Reproductive ecology and genetic structure of understorey herbs in the Atlantic Rain Forest of Brazil

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[A system] also ought to consider the relationship to the pollinators, if it is at all to get at the essence of floral biology.

Vogel (1954)

“One must not be annoyed at having to spend a long time near a flowering plant, and at having often to repeat the same observations on any species of flower, for it is not always visited forthwith by the particular insect which is designed to fertilize it.”

Sprengel (1793) quoted in Knuth (1906)

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K. S

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Summary

Species interactions are a key component of biodiversity. Plant-animal mutualisms such as pollination link plant reproduction and ecosystem functioning, and maintain gene flow in plant populations.

Plant reproduction is particularly pollination-dependent in the tropics relative to the temperate zone due to the greater biodiversity in the tropics where up to 98 % of all flowering plant species are pollinated by animals. Hummingbird-pollination is most important in the understorey of neotropical forests and is common predominantly among non-woody plants. Understorey plants constitute an important component of plant diversity in neotropical forests, representing 20-50 % of the local diversity of vascular plants.

Since little is known about plant-animal-interactions of understorey plants in tropical rainforests, this thesis aimed to investigate the pollination ecology and reproductive biology of three herbaceous species showing an ornithophilous flowering syndrome: *Heliconia angusta* (Heliconiaceae), *Besleria melancholica* (Gesneriaceae) and *Stenostephanus lobeliiformis* (Acanthaceae). These species, the first two being endemic, are typical components of the understorey community of the Atlantic Rain forest of South-Eastern Brazil, a highly endangered biodiversity hotspot, which suffers from forest degredation and fragmentation.

I investigated the composition of floral visitors to the study species and observed their behaviour to determine potential pollinators and robbers, their activity patterns in the course of a day, their behaviour in differing weather conditions, as well as their response to floral density. Nectar samples were analysed since a particular nectar sugar composition has often been related to the pollinators of a plant. Many studies found that flowers often attract a broader spectrum of visitors than that predicted from their respective syndromes, and that generalist insects often visit more specialized plants. Flower visitor observations revealed similar results for *H. angusta* and *S. lobeliiformis*. Both plant species were visited mostly by Hymenoptera (in particular stingless bees, Meliponini), whereas hummingbirds only accounted for a minor part of all visits in both species. The flowers of *B. melancholica,* however, were predominantly visited by hummingbirds. I observed stingless bees of the genus *Trigona* visiting the flowers of all three study species, sometimes as nectar or pollen robbers as well as acting as legitimate flower visitors, and thus being potential pollinators. In *H. angusta,* time of day had a significant influence on the number of visitors of the different floral visitor groups, with most visitors appearing between 9:00 am and 1:00 pm; better light conditions promoted higher visitor frequencies.

The nectar of all study species was found to be sucrose-rich and diluted, whereas it was poor in the hexoses glucose and fructose. These nectar features fit well to the preferences of the observed floral visitors of the study species. In addition to the floral reward, flower attractiveness may have a positive influence on the abundance of the flower visitors. With an increasing number of flowers displayed, more visits were recorded to the flowers of *S. lobeliiformis* and *H. angusta*. In contrast, no correlation between floral display size and number of visitors was found for *B. melancholica.*

Female reproductive success also depends on the breeding system of a species, which I investigated via pollination experiments. *Stenostephanus lobeliiformis* is partially and *B. melancholica* is mostly self-incompatible. Breeding system studies in *H. angusta* mostly failed because plants were rotten or withered before fruit set. However, there is indication that *H. angusta* is partially self-compatible like the majority of *Heliconia* species. All three study species are primarily outcrossing and strongly depend on pollinators for reproduction. An increased fruit set after pollen supplementation treatments, compared with natural pollination in *S. lobeliiformis,* suggests pollen limitation for this mass-flowering species probably due to competition for pollination. While outcrossed and control seeds germinated well, no seeds of *S. lobeliiformis* resulting from selfing germinated at all, indicating inbreeding depression due to increased geitonogamy or biparental inbreeding in the dense stands of this plant.

In the next project, data on *B. melancholica* were combined with data on *Heliconia metallica* from Peru, provided by one of the co-authors of the respective publication. To investigate pollinator-mediated and abiotic effects (i.e. light availability) on plant reproduction, path analyses were used to disentangle direct and indirect effects of canopy openness and floral density on fruit set. Furthermore, the relationship between stigmatic pollen load and floral density was analysed for these two neotropical understorey plants. Our study revealed negative density-dependence in the reproduction of the two study species. In both species, fruit set was not directly related to canopy openness but decreased with increasing floral density. In *H. metallica*, canopy openness had an indirect negative effect on reproduction mediated by its effects on floral density. In *B. melancholica*, stigmatic pollen loads linearly decreased with increasing floral density, indicating competition for pollination at high densities. In *H. metallica*, stigmatic pollen loads were reduced at both low and high densities, indicating an interplay of facilitative and competitive effects of floral density on pollen deposition.

Finally this thesis explored the genetic consequences of anthropogenic habitat fragmentation in *H. angusta* using AFLP-markers. Clonal diversity of patches of plants was lower in forest fragments than in the continuous forest, albeit the differences were only marginally significant. Climate data revealed that in forest fragments temperatures were increased whereas relative humidity decreased in comparison to continuous forest. Such suboptimal conditions, which can result in environmental stress for plants, can enhance clonal propagation but might result in the loss of clonal diversity due to increased geitonogamy and inbreeding depression. Contrary to expectations, no evidence was found that genetic diversity decreased in patches of forest fragments. A PCoA as well as a STRUCTURE analysis did not show any clear clustering of patches in the continuous forest and forest fragments, and a Mantel-test did not detect a significant relation between genetic and geographical distance. The similar and moderate level of genetic diversity found within the patches of the continuous forest and forest fragments suggests that *H. angusta* has not suffered yet from the expected negative effect of fragmentation.

Given that *Heliconia* species are long-lived perennials, the expected effects of fragmentation on genetic diversity may take longer to express.

In conclusion, this thesis has shown that understorey plants of tropical rainforests, despite their associated floral syndrome, attract a broader spectrum of mutualistic and antagonistic visitors than might be expected. Furthermore, it is important to consider abiotic and biotic effects such as conspecific floral density when investigating floral visitors and plant reproduction. Plants at low densities presenting few flowers do not necessarily suffer from pollen limitation. In contrast, plants growing at high densities may attract more visitors, but at the same time receive fewer visits, and thus less pollen due to competition for pollination. Increased geitonogamy in dense stands may lead to inbreeding depression, which should be tested not only via germination studies, but also via progeny fitness traits at the stage of saplings. Field observations and experiments remain essential to investigate the pollination and reproductive ecology of plant species, thus contributing to the knowledge on complex plant-animal-interactions. This might particularly be important for endemic or vulnerable species in a threatened biodiversity hotspot like the Atlantic Coastal Rainforest of Brazil. In addition, molecular markers provide a fast and convenient tool to assess the condition of populations and are a powerful tool in conservation biology for understanding the consequences of fragmentation on the survival and performance of species.

Zusammenfassung

Art-Interaktionen stellen eine Schlüsselkomponente der Biodiversität dar. Mutualistische Tier-Pflanze-Interaktionen wie z.B. Bestäubung verbinden die Fortpflanzung von Pflanzen und Ökosystemfunktionen und erhalten den Genfluss zwischen Pflanzenpopulationen.

Im Vergleich zu temperaten Gebieten ist die sexuelle Reproduktion von Pflanzen besonders in den Tropen bestäuberabhängig; zum einen aufgrund der höheren Biodiversität der Tropen und zum anderen, da bis zu 98 % aller Angiospermen tierbestäubt sind. Bestäubung durch Kolibris (Trochilophilie als Unterform der Ornithophilie) ist besonders im Unterwuchs neotropischer Wälder von großer Bedeutung und verbreitet vor allem unter krautigen Pflanzen. Unterwuchspflanzen repräsentieren 20-50 % der lokalen Diversität vaskulärer Pflanzen und stellen somit eine wichtige Komponente der Pflanzendiversität neotropischer Wälder dar.

Bis dato ist das Wissen über Tier-Pflanze-Interaktionen von Unterwuchspflanzen in tropischen Regenwäldern begrenzt, weshalb das Ziel dieser Dissertation war, die Bestäubungsökologie und Reproduktionsbiologie dreier krautiger Pflanzenarten mit trochilophilem Blütensyndrom zu untersuchen: *Heliconia angusta* (Heliconiaceae), *Besleria melancholica* (Gesneriaceae) und *Stenostephanus lobeliiformis* (Acanthaceae). Diese Arten, davon die ersten beiden endemisch, sind typische Bestandteile der Unterwuchsvegetation des Atlantischen Küstenregenwaldes Süd-Ost Brasiliens, einem durch Walddegradierung und Fragmentierung hochgradig gefährdeten Biodiversitäts-hotspot.

Für diese ausgewählten Pflanzenarten untersuchte ich das jeweilige Artenspektrum der Blütenbesucher, ich determinierte anhand von Beobachtungen deren Verhalten als potentielle Bestäuber oder Räuber, deren Aktivitätsmuster im Tagesverlauf und unter verschiedenen Wetterbedingungen sowie deren Reaktion auf variierende Blütendichten. Weiterhin wurden Nektarproben analysiert, da bestimmte Zuckerzusammensetzungen von Nektar oft mit den Bestäubern einer Pflanzenart in Verbindung gebracht werden.

Wie von einer Vielzahl von Studien berichtet, ziehen Blüten oftmals ein viel breiteres Spektrum an Besuchern, als das allein vom Blütensyndrom vorhergesagte, an. Zudem besuchen generalistische Insekten oftmals spezialisierte Pflanzen.

Ähnliche Ergebnisse erzielten die Beobachtungen der Blütenbesucher von *H. angusta* und *S. lobeliiformis*: beide Arten wurden hauptsächlich von Hymenopteren (insbesondere Stachellose Bienen, Meliponini) besucht, wohingegen Kolibris nur einen kleineren Part der Gesamtbesuche ausmachten. Die Blüten von *B. melancholica* wurden vorwiegend von Kolibris besucht.

Stachellose Bienen der Gattung *Trigona* wurden als Blütenbesucher an allen drei untersuchten Pflanzenarten beobachtet; zum Teil als Nektar- oder Pollenräuber, aber ebenso als reguläre Blütenbesucher und somit potentielle Bestäuber.

Die Tageszeit hatte einen signifikanten Einfluss auf die Anzahl der Blütenbesucher der verschiedenen Besucher-Gilden von *Heliconia angusta*. Die meisten

Blütenbesucher wurden zwischen 9 und 13 Uhr gesichtet; bessere Lichtbedingungen resultierten in höheren Besuchsfrequenzen.

Der Nektar aller drei untersuchten Pflanzenarten ist reich an Sucrose und dünnflüssig, wohingegen die Hexosen Glucose und Fructose nur in geringen Mengen nachweisbar waren. Diese Nektar-Charakteristika passen gut zu den Präferenzen der beobachteten Blütenbesucher aller drei Pflanzenarten. Neben der Verköstigung durch die Blüten, kann auch die Blütenattraktivität einen positiven Einfluss auf die Abundanz der Blütenbesucher ausüben. Mit steigender Anzahl präsentierter Blüten wurden mehr Besuche an den Blüten von *S. lobeliiformis* und *H. angusta* verzeichnet. Im Gegensatz dazu konnte keine Korrelation zwischen der Blüten- und Besucheranzahl bei *B. melancholica* festgestellt werden.

Der weibliche Reproduktionserfolg hängt in keinem geringeren Maße auch vom Fortpflanzungssystem einer Art ab, welches ich mittels Bestäubungs-Experimenten untersuchte. *Stenostephanus lobeliiformis* ist teilweise, *B. melancholica* vorwiegend selbst-inkompatibel. Untersuchungen zum Fortpflanzungssystem von *H. angusta* sind zum Großteil gescheitert, da die Pflanzen bereits vor dem Fruchtansatz vertrocknet beziehungsweise verfault waren. Nichtsdestotrotz gibt es Hinweise, dass *H. angusta*, wie auch der Großteil der *Heliconia*-Arten, partiell selbst-kompatibel ist. Alle drei untersuchten Arten sind vorwiegend auskreuzend und sind stark auf Bestäuber für die sexuelle Reproduktion angewiesen. Ein erhöhter Fruchtansatz bei *S. lobeliiformis* nach Pollenzufuhr mittels Handbestäubung, verglichen mit natürlicher Bestäubung, weist auf Pollenlimitierung hin. Dies ist wahrscheinlich auf innerartliche Konkurrenz um Bestäubung dieser zeitgleich in großer Dichte blühenden Art zurück zu führen.

Im nächsten Projekt wurden Daten von *B. melancholica* kombiniert mit denen von *Heliconia metallica* aus Peru, welche von einem der Ko-Autoren der entsprechenden Publikation zur Verfügung gestellt wurden. Um bestäubervermittelte und abiotische Effekte (z.B. Lichtverfügbarkeit) auf die Pflanzenreproduktion zu untersuchen, wurden Pfadanalysen angewendet, um direkte und indirekte Effekte der Kronenöffnung und innerartlicher Blütendichte auf den Fruchtansatz zu ermitteln. Weiterhin wurde der Zusammenhang zwischen der Anzahl an Pollen auf den Stigmata und der Blütendichte bei diesen zwei neotropischen Unterwuchsarten analysiert. Die Studie kommt zu dem Ergebnis, dass die Reproduktion der zwei untersuchten Pflanzenarten negativ von der Blütendichte beeinflusst wird. Bei beiden Arten war der Fruchtansatz nicht direkt mit der Kronenöffnung in Verbindung zu bringen, nahm jedoch mit zunehmender Blütendichte ab. Bei *H. metallica* hatte die Kronenöffnung, aufgrund des Effektes auf die Blütendichte, einen indirekten negativen Effekt auf die Reproduktion. Bei *B. melancholica* nahm die Anzahl an Pollen auf den Stigmata linear mit zunehmender Blütendichte ab, was auf Konkurrenz um Bestäubung bei hohen Blütendichten hinweist. Bei *H. metallica* war die Anzahl an Pollen auf den Stigmata sowohl bei geringen als auch bei hohen Blütendichten reduziert, was ein Zusammenspiel fördernder und competitiver Effekte der Blütendichte auf die Pollenzufuhr indiziert.

Abschließend wurden im Rahmen dieser Dissertation die genetischen Konsequenzen anthropogener Habitatfragmentierung bei *H. angusta* mittels AFLP-Markern

untersucht. Die klonale Diversität der Pflanzengruppierungen war in Waldfragmenten geringer als im zusammenhängenden Waldgebiet, wobei dieser Unterschied aber nur marginal signifikant war. Die Auswertung von Klimadaten ergab, dass in den Waldfragmenten, im Vergleich zum zusammenhängenden Wald, höhere Durchschnittstemperaturen aber eine geringere Luftfeuchtigkeit herrschen. Solch suboptimale Umweltbedingungen, die für Pflanzen Stress darzustellen vermögen, können zu gesteigertem klonalen Wachstum führen, aber können zugleich aufgrund erhöhter Geitonogamie und Inzucht-Depression in einem Verlust klonaler Diversität resultieren. Im Gegensatz zu den Erwartungen konnte nicht nachgewiesen werden, dass die genetische Diversität in Waldfragmenten verringert ist. Eine PCoA sowie eine STRUCTURE-Analyse zeigen keine klare Strukturierung der Pflanzengruppierungen im zusammenhängenden Wald und in den Waldfragmenten; ein Mantel-Test detektierte keinen signifikanten Zusammenhang zwischen genetischer und geographischer Distanz. Das ähnliche und moderate Maß der genetischen Diversität innerhalb der Pflanzengruppierungen im zusammenhängenden Wald und den Waldfragmenten weist darauf hin, dass *H. angusta* noch nicht unter den negativen Effekten der Fragmentierung leidet. Da Heliconien langlebende perenne Arten sind, könnte es längere Zeiträume in Anspruch nehmen, bis sich die erwarteten Effekte der Fragmentierung auf die genetische Diversität auswirken.

Zusammenfassend hat diese Dissertation gezeigt, dass Unterwuchspflanzen tropischer Regenwälder, trotz ihres assoziierten Blütensyndroms, ein breiteres Spektrum an mutualistischen und antagonistischen Blütenbesuchern anziehen, als erwartet werden könnte.

Von großer Bedeutung ist es, dass abiotische und biotische Effekte wie z.B. innerartliche Blütendichte berücksichtigt werden, wenn Blütenbesucher und die Reproduktion von Pflanzen untersucht werden sollen. Pflanzen, die in geringen Dichten wachsen und wenige Blüten präsentieren, müssen nicht notwendigerweise unter Pollenlimitierung leiden. Im Gegensatz dazu ziehen Pflanzen in dichten Beständen potentiell mehr Besucher an, aber erhalten zugleich weniger Besuche und damit weniger Pollen aufgrund von Konkurrenz um Bestäubung. Gesteigerte Geitonogamie in dichten Beständen kann zu Inzucht-Depression führen, was nicht nur mittels Keimungsversuchen, sondern auch mittels Fitnessmerkmalen der Nachkommen auf der Ebene von Keimlingen und Jungpflanzen getestet werden sollte. Feldobservationen und -Experimente bleiben essentiell, um die Bestäubungs- und Reproduktionsökologie von Pflanzenarten zu untersuchen, und um damit zum Wissen über die komplexen Tier-Pflanze-Interaktionen beizutragen. Das mag besonders wichtig sein für endemische oder gefährdete Arten in einem bedrohten Biodiversitäts-Hotspot wie der Atlantische Küstenregenwald Brasiliens.

Außerdem stellen molekulare Marker eine schnelle und zweckdienliche Methodik dar, um den Zustand von Pflanzenpopulationen zu bewerten und sind zudem von großer Bedeutung für den Naturschutz, um die Konsequenzen der Fragmentierung für das Überleben und die Performanz der Arten besser zu verstehen.

Resumo

As interações de espécies representam um componente chave da biodiversidade. As interações mutualistas entre animais e plantas, como p. ex. a polinização, relacionam a reprodução sexuada e o funcionamento do ecossistema e mantêm o fluxo gênico entre populações vegetais.

Em comparação com zonas temperadas, a reprodução vegetal nos trópicos depende particularmente de polinizadores. Por um lado, devido à maior biodiversidade nos trópicos e, por outro lado, devido ao fato de até 98 % de todas as angiospermas serem polinizadas por animais. A polinização por beija-flores é importante sobretudo no sub-bosque de florestas neotrópicas e é mais comum principalmente entre plantas herbáceas. As plantas de sub-bosque representam 20 % a 50 % da diversidade local de plantas vasculares e são, assim, um componente importante da diversidade vegetal em florestas neotrópicas.

Até à data, o conhecimento sobre as interações entre plantas e animais de plantas de sub-bosque em florestas trópicas é limitado. Por isso, esta tese teve como objetivo a investigação da ecologia de polonização e da biologia reprodutiva de três espécies herbáceas com síndrome floral da ornitofilia: *Heliconia angusta* (Heliconiaceae), *Besleria melancholica* (Gesneriaceae) e *Stenostephanus lobeliiformis* (Acanthaceae). Estas espécies, das quais as duas primeiras são endêmicas, são componentes típicas da vegetação de sub-bosque da Mata Atlântica no Sudeste do Brasil, um *hotspot* de biodiversidade em elevado perigo devido à degradação e à fragmentação florestal.

Pesquisei a gama de visitantes florais nas espécies escolhidas. Através de observações determinei o comportamento destas espécies como potenciais polinizadores ou predadores, os seus padrões de atividade ao longo do dia e com diferentes condições climáticas, bem como a sua reação a diferentes densidades florais. Além disso, foram analisadas amostras de néctar, uma vez que determinadas composições de açúcar no néctar são freqüentemente relacionadas aos polinizadores de uma espécie vegetal.

Segundo um grande número de investigações, as flores atraem freqüentemente uma gama de visitantes bastante mais vasta do que o previsto apenas pela síndrome floral. Para além disso, os insetos generalistas visitam com freqüência plantas especializadas.

A observação dos visitantes florais da *H. angusta* e da *S. lobeliiformis* apresentou resultados semelhantes: ambas as espécies foram visitadas principalmente por hymenoptera (particularmente por abelhas sem ferrão, Meliponini) e os beija-flores constituíram apenas uma pequena parte do total de visitantes florais. As flores da *B. melancholica* foram visitadas principalmente por beija-flores.

As abelhas sem ferrão do gênero *Trigona* foram observadas como visitantes florais nas três espécies investigadas, em parte como predadores de néctar ou pólen, mas também como visitantes florais regulares e, portanto, como potenciais polinizadores.

A hora do dia tinha uma influência significativa no número de visitantes florais das diferentes guildas visitantes da *Heliconia angusta*. A maioria dos visitantes florais foram vistos entre as 09h00 e as 13h00. Melhores condições de luminosidade resultaram numa freqüência de visita mais elevada.

O néctar das três espécies investigadas é fluido e rico em sacarose. No entanto, as hexoses glucose e frutose foram detetáveis somente em pequenas quantidades. Estas caraterísticas do néctar adaptam-se às preferências dos visitantes florais observados das três espécies de plantas. Para além do alimento, a atratividade das flores pode ter também alguma influência positiva na abundância dos visitantes florais. Com o aumento do número de flores disponíveis, foram registradas mais visitas às flores da *S. lobeliiformis* e da *H. angusta*. Pelo contrário, não foi registrada uma correlação entre o número de flores e de visitantes da *B. melancholica*.

O sucesso reprodutivo da fêmea depende, na mesma dimensão, também do sistema reprodutivo de uma espécie, o que investiguei através de experiências de polinização. A *Stenostephanus lobeliiformis*, em parte, e a *B. melancholica*, habitualmente, são auto-incompatíveis. As experiências relativas ao sistema reprodutivo da *H. angusta* não resultaram, em grande parte, porque as plantas murcharam ou apodreceram antes da entrada em frutificação. Apesar disso, existem indicações de que a *H. angusta*, assim como a maioria das espécies da *Heliconia*, é parcialmente auto-compatível. As três espécies pesquisadas são principalmente cruzadas e dependem bastante do polinizador para a reprodução sexuada. O aumento da frutificação na *S. lobeliiformis* após receber o pólen por polinização manual, comparativamente com a polinização natural, sugere uma limitação de pólen. Este fato deve-se provavelmente à competição intra-específica pelos serviços de polinização desta espécie simultaneamente ao florescimento de alta densidade.

No projeto seguinte os dados da *B. melancholica* foram conjugados com os da *Heliconia metallica* do Peru, os quais foram disponibilizados por um dos co-autores da publicação respectiva. Foram utilizadas análises de caminhos para investigar os efeitos abióticos (p. ex. a disponibilidade de luz) e os efeitos relacionados com o polinizador na reprodução de plantas e para os averiguar efeitos diretos e indiretos da antese e da densidade floral intra-específica na frutificação. Além disso, foi investigada a conexão entre o número de pólen nos estigmas e a densidade floral destas duas espécies neotrópicas de sub-bosque. Verificando-se que a reprodução das duas espécies investigadas é afetada negativamente pela densidade floral. Não foi possível relacionar diretamente, no caso de nenhuma das duas espécies, a frutificação com a abertura da flor, mas diminuiu com a densidade crescente de flores. No caso da *H. metallica*, a abertura da flor teve um efeito negativo indireto na reprodução devido à densidade floral. A quantidade de pólen nos estigmas da *B. melancholica* diminuiu proporcionalmente ao aumento da densidade floral, indicando competição pela polinização em densidades florais elevadas. A *H. metallica* apresentou uma quantidade reduzida de pólen nos estigmas, em densidades florais baixas e altas, indicando uma interação de efeitos facilitadores e competitivos da densidade floral na recepção de pólen.

Por fim, foram pesquisadas no âmbito desta tese as conseqüências genéticas da fragmentação antrópica do habitat na *H. angusta* mediante marcadores AFLP. A diversidade clonal dos agrupamentos de plantas em fragmentos florestais foi tendencialmente menor do que na área florestal contínua. A interpretação de dados climáticos revelou que os fragmentos florestais, em comparação com a floresta contínua, têm uma temperatura média mais elevada, mas uma umidade inferior. Estas condições ambientais não ótimas, potencialmente causadoras de estresse para as plantas, podem levar a um crescimento clonal superior, mas causar também uma diminuição da diversidade clonal devido à geitonogamia elevada e à diminuição de consangüinidade. Contrariamente às expectativas, não foi possível provar a existência de uma diversidade genética reduzida em fragmentos florestais. A análise das Coordenadas Principais (inglês: PCoA), bem como a análise de STRUCTURE não revelaram uma estruturação clara dos agrupamentos vegetais na floresta contínua nem nos fragmentos florestais. Um teste de Mantel não detectou uma relação significativa entre a distância genética e geográfica. O nível semelhante e moderado de diversidade genética encontrado nos agrupamentos de plantas da floresta contínua e dos fragmentos florestais indica que a *H. angusta* ainda não é afetada pelos efeitos negativos da fragmentação. Como as *Heliconias* são espécies perenes e com um longo período de vida, os efeitos expectáveis da fragmentação na diversidade genética poderão demorar mais tempo a manifestar-se.

Resumindo, esta tese mostrou que as plantas de sub-bosque das florestas trópicas, apesar da sua síndrome floral associada, atraem uma gama mais vasta de visitantes florais mutualistas e antagonistas do que seria provável.

É bastante importante ter em conta os efeitos abióticos e bióticos, como p. ex. a densidade floral da mesma espécie, durante a investigação de visitantes florais e da reprodução de plantas. As plantas que crescem com densidades baixas e com poucas flores não experienciam necessariamente uma limitação de pólen. Pelo contrário, as plantas que crescem com densidades altas atraem potencialmente mais visitantes, mas recebem menos visitas e, conseqüentemente, menos pólen, devido à competição pela polinização. A geitonogamia intensificada em densidades altas pode causar a diminuição da consangüinidade. Este fato deveria ser investigado não só através de testes de germinações, mas também mediante as características de aptidão dos descendentes ao nível das plântulas e plantas jovens. As observações e experiências de campo continuam a ser essenciais na investigação da ecologia de polinização e reprodutiva de espécies vegetais, contribuindo assim para o conhecimento das complexas interações animais-plantas. Isto pode ser particularmente importante no caso de espécies endêmicas ou em perigo dentro de um *hotspot* de biodiversidade em perigo, como a Mata Atlântica no Brasil.

Além disso, os marcadores moleculares representam um método rápido e eficiente para avaliar o estado de populações de plantas e, adicionalmente, são bastante importantes na biologia da conservação, para uma melhor compreensão das conseqüências da fragmentação na sobrevivência e desempenho das espécies.

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General introduction

Species interactions are a key component of biodiversity. Without the diversity of antagonistic, mutualistic, comensalistic, and amensalistic interactions, ecosystems as we know them would simply cease to function (Ollerton et al. 2006). One obvious form of these interactions is that between flowering plants and their pollinators. Recent estimates assume that 90 % or more of angiosperm species rely in whole or partly on animals for pollination and sexual reproduction, rather than on abiotic agents such as wind or water (e.g. Nabhan & Buchmann 1997, Renner 1998, Ollerton et al. 2011).

Truly mutualistic pollination interactions are beneficial to plants and animals, but this does not imply cooperation (Waser & Price 1983, Westerkamp 1997). Mutualism also directly benefits humanity through crop productivity, and indirectly, through ecosystem health. Hence, pollination is an important (and gratis) ecosystem service (Costanza et al. 1997). As is true for other ecosystem services, pollination by animals is not replaceable to any appreciable degree by technology. Thus, it is of great concern that this mutualism is under threat e.g. from habitat alteration, degradation, and fragmentation (Kearns et al. 1998, Steffan-Dewenter et al. 2001). There is enough cause for alarm that conservation biologists – not only “pure ecologists”- seek a deeper understanding of plant-pollinator interactions.

What affects sexual reproduction – an insight

Sexual reproduction is an important stage of a plant´s life cycle and has major implications for the dynamics of plant populations (e.g. Bruna 2003, Liston et al. 2003). Sexual reproduction enables a population to adapt to a changing environment by producing recombinants (Crow 1994) and it promotes gene flow between existing populations via pollen and seeds (Ghazoul 2005), increasing the genetic diversity of populations (Heinrich & Raven 1972, Grüttner & Heinze 2003).

Pollination plays a major role in sexual reproduction of plants (Baker 1983), and is able to cause the loss of species if affected (Wunderlee 1997). Tropical flowering plants depend overwhelmingly on animals as vectors of pollen transfer (Kay & Schemske 2003). Most pollinators are drawn from the insect orders Hymenoptera, Diptera, Lepidoptera, and Coleoptera and from vertebrates - in particular birds and bats (Proctor et al. 1996, Machado & Oliveira 2000). Hummingbird-pollination is common among non-woody plants in neotropical forests because it is closely associated with four mostly herbaceous and widespread plant families, e.g. Bromeliaceae, Gesneriaceae, Passifloraceae, and Heliconiaceae (Proctor et al. 1996). Hummingbird pollination of understorey plants including Acanthaceae, Marantaceae, Musaceae, Rubiaceae and Zingiberaceae (Bawa 1990) supports the finding of a scarcity of hummingbird pollination in the canopy (Schatz 1990).

Understorey plants constitute an important component of plant diversity in neotropical forests (Gentry 1990), representing 20-50 % of the local diversity of vascular plants (Andreata et al. 1997, Duque et al. 2002). However, little is known about the ecology, in particular the pollination and reproductive biology, of understorey plants in tropical rainforests (Horvitz & Schemske 1988, Freiberg & Gottsberger 2001, Bruna et al. 2004, Drucker et al. 2008). Thus, developing basic knowledge about the reproductive ecology of important biodiversity components of these forests, such as understorey species, might contribute to a better understanding of ecosystem functions and services and can lead to the development and implementation of conservation approaches.

Angiosperm species have flowers with many morphologic and functional features, which can be associated with pollination syndromes (Faegri & van der Pijl 1979, Borges 2000). These syndromes are composed of a set of attributes (e.g. colour, shape, reward, anthesis), which are common or specific to plant species adapted to a certain type of pollinator (Waser et al. 1996, Bosch et al. 1997, Leonard et al. 2011). Pollination syndromes take into account these set of floral characters, which determines the likeliest group of pollinators on certain species (Bawa et al. 1985). As an example, ornithophilous flowers tend to have traits that facilitate bird pollination (Smith et al. 1996), such as long, narrow, tubular corollas, often vivid coloration and diluted nectar (Willmott & Burquez 1996).

The pollination syndrome concept, however, has recently been criticized as researchers have found that flowers often attract a broader spectrum of visitors than that assumed by their respective syndromes (Fenster et al. 2004, Dias da Cruz 2006, Ollerton et al. 2009, Schmid et al. 2011) and that many plants have more than one type of pollinator (Waser et al. 1996, Waser & Ollerton 2006). Ollerton et al. (2003) reported that generalist insects often visit more specialized plants - a situation that was also found for bird-adapted mistletoe flowers being visited by bees (Robertson et al. 2005). However, the most abundant visitor does not necessarily act as the most efficient pollinator (Fenster et al. 2004, Schmid et al. 2011). In addition to representing secondary or alternative pollinators for more specialized plants (Canela & Sazima 2005, Schmid et al. 2011), other studies have shown that more generalist insects may frequently be regarded as pollen and nectar robbers - in particular to flowers of many long-tubed, nectar rich, hummingbird pollinated species (McDade & Kinsman 1980, Renner 1983).

The principle reward for most flower visitors is nectar (Proctor et al. 1996), and the particular nectar sugar composition has often been related to the pollinators of a plant. Flowers pollinated by hummingbirds, butterflies or long-tongued bees often secrete sucrose-rich nectar (Baker & Baker 1990, Perret et al. 2001, Krömer et al. 2008).

In addition, weather and light conditions influence the flower visitors´ behaviour. Studies by Renner (1983), Stone and Jenkins (2008), Vicens and Bosch (2000) and Döll et al. (2007) revealed impacts of temperature, solar radiation and time of day on the number of floral visitors and their frequency.

Moreover, female reproductive success is also conditioned by the breeding system of the species (Byers & Meagher 1992, DeMauro 1993), affecting the structure of genetic diversity (e.g. Hamrick & Godt 1990, Les et al. 1991) and the fitness of individuals (Kittelson & Maron 2000). For self-incompatible plants, outcross pollen is the only source of fertilization of ovules (Feinsinger et al. 1991), whereas self-compatible plants can achieve sexual reproduction in the absence of other local pollen sources by geitonogamy, and for self-pollinating plants, even in the absence of pollinators (Ghazoul 2005). However, geitonogamous selfing can incur a number of fitness costs in both self-compatible and self-incompatible species (e.g. Harder & Barrett 1995, Harder & Barrett 1996, Snow et al 1996). If selfed offspring are less fit than those resulting from outcrossing, then geitonogamy will result in inbreeding depression (Husband & Schemske 1996). Deposition of self-pollen on stigmas may interfere with outcross pollen deposition, germination, growth, and fertilization, usurping ovules that might otherwise be outcrossed (e.g. Klinkhamer & de Jong 1993, Harder et al. 2001). Finally, self-pollen deposited on stigmas is unavailable for male reproductive success through outcrossing, resulting in pollen discounting (e.g. Lloyd 1992, Harder & Barrett 1996).

Furthermore floral density may have an effect on reproduction (Kunin 1993, Ågren 1996). Understorey plants in tropical rain forests are often patchily distributed, with densities ranging from dense aggregations of reproductive conspecifics to spatially isolated individuals (Kay & Schemske 2003, Bruna et al. 2004, Schleuning et al. 2008). One reason for this patchiness is that in many species, inflorescences are formed only in canopy gaps (Stiles 1979, Horvitz & Schemske 1995, Bruna 2003) because an increase in light availability is beneficial for growth and reproduction of many understorey species (Souza & Martins 2004, Bruna & Ribeiro 2005, Schleuning et al. 2008). Many tropical understorey plants are clonal, in particular herbs (Villegas 2001) and palms (Souza & Martins 2006), and reproduce sexually only in small canopy gaps (Schleuning et al. 2008).

Plants growing in dense floral neighbourhoods of conspecifics or producing large numbers of new flowers each day are often more attractive for pollinators. Augmented attractiveness likely attracts numerous and diverse flower visitors (Gentry 1974b, Franckie et al. 1976), leading to an increased pollen deposition and fruit set (Kunin 1997, Roll et al. 1997, Burd 1994). However, at high densities plants/flowers may also compete for resources and pollination services, leading to reduced visitation rates per flower, reduced pollen deposition and a decreased fruit set (Weiner 1982; Rathcke 1983; Ghazoul 2005). Thus, the positive effect of higher attractiveness in dense stands might be diminished (Corbet 1998, Mustajärvi et al. 2001).

Isolated plants or plants that produce only small numbers of new flowers usually attract few or low quality pollinators (Jennersten & Nilsson 1993, Kunin 1997) possibly resulting in pollen limitation and compromising fruit and seed production (Burd 1994, Larson & Barrett 2000, Ashman et al. 2004). For example, the most recent survey considered 482 studies of fruit production and concluded that 63 % of species exhibit pollen limitation at some sites, or during some years (Knight et al. 2005). Thus, pollen limitation may commonly hamper the ability of plants to realize their reproductive capacity.

Pollen limitation may not only result from insufficient pollen transfer (quantity limitation), but also from deposition of low-quality pollen (quality limitation), such as genetically related, incompatible pollen (Aizen & Harder 2007). However, pollen quality effects associated with both self-fertilization and mating between related plants can also reduce seed production, which is a sign of inbreeding depression (Aizen & Harder 2007). Given that predominantly outcrossing species commonly exhibit strong inbreeding depression (Husband & Schemske 1996), self-pollination and/or biparental inbreeding has frequently a negative impact even on early fitness traits such as seed production and germination (Lienert & Fischer 2004).

Genetic consequences of anthropogenic habitat fragmentation

Genetic diversity has been shown to positively influence individual fitness and reproduction (Reed & Frankham 2003, Leimu et al. 2006). Hence, the protection of genetic diversity has high priority for conservation (Bowman 1996, Frankham et al. 2009).

However, the ongoing fragmentation of most tropical forests in the world constitutes one of the major threats to the conservation of biodiversity (Chazdon et al. 2009). For many species, the isolation and reduction in habitat size after fragmentation disrupts several ecological and genetic processes that occur at the population level (Aguilar et al. 2006, Aguirre & Dirzo 2008). Many studies report that habitat fragmentation often disrupts mutualistic plant-animal interactions such as those between plants and their pollinators (e.g. Ghazoul 2005, Kolb 2008, Kiers et al. 2010), inter alia leading to reductions in pollinator abundance, species richness, limited pollinator movement among patches (e.g. Steffan-Dewenter & Tscharntke 1999, Lennartsson 2002), and thus further reduced fitness in small populations (e.g. Ågren 1996).

Hence, fragmented populations are usually exposed to the negative effects of reduced gene flow (Fischer & Lindenmayer 2007), increased inbreeding, genetic drift, and population differentiation (Lynch et al. 1995, Young et al. 1996). These effects result in the collective loss of genetic variation, reducing the probability of population adaptation to new environmental conditions, and increasing the risk of extinction (Ellstrand & Elam 1993, Pertoldi et al. 2007).

Furthermore, suboptimal site conditions, low probabilities of sexual reproduction, and seedling recruitment might result in disequilibrium between sexual and clonal (vegetative) reproduction in clonally growing plant species leading to a shift towards an enhanced clonal reproduction (Eckert 2002). Many studies report that forest fragments commonly exhibit e.g. a lower relative humidity and increased air temperature due to the edge effect (Laurance et al. 2002). The potential advantages of clonal growth include facilitation of resource uptake in heterogeneous environments (Hutchings & Wijesinghe 1997), persistence under suboptimal environmental conditions (Erikson & Ehrlen 2001), and increased attraction of pollinators by increased floral display size (Harder & Barrett 1996). Nevertheless, clonal growth incurs fitness costs by increased selfing and inbreeding depression in self-compatible species, and reduced mate availability in self-incompatible species (Eckert 2000, Honnay & Jacquemyn 2008). As a consequence, clonal diversity decreases (Watkinson & Powell 1993).

Despite the unfortunate confluence in the tropics of high biodiversity and the highest rate of species loss by habitat destruction, most studies assessing the genetic consequences of fragmentation focus on temperate rather than tropical species (Lowe et al. 2005, but see Kramer et al. 2007). Aguilar et al. (2008) report in their meta-analysis, that only 20 out of 102 studied plant species are tropical species and out of these only three species are herbaceous. Hence, there is a lack of knowledge on the genetic consequences of forest fragmentation on tropical herbaceous species.

Study objectives

The aim of this thesis was to investigate the pollination ecology and reproductive biology of neotropical understorey plants as well as to evaluate the influence of habitat fragmentation on the genetic diversity and structure of plant populations.

I chose three neotropical herbaceous understorey plant species as study objects, since they are a typical component of the understorey and represent important plant families of tropical forests. Two out of the three species are endemic and one species is considered as vulnerable in the Atlantic Rainforest of Brazil (see chapter ‘study species’ below). Hence, researching and developing knowledge about their reproductive ecology in particular in the face of ongoing habitat destruction is crucial to maintain vigorous populations and overall biodiversity, as well as to better understand the complex plant-animal interactions in tropical forests.

I observed the diversity and abundance of floral visitors of the three plant species to investigate the whole visitor spectrum of the flowers despite their respective pollination syndrome. In addition, nectar samples were analysed in order to test if the nectar composition is related to the preferences of the observed flower visitors (Publications 1 and 2). Furthermore, I investigated the behaviour of the floral visitors to determine if they are potential pollinators or robbers, if the flower visitation rate is influenced by the time of day, weather and/or light conditions (Publication 1), and to explore the influence of the floral display size on visitor number and frequency (Publications 1 and 2).

Furthermore, I investigated the breeding system and female reproductive success via pollination experiments to indicate whether the species are self-compatible (or not) and pollen-limited, (or not). Germination experiments were carried out as an initial look at inbreeding depression (Publication 2).

Since I wanted to gain a better understanding of abiotic and pollinator-mediated effects on plant reproduction, path analyses were used to disentangle if light availability in the understorey has direct abiotic or indirect biotic effects on the plants´ reproductive success. In addition, I investigated if conspecific floral density has negative, neutral or positive effects on pollen load and fruit set of the study species or if there is a trade-off between facilitative and competitive effects of conspecific density on plant reproductive success (Publication 3).

Finally, I focused on the anthropogenic habitat fragmentation and its influence on the genetic diversity and structure of patches of plants of one of the study species (Publication 4). I wanted to investigate whether the genetic diversity in forest fragments is decreased and if plants in forest fragments are genetically differentiated from conspecifics in the continuous forest. The clonal diversity, genetic diversity, and structure of patches of the species in the continuous forest and five forest fragments were assessed using AFLP-markers. Furthermore, climate data from the continuous forest were compared with those from forest fragments, since in forest fragments climatic conditions often are worse due to edge-effects, e.g. temperatures often increases whereas air humidity decreases.

Study region

The Atlantic Rainforest – status of a hotspot

The Brazilian Atlantic Rainforest (“Mata Atlântica”) is one of the biodiversity hotspots for conservation priorities in the world (Myers et al. 2000). Despite the fact that the original vegetation cover has been reduced at least by 70 %, more than 60 % of all terrestrial species live in this region in an area constituting less than 2 % of earth surface (Galindo-Leal & Gusmão Câmara 2003).

The dense ombrophilous forests along the coast have vigorous and diverse vegetation, because of the constant humidity from the ocean. Here the precipitation can reach 4000 mm/year, the highest level in Brazil. This forest can be classified into three formations: coastal plain-, abrupt montane-, and high altitude forest (Veloso et al. 1991). In general, the floristic diversity is greatest in these formations of the biome: ca. 20.000 plant species exist here of which 40 % are endemic (Galindo-Leal & Gusmão Câmara 2003). The Atlantic Forest region is probably the highest in species diversity and degree of endemism in South America (Silva & Casteleti 2003, Tabarelli et al. 2005) and was once one of the largest rainforests of the subcontinent. Its remnants cover nowadays, depending on the definition of “Atlantic Forest” and the spatial methods employed, about 7-16 % of its original extent (Galindo-Leal & Câmara 2003, Tabarelli et al. 2005, Ribeiro et al. 2009). According to Ribeiro et al. (2009), the remnants of the Mata Atlântica are currently distributed in more than 245.000 forest fragments of which 83.4 % are smaller than 50 ha and only 0.03 % are larger than 10.000 ha. In addition to their degree of fragmentation, these remaining forests are frequently to be found in varying stages of degeneration caused by anthropogenic disturbance. Forest types with different disturbance histories are very heterogeneous habitats, continuously changing in structure and varying in species composition (e.g. Williams-Linera et al. 1998, Laurance et al. 2002).

Study area

The study was conducted in the Atlantic Rainforest (“Mata Atlântica”) of the state of Rio de Janeiro, Brazil in the private reserve “Reserva Ecológica de Guapiaçu” (REGUA – 22°25’53”S, 42°45’20”W) in the municipality of Cachoeiras de Macacu (Fig. 1). The 5500 ha reserve is located on the south-facing slopes of the Serra dos Órgãos Mountain range, about 100 km from the city of Rio de Janeiro. The mean annual temperature for this region is about 23 °C with a mean annual rainfall of about 2560 mm. There is a hot and rainy season from October to March and a cooler and drier season from April to September (Kurtz & de Araújo 2000). The vegetation can be classified as “evergreen dense ombrophilous forest” (Veloso et al. 1991), which is typical for the lower and medium elevations of the coastal mountain range (Morellato & Haddad 2000, Oliveira-Filho & Fontes 2000). The study area is characterized by continuous forest and forest fragments of different sizes in a matrix of agricultural land.

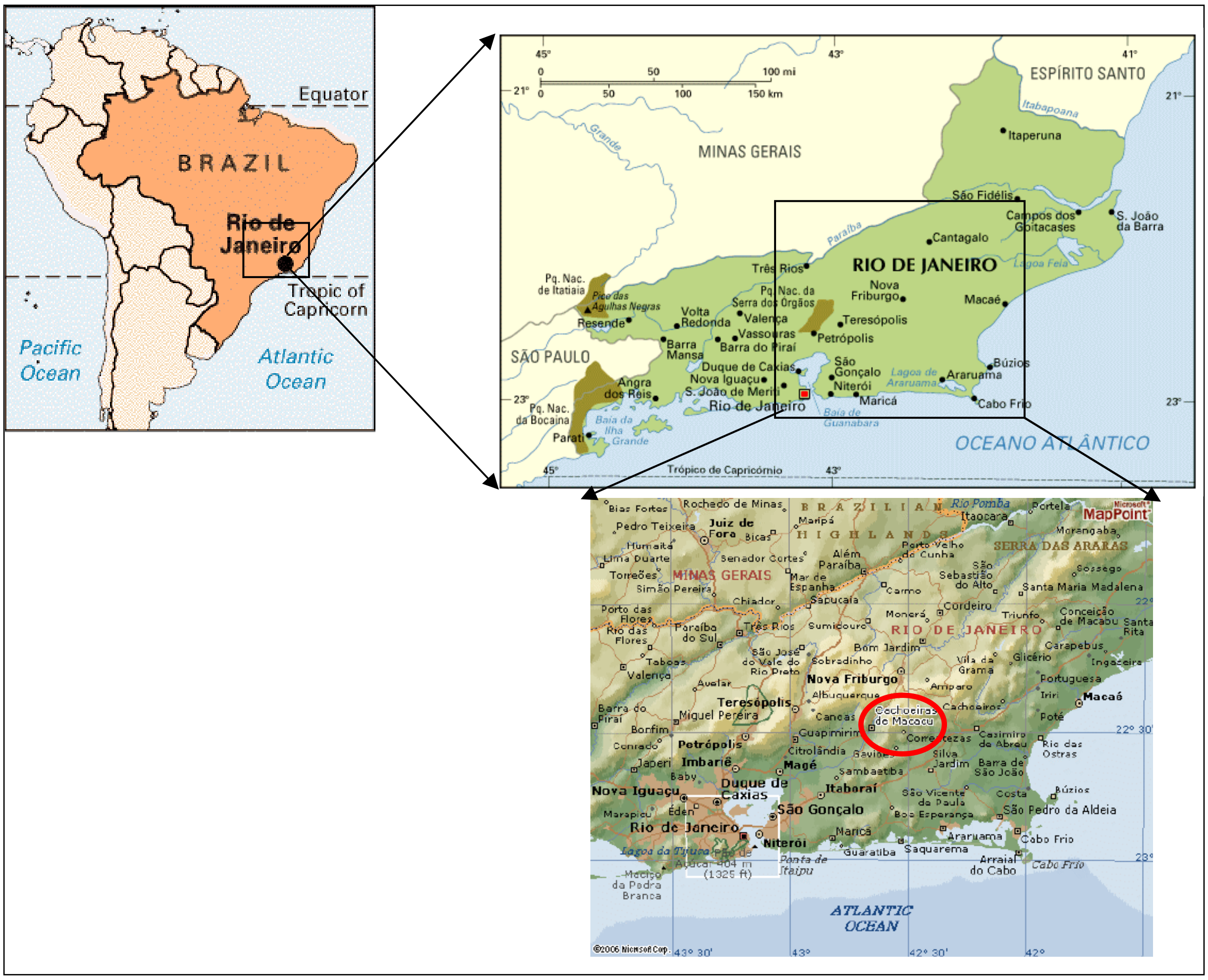

Figure 1. Map of the study area in south-eastern Brazil in the state of Rio de Janeiro.

Study species

Heliconia angusta L. (Heliconiaceae)

The family Heliconiaceae comprises a single genus, *Heliconia* L., with 250-300 species distributed mainly throughout neotropical areas from northern Mexico to southern Brazil (Dahlgren et al. 1985, Kress 1990). In Brazil, there are approximately 40 species covering two primary areas of distribution: the Amazon basin and the Atlantic coastal rainforest (Kress 1990). *Heliconia* is a highly diverse tropical genus, which is considered a potential keystone mutualist that provides resources for many animal species (Price 2002).

Figure 2. *Heliconia angusta L. (Heliconiaceae)*

Heliconia angusta is an ornithophilous, perennial, clonally growing, understorey herb endemic to the Atlantic Rainforest in south-eastern Brazil. Like other *Heliconia* spp., it is a common component of the understorey of Neotropical forests and has a patchy distribution. Sympodial rhizomes produce erect above-ground shoots (ramets) of up to 1.75 m height that can develop a single terminal inflorescence (Guimarães Simão & Scatena 2001). *H. angusta* displays a “steady state” flowering strategy (Gentry 1974b, Stiles 1975) producing flowers from April to October, making it a crucial nectar resource for hummingbirds (De Castro & Araujo 2004). The single-terminal inflorescence consists of five to eight red coloured bracts and about five hermaphroditic flowers per cincinnus (Fig. 2). The approximately 4 cm-long tubular corolla of the flowers is white, while the ovary and pedicel are orange in colour.

Flower anthesis usually lasts for only one day; flowers open in the early morning and are abscised in the evening, or at the latest, the next morning. Over a period of 2 – 4 months (between June and October), each ramet produces one or two new flowers roughly every 2nd day. In the Atlantic Rainforest, *H. angusta* is found mainly in shaded, moist places inside the forest, sometimes at the forest edge close to rivers (Simão & Scatena 2001).

Species of *Heliconia* are commonly pollinated by hummingbirds; the mature blue fruits are dispersed by birds. *H. angusta* is considered, like the majority of *Heliconia* species, also to be partially self-compatible, i.e. selfing is possible to a low amount, the species is primarily outcrossing (Bruna et al. 2004, Schleuning et al. 2011a, Suárez-Montes et al. 2011).

Besleria melancholica (Vell.) C.V. Morton (Gesneriaceae)

Many species of the family of Gesneriaceae are endemic to geographically small areas or, the majority, restricted to specific habitats (Denham 2004) and generally disappear when habitats face sustained disturbance. They can be regarded as indicators for the diversity of flowering plants and as representatives of the status of the whole flora of a given area (Skog 2005).

Figure 3. *Besleria melancholica* (Vell.) C.V. Morton (Gesneriaceae)

In the Neotropics, the genus is one of the most diverse genera within the Gesneriaceae, comprising about 160 species, of which at least 16 of them occur in Brazil (Chautems 1991). The genus *Besleria* is one of only a few genera of Gesneriaceae whose species grow as sub-shrubs or even small trees.

Besleria melancholica (Fig. 3) is a perennial sub-shrub reaching a height of up to 1.50 m (mean height 1.20 m) with a patchy distribution. It is endemic to the coastal Atlantic Rain Forest of the state of Rio de Janeiro and grows at elevations between 150 and 1200 m asl. The genus is common in the forest understorey in shady habitats near slopes and ways (Lopes et al. 2005, Kriebel-Haehner 2006). The hermaphroditic, protandrous, scentless flowers are tubular; the 20-25-mm-long corolla is of white colour with a rather wide opening of 6-9 mm. The calyx is bright yellow. The axial inflorescences consist of approximately eight flowers. Usually only one to six (mean four) open flowers are presented per individual at the same time. Each flower anthesis lasts for 8 -13 days, a single individual plant bears flowers over a period of about 4 months from late June until October (steady-state flowering, Gentry 1974b). Four to six weeks after anthesis fleshy, berry-like, orange-coloured fruits with thousands of tiny seeds are formed, which are dispersed by birds.

Stenostephanus lobeliiformis Nees (Acanthaceae)

Due to the species richness within the family of Acanthaceae (approximately 85 genera and 2000 known species in the Neotropics) there is a huge lack of knowledge about the ecology of individual species (Wasshausen 2005).
The genus contains about 39 species that are common within cloud forests, along riversides and in wet dips (Daniel 1999). Its distribution is exclusively neotropical and ranges from southern North America to Colombia and Brazil.

Figure 4. *Stenostephanus lobeliiformis* Nees (Acanthaceae)

S. lobeliiformis (Fig. 4) is an erect perennial ramified herb, which can reach a height of up to 1.50 m. The terminal inflorescences of 10-22 cm in length consist of 18-40 flowers. The ornithophilous, hermaphroditic, protandrous, scentless flowers are vivid scarlet in color, the corolla is a 18-mm-long tube with a narrow opening of 2 mm. The two stamens and the stigma exceed the corolla by 7-11 mm without any spatial separation. Flower anthesis lasts for 3-4 days; the whole inflorescence flowers for approximately 4 weeks. All of the individuals in the habitat flower over a period of 4-5 weeks between late June and August (mass flowering). The fruits are dry capsules; the seeds (maximum four per fruit) are dispersed autochorously. This species grows in dense populations near river sites (Lindner et al. 2010). *Stenostephanus lobeliiformis* is considered as a vulnerable species in the state of Espirito Santo in Brazil.

1.

Potential pollinators and robbers: a study of the floral visitors of *Heliconia angusta* (Heliconiaceae) and their behaviour

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1.1 Abstract

Floral syndromes are traditionally thought to be associated with particular pollinator groups. Ornithophilous flowers tend to have traits that facilitate bird pollination such as having long, narrow, tubular corollas, often vivid coloration and diluted, sucrose-rich nectar. However, recent studies have shown that flowers attract a broader spectrum of visitors than might be expected. Furthermore, the classification of floral visitors as ‘robbers’ or ‘pollinators’ often is not as simple as it seems, as pollinators can at times act as robbers and vice versa. We studied the species composition, behaviour and ecology of floral visitors, including potential pollinators and robbers, of *Heliconia angusta* (Heliconiaceae), an endemic understorey herb of the Atlantic Rainforest of Brazil. In addition, the impact of the plant inflorescence attractiveness and of weather and light conditions on visitor abundance and frequency was investigated.

Flower visitors were found to be scarce with a total of only 151 visits being observed during 120 h of field observations. A stingless bee species *(Trigona* sp.) appeared to be the most abundant visitor to the ornithophilous flowers of *H. angusta*, along with four different species of hummingbirds and two species of butterflies. We consider *Trigona* sp. rather as pollen robber, but which still has the potential to be a secondary pollinator, whereas the hummingbirds were the principle legitimate visitors. Most flower visitors were recorded between 9.00 am and 1.00 pm with a higher number visiting under semi-shaded conditions than in full shade. Hummingbird numbers increased with flower abundance while the other visitor group numbers were not affected.

Keywords: hummingbirds, stingless bees, *Trigona*, pollen robbers, nectar, inflorescence attractiveness

2.

The reproductive biology of two understorey plants in the Atlantic Rain Forest, Brazil

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2.1 Abstract

Outcrossing and sexual reproduction of most flowering plants depends on pollinators. Plant traits likely to be involved in pollinator attraction include flower colour, shape, and size. Furthermore plant or flower density and the temporal flowering pattern may have an effect on reproduction. In this study we examine the pollination ecology, breeding system, female reproductive output and germination of two tropical understorey species, *Stenostephanus lobeliiformis* (Acanthaceae) and *Besleria melancholica* (Gesneriaceae), which differ in these traits. Pollinator observations revealed that the dense flowering *S. lobeliiformis* with pinkish flowers received a higher diversity of pollinators, whereas visitor frequency to one flower per hour was much less ($0.1\ h^{-1}$) than that to *B. melancholica*, which has a smaller floral display of dull coloured flowers ($1.5\ h^{-1}$). Pollination experiments revealed that *S. lobeliiformis* but not *B. melancholica* is pollen limited. In addition, both species are partially self-incompatible and depend on pollinators for outcrossing. Natural fruit set of open-pollinated unmanipulated flowers (control treatment) in both species is 22 - 26 %. Germination studies indicated inbreeding depression in *S. lobeliiformis.* We conclude that the pollination ecology of these species is influenced by a broad set of traits and that very different combinations of these traits can be successful in terms of reproduction.

Keywords: Besleria melancholica, floral visitors, nectar, pollination experiments, reproductive biology, *Stenostephanus lobeliiformis*

3.
Negative effects of conspecific floral density on fruit set of two neotropical understorey plants

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3.1 Abstract

Plant reproductive success is usually positively related to conspecific floral density but neutral or negative effects of floral density on reproduction have also been reported. Differences in the relationship between reproduction and floral density largely originate from a trade-off between increasing attractiveness versus increasing competition for pollinators at high floral densities. Although floral densities strongly vary in the understorey of tropical forests, for instance due to variation in light availability, little is known about the density-dependence of reproduction in tropical understorey plants. We used path analyses to disentangle direct and indirect effects of canopy openness and floral density on fruit set and analyzed the relationship between pollen load and floral density for two neotropical understorey plants, *Heliconia metallica* and *Besleria melancholica*. In both species, fruit set was not directly related to canopy openness but decreased with increasing floral density. In *H. metallica*, canopy openness had an indirect negative effect on reproduction mediated by its effects on floral density. Effects of floral density on pollen loads were species-specific. In *B. melancholica*, pollen loads linearly decreased with increasing floral density, indicating competition for pollinators at high densities. In *H. metallica*, pollen loads were reduced at both low and high densities, indicating an interplay of facilitative and competitive effects of floral density on pollen deposition. In contrast to other studies, we found negative density-dependence of reproduction in both understorey species. Negative effects of floral density on reproduction appear to be related to pollinator-mediated effects on reproduction rather than to variation in abiotic conditions.

Key-words: *Besleria melancholica*; Brazilian Atlantic Forest; canopy openness; competition for pollination; facilitation, *Heliconia metallica;* Peruvian Amazon Forest; pollen load.

4.
The influence of fragmentation on clonal diversity and genetic structure of *Heliconia angusta*, an endemic understorey herb of the Brazilian Atlantic Rainforest

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4.1 Abstract

Fragmented populations are usually exposed to the negative effects of reduced gene flow, increased inbreeding, genetic drift, and population differentiation. These effects result in the collective loss of genetic variation, reducing the probability of population adaptation to new environmental conditions, and increasing the risk of extinction. Compared to continuous forest, forest fragments commonly exhibit e.g. a lower relative humidity and an increased air temperature due to edge effects. These suboptimal site conditions might result in enhanced clonal reproduction, potentially reducing clonal diversity due to increased selfing and inbreeding depression. In this study, clonal diversity, genetic diversity and structure of 14 patches of *Heliconia angusta* (Heliconiaceae) in the continuous forest and five forest fragments were assessed using AFLP-markers. We found that clonal diversity of patches was indeed lower in forest fragments in comparison to continuous forest, albeit the differences were only marginally significant. Genetic diversity of patches in forest fragments did not differ from those in the continuous forest. A PCoA as well as a STRUCTURE analysis did not show any clear clustering of patches in the continuous forest and forest fragments. Our results suggest that *H. angusta* has not suffered yet the expected negative effects of fragmentation.

Key words: AFLP, climate data, genetic differentiation, genetic diversity, forest fragments, tropical rain forest

Synthesis

This thesis aimed to investigate the pollination ecology and reproductive ecology of three neotropical herbaceous understorey species of the Atlantic Coastal Rain Forest of south-eastern Brazil: *Heliconia angusta* (Heliconiaceae), *Besleria melancholica* (Gesneriaceae) and *Stenostephanus lobeliiformis* (Acanthaceae). In addition, the genetic consequences of anthropogenic habitat fragmentation were studied for *H. angusta*.

General discussion

Since the great majority of tropical angiosperms depend on animals as vectors of pollen transfer (Kay & Schemske 2003) for sexual reproduction (Buchmann & Nabhan 1996), the first step of investigation must be field observations to determine the floral visitors of a particular plant species, their behaviour and reward requirements.

Despite the great variety of plant-pollinator systems, it is possible to associate floral traits with particular pollinator groups as a series of pollination syndromes (e.g. Campbell et al. 1996, Galetto 1998). These syndromes have been, and still are, used to infer what pollinates plant species in the absence of direct obervations (e.g. Pérez et al. 2006, Whittall & Hodges 2007). However, the pollination syndrome concept has recently been criticized. Ollerton et al. (2009), for example, studied the expression of floral traits and pollinator species of 482 plant species in six communities on three continents. The study revealed that in approximately two-thirds of the plant species, the most common pollinator could not be successfully predicted by the respective syndrome.

Pollinator-plant specialization, in general, is rare. Pollination systems with a high level of specialization are exceptions; they often vary from less specialized to more generalized systems (Waser et al. 1996). Ollerton et al. (2003) reported that generalist insects often visit more specialized plants, a situation that was also found for bird-adapted mistletoe flowers being visited by bees (Robertson et al. 2005).

Flower visitor observations revealed similar results for the ornithophilous flowers of the two study species *Heliconia angusta* (Fig. 2) and *Stenostephanus lobeliiformis* (Fig.4). Both of these plant species were visited mostly by Hymenoptera (in particular stingless bees, Meliponini), whereas hummingbirds only accounted for 5.5 % of all visits in *S. lobeliiformis,* and for 40 % in *H. angusta.* In contrast, the dull-coloured, rather inconspicuous flowers of *Besleria melancholica* (Fig. 3) were predominantly visited by hummingbirds.

However, care has to be taken when interpreting the most common visitor as the most important pollinator (Fenster et al. 2004). Schmid et al. (2011) reported that although bees most frequently visited the ornithophilous flowers of *Aechmea nudicaulis* (Bromeliaceae), a rare visiting hummingbird had the highest relative pollination effectiveness and was significantly more effective than all the bees combined.

Hummingbirds and bees also varied in their effectiveness on different *Penstemon* species (Castellanos et al. 2003).

Even species that appear to exploit a range of different pollen vectors may in practice be pollinated effectively by only one or two species (Bawa 1990). In pollination biology, this view has been formalized as the most effective pollinator principle (MEPP), which states that a plant should evolve specializations to its most effective pollinators at the expense of less effective ones (Stebbins 1970). The paradox is that, although flowers appear phenotypically specialized, they tend to be ecologically generalized (Aigner 2006).

Only experimental tests can reveal the pollination effectiveness of each visitor species (Freitas & Paxton 1998, Botes et al. 2009). Hence, further studies should focus on the pollination effectiveness of each visitor group separately in terms of e.g. fruit set, seed set and pollen deposition rate.

Further reports on many tropical long-tubed, hummingbird-pollinated species suggest that a more complicated relationship frequently exists involving floral parasitism, e.g. by pollen and nectar collecting insects (Gentry 1974a, Janzen 1975, McDade & Kinsman 1980). Many species of stingless bees, in particular of the genus *Trigona*, are known to rob flowers (Almeda 1977, Roubik 1982, Renner 1983) by collecting nectar and pollen. In fact, we observed stingless bees of the genus *Trigona* visiting the flowers of all three study species. However, they differed in their behavior. When entering an open flower, or touching the anthers/stigma respectively, they were counted as legitimate flower visitors (potential pollinators). When piercing the corolla to steal nectar without touching reproductive parts of a flower (primary nectar robbers), or using already existing holes made by primary nectar robbers (secondary nectar robbers), they were recorded as nectar robbers (definition following Inouye 1980). Both types of behaviour were observed in *B. melancholica* and *S. lobeliiformis*. In contrast, in *H. angusta* the nectar robbing behaviour was not observed. *Trigona* sp. was observed at times touching the stigmas of *H. angusta* flowers when collecting pollen. Although these legitimate visits only account for 16.05 % of all visits of the bees, they might have resulted in a transfer of pollen to the stigma and the pollination of flowers on occasion. These findings confirm the importance of direct field observations to investigate the actual visitor spectrum of a particular plant species. In either case, prediction of pollinators from the traditional syndromes alone, as various workers have done (e.g. Perret et al. 2001, Carpenter et al. 2003) seems a risky business (Ollerton et al. 2009). Furthermore, only direct observations can reveal potential pollinators and antagonistic floral visitors, since the classification of floral visitors as either ‘robbers’ or ‘pollinators’ may be simplistic (Arizmendi et al. 1996) as some robbers can at times be considered potential pollinators and vice-versa (e.g. Roubik 1989, Slaa et al. 2006).

The principle reward for most flower visitors is nectar (Proctor et al. 1996). It has been frequently proposed that specific nectar sugar compositions represent adaptations to dietary preferences of the respective pollinators, and thus forms part of the pollination syndrome (Baker et al. 1998, Schmidt-Lebuhn et al. 2007). The nectar of all study species was found to be sucrose-rich and diluted, whereas it was poor in hexoses glucose and fructose. Relatively diluted but sucrose-dominant nectar is a common feature in flowers pollinated by hummingbirds, butterflies and long-tongued bees (Baker & Baker 1990, Stiles & Freeman 1993, Baker et al. 1998, Krömer et al. 2008). Stingless bees are usually included in the sucrose-preferring group (Schwerdtfeger 1996, Biesmeijer et al. 1999a,b). Similar results were found for other ornithophilous neotropical plant species, e.g. by Perret et al. (2001) for species of Gesneriaceae and by Schmidt-Lebuhn et al. (2007) for species of Acanthacea: e.g. *Aphelandra sinclairiana, Justicia appendiculata* and *Sanchezia oblonga.*

In addition to the floral reward, in terms of number of flowers displayed or open, flower attractiveness may have a positive influence on the abundance and frequency of the flower visitors (Bosch & Waser 2001, Steven et al. 2003, Harder et al. 2004). With an increasing number of flowers displayed, more visits by Hymenoptera and hummingbirds were recorded to the flowers of *S. lobeliiformis* and more visits by hummingbirds to *H. angusta*. A positive correlation between an increasing attractiveness of the plant and the number of floral visitors was also reported e.g. by Brody and Mitchell (1997) for the ornithophilous *Ipomopsis aggregata* (Polemoniaceae). By contrast, no correlation between floral display size and number of visitors was found for *B. melancholica.*

The time of day had a significant influence on the number of visitors in *H. angusta* with most visitors appearing between 9:00 am and 1:00 pm. In addition, better light conditions promoted higher visitor frequencies. Higher temperatures, co-correlated with time of day, and sunlight also increased the visitation rates of the Florida scrub, belonging to the family of Lamiaceae, *Dicerandra frutescens* (Deyrup & Menges 1997) and for the understorey herb *Justicia rusbyi* (Acanthaceae) in eastern Bolivia (Döll et al. 2007). The number of visits by hummingbirds showed a clear peak between 9:00 am and 11:00 am and then decreased during the day. A similar pattern of foraging activity was also reported by Garrison and Gass (1999) for the traplining hermit hummingbird *Phaethornis longirostris* that are attracted to highly productive flowers such as *Heliconia pogonantha* (Heliconiaceae) in Costa Rica.

Since the breeding system of the species affects female reproductive success (Byers & Meagher 1992, DeMauro 1993), the structure of genetic diversity (e.g. Hamrick & Godt 1990, Les et al. 1991) and the fitness of individuals (Kittelson & Maron 2000), I continued my research by investigating the mating system of the study species.

Pollination experiments revealed that *S. lobeliiformis* is partially and *B. melancholica* mostly self-incompatible. Breeding system studies failed in *H. angusta* because most study plants withered or were rotten before fruit set. However, one can assume that *H. angusta* is partially self-compatible like the majority of *Heliconia* species (Bruna et al. 2004, Schleuning et al. 2011a, Suárez-Montes et al. 2011). Overall, all three study species are primarily outcrossing and strongly depend on pollinators for reproduction. An increased fruit set after pollen supplementation treatments, compared with natural cross pollination in *S. lobeliiformis,* suggests pollen limitation. Due to competition of flowers for pollination within the mass flowering dense population, single flowers might receive an insufficient amount of pollen. In contrast to our expectations, no pollen limitation could be found in *B. melancholica,* which only presents few flowers at a time and grows in low densities of individuals. Flowers of *B. melancholica* were visited more frequently than flowers of the dense flowering *S. lobeliiformis*, and thus potentially receive more pollen.

Pollen limitation may not only result from insufficient pollen transfer (quantity limitation), but also from deposition of low-quality pollen (quality limitation), such as genetically related, incompatible pollen (Aizen & Harder 2007). Pollen quality effects can additionally reduce seed production, which is a sign of inbreeding depression (Aizen & Harder 2007). Even early fitness traits such as seed germination can reveal inbreeding depression (Lienert & Fischer 2004). Hence, germination studies were conducted with the seeds resulting from different pollination treatments of *S. lobeliiformis* and *B. melancholica*.

No seeds of *H. angusta* were available since pollination experiments failed, and no seeds of *S. lobeliiformis* from the selfing treatments germinated at all, while the outcrossed and control seeds germinated well, which can be interpreted as a sign of inbreeding depression. In contrast, the seeds of *B. melancholica* resulting from the geitonogamy treatment (HS) germinated best, whereas germination of the outcrossed and control seeds was low. A possible explanation of the low germination might be that the fruits of both species were collected too early. Although the fruits appeared to be mature, the seeds might still have been unripe. Due to the ornithochorous fruit dispersal in the case of *B. melancholica*, the decision had to be made whether to sample the fruits early or to take the risk that they will be eaten by birds and thus lost to the data set. A similar problem arose for *S. lobeliiformis*: since mature capsules dehisce explosively, we had to avoid seed loss and thus may have collected them before they were mature.

The initial plan was to cultivate the seedlings for a certain time in the green house and to measure other fitness traits such as height, number of leaves, leaf area and biomass. However, since all seedlings died due to parasites (larvae of fungus gnat, Sciaridae), the germination studies only remain an initial look at inbreeding depression.

One general challenge during fieldwork was the scarcity of flowering individuals of the investigated species in the study area. Flowering individuals used for pollination treatments were (partially) bagged, and thus were not available for flower visitor observations. Pollen for the hand-crossing experiments had to be collected from plants which were not subjected to any treatment or experiment. The outcrossing treatment, for example, required emasculation of the flowers when the anthers were still closed to prevent a contamination with pollen. These collected anthers could not be used for the pollen supplementation treatments because the pollen they contained was still immature. Emasculated flowers could not be used for pollinator observations, because the damage might influence the visitor behavior or assemblage. Pollen collecting bees, for example, would not be attracted, whereas only nectar collecting visitors might not be influenced. Thus, the data on the floral visitors would have been biased.

The pollination bags (pollen-proof) are easy to handle and effective for breeding system analysis. However, they have some disadvantages when it is raining or stormy. I experienced that bags, despite being perforated, accumulated water at the bottom, leading the bag getting heavy, curving down, and sometimes breaking the inflorescence.

To investigate pollinator-mediated and abiotic effects on plant reproduction we used path analyses to disentangle direct and indirect effects of canopy openness and floral density on fruit set, and analyzed the relationship between pollen load and floral density for two neotropical understorey plants, *Heliconia metallica* and *Besleria melancholica*. The data for *H. metallica* from Peru were provided by Mathias Templin, one of the co-authors of the third publication. An increase in light availability is beneficial for growth and reproduction of many understorey species (Souza & Martins 2004, Bruna & Ribeiro 2005, Schleuning et al. 2008) and inflorescences are often formed only in canopy gaps (Stiles 1979, Horvitz & Schemske 1995, Bruna 2003). Plant reproductive success is usually positively related to conspecific floral density (Burd 1994, Kunin 1997, Roll et al. 1997, Corbet 1998), but neutral or negative effects of floral density on reproduction have also been reported (e.g. Weiner 1982, Rathcke 1983, Ghazoul 2005).

Our study revealed negative density-dependence in the reproduction of the two study species. In both species, fruit set was not directly related to canopy openness but decreased with increasing floral density. Conspecific floral density of *H. metallica* increased significantly with increasing light availability. Consistently, Schleuning et al. (2008) observed that growth and clonal expansion of *H. metallica* was rapid and reproduction was frequent in canopy gaps, whereas under closed canopy, plants grew slowly and rarely flowered. Thus, the clonal *H. metallica* is able to rapidly exploit increased light availability. In contrast, canopy openness did not affect conspecific floral density in the non–clonal *B. melancholica*. Similar to the species-specific effects found in our study, the growth response of other understorey plants to increased light availability also strongly differs among species (Denslow et al. 1990, Svenning 2001). For instance, Souza and Martins (2004) could not detect any influence of canopy openness on the density of the understorey palm *Geonoma brevispatha* in Brazil.

Effects of floral density on pollen loads were species-specific. In *B. melancholica*, pollen loads linearly decreased with increasing floral density, indicating competition for pollination at high densities. In *H. metallica*, pollen loads were reduced at both low and high densities, indicating interplay of facilitative and competitive effects of floral density on pollen deposition.

Since negative density-dependence could also be related to competition for abiotic resources (Rathcke 1983, Mustajärvi et al. 2001), we found that floral densities affected fruit set more strongly than overall densities of conspecifics in both species. This suggests that the negative density dependence of reproduction in *H. metallica* and *B. melancholica* is mediated by density-dependent plant-animal interactions rather than by effects of vegetative competition. Similarly, Feinsinger et al. (1991) also did not find any effects of vegetative competition on the fruit set of three bird-pollinated understorey plants at varying floral densities. Further pollination studies in the neotropical forest understorey are required to test whether negative density-dependence of reproduction is a general pattern for hummingbird-pollinated understorey plants.

In the fourth project, the genetic consequences of anthropogenic habitat fragmentation were studied for the clonally growing *H. angusta* using AFLP-markers. This species was the only study species that occurred both in the continuous forest (CF) and in forest fragments (FF). In addition to low probabilities of sexual reproduction and seedling recruitment in small fragments (Bruna 1999) suboptimal site conditions are considered to result in an enhanced clonal reproduction in clonally growing plant species (Eckert 2002). *Heliconia* is intolerant to water stress (Skillman et al. 1999), an environmental condition that usually increases after fragmentation.

Although clonally growing plant species can persist even under environmental stress (Honnay & Bossuyt 2005), it may lead to increased geitonogamy and inbreeding depression in self-compatible species, and reduced mate availability in self-incompatible species (Eckert 2000, Honnay & Jacquemyn 2008). As a consequence, clonal diversity decreases (Watkinson & Powell 1993). Our study revealed that the clonal diversity of patches is lower in FFs than in the CF, albeit the differences were only marginally significant.

This finding is supported by our climate data measured in the CF and in three FFs. In the FFs average temperatures were significantly higher and relative humidity was significantly lower than in the CF.

Fragmented populations are usually exposed to the negative effects of reduced gene flow (Fischer & Lindenmayer 2007) due to disrupted plant-animal interactions such as pollination and seed dispersal (e.g. Kolb 2008, Kiers et al. 2010), increased inbreeding, genetic drift, and population differentiation (Young et al. 1996). These effects result in the collective loss of genetic variation (Pertoldi et al. 2007).

In contrast to our expectations, no evidence was found that genetic diversity is decreased in patches of FFs. The mean expected heterozygosity He amounts for 0.103. Murawski and Hamrick (1990) investigated the clonally growing, terrestrial, hummingbird pollinated bromeliad *Aechmea magdalenae* in Panama and Suárez-Montes et al. (2011) studied *Heliconia uxpanapensis*, endemic to Mexico. Both report similar amounts of genetic diversity; they found no difference between continuous forest and forest fragments. Just like *H. uxpanapensis*, *H. angusta* may be naturally exposed to high levels of isolation, resulting in the moderate level of genetic diversity. In turn, this may partially explain the absence of an effect of forest fragmentation on the genetic diversity of both species.

Patches in FFs were not more genetically differentiated than patches in the CF. A PCoA as well as a STRUCTURE analysis did not show any clear clustering of patches in the continuous forest and forest fragments, and a Mantel-test did not detect a significant relation between genetic and geographical distance. The similar and moderate level of genetic diversity found within the patches of the continuous forest and forest fragments suggests that *H. angusta* has not yet suffered the expected negative effect of fragmentation.

One possible explanation is that actual rate of outcrossing and gene flow are sufficient to maintain observed levels of genetic variation within fragmented populations (Suárez-Montes et al. 2011). Another explanation might be the unique and often slow response of plant species to fragmentation, which is related to specific plant life history traits such as long generation times or potential for clonal growth (Eriksson & Ehrlen 2001). It may take many generations for genetic drift to have a significant impact on population genetic structure (Young et al. 1996, Tomimatsu & Ohara 2003). Given that *Heliconia* species are long-lived perennials and fragmentation has been more intense during the past 20-30 years, the expected effects of fragmentation on genetic diversity may take longer to express (Suárez-Montes et al. 2011).

Our results for *H. angusta* provide little support for the expectation that forest fragmentation has affected the amount and distribution of its genetic variation. However, since *H. angusta* plants did not flower in the forest fragments, we could neither investigate the flower visitor diversity and abundance nor the reproductive success of plants in the forest fragments. Future studies should test for the assumption that in FFs, pollinators are scarce or less effective, leading to a decrease in outcrossing rates and reproductive success, increased inbreeding, and thus a loss of genetic diversity of *H. angusta* plants.

Given the ongoing high rate of deforestation and fragmentation in the Brazilian Atlantic Rain Forest, *H. angusta* constitutes a suitable system to monitor the expected negative effect of fragmentation on genetic diversity. Due to its potential vulnerability, future conservation efforts should be directed toward ensuring the maintenance of pollen and seed dispersal among the fragmented and the continuous forests. Hence, genetic progeny surveys in this species will provide useful information to determine if current conditions of fragmentation are affecting the status of conservation of the interaction with pollinators and dispersers.

Implications for nature conservation

The ongoing loss of biodiversity is also an ongoing loss of ecosystem functioning, whereas the specific causes range from short-term and local to general and global-scale (Naeem et al. 1994).

Research approaches and knowledge have improved significantly over the past 15 years, yet greater baseline knowledge about the complexity of ecosystems and their functioning is essential for creating strategic conservation actions in a transparent economically and environmentally sustainable way. Plant-animal mutualisms such as pollination and seed dispersal link plant productivity and ecosystem functioning, and maintain gene flow in plant populations (Davidar 2010). Plant reproduction is particularly pollination-dependent in the tropics relative to the temperate zone (Vamosi et al. 2006) due to the greater biodiversity in the tropics where up to 98 % of all flowering plant species are pollinated by animals (Bawa 1990). Pollination is a critical ecosystem function for the continued persistence of the most biodiverse terrestrial habitats on Earth (Sekercioglu 2010). About 1200 vertebrate and about 289 000 invertebrate species are involved in pollination (Roubik 1995, Buchmann & Nabhan 1996, Nabhan & Buchmann 1997). Pollinators are estimated to be responsible for 60-90 % of the reproduction of wild plants (Kremen et al. 2007) and for 35 % of global crop production (Klein et al. 2007).

It is estimated that globally, about 200 species of wild vertebrate pollinators might be on the verge of extinction (Allen-Wardell et al. 1998). The widespread decline of pollinators and consequently pollination services is a cause for concern and is expected to contribute towards loss of biodiversity in natural ecosystems (Buchmann & Nabhan 1996, Kevan & Viana 2003).

This alarming trend has led to the creation of the "International Initiative for the Conservation and Sustainable use of Pollinators", a key element under the Convention on Biodiversity, and the International Union for the Conservation of Nature, which has a task force on declining pollination in the Survival Service Commission (Davidar 2010). Furthermore, the importance of a species as a critical resource may be a primary determinant of the consequences of a disruption in plant-pollinator mutualism. Fluctuations in populations of keystone mutualists (Terborgh 1986) that provide resources when other resources are scarce (or not available) are expected to have a drastic effect on the community (Bawa 1990).

The tropical genus *Heliconia* is considered a potential keystone mutualist that provides resources for several animal species (Price 2002), e.g, their primary pollinators: hummingbirds (Kolb 2008, Figueroa-Esquivel et al. 2009). The study species *Heliconia angusta* is an endemic understorey herb of the Brazilian Atlantic Rain Forest (Guimarães Simão & Scatena 2001). The second study species, *Besleria melancholica* (Gesneriaceae), is endemic in the coastal rainforest of the state of Rio de Janeiro (Lopes et al. 2005). Many species of the family of Gesneriaceae are endemic to geographically small areas and the majority of species in this family are restricted to specific habitats (Denham 2004), and generally disappear when habitats face sustained disturbance. They can be regarded as indicators for the diversity of flowering plants and as representatives of the status of the whole flora of a given area (Skog 2005).

Numerous studies show that narrow endemics are susceptible to extinction for a variety of reasons, including habitat destruction, biotic interactions, and genetic collapse (Schemske et al. 1994). Many of these factors increase the vulnerability of the species by lowering reproductive success. Consequently, the study of factors that critically affect reproductive success needs to be considered in the design of conservation strategies (Godt & Hamrick 1995).

Like most of the understorey plants in tropical rain forests, all three study species are patchily distributed. *Heliconia angusta* and *Besleria melancholica* typically grow in low densities of spatially isolated individuals, and *Stenostephanus lobeliiformis* grows in rather dense aggregations of reproductive conspecifics at scattered sites in the forest. Small populations are especially predicted to face negative genetic (Lynch et al. 1995, Young et al. 1996) and ecological consequences of disturbed plant-animal-interactions (Ǻgren 1996), which may reduce their fitness (Ellstrand & Elam 1993).

The on-going fragmentation of most tropical forests in the world constitutes one of the major threats to the conservation of biodiversity (Chazdon et al. 2009). Habitat fragmentation can disrupt mutualistic interactions by reducing the diversity and abundance of pollinators and seed dispersal agents, creating barriers to pollen and seed dispersal (Cordeiro & Howe 2003, Aguilar et al. 2006) and causing declines in plant reproduction (Kearns & Inouye 1997, Kearns et al. 1998, Cunningham 2000a). Hence, fragmented populations are usually exposed to the negative effects of reduced gene flow (Fischer & Lindenmayer 2007). As a result genetic variation decreases, reducing the probability of population adaptation to new environmental conditions, and increasing the risk of extinction (Pertoldi et al. 2007). Investigating plant-pollinator interactions and the resulting pollen dispersal patterns is therefore particularly relevant for understanding processes ensuring long-term viability of fragmented plant populations (Burkle & Alarcon 2011, Mayer et al. 2011).

Vamosi et al. (2006) report in their meta-analysis of fruit-set effect sizes of pollen-supplementation experiments conducted on 241 species in different biodiversity zones of the world that plants occurring in species-rich communities may be more prone to pollen limitation because of interspecific competition for pollination. These regions are the same areas where the fewest pollen supplementation studies have been conducted. As a consequence, plants in biodiversity hotspots may have a higher risk of extinction due to higher pollen limitation and habitat destruction.

The Brazilian Atlantic Rain Forest (“Mata Atlântica”) has been identified as one of the global biodiversity hotspots for conservation priorities (Myers et al. 2000) and is probably the region with the highest species diversity and degree of endemism throughout South America (Silva & Casteleti 2003, Tabarelli et al. 2005). However, this forest is highly fragmented and nowadays, its remnants cover only about 7-16 % of its original extent (Galindo-Leal & Câmara 2003, Ribeiro et al. 2009).

Despite the fact that several species of *Heliconia* are included in the Red List of Threatened Species (IUCN 2011), so far, only one study by Suárez-Montes et al. (2011) on *H. uxpanapensis* in Mexico and the study on *H. angusta* (publication 4 of this thesis) investigated the effects of fragmentation on the genetic variation of the species. Given the on-going rate of deforestation and fragmentation of their natural habitats, neotropical rain forests, and their potential vulnerability to increments in inbreeding within fragmented populations, future conservation efforts for this species should be directed to ensure that levels of gene flow among patches are sufficient to mitigate the potential loss of genetic diversity, and thus threat of extinction of this endemic species within fragments.

Molecular markers provide a fast and convenient tool to assess the condition of populations (Spielman et al. 2004) and are a powerful tool in conservation biology for understanding the consequences of fragmentation on the survival and performance of species.

Hence, the obvious overall conclusion must be: maintenance of contiguous forests and intact functioning ecosystems is needed to sustain mutualistic interactions such as pollination, to maintain vigorous populations, and thus overall biodiversity. The decline of pollination in commercial crops (Buchmann & Nabhan 1996, Allen-Wardell et al. 1998) is likely to receive attention because of the substantial economic risks involved, but unless comparable research effort is made for wild plants, we may remain oblivious to declines in pollination and reproduction of wild plants that place biodiversity and other natural resources at risk (Cunningham 2000b).

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Curriculum Vitae and full publication list

Dipl.- Biol. Katharina Stein

Date and place of birth: 17.04.1982, Leipzig
Nationality: German
Address: Karl-Heine-Strasse 47, 04229 Leipzig

12.2012 – ongoing	Scientific associate at the University of Würzburg, Institute of Animal Ecology and Tropical Biology, Member of the BMBF-project WASCAL, field-research in West-Africa
07. – 11.2012	Tutor of the international Master course ”Tropical Forestry and Management”, Dresden University of Technology
03. – 06.2012	Scientific assistant in the University Colaboration Project ”Implementation of the Convention on Biodiversity at a local level” in Peru, Bolivia and Paraquay, Dresden University of Technology (including an international workshop in Peru)
05. – 08.2010	Fieldwork in Brazil for data gathering (PhD-project)
03. – 04.2010	Study trip to the Philippines ("Interdisciplinary case studies: Land-use conflicts and conservation of natural resources", Department of Community Ecology within the HiGrade-Program of the UFZ Halle
05. – 12.2009	Fieldwork in Brazil for data gathering (PhD-project)
04.2009 – ongoing	PhD-student at Martin-Luther-University Halle/Saale (PhD-scholarship of ”Studienstiftung des Deutschen Volkes” until 03.2012)
06.2008 – 03.2009	Project collaborator at „Rain Forest Center“ of NABU Sachsen e.V. (environmental education for children and adults)
03.2008	Final degree – diploma
04.2007 – 02.2008	Diploma thesis „The influence of environmental gradients in a tropical montane rain forest on the population structure and vigour of *Nematanthus crassifolius* (Schott.) Wiehler, *Besleria melancholica* (Vell.) C.V. Morton und *Stenostephanus lobeliaeformis* Nees“; (including 6 month fieldwork in Brazil)
04.2005	Study trip and workshop in tropical ecology in southeast Brazil

08.2004	German-Russian Summer School at the University of Petrozavodsk (Karelia, Russia) within the DAAD "Go East"-program, topic: "Biology of plants. Biodiversity in a changing environment in Northern Europe"
10.2003 – 03.2008	Advanced study period of biology at the University of Leipzig
10.2003	Pre-degree – intermediate diploma
10.2001 – 10.2003	Basic study period of biology at the University of Leipzig
03.2001 – 08.2001	„Future Capital Project“ in Copenhagen (production of a documentary on the European Voluntary Service)
09.2000 – 03.2001	European Voluntary Service (EVS) at a ”Nature-School” in Padborg, Denmark (environmental education)
07.2000	University-entrance-diploma at Martin-Rinckart-Gymnasium, Eilenburg, Saxony

Conferences

- Annual meeting of „Gesellschaft für Tropenökologie e.V.“ (GTÖ, Society of Tropical Ecology), 22.02. - 25.02.2012, Erlangen (oral presentation)

- Annual meeting of the „Association of Tropical Biology and Conservation“(ATBC) “Adaptability to climate change and attaining the millennium development goals for tropical ecosystems”, 12. – 16. 06. 2011, Arusha, Tanzania (oral presentation)

- Annual meeting of „Gesellschaft für Tropenökologie e.V.“ (GTÖ, Society of Tropical Ecology), 21.02. - 24.02.2011, Frankfurt/Main (oral presentation)

- Annual meeting of „Gesellschaft für Ökologie“ (GFÖ, Society of Ecology), 30.08. – 03.09.2010, Gießen (poster)

- 12th Nordic Meeting on Neotropical Botany, 07. – 09. August 2008, Turku, Finland (oral presentation)

- Annual meeting of the „Association of Tropical Biology and Conservation“ (ATBC) “Past and Recent History of Tropical Ecosystems“, 09. – 13. juni 2008, Paramaribo, Surinam (oral presentation)

- 4th International Canopy Conference „Tropical versus Temperate Forests“, 10. – 17.Juli 2005, Leipzig

Teaching and supervised theses

- 2012 practical course “Integrated land use management“ in Tharandt, Dresden University of Technology
- Bachelor thesis – Julia Köhler “Genetische Diversität und Struktur von *Heliconia angusta* Vell. (Heliconiaceae) an Waldstandorten im Atlantischen Küstenregenwald, Brasilien“
- Bachelor thesis – Anke Kindermann ”Genetische Diversität und Struktur von *Heliconia angusta* Vell.(Heliconiaceae) aus Fragmentstandorten in Brasilien“
- 2011 international workshop within the United Nations Environment Program (UNEP) on “integrated land use management” in Tharandt, Professorship of Tropical Forestry, Dresden University of Technology
- 2010, 2011 practical course (botany and ecology) at ”Müritznationalpark“
- 2008, 2009, 2010 student excursions to the floodplain forest in Leipzig and Botanical Garden
- 2006 advanced practical course and seminar "systematic botany and ecology of tropical plants"
- 2005, 2006 practical course ”botany and ecology” in Zingst

Publications (peer-reviewed)

Publications marked with asterisks (*) are part of the PhD-thesis

Lindner A, Stein K and M Freiberg (2010) Abundance and vigor of three selected understorey species along environmental gradients in south-eastern Brazil. *Ecotropica* 16(2): 101-112

*Stein K and I Hensen (2011) Potential pollinators and robbers: a study of the floral visitors of *Heliconia angusta* (Heliconiaceae) and their behaviour. *Journal of Pollination Ecology* 4(6): 39-47

*Stein K, Templin M, Hensen I, Fischer M, Matthies D and M Schleuning (2013) Negative effects of conspecific floral density on fruit set of two neotropical understory plants. *Biotropica* 45(3): 317-324

*Stein K and I Hensen (2013) The reproductive biology of two understory plants in the Atlantic Rainforest, Brazil. *Ecological Research* 28(4): 593-602

*Stein K, Hirsch H, Kindermann A, Köhler J, Rosche C and I Hensen (2013) The influence of fragmentation on clonal diversity and genetic structure of *Heliconia angusta*, an endemic understorey herb of the Brazilian Atlantic Rainforest. (submitted to *Journal of Tropical Ecology*, minor revision)

Rinawati F, Stein K and A Lindner (2013) Climate change impacts on biodiversity – The setting of a lingering global crisis. *Diversity 5*: 114-123