphological structures, their function, and their ecological role (Jacob, 2004; Valladares and Niinemets, 2007). In trees, the relationship between stem diameter and height determines the structural stability of aerial parts and the optimal distribution of leaf area (McMahon, 1973; King *et al.*, 2009). Shape and habitat use are interdependent and directly related with the physiology and energetic requirements of any plant species, determining the recruitment and establishment strategy (Poorter *et al.*, 2005; Poorter, 2006). Variation in the relationship between diameter and height across ontogenetic stages has been reported (e.g. King, 1996; Avalos and Fernández Otárola, 2010; King and Clark, 2011), and subtle differences in these factors expressed over the life cycle have important implications in the final shape and functional characteristics of the species (Bohlman and O'Brien, 2006; Kohyama, 1987).

Light is a limiting factor for plants in tropical forests (Chazdon and Pearcy, 1986; King, 1994) and plants adjust their shape to satisfy their energetic requirements (Schwinning and Weiner, 1998). Taller plants have access to higher light input, but also their energetic requirements for growth and maintenance increase (Givnish, 1988; King, 1990a). Trees with different vertical habitat use have been found to present different crown structure and diameter-height relationships as influenced by differences in light availability and their interception (King, 1990b, 1996). Tall canopies should impose stronger light limitation on lower strata and favour an increase in height in order to achieve the energetic requirements for reproduction (see Falster and Westoby, 2003), but how these different pressures affect trunk and crown allometric relationships in related species inhabiting different environments is not clear.

Related sympatric species are expected to show different allometric characteristics which will reflect the differential use of the vertical space, but phylogenetic relationships can limit allometric variability (Niklas, 1994). The role of phylogenetic relatedness in the determination of allometric strategies and related ecological characteristics remains poorly explored and should be tested in related groups of species (see Sposito and Santos, 2001; Yamada *et al.*, 2006). Studies about competition, coexistence, and use of the habitat can benefit of the application of an allometric approach (Schwinning and Weiner, 1998), and the understanding of morphological relationships could help explain the great diversity of tree species coexisting in high densities with limited resources (Kohyama, 1996).

Other factors could also impose physiological and ecological restrictions on the use of resources by groups of individuals inside populations. For example, different reproductive effort among the sexes in dioecious species (Armstrong and Irvine, 1989; Queenborough *et al.*, 2007), could affect their resource allocation and allometric relationships. However, sexual dimorphism in the allometry of plants has not been analyzed before. Differential reproductive effort is dependent on resource acquisition and allocation which is related with structure and shape (Schwinning and Weiner, 1998). If highly unequal reproductive effort exists between the sexes in tropical trees it is expected to be reflected in different strategies for the use of the space, probably affecting stem and crown allometry. If sexual dimorphism in allometry exists, the sexes in one species might have different ecological properties and act as functional different species in the community.

To understand the relationship between forest structure, plant morphology, sex, and use of the habitat in related sympatric species we studied the allometric characteristics of four dioecious species of the genus *Virola* (Myristicaceae) in two tropical rain forests areas; two species in Brazil and two in Costa Rica. We test for similarity in allometry within the

genus using species at dissimilar latitudes (separated for $\approx 5\,500\text{km}$) and in dissimilar and highly diverse tropical rain forest. We tested (1) whether differences in allometric relationships among ontogenetic stages (juveniles and adults) exist, (2) whether these congeneric species differ in their trunk and crown allometry, and (3) whether sexual dimorphism in allometric relationships is present. We also determine the initial reproductive size of each species and its relationship to canopy height. We expect crown and trunk differences among the species sharing the same habitat as adaptations for differential use of the space, and between the environments in response to their structural differences (e.g. different canopy height). We also expect a direct influence of canopy height on initial reproductive size. Finally, we discuss the effect of mechanical and ecological factors on stem and crown allometry in these species.

MATERIALS AND METHODS

Study Site

This study was conducted in two neotropical rain forest sites, one in the southeastern coast of Brazil, and the other in southwestern Costa Rica. In Brazil we worked in the Atlantic Rainforest near the city of Ubatuba, São Paulo. Our sampling site is located on middle elevations (250–450 m a.s.l.) of the Serra do Mar mountain range, in the Fazenda Capricórnio area (23°22'S, 45°05'W). The vegetation at this site is classified as tropical moist evergreen forest (Holdridge 1947), or submontane rain forest (Oliveira-Filho & Fontes 2000). Up to 206 species/ha ($> 4.8\text{ cm DBH}$) have been reported, with the families Myrtaceae and Rubiaceae being the most abundant and diverse (Gomes, Bernacci & Joly 2011; Rochelle *et al.* 2011). Annual temperature varies between 18 and 27 °C. Annual

precipitation can reach 3200 mm, with an average of 2918mm for the period 2008-2010 (Ubatuba climatic station, IAC). The total sampled area was 10 ha of primary forest, using as a starting point the plots I and J of the Biota Functional gradient project (FAPESP). A detailed description of soil physical and chemical characteristics, and aboveground biomass in the area is present in Alves *et al.* (2010).

In Costa Rica, we worked on the Osa Peninsula, at Los Charcos de Osa Station (8°40'24N, 83°30'30W) at 50-150 m a.s.l.. Average temperature varies between 21 and 33.5 °C and annual precipitation reaches 4940 mm (Río Claro weather station, Instituto Meteorológico Nacional). The area encompasses 4 ha along a gradient of forests under selective logging left to natural regeneration, and primary forests.

Studied species

The genus *Viola* (Nutmeg trees, Myristicaceae) includes approximately 46 species in America. All species are dioecious, and restricted to the neotropics. In Brazil, we worked with *Viola bicuhyba* Schott ex Spreng. and *V. gardneri* (A. DC.) Warb., both endemic to the Atlantic rain forest. *Viola bicuhyba* is found from the states of Bahia (11°30'S) to Rio Grande do Sul (29°40'S), whereas *V. gardneri* is distributed between the states of Pernambuco (7°30'S) to São Paulo (25°15'S; Rodrigues 2011). In Costa Rica, we worked with *V. surinamensis* (Rol. ex Rottb.) Warb., and *V. koschny* Warb. Both species are distributed from Costa Rica to the Brazilian Amazon. *Viola surinamensis* is also found in Trinidad and Tobago and the Lesser Antilles (Jiménez 2007).

Tree measurements

For all individuals with a stem height > 3 m we measured: a) stem height using trigonometry; angles were measured with a Suunto Tandem clinometer; b) diameter at breast height (DBH) measured at approximately 1.5 m above the ground, or above the topmost buttresses when present. Crown amplitude was measured only for reproductive individuals in three directions: a) horizontal diameter 1 (measured in the direction of the prevalent terrain slope); b) horizontal diameter 2 (perpendicular to the previous measurement) and c) vertical length (vertical distance between the lowest branch and the top of the crown). Horizontal crown amplitude was measured by projecting the extremes of the crown periphery on the ground. The distance between the extremes was measured and corrected for terrain slope. Trees exhibiting structural damages (13 individuals) or morphological anomalies (i.e., development of a second stem, 3 individuals) were excluded from the analysis.

Sex and reproductive status were determined from monthly phenological observations of each individual for a period of two years in Brazil (see Chapter 1 for details), and observations during the flowering season for three years in Costa Rica. No sex change was observed and has not been reported for the genus before. The sex of non-reproductive individuals is unknown.

Data Analysis

We analyzed the relationship between Log DBH and Log height for the four species using standardized major axis (SMA) regression models. SMA regression is adequate when the objective is to summarize the relationship between two variables, as is the case in allometric studies (see Warton *et al.* 2006). We compared for all the species the slope of the fitted line, the elevation of these fitted lines and its shift (displacement) along the common

axis. For example, considering the stem height/DBH relationship, different slopes across species indicate different increments in height for additional increments in DBH. Also, different elevations indicate that individuals of different species with similar DBH have different heights. Finally, shifts along the common axis indicate that the relationships between height-DBH are the same across species but some species are taller than others (Warton *et al.* 2006). If differences among the species were found, pair-wise comparisons were done in order to establish the specific pairs of species that differed. The slope among the lines was compared with the likelihood-ratio test, and the elevation and shift with the Wald test.

To determine the best model following the species ontogeny we analyzed the regression between DBH and height for juveniles and adults, jointly and separately. We also looked for differences among sexes for reproductive individuals in the Brazilian species, for which the sample size was large enough. Analyses were performed using R (R Development Core Team 2011). To fit the SMA regression model and perform the pair-wise comparisons we used the R package *Smatr* (Warton *et al.* 2011)

Allometric models have been proposed in order to study trunk dimensions and mechanical stability. McMahon & Kronauer (1976) proposed three models for the relationship between tree height and diameter based on a cylindrical shape and their security margin against structural collapse. The stress similarity model considers a constant maximum bending stress throughout the entire trunk height, that is, height scales to the square root of the diameter (height \propto DBH^{1/2}, slope=0.5). The elastic similarity model stipulates the same margin of safety against mechanical failure for structures of different sizes, the maximum height an erect cylinder can reach is proportional to its diameter to the power of 2/3 (height \propto DBH^{2/3}, slope=0.67). Finally, in the geometric similarity model stem

height changes as a simple function of diameter (height \propto DBH, slope=1). We tested adjustment of the allometric relationship between height and DBH to these models of stress, in order to analyze their fit to the genus *Virola*.

Crown structure was analyzed using principal components analysis (PCA) for each species to reduce the three variables related to the crown dimensions (horizontal diameter 1 and 2, and vertical length) to a single variable which represents the crown size. This variable corresponds to the values on the first axis of the PCA (hereafter PC1). We did a PCA specific for each species which was used to analyze crown structure relationships within the species and an additional PCA combining all the species in order to have a common scale to make comparisons among them. For all cases the PC1 explained a high proportion of the variance ($> 54\%$) and the three variables used had very similar positive eigenvectors for this axis (Table 1), being an adequate descriptor of crown size (a high value in this scale indicates a large crown in the vertical and horizontal plane). Crown shape is variable and no geometric figure is in general applicable for all individuals without including great error which makes this PCA approach better than using an estimation of the perimeter. The combination of the three crown variables into a single variable gives a better analysis of crown dimensions than the use of a single crown measure, as it considers the variation in size in a tridimensional space. The influence of DBH and stem height on crown structure (PC1 specific for each species) was explored using GLM models (stem height was a covariate). The allometric relationship between crown size and DBH was compared among the species using SMA regressions using the PC1 which combines the data for all species in order to make comparisons among the fitted lines. Finally, we used logistic regressions models with a binomial distribution to determine the likelihood of transition

from juvenile to reproductive stages according to the height of the individuals for all four species.

RESULTS

Adults and juveniles scaling relationships

Maximum height reached differed between the Brazilian species; *V. bicuhyba* can reach higher sizes than *V. gardneri*, whereas maximum height was very similar between the Costa Rican species (Appendix I - Table S1). No change in the allometric relationship between DBH and height was detected from juveniles to adults. The model combining these ontogenetic stages had the best adjustment to the data for all the species and was used instead of less explanatory models specific for each stage. The slopes and elevations of the regression lines between DBH and height were very similar among the four species and highly significant in all cases, explaining a high proportion of the variance ($\geq 92\%$, Table 2). Significant differences were found between these slopes (Likelihood ratio: 25.89, $df=3$, $p<0.0001$), but pair-wise comparisons showed that this difference was exclusive to *V. gardneri*, which differed from all other species (Table 2, Fig. 1). *Virola bicuhyba* and *V. surinamensis* adjusted to the elastic similarity model, but *V. gardneri* slope is between the stress and elastic similarity models, and *V. koschnyi* is between the elastic and geometric similarity allometric models (Table 2). A general model for the genus *Virola* combining data for the four species match exactly the elastic similarity model (slope = 0.67, $95\%CI=0.65-0.69$, $r^2=0.93$, $p<0.0001$, $n=254$, Appendix I - Fig. S1).

As *V. gardneri* presented a different slope it was excluded for the elevation and shift analyses. Elevation and shift along the common axis of the fitted lines differed among

species (Wald: 13.31, df=2, p=0.001; Wald: 21.03, df=2, p<0.0001, respectively), *V. bicuhyba* being lower than the Costa Rican species in both cases (Fig. 1).

Crown structure

The crown variables measured were correlated (Appendix I - Table S2) and show that the average horizontal crown length is highly correlated with DBH, but crown vertical length is more correlated with stem height. The GLM models show that the DBH has a large significant effect on crown structure, but height has a significant effect only for *V. bicuhyba* (Table 3). The slope of the scaling relationship between crown size (PC1) and DBH was different among the species (Likelihood ratio =12.3, df=3, p=0.006), this difference being among *V. koschnyi* and the other species (Table 4, Fig. 2), thus the former was excluded from the other analyses. The slope between *V. koschnyi* and *V. gardneri* was not significantly different. The elevations of the fit lines also differed (Wald=50.9, df=2, P<0.0001), and the pair-wise comparisons showed that *V. gardneri* is different from the other species. *Virola surinamensis* also showed a significant shift in their position along the common axis in relation to the other species (Wald=19.05, df=2, P<0.0001).

Reproductive size

The logistic regressions showed that tree height has an important effect on reproductive transition in these species ($\chi^2=202.9$, df=257, p<0.0001), and the initial reproductive size was different among the species ($\chi^2=61.3$, df=254, p<0.0001). *Virola bicuhyba* and *V. gardneri* showed the smallest initial reproductive size with an approximate height of 17m for both species. *Virola koschnyi* starts its reproduction at approximately 23m and in *V.*

surinamensis the reproductive activity starts when 32m are attained (Fig. 3). The slope of the logistic regressions did not vary among species ($\chi^2=1.2$, df=251, p=0.75).

Sexual dimorphism

No sexual dimorphism in the allometric relationships was observed in the Brazilian species. No differences in the slope, elevation, and shift for the relationship between DBH and height were found (Table 5, Fig. 4A,B). No differences were found either when the relationship between crown size (PC1) and DBH was analyzed (Table 5, Fig. 4C,D).

DISCUSSION

Ontogeny and scaling relationships

Plant establishment strategies are diverse and variable among species. Allometric variation between juveniles and adults have been frequently found in tropical trees (e.g. King 1996; King & Clark 2011), probably related with a strategy of differential resource allocation since part of the resources used for growth in juveniles are allocated to reproduction in adults. Nevertheless, the species here studied share the same allometric strategy among juveniles and adults, indicating that the investment in stem width in relation to height is constant along these stages.

All four species studied showed very similar architecture and use of the habitat as juveniles, with the same establishment strategy in the two distant and structurally complex tropical rain forests studied. The ecological characteristics of saplings of the four species are similar. All are shade tolerant and gaps can favor the establishment of the individuals as has been reported for *V. surinamensis* by Howe (1990), but they seem not to be adapted to

excessive illumination levels (Fetcher *et al.* 1987). The similarity among species can be related to phylogenetic and architectonic restrictions in order to maintain structural stability (McMahon & Kronauer 1976). Mean wood density values are similar among the four species (Chave *et al.* 2006), and could help explain their similar allometric relationships and according to Gelder *et al.* (2006) this property is related to the life strategy of tropical trees.

The match between the model combining the four species and the elastic similarity model also gives insights about this relationship. As a genus, *Virola* supports this theoretical model which is related with mechanical restrictions on tree growth. Nevertheless, each species can deviate to some degree from this pattern. Data from tropical trees show great variability in the adjustment to these theoretical models and many species do not adjust to any of them as is the case of *V. gardneri* and *V. koschnyi* in this study. For example, in the Brazilian Atlantic forest Alves & Santos (2002) showed that three sub-canopy and canopy species adjusted to the elastic similarity model, while an emergent species adjusted to the stress similarity model. In other study five out of eight tropical tree species adjusted to the elastic similarity meanwhile the others do not adjust to any model (O'Brien *et al.* 1995).

As theoretical model, elastic similarity seems to be the one with more applicability for tropical trees. The scaling relationship predicted by this model describes the maximum theoretical limit against mechanical failure (buckling limit). Competition for light related with the capacity of increase in height may create a strong selective pressure for many species, and favors the achievement of this theoretical maximum scaling relationship.

Different models can offer the best adjustment for different ontogenetic stages in the same species (e.g. Niklas 1995; Avalos & Fernández Otárola 2010). King (1996) found a change in trunk allometry at the height of 2.5m for *V. sebifera* in Costa Rica. Scaling changes are more probable on small ontogenetic stages as tissue composition and proportional importance can vary during plant development, and the mechanical properties of each tissue are different (Niklas 1993). However, individuals lower than 3 m are not considered in this study and these changes if present were not sampled.

Virola gardneri is the only species showing a partially different use of the habitat, during its reproductive size. This is clear for the use of the canopy in this species which can be influence the allometric differences observed. *Virola bicuhyba* invest more resources in vertical growth than in widening of the trunk, occupying a higher stratum than *V. gardneri*, but the two species are main components of the canopy in the Atlantic rain forest (Guillerme *et al.* 2004; Rochelle *et al.* 2011). The Costa Rican species develop slender stems in order to reach the taller canopy where they are present, as has been found for other tropical tree species (Poorter *et al.* 2003), but do not differ in their use of the canopy, and present similar trunk allometry.

Canopy height was related with the initial reproductive size and stem height was the best descriptor of the reproductive stage. Reaching a certain illumination threshold in the forest vertical structure probably triggers the reproductive activity of these trees, but the light requirements can be different among the species and dependent on particular energetic demands. The lower canopy in the Atlantic forest (20.7 ± 5.7 m SD at the studies area; pers. obs.) explains the shared low reproductive size in *V. bicuhyba* and *V. gardneri*. A taller canopy in Costa Rica (27.5 ± 15.1 m SD; pers. obs.) gives rise to a more complex vertical

structure in the forest and trees under these conditions should grow taller in order to achieve the light input necessary for their reproductive activities.

Crown structure

Light availability in tropical forest increases vertically from the floor to the canopy and taller trees have the possibility to extend their crowns to intercept more light. The counter-argument can also be made; smaller trees should need larger crowns in order to intercept more light in the lower vertical forest strata where light irradiance is limited. Crown structure seems to be directly related with the ecological strategy of each species (King 1996). Some studies have reported that crown size in forest species is negatively related with tree height, but not with light requirements (Poorter *et al.* 2003). Various studies have reported smaller crowns and thinner trunks on canopy species than in equivalent size individuals of species adapted to lower canopy strata (King 1996; Osunkoya *et al.* 2007; Sterck & Bongers 1998). These characteristics are supposed to reduce the cost of vertical growth in tall species (King & Clark 2011). Our data does not support this pattern when analyzed at the intrageneric level. In Brazil the taller *V. bicuhyba* presented larger crown than *V. gardneri* along all their reproductive size range and in Costa Rica *V. koschnyi* and *V. surinamensis* occupy the same forest vertical strata, but differ in their crown size.

Crown expansion cannot be attained without a mechanical tradeoff between tree height and stem diameter, the first decreasing and the second increasing stability (Gelder *et al.* 2006). Tall trees are subject to more extreme environmental conditions as wind speed increases with height (Niklas 2000), and small crowned trees would be more stable offering less mechanical pressure under extreme conditions. In fact, *V. koschnyi* presents the highest trunk (height-DBH) scaling slope, surpassing the elastic similarity model, but this species

presents the smallest crown. Our data showed that crown size is better explained by DBH than height. This is related with the mechanical stability of the trees as the buckling safety factor is positively related with DBH (Gelder *et al.* 2006). Wide stems can support large crowns while conserving structural safety. Only for the *V. bicuhyba* stem height influences crown size. *Virola bicuhyba* is a canopy species in the studied area, but very large individuals are emergent and probably experience some crown structural modification. Crown size increments with increasing in size has been found for emergent species in Costa Rica (King & Clark 2011). In fact *V. bicuhyba* is the species with the largest crown among the four studied species.

Sexual dimorphism

As far as we know, this is the first study evaluating sexual dimorphism in the allometric relationships of tropical trees. Sexual dimorphism in tree structure would be expected in cases where differential use of resources between the sexes exists and different structure would favor differential resource acquisition and use. The lack of structural and allometric differences between the sexes for the two Brazilian species here studied and for any of the variables tested does not support the existence of different ecological strategies and differential use of resources between the sexes in this dioecious tropical genus.

Concluding remarks

Our study supports the idea of the existence of a phylogenetic base at the genus level for the allometric characteristics of these four species, nevertheless, a strong capacity to adapt to the characteristics of each environment is clear. Phylogeny is not a restriction to adaptation and enough variability is present to allow a slightly different use of the habitat

for these species and its coexistence. Our data helps explain the mechanisms allowing the sympatric coexistence of many congeneric species in tropical forest. The idea of ecological convergence in the allometric characteristics cannot be discarded, but the wide geographic separation between the study sites and the different characteristics of the studied forest make more difficult this possibility.

Crown characteristics were the most variable among the species and future studies should focus in the ecological and functional significance of crown structure for congeneric species. Also tridimensional crown variation during the ontogeny of the individuals should be studied in order to help explain the effect of their variability in the life history of the species.

The absence of sexual dimorphism in allometric characteristics give insights about similar requirement and use of resources for these dioecious species and conflicts with the expected differences based on reports of unequal reproductive investment between the sexes. This supports the necessity of improvements in the quantification of reproductive investment between the sexes in dioecious species (see Chapter 1). The existence of branching and leafing patterns between the sexes should be also considered in future studies concerning structural sexual dimorphism in dioecious species.

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Table 1. Eigenvectors and other parameters for the first axis of five principal components analyses for the crown variables measured. The first PCA combines the individuals of the four species, and the other ones are specific for each species of *Virola*.

Variable	All species	Species specific			
	combined	<i>V. bicuhyba</i>	<i>V. gardneri</i>	<i>V. koschnyi</i>	<i>V. surinamensis</i>
Horizontal diameter 1	0.58	0.59	0.56	0.65	0.58
Horizontal diameter 2	0.59	0.61	0.56	0.57	0.59
Vertical length	0.56	0.53	0.60	0.49	0.56
n	105	41	30	16	18
Eigenvalue	2.35	2.38	1.93	1.62	2.45
Variance explained (%)	78.40	79.51	64.61	54.01	81.56

Table 2. Standardized major axis (SMA) regression slope for the fit between DBH and height for individuals of four species of *Virola* in Brazil and Costa Rica. Values in parentheses are the 95% confidence intervals. $P \leq 0.0001$ in all cases.

Species	n	Slope	r^2
<i>V. bicuhyba</i>	108	0.68 (0.64-0.72)	0.92
<i>V. gardneri</i>	78	0.59 (0.56-0.62)	0.94
<i>V. koschnyi</i>	31	0.72 (0.68-0.77)	0.97
<i>V. surinamensis</i>	37	0.68 (0.63-0.74)	0.94

Table 3. Generalized linear model results for the influence of DBH and stem height on crown size (axis 1 from a PCA specific for each species) for four species of *Virola* in Brazil and Costa Rica. Each species was tested independently.

Species	Factor	χ^2	p
<i>V. bicuhyba</i>	DBH	38.66	<0.001
	Height	9.88	0.002
	DBH*Height	1.02	0.31
<i>V. gardneri</i>	DBH	25.76	<0.001
	Height	1.24	0.27
	DBH*Height	1.87	0.17
<i>V. koschnyi</i>	DBH	28.69	<0.001
	Height	0.014	0.90
	DBH*Height	1.06	0.30
<i>V. surinamensis</i>	DBH	15.63	<0.001
	Height	2.68	0.10
	DBH*Height	0.004	0.95

Table 4. Standardized major axis (SMA) regression slope for the fit between DBH and crown size (PC1) for four species of *Virola* in Brazil and Costa Rica. Values in parentheses are the 95% confidence intervals. $P < 0.0001$ in all cases. For sample size see table 2.

Species	Slope	r^2
<i>V. bicusbyba</i>	0.09 (0.08-0.10)	0.81
<i>V. gardneri</i>	0.07 (0.06-0.09)	0.67
<i>V. koschnyi</i>	0.06 (0.05-0.07)	0.89
<i>V. surinamensis</i>	0.08 (0.07-0.10)	0.89

Table 5. Standardized major axis (SMA) regression slopes for the fit between height or crown size (PC1) and DBH for males and females of two sympatric species of *Virola* in Brazil. Values in parentheses are the 95% confidence intervals. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$, ns – non significant.

Species		Height vs. DBH			Crown Size (PC1) vs. DBH		
		n	Slope	r^2	n	Slope	r^2
<i>V. bicusbyba</i>	Males	23	0.62 (0.46-0.84)	0.55***	22	0.10 (0.09-0.12)	0.85***
	Females	22	0.61 (0.41-0.91)	0.24*	20	0.08 (0.06-0.10)	0.77***
<i>V. gardneri</i>	Males	15	0.65 (0.40-1.04)	0.32*	14	0.11 (0.08-0.16)	0.66**
	Females	15	0.54 (0.32-0.91)	0.16 ^{ns}	15	0.09 (0.06-0.13)	0.65**

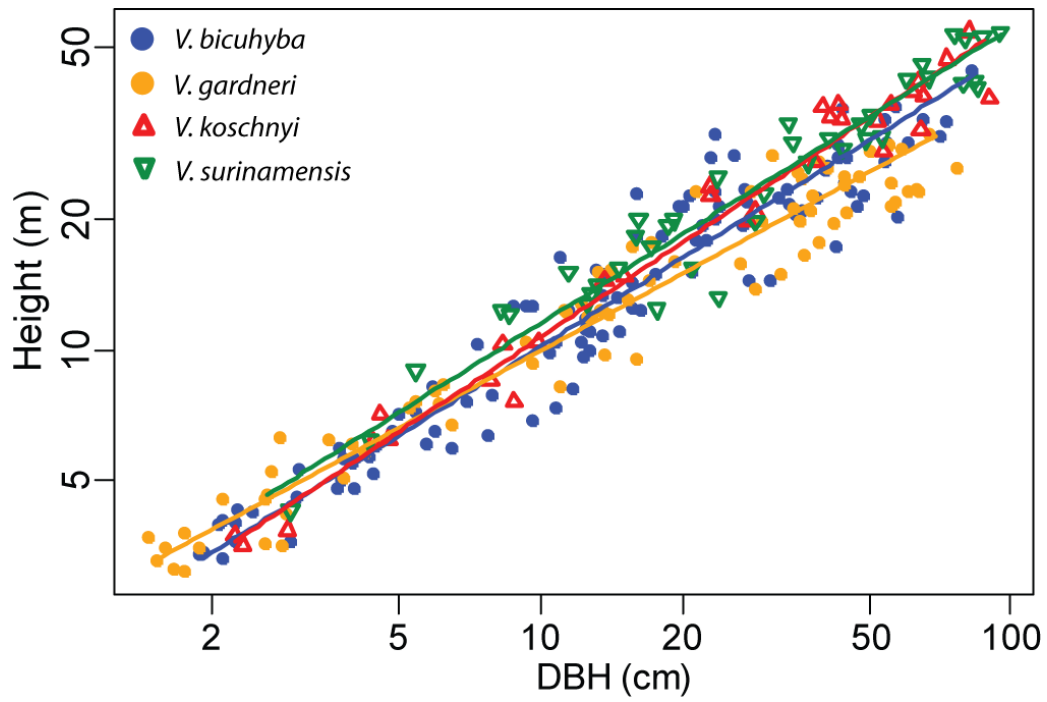


Figure 1. Fitted scaling relationship between log DBH and log tree height using SMA regression models for juvenile and reproductive individuals of species of *Virola* in Brazil (dots) and Costa Rica (triangles).

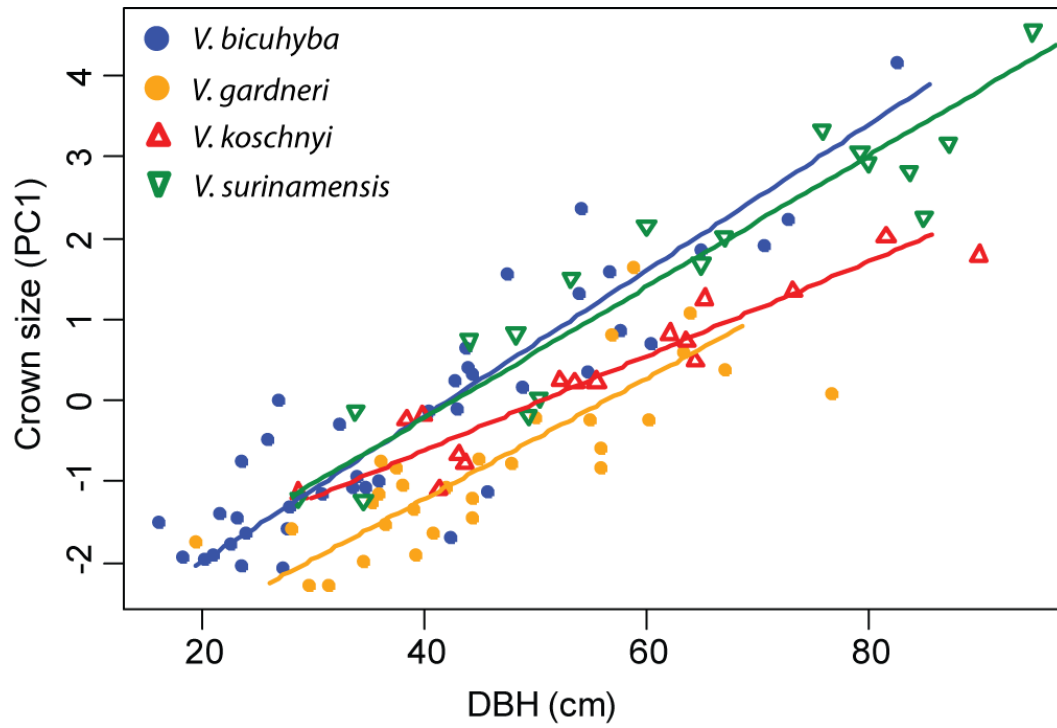


Figure 2. Fitted scaling relationship between DBH and crown size (first ex of a PCA combining three crown dimension variables for the four species) using SMA regression models for reproductive individuals of various *Virola* species in Brazil (dots) and Costa Rica (triangles).

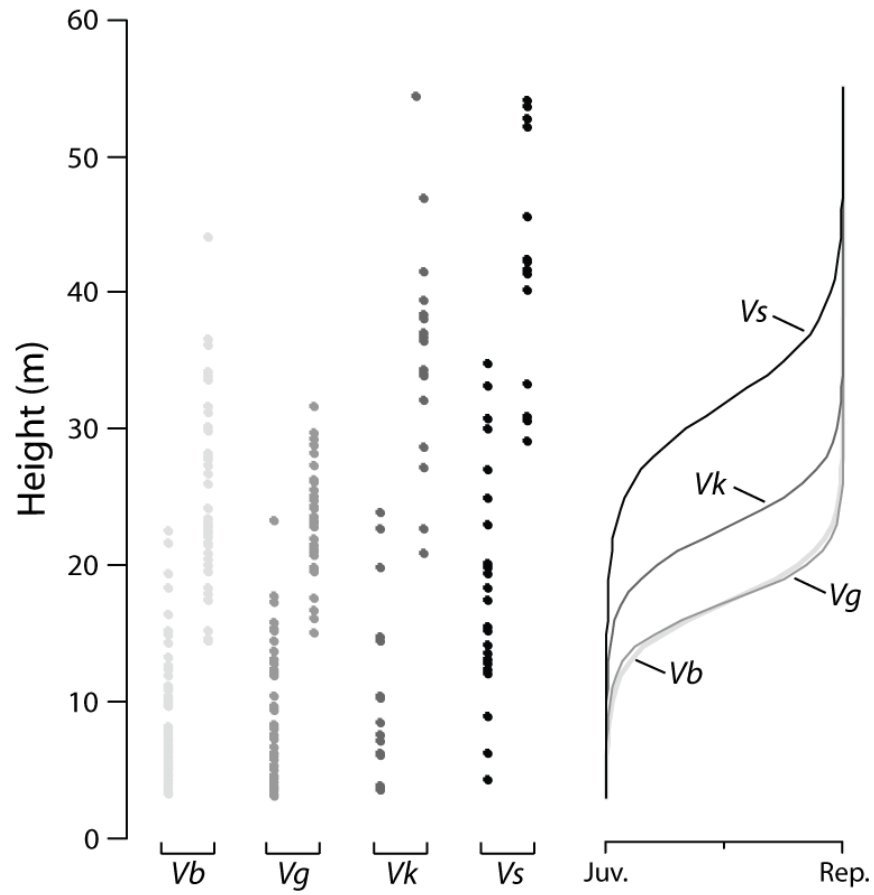


Figure 3. Transition from juvenile to reproductive in relation to height for four species of *Virola* in Brazil (Vb and Vg) and Costa Rica (Vk and Vs). For each species points to the left are juveniles and points to the right are reproductive individuals. Fit lines for logistic regressions are depicted; $p < 0.0001$ for all cases. Vb – *V. bicuhyba*, Vg – *V. gardneri*, Vk – *V. koschnyi*, and Vs – *V. surinamensis*.

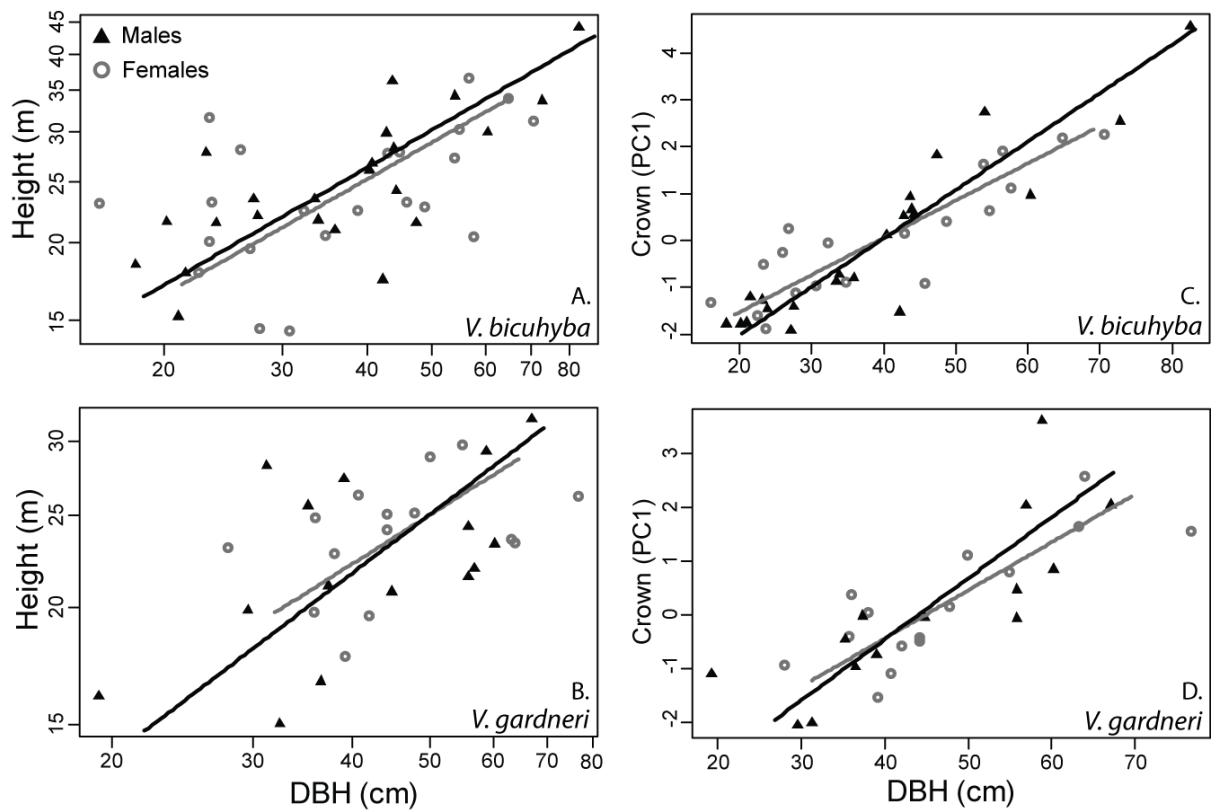


Figure 4. Fit lines for the scaling relationship between DBH and tree height (in log scales) and for the relationship between crown (PC1) and DBH (normal scale) using SMA regression models for reproductive individuals of *Virola bicusbyba*, and *V. gardneri* by sex.

APPENDIX I

Table S1. Maximum dimensions measured for stem and crown variables of four species of *Virola* in Brazil (Br) and Costa Rica (CR).

Species	Height (m)	DBH (cm)	Average crown diameter (m)	Crown vertical length (m)
<i>V. bicuhyba</i> - Br	44.1	82.6	17.7	25.1
<i>V. gardneri</i> - Br	31.7	76.7	12.8	14.2
<i>V. koschnyi</i> - CR	54.5	90	15.6	21.6
<i>V. surinamensis</i> - CR	54.2	94.8	17.8	29.3

Table S2. Correlation among the morphological variables of four species of *Virola* in Brazil (Br) and Costa Rica (CR). One matrix is shown for each species. Variables are: Stem height (Height), DBH, mean crown horizontal diameter (CHD), and crown vertical length (CVL).

Species	Variable			
		DBH	Height	CVL
<i>V. bicuhyba</i> Br	Height	0.68		
	CVL	0.66	0.91	
	CHD	0.89	0.60	0.61
<i>V. gardneri</i> Br	Height	0.47		
	CVL	0.63	0.63	
	CHD	0.78	0.23	0.59
<i>V. koschnyi</i> CR	Height	0.72		
	CVL	0.51	0.72	
	CHD	0.92	0.60	0.32
<i>V. surinamensis</i> CR	Height	0.87		
	CVL	0.81	0.86	
	CHD	0.92	0.79	0.72

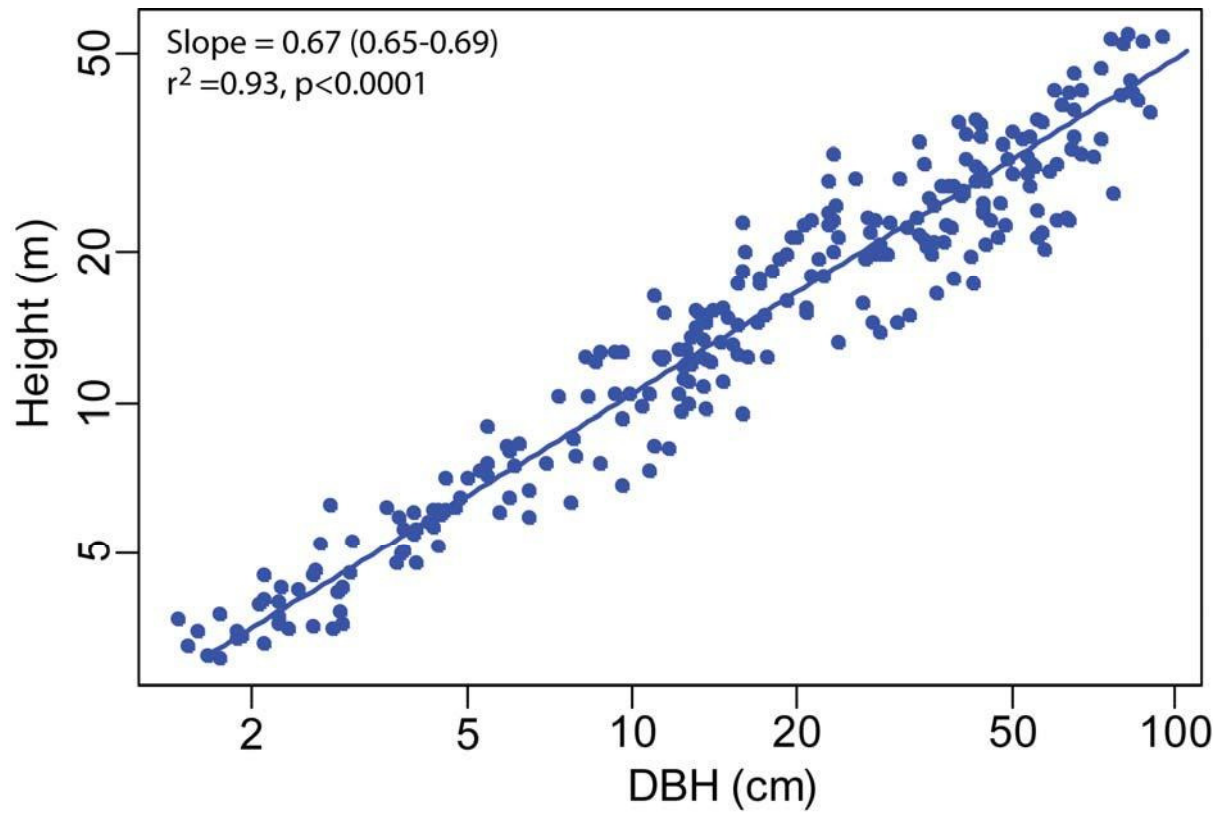


Fig. S1. Fit line for the scaling relationship between DBH and tree height (log scales) using SMA regression models for the combined data of four *Virola* species from Brazil and Costa Rica. Data in parentheses are 95% CI.

CHAPTER 3

Spatial organization of two sympatric tree species of *Virola* (Myristicaceae) in the Brazilian Atlantic Rain forest

Mauricio Fernández Otárola, Marlies Sazima, and Vera Nisaka Solferini

Abstract

Spatial distribution in plants is dependant of processes acting during ontogeny, from seed dispersal to later thinning effects mediated by intra and interspecific interactions and environmental factors. Intrapopulation genetic analyzes facilitate the understanding of the mechanisms responsible for the species spatial organization, as the spatial genetic structure reflects the effect of ecological processes. To determine the relationship between fruiting phenology, seed dispersal, and secondary intra and interspecific interactions, and their effect on the organization of populations of tropical trees we analyze the spatial and genetic structure of two dioecious species (*Viola*, Myristicaceae), using univariate and bivariate point pattern analysis and microsatellites genetic data for fully sampled, sympatric populations. The species differed in their habitat tolerances. *Viola gardneri* is predominantly found in highly illuminated hill tops, while *V. bicuhyba* is homogeneously distributed in the area. A general pattern of spatial aggregation similar between the species exist. Adults are clumped at distances of 20-40 m; large juveniles in both species are clumped over distances up to 80 m from each other; small juveniles are significantly clumped at very short distances (up to 10 m). For all cases *V. gardneri* showed higher degree of aggregation than *V. bicuhyba*. Bivariate analyzes showed no interaction between species, and between sexes in their spatial organization. Small and large juveniles are aggregated under the crown of reproductive individuals in *V. gardneri*, but to larger distances in *V. bicuhyba*. Despite similar patters of clumping, the genetic structure analyzes showed strong genetic structure in *V. gardneri* for all the size classes, while no clear genetic structure is observed in *V. bicuhyba*. Similar patterns of spatial organization are related to similar dispersers of the species, but the much larger seeds in *V. gardneri* and their concentrated season of fruit ripening in comparison to *V. bicuhyba* explain their stronger aggregation and genetic structure. Fruiting phenology, fruit characteristics, and seed dispersers' assemblage explain the observed spatial and

genetic organization in these sympatric congeneric species. The combination of spatial and genetic analyzes are a powerful tool to disentangling the processes affecting populations organization, their ecological characteristics, and their interaction with other species.

INTRODUCTION

Tropical forests are the most diverse ecosystems in the world and trees define its structure and complexity with hundreds of species coexisting in small areas in these environments (Phillips *et al.* 1994; Turner 2001). The basic ecological question when the spatial organization of individuals within populations of tropical trees has been analyzed is if a random process can explain their distribution. Previous studies have found a general tendency for aggregation in tree species, consistent when individuals of different sizes were compared (Hubbell 1979; Condit *et al.* 2000; Li *et al.* 2009).

The factors acting on the distribution of a species are complex and vary along the ontogeny. The shape of the seed shadow together with seed dispersal define the initial distribution and is highly variable among species (Willson 1993). Secondary processes acting on the dispersed seeds alter the original dispersal pattern with major consequences on the subsequent ontogenetic stages (Nathan & Muller-Landau 2000): density dependence mortality (Janzen 1970; Connell 1971; Comita & Hubbell 2009), pathogens (Mangan *et al.* 2010), soil characteristics (Clark *et al.* 2002), light and other physiological requirements (Dawson & Ehleringer 1993), all have important effects on the population's final spatial organization and genetic structure.

Intrapopulational genetic analyzes can improve the understanding of the mechanisms responsible for their spatial organization. Spatial genetic structure exists when the genotypes are not homogeneously distributed in the area, forming clumps of related individuals. The factors

responsible for this process are diverse, but the patterns of seed dispersal are important in many cases (Vekemans & Hardy 2004).

Of special interest are the intra and interspecific interactions in the establishment of the individuals and on their final distribution. These factors may have important implications for coexistence and space use of related species. Intraspecific interactions are expected to generate a population thinning along the life history of the individuals. Different habitat preferences can favor the coexistence of related species (Cox 1981; Queenborough *et al.* 2007b) and the spatial distribution can finally reflect the strategies favoring coexistence and the different kind of interactions at the intra and interspecific level.

Congeneric dioecious tree species present various similar ecological characteristics as similar flowers and fruits, shared seed dispersers, and probably also herbivores and pathogens which are expected to generate some level of interference among them (Renner & Ricklefs 1995). These factors are directly related with the spatial arrangement of the individuals. In dioecious species the sexes can differ in their physiological demands, mortality rate, initial reproductive size, maximum plant size, habitat selection, and consequently, can differ in their final distribution (Bierzychudek & Eckhart 1988; Wheelwright & Logan 2004). Therefore, the sex ratio can influence the spatial organization of the population (Grant & Mitton 1979).

Various statistical techniques have been developed to analyze the spatial arrangement of trees. Point pattern analyses are appropriate to characterize plants intrapopulational distribution. The combination of these techniques and null model has allowed to test specific hypotheses about the ecological factors contributing to the observed spatial patterns (Wiegand & Moloney 2004). In fact, the analysis of the distribution of different ontogenetic stages allows the study of the effects of different processes on spatial organization along the life history of plant species. Genetic

spatial analyses are the ideal tool to corroborate the patterns deduced from the traditional spatial analyzes.

In this study we analyze the spatial and genetic structure of two sympatric dioecious tree species of the genus *Virola* (Myristicaceae), using univariate and bivariate point pattern analysis and microsatellites genetic data. We hypothesize the species will show particular recruitment capabilities in different microenvironments in the forest which will determine unequal final distributions. The observed distribution of reproductive individuals should be the result of processes occurring along the ontogeny, and we compare the spatial genetic structure and spatial organization of individuals in different size categories in order to interpret the dispersal, recruitment and establishing dynamics in these species. We also explored the interaction between different species, sexes within species, and ontogenetic stages in order to determine if these processes affect the final distribution of individuals in the population. We expect segregating effects among conespecifics, but not among heterospecifics. Homogeneous distribution of the sexes is also expected because this will facilitate the reproductive interactions in these dioecious species. We use different null models to simulate the spatial organization of the individuals and determine the possible processes defining their distribution and favoring their coexistence.

MATERIALS AND METHODS

Study Site

This study was conducted in the southeastern coast of Brazil in the Atlantic Rain forest near the city of Ubatuba, São Paulo. Our sampling site is located on the middle elevations (250-450 m a.s.l.) of the Serra do Mar, at the Fazenda Capricórnio, (23°22'03S, 45°05'03W). Vegetation is classified as tropical moist evergreen forest (Holdridge 1947), or submontane rain forest

(Oliveira - Filho & Fontes 2000). Up to 206 species (> 4cm DBH) have been found per hectare (Gomes, Bernacci, & Joly 2011; Rochelle, Cielo-Filho, & Martins 2011) in the area. Annual temperature varies between 18 to 27°C and mean annual precipitation is 2600 mm. The total sampled area was 10 ha of primary forest. The area here studied is composed by a hill with a well defined eastern and western slope

Studied Species

The genus *Virola* (Nutmeg trees, Myristicaceae) includes approximately 46 species in America. All species are dioecious trees, distributed in the Neotropics. We worked with *Virola bicuhyba* Schott ex Spreng. and *V. gardneri* (A. DC.) Warb., both species are endemic, and are the only species of the genus found in the Atlantic rain forest. *Virola bicuhyba* is found from Bahia to Rio Grande do Sul, whereas *Virola gardneri* is found between the states of Pernambuco to São Paulo (Rodrigues 2011). These two species present similar yellowish small flowers. Fruits are dehiscent having only one seed covered by a red aril. Seeds are dispersed by large birds (mainly toucans; Galetti *et al.* 2000), as other *Virola* species (Howe & Kerckhove 1981; Howe 1981; Howe *et al.* 1985; Holbrook & Loiselle 2009; Kays *et al.* 2011).

Sampling strategy and population structure

For each species we looked for all reproductive individuals and juveniles (≥ 1 m height) in the full studied area. Additionally, we considered all juveniles >75 cm in a subsample of 2.5 ha (hereafter subplot), located in the middle of the studied area. For each individual we measured the diameter at breast height (DBH) at 1.3 m (only for individuals taller than 3 m), and tree height. Height was measured directly or estimated using trigonometry (angles were measured with a Suunto Tandem

clinometer). The sex and reproductive status was determined during monthly phenological observations of each individual for a period of two years. The sex of non-reproductive individuals is unknown.

Individuals were mapped. A high-sensitivity GPS (GPSmap76Cx, Garmin) was used to mark the location of specific individuals in the population. All points marked using the GPS have an error <2 m, which is inside the range of the crown of any tree in the population. These trees were used as base points to map all nearer individuals reducing mapping error. To do this we measured the azimuth and the distance between the mapped trees and all neighboring individuals. Azimuths were measured with a Suunto Tandem compass, and distances with a Leica Disto D2 laser distance meter or using traditional measuring tapes. The sampled area had an irregular shape due to topographic complexity making not possible the establishment of a rectangular area (fig. 1A,B).

Genetic sampling

We collected tissue samples for all the individuals measured and mapped in the plots in order to perform DNA extractions. We also collected samples from 6 additional reproductive *V. bicuhyba* outside the 10 ha plot (not considered in the spatial distribution analyses). Leaves were collected always than possible, but for large individuals a Suunto increment borer (25cm/2-thread) was used to extract a small cylindrical sample of the external stem tissue (5mm wide, 2-3cm length). After collection of each sample the borer was cleaned, washed with water, and with alcohol 70%, and finally sterilized with fire in order to prevent contamination of the samples and transmission of tissue among the individuals. The small hole left on the stem was naturally filled by the red sap emerged from the stem in some cases or by the natural growth of the tree after

some months especially for juveniles. Two years after the extraction no individual showed any sign of infection in the extracted area. Leafs and stem samples were stored in ice in the field and then in liquid nitrogen. DNA extraction was done using the DNeasy plant mini kit (QIAGEN).

We used primers previously developed for *V. flexuosa* (Holbrook *et al.* 2007) and *V. surinamensis* (Draheim *et al.* 2009) to genotype the populations. The combination of primers was different for each species (Table 1). Published PCR protocols were modified in order to allow amplification of all species with a unique reaction for both species. PCR was carried out in a volume of 10 μ L containing 1 μ L template DNA (2-5 ng approximately), 1X $(\text{NH}_4)_2\text{SO}_4$ Taq buffer (Fermentas), 1 mM MgCl_2 (2.125 mM for the primer VF1I only), 0.2 mM each dNTP, 0.5% BSA, 0.2 μ M each primer, and 0.5 U *Taq* polymerase (Fermentas). All PCRs began with 4 min at 95 °C, followed by 30 cycles of 30 s at 95 °C, 45 s at a primer-specific annealing temperature (annealing temperatures did not differ between the species; Table 1), 45 s at 72 °C, and a final extension at 72 °C for 5 min.

Primers were labeled with fluorescence (6-FAM, VIC, NED or PET) and the fragments separated in an ABI3130XL (Applied Biosystems) genetic analyzer. Genotypes were determined by comparison with a GeneScan 500LIZ size standard (Applied Biosystems) in GeneMarker 1.95 (SoftGenetics LLC).

Data Analysis

- Sex ratio

Sex ratios were compared using contingency tables. To test sex ratios across different reproductive sizes, we categorized the individuals into three groups according to their DBH (15 - \leq 30 cm; >30 - \leq 50cm; >50 cm). The sex of seven *V. bicuhyba* and five *V. gardneri* with

reproductive size was not determined because either they suffered severe physical damage that precludes their reproduction during the study period or they produced a minimum quantity of flowers on inaccessible areas of the crown. These trees were excluded from the sex ratio analyses.

- Spatial distribution

We divided the studied area in three sections: hill top (25 m at each side of the line defining the slope division; 31% of the sampled area), eastern slope (31% of the area), and western slope (38% of the area). We standardized the amount of individuals correcting for the area of each section and applied one way chi tests for each species.

Point pattern analyses are widely used to study the distribution of plants which can be represented as points in the space. We used the univariate and bivariate pair correlation function $g(r)$ to analyze the spatial distribution of the trees at different spatial scales in the studied area. This function uses rings (width r) centered on each individual in the population to analyze spatial patterns. It is also a normalized neighborhood density function, defined as the expected density of trees at distance r , divided by the mean density λ of trees in the study region (Wiegand *et al.* 2009).

Null models test if the observed spatial patterns (g function adjusted to the observed point pattern) are random or determined by ecological processes by using Monte Carlo simulations and comparing the observed distributions with the confidence intervals which act as limits of significance. The simplest and most widely used null model for univariate point pattern analysis is complete spatial randomness (CSR) where each point has the same probability to occupy any place in the studied area independently of any other point (λ is constant in the full area). This

allow us determine if there exist any first order effect (deviations from a homogeneous distribution).

If first order effects are found, they should be considered when testing for second order effects (interaction between two different kinds of points) or erroneous interpretations of the spatial distribution could be done. A heterogeneous Poisson null model can simulate the first order effects. This model applies an intensity function $\lambda(x,y)$ to determine the probability of occurrence of each simulated individual which varies according with the coordinates (x,y) in the studied area. An adequate estimate of the intensity function should be based on the observed pattern of points and the simulated pattern in the area would follow a Poisson distribution. For details on the implementation of these models see Wiegand & Moloney (2004) and Wiegand *et al.* (2007).

The CSR and Poisson models assume that different processes act in the initial definition of the spatial patterns of each kind of point. Nevertheless, when it is known that the same processes determine the initial distribution for a bivariate group of points, another model should be used. A bivariate random labeling null model preserves the position of each point in the area, but assigns their identity randomly (e.g. males and females in the same species). In this way it does not answer the question of which process generated the observed pattern, but helps determine if this process is homogeneous along the area.

In order to determine the spatial patterns in the population, their relationship with the size of the individuals, and if different size classes interact with each other we classified the individuals in three categories according to their height and reproductive stage: small juveniles ($>0.75\text{m}$ and $\leq 2.5\text{m}$), large juveniles ($>2.5\text{m}$ - non reproductive), and reproductive. We used a grid-based analysis (Wiegand & Moloney 2004) to implement spatial analyses; the full studied

area was divided into a grid of square cells of 10 m side for adults and large juveniles, and 5 or 3 m side for small juveniles (depending on the analysis). The ring width was 1 cell for all analyses; the full sample area was considered to analyze large juveniles and reproductive individuals, but only the subplot was used in the analysis of small juveniles (fig. 1B).

We first analyzed the univariate point pattern (the within species distribution), comparing the observed distribution of each size category of each species against the CSR null model. This allows us to determine if there exists spatial heterogeneity in the distribution of each category per species. We then analyzed the bivariate point pattern comparing pairs of different kinds of points in order to determine the interaction between them. We compared the intraspecific effect of reproductive individuals on other size categories (large and small juveniles), the intraspecific effect of large juveniles on small juveniles, and finally, the interspecific effect of each size categories on their sympatric equivalent. To do this, we fixed the position of the first category in the pair and used a heterogeneous Poisson null model to randomize the second type of points. This process accounts for the first order effects found in the species distribution (spatial heterogeneity), in order to reveal true second order effect (interactions among categories). In this way we can determine if the distribution of the first category influences the second one. In the case of paired comparisons between equal size categories each kind of point was fixed in successive analysis, in case the effect among them was not symmetrical. Finally, we used the random labeling null model to analyze the distribution of the two sexes within the population for each species.

We performed 1000 simulations of each null model and calculated the 2.5 and 97.5 quantiles which were compared to the observed distribution at any given distance following Li *et al.* (2009). When the observed distribution was not within the interval defined by these quantiles it was considered as significantly different from the null model at that distance. We then

performed a goodness of fit test for the distance interval where these significant deviations were observed in order to assign an exact probability value. All analyses were performed in the Programita software (version July 31st, 2010; Wiegand & Moloney 2004).

- Genetic structure

Deviation from Hardy-Weinberg equilibrium were calculated using the Markov chain test in Arlequin (Excoffier *et al.* 2007). Chain length was 1000000 with 100000 dememorization steps. The genetic structure within the population was analyzed based on the estimation of F_{st} and F_{is} calculated according to Weir and Cockerham (1984). Pairwise F_{st} was calculated among the size classes (small juveniles, large juveniles, and reproductives) within the population in the program Fstat 2.9.3.2 (Goudet 1995).

The spatial genetic structure between individuals at given distance classes was analyzed based on the average kinship coefficient (ρ_{ij}) between all pairs of individuals i and j in the population (Loiselle *et al.* 1995). We defined distance classes among individuals of 10 m in the case of small juveniles and 20 m for large juveniles and reproductive. The coefficient of variation was calculated based on 20 000 permutations of loci and individual spatial locations and the 95% confidence intervals (CI) were used as significance limits. Average kinship coefficient values larger than the upper CI indicate more genetic similarity than the expected by chance among the individuals at that specific distance. Calculations were done in the program SPAGeDI 1.3 (Hardy & Vekemans 2002).

RESULTS

Size structure and sex ratios across species

A total of 195 individuals (61 small juveniles, 79 large juveniles and 55 reproductive) of *V. bicuhyba* and 136 of *V. gardneri* (45, 58, and 33 individuals respectively) were found in the

studied area. When only the individuals taller than 1 m (sampled in the full area) are considered the cumulative population size structure follows an inverted J-shape since they are dominated by small individuals for both species and few large individuals are present (Table 2). There is no difference in the sex ratio for each species or between them (*V. bicuhyba* 54.5% males, n=55; *V. gardneri* 51.5% males, n=33; $\chi^2=0.076$, df=1, p=0.78). No differences were found in the frequency of the sexes across DBH categories (*V. bicuhyba* $\chi^2=1.639$, df=2, p=0.44; *V. gardneri* $\chi^2=1.596$, df=2, p=0.45).

Univariate spatial analyses – Within species distribution

The species show different micro-habitat preferences in their distribution. *Virola gardneri* is distributed preferentially near the hill top along the studied area. 68% of the reproductive and large juveniles are within the 25 m at each side of the linear hill top, which corresponds to the 26% of the sampled area approximately, and 94% of the individuals are in this area plus the eastern slope of the hill (62% of the area; $\chi^2=64.75$, df=2, p<0.0001; Fig. 1). *Virola bicuhyba* does not show any preference for this environment and 29.1% of the individuals are within the 25 m at each side of the hill top, and the rest of the population is distributed equally at both sides of the hill (32.6% E side, 38.3% W side; $\chi^2=3.86$, df=2, p=0.145; Fig. 1).

There is a general pattern of spatial aggregation of the individuals in the three size categories. The distance at which this aggregation occurs is similar between the two species for each size category. Adults are clumped at short distances, having more individuals than expected under CSR at distances of 20-40 m from each other in *V. bicuhyba*, and 20-30 m in *V. gardneri*. Nevertheless, large juveniles in both species are clumped over distances up to 80 m from each other. Finally, small juveniles are significantly clumped in very short distances in both species

and irregular patterns are observed at larger distances probably related with neighboring groups of individuals (Fig. 2A and Appendix II – Fig. S1). In all cases the degree of aggregation showed by *V. gardneri* was higher than in *V. bicuhyba*.

Bivariate spatial analyses – Species, sexes, and size classes

No interaction was identified between the sexes which are homogeneously distributed for both species (Appendix II - Fig. S2). Large juveniles are aggregated around reproductive individuals, being found near to the crown's limit in *V. gardneri*, but not a clear pattern exist in *V. bicuhyba* (Fig. 2B, Appendix II - Fig. S3). Small and large juveniles tend to be aggregated at distances up to 12 m in *V. bicuhyba*, but distribution is random among them in *V. gardneri* (Fig. 2B, Appendix II - Fig. S4). Finally, when interspecific effects within each size category are compared no significant interactions between reproductive individuals between the two species, or large juveniles between the two species were found, but a clear overlap in the distribution of small juveniles was detected at very short distances (Fig. 2C, and Appendix II – Fig. S5).

Population genetic structure

Virola gardneri showed a higher average number of alleles per loci. Both species show a high genetic diversity presenting high levels of heterozygosity (Table 1). F_{is} estimates did not differ from zero for any species as evidenced by the 95% CI (Table 1). Pairwise F_{st} estimates among size classes were minimal for the two species, and in both cases the highest difference was between small juveniles and adults (Table 3). Nevertheless, the genetic characteristics are similar for the studied loci among size classes in both species (Table S1 and S2).

Virola bicuhyba show a weak spatial genetic structure (SGS) only at very short distances (20-40 m in large juveniles and adults; Fig. 3). Small juveniles do not show any clear SGS. When

the full population is considered individuals show SGS up to distances of 60 m from each other. *Virola gardneri* shows strong SGS up to distances of 70 m for small juveniles, and 80 for the other size classes as well as for the full population (Fig. 3).

DISCUSSION

Sex ratios

Unbiased sex ratios were found in the populations of the two analyzed species. Biased sex ratios in plants can appear as a result of sex specific life history traits (Nicotra 1998). Reasons include the differential reproductive effort between the sexes; males can start their reproduction with smaller sizes while females delay the beginning of their reproduction until reach bigger dimensions (Opler & Bawa 1978; Bullock & Bawa 1981; Nicotra 1998). Also higher mortality has been attributed to females, contributing to the higher abundance of males in large size categories (Lloyd 1973; Opler & Bawa 1978). Nevertheless, the sex ratio in *V. bicuhyba* and *V. gardneri* is unbiased when partitioned in size categories. Similar results were found by Wheelwright & Logan (2004) for *Ocotea tenera* in Costa Rica indicating that these factors are more species specific.

Sex in most Myristicaceae seems to be genetically determined, but the exact mechanism of sex determination is still unknown. Most studied species in the family are dioecious; the only exceptions to this pattern are some species of *Iryanthera* which are monoecious (Smith & Woodhouse 1937; Ackerly, Rankin-De-Merona, & Rodrigues 1990) or androdioecious (Queenborough *et al.* 2007a). Equal cost to produce each sex and the absence of large differences in their specific requirements can be responsible for the similar sex ratios found here. No difference in habitat preference between the sexes supports this view.

Spatial distribution

Studies on tropical trees spatial structure have found a general pattern of aggregation at the intraspecific level on variable scales (e.g. Condit *et al.* 2000, Hubbell 1979, Li *et al.* 2009).

Virola bicuhyba and *V. gardneri* are consistent with this pattern and aggregation was observed for all size categories. The patterns of aggregation between the two species are remarkably similar along all the size classes and may be related with the analogy in fruit characteristics and dispersers (Seidler & Plotkin 2006). However, fruiting in these species occurs at different times during the year (see Chapter 1), and only a subset of the dispersers of *V. bicuhyba* can disperse *V. gardneri*. Similar ecological processes seem to affect these species generating similar distributions, but the species seems to tolerate partially different micro-environments with implications for their establishment and persistence, partially explaining the aggregated patterns. The area here studied is composed by a hill with a well defined eastern and western slope. *Virola gardneri* seems to be favored in areas with high illumination near the hill top, which is the area most exposed to sun light during all the day, as well as the eastern slope of the hill, which receives a higher light incidence during the day than the western side. The western side is exposed to the sun during the afternoon, but cloud coverage and rain are a common phenomenon during this period reducing light incidence. *Virola gardneri* uses lower canopy strata in comparison to *V. bicuhyba* (Chapter 2). The combination of using less illuminated lower canopy strata and lower natural light incidence in the western side might preclude the establishment of this species in this area of the forest. No clear preferences for particular conditions are evident for *V. bicuhyba*, and the species is widespread in the forest colonizing disturbed as well as pristine areas (personal observations). Queenborough *et al.* (2007b) studied the spatial distribution of 16 sympatric species of Myristicaceae in the Amazonian Ecuador and found habitat specific associations based on topographic characteristics. Topography by itself does not seem to

determine the distribution of *V. gardneri*, but its effects on environmental conditions in the study area are important. Soil characteristics does not seem to have an important effect on the species spatial distribution in the studied area, as no soil differences were found within the subplot, and probably the same pattern is found for all the sampled area (P. Camargo com. pers.).

The lack of spatial segregation between the sexes supports the absence of sex specific habitat requirements, which could influence their spatial distribution. Despite spatial segregation has been found in dioecious species (see Bierzychudek & Eckhart 1988), other studies with tropical trees did not find evidence of spatial segregation of the sexes (e.g. Bawa & Opler 1977; Bullock 1982). Spatial segregation could have negative effects on pollen dispersal especially in dioecious species.

Few studies have analyzed the interspecific effects in tree species distribution and most have found no effect on the spatial structure of other species (e.g. Wiegand *et al.* 2007). In our case only small juveniles in both species show aggregation at very short distances probably related with the common dispersers of the species. The most important disperser for *Viola* are large birds mainly toucans (Galetti *et al.* 2000). *Viola* seeds are also eaten by smaller birds as cotingas (Pizo *et al.* 2002), or rarely by other large birds as cracids (e.g. Galetti *et al.* 1997). Toucans and cotingas eat the fruits and regurgitate the intact seed after the removal of the aril. By their high mobility and the short time elapsed between seed ingestion and regurgitation these dispersers are not expected to cause clumping of seeds in the forest (Kays *et al.* 2011), but clumping combining the two species could be happening if they present favorite resting sites where regurgitation frequently occur after feeding pulses. Other *Viola* dispersers as tapirs and monkeys are expected to create aggregated patterns of seed deposition and recruitment (Strier 1991; Russo & Augspurger 2004; O’Farrill *et al.* 2012), but these dispersers are very rare or absent in the studied area. The small juveniles category here defined includes individuals from

different reproductive episodes. If germination of both species is positively affected by similar micro-environmental conditions the clumped establishment would be favored. In this way, the maintenance of particular conditions favoring the germination of both species could create the clumping pattern for small juveniles. Subsequent differences in microhabitat tolerance during growth may preclude the appearance of clumps combining the two species in the large juveniles and adults categories. As *V. bicuhyba* shows no clear preferences for particular habitat characteristics recruitment in this initial point seems to occur, generating the strong pattern of aggregation of small and large juveniles. Nevertheless, *V. gardneri* recruits only in particular favorable areas, not necessarily the places of high initial establishment, and small and large juveniles show a random distribution.

No species showed evidence of negative intraspecific interactions since the distribution of the individuals was not segregated. Ziparro & Morellato (2005) found that seed predation was not related with the distance from the source trees in *V. bicuhyba*, but Howe (1985) found an advantage in the survival of seeds dispersed at larger distances from mother trees in *V. surinamensis*. No information for seedling distribution and mortality is available and we cannot discard negative effects from conspecifics on this ontogenetic stage. In *V. bicuhyba* the aggregation of individuals occurred only at large distances from reproductive individuals, out of the range of <30 m that has been reported as important for intraspecific interactions in tropical trees (Hubbell *et al.* 2001; Wiegand *et al.* 2007).

Only large juveniles in *V. gardneri* show a clear pattern of aggregation at a short range from reproductive individuals. Small juveniles show a weak aggregation at longer distances, and these two juvenile classes are not aggregated. Aggregation at short distances from reproductive trees indicates that establishment and probably recruitment in this species can occur under the

crown or just outside it, which can be related with limited dispersal generating accumulation near the source trees.

Genetic spatial structure

The genetic information supports the idea of dispersal at shorter distances in *V. gardneri* in comparison to *V. bicuhyba*. As sampling design and sampling area were the same for the two species, genetic data is directly comparable between these species. Also, as sampling of all individuals was carried out, interpretation of the fine scale genetic patterns can be made directly from the spatial correlograms shown in Fig. 3. The pattern observed for the two species match the findings of other studies, where SGS is commonly found at short spatial scales (see Vekemans & Hardy 2004). This pattern can be related in most cases to dispersal limitation creating the previously discussed aggregated patterns common for tree species in tropical forest (Hubbell 1979; Condit *et al.* 2000; Li *et al.* 2009). In fact, the distances where SGS is detected are similar to those where the individuals of the three size classes are aggregated for the two species.

Our data allow the comparison of the ecological factors mediating the differences in the spatial distribution and SGS between these species. The limitation of SGS to very short distances in *V. bicuhyba* supports the idea of long distance seed dispersal in this species, as genotypes arriving from distant areas reduce the genetic structure found at very short spatial scales. This explains the absence of SGS in small juveniles and the weak SGS found at the border of the crown area for large juveniles and reproductive individuals, and can be related to the large period of fruit availability in this species and its small seeds capable of being dispersed by a large number of bird species.

Virola gardneri has a stronger aggregation and a SGS extending at longer distances than *V. bicuhyba*. Recruitment occurs at shorter distances from parent trees in *V. gardneri* than in *V.*

bicuhyba, and the spatial aggregation is probably the result of short seed dispersal distances. This short dispersal distance can be the result of the large seeds of *V. gardneri* and the short period of fruit ripening (see Chapter 1). The large seeds of *V. gardneri* may satiate the dispersers more quickly, resulting in less seeds dispersed per visit. Large fruit availability in a short period can also result in narrow foraging areas; under this scenario, many seeds can fall under the mother trees or be dispersed to short distances, creating the clumping distribution pattern observed in this species and their strong SGS.

The biennial flowering pattern in *V. gardneri* (see Chapter 1) can also contribute to their strong SGS, since the effective population size available at each year is approximately half the actual population, limiting the number of possible mating partners of each individual. This biennial flowering may favor longer distance pollen flow because density of reproductive individuals is reduced (see Ellstrand 1992), but even under this scenario, the limited seed dispersal would create the observed levels of SGS. The number of loci used in the analysis is small, but they are highly polymorphic, especially for *V. gardneri*. The inclusion of more loci will test the confidence in these patterns. .

Concluding remarks

Virola gardneri is more clumped than *V. bicuhyba* and positive intraspecific interaction between adults and other size classes were found. Limited seed dispersal could generate accumulation of non-dispersed seeds around mother trees, but other factors such as more abundant, or more aggregated seed dispersal could not be discarded. Genetic information supports this scenario, as SGS is present, especially among small individuals.

We hypothesize that density dependent mortality in *V. gardneri* is lower than in their sympatric congener and the thinning effect during time is not as strong as in *V. bicuhyba*. This

could be related with the much larger seeds in *V. gardneri* (see Chapter 1), which could provide more resources for the initial growth of the individuals favoring their establishment (Dalling & Hubbell 2002). All these facts can contribute to the more aggregated spatial pattern in this species. The species show similar intraspecific spatial organization probably related with similar fruit characteristics and dispersal vectors. The sexes in each species do not differ in their environmental requirements and do not show spatial segregation.

Small scale distribution of these species is determined by particular environmental conditions. Specially *V. gardneri* seems to be more restricted in their habitat tolerances than *V. bicuhyba*, preferring areas with a higher light input. Interspecific interactions did not explain the distributional patterns. Partially different use of the habitat and micro-environmental specialization can favor the coexistence of *V. bicuhyba* and *V. gardneri* in the Atlantic forest.

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Table 1. Microsatellites' profiles for populations of *Virola* spp. in the Brazilian Atlantic forest, including sample size (n), annealing temperature °C (t), number of alleles (k), observed heterozygosity (Ho), expected heterozygosity (He), frequency of null alleles (Null), F_{is} estimate (f), and probability for Hardy-Weinberg equilibrium test (HW); ns = non significant.

Loci	<i>V. bicusbyba</i>								<i>V. gardneri</i>						
	n	t	k	Ho	He	Null	f	HW	n	k	Ho	He	Null	f	HW
Vmul2-65	210	52	6	0.309	0.305	-0.006	-0.016	ns	151	11	0.702	0.706	0.000	0.001	ns
Vsur2-35	210	52	21	0.890	0.879	-0.010	-0.032	0.041	151	26	0.954	0.911	-0.026	-0.049	ns
Vmul68	209	50	7	0.675	0.711	0.022	0.044	ns	151	13	0.887	0.836	-0.033	-0.064	ns
Vsur34	210	52	15	0.738	0.756	0.009	0.013	0.005	151	14	0.735	0.826	0.063	0.107	<0.0001
VF11	210	58	3	0.586	0.545	-0.044	-0.073	0.043	-	-	-	-	-	-	-
Vsur45	210	52	4	0.562	0.525	-0.038	-0.074	0.0007	-	-	-	-	-	-	-
All loci	-	-	9.33	0.627	0.620		-0.019*		-	16	0.82	0.82		-0.004	

* 95% CI (-0.055 - 0.015), ns different from zero.

Table 2. Cumulative structure of the populations according to their size for populations of *Virola* spp. in the Brazilian Atlantic forest. Only individuals taller than 1m were considered.

Quantil (%)	Height (m)	
	<i>V. bicusbyba</i>	<i>V. gardneri</i>
25	2.2	2.3
50	6.9	5.9
75	19.4	17.1
97.5	36.4	29.5
100	44.4	35.5

Table 3. Pairwise F_{st} among size classes for populations of *Virola* species in the Brazilian Atlantic forest. Size classes are small juveniles (1), large juveniles (2), and reproductive individuals (3); n is the sample size for each class.

Species	n	Size class	
		1	2
<i>V. bicusbyba</i>	65	1	
	80	2	0.007*
	65	3	0.015***
<i>V. gardneri</i>	35	1	
	61	2	0.002ns
	55	3	0.011*

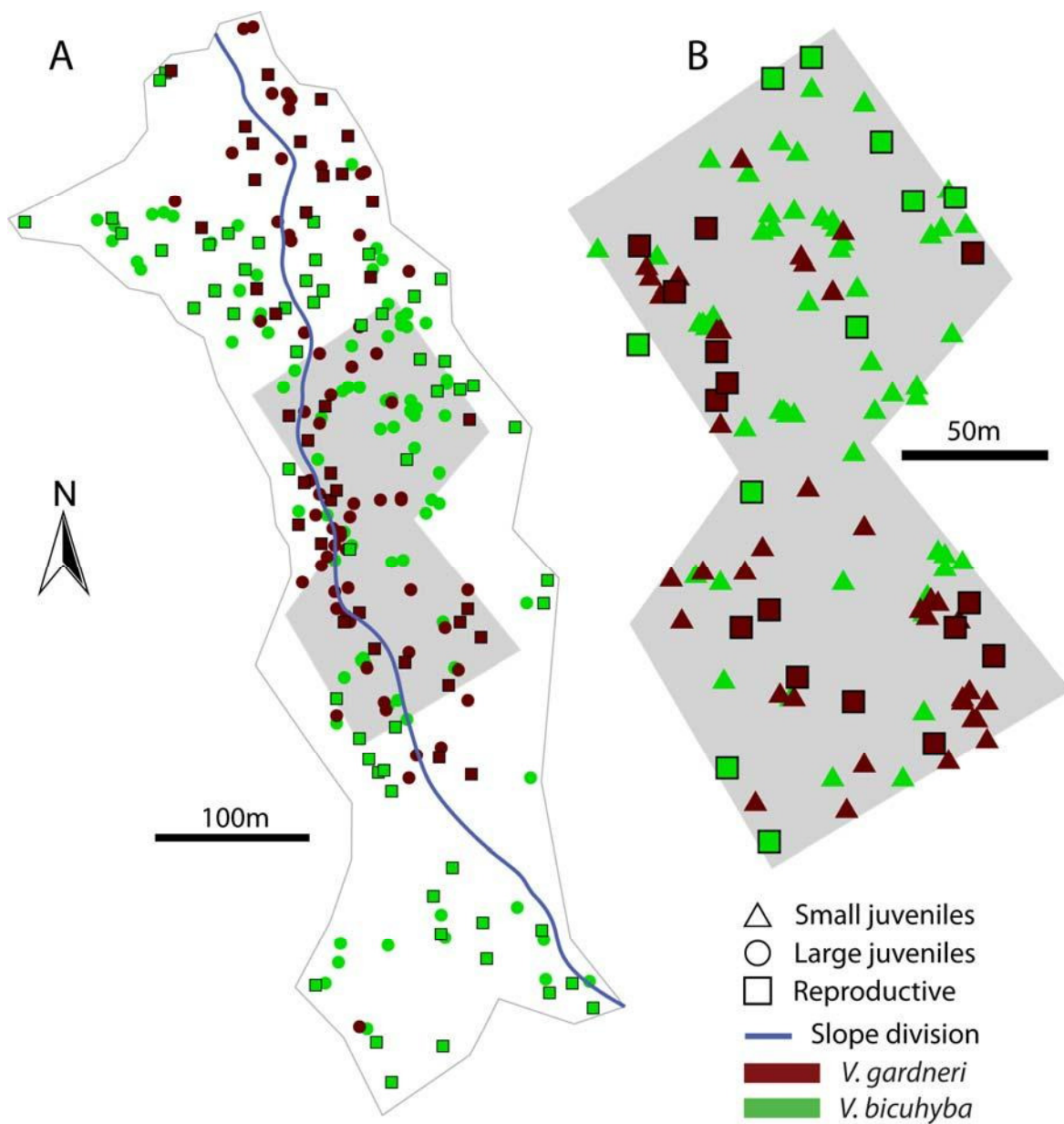


Figure 1. Distribution of the individuals in the studied area. A- Full area showing the location of adults, large juveniles, and the subplot in gray. B- Detail of the subplot showing adults and small juveniles (large juveniles are not shown to improve clarity).

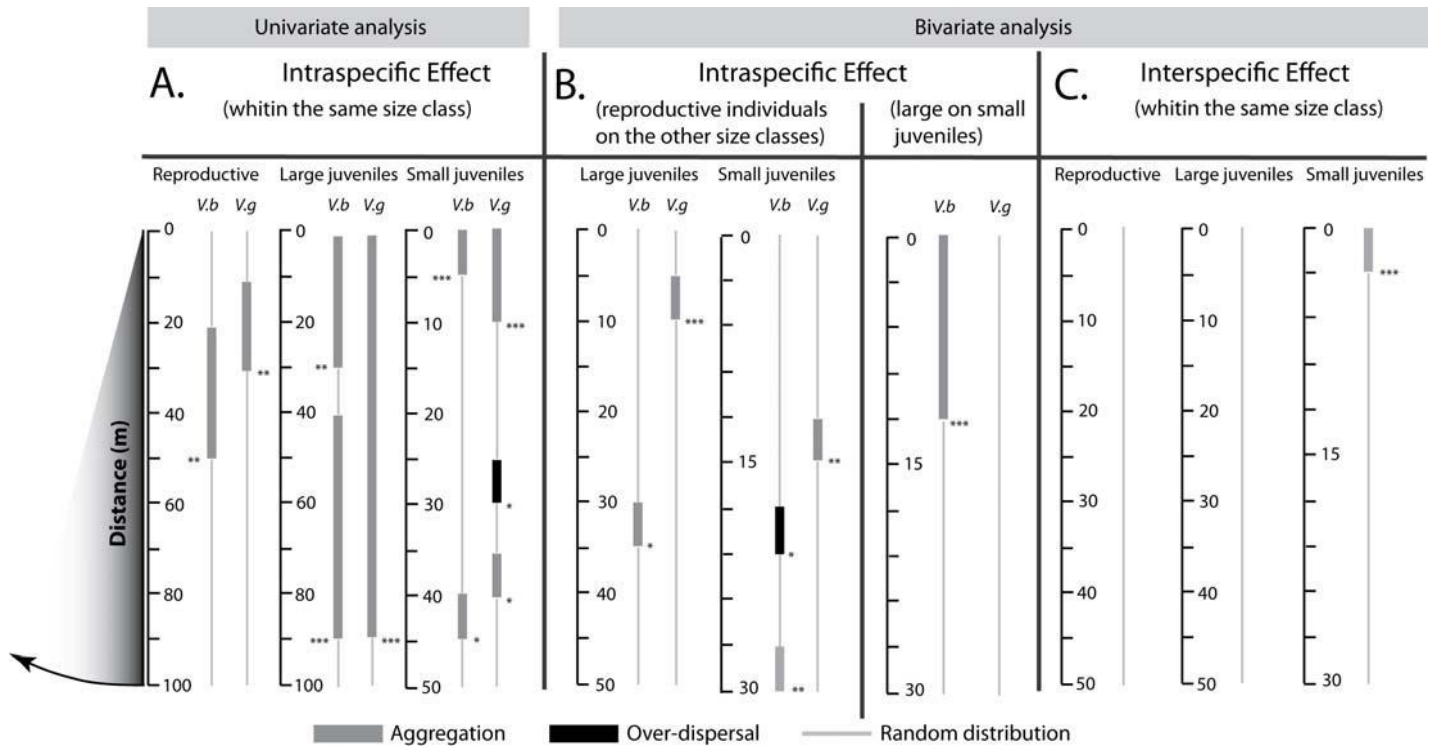


Figure 2. Spatial distribution of individuals of *Virola bicuhyba* (*V.b*) and *V. gardneri* (*V.g*), showing significant deviations from a pair correlation function (*g*) from a complete spatial randomness (CSR) null model in A, and for a heterogeneous Poisson null model in B and C, using 1000 randomizations and 95% CI as limits of significance. A) intraspecific effects of individuals on the same size class; B) intraspecific effect of reproductive individuals and large juveniles on other size classes; C) interspecific effect of individuals on the same size class. Significance values correspond to a goodness of fit test applied to the distance intervals where the null model was rejected based on the CI; * $p < 0.05$, ** $p < 0.01$, *** $p \leq 0.001$. This figure resumes the information shown in the figures S1 to S5.

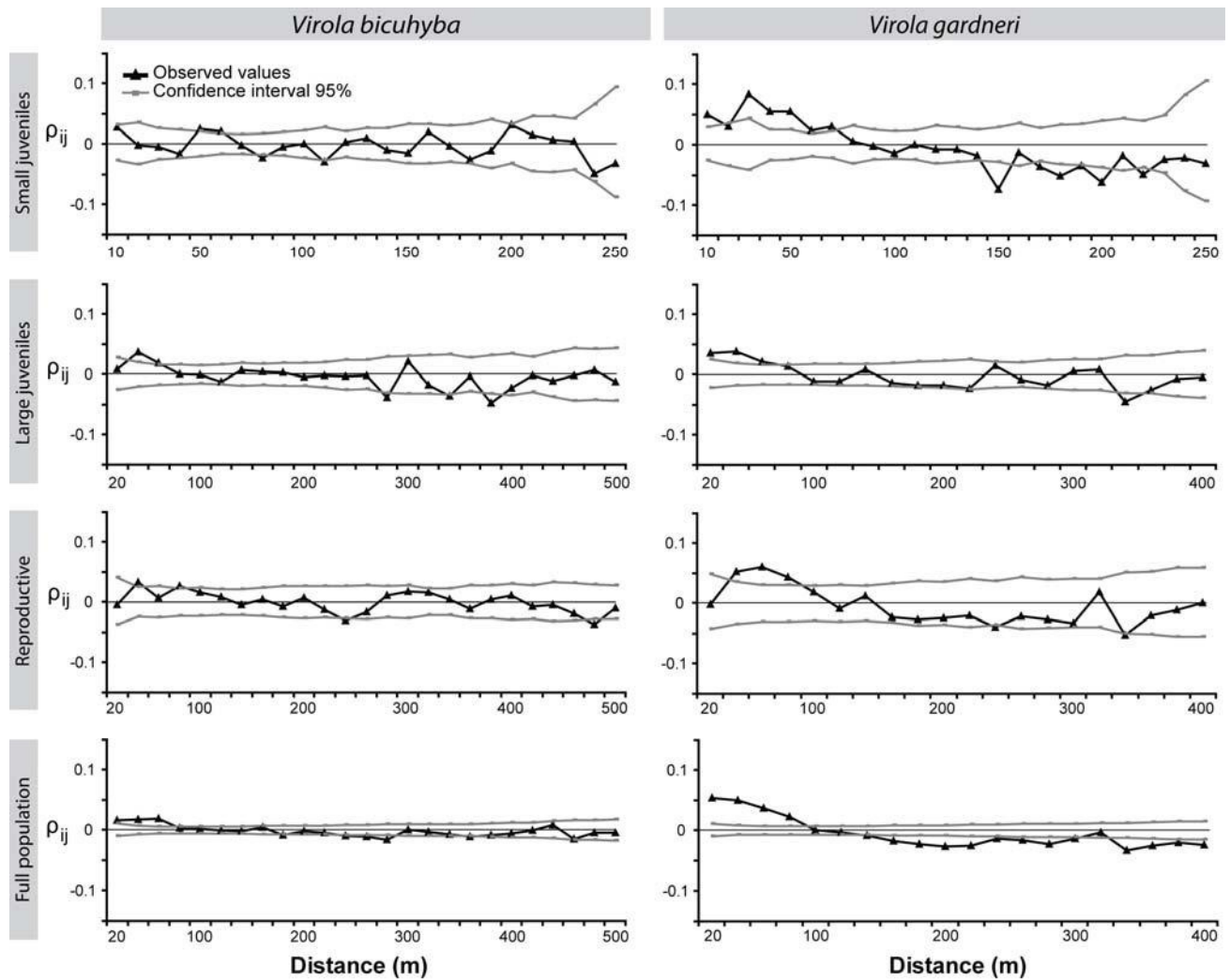


Figure 3. Spatial genetic autocorrelation within each size category, based on the kinship coefficient (dark line) proposed by Loiselle *et al.* (1995). Gray lines are 95% confidence intervals (CI) for 20000 spatial location iterations. Distance classes are 10 m for small juveniles and 20 m for the other size classes. Gray continuous lines represent the value of 0, expected for a random distribution of genotypes. Data above the upper CI indicates significant genetic structure (more genetic similarity than the expected by chance) or the contrary for values under the lower CI.

APPENDIX II

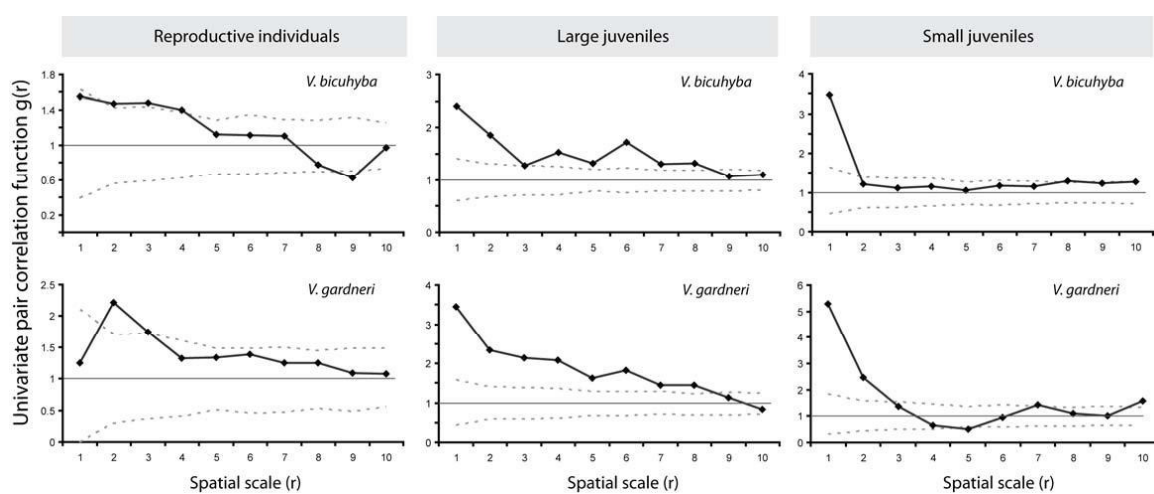


Figure S1. Univariate analysis of the spatial distribution of each size category per species. Pair correlation function for the observed data (dark line), and confidence limits (quantils 2.5 and 97.5%, gray dashed lines) for 1000 iterations of a complete spatial randomness null model over spatial scale r ($r=5\text{m}$ for small juveniles and 10m for the other categories). Gray continuous lines represent the value of 1, expected for a random distribution. Data above this line indicates aggregation and below it segregation.

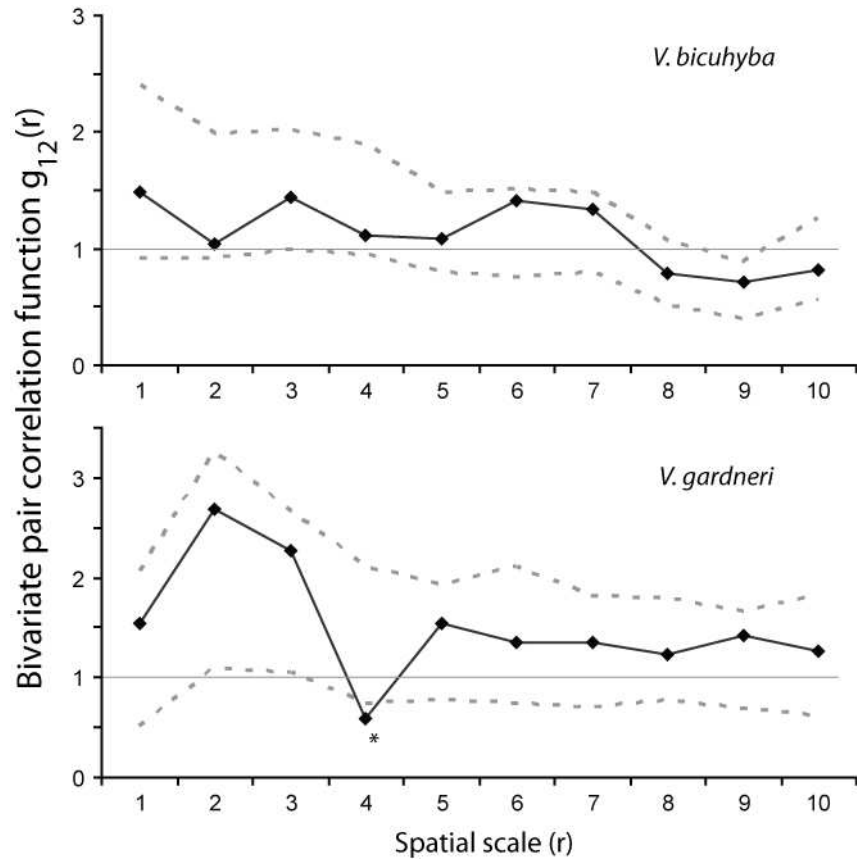


Figure S2. Bivariate analysis of the intraspecific effect of the sexes in their spatial distribution per species. Pair correlation function for the observed data (dark line), and confidence limits (quantil 2.5 and 97.5%, gray dashed lines) for 1000 iterations of a random labeling model over spatial scale r . Gray continuous lines mark the value of 1, expected for a random distribution. Data above this line indicates aggregation and below it segregation. $r=10m$; $*p=0.05$.

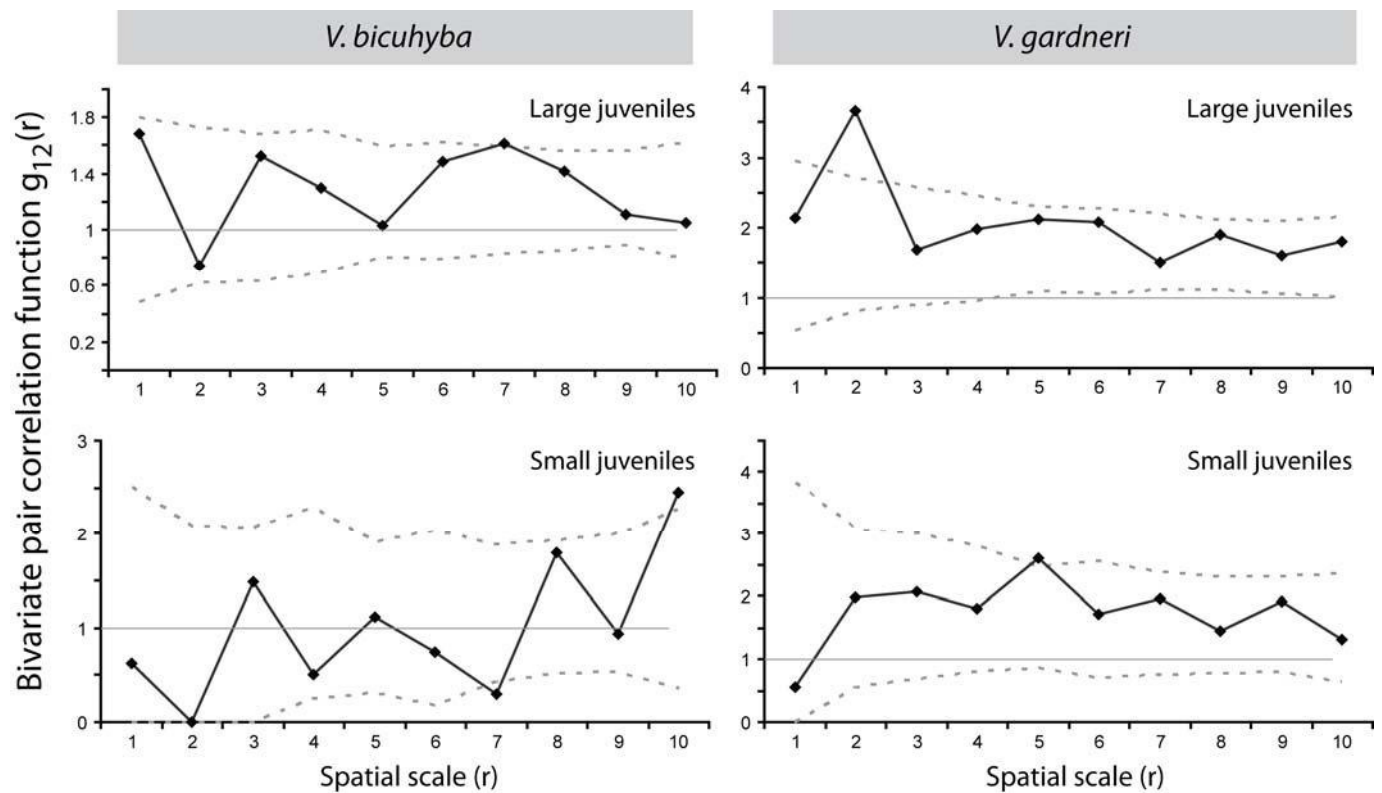


Figure S3. Bivariate analysis of the intraspecific effect of reproductive individuals on the distribution of the other size categories per species. Pair correlation function for the observed data (dark line), and confidence limits (quantil 2.5 and 97.5%, gray dashed lines) for 1000 iterations of a heterogeneous Poisson null model which fixes the position of one point kind. $r = 5$ m for large juveniles and 3 m for the small juveniles. The gray continuous line represents the value of 1, expected for a random distribution. Data above this line indicates aggregation and below it segregation.

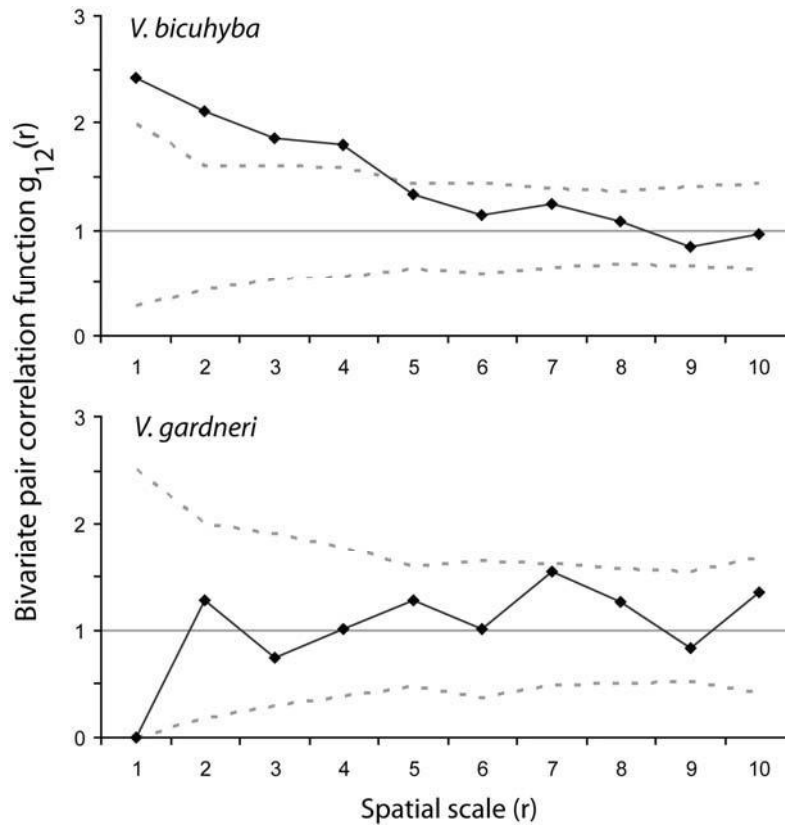


Figure S4. Bivariate analysis of the intraspecific effect of large juveniles on small juveniles for *Virola bicuhyba* and *V. gardneri*. Pair correlation function for the observed data (dark line), and confidence limits (quantils 2.5 and 97.5%, gray dashed lines). The gray continuous line represents the value of 1, expected for a random distribution. Data above this line indicates aggregation and below it segregation. $r = 3$ m.

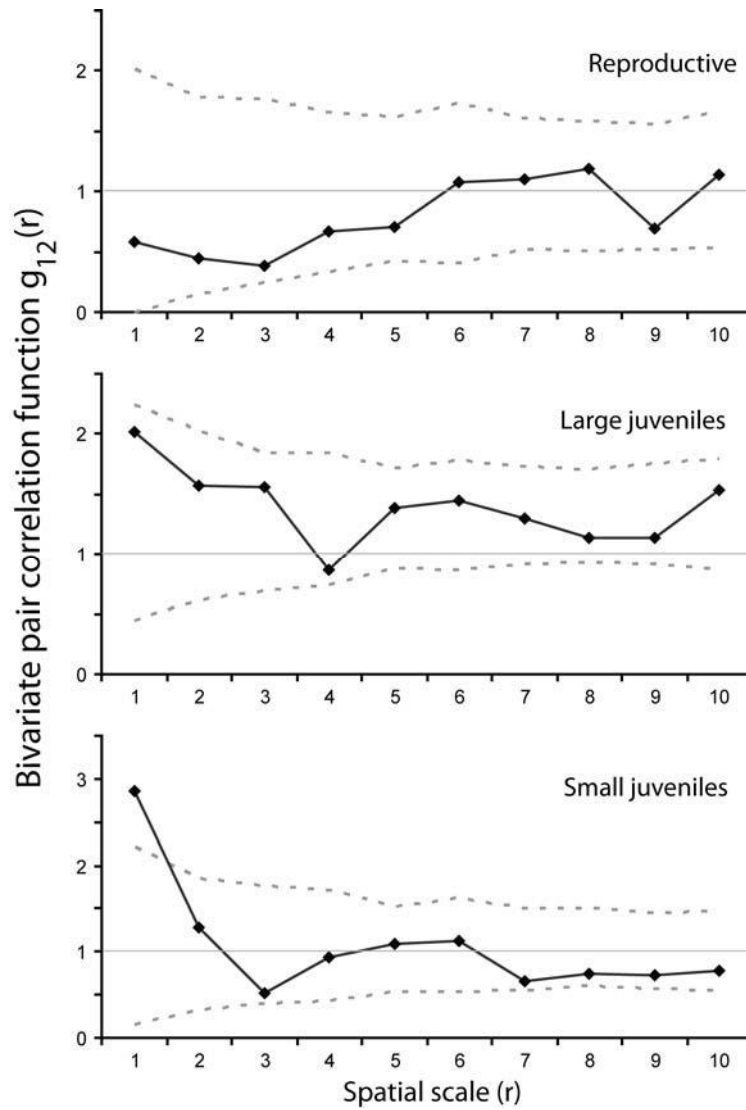


Figure S5. Bivariate analysis of the interspecific effect in distribution of the trees according to size category. Pair correlation function for the observed data (dark line), and confidence limits (quantils 2.5 and 97.5%, gray dashed lines). $r=5\text{m}$ for large juveniles and 3m for the small juveniles. The gray continuous line represents the value of 1, expected for a random distribution. Data above this line indicates aggregation and below it segregation.

Table S1. Microsatellites' profiles for populations of *Virola bicuhyba* according to size classes in the Brazilian Atlantic forest, including sample size (n), number of alleles (k), observed heterozygosity (Ho), expected heterozygosity (He), F_{is} estimate (f), and probability for Hardy-Weinberg equilibrium test (HW); ns = non significant.

	Locus	n	k	Ho	He	f	HW
Reproductive	Vmul2-65	65	5	0.338	0.327	-0.036	ns
	Vsur2-35	65	19	0.877	0.909	0.035	ns
	VF1la	65	3	0.569	0.558	-0.02	ns
	Vsur45	65	4	0.600	0.542	-0.108	0.034
	Vmul68	64	7	0.750	0.707	-0.061	0.009
	Vsur34	65	12	0.708	0.705	-0.003	ns
	Mean		8.33	0.640	0.625	-0.025	
Large juveniles	Vmul2-65	43	4	0.372	0.376	0.012	ns
	Vsur2-35	43	14	0.884	0.873	-0.012	ns
	VF1la	43	3	0.651	0.573	-0.139	ns
	Vsur45	43	3	0.558	0.524	-0.066	ns
	Vmul68	43	7	0.465	0.737	0.371	<0.0001
	Vsur34	43	12	0.721	0.811	0.112	ns
	Mean		7.17	0.609	0.649	0.063	
Small juveniles	Vmul2-65	102	5	0.265	0.260	-0.017	ns
	Vsur2-35	102	18	0.902	0.830	-0.087	ns
	VF1la	102	3	0.569	0.528	-0.078	ns
	Vsur45	102	3	0.539	0.512	-0.054	ns
	Vmul68	102	5	0.716	0.691	-0.036	ns
	Vsur34	102	12	0.765	0.748	-0.023	ns
	Mean		7.67	0.626	0.595	-0.052	

Table S2. Microsatellites' profiles for populations of *Virola gardneri* according to size classes in the Brazilian Atlantic forest, including sample size (n), number of alleles (k), observed heterozygosity (Ho), expected heterozygosity (He), F_{is} estimate (f), and probability for Hardy-Weinberg equilibrium test (HW); ns = non significant.

	Locus	n	k	Ho	He	f	HW
Reproductive	Vmul2-65	34	8	0.676	0.669	0.005	ns
	Vsur2-35	34	20	0.882	0.883	0.001	ns
	Vmul68	34	9	0.912	0.843	-0.082	ns
	Vsur34	34	11	0.794	0.861	0.078	ns
	Mean		12	0.816	0.814	-0.002	
Large juveniles	Vmul2-65	61	10	0.721	0.714	-0.010	ns
	Vsur2-35	61	21	0.984	0.925	-0.064	0.002
	Vmul68	61	12	0.918	0.865	-0.062	ns
	Vsur34	61	14	0.688	0.829	0.170	0.0003
	Mean		14.25	0.828	0.833	0.006	
Small juveniles	Vmul2-65	55	7	0.709	0.716	0.010	ns
	Vsur2-35	55	20	0.964	0.907	-0.063	ns
	Vmul68	55	11	0.836	0.793	-0.055	ns
	Vsur34	55	12	0.745	0.788	0.054	ns
	Mean		12.5	0.814	0.801	-0.016	

CONCLUSÕES FINAIS

Virola bicuhyba e *V. gardneri* são filogeneticamente próximas. Essa relação determina características comuns ao gênero e pode impor limitações à capacidade das espécies de diferir uma da outra. Entretanto, neste trabalho demonstramos que mesmo pequenas diferenças entre as espécies podem gerar uso diferenciado do ambiente, que é suficiente para as espécies coexistirem sem interagir negativamente.

Fenologia reprodutiva:

O início sincrônico da floração destas espécies de *Virola* na Mata Atlântica pode ter origem filogenética ou ser determinado por processos de convergência ecológica, sendo uma resposta em conjunto às mesmas variáveis ambientais (ou combinação delas). Isto pode estar relacionado com a disponibilidade de insetos polinizadores e a floração em conjunto poderia ser positiva para essas espécies ao atrair uma maior quantidade destes. Espécies do gênero *Virola* são dióicas e não apresentam adaptações para anemofilia, portanto são completamente dependentes dos polinizadores para sua reprodução. Em outro cenário, a interferência na reprodução gerada por cargas de pólen interespecífico poderia favorecer a separação do período de floração em um processo de deslocamento temporal da floração. Apesar das duas espécies iniciarem a floração ao mesmo tempo, sua intensidade e distribuição temporal diferem grandemente. A separação do período de frutificação das espécies evita a competição por dispersores. Estudos futuros deveriam analisar os padrões de floração e frutificação em populações nas quais *V. bicuhyba* e *V. gardneri* não sejam simpátricas e assim determinar se a floração em conjunto e a frutificação em períodos diferentes têm relação com a coexistência das espécies, ou se são determinados por outros fatores.

O padrão de floração anual dos indivíduos de *V. bicuhyba* é comum em árvores tropicais, mas não encontramos menção ao padrão bienal de *V. gardneri* (ocorrendo nos dois sexos) em outras espécies. A intensidade e o período de floração em *V. bicuhyba* estão relacionados com o tamanho dos indivíduos; indivíduos maiores apresentam maior cobertura total e menor sincronia na floração devido ao seu período de floração estendida. Isto provavelmente está relacionado com maior disponibilidade de recursos, uma vez que esses indivíduos recebem maiores níveis de iluminação no dossel. Entretanto, indivíduos de tamanhos semelhantes apresentam grande variação na sua cobertura por flores e tempo de floração. Isto poderia ter implicações em processos de seleção sexual mediada por polinizadores, caso a maior cobertura e maior tempo de floração atuem diferencialmente na atração dos polinizadores, favorecendo o fluxo de pólen de alguns indivíduos. O padrão bienal em *V. gardneri* parece eliminar qualquer tipo de relação com o tamanho, possivelmente devido ao acúmulo de recursos durante o longo período entre florações permitindo à maioria dos indivíduos realizarem florações maciças em um período restrito de tempo.

Alometria e morfologia:

Existe uma estratégia alométrica no crescimento do tronco para o gênero *Virola* que é evidenciada na similaridade entre as quatro espécies estudadas (*V. bicuhyba* e *V. gardneri* no Brasil; *V. koschnyi* e *V. surinamensis* na Costa Rica). Entretanto, pequenas variações no caso das espécies brasileiras permitem diferir no seu uso dos estratos verticais da floresta, investindo diferencialmente entre altura e diâmetro do tronco. Contrário ao encontrado na relação altura diâmetro, a relação entre o tamanho da copa e o diâmetro do tronco foi muito variável entre as espécies e não foi encontrada relação entre essa característica e o uso do estrato vertical para cada espécie. Os sexos não apresentaram dimorfismo nas características alométricas do tronco e copa o que sugere que o uso do habitat e as necessidades energéticas não são diferentes.

Distribuição e estrutura genética espacial:

Virola bicuhyba e *V. gardneri* mostraram os mesmos padrões de distribuição espacial com agregação de indivíduos em escalas espaciais similares ao comparar cada categoria de tamanho. Entretanto, as espécies apresentam diferentes tolerâncias microambientais na floresta, possivelmente relacionadas com os níveis de iluminação.

Não foi encontrada interação entre os sexos para estas espécies o que implica que sua distribuição é homogênea na área estudada, não existindo diferenças nas preferências de hábitat entre eles. Isto concorda com ausência de dimorfismo sexual nas relações alométricas e seu uso do mesmo estrato vertical e com a hipótese aqui proposta de similaridade no esforço reprodutivo entre os sexos.

A única interação entre as espécies em relação a sua distribuição espacial foi para a agregação dos juvenis pequenos em escalas espaciais muito curtas. Isto pode se dever ao compartilhamento dos mesmos grupos de dispersores de sementes entre as duas espécies, os quais gerariam sombras de sementes equivalentes, porém em momentos diferentes dado que o amadurecimento dos frutos ocorre em períodos diferentes. O padrão espacial mais agregado em *V. gardneri* pode ter relação com sua semente grande que restringe a poucas espécies de aves capazes da sua dispersão, contrário do que ocorre com *V. bicuhyba* com sua semente de menor tamanho. Isto é corroborado pela forte estruturação genética espacial observada para *V. gardneri*, mas quase ausente em *V. bicuhyba*.

Relação entre as distintas áreas:

Tanto a fenologia reprodutiva, quanto a alometria das espécies e sua distribuição espacial estão fortemente relacionadas, sendo possível explicar vários dos processos observados apenas quando se consideram os três tópicos conjuntamente. A alometria das espécies influi na alocação e disponibilidade de recursos, estando relacionada com o uso do espaço vertical. Essa

disponibilidade de recursos influi nas estratégias reprodutivas das espécies, tanto na sua floração como nas características dos frutos e sementes.

Virola bicuhyba que apresenta florações anuais e por longos períodos ocupa um estrato vertical mais alto e apresenta uma copa maior o que implica em maior captação de luz e disponibilidade de energia. Entretanto, *V. gardneri* faz uso de um estrato ligeiramente mais baixo na floresta e possivelmente tem acesso a menor quantidade de recursos, o que poderia influir na sua estratégia de floração bienal e no seu longo período de amadurecimento dos frutos. Assim, as estratégias alométricas e fenológicas observadas estariam relacionadas.

Virola gardneri, que apresenta copa menor e uso do estrato vertical ligeiramente inferior ao de *V. bicuhyba*, recruta preferencialmente em áreas com maior exposição à luz. O tamanho dos frutos e o tempo do seu amadurecimento determinam as interações com os organismos dispersores, os quais definem a organização espacial inicial e estrutura genética das populações. Assim, *V. gardneri* apresenta floração bienal, demora um ano em amadurecer seus frutos sendo esses 50% maiores que os de *V. bicuhyba*, espécie com floração anual e período de amadurecimento menor. Frutos maiores e com amadurecimento em curtos períodos em *V. gardneri* possuem menos dispersores e menores distâncias de dispersão gerando populações mais agregadas e fortemente estruturadas geneticamente. Essa distribuição inicial é modificada por eventos relacionados com diferentes tolerâncias por microambientes particulares e com mortalidade relacionada com interações interespecíficas. De novo, a tolerância por condições particulares está relacionada com a estratégia de alocação de recursos para o crescimento (relações alométricas) e a mortalidade pode variar entre as espécies segundo as características dos frutos, sendo que seu tamanho está relacionado com a disponibilidade de nutrientes para o embrião e sua capacidade de estabelecimento.