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ANDRÉ RODRIGO RECH

WALKING THROUGH THE FLOWER FIELDS: THE ROLE OF TIME AND SPACE ON THE EVOLUTION OF POLLINATION STRATEGIES

CAMINHANDO ENTRE FLORES: O PAPEL DE VARIAÇÕES NO TEMPO E ESPAÇO NA EVOLUÇÃO DE ESTRATÉGIAS DE POLINIZAÇÃO

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INSTITUTO DE BIOLOGIA

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Abstract

Patterns in ecology are the products of current factors interacting with a longstanding history of contingency. Nevertheless, few studies have attempted to disentangle the contribution of past and current factors on plant reproduction patterns. Here we studied pollination considering both, spatial and temporal dimensions. Time variation goes from hours to millennia as well as space, whose importance was considered from meters to the whole planet. The chapter's sequence within the thesis is planned to go from the small to the large scale. We show the importance of fine grained variations such as hours and meters in the flower differentiation and pollination of two *Davilla* species in the chapter 1. In the chapter 2 we studied pollen deposition and visitation frequency in *Knautia arvensis* considering a year scale and showed the most important pollinator changing every year. In the Chapters 3 we used *Curatella americana* with populations studied across Brazilian Cerrado and show spatial variation in flower and leaf morphology and pollen tube growth. Chapter 4 also using *C. americana* shows the variation on the reproductive system across space, with cross-pollination related to pollinator availability and the level of autogamy underpinned by past climate. To finish, Chapter 5 deal with 50 community-based assessments of wind and animal pollination over the world and show the importance of precipitation (current and past) and plant species richness as major drivers of these proportion. As a general conclusion, it is clear that temporal and spatial factors cannot be ignored in spite to understand floral evolution and the interactions between plant and pollinators.

Resumo

Os padrões encontrados em ecologia são resultado de processos contemporâneos interagindo com uma longa história de contingência. No entanto, poucos estudos têm buscado entender o papel relativo de fatores contemporâneos e pretéritos sobre padrões reprodutivos de plantas. No decorrer dessa tese foram consideradas essas duas dimensões (temporal e espacial) em estudos sobre polinização. A amplitude do estudo em relação ao tempo foi de horas até milênios, da mesma forma que para o espaço, para o qual se considerou desde metros até variações entre diferentes continentes na escala planetária. Os capítulos estão organizados em uma escala crescente de tempo e espaço. No primeiro capítulo foi considerada a variação fina de horas e metros no estudo sobre a polinização de algumas espécies de *Davilla*; nesse capítulo também são apresentados outros aspectos da história natural na família Dilleniaceae e uma abordagem filogenética para a evolução de algumas características florais. No capítulo 2, ao longo de vários anos, foi verificada a habilidade de visitantes florais depositar pólen, sua frequência e a importância de cada grupo de visitante nas flores de *Knautia arvensis* nesse período. O capítulo 3 demonstra variações no espaço tanto na morfologia floral e foliar como no crescimento do tubo polínico em diferentes testes de polinização, utilizando *Curatella americana* com populações distribuídas no Cerrado Brasileiro. No capítulo 4 é apresentada a variação espacial no sistema reprodutivo e a relação dos polinizadores com o nível de polinização cruzada e do passado climático com o nível de autopolinização espontânea, também tratando de *C. americana*. Para finalizar o capítulo 5 considera 50 inventários distribuidos ao redor do planeta categorizando as plantas em anemófilas ou zoófilas e demonstra o papel da precipitação (presente e passada) e da riqueza de espécies vegetais na prevalência de cada um dos modos de polinização. Como conclusão geral, fica clara a importância de se considerar as dimensões temporal e espacial nas interações entre plantas e polinizadores, a fim de entender como essas evoluem e como impactam na evolução da morfologia floral e nos sistemas de polinização.

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Introdução Geral

Em suas correspondências Darwin classificou o caminho evolutivo que conduziu à intensa especiação em Angiospermas como um "mistério abominável" (Darwin and Seward 1903). Dentre os vários mecanismos potencialmente geradores de diversidade, destacam-se aqueles relacionados a reprodução (polinização e dispersão). Por serem decisivas para a continuação das linhagens essas fases da vida das plantas demandam características e estratégias que assegurem a atração de polinizadores e dispersores (Crepet & Niklas, 2009).

Acredita-se que a grande maioria das Angiospermas dependa de animais para sua polinização (Ollerton et al. 2011). Essa estimativa, no entanto, não considera o fato de que muitas espécies, embora polinizadas por animais podem possuir populações reproduzindo-se de forma autogâmica, ou com sistemas mistos, utilizando ao mesmo tempo animais e vetores abióticos (Levin 2011, Friedman e Barrett 2009). Essa desconsideração se deve em grande parte ao fato de que em geral na literatura, se considerou o sistema de polinização e o de reprodução como atributos fixos das espécies (Levin 2011). Abordagens intraespecíficas considerando variações no tempo e espaço, são escassas nos estudos de polinização. No entanto, na medida em que essas abordagens têm sido utilizadas ocorreram avanços significativos na forma com que se entende a evolução das estratégias de polinização (Kalisz et al. 2004).

Estudos realizados por períodos tão curtos quanto dias ou estações (Gomez, 2000a; b; Varassin 2002; Araújo *et al*. 2004) até intervalos supra-anuais encontraram variação temporal na importância dos polinizadores (Fenster & Dudash, 2001; Herrera, 2005; Alarcón *et al*., 2008). Semelhante aspecto se aplica ao espaço, onde a guilda de polinizadores de uma determinada espécie pode variar entre ambientes geograficamente distintos (Herrera, 2005), ou entre indivíduos crescendo em diferentes microhábitats (Herrera, 1997, Biesmeijer *et al.* 1999).

Enquanto as flutuações no tempo tendem a favorecer sistemas de polinização mais resistentes ou resilientes (Bartomeus et al. 2013), a variação espacial, gerando adaptação local aos polinizadores ou à ausência deles (Kalisz et al. 2004, Sun et al. 2014), pode gerar espécies cujas populações formam um mosaico geográfico de interações (Thompson 2005). Além disso, a disponibilidade de polinizadores pode ter impacto direto sobre a composição de espécies de determinadas comunidades (Regal 1982).

No desenvolvimento da presente tese, buscou-se avaliar o possível efeito de variações em diferentes escalas de tempo e espaço sobre diferentes estratégias de polinização. Assim, os capítulos encontram-se em uma sequência crescente na escala temporal ou espacial. No primeiro capítulo descreveu-se alguns aspectos da história natural na família Dilleniaceae, o sinal filogenético em alguns atributos florais de *Davilla* e o papel de variações espaciais, na escala de metros, e temporais, na escala de horas, na polinização de duas espécies desse gênero. No segundo capítulo, estudou-se a polinização de *Knautia arvensis* (Caprifoliaceae) no Reino Unido, considerando a escala temporal de anos na avaliação da composição da guilda de potenciais polinizadores. No terceiro capítulo estudou-se *Curatella americana* e o papel da variação espacial, na escala de centenas de quilômetros, no padrão reprodutivo e a maneira com que se dá esse processo, bem como, sua importância sobre a variação na morfologia floral e vegetativa. O quarto capítulo também se refere a *C. americana*, no qual se verificou o papel de largas distâncias e variações paleoclimáticas na determinação do sistema reprodutivo e de polinização desta espécie. No quinto capítulo, utilizou-se larga escala espacial (planeta terra) e temporal (milênios) no estudo da importância relativa da polinização biótica e anemófila.

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Capítulo 1 - Polinização na família Dilleniaceae: estudo dos gêneros Neotropicais e uma hipótese ecológica para diferenciação floral em *Davilla* baseada na filogenia

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Resumo: As variações morfológicas nas estruturas florais em Dilleniaceae desde há muito tempo chamam atenção de sistematas e morfologistas. No entanto, apesar do amplo enfoque na morfologia pouco se sabe sobre a polinização dessas plantas, especialmente para os gêneros neotropicais, para os quais apenas anotações sobre visitantes florais estão disponíveis na literatura. Nesse trabalho, estudamos a polinização e o sistema reprodutivo de espécies dos dois principais gêneros Neotropicais de Dilleniaceae (*Davilla* e *Doliocarpus*). Utilizamos a hipótese filogenética disponível para *Davilla*, para testar o sinal filogenético de alguns atributos florais. Apenas o número de flores por inflorescência apresentou sinal filogenético entre os atributos testados, no entanto, essa variável correlacionou-se negativamente com o tamanho das flores. No campo, observamos que espécies cujas inflorescências têm menos flores possuem flores maiores, permanecem mais tempo floridas, tendem a ser autoincompatíveis e são polinizadas por abelhas maiores. Sugerimos que o número e o tamanho das flores no gênero *Davilla* pode ser uma variação adaptativa de duas estratégias de polinização. As únicas duas espécies estudadas em simpatria (*D. lacunosa* e *D. grandiflora*) apresentavam cada qual uma estratégia diferente. A família Dilleniaceae é majoritariamente polinizada por abelhas, mas moscas e besouros participam ocasionalmente da polinização de algumas espécies.

Key-words: biologia floral, visitantes florais, sinal filogenético, evolução

Introdução

A família Dilleniaceae apresenta variação nos caracteres florais entre os gêneros em tal amplitude que chegou a ser considerada como uma possível ligação entre as “magnolideas” e as dicotiledôneas mais derivadas (Cronquist, 1988). Entre os gêneros da família, o androceu, por exemplo, pode apresentar de 1 até 900 estames e o gineceu de 1 até 20 carpelos com diferentes níveis de fusão, variação que também pode ser percebida na simetria floral (Endress, 1997). A família é monofilética com base em dados morfológicos e moleculares e, tradicionalmente foi reconhecida como um modelo importante para estudos evolutivos entre as angiospermas (Takhtajan, 1991; Tuker & Bernhardt, 2000).

Em termos eco-morfológicos existem clados na família que possuem morfologia floral bastante especializada e compatível com adaptações à visitantes florais especializados como, por exemplo, anteras poricidas (Endress, 1997). No entanto, as espécies que ocorrem na região neotropical apresentam flores abertas do tipo “prato” (*sensu* Faegri & van der Pijl, 1979), simetria radial, com cores variando de branco ao amarelo (Kubitzki 2004). O único estudo de biologia reprodutiva realizado com uma espécie neotropical de Dilleniaceae (*Davilla kunthii*), registrou abelhas como principais polinizadores e sistema reprodutivo autocompatível, preferencialmente xenógamo mas com autopolinização espontânea (Rech et al. 2011). Embora todos os outros registros acerca da polinização e reprodução das Dilleniaceae neotropicais sejam anotações de observações esporádicas, tradicionalmente sugeriu-se que o sistema de polinização fosse generalista (Ducke, 1902; Kuhlmann & Kuhn, 1947; Skutch, 1971; Bawa, 1974; Gottsberger, 1977; Croat, 1978).

Nesse estudo buscou-se caracterizar e comparar a polinização e a reprodução de espécies de *Davilla* e *Doliocarpus* procurando evidenciar as variações eco-morfológicas relacionadas aos mecanismos de polinização. Testamos o sinal filogenético e a correlação entre alguns atributos em *Davilla* e avaliamos a importância funcional de alguns atributos florais em condições naturais. Testamos a hipótese de que abelhas sociais atuam como pilhadores de pólen em espécies predominantemente autoincompatíveis.

Material e Métodos

Espécies e locais estudados

Foram estudadas a biologia floral, sistema reprodutivo e polinizadores de *Davilla nitida*, *D. grandiflora* e *Doliocarpus brevipedicellatus* (Tabela 01). Além disso, foram feitas observações não sistematizadas de visitantes florais em outras duas espécies de *Davilla* (*D. lacunosa* e *D. pedicellaris*), bem como em *Doliocarpus dentatus*, *Tetracera wildenoviana* e *Pinzona coriaceae*. Para o estudo do sinal filogenético em estruturas florais considerou-se todas as espécies no gênero *Davilla*.

Biologia Floral e Reprodutiva

Para o estudo sobre biologia floral foram registradas informações em campo como: horário, sequência e duração da antese, número e longevidade das flores, emissão de odor e disponibilidade de pólen (Kearns & Inouye, 1993; Dafni et al. 2005). Além disso, foram coletadas e fixadas flores, inflorescências e frutos (álcool 70% glicerinado) para análise estrutural e ilustrações. A receptividade estigmática foi avaliada com peróxido de hidrogênio em flores previamente ensacadas (Kearns & Inouye, 1993). A localização de osmóforos foi verificada mediante imersão de flores (n=20) em solução de vermelho neutro (Dafni, 2005). Foram tomadas medidas das sépalas, sendo o tamanho da maior sépala considerado como medida indicativa do tamanho floral para fins de comparação entre espécies de *Davilla*.

Para determinar o sistema reprodutivo foram realizados testes de polinização ensacando flores em pré-antese: polinização cruzada, autopolinização manual, autopolinização espontânea. O número de flores utilizado para cada espécie e tratamento dependeu do tempo de antese e da oferta de flores, portanto, foi variável entre tratamentos e espécies. Para *D. nitida* não foi realizado teste para autopolinização manual. Para *Davilla lacunosa* e *Doliocarpus dentatus* foi testada apenas a dependência por vetores de pólen ensacando inflorescências e avaliando a formação de frutos (autopolinização espontânea). Dado o tamanho pequeno das flores e a grande quantidade de estames, não foi realizada emasculação e portanto, não foram obtidos resultados sobre apomixia. Foram marcadas flores que permaneceram expostas aos visitantes e acompanhadas para verificar a taxa de frutificação em condições naturais.

Os visitantes florais foram observados durante o pico de floração de cada espécie, embora o período total de observação tenha sido variável nas diferentes espécies, porém nunca foi inferior à 50 horas. No primeiro dia de estudo em cada local as observações e contagens foram iniciadas antes do amanhecer (por volta de 04:00h), a seguir as observações foram adaptadas ao horário de início da atividade dos visitantes. A quantificação da intensidade de visitação foi feita durante 10 minutos a cada meia hora até cessarem as visitas. Nos 20 minutos restantes foi registrado o comportamento da maioria dos visitantes como, período e duração das visitas, número de flores visitadas e número de visitas de indivíduos/inflorescência. A distinção entre polinizadores e pilhadores foi baseada no comportamento de forrageio durante a coleta do recurso floral, contato com anteras e estigmas (legitimidade das visitas) associado ao sistema reprodutivo da planta.

Em *Davilla nitida* foi registrado o tempo de manipulação das flores por operárias de *Apis mellifera* a cada visita (30 aferições), repetindo o procedimento durante 10 minutos a cada meia hora em dias consecutivos. Os dados foram analisados por meio de análise de variância e correção utilizando teste de Bonferroni ($p<0,05$). Em *D. grandiflora* 50 flores foram ensacadas após uma visita de *A. mellifera* para verificar a importância dessas abelhas na polinização da espécie. O pólen aderido ao corpo de alguns visitantes de *D. grandiflora* foi analisado a fim de verificar a riqueza de espécies visitadas por cada um dos indivíduos. As espécies de abelhas mais abundantes foram marcadas com corretor de texto líquido atóxico (Faber-Castell) e o comportamento de vôo foi então acompanhado marcando-se o tempo entre a saída e retorno a um determinado indivíduo. As observações de visitantes em *Pinzona coriaceae* e *Tetracera wildenoviana* foram curtas e não sistematizadas.

O material testemunho foi depositado nos herbários do Instituto Nacional de Pesquisas da Amazônia (INPA) e da Universidade Estadual de Campinas (UEC) e os visitantes florais foram depositados no Museu de Zoologia da Universidade de São Paulo – *Campus* de Ribeirão Preto.

Sinal filogenético

Foi estimado o sinal filogenético para atributos florais das espécies de *Davilla*. Os atributos considerados foram: número de estames, tamanho da antera, tamanho da sépala, número de flores por inflorescência, comprimento da inflorescência e diâmetro do fruto. Para o cálculo, foi considerado o autovetor da regressão transformado logaritmicamente (PVR, Diniz-Filho *et al.* 1998). A informação filogenética foi extraída de Fraga (2013). Para o cálculo do PVR foi realizada uma análise de componentes principais (PCoA) reduzindo as relações filogenéticas em eixos que foram usados para os cálculos subsequentes. Os coeficientes de determinação dessas regressões (R^2) são estimativas do sinal filogenético de cada atributo

(Cheverud et al. 1985, Diniz-Filho et al. 2012). O método de Moran foi utilizado para selecionar os autovetores (Diniz-Filho et al. 2012). A significância do sinal filogenético obtido em cada atributo foi testada com 1000 iterações. Foi testada também a correlação entre os atributos utilizando os valores dos resíduos dos PVRs, considerando essa correlação como independente da filogenia (Diniz-Filho et al. 2011). A análise foi realizada utilizando o pacote PVR/PSR para R (http://cran.r-project.org/web/packages/PVR/index.html).

Resultados

Período de Floração

A floração de *Davilla grandiflora* e de *D. lacunosa* iniciou na segunda quinzena de abril e prolongou-se até início de junho, apresentando pico de floração em abril e maio, respectivamente. A floração de *D. nitida* ocorreu entre a segunda quinzena de maio e a primeira de junho. *Doliocarpus dentatus* floriu no mês de julho com uma florada de apenas uma semana, enquanto *D. brevipedicellatus* floriu na primeira quinzena de outubro.

Biologia floral

Todas as espécies de Dilleniaceae possuem antese no período da manhã e poucas horas (entre uma e duas) após as pétalas são destacadas. Os órgãos reprodutivos estão dispostos no centro da flor, caracterizando o tipo aberto *sensu* Faegri & van der Pijl (1979), o que os torna acessíveis aos visitantes. O único recurso floral disponível aos visitantes é o pólen. As flores de *Davilla* apresentaram odor adocicado, enquanto nas de *Doliocarpus* não foi percebido odor. As espécies de *Davilla* possuem flores amarelas, ao passo que as flores de *Doliocarpus* são completamente brancas, com exceção dos filetes de *D. dentatus* que são vermelhos.

Sistema reprodutivo

As espécies de *Davilla* estudadas apresentaram algum nível de autocompatibilidade. Em *D. grandiflora* não ocorreu autopolinização espontânea e as polinizações em condições naturais produziram mais frutos que as polinizações cruzadas (Tabela 2). Em *D. nitida* os valores de autopolinização manual e espontânea, assim como de polinização em condições naturais foram similares entre si e menores que o resultado de polinização cruzada (Tabela 2).

A população de *Doliocarpus brevipedicellatus* estudada é auto-incompatível e apresentou limitação de pólen, uma vez que a produção de frutos por polinização cruzada manual foi maior que a aquela mediada por polinizadore. Apenas os tratamentos de polinização cruzada e polinização em condições naturais (mediada pelo polinizador) resultaram na formação de frutos (Tabela 2). Em *Doliocarpus dentatus* a polinização em condições naturais resultou na formação de quatro vezes mais frutos do que a autopolinização espontânea, indicando a importância dos visitantes florais para polinização (Tabela 2).

Visitantes florais

Davilla grandiflora, *D. lacunosa* e *D. nitida*

O visitante floral mais frequente de *D. grandiflora* foi *Apis mellifera* (Apidae) que iniciava o forrageio por volta de 06:00h e encerrava as visitas por volta de 09:00h quando não havia mais pólen. Apesar de *A. mellifera* ser bastante ativa as flores visitadas apenas por esta espécie de abelha não formaram frutos. Aproximadamente uma hora depois do início do forrageio de *A. mellifera* foram registradas também visitas de Halictidae, de Apidae-Meliponini (*Tetragona clavipes*, *Trigonisca meridionalis*) e duas espécies de moscas (Diptera), cuja frequência de visitas representou menos que 2% do número total de visitas. Duas espécies de *Euglossa* (Apidae) visitaram as flores em rota de forrageamento ("*trapline*"), em dias consecutivos e aproximadamente no mesmo horário. As visitas dessas espécies de *Euglossa* foram rápidas (três segundos) e visitaram apenas algumas flores de cada indivíduo percorrendo todos os indivíduos da população focal (n = 15). A análise da carga polínica indicou que os indivíduos de *Euglossa* spp. transportavam apenas pólen de *Davilla*.

A visitação em *D. lacunosa* iniciou entre 06:30-07:00h e os principais visitantes foram Apidae: *Tetragona clavipes*, *Scaura longula*, *Apis mellifera*, *Euglossa* sp. e Halictidae. A maior parte dos visitantes parecia deslocar-se pouco entre os indivíduos floridos durante o forrageamento. Indivíduos marcados de abelhas sociais (*A. mellifera* e *T. clavipes*) deixavam a planta focal com as corbículas cheias de pólen e retornavam ao mesmo indivíduo focal ao longo do período de duração da flor. Os picos das visitas às flores das espécies sincronopátricas *D. grandiflora* e *D. lacunosa* foram divergentes, sendo às 0700h e 0900h, respectivamente (Figura 2).

Os visitantes de *D. nitida* foram abelhas Apidae (*Celetrigona longicornis*, *Ptilotrigona lurida*, *Scaptotrigona* sp. aff. *xantotricha* e *Apis mellifera*) e quatro espécies de moscas Syrphidae. Os dois principais visitantes florais, *A. mellifera* e *Ptilotrigona lurida*, apresentaram padrões divergentes de forrageamento (Figura 3). A duração do período de manipulação de *A. mellifera* nas flores de *Davilla nitida* foi diferente ($p< 0{,}001$) entre o primeiro intervalo de observação (06:00h) e os demais que foram similares entre si ($p>0{,}05$). As médias desses períodos (segundos) de manipulação em cada intervalo foram: 06:00h=10,9 (±7,8); 06:30=7,8 (±6,8); 07:00=5,6 (±4,2); 07:30=5,8±4,5; 08:00=5,5 (±4,9).

Todas as espécies de *Davilla* receberam visitas de besouros ao final da antese em cujas flores copulavam e ovipositavam (Figura 4). No entanto, não foi observado de besouros alimentando-se das partes florais. Além de besouros foram encontradas larvas de lepidópteros e dípteros no interior das câmaras formadas pelas sépalas persistentes de *Davilla grandiflora*. Ainda, em câmaras florais de outra espécie de *Davilla* da região de Santarém foram encontradas larvas de pernilongos (Diptera), indicando que as flores são utilizadas como abrigos para desenvolvimento larval de insetos.

Doliocarpus

Os visitantes florais de *Doliocarpus brevipedicellatus* foram abelhas: *Megachile* sp. (Megachilidae) e as Apidae; *Trigona* sp. aff. *pallens*, *Partamona epiphytophila* e *Xylocopa* (*Schonnherria*) *muscaria*. As abelhas *Megachile* sp. e *Xylocopa muscaria* realizavam visitas rápidas e deslocavam-se bastante entre indivíduos durante o forrageamento, ao passo que as abelhas sociais permaneciam muito tempo manipulando as flores de modo que se deslocavam pouco entre indivíduos. Foram registradas ainda visitas de moscas e hemípteros, os quais, dado o comportamento e frequência da visita, foram considerados polinizadores ocasionais e acidentais, respectivamente. Em *D. dentatus* apenas moscas foram registradas visitando as flores.

Pinzona coriaceae e *Tetracera wildenoviana*

Foram observadas apenas abelhas Apidae-Meliponini visitando as flores dessas espécies. O padrão de visitação observado foi exatamente o mesmo registrado para as espécies de *Davilla* com flores menores (*D. lacunosa* e *D. nitida*). No entanto, uma vez que o período de observações foi curto é provável que existam outros visitantes atuando como polinizadores.

Sinal filogenético

Entre as estruturas analisadas quanto ao sinal filogenético apenas o número de flores na inflorescência apresentou sinal filogenético (R^2 = 0.36) maior que 0,1. Após controlar para o efeito da filogenia, apenas os resíduos do tamanho da inflorescência e do número de flores apresentaram correlação negativa, o que indica que há correlação entre as duas variáveis (inflorescências com mais flores tendem a ter flores menores) independente da relação filogenética entre as espécies consideradas. A figura 1 representa duas possíveis estratégias de polinização relacionadas com tamanho, número de flores e potenciais polinizadores representadas pelas duas espécies simpátricas que aparentemente contrastam essas duas possibilidades. Nessa figura percebe-se *Davilla* lacunosa à esquerda com muitas flores pequenas, polinizada especialmente por abelhas sociais e muitos frutos pequenos (boa parte resultante de autopolinização). À direita, *D. grandiflora* com menos flores de tamanho maior, polinizada especialmente por abelhas Euglossini, produzindo poucos frutos também maiores e com maior nível de autoincompatibilidade.

Discussão

Os dados apresentados aqui somados aos de Rech *et al*. (2011) para *D. kunthii* e Rech *et al*. (in prep.) para *Curatella americana*, preenchem uma lacuna de informações acerca da biologia floral, reprodução e polinizadores dos gêneros de Dilleniaceae Neotropicais. Com esse conjunto de dados é possível discutir os aspectos relacionados à polinização na abrangência da família embasados na filogenia (Horn *et al*. 2009). Flores que duram apenas algumas horas e as pétalas que se desprendem nas primeiras horas da antese parecem emergir como características omnipresentes nas espécies de Dilleniaceae. Essas características foram registradas para *Dillenia* (Burkill 1916, Corner 1940, 1988; Raju 1988), *Curatella* (Rech *et al*, in prep., Frankie 1976, Frankie *et al*. 1983), *Davilla* (Rech *et al*. 2011, Skutch 1971, Gottsberger 1977), *Doliocarpus* (Gottsberger 1977), *Tetracera* (de Voogd 1929, Willemstein 1987) e *Hibbertia* (Keighery 1991). O controle fisiológico da queda das pétalas e o significado adaptativo, caso haja, são tópicos interessantes para estudos das funções da corola na reprodução dessas plantas.

Outra característica compartilhada por todas as Dilleniaceae estudadas é a ausência de néctar (Endress 1997). Dessa forma o pólen é o único recurso disponível aos visitantes florais. A maior parte das espécies estudadas anteriormente possui anteras poricidas com heteranteria, indicando abelhas como um grupo de polinizadores importantes na história da evolução floral da família (Tuker e Bernhardt 2000, Endress 1997). As anteras das Dilleniaceae Neotropicais apresentam deiscência rimosa e as flores em geral apresentam um *bauplan* (“jeito”) bastante similar entre si, com flores do tipo aberto e pólen acessível aos visitantes (Figura 05). Dado que *Tetracera* e os gêneros Neotropicais tem derivação basal na família (Horn 2009) é bastante plausível pensar que a evolução de anteras poricidas tenha ocorrido dentro de um grupo polinizado por abelhas em geral.

Os dados aqui encontrados reforçam a perspectiva de abelhas como polinizadores importantes na família (Tuker e Bernhardt 2000). No entanto, baseando-se na atividade de *Apis mellifera*, e possivelmente outras espécies, nas flores de *Davilla grandiflora* é possível concluir que nem todas as abelhas são bons polinizadores (Rech *et al.* in prep., Westerkamp 1991, Garibaldi et al. 2013). Além disso, a diferença na duração do período de manipulação das anteras de *D. nitida* por *A. mellifera*, sugere que grande parte do pólen é retirada na primeira meia hora de visitação corroborando Rush *et al.* (1995) quanto ao papel de abelhas sociais na depleção de pólen. Essa retirada de pólen provavelmente tem impacto sobre a atividade dos demais visitantes que iniciam a visitação mais tarde (Dupont et al. 2004, Paini e Roberts 2005) e sobre o pólen necessário para polinização (Gross e Mackay 1998, Gross 2001). Nesse sentido, hipotetizamos que a evolução das anteras poricidas em Dilleniaceae pode ter reduzido não apenas a pilhagem por visitantes florais, mas inclusive a atividade de abelhas ineficientes na polinização (Hargreaves et al. 2009, Renner 1989). Mesmo nas espécies com anteras poricidas, ocorre retirada de pólen por algumas espécies de abelhas, no entanto, apenas aquelas com maiores tamanhos corporais podem atuar como polinizadores efetivos (Endress 1997).

Comparando a floração entre gêneros na família Dilleniaceae, Endress (1997) sugeriu que existem basicamente duas estratégias de floração: 1. *steady-state*, com poucas flores abertas concomitantemente e floradas que podem durar meses, como em *Dillenia suffruticosa* e, 2. *big-bang flowers*, que concentram a florada em eventos massivos e curtos como em *Tetracera nordtiana*. No caso das espécies estudadas, floradas massivas predominaram, sendo a única estratégia encontrada para *Doliocarpus*, *Pinzona* e *Tetracera*. Floradas massivas foram registradas também em *Curatella americana* (Rech *et al.* in prep.). Em *Davilla* ambas as estratégias foram registradas sendo a estratégia *steady-state* reportada para *D. grandiflora* e *D.*

pedicellaris e a estratégia massiva registrada em *D. nitida*, *D. lacunosa* nesse estudo, e também em *D. kunthii* (Rech *et al*. 2011). As duas estratégias parecem diretamente relacionadas ao número de flores por inflorescência e o tamanho dessas flores, características negativamente correlacionadas nesse estudo.

O número de flores em *Davilla* parece ser parcialmente explicado pela filogenia, ou seja, espécies proximamente relacionadas tendem a ter número de flores similar. No entanto, o tamanho das flores apresentou sinal filogenético muito fraco, o que sugere um potencial papel da polinização na seleção dessa característica, independente das relações filogenéticas. Corroborando essa hipótese, espécies com flores maiores e em menor número foram polinizadas por abelhas de tamanho médio a grande, enquanto as espécies de floração massiva e flores pequenas foram polinizadas por abelhas sociais (predominantemente), moscas e vespas (Rech *et al*. 2011, Croat 1978). O padrão morfológico e de visitantes florais teve apoio também no sistema reprodutivo, uma vez que a espécie com flores maiores é mais autoincompatível. No entanto, há que se ter cautela com essa explicação, pois mais espécies precisam ser estudadas.

Besouros possivelmente tiveram papel importante na evolução de Dilleniaceae, atuando como polinizadores que se alimentavam de partes florais e ovipositavam nas flores. Alguns autores propuseram besouros como principais polinizadores de diferentes espécies de Dilleniaceae, várias delas hoje sabidamente polinizadas por abelhas (Gottsberger 1977, Tuker e Bernhardt 2000). Em *Curatella* os besouros se alimentam de partes florais e copulam nas flores, no entanto, não ovipositam (Rech, unpublished data). Em *Davilla* os besouros apenas copulam e ovipõem entre os estames quando as sépalas se encontram em processo de fechamento. Após o fechamento das sépalas, que em *Davilla* formam uma câmara, as larvas se desenvolvem protegidas alimentando-se dos frutos/sementes em desenvolvimento. Parece haver, nesse caso, uma transição de florivoria para frugivoria. No entanto, vale ressaltar que as câmaras formadas pelas sépalas de *Davilla* servem como local de desenvolvimento de várias espécies de insetos e nem todas se alimentam dos frutos/sementes em desenvolvimento.

Concluímos com esse estudo que Dilleniaceae é uma família de plantas polinizadas majoritariamente por abelhas. A evolução de anteras poricidas e polinização por abelhas vibradoras, que ocorre nos gêneros mais derivados, ocorreu muito provavelmente a partir de espécies com anteras rimosas cuja polinização era feita por abelhas em geral. Na família são encontradas duas estratégias de floração e reprodução que se relacionam com os polinizadores e possivelmente representem o resultado de processos de divergência floral simpátricos, o que carece de ser testado apropriadamente.

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Tabelas

Tabela 01. Locais de estudo, coordenadas geográficas, temperatura e precipitação para cada uma das espécies de Dilleniaceae estudadas. TºC representa a temperatura dada em graus Celcius e Prec. a precipitação anual na área em milímetros.

Espécie	Local	Latitude	Longitude	TºC	Prec.
Tetracera wildenoviana	Manaus - Amazonas	3º 05’S	59º 58’ W	26.5	2194
Pinzona coriaceae	Presidente Figueiredo - Amazonas	2º 03’S	60º 06’ W	27.2	2939
Davilla rugosa	Chapada dos Guimarães – Mato Grosso	15°04' S	56°05' W	21.5	1517
Davilla lacunosa	Chapada dos Guimarães – Mato Grosso	15°04' S	56°05' W	21.5	1517
Davilla nitida	Ipiranga do Norte – Mato Grosso	12°15’ S	56°07’W	24	2000
Davilla pedicellaris	Santarém – Pará	2°29' S	54°57' W	25.4	2083
Doliocarpus brevipedicellatus	Santarém – Pará	2°29' S	54°57' W	25.4	2083
Doliocarpus dentatus	Ipiranga do Norte – Mato Grosso	12°15’ S	56°07’W	24	2000

Tabela 02. Resultados dos testes de polinização (%) para espécies de Dilleniaceae. Cross: polinização cruzada, self: autopolinização manual, autonomous: autopolinização espontânea, natural: polinização em condições naturais.

	cross	self	autonomous	natural
Davilla nitida	45	8	14	9
Davilla grandiflora	21	7	0	37
Davilla lacunosa	-	-	-	31
Doliocarpus brevipedicellatus	12	0	0	2.9
Doliocarpus dentatus	-	-	8	31

Figuras

Figura 1. Ilustração comparativa de *Davilla lacunosa* (à esquerda) e *D. grandiflora* (à direita). Em detalhe na parte superior os seus respectivos principais polinizadores, *Tetragona clavipes* (à esquerda) e *Euglossa* sp. (à direita) e na parte inferior o padrão contrastante em número e tamanho dos frutos. Ilustrador Pedro Lorenzo.

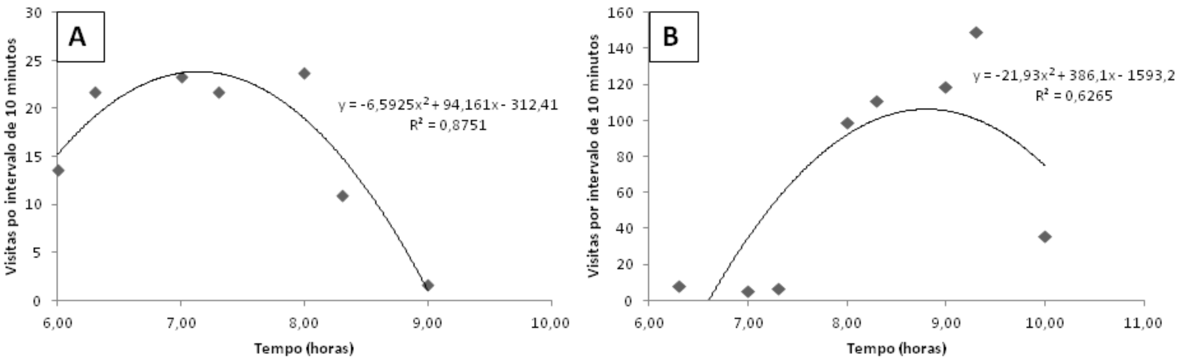

Figura 02. Padrão de visitação a intervalos de 10 minutos de observação em populações sincronopátricas de (A) *Davilla grandiflora* e (B) *D. lacunosa* em Mato Grosso

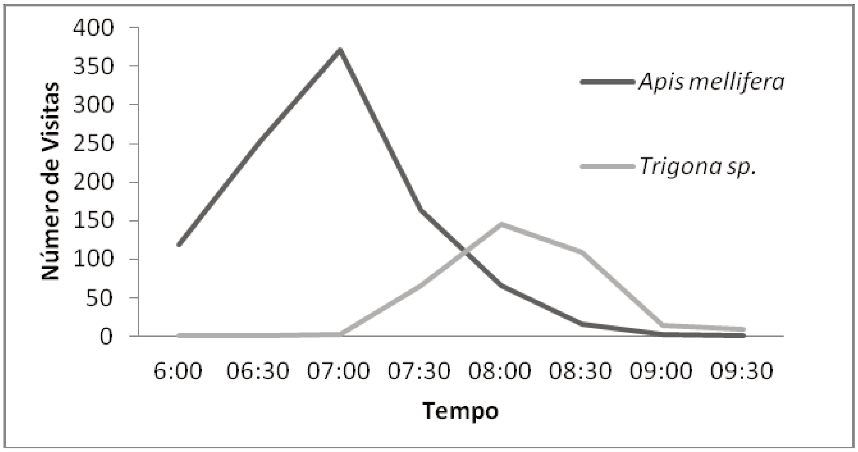

Figura 03. Padrão de forrageamento dos dois principais visitantes florais de *Davilla nitida* em Ipiranga do Norte – Mato Grosso.

Figura 04. Besouro curculionídeo visitando flor de *Davilla nitida* na qual oviposita em Mato Grosso

Capítulo 2. The effectiveness of different groups of insects as pollinators of *Knautia arvensis* (Caprifoliaceae), a generalist plant species with compound inflorescences

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Short title: Pollinator effectiveness in *Knautia arvensis*

Abstract: Interactions among plants and pollinators vary on a continuum from high specialization (a single pollinator species) to complete generalisation, in which many different groups of pollinators perform equivalent services. Moreover, these interactions vary in time and space, giving rise to very complex scenarios. Here we studied the pollination of *Knautia arvensis*, assessing pollinator effectiveness (proportion of stigmas with pollen after a single visit) combined with proportional visitation frequency in five different years. We also compared pollinator behaviour (time spent on flowers and flight distance) in two areas with different flower densities. *Knautia arvensis* flowers over a long period and produces very accessible and concentrated nectar. Pollination effectiveness varied between pollinator groups and the importance of the main pollinator group varied considerably between years. Different groups of pollinators showed different flight distances, and all were influenced by flower density. Butterflies were the best pollinators according to proportion of stigmas pollinated and flight distance, although their variable frequency prevented them from being the most important pollinators in all years. None of the proxies were equally good at predicting pollinator effectiveness nor are all of them universally applicable to all plant species. These traits makes the species attractive to many different groups of pollinators and reinforce the adaptive meaning of generalised pollination strategies when time is taken into account.

Introduction

Interactions between plants and flower visitors are highly complex and dynamic over space and time (Lamborn and Ollerton, 2000; Gómez 2002; Muchhala *et al.*, 2008; Alarcon *et al.*, 2008; Amorim *et al.*, 2012). For most plants, a number of species of flower visitors can potentially act as pollinators, though visitation frequency *per se* is no guarantee of pollinator effectiveness (e.g. Watts *et al.*, 2012, Sakamoto and Morinaga 2013). Quantifying the relative effectiveness of each flower visitor as a pollinator is difficult for small flowered species and studies have mainly been undertaken in plants that have relatively large flowers, presented singly or in few-flowered inflorescences (Waser et al. 1996, Ollerton et al. in prep.). Assessing pollinator effectiveness in plants with compound inflorescences is even more difficult, yet such plants belong to diverse, ecologically important families such as Asteraceae and Apiaceae (Olsen, 1997).

Plants within these families often (though not always - see Sazima and Machado, 1983) possess what appears to be highly generalised pollination systems involving diverse groups of flower visitors (Schemske, 1983; Torres and Galleto, 2002; Zych, 2007). Previous studies have tried to understand the role of these different taxa as pollinators in generalized systems and often used indirect proxies of effectiveness such as visitation rate (e.g. Lindsey, 1984; Lamborn and Ollerton, 2000) and insect pollen loads (Ollerton *et al.*, 2007a; Zych, 2002; 2007; Niemirski and Zych, 2011). There are few published studies which have assessed the comparative direct consequences of insect visitation for pollen deposition on stigmas in plants with compound inflorescences (though see King et al. 2013 which we discuss later).

Knautia arvensis (L.) Coult. (Caprifoliaceae) is a common European herb with compound inflorescences. Previous studies have indicated many different groups of flower visitors potentially working as pollinators (Knuth 1898a-c, Lack 1982, Jennersten 1984, Larsson 2005). The flowers of *K. arvensis* are protandrous and produces only a single seed (Larson 2005, Tutin et al. 1976). Studying the pollination of *K. arvensis*, Lack (1982) found strong differences in flower visitor assemblages between two years of observation and Larsson (2005) demonstrated differences in pollinator's capacities for pollen deposition. Based on these two studies here we have directly measured pollinator effectiveness of the diverse assemblage of visitors to the compound inflorescences of *Knautia arvensis* (L.) Coult. (Caprifoliaceae:

Dipsacoideae). We then combined this proxy for quality with visitation frequency in different years, aiming to understand the extent of inter-annual relative variation in pollinator importance of *K. arvensis*. We also discuss pollinator importance regarding to residence time on inflorescences, pollinator movements, flower density and flowering phenology.

Methods

Field observations and experimental manipulations were conducted during the late summers of 2001, 2006-2009, and 2012 on the Quarry Field and Scrub Field areas of the Bradlaugh Fields site in Northampton, central England (Ordnance Survey grid reference SP 765639). These sites are a matrix of mesotrophic grassland with calcareous floristic elements, overlying Jurassic (Great Oolite) limestone. The site is managed by the Wildlife Trust for Bedfordshire, Cambridgeshire and Northamptonshire.

Flowering time and flower visitor observations

Flowering season was monitored each year when quantitative data was taken. Flowers were collected, inflorescence diameter measured and the number of florets counted. We also took nectar from the flowers to measure the sugar content using a hand-held refractometer and microcapillary tubes (see Dafni et al. 2005).

Pollinator effectiveness

In order to calculate pollinator effectiveness we measured the frequency of insect visitation and visitation efficacy given by pollen deposition on virgin stigmas (Freitas 2013 and references therein.). Virgin flowers were obtained by bringing cut inflorescences in bud to the lab and waited their anthesis and the receptive female phase (Larsson 2005). Then the inflorescences were transported back to field and presented to the visitors for one single visit at the end of a 0.8 metre hand-held pole. The identity of the insect was recorded and duration of the visit was measured with a stopwatch. After visitation, flowers were checked for pollen deposition under a stereomicroscope in the field. We counted the number of stigmas per inflorescence with pollen as a measure of pollen deposition. Flower visitors were categorised according their broad taxonomical groups to test their collective ability for pollen deposition, as the focus of much discussion about the effectiveness of visitors to generalist flowers has been on “functional groups” of pollinators (e.g. Lamborn and Ollerton 2000, Fenster et al. 2004, Brunet and Sweet 2006).

We used the abundance of the different groups of insects in each year (I) and the proportion of stigmas with pollen after a single visit to an inflorescence (E) as components of pollinator quantity and quality (Herrera 1987, Olsen 1997, Ne’eman et al. 2010, King et al. 2013). Considering these two parameters we calculated an index of pollinator effectiveness (PE) for each one of the groups of insects as follows:

$$PE = I * E$$

Results are shown as the proportional annual contribution of each group to *K. arvensis* pollination.

Flight distances and residence times of flower visitors

For the three main groups of flower visitors (see Results) we separately measured the time spent on an inflorescence and the distance they flew when they departed from one inflorescence to another. The flight distance was categorized as a scale from 0 to 4 representing the following: 1) 0 - 30cm; 2) 31 - 100cm; 3) 101 - 500cm; and 4) more than 500cm. We also recorded the movement of visitors to another inflorescence of *Knautia arvensis*, and to the inflorescences of other species. In order to test whether the results are a property of the flower visitors' behaviour and not due to flower density, we collected the same data in two meadows with different overall floral densities. To quantify the floral density at each site, we took 24 photographs (covering around 1.5 m^2 each) of the plants on the ground associated with a reference rule. Later, using ImageJ software (Abramoff et al. 2004), we calculated the precise photographed area and the number of flowers per unit area.

Data analysis

We compared floral density between areas using a t-test. To model time spent on flowers and flight distance, we used Generalized Linear Models (GLM), according to both, the site and the functional group of pollinator. As time on flowers was highly skewed, we used the logarithm of time in a Gaussian model. In addition, since the data for distance were collected according to four categories, we used a Poisson distribution. Models were also tested for single visit deposition, using stigmas with pollen as the response variable and pollinator functional group, time (log transformed) spent on inflorescence and number of flowers within one inflorescence as predictors. The intercept was considered the null model. Again GLM was used to model the number of stigmas with pollen after a single visitation and data distribution was better adjusted to a negative binomial curve. In order to compare the generated models we used the Akaike information criterion (AIC), and to distinguish differences between levels of each effect inside the best model we used a Tukey multiple comparisons test. All means are presented as ± standard deviation. All analyses were performed in R (RDCT 2008).

RESULTS

Flowering time, floral biology and reproductive success

In all years *Knautia arvensis* flowered in the study area from July to October, with the peak of open flowers in the middle of this period. Inflorescences were on average 43.4±3.8mm in diameter and each capitulum had a mean of 23.7±5.7 ray florets and 58.9± 13.6 disc florets. Inflorescence density was approximately 1.5±2.1 inflorescences per m^{-2} within areas with low flower density (Quarry Field) and 4.7±4.4 within areas of high flower density (Scrub Field); this three-fold average difference was statistically significantly different ($t = 5.68$, $df = 47$, $p< 0.0001$) and we used this natural variation in inflorescence density as the basis for our observations of pollinator movements (see below). Mean nectar production changed from 0.29 ±0.07 µl and sugar concentration greater than 50% (n:10) at the first day of the anthesis, to 0.36 ±0.15 µl and 44% (n: 13) at the second day and 0.48 ±0.32 and 45% (n: 17) at the third day (female phase).

Flower visitors

More than 20 species of visitors were classified as potential pollinators and they represented six different orders: Hymenoptera, Lepidoptera, Diptera, Coleoptera, Hemiptera and Mecoptera (Figure 1). Bumblebees, hoverflies, and butterflies and day-flying moths had the greatest visitation frequency in most years, although their relative proportions varied significantly between years (Figure 2). The large proportion of other visitors in 2007 was due to an increase in numbers of soldier beetles (*Rhagonycha fulva*).

Pollinator effectiveness

The model that best explained the variation in the number of stigmas with pollen after a single visit was the one including number of stigmas and visitor functional group (Table 1). This means that the main three groups of visitors were not equally efficient in the proportion of receptive stigmas pollinated (Figure 3). Combining the ability of depositing pollen (pollinator effectiveness - E) with the data on relative abundance of pollinators (I), it is clear that the relative importance of the main groups of flower visitors as pollinators varies between years (Figure 4).

Flight distances and residence times of insects in sites with contrasting *Knautia* inflorescence density

Flight distance between inflorescences was more likely explained by the statistical models considering flower density and functional group of pollinator without interaction (Table 2). Therefore bees, hoverflies and butterflies have different flight distances and they fly different distances in sites of low and high flower density. Moreover, changes in flower density do not change the general trend (Figure 5 A and B), which is why interaction between the two factors was not included in the best model. Bumblebees and hoverflies, in particular, concentrated more than 50% of their flights to flowers closer than 30cm. Meanwhile, Lepidoptera flew the larger distances between flowers. Residence time on flowers was included into the second most likely model, but when considered alone it performed worse than the null model to explain flight distance.

Bumblebees were the group with the shortest residence times and butterflies spent the longest time on inflorescences (Figure 6). For the analysis of residence time on inflorescences the model taking only the pollinator functional group into account was the most likely, with the models taking also the location with and without interaction almost as likely (Table 3). Also, when taken alone, site was very unlikely, therefore we considered only the pollinator group effect as significant and took the simplest model as the most likely.

Discussion

The flowering period of *Knautia arvensis* lasts for three months and inflorescences provide a rich nectar reward. These two aspects make the flowers of *K. arvensis* very attractive to a wide range of different groups of pollinators (Knuth, 1898a, b, c; Lack 1982; Larsson 2005, Ebeling *et al*., 2008). In relation to the classification of pollination systems provided by Ollerton et al. (2007b), *K. arvensis* could be considered generalised according to all parameters: phenotypicaly generalised because flower morphology, although tubular, allows all visitors to access nectar; ecologically generalised because the number of pollinator species is high; and functionally generalised because they belong to several groups of unrelated taxa.

Although traditionally Lepidoptera have been regarded as ineffective pollinators (Morse, 1982; Percival, 1965; Wiklund *et al*., 1979) we found them as an important group of pollinators of *K. arvensis* in 2008. Jennersten (1984) discussed the poor value of Lepidoptera as pollinators in Sweden but pointed out that this might not be true for *Knautia arvensis*, where butterflies accounted for around 60% of the flower visitors. He also recorded Coleoptera (around 10%), Diptera (around 5%) and bees (around 25%) as flower visitors of *K. arvensis*. Given the protandry of *K. arvensis*, the flower visitors collecting nectar will also be more likely to be pollinators, because they visited more equally male and female phase flowers than the exclusively pollen eaters (Jennersten, 1984, Larsson 2005). Larsson (2005) hypothesised that female flower should produce more nectar to compensate the lack of pollen and consequent decrease in visitation rate, and this is exactly the trend we found here, with nectar production increasing over the inflorescence's life span.

Comparing pollinators of *K. arvensis* in Sweden, Larsson (2005) found differences between groups of flower visitors and even within groups, between bumblebees and solitary bees. Although solitary bees deposited larger amounts of pollen per visit, they visited mainly male flowers and also showed a general low visitation frequency. Moreover, bumblebees and hoverflies were much more frequent and visited male and female flowers more equally, which resulted in them being better pollinators. Lack (1982) found the flowers of *K. arvensis* visited mainly by bumblebees, and also by Diptera, Coleoptera and Lepidoptera. Moreover, in one year of his study (1979), he found almost exclusively butterflies visiting the flowers of *K. arvensis* (Lack, 1982). We also showed here that groups of visitors perform differently as pollinators and that their importance changes over time. These results strongly corroborate the adaptive value of generalized pollination systems improving the resistance to failure of the plants using this reproductive strategy (Waser et al. 1996, Wolowski et al. 2014).

In *Knautia arvensis* the mean inbreeding depression was 58% in offspring produced by self-pollination (Vange, 2002). Self-pollination is prevented in hermaphroditic blossoms by protandry, but the possibility of geitonogamy remains, as different inflorescences within an individual could be in different sexual phases at a given time (Eckert, 2000, Vange 2002). Therefore pollinator behaviour becomes crucial as the increase in flight distance among visited inflorescences will improve the probability of cross-pollination (Larsson, 2005). Our study showed that functional groups of pollinators differed according to their flight distance among flowers. Day-flying Lepidoptera were the group flying further, and bumblebees the ones that flew shorter distances, normally to the closest flowers (Figure 5 A and B). The same tendency was also recorded by Larsson (2005), studying *K. arvensis* in Sweden. Therefore, any interpretation of pollinator importance based only on visitation frequency and pollen deposition still needs to be treated with caution (Ivey et al. 2003, Larsson 2005).

Previous studies have suggested that lepidopterans are the flower-visiting European insects that fly the largest distances between flowers, therefore achieving a higher quality of pollen dispersal for the recipient plants (Murawski and Gilbert, 1986; Herrera, 1987; Larsson 2005). In contrasting, bumblebees have been noted often visiting the closest flowers (Zimmerman, 1979; Collevati et al., 2000), increasing geitonogamy and lessening their effectiveness as good quality pollinators for self-incompatible species (Larsson 2005). The differing natural histories of these visitors could explain their different flight distances. Bees are central-place foragers that use nectar and pollen for provisioning nests, and therefore optimal foraging could explain their behaviour of stopping and checking all inflorescences within a given area. Lepidopterans in contrast are nomad insects that use flowers as refuelling places during their journeys to find sexual partners and places to lay eggs.

Relatively few studies have experimentally compared pollination effectiveness among flower visitors in generalised pollination systems (Rech and Ollerton unpublished data). Some of these studies have found differences among pollinators, although they did not find morphological matches to support the most effective pollinator principle (Stebbins 1970; Aigner, 2001). King et al. (2013) claimed they found such a correspondence between the predicted group according to flower morphology and the effective pollinator observed. In their work they defined a bee/hoverfly syndrome for *K. arvensis* even though this syndrome has never been formally described (Faegri and van der Pijl 1979). Whilst we agree with King et al. (2013 and many others who have discussed this) that effective pollinator identity must be determined in order to understand floral evolution, we believe that they under-estimate the difficulty of assessing every species of pollinator for highly generalised plants, especially if time and space will be taken into account. For example, for *Knautia arvensis* they included only eight species of flower visitor from three functional/taxonomic groups, whereas we know that the flower visitors to *K. arvensis* include at least 30 species from six taxonomic groups. Likewise, King et al. (2013) recorded only 10 visitors species for *Heracleum sphondylium*, though this is one of the most generalised plants in the European flora with a list of flower visitors that contains at least 100 species, with considerable spatio-temporal variability (Zych 2007, Ollerton unpublished data). Considering that flower phenotype is a result of processes operating over both time and space, a single picture of one year (with poor weather as the authors pointed out in the paper) and a single population need to be taken very carefully when used to understand the evolution of floral phenotype.

In conclusion, we consider *K. arvensis* to be a species with a highly generalised pollination system that cannot be fitted into one of the classical syndromes. Moreover, our study shows that different proxies of pollinator effectiveness must be used for different species. For example, time spent on inflorescences was a good predictor of pollinator effectiveness in *Asclepias incarnata* (Ivey et al. 2003), while it is not for *K. arvensis* and other species (King et al. 2013). Flower visitor frequency correlated with effectiveness in *R. raphanistrum* while it was a poor predictor in many other species (Johnson and Steiner 2000). Nonetheless, the amount of pollen deposited on stigmas (normally measured as single visit pollen deposition) will only be useful for plants with more than one ovule, otherwise, number of stigmas with pollen (as we measured here) will be more informative. Understanding the pollination ecology of highly generalised and ecology important species such as *K. arvensis* requires careful study over multiple populations and years using adequate proxies for pollinator effectiveness and frequency, which is not a simple task.

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Tables

Table 01. Results of the model selection for factors determining pollen deposition on stigmas of *Knautia arvensis*. Visitor are grouped as bumblebees, flies, day flying Lepidoptera, and other visitors are grouped as others, time is log transformed and number of stigmas per inflorescence is used as control for different sized inflorescences. The full model included the fixed factors: visitor group, time spent on flower, number of stigmas and the possible interaction between time and visitor group. Null model is only the intercept.

Model	dAIC	Degrees of freedom
Visitor + Number of stigmas	0.0	7
Full model (visitor, stigmas, time and interaction)	1.1	11
Stigmas + visitor + time	1.9	8
Only time	20.4	3
Visitor + time	24.3	7
Only visitor group	24.6	6
Only number of stigmas	25.4	10
Null model	128.0	1

Table 02. Results of the model selection for pollinator flight distance after visiting inflorescences of *Knautia arvensis*. Visitor are grouped as bumblebees, flies and day flying Lepidoptera; site refers to places with low and high flower densities and residence time refers to the time spent on flowers before flight departure.

Model	AIC	Degrees of Freedom
Site and visitor without interaction	0.0	4
Site, visitor and residence time	0.8	5
Site	1.3	2
Visitor	3.7	3
Site and visitor with interaction	4.0	6
Null	4.6	1
Distance and residence time	6.5	2

Table 03. Results of the model selection for residence time on inflorescences of *Knautia arvensis*. Visitor are grouped as bumblebees, flies and day flying Lepidoptera, and site refers to two sites with low and high flower densities.

Model	AIC	Degrees of Freedom
Only visitor	0.0	4
Visitor and site plus interaction	0.5	7
Visitor and site without interaction	0.6	5
Only site	85.6	3
Null	85.6	2

Figures

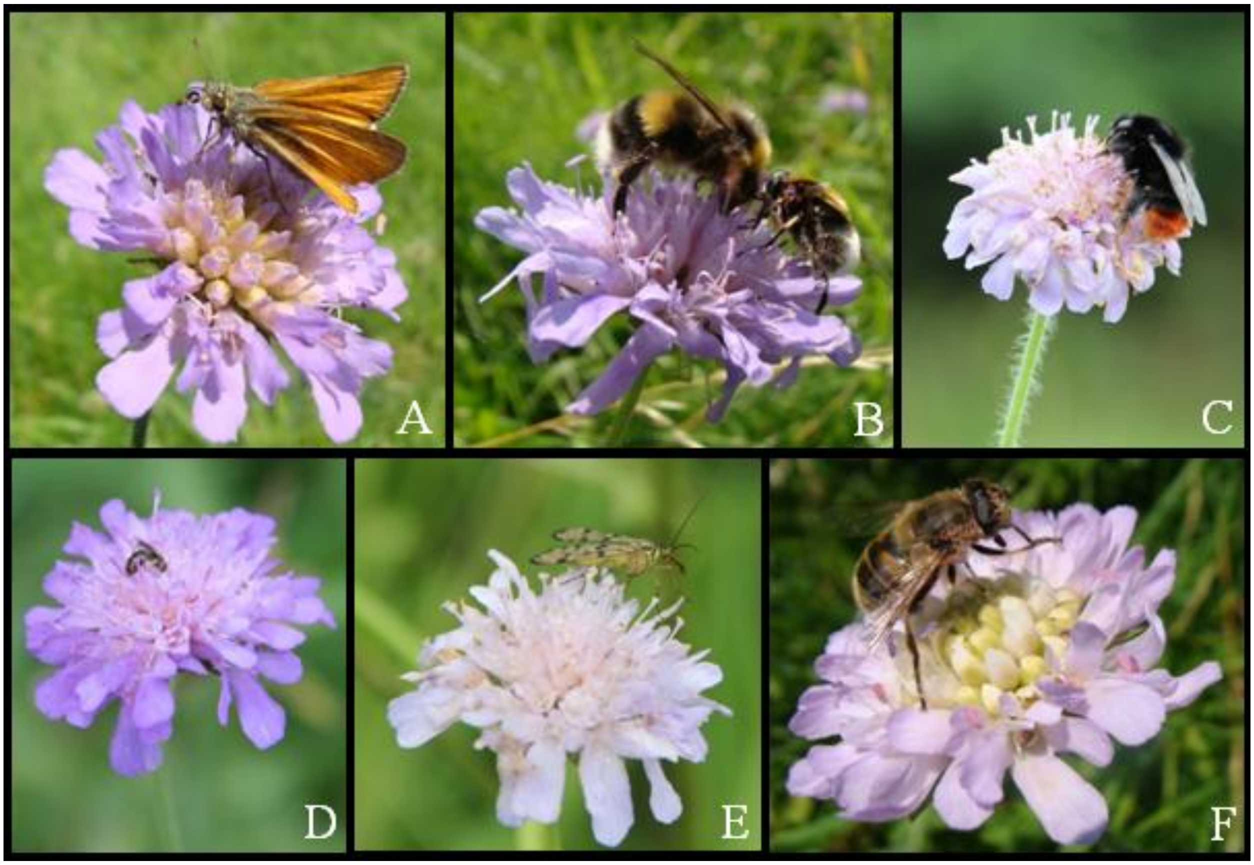

Figure 1 – Examples of flower visitors of *Knautia arvensis* in Northampton,UK. A. A butterfly, B. A bumblebee at the left and a hoverfly at the right side. C. A bumblebee, D. A Halictidae, E. A Mecoptera and, F. A hoverfly.

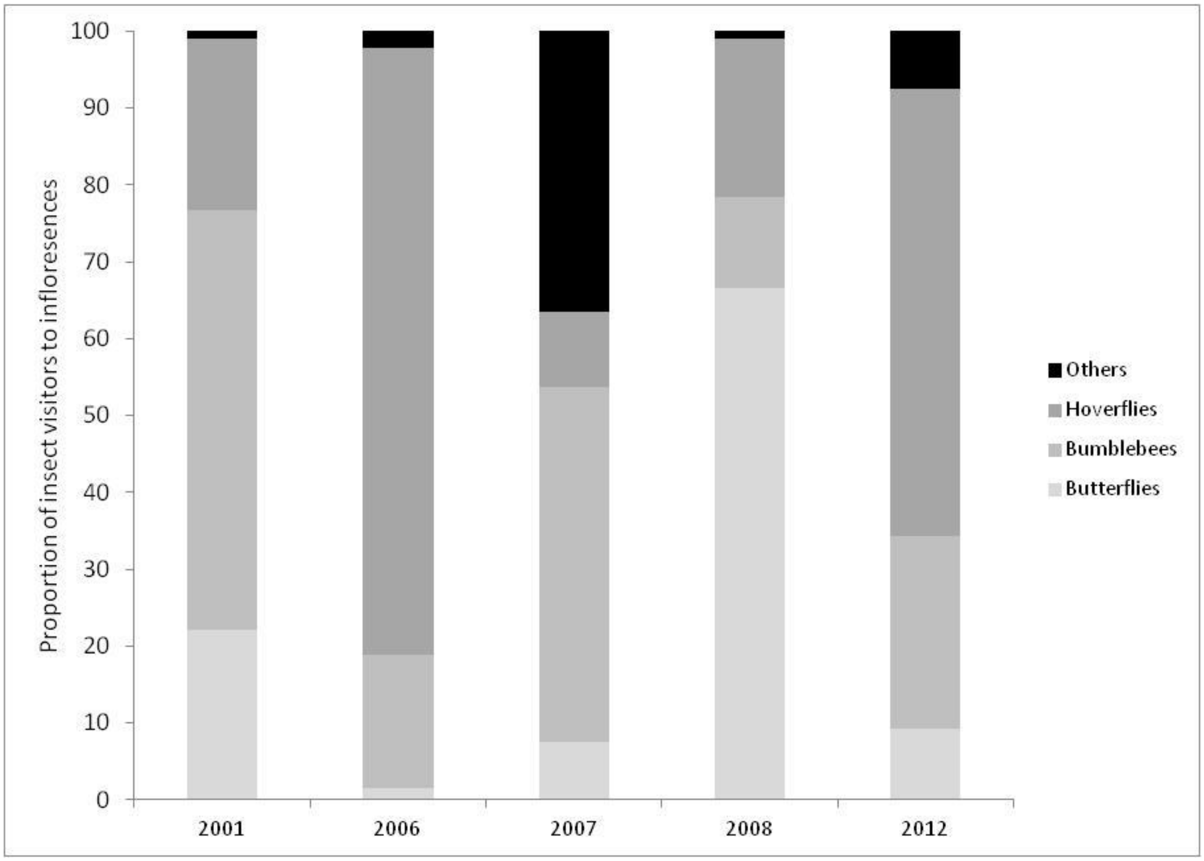

Figure 2 - Variation in frequency of the most common flower visiting groups to *Knautia arvensis* over the five years of sampling in Northampton - UK.

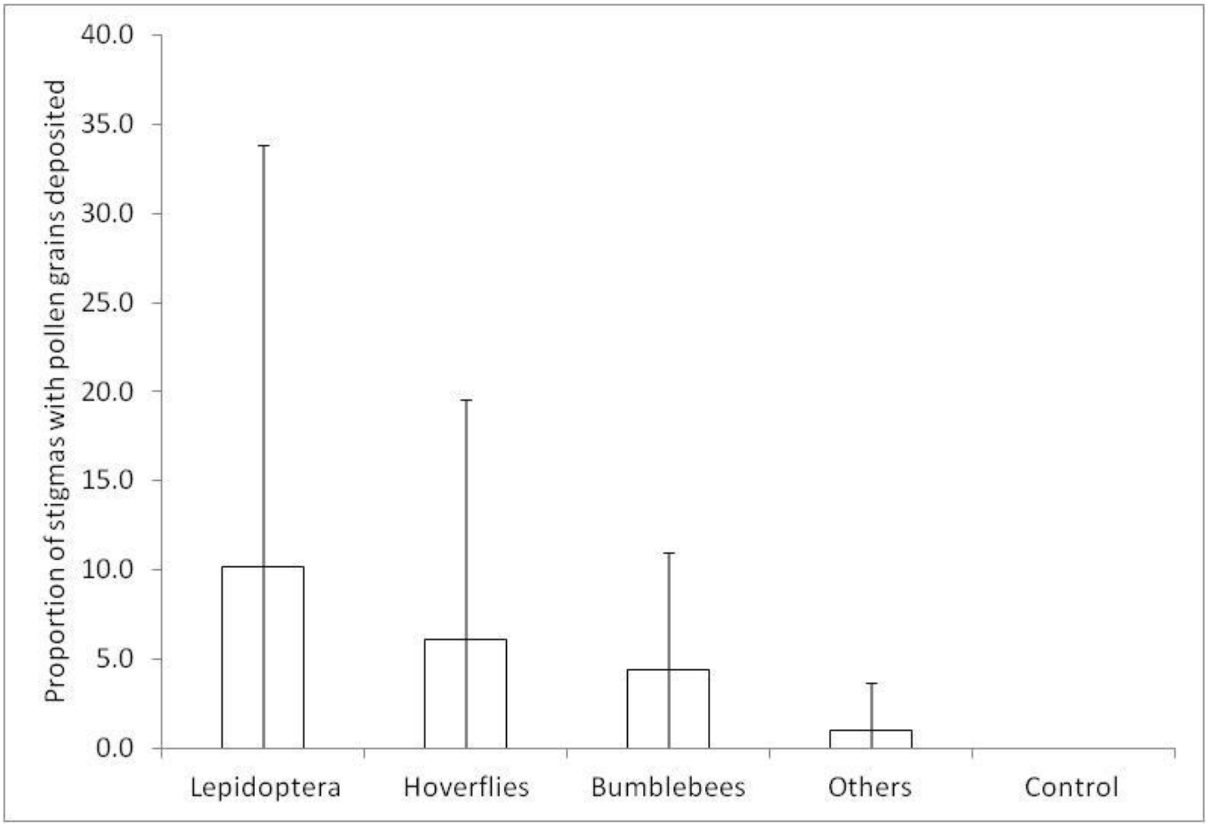

Figure 3 - Average proportion of stigmas with pollen grains after a single visit to an inflorescences of *Knautia arvensis* in Northampton – United Kingdom.

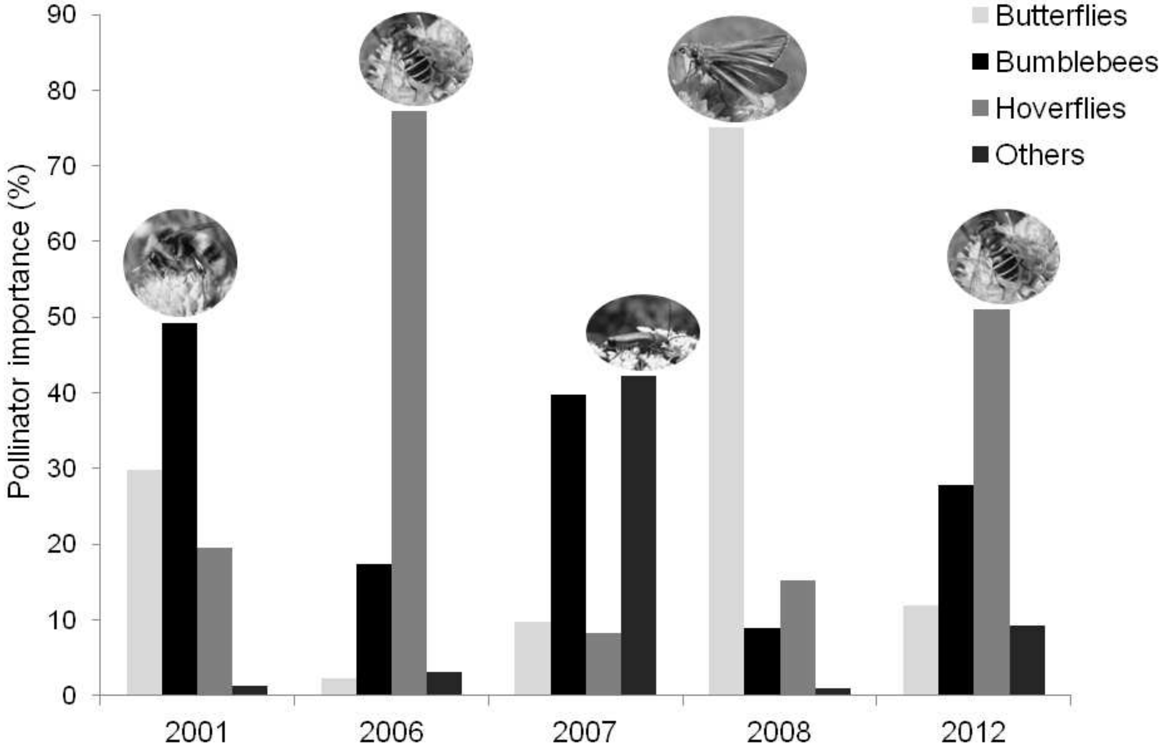

Figure 04 – Relative annual importance of each pollinator group to the pollination of *Knautia arvensis* in Northampton – United Kingdom. Importance is given by pollinator abundance and effectiveness (number of stigmas with pollen after a single visit). The most important pollinator in each year is shown with a picture.

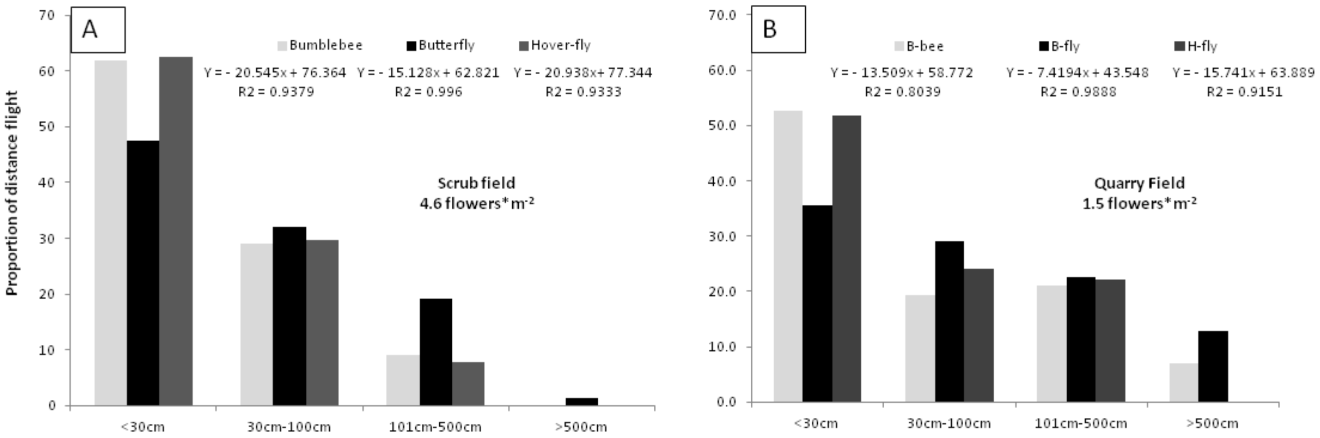

Figure 05 – Proportion of the average flight distances of the three major groups of pollinators in an area with (A) high and (B) low density of inflorescences of *Knautia arvensis*.

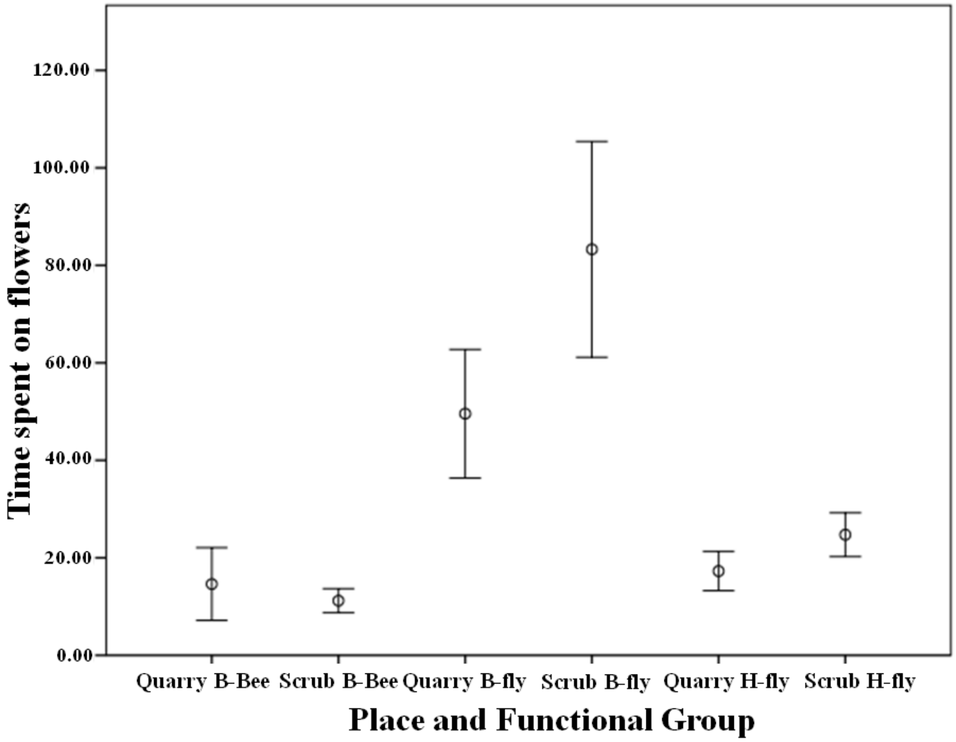

Figure 06 - Average time spent on flowers by the functional groups of pollinators of *Knautia arvensis* in two sites with different inflorescence densities in Northampton – United Kingdom. Quarry Field is the place with low flower density opposite to Scrub Field. B-bee: bumblebee, B-fly: Butterfly and H-fly: Hoverfly.

Capítulo 3. Geographical variation in flower morphology and reproduction in the Neotropical savannah tree *Curatella americana* (Dilleniaceae)

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Abstract: Widely distributed organisms face different ecological scenarios over their range, potentially leading to inter-population micro-evolutionary differentiation. In animal pollinated plants, flower morphology and mating systems are supposed to evolve in association with spatial variation on pollinator assemblages. When pollinators are scarce, autogamy (spontaneous self-pollination) has been claimed to assure reproduction, allowing plant colonization and population establishment, but this has rarely been tested over large spatial scales for trees. Here we used a tropical tree (*Curatella americana*) in order to test whether mating system and flower morphology are related to the pollination context across Brazilian savannahs. We compared the fruit set with and without pollinators in the field, and analysed pollen tube growth from self and cross-pollinated flowers. Higher natural fruit set was associated with lower fruit set in bagged flowers. Also, higher levels of autogamy in the field experiments were shown to be the result of larger numbers of pollen tubes growing in self-pollinated flowers, indicating more self-compatibility in these populations. Morphometric studies of floral and leaf traits indicate that populations with more autogamy had less herkogamy and a larger stigma area, while populations with higher natural pollination had larger anthers. We found a similar pattern of spatial variation in mating system, flower morphology and pollination as previously described for herbs, reemphasizing that mating system is a population based attribute varying according to the ecological situation where plants occur.

Keywords: Cerrado savannah, mating system, reproductive insurance, self-compatibility

Introduction

The ecological interactions established by a given organism can be highly variable over space and through the time (Thompson 2005). Plants, for instance, can interact with contrasting assemblages of pollinators over their geographical range (Waser et al. 1996, Herrera 2005, Gómez *et al*. 2014). These patterns can vary from specialised to generalised for both plants and animals, and can have strong implications to the plant mating system (Gómez 2002, Dart *et al*. 2012, Barrett 2013). However, an overview of the literature reveals that mating systems are normally considered an attribute of species (Brys and Jacquemyn 2011, Levin 2012, Rosa-Guerreros 2014). Meanwhile, it is reasonable to consider the mating system as a result of opposing demands for genetic variability, promoted by cross-pollination, and the reproductive assurance given by self-pollination (Herlihy and Eckert, 2002). The result is normally a mixed mating system, using the "best of two worlds"; and populations facing different ecological scenarios commonly show different levels of autogamy (Kalisz and Vogler 2003, Goodwillie 2005, Barret 2013).

To rely upon the "best of two worlds" and oscillate between the two extremes as the environment demands is a part of the reproductive assurance hypothesis proposed by Darwin (1876) and later re-elaborated (Baker and Stebbins 1965, Baker 1967). According to this hypothesis, mechanisms ensuring autogamy should be favoured in areas of low pollinator availability (Herlihy and Eckert 2002). This idea was first formally applied to islands, where there is expected to be low colonization by self-incompatible or dioecious plants, and has been called "Baker's rule" (Baker 1967, Cheptou 2011). The rule was later broadened including any area with unpredictable pollinators (Kalisz and Vogler 2003). It is known that factors such as small population size (normally found in populations growing on the edge of the species distribution) are likely to promote higher levels of autogamy (Griffin and Willi 2014, Ivey and Carr 2012, Levin 2012). Moreover, not only mating systems but even the sex of an individual can also vary through its life, showing how flexible plant reproductive strategies can be (Ivey and Carr, 2012; Eckert et al. 2009; Ehlers and Bataillon, 2007; Carroll *et al* 2001). Therefore, because climate and population size change over time, mating system and sex expression should also track these changes at the population level.

Changes in mating system are normally associated with variation in a group of flower traits (Berg 1960, Armbruster et al. 1999); for example, transitions to higher levels of autogamy are usually primarily related to reductions in flower size and herkogamy (Wyatt, 1988), flower development time (Armbruster et al., 2002; Mazer et al., 2004) and early flowering in the season (Mazer et al., 2004; Martin and Willis, 2007). Other flower traits, such as reduction in rewards, attractiveness, flowering time and display (Ornduff, 1969; Dudley et al., 2007), are hypothesised to follow the primary modifications. However, most of these variation trends were detected by comparing different species and not populations of the same species (though see Ollerton et al. 2012). Therefore, widely distributed species with flower morphology variation are potentially good models to add to our understanding of the evolution of autogamy and flower morphology (Brunet and Eckert, 1998; Brunet and Sweet, 2006; Herlihy and Eckert, 2007).

Curatella americana L. (Dilleniaceae) is a widely distributed species in Neotropical open vegetation areas such as the Brazilian cerrado (Bruniera and Groppo 2010). The species is reported from southern Mexico to the southern limits of the tropics, and is one of the most common trees across its distribution (Ratter *et al*. 2003). Here, we studied populations of *C. americana* from three disjunct areas of savannah in Brazil and described them according to floral morphology and the level of autogamy and self-compatibility. We tested the following hypotheses: 1. the level of autogamy will be highest in populations with lower levels of natural pollination (indication of pollinator availability and pollen limitation); 2. populations in the smaller areas or at the distribution edge will have higher levels of autogamy; 3. populations with higher levels of autogamy will show less herkogamy and smaller flowers.

Material and Methods

Study site

We studied floral biology and morphology, mating system and pollen tube growth following controlled pollination tests in the Neotropical savannah tree *Curatella americana*. Six out of ten studied populations were in the larger and continuous area of Brazilian savannah (cerrado), hereafter called Central Brazil; three belonged to the disjunct area of Roraima, hereafter referred as North; and one is a fragment of savannah surrounded by Amazon forest hereafter called Santarém (Table 1, Figure 1). Populations nine and ten (Jatai and Caldas Novas;

Figure 1) belong to the Central Brazil region but occurred at the southern edge of *C. americana* distribution in Brazil.

Floral biology and autogamy were studied in all populations, but some of the other experiments were carried in a subset of the populations. Morphology was studied in five population in the three regions (2, 3, 4, 5 and 6; Figure 1). The closest populations were separated by 100 kilometres and the furthest ones were more than 2,700 kilometres apart from each other.

Floral biology

The floral biology of *C. americana* was observed *in situ*, and we recorded time, sequence and duration of anthesis, flower longevity, odour emission, and pollen availability following Dafni *et al.* (2005). Hydrogen peroxide was dropped onto the stigmas, then checked for bubbles as an indication of viability (Dafni *et al.* 2005). The location of the osmophores was checked visually after immersing flowers in a solution of neutral red (Dafni *et al.* 2005).

Flowers were collected and fixed in 70% alcohol for later measurements in the lab. Flower variation in morphology was evaluated in five populations (2, 3, 4, 5 and 6; Figure 1). The length of petal, sepal, stamen (total and anther), pistil (total and stigma area) and the distance between the two stigmas in one flower were measured in 15 individuals per population (one measure per individual) using a digital calliper. Shape of petal, sepal, ovary and leaf (as control) was analysed using geometric morphometrics (Bookstein, 1991). Landmarks and semi-landmarks were placed on scanned structures using the software TPS Dig (Figure 02 – Rohlf, 2000). We used six leaves per individual and 10 individuals in each population. For the floral traits, we took three measures for each individual and five individuals per population. Other aspects of floral biology were observed and noted in the field.

Pollinator importance

In order to check for fruit set without pollinators (autogamy) we bagged inflorescences (minimum 20 flowers each) in at least 10 individuals per population. The fruit set from autogamy was compared to the fruit set from opened flowers exposed to flower visitation (natural pollination). Given that natural pollination and visitation rate were highly correlated (Rech *et al.* in prep.), we used the difference between both treatments (NP – AU) as a proxy of pollinator importance in each population. The standard deviation in the results of natural pollination and autogamy was used as a measure of inter-individual variation in each area. In order to check for the possibility of self-pollen deposition, we also bagged flowers one day before anthesis and then collected one stigma per flower as soon as it opened and another one four hours later. Each flower has two stigmas at the same height, which makes the test perfectly paired. The stigmas were directly deposited on slides with stained jelly, covered with cover slips and later had pollen quantified using microscopy.

Pollen tubes

In order to check the pollen tube growth through the gynaecium, we collected hand-pollinated pistils (cross and self-pollinated) from ten individuals in each population. The pistils were fixed in FAA (Formalin-Acetic-Alcohol) + 50% ethanol, 24 hours after hand pollination. The pistils were prepared according to the technique proposed by Martin (1959), stained using a solution of aniline blue and observed under fluorescent microscopy. Because the pistils were covered by trichomes, we pre-treated them in a solution of sodium hypochlorite in order to facilitate trichome removal. The results were analysed visually under optical fluorescence microscopy.

Data analysis

We decomposed the form of all the landmark configurations into shape and size by means of geometric morphometrics (Bookstein, 1991). Size was measured as centroid size (CS), the square root of the sum of the squared distances of each landmark from the centroid, or gravity center, of the landmark configuration. To measure shape, all configurations were scaled to unit CS, and superimposed by a generalized least squares (GLS) procrustes procedure. Given the presence of semi-landmarks, a sliding procedure minimizing the GLS residuals was used, as the exact location of the semi-landmarks along the structure outline is arbitrary. A mean shape was calculated and the differences between its landmarks and the ones of each individual were the residuals of the GLS procedure. We used the Relative Warps as shape variables (the axes of a Principal Components Analysis - PCA) on the covariance matrix of the GLS residuals. The four last axes are null, given that dimensionality lost in the procrustes superimposition. This superimposition was held using the software TPS relative warps version 1.53 (Rohlf 1998) whose axis were then used to evaluate the interpopulation variation. To test for differences in shape among the regions we performed a Multivariate Analysis of Variance (MANOVA) on the N relative warps using a hierarchically nested design, with replications nested within individuals, nested in populations and regions. For the linear measurements we used an Analysis of Variance (ANOVA) and when the result were significant, the means were further differentiated using a Tukey test.

In order to compare the number of pollen grains on the stigmas we used a paired t-test. Because flowers had two pistils, one stigma was collected as soon as the flower opened and another one at the end of anthesis, giving a controlled paired situation. The analyses were carried out using R software (RDCT 2008).

Results

Floral biology

Curatella americana exhibited a mass flowering pattern from June to September in Central Brazil (populations 5-10; Figure 1), from September to October in Santarém (population 4), and from October to November in the North (populations 1-3). Flowers are pentamerous, actinomorphic and open (plate type), grouped in panicles, white to the human eyes, and do not reflect ultra-violet light (Figure 3). The odour is sweet and the osmophores are in the margin of the petals. Flowers opened between 04:30h and 06:30h, but temperatures below 18ºC delayed the process, and below 15ºC prevented flower opening. Pollen was released between 30 minutes and an hour after flower opening. Stigmas were receptive during all the time flowers were open (around seven hours). Sepals do not close as a chamber and most of the petals remain attached to the flower over anthesis. By the first time for *C. americana* we registered: in the North (population 3, Boa Vista) one female flowered individual showing only rudiments of anthers without pollen; in Central Brazil (Nova Xavantina) two individuals with staminate and hermaphrodite flowers.

Pollination and pollen tubes

The difference in fruit set between autogamy and natural pollination was higher in Central Brazil ($\overline{x}$ = 39%), than in Santarém (x = 23%) or in the North ($\overline{x}$ = 0.06, see Table 2 for population average values). Inter-individual variation (standard deviation) was higher for autogamy than in natural pollination. The amount of pollen on stigmas increased from the beginning to the end of the anthesis in bagged flowers (t = -4.962, $p < 0.001$; Figure 4) indicating spontaneous self-pollination in all populations. Nevertheless the level of autogamy varied considerably among populations (Table 2). The reason behind fruit set differences could be clearly seen in the way pollen tubes grew through the style. Higher numbers of pollen tubes grew regularly and continuously through the styles of cross pollinated flowers, whilst there were fewer pollen tubes growing in self-pollinated flowers. Pollen tubes in self-pollinated flowers grew irregularly (strong callose reactions) and clearly more contrasting with cross-pollination in Central Brazil than in the North (Figure 5a-5d). Most of the pollen tubes in self-pollinated flowers stopped growing in the first two thirds (2/3) of the style. Only in self-pollinated flowers from the North was it possible to see pollen tubes in self-pollinated flowers reaching the ovule (Figure 5e-5f).

Morphometrics

Geometric morphometrics revealed regional differences in shape for all traits analysed (petal, sepal, ovary and leaf) even though the proportion of the total variation that was among regions was different for each trait (check F values, Table 3). Petals and sepals, for instance, varied less than leaves and ovaries, as can be seen in Figure 6. Considering only the first two axes of the PCA, it is not possible to visualize the differences in shape for petals and sepals according to the region they come from (Figure 6). For leaves and ovaries it is possible to see a gradient of variation, following latitude with Santarém, situated in the middle in terms of geography, also in between for shape variation (Figure 6).

The number of stamens did not vary among regions (Table 4). There was a difference in ginoecium lenght, but only the North and Central Brazil could be separated, Santarém remained in between and similar to both (Figure 7a). Stigma area was larger in the North, whereas anthers were larger in Central Brazil (Figure 7b and 7c). Santarém was similar to Central Brazil regarding to anther size and to the North regarding stigma area (Table 4, Figure 7b and 7c).

Discussion

Floral biology

The mass flowering pattern showed by *C. americana* was already reported for *Davilla kunthii*, another species of Neotropical Dilleniaceae studied in central Amazon (Rech et al. 2011). Apart from the colour, white in *C. americana* and yellow in *D. kunthii*, both have similar floral biology, pollination ecology, and mixed mating systems (Rech et al. 2011). The open flower morphology with exposed anthers makes it easy for the flower visitors to contact anthers and stigmas in a single visit, what can be regarded as a phenotypicaly generalized pollination system (*sensu* Ollerton et al. 2007). Pollen as the unique reward and the white UV absorbing pattern found in the petals, indicates a flower attractive to bees (Lunau et al. 2011). Field observations actually revealed bees as the main flower visitors of *C. americana* in all sites where visitors were present (Rech *et al.* in prep.). Some populations showed a very low level of natural pollination indicating low contemporary availability of pollinators. Even though the floral biology of *C. americana* suggest adaptations to animal pollination backing the idea that autogamy probably has increased as pollinator availability has decreased.

Pollination and pollen tubes

The populations with higher levels of autogamy showed more inter-individual variation, though the same pattern was not found for natural pollination, with all populations showing a similar inter-individual variation (see SD in Table 2). We suggest this as an outcome of the way autogamous populations were established through time. In these populations, a requisite to colonize and keep reproducing was the ability to self-pollinate, and therefore, because of the subsequent lower levels of gene flow, individuals remain more different from each other (Kalisz and Vogler 2003, Dart et al. 2012, Levin 2012). On the other hand, in mainly cross-pollinated populations, the allele exchange can act as a genetic stabilizer. This gene flow will keep basal rates of self-compatibility and not a complete reversion to self-incompatibility, only because of the complex genetic changes demanded for that (Igic and Busch 2013, Barrett 2013). Nevertheless, the report of one functionally dioecious and two andromonoecious individuals point *C. americana* out as an interesting model to address questions about gender variation and evolution in plants. We do not have any genetic evidence supporting the phenotypic trend found, and ecological factors related to way autogamy and cross-pollination occur, such as weather, cannot be excluded as concurrent explanations to the patterns found.

Abiotic factors, such as temperature, light and salinity may also affect the level of self-compatibility in plants (Levin 2012). Temperature-related increments in self-compatibility have been observed in several species: *Lilium longiflorum* (Ascher and Peloquin, 1970), *Brassica oleracea* (Johnson, 1971), *Lycopersicon peruvianum* (Hogenboom, 1972), *Petunia hybrida* (Takahashi, 1973), *Cichorium sativum* (Eenick, 1981) and *Convolvulus arvensis* (Westwood et al. 1997). The temperatures in the Northern populations of *C. americana* are in average 2ºC higher than in the other studied regions. However, populations in the Southern edge of the distribution had the lowest temperatures and equivalent levels of autogamy as the populations in the North, suggesting that temperature is not the main factor influencing the level of autogamy in *C. americana*.

Contrary to hypothesis one, not all populations with high levels of natural pollination had low levels of autogamy. The two populations on the Southern edge of the distribution (Jatai and Caldas Novas) had equivalent fruit set as other populations in Central Brazil, but the levels of autogamy were similar to the ones found in the North, suggesting a possible role of the historical mechanisms determining the contemporary mating system of *C. americana* (Rech *et al*. in prep.). Mating systems can be very flexible as already shown in artificial selection experiments (Levin, 2011). Only two generations of directional selection were enough to elevate the autogamy level from 4% to 56% in *Phlox drummondii* (Bixby and Levin, 1996). Therefore, past events could be underpinning the contemporary reproductive pattern found in *C. americana* (Rech *et al*. in prep.).

Individuals of *C. americana* can live for more than 180 years (Costa, 2013) slowing the process of recruitment and replacement when compared to herbs such as *P. drummondii*. Therefore, if there is an ecological filter preventing colonization by species relying exclusively upon animal pollination, the establishment of a mixed mating, predominantly autogamous population can be very fast. Nonetheless, the recovering of higher rates of self-incompatibility when population size increases and pollinators come back into place can be very slow, specially for individuals living more than 100 years like *C. americana*. Therefore, the poorly documented reversion from self-compatibility could be also related to the speed of the process generating it. It is also important to take into account that populations are normally not exclusively self-pollinated, so the unfavourable effects of a pure strategy can be reduced (Goodwillie *et al*. 2005).

Morphometrics

Populations of *Curatella americana* differed in many morphological traits. However, caution is needed in the interpretation of this variation for two reasons. First, floral traits varied in the same way as vegetative traits, and second, most of the traits varied as a gradient. Similarly to the levels of autogamy, the size of anthers was also the same in the population from Santarém and in Central Brazil populations. In contrast, stigma area in Santarém was similar to the North populations, as well as its level of natural pollination. It is clear that Santarém has an intermediate position in morphology regardless of the trait or the pattern of variation. We interpret the intermediary position of Santarém, even for traits varying in opposite ways, as a consequence of the latitudinal gradient.

The synchronized variation in floral and vegetative traits in plants with ecologically and phenotypically generalized pollination systems (open flower morphology and many unrelated species of pollinators) is one of the expectations of "Berg's rule" (Berg, 1960, Armbruster *et al.* 1999, Fenster 1991, Conner and Sterling 1995). As floral traits derive from vegetative ones, decoupled patterns of variation will not be expected unless clearly different regimes of selection drive the evolution of different groups of traits in different ways (Berg, 1960; Armbruster *et al.* 1999). Population studies using neutral markers are necessary to better understand what is genetically underlining the morphological differentiation in *Curatella americana*. If it is the result of neutral differentiation along the isolation time among populations, the same gradient pattern found for most traits should also be observed for neutral genetic markers.

Considering potentially different rates of differentiation working on flower traits opposite to vegetative ones, Berg (1960) called each group collective as a pleiad. In our study, not all traits varied in the same direction as pleiades (Berg 1960). Stigma area was larger and herkogamy lower in the North. These two traits can be related to a possible process of reproductive insurance, as both increase the chance of a flower being self-pollinated (see review in Levin 2012). The same pattern was also reported in *Nicotiana glauca* after introduction in sites where native pollinators were not present, reinforcing the role of pollinators mediating the whole process (Ollerton *et al*. 2012). On the other hand, anthers were larger in Central Brazil. Considering that: 1. anther size and pollen production are positively correlated (Harder and Thompson 1989, Philipp *et al*. 1990), and 2. pollen removal can be related to seed set (Broyles and Wyatt 1991), it seems that there is a selective pressure on the male component (pollen production) of the fitness in areas with higher levels of cross-pollination (Lloyd 1984).

Although trees have longer lives and therefore slower individual substitution rates, we showed here a similar pattern of variation in mating system and flower morphology as previously demonstrated for herbs and shrubs, indicating colonization filters and pollinator availability as important drivers of the flower morphology and mating system. The results discussed here re-emphasize mating system as a property of populations and not of species. Our hypotheses were partially supported. Specifically, for hypothesis one autogamy was higher in areas of low natural pollination as predicted. However, at the edge populations in the South, both natural pollination and autogamy were high. Higher levels of autogamy were not found in the smallest population, contrary to our second hypothesis. The third hypothesis was also partially corroborated: flowers as a whole did not vary in size as predicted, but there was less herkogamy in populations with higher levels of autogamy. Interpretations of these findings are discussed in the three following sections.

Acknowledgment

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Tables

Table 1. Geographic location and abiotic parameters (AT – Mean Annual Temperature -ºC. FT – Mean Temperature measured during field work -ºC and Mean Annual Precipitation - mm) for the studied populations of *Curatella americana* in the Brazilian Cerrado (Neotropical Savannah). Data on AT and Rainfall were obtained from Worldclim (Hijmans. 2005)

	Population	Region	Latitud	Longitud	AT ºC	FT ºC	Rainfall
1	Amajari	North	3° 39' 24.86" N	61° 19' 3.75" W	26.9	27	1524
2	Faz. Bamerindus	North	3° 20' 53.65" N	60° 59' 1.63" W	27	25.7	1468
3	Boa Vista	North	2° 43' 8.80" N	60° 37' 55.33" W	27.2	26.4	1537
4	Santarém	Santarém	2° 29' 12.35" S	54° 48' 26.84"W	25.8	25.4	2083
5	Manso	Central Brazil	14° 49' 42.48" S	55° 43' 19.68" W	24.6	21.5	1517
6	Cuiabá	Central Brazil	15° 24' 34.05" S	56° 1' 30.46" W	25.1	23.1	1370
7	Poconé	Central Brazil	16° 17' 9.87" S	56° 38' 35.80" W	25.9	23.9	1244
8	Nova Xavantina	Central Brazil	14° 47' 43.12" S	52° 32' 20.01" W	24.5	21.5	1453
9	Jatai	Central Brazil	17° 51' 21.01" S	51° 45' 51.04" W	23.5	18.1	1097
10	Caldas Novas	Central Brazil	17° 46' 17.11" S	48° 39' 36.11" W	23.4	19.4	1424

Table 2. Average fruit set plus standard deviation for each of the ten studied populations of *Curatella americana* in Brazil. Autogamy refers to bagged inflorescences with no pollinator access while natural pollination is the fruit set in exposed flowers. Population numbers follow Table 1.

Mean	1	2	3	4	5	6	7	8	9	10
Autogamy	**0.21**	**0.23**	**0.15**	**0.05**	**0.06**	**0.03**	**0.05**	**0.02**	**0.2**	**0.29**
Standard deviation	0.19	0.25	0.16	0.04	0.07	0.03	0.05	0.02	0.18	0.21
Natural Pollination	**0.32**	**0.23**	**0.21**	**0.28**	**0.48**	**0.65**	**0.62**	**0.63**	**0.34**	**0.72**
Standard deviation	0.14	0.13	0.12	0.10	0.09	0.11	0.12	0.14	0.17	0.12
Difference (NP-A)	**0.11**	**0.0**	**0.06**	**0.23**	**0.48**	**0.58**	**0.57**	**0.61**	**0.14**	**0.43**

Table 3. Multivariate Analysis of Variance (MANOVA) considering the geometric morphometric PCA axes for floral and leaf traits of *C. americana* in the three geographical regions studied (Central Brazil. Santarém and Roraima).

		DF	F	p
Leaf	Region	2	13.9828	< 0.0001
	Individual	98	1.7766	< 0.0001
	Residual	494		
Ovary	Region	2	5.3359	<0.0001
	Individual	20	1.3424	0.00268
	Residual	49		
Petal	Region	2	2.3023	0.002331
	Individual	22	1.4076	0.000156
	Residual	49		
Sepal	Region	2	1.7413	0.03065
	Individual	22	1.1943	0.03136
	Residual	49		

Table 4. Analysis of Variance (ANOVA) for counted and linearly measured floral traits of *Curatella americana*. Populations are compared among different regions (Central Brazil. Santarém and Roraima).

Anova	Residual	DF	F	p
Number of stamens	69	2	0.6220	0.5398
Stigma area	69	2	6.4097	0.00279
Anther size	69	2	12.1346	0.00003
Distance between stigmas	69	2	3.9929	0.02286*
Gynaecium length	69	2	6.1636	0.00344
Androecia length	69	2	1.6270	0.2039
Petal size	69	2	1.5381	0.2221

*non-significant after Bonferroni correction

Figures

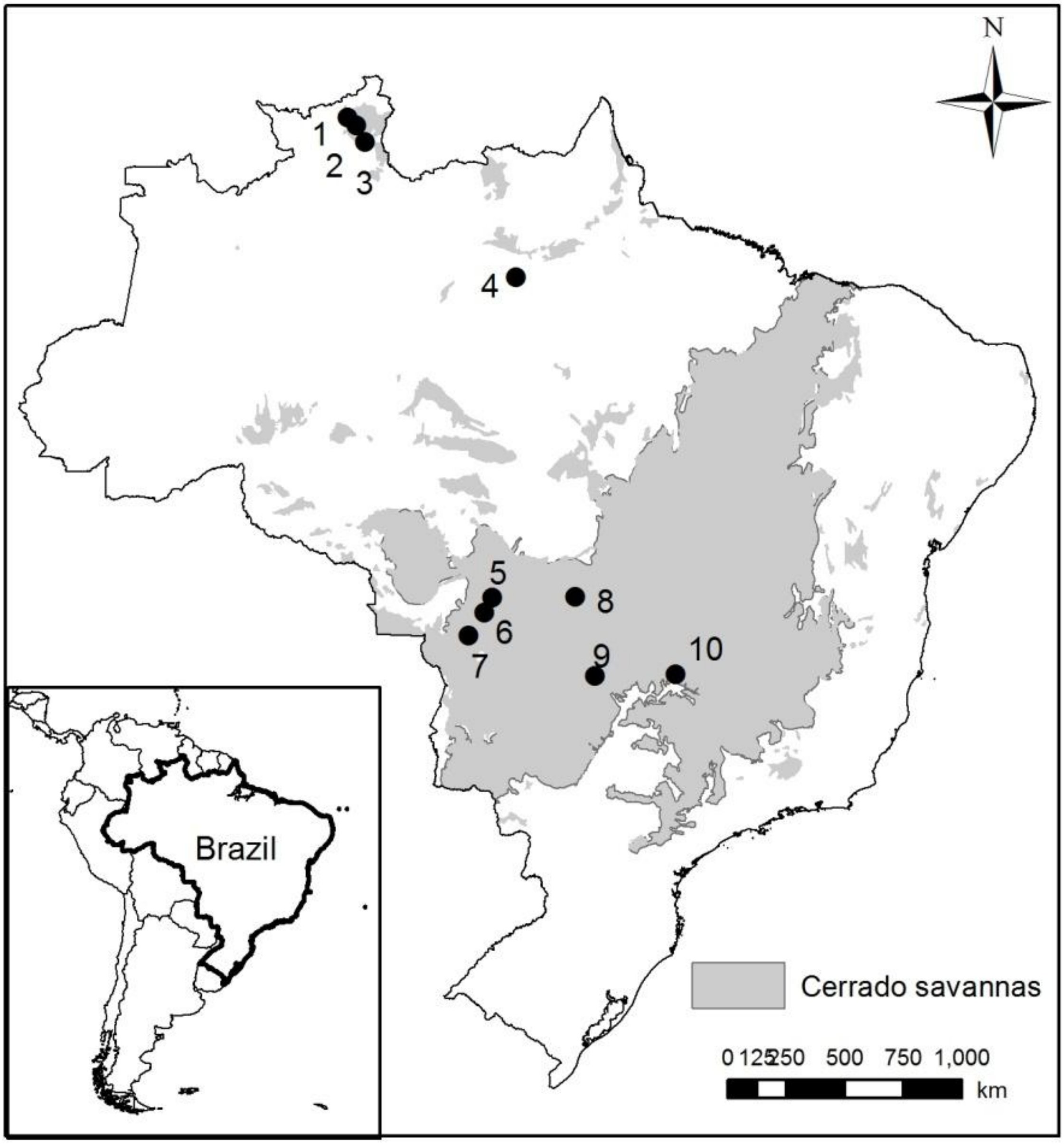


Figure 1 – Map of the Brazilian original area of Cerrado (Neotropical Savannah) and indication of the studied populations. Numbers follow Table 1.

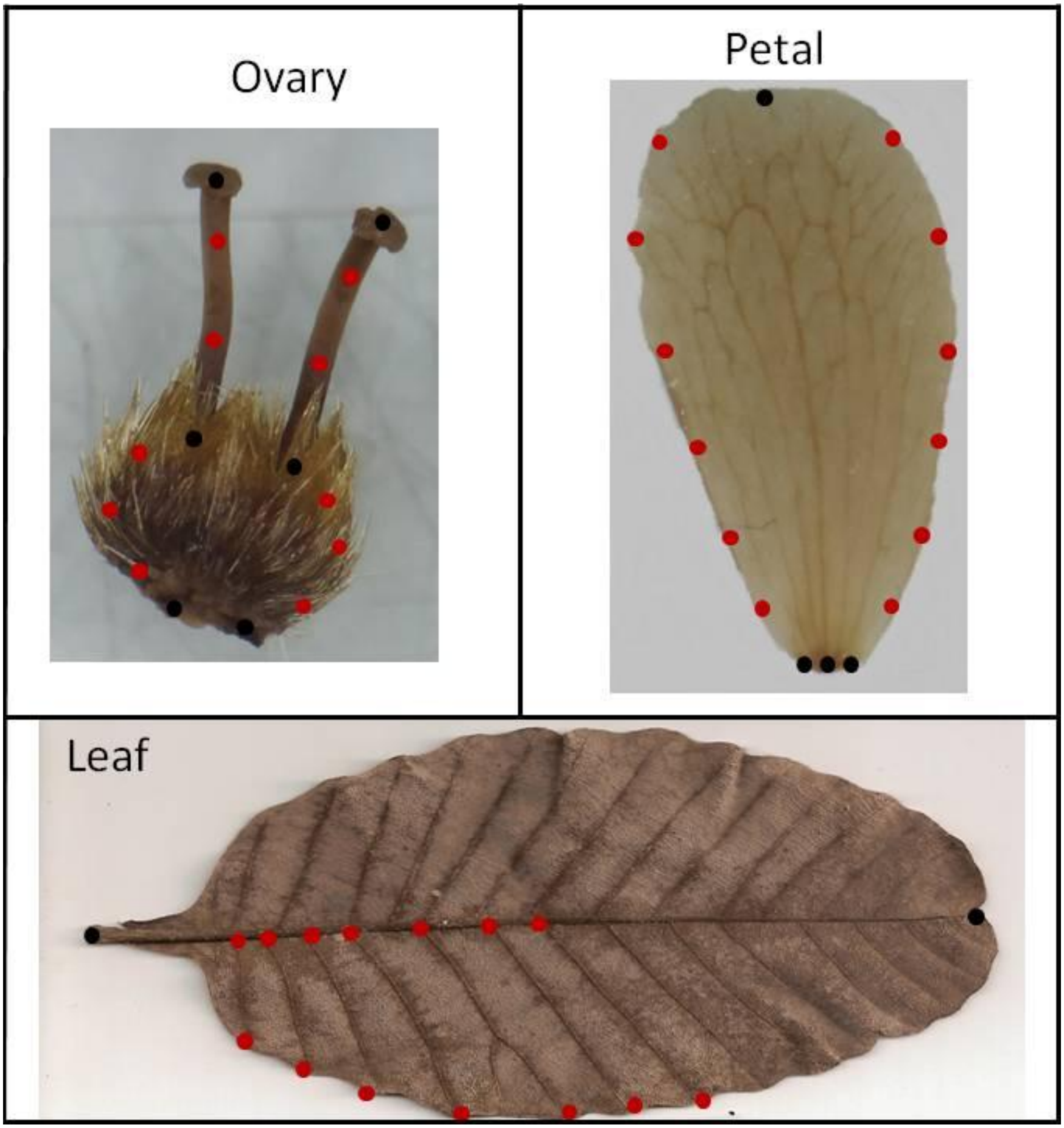


Figure 02. Schematic representation of landmark (black dots) and semi-landmark (red dots) positions of flower and leaf traits of *Curatella americana*. Sepals (not shown) follow the same pattern used for petals.

Figure 3. Inflorescence of *Curatella americana* and flower visitors in detail. A. Syrphidae (fly), B. *Ptiloglossa* sp. (large-bee), *Melipona quinquefasciata* (small-bee), D. Curculionidae beetle and E. Inflorescence with a *Bombus* cf. *morio* (large-bee) visiting the flower. Illustrated by Pedro Lorenzo.

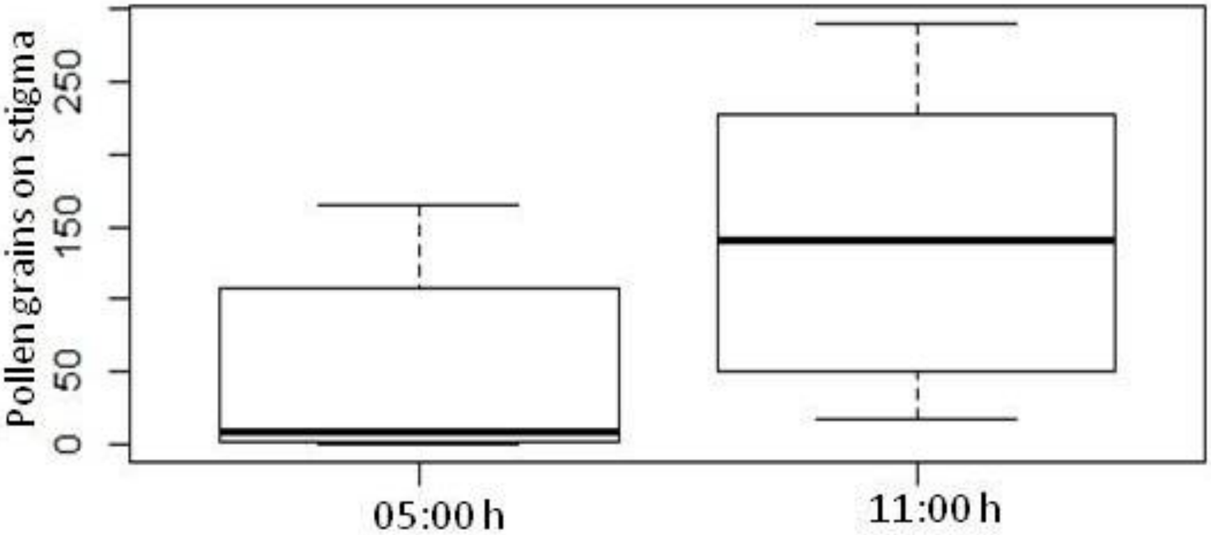


Figure 4. Box plot showing the average number of pollen grains deposited on single virgin stigmas of *Curatella americana* at the beginning (control) and end of anthesis indicating spontaneous self-pollen deposition.

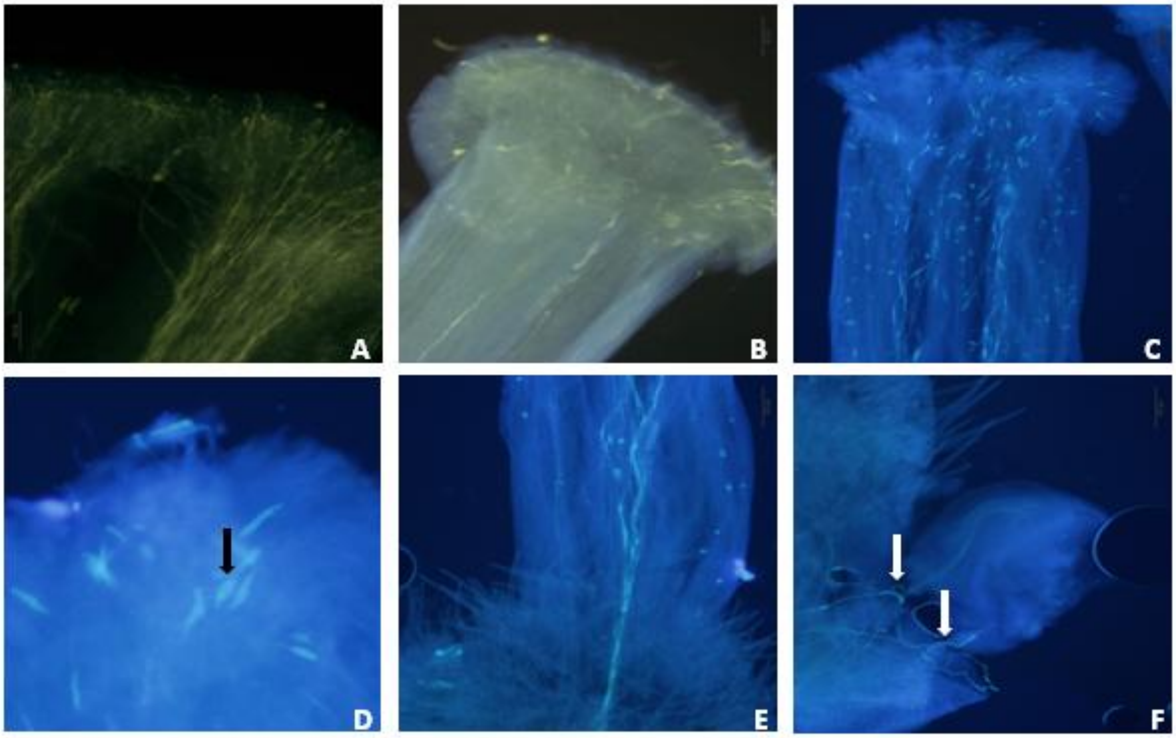
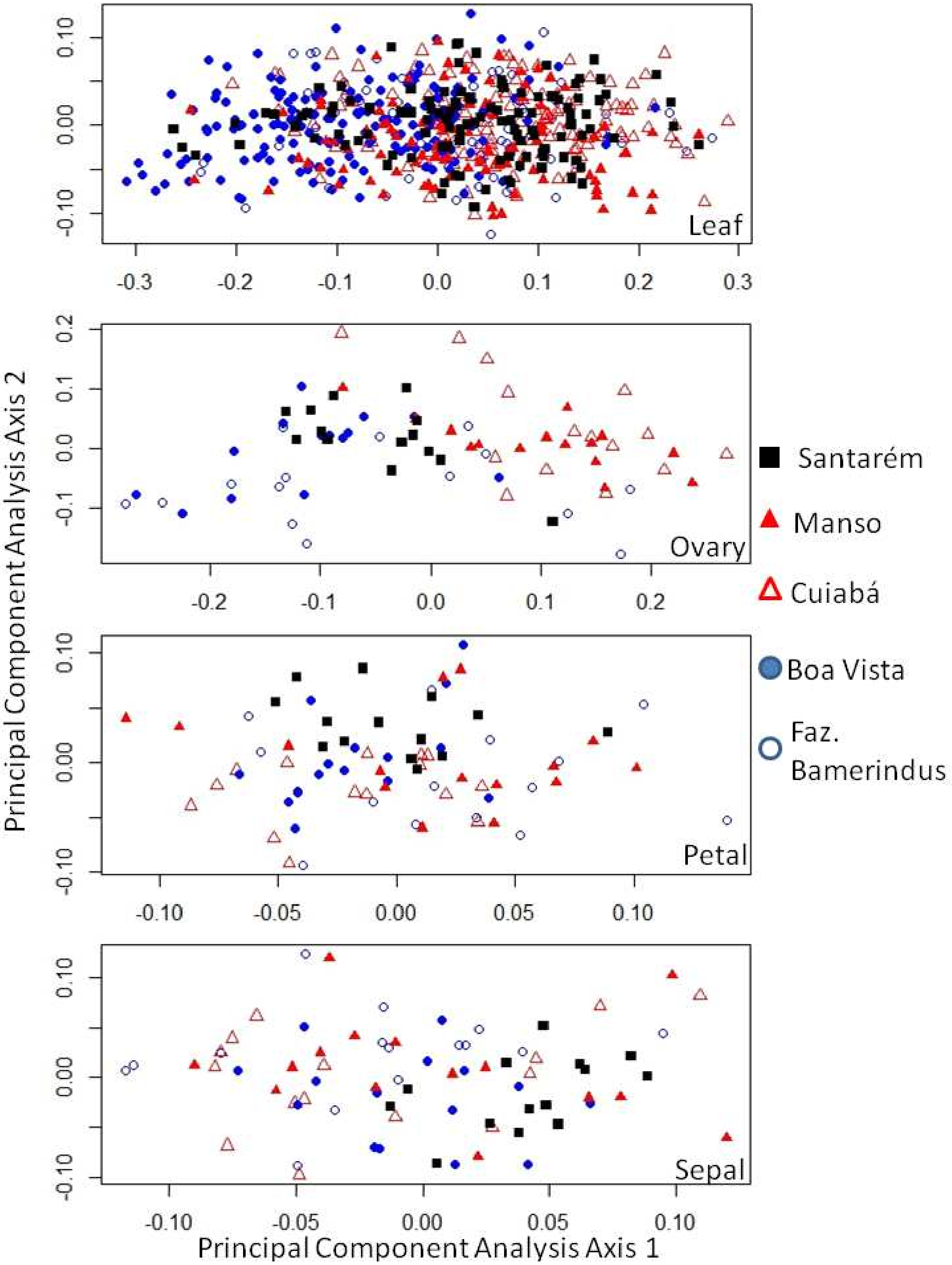


Figure 5. Pollen tube growth in flowers of *Curatella americana* after different pollination tests. A and B – Cross and self-pollination in one population from Central Brazil (Cuiabá). C – Self-pollination in one population from the North (Boa Vista). D – Self-incompatiblity reaction (black arrow) in one self-pollinated flower from Central Brazil (Manso). E - pollen tube growing through the base of the style and (F) reaching the ovule (white arrow) at the base of ovary (Boa Vista).

Figure 06. Principal component analysis (PCA) using geometric morphometric coordinates for leaves and floral traits of *Curatella americana* L. Each dot in the graphs is an individual measure of one trait projected according to the two first axes of the PCA. Colours indicate regions: black is Santarém. red is Central Brazil. and blue is the North. Different shapes indicate different populations within each region as indicated.



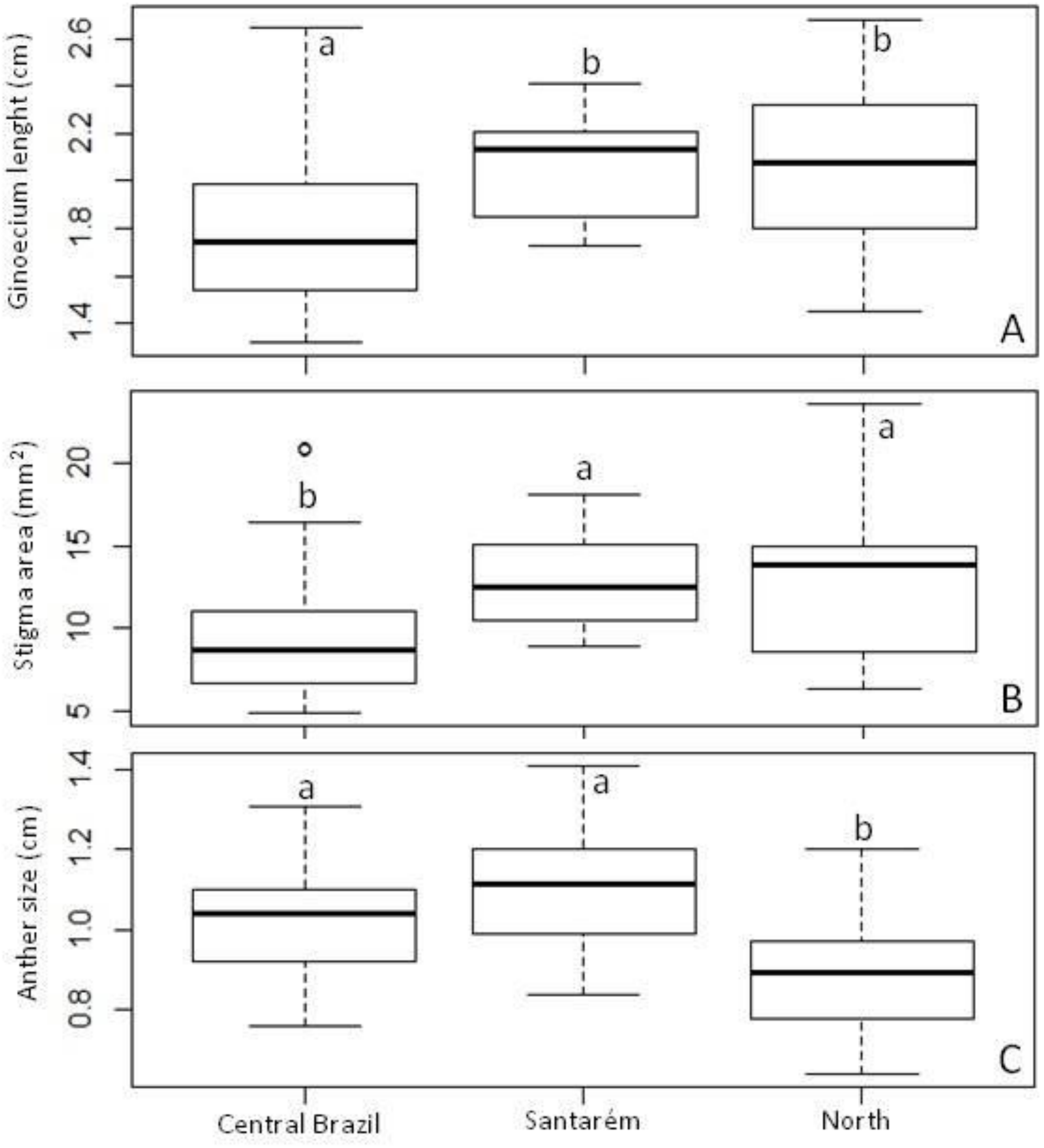


Figure 7. Comparison of the linear measures of floral structures of *Curatella americana* from different regions of Brazilian cerrado (Neotropical savannah).

Capítulo 4. Pollination and reproduction of *Curatella americana*: a biogeographical pattern influenced by Quaternary climate and contemporary pollinators

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Abstract - Patterns in ecology are the products of current factors interacting with a longstanding history of contingency. Nevertheless, few studies have attempted to disentangle the contribution of past and current factors on plant reproduction patterns. Here, we described the geographical pattern of reproduction in the mixed mating species *Curatella americana* and attempted to separate the relative importance of current and past processes on those patterns. The questions asked were: 1) Do cross-pollinated flowers set more fruit than self-, natural or autogamously pollinated flowers? 2) How does pollinator abundance and functional diversity relate to the level of cross-pollination? 3) How do Quaternary and current climate affect pollination mode? We recorded pollinators (richness, frequency and body size) and also performed pollination tests (cross, self, autogamy and natural pollination) with bagged flowers in ten different populations of *C. americana* spread over the Brazilian savannah (cerrado). Autogamy was related to past climate dynamics and not to present pollinator variables. Meanwhile, hand-cross and natural pollination related to pollinators (specially large bees) and temperature, indicating the importance of current factors. Populations at the Southern edge of the distribution showed high levels of hand-cross-pollination and autogamy backed by high visitation by large bees and recent colonization events. Our results indicate that past climate has favoured autogamy as a

reproductive insurance strategy facilitating colonization and population maintenance over time, while pollinators are currently modulating the level of cross-pollination.

Introduction

Flowering plants need pollen vectors to be cross-pollinated (Aizen & Harder 2007). These vectors can be abiotic, such as wind and water, or biotic, and an estimated 87.5% of angiosperms use animal pollinators as pollen vectors (Ollerton *et al.* 2011). In general, the greater the number of pollen vectors used by a given plant species, the lower the level of pollen limitation as more generalized pollination systems have a higher probability of pollen being delivered onto conspecific stigmas (Knight *et al.* 2005). Generalized pollination systems are therefore more resistant to pollinator species loss and, hence, they are hypothesised to predominate in environments where the pollinator fauna is highly variable (Waser *et al.* 1996) and/or not immediately fitted to the ancestral pollination mode, such as on islands (Armbruster & Baldwin 1998, Rivera-Marchand & Ackerman 2006, Dalsgaard *et al.* 2009, Martén-Rodrigues & Fenster 2010). More diverse arrays of pollinators can also be more stable over time and space because of the buffering effect of different species responding in different ways to environmental changes, what is called the "biodiversity insurance hypothesis" (Loreau *et al.* 2001, Bartomeus *et al.* 2013, Fründ *et al.* 2013).

Plants can also show diverse and complex reproductive strategies related to their mating systems (Goodwillie *et al.* 2005). Although self-incompatibility can result in higher quality progeny and an increase in genetic diversity (Kalisz & Vogler 2003, but see Dart & Eckert 2013) a reproductive assurance strategy of autogamous self-pollination (hereafter called autogamy) may allow species to colonize new areas or survive within the ones where conditions are non-optimal (Lloyd & Schoen 1992, Fausto *et al.* 2001). The idea of autogamy assuring reproduction was originally proposed by Darwin (Darwin 1877, p. 58) and formalized by Baker (1955, 1967) and latterly named "Baker's rule". The same principle was later expanded to small populations living at the edges of species distributions, where the lower plant density is likely to reduce cross-pollination (Randle *et al.* 2009, Levin, 2011).

Historically, mating systems were discussed as species' properties and most comparisons have been done among species rather than among populations or individuals of a given species (Levin 2011). However, mating systems can vary according to local environmental conditions and an appreciation of this may improve our understanding of the evolution of plant reproductive strategies (Dart *et al.* 2012, Ivey & Carr 2012). Moreover, to consider the spatial and temporal variation of mating systems can help to better understand the impact of past climate change on plant reproductive systems, which may also help predict the impact of future changes (Dawson *et al.* 2011).

Past climate dynamics have affected species distribution and diversity patterns (Svenning & Skov 2007, Cárdenas *et al.* 2011, Sandel *et al.* 2011, Kissling *et al.* 2012), population demography and genetic structure (Grazziotin *et al.* 2006, Cabanne *et al.* 2007), and recent studies have suggested an influence of past climate stability on the structure of mutualistic plant-pollinator assemblages (Dalsgaard *et al.* 2011, 2013). Frequently the reconstruction of Quaternary paleo-environments has been done by using pollen records (Anhuf *et al.* 2006). In South America, for instance, there is considerable debate about whether currently forested areas such as the Amazon basin may previously have been savannah, and the consequences for species diversification in the area (Colinvaux & Oliveira 2001, Richardson *et al.* 2001, Pennington *et al.* 2004, 2006).

One of the principal pollen types used to reconstruct the history of South American savannah environments is *Curatella americana* L. (Dilleniaceae) (Absy *et al.* 1997). The intrinsic association of this species with savannahs and the mixed mating system of outcrossing and selfing that the species possesses makes *C. americana* a suitable model to address ecological questions about spatial variability in plant reproduction, and relationships with changes in savannah distribution and climate stability over time. Previous studies have shown that areas of savannah have varied in size throughout the Neogene (Ledru *et al.* 2006, Pennington *et al.* 2006), and that the disjunct areas of savannah present nowadays in Pará, Roraima and other areas of Brazil were probably connected and separated many times over the Quaternary (Quijada-Mascareñas *et al.* 2007, Werneck 2011). At the present time, *C. americana* is likely to be found in most of the areas of savannah, also known as cerrado, in Brazil (Ratter *et al.* 2003). It is reported even in small areas of savannah surrounded by forest at the Amazon region (Ratter *et al.* 2003, Magnusson *et al.* 2008) which are supposed to be isolated at least from the mid Holocene onwards (Mayle & Power 2008, Werneck *et al.* 2011).

Regardless of the potential relationship, to the best of our knowledge no study has shown, based on empirical data, the past and current climatic mechanisms underlying biogeographical patterns of plant-pollinator interactions and the consequent implications for plant reproduction. All the information available is based on phylogenetic based inference and most of the hypotheses remain untested. In this study, we investigate the spatial structure and the determinants of the pollination system of *C. americana* in savannah areas of Brazil, considering both past and current climate, mating system, and pollinators. Specifically, we ask: 1) Do cross-pollinated flowers set more fruit than self-, natural or autogamously pollinated flowers? 2) How does pollinator abundance and functional diversity relate to the level of cross-pollination? 3) How does Quaternary and current climate affect pollination mode?

Material and Methods

Study sites and species: We studied ten populations of *Curatella americana* distributed among three disjunct areas of savannah (cerrado - Table 1, Figure 1). Vegetation physiognomies are very similar among places, but in general plant species diversity decreases northwards (Ratter *et al*. 2003, Bridgewater *et al.* 2004). We observed animal pollinators and performed experiments on *C. americana* at all the studied sites. The species flowers from June to September in Central Brazil, mid-August to early October in Pará state, and October and November at Roraima state. Flowers are white, pentamerous and grouped into dense inflorescences (Rech *et al*. in prep.). Each flower stays receptive for three to five hours for one single day (Rech unpublished).

Mating system: In order to study the reproductive system of *C. americana in situ* we applied the following pollination tests: hand-cross-pollination, hand-self-pollination, autogamous self-pollination and natural pollination. All pollination tests were performed with flowers previously bagged using cloth insect exclusion bags, except for natural pollination, which involved counting and tagging flowers exposed to flower visitors. The number of tested flowers was always higher than 20 flowers per individual and a mean of 15 different individuals per test per population. In two of the studied areas (Nova Xavantina and Caldas Novas) we chose 12 individuals and compared the fruit weight from self (n = 107) and cross (n = 102) pollinated flowers (as a proxy to seed quality - Coomes & Grubb 2003).

Flower visitation and pollination: For all populations we recorded flower visitors (species richness and abundance) from anthesis until the end of visitation. In order to quantify visitation we counted all visits to an observable (and counted) set of flowers for ten minutes each half an hour for at least 20 hours (120 ten minute sections) in each population. All the visitors able to touch anthers and stigmas were considered and scored as potential pollinators. After observing behaviour, flight distance and pollinator size, we grouped the pollinators into two categories: 1) Large-sized bees, and 2) Others, which includes bees the same size or smaller than *Apis mellifera*, beetles, flies and wasps. We separated pollinators according to size because flight range correlates with body size (Greenleaf *et al.* 2007, Araujo *et al.* 2004, Gathmann & Tscharntke 2002). Using this premise we expected a correlation between higher level of cross-pollination and potential farther pollen transfer.

Statistical analysis

Mating system analysis: To test for differences in fruit set related to the mating system and the regions, we used a Generalised Linear Mixed Model assuming a binomial distribution. The fixed factors were region, pollination experiment treatment, and the interaction between them. The random factors were the individuals nested within sites and these nested within regions. Our response variable was the production of a fruit from each flower. We tested the models with all fixed factor combinations and only a fixed intercept (Null Model), always keeping the random factor. For the fruit weight comparison we used pollination treatment (self and cross-pollination) as predictors and generated models using individuals as random factors. All the alternative models were built removing factors or interactions between factors from the full model. In order to compare the generated models we used the Akaike Information Criterion (AIC – Burham & Anderson 2002). All tests were done using R software (R Development Core Team 2008).

Climate modelling analyses: for each studied site, we modelled the climate changes since Last Glacial Maximum (LGM) by estimating the mean annual temperature (MAT_LGM) and annual precipitation (MAP_LGM) at each location for 21ky, according to the Community Climate System Model (CCSM). We also extracted the current values of temperature (MAT_Current) and precipitation (MAP_Current) from the Global Climate Data (Worldclim 1.4 - http://www.worldclim.org/). For each site, we calculated the velocities of change in temperature (MAT_Velocity_21) and precipitation (MAP_Velocity_21), as the long-term average over the last 21,000 years. All calculations were based on a 2.5 minute geographical resolution.

We then estimated the effect of climate and pollinator activity on pollination mode. Due to the modest sample size of populations (n = 10) and some predictor variables being strongly correlated (i.e. $r \geq 0.6$; Appendix 1), we took the following modelling approach. First, we modelled the pure effect of climate on pollination mode using current and past climate predictors, identifying minimum adequate models (MAMs) using the approach outlined in Diniz-Filho *et al.* (2008). As the measure of past climate stability summarised as temperature and precipitation anomaly were strongly correlated, we modelled the effect of temperature and precipitation anomaly separately. The effect of past climate stability was also tested using modelled temperature and precipitation velocity instead of anomaly, giving qualitatively the same results (see Appendix 2). Second, no matter whether the four pollinator variables (pollinator richness, visitation frequency, and proportion of large bee visitation calculated both with and without the honey bee) were significantly related to pollination mode in single correlation test using both traditional non-spatial correlation analysis and correcting the degrees of freedom using Dutilleul's (1993) method, we tested whether each of these pollinator activity variables may have other or additional effects to climate. We examined this by again following the approach of Diniz-Filho *et al.* (2008) to identify MAMs, but this time only considering climate variables included in the above-identified MAMs and each of the four pollinator variables.

For all analyses, MAP, MAP anomaly, MAP velocity and MAT velocity were Log_{10}-transformed, pollination visitation frequency was square root transformed, and all proportional measures (i.e. pollination mode variables and large bee predictors) were arcsine-square root transformed. All other variables were left untransformed. All analyses were conducted using the software Spatial Analysis in Macroecology, SAM 4.0 (Rangel *et al.* 2010).

Results

The main flower visitors and potential pollinators of *C. americana* flowers were bees of different sizes. Beetles were also recorded at all populations but they only ate anthers and copulated on the flowers, with small, if any, importance as pollinators. In eight out of ten populations flies and wasps were also recorded as flower visitors; however, they were visiting with a very low frequency; only in Jatai, Caldas Novas and Santarém they performed more than 1% and never more than 5% of total visits. During the visits they ate pollen directly from the anthers (flies) and not always touching anthers and stigmas (flies and wasps). Bees therefore seem to be the main potential pollinators.

In all populations, cross-pollinated flowers set more fruit than self-, natural- or autogamously-pollinated flowers (Table 2). Cross- and self-pollination (measured by fruit set) were negatively correlated (r = -0.87, p = 0.009. Fruit set from cross- and self-pollination were more contrasting in the South and more similar in the Northern populations (Table 2). In other words, out-crossing decreases from South towards North. The analysis of fruit weight according to pollination test and site showed that only pollination treatment was important, with cross pollination producing heavier fruit (Figure 2, Table 3).

The reproductive system of *C. americana* was more likely explained by the full model (considering region, pollination treatments, and the interaction between them) (Table 4). Considering only the additive effects of region and reproductive system makes the model nearly as likely as including only the reproductive system regardless of region. This result shows that the reproductive system of the species is structured on a regional scale, and although there are differences among populations inside a region, differences among regions are much greater. The model considering only the regions was just slightly less probable than the null model. Although there is a strong difference among regions, it is possible to see that the level of autogamous pollination is highly variable among individuals within a given region and, even in the North region it is possible to find some individuals with very low fruit set inside bagged inflorescences (Figure 3).

Past and current climate analyses

Both current and past climate had an effect on the reproductive system of *C. americana*. Natural pollination was highly related to the yearly current fluctuations in temperature (i.e. current seasonality), and was significant both in non-spatial and spatial correlation analysis, alone explaining 83% of the variation in natural pollination (Table 5 and 6). Natural pollination was also positively related to visitation by large bees (79%), negatively related to mean annual temperature (63%) and mean annual temperature velocity (76%). Autogamous pollination occurs especially in areas with high temperature anomaly, i.e. historical climatically unstable areas.

Hand-self-pollination: none of the climate variables or pollinator activity variables were related to this variable. Hand-cross-pollination: the best fitted model was the one including proportion of large bee visits (removing the abundance of honey bees). Nonetheless, a climate model containing temperature seasonality and precipitation anomaly performed nearly as well (ΔAIC_c= 1.971). The predicting variable "proportion of large bee visits" was correlated with five climate variables (MAT, MAP, MAT seasonality, MAP anomaly and MAT velocity).

Discussion

The current pollination mode and reproduction of *C. americana* in the Brazilian cerrado is the result of both historical and contemporary factors: Quaternary climate largely determined the level of autogamous self-pollination, whereas contemporary temperature seasonality and proportion of large bee visitation determined the level of natural and cross-pollination. This indicates that autogamous self-pollination is likely to occur in areas of higher climate dynamics and, hence, probably re-colonized many times in the past, as pollen records seems to indicate in the Northern (Caracarana lake – Rodrigues 2006) and Southern edge of *C. americana* distribution in Brazil (Crominia - Salgado-Laboriau 1997). In agreement with this, genetic data on the phylogeography of *C. americana* indicated recent expansion in most populations (Canuto 2011, Canuto *et al.* in prep.). Most of the literature on Brazilian savannah biogeography agrees that its area varied considerably during the Quaternary (e.g. Ledru *et al.* 2006, Pennington *et al.* 2006, Quijada-Mascareñas *et al.* 2007, Werneck 2011), and this has impacted *C. americana* autogamy.

Higher levels of autogamy in the Northern populations were caused by a weaker restriction to self-pollen germination and less herkogamy, probably in response to the lack of pollinators influenced by past climate (Rech *et al.* in prep.). The occurrence of autogamous self-pollination as a reproductive assurance mechanism has been already shown in many other species (reviewed in Eckert *et al.* 2006). For both natural and hand-cross-pollination the proportion of the total visits carried out by large bees and temperature were more important factors. Moreover, the proportion of large bees was correlated to many variables (MAT, MAT seasonality and velocity) preventing us of separating the effect of temperature on pollinators or directly on the results of pollination tests.

Most of the studied populations in central Brazil (Populations 5, 6, 7 and 8 – Figure 01) are growing in a supposedly old savannah area (Terribile *et al*. 2012), where the longer distance pollen flow mediated by large bees and climate stability can be acting to promote the reproduction of individuals better able to cross-pollinate. Considering that cross-pollination produces heavier - and presumably higher quality - fruit, the progeny from this fruit will be expected to outcompete or survive longer periods of unfavourable conditions than the ones from self-pollination (Coomes & Grubb 2003). In line with this rationale, the two populations in the Southern edge of the cerrado (Caldas Novas and Jatai) showed both moderate levels of autogamous self-pollination, indicating recent colonization events (Salgado-Laboriau 1997), and high levels of cross-pollination, which is backed by great proportion of large bee pollination found there. The population from Jatai (number 9 - Figure 1) presented a high hand-cross-pollinated fruit set but natural pollination was low. We related this to the large amount of honey bee visits, which totalled around 90% of the visits and are known as poor pollinators (Westerkamp 1991, Garibaldi *et al*. 2013).

The gradient of pollinator species richness and abundance reducing from South to North was previously reported for woody plants in Brazilian savannahs (Bridgewater *et al*. 2004). There is a suggestion that this pattern, which contrasts to the expected tendency of increasing diversity towards the Equator, could be related to the climate instability in the past (Werneck *et al*. 2012). Our results for pollinator richness also point out the importance of past climate on the number of bee species. Therefore, perhaps the patterns observed for woody plant species diversity in Cerrado could also be applicable to other groups of organisms, such as the ones that interact with plants (pollinators, seed dispersers and herbivores), an idea deserving further investigation. Another identified trend concerns flies; although they never surpassed 5% of the visits they were more frequent at the wettest (Santarém) and coldest (Caldas Novas and Jatai) places (Martin-Gonzales *et al.* 2009, Devoto *et al.* 2005).

Higher cross-pollination in cooler and more seasonal places is in accordance with the pattern of global bee diversity, which peaks in subtropical areas with higher seasonality (Michener 2007). Reinforcing the idea of the mediating role of bees to promote cross-pollination, both hand-self- and autogamous-pollination showed no relationship with any of the variables related to the pollinators. Moreover, cross- and natural- pollination were related to the proportion of large bees, and not to pollinator species richness and visitation frequency, indicating that not all visitors are equally good pollinators and not all proxies are equally realistic for pollinator quality (Popic *et al.* 2013, Sakamoto & Morinaga 2013). Moreover, it was already experimentally shown that functional complementarity is far more important than the simple increment in species number for pollination (Fründ *et al.* 2013).

In conclusion, the whole pattern of flower morphology, pollination and mating system in *C. americana* suggests a reproductive assurance strategy that has benefitted the species in the past. This strategy could be one of the key factors explaining *C. americana* being one of the most conspicuous and widely distributed woody species in Neotropical savannahs (Ratter *et al*. 2003). We also corroborate here the already proposed effect of high functional diversity of pollinators buffering effects of climate dynamics, since places with more species of large sized bees were more likely to remain functional when the environment changed and provide current higher levels of cross pollination (Bartomeus *et al*. 2013). Although there are many aspects of pollination and past climate relationships to be clarified, we think that the incorporation of past dynamics is fundamental, especially because interactions should be much more sensitive to climate dynamics than the species itself seems to be (Bartomeus *et al*. 2013).

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Tables

Table 1. Studied populations of *Curatella americana* and their respective geographical coordinates. Regions refer to the large portions of Brazilian savannah (cerrado). The southern ones in central Brazil are called South, the population inside the Amazon forest is referred to as Middle and the northernmost Brazilian areas are referred to as North.

Population	Region	Site	Latitude	Longitude
01	North	Boa Vista	2° 43' 8.80" N	60° 37' 55.33" W
02	North	Faz. Bamerindus	3° 20' 53.65" N	60° 59' 1.63" W
03	North	Amajari	3° 39' 24.86" N	61° 19' 3.75" W
04	Middle	Santarém	2° 29' 12.35" S	54° 48' 26.84"W
05	South	Manso	14° 49' 42.48" S	55° 43' 19.68" W
06	South	Cuiabá	15° 24' 34.05" S	56° 1' 30.46" W
07	South	Poconé	16° 17' 9.87" S	56° 38' 35.80" W
08	South	Nova Xavantina	14° 47' 43.12" S	52° 32' 20.01" W
09	South	Jatai	17° 51' 21.01" S	51° 45' 51.04" W
10	South	Caldas Novas	17° 46' 17.11" S	48° 39' 36.11" W

Table 2. Result of the model selection using ΔAIC for fruit weight considering pollination treatment (cross and self-pollination) and site (Nova Xavantina and Caldas Novas). Individuals were considered random factors.

Model	ΔAIC value	Degrees of Freedom
Pollination treatment	0.0	4
Null model	6.3	3
Pollination treatment + site	6.4	5
Site	12.3	4
Pollination treatment + Site + Interaction	15.0	6

Table 3. Results of the selection of models using ΔAIC for fruit set in *Curatella americana*. The full model included region (South, Middle and North) and reproductive systems (cross, self, autogamous and natural pollination) as fixed factors, the interaction between them and individuals and sites (replication) as random factors. “Full without interaction” was similar to the full model except for the interaction between fixed factors. “Only reproductive system” did not considered region, whilst “Only region” did not considered reproductive system, and the null model is only the intercept and the random factors (individual and population).

Model	AIC	Degrees of Freedom
Full	0.0	13
Full without interaction	1523.9	7
Only reproductive system	1545.7	5
Only region	4813.5	4
Null	4846.8	2

Table 4. Population means of the proportion of fruit set in the pollination treatments of *Curatella americana* L. at all studied populations in Brazil. At Roraima - BV: Boa Vista, Faz: Fazenda Bamerindus, Ama: Amajari; Pará - Stm: Santarém; Mato Grosso - Cui: Cuiabá, Man: Manso, Poc: Poconé, Nxav: Nova Xavantina and Goiás – Jat: Jatai, Cnov: Caldas Novas.

	Ama	Faz	BV	Stm	Cui	Man	Poc	Nxav	Cnov	Jat
Cross-pollination	0.52	0.54	0.33	0.66	0.66	0.83	0.81	0.79	0.82	0.73
Hand selfing	0.53	0.29	0.22	0.06	0.13	0.37	0.08	0.17	0.43	0.20
Autogamous self	0.21	0.24	0.23	0.05	0.06	0.06	0.05	0.02	0.29	0.20
Natural pollination	0.32	0.15	0.23	0.28	0.65	0.48	0.62	0.63	0.72	0.34

Table 5 Single correlations of climate and pollinator visitation with pollination mode. Statistically significant relationships are marked in bold.

	Hand-cross-pollination	**Hand-self-pollination**	**Spontaneous self-pollination**	**Natural pollination**
MAT	**-0.78**[†]	+0.00[NS]	+0.19[NS]	**-0.63**[†]
MAP	-0.22[NS]	-0.24[NS]	-0.16[NS]	-0.50[NS]
MAT seas	**+0.78**[†]	-0.24[NS]	-0.36[NS]	**+0.91**[*]
MAP seas	-0.36[NS]	+0.51[NS]	**+0.62**[†]	-0.23[NS]
MAT anomaly	-0.37[NS]	+0.59[NS]	**+0.74**[†]	-0.23[NS]
MAP anomaly	**+0.72**[†]	-0.32[NS]	-0.49[NS]	+0.47[NS]
MAT velocity	**-0.65**[†]	+0.07[NS]	+0.06[NS]	**-0.76**[†]
MAP velocity	**+0.68**[†]	-0.50[NS]	-0.52[NS]	+0.45[NS]
Pollinator richness	+0.52[NS]	-0.30[NS]	-0.47[NS]	+0.54[NS]
Pollinator visitation frequency	+0.40[NS]	-0.13[NS]	+0.09[NS]	+0.17[NS]
% Large bee visits, incl. honey bee	**+0.70**[†]	-0.37[NS]	-0.53[NS]	**+0.79**[†]
% Large bee visits, natives only	**+0.84**[†]	-0.15[NS]	-0.25[NS]	**+0.79**[†]

[*] P < 0.05 both when using non-spatial statistics and when significance level is based on degrees of freedom corrected for spatial auto-correlation using Dutilleul's (1993) method; [†] P < 0.05 when using non-spatial statistics, but non-significant when using Dutilleul's (1993) method; [NS] non-significant.

Table 6. Multiple regression models using climate to predict pollination mode. The standardized regression coefficients are reported for ordinary least square (OLS) regression, and reported for both an averaged model based on weighted *wi* and minimum adequate models (MAMs) (Diniz-Filho et al. 2008). For all MAMs, we give AIC_c, the Condition Number (CN), Moran's I (significance tested using 5 distance classes and applying a permutation test with 10,000 iterations), and coefficients of determination (R^2 and R^2_{adj}). We did not assign any MAM if all variables in the best-fit model were non-significant. Notice that climate stability is represented by temperature and precipitation anomaly between 21000 years ago and now. See Appendix 2 for similar calculations when using temperature and precipitation velocity.

	Hand-cross-pollination						**Hand-self-pollination**					
	Σw_i	Averaged	MAM†	Σw_i	Averaged	MAM$^{£}$	Σw_i	Averaged	MAM	Σw_i	Averaged	MAM
MAT	0.56	-0.72	-0.78^{**}	0.31	-0.66	–	0.10	-0.06	–	0.14	-0.08	–
MAP	0.09	+0.14	–	0.08	-0.10	–	0.13	-0.25	–	0.19	-0.31	–
MAT seas	0.51	+0.74	–	0.64	+0.64	$+0.59^{*}$	0.13	-0.29	–	0.18	-0.31	–
MAP seas	0.06	-0.21	–	0.04	-0.12	–	0.29	+0.47	–	0.47	+0.50	–
MAT anomaly	0.14	-0.32	–				0.53	+0.58	–			
MAP anomaly				0.59	+0.54	$+0.51^{*}$				0.19	-0.29	–
AIC_c			-6.997			-8.84						
Moran's I			$\leq 0.27^{NS}$			$\leq 0.22^{NS}$						
CN			1			1.5						
R^2			0.61			0.82						
R^2_{adj}			0.61			0.80						

Table 2. Continued.

	Autogamous pollination						**Natural pollination**					
	Σw_i	Averaged	MAM	Σw_i	Averaged	MAM	Σw_i	Averaged	MAM	Σw_i	Averaged	MAM
MAT	0.06	+0.14	–	0.09	+0.11	–	0.04	-0.04	–	0.04	-0.04	–
MAP	0.08	+0.05	–	0.11	-0.23	–	0.05	+0.04	–	0.05	+0.03	–
MAT seas	0.11	-0.30	–	0.15	-0.36	–	0.99	+0.91	$+0.91^{**}$	0.99	+0.91	$+0.91^{**}$
MAP seas	0.21	+0.53	–	0.61	+0.62	–	0.05	+0.09	–	0.05	+0.09	–
MAT anomaly	0.79	+0.73	$+0.74^{*}$				0.06	-0.12	–			–
MAP anomaly				0.22	-0.45	–				0.08	+0.16	
AIC_c			-3.821						-11.098			-11.098
Moran's I			$\leq 0.39^{NS}$						$\leq 0.01^{NS}$			$\leq 0.01^{NS}$

CN	1	1	1
R^2	0.55	0.83	0.83
R^2_{adj}	0.55	0.83	0.83

**P < 0.01; *P < 0.05; ^{NS}non-significant. † One model was equally fit (i.e. $\Delta AIC_c \leq 2$) containing the following variables: 1) MAT seas. $^{\pounds}$two models were equally fit: 1) MAT; 2) MAT Seas.

	MAT anomaly	MAP anomaly	MAT velocity	MAP velocity	MAT	MAP	MAT seas	MAP seas	Pollinator richness	Flower visitation frequency	% Large bee visits, incl. honey bee
MAT anomaly	-										
MAP anomaly	**-0.75**†	-									
MAT velocity	+0.19^{NS}	-0.59^{NS}	-								
MAP velocity	-0.56^{NS}	**+0.77**†	-0.30^{NS}	-							
MAT	+0.06^{NS}	-0.52^{NS}	**+0.79**†	-0.37^{NS}	-						
MAP	-0.45^{NS}	+0.26^{NS}	+0.03^{NS}	-0.12^{NS}	+0.10^{NS}	-					
MAT seas	-0.14^{NS}	+0.37^{NS}	**-0.64**†	+0.54^{NS}	**-0.69**†	-0.59^{NS}	-				
MAP seas	+0.59^{NS}	-0.54^{NS}	-0.06^{NS}	**-0.79**†	+0.18^{NS}	+0.06^{NS}	-0.33^{NS}	-			
Pollinator richness	-0.61^{NS}	**+0.68**†	**-0.66**†	+0.33^{NS}	-0.68^{NS}	+0.25^{NS}	+0.45^{NS}	-0.42^{NS}	-		
Pollinator visitation frequency	+0.14^{NS}	+0.15^{NS}	-0.55^{NS}	-0.06^{NS}	**-0.75**†	+0.13^{NS}	+0.35^{NS}	+0.19^{NS}	+0.46^{NS}	-	
% Large bee visits + honey bee	**-0.65**†	**+0.78**†	**-0.74**†	+0.57^{NS}	**-0.66**†	**+0.78**†	**+0.75**†	-0.45^{NS}	**+0.85***	+0.29^{NS}	-
% Large bee visits natives only	-0.35^{NS}	**+0.71**†	**-0.84***	+0.56^{NS}	**-0.91**†	**+0.71**†	**+0.80**†	-0.33^{NS}	**+0.78**†	+0.58^{NS}	**+0.88***

Appendix 1. Correlations between putative predictor variables.

[*] $P < 0.05$ both when using non-spatial statistics and when significance level is based on degrees of freedom corrected for spatial auto-correlation using Dutilleul's (1993) method; [†] $P < 0.05$ when using non-spatial statistics, but non-significant when using Dutilleul's (1993) method; [NS] non-significant.

Appendix 2. Multiple regression models using climate to predict pollination mode. The standardized regression coefficients are reported for ordinary least square (OLS) regression, and reported for both an averaged model based on weighted *wi* and minimum adequate models (MAMs) (Diniz-Filho et al. 2008). For all MAMs, we give AIC_c, the Condition Number (CN), Moran's I (significance tested using 5 distance classes and applying a permutation test with 10,000 iterations), and coefficients of determination (R^2 and R^2_{adj}). Notice that climate stability is represented by temperature and precipitation velocity between 21000 years ago and present. See Table 2 for similar calculations when using temperature and precipitation anomaly.

Figures

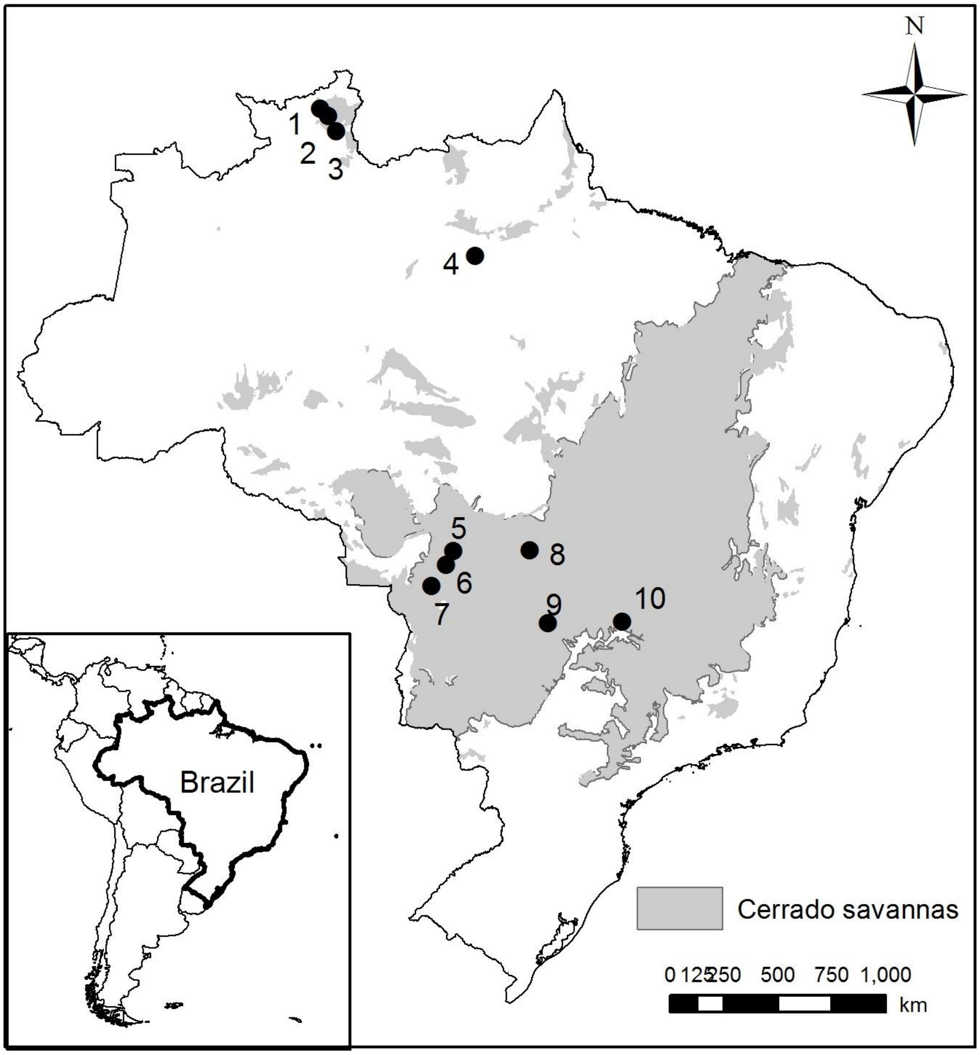


Figure 1. Map of the sampled sites of *Curatella americana* in Brazil. Numbers follow table 01.

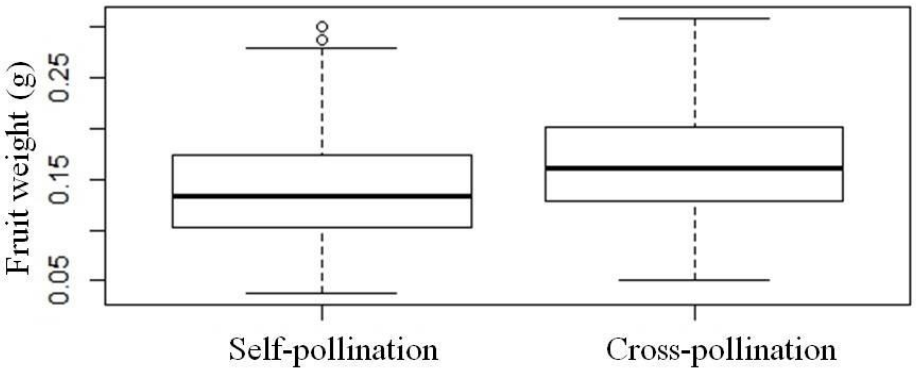
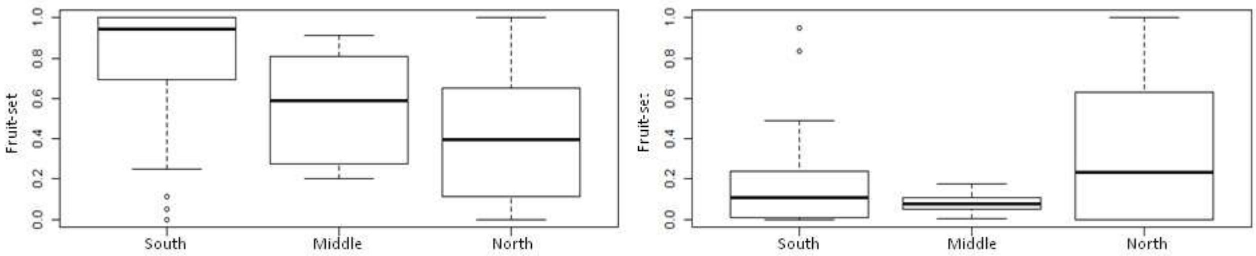
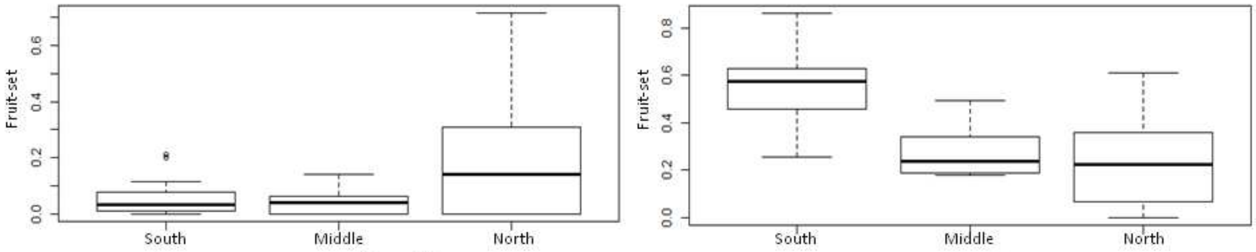


Figure 2. Fruit weight comparison between self and cross-pollinated fruit in *Curatella americana*.



Cross-pollination

Self-pollination



Spontaneous self-pollination

Natural pollination

Figure 3. Box plot comparing mean fruit set according to the mating system of *Curatella americana* in controlled pollination tests. Region names follows table 1.

Chapter 5. **The macroecology of animal *versus* wind pollination**

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Key-words: abiotic, biotic, climate, precipitation, species richness, forest, open vegetation, pollen dispersal.

Short running-title: The macroecology of pollination

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ABSTRACT

Aim: To test the underlying biotic and abiotic determinants of global variation in animal *versus* wind pollination in plant communities.

Location: Globally, we analysed 50 plant communities ranging from 54º North to 34º South.

Methods: For each community, the proportion of animal and wind pollinated plants was estimated, then scored as: forest or open vegetation; mainland or island; topographic heterogeneity and plant species richness. We also determined current and Quaternary climate-change variables from Worldclim. To test which of these biotic and abiotic determinants best explain the proportion of pollination modes, we used ordinary least squares (OLS) regression with information-theoretic model selection.

Results: Overall the proportion of animal-pollinated plant species was positively associated with plant species richness and current temperature. In addition, animal pollination was related to both current and historical precipitation: in open vegetation, animal pollination was more pronounced in areas that had wetter glacial climates than today, whereas in forest communities, animal pollination increased strongly and positively with current precipitation. Climate stability *per se*, as measured by glacial-interglacial velocity of climate change (precipitation and temperature) had no measurable effect on the proportion of animal *versus* wind pollination.

Conclusion: The relative frequencies of the two major pollination modes in plant communities around the world are related to local plant species richness, temperature, current and - in some settings - historical precipitation. This possibly reflects primary production and resource availability to produce nectar in warm and wet habitats. Moreover, wind pollination probably become less efficient due to pollen clumping by water in high humidity areas and in diverse and structurally closed plant communities. Irrespectively of exact mechanism, our study shows that

both ecological and historical processes determine the global distribution of animal versus wind pollination.

INTRODUCTION

Pollination is a fundamental process in plant reproduction and the maintenance of plant communities and, hence, is crucial for the functioning of most terrestrial ecosystems (Ollerton *et al.*, 2011; Lever *et al.*, 2014). Plants can achieve pollen transfer among conspecifics using biotic (animal) and abiotic (wind and, rarely, water) pollen vectors. Wind pollination is found in about 20% of the families of angiosperms and in many of the gymnosperms (Ackerman, 2000). For angiosperms, it is estimated that around 12.5% of all species are wind pollinated (Ollerton *et al.*, 2011). Wind pollination was originally considered a basal pollination mode for angiosperms, but is actually a derived condition that has evolved independently more than 60 times within various clades of otherwise animal pollinated species (Ackerman, 2000; Friedman & Barrett, 2008; 2009; Hu *et al.*, 2012), with occasional reversions back to animal pollination (*e.g.* Wragg & Johnson, 2011).

Wind pollination is currently understood as a reproductive strategy that evolves when pollinators become unavailable, for instance, caused by natural pollinator extinctions or when plants colonize pollinator depauperate areas such as islands (Weller *et al.*, 1998; Goodwillie, 1999; Culley *et al.*, 2002; Olesen & Jordano, 2002; Friedman & Barrett, 2009). Some evidence to support this idea is the observation that the proportion of wind-pollinated plants is higher in the temperate region (Culley *et al.*, 2002), where climate is more dynamic, both with respect to within-year seasonal fluctuations (Cramer *et al.*, 1999; Swenson *et al.*, 2012) and long-term changes over the Quaternary (Sandel *et al.*, 2011). In temperate regions a maximum of about 80% of the plant species within a given community are animal pollinated, whereas animal pollination is more common in the climatically more stable tropics where ≥90 % of all locally co-occurring plant species are pollinated by animals (Whitehead, 1969; Regal, 1982; Ollerton *et* *al*., 2011). Although this pattern is already known, no study has hitherto quantified the underlying factors causing the global variation in community level differences in wind *versus* animal pollination (Barrett, 1996; Schemske *et al.*, 2009; Ollerton *et al*., 2011).

It is plausible to think that the efficiency of wind and animal pollination depends on the biotic and abiotic environment where the plants are occurring, and indeed a number of testable hypotheses for geographic variation in wind *versus* animal pollination have been put forward (Whitehead, 1969; Culley *et al*., 2002). For instance, extensive dense and species-rich forests with high canopy and low wind speeds are supposed to be unfavourable places for wind pollination simply because the chance of wind transporting pollen to a conspecific should be rather low (Whitehead, 1969; Regal, 1982; Ollerton *et al*., 2006; 2011; Culley *et al*., 2002). Climate may also affect the efficiency of wind pollination as areas of high humidity and precipitation should make pollen grains heavier and clumped, limiting dispersal distance and the efficacy of the strategy (Whitehead, 1969; Niklas, 1985). Additionally, high temperature and precipitation may increase productivity and support more nectar production for animal pollination. On the other hand, high temperature and precipitation seasonality may promote wind pollination as animal pollinator abundances may fluctuate through the year and therefore become less reliable (Regal, 1982) or, alternatively, pollination may be in synchrony with these seasonal fluctuations in pollinator abundances thereby causing no measurable effect on pollination mode (McKinney *et al*., 2012).

Past climatic conditions may also influence current pollination systems (Dalsgaard *et al*., 2011; 2013; Groom *et al*., 2014). For instance, one explanation for the dominance of wind pollination among gymnosperms is related to the global climate (drier and hotter) when these plants first appeared on Earth 280-260 mya (Willis & McElwain, 2002). Paleoclimate has been used to explain contemporary ecological patterns such as species distributions and endemism (Svenning & Skov, 2007; Cárdenas *et al.*, 2011; Sandel *et al.*, 2011; Kissling *et al.*, 2012), and the structure of interactions within communities of plants and their animal pollinators (Dalsgaard *et al.*, 2011; 2013). It has been suggested that past climatic instability may have broken up mutualistic associations between plant species and their pollinating animals (Memmott *et al.*, 2007; Dalsgaard *et al.*, 2011; 2013; McKinney *et al.*, 2012) and, hence, instability may have favoured wind pollination. Finally, in islands and areas of low topography, such as coastal plains, wind pollination is supposed to be favoured due to high wind speeds and because wind pollinated plants are independent of pollinator colonization to maintain reproduction (Kühn *et al.*, 2006; Crawford *et al.*, 2011).

Here, we use a global dataset to quantify the effect of these putative contemporary and historical drivers of the current proportion of animal *versus* wind pollinated species in plant communities around the world.

METHODS

Animal versus *wind pollination datasets*

The dataset used here was taken from 50 published and unpublished community-based independent assessments of the proportion of wind and animal pollinated species (Figure 1). The 50 studies are censuses of all angiosperm species in flower in a given community at a specific time. The latitudes range from 34º South (La Floresta – Uruguay), to the equator (Flooded Rainforest in Colombia), and to 54º North (Grampian Mountains – Scotland).

For the published studies we followed the judgement of the original authors as to the pollination mode (wind or animal) of the plants they surveyed. For our own data collection flowers were assessed in terms of presence or absence of: mass pollen release when an inflorescence was shaken; feathery stigmas; flower colour and size; presence of scent or nectar; and the presence and behaviour of potential flower visitors. Therefore, our dataset deals with the prevalence of species whose floral traits and ecological interactions indicate either wind or animal pollination.

The proportion of animal-pollinated plant species could be underestimated because some plant species possessing wind-pollination traits can also be visited and sometimes pollinated by animals (Figueredo & Sazima, 2000). There might also be possible biases related to misclassification of the obligatory self-pollinating and non-sexually reproducing species, though this should be a relatively minor bias (Ollerton *et al*., 2011) and is unlikely to be correlated spatially and, hence, it should not affect our results. Similar to Ollerton *et al*. (2011), we took a community-level approach (rather than a phylogenetic one) as we were interested in the ecological question of how large a proportion of plant species in terrestrial communities are animal- or wind-pollinated, and the possible processes generating these proportions.

Determinants of animal pollination

For each site, we extracted a suite of variables describing current climate conditions and paleoclimatic history hypothesised to affect the degree of wind *versus* animal pollination. Current climate descriptors included mean annual temperature (MAT), annual precipitation (MAP), temperature seasonality (MAT seasonality) and precipitation seasonality (MAP seasonality) extracted using the 2.5 arc-minute resolution (approximately 4 km^2) from the

Worldclim dataset (www.worldclim.org; Hijmans *et al*., 2005). As different climate change metrics may capture different effects of climate change (Garcia *et al*., 2014), we considered two measures of historical climate stability: 1) the velocity of mean annual temperature (MAT velocity) and mean annual precipitation (MAP velocity) between the Last Glacial Maximum (LGM) and the present; 2) the anomaly of mean annual temperature (MAT anomaly) and mean annual precipitation (MAP anomaly) between the LGM and the present, calculated as current minus LGM precipitation/temperature (i.e. positive values indicate that conditions are wetter or warmer at present than at LGM). Velocities reflect the speed of climate change irrespective of the direction of the change, whereas anomalies also reflect the direction of climate change. Paleoclimate estimates were obtained from the CCSM3 model (Collins *et al*., 2006; Otto-Bliesner *et al*., 2006), statistically downscaled to 2.5 arc-minute resolution (Hijmans *et al*., 2005) to match the current climate data.

In addition, we determined various non-climate descriptors of local conditions at each site, including whether the site was on the mainland or on an island, topographic heterogeneity, regional proportion of tree cover, plant species richness, and whether the vegetation was forested or of a more open vegetation. Topographic heterogeneity was calculated as the range of elevation values observed in a 0.2° × 0.2° window (approximately 20 × 20km) centered on the sampling location, using the Worldclim 1km DEM (which is derived from the SRTM DEM - Farr *et al.* 2007). The regional proportion of tree cover was calculated as the average tree cover observed within a window of 64 x 64km around each site, based on the Vegetation Continuous Fields product applied to MODIS data (Hansen *et al*., 2003). Vegetation structure was based on site descriptions, as was whether a given study was conducted in forest (n

= 14), or in open vegetation such as grassland, scrubland, and other low and open vegetation types (n = 36).

For all analyses, plant species richness, MAT anomaly, precipitation and temperature velocities were Log_{10}-transformed; contemporary mean annual precipitation and topography were square-root transformed. The proportions of animal and wind pollinated plant species in each community were arcsine square-root transformed. All other variables were left untransformed. We examined the correlations between all predictor variables, and excluded temperature seasonality from all analyses as it was strongly correlated with current temperature, and the proportion of regional tree cover as it was strongly correlated with contemporary precipitation (see Appendix Table S1 in Supporting Information). Due to the strong positive relationship between precipitation and the proportion of regional tree cover, we adjusted our above hypothesis of a unidirectional positive effect of precipitation on animal pollination. For forest, we therefore expect a strong positive correlation between precipitation and the proportion of animal pollination, whereas for open vegetation types we expect a less strong (even zero) effect of precipitation due to the possible opposite effects of precipitation and fragmentation on the proportion of animal pollination.

Statistical analyses

In order to check for a latitudinal trend, the relationship of pollination mode proportions and latitude was tested with a quadratic regression.

To test the study hypotheses, we used ordinary least squares (OLS) regression and conducted information-theoretic model selection as outlined by Diniz-Filho *et al.* (2008). In doing this, we tested the effect of both temperature/precipitation velocity and anomaly; the anomaly models generally performed the best (highest R^2 and lowest AIC), so we focused on these models. First, we fitted models with all combinations of the explanatory variables: forest *versus* open vegetation, insularity, plant species richness, contemporary precipitation, contemporary temperature, precipitation seasonality, topography and historical climate stability as measured by temperature and precipitation anomaly. We then identified minimum adequate models (MAMs) among these as any model with $\Delta AIC_c < 2$ (Table 1). We identified MAMs both for the global dataset (n = 50, 255 alternative models), and separately for open vegetation types (n = 36, 127 models, excluding the forest/open variable) and forests (n = 14, 127 models). When using temperature and precipitation velocity as a measure of climate stability, we excluded topography as it is strongly correlated with velocities (Appendix S1), i.e. when using velocity as our historical climate variable we compared 127 models for all vegetation types and 63 models for the separate analysis of open vegetation types and forest.

Standardized regression coefficients are reported for both a multi-model average regression model based on weighted w_i and the MAMs (Diniz-Filho *et al*., 2008). We tested whether significant positive spatial autocorrelation remained in model residuals (i.e., whether $p < 0.05$, tested using 10 distance classes and applying a permutation test with 10,000 iterations). No significant positive spatial autocorrelation was recorded; hence, we did not use spatial models such as spatial autoregressive models. All analyses were conducted using the software Spatial Analysis in Macroecology 4.0 (Rangel *et al*., 2010).

RESULTS

The proportion of wind-pollinated plants in a community increases and, hence, the proportion of animal-pollinated plant species decreases with latitude (Figure 1). This pattern is broadly symmetrical across the Equator, though a larger sample of communities from southern parts of the Southern Hemisphere is required for a better assessment.

The MAMs for open vegetation (n = 36, $R^2 = 0.39$, $R^2_{adj} = 0.37$) and forest (n = 14, $R^2 = 0.78$, $R^2_{adj} = 0.78$) separately had higher explanatory power than the MAM for all vegetation types pooled (n = 43, $R^2 = 0.37$, $R^2_{adj} = 0.34$; Table 1 and Appendix S2 in Supporting Information). Across all vegetation types, the proportion of animal-pollinated plant species correlated positively with plant species richness and current temperature (Figure 2). Additionally, there was a tendency for forest sites to have a higher proportion of animal-pollinated species than open vegetation (Table 1 and Appendix S2). For open vegetation, animal pollination was relatively more frequent in species-rich plant communities and in areas that were wetter during LGM than today (n = 36, $R^2 = 0.39$, $R^2_{adj} = 0.37$; Table 1). Models including climate-change anomalies performed better than models using velocities as historical climate-change measures (n = 36, $R^2 = 0.31$, $R^2_{adj} = 0.29$; Appendix S2, Figure 3). For forest communities, animal pollination increased strongly and positively with current precipitation, while there was no relation in open vegetation (Table 1). Insularity, seasonality, temperature anomaly, precipitation and temperature velocities were not included in any of the MAMs (Tables 1, Appendix S2).

DISCUSSION

In this study we have shown that the relative proportions of wind- and animal-pollinated plant species in a community are associated with both current and historical determinants. Animal pollination dominates in the tropics and in areas with high precipitation. Open vegetation areas where LGM precipitation was higher than today had a larger proportion of animal pollinated species, as did forest sites with high contemporary precipitation. The influence of contemporary precipitation on the frequency of animal pollination supports the hypothesis that wind pollination is physically disfavoured in wetter areas (Whitehead, 1969; Niklas, 1985). Similarly, we interpret the historical precipitation effects for open vegetation types as a legacy of past high precipitation favouring animal pollination – and perhaps reflecting greater persistence and thus better survival of species with this pollination mode through the glacials (Groom *et al*. 2014) – rather than an effect of climate stability *per se*, as also illustrated by the lack of effects of climate-change velocities on animal *versus* wind pollination.

Compared to the strong effect of precipitation, we found weaker support for the hypothesis that high temperature promotes animal pollination. Similarly, the structure of plant-pollinator networks is clearly related to precipitation, but less strongly to temperature (Dalsgaard *et al*., 2011; 2013). Furthermore, current precipitation was strongly related to the percentage of regional tree cover, hence, high precipitation promoting forests. This and poor conditions for pollen dispersal by wind in closed forests may also explain why we found a trend for forests to have more animal pollinated species than open vegetation (Friedman & Barrett, 2008). Using a phylogenetic framework, Friedman & Barrett (2008) illustrated that while open vegetations have higher transition rates from animal to wind pollination, forest species are more likely to experience reverse transitions, probably because they offer poor conditions for pollen dispersal by wind (Friedman & Barrett, 2008).

Interestingly, climate seems to play a similar role for seed dispersal as there is also a pattern of higher importance of animal dispersers in rainforests, whereas in dry forests wind and self-dispersal are more common (Howe & Smallwood, 1982; Jordano, 2000 and references therein). For instance, current precipitation largely explains the proportion of endozoochory across the Atlantic Forest of Brazil, animals being more important dispersers at the wetter areas (Almeida-Netto *et al*., 2008). One tentative joint explanation for the lower frequency of animal pollinated and animal seed dispersed plants at drier sites could be the higher metabolic costs to produce nectar and fleshy fruit structures, as was originally proposed only for fruit structures (Wilson *et al*., 1989; Almeida-Neto *et al*., 2008). This may apply not only to contemporary precipitation, but also to past precipitation. Therefore, wetter areas in the past could have offered better conditions for animal pollinators and the current higher proportion of animal pollination in such areas could be a legacy of those former times. Moreover, this suggests that historical climate could also affect seed dispersal in a similar manner as the one observed here for pollination, a testable hypothesis for future study. In forests, on the other hand, the current climate seems to override any historical signal.

Another important factor positively associated with animal pollination was plant species richness. Trophic interactions between plants and animals respond to the bottom-up effect of plant species richness (Scherber *et al*., 2010); for instance, in the Neotropics the diversity of pollinating birds and bats is associated with their food plant species richness (Fleming, 2005). Meanwhile, as plant species richness increases, the density of conspecifics per unit area tends to decrease (Commita *et al*., 2010) and this also may reduce the efficiency of pollen dispersion by wind (Whitehead, 1969; Regal, 1982). Moreover, plant species richness may play an important role in sustaining pollinator communities (Ebeling *et al*., 2008; Dorado &

Vázquez, 2014) via two main mechanisms: 1) more plant species per unit area may ensure a more predictable and continuous food supply via complementarity in resource composition and production over time (Waser & Real, 1979; Rathcke, 1983; Buzato *et al*., 2000; Blüthgen & Klein, 2011; Yang *et al*., 2013); 2) via the "biodiversity insurance effect" as more plant species increases species redundancy within a functional group and consequently reduces the risk of an animal group going extinct after a single plant species is lost (Bartomeus *et al*., 2013; Fründ *et al*. 2013; Thibaut & Connolly, 2013). For instance, a manipulative experiment had shown that high functional diversity of plants enhances the number of pollinators, and the functional diversity of pollinators increases community persistence over time (Fontaine *et al*., 2006).

Island plant-pollinator networks are known to have a lower animal to plant ratio and be less modular and specialized than mainland networks (Olesen & Jordano, 2002; Dalsgaard *et al*., 2013). A paucity of pollinators suggests that wind pollination should be more frequent on islands though this pattern is by no means as clear as expected (Barrett 1996). Our results also offer no support for insularity as an important determinant of the relative importance of animal and wind pollination in plant communities. One site (Juan Fernandez Islands) has an extremely high proportion of wind pollination (Bernardello *et al*., 2001), but this may be unusual as other islands are very similar to mainland communities at similar latitudes.

In summary, we found that contemporary and historical precipitation, vegetation structure, and plant species richness play major roles in determining global patterns of the relative frequencies of animal *versus* wind pollination. The finding that historical climate (here represented by Quaternary precipitation anomaly) influences the contemporary prevalence of animal and wind pollination, at least in open areas, reinforces the importance of historical processes shaping ongoing processes in plant-pollinator interactions (Dalsgaard *et al*., 2011;

2013) and the global distribution of animal and plant life on earth (Svenning & Skov, 2007; Cárdenas *et al.*, 2011; Sandel *et al.*, 2011; Kissling *et al.*, 2012). We hope our study will stimulate similar large-scale studies examining both historical and contemporary ecological drivers of other kinds of dispersal mechanisms, such as between plants and their frugivores *versus* wind and self-dispersal.

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Biosketch: The authors are based in Brazil (Rech), Denmark (Dalsgaard, Sandel and Svenning) and the United Kingdom (Ollerton), and this manuscript is the result of a collective effort aimed to understand the underlying determinants of the relative importance of the two major pollination modes at the global scale. Rech (http://oldweb.northampton.ac.uk/aps/env/lbrg/members/rech.html) and Ollerton (http://oldweb.northampton.ac.uk/aps/env/lbrg/members/ollerton.html) are mainly interested in the evolution and ecology of pollination strategies as well as its relationship to community structure. Dalsgaard is especially interested in linking community ecology, plant-animal interaction networks and macroecology (http://research.ku.dk/search/profil/?id=270366&f=1). Sandel (http://brodysandel.wordpress.com/) and Svenning (http://pure.au.dk/portal/en/svenning@biology.au.dk) are interested in macroecology and community ecology.

Supporting information

Appendix Table S1. Correlations between predictor variables

Appendix Table S2. Contemporary and historical determinants of the pollination mode

Table and Figures

Table 1. Contemporary and historical determinants of the proportion of animal pollinated plant species in plant communities worldwide. The analysis was conducted for all vegetation types (n = 50), and separately for open vegetation types (n = 36) and forest (n = 14). The standardized regression coefficients are reported for ordinary least square (OLS) regression, and reported for both an averaged model based on weighted *wi* and minimum adequate models (MAMs) (Diniz-Filho *et al*. 2008). For all MAMs, we give AIC_c, Condition Number (CN), Moran's I, and coefficients of determination (R^2 and R^2_{adj}). Notice that historical climate stability is represented by temperature and precipitation anomaly between 21000 years ago and now (current minus LGM precipitation/temperature, i.e. positive values reflect areas having been drier or colder at LGM than at present). See Appendix Table 2 for similar calculations when using temperature and precipitation velocities as historical climate stability measures.

	All vegetation types (n = 50)			Open vegetation (n = 36)			Forest (n = 14)		
	Averaged	Σw_i	MAM[†]	Averaged	Σw_i	MAM[¶]	Averaged	Σw_i	MAM[£]
Open vegetation vs forest	+0.21	0.52	$+0.21^{NS}$						
Insularity	+0.06	0.23		+0.02	0.19		+0.14	0.10	
Plant species richness	+0.40	0.98	$+0.39^{**}$	+0.49	0.98	$+0.51^{**}$	+0.01	0.09	
MAT	+0.48	0.97	$+0.36^{**}$	+0.16	0.28		+0.49	0.19	
MAP	-0.06	0.23		-0.21	0.36		+0.97	0.96	$+0.86^{**}$

MAP seasonality	-0.09	0.27		-0.03	0.21		+0.25	0.24	
Topography	+0.24	0.53		+0.22	0.45		-0.24	0.18	
MAT anomaly	+0.13	0.29		+0.01	0.20		+0.53	0.45	
MAP anomaly	-0.06	0.24		-0.37	0.88	-0.40^{**}	+0.22	0.16	
AIC_c			-36.11			-32.42			-16.57
Moran's I			$\leq 0.10^{NS}$			$\leq 0.13^{NS}$			$\leq 0.49^{NS}$
CN			1.3			1.1			1.0
R^2			0.37			0.39			0.74
R^2_{adj}			0.34			0.37			0.78

$^{**}P < 0.01$; $^{*}P < 0.05$; ^{NS}non-significant. † Seven models were equally fit (i.e. $\Delta AIC_c \leq 2$) containing the following variables, 1) Open vegetation vs forest, plant species richness, MAT, topography; 2) plant species richness, MAT, topography; 3) plant species richness, MAT; 4) plant species richness, MAT, topography, MAT anomaly; 5) plant species richness, MAT, MAP seasonality, topography; 6) Open vegetation vs forest, plant species richness, MAT, topography, MAT anomaly; 7) Open vegetation vs forest, plant species richness, MAT, MAP anomaly. ¶ Two other models were equally fit, 1) Plant species richness, topography, MAP anomaly; 2) Plant species richness, MAP, MAP anomaly. $^{£}$One other model was equally fit, 1) MAP, MAT anomaly. Notice that none of these MAMs contained significant variables not already contained in the best-fit MAM.

Figure 1. Geographic patterns of wind and animal pollination in 50 communities surveyed across the world. Fitted curves are second order polynomials; $r^2 = 0.33$, $F_{2, 47}$, 11.66, $p < 0.001$. On the map, dots were randomly moved within a small range to reduce the overlap of nearby sites.

Figure 2. The relationship between the proportion of animal pollinated plant species per community and the most important predictors, a) plant richness; b) temperature; c) precipitation; d) precipitation anomaly. Filled symbols illustrate open vegetation types whereas open symbols illustrate forest communities. Only significant relationships in minimum adequate models (MAMs) are shown, all vegetation types ($n = 50$; full line); open vegetation types ($n = 36$, dashed line); and forest ($n = 14$, dotted line). See Table 1 for statistics, and Figure 3 for partial residual plots.

Figure 3. Partial residual plots illustrating the relationship between the proportion of animal pollinated plant species per community and the most important predictors in minimum adequate models (MAMs), a) plant species richness and b) temperature for all vegetation types ($n = 50$); and c) plant species richness and d) precipitation anomaly for open vegetation types ($n = 36$). The shown fits illustrate the relationship with the residuals when all other variables included in the MAMs are accounted for in multiple regression models. For forests, the identified MAM only contained precipitation and, hence, no residual plot is shown. For statistics, see Table 1.

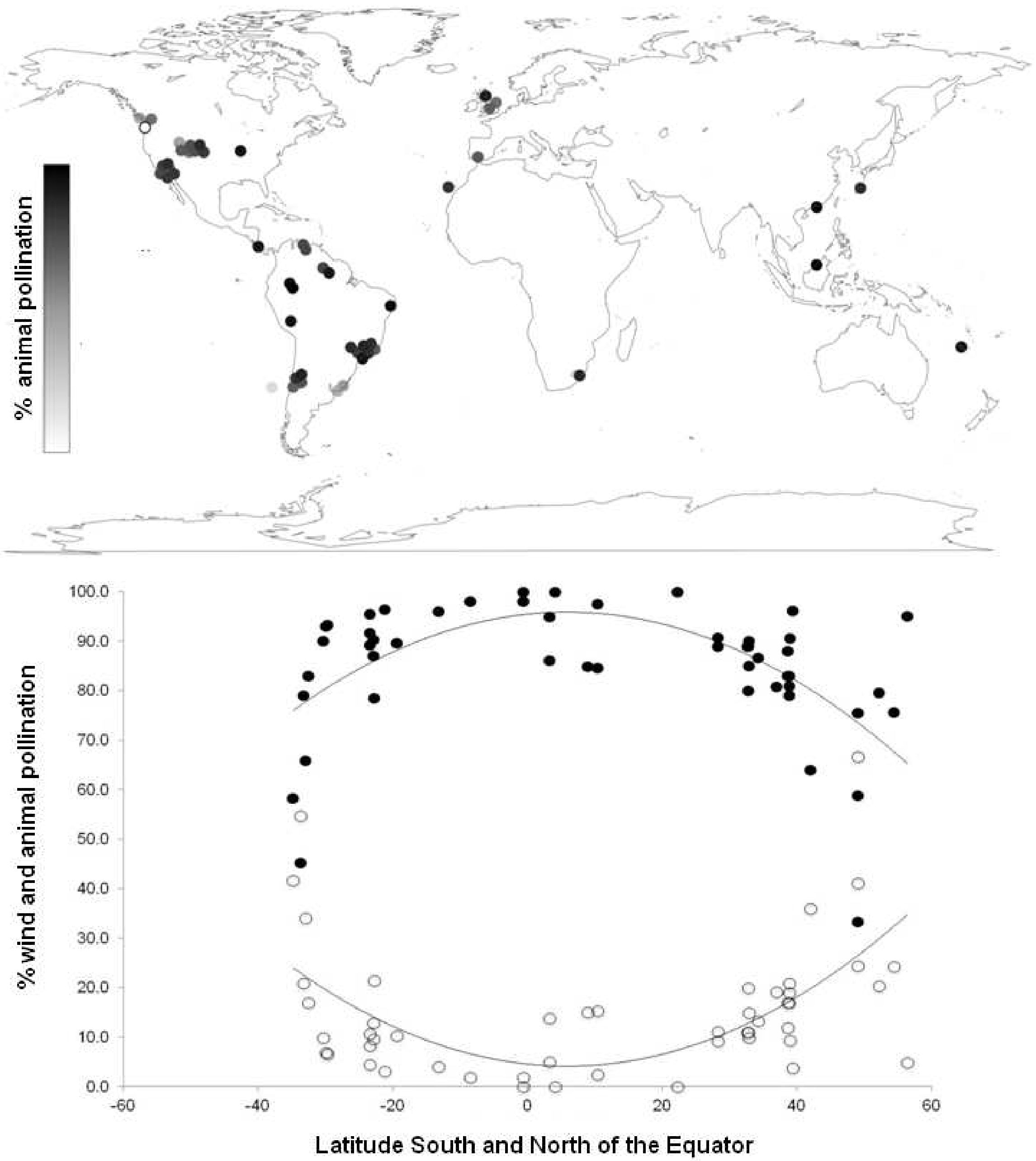
% animal pollination
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
% wind and animal pollination
-60
-40
-20
0
20
40
60
Latitude South and North of the Equator

Figure 1.

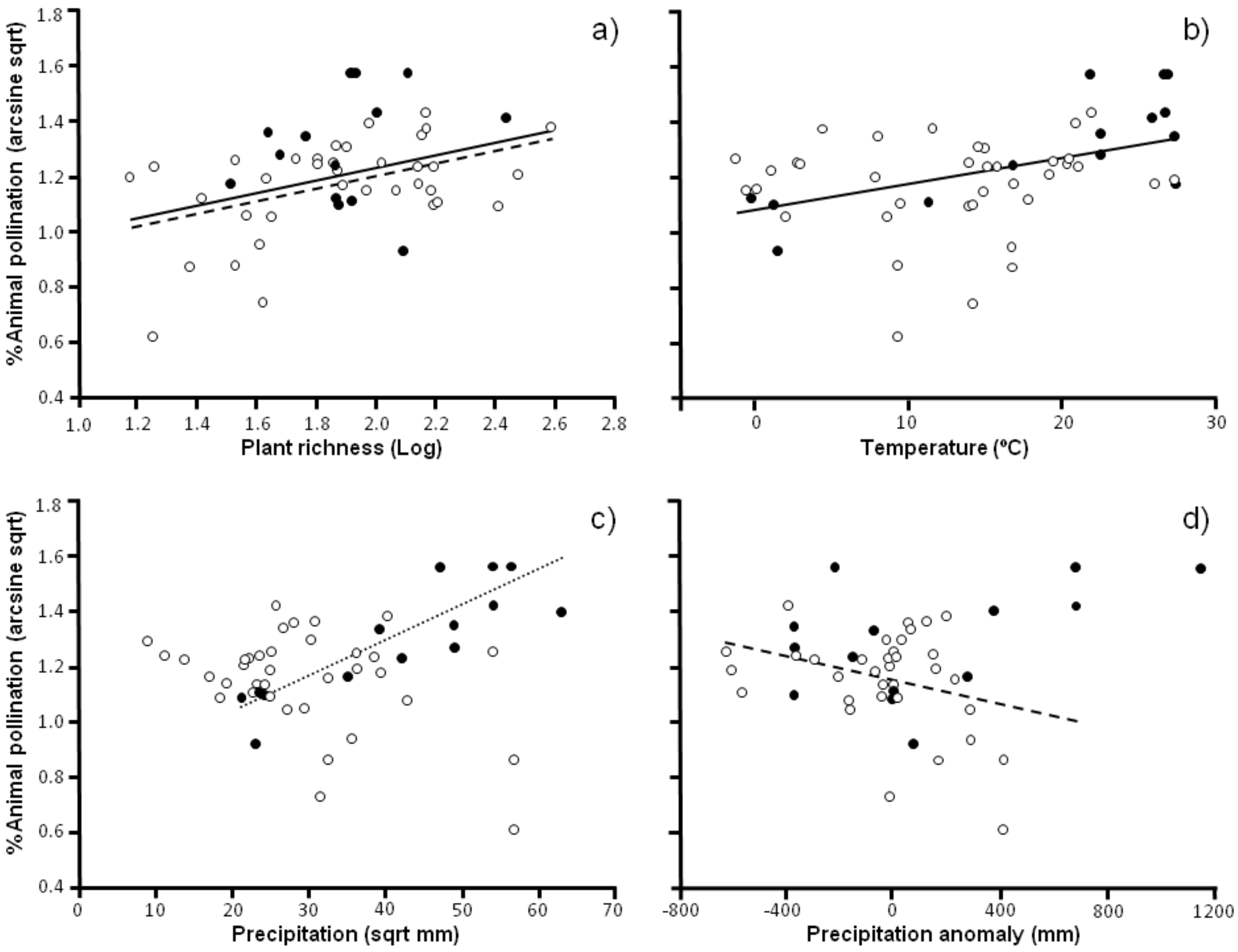
a)
b)
c)
d)
%Animal pollination (arcsine sqrt)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
Plant richness (Log)
0
10
20
30
Temperature (ºC)
%Animal pollination (arcsine sqrt)
0
10
20
30
40
50
60
70
Precipitation (sqrt mm)
-800
-400
0
400
800
1200
Precipitation anomaly (mm)

Figure 2.

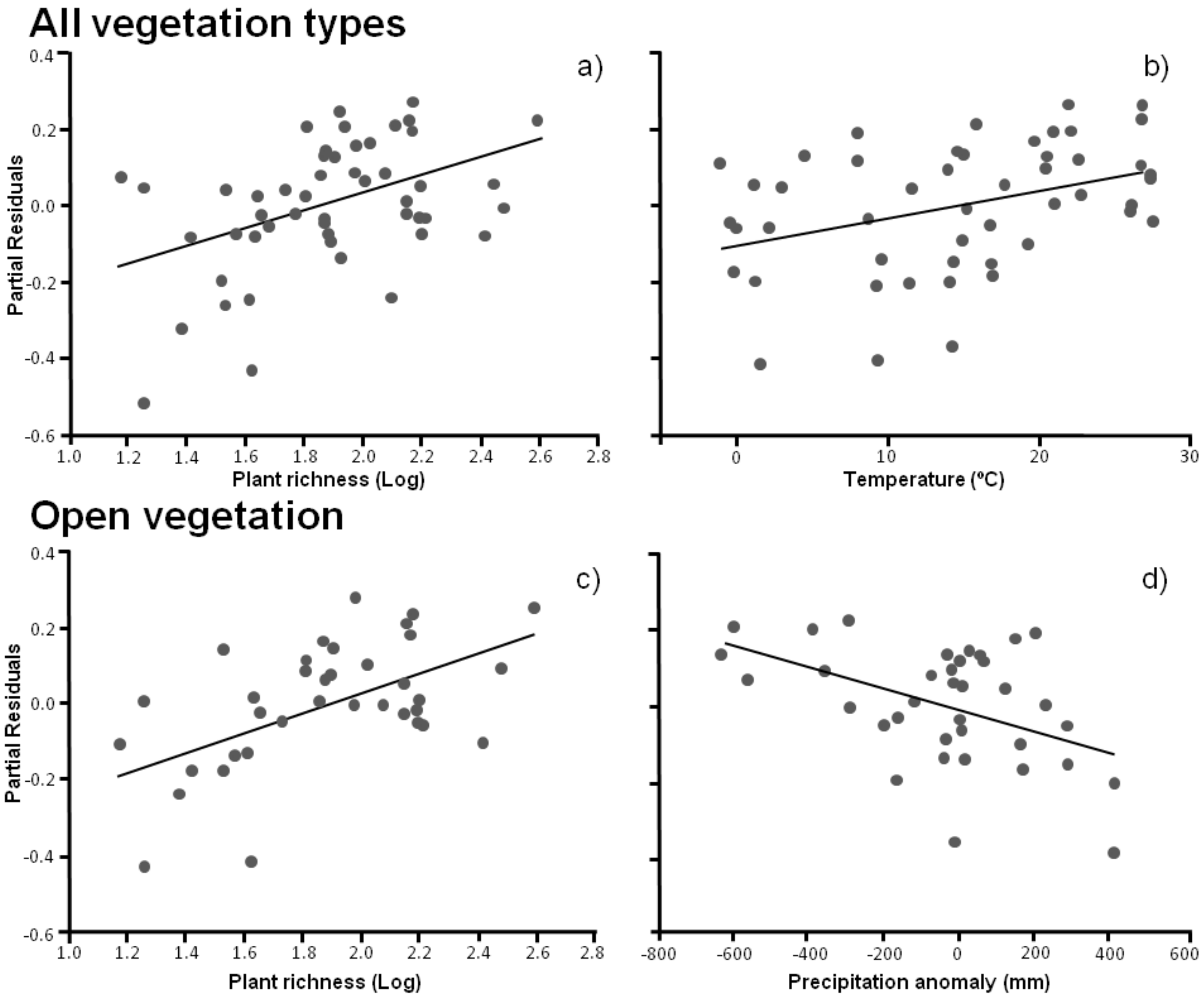
All vegetation types
a)
b)
Partial Residuals
0.4
0.2
0.0
-0.2
-0.4
-0.6
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
Plant richness (Log)
0
10
20
30
Temperature (ºC)
Open vegetation
c)
d)
Partial Residuals
Plant richness (Log)
-800
-600
-400
-200
0
200
400
600
Precipitation anomaly (mm)

Figure 3.

Appendix Table S1. Correlations between predictor variables for all vegetation types (n = 50) above the diagonal, and separately for open vegetation types (n = 36) below the diagonal.

	Plant richness	% Tree cover	MAT	MAP	MAT seasonality	MAP seasonality	Topography	MAT anomaly	MAP anomaly	MAT velocity	MAP velocity
Plant richness		-0.10^{NS}	-0.01^{NS}	-0.11^{NS}	$+0.05^{NS}$	-0.01^{NS}	-0.04^{NS}	$+0.23^{NS}$	$+0.17^{NS}$	$+0.17^{NS}$	-0.04^{NS}
% Tree cover	-0.20^{NS}		$+0.32^{\dagger}$	$+0.79^{**}$	$-0.37^{\dagger}$	$-0.27^{\dagger}$	-0.03^{NS}	-0.11^{NS}	$+0.45^{**}$	$+0.07^{NS}$	$+0.06^{NS}$
MAT	-0.01^{NS}	$+0.03^{NS}$		$+0.52^{\dagger}$	-0.78^{*}	$+0.33^{\dagger}$	-0.41^{*}	$-0.50^{\dagger}$	$+0.15^{NS}$	$+0.03^{NS}$	$+0.59^{**}$
MAP	-0.28^{NS}	$+0.71^{**}$	$+0.23^{NS}$		$-0.48^{\dagger}$	-0.26^{*}	$-0.29^{\dagger}$	-0.20^{NS}	$+0.41^{*}$	$+0.18^{NS}$	$+0.32^{\dagger}$
MAT seasonality	$+0.07^{NS}$	-0.14^{NS}	-0.64^{*}	-0.24^{NS}		$-0.42^{\dagger}$	$+0.14^{NS}$	$+0.58^{*}$	-0.27^{NS}	$+0.15^{NS}$	-0.43^{*}
MAP seasonality	$+0.09^{NS}$	-0.18^{NS}	$+0.41^{*}$	-0.28^{*}	-0.54^{*}		$+0.25^{NS}$	-0.46^{*}	$-0.32^{\dagger}$	-0.43^{*}	$+0.16^{NS}$
Topography	-0.01^{NS}	$+0.13^{NS}$	-0.30^{*}	-0.21^{NS}	-0.07^{NS}	$+0.35^{*}$		-0.14^{NS}	$-0.30^{\dagger}$	-0.77^{**}	-0.59^{**}
MAT anomaly	$+0.29^{NS}$	-0.01^{NS}	-0.46^{*}	-0.05^{NS}	$+0.55^{*}$	-0.53^{*}	-0.30^{NS}		-0.06^{NS}	$+0.55^{**}$	-0.26^{NS}
MAP anomaly	$+0.06^{NS}$	$+0.29^{NS}$	-0.16^{NS}	$+0.29^{NS}$	-0.08^{NS}	-0.24^{NS}	-0.05^{NS}	$+0.09^{NS}$		$+0.30^{\dagger}$	$+0.13^{NS}$

MAT velocity	$+0.15^{NS}$	$+0.01^{NS}$	-0.05^{NS}	$+0.17^{NS}$	$+0.27^{NS}$	-0.49^{*}	-0.76^{**}	$+0.66^{**}$	$+0.17^{NS}$		$+0.42^{*}$
MAP velocity	-0.09^{NS}	-0.14^{NS}	$+0.52^{**}$	$+0.19^{NS}$	-0.27^{NS}	$+0.15^{NS}$	-0.54^{**}	-0.16^{NS}	-0.17^{NS}	$+0.42^{*}$	

$^{**}P < 0.01$; $^{*}P < 0.05$ when P-values based on degrees of freedom corrected for spatial autocorrelation using Dutilleul's (1993) method; †significant when using traditional non-spatial statistics, but non-significant when corrected for spatial autocorrelation; ^{NS}non-significant.

Appendix Table S1, continued. Correlations between predictor variables separately for forest (n = 14).

	Plant richness	% Tree cover	MAT	MAP	MAT seasonality	MAP seasonality	Topography	MAT anomaly	MAP anomaly	MAT velocity	MAP velocity
Plant richness		$+0.13^{NS}$	-0.07^{NS}	$+0.31^{NS}$	$+0.02^{NS}$	-0.49^{NS}	-0.16^{NS}	-0.25^{NS}	$+0.45^{NS}$	$+0.26^{NS}$	$+0.10^{NS}$
% Tree cover			$+0.60^{\dagger}$	$+0.82^{\dagger}$	$-0.68^{\dagger}$	-0.37^{NS}	-0.35^{NS}	-0.50^{NS}	$+0.57^{\dagger}$	$+0.33^{NS}$	$+0.45^{NS}$
MAT				$+0.84^{*}$	-0.96^{**}	$+0.41^{NS}$	$-0.66^{\dagger}$	-0.91^{*}	$+0.37^{NS}$	$+0.28^{NS}$	$+0.77^{\dagger}$
MAP					-0.84^{*}	-0.04^{NS}	$-0.53^{\dagger}$	$-0.82^{\dagger}$	$+0.46^{NS}$	$+0.33^{NS}$	$+0.64^{\dagger}$
MAT seasonality						-0.30^{NS}	$+0.59^{\dagger}$	$+0.87^{*}$	-0.44^{NS}	-0.20^{NS}	$-0.69^{\dagger}$
MAP seasonality							-0.09^{NS}	-0.18^{NS}	-0.42^{NS}	-0.25^{NS}	$+0.20^{NS}$
Topography								$+0.64^{\dagger}$	$-0.75^{\dagger}$	-0.83^{*}	$-0.74^{\dagger}$
MAT anomaly									-0.43^{NS}	-0.24^{NS}	$-0.68^{\dagger}$
MAP anomaly										$+0.74^{*}$	$+0.50^{NS}$
MAT velocity											$+0.48^{NS}$

MAP velocity

$^{**}P < 0.01$; $^{*}P < 0.05$ when P-values based on degrees of freedom corrected for spatial autocorrelation using Dutilleul's (1993) method; †significant when using traditional non-spatial statistics, but non-significant when corrected for spatial autocorrelation; ^{NS}non-significant.

Appendix Table S2. Contemporary and historical determinants of the proportion of animal pollinated plant species. The analysis was conducted for all vegetation types (n = 50), and separately for open vegetation types (n = 36) and forest (n = 14). The standardized regression coefficients are reported for ordinary least square (OLS) regression, and reported for both an averaged model based on weighted *wi* and minimum adequate models (MAMs) (Diniz-Filho *et al*. 2008). For all MAMs, we give AIC_c, the Condition Number (CN), Moran's I, and coefficients of determination (R^2 and R^2_{adj}). Notice that historical climate stability is represented by temperature and precipitation velocity between 21000 years ago and now, and that topography is not included as strongly correlated with velocities. See Table 1 for similar calculations using precipitation and temperature anomalies as historical climate stability measures.

	All vegetation types (n = 50)			Open vegetation (n = 36)			Forest (n = 14)		
	Averaged	Σw_i	MAM†	Averaged	Σw_i	MAM¶	Averaged	Σw_i	MAM$^{\pounds}$
Open vegetation vs forest	+0.23	0.59	$+0.21^{NS}$						
Insularity	+0.06	0.24		+0.08	0.22		+0.15	0.14	
Plant species richness	+0.40	0.98	$+0.39^{**}$	+0.44	0.93	$+0.40^{*}$	-0.05	0.11	

MAT	+0.39	0.92	+0.36**	+0.16	0.31		+0.36	0.15	
MAP	-0.06	0.24		-0.31	0.65	-0.29^{NS}	+0.81	0.94	+0.86**
MAP seasonality	-0.01	0.24		+0.06	0.23		+0.13	0.16	
MAT velocity	-0.08	0.25		-0.16	0.30		+0.22	0.26	
MAP velocity	+0.05	0.25		+0.05	0.22		+0.16	0.12	
AIC_c			-36.11			-27.82			-16.57
Moran's I			≤0.10^{NS}			≤0.11^{NS}			≤0.49^{NS}
CN			1.3			1.3			1.0
R^2			0.37			0.31			0.74
R^2_{adj}			0.34			0.29			0.78

$^{**}P < 0.01$; $^{*}P < 0.05$; ^{NS}non-significant. † One model was equally fit (i.e. $\Delta AIC_c \leq 2$) containing the following variables, 1) plant species richness, MAT. ¶ Three other models were equally fit, 1) Plant species richness, MAT, MAP; 2) Plant species richness; 3) Plant species richness, MAP, MAT velocity. £One other model was equally fit, 1) MAP, MAT velocity. Notice that none of these MAMs contained significant variables not already contained in the best-fit MAM

Conclusão Geral

Ao longo dessa tese foi demonstrada a importância de se considerar as dimensões tempo e espaço no estudo da evolução de interações planta-polinizador e potenciais implicações dessa relação para a morfologia floral. Demonstramos ao longo dos capítulos que variações nessas dimensões podem afetar os mecanismos de polinização e reprodução de plantas. Fica clara a necessidade de se conhecer ao mesmo tempo a biologia básica dos organismos em questão e o contexto ecológico atual e pretérito no qual sua linhagem evoluiu para se entender os padrões dos quais faz parte. Nem todas as características florais apresentam-se conservadas filogenéticamente como demonstrado para *Davilla* e espécies sincronopátricas podem apresentar diferentes formas de evitar compartilhamento de polinizadores. Com base nos resultados do capítulo 2, percebe-se que ao estudar os polinizadores de uma determinada planta com intuito de verificar a relação daqueles mais efetivos com a morfologia floral é preciso considerar uma janela temporal compatível com o tempo de vida da planta estudada. Não considerar o tempo de vida da planta em estudo pode incorrer na detecção de padrões completamente artificiais em anos atípicos ou em sistemas que naturalmente oscilam ciclicamente. A mesma conclusão é pertinente quanto à espécies de ampla distribuição, cujas populações podem estabelecer padrões bastante diferentes de interações ao longo de sua ocorrência como demonstrado para *Curatella americana*. Pela primeira vez foi demonstrado que a história do ambiente, nesse caso representada pelas variações no clima pretérito, tem relação direta com o nível de autopolinização espontânea enquanto o nível de polinização cruzada relaciona-se muito mais fortemente com a diponibilidade de abelhas grandes nos locais estudados. Além disso, foi demonstrado o carácter de segurança reprodutiva, conferido pelos exemplos de polinização anemófila, que se torna mais eficiente em áreas de baixa riqueza de espécies e que apresentaram períodos de seca no passado. Em síntese, os resultados apresentados ao longo dos capítulos chamam atenção para questões clássicas da ecologia, as quais, embora amplamente reconhecidas, pouco tem sido consideradas até o presente no estudo da evolução de sistemas de polinização e reprodutivos. Esperamos com esses resultados motivar estudos que busquem situar os padrões interativos entre plantas e polinizadores em seu cenário ecológico e histórico e acreditamos que esse tipo de abordagem pode contribuir significativamente para entender, por exemplo, como as diversas espécies vegetais atuais enfrentarão cenários como o de mudanças globais, uma vez que a permanência e o sucesso de qualquer linhagem passa necessariamente pela sua reprodução.