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POLINIZAÇÃO E QUALIDADE DE SEMENTES PRODUZIDAS POR *Psychotria tenuinervis* (RUBIACEAE) EM FRAGMENTOS DE MATA ATLÂNTICA: EFEITO DA DISTÂNCIA DE BORDAS ANTRÓPICAS E NATURAIS

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RESUMO DA TESE

A variabilidade climática espacial e temporal entre áreas pode provocar mudanças nos eventos reprodutivos de populações de animais e plantas. O isolamento de manchas florestais e a criação de bordas pela fragmentação florestal podem ocasionar mudanças nas condições abióticas e bióticas tanto entre quanto dentro de fragmentos fllorestais, podendo afetar alguns aspectos relacionados à reprodução e fluxo gênico das plantas e conseqüentemente diminuir a qualidade de sementes devido ao aumento do endocruzamento. Essas mudanças também podem ser encontradas em bordas naturais (limites entre florestas e rios ou riachos). Tanto bordas antrópicas, criadas pela fragmentação, quanto bordas naturais, podem apresentar perturbação no fluxo gênico e conseqüentemente na qualidade das sementes produzidas pelas plantas lá localizadas. O objetivo desta tese foi investigar se, em escala regional, (i) houve diferenças climáticas entre fragmentos florestais; (ii) a fenologia reprodutiva de *P. tenuinervis* seria influenciada pelas condições climáticas; (iii) existiriam diferenças na fenologia reprodutiva de *P. tenuinervis* entre fragmentos com diferentes distâncias. E em escala local foi investigar se (iv) houve diferenças no microclima; (v) na fenologia reprodutiva; (vi) nas comunidades de visitantes florais; na freqüência de suas visitas; e na produção de frutos e sementes; (vii) variabilidade e estrutura genética; (viii) massa, taxa e velocidade de germinação de sementes produzidas por indivíduos de *Psychotria tenuinervis* localizados em bordas antrópicas (BA), bordas naturais (BN) e interior do fragmento (IF). O estudo foi conduzido em cinco fragmentos (em escala regional) no Rio de Janeiro, sudeste do Brasil, e em escala local, dentro de um deles. Houve diferenças no clima entre os cinco fragmentos, porém o padrão fenológico de *P. tenuinervis* encontrado nos dois anos foi similar entre eles. Esses

resultados indicam que o padrão geral da fenologia reprodutiva desta espécie, em uma escala regional, pode ser influenciado por fatores evolutivos. Em escala local, não houve diferenças no microclima; padrões fenológicos; taxa de visitação floral (só em 2002, BN apresentou mais visitas e BA menos), produção de frutos e sementes; variabilidade e estrutura genética; nem na taxa e velocidade de germinação, entre os três ambientes devido a grande variação entre as parcelas dentro deles. A indicação dessa heterogeneidade e a provável importância de outros fatores, como clareiras ou idade das bordas, ao invés da distância de bordas, nos fragmentos estudados, podem ser muito importantes para programas de conservação.

THESIS ABSTRACT

Spatial and temporal climatic variability among areas may affect the reproductive events of plant and animal populations. Habitat changes or abrupt limits between habitats can affect the interactions between plants and their pollen and seed vectors and lead to a decrease in seed quality because of increased inbreeding. The isolation of forest patches and the edges created by fragmentation may change the abiotic and biotic conditions among and within forest fragments; they also could affect some aspects related to plant reproduction and gene flow, decreasing seed quality due to the inbreeding. These changes could also occur at natural edges (limits between forests and rivers or streams). Plants near anthropogenic and natural edges could present alterations of their gene flow and consequently in the quality of their seeds. The aim of this thesis was to investigate whether, on a regional scale, there were (i) climatic differences among forest fragments, (ii) influences of climatic conditions on the reproductive phenology of *P. tenuinervis*, or

differences in (iii) reproductive phenology of *P. tenuinervis* among forest fragments, and on a local scale, whether there were differences (iv) in microclimate, (v) reproductive phenology, (vi) community of flower visitors, (vii) genetic variability and structure, and (viii) mass, rate and velocity of germination of seeds produced by *Psychotria tenuinervis* located on anthropogenic edges (AE), natural edges (NE) and in the forest interior (FI). The study was carried out, on a regional scale, in five fragments in Rio de Janeiro, Brazil, and on a local scale, within one of them. In spite of differences in climatic conditions among the five fragments, the phenological pattern of *P. tenuinervis* found in the two years was similar among them. These results indicated that the general pattern of reproductive phenology of this plant species, on a regional scale, could be influenced by evolutionary factors. On a local scale, there were no differences, among the three habitats, in microclimate, phenological pattern, rate of flower visits (only in 2002, NE with more and AE fewer visits), fruit and seed production, genetic variability and structure, and rate and velocity of seed germination. These pattern may occurr due to the great variation among the sample plots within each habitat. The heterogeneity found within each habitat, and the probable greater importance of gaps or edge age instead of the distance from the edges, could be very important for conservation programs of forest habitats.

INTRODUÇÃO GERAL

Atualmente, a fragmentação florestal é um problema mundialmente conhecido, havendo muitas pesquisas sobre comunidades e populações de plantas e animais que ocorrem nestes ambientes. Quando e como começaram os estudos sobre fragmentação? Quais os primeiros aspectos estudados na fragmentação de habitat? Olhar para o passado e compreender o histórico do assunto que se trabalha, ajuda a caminhar em direção ao futuro com muito mais competência para contribuir para a ciência, pois sabendo o que já foi feito, é possível identificar os caminhos que ainda faltam percorrer e quais são os passos que faltam para aumentar a sua compreensão.

a) O desenvolvimento do estudo da fragmentação de habitat

Vários autores (Wilcove *et al.* 1986, Simberloff 1988, Hanski & Simberloff 1997, Laurance & Bierregaard Jr. 1997b) indicaram que os estudos de fragmentação começaram após os trabalhos pioneiros de MacArthur e Wilson sobre biogeografia de ilhas, cuja teoria foi reunida e publicada no livro intitulado: *A Teoria da Biogeografia de Ilhas* (MacArthur & Wilson 1967). Tal teoria sugere que o número de espécies em uma ilha oceânica representa um balanço, ou equilíbrio dinâmico, entre processos de imigração e extinção. O equilíbrio do número de espécies em uma ilha depende tanto da característica da ilha, de seu tamanho e do isolamento das fontes potenciais de colonizadores, quanto das características das próprias espécies, como suas habilidades de dispersão e a densidade de suas populações. Essa teoria, sem dúvida, ganhou força e atraiu a atenção de muitos pesquisadores devido à sua simplicidade e sua universalidade, onde o número de espécies

em uma ilha seria um balanço entre imigração e extinção, os quais, seriam dependentes do tamanho e do isolamento das ilhas (Williamson 1989).

Com a crescente divulgação e preocupação com o desmatamento e a destruição de habitats em todos os continentes do globo terrestre no final dos anos 70 e começo dos 80, as idéias e modelos de biogeografia de ilhas começaram a ser aplicados e transferidos para fragmentos de habitat, com o objetivo de tentar preservar as espécies nesses habitats, cuja maioria se encontrava ameaçada de extinção devido ao contínuo desmatamento (Wilcove *et al.* 1986). Devido a analogia dos habitat fragmentados com as ilhas oceânicas, a teoria de MacArthur e Wilson começou a ser aplicada nestes ambientes. A fragmentação ocorre quando uma grande extensão do habitat é transformada em alguns "pedaços" ou partes de menor área, isolados entre si por uma matriz de habitat diferente da original. Quando a paisagem que circunda os fragmentos é inóspita para as espécie do habitat original e quando a dispersão dessas espécies é pequena, os fragmentos remanescentes podem ser considerados verdadeiras "ilhas de habitat" onde a comunidade local estará isolada (Preston 1962). O processo de isolar formações através da fragmentação foi denominada "insularização" por Wilcox (1980).

Os primeiros estudos sobre a fragmentação de habitat, baseados na teoria do equilíbrio dinâmico da biogeografia de ilhas, tinham o objetivo de propor princípios gerais de delineamento de refúgios, como formato, tamanho e conectividade, com o intuito de reduzir a taxa de extinção nos refúgios isolados (Simberloff 1988). Em meados dos anos 70 foram publicados alguns trabalhos baseados nesta teoria (Terborgh 1974, Diamond 1975, Wilson & Willis 1975 *apud* Hanski & Simberloff 1997) com sugestões de regras e critérios para a delimitação de refúgios, baseados na relação área-volume e na ligação dos fragmentos pequenos através de corredores. O debate sobre estas regras: um único grande

refúgio é melhor ou pior do que vários refúgios menores com o mesmo tamanho do único grande refúgio (SLOSS), gerou vigorosas discussões e, conseqüentemente, uma enorme quantidade de trabalhos (Simberloff 1988).

No começo da década de 1980 a ênfase dos estudos em habitats fragmentados começou a mudar. Até então, a maioria dos trabalhos focava a comunidade de animais e plantas em fragmentos, com base na teoria do equilíbrio da biogeografia de ilhas. Porém, com o aparecimento dos estudos de genética em fragmentos, principalmente sobre a depressão por endocruzamento devido à redução do tamanho da população e à deriva genética (Hooper 1971 *apud* Simberloff 1988), os estudos de populações de espécies remanescentes passou a ganhar um maior enfoque (Simberloff 1988).

Os estudos sobre genética de populações começaram a ser incluídos no estudo de fragmentação de habitat e, conseqüentemente, nos estudos de conservação, devido ao problema que pode ocorrer com plantas e animais remanescentes em refúgios ou pequenas porções de habitat: a endogamia (Moore 1962). A depressão por endocruzamento ameaça severamente as populações em refúgios, e a deriva genética pode empobrecê-las geneticamente, aumentando a endogamia (Franklin 1980). Franklin (1980) e Soulé (1980) sugeriram que um tamanho populacional mínimo (TPM) de 50 indivíduos seria necessário para impedir a depressão por endocruzamento, assim como um TPM de 500 indivíduos preveniria uma erosão da variabilidade genética a longo prazo. Porém estes números irão variar de acordo com algumas características das espécies, como por exemplo a sua abundância e ciclo de vida.

Na discussão sobre qual seria o melhor formato das reservas (SLOSS) ainda existem argumentos favoráveis à preservação de vários fragmentos pequenos (preservando maior diversidade de habitats), pois vários trabalhos encontraram mais espécies em conjuntos de

ilhas e de fragmentos pequenos do que em fragmentos grandes com o mesmo tamanho (Simberloff & Abele 1982). Após a ênfase nos estudos genéticos e a mudança do enfoque de comunidades para populações, em vários casos se verificou que manter várias reservas pequenas pode ser prejudicial a longo prazo para as populações de algumas espécies remanescente, devido ao endocruzamento e à deriva genética, que aumentam a chance de extinção de pequenas populações. Lógico que tudo isso irá depender da espécie em questão, seu tamanho, ciclo de vida, tempo de geração, sistema reprodutivo, assim como, seus requisitos ecológicos.

Portanto, devido à contribuição da genética aos estudos de fragmentação de habitat, o destino e a heterogeneidade das populações das espécies passaram gradativamente a ter tanta importância quanto os estudos relacionados a diversidade de espécies nos fragmentos (Simberloff 1988). Com isso, os trabalhos passaram a ter como foco de interesse, o conjunto de relações entre a diversidade de habitats, ou número de refúgios, e a dinâmica de colonização e extinção das populações de plantas e animais numa escala mais ampla, de paisagem, resgatando a migração como um componente importante na dinâmica dessas populações. Surge, então, os estudos enfocando metapopulações (Hanski & Simberloff 1997). Cada vez mais aumentava a compreensão de que a conservação requer conhecimentos sobre a autoecologia das espécies, especialmente requerimentos de habitat de uma espécie de interesse, pois assim, preservando o máximo possível do habitat de uma espécie, a chance de conservá-la aumentaria (Simberloff 1988), voltando a se dar importância aos pequenos remanescentes de habitats, que eram considerados sem importância por não poderem proteger muitas espécies ou muitos indivíduos de uma população.

Uma das prioridades em estudos sobre fragmentação passou a ser a definição do tamanho mínimo do fragmento ou reserva para se conservar, à longo prazo, o maior número de espécies possíveis, levando em consideração os conceitos de genética de populações (Simberloff & Abele 1982). Porém, o conhecimento e a definição do tamanho mínimo de um fragmento que garanta a conservação de uma espécie a longo prazo, vai depender da espécie que esteja sendo considerada. Diamond (1976) citou que espécies devem ser diferenciadas quanto as suas importâncias e não apenas contadas, sendo que as estratégias de conservação não devem tratar todas as espécies como iguais, mas devem focar em espécies e habitats ameaçados por atividades humanas, e/ou espécies que tenham diferentes requerimentos e histórias de vida.

Hoje em dia, estão sendo realizados vários tipos de estudos envolvendo a fragmentação com enfoques diferentes. Além dos estudos sobre a relação entre a área do refúgio e o número de espécies e suas abundâncias, estão sendo adicionados ao conhecimento geral sobre a fragmentação, o isolamento e a conectância entre refúgios, os estudos sobre bordas (ver exemplo em Metzger 1999, Debinski & Holt 2000, Hobbs & Yates 2003), estudos genéticos e demográficos, estudos sobre a influência do tipo de matriz, e a complexidade das bordas nos fragmentos (ver exemplo em Metzger 1999), assim como contribuições mais práticas e aplicadas, como o manejo de fragmentos (Schelha & Greenberg 1996, Laurance & Bierregaard Jr. 1997a).

b) O estudo da fragmentação no Brasil

Os primeiros estudos sobre fragmentação no Brasil ocorreram na década de 1980 na Amazônia, através do Projeto do Tamanho Mínimo Crítico de Ecossistemas, atualmente conhecido por Projeto de Dinâmica Biológica de Fragmentos Florestais (PDBFF). Em

1979, o Fundo Mundial para Vida Selvagem (WWF) em conjunto com o Instituto Nacional de Pesquisa na Amazônia (INPA) implantaram o PDBFF, para investigar e tentar compreender os fatores que desencadeiam a perda de espécies em fragmentos florestais após o seu isolamento, com o objetivo de definir o tamanho mínimo de fragmentos que mantenha a comunidade animal e vegetal dos fragmentos perto da sua diversidade característica (Lovejoy *et al.* 1983, Bierregaard Jr *et al.* 1992). A partir de 1989, o Museu Nacional de História Natural de Smithsonian passou a administrar o PDBFF (Bierregaard Jr *et al.* 1992).

O desenho experimental do PDBFF é baseado na comparação de uma série de réplicas de fragmentos florestais, ou reservas, de diferentes tamanhos antes e depois deles terem sidos isolados da floresta contínua. Os estudos mais básicos consistem em inventários através do tempo de grupos seletos de plantas e animais nas parcelas experimentais (Bierregaard Jr *et al.* 1992), porém estudos mais detalhados sobre comportamento e ecologia de determinados grupos de espécies, assim como as mudanças físicas, têm sido desenvolvidos. Até hoje, o PDBFF é o único projeto brasileiro que coletou dados quantitativos e qualitativos de espécies vegetais e animais antes e depois da fragmentação (Debinski & Holt 2000).

Um grande volume de artigos e capítulos de livros publicados no Brasil sobre aspectos da influência da fragmentação em grupos de animais e vegetais é proveniente de pesquisas realizadas na Amazônia, principalmente originadas no PBDFF. Na Mata Atlântica, os trabalhos sobre fragmentação só surgiram no começo da década de 1990, se acentuando no final da mesma. No Cerrado, poucos trabalhos sobre fragmentação foram desenvolvidos (Figura 1), enquanto não existem trabalhos publicados sobre os outros biomas brasileiros.

Os estudos realizados sobre fragmentação no Brasil têm abordado os seguintes temas: (1) características dos fragmentos na paisagem (estudos de ecologia da paisagem), como tamanho, formato e posicionamento do fragmento, com diversidade de plantas e animais, e distribuição geográfica de fragmentos, levando em consideração seus tamanhos e formatos; (2) comparações de diferentes características entre fragmentos, como a diversidade e densidade antes e depois da fragmentação, e estudos sobre a influência do tamanho do fragmento na germinação de sementes, dispersão e diversidade de animais e plantas; (3) comparações de diferentes características dentro de um mesmo fragmento, como a diferença entre habitats, comparando diversos aspectos de populações e comunidades de plantas e animais entre eles, como borda-interior, matriz-interior, tipos de matrizes, assim como diferenças de microclima (Tabela 1).

Como a maior parte do conhecimento sobre os efeitos da fragmentação de florestas tropicais no Brasil (e também no mundo) provém do PDBFF desenvolvido na Amazônia, torna-se difícil generalizar os resultados e padrões encontrados na Amazônia, para fragmentos da Mata Atlântica. As paisagens desta são completamente diferentes da paisagem artificial que foi criada para ser estudada em Manaus, onde além de diferirem no tempo em que seus fragmentos foram criados, as bordas dos fragmentos criados pelo PDBFF possuem a mesma idade e tipo de perturbação, e não possuem a longa história de perturbação antrópica (caça, queimadas, corte de lenha) como os fragmentos da Mata Atlântica.

c) O estudo sobre bordas de fragmentos

Os estudos sobre bordas antrópicas e seus efeitos nos fragmentos ainda são muito recentes, especialmente nos trópicos (Laurance & Bierregaard Jr. 1997c). A revisão de

Murcia (1995), por sua vez, foi marcante no estudo da fragmentação, organizando os conhecimentos sobre os possíveis efeitos da formação de uma borda antrópica nos organismos remanescentes de fragmentos florestais.

Os primeiros trabalhos sobre borda e seus efeitos na fragmentação, por várias décadas, foram descritivos e não inquisitivos sobre os mecanismos que causam as modificações relacionadas com a borda nas florestas (Murcia 1995). As primeiras abordagens para quantificar a importância das bordas nos fragmentos florestais avaliavam a razão perímetro/área (Formam e Godron 1986, Didham 1997). Atualmente, a razão perímetro/área tem dado lugar ao modelo centro/área de Laurance & Yensen (1991) que se baseia na quantificação da distância da penetração da borda (d), com o objetivo de calcular a área central, não afetada pela borda, de um fragmento de tamanho ou formato qualquer. Malcolm (1994) apresentou um modelo mais realista da natureza aditiva dos efeitos de borda afetando um único ponto dentro da zona de borda, d.

Segundo Murcia (1995), a formação de bordas florestais causa mudanças abióticas, mudanças bióticas diretas, e mudanças bióticas indiretas, como as interações entre plantas e animais que são muito pouco estudadas: predação, herbivoria, dispersão de sementes e polinização. A primeira modificação ocasionada pela criação de uma borda é a mudança nas condições abióticas, ou seja, alterações no microclima nas áreas próximas a ela (Bierregaard Jr. *et al.* 1992, Murcia 1995). Comparada às florestas, pastagens e plantações permitem que maiores quantidades de radiação solar alcancem o solo durante o dia, e permitem maior reirradiação para a atmosfera à noite. Conseqüentemente, a temperatura nas pastagens e plantações tende a ter máximas mais altas e a apresentar amplas flutuações. O ambiente no interior da floresta, em contraste, é mais ameno e úmido do que a matriz (Murcia 1995).

As mudanças microclimáticas na borda do fragmento podem estimular alterações bióticas diretas, como por exemplo, mudanças na estrutura florestal da borda, uma vez que o crescimento, a mortalidade, a abundância e a distribuição das plantas neste novo ambiente, podem ser afetados pelas mudanças abióticas (Murcia 1995). A densidade e a atividade de algumas espécies de animais florestais também podem ser afetados. Com isso, podem ocorrer mudanças na composição de espécies de animais na borda, resultantes da atração de algumas espécies novas no fragmento, provenientes da matriz e/ou do desaparecimento de espécies de animais florestais, devido à competição com outras espécies de animais cujas densidades tenham aumentado, ou devido a mudança nas condições abióticas ideais necessárias para a espécie (Saunders *et al.* 1991). Alterações em vários dos aspectos da história de vida de plantas e animais na borda dos fragmentos, podem resultar em mudanças bióticas indiretas (Murcia 1995), como as interações entre espécies: herbivoria, polinização, predação e dispersão de sementes (Saunders *et al.* 1991, Aizen & Feinsinger 1994a,b).

Apesar desses 3 efeitos da borda antrópica nos fragmentos (abióticos, bióticos diretos e indiretos), Murcia (1995) concluiu que a falta de uma grande generalização sobre os padrões de efeitos de borda pode ser atribuída ao pobre delineamento de alguns estudos, e a falta de consistência na metodologia dos trabalhos sobre bordas e os seus efeitos nos organismos. A falta de replicação adequada é outra importante limitação de vários estudos. Murcia (1995) ressaltou também, que existem poucos trabalhos investigando o efeito de bordas na interação de espécies.

d) Questões específicas da tese

O clima pode influenciar vários aspectos da biologia de organismos tropicais, como o crescimento e a reprodução de plantas (Corlett & LaFrankie Jr 1998). A variabilidade climática e/ou microclimática espacial e temporal entre áreas pode modificar eventos reprodutivos de populações de plantas e animais (van Schaik *et al.* 1993). Padrões fenológicos apresentados pelas plantas são adaptações ao ambiente biótico e abiótico que as circundam e as variações fenológicas geralmente refletem a influência de sinais ambientais proximais, que iniciam as fases reprodutivas (precipitação, estresse hídrico e irradiância) e fatores finais, que selecionam para fenologias reprodutivas particulares (necessidade de reprodução cruzada, polinizadores, dispersores e predadores de sementes) (Piñero & Sarukhan 1982, Adler & Kielpinski 2000). O primeiro passo para estudar a performance reprodutiva de plantas é identificar padrões temporais e espaciais de atividades reprodutivas, para tentar identificar os fatores proximais e finais que influenciam os padrões fenológicos (Adler & Kiespinki 2000). Portanto, plantas de diferentes populações podem apresentar diferentes fenologias se os fatores proximais forem importantes, porque essas populações estarão sob diferentes climas, como precipitação e temperatura. Porém, se forem os fatores finais que influenciam esses padrões, eles devem apresentar florações e frutificações similares entre as populações.

A maioria das espécies de árvores tropicais apresenta sistema reprodutivo autoincompatível e geralmente depende de animais polinizadores para produzir frutos e sementes (Bawa 1990). Distúrbios que afetem vetores de transferência de pólen podem apresentar impactos importantes na reprodução de espécies de plantas (Ghazoul & McLeish 2001). A produção de sementes de flores de angiospermas depende da quantidade e qualidade (grãos de pólen incompatíveis ou de indivíduos aparentados) do pólen que chega

ao seu estigma (Waser & Price 1991). Em espécies de plantas, o fluxo gênico, através da polinização e da dispersão de sementes, determina tanto a produção de sementes quanto o grau de isolamento genético de suas populações (Dewey & Heywood 1988). Por exemplo, a produção de sementes pelas plantas poderia mudar bastante se a quantidade ou a constância de visita de algum animal polinizador declinasse em habitats perturbados (Aizen & Feinsinger 1994a, b). Regimes de polinização que diferirem na composição e abundância de polinizadores irão provocar reprodução diferencial (Herrera 2000). A limitação de pólen é um fator que afeta a produção de frutos e sementes (Kato & Hiur 1999), a perda da qualidade da semente, a diminuição de sua massa, e da taxa e velocidade de germinação, podendo influenciar a dinâmica populacional e, conseqüentemente, suas chances de extinção local. A massa das sementes, assim como sua taxa e velocidade de germinação, podem influenciar a probabilidade do estabelecimento de plântulas, afetando a distância nas quais as sementes serão dispersas e o tempo de recrutamento das plântulas e, conseqüentemente, influenciando a probabilidade delas alcançarem habitats disponíveis para germinação e sobrevivência (Fenner 1985, Paz *et al.* 1999).

O comportamento do animal mutualista pode gerar um movimento extensivo ou restrito de pólen e sementes (Loiselle *et al.* 1995). Visitantes florais com distância de vôo curta, podem aumentar o endocruzamento, diminuindo o fluxo gênico (Shapcott 1998). O número e principalmente a qualidade das sementes produzidas por algumas populações pode diminuir devido ao aumento do endocruzamento (perda de heterozigozidade) (Templeton *et al.* 1990, Waser & Price 1991) e com a diminuição da variabilidade genética de subpopulações (Ellstrand & Elam 1993, Alvarez-Buylla *et al.* 1996).

A fragmentação florestal, em uma escala regional, pode isolar e suportar populações discretas de plantas (Metzger 1999) e, portanto, facilitar a identificação de diferenças e

mudanças no padrão de atividades reprodutivas entre populações que podem estar sob diferentes condições climáticas. Em escala local, a formação de limites abruptos, ou bordas, entre a floresta e as áreas desmatadas (matriz) pode ocasionar mudanças nas condições abióticas dentro dos habitats (Poulin *et al.* 1999, Debinski & Holt 2000). Além das mudanças nas condições ambientais que resultam da proximidade de uma matriz estruturalmente diferente (Bierregaard Jr *et al.* 1992), a criação de bordas antrópicas pode também estimular modificações bióticas diretas, como alterações na presença e na quantidade de flores e frutos de plantas (Aizen & Feinsinger 1994a,b, Murcia 1995), assim como na época e duração da floração e frutificação (Rathcke & Lacey 1985). Por sua vez, bordas naturais (limites entre florestas e rios, riachos, lagos ou campos naturais) também podem apresentar diferenças abióticas e bióticas do interior da floresta (Corbet 1990, Matlack 1994). Portanto, tanto mudanças abióticas quanto alterações na composição e comportamento de visitantes florais pode afetar, diretamente, a reprodução de plantas e a variabilidade genética próximo a bordas antrópicas e naturais.

O objetivo deste trabalho foi investigar se (1) a fenologia reprodutiva de *Psychotria tenuinervis* (Rubiaceae) é influenciada por condições climáticas atuais (pluviosidade, temperatura); (2) existem diferenças na fenologia; (3) na polinização e (4) na qualidade das sementes produzidas (taxa e velocidade de germinação e a variabilidade e estrutura genética da safra de sementes produzidas) por indivíduos de *P. tenuinervis* localizados em bordas naturais, antrópicas e no interior de fragmento florestal.

As poucas investigações realizadas até o momento sobre aspectos reprodutivos de plantas em fragmentos florestais, apenas relacionam aspectos da reprodução com a redução do tamanho do fragmento (Aizen & Feinsinger 1994a,b, Murcia 1996) ou com o seu grau de isolamento (Steffan-Dewenter & Tscharntke 1999). Nenhum estudo foi realizado

analisando variações espaciais no comportamento reprodutivo e na polinização de plantas em fragmentos, como por exemplo diferenças entre bordas antrópicas e bordas naturais. É importante, para programas de conservação e de manejo de espécies de plantas, saber avaliar até que ponto as alterações na sua reprodução em fragmentos florestais são conseqüência da ação antrópica ou refletem as variações naturais relacionadas à heterogeneidade da floresta (Casenave *et al.* 1998).

Esta tese foi dividida em capítulos, cujos objetivos específicos estão listados a seguir, e as justificativas e expectativas em relação a cada objetivo se encontram nos respectivos capítulos. O objetivo geral deste trabalho foi investigar se, em escala regional, (i) houve diferenças climáticas (precipitação e temperatura) entre fragmentos florestais (capítulo 1); (ii) a fenologia reprodutiva de *P. tenuinervis* foi influenciada pelas condições climáticas; e (iii) existem diferenças na fenologia reprodutiva de *P. tenuinervis* entre fragmentos (capítulo 2). Em escala local, foi investigar se haveriam diferenças (iv) no microclima (temperatura, umidade do solo e abertura da dossel) (capítulo 1); (v) na fenologia reprodutiva (capítulo 2); (vi) nas comunidades de visitantes florais; na freqüência de suas visitas; e na produção de frutos e sementes (capítulo 3); (vii) na variabilidade e estrutura genética; (viii) na massa, taxa e velocidade de germinação de sementes (capítulo 4) produzidas por indivíduos de *P. tenuinervis* localizados em bordas antrópicas (BA), bordas naturais (BN) e interior do fragmento (IF).

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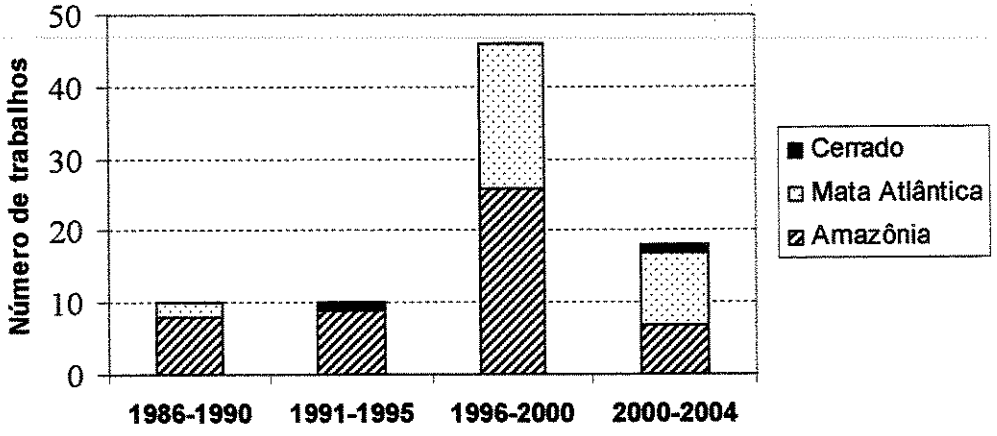


Figura 1: O número de trabalhos sobre fragmentação no Brasil em cada bioma, desde a década de 1980.

Tabela 1: Temas dos estudos realizados sobre fragmentação no Brasil.

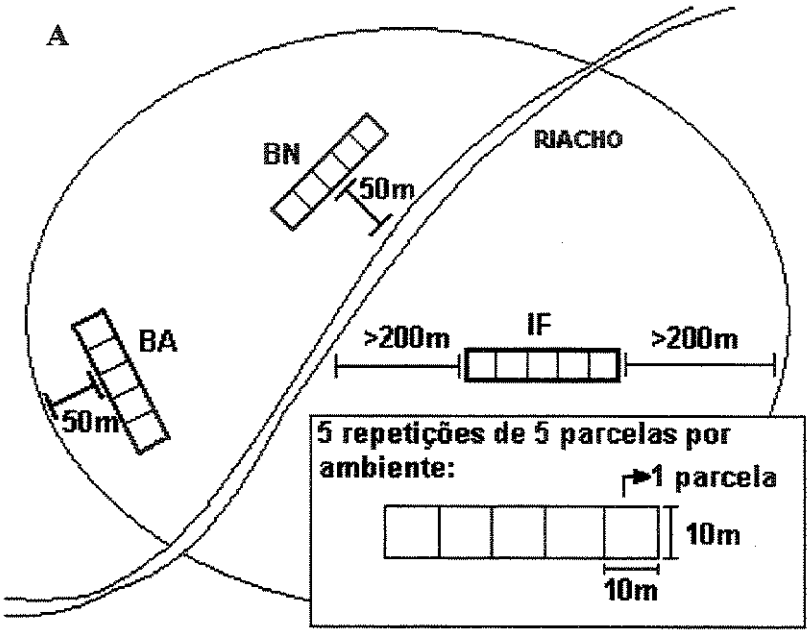
Assuntos	Referencias (*)
1) Características dos fragmentos na paisagem	
Estudos de paisagem:	(5, 8, 33, 37, 50, 51, 52, 56)
Distribuição geográfica de fragmentos:	(60, 67)
2) Comparações de diferentes características entre fragmentos	
Comparação antes e depois da fragmentação:	(15, 21, 26, 27, 28, 72)
Comparação entre fragmentos:	(1, 2, 3, 4, 6, 7, 9, 11, 13, 14, 15, 16, 17, 18, 20, 22, 23, 32, 35, 38, 39, 40, 41, 42, 45, 44, 46, 47, 48, 53, 57, 58, 59, 61, 62, 63, 64, 68, 69, 70, 76, 73, 75, 78, 79, 80, 81, 84)
3) Comparações de diferentes características dentro de um mesmo fragmento	
Diferença entre habitats: borda-interior, matriz-interior, entre tipos de matrizes	(4, 10, 11, 12, 18, 19, 20, 24, 25, 26, 27, 29, 30, 31, 36, 34, 40, 43, 49, 54, 55, 65, 66, 71, 74, 77, 79, 82, 83).

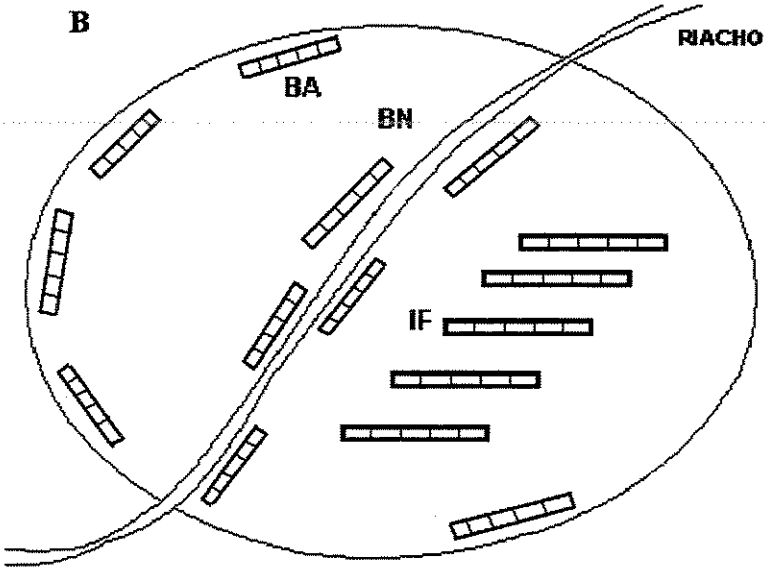
Referencia (*)

1) Anciaes & Marini 2000.
2) Andresen 2003.
3) Becker *et al.* 1991.
4) Benitez 1998.
5) Bernacci *et al.* 1998.
6) Bierregaard Jr. & Lovejoy 1989.
7) Bierregaard Jr. & Stouffer 1997.
8) Brito & Fernandez 2002.
9) Bruna 1999.
10) Camargo & Kapos 1995.
11) Carvalho & Vasconcelos 1999.
12) Cavalcanti 1992.
13) Chiarello 1999.
14) Chiarello & Melo 2001.
15) Christiansen & Pitter 1997.
16) Cullen Jr. *et al.* 2000.
17) Da Fonseca & Robinson 1990.
18) Didham 1998.
19) Didham & Lawton 1999.
20) Didham *et al.* 1998.
21) Ferraz *et al.* 2003.
22) Ferreira & Laurance 1997.
23) Funk & Mills 2003.
24) Galetti *et al.* 2003.
25) Gascon 1993.
26) Gascon 1998.
27) Gascon *et al.* 1999.
28) Harper 1989.
29) Kapos 1989.
30) Kapos *et al.* 1993.
31) Kapos *et al.* 1997.
32) Klein 1989.
34) Laurance *et al.* 1997d.
35) Laurance *et al.* 1998a.
36) Laurance *et al.* 1998b.
37) Laurance *et al.* 1998c.
38) Laurance *et al.* 1999.
39) Laurance *et al.* 2001a.
40) Laurance *et al.* 2001b.
41) Leite & Marini 1999.
42) Lima & Gascon 1999.
43) Malcolm 1994.
44) Maldonado-Coelho & Marini 2000.
45) Maldonado-Coelho & Marini 2004.
46) Marini 2001.
47) Marsden *et al* 2001.
48) Martins 1989.
49) Mesquita *et al.* 1999.
50) Metzger 1997a.
51) Metzger 1997b.
52) Metzger 2000.
53) Morato & Campos 2000.
54) Nascimento *et al.* 1999.
55) Oliveira *et al.* 1997.
56) Pires *et al.* 2002.
57) Pizo 1997.
58) Powell & Powell 1987.
59) Quental *et al.* 2001.
60) Ranta *et al.* 1998.

61) Scariot 1999.
62) Scariot 2000.
63) Schwarzkopf & Rylands 1989.
64) Silva & Tabarelli 2000.
65) Sizer & Tanner 1999.
66) Sizer *et al.* 2000.
67) Skole & Tucker 1993.
68) Souza & Brown 1994.
69) Souza *et al.* 2000.
70) Souza & Martins 2003.
71) Stevens & Husband 1998.
72) Stoufer& Bierregaard Jr., 1995.
73) Stratford & Stouffer 2001.
74) Tabanez *et al.* 1997.
75) Tabanez & Viana 2000.
76) Tabarelli *et al.* 1999.
77) Tabarelli & Mantovani 1997.
78) Tocher *et al.* 1997.
79) Tonhasca *et al.* 2002a.
80) Tonhasca *et al.* 2002b.
81) Vasconcelos 1988.
82) Viana *et al.* 1997.
83) Werneck *et al.* 2000.
84) Zimmerman & Bierregaard Jr.1986.

APÊNDICE





Esquema do desenho amostral no fragmento de Saquarema no Hotel Fazenda Serra da Castelaña, mostrando a disposição das parcelas em cada repetição de cada ambiente. A. Detalhe das 5 parcelas dentro das repetições e B. Visão geral das 5 repetições dentro do fragmento. BA = borda antrópica, BN = borda natural e IF = interior do fragmento.

CAPÍTULO 1

MICROCLIMATE OF ATLANTIC FOREST FRAGMENTS: REGIONAL AND LOCAL SCALE HETEROGENEITY[1]

Flavio Nunes Ramos & Flavio A. M. Santos

[1] Nos moldes da revista Brazilian Archives of Biology and Technology

ABSTRACT

Spatial and temporal climatic variability among areas may affect the reproductive events of plant and animal populations. In this work we investigated whether there were differences (i) in the long term rainfall and temperature among forest fragments (regional scale), and (ii) in the canopy cover, air temperature and soil humidity among anthropogenic edges (AE), natural edges (NE) and forest interior (FI) (local scale). The study was carried out in five forest fragments (regional scale) in the state of Rio de Janeiro, south-eastern Brazil, and among habitats within one of them (local scale). On a regional scale, rainfall and temperature varied among the fragments. On a local scale, there were no significant differences in the minimum temperature, soil moisture or canopy openness among the three habitats (AE, NE and FI), because of the great variation in these parameters within each habitat. Only maximum and amplitude of temperature differed among habitats, with AE showing the highest average values and NE, the lowest. The heterogeneity found within habitats suggests that other factors, such as gaps or edge age, could have a greater influence on the microclimate than the distance from the edges, and could be very important in conservation programs.

Key words: Atlantic forest, canopy openness, edges, fragmentation, soil moisture, temperature.

RESUMO

A variabilidade espacial e temporal no clima pode provocar mudanças nos eventos reprodutivos de populações de animais e plantas. O objetivo deste trabalho foi investigar se (i) existe diferença na pluviosidade e temperatura entre fragmentos florestais (escala regional), e se (ii) existe diferença na abertura de dossel, temperatura do ar e umidade do solo em bordas antrópicas (BA), bordas naturais (BN) e interior do fragmento (IF) (escala local). O estudo foi conduzido em cinco fragmentos (escala regional) no Rio de Janeiro, sudeste do Brasil, e em escala local, dentro de um deles. Em escala regional, houve diferença na pluviosidade e temperatura entre os fragmentos. Em escala local, não houve diferenças na temperatura mínima, umidade do solo e abertura de dossel entre os três ambientes, devido à grande variação entre as parcelas dentro dos ambientes. Apenas a temperatura máxima e sua amplitude diferiram entre os ambientes, sendo que BA apresentou os maiores valores médios e BN os menores. Esta heterogeneidade encontrada dentro dos ambientes sugere que outros fatores, como clareiras ou idade da borda, podem ter mais influência no microclima do que a distância da borda, e isso pode ser muito importante para programas de conservação.

Palavras chave: Abertura de dossel, bordas, fragmentação, Mata Atlântica, temperatura, umidade do solo.

INTRODUCTION

Climate may influence many aspects of the biology of tropical organisms, including plant growth and reproduction (Corlett & LaFrankie Jr., 1998). The timing of periodic events in relation to climatic seasonality is of obvious importance in strongly seasonal areas, although even in the aseasonal tropics, synchronisation at the population level may be essential, for example, for cross-pollination and for escaping from herbivores (Aide, 1993) or seed predators (Augspurger, 1981). Spatial and long-term climatic and/or microclimatic variability among areas, such as in rainfall and temperature, could alter the reproductive events of plant and animal populations (van Schaik *et al.*, 1993), including pollinator abundance (Augspurger, 1980) and plant phenology (Smith-Ramirez & Armesto, 1994). Animal or plant populations of the same species in distant areas may show different reproductive patterns depending on the climatic conditions. The knowledge of the spatial and temporal variations in climatic conditions among areas on a regional scale is therefore very important to understand the phenological patterns of plant and animal populations.

The periodicity of plant growth and reproduction has a profound impact on most of the animal species that depend on periodically available plant resources: young leaves, pollen, nectar, fruits and seeds (Corlett & LaFrankie Jr., 1998). Thus, temporal variation in flowering season can influence the seed-set success if pollinator activity varies with the flowering of individual species (Kudo *et al.*, 2004). The success of pollination under fluctuating conditions would thus depend on the mating system and type of pollinators (e.g. Motten, 1986; Gugerli, 1997).

On a local scale, large variation in understorey micro-environmental factors including light availability (Nicotra *et al.*; 1999, Bianchini *et al.*, 2001), temperature (Young & Mitchell, 1994) and moisture (Camargo & Kapos, 1995) may be related to gaps

and to the structural complexity and/or deciduousness of the canopy. The frequency of natural disturbance events in a forest varies among localities and variations in forest microclimate distribution within and among stands profoundly influences overall understorey light availability and its spatial distribution (Nicotra *et al.*, 1999).

The formation of abrupt limits, or edges, between forested and deforested areas (matrix) by forest fragmentation changes the abiotic conditions and could affect the remnant organisms (Bierregaard Jr. *et al.*; 1992, Metzger, 1999; Poulin *et al.*, 1999; Debinski & Holt, 2000). The microclimatic changes at the edges of fragments could stimulate direct biotic modifications, such as alterations in the forest structure of the edge, because the growth, mortality, and distribution of the plants in this new environment may be directly affected by the physical conditions, and by the density and activity of some animal species (Murcia, 1995). Consequently, changes in many aspects of the life histories of plants and animals at the edges may cause alterations in species interactions, including herbivory, seed predation, pollination and seed dispersion (Saunders *et al.*, 1991, Aizen & Feinsinger, 1994). Natural edges (limits between forests and rivers, streams, lakes or natural fields) may also show abiotic and biotic differences in relation to the forest interior (Corbet, 1990; Matlack, 1994; Casenave *et al.*, 1998; Meleason & Quinn, 2004).

So far, no study has analysed the heterogeneity among fragments (regional scale) or the heterogeneity in abiotic factors among anthropogenic edges, natural edges and forest interior within a fragment (local scale). The aim of this paper was to investigate whether there were differences (i) in the long-term rainfall and temperature among forest fragments separated by up to 100 km (regional scale), and (ii) in the canopy cover, air temperature and soil humidity among anthropogenic edges, natural edges and forest interior (local scale).

MATERIAL AND METHODS

Study sites

Regional scale

Five forest fragments, classified as evergreen forests or ombrophilous dense forest (Radambrasil, 1983) were selected in State of Rio de Janeiro, southeastern Brazil. Four of the fragments were located in conservation units: Parque Estadual da Pedra Branca (PB) (elevation 202 m, 22°55'S, 43°26'W), Parque Estadual do Mendanha (ME) (elevation 23 m, 22°49'S, 43°33'W), Parque Estadual da Serra da Tiririca (ST) (elevation 215 m, 22°56'S, 43°00'W), Parque Nacional da Floresta da Tijuca (FT) (elevation 13 m, 22°58'S, 43°13'W), and one was a private area: Hotel Fazenda Serra da Castelaña (SC) (elevation 160 m, 22°50'S, 42°28'W). We selected fragments along the main highways in the south and southwest of the State in order to facilitate access to them. The distances among fragments ranged from about 16 to 110 km (Table 1).

Climate was compared for the five fragments by constructing climatic diagrams using long-term rainfall and temperature data (more than 30 years). The precipitation data were obtained from Serla (Secretaria Estadual de Rios e Lagoas) and the temperature data were obtained from InMet (Instituto Nacional de Meteorologia) (PB: 22°55' S, 43°25' W; ME : 22°51' S, 43°32' W; ST: 22°52' S, 43°14' W; FT: 22°57' S, 43°16'; SC: 22°51'S, 42°33' W).

Local scale

The study was carried out in the forest fragment of the Hotel Fazenda Serra da Castelaña (SC), city of Saquarema, RJ, including 1200 ha of Atlantic forest with a hilly

topography, with altitudes varying from 30 to 400 m. The fragment has probably not been deforested because its topography is not appropriate for cropland and cattle pasture. The study was done in a 180-ha sector (22° 50' S e 42° 28' W) of this area in order to facilitate access to the habitats. The forest studied was surrounded by pasture and cropland, thus creating anthropogenic edges. Within the forest, there was a stream 2-5 m wide and 700 m long that created a natural edge with the forest. Three habitats were investigated at the study site: (1) the edge of the forest with pasture and cropland (AE = anthropogenic edges ~50 m from the pasture), (2) the edge of the forest with the stream (NE = natural edges ~50 m from the stream), and (3) the forest interior (FI = 200 m or more from any edge). Five sample plots of 10 x 50 m in each habitat were non-systematically located, and the distances among sample plots ranged from 150 to 883 m (see appendix). The climate was classified as Cwa based on the Köppen system (Veanello & Alvez, 1991).

Microclimatic differences

a) *Temperature measurements*

The maximum and minimum air temperatures were recorded once a month from March 2003 to February 2004, using maximum and minimum thermometers placed 1.2 m above the ground in each of the 15 sample plots.

b) *Soil moisture measurements*

At monthly intervals from March 2003 to February 2004, three 40 g samples of the 0-20 cm soil layer (excluding litter) were taken from each sample plots in each habitat. The samples were double wrapped in plastic bags and weighed fresh in the lab (digital balance),

them dried in a oven at *ca.* 65°C for 48 h and weighed again when dry. The percent water content was calculated as: (fresh weight – dry weight) / fresh weight.

c) *Canopy openness*

Five canopy openness measurements were taken in each sample plot in each habitat twice in 2003, in the summer (wet season) and winter (dry season) (January and September, respectively). To measurements it was used hemispherical photographs taken with a Nikon Coolpix 950 with fish-eye lens autofocus Nikon 8mm (180°), placed 60 cm above the ground. The hemispherical photographs were analyzed for canopy openness (percentage of the hemispherical image not covered by vegetation) using the software Gap Light Analyzer 2.0 (GLA) (Frazer *et al.*, 1999). This program transforms the colors from the photos to black and white in order to quantify the pixels before calculation of canopy openness. To minimize subjectivity, three different persons transformed independently the colored images to black and white, and the mean among these was used for the calculation of canopy openness.

Statistical analysis

The differences in canopy openness, temperature (minimal, maximal and amplitude) and soil moisture among the three habitats (AE, NE and FI) within the fragment were tested by two-way nested ANOVA (Zar, 1996). Time was the second factor tested: seasons (canopy openness) and months (temperature and soil moisture). To improve the homoscedasticity and normality of the distributions, the data for canopy openness measurements and soil moisture were arcsine transformed before analysis (Zar, 1996). Means were back-transformed for use in the figures.

In the nested analyses of variance, the tested factor was the habitat. The five sample plots (nested within each habitat) were randomly sampled and were considered as random effects. Habitat, sample plots and canopy openness, and temperature and soil moisture were tested against the corresponding next lower hierarchical level (Sokal & Rohlf, 1995).

RESULTS

Regional scale

None of the five fragments showed dry months, i.e. the temperature and rainfall lines did not overlap each other. However, all areas except FT had 1-3 months of low rainfall. FT had an annual rainfall almost twice as high as the other four areas (1200 mm). Additionally, FT had the lowest minimum and maximum temperatures while ME had the highest values (Figure 1). It seems that the climatic patterns were not related to the distances among fragments (Table 1, Figure 1).

Local scale

a) *Temperature*

The minimum temperature during the year (2003) ranged from 12.6-19.0 °C, the maximum temperature ranged from 23.8-34.2°C, and the temperature amplitude was 10.6-17.6°C, regardless of the habitats (Figure 2). There were no significant differences in the minimum temperature ($F_{2,12} = 1.8$; $p = 0.20$) among the habitats, probably because of the great variation among the sample plots within habitats ($F_{12,132} = 5.2$; $p = 0.0001$). However, AE showed the greatest average maximum temperature ($F_{2,12} = 12.3$; $p = 0.0001$) and amplitude ($F_{2,12} = 5.3$; $p = 0.02$), while NE had the lowest values. There were differences in

the minimum temperature among the months, independently of the habitats ($F_{11,132} = 35.8$; $p = 0.0001$). However, the interaction between months and habitat was significant, both for the maximum temperatures ($F_{22,132} = 1.9$; $p = 0.02$) and amplitude ($F_{22,132} = 1.8$; $p = 0.03$).

b) *Soil moisture*

The soil moisture of all habitats during 2003 ranged from 7.2% to 19.9% (Figure 3). There were no differences in soil moisture ($F_{2,12} = 1.6$; $p = 0.25$) among the habitats, probably because of the great variation among the sample plots within habitats ($F_{12,132} = 14.3$; $p = 0.0001$). However there was a significant interaction between months and habitat ($F_{22,132} = 3.0$; $p = 0.0001$).

c) *Canopy openness*

Canopy openness in the winter was greater than in summer for all habitats, probably because of the deciduousness of many tree species in the dry season (Figure 4). Canopy openness ranged from 4.0% to 18.9% for all habitats in both seasons. There were no differences in canopy openness among habitats ($F_{2,12} = 2.9$; $p = 0.10$), but there was a significant interaction between season and habitat ($F_{2,132} = 3.6$; $p = 0.03$). The sample plots within the habitats showed great heterogeneity ($F_{12,132} = 15.4$; $p = 0.0001$) in both seasons, although the greatest variations among them were seen in the winter. NE3 showed the lowest medians for canopy openness in the summer (5.7%) and winter (4.6%), whereas the greatest medians were displayed by FI4 in the summer (12.0%), and by AE3 in winter (15.9%) (Figure 5).

DISCUSSION

On a regional scale, some fragments showed drier periods in the year, although this did not imply hydric deficit, and others displayed more constant precipitation throughout the year. The variations in rainfall and temperature seen here, among fragments separated by up to 100 km, could be sufficient to influence some populations of organisms living in these fragments. For instance, the reproduction among different populations of some plant and animal species would be affected by climatic variations (Silvertown & Lovelt-Doust, 1993), and the synchronisation of reproduction among populations may be essential for their long-term success, especially in self-incompatible plants, and for the satiation of seed predators (van Schaik *et al.*, 1993).

On a local scale, the microclimatic variables showed spatial and temporal variations in the area studied in agreement with other reports (Murcia, 1995; Didham, 1997; Renhorn *et al.*, 1997; Restrepo & Vargas, 1999; Gehlhausen *et al.*, 2000). There were no differences in the minimal temperature, soil moisture and canopy openness among anthropogenic edges, natural edges and forest interior. The low maximal temperature seen at NE was probably caused by the stream water that buffered or lowered the high temperature in this habitat, as recorded in New Zealand (Meleason & Quinn, 2004). Other studies have shown spatial variability in some microclimatic variables between edges and the forest interior, depending on the orientation of the forest fragment, relative to the angle of incidence of the sun (Young and Mitchell, 1994; Renhorn *et al.*, 1997), or because some edges were buffered by the heterogeneity of the vegetation structure in adjacent habitats (Williams-Linera *et al.*, 1998; Gehlhausen *et al.*, 2000; Mourelle *et al.*, 2001; Newmark 2001). The age of the fragment formation (Turton & Freiburger, 1997) and the extent of deforestation (Giambelluca *et al.*, 2003) along the edge may also influence the microclimatic variables.

All of the microclimate variables examined here showed temporal variations. Other studies have also reported temporal microclimatic differences in fragmented edges over years (Camargo & Kapos, 1995; Kapos *et al.*, 1997), seasons (Murcia, 1993 *apud* Restrepo & Vargas, 1999) and even hours (Newmark, 2001; Giambelluca *et al.*, 2003) caused by the grown of vegetation and natural oscillation within a day. The seasonal variation in sunlight could contributed to seasonal microclimatic variations (Young & Mitchell, 1994), as could oscillations in vegetation growth in the daily light intensity.

The canopy openness did not differ among the habitats, probably because of the great variation among the sample plots within the habitats. The forest canopy may vary in species composition, deciduousness, height above the soil, and in thickness and foliage density (Lieberman *et al.*, 1989; Bianchini *et al.*, 2001). The heterogeneity observed probably reflected variation in the forest structure (tree diameter and height) in each sample plot (M. T. Ribeiro *et al.*, unpublished data), in the number of deciduous tree species and in the presence of small gaps. According to Smith *et al.* (1989), the aggregation of crowns in the canopy depends on the spatial distribution of the individuals and on gap formation.

The variability of microclimatic gradients found in this study is probably common to forest edge microclimatic gradients in general. However, the insufficient number of replicates in most studies probably accounts for the reported lack of microclimatic heterogeneity in the fragments. According to Murcia (1995), the lack of consensus in the microclimatic differences between edges and the fragment interior reflect differences in methodology used and the absence of replicates and adequate controls. However, the greater heterogeneity in microclimate variables seen among the sample plots of each habitat in this study could indicate that other factors, such as edge age, matrix type, or the proximity of gaps, may have more influence on these variables than the proximity to the

edges. The results of this work show the need to incorporate more edges and/or fragment replications in future studies of fragmentation, in order to obtain more natural heterogeneity within fragments and to avoid erroneous conclusions about the influence of fragmentation on resident or persistent organisms.

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Table 1: Distances (km) among the studied Atlantic forest fragments in Rio de Janeiro. Parque Estadual do Mendanha (ME), Parque Estadual da Pedra Branca (PB), Hotel Fazenda Serra da Castelhaña (SC), Parque Estadual da Serra da Tiririca (ST), Parque Nacional da Floresta da Tijuca (FT).

	PB	**SC**	**ST**	**FT**
ME	15.8	109.9	57.6	36.7
PB	-	99.1	44.9	22.4
SC	-	-	54.9	77.8
ST	-	-	-	23.0

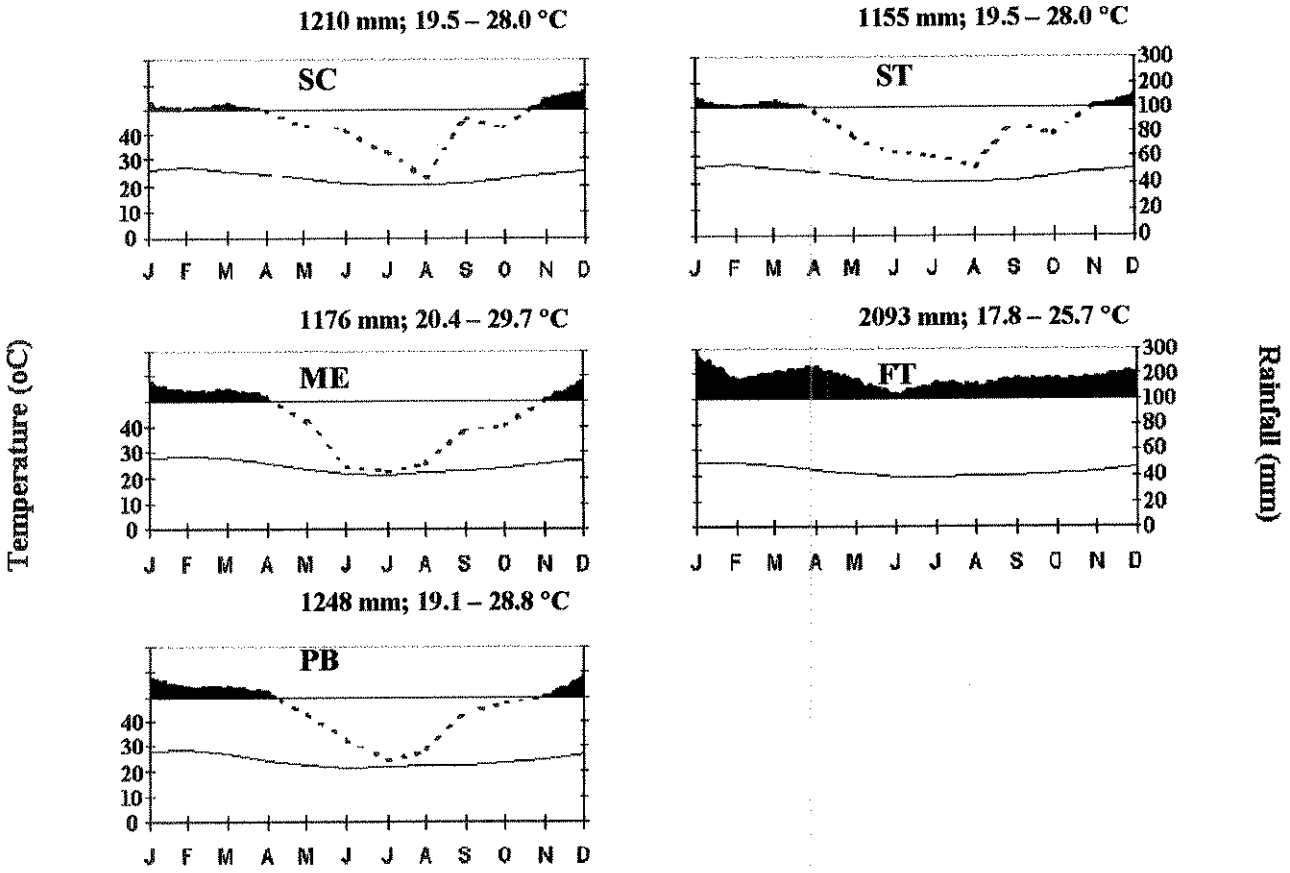

Figure 1: Climatic diagrams for five forest fragments of Rio de Janeiro Atlantic forest. The annual rainfall and the minimum and maximum mean temperatures are shown at the top of each diagram. Hotel Fazenda Serra da Castelhaña (SC), Parque Estadual da Serra da Tiririca (ST), Parque Estadual do Mendanha (ME), Parque Nacional da Floresta da Tijuca (FT), Parque Estadual da Pedra Branca (PB).

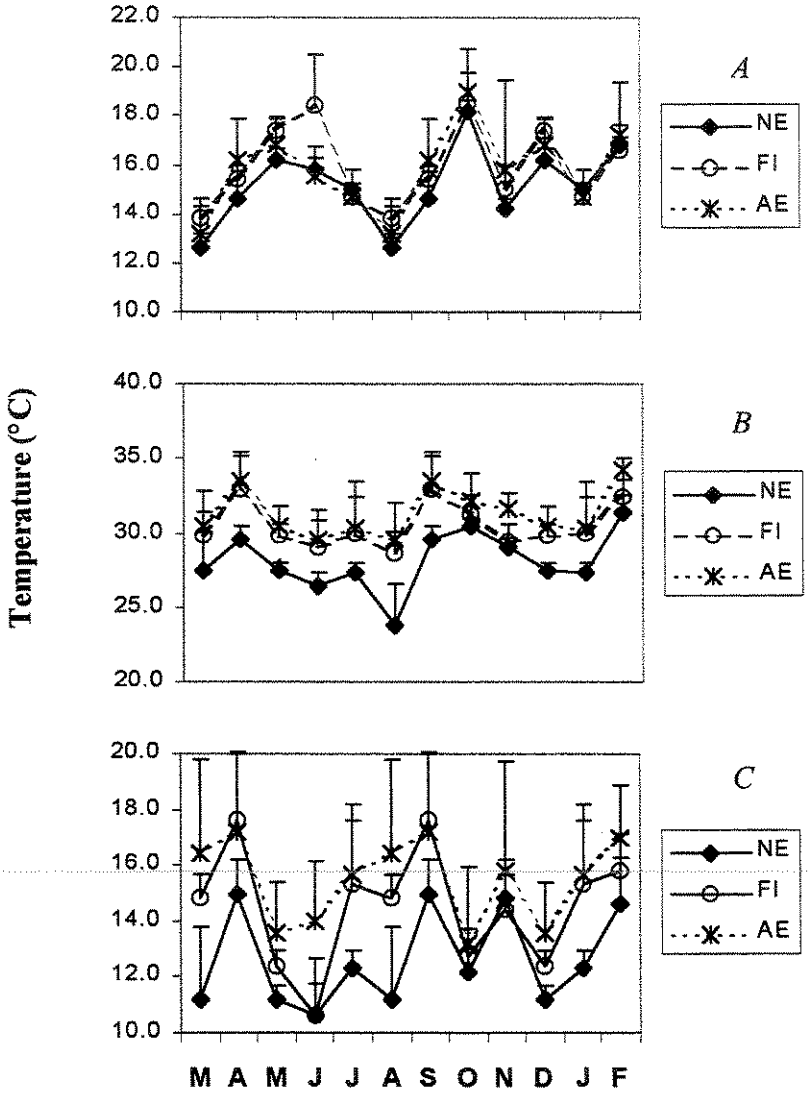

Figure 2: Mean (and 1 standard deviation) of the minimum (A), maximum (B) and amplitude (C) temperatures for a natural edge (NE), forest interior (FI) and anthropogenic edge (AE) at Hotel Fazenda Serra da Castelhaña (SC) (March 2003 to February 2004).

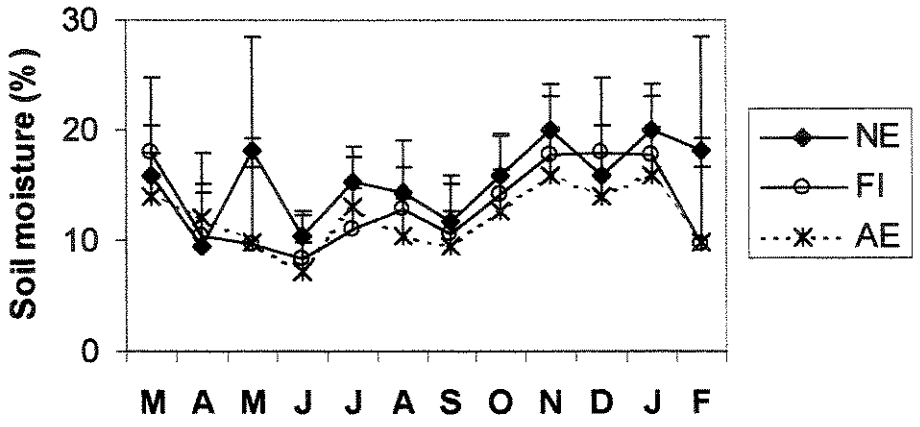

Figure 3: Mean (and 1 standard deviation) of soil moisture (%) for a natural edge (NE), forest interior (FI) and anthropogenic edge (AE) at Hotel Fazenda Serra da Castelhaña (SC) (March 2003 to February 2004) (back-transformed means and standard deviations).

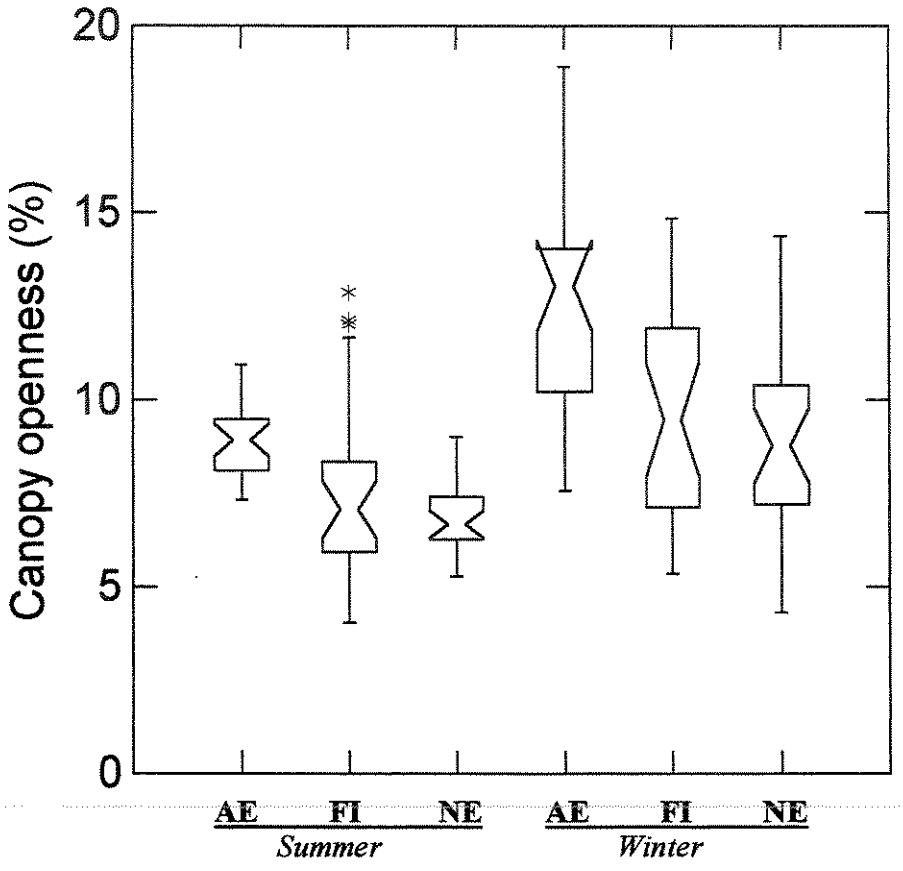

Figure 4: Canopy openness (%) for an anthropogenic edge (AE), forest interior (FI) and natural edge (NE), at Hotel Fazenda Serra da Castelhaña (SC) (summer and winter of 2003). The box plot presents the median, 25th and 75th percentiles (box), and the minimum and maximum values (whiskers). The asterisks indicate values outside the acceptable range. The boxes are notched at the median values and return to full width at the lower and upper confidence interval (95%) values.

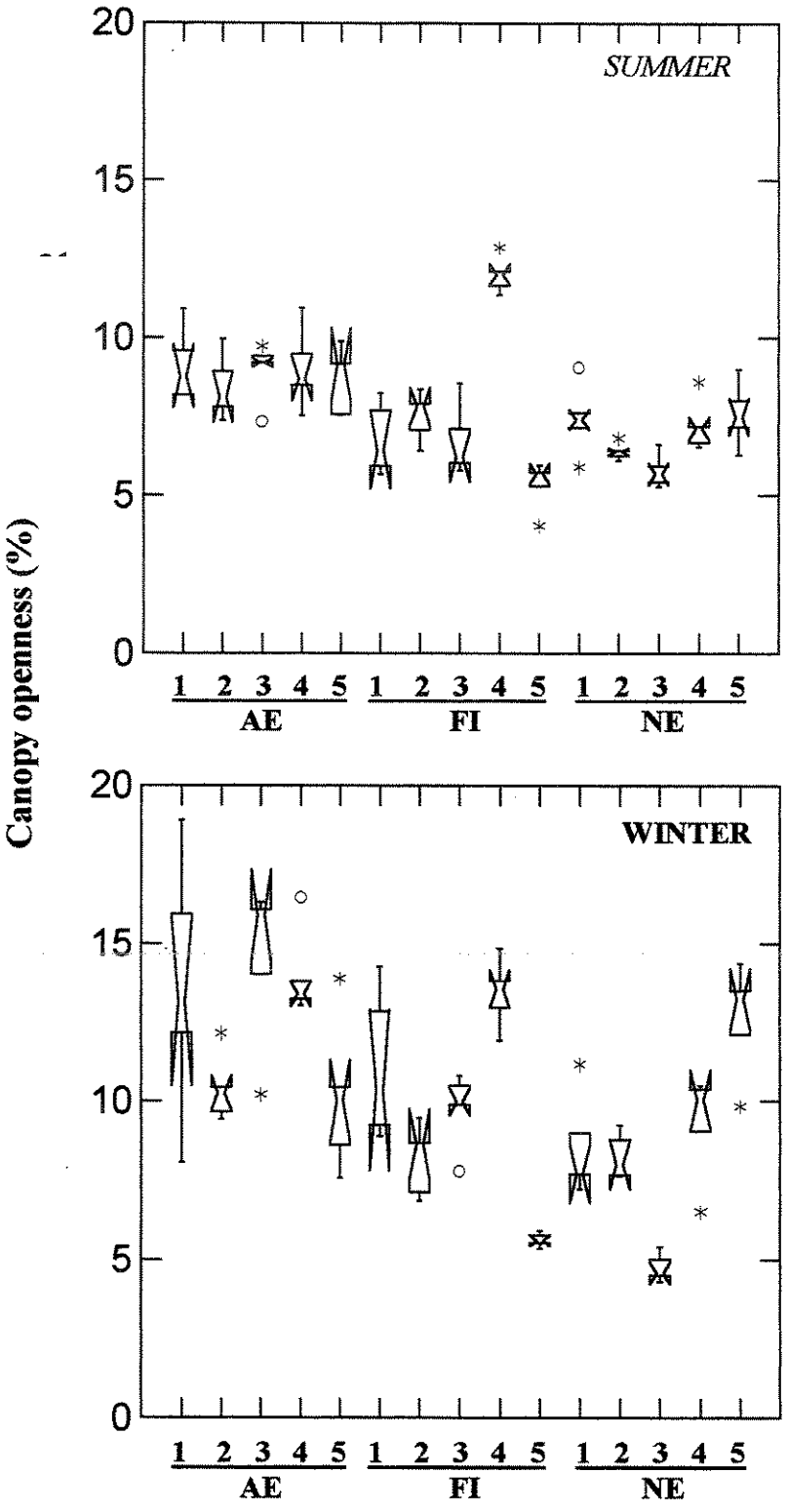

Figure 5: Canopy openness (%) in each sample plot of an anthropogenic edge (AE), forest interior (FI) and natural edge (NE) in the summer and winter of 2003. The legends for the boxes are given in figure 4.

CAPÍTULO 2

PHENOLOGY OF *Psychotria tenuinervis* (RUBIACEAE) IN ATLANTIC FOREST FRAGMENTS[1]

Flavio Nunes Ramos & Flavio A. Maës dos Santos

[1] Nos moldes da revista Plant Ecology

ABSTRACT

Phenological patterns displayed by plants are adaptations to the surrounding abiotic and biotic environment. The aim of this study was to investigate whether: (1) the reproductive phenology of *P. tenuinervis* is influenced by climatic conditions (precipitation and temperature); (2) there are differences in the reproductive phenology of *P. tenuinervis* among fragments (regional scale); and (3) there are differences in the reproductive phenology of *P. tenuinervis* among anthropogenic edges, natural edges and in the forest interior within a fragment (local scale). The patterns (curve, peak, concentration, seasonality) of flowering and fruiting found in 2002 and 2003 were similar among the forest fragments. These results indicate that the regional scale pattern of reproductive phenology of *P. tenuinervis* can be influenced by evolutionary or ultimate factors, since there was no consistent relationship with abiotic or proximate factors tested. There was phenological similarity among the three habitats, on a local scale, probably because of the extensive heterogeneity within each habitat with the percentage of flowering and fruiting individuals and the intensity and duration of these phenophases varying among the sample plots. This high variability within habitats indicated that factors other than the distance from the edges probably had a greater influence on the reproductive phenology of *P. tenuinervis*. Such factors include the occurrence of gaps, matrix composition, and edge age. These results also indicate that the heterogeneity within fragmented habitats needs to be considered in conservation and management programs for fragmented landscapes.

Key words: Edges, flowering, fragmentation, fruiting, heterogeneity, phenology.

RESUMO

Padrões fenológicos apresentados por plantas são adaptações ao ambiente abiótico e biótico. Os objetivos deste estudo foram investigar, se: (1) a fenologia reprodutiva de *P. tenuinervis* é influenciada pelas condições climáticas (precipitação e temperatura); (2) existem diferenças na fenologia reprodutiva de *P. tenuinervis* entre fragmentos (escala regional); (3) existem diferenças na fenologia reprodutiva de *P. tenuinervis* entre bordas antrópicas, bordas naturais e interior dentro de um fragmento (escala local). O padrão (curva, pico, concentração e sazonalidade) da floração e frutificação encontrado em 2002 e 2003 foi similar entre os fragmentos. Esses resultados indicam que o padrão geral da fenologia reprodutiva de *P. tenuinervis*, em uma escala regional, pode ser influenciado por fatores evolutivos, uma vez que não houve relação consistente com os fatores abióticos ou proximais testados. Houve uma grande similaridade fenológica entre os três ambientes, em escala local, provavelmente devido a grande heterogeneidade dentro de cada ambiente, uma vez que a porcentagem de indivíduos florescendo e frutificando e a intensidade e a duração das fenofases foram diferentes entre as parcelas dentro de cada ambiente. Essa alta variabilidade dentro dos ambientes pode indicar que outros fatores podem ter maior influência na fenologia reprodutiva de *P. tenuinervis* do que a distância de bordas. Estes fatores incluem a presença de clareiras, a composição da matriz e a idade da borda. Estes resultados indicam que a heterogeneidade dentro de habitats fragmentados deve ser considerada em programas de conservação e manejo de paisagens fragmentadas.

Palavras chave: bordas, fenologia, floração, fragmentação, frutificação, heterogeneidade.

INTRODUCTION

The phenological patterns displayed by plants are adaptations to the surrounding abiotic and biotic environment (van Schaik *et al.*, 1993). Phenological variations generally are a consequence of the influence of proximate environmental cues (precipitation, water stress and radiation) that initiate reproductive phases, and ultimate factors that select for particular reproductive phenologies (the need for outcrossing among individuals, as well as pollinators, seed dispersers and predators) (Piñero and Sarukhan, 1982; Adler and Kielpinski, 2000).

Abiotic factors can influence reproductive seasons either directly by affecting the ability to produce flowers and fruits or indirectly by affecting pollen and seed vectors (Rathcke and Lacey, 1985). For instance, many plant species flower in response to temperature, which usually acts through cumulative heatsums above a certain threshold. Flowering is also often induced by rainfall in seasonal tropical forests, with heavier rains increasing the synchronization of flowering within populations of some tropical trees (Rathcke and Lacey, 1985). On the other hand, biotic processes such as the availability of biotic agents in some seasons, the attraction of pollinators and dispersers, and predator satiation (van Schaik *et al.*, 1993) can influence phenological responses. Thus, the arrival of migratory birds may be timed to the flowering of hummingbird-pollinated or bird-dispersed plants (van Schaik *et al.*, 1993).

Flowering and fruiting often vary not only seasonally (Foster, 1996) but also within and among populations (Smith and Bronstein, 1996). The first step in studying reproductive performance is to identify spatial and temporal patterns of reproductive activity. Such studies are important because they lay the foundation for identifying proximate cues and ultimate factors that underlie phenological patterns (Adler and Kielpinski, 2000). Hence,

plants of different populations should display different phenologies if proximate cues are important because these populations will be under distinct climates, such as rainfall and temperature. However, if ultimate factors underlie these patterns, the patterns of flowering and fruiting among the populations should be similar.

Forest fragmentation on a regional scale can isolate and support discrete populations of plants (Metzger, 1999) and facilitate the identification of differences and changes in patterns of reproductive activity among populations that may be under distinct climatic conditions. On a local scale, the formation of abrupt limits, or edges, between the forest and deforested areas (matrix) may alter the abiotic conditions within patches (Poulin *et al.*, 1999; Debinski and Holt, 2000). In addition to the change in the environmental conditions that result from the proximity to a structurally dissimilar matrix (Bierregaard Jr *et al.*, 1992), the creation of an anthropogenic edge may also stimulate direct biotic modifications, such as changes in the presence and quantity of flowers and fruits (Aizen and Feinsinger, 1994a; 1994b; Murcia, 1995) as well as in the time and duration of flowering and fruiting (Rathcke and Lacey, 1985). Natural edges (limits between forests and rivers, streams, lakes or natural fields) may also present abiotic and biotic differences in relation to the forest interior (Corbet, 1990; Mattlack, 1994). Thus, both abiotic changes and alterations in the composition and behavior of floral visitants may directly affect plant reproduction near anthopogenic and natural edges.

The aim of this study was to investigate whether: (1) the reproductive phenology of *P. tenuinervis* was influenced by climatic conditions (precipitation and temperature); (2) there were differences in the reproductive phenology of *P. tenuinervis* plants among forest fragments (regional scale); and (3) there were differences in the reproductive phenology of *P. tenuinervis* plants within a fragment, at the anthropogenic edges, natural edges and in the

forest interior (local scale). *P. tenuinervis* was chosen because it was very abundant in the study areas, it occurred in the understory of the three habitats (anthropogenic edge, natural edges and forest interior) within the fragment and it produced flowers and fruits at a relatively low height above the ground.

MATERIAL AND METHODS

Study site

Regional scale

Five forest fragments, classified as evergreen forests or ombrophilous dense forest (Radambrasil, 1983) were selected in State of Rio de Janeiro, southeastern Brazil. Four of these were in conservation units, namely, Parque Estadual da Pedra Branca (PB) (22°55'S, 43°26'W), Parque Estadual do Mendanha (ME) (22°49'S, 43°33'W), Parque Estadual da Serra da Tiririca (ST) (22°56'S, 43°00'W), Parque Nacional da Floresta da Tijuca (FT) (22°58'S, 43°13'W), and one was in a private area, Hotel Fazenda Serra da Castelaña (SC), at Saquarema (22°50'S, 42°28'W). The study areas were selected by visiting ten fragments close to the main highways of southern and southwestern Rio de Janeiro State, and choosing those with an easy access. Only the areas cited above showed populations of *P. tenuinervis*. The areas were visited based on herbarium records of collected vouchers or on their similarity to such collection sites. The distances among the fragments varied from about 16 to 110 km (Table 1).

Local scale

This study was done in the coastal Serra de Palmital, at Saquarema, in the state of Rio de Janeiro, Brazil. This area consists of about 1200 ha of Atlantic forest with hills

varying from 30 to 400 m in height and has not been deforested, probably because its rough topography is not appropriate for cropland and cattle pasture. The study was done in 180 ha (22° 50' S; 42° 28' W) of this area to facilitate access to the habitats studied. The forest of the study area was surrounded by pasture and cropland, which created anthropogenic edges. Within the forest there was a stream 2 - 5 m wide and 700 m long that created a natural edge with the forest. The study was done in three habitats: (1) the edge of the forest with pasture and cropland (AE = anthropogenic edges ~50 m from the pasture), (2) the edge of the forest with the stream (NE = natural edges ~50 m from stream), and (3) the forest interior (FI = 200 m or more from any edge). Five sample plots of 10x50 m in each habitat were non-systematically located (see appendix). The distances among sample plots varied from 150 to 883 m. The climate was classified as Cwa based on the Köppen system (Veanello and Alvez, 1991).

Methods

The observations on reproductive phenophases were done monthly in all areas, from January 2002 to December 2003. The phenology of *P. tenuinervis* individuals higher than 1 m (smallest observed height for reproductive individuals of *this species*) was monitored in sample plots of 10 x 50 m non-systematically located in each area. On a regional scale areas, each fragment had one plot with 50 *P. tenuinervis* individuals. In local scale areas, five sample plots, containing 20 *P. tenuinervis* individuals each per habitat were non-systematically located. The distances among sample plots varied from 150 to 883 m. Flowering was defined as the presence of flower buds and/or open flowers and fruiting was defined as the presence of unripe and ripe fruits. Fournier (1974) methods were used to

score the intensity of phenological events and to calculate the monthly percentage of activity for each area.

Statistical analysis

The phenological patterns of the reproductive activity of *P. tenuinervis* in each forest fragment and in each habitat were compared and analyzed using circular statistics (Batschelet, 1981; Milton, 1991; Davies and Ashton, 1999; Morellato *et al.*, 2000). The dates of the phenological events were converted to angles, from 0° = January to 330° = December at intervals of 30°. The mean angle (***a***), the mean date (time converted from mean angle), the vector length (r) (the concentration around the mean angle) and the circular standard deviation were estimated for each forest fragment (Zar, 1996).

The mean angle (***a***) or mean date indicates the average date of peak reproductive activity among the individuals. The vector ***r*** has no units and indicates the degree of aggregation among individuals or synchrony of reproductive activity. The length of this vector may vary from 0 (when phenological activity is distributed uniformly throughout the year) to 1 (when phenological activity is concentrated around a single date or time of the year) Although high ***r*** values generally indicate aggregated phenological behavior, the Rayleight test (***z***) was used to determine whether the distribution of phenological activity was significantly nonrandom, or if there was seasonality (Batschelet, 1981). When the mean angle was significant, we used two-sample Watson-Williams tests (F) to compare the mean angle (***a***) of each phenological variable among forest fragments in order to determine whether the fragments showed the same seasonal pattern.

Differences in the mean duration of flowering and fruiting of *P. tenuinervis* individuals among forest fragments were tested by one-way ANOVA, while differences among the three habitats (anthropogenic edges, natural edges and forest interior) were tested with a two-level nested ANOVA (Zar, 1996), with habitat as the fixed effect. To improve the homoscedasticity and normality of the distributions, the data were square-root transformed (Zar, 1996). The back-transformed means are shown in the tables and figures.

Correlation coefficients were calculated between the phenophase (intensity of flowering and fruiting) of each fragment and its climatic factors: normal rainfall, monthly total rainfall for the same and previous years, normal mean, minimal and maximal temperatures and mean, minimal and maximal monthly temperature. Since most of the distributions were not normal, even after many kinds of transformations, Spearman's rank correlation test was used. The precipitation data were obtained from Serla (Secretaria Estadual de Rios e Lagoas) and the temperature data from InMet (Instituto Nacional de Meteorologia) (PB: 22°55' S, 43°25' W; ME : 22°51' S, 43°32' W; ST: 22°52' S, 43°14' W; FT: 22°57' S, 43°16'; SC: 22°51'S, 42°33' W).

RESULTS

Regional scale

The percentage of flowering was almost always below 50% for all fragments in both years (Figure 1), and the flowering in almost all fragments displayed seasonality with a low degree of concentration ($r < 0.20$) in 2003 but a high degree in 2002 ($r > 0.95$) in three fragments. The other fragments showed low r values because of two flowering peaks (Table 2). Although the duration of flowering was not different among the fragments in 2003 ($F_{4;47} = 1.8$; $P = 0.15$), in 2002 SC showing the longest duration ($F_{4;120} = 11.9$; $P =$

0.0001) (Table 3). Additionally, the mean dates or mean angles of flowering were different among the forest fragments both in 2002 (P from 0.049 to 0.0001) and 2003 (P from 0.02 to 0.0001) (Table 2). Nevertheless, the flowering patterns were similar among the five forest fragments since all of them displayed a flowering peak during the last months of the year, from October to December in both years; SC and FT also displayed a preliminary early peak in both years (Figure 1). Despite the similarity in flowering pattern, three forest fragments in 2002 ($\chi^2_4 = 21.1$; P= 0.0003) and four in 2003 ($\chi^2_4 = 152.9$; P= 0.0001) had a low percentage (<50%) of flowering individuals, compared to the others (>50%) (Table 3).

The percentage of fruiting was almost always <40% for all fragments in both years (Figure 1) and the fruiting of almost all fragments showed seasonality, with a lower fruiting concentration (low values of ***r***) in all fragments in both years (Table 2), mainly because of the long duration of this period in the year (Table 3). SC and FT had the shortest duration of fruiting in 2002 ($F_{4, 118} = 9.4$; P = 0.0001), whereas ME and SC had the shortest periods in 2003 ($F_{4, 131} = 3.8$; P = 0.006). Thus, SC showed the shortest duration of fruiting in both years (Table 3). The mean dates of fruiting differed among the forest fragments in 2002 and 2003 (P from 0.049 to 0.0001 in both years), three forest fragments in 2002 ($\chi^2_4 = 10.0$; P = 0.04) and one in 2003 ($\chi^2_4 = 20.0$; P = 0.0005) had a low percentage of fruiting individuals (<50%), while two and four fragments, respectively, had a high percentage (>50%) (Table 3). In 2002, the population of the fragments showed a fruiting peak from November to December after the flowering peak, except for ME which had a low fruiting peak. In 2003 the fragments had a fruiting peak from January to June (Figure 1).

Only in SC and FT fragments was there a significant correlation between the flowering patterns and climatic conditions. At SC, the flowering in 2003 was negatively correlated with the monthly maximal temperature at the same year ($r_s = -0.74$, P = 0.01),

while at FT, the flowering in 2002 was negatively correlated with the monthly minimal temperature in 2001 (r_s = -0.65, P = 0.02). A significant correlation between the fruiting patterns and climatic conditions was found only for SC and ST. In ST, the fruiting in 2003 was positively correlated with the monthly maximal temperature in the same year (r_s = 0.69, P = 0.02), while at SC, there was a positive correlation between the fruiting in 2003 and the monthly maximal (r_s = 0.91, P = 0.0001), minimal (r_s = 0.71, P = 0.01) and mean (r_s = 0.87, P = 0.001) temperatures in the same year. There was a positive correlation between the fruiting in 2003 and the normal maximal (r_s = 0.70, P = 0.01) minimal (r_s = 0.68, P = 0.01) and mean (r_s = 0.66, P = 0.02) temperature. There were no significant correlations between flowering or fruiting and the other climatic factors tested.

Local scale

Among habitats

All of the three habitats showed seasonality in flowering, with a low concentration (r < 0.50) in both years (Table 4). The percentage of individuals flowering per habitat was high (>50%), and there were not differences among them both in 2002 (χ^2_2 = 0.96; P= 0.62) and 2003 (χ^2_2 = 0.67; P= 0.72) (Table 5). Additionally, the intensity of flowering was low (< 50%) for all habitats in 2002, but high (>50%) for AE and FI in 2003. AE showed the highest value in both years (Figure 2). In both years, the populations of all habitats displayed two flowering peaks, with the first being greater than the second in 2002 and the second greater than the first in 2003 (Figure 2). Additionally, AE displayed the longest duration of flowering both in 2002 ($F_{2;\ 12}$ = 4.0; P = 0.045) and 2003 ($F_{2;\ 187}$ = 13.7; P = 0.001) (Table 5).

All of the three habitats displayed seasonality in fruiting, with a low concentration ($r < 0.50$) in both years, except for FI in 2002 (Table 4). The percentage of individuals fruiting per habitat was high (>50%), and there was no difference among the habitats in 2002 ($\chi^2_2 = 2.1$; P= 0.35) and 2003 ($\chi^2_2 = 0.7$; P= 0.72) (Table 5). The intensity of fruiting was always < 30% in all habitats in both years (Figure 2). The fruiting patterns observed within each year were similar among the three habitats, but the opposite between years. In 2002, the populations showed a fruiting peak from September to December, after the flowering peak (Figure 2), whereas in 2003, they displayed a fruiting peak from January to June, resulted from the flowering period of the previous year (Figure 2). There was no significant difference among the habitats ($F_{2, 192} = 0.8$; P = 0.43) in 2003, but AE displayed the longest duration of fruiting and FI, the shortest in 2002 ($F_{2, 12} = 11.9$; P = 0.001) (Table 5).

Within habitats

All sample plots in each habitat displayed seasonality in flowering, with a low concentration ($r < 0.50$), but the mean dates of flowering differed among them in both years (Tables 6, 7 and 8). The percentage of individuals flowering was high (>50%) in sample plots within each habitat in 2002 and there were no differences among them (Table 9), whereas differences were seen in 2003 (Table 10). The percentage of flowering was < 50% for almost all sample plots of the three habitats in both years and the patterns of flowering were similar among them in both years. However, the number of flowering peaks changed between 2002 and 2003 (two and one, respectively), as did, the intensity, which was variable among the sample plots within each habitat (Figure 3). Only the NE sample plots

showed differences in the duration of flowering in 2002, while FI and NE sample plots showed differences in 2003 (Tables 9 and 10).

All sample plots of each habitat showed seasonality in fruiting in both years. However, in 2002 there was a greater range in the fruiting concentration (values of r) in most cases, because some sample plots displayed fruiting throughout the year (low r values), while others displayed fruiting that was more concentrated in the second semester (high r values). On the other hand, in 2003 there was a lower fruiting concentration as indicated by the r values < 0.50 (Tables 6, 7 and 8, Figure 4). Whereas most AE and NE sample plots showed a high (>50%) percentage of individuals with fruit in 2002, there was a marked variation among IF sample plots, in 2003, there was greater variation among the sample plots of all three habitats (Tables 9 and 10). The percentage of fruiting was <50% for almost all sample plots in each habitat in both years (Figure 4). The fruiting patterns were similar among the five sample plots of the three habitats, with all populations showing two fruiting peaks in 2002 and one in 2003, although the intensity varied among the sample plots (Figure 4). The sample plots within the three habitats differed in the duration of fruiting in 2003, but only NE sample plots differed among them in 2002 (Tables 9 and 10).

DISCUSSION

According to the classification of Newstrom *et al.* (1994), *P. tenuinervis* has an annual phenological cycle which is continuous, except for fruiting. This was categorized as intermediate flowering, species where flowering lasts from 1 to 5 months. In general, the phenological pattern of *P. tenuinervis* was similar to that reported for 12 other *Psychotria* species: six in São Paulo (Martin-Gajardo, 1999) and two in Rio de Janeiro (Almeida and Alves, 2000), southeastern Brazil and four on Barro Colorado Island, Panama (Wright,

1991). Some of these species also displayed more than one flowering peak and showed a long period of fruiting with only four months without fruit, as seen with *P. tenuinervis*. Poulin *et al.* (1999) also found low concentration of fruiting in 19 *Psychotria* species in Panama, with 4-12 months of fruiting. This coincidence of phenological patterns among many *Psychotria* species may indicate that these patterns are constrained by phylogenetic inertia, at least at the genus level. For example, Smith-Ramirez *et al.* (1998) found phylogenetic inertia at the family level in Chilean Myrtaceae.

The synchronous production of flowers from many individuals of *P. tenuinervis* during a short period is typical of entomophilous species (Morellato, 1991; Smith-Ramirez and Armesto, 1994) and provides a greater attraction for pollinators (Kato and Hiura, 1999). Additionally, the long period of fruiting in this species, which bears fruit with two medium/large seeds (about 18 g each, Chapter 4), agrees with the McKey (1975) hypothesis that tropical plants with long fruiting periods produce a rich fruit pulp and a few large seeds, thus directing their fruit to consummation by less generalist bird species. An assessment of the dispersion of *P. tenuinervis* seeds by birds is necessary to confirm this relationship.

The pattern (curve, peak, concentration, seasonality) of flowering and fruiting found in the two years, as well as the duration of flowering, were similar among the forest fragments. However, the intensity of each reproductive phenophase, the number of individuals presents in each one, and the duration of fruiting was variable, even in some forest fragments with different climatic patterns (chapter 1). These results suggest that the general pattern of reproductive phenology of *P. tenuinervis* could be influenced by evolutionary factors (ultimate factors), since there was no consistent relationship with abiotic factors (proximate factors). Perhaps the distances among the fragments (from 16 to

110 km), or other characteristics, such as elevation or sun orientation were not sufficiently great to allow the detection of climatic differences enough to influence the reproductive phenology of *P. tenuinervis*.

There were few differences among the three habitats within a fragment (SC). Only the anthropogenic edge (AE) showed greater flowering and fruiting intensity than the other two habitats, perhaps because of the higher maximal temperatures present these (chapter 1), since other microclimatic factors, such as minimal temperature, canopy openness and soil moisture, did not differ among the habitats (chapter 1). The different climatic conditions at AE apparently only influenced the intensity but not the timing of the phenological response in relation to the other two habitats. Other studies have also found similar patterns of phenology between edges and interior habitats, with greater intensity on edge habitats, for five species in Mexico, even when different distances from the edges were used (10 and 50 m, respectively) (Williams-Linera, 2003). In the present study, the marked variation or heterogeneity in the reproductive patterns and variables within each habitat probably masked any differences among the habitats. This finding suggests that factors other than the distance from the edges could influence the reproductive phenology of *P. tenuinervis*. Factors such as the presence of gaps (Piñero and Sarukhan, 1982; Kursar and Coley, 1992), the matrix composition (Mesquita *et al.*, 1999), and edge age (Restrepo *et al.*, 1999), can influence plant survival and reproduction and may be responsible for the marked variation among sample plots within habitats. Although a few studies have examined flower and fruit production in fragmented habitats of different sizes (Ghazoul and McLeish, 2001; Bruna and Kress, 2002; Tomimatsu and Ohara, 2002), we found no suitable studies in the literature that would allow comparison with the results describe here.

The results of this work suggest that the general regional scale pattern of reproductive phenology in *P. tenuinervis* is influenced by evolutionary factors (ultimate factors). On a local scale, the marked heterogeneity and the probable importance of factors other than proximity to an edge could be very important in conservation programs. Thus, other factors should be taken into consideration when modeling and evaluating the availability of edge and interior habitats in fragmented landscapes and in designing natural reserves (Restrepo *et al.*, 1999).

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Table 1: Distances (km) among the studied Atlantic forest fragments in Rio de Janeiro. Parque Estadual do Mendanha (ME), Parque Estadual da Pedra Branca (PB), Hotel Fazenda Serra da Castelhaña (SC), Parque Estadual da Serra da Tiririca (ST), and Parque Nacional da Floresta da Tijuca (FT).

	PB	**SC**	**ST**	**FT**
ME	15.8	109.9	57.6	36.7
PB	-	99.1	44.9	22.4
SC	-	-	54.9	77.8
ST	-	-	-	23.0

Table 2: Phenological patterns of *P. tenuinervis* individuals from five Atlantic forest fragments in the State of Rio de Janeiro in 2002 and 2003. Different letters in the rows indicate a significant difference. Parque Estadual do Mendanha (ME), Parque Estadual da Pedra Branca (PB), Hotel Fazenda Serra da Castelhaña (SC), Parque Estadual da Serra da Tiririca (ST), and Parque Nacional da Floresta da Tijuca (FT).

	ME	PB	SC	ST	FT
2002					
Flower					
Observation (N)	9	110	98	42	92
Mean Angle (*a*)	324.18°e	271.47°c	229.67°a	265.67°b	282.58°d
Mean Date	25/11/02	02/10/02	21/08/02	26/09/02	14/10/02
Length of mean vector (r)	0.95	0.97	0.37	0.95	0.70
Circular standard deviation	18.48°	14.87°	80.63°	19.22°	48.46°
Rayleight test of uniformity (P)	0.01	0.01	0.01	0.01	0.01
Fruit					
Observation (N)	113	131	102	60	99
Mean Angle (*a*)	76.99°b	62.14°b	274.23°c	31.44°a	24.77°a
Mean Date	19/03/02	04/03/02	05/10/02	01/02/02	25/01/02
Length of mean vector (r)	0.54	0.42	0.66	0.38	0.36
Circular standard deviation	63.61°	75.97°	52.70°	79.70°	81.75°
Rayleight test of uniformity (P)	0.01	0.01	0.01	0.01	0.01
2003					
Flower					
Observation (N)	10	13	298	2	52
Mean Angle (*a*)	306.21°c	251.14°a	254.4°a	315.00°c	276.07°b
Mean Date	7/11/03	12/09/03	16/09/03	15/11/03	7/10/03
Length of mean vector (r)	0.07	0.11	0.15	0.03	0.16
Circular standard deviation	22.62°	27.89°	33.13°	15.09°	34.33°
Rayleight test of uniformity (P)	0.01	0.01	0.01	0.16	0.01
Fruit					
Observation (N)	56	321	166	297	584
Mean Angle (*a*)	114.24°c	98.67°c	55.22°a	85.42°b	76.7°b
Mean Date	25/04/03	09/04/03	26/02/03	26/03/03	17/03/03
Length of mean vector (r)	0.46	0.38	0.43	0.58	0.44
Circular standard deviation	63.14°	56.35°	60.71°	59.66°	61.52°
Rayleight test of uniformity (P)	0.01	0.01	0.01	0.01	0.01

Table 3: Percentage of flowering and fruiting *P. tenuinervis* individuals and the mean duration (and standard deviation) of these phenophases in five Atlantic forest fragments in the State of Rio de Janeiro in 2002 and 2003. See Table 2 for forest fragments legend. Different letters in the same column indicate significant different means ($p<0.05$).

Forest Fragments	Flowering (%)	Mean duration (SD)	Fruiting (%)	Mean duration (SD)
2002				
ME	17	1.1 (0.4)^{a}	36	6.5 (0.7)^{c}
PB	48	1.3 (0.5)^{a}	36	5.3 (3.2)^{bc}
SC	66	2.5 (1.0)^{b}	56	2.9 (1.8)^{ab}
ST	48	1.5 (0.5)^{a}	49	5.5 (3.0)^{bc}
FT	79	1.7 (0.9)^{a}	74	2.8 (2.9)^{a}
2003				
ME	4	3.0 (0.0)^{a}	24	3.7 (2.3)^{a}
PB	10	1.8 (0.8)^{a}	54	5.2 (1.8)^{ab}
SC	70	2.9 (1.0)^{a}	56	3.9 (1.7)^{a}
ST	2	2.0(0.0)^{a}	58	4.6 (2.1)^{b}
FT	18	2.6 (1.2)^{a}	80	5.5 (2.3)^{ab}

Table 4: Phenological patterns of *P. tenuinervis* individuals on anthropogenic edges (AE), natural edges (NE) and in forest interior (FI) in the Serra da Castelaña site in 2002 and 2003. Different letters in the rows indicate a significant difference.

	AE	FI	NE
2002			
Flower			
Observation (N)	195	139	195
Mean Angle (*a*)	204.64°a	234.41°b	238.68°b
Mean Date	26/07/02	26/08/02	30/08/02
Length of mean vector (r)	0.49	0.45	0.44
Circular standard deviation	68.19°	72.39°	73.6°
Rayleight test of uniformity (P)	0.01	0.01	0.01
Fruit			
Observation (N)	296	148	233
Mean Angle (*a*)	294.46°a	284.27°a	295.10°a
Mean Date	26/10/02	15/10/02	26/10/02
Length of mean vector (r)	0.38	0.76	0.47
Circular standard deviation	79.18°	42.39°	70.00°
Rayleight test of uniformity (P)	0.01	0.01	0.01
2003			
Flower			
Observation (N)	534	494	289
Mean Angle (*a*)	264.91°b	253.19°a	263.06°b
Mean Date	25/09/03	14/09/03	24/09/03
Length of mean vector (r)	0.14	0.12	0.08
Circular standard deviation	32.03°	28.93°	23.89°
Rayleight test of uniformity (P)	0.01	0.01	0.01
Fruit			
Observation (N)	280	341	493
Mean Angle (*a*)	56.94°b	43.26°a	66.27°b
Mean Date	27/02/03	14/02/03	07/03/03
Length of mean vector (r)	0.48	0.41	0.50
Circular standard deviation	65.07°	59.27°	67.54°
Rayleight test of uniformity (P)	0.01	0.01	0.01

Table 5: Percentage of flowering and fruiting *P. tenuinervis* individuals and the mean duration (and standard deviation) of these phenophases, on anthropogenic edges (AE), natural edges (NE) and in forest interior (FI) in the Serra da Castelaña site in 2002 and 2003.

Habitat	Flowering (%)	Mean duration (SD)	Fruiting (%)	Mean duration (SD)
2002				
AE	77	2.6 (1.2)^{c}	71	4.3 (2.4)^{b}
FI	67	2.3 (1.0)^{b}	51	3.1 (1.6)^{a}
NE	84	2.5 (1.3)^{bc}	71	3.5 (2.3)^{ab}
2003				
AE	69	2.8 (0.8)^{c}	52	3.4 (1.9)^{ab}
FI	61	2.5 (0.8)^{b}	62	3.6 (1.9)^{b}
NE	61	2.1 (0.6)^{a}	81	3.8 (2.2)^{b}

Table 6: Phenological patterns of *P. tenuinervis* individuals in the five replicates of anthropogenic edges (AE) at the Serra da Castelaña site in 2002. Different letters in the rows indicate a significant difference.

	AE 1	AE 2	AE 3	AE 4	AE 5
2002					
Flower					
Observation (N)	52	44	27	41	33
Mean Angle (*a*)	190.49°a	243.03°b	183.38°a	204.40°a	191.50°a
Mean Date	12/07/02	03/09/02	05/07/02	26/07/02	13/07/02
Length of mean vector (r)	0.44	0.57	0.63	0.50	0.61
Circular standard deviation	73.10°	60.66°	55.26°	67.48°	57.16°
Rayleight test of uniformity (P)	0.01	0.01	0.01	0.01	0.01
Fruit					
Observation (N)	86	32	41	71	38
Mean Angle (*a*)	305.57°b	346.24°b	294.96°a	278.95°a	286.66°a
Mean Date	06/11/02	17/12/02	26/10/02	10/10/02	18/10/02
Length of mean vector (r)	0.26	0.47	0.56	0.78	0.82
Circular standard deviation	94.62°	70.13°	61.78°	39.98°	36.49°
Rayleight test of uniformity (P)	0.01	0.01	0.01	0.01	0.01
2003					
Flower					
Observation (N)	223	72	68	60	66
Mean Angle (*a*)	261.14°ab	271.03°c	256.52°a	270.00°c	269.03°bc
Mean Date	22/09/03	02/10/03	17/09/03	01/10/03	30/09/03
Length of mean vector (r)	0.13	0.22	0.12	0.09	0.10
Circular standard deviation	29.64°	40.77°	28.88°	24.45°	26.90°
Rayleight test of uniformity (P)	0.01	0.01	0.01	0.01	0.01
Fruit					
Observation (N)	57	87	22	82	32
Mean Angle (*a*)	41.59°a	87.95°b	47.98°a	53.64°a	33.47°a
Mean Date	12/02/03	28/03/03	18/02/03	24/02/03	04/02/03
Length of mean vector (r)	0.51	0.49	0.38	0.46	0.17
Circular standard deviation	68.04°	66.96°	56.23°	63.32°	35.52°
Rayleight test of uniformity (P)	0.01	0.01	0.01	0.01	0.01

Table 7: Phenological patterns of *P. tenuinervis* individuals in the five replicates of forest interior (FI) in the Serra da Castelaña site in 2002. Different letters in the rows indicate a significant difference.

	FI 1	FI 2	FI 3	FI 4	FI 5
2002					
Flower					
Observation (N)	21	24	31	34	36
Mean Angle (*a*)	268.48°c	268.00°c	191.75°a	255.88°bc	235.24°b
Mean Date	29/09/02	29/09/02	13/07/02	16/09/02	27/08/02
Length of mean vector (r)	0.66	0.60	0.55	0.36	0.41
Circular standard deviation	52.34°	58.15°	62.75°	81.71°	76.26°
Rayleight test of uniformity (P)	0.01	0.01	0.01	0.01	0.01
Fruit					
Observation (N)	9	15	64	20	40
Mean Angle (*a*)	279.21°ab	290.32°ab	270.43°a	289.69°ab	303.00°b
Mean Date	10/10/02	21/10/02	01/10/02	21/10/02	03/11/02
Length of mean vector (r)	0.86	0.83	0.76	0.87	0.73
Circular standard deviation	32.04	34.81	42.06	30.82	45.06
Rayleight test of uniformity (P)	0.01	0.01	0.01	0.01	0.01
2003					
Flower					
Observation (N)	36	54	195	73	136
Mean Angle (*a*)	283.71°d	271.17°c	246.42°a	259.84°b	244.21°a
Mean Date	14/10/03	02/10/03	07/09/03	20/09/03	05/09/03
Length of mean vector (r)	0.06	0.09	0.12	0.11	0.08
Circular standard deviation	20.60°	25.47°	29.00°	27.26°	22.74°
Rayleight test of uniformity (P)	0.01	0.01	0.01	0.01	0.01
Fruit					
Observation (N)	70	67	78	27	99
Mean Angle (*a*)	89.38°c	58.40°b	18.85°a	13.92°a	50.20°b
Mean Date	01/04/03	29/02/03	19/01/03	14/01/03	21/02/03
Length of mean vector (r)	0.52	0.41	0.09	0.12	0.46
Circular standard deviation	69.29°	58.99°	24.88°	28.66°	63.80°
Rayleight test of uniformity (P)	0.01	0.01	0.01	0.01	0.01

Table 8: Phenological patterns of *P. tenuinervis* individuals in the five replicates of natural edges (NE) in the Serra da Castelaña site in 2002. Different letters in the rows indicate a significant difference.

	NE 1	NE 2	NE 3	NE 4	NE 5
2002					
Flower					
Observation (N)	23	44	51	43	50
Mean Angle (*a*)	217.91°b	201.85°a	247.16°b	285.29°c	282.82°c
Mean Date	09/08/02	24/07/02	08/09/02	16/10/02	14/10/02
Length of mean vector (r)	0.43	0.45	0.51	0.55	0.47
Circular standard deviation	74.28°	72.04°	66.65°	63.00°	70.25°
Rayleight test of uniformity (P)	0.01	0.01	0.01	0.01	0.01
Fruit					
Observation (N)	37	195	48	66	47
Mean Angle (*a*)	299.82°c	282.76°b	301.72°c	0.29°a	315.36°c
Mean Date	31/10/02	14/10/02	02/11/02	01/01/02	16/11/02
Length of mean vector (r)	0.84	0.72	0.62	0.30	0.47
Circular standard deviation	33.39°	46.28°	55.61°	89.12°	70.79°
Rayleight test of uniformity (P)	0.01	0.01	0.01	0.01	0.01
2003					
Flower					
Observation (N)	64	31	103	75	21
Mean Angle (*a*)	257.68°a	285.21°c	254.63°a	267.88°b	288.32°c
Mean Date	18/09/03	25/10/03	15/09/03	28/09/03	19/10/03
Length of mean vector (r)	0.08	0.08	0.07	0.10	0.09
Circular standard deviation	24.08°	23.69°	21.18°	26.34°	25.01°
Rayleight test of uniformity (P)	0.01	0.01	0.01	0.01	0.01
Fruit					
Observation (N)	42	72	168	144	72
Mean Angle (*a*)	29.71°a	37.65°a	85.64°b	98.49°b	36.19°a
Mean Date	30/01/03	08/02/03	26/03/03	09/04/03	07/02/03
Length of mean vector (r)	0.20	0.31	0.52	0.51	0.32
Circular standard deviation	38.05°	49.26°	69.65°	68.83°	50.14°
Rayleight test of uniformity (P)	0.01	0.01	0.01	0.01	0.01

Table 9: Percentage of flowering and fruiting *P. tenuinervis* individuals and the mean duration (and standard deviation) of these phenophases for all five replicates of anthropogenic edges (AE), natural edges (NE) and forest interior (FI) in the Serra da Castelaña site in 2002.

Habitat	Flowering (%)*[1]	Mean duration (SD)*[3]	Fruiting (%)*[2]	Mean duration (SD) *[4]
AE				
1	85	2.9 (1.3)^{a}	85	5.1 (3.4)^{b}
2	79	2.8 (1.5)^{a}	58	5.4 (2.9)^{a}
3	60	2.5 (1.1)^{a}	55	3.7 (2.0)^{a}
4	89	2.4 (1.2)^{a}	89	4.2 (0.9)^{a}
5	70	2.3 (0.7)^{a}	70	2.9 (1.3)^{a}
FI				
1	59	1.9 (1.0)^{a}	24	1.5 (1.0)^{a}
2	63	2.1 (1.0)^{a}	31	2.2 (0.8)^{b}
3	75	2.0 (0.7)^{a}	85	3.8 (1.5)^{c}
4	60	2.7 (1.2)^{a}	35	3.1 (1.5)^{bc}
5	76	2.6 (1.2)^{a}	76	3.1 (1.9)^{bc}
NE				
1	63	1.8 (0.8)^{a}	42	2.6 (1.1)^{a}
2	88	2.9 (1.1)^{ab}	94	3.8 (0.5)^{ab}
3	84	3.1 (1.4)^{a}	84	2.9 (2.5)^{a}
4	100	2.1 (1.5)^{b}	56	5.0 (3.5)^{b}
5	84	2.6 (1.3)^{a}	79	3.1 (2.4)^{a}

*[1] = AE: χ^2_4= 1.4; P= 0.84, FI: χ^2_4 = 0.7; P= 0.95, NE: χ^2_4 = 1.7; P= 0.79; *[2] = AE: χ^2_4 = 2.6; P= 0.62, FI: χ^2_4 = 12.6; P= 0.01, NE: χ^2_4 = 5.1; P = 0.27; *[3] = AE: $F_{4, 85}$ = 0.91; p = 0.54, FI: $F_{4, 85}$ = 1.99; p = 0.10, NE: $F_{4, 85}$ = 2.86; p = 0.003; *[4] = AE: $F_{4,85}$ = 1.89; P = 0.12, FI: $F_{4,85}$ = 4.7; P = 0.002, NE: $F_{4,85}$ = 3.35; P = 0.014; Different letters indicate significant differences in the column for each habitat.

Table 10: Percentage of flowering and fruiting *P. tenuinervis* individuals and the mean duration (and standard deviation) of these phenophases for all five replicates of anthropogenic edges (AE), natural edges (NE) and forest interior (FI) at the Serra da Castelaña site in 2003.

Habitat	Flowering (%)*[1]	Mean Duration (SD)*[3]	Fruiting (%)*[2]	Mean duration (SD)*[4]
AE				
1	100	3.2 (0.9)^{b}	50	3.8 (1.6)^{ab}
2	60	3.2 (0.7)^{ab}	55	4.5 (2.8)^{b}
3	55	2.7 (0.8)^{ab}	35	2.7 (1.7)^{ab}
4	65	2.5 (0.7)^{ab}	70	3.4 (1.4)^{ab}
5	65	2.4 (0.6)^{a}	50	2.0 (0.7)^{a}
FI				
1	35	2.3 (0.5)^{ab}	65	4.5 (2.2)^{b}
2	45	2.3 (0.7)^{ab}	60	3.5 (1.8)^{ab}
3	85	3.0 (0.8)^{b}	80	2.2 (0.9)^{a}
4	50	2.6 (0.7)^{ab}	35	1.9 (0.9)^{a}
5	90	2.2 (0.9)^{a}	70	4.7 (1.6)^{b}
NE				
1	50	2.0 (0.7)^{ab}	50	2.7 (1.3)^{a}
2	40	2.2 (0.3)^{ab}	90	2.9 (2.0)^{a}
3	100	2.2 (0.5)^{b}	90	4.8 (2.3)^{b}
4	75	2.3 (0.6)^{b}	100	4.8 (2.2)^{b}
5	40	1.7 (0.9)^{a}	75	3.1 (1.9)^{ab}

*[1] = AE: χ^2_4= 18.4; P= 0.001, FI: χ^2_4 = 40.5; P= 0.0001, NE: χ^2_4 = 44.6; P= 0.0001; *[2] = AE: χ^2_4 = 12.1; P= 0.02, FI: χ^2_4 = 18.2; P= 0.001, NE: χ^2_4 = 18.8; P = 0.0009; *[3] = AE: $F_{4,68}$ = 3.21; P = 0.02, FI: $F_{4,60}$ = 1.99; P = 0.42, NE: $F_{4,59}$ = 3.21; P = 0.57; *[4] = AE: $F_{4,51}$ = 3.1; P = 0.02, IF: $F_{4,61}$ = 8.0; P = 0.0001, NE: $F_{4,80}$ = 4.4; P = 0.003; Different letters indicate significant differences in the column for each habitat.

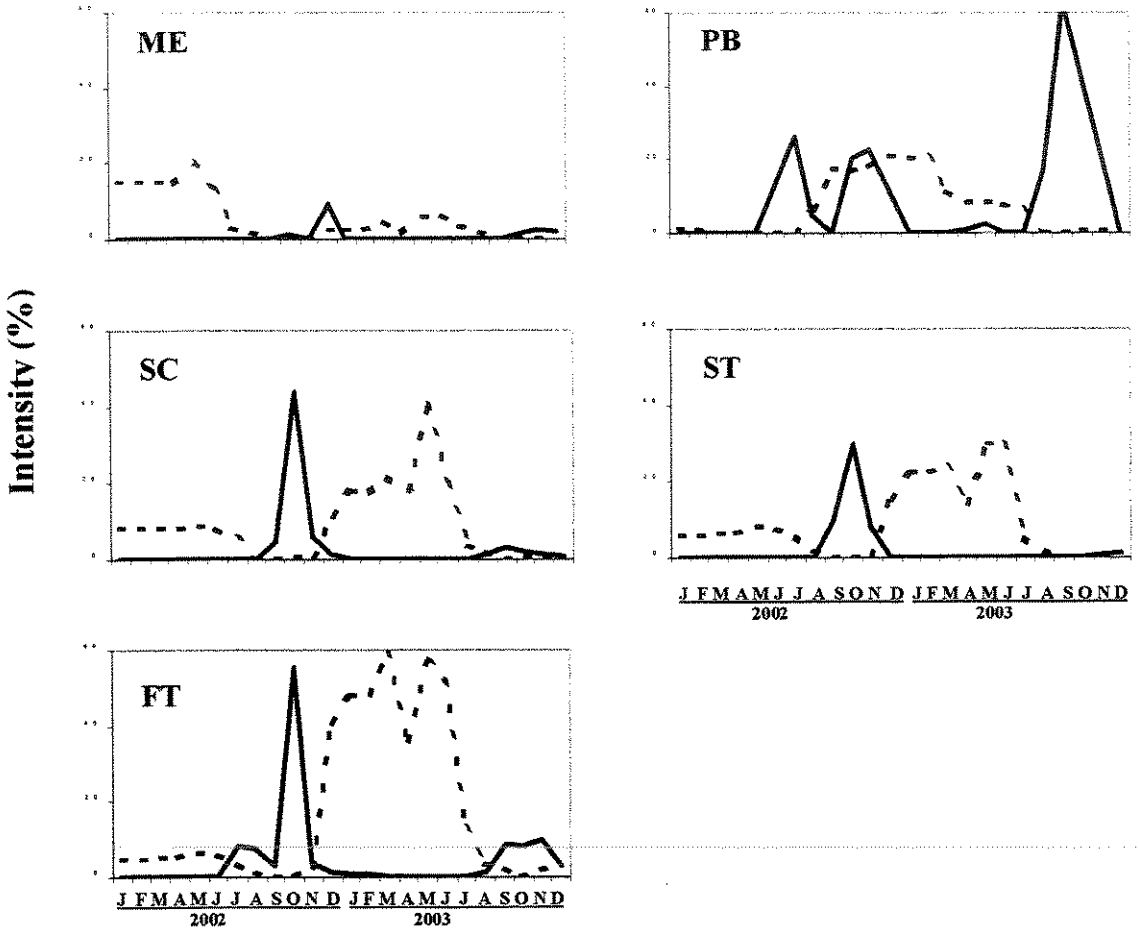

Figure 1: Reproductive phenology (expressed as a Fournier index, in %) of *P. tenuinervis* individuals in five Atlantic forest fragments in Rio de Janeiro State in 2002 and 2003. Solid line - flowering and dashed line - fruiting. The forest fragments were Parque Estadual do Mendanha (ME), Parque Estadual da Pedra Branca (PB), Hotel Fazenda Serra da Castelhaña (SC), Parque Estadual da Serra da Tiririca (ST) and Parque Nacional da Floresta da Tijuca (FT).

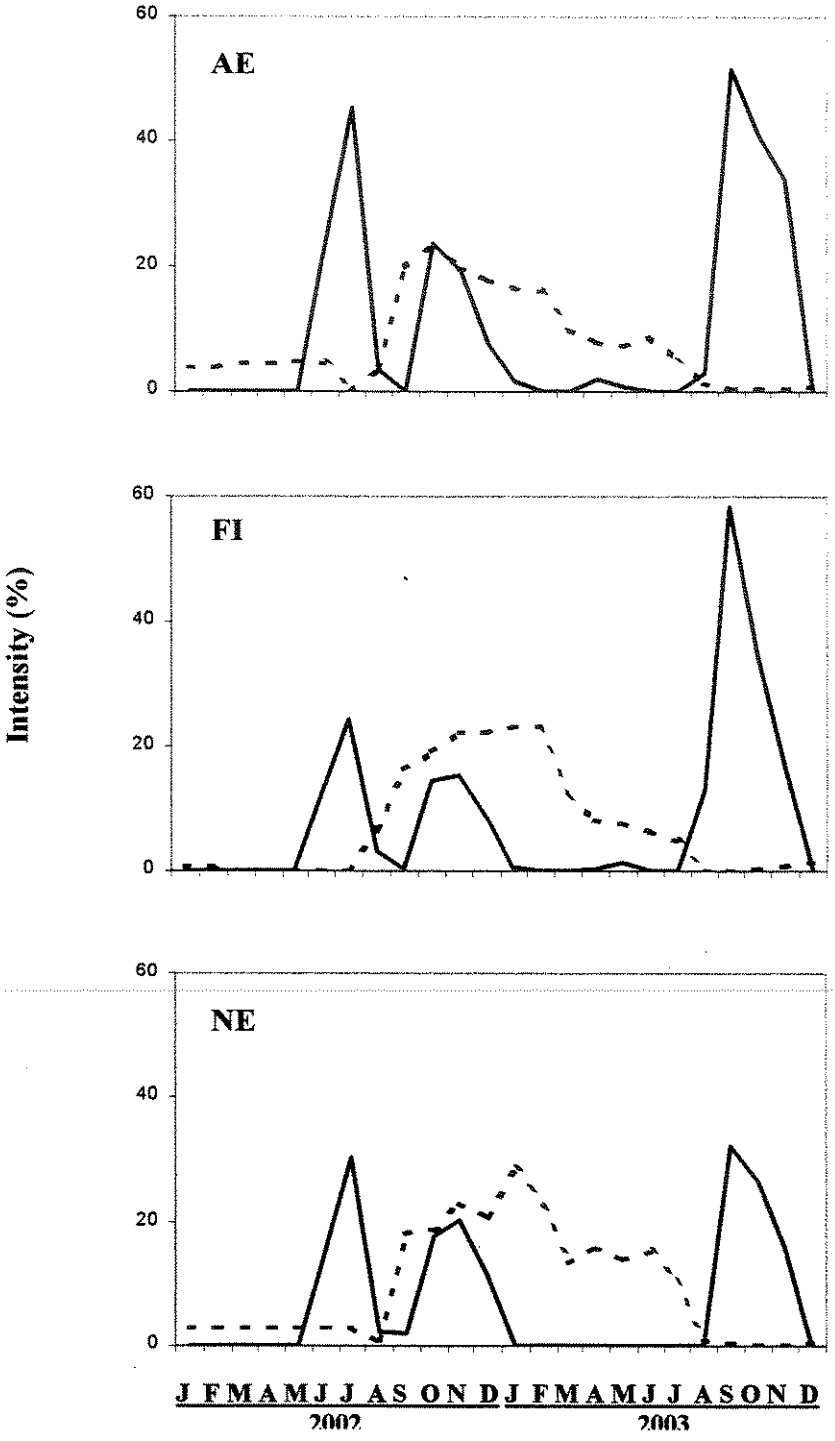

Figure 2: Reproductive phenology (expressed as a Fournier index, in %) of *P. tenuinervis* individuals in three habitats, anthopogenic edge (AE), natural edge (NE) and forest interior (FI), in the Serra da Castelaña site (SC) in 2002 and 2003. Solid line - flowering and dashed line - fruiting.

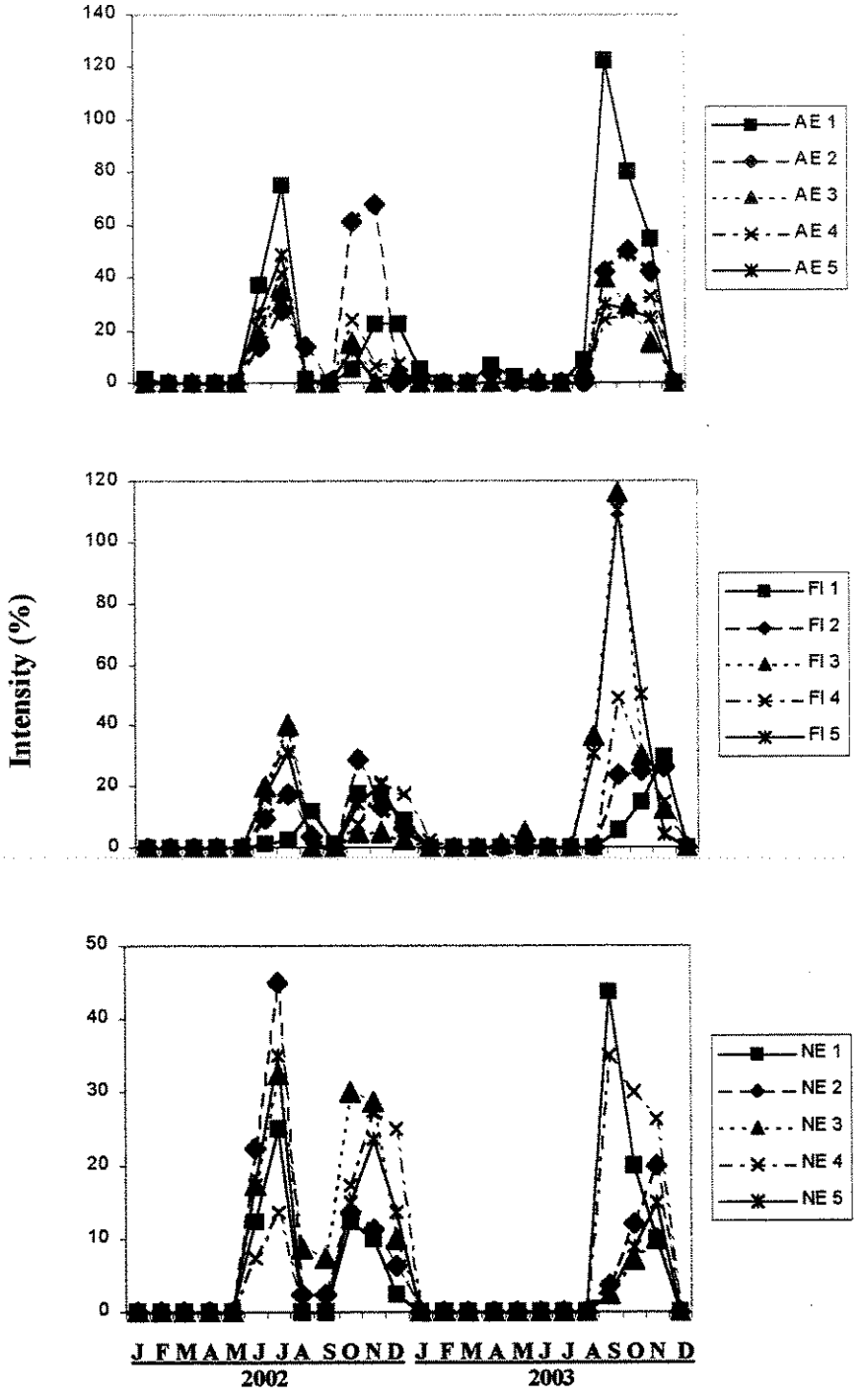

Figure 3: Flowering phenology (expressed as a Fournier index, in %) of *P. tenuinervis* individuals of each habitat replicate of anthopogenic edge (AE), natural edge (NE) and forest interior (FI), in the Serra da Castelaña site (SC) in 2002 and 2003.

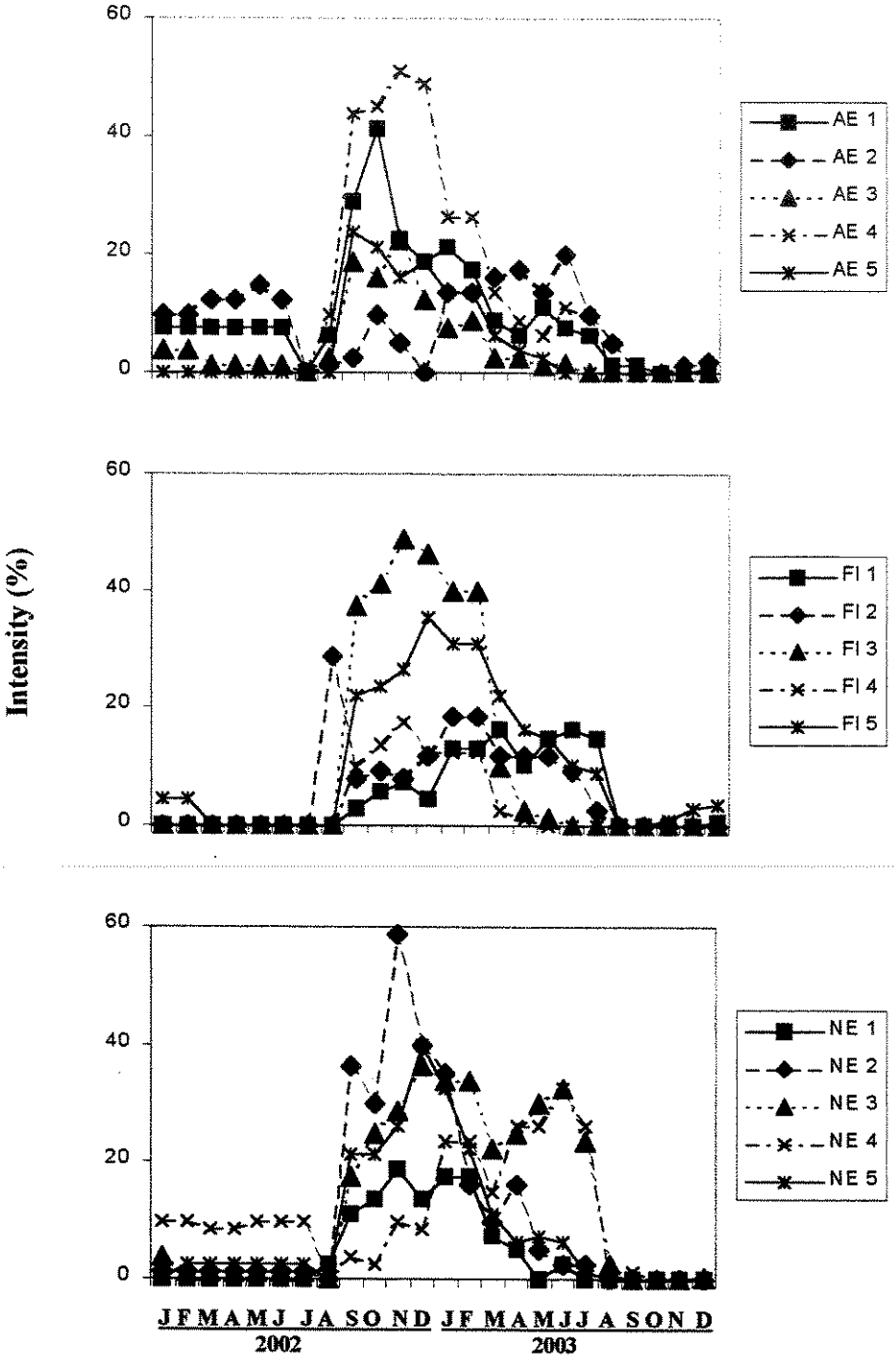

Figure 4: Fruiting phenology (expressed as a Fournier index, in %) of *P. tenuinervis* individuals of each habitat replicate of anthopogenic edge (AE), natural edge (NE) and forest interior (FI), in the Serra da Castelaña site (SC) in 2002 and 2003.

CAPÍTULO 3

FLORAL VISITORS AND POLLINATION OF *Psychotria tenuinervis* (RUBIACEAE): DISTANCE FROM THE ANTHROPOGENIC AND NATURAL EDGES OF AN ATLANTIC FOREST FRAGMENT[1]

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[1] Nos moldes da revista American Journal of Botany

ABSTRACT

Pollinators, especially insects, could be influenced by forest fragmentation. The aim of this paper was to examine whether there were differences in (1) the communities of floral visitors; (2) the frequency of visits; and (3) the fruit and seed sets of individuals of *Psychotria tenuinervis* occurring at anthropogenic edges (AE), natural edges (NE) and in the forest interior (FI) in two years of study. In 2002, the total number of flower visits was greater in NE and lower in AE, while there was no difference among the habitats in 2003. There were differences among sample plots within the habitats in both years. Bees were the most frequent visitors of *P. tenuinervis* flowers, and the introduced honeybee *Apis mellifera* was the most common species observed. There were no differences in the fruit and seed sets, or in the density of reproductive individuals of *P. tenuinervis* among the habitats, although in 2002, NE showed the greatest proportion of fruits per flower and AE the smallest. The similarity among the habitats probably resulted from the marked variation or heterogeneity among the sample plots and among the plants within the habitats, which masked any inter habitat differences. The observed heterogeneity and the probable importance of other factors, such as gaps or edge ages in the fragment studied, could be very important for conservation programs.

Key-words: Bees, edge, floral visitors, fragmentation, fruit set, pollination, seed set.

RESUMO

Espécies de polinizadores, principalmente insetos, podem ser influenciados pela fragmentação florestal. O objetivo deste trabalho foi verificar se existem diferenças: (1) nas comunidades de visitantes florais; (2) na freqüência de suas visitas; e (3) na produção de frutos e sementes de indivíduos de *Psychotria tenuinervis* localizados em bordas antrópicas (BA), bordas naturais (BN) e no interior do fragmento (IF). Em 2002, ocorreram mais visitas florais em BN e menos em BA, enquanto em 2003, não houve diferença entre os ambientes. Houve diferença entre as parcelas dentro dos ambientes em ambos os anos. Abelhas foram os visitantes florais mais freqüentes de *P. tenuinervis*, sendo *Apis mellifera* a espécie mais comum. Não houveram diferenças na produção de frutos e sementes nem na densidade de indivíduos reprodutivos entre os ambientes, apesar de em 2002, BN ter apresentado a maior produção de frutos e BA a menor. Essa similaridade entre os ambientes proavelmente ocorreu devido a grande variação ou heterogeneidade entre as parcelas e entre plantas dentro dos ambientes, que mascarou as diferenças entre ambientes. A indicação dessa heterogeneidade e a provável importância de outros fatores, como clareiras ou idade das bordas, nos fragmentos estudados, podem ser muito importantes para programas de conservação.

Palavras chave: abelhas, borda, fragmentação, polinização, produção de frutos, produção de sementes, visitantes florais.

INTRODUCTION

Tropical trees are mostly self-incompatible and are generally dependent on animal-mediated pollination for seed production (Bawa, 1990). Disturbances that affect the vectors of pollen transfer can therefore have an important impact on the reproductive output of tree species (Ghazoul and McLeish, 2001). For instance, the seed production of an angiosperm flower depends on the pollen quantity and "quality" (pollen grains that are incompatible or are from related individuals) reaching its stigma (Waser and Price, 1991).

The first modification caused by edge creation during forest fragmentation is a change in abiotic conditions resulting from the proximity to a structurally dissimilar matrix (deforested areas) (Bierregaard Jr *et al.*, 1992; Metzger, 1999; Poulin *et al.*, 1999). The microclimatic changes at the edges of a fragment may in turn stimulate direct biotic modifications such as changes in the composition of animal species at the edges, perhaps by attracting some exotic species, the loss of species originally present in the forest (Saunders, Hobbs and Margules, 1991), and shifts in the interactions between species (Murcia, 1995), such as in seed dispersion and pollination (Saunders, Hobbs and Margules, 1991; Aizen and Feinsinger 1994a). Boundaries between habitats, such as natural edges (limits between forests and rivers, streams, lakes or natural fields), may also show abiotic and biotic differences compared to the forest interior (Corbet, 1990; Mattlack, 1994). These differences among habitats could alter important characteristics and processes of the plant and animal populations (Aizen and Feinsinger 1994a; 1994b; Murcia, 1996). However, there have been few studies relating the impact of fragmentation on pollination (Herrera, 1995; Murcia, 1996; Debinski and Holt, 2000), one of the most influential interactions affecting plant demography (Silvertown and Lovelt-Doust, 1993; Murcia, 1996).

Pollinator species, mainly insects, are influenced by microclimatic variations in temperature and humidity (Herrera, 1995) because their activities rely on an appropriate microclimate in the flower and in the environment (Corbet, 1990). As a result, the number of attracted species, their behaviour in the flowers and the rate of visitation may be altered in anthropogenic and natural edges compared to the forest interior. According to Herrera (1995), the microclimate can influence the behaviour of flower visitors either by affecting the nature and availability of floral rewards or by a direct effect on the activity of the visitor. Alteration in the pattern of plant pollination at forest edges may change the reproductive success of plants because the quantity and quality of pollen received by the stigma can affect the total fruit and seed crop (Waser and Price, 1991; Aizen and Feinsinger 1994a; Herrera 2000).

The aim of this study was to examine whether there were differences in (1) the communities of floral visitors; (2) the frequency of visits; and (3) the fruit and seed sets of individuals of *Psychotria tenuinervis* located at anthropogenic edges, natural edges and in the forest interior in a forest fragment in the state of Rio de Janeiro, southeastern Brazil. *Psychotria tenuinervis* was chosen because it occurred in the three habitats, and produces flowers and fruits at a relatively low height.

A few studies have examined the reproduction and pollination of plants in forest fragments and have suggested a relationship between the extent of pollination and the fragment size (Aizen and Feinsinger 1994a; 1994b; Murcia, 1996) and its degree of isolation (Steffan-Dewenter and Tscharntke, 1999). However, to our knowledge, no study has examined the spatial variation in reproductive behaviour and in plant pollination at anthropogenic and natural edges of forest fragments. Such an assessment is important in order to determine whether the changes in pollination in a fragment are the consequences of

anthropogenic actions or are simply natural variations related to the forest heterogeneity (Casenave *et al.*, 1998).

MATERIAL AND METHODS

Study species

The family Rubiaceae has a wide and mainly typical distribution and contains 400-500 genera (Barroso, 1991). The genus *Psychotria* is pantropical, and contains mainly shrubs (Gentry and Dodson, 1987 *apud* Valladares *et al.*, 2000) that have distylous flowers (pin and thrum), with most species showing incompatibility within the same individual and morph (Bawa and Beach, 1983; Hamilton, 1990). *Psychotria tenuinervis* Muell. Arg. is a non-clonal shrub 1-5 m high that is typical of the Atlantic forest understory in the state of Rio de Janeiro (Gomes, Mantovani, and Vieira, 1995). This species has small white flowers (8 mm) that last just one day (C. B. Virillo *et al.*, unpublished), flowering occurs from 2 to 4 months per year whereas fruits are produced in almost all months of the year (chapter 2).

Study site

This study was done in the coastal Serra de Palmital, at Saquarema, in the state of Rio de Janeiro, Brazil. This area consist of about 1200 ha of Atlantic forest with hills varying from 30 to 400 m in height and has not been deforested, probably because its rough topography is not appropriate for cropland and cattle pasture. The study was done in 180 ha (22° 50' S; 42° 28' W) of this area to facilitate access to the habitats studied. The forest of the study area was surrounded by pasture and cropland that created anthropogenic edges. Within the forest there is a stream 2 - 5 m wide and 700 m long that created a natural edge with the forest. The study was done in three habitats: (1) the edge of the forest with pasture

and cropland (AE = anthropogenic edges ~50 m from the pasture), (2) the edge of the forest with the stream (NE = natural edges ~50 m from stream), and (3) the forest interior (FI = 200 m or more from any edge). Five sample plots of 10x50 m in each habitat were non-systematically located (see Appendix). The distances among sample plots varied from 150 to 883 m. The vegetation of the study area was classified as evergreen forest, or Ombrophilous Dense Forest (Radambrasil 1983) and the climate was classified as Cwa according to the Köppen system (Veanello & Alvez 1991).

Methods

Five sample plots (10 x 50 m) were non systematically selected in each habitat (AE, NE and FI). The distances among the sample plots varied from 150 to 883 m. To try to minimize the possibility that the number of flowers could influence the attraction of visitors, 10 *P. tenuinervis* individuals with a similar number of flowers (25-50% of the crown with flowers), that represented the most frequently flowering individuals in the area (see chapter 2), were selected in each one of the five sample plots of each habitat. These plants were used for the following studies:

Visitor community and frequency of visits

The number of visits to *P. tenuinervis* flowers was recorded on 10 sunny to slightly cloudy days in July and 5 days in November, which corresponded to the two months of the flowering period in 2002. In 2003, the visits were recorded during 15 days in September-October, which corresponded to the two months of the flowering period in this year (chapter 2). The observations were made from 7:00 to 16:00 hs, with 60 min being spent in each sample plot in each habitat (AE, NE and FI) per day. To avoid systematic biases

introduced by time-dependent changes in insect behaviour, no sample plot was surveyed twice during the same hours of the day. During the hour spent per plot, three plants with similar numbers of flowers were randomly selected and each was observed for 10 min. During this 10 min period, the observations were restricted to a branch or part of the crown (with 30-80 open flowers) for which the number of insect visitors and the frequency of visit (sensu Aizen and Feinsinger, 1994b) were recorded. The flower visitors were monitored nine hours per day, for a total of 135 h in 15 days or 405 10 min periods of observation in both years. The observations were made in both pin and thrum individuals (50% of each morph).

Fruit and seed set and density of reproductive individuals

In *P. tenuinervis*, aborted flowers leave distinctive scars in the infrutescences. To determine the fruit set per inflorescence, we counted the immature fruits and divided this number by the number of flowers originally produced (total of immature fruits plus number of scars) in five randomly sampled infrutescences of five shrubs in each sample plot in each habitat. To determine the seed set per fruit, we counted the fully developed seeds in 15 randomly selected fruits per individual per sample plot in each habitat.

To estimate the density of reproductive *P. tenuinervis* plants, the distance of the four nearest reproductive individuals of *P. tenuinervis* (one in each Cartesian directions: N, S, E and W) to a randomly selected reference or central individual was measured. Five reproductive individuals at least 10 m from each other were chosen as the central or reference individuals in each sample plot of each habitat (one per plot). The same reference individuals were used in 2002 and 2003.

Statistical analysis

The differences in the community of floral visitors, the frequency of visits, the number of fruit per flower and seeds per fruit of *P. tenuinervis*, and the density of reproductive plants among the three habitats were tested by three-level nested ANOVA (Zar, 1996). To improve the homoscedasticity of the data and ensure the normality of the distributions, the data for the frequency of floral visit and the number of seeds per fruit were square-root transformed while data for the number of fruits per flower were arcsin transformed before analysis (Zar, 1996). The back-transformed means are shown in the tables and figures.

In the nested ANOVA, the factors tested were the habitat (fixed factor), its five sample plots (nested within habitat), the individuals of *P. tenuinervis* (nested within sample plots, within habitat) and the data for floral visitors, frequency of visits, the number of fruits per flower and seeds per fruit (nested within individuals, within sample plots, within habitat). Habitat, sample plots and the individuals of *P. tenuinervis* were tested against the corresponding next lower hierarchical level (Sokal and Rohlf, 1995). The five sample plots and the individuals were randomly sampled and both were therefore considered as random effects.

RESULTS

Visitor community and the frequency of visits

In almost half of the 405 periods of observation (46.4%) there were no visits in 2002, whereas this value was 12.2% in 2003. There were a total of 1359 visits in 2002 and 3146 in 2003, with no significant difference in the number of visits between morphs in both years ($t_{217} = 0.54$; $p = 0.59$ and $t_{400} = 1.10$; $p = 0.28$).

The frequency of visits differed between years. In 2002, most of the flower visits occurred at NE (70.7%), followed by FI (19.5%) and AE (9.9%) ($F_{2;12} = 4.0$; $p = 0.04$) and there were differences among sample plots within the habitats ($F_{12;316} = 6.9$; $p = 0.0001$). In 2003, there was no significant difference in the total number of visits among habitats ($F_{2;12} = 0.54$; $p = 0.60$), but there was a significant difference among sample plots within the habitats ($F_{12;348} = 27.9$; $p = 0.0001$, Fig. 1).

Although there was a difference in the number of flower visitors between the years, there was no difference among the habitats in 2002 and 2003 ($\chi^2_2 = 3.3$; $p = 0.19$ and $\chi^2_2 = 1.2$; $p = 0.56$). Insects were the only group that visited *P. tenuinervis* flowers. There were 12 species of floral visitors in 2002 and 21 species in 2003, giving a total of 26 species of visitors observed. Among the visitors, 15 were Hymenoptera (7 in 2002 and 13 in 2003), that consisted of 12 bees (5 in 2002 and 11 in 2003) and three wasps (2 in 2002 and 3 in 2003), seven were Lepidoptera (4 in 2002 and 5 in 2003) and four were Diptera (1 in 2002 and 3 in 2003) (Table 1). Bees were the most frequent flower visitors and accounted for about 95.0% and 95.3% of all visits in 2002 and 2003, respectively. The introduced honeybee, *Apis mellifera* (52.8% and 83.3% of the visits in 2002 and 2003) was the most common species seen during the 10 min observation periods.

The visits of *Apis mellifera* and all native visitors (bees, wasps, dipterans and lepidopterans) differed spatially and temporally. In 2002, NE had the greatest visitation rate for *Apis mellifera* while AE had the smallest ($F_{2;12} = 4.1$; $p = 0.04$) (Fig. 2), but there was no difference in 2003 ($F_{2;12} = 0.48$; $p = 0.63$). The sample plots within the habitats differed in both years ($F_{12;301} = 12.8$; $p = 0.0001$ and $F_{12;372} = 40.3$; $p = 0.0001$, respectively). However, there was no significant difference in the number of visits by native visitors to *P. tenuinervis* individuals among the three habitats, in 2002 and 2003 ($F_{2;12} = 1.5$; $p = 0.27$ and

$F_{2;12} = 0.92$; $p = 0.43$, respectively), although there was a difference among the sample plots within the habitats in both years ($F_{12;294} = 4.7$; $p = 0.0001$ and $F_{12;370} = 10.8$; $p = 0.0001$, respectively).

The proportion of visits to *P. tenuinervis* flowers also differed with the time of day. The insects visited more frequently in the morning (7:00-12:00 a.m.) in 2002 ($H_8 = 81.9$; $p = 0.0001$) and 2003 ($H_8 = 33.8$; $p = 0.0001$) (Fig. 3). Additionally, considering all observations for each plant (regardless of the habitat), there was no relationship between the number of native insects and the number of honeybees foraging on *P. tenuinervis* flowers in 2002 ($r_s = -0.11$; $n = 1113$; $p = 0.42$), but there was a significant difference in 2003 ($r_s = -0.48$; $n = 2106$; $p = 0.0001$). Some aggressive or agonistic interactions between native species and *A. mellifera* were observed in both years.

Fruit and seed set and density of reproductive individuals

In 2002, the number of fruit per flower differed among the three habitats ($F_{2;12} = 3.9$; $p = 0.049$), with NE having the greatest proportion and AE, the smallest one. However, there was no significant difference in the fruit per flower among habitats in 2003 ($F_{2;12} = 0.03$; $p = 0.97$) (Table 2). In 2002 and 2003, there was marked variation in the fruit per flower among sample plots within the habitats ($F_{12;60} = 1.9$; $p = 0.046$ and $F_{12;60} = 2.0$; $p = 0.004$, respectively) and among plants within the sample plots within the habitats ($F_{60;300} = 4.9$; $p = 0.001$ and $F_{60;300} = 4.2$; $p = 0.001$, respectively). There was no significant difference in the seeds per fruit among the three habitats in both years ($F_{2;12} = 0.07$; $p = 0.93$ and $F_{2;12} = 0.10$; $p = 0.90$) (Table 2). However, there was variation among the plants within the sample plots within the habitats in both years ($F_{60;300} = 3.7$; $p = 0.0001$ and $F_{60;300} = 3.5$; $p = 0.001$), but not among the sample plots within the habitats ($F_{12;60} = 1.7$; p

= 0.13 and $F_{12;60}$ = 1.5; p = 0.18). There was no significant difference in the density of reproductive *P. tenuinervis* among the three habitats in the two years ($F_{2;12}$ = 3.1; p = 0.08 and $F_{2;12}$ = 2.2; p = 0.15) (Table 2), probably because of the marked variation among sample plots within the habitats ($F_{12;30}$ = 3.4; p = 0.0003 and $F_{12;30}$ =4.3; p = 0.0005).

DISCUSSION

Bees are the principal and most frequent floral visitors and pollinators in tropical forests (Roubik, 1989; Cane, 2001). For *Psychotria* species, one of the most common genera in tropical forests (Hamilton, 1990), the most frequent floral visitors in addition to bees are lepidopterans and hummingbirds (Augspurger, 1983; Bawa and Beach, 1983; Stone, 1996; Altshuler, 1999; Castro and Oliveira, 2002).

It is not surprising that honey bees (*A. mellifera*) were one of the most frequent visitors to *P. tenuinervis*, since this exotic species is always among the principal floral visitors of many plant communities in the America, probably because of its large number of workers, large foraging area and limited food and nesting requirements (Roubik, 1989). Africanized bees (*A. mellifera*) were introduced to the Americas about 50 years ago. However, the real impact of this exotic species on native bee communities is difficult to assess since no early studies examined this interaction in the beginning of its introduction (Wilms, Imperatriz-Fonseca and Engels, 1996).

The greater frequency of visit by honeybees to flowers of *P. tenuinervis* in the forest interior was unexpected since many studies have found that *A. mellifera* is more common at the matrix and edges of fragments and less common within forests (Aizen and Feinsinger, 1994b). Additionally, this species was responsible for the differences in the frequency of visits among the habitats in 2002, probably because of variations in its abundance between

years, as is common among pollinator species (Roubik, 2001). There were also variations in the total numbers of flower visits (by exotic and native insects) among the sample plots and among plants within habitats in both years.

Our results suggest that there was little or no competition between the exotic and native insects that visited *P. tenuinervis* flowers, since there were few agonistic interactions between them and this plant is very abundant in the study area (V. Rosseto *et al.*, unpublished). There was also no observed competition between honey and native bees, at Boraceia, in southeastern Brazil, since workers of many species of native stingless bees and honeybee workers were found foraging together on flowers or inflorescences of the same trees (Wilms, Imperatriz-Fonseca and Engels, 1996). These authors suggested that although honeybees are the main competitors in bee communities at Boraceia, this impact on native bee species is apparently buffered or minimized by the massive flowering of the trees that are the most important food source for eusocial bees. However, Aizen and Feisinger (1994b), in a study of fragmented habitats in Argentina, found a negative correlation between the number of visits by honeybees and those by other native insects to flowers of two plant species, thus indicating competition among them. Thomson (2004) found that *Bombus occidentalis* colonies exposed to competition with *A. mellifera* experienced nectar scarcity and responded by reallocating foragers from pollen to nectar collection. This action resulted in lowered rates of larval production. Other studies have found conflicting and contradictory results regarding competition with honeybees and their ability to reduce the number of native floral visitors (Roubik, 1989; Aizen and Feinsinger, 1994b; Gross, 2001; Roubik and Wolda, 2001).

There were no differences in the fruits per flower, seeds per fruit, or reproductive individuals among the habitats, although a significant difference in fruits per flower was

seen in 2002. As with flower visitation, the differences in reproductive capacity among the habitats were slight because of the marked differences and heterogeneity among the sample plots and among the plants within the habitats. Another cause of the similarities in the seed set among the habitats was probably the lower number of seeds produced per fruit (1 to 3 seeds). These findings suggest that factors other than the distance from the edges could influence the pollination of *P. tenuinervis*, and possibly of other plant species as well. Factors such as gaps (Piñero and Sarukhan, 1982; Kursar and Coley, 1992), matrix composition (Mesquita, Delamonica and Laurance, 1999) and edge age (Restrepo, Gomez, and Heredia, 1999) are known to influence plant survival and reproduction and may be responsible to the marked variation among habitat sample plots.

Most studies of fragmented habitats have concentrated on the difference in plant pollination in many fragments of different sizes. Only a few studies have investigated the direct (Murcia, 1996) and indirect (Jules and Rathcke, 1999; Restrepo, Gomez, and Heredia, 1999) causes of differences in plant pollination in relation to the distance from edges. As in the present study, Murcia (1996) found no differences in floral visitation in different distances from the edges among 16 plant species studied. Restrepo, Gomez, and Heredia (1999) found that the fruit production of plant communities varied among edges and according to edge age. Fruit production at young edges (< 12 years) did not differ from that at old edges (> 40 years) but was greater than in the forest interior. These authors suggested that the new abiotic conditions at recently formed edges, such as an increase in light and temperature, could account for the increase in fruit set with time, as the abiotic conditions become more similar to that in the interior, during forest succession, the fruit production also becomes more similar to that in the interior. In contrast to these studies,

Jules and Rathcke (1999) found that seed production of *Trillium ovatum* decreased along edges, due to lower pollen deposition in the flowers.

In conclusion, the results of this study show that there was marked heterogeneity among the sample plots of each habitat, and that other factors, such edge age, matrix type or the proximity of gaps may be more important than the proximity of anthropogenic or natural edges. Clearly, factors other than edges should be taken into consideration when modeling and evaluating the availability of "edge" and "interior" habitats in fragmented landscapes and designing natural reserves (Restrepo, Gomez, and Heredia, 1999). The use of more sample plots on edges in future studies would help to demonstrate the natural heterogeneity of fragments and would prevent wrong conclusions about the influence of edges on resident or persistent organisms.

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Table 1: Species (and families) of visitors to *P. tenuinervis* flowers in each habitat (anthropogenic edges = AE; natural edges = NE; forest interior = FI) in 2002 and 2003.

	AE		FI		NE	
Species	**2002**	**2003**	**2002**	**2003**	**2002**	**2003**
Hymenoptera						
Apis mellifera (Apidae)	X	X	X	X	X	X
Augochlora sp. (Apidae)		X		X		
Cephalotrigona capitata (Apidae)		X		X		X
Eulaema nigrita (Apidae)	X				X	
Eulaema sp. (Apidae)				X		
Euglossa sp. (Apidae)				X		
Melipona bicolor (Apidae)	X	X		X	X	X
Melipona quadrifasciata (Apidae)				X		
Paratetrapedia sp. (Apidae)				X		
Partamona sp. (Apidae)		X		X	X	X
Trigona fulviventris (Apidae)	X	X	X	X	X	X
Trigona spinipes (Apidae)				X		X
Epiponinae sp1 (Vespidae)	X			X		
Epiponinae sp2 (Vespidae)		X			X	
Epiponinae sp3 (Vespidae)						X
Total	5	7	2	12	6	7
Diptera						
Syrphidae sp1		X		X		X
Syrphidae sp2				X		
Syrphidae sp3			X		X	
Philopotinae sp1		X				
Total	0	2	1	2	1	1
Lepidoptera						
Urbanus dorantes (Hesperidae)		X			X	
Catasticta sp. (Nymphalidae)		X				
Pseudoscada erruca (Nymphalidae)				X		
Parides sp. (Papilionidae)		X				X
Eurema sp. (Pieridae)					X	
Melete lycimnia (Pieridae)	X	X			X	
Melete sp. (Pieridae)					X	
Total	1	4	0	1	4	1
General total	6	13	3	15	11	9

Table 2: Number of fruits per flower, number of seeds per fruit (mean, and lower and upper standard deviation) and density of reproductive *P. tenuinervis* (indiv/ha) (mean ± standard deviation) in each habitat (anthropogenic edges = AE; natural edges = NE; forest interior = FI) in 2002 and 2003. There was significant difference only for the number of fruits per flower among habitats in 2002. Columns with the same letters do not differ significantly in nested ANOVA.

	AE	**NE**	**FI**
Fruit / flower			
2002	0.27 (0.08-0.09)**a**	0.40 (0.03-0.03)**b**	0.32 (0.09-0.10)**ab**
2003	0.35 (0.12-0.13)	0.34 (0.04-0.04)	0.34 (0.08-0.09)
Seed / fruit			
2002	1.94 (0.27-0.30)	1.95 (0.26-0.31)	1.95 (0.25-0.29)
2003	1.91 (0.26-0.34)	1.90 (0.23-0.33)	1.92 (0.22-0.30)
Density			
2002	462 (±307)	1003 (±1100)	625 (±464)
2003	503 (±460)	1106 (±1203)	650 (±620)

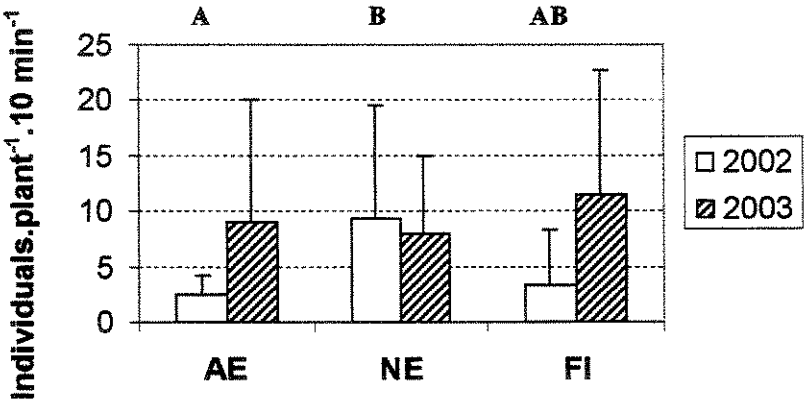

Figure 1: Frequency of visits to *P. tenuinervis* flowers in each habitat (anthropogenic edges = AE; natural edges = NE; forest interior = FI) in 2002 and 2003 (back-transformed means and standard deviations). Bars topped by the same letters do not differ significantly by nested ANOVA. Only visits in 2002 showed a significant difference among the habitats.

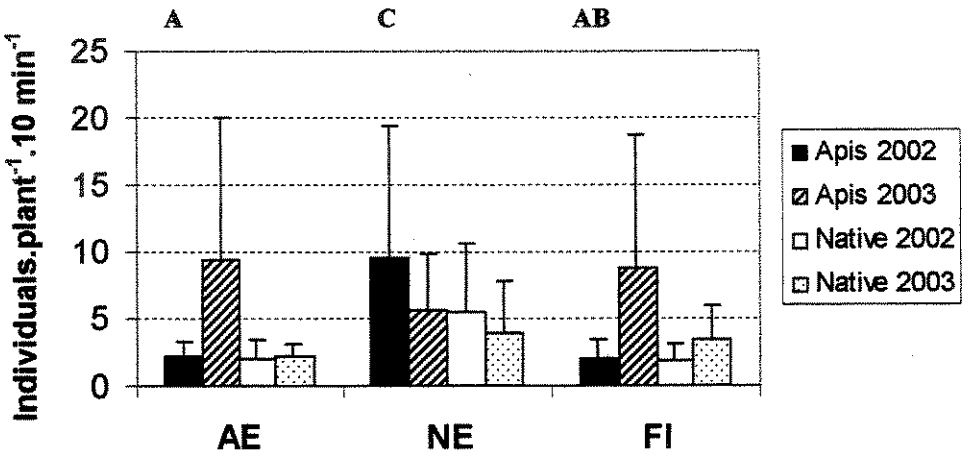

Figure 2: Frequency of visits by *Apis mellifera* and native visitors to *P. tenuinervis* flowers in each habitat (anthropogenic edges = AE; natural edges = NE; forest interior = FI), in 2002 and 2003. Means and standard deviations were back-transformed. Bars topped by the same letters do not differ significantly by nested ANOVA. A significant difference among the habitats was seen only for visit by *Apis mellifera* in 2002 (black bars).

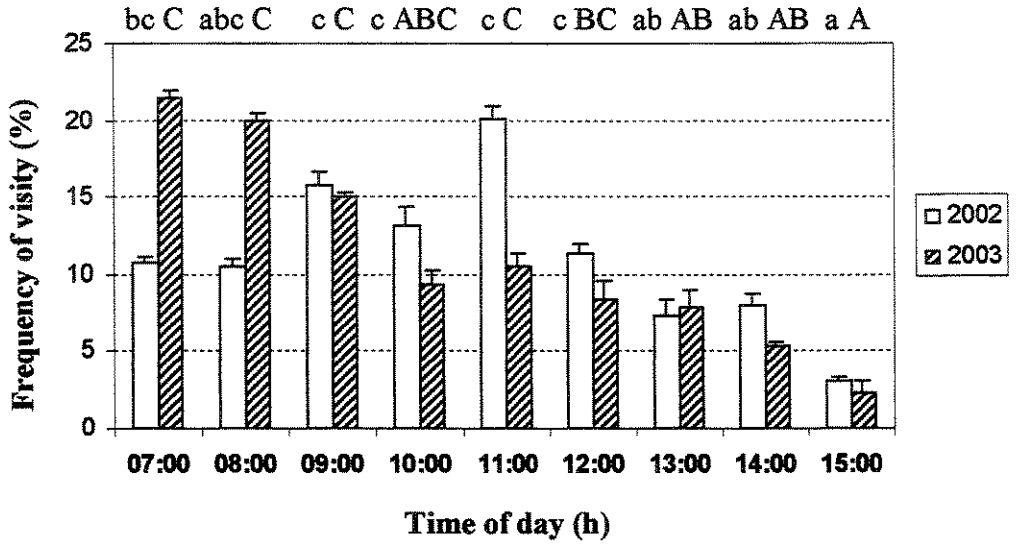

Figure 3: Frequency of visits (%) to *P. tenuinervis* flowers during the day in 2002 and 2003 (back-transformed means and standard deviations). Bars topped by the same lower-case (2002) or capital (2003) letters do not differ significantly by the Kruskal-Wallis test.

CAPÍTULO 4

QUALITY OF SEEDS PRODUCED BY *Psychotria tenuinervis* (RUBIACEAE): DISTANCE FROM ANTHROPOGENIC AND NATURAL EDGES OF ATLANTIC FOREST FRAGMENT[1]

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ABSTRACT

Gene flow through pollen and seed dispersal determines seed production and its quality, as well as the degree of genetic isolation in plant populations. Habitat changes or abrupt limits between habitats can affect the interactions between plants and their pollen and seed vectors and lead to a decrease in seed quality because of increased inbreeding. Anthropogenic edges created by fragmentation and natural edges may disrupt gene flow and affect the quality of seeds produced by plants located in these habitats. The aim of this study was to investigate whether there were differences in the (1) genetic variability, (2) genetic structure, (3) seed mass, and (4) germination rate and velocity of the seeds produced by *Psychotria tenuinervis* individuals located at anthropogenic edges (AE), natural edges (NE) and forest interior (FI). Among the three habitats, the populations of *P. tenuinervis* showed no differences in genetic variability or genetic structure (G_{ST}=0.07±0.09). However, there was an indication of inbreeding (G_{IS}=0.71±0.08), which was significantly higher on NE (0.82) than on AE (0.74) and FI (0.64). There were no differences in the seed mass, germination rate and velocity among the three habitats, probably because most of them showed within-habitats variation. These results suggest that other characteristics of the fragment, such as gaps, edge age and type of matrix exert more influence on seed mass and germination than the distance from the edges. Seed characteristics were not influenced by the genetic pattern of *P. tenuinervis*, since there was little difference in the genetic variability and structure among and within habitats.

Key words: allozymes, edges, fragmentation, genetic variability, germination, seeds, seed quality.

RESUMO

O fluxo gênico, através da polinização e dispersão de sementes, determina tanto a aptidão quanto o grau de isolamento genético em populações de plantas. Alterações de habitats ou limites abruptos entre habitats podem afetar a interação entre plantas e seus vetores de pólen e sementes diminuindo a qualidade de sementes devido ao aumento do endocruzamento. Tanto bordas antrópicas criadas pela fragmentação quanto bordas naturais podem apresentar perturbação no fluxo gênico e conseqüentemente na qualidade das sementes produzidas pelas plantas que ocupam tais ambientes. O objetivo deste trabalho foi investigar se existem diferenças na: (1) variabilidade genética; (2) estrutura genética; (3) massa e (4) taxa e velocidade de germinação de sementes produzidas por indivíduos de *Psychotria tenuinervis* localizados em bordas antrópicas (BA), bordas naturais (BN) e interior de um fragmento florestal (IF). *P. tenuinervis* não apresentou diferenças significativa na variabilidade genética, nem significante estruturação genética (G_{ST}=0.07 ± 0.09) entre os três ambientes. Porém houve uma indicação de endocruzamento (G_{IS}=0.71±0.08), que foi significativamente maior em BN (0,82) do que em BA (0,74) e IF (0,64). Não houve diferença significativa na massa das sementes, nem na taxa e velocidade de germinação entre os três ambientes, provavelmente devido as diferenças observadas dentro deles. Portanto, parece que outras características do fragmento, como clareiras, idade das bordas, e tipo de matriz, exercem mais influência na massa e germinação de sementes do que apenas a distancia das bordas, porém essas características das sementes não foram influenciadas pela variabilidade e estrutura genética da espécie, uma vez que não houve diferença entre e dentro dos habitats.

Palavras chave: bordas, fragmentação, variabilidade genética, geminação, isoenzimas, sementes, qualidade das sementes.

INTRODUCTION

In plants, the gene flow through pollination and seed dispersal determines the seed production and the degree of genetic isolation among populations (Dewey & Heywood 1988, Ellstrand & Elam 1993). For instance, seed production by plants may change greatly if some animal pollinators decline in number or consistency in a disturbed habitat (Aizen & Feinsinger 1994a, 1994b). The behaviour of the pollinator may determine the quantity and distance that the pollen will be transported and, consequently its quality (Silvertown & Lovelt-Doust 1993, Kato & Hiur 1999). Pollination regimes that vary in the composition and abundance of pollinators will result in differential reproduction (Herrera 2000). A limited availability of pollen can affect the fruit and seed set (Kato & Hiur 1999) and the loss of seed quality, including a decrease in seed mass, germination rate and velocity, all of which may influence the population dynamics and the chances of local extinction. Seed mass and germination can, in turn, influence the probability of seedlings to become established by affecting the distance that which seeds disperse and the time of seedling recruitment. These factors will influence the likelihood that the seeds will reach suitable habitats for germination and will affect the probability of early survival (Fenner 1985, Paz *et al.* 1999).

The characteristics of the reproductive systems of plants, especially the pollination and the seed dispersal systems, also have an important role in determining the variability within and among populations (Loveless & Hamrick 1984). In tropical plants, whose flowers are pollinated by insects and whose fruits are dispersed by birds, the opportunity for gene flow may be extensive, but can also result in restricted pollen and seed movement, depending on the behaviour of the mutualistic animals (Loiselle *et al.* 1995b). Floral visitors with a short flying distance may increase inbreeding by decreasing gene flow

(Shapcott 1998). The number and quality of seeds produced by some populations can decrease because of increased inbreeding (loss of heterozygosity) (Templeton *et al.* 1990, Waser & Price 1991) and decreased genetic variability within sub-populations (Ellstrand & Elam 1993, Alvarez-Buylla *et al.* 1996). For example, the deposition of seeds next to parental plants leads to divergence among populations and even to subdivision within populations (Foré *et al.* 1992). In some species, the consequence of restricted dispersal may be modified by pollinators, frugivores or by persistence of the seeds in the seed bank, mainly because gene flow reduces the genetic differentiation of the population and may introduce new genetic variations (Nason *et al.* 1997).

Habitat changes or abrupt limits between habitats can affect the interactions between plants and their pollinators or seed dispersers (Ellstrand & Elam 1993, Alvarez-Buylla *et al.* 1996, Nason *et al.* 1997). For example, habitat fragmentation may alter the composition of animal assemblages and the relative contribution of some species as pollinators or seed dispersers (Murcia 1995, 1996, Nason & Hamrick 1997). Habitat fragmentation can produce small, isolated populations for which losses in genetic variability are likely (Menges 1991), or can produce internally subdivided populations because of the creation of an edge or increased habitat heterogeneity. Many tropical tree species are particularly vulnerable to this landscape transformation because of their own low densities and the disruption of their pollen and seed vector associations (Nason *et al.* 1997). Natural edges (limits between forests and rivers, streams, lakes or natural fields) may also disrupt or alter gene flow and lead to an increase in habitat heterogeneity. Reductions in fitness caused by a decrease in genetic variation could occur early in the life of the sporophyte plant (eg. fruit set, seed set, and germination). Most studies of inbreeding

depression have focused on fruit and seed set. However, differences on seed mass and germination among populations could well reflect inbreeding effects (Menges 1991).

The aim of this work was to investigate whether there were differences in the: (1) genetic variability; (2) genetic structure; (3) seed mass, and (4) germination rate and velocity of seeds produced by individuals of *Psychotria tenuinervis* located at anthropogenic edges, natural edges and in the forest interior.

MATERIAL AND METHOD

Study species

Psychotria tenuinervis Muell. Arg. is a non-clonal species with distylous flowers that shows incompatibility among flowers of the same individual and the same morph (Bawa & Beach 1983; Hamilton 1990). The flowers of this species are pollinated by insects, mainly bees (especially *Apis mellifera* in the area studied), and the fruit are fleshy, with one to three seeds (chapter 3) that are probably dispersed primarily by birds, like other *Psychotria* species (Paz *et al.* 1999, Loiselle *et al.* 1995b). This species was chosen because it was present in the three habitats studied and its flowering and fruiting occur at a relatively short height.

Study site

This study was carried out in the coastal Serra de Palmital, at Saquarema, in the state of Rio de Janeiro, Brazil. This area consist of about 1200 ha of Atlantic forest with hills varying from 30 to 400 m in height and has not been deforested, probably because its rough topography is not appropriate for cropland and cattle pasture. The study was done in 180 ha (22° 50' S; 42° 28' W) of this area to facilitate access to the habitats studied. The

forest of the study area was surrounded by pasture and cropland, which created anthropogenic edges. Within the forest there was a stream 2 - 5 m wide and 700 m long that created a natural edge with the forest. The study was done in three habitats: (1) the edge of the forest with pasture and cropland (AE = anthropogenic edges ~50 m from the pasture), (2) the edge of the forest with the stream (NE = natural edges ~50 m from stream), and (3) the forest interior (FI = 200 m or more from any edge). Five sample plots of 10x50 m in each habitat were randomly located (see appendix). The distances among sample plots varied from 150 to 883 m. The vegetation of the study area was classified as evergreen forest, or Ombrophilous Dense Forest (Radambrasil 1983) and the climate was classified as Cwa according to the Köppen system (Veanello & Alvez 1991).

Seed quality

Ten individuals in each of the five sample plots in each habitat (AE, NE and FI) were numbered and monitored in order to evaluate the quality of their seeds. The rate and velocity of germination, the seed mass, the genetic variability and the genetic structure were used to assess the seed quality.

1 – **Genetic analyses**

To quantify and compare the genetic variability of *P. tenuinervis*, 2 - 10 immature seeds from 2 - 10 *P. tenuinervis* shrubs from each sample plot in the three habitats were collected, kept on ice during the period in the field, and frozen in liquid nitrogen until electrophoresis was done. Lott & Jackes (2001) recommended the use of immature seeds for genetic work because they are easier to collect, can be obtained earlier and in greater

quantity than mature seeds, and can be collected from the crowns of the plants prior to seed loss through fruit dehiscence, predation or dispersal.

Allozyme analyses were done as described by Soltis *et al.* (1983), with modifications. For electrophoretic analyses, each immature seed was mashed with 30.0 µl of extraction buffer [0.1 M Tris, 0.2 M sucrose, 0.6% PVP, 1 mM EDTA, 0.15% bovine serum, 0.06 M DIECA (diethyl sodium carbamate), 0.03 M sodium tetraborate ($Na_2B_4O_7$), and 0.1% β-mercaptoethanol, pH 7.0 (Sun & Ganders 1990, with modifications)], and adsorbed onto filter paper wicks (Whatman No. 3), that were loaded onto 8.5% starch gels (Sigma hydrolyzed potato starch). The buffer systems used and the 10 enzyme systems investigated are described in Table 1.

2 – Seed mass and germination

Ten seeds were collected from up to 10 *P. tenuinervis* individuals (not necessarily the same individuals as used in the genetic experiments), from each sample plots in the three habitats. The germination tests were done at 25°C, the temperature most recommended for the germination of seeds from most Brazilian species (Oliveira *et al.* 1989). The germination was carried out transparent plastic boxes ("gerbox") filled with heat-sterilized vermiculite, in a temperature and light controlled chamber, with temperature kept constant within ±1°C and a 12 h photoperiod, according to germination protocols used in the Department of Plant Physiology at Unicamp. Measurements were taken three times a week and the number of germinated seeds was recorded until one month had passed without any new germination. Germination was considered as visible radicle protrusion. The rate of seed germination was estimated using the index of germination velocity (IGV),

according to Labouriau (1970): IGV = $1/\bar{t} = \sum ni / \sum ni \cdot ti$, where $\bar{t}$ is the average germination time, *ti* is the number of days between the beginning of the experiment and the i^{th} observation, and *ni* is the total number of seeds germinated within the time interval t_{i-1} |—| t_i.

The dry weights of ten seeds from 10 *P. tenuinervis* individuals from each sample plot in the three habitats (500 per habitat) were compared in order to determine whether there was any difference among the habitats. The seeds were collected and dried in an oven at *ca.* 65°C for 48 hr.

Statistical analysis

In the genetic analysis, banding patterns were genetically interpreted by direct observation of the gels. Alleles were identified by their mobility relative to the most common allele in the population. The genetic variation within each habitat was estimated by the percentage of polymorphic loci (P), the average number of alleles per locus (A), and the observed (H_o) and expected (H_e) heterozygosities with the latter calculated according to the unbiased estimate of Nei (1978). Deviations from Hardy-Weinberg expectations were tested using an exact probability test (conventional Monte Carlo) and the statistical significance of the values were checked by bootstrapping procedures (over loci, 5000 permutations) with a sequential Bonferroni correction (Rice 1989). The softwares Fstat (Goudet 1995) and Genetix (Belkhir *et al.* 2001) were used to the above calculation. The patterns of variation among samples were assessed by hierarchical gene diversity analysis (Chakraborty *et al.* 1982) in subdivided populations (Nei 1973). The total genetic diversity (H_T) was partitioned into its components within populations (H_S). The genetic differentiation among ($G_{ST} = H_t - H_s / H_t$) and within ($G_{IS} = H_s - H_o / H_s$) populations were

calculated, with H_o being the observed heterozygosity. G_{ST} is an estimate analogous of Wright's F_{ST} and G_{IS} of Wright's F_{IS}. All calculations were done using the program NEGST (Chakraborty *et al.* 1982). To compare the G-estimates between habitats, a Z test was used (Sokal & Rohlf 1995) since these estimates could be treated as correlation coefficients (Crow & Kimura 1970). As suggested by Nei (1973), the statistical average of absolute gene diversity (H_T) was obtained over all monomorphic and polymorphic loci to clarify a general form of differentiation among populations. The genetic identity (Nei 1978) and the geographic distance matrices were compared by the Mantel test, with 5000 randomized runs.

The software BOTTLENECK 1.2.02 (Piry *et al.* 1999) was used to determine whether the *P. tenuinervis* population in each habitat was at mutational and genetic drift equilibrium (according to Cornuet & Luikart 1996). The significance of the excess of genetic diversity ($H_e > H_{eq}$) was estimated by the Wilcoxon signed rank test, with 5000 randomized runs (Moraes 2003), where H_e is the expected heterozigosity under Hardy Weinberg equilibrium and H_{eq} is the expected heterozigosity under mutation and genetic drift equilibrium. This is considered the most appropriate test when few loci (< 20) are used (Piry *et al.* 1999).

The multilocus (t_m) and single-locus (t_s) outcrossing rate and the inbreeding coefficients (f) of maternal parents were estimated using the model of mixed mating in order to maximize the likelihood equation, with the algorithm of expectation-maximization methods (EM) proposed by Ritland & Jain (1981), using the programs MLT (Ritland 1990) and MLTR v 1.1 (Ritland 2002). Additionally, the software GenAlEx v.5.1 (Peakall & Smouse 2001) was used to determine the spatial autocorrelation in the genetic results. This analysis was based on genetic distance methods using multiallele and multilocus

autocorrelation (Smouse & Peakall 1999, Peakall *et al.* 2003). The autocorrelation coefficient "*r*" that was used in this analysis is a proper correlation coefficient, bounded by (-1, +1), is closely related to Moran's-I, with its significance tested for each allele through a 95% confidence interval generated by 1000 spatial permutations. According to Smouse and Peakall (1999) and Peakall *et al.* (2003) unlike classical spatial autocorrelation analysis that is usually executed one allele at a time the procedure is intrinsically multivariate, avoiding the need for allele-by-allele, locus-by-locus analysis. By combining alleles and loci, we strengthen the spatial signal by reducing stochastic (allele to allele and locus to locus) noise.

Differences in seed mass, seed germination rate and velocity and the mean expected heterozygosity among the three habitats (AE, NE and FI) were tested by a three-level nested ANOVA (Zar 1996). To improve the homoscedasticity and ensure normal distributions, the seed mass data were log-transformed and the data for percentage and velocity of seed germination were arcsine transformed before analysis (Zar 1996). The back-transformed means are reported in the tables and figures.

In the nested ANOVA, the factors tested were the habitat (fixed effect), the five sample plots (nested within habitat), the individuals of *P. tenuinervis* (nested within sample plots within habitat) and the data from seed mass, percentage and rate of germination, and mean expected heterozygosity (nested within individuals within sample plots within habitat). Habitat, sample plots and the individuals of *P. tenuinervis* were tested against the corresponding next lower hierarchical level (Sokal & Rohlf 1995). The five sample plots and the individuals were randomly sampled and were therefore considered as random effects.

RESULTS

Genetic analyses

Among habitats

Resolution was obtained with 10 different enzyme systems and 14 putative loci were scored. Among the three habitats, there were no differences in the genetic variability of *P. tenuinervis* populations (Table 2). Of the 14 loci scored, 50% or more were polymorphic within each habitat. Overall, 11 of the 14 loci were polymorphic in at least one habitat. The mean number of alleles per locus was about 2.5 within each habitat; however, five loci had more than three alleles in at least one habitat. Genetic diversity measures indicated that the *P. tenuinervis* populations in the three habitats had similar levels of genetic variation. Additionally, the mean expected heterozygote frequency was not significantly different among the three habitats ($F_{2;12} = 0.82$; $p = 0.46$) or among the sample plots within the habitats ($F_{12;179} = 1.38$; $p = 0.18$).

The populations of *P. tenuinervis* showed no genetic structure among habitats ($G_{ST} = 0.07 \pm 0.09$) since the individuals in sample plots of each habitat were responsible for 70.4% of the total genetic variation, indicating greater variability within the habitats than among them. However, there was a strong indication of inbreeding in the overall *P. tenuinervis* population ($G_{IS} = 0.71 \pm 0.08$), which was significantly higher in NE (0.82 G_{IS}=0.71±0.16) than in AE (0.74±0.14) and FI (0.64±0.19) ($Z_2 = 0.96$; $p = 0.011$). Additionally, the frequencies of almost all loci in the sample plots of each habitat were below Hardy-Weinberg expectation (except FUM in the NE habitat).

Since the high fixation indices (G_{IS}) found here could be the effect of "sister" seeds there were many seeds from the same mother shrub, these indices were calculated from just one seed per shrub (randomly selected) in order to eliminate this effect. Despite this

precaution, the indices remained high and significant (G_{IS} = 0.55±0.13), thus reinforcing the conclusion of non-random mating. There were three further results that reinforced the indication of mating among related individuals: i) all habitats showed evidence of a recent bottleneck (P < 0.001), with a multilocus outcrossing estimate (t_m) of the progeny that was significantly greater than the single-locus (t_s) value (Table 2); ii) the inbreeding coefficient of maternal parents (f = 0.20) was much lower than that for the progeny in all habitats (Table 2), and (iii) closer *P. tenuinervis* individuals were more related with each other than the more distant ones, as indicated by the spatial autocorrelation that showed an isolation by distance pattern (Figure 1).

Within habitat

Even within habitats there was not much heterogeneity in the genetic variability among *P. tenuinervis* individuals within the sample plots. The expected heterozygosity was not significantly different among *P. tenuinervis* individuals within AE ($F_{4;62}$ = 0.81; p = 0.52), NE ($F_{4;62}$ = 1.33; p = 0.27) and FI ($F_{4;62}$ = 2.37; p = 0.06). The percentage of polymorphic loci was similar among *P. tenuinervis* individuals within the sample plots in AE, and FI, as was the mean number of alleles per locus. Only one sample plot in NE showed a low expected frequency of heterozygosity, whereas the others were higher and similar to each other (Table 3). There was no sub-structuring within NE (G_{ST} = 0.07±0.08) and FI (G_{ST} = 0.06±0.08), but there was within AE (G_{ST} = 0.11±0.03). Additionally, there were inbreeding patterns within all habitats (G_{IS}, NE = 0.72, AE = 0.67 and FI = 0.66). The Mantel test revealed no correlation between the genetic identities and geographic distances within AE (r = -0.79; p=0.87), NE (r = -0.01; p=0.51), and FI (r = -0.12; p = 0.47).

Seed mass and germination

Among and within habitat

There were no differences in the seed mass, germination rate and velocity among the three habitats ($F_{2;12} = 0.74$; $p = 0.50$; $F_{2;12} = 0.88$; $p = 0.44$; $F_{2;12} = 0.48$; $p = 0.63$; respectively) (Table 4). However, there were differences in the seed mass ($F_{12;1433} = 6.66$; $p = 0.0001$), but not in the germination rate and velocity ($F_{12;29} = 0.52$; $p = 0.88$; $F_{12;27} = 0.59$; $p = 0.82$; respectively), among the sample plots within habitats (Table 5).

DISCUSSION

Our data for G_{ST} indicate little spatial differentiation among and within habitats, as has been documented in several tropical species (Heywood & Fleming 1986, Hamrick & Loveless 1989), probably because of the absence of an ecological barrier against gene flow. Trees and shrubs usually have high levels of genetic variation, most of which occurs within populations, with little genetic differentiation among populations (Perez-Nasser *et al.* 1993). Additionally, all studies with other *Psychotria* species have also found low to moderate genetic differentiation among subpopulations (Hamrick & Loveless 1989, Perez-Nasser *et al.* 1993, Loiselle *et al.* 1995a, 1995b), which could indicate that gene flow systems, seed dispersal by birds and/or pollination by bees allowed sufficient levels of gene flow among their populations to maintain the homogeneity.

Despite considerable diversity within populations of *P. tenuinervis* and no sub-structure among habitats (non-significant G_{ST}), the mean fixation index ($G_{IS} = 0.71$) of the progeny was significantly greater than zero, indicating inbreeding within each habitat. Besides, the inbreeding coefficient (f) of maternal parents was lower than for the progeny but was still high ($f = 0.20$). This contrasts with results for other rain forest species (mean

F_{IS} = 0.048), that have been shown to be predominantly outcrossed plants (Hamrick *et al.* 1992, Rocha & Lobo 1996), and for other *Psychotria* species that seem to have random mating in their populations (Hamrick & Loveless 1989, Perez-Nasser *et al.* 1993, Loiselle *et al.* 1995a, 1995b). Thus, the results of this study indicate that there were many matings among related shrubs, since this is a non-clonal (V. Rosseto *et al.* unpubl.) and self-incompatible species (C. B. Virillo *et al.* unpubl.). The difference between the single and multilocus outcrossings and the autocorrelation analysis showed that the closest *P. tenuinervis* individuals were more related to each other, reinforcing the indication of inbreeding. The pollinators were probably responsible for most of the mating among related shrubs, and the seed dispersers were probably responsible for most of the gene flow over long distances, thereby preventing a spatial differentiation within the population. The genic flow reached about 300 m (fig 1).

In Costa Rica, Loiselle *et al.* (1995b) found that individuals of *Psychotria officinalis* located within 5 m of each other, were more related and that there were low but significant values of genetic differentiation among subpopulations. These authors indicated that this pattern of genetic correlation is observed in species with high outcrossing and presumably effective pollen flow. Since the high level of gene flow through pollination would result in neighborhood areas much greater than the 5-10 m scale of autocorrelation reported, the relatedness among neighbor individuals was expected to reflect localized seed dispersal rather than isolation by distance resulting from localized dispersal of both pollen and seeds. Some of the birds that disperse *P. officinalis* in Costa Rica consume the fruit and drop seeds in the immediate vicinity of the parent while others carry the fruits elsewhere. The net result would be a mixing of the seedling pool with some inclusion of localized family clusters (Loiselle *et al.* 1995b).

In the present study, the fixation indices found for *P. tenuinervis* were probably a consequence of the inbreeding caused by a fail in gene flow, probably related to a pollination deficit. Additionally, the genetic diversity in *P. tenuinervis* was high, and occurred at a high density within sample plots in each habitat (V. Rosseto *et al.* unpublished data). Many canopy trees produce numerous flowers per tree over a short period of time, in a synchronized event (van Schaik *et al.* 1993) as also observed in *P. tenuinervis* (chapter 2). Although this short and synchronous period of flowering among many individuals within a large area attracts pollinators, such a pattern reduces pollinator movements and hence reduces outcrossing (Shapcott 1998). It seems likely that pollinators move primarily among flowers within single trees or among close individuals with relatively little movement between distant plants, leading to a greater proportion of mating among relatives.

The actual main visitor to *P. tenuinervis* flower individuals, *Apis mellifera* (chapter 3), could contribute to the decrease in gene flow. This exotic bee is considered a poor pollinator because its workers can dominate the entire crown of a flowering tree or shrub, thereby passively and/or actively excluding other flower visitors that could produce more outcrossing (Roubik 1991 *apud* Aizen & Feinsinger 1994a). *Apis mellifera* workers tend to forage longer within a tree canopy, and to move among trees more rarely than do individuals of native species (Aizen & Feinsinger 1994a). Such behaviour could increase the inbreeding of the plant species (Waser & Price 1991). This high frequency of visits to flowers by *A. mellifera* could be the main difference between the present study and the other *Psychotria* studies that did not report a high G_{IS}.

The dispersal of *Psychotria* seeds by birds could generate some sibling mating and contribute to a positive G_{IS}, significant in some cases because many seeds fall near mother

shrubs. In plants with large fruit crops, the birds remain in the tree or nearby for several minutes. As defecation rates for birds are rapid, many seeds are likely to be defecated near the maternal plant. Moreover, many *Psychotria* seeds are often deposited in the same fecal clump (Loiselle *et al.* 1995a). Such dispersal events would tend to restrict gene flow. However, some bird species, such as female manakins, could create more extensive gene flow by dispersing seeds over a long distance in the forest understory (Loiselle *et al.* 1995a). The net result would be a mixing of the seedling pool with some inclusion of localized family clusters (Loiselle *et al.* 1995b). This would avoid the formation of a substructure within the population, but with an excess of homozygote excess (Godoy & Jordano 2001).

Modeling studies have shown that family aggregates develop quickly in populations with a limited gene flow, such as among self-compatible insect-pollinated species or where most seeds fall beneath the parent plant (Ennos & Clegg 1982 *apud* Shapcott 1998). Rare, long-distance dispersal events have little effect on the development of this family structure (Shapcott 1998). The contrast in the results for congeners with similar mating systems demonstrates the need to incorporate ecological observations and to evaluate the genetic structure of a range of populations and species in order to gain more insight on the patterns of genetic variation in plant populations (Loiselle *et al.* 1995b). As shown here, there was genetic structure among the AE sample plots (G_{ST} 0.12). This difference may have occurred because of problems in gene flow that were probably related to seed dispersal since there was no difference in pollination among habitats (chapter 3).

The seed mass and germination of *P. tenuinervis* were similar to those of other *Psychotria* species. The germination of seeds from many *Psychotria* species occurs at a low rate and is highly delayed by about 3-5 months (Paz *et al.* 1999, Sassaki *et al.* 1999, Rosa

& Ferreira 2001). There was much heterogeneity in seed mass and in the rate and velocity of germination within each habitat so that possible differences among habitats could have been masked. In other words, other characteristics of the fragment, such as gaps, edge age, and type of matrix may have a greater influence on seed mass and germination than simply the distance from the edges. However, it seems that seed germination was apparently not influenced by genetic pattern of *P. tenuinervis*, because of the great variability in the seed mass, and in the rate and velocity of germination within habitats.

In conclusion, the isolation of *P. tenuinervis* populations by fragmentation did not appear to influence the species' genetic variability, although this species has a short life and generation (compared with trees). In addition, the level of genetic variation of this species was low to moderate (Wright 1978), and was comparable to that reported for other *Psychotria* species (Hamrick & Loveless 1989, Perez-Nasser *et al.* 1993, Loiselle *et al.* 1995a, 1995b), and for tropical trees in general (Hamrick & Loveless 1986, 1989, Hamrick & Godt 1989).

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Table 1: Enzymatic systems, their respective loci and the electrophoretic systems used for the genetic analyses of *Psychotria tenuinervis* seeds.

Enzymes	*ECN*	*Loci*	*Systems*
6-Phospho glucodehydrogenase	1.1.1.44	6Pgd-1	(a)
Acid phosphatase	3.1.3.2	Acph-1	(c)
Adenylate kinase	1.1.1.1	Adh-1	(a)
		Adh-2	(a)
		Adh-3	(a)
Alkaline phosphatase	3.1.3.1	Alp-1	(c)
Esterase	3.1.1.2	Est-1	(c)
		Est-2	(c)
Fumarase	4.2.1.2	Fum-1	(c)
Leucine aminopeptidase	3.4.11.1	Lap-1	(c)
Malate dehydrogenase	1.1.1.37	Mdh-1	(b)
Malic enzyme	1.1.1.40	Me-1	(b)
Phosphoglucose isomerase 6-phosphato	5.3.1.9	Pgi-1	(a)
		Pgi-2	(a)

(a) Electrode: 10 mM Litium hidroxide, 90 mM boric acid, 3 mM EDTA, pH 8.0. Gel: electrode solution diluted 1:10.
(b) Electrode: 0.25 M Tris and 0.057 M citric acid, pH 8.0. Gel: electrode solution diluted 1:25 (Ward and Warwick 1980)
(c) Electrode: 30 mM boric acid, 6 mM sodium hydroxide, pH 8.0. Gel: 1 mM Tris adjusted to pH 8.5 with 1 N HCl.

Table 2: Genetic variability, the multi (t_m) and single locus (t_s) outcrossing estimates and the inbreeding coefficient (f) of maternal parents for *P. tenuinervis* seeds among the five sample plots of an anthropogenic edge (AE), natural edge (NE) and forest interior (FI). NSh = number of shrubs sampled, NS = number of seeds sampled, H_e = mean expected heterozygosity, unbiased estimate (Nei 1978), H_o = mean observed heterozygosity, P (%) = mean percentage of polymorphic loci, A = mean number of alleles per locus. The standard error is shown in parentheses.

Habitat	NSh	NS	H_e	H_o	P (%)	A	t_m	t_s	f
AE	24	118	0.17 (0.15)	0.05 (0.05)	71	2.5 (1.2)	0.37 (0.08)	0.32 (0.09)	0.20 (0.20)
NE	29	117	0.13 (0.14)	0.03 (0.05)	50	2.5 (1.6)	0.39 (0.13)	0.34 (0.12)	0.20 (0.25)
FI	18	117	0.16 (0.15)	0.05 (0.05)	50	2.6 (1.4)	0.52 (0.11)	0.44 (0.08)	0.20 (0.00)
Overall	71	382	0.15 (0.13)	0.05 (0.05)	57	3.1 (1.6)	-	-	-

Table 3: Genetic variability of *P. tenuinervis* among the five sample plots of the anthropogenic edge (AE), natural edge (NE) and forest interior (FI). NSh = number of shrubs sampled, NS = number of seeds sampled, He = mean expected heterozygosity, unbiased estimate (Nei 1978), Ho = mean observed heterozygosity, P (%) = mean percentage of polymorphic loci, A = mean number of alleles per locus.

Habitat	NSh	NS	H_e	H_o	P (%)	A
AE						
1	3	24	0.16 (0.42)	0.03 (0.07)	38	1.50 (0.9)
2	3	22	0.19 (0.30)	0.11 (0.16)	57	1.80 (0.7)
3	7	24	0.07 (0.40)	0.01 (0.03)	31	1.40 (0.5)
4	4	24	0.11 (0.42)	0.05 (0.14)	31	1.40 (0.5)
5	7	24	0.16 (0.31)	0.03 (0.06)	50	1.60 (0.3)
NE						
1	5	24	0.02 (0.41)	0.00 (0.00)	08	1.08 (0.8)
2	8	24	0.14 (0.28)	0.03 (0.07)	57	1.64 (1.0)
3	7	23	0.08 (0.30)	0.02 (0.05)	21	1.21 (0.7)
4	3	24	0.14 (0.51)	0.03 (0.05)	42	1.67 (0.5)
5	6	22	0.11 (0.29)	0.07 (0.13)	43	1.79 (0.5)
FI						
1	2	24	0.07 (0.51)	0.04 (0.07)	33	1.42 (0.3)
2	2	22	0.08 (0.29)	0.03 (0.06)	36	1.50 (0.9)
3	6	24	0.09 (0.52)	0.05 (0.11)	25	1.33 (0.7)
4	3	24	0.16 (0.49)	0.08 (0.16)	50	1.75 (0.9)
5	5	24	0.24 (0.46)	0.06 (0.15)	75	1.83 (0.9)

Table 4: Seed mass (g), percentage and velocity (days) of seed germination (mean, lower and upper standard deviations) in each habitat (FI, NE and AE). There were no significant differences among the habitat. The data were back transformed from arcsine.

Habitat	Mass (g)	Germination (%)	Velocity (days)
AE	17.3 (±3.8)	21.7 (13.4 – 13.6)	82.6 (16.0 – 16.2)
NE	17.4 (±3.6)	25.1 (31.4 – 31.7)	79.4 (31.5 – 31.6)
FI	18.0 (±3.6)	9.7 (7.7 – 7.9)	142.9 (58.6 – 58.9)

Table 5: Seed mass (g, mean and standard deviation), and percentage and velocity (days) of seed germination (mean, lower and upper standard deviations) among the five sample plots in each habitat (FI, NE and AE). The data were back transformed from arcsine.

Habitat	Mass (g)	Germination (%)	Velocity (days)
AE			
1	16.4 (±3.7)	20.0 (28.1 - 28.3)	61.0 (30.1 -30.4)
2	16.8 (±3.4)	0.0 (0.0)	0.0 (0.0)
3	17.6 (±3.6)	0.0 (0.0)	0.0 (0.0)
4	18.4 (±3.3)	33.0 (40.6 - 41.6)	88.0 (69.1 - 69.5)
5	17.5 (±4.1)	33.0 (46.8 - 47.1)	99.0 (64.6 – 64.9)
NE			
1	16.8 (±3.8)	0.0 (0.0)	0.0 (0.0)
2	18.4 (±3.5)	4.0 (4.9 - 5.3)	59.0 (27.8 - 28.1)
3	17.0 (±3.0)	24.0 (31.6 - 31.9)	54.0 (75.1 - 75.4)
4	19.1 (±4.0)	86.0 (1.0 –1.3)	133.0 (95.6 - 95.8)
5	15.7 (±3.5)	12.0 (19.0 - 19.4)	72.0 (35.2 - 35.5)
FI			
1	17.5 (±3.8)	0.0 (0.0)	0.0 (0.0)
2	17.4 (±2.9)	13.0 (17.5 - 17.7)	178.0 (81.2 - 81.4)
3	18.0 (±4.1)	23.0 (35.1 - 35.5)	49.0 (57.1 - 57.6)
4	18.2 (±3.3)	3.0 (5.3 - 5.8)	205.0 (97.8 - 97.9)
5	19.0 (±3.8)	10.0 (9.7 – 10.0)	140.0 (59.1 - 59.4)

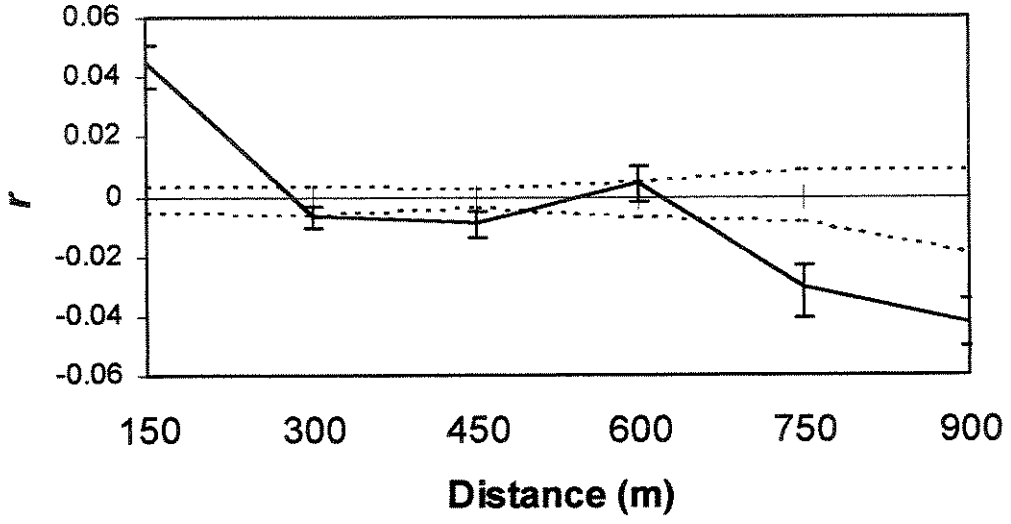

Figure 1: Coefficient (mean and standard error) of spatial analysis for multiallele and multilocus autocorrelation of *P. tenuinervis* for 5 classes of 150 m of distance (entire line). The dashed lines represent upper and lower 95% confidence intervals around the zero relationship.

CONSIDERAÇÕES FINAIS

O padrão geral da fenologia reprodutiva de *P. tenuinervis*, em uma escala regional, parece ser influenciado por fatores evolutivos, uma vez que houveram diferenças no clima (precipitação e temperatura) entre os cinco fragmentos estudados. Porém, o padrão fenológico de *P. tenuinervis* encontrado nos dois anos foi similar. Futuros estudos poderão verificar quais fatores evolutivos podem influenciar ou ter influenciado a fenologia desta espécie, talvez comparando a fenologia de populações de grupos de espécies com diferentes síndromes de polinização e dispersão, a fim de verificar se as espécies que possuem as mesmas síndromes apresentam padrões fenológicos similares entre si, mas diferente entre grupos.

Os resultados dos estudos de comparação entre ambientes dentro do fragmento mostraram que não houve diferenças: no microclima; padrões fenológicos; taxa de visitação floral, produção de frutos e sementes, e na taxa e velocidade de germinação entre bordas antrópicas, bordas naturais e o interior do fragmento. Provavelmente, esta similaridade entre os ambientes ocorreu devido a grande variação ou heterogeneidade entre as repetições ou as parcelas dentro deles, o que pode indicar que outros fatores estão agindo, ou possuem mais influência nas variáveis estudadas do que a distância entre bordas. Outros estudos já demonstraram a importância e a influencia direta e indireta de fatores, como clareiras (Piñero & Sarukhan 1982, Kursar & Coley 1992), idade das bordas (Restrepo *et al.* 1999), ou tipo de matrizes (Mesquita *et a*l. 1999), nos organismos remanescentes de bordas de fragmentos florestais. Portanto algum destes fatores ou uma combinação entre eles está possivelmente atuando nas características reprodutivas de *P. tenuinervis* no local de estudo, de modo a provocar a heterogeneidade das respostas reprodutivas obtidas no presente

estudo. A partir destes resultados fica evidente que é importante investigar a heterogeneidade dentro de habitats fragmentados, para a execução de programas de conservação melhores e mais específicos para estas paisagens, e que estes fatores devem ser levados em consideração ao se modelar e avaliar "bordas" e "interiores" em paisagens fragmentadas e também para o desenho de reservas florestais.

Talvez, se tivéssemos escolhido distâncias diferentes das utilizadas, sendo as parcelas de borda mais próximas ao limite da floresta e as de interior mais distantes da borda, os resultados fossem diferentes. Porém, vários trabalhos na literatura (Murcia 1995, Laurance 2000, Williams-Linera 2003), apresentam diferentes distâncias da borda e interior e mesmo assim muitos deles apresentaram uma grande heterogeneidade de resultados, enfraquecendo a questão de que os limites de efeito de borda nesse fragmento seriam menores do que os usados para definir uma parcela de borda (50 m) ou maiores do que os usados para definir uma parcela de interior (200 m).

É importante enfatizar que o isolamento da população estudada de *P. tenuinervis* pela fragmentação parece não ter tido influência na variabilidade genética desta espécie, uma vez que ela apresentou uma variabilidade de alta a moderada, parecida com a variabilidade de outras espécies de árvores tropicais (Hamrick & Loveless 1986, 1989, Hamrick *et al.* 1989), e de outras espécies do genero *Psychotria* (Hamrick & Loveless 1989, Perez-Nasser *et al.* 1993, Loiselle *et al.* 1995a, 1995b). Estudos em florestas com algumas espécies de árvores que possuem longo tempo de vida e de geração, normalmente não encontram diminuição ou outros problemas relacionados a variabilidade genética após à fragmentação dos habitats (Bierregaard Jr. 1992). Somente depois de muitas gerações e portanto muitos anos, ou décadas após a fragmentação é que será notado algum efeito. Já a similaridade na estrutura genética entre ambientes, encontrada neste estudo, pode indicar

que não há barreiras para o fluxo gênico dentro do fragmento, entre os ambientes estudados. Porém, o índice de endogamia indica que pode estar havendo uma curta distância de polinização, provocando a reprodução entre indivíduos aparentados, apesar de uma melhor distribuição genética dentro do fragmento, proporcionado pelos dispersores de sementes e impedindo a subestruturação da população. Seria interessante tentar investigar se esta hipótese está de fato ocorrendo, estudando as distâncias de dispersão de pólen e sementes desta espécies de planta, neste fragmento, e comparar com outra floresta que não seja fragmentada, ou um fragmento de tamanho bem superior a este.

Devemos prestar atenção nas bordas naturais, pois apesar delas não apresentarem diferenças em relação ao interior do fragmento no presente estudo, acredito que principalmente as florestas próximas a grandes corpos d'água, como lagos, lagoas e grandes rios possam apresentar diferenças com o interior da floresta, apesar da heterogeneidade que elas possam ter. Por isso, é importante tentar evitar ou manejar os possíveis efeitos prejudiciais aos organismos que a utilizam, como por exemplo formação de grandes clareiras, próximo a elas, e distúrbios antrópicos da vegetação na borda.

Outro fator de extrema importância para os próximos trabalhos e projetos com fragmentos é procurar se preocupar com suas imediações, fora da área florestada. Um dos objetivos e preocupações dos pesquisadores nesta área, além de conhecer o que está ocorrendo dentro do fragmento, tem que ser o de evitar que ele se degrade e fazer com que ele aumente de área e melhore a qualidade de sua borda. Devemos então trabalhar para melhorar a qualidade dessas bordas e da percolação da matriz, o que tamponaria os efeitos prejudiciais nos fragmentos e aumentaria o fluxo gênico entre eles, respectivamente. Uma possível direção seria pesquisar que tipos de culturas, tanto de pequenos quanto de grandes

proprietários de terra, estariam melhorando a qualidade da borda e da matriz e estimular este tipo de cultura junto a estes agricultores.

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