UNIVERSIDADE ESTADUAL DE CAMPINAS
Instituto de Biologia

PEDRO JOAQUIM BERGAMO

EFEITOS INDIRETOS MEDIADOS POR POLINIZADORES EM COMUNIDADES DE PLANTAS

POLLINATOR-MEDIATED INDIRECT EFFECTS ON PLANT COMMUNITIES

CAMPINAS
2020

PEDRO JOAQUIM BERGAMO

POLLINATOR-MEDIATED INDIRECT EFFECTS ON PLANT COMMUNITIES

EFEITOS INDIRETOS MEDIADOS POR POLINIZADORES EM COMUNIDADES DE PLANTAS

Thesis presented to the Institute of Biology of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Ecology.

Tese apresentada ao Instituto de Biologia da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do Título de Doutor em Ecologia.

Orientadora: Profa. Dra. Marlies Sazima

Coorientadora: Profa. Dra. Marina Wolowski Torres

ESTE TRABALHO CORRESPONDE À VERSÃO FINAL DA TESE DEFENDIDA PELO ALUNO PEDRO JOAQUIM BERGAMO E ORIENTADA PELA PROFA. DRA. MARLIES SAZIMA.

CAMPINAS

2020

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca do Instituto de Biologia
Mara Janaina de Oliveira - CRB 8/6972

B452p
Bergamo, Pedro Joaquim, 1992-
Pollinator-mediated indirect effects on plant communities / Pedro Joaquim Bergamo. – Campinas, SP : [s.n.], 2020.

Orientador: Marlies Sazima.
Coorientador: Marina Wolowski Torres.
Tese (doutorado) – Universidade Estadual de Campinas, Instituto de Biologia.

1. Biologia reprodutiva. 2. Campos de altitude. 3. Ecologia de comunidades. 4. Coexistência de espécies. 5. Polinização. I. Sazima, Marlies, 1944-. II. Wolowski, Marina. III. Universidade Estadual de Campinas. Instituto de Biologia. IV. Título.

Informações para Biblioteca Digital

Título em outro idioma: Efeitos indiretos mediados por polinizadores em comunidades de plantas
Palavras-chave em inglês:
Reproductive biology
High-altitude grasslands
Community ecology
Coexistence of species
Pollination
Área de concentração: Ecologia
Titulação: Doutor em Ecologia
Banca examinadora:
Marina Wolowski Torres [Coorientador]
Camila de Toledo Castanho
Isabela Galarda Varassin
Leandro Freitas
Thomas Michael Lewinsohn
Data de defesa: 27-03-2020
Programa de Pós-Graduação: Ecologia

Identificação e informações acadêmicas do(a) aluno(a)
- ORCID do autor: https://orcid.org/0000-0002-4984-4410
- Currículo Lattes do autor: http://lattes.cnpq.br/9996095159810861

Campinas, 27 de Março de 2020

COMISSÃO EXAMINADORA

Prof.(a) Dr.(a) Marina Wolowski Torres

Prof.(a) Dr.(a) Camila de Toledo Castanho

Prof.(a) Dr.(a) Isabela Galarda Varassin

Prof.(a) Dr.(a) Leandro Freitas

Prof.(a) Dr.(a) Thomas Michael Lewinsohn

Os membros da Comissão Examinadora acima assinaram a Ata de Defesa, que se encontra no processo de vida acadêmica do aluno.

A Ata da defesa com as respectivas assinaturas dos membros encontra-se no SIGA/Sistema de Fluxo de Dissertação/Tese e na Secretaria do Programa de Pós-Graduação em Ecologia do Instituto de Biologia.

Dedico esta tese aos meus pais por terem me transmitido valores inestimáveis sobre o respeito ao conhecimento.

AGRADECIMENTOS

A leitura dos capítulos não abrange as relações pessoais-profissionais que me influenciaram profundamente ao longo do doutorado. Portanto, utilizo este espaço para expressar a importância destas relações e agradecer imensamente:

À Marlies Sazima, minha orientadora ao longo de oito anos, pelo melhor exemplo que poderia ter na carreira científica.

À Marina Wolowski, minha coorientadora ao longo de seis anos, pelo zelo com meu desenvolvimento profissional.

À Nathália Streher, companheira de tese, pelo apoio contínuo em todas as etapas do doutorado.

À Anna Traveset, que me acolheu em Mallorca, por maximizar meu estágio de pesquisa no exterior.

À Amparo Lázaro, que se tornou minha “coorientadora” em Mallorca, por partilhar tanto conhecimento comigo.

À Giovana Martins, Pamela Medina, Petra Ferronato, Nicoll Escobar, Rebeca Politano e Thamiris Smania, pela ajuda (quase) infindável em campo e em laboratório.

Aos membros do comitê de acompanhamento, Leandro Freitas e Martin Pareja, pelas dicas valiosas na escolha dos caminhos da pesquisa.

À Judith Bronstein, Goggy Davidowitz e Mario Vallejo-Marín, pelo crescimento proporcionado através de suas visitas acadêmicas ao Brasil.

Aos membros da pré-banca, Isabela Varassin, Leandro Freitas e Pietro Maruyama, pelas correções e apontamentos na versão prévia da tese.

Aos membros da banca, Camila Castanho, Isabela Varassin, Leandro Freitas, Thomas Lewinsohn, Anselmo Nogueira, Patrícia Morellato, Mathias Pires, pelas correções e apontamentos na versão de defesa da tese.

Aos professores do PPG Ecologia/Unicamp, pelos diversos ensinamentos ao longo do doutorado.

À Unicamp, especialmente aos funcionários e estudantes, por defenderem uma instituição de ensino superior pública e de qualidade.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, processo nº140254/2016-1) e à Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, processo no país nº 2016/06434-0 e BEPE 2018/02996-0), pelo financiamento contínuo deste doutorado. O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

Ao Parque Nacional do Itatiaia, especialmente aos funcionários, pela fonte inesgotável de inspiração.

Ao grupo da Marlies e agregados (Vivian, João, Coquinho, Vinícius, André, Pietro, Jeferson, Kayna, Felipe, Meire, Pamela, Camila), por fazerem na prática uma ciência sensível e coletiva.

Ao grupo de Ecología Terrestre do IMEDEA (Joisiane, Pau Colom, Pau Serra, Raquel, Sandra e Silvia), por transformarem Mallorca em um lar para mim.

Aos amigos de Campinas (Bianca, Chubs, Cuca, Karine, Kidó, Lana, Lembi, Lui, Maiona, Melô, Narra, Petra, Perse, Privê, Provs, Villa), da Periquita e agregados (Bruno, Fer, Jana, Miriam, Mylena, Pablo, Tiago, Thamy, Wandy) e da pós (Cristina, Chip, Giselle, Lufe, Tamilie, Verônica), com os quais compartilhei lares e momentos, por proporcionarem uma rede de afeto e compreensão sem igual.

Aos meus pais, Regina e Geraldo, meus irmãos André, Bruno e Dani e minhas sobrinhas Laura e Luísa, pelo apoio e amor incondicional.

Ao Glauco, meu companheiro ao longo de oito anos, por tornar minha vida serena e repleta de amor.

RESUMO

As plantas com flores frequentemente compartilham polinizadores, levando a efeitos indiretos mediados por estes. Apesar de muitos estudos terem avaliado estes efeitos entre pares de espécies de plantas, ainda não é clara a relação entre efeitos indiretos e processos ecológicos ao nível da comunidade. Assim, é incerto que fatores ecológicos determinam a direção dos efeitos indiretos mediados por polinizadores (de competição à facilitação) e como estes efeitos influenciam padrões e processos em comunidades de plantas. Neste sentido, caracterizar a denso-dependência na polinização e incorporar medidas de sucesso reprodutivo podem elucidar como os efeitos indiretos atuam em comunidades de plantas. Avaliamos os efeitos indiretos mediados por polinizadores ao nível da comunidade a partir da denso-dependência coespecífica e heteroespecífica na polinização e suas implicações na coexistência (Capítulo 1) e como esses efeitos indiretos afetam a estrutura e dinâmica (Capítulo 2) de uma comunidade de plantas de campos de altitude. Além disso, desenvolvemos um arcabouço teórico e analítico a partir da teoria de redes ecológicas para estudar efeitos indiretos mediados por polinizadores e o aplicamos à uma comunidade de plantas de dunas mediterrâneas (Capítulo 3) e à flora ornitófila de floresta tropical montana (Capítulo 4). Encontramos denso-dependência negativa (evidenciando competição intraspecífica em espécies abundantes) e facilitação interespecífica principalmente para espécies raras na polinização dos campos de altitude, uma combinação que favorece a coexistência nesta comunidade com baixa atividade de polinizadores. Além disso, especialização, *display* floral e grupo funcional de polinizador foram características ecológicas importantes que determinaram a direção da denso-dependência e de interações indiretas interespecíficas. Além disso, períodos com alta abundância de flores e baixa diversidade funcional de atributos reprodutivos estavam associados a maior sucesso reprodutivo nos campos de altitude. Estes padrões estruturais da comunidade evidenciam a prevalência de facilitação entre espécies com atributos reprodutivos similares entre si. Contudo, identificamos predominância de competição interespecífica na polinização influenciando o sucesso reprodutivo das plantas nas dunas mediterrâneas e facilitação interespecífica na polinização diminuindo a limitação polínica na flora ornitófila de floresta montana. Nestas duas comunidades os efeitos indiretos foram assimétricos: espécies de plantas generalistas e atrativas atuaram como as que causam os efeitos indiretos, enquanto que espécies especialistas e menos atrativas como as que recebem estes efeitos. Com isso, demonstramos como os efeitos indiretos mediados por polinizadores influenciam padrões e processos em comunidades de plantas. Em conclusão, fornecemos evidências que a polinização é um eixo importante do nicho, mediando interações entre plantas ao nível da comunidade.

ABSTRACT

Flowering plants often share pollinators, leading to pollinator-mediated indirect effects. Although several studies have evaluated pollinator-mediated effects between plant species pairs, it is still unclear how indirect effects determine ecological processes at the community-level. It is uncertain which ecological factors determine the direction of the pollinator-mediated indirect effects (from competition to facilitation) and how such effects influence patterns and processes in plant communities. In this context, assessments of density-dependence in pollination and the incorporation of fitness estimates may improve the understanding about how pollinator-mediated indirect effects act on plant communities. We evaluated pollinator-mediated indirect effects at the community-level by characterizing conspecific and heteroespecific density-dependence in pollination and its implications in coexistence (Chapter 1) and how such effects influence the structure and dynamics (Chapter 2) of a tropical highland grassland plant community. We also developed a theoretical and analytical framework using network theory to study pollinator-mediated indirect effects and applied it to a mediterranean dune plant community (Chapter 3) and to an ornithophilous flora of a tropical montane forest Park (Chapter 4). We found negative density-dependence (showing intraspecific competition for abundant plant species) and interspecific facilitation most for rare plant species in pollination of the highland grasslands, a combination that fosters coexistence in this community marked by low pollinator activity. Moreover, ecological factors such as specialization, floral display and pollinator functional group determined the direction of the density-dependence and interspecific indirect interactions. Otherwise, periods with high floral abundance and low functional diversity of reproductive traits were associated with high fitness in the highland grasslands. These structural community patterns highlight the prevalence of facilitation among species with similar reproductive traits. Nevertheless, we identified a predominance of interspecific competition in pollination influencing plant fitness in the mediterranean dunes and interspecific facilitation in pollination alleviating pollen limitation of the ornithophilous floral in the montane forest. The indirect effects were asymmetrical in these two communities: generalized and attractive plant species acted as the ones causing the indirect effects, whereas specialized and less attractive species as the ones receiving such effects. Thereby, we showed how pollinator-mediated indirect effects influence patterns and processes in plant communities. In summary, we provided evidence on how pollination is an important niche axis, mediating plant-plant interactions at the community-level.

SUMÁRIO

INTRODUÇÃO GERAL

Na natureza, as espécies frequentemente compartilham o mesmo recurso. A partilha deste recurso implica em sobreposição de nicho, com consequências para as populações e sucesso reprodutivo de cada espécie envolvida (MacArthur & Levins 1967). Desta maneira, a presença de uma espécie pode afetar indiretamente uma segunda espécie devido a alterações nas condições ambientais, nos recursos ou nas interações que compartilham (Wootton 1994). Em animais, a definição de efeitos indiretos frequentemente envolve o fato dos indivíduos se afetarem sem haver contato físico, abrangendo todo tipo de competição por exploração (Strauss 1991). Porém, como plantas são imóveis e por definição, não terão contato físico (excluindo os casos de epifitismo), esta definição se torna incompleta para estes organismos. Para alguns autores, o conceito de efeitos indiretos para plantas necessariamente envolve uma terceira espécie mediando os efeitos entre outras duas espécies de planta (Strauss 1991). Aqui, adotarei o conceito mais abrangente, no qual a presença de uma espécie afeta uma segunda espécie sem haver contato físico entre elas.

Os efeitos indiretos podem variar de negativos, como no caso da competição por recursos (Tilman 1982) a positivos, como quando uma espécie altera as condições abióticas e disponibilidade de recursos de modo favorável a uma segunda espécie (facilitação, Callaway & Walker 1997). Os efeitos indiretos possuem consequências ecológicas importantes determinando a dinâmica de populações e a estrutura de comunidades (Wootton 1994). Além disso, os efeitos indiretos também levam a consequências evolutivas como a seleção fenotípica e o deslocamento de caráter (Schluter 2000).

Os efeitos indiretos podem ser mediados por uma terceira espécie que provê um recurso compartilhado por outras duas espécies (Strauss 1991). Esta terceira espécie pode ser o recurso em si, quando uma presa é compartilhada por duas espécies de predadores, uma planta é compartilhada por dois herbívoros ou um hospedeiro por dois parasitas (Strauss & Irwin 2004).

Todos os casos anteriormente mencionados implicam em competição por recursos. Ainda, os casos "reversos" também são comuns na natureza: duas espécies podem interagir indiretamente ao compartilhar um inimigo natural. Isto pois uma espécie pode levar a um aumento na população do inimigo natural e desta maneira, aumentar a taxa de ataque à segunda espécie ("competição aparente", Holt 1977). Por outro lado, uma espécie pode saciar a população do inimigo natural e levar a menores taxas de ataque à segunda espécie, gerando efeitos indiretos positivos ("mutualismo aparente", Abrams & Matsuda 1996).

Os efeitos indiretos não estão restritos a trios de espécies, o que leva a padrões mais complexos. Diversas espécies podem compartilhar o mesmo recurso e gerar um padrão de competição intransitiva, na qual não há uma hierarquia competitiva entre o grupo de espécies que compartilha recurso ("A vence B, B vence C, C vence A", Soliveres et al. 2015). Além disso, as interações diretas entre um par de espécies podem gerar efeitos indiretos na interação entre outro par de espécies. Este é o caso das cascatas tróficas, no qual interações tróficas (por exemplo, predador-presa) alteram o balanço da competição por recursos ou mesmo de outras interações tróficas (Pace et al. 1999).

Quando mediados por uma terceira espécie, existem dois mecanismos que mediam os efeitos indiretos: densidade e atributos (Wootton 1994). Efeitos indiretos mediados por densidade acontecem quando mudanças na densidade da espécie compartilhada governam as interações indiretas. Por exemplo, o aumento na densidade de um inimigo natural compartilhado pode causar competição aparente entre presas (Luttbeg et al. 2003). Já efeitos indiretos mediados por atributos ocorrem quando mudanças no fenótipo da espécie compartilhada determinam as interações indiretas. Por exemplo, uma espécie de herbívoro pode induzir defesas em uma planta e assim, diminuir o consumo desta planta por uma segunda espécie de herbívoro (Ohgushi et al. 2012). Estes mecanismos não são excludentes entre si, e densidade e atributos podem mediar em conjunto o balanço dos efeitos indiretos (Luttbeg et al. 2003).

Na natureza, espécies de plantas frequentemente alteram as condições abióticas ou compartilham recursos e assim, causam efeitos indiretos entre si. Alguns dos modelos de competição por recursos mais bem desenvolvidos e suas consequências ecológicas envolvem espécies de plantas (Tilman 1982). Ainda, uma espécie de planta pode melhorar as condições climáticas ou do solo para outra espécie, conhecido como facilitação, com consequências ecológicas e evolutivas (Callaway & Walker 1997). Nos casos que envolvem uma terceira espécie, as plantas frequentemente estão sob competição aparente ao compartilhar inimigos naturais como herbívoros (Morris et al. 2004). Porém, as plantas também podem compartilhar espécies mutualistas como polinizadores, dispersores de sementes e fungos micorrízicos (Johnson & Bronstein 2019). No caso dos mutualismos, os efeitos indiretos variam de negativos (competição por mutualistas) a positivos (facilitação ao compartilhar mutualistas) (Vandermeer et al. 1982). Esta tese enfoca um dos casos de plantas compartilhando mutualistas: quando espécies de plantas compartilham polinizadores.

1. A NATUREZA DOS EFEITOS INDIRETOS MEDIADOS POR POLINIZADORES

As plantas com flores frequentemente compartilham polinizadores. Os polinizadores podem ser recursos essenciais para a reprodução das plantas e assim, parte do nicho ecológico das plantas (Pauw 2013, Benadi 2015). Desta maneira, a sobreposição no uso de polinizadores (e por consequência, de recursos reprodutivos) representa uma sobreposição de nicho entre as plantas (Pauw 2013, Benadi 2015). Portanto, interações indiretas entre plantas podem ocorrer ao compartilhar polinizadores, com consequências para reprodução de cada indivíduo. As consequências destas interações indiretas são conhecidas como efeitos indiretos mediados por polinizadores (Rathcke 1983). Os efeitos indiretos podem ocorrer entre plantas da mesma espécie que compartilham polinizadores ou entre plantas de espécies diferentes (Rathcke 1983). Além disso, os efeitos indiretos podem variar em intensidade, direção (positivo, neutro ou negativo) e tipo (via atração de polinizadores, manutenção de polinizadores ou transferência de pólen interspecífica) (Rathcke 1983).

Os efeitos indiretos mais estudados são entre espécies de planta que florescem ao mesmo tempo, chamados de interações entre plantas via atração de polinizadores (Moeller 2004). As taxas de visitação por polinizadores (e por consequência, o sucesso reprodutivo) de uma planta podem diminuir na presença de uma segunda espécie de planta florindo – fenômeno conhecido como *competição via atração de polinizadores* (Mitchell et al. 2009). Na competição via atração de polinizadores, as duas espécies de plantas podem ter prejuízos caso haja uma diluição das visitas de polinizadores entre elas (Waser 1978). Ou então, uma das espécies de planta pode ter suas taxas de visitação reduzidas quando na presença de outra altamente atrativa (por exemplo, por produzir mais recursos ou de melhor qualidade), a qual se beneficia ao manter altas taxas de visitação de polinizadores (Caruso 2000). Estes mesmos efeitos podem ocorrer entre indivíduos de uma mesma espécie de planta, levando a competição intraspecífica por polinizadores (Ward et al. 2013).

As taxas de visitação (e o sucesso reprodutivo) de uma planta também podem aumentar na presença de uma segunda espécie de planta, fenômeno conhecido como *facilitação via atração de polinizadores* (Braun & Lortie 2019). Neste tipo de facilitação, as duas espécies de plantas podem se beneficiar caso recebam mais visitas de polinizadores ao florir em conjunto do que quando florindo em separado (Ghazoul 2006). Ou então, uma das espécies de planta recebe mais visitas de polinizadores na presença da outra, enquanto não há prejuízos para esta segunda espécie (Feldman et al. 2004). Este segundo caso é comum quando uma espécie de planta atrativa (produzindo mais recursos ou de melhor qualidade) atua como um ‘ímã’ de polinizadores a espécies menos atrativas, conhecido como *efeito magnético* (Johnson et al.

2003). De maneira similar à competição, os efeitos indiretos positivos também podem ocorrer entre indivíduos de uma mesma espécie de planta (Rathcke 1983).

Dentre os outros tipos de efeitos indiretos, a troca de pólen entre espécies de plantas ocorre quando os polinizadores se movimentam entre indivíduos de espécies diferentes (Murcia & Feinsinger 1996). Este fenômeno é conhecido como transferência imprópria de pólen e possui consequências para o componente feminino e masculino da reprodução das plantas (Morales & Traveset 2008). A deposição heterospecífica de pólen nos estigmas pode influenciar o componente feminino (Ashman & Arceo-Gómez 2013). A maior parte dos estudos reporta consequências negativas do pólen heterospecífico ao bloquear o espaço do estigma para o pólen coespecífico, competição por espaço entre tubos polínicos no estilete ou pela liberação de substâncias alelopáticas (Arceo-Gómez & Ashman 2016). Porém, um estudo reportou efeitos positivos do pólen heterospecífco quando este afetou negativamente o pólen advindo de autopolinização mas não o advindo de polinização cruzada (Arceo-Gómez & Ashman 2014a). Portanto, as consequências da deposição heterospecífica de pólen devem ser dependentes de contexto. Por outro lado, os efeitos no sucesso masculino sempre são sempre assumidos como negativos devido a perda coespecífica de pólen (Muchhala et al. 2010). A planta doadora de pólen perde este pólen na parte reprodutiva feminina de outra espécie, e esse pólen invariavelmente torna-se indisponível para cumprir sua função reprodutiva. Apesar disso, conhece-se menos sobre a ecologia e consequências evolutivas da perda coespecífica de pólen em comparação a deposição heteroespecífica de pólen (Morales & Traveset 2008).

Além disso, plantas podem se afetar indiretamente através de polinizadores compartilhados mesmo quando não estão florescendo juntas. Estes efeitos ocorrem quando uma espécie de planta provê recursos florais para os polinizadores, mantendo as populações destes polinizadores na comunidade. As próximas plantas a florescerem na comunidade são beneficiadas pela manutenção de populações abundantes de polinizadores (Waser & Real 1979, Ogilvie & Thomson 2016). Apesar de pouco reportado, estudos experimentais e observacionais demonstraram que quando uma planta deixa de florescer, as próximas podem ser prejudicadas pela consequente redução nas abundâncias dos polinizadores (Waser & Real 1979, Ogilvie & Thomson 2016). Este fenômeno é conhecido como *facilitação via manutenção de polinizadores* (Rathcke 1983, Moeller 2004).

Os efeitos indiretos mediados por polinizadores podem ter consequências ecológicas ao determinar a produção de sementes e assim, influenciar a demografia de populações de plantas (Knight 2003). Desta maneira, estes efeitos indiretos atuam como filtros ecológicos ao selecionar quais espécies persistem em um local, representando processos estruturadores da comunidade de plantas (Sargent & Ackerly 2008, Wolowski et al. 2017). Os efeitos indiretos também têm consequências evolutivas, pois alteram a seleção fenotípica de atributos reprodutivos de plantas em cofloração em comparação a quando estão florescendo sozinhas (Fishman & Wyatt 1999). Além disso, plantas interagindo indiretamente via polinizadores podem passar por processos de evolução *in situ* como o deslocamento de caráter (Eisen & Geber 2018). Desta maneira, os efeitos indiretos mediados por polinizadores tem o potencial de influenciar padrões de especiação (Moreira-Hernandéz & Muchhala 2019). Há também interesses aplicados neste fenômeno: plantas exóticas podem influenciar a polinização de plantas nativas (Lopezaraiza-Mikel et al. 2007) e tanto nativas como exóticas podem influenciar a polinização de espécies cultivadas de interesse econômico, com consequências para a produção de alimentos (Badillo-Montaño et al. 2019). Portanto, entender os efeitos indiretos mediados por polinizadores é importante para avançar o conhecimento sobre processos em populações e comunidades de plantas desde pontos de vista ecológicos, a evolutivos e aplicados.

2. ESTUDOS ENTRE PARES DE ESPÉCIES DE PLANTAS

A maior parte do que conhecemos sobre efeitos indiretos mediados por polinizadores advém de estudos entre pares de espécies de planta (Rathcke 1983, Charlebois & Sargent 2017). Estes estudos demonstraram algumas características gerais dos efeitos indiretos mediados por polinizadores. Em geral, os efeitos mediados por polinizadores são assimétricos. A intensidade da competição é geralmente maior para uma das espécies (Briscoe Runquist & Stanton 2013). De maneira similar, a intensidade dos benefícios da facilitação também difere entre os pares de espécie de planta interagindo (Ghazoul 2006). Fatores ecológicos devem determinar a direção dessa assimetria, levando uma espécie a atuar mais como a que causa os efeitos indiretos e outra como a que mais recebe estes efeitos. As assimetrias podem ocorrer devido a relações dependentes de densidade: a espécie com maior densidade de flores atua reduzindo ou aumentando as taxas de visitação de polinizadores a espécie com menor densidade (Ghazoul 2006, Runquist & Stanton 2013). Ao nível intraespecífico, a maior parte dos estudos relata intensificação da competição intraspecífica por polinizadores com o aumento da densidade (Ghazoul 2005, Heystek & Pauw 2014). Porém, efeitos intraspecíficos positivos mediados por polinizadores também podem ocorrer com o aumento da densidade coespecífica (Pauw & Johnson 2018).

De modo geral em Ecologia, a densidade do recurso determina a intensidade da competição e facilitação entre as espécies. De fato, a densidade das populações de polinizadores também importa: densidades baixas de polinizadores podem levar tanto a maiores benefícios da atração conjunta via facilitação (Lázaro et al. 2014) como a aumento da competição (Lundgren et al. 2016). As densidades das plantas e polinizadores podem interagir entre si determinando os efeitos indiretos. Quando os polinizadores são abundantes, o aumento na densidade das plantas (coespecíficas e heterospecíficas) leva a maior intensidade de efeitos positivos intraspecíficos e interspecíficos (Ye et al. 2014). Porém, quando os polinizadores são escassos, os efeitos positivos intraspecíficos se mantém com a maior densidade de plantas coespecíficas enquanto a competição interspecífica se intensifica com o aumento da densidade de vizinhos heterospecíficos (Ye et al. 2014).

A distribuição espacial de plantas é vista tanto como resultado de competição ou de facilitação (“response trait”) mas também como um promotor destes efeitos indiretos (“effect trait”). No contexto da polinização, características espaciais das populações de plantas também influenciam os efeitos indiretos mediados por polinizadores. Menores distâncias entre as espécies de plantas e uma composição mista das manchas de plantas vizinhas (manchas de heterospecíficos) aumenta a intensidade dos efeitos indiretos (Bruckman & Campbell 2016). No nível intraspecífico, menores distâncias entre indivíduos levam a maior atração de visitantes florais e consequentemente, efeitos indiretos positivos do compartilhamento de polinizadores (Kunin 1997). Porém, estes agregados coespecíficos podem levar a maior competição com outras espécies de plantas (Seifan et al. 2014). Já composições mistas de manchas heterospecíficas podem resultar tanto em facilitação (Seifan et al. 2014), como ao mesmo tempo aumentar a chance de efeitos competitivos devido a maior probabilidade de transferência imprópria de pólen entre espécies (Campbell & Motten 1985, Thomson et al. 2019). Em um estudo, a densidade foi mais importante que padrões espaciais, pois o aumento da abundância de uma espécie de planta intensifica a competição com os vizinhos independentemente de seu padrão espacial (Seifan et al. 2014).

Além da densidade, os atributos das espécies também determinam suas habilidades competitivas e o quanto podem tolerar ou se beneficiar de efeitos indiretos. As espécies com maior número de flores por indivíduo (*display* floral) geralmente concentram as visitas dos polinizadores e podem prejudicar as outras plantas com as quais interagem indiretamente (Brown et al. 2002). Dentro de uma espécie, indivíduos com maior *display* floral também atraem mais polinizadores e podem prejudicar outros indivíduos em cofloração (Ghazoul 2005).

Porém, a competição intraspecífica também pode acarretar na diminuição das taxas de visitação por polinizadores em indivíduos com maior *display* floral, pois a maior oferta de flores por indivíduo pode saciar mais rapidamente os polinizadores (Ward et al. 2013). Além disso, o tipo polinizador também pode ser importante pois cada grupo de polinizador responde de maneiras diferentes a distribuição espacial dos recursos florais (Kunin 1993, Albrecht et al. 2016). Porém, não é conhecido se intensidade e a direção dos efeitos indiretos varia dependendo do tipo de polinizador compartilhado.

Por fim, a intensidade dos efeitos indiretos pode depender de características intrínsecas ao par de espécies de planta. Os efeitos negativos da deposição heterospecífica de pólen são maiores entre pares de plantas filogeneticamente próximas entre si (Arceo-Gómez & Ashman 2016). Além disso, existe a expectativa da similaridade de atributos florais entre as espécies de planta levar a maior compartilhamento de polinizadores e por consequência, de efeitos indiretos mediados por polinizadores (Gibson et al. 2012). Porém, resultados de meta-análises apontam conclusões contraditórias: parentesco filogenético e similaridade nos atributos florais intensificaram a competição mediada por polinizadores (Morales & Traveset 2009). Já a meta-análise mais recente e com maior volume de dados não encontrou efeito da filogenia e dos atributos florais (Charlebois & Sargent 2017).

De modo geral, a presença de uma espécie pode afetar o sucesso reprodutivo e, por consequência, a evolução de outras espécies. Estudos com pares de espécies demonstraram consequências evolutivas dos efeitos indiretos mediados por polinizadores. As consequências evolutivas vão desde seleção fenotípica de caracteres reprodutivos a especiação (Pauw 2019). Além disso, uma espécie de planta pode interferir no fluxo de pólen de outra ao influenciar os movimentos dos polinizadores compartilhados (Campbell 1985). Como consequência, uma espécie de planta pode levar a diminuição nas taxas de polinização cruzada nas populações de outra espécie (Bell et al. 2005). A intensidade da seleção fenotípica é mais forte quanto maior a limitação polínica das plantas interagindo indiretamente (Caruso 2000). Quando há competição interspecífica, a seleção fenotípica pode atuar favorecendo a divergência nos atributos florais de forma a reduzir efeitos negativos do compartilhamento dos polinizadores (Muchhala & Potts 2006). Foi demonstrado que a competição interspecífica por polinizadores leva a evolução de divergências nas fenologias de floração entre pares de espécies de plantas (Waser 1978) e de mecanismos de independência de polinizadores para a reprodução (autopolinização, Fishman & Wyatt 1999). Em contrapartida, a facilitação também leva a seleção de convergência nas fenologias de floração (Eisen & Geber 2018). Porém, é importante notar que a competição por polinizadores deve aumentar a intensidade de seleção por ampliar as diferenças de aptidão dentro da população, enquanto o contrário é esperado sob facilitação (Eisen et al. 2020). Entre espécies de plantas aparentadas, a deposição heterospecífica de pólen pode levar a hibridação, com consequências para especiação (Moreira-Hernandéz & Muchhala 2019). Nestes casos, os efeitos indiretos mediados por polinizadores podem ser pressões influenciando a evolução de mecanismos de isolamento reprodutivo (Kay 2006).

3. EFEITO DA COMUNIDADE EM COFLORAÇÃO EM ESPÉCIES FOCAIS

Estudos com pares de espécies são importantes para entender por quais mecanismos os efeitos indiretos mediados por polinizadores operam em escalas mais locais (entre plantas vizinhas). Porém, distintas espécies de plantas podem compartilhar os mesmos polinizadores em diferentes graus (Carvalheiro et al. 2014, Bergamo et al. 2017). Portanto, os efeitos indiretos mediados por polinizadores não estão restritos a pares de espécies de plantas em uma comunidade. Esta mudança de escala (de pares à comunidade) implica que a função de atração dos polinizadores provavelmente é resultado da atratividade geral da comunidade em cofloração. Desta maneira, a composição da comunidade em cofloração pode influenciar a polinização de cada espécie de planta.

Alguns estudos foram delineados para testar como a composição da comunidade afeta a polinização e reprodução de espécies focais. De modo geral, espécies de plantas que ocorrem em comunidades mais ricas tem menor sucesso reprodutivo, sugerindo efeitos de competição interspecífica por polinizadores (Vamosi et al. 2006). Uma espécie focal recebeu menos visitas de polinizadores e teve menor sucesso reprodutivo quando ocorria em comunidades mais diversas em comparação com comunidades menos diversas (Arceo-Gómez & Ashman 2014b). Este efeito de competição pode ser mais severo caso a espécie focal seja rara (Evans et al. 2017). Porém, maior riqueza de vizinhos pode levar a atração de maior diversidade de polinizadores para a espécie focal (Lázaro et al. 2009), indicando que a riqueza de plantas também pode levar a efeitos indiretos positivos. Neste sentido, não só a riqueza de vizinhos como o maior número de espécies na comunidade também pode intensificar efeitos de competição por polinizadores (Eisen et al. 2020). Além disso, não só a riqueza, mas uma maior diversidade funcional da comunidade também eleva a diversidade de polinizadores visitando espécies focais (Albor et al. 2019). Apesar deste efeito positivo, a diversidade funcional também está associada a redução das taxas de visitação de espécies focais ao diluir as visitas dos polinizadores em mais plantas vizinhas (Fornoff et al. 2017, Albor et al. 2019).

Estudos avaliando o papel da composição da comunidade também apontam efeitos dependentes de densidade. Além disso, o efeito da densidade é dependente de escala (Hegland 2014). Um estudo demonstrou facilitação na escala local (entre plantas vizinhas): maior densidade co- e heterospecífica levou a efeitos indiretos positivos na reprodução de uma espécie (Hegland 2014). Já na escala da comunidade o aumento da densidade co- e heterospecífica resultou em competição, com efeitos indiretos negativos na reprodução (Hegland 2014). Um outro estudo não encontrou efeito da densidade co- e heterospecífica na escala local, porém, observou aumento da competição interspecífica com a maior densidade de heterospecíficos na escala da comunidade (Albor et al. 2019).

Além de efeitos gerais da composição e densidade, existe muita variação na configuração da vizinhança com a qual os indivíduos de uma espécie de planta coocorre em uma comunidade. O efeito indireto mediado por polinizadores em uma espécie focal variou de positivo a negativo dependendo da composição de espécies na vizinhança (Lachmuth et al. 2018). Esta variação dependeu da combinação de atributos da espécie focal com as espécies vizinhas: o efeito positivo foi maior quando vizinhos coespecíficos eram maiores e heterospecíficos menores (Lachmuth et al. 2018). Estudos de facilitação planta-planta via recursos abióticos já haviam apontado que o efeito indireto pode ser positivo quando os vizinhos são menores ou mais jovens e mudar para negativo conforme os vizinhos crescem e tornam-se adultos (Callaway & Walker 1997), demonstrando um paralelo com a facilitação planta-planta via polinizadores. Portanto, a variação espacial na composição dentro da comunidade também influencia a reprodução de uma espécie. Além disso, a composição da comunidade em cofloração também varia temporalmente. Um estudo demonstrou que em períodos do ano em que a comunidade em cofloração era mais próxima filogeneticamente da espécie focal, as plantas apresentaram maior limitação polínica (Sargent et al. 2011). Isto sugere que a intensidade e direção dos efeitos indiretos mediados por polinizadores em uma espécie focal também variam ao longo do tempo.

Outros estudos observacionais e experimentais aplicaram uma lógica reversa: qual é o papel de uma espécie de planta na polinização das outras da comunidade? Nestes estudos, uma espécie altamente abundante e atrativa (nativa ou invasora) era escolhida para ser removida e comparada com áreas na qual estava presente. Então, comparava-se as taxas de visitação ao nível da comunidade na presença e ausência da espécie dominante. O primeiro estudo deste tipo reportou efeitos positivos na taxa de visitação geral da comunidade, sugerindo um efeito facilitador da espécie dominante (Lopezaraiza-Mikel et al. 2007). Outro estudo, utilizando duas espécies dominantes em uma mesma comunidade, demonstrou que uma espécie dominante pode ter efeitos facilitadores enquanto a segunda espécie dominante pode causar competição (Bartomeus et al. 2008). Além disso, os efeitos de uma espécie no resto da comunidade podem ser dependentes de densidade: facilitatora quando em menores densidades e competidora quando em altas densidades (Seifan et al. 2014).

O efeito da espécie dominante deve variar de acordo com as características das outras espécies em cofloração. Portanto, além de efeitos gerais nas taxas de visitação ao nível da comunidade, deve existir variação em como cada espécie em cofloração é afetada por uma outra dominante. Apesar de não possuir um efeito geral na comunidade, uma espécie dominante teve efeito facilitador nas que possuíam cores florais similares às suas (Hegland & Totland 2012). Em outro estudo, a espécie dominante teve efeito competidor fraco ao nível da comunidade, porém afetou mais intensamente a polinização de plantas com simetria bilateral e cores florais similares às suas (Goodell & Parker 2017). Portanto, estes estudos indicam que o efeito (seja facilitação ou competição) deve ser mais forte se as espécies em cofloração possuem atributos similares à espécie de planta dominante.

4. ESTUDOS AO NÍVEL DE TODA A COMUNIDADE

Poucos estudos avaliaram de fato a atração conjunta de polinizadores ao nível de toda a comunidade. A teoria geral de montagem de comunidades prevê que se competição ou facilitação prevalece, deve-se observar padrões resultantes dos efeitos destes processos na estrutura das comunidades. Portanto, a primeira pergunta a surgir é: existe algum efeito indireto mediado por polinizadores prevalente em cada comunidade de planta? O primeiro estudo nesta direção demonstrou que em uma comunidade alpina, a densidade de vizinhos heterospecíficos e cospecíficos não afetava a visitação de polinizadores para a maior parte das espécies de plantas (Hegland et al. 2009). Porém, dentre os efeitos dependentes de densidade, predominaram efeitos positivos de vizinhos coespecíficos e facilitação entre espécies (Hegland et al. 2009). Portanto, este estudo pioneiro demonstrou que para uma comunidade com baixa abundância de polinizadores (ou seja, escasso em recursos reprodutivos), efeitos positivos de plantas vizinhas na atração de visitantes florais são mais comuns que efeitos negativos. Novamente, aqui há um paralelo com a facilitação planta-planta via recursos abióticos: maior prevalência de interações positivas entre plantas quando os ambientes são estressantes (escasso em recursos, Callaway & Walker 1997).

As taxas de visitação por polinizadores nem sempre estão linearmente relacionadas com o sucesso reprodutivo das plantas (Lázaro et al. 2015). Desta maneira, inferir competição ou facilitação entre plantas apenas a partir do efeito de vizinhos nas taxas de visitação pode levar a interpretações errôneas sobre qual efeito indireto prevalece nas comunidades. Outro estudo em ecossistema alpino avaliou o efeito de plantas vizinhas nas taxas de visitação e produção de sementes de duas comunidades (Lázaro et al. 2013). A densidade de vizinhos heterospecíficos teve efeito geral negativo nas taxas de visitação, porém, a densidade de vizinhos coespecíficos e heterospecíficos teve efeito geral positivo na produção de sementes (Lázaro et al. 2013). Desta maneira, este estudo reforça que em ecossistemas alpinos deve haver prevalência de facilitação mediada por polinizadores. Os resultados contrastantes entre taxas de visitação e produção de sementes exemplificam a dificuldade em determinar quais efeitos indiretos ocorrem entre as espécies de plantas.

Além da discordância entre o tipo de variável resposta, este estudo também revelou que existem variação entre comunidades: foi encontrado efeito da densidade de vizinhos para apenas uma de duas comunidades amostradas (Lázaro et al. 2013). Desta maneira, o próximo passo é avaliar se há fatores que possam determinar esta variação entre comunidades. Torna-se então necessário avaliar distintas comunidades com o mesmo protocolo. Neste sentido, outro estudo amostrou redes de deposição de pólen em três comunidades andinas ao longo de um gradiente altitudinal (Tur et al. 2016) A ocorrência de facilitação entre plantas, inferida a partir do aumento de deposição pólen coespecífico em função do pólen heterospecífico, aumentou ao longo da altitude (Tur et al. 2016). Os autores atribuem esta predominância de facilitação à escassez de polinizadores em altitudes mais elevadas, o que aumentaria os benefícios da atração conjunta de polinizadores (Tur et al. 2016). Portanto, características do ambiente e, potencialmente da comunidade de plantas e polinizadores, podem determinar a predominância de cada efeito indireto mediado por polinizadores em comunidades.

Estudos com espécies focais demonstraram ampla variação espacial dentro das comunidades nos efeitos indiretos mediados por polinizadores (Seifan et al. 2014, Lachmuth et al. 2018). Portanto, as comunidades devem ser compostas por mosaicos nos quais a intensidade e sinal dos efeitos indiretos variem de acordo com a composição das vizinhanças de plantas interagindo entre si. Utilizando comunidades de espécies de *Protea*, Nottebrock e colaboradores (2017) identificaram alguns fatores associados a variação espacial dos efeitos indiretos dentro da comunidade. Se destacaram a quantidade e qualidade de recursos oferecidos por vizinhos e se a mancha de vizinhos era composta por coespecíficos ou heterospecíficos. Ainda, este estudo também demonstrou dependência de escala: efeitos negativos da densidade de vizinhos coespecíficos e heterospecíficos na escala da vizinhança, enquanto que efeitos positivos da densidade plantas heterospecíficas na escala de toda a comunidade (Nottebrock et al. 2017). Portanto, ainda que exista variação dentro da comunidade, é possível observar um efeito indireto prevalente.

Por ser um fenômeno pouco estudado ao nível da comunidade, os estudos focaram mais em identificar a direção do efeito indireto prevalente e possíveis fatores ecológicos associados à competição ou à facilitação. Porém, o compartilhamento de polinizadores significa uma sobreposição no nicho das plantas (Pauw 2013, Benadi 2015). Portanto, os efeitos indiretos mediados por polinizadores devem ter consequências ao nível de comunidade similares às observadas quando espécies sobrepõem seus nichos. Em um esforço para conectar a ecologia da polinização e teoria de coexistência, Benadi e Pauw (2018) mediram o quanto as taxas de visitação variam dependendo da densidade coespecífica ao nível da comunidade. Estes autores encontraram que as plantas recebem mais visitas de polinizadores em densidades intermediárias em comparação a densidades baixas ou altas. Desta maneira, sugerem denso-dependência negativa na polinização decorrente de competição intraspecífica por polinizadores, um mecanismo importante que pode levar a coexistência entre espécies com baixas e com altas densidades em uma comunidade (Benadi & Pauw 2018). Portanto, é hora de focar os estudos sobre como os efeitos indiretos mediados por polinizadores podem ser processos importantes determinando a estrutura e dinâmica das comunidades de plantas.

5. LACUNAS NO CONHECIMENTO E ESCOPO DA TESE

Identificar a direção dos efeitos indiretos mediados por polinizadores é importante pois efeitos positivos e negativos possuem consequências distintas para a estrutura e dinâmica das comunidades. Neste sentido, esforços recentes têm demonstrado como a polinização afeta a coexistência entre plantas (Pauw 2013, Benadi 2015, Lanuza et al. 2018, Johnson & Bronstein 2019). A teoria prevê que relações denso-dependentes na polinização podem alterar o balanço da interação entre plantas e (des)estabilizar a coexistência (Ghazoul 2005, Essenberg 2012). Por exemplo, uma comunidade marcada por denso-dependência positiva na polinização significa que espécies mais abundantes estão em vantagem (recebem mais polinização) que espécies raras. A denso-dependência positiva leva a efeitos Allee ao nível da comunidade (“community-level Allee effects”, Lachmuth et al. 2018): desestabiliza a coexistência pois as espécies abundantes estão sob vantagem e assim, aumenta as chances das espécies raras se extinguirem localmente (Benadi 2015). Já a denso-dependência negativa significaria uma vantagem a espécies mais raras ao implicar em maior competição intraspecífica nas espécies mais abundantes. Portanto, denso-dependência negativa estabiliza a coexistência de espécies abundantes e raras (Benadi 2015). Desta maneira, a denso-dependência negativa na polinização poderia contribuir para a manutenção da alta diversidade observada em comunidades tropicais.

Apesar de alguns estudos terem identificado denso-dependência na polinização ao nível da comunidade (Lázaro et al. 2013, Nottebrock et al. 2017, Benadi & Pauw 2018), nenhum destes abordou como os efeitos indiretos interspecíficos podem modificar as consequências da denso-dependência intraspecífica. Isto é crítico pois pode alterar completamente as implicações para a coexistência entre plantas. Por exemplo, um cenário estável de denso-dependência negativa pode desaparecer caso as espécies raras sofram maior competição interspecífica na polinização em comparação a espécies abundantes (Johnson & Bronstein 2019). De maneira similar, a coexistência é estabilizada caso espécies raras recebam mais facilitação que abundantes e desestabilizada no caso reverso (Feldman et al. 2004). Portanto, no **Capítulo 1,** utilizamos uma amostragem intensiva para avaliar como a densidade de coespecíficos afeta a polinização de 67 espécies de plantas ocorrendo nos campos de altitude do Parque Nacional do Itatiaia, Brasil. Avaliamos ainda como a densidade de heterospecíficos afeta a polinização destas espécies, identificando a direção do efeito indireto (facilitação vs. competição) para espécies raras e espécies abundantes.

Ao longo da Introdução, apresentamos diversos exemplos de estudos e seus principais resultados. Por diversas vezes, um estudo encontrou um efeito negativo de um fator (e.g. efeito negativo da densidade de heterospecíficos, Runquist & Stanton 2013) enquanto que outro estudo reportou efeito positivo deste mesmo fator (e.g. Ghazoul 2006). O que determina a direção dos efeitos indiretos mediados por polinizadores deve então depender de características intrínsecas a cada espécie, do par de espécies interagindo e do contexto da comunidade. A meta-análise mais recente não identificou quase nenhum fator como determinante da direção dos efeitos indiretos mediados por polinizadores entre espécies (Charlebois & Sargent 2017). De maneira similar, outra revisão não identificou fatores determinantes da direção dos efeitos indiretos ao nível intraespecífico (Ghazoul 2005). Uma possível interpretação é que para um mesmo fator (e.g. densidade), certos estudos identificaram competição enquanto que outros reportaram facilitação. Portanto, ao realizar a meta-análise ou revisão, os efeitos negativos “cancelariam” os positivos, levando a um aparente resultado de neutralidade. Portanto, são necessários esforços para identificar fatores que determinem a direção de efeitos indiretos mediados por polinizadores.

A abordagem ao nível da comunidade é promissora para identificar características ecológicas e atributos das espécies determinando a prevalência de efeitos indiretos positivos *vs*. negativos. Esta abordagem elimina a variação inerente a diferentes habitats e com histórias biogeográficas distintas quando se compara espécies de diferentes locais. Além disso, as plantas em um mesmo local estão sujeitas ao mesmo *pool* de polinizadores e, portanto, as diferenças interspecíficas devem ser atribuídas a características intrínsecas às espécies de planta (Herrera 2020). Desta maneira, no **Capítulo 1,** o objetivo foi determinar a direção da denso-dependência na polinização de coespecíficos e heterospecíficos para 37 espécies de planta ocorrendo nos Campos de altitude do Parque Nacional do Itatiaia, Brasil. Portanto, pudemos agrupar as espécies de plantas de acordo com seus atributos funcionais e características ecológicas e assim, analisar a prevalência de efeitos indiretos positivos *vs*. negativos dentro destes grupos.

A teoria geral de montagem de comunidades prevê que um processo estruturador dominante deve levar a padrões observáveis na estrutura da comunidade (Webb et al. 2002). Ao abordar a polinização como um processo estruturador, são esperados efeitos discrepantes na comunidade de plantas dependendo da direção do efeito indireto mediado por polinizadores (competição *vs*. facilitação, Sargent & Ackerly 2008, Wolowski et al. 2017, Bergamo et al. 2018). De fato, diversos trabalhos reportaram a estrutura filogenética e funcional de comunidades em cofloração para inferir como a polinização estrutura comunidades (Armbruster et al. 1994, Gumbert et al. 1999, McEwen & Vamosi 2010, Muchhala et al. 2014, Ishii et al. 2019, revisto em E-Votjkó et al. 2020). Porém, diversas críticas foram feitas à teoria geral por tentar inferir um processo estruturador a partir de um padrão. Isto pois um mesmo padrão pode ser gerado por distintos processos (Gerhold et al. 2015). Por exemplo, agregação funcional pode ser gerada por competição quando há hierarquia competitiva ou facilitação que promove convergência de atributos (Gerhold et al. 2015). Esta crítica também pode se aplicar a polinização como processo estruturador, na qual competição e da facilitação na polinização poderiam gerar agregação funcional. Portanto, é necessário utilizar evidências que auxiliem ligar o padrão da comunidade ao seu processo estruturador.

Os estudos inferindo a polinização como um processo estruturador não trazem evidências prévias se competição ou facilitação de fato ocorrem nas comunidades modelo (e.g. Armbruster et al. 1994, Gumbert et al. 1999, McEwen & Vamosi 2010, Muchhala et al. 2014, Ishii et al. 2019). Um dos principais resultados do **Capítulo 1** demonstrou que facilitação é mais importante que competição na polinização dos campos de altitude do Parque Nacional do Itatiaia. Desta maneira, no **Capítulo 2,** investigamos padrões estruturais (estrutura filogenética e funcional) desta mesma comunidade de plantas utilizando dados de fenologia de floração e atributos reprodutivos. Assim, tomamos como vantagem as evidências prévias demonstrando facilitação na polinização para testar se os padrões esperados sob este processo de fato ocorrem na comunidade estudada.

Além de possuírem poucas evidências demonstrando de fato a ocorrência do efeito indireto mediado por polinizadores, a maioria dos estudos de montagem de comunidades também não incorporaram medidas de polinização relacionadas ao sucesso reprodutivo (porém, veja Wolowski et al. 2017). Isto é crítico pois competição e facilitação só podem ser inferidas caso haja um efeito na reprodução das espécies de planta compartilhando polinizadores. Assim, no **Capítulo 2** também utilizamos medidas de sucesso reprodutivo das espécies em cofloração para melhor determinar como a facilitação atua na montagem das comunidades. Para isso, associamos as medidas de sucesso reprodutivo com medidas de diversidade funcional. Portanto, pudemos demonstrar relações entre a estrutura da comunidade (diversidade funcional) com a funcionalidade da polinização no ecossistema (sucesso reprodutivo).

Avaliar a ocorrência de efeitos indiretos mediados por polinizadores ao nível da comunidade é desafiador. O trabalho de campo demanda amostrar muitas espécies de planta em diferentes densidades e configurações de vizinhanças. Esta tarefa intensiva talvez seja um dos motivos de haver poucos estudos com este enfoque. Por outro lado, a abordagem de redes ecológicas tem sido amplamente utilizada para caracterizar interações ao nível da comunidade (Dormann et al. 2017). Estudos com redes ecológicas também caracterizam os efeitos indiretos entre espécies de um nível trófico causados pelo compartilhamento de interagentes de outro nível trófico (Müller et al. 1999). Porém, a abordagem de redes ainda é muito restrita a efeitos indiretos em sistemas predador-presa, parasita-hospedeiro e planta-herbívoro (Müller et al. 1999, Morris et al. 2004, Frost et al. 2016). Portanto, existe potencial em aplicar a abordagem de redes ecológicas para investigar efeitos indiretos mediados por polinizadores ao nível da comunidade.

Estudos recentes aplicaram índices de redes ecológicas para caracterizar efeitos indiretos entre plantas compartilhando polinizadores (Carvalheiro et al. 2014, Bergamo et al. 2017, Badillo-Montaño et al. 2019). No entanto, estes estudos não puderam identificar a direção dos efeitos indiretos (competição *vs*. facilitação) pela falta de dados de sucesso reprodutivo das plantas. Para superar estas limitações, nos **Capítulos 3** e **4,** desenvolvemos um arcabouço analítico e teórico utilizando redes ecológicas para identificar efeitos indiretos mediados por polinizadores ao nível da comunidade. Incorporamos medidas de sucesso reprodutivo para predizer cenários previstos por competição *vs*. facilitação. Desta maneira, caracterizamos a direção dos efeitos indiretos prevalentes em duas comunidades de plantas ao relacionar índices de redes ecológicas e o sucesso reprodutivo das plantas. No arcabouço proposto não é necessário amostrar as densidades e vizinhança das espécies de plantas e tem potencial para ser aplicado em outros ecossistemas.

A abordagem de redes ecológicas também permite caracterizar a assimetria inerente aos efeitos indiretos mediados por polinizadores. Estas assimetrias pressupõem que uma espécie de planta atua mais como a que causa os efeitos enquanto que outra atua mais como a que recebe (Carvalheiro et al. 2014, Bergamo et al. 2017). Portanto, ao trazer para o nível da comunidade, espera-se que um conjunto de espécies atue mais como a que causa os efeitos indiretos e outro conjunto como o que receba. Sabe-se que a densidade pode determinar a assimetria nos efeitos indiretos (Briscoe Runqist & Stanton 2013). Porém, não há evidências se outras características ecológicas e atributos das espécies também determinam estes dois papéis. Assim, nos **Capítulos 3** e **4** também desenvolvemos índices de redes ecológicas que caracterizam estes papéis ao nível da espécie. E, em seguida, identificamos que padrões de interação e atributos reprodutivos estavam associados com cada um destes papéis.

6. OBJETIVOS

O objetivo geral desta tese foi identificar *fatores ecológicos* que determinam a direção dos efeitos indiretos mediados por polinizadores e como estes efeitos influenciam a *estrutura* e *dinâmica* das comunidades de plantas. Os objetivos específicos foram:

(i) Determinar a direção das relações de denso-dependência na polinização entre plantas coespecíficas e heterospecíficas e suas implicações na coexistência (Capítulo 1);

(ii) Identificar características ecológicas e atributos das plantas que influenciam a direção dos efeitos indiretos denso-dependentes (Capítulo 1);

(iii) Investigar como efeitos indiretos mediados por polinizadores determinam a montagem de comunidades (Capítulo 2);

(iv) Desenvolver um arcabouço teórico e analítico para o estudo de efeitos indiretos mediados por polinizadores ao nível de comunidade (Capítulos 3 e 4);

(v) Identificar características ecológicas e atributos das plantas que determinam as assimetrias nos efeitos indiretos mediados por polinizadores. (Capítulos 3 e 4).

7. SISTEMAS DE ESTUDO

(i) Campos de altitude do Parque Nacional do Itatiaia (PNI)

A comunidade modelo dos Capítulos 1 e 2 se localiza no planalto do Parque Nacional do Itatiaia (PNI), Brasil (~2.300 m, 22º21'S, 44º40'O). A vegetação é classificada como campos de altitude, caracterizada por componente predominantemente graminoide em solos rasos e outro componente herbáceo diverso em afloramentos rochosos. O clima possui sazonalidade marcada, sendo uma estação chuvosa e quente e outra estação seca e fria. Portanto, é classificado como clima tropical de montanha (Cwb no sistema Köppen). É um ecossistema altamente diverso e caracterizado por muitos endemismos (Ribeiro et al. 2007). Além disso, é um ecossistema de topo de montanha, com uma topografia, pedologia e climas únicos para a região (Ribeiro et al. 2007). Portanto, é um ecossistema severamente ameaçado pelas mudanças climáticas devido a falta de locais alternativos nos quais as espécies adaptadas às condições específicas dos Campos de altitude possam utilizar como habitat alternativo.

Ao longo de duas estações reprodutivas (outubro 2016 – maio 2017 e setembro 2017 – maio 2018) realizamos excursões mensais a campo para registrar aspectos básicos da biologia reprodutiva das espécies de plantas polinizadas por animais. Estabelecemos três transectos de 1,5 a 2 km de extensão cada. Os transectos seguiram trilhas pré-estabelecidas do PNI. Em cada transecto posicionamos parcelas de 2 x 1 m com uma distância mínima de 50m entre parcelas para minimizar a interferência entre plantas de parcelas diferentes. No total, estabelecemos 101 parcelas (29 na trilha ao Morro do Couto, 29 na trilha dos 5 Lagos e 43 na estrada para o Abrigo Rebouças), totalizando 0,02 hectares de área amostral.

Em cada parcela, registramos a fenologia de floração ao contar o número de flores por indivíduo de cada espécie. Para espécies com flores pequenas organizadas em inflorescências agregadas (Apiaceae, Asteraceae, Eriocaulaceae), utilizamos o número de inflorescências como unidade de contagem. Além disso, a cada mês selecionamos um subconjunto das parcelas contendo plantas em flor para realizar sessões de observação de 1 h por parcela devido à baixa atividade dos polinizadores nos campos de altitude (Freitas & Sazima 2006). Fizemos observações das 9h00-15h00, englobando o pico de atividade dos polinizadores na área. Ao final do dia, coletamos pistilos de flores em final de antese para contagem dos grãos-de-pólen no estigma e tubos polínicos no estilete em laboratório como medidas de sucesso reprodutivo. As medidas de sucesso reprodutivo foram coletadas para 50 espécies. Para 63 espécies, coletamos os seguintes atributos florais: reflectância das pétalas (com um espectrofotômetro USB4000 acoplado a lâmpadas de deutério-halogênio), tamanho da flor (comprimento para flores tubulares e diâmetro para flores/inflorescências radiais) e altura das estruturas reprodutivas da flor. As medidas morfológicas foram feitas com um paquímetro digital. Por fim, avaliamos a dependência de polinizadores para a reprodução de 50 espécies de plantas. Para isso, ensacamos flores/inflorescências contendo apenas botões de cinco indivíduos por espécie. Posteriormente, registramos se houve formação de frutos e sementes a partir destas flores/inflorescências que não tiveram contato com polinizadores. A formação de frutos e sementes indica capacidade de autopolinização e autofertilização, sendo então uma evidência de independência de polinizadores para a reprodução. Detalhes dos métodos estão descritos nos Capítulos 1 e 2.

Registramos 76 espécies polinizadas por animais nos campos de altitude do PNI, destacando-se as famílias Asteraceae (20 espécies), Ericaceae (6), Lamiaceae (4), Fabaceae (3), Iridaceae (3) e Melastomataceae (3). A biologia reprodutiva dos campos de altitude do PNI é caracterizada por um pico de floração marcado no verão (Janeiro – Março). Este pico de floração reflete uma estação altamente favorável para a reprodução e provavelmente coincide com a atividade de polinizadores na área. Portanto, a sazonalidade geral do ambiente também se reflete na atividade de floração da maioria das espécies polinizadas por animais. Registramos interações para 63 espécies de plantas. As observações de interações planta-polinizador evidenciam a baixa atividade de polinizadores (0,83 visitas.flor^{-1}.hora^{-1}). Esta atividade reflete a baixa abundância de polinizadores nos campos de altitude (Freitas & Sazima 2006) e pontuamos como uma pressão ecológica importante influenciando a reprodução das espécies de plantas. De 63 espécies, 33,3% são altamente generalistas (polinizadas por abelhas, moscas, vespas, besouros e formigas em variadas intensidades), demonstrando alta generalização na polinização nos campos de altitude (Freitas & Sazima 2006). Porém, registramos espécies polinizadas apenas por beija-flores (19%), abelhas (17,5%) e moscas (6,3%), o que pode se relacionar com atributos específicos destas flores (Danieli-Silva et al. 2012). Ainda, diversas espécies apresentaram sistemas mistos: abelhas e moscas (15,9%) e abelhas e beija-flores (4,8%). A alta porcentagem de plantas polinizadas por beija-flores contrasta com as de campos de altitude do Parque Nacional da Bocaina (Freitas & Sazima 2006). Mais além, destacamos as moscas, principalmente Syrphidae, como um componente importante da fauna de polinizadores para as espécies generalistas e por consequência, para a comunidade de plantas em geral.

Reportamos grande variação no sucesso reprodutivo, demonstrando que fatores ecológicos devem atuar mediando estas diferenças entre espécies. Além disso, muitas espécies exibiram flores pequenas e de cores claras. Portanto, processos relacionados à similaridade nos atributos florais entre espécies devem ser importantes nesta comunidade. Ainda, destacamos que esta similaridade está relacionada principalmente à generalização na polinização, pois muitas espécies apresentam morfologias abertas, atrativas para diversos grupos de insetos

(Waser et al. 1996). Este misto de similaridade e generalização contrasta com o pensamento clássico que a similaridade nos atributos florais estaria restrita a espécies de plantas especializadas ao mesmo grupo de polinizador. A baixa atividade de polinizadores leva a expectativa de menor dependência de polinizadores para a reprodução (Baker 1967). Verificamos que 18 de 54 espécies (33%) são capazes de produzir sementes sem polinizadores. Porém, não sabemos o nível desta dependência e, portanto, especulamos que diversas dessas 18 espécies devem produzir mais sementes quando expostas à atividade dos polinizadores. Portanto, concluímos que a comunidade dos campos de altitude do PNI apresenta dependência de polinizadores de modo geral. Desta maneira, estratégias que assegurem a polinização por animais são importantes para a comunidade de plantas. A partir dos resultados apresentados nesta tese, indicamos a facilitação como um processo importante garantindo a polinização desta comunidade. Em resumo, as interações planta-polinizador são um processo estruturador importante da vegetação de campos de altitude.

(ii) Dunas mediterrâneas do *Parc Natural de s'Albufera*

A comunidade modelo do Capítulo 3 se localiza nas dunas de *Son Bosc, Parc Natural de S'Albufera*, Mallorca, Espanha (nível do mar, 39º46'28.1" N, 3º07'45.34" L). A vegetação é classificada como duna mediterrânea, caracterizada por um componente herbáceo e poucos arbustos em solos arenosos. O clima é marcado por sazonalidade, sendo uma estação quente e seca e uma estação fria e chuvosa. Portanto, é um clima mediterrâneo típico (Csb no sistema Köppen). É um ecossistema altamente diverso e com alta abundância de polinizadores devido às características favoráveis ao estabelecimento de sítios de nidificação em solos arenosos (Castro-Urgal & Traveset 2014). Porém, a ação antrópica é uma grande ameaça às comunidades planta-polinizador na região principalmente devido à pressão do turismo em ambientes costeiros (Traveset et al. 2018).

Utilizamos dados previamente coletados por Lázaro et al. (2020) em duas estações reprodutivas (março – julho 2016 e março – julho 2017). Foram observadas as interações entre plantas e polinizadores a partir de censos de cinco minutos por planta. As sessões de observação foram realizadas das 10h às 17h, cobrindo o período de maior atividade dos insetos na comunidade. O sucesso reprodutivo foi estimado a partir da produção de sementes por indivíduo. Para isso, foi quantificada a taxa de frutificação (número de frutos/infrutescências dividido pelo número de flores/inflorescências) e o número de sementes por fruto. A estimativa final se deu pela multiplicação da taxa de frutificação e o número de sementes por fruto, resultando no número de sementes produzidas pelo número de flores produzidas naquele indivíduo. Por fim, foram medidos diversos atributos da população e das flores: abundância de flores (por m2); duração da fenologia de floração da população; simetria floral (zigomórfica vs. actinomórfica); tamanho da flor e do tubo floral, medidos com um paquímetro digital; volume de néctar, quantificado com microcapilares e avaliação da dependência de polinizadores para a reprodução a partir do isolamento de flores ao contato com visitantes florais. Detalhes dos métodos estão descritos no Capítulo 3.

A biologia reprodutiva das dunas mediterrâneas de Son Bosc evidenciam os meses de outono e primavera (abril – junho) como a estação favorável para floração. Portanto, a sazonalidade geral do ambiente também se reflete na floração da comunidade. Abelhas, vespas, moscas, besouros e borboletas foram os principais grupos de polinizadores desta comunidade. Dentre estes grupos, as abelhas se destacaram por sua alta riqueza e abundância, provavelmente reflexo do mediterrâneo ser um *hotspot* de diversidade deste grupo (Westphal et al. 2008). Além disso, a comunidade de plantas é altamente rica e apresentou generalização moderada (Castro-Urgal & Traveset 2014). Por ser uma comunidade insular costeira e próxima ao continente, muitas espécies podem imigrar às dunas e se estabelecer, aumentando a riqueza local e o número de espécies generalistas (Traveset & Navarro 2018). Além disso, é localizada em uma ilha continental e portanto, com história biogeográfica antiga, o que pode explicar a ocorrência de interações especializadas e resultar na generalização moderada observada (Traveset & Navarro 2018). As taxas de visitação foram relativamente altas (1,45 visitas.flor^{-1}.hora^{-1}), o que reforça o padrão de alta abundância de polinizadores na área. Observamos ampla variação no sucesso reprodutivo, sugerindo que fatores ecológicos possam causar estas diferenças entre as espécies. No Capítulo 3, evidenciamos a competição por polinizadores como um processo importante determinando esta variação. Além disso, atributos florais também influenciaram o sucesso reprodutivo nesta comunidade, pois espécies com flores grandes produziram mais sementes por flor que espécies com flores de tamanhos menores (Lázaro et al. 2020). Uma expectativa comum para comunidades insulares é a alta incidência de plantas generalistas ou independentes de polinizadores devido às vantagens na colonização (Baker 1967). Porém, verificamos alta dependência de polinizadores na reprodução (0,76 em média, sendo que 1 indicaria total dependência). Provavelmente o fato de ser uma ilha continental e com grande área contribuiu para o estabelecimento de populações relativamente estáveis de plantas e polinizadores, favorecendo a evolução de mecanismos de polinização cruzada (Traveset & Navarro 2018). Desta maneira, a polinização é um processo fundamental influenciando o sucesso reprodutivo da vegetação de dunas mediterrâneas do Parc Natural de s'Albufera.

(iii) Floresta montana do Parque Nacional do Itatiaia (PNI)

A comunidade modelo do Capítulo 4 se localiza na floresta montana do PNI, Brasil (~1100, 22º27'S, 44º36'O). A vegetação é classificada como floresta ombrófila montana (Veloso et al. 1991). O clima possui sazonalidade, sendo uma estação úmida e quente e uma estação seca e fria. Portanto, é um clima classificado como subtropical úmido (Cwa no sistema Köppen). A Mata Atlântica é um *hotspot* de biodiversidade global devido à alta riqueza de diversos grupos e ameaças antrópicas (Myers et al. 2000). Para este estudo, restringimos a comunidade de plantas apenas àquelas polinizadas por beija-flores. A polinização por beija-flores é um importante componente em comunidades da Mata Atlântica (Buzato et al. 2000).

Utilizamos dados previamente coletados nesta comunidade de plantas ornitófilas ao longo de mais de dez anos (Canela 2006, Wolowski et al. 2013a,b, Wolowski et al. 2017, Bergamo et al. 2018, 2019). Os dados de interação com beija-flores foram obtidos a partir de observação focal e contagem do número de visitas nas plantas ornitófilas em flor, totalizando um mínimo de 15h por espécie de planta (Canela 2006). Foram realizadas polinizações controladas para avaliar a dependência de polinizadores para a reprodução e a presença de sistema de autoincompatibilidade (Wolowski et al. 2013a). Além disso, comparações entre a reprodução natural e a reprodução a partir de polinizações cruzadas controladas permitiram a avaliação da limitação polínica (Wolowski et al. 2013b). A limitação polínica foi utilizada como medida de sucesso reprodutivo por indicar o quanto da reprodução naquela população é limitada devido a menor quantidade e/ou qualidade da polinização (Wolowski et al. 2013b). O acompanhamento da fenologia de floração se deu por meio de parcelas estabelecidas em trilhas do PNI, nas quais contava-se o número de indivíduos floridos por espécie e o número de flores por indivíduo (Wolowski et al. 2017). Por fim, foram coletados diversos atributos florais destas espécies: volume e concentração do néctar (Canela 2006, Bergamo et al. 2018), tamanho efetivo da corola e altura das estruturas reprodutivas na flor (Bergamo et al. 2018) e reflectância das pétalas com posterior cálculo de parâmetros de cor relevantes na visão de beija-flores (Bergamo et al. 2018, 2019). Detalhes dos métodos estão descritos no Capítulo 4.

Incluímos 21 espécies de plantas para as quais obtivemos todos os dados de interação, biologia reprodutiva e atributos florais. Isto representa 58% da flora polinizada por beija-flores registrada nesta comunidade (Wolowski et al. 2013a). As famílias mais representativas foram Bromeliaceae (10 espécies), Gesneriaceae (4) e Acanthaceae (3). Foram registradas cinco espécies de beija-flor (Canela 2006): *Phaethornis eurynome* (Lesson, 1832), *Phaethornis* *squalidus* (Temminck, 822), *Clytolaema rubricauda* (Boddaert, 1783), *Leucochloris albicollis* (Vieillot, 1818) e *Thalurania glaucopis* (Gmelin, 1788). A riqueza de plantas é comparável a outras comunidades de plantas polinizadas por beija-flores da Mata Atlânica, porém a de beija-flores é menor, seguindo um padrão altitudinal (Wolowski et al. 2017). O suprimento de flores é relativamente constante ao longo do ano, não havendo uma sazonalidade marcada (Canela 2006), o que está de acordo com estudos em outras comunidades de plantas polinizadas por beija-flores (Buzato et al. 2000). Muitas flores possuem características típicas da ornitofilia: néctar volumoso e pouco concentrado, flores com morfologia tubular e cores alaranjadas e vermelhas (Canela 2006, Bergamo et al. 2018). Porém, existe variação entre as espécies em todos estes atributos, o que media diferenças no nicho de polinização das plantas (Bergamo et al. 2018).

Mais da metade das espécies de plantas apresentou limitação polínica, variando de baixa a alta intensidade (Wolowski et al. 2013b). Portanto, a interação com beija-flores é fundamental para a manutenção das populações de diversas espécies. A alta incidência de limitação polínica indica dependência de polinizadores (Ashman et al. 2004). Por outro lado, a limitação polínica crônica favorece a evolução de mecanismos de independência de polinizadores (Ashman et al. 2004). A dependência de polinizadores foi alta na comunidade (baixos índices de autogamia - 0,15 em média, sendo que 0 indica total dependência de polinizadores), porém o índice de autoincompatibilidade revelou que apenas um terço das espécies (7) são autoincompatíveis (Wolowski et al. 2013a). A alta dependência de polinizadores pode favorecer a ocorrência de limitação polínica nesta comunidade. Porém, as espécies devem experimentar diferentes intensidades de limitação polínica dependendo dos padrões de interação com os beija-flores e sistema de reprodução. No Capítulo 4, demonstramos que a facilitação é um processo importante diminuindo a limitação polínica nas espécies, corroborando estudos prévios nesta comunidade (Wolowski et al. 2017, Bergamo et al. 2018). Atributos florais e o sistema de reprodução foram fatores importantes mediando as relações de facilitação entre as espécies de plantas. Portanto, a polinização configura como um processo importante determinando a estrutura e a dinâmica da flora ornitófila de floresta montana do PNI.

CAPÍTULO 1.

Pollination outcomes reveal negative density-dependence coupled with interspecific facilitation among plants

Pedro Joaquim Bergamo[*1,2], Nathália Susin Streher[3], Anna Traveset[2], Marina Wolowski[4], Marlies Sazima[5]

[1]Graduate Program in Ecology, University of Campinas, Campinas, Brazil

[2]Mediterranean Institute for Advanced Studies, CSIC-UIB, Mallorca, Spain

[3]Graduate Program in Plant Biology, University of Campinas, Campinas, Brazil

[4]Institute of Natural Sciences, Federal University of Alfenas, Alfenas, Brazil

[5]Plant Biology Department, Institute of Biology, University of Campinas, Campinas, Brazil

Published in *Ecology Letters* (23):1, 129-139.

Abstract

Pollination is thought to be under positive density-dependence, destabilizing plant coexistence by conferring fitness disadvantages to rare species. Such disadvantage is exacerbated by interspecific competition but can be mitigated by facilitation and intraspecific competition. However, pollinator scarcity should enhance intraspecific plant competition and impose disadvantage on common over rare species (negative density-dependence, NDD). We assessed pollination proxies (visitation rate, pollen receipt, pollen tubes) in a generalized plant community and related them to conspecific and heterospecific density, expecting NDD and interspecific facilitation due to the natural pollinator scarcity. Contrary to usual expectations, all proxies indicated strong intraspecific competition for common plants. Moreover, interspecific facilitation prevailed and was stronger for rare than for common plants. Both NDD and interspecific facilitation were modulated by specialization, floral display and

pollinator group. The combination of intraspecific competition and interspecific facilitation fosters plant coexistence, suggesting that pollination can be a niche axis maintaining plant diversity.

Introduction

Negative density-dependence (NDD), i.e., a fitness disadvantage when a species becomes relatively abundant, prevents total dominance in a community (Adler et al. 2018). In species-rich tropical plant communities, NDD often fosters coexistence and promotes co-occurrence of rare and abundant species (Murphy et al. 2017). The abundant plant species often face higher intraspecific competition for space or abiotic resources and stronger interactions with natural enemies than rare ones, stabilizing interspecific competition (Comita et al. 2014). On the other hand, interactions with mutualists such as pollinators are often thought to be under positive density-dependence (PDD), and thus, less likely to contribute to coexistence (Ghazoul 2005). However, intraspecific competition for pollinators is common (Rathcke 1983, Ward et al. 2013) and NDD in flower visitation rates could make pollination a driver of plant coexistence in species-rich communities (Benadi & Pauw 2018). In fact, the relationship between pollinator visitation and pollination outcomes is often density-independent (Harder et al. 2016). Thus, it is necessary to measure visitation and its outcomes (pollen deposition on stigmas and pollen tubes) to better understand how pollination fosters plant coexistence.

Although NDD may be perceived at the landscape-level, flower abundance influences pollination outcomes at small spatial scales, i.e., between neighboring plants (Nottebrock et al. 2017). Plants may exhibit negative or positive intraspecific responses (when high conspecific density attracts less or more pollinators, respectively, Totland 1993), interspecific facilitation (when plants of different species jointly attract more pollinators, Moeller 2004) and interspecific competition (when visitation rates are reduced by plants of other species, Mitchell et al. 2009). Moreover, interaction between neighbors for pollination may be widespread and one interaction sign may prevail in a community (Rathcke 1983). Studies with focal plant species have shown that the effect sign of conspecific and heterospecific flower density on pollination is scale-dependent (plot- vs. landscape-level; Hegland 2014, Albrecht et al. 2016). Furthermore, plant species may vary in their responses to plot- (Lázaro et al. 2014) and landscape-level density (Benadi & Pauw 2018), hampering our ability to extend patterns from focal species to the whole community.

Theoretical models and empirical data suggest a hump-shaped relationship for visitation rates in response to conspecific and heterospecific flower density (Rathcke 1983, Seifan et al. 2014, Benadi & Pauw 2018). This is because rare species and low availability of floral resources at the local scale attract few pollinators. Thus, an increase in floral resources at this point generates a strong positive response in visitation rates. On the other hand, abundant species and extremely high availability of floral resources at local scales may lead to strong intra and interspecific competition for pollination, decreasing again the visitation rates (Rathcke 1983). When interspecific competition is prevalent over facilitation, coexistence between rare and abundant plants is only fostered when there is some degree of pollination specialization in the community. In this situation, specialization leads intraspecific competition to be stronger than interspecific competition (Pauw 2013, Benadi 2015). Nevertheless, it is unclear which coexistence mechanisms are required to operate in generalized plant communities.

The ecosystems that exhibited flower visitation hump-shaped patterns were characterized by relatively high specialization and pollinator abundance (Rathcke 1983, Benadi & Pauw 2018). On the other hand, pollinator scarcity should lead to strong intraspecific competition, potentially generating advantages of rarity (NDD). However, the shape that density-dependence assumes in generalized and pollinator-depauperated communities is unclear. In addition to NDD, prevalence of interspecific facilitation is also expected, as the importance of joint attraction is higher when pollinators are scarce (Moeller 2004, Tur et al. 2016). Furthermore, facilitation can foster coexistence if rare plants benefit more than abundant plant species, weakening Allee effects (Feldman et al. 2004).

Besides community-level patterns of density-dependence, little is known about the ecological drivers influencing density-dependence and interspecific interactions for pollination. For instance, generalized plant species exhibit higher visitation rates than specialists and may be less prone to exhibit NDD (Benadi & Pauw 2018). Moreover, heterospecific neighbors may enhance the diversity of pollinators visiting the focal plant species (Lázaro et al. 2009). Attracting a diverse array of visitors should translate into interspecific facilitation for generalized species. Conversely, specialized species may not experience such benefit, and may suffer stronger interspecific competition. Secondly, species that produce few flowers (small floral display) may face disproportional effects from other plants, since changes on pollinator attraction will strongly affect their visitation rates (Carvalheiro et al. 2014). We expect these species to be more subjected to density-dependence than species with large floral displays. Lastly, each pollinator functional group (e.g. bees, flies and hummingbirds) may respond differently to floral density (Albrecht et al. 2016). Indeed, factors influencing potential plant-plant interactions vary among plants pollinated by these different functional groups of pollinators (Carvalheiro et al. 2014, Bergamo et al. 2017). However, the possibility that plant specialization, floral display and pollinator group are associated with the prevalence of NDD vs. PDD or of facilitation vs. competition has never been tested.

In this study, we investigated landscape and plot density-dependence on visitation rates, pollen deposition and pollen tube number in a biodiverse tropical community. We conducted our study in a tropical mountaintop ecosystem characterized by high generalization and low pollinator visitation rates (Freitas & Sazima 2006, Danieli-Silva et al. 2012). To achieve a comprehensive community-wide assessment, we collected data for 67 animal-pollinated plant species. We expected 1) disadvantage of the most abundant species due to intraspecific competition for pollination, generating NDD and 2) positive heterospecific density-dependence (interspecific facilitation) especially for the rare species, both driven by the low availability of pollinators. We predicted that NDD and prevalence of interspecific facilitation would be modulated by plant generalization, floral display size and pollinator group. Our results showed prevalence of NDD and interspecific facilitation on pollination outcomes, a combination that could foster plant coexistence.

Materials and methods

Study system

The study was conducted in the highland grassland ecosystem (*Campos de altitude*) in the Itatiaia National Park, southeastern Brazil (~ 2,300 m a.s.l., 22º21'S, 44º40'W). This ecosystem is characterized by marked seasonality, classified as tropical mountain climate (Cwb in Köppen system, Ribeiro et al. 2007). Mean annual temperature is 14.4 ºC, with minimum temperatures reaching -10 ºC, and mean annual precipitation is 2,400 mm (Ribeiro et al. 2007). Grasses, herbs and shrubs dominate the vegetation, growing in rocky outcrops and shallow soils. More than 150 plant species were previously recorded in the study area (Brade 1956) and our study included the 67 species (45%) for which at least one pollination metric was measured (Table S1).

Field sampling

We collected data in monthly censuses during two consecutive warm and humid seasons (October/2016 to May/2017 and September/2017 to May/2018) totaling 17 censuses. Sampling was conducted in 101 plots of 2 m^2 established on three transects along pre-existing trails in the park. We placed 30-40 plots per transect, with a minimum distance of 50 m between plots, totaling 2 km per transect and 0.02 ha of total area sampled. The minimum distances between plots of distinct transects were 1.2-2.5 km. On each census, we registered the number of individuals flowering and the number of flowers on each individual for all plots. For species with large inflorescences, we estimated the number of flowers per inflorescence. For species with flowers arranged in small capitula or other similar arrangements (i.e. individual flowers with less than one centimeter in size), we used the number of inflorescences as a surrogate of abundance (Benadi & Pauw 2018). Although the amount of resources per flower varied among species, we used abundance as a feasible first approximation of floral resources. Then, we obtained the landscape conspecific density (sum of the number of conspecific flowers of all transects for each census), plot conspecific density (number of conspecific flowers within the plot) and plot heterospecific density (number of heterospecific flowers within the plot). Landscape and plot conspecific densities were not correlated (see Results), indicating within species plot-variation. Therefore, we had to sum up all plots to achieve a comparable measurement of conspecific density over a larger scale and, consequently, broad density-dependent responses.

We registered the visitation rate per focal plant (number of visits/flowers observed/observation duration) via direct observations. We considered only visits with potential for pollination (i.e. when the visitor touched stigmas and anthers). On each monthly census, we observed a random subset of the plots. Each session lasted from 30-60 min due to the low visitation rates of this ecosystem (Freitas & Sazima 2006). We observed 4-6 plots per day; the same plot was observed from 1-9 times throughout the whole study. Sessions were conducted from 0900h to 1500h (peak visitation time) on sunny days. We conducted 134h of observation for 222 focal plants of 65 plant species (two species were not censused because of their rarity and short flowering, Table S1). At the end of the day, we collected pistils from open flowers of each individual of all 101 plots and stored them in 70% alcohol. We collected 24 samples on average per species, each sample from a different plant individual (Table S1). We selected flowers starting to wilt to guarantee that all potential pollination events could have occurred. We collected 994 pistil samples from 44 species, which had flowers large enough to be manipulated without promoting artificial self-pollination (Table S1). For the other 23 species we could only calculate visitation rates by observing pollinator activity.

Pollen receipt and pollen tubes

To quantify the pollination outcomes, we counted the number of pollen grains on stigmas (pollen receipt - quantitative component of female fitness) and the number of pollen tubes in styles (qualitative component of female fitness) (Alonso et al. 2012). We stained pistils following Martin (1959) and performed counting with epifluorescence microscopy. Only conspecific pollen grains were counted (recognized due morphological similarity with a pollen reference collection). Pollen receipt and pollen tubes are commonly used as proxies of the pollinators’ contribution to female fitness. Moreover, it allows a comparison of quantitative vs. qualitative effects mediated by pollinators (Alonso et al. 2012, Tur et al. 2016). Plant species vary in pollen receipt and pollen tubes due to intrinsic differences in pollen and pistil traits. To achieve comparable values among species, we calculated z-scores (scaled to a mean of 0 and standard deviation of 1) within species for pollen receipt and pollen tubes.

Statistical analyses

Landscape and plot density-dependence

To assess landscape and plot density-dependence on visitation rate and pollination outcomes, we fitted generalized linear mixed models (GLMMs). For visitation rate (of the focal plant), we used the raw counts as a response variable and included the number of flowers per hour observed as offset, using Poisson error-structure and log link. For pollination outcomes, we used the z-scores of pollen receipt and number of pollen tubes as response variables. Then, each pollination outcome was fit separately using Gaussian error-structure and identity link. All models had the flower count variables (landscape and plot conspecific density; and plot heterospecific density, all log- and z-transformed to improve model convergence) as fixed effects. Observation time was included as fixed effect in the visitation model. We also included interaction terms between plot heterospecific density with landscape and plot conspecific density. Monthly census, focal plant species identity and plot within transect were included as random effects to account for changes in the community over the flowering season. We used two variables to describe plot heterospecific density: one using the total number of heterospecific flowers in the plot, and a second using the number of heterospecific flowers with potential pollinator sharing. Potential pollinator sharing was assumed when the focal and heterospecific plants were pollinated by the same pollinator functional group (e.g. bees, flies, beetles, hummingbirds), determined for each plant species based on observation data. Furthermore, we fitted the same models using quadratic terms for the fixed effects, since hump-shaped relationships are common (Benadi & Pauw 2018). We then compared linear and quadratic models based on their AIC values. We checked multicollinearity between the fixed factors by computing Variation Inflation Factor (VIF). Factors had VIF < 3 in all models, and thus we assumed robustness to collinearity (Zuur et al. 2010).

Determinants of density-dependence

We investigated ecological determinants of density-dependence. For this, we used a subset of 37 species for which we had enough pollen receipt and pollen tubes sample sizes per species (at least 8 samples from different individuals, Table S1). We did not use visitation rates since few species had enough sample size for this variable. For landscape-level conspecific density-dependence, we restricted our analysis to 28 species that had flower counts over several months, and thus, enough variation in flowering density (Table S1). We fit two GLMMs: the first with pollen receipt and the second with pollen tubes as response variables. All models had landscape conspecific density, plot conspecific density, plot heterospecific density (with potential pollinator sharing), their interactions, and monthly census as fixed effects. We log transformed all flower count variables due their skewed distribution. Focal plant species was included as a random effect (random slope and intercept), as well as plot within transect. We extracted the slope (β) per species from the models as evidence for the sign of density-dependence and interspecific interactions for pollinators (following Tur et al. 2016). We considered evidence of landscape- and plot-level advantages of abundance (PDD), or interspecific facilitation when $\beta \pm 2SE > 0$, evidence of landscape- and plot-level advantages of rarity (NDD), or interspecific competition when $\beta \pm 2SE < 0$, and no overall effect when $\beta \pm 2SE$ overlapped 0. In these models, we could extract the slope separately for each plant species. Thus, we preferred to use the response variables of pollen receipt and tube counts without z-transformation, fitting models with Poisson error-structure and link log. Then, we calculated the percentage of interaction signs per species for each response. The overall effect was calculated by weighted meta-analysis (with the inverse of the variance as weight) using the slope of each species as effect size and its associated error as the standard error (Hedges & Olkin 1985).

To investigate which factors modulate density-dependence, we classified each species into three attribute categories: 1) *functional specialization*: specialized (pollinated by one functional group – bees, or flies, etc.) vs. generalized (pollinated by more than one group, Ollerton et al. 2007). 2) *Floral display size*: small (individuals displaying < 25 open flowers) vs. large (> 25 open flowers) (Fig. S1) and 3) *main pollinator group* (bees, flies or hummingbirds), considering the group that accounted for the majority of visits (> 70%) to the plant species. Appendix S1 contains details on plant classification. Then, we used these attributes as moderators in weighted fixed-effects meta-analytical models. We applied *post hoc* contrasts to inspect the differences within categories. We also calculated the proportion of species with positive, negative and no effects for all categories. All GLMMs were fitted with the R-package *lme4* (Bates et al. 2015) and meta-analytical procedures were done with the R-package *metafor* (Viechtbauer 2010).

Results

We registered 4,455 visits to 6,267 observed flowers. Visitation rates were relatively low with less than one visit per flower per hour on average (0.85 ± 1.92 visits.flower^{-1}.hour^{-1}). We counted 19,345 conspecific pollen grains on stigmas, with 19.46 ± 40.47 grains on average per stigma and 12,965 pollen tubes, with 13.06 ± 23.80 tubes on average per style.

The effect of conspecific density depended on spatial scale and on heterospecific density (i.e. interactions between variables were significant, Table 1). The effect of landscape conspecific density was mostly negative, being most accentuated when heterospecific density was high (Fig. S2a, c, e). This gives strength to the expectation that rare species had advantages (negative density-dependence, NDD). Positive effects were only detected for visitation rate when heterospecific density was high (Fig. S2a). On the other hand, the effect of plot conspecific density was only negative for visitation rate and only when heterospecific density was low (Fig. S2b, d, f). Thus, contrary to expectations, the effect of plot conspecific density was mostly positive, revealing advantages of abundance (positive density-dependence, PDD). The effect of heterospecific density depended on conspecific density (Table 1). The increase of heterospecific density increased the different pollination metrics when landscape conspecific density was low (Fig. 1a, c, e) and when plot conspecific density was high (Fig. 1b, d, f). This suggests that facilitative effects were stronger at low landscape (rare species) and high plot conspecific density (abundant species).

The effect of heterospecific density disappeared when we considered the density of all species regardless of pollinator sharing (Table S2). The models with quadratic terms had higher AIC values than the linear models, indicating that linear relationships provided a better fit (Tables S3-S4). Flower count variables were weakly correlated (Tables S5-S6), allowing us to interpret their effects separately.

Table 1. GLMM coefficients of the visitation rate (Poisson error structure), pollen receipt and pollen tubes (Gaussian error structure) models. Landscape conspecific density was estimated as the sum of conspecific flowers at landscape-level whereas plot conspecific density was measured as the number of conspecific flowers at plot level. Heterospecific density refers to the total number of heterospecific flowers at plot level (with potential pollinator sharing). β = effect estimate, SE = standard error, χ_2 = equivalent F statistic. Bold values indicate significant effects at $p < 0.05$.

Fixed effects	Visitation rate		Pollen receipt		Pollen tubes	
	β ± SE	χ_2 (p-value)	β ± SE	χ_2 (p-value)	β ± SE	χ_2 (p-value)
Landscape conspecific density	**-0.34 ± 0.03**	**4.20 (0.022)**	**-0.09 ± 0.04**	**4.21 (0.040)**	-0.08 ± 0.04	3.69 (0.053)
Plot conspecific density	**-0.11 ± 0.20**	**13.66 (<0.001)**	**0.15 ± 0.04**	**15.54 (<0.001)**	**0.17 ± 0.04**	**18.14 (<0.001)**
Heterospecific density	**0.91 ± 0.02**	**22.23 (<0.001)**	**0.10 ± 0.03**	**9.19 (0.002)**	**0.09 ± 0.03**	**8.76 (0.003)**
Landscape conspecific density * Heterospecific density	**-0.22 ± 0.20**	**20.79 (<0.001)**	**-0.04 ± 0.04**	**6.85 (0.011)**	**-0.05 ± 0.04**	**6.44 (0.027)**
Plot conspecific density * Heterospecific density	**-0.17 ± 0.21**	**35.87 (<0.001)**	**0.10 ± 0.04**	**6.08 (0.014)**	**0.08 ± 0.04**	**4.13 (0.042)**
Observation time	**-0.10 ± 0.08**	**5.11 (0.024)**	-	-	-	-

Random effect among-group variances: visitation rate model - 0.10 for monthly census, 0.11 for plant species and 0.17 for plot within transect. Pollen receipt model – 0.07 for monthly census, 0.02 for plant species and 0.04 for plot within transect. Pollen tubes model - 0.07 for monthly census, 0.01 for plant species and 0.04 for plot within transect.

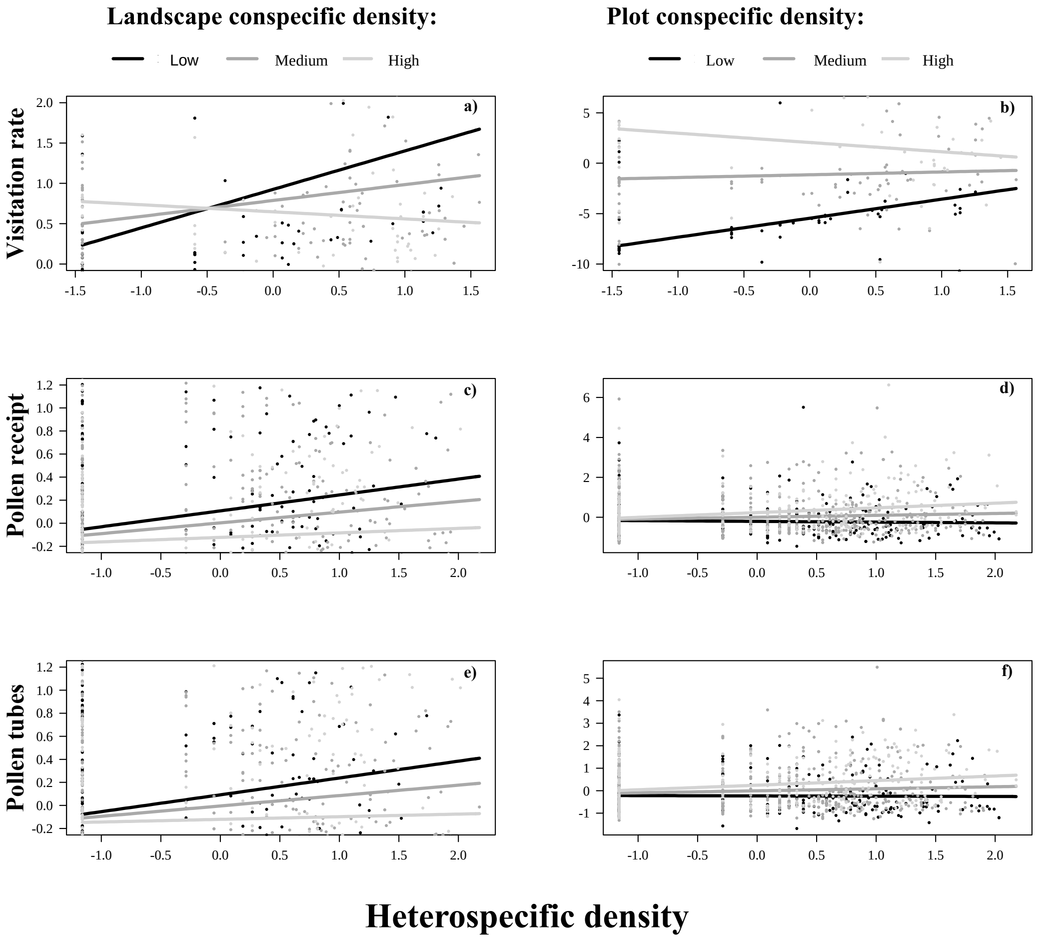

Figure 1. Interactive effects between heterospecific density with landscape conspecific density (left panels) and with plot conspecific density (right panels) on visitation rates and pollination outcomes. Each dot represents the partial residuals (after removing variation explained by other variables in each model) for each focal plant species-date combination (panels a-b) and individual stigma samples (panels c-f). Black dots and lines represent the heterospecific density effect for species with low landscape and plot conspecific density (below the first quartile), dark grey for species with intermediate landscape and plot conspecific density (between the first and third quartile) and light grey for high landscape and plot conspecific density (above the third quartile). Visitation rates are on visits.flower$_{-1}$.hour$_{1}$. The variables ‘pollen receipt’ and ‘pollen tubes’ were z-transformed (within plant species) to achieve comparable values among different species.

Determinants of density-dependence

The effects detected when running analyses per species reinforced the overall effects: prevalence of landscape-level NDD (advantages of rarity), plot-level PDD (advantages of abundance) and interspecific facilitation (Tables S7-S9, Fig. 2, Fig. S3). The proportions of species showing each effect also generally followed these patterns, with most species showing landscape-level NDD, plot-level PDD and interspecific facilitation (Tables S10-S12, Fig. 3). Exceptions were 11 species showing landscape-level NDD and PDD on pollen tubes and 15 species showing interspecific facilitation and neutral effects on pollen tubes (Fig. 4).

The prevalence of density-dependence was modulated by functional specialization, floral display and pollinator group as expected (Table 2, Fig. 2). The exception was the lack of effect of floral display for landscape density-dependence on pollen tubes. The direction of landscape density-dependence varied between attribute categories: as expected, generalists showed no landscape density-dependence, while specialists showed landscape-level NDD on pollen receipt ($\chi_2 = 18.81$, $p < 0.001$). For pollen tubes, generalists showed landscape-level PDD, while specialists showed landscape-level NDD ($\chi_2 = 28.96$, $p < 0.001$). Regarding floral display, species with large displays exhibited landscape-level PDD, while small display ones showed landscape-level NDD on pollen receipt ($\chi_2 = 122.08$, $p < 0.001$). There was no landscape-level density-dependence on pollen tubes for either group ($\chi_2 = 0.05$, $p = 0.832$). Between pollinator groups, bee- and fly-pollinated plants had no landscape density-dependence on pollen receipt and showed similar effects ($\chi_2 = 0.46$, $p = 0.500$), while both were different from the prevalent landscape-level NDD of hummingbird-pollinated plants ($\chi_2 = 22.87$, $p < 0.001$ and $\chi_2 = 15.94$, $p < 0.001$, respectively). For pollen tubes all pollinator groups differed: bee-pollinated plants had no landscape density-dependence, while the magnitude of the landscape-level NDD effect differed between fly- and hummingbird-pollinated plants.

Contrary to expectations, plot-level PDD (advantages of abundance) prevailed for all attribute categories, with distinct effect sizes (Fig. 2). Lastly, the direction of interspecific interactions varied between attribute categories (Fig. 2): as expected, generalists were facilitated, while competition prevailed for specialists on pollen receipt ($\chi_2 = 270.88$, $p < 0.001$) and pollen tubes ($\chi_2 = 79.54$, $p < 0.001$). Facilitation prevailed for both species with large displays and small displays. However, on pollen receipt, the effects were stronger for large displays ($\chi_2 = 14.90$, $p < 0.001$), while both groups were facilitated in similar strength on pollen tubes ($\chi_2 = 0.03$, $p = 0.862$). Finally, facilitation on pollen receipt prevailed in all pollinator groups. Bee- and fly-pollinated plants had similar positive effects ($\chi_2 = 0.91$, $p = 0.340$), both higher than hummingbird-pollinated plants ($\chi_2 = 31.72$, $p < 0.001$ and $\chi_2 = 7.87$, $p = 0.005$, respectively). For pollen tubes, facilitation remained prevalent in bee-pollination and changed to neutral for fly-pollination, although both showed similar overall effect ($\chi_2 = 2.96$, $p = 0.085$). In contrast to results on bees ($\chi_2 = 22.84$, $p < 0.001$) and flies ($\chi_2 = 18.07$, $p < 0.001$), competition was prevalent for hummingbird-pollination.

Table 2. Meta-analytical coefficients of the analyses with the slopes per species. Slopes were extracted from the pollen receipt and pollen tubes' (Poisson error structure) models using landscape conspecific density, plot conspecific density and heterospecific density as fixed effects. Landscape conspecific density was estimated as the sum of conspecific flowers at landscape-level whereas plot conspecific density was measured as the number of conspecific flowers at plot level. Heterospecific density refers to the total number of heterospecific flowers at plot level (with potential pollinator sharing). Values indicate QM coefficients and associated p levels for each attribute category on each model. Bold values indicate significant effects at $p < 0.05$.

	Pollen receipt			Pollen tubes		
Attribute category	Landscape conspecific density	Plot conspecific density	Heterospecific density	Landscape conspecific density	Plot conspecific density	Heterospecific density
Functional specialization	**29.48** **p < 0.001**	**1000.48,** **p < 0.001**	**196.74** **p < 0.001**	**46.74** **p < 0.001**	**428.80** **p < 0.001**	**42.12** **p < 0.001**
Floral display	**112.07** **p < 0.001**	**882.45** **p < 0.001**	**58.01** **p < 0.001**	0.82 p = 0.36	**496.93** **p < 0.001**	**10.05,** **p = 0.002**
Pollinator group	**57.83,** **p < 0.001**	**1104.23,** **p < 0.001**	**31.84** **p < 0.001**	**46.41,** **p < 0.001**	**663.62,** **p < 0.001**	**18.60** **p < 0.001**

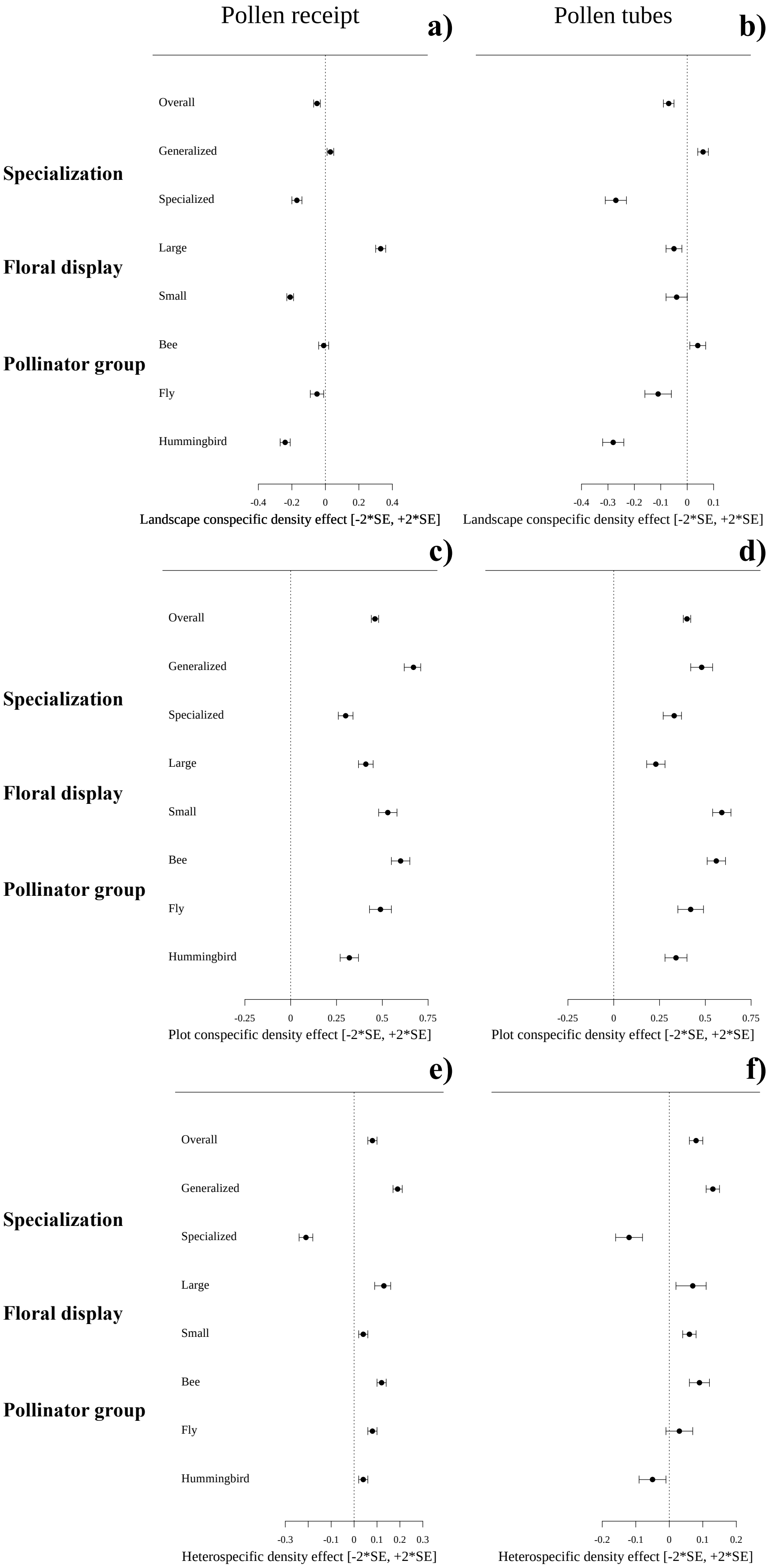
Pollen receipt
a)
Pollen tubes
b)
Overall
Generalized
Specialization
Specialized
Large
Floral display
Small
Bee
Pollinator group
Fly
Hummingbird
-0.4
-0.2
0
0.2
0.4
Landscape conspecific density effect [-2*SE, +2*SE]
-0.4
-0.3
-0.2
-0.1
0
0.1
Landscape conspecific density effect [-2*SE, +2*SE]
c)
d)
-0.25
0
0.25
0.5
0.75
Plot conspecific density effect [-2*SE, +2*SE]
-0.25
0
0.25
0.5
0.75
Plot conspecific density effect [-2*SE, +2*SE]
e)
f)
-0.3
-0.1
0
0.1
0.2
0.3
Heterospecific density effect [-2*SE, +2*SE]
-0.2
-0.1
0
0.1
0.2
Heterospecific density effect [-2*SE, +2*SE]

Figure 2. Estimated slopes (βj ± 2 SE) for the effect of landscape conspecific density, plot conspecific density and plot heterospecific flower density (with potential pollinator sharing at the plot level) on a, c, and e) pollen receipt (quantitative female component) and b, d, and f) pollen tubes (qualitative female component). Since all predictor variables were centered, the slopes represent effects when the other predictor in the interaction is average. We interpreted as positive effects (landscape- and plot-level positive density-dependence and interspecific facilitation) when βj ± 2 SE > 0, negative (landscape- and plot-level negative density-dependence and interspecific competition) when βj ± 2 SE < 0 and neutral when βj ± 2 SE overlapped 0. Specialization category includes species pollinated by one pollinator group and generalization by more than one group (i.e. functional specialization *sensu* Ollerton et al. 2007). Floral display size categories were based on the distribution of open flowers per species in the community (Fig. S1). Small display includes species with < 25 open flowers per individual and large > 25 flowers. Pollinator group categories were based on the main pollinator group visiting the species. Some species had two groups visiting in similar proportions and were thus represented in both categories (e.g. bees and flies).

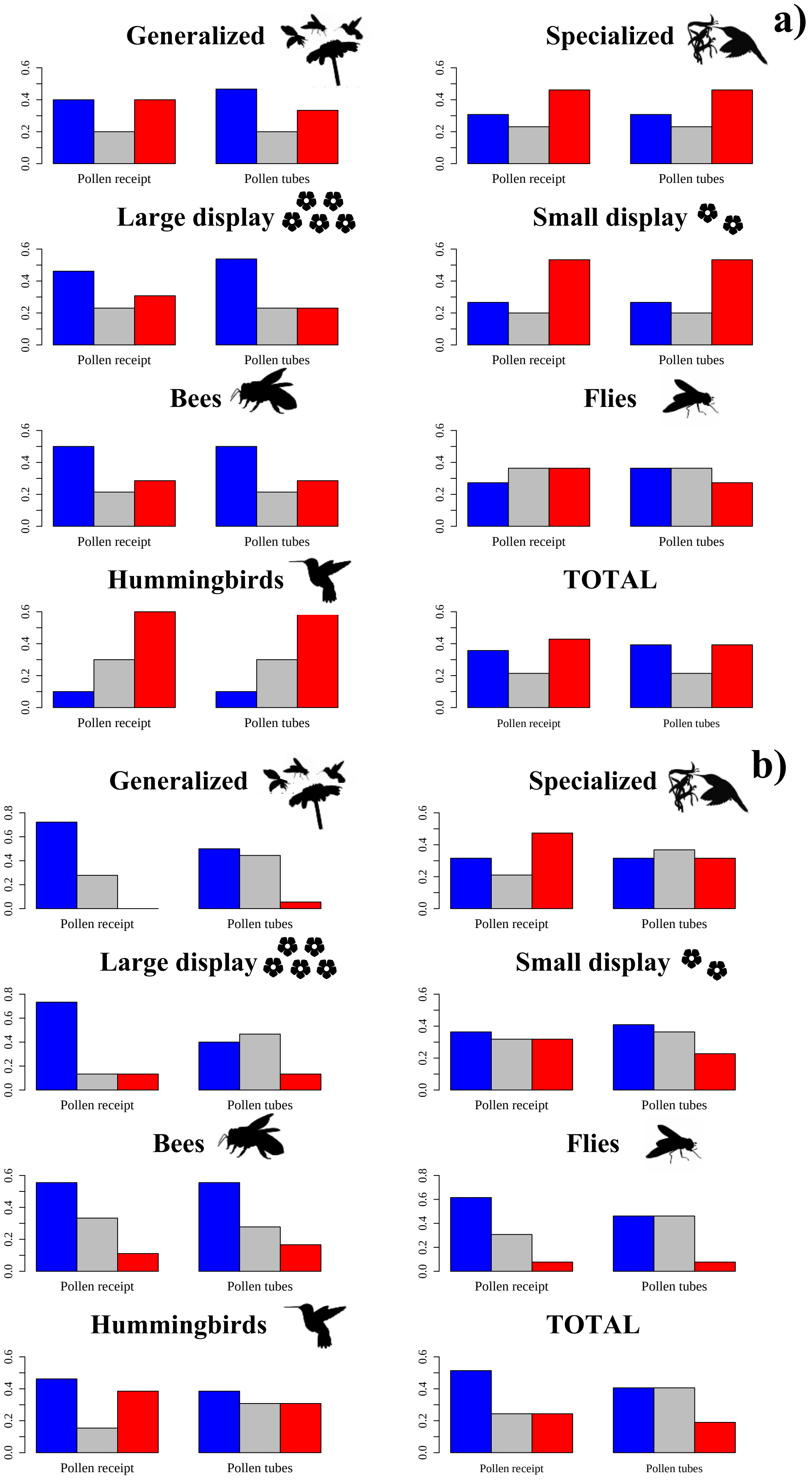
a)
Generalized
Specialized
Large display
Small display
Bees
Flies
Hummingbirds
TOTAL
0.0
0.2
0.4
0.6
Pollen receipt
Pollen tubes
b)
Generalized
Specialized
Large display
Small display
Bees
Flies
Hummingbirds
TOTAL
0.0
0.2
0.4
0.6
0.8
Pollen receipt
Pollen tubes

Figure 3. Colored bar charts represent the proportion of each landscape-level density-dependence sign (A) and interspecific interaction sign (B) in the community and per each plant attribute category. Blue represents positive effects (evidence of landscape-level positive density-dependence or interspecific facilitation), grey neutral effects, and red negative effects (evidence of landscape-level negative density-dependence or interspecific competition). Specialization category includes species pollinated by one pollinator group and generalization by more than one group (i.e. functional specialization *sensu* Ollerton et al. 2007). Floral display size categories were based on the distribution of open flowers per species in the community (Fig. S1). Small display includes species with < 25 open flowers per individual and large > 25 flowers. Pollinator group categories were based on the main pollinator group visiting the species. Some species had two groups visiting in similar proportions and were thus represented in both categories (e.g. bees and flies).

Discussion

We found negative density-dependence in visitation and pollination outcomes, indicating advantages of rare species when compared to abundant ones. Landscape-level NDD was stronger at high heterospecific density. Therefore, interspecific interactions played a critical role in generating advantages of rarity in pollination. Interspecific competition from highly abundant heterospecifics could lead to disadvantages to rare species and destabilize plant coexistence (Pauw 2013), but instead, we found facilitation for all outcomes. We argue that interspecific facilitation contributed to generate NDD because rare species benefited more from heterospecific neighbors than abundant ones (see Fig. 1a, c, e). Facilitation can only foster coexistence if rare species benefit disproportionately in relation to the abundant species (Soliveres et al. 2015). Thus, plants' pollination niches may represent an axis stabilizing plant interspecific competition (Benadi & Pauw 2018, Lanuza et al. 2018, Johnson & Bronstein 2019). Nevertheless, landscape-level NDD was only marginally significant for pollen tubes. Some abundant species, although receiving less pollen, may have received enough to produce as many viable pollen tubes as rare species. Even though the benefit of rarity is weak when measured in terms of pollen tubes, the fact that landscape-level NDD is stronger at high heterospecific density and facilitation was stronger for rare species than common ones, may still contribute to plant coexistence.

High conspecific density at the plot-level was linked to a decline in visitation rates, but to an increase in pollen receipt and pollen tubes. These contrasting results may be explained by previous evidence on pollen carryover: low visitation rates at high conspecific density still lead to higher pollen carryover on pollinators and, consequently, to more pollen deposited per stigma than at low conspecific density (Robertson 1992). Interestingly, plot-level advantages of rarity (NDD) in visitation were stronger at low heterospecific density. This suggests that at the plot-level, intraspecific competition in visitation occurs when interspecific interactions are weaker. The plot-level advantages of abundance (PDD) in pollen receipt and pollen tubes were also reinforced at high heterospecific density and due to stronger benefits from heterospecific neighbors to abundant species. Such relationships could destabilize interspecific competition, leading to the exclusion of rare species (Heystek & Pauw 2014, Nottebrock et al. 2017). However, our results show that plot-level advantages of abundance are compensated at the landscape-level. While high density at the plot-level promotes better pollination outcomes, high density at the landscape-level may cause pollinator dilution and result in more flowers left unpollinated (Hegland 2014).

The effect of plot-level heterospecific density was consistently positive for all variables measured, indicating that interspecific facilitation in pollinator attraction also leads to benefits in pollination outcomes. There were no relationships with heterospecific density regardless of pollinator sharing, reinforcing the idea that interactions via pollinator sharing among neighbors accounted for this pattern. Although the identity of the heterospecific neighbor can determine the outcome of plant-plant interactions (Arceo-Gómez et al. 2019), overall heterospecific density was shown to affect the pollination of a single species (Albor et al. 2019). The prevalence of interspecific facilitation is expected when pollinators are scarce (Rathcke 1983), which was demonstrated experimentally (Lázaro et al. 2014) and in pollinator-depauperate communities (Tur et al. 2016). In these communities, plant species are under scarce pollination environments and, thus, jointly benefit from increases in pollinator attraction. The combination of interspecific facilitation with stronger benefits for rare species, and intraspecific competition is thought to maintain biodiversity (Feldman et al. 2004). Interestingly, we found empirical evidence for such mechanisms in a relatively generalized community, despite the theoretical requirement of niche partitioning (pollination specialization) for NDD (Pauw 2013, Benadi 2015). We propose that strong facilitation fosters coexistence in generalized communities. In this scenario, the reduced pollination partitioning due to generalization generates positive effects in rare species while abundant species are prevented from dominating due to intraspecific competition. This assumption still needs theoretical and experimental exploration.

We found evidence for linear relationships instead of the predicted theoretical hump-shaped patterns in pollinator visitation (Benadi & Pauw 2018). Also in alpine systems, linear relationships between visitation rates or seed set with flower conspecific and heterospecific density have been detected (Hegland et al. 2009, Lázaro et al. 2013). We attributed this to the low availability of pollinators, a feature shared by alpine communities and the tropical highland grassland studied here. In such systems, increases in conspecific floral resources are not followed by the same magnitude of increasing in visitation rates (Arroyo et al. 1985), generating negative linear relationships. Moreover, high heterospecific density will still lead to increases in pollinator abundance in the area and benefits of joint attraction, in contrast to the expected interspecific competition generating hump-shaped patterns.

We included only immediate pollination outcomes and, thus, proximately linked to the contribution of pollination to plant coexistence. However, we acknowledge that seed set is commonly used as a fitness proxy in coexistence studies. By comparing immediate outcomes and subsequent fitness estimates, one may assess how pollination influences plant coexistence across the reproductive dynamics of the community.

Determinants of landscape and plot density-dependence

The landscape-level advantages of rarity (NDD) hold when accounting for only intraspecific changes in density. Thus, several species experienced disadvantages when they became abundant, making community-level Allee effects unlikely (Lachmuth et al. 2018). The low pollinator availability in this community likely strengthened intraspecific competition and fostered NDD (Ye et al. 2014). As expected, specialists showed landscape-level NDD, since it is more likely that specialized plants have stronger intraspecific competition for the same pollinator species (Johnson et al. 2012). Conversely, for generalists, some individuals may be ‘rescued’ by pollinator species that plants compete for less frequently (Ghazoul 2005). Regarding floral display, patterns matched expectations based on pollinator behavior. Pollinator foraging models have shown that flowers on plant species with small displays are visited less per plant when at high density, potentially generating NDD, while species with large displays are visited less per plant at low density, diminishing pollen receipt and favoring PDD (Ohashi & Yahara 2001). The landscape density-dependence disappeared for pollen tubes. This can be explained by the same pollinator foraging models: at high density, geitonogamy decreases for small-display species while it increases for large-display species (Ohashi & Yahara 2001). Thus, the pollination quality may counteract the density-dependence on pollen receipt.

Advantages of abundance (PDD) were often reported for both bee- and fly-pollinated plants (Ghazoul 2005, Inouye et al. 2015). On the other hand, hummingbird-pollinated plants were shown to be under low (Caruso 1999) and strong intraspecific competition (Aldrich & Hamrick 1998), leading to no clear expectation about density-dependence. The low pollinator abundance may be similar across pollinator groups in our field site, leading to intraspecific competition within each of these pollination guilds, and to the observed lack of density-dependence for bee- and landscape-level NDD for fly- and hummingbird-pollinated species.

Pollination outcomes often increase with conspecific floral density at small spatial scales (Essenberg 2012), consistent with the plot-level PDD found for all attribute categories. This has been used to explain why pollination generates Allee effects and destabilizes plant coexistence (Lachmuth et al. 2018). However, landscape-level advantages of rarity (NDD) prevailed, creating a scale-dependent relationship between pollination outcomes and floral abundance. Our results stress the importance of broader evaluations to assess the contribution of pollination to plant coexistence.

The fact that prevalence of interspecific facilitation also depended on plant attribute categories could be explained by functional mechanisms. Moreover, no interspecific interactions prevailed in qualitative outcomes for some categories, suggesting that some functional mechanisms lead to facilitation and competition to be equally represented (Thomson et al. 2019). Most facilitated species were generalists, which interact with a diverse array of pollinators. Heterospecific patches often attract a high diversity of pollinators (Lázaro et al. 2009), making benefits of joint attraction of distinct pollinators more likely to occur for generalists. On the other hand, all competitive effects were represented in specialized species. In general, specialists strongly depend on their pollinators (Vázquez et al. 2009). Thus, pollinator preference for an attractive heterospecific neighbor will negatively impact the pollination of specialists, as they cannot rely on alternative pollinators. Facilitation prevailed for both display categories, with large display species accounting for most of the change to neutral on pollen tubes. Although large displays benefit from high pollinator attraction, they also incur high levels of geitonogamy (Klinkhamer & de Jong 1993). Thus, the benefit of interspecific facilitation in pollinator attraction may be partly offset by costs of self-pollen deposition in species with large displays.

Regarding pollinator groups, bee-pollinated plants showed prevalence of facilitation on both pollination outcomes. We attribute this to the strong positive responses of bees to increases in floral densities at the local scale (Kunin 1997, Makino et al. 2007). Moreover, bees often show floral constancy even in heterospecific patches (Gegear & Laverty 2001, Lázaro et al. 2009), which maintains the visit quality. Facilitation on pollen receipt prevailed for fly-pollinated plants but showed no facilitation on pollen tubes. Although these plants were primarily pollinated by flies, many were also visited by other insect groups at low proportions. Thus, these plants tend to be generalized in the studied community and may benefit from pollinator sharing. However, flies may not be as constant as bees (Inouye et al. 2015), which may offset the benefits of higher attraction in fly-pollinated plants. Hummingbird-pollinated plants experienced facilitation and competition in similar proportions. Previous studies suggested an interplay of facilitation and competition on hummingbird-pollinated plant communities (Wolowski et al. 2017, Bergamo et al. 2018), and our results confirm that both interaction signs are similarly important for these plants.

Conclusion

Pollination success was greatest when a plant was rare on a landscape scale but occurred in locally dense patches of conspecific or heterospecific flowers. The combination of negative density-dependence (NDD, advantage of rarity) detected at the landscape scale and interspecific facilitation detected at the local scale found here reduces interspecific competition and fosters plant coexistence. In this context, the benefit of positive interspecific interactions would maintain rare species in the community at the same time that abundant species are prevented from reaching total dominance due to intraspecific competition. We provided empirical evidence that pollination is a component of a species' niche, playing an important role in assembling communities. Moreover, we identified ecological drivers of conspecific density-dependence and interspecific interactions for pollination, namely functional specialization, floral display size and pollinator group. Our feasible, community-wide methodology may be useful for exploring other communities in the same way, as more data from different communities would allow us to better understand mechanisms of coexistence and maintenance of biodiversity in ecological communities.

Acknowledgements

We thank the Instituto Chico Mendes de Biodiversidade and the Itatiaia National Park for research permit in protected areas (ICMBio/SISBIO nº 58349-1). P. Medina and V. Zambon for help in the field. N. Escobar, P. Ferronato, G. Martins, P. Medina, R. Romanini and T. Smania for help with pollen counting. J. Amaral, L. Bacci, J. A. M. Carmo, J. Dutilh, M. Egea, C. D. Inácio, L. Meireles, A. Scatigna, G. Shimizu and J. Semir for plant identification. Three anonymous reviewers greatly improved earlier drafts of the manuscript. This work was supported by FAPESP (grants 2016/06434-0 and 2018/02996-0 to P.J.B.), CNPq (grants 436335/2018-2 to M.W. and 302781/2016-1 to M.S.) and CAPES (Ph.D. scholarship to N.S.S., Financial Code 001).

References

Adler, P.B., Smull, D., Beard, K.H., Choi, R.T., Furniss, T., Kulmatiski, A., Meiners, J.M., Tredennick, A.T. & Veblen, K.E. (2018). Competition and coexistence in plant communities: intraspecific competition is stronger than interspecific competition. *Ecol. Lett.*, 1319-1329.

Albor, C., García-Franco, J.G., Parra-Tabla, V., Díaz-Castelazo, C., Arceo-Gómez, G. (2019). Taxonomic and functional diversity of the co-flowering community differentially affect *Cakile edentula* pollination at different spatial scales. *J. Ecol.*, doi:10.1111/1365-2745.13183.

Albrecht, M., Ramis, M.R. & Traveset, A. (2016). Pollinator-mediated impacts of invasive plants on the pollination of native plants: the role of spatial scale and distinct behaviour among pollinator guilds. *Biol. Invasions*, 18, 1801-1812.

Aldrich, P.R. & Hamrick, J.L. (1998). Reproductive dominance of pasture trees in a fragmented tropical forest mosaic. *Science*, 281, 103-105.

Alonso, C., Herrera, C.M. & Ashman, T-L. (2012). A piece of the puzzle: a method for comparing pollination quality and quantity across multiple species and reproductive events. *New Phytol.*, 193, 532-542.

Arceo-Gómez, G., Kaczorowski, R.L., Patel, C. & Ashman, T-L. (2019). Interactive effects between donor and recipient species mediate fitness costs of heterospecific pollen receipt in a co-flowering community. *Oecologia*, 189, 1041-1047.

Arroyo, M.T.K., Primack, R. & Armesto, J. (1985). Community studies in pollination ecology in the high temperate Andes of Central Chile. 2. Effect of temperature on visitation rates and pollination possibilities. *Plant Syst. Evol.*, 149, 187-203.

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Soft.*, 67, 1–48.

Benadi, G. (2015). Requirements for plant coexistence through pollination niche partitioning. *P. Roy. Soc. B-Biol. Sci.*, 282, rspb.2015.0117.

Benadi, G. & Pauw, A. (2018). Frequency dependence of pollinator visitation rates suggests that pollination niches can allow plant species coexistence. *J. Ecol.*, 106, 1982-1901.

Brade, A. C. (1956). A flora do Parque Nacional do Itatiaia. *Bol. do Parque Nacional do Itatiaia*, 5.

Bergamo, P.J., Wolowski, M., Maruyama, P.K., Vizentin-Bugoni, J., Carvalheiro, L.G. & Sazima, M. (2017). The potential indirect effects among plants via shared hummingbird pollinators are structured by phenotypic similarity. *Ecology*, 98, 1849-1858.

Bergamo, P.J., Wolowski, M., Maruyama, P.K., Vizentin-Bugoni, J. & Sazima, M. (2018). Trait patterns across space and time suggest and interplay of facilitation and competition acting on Neotropical hummingbird-pollinated plant communities. *Oikos*, 127, 1690-1700.

Carvalheiro, L.G., Biesmeijer, J.C., Benadi, G., Fründ, J., Stang, M., Bartomeus, I. et al. (2014). The potential for indirect effects between co-flowering plants via shared pollinators depends on resource abundance, accessibility and relatedness. *Ecol. Lett.*, 17, 1389-1399.

Caruso, C.M. (1999). Pollination of *Ipomopsis aggregate* (Polemoniaceae): effects of intra- vs. interspecific competition. *Am. J. Bot.*, 86, 663-668.

Comita, L.S., Queenborough, S.A., Murphy, S.J., Eck, J.L., Xu, K., Krishnadas, M. et al. (2014). Testing predictions of the Janzen-Connell hypothesis: a meta-analysis of experimental evidence for distance- and density-dependent seed and seedling survival. *J. Ecol.*, 102, 845-856.

Danieli-Silva, A., Souza, J.M.T., Donatti, A.J., Campos, R.P., Vicente-Silva, J., Freitas, L. et al. (2012). Do pollination syndromes cause modularity and predict interactions in a pollination network in tropical high-altitude grasslands? *Oikos*, 121, 35-43.

Essenberg, C.J. (2012). Explaining variation in the effect of floral density on pollinator visitation. *Am. Nat.*, 180, 153-166.

Feldman, T.S., Morris, W.F. & Wilson, W.G. (2004). When can two plant species facilitate each other's pollination? *Oikos*, 105, 197-207.

Freitas, L. & Sazima, M. (2006). Pollination in a tropical high-altitude grassland in Brazil: interactions at the community level. *Ann. Mo. Bot. Gard.*, 93, 465-516.

Gegear, R.J. & Laverty, T.M. (2001). The effect of variation among floral traits on the flower constancy of pollinators. In: Cognitive Pollination Ecology {eds. Chittka, L. & Thomson, J.D.}. Cambridge University Press, Cambridge, UK, pp 1-21.

Ghazoul, J. (2005). Pollen and seed dispersal among dispersed plants. *Biol. Rev.*, 80, 413-443.

Harder, L.D., Aizen, M.A. & Richards, S.A. (2016). The population ecology of male gametophytes: the link between pollination and seed production. *Ecol. Lett.*, 19, 497-509.

Hedges, L.V. & Olkin, I. (1985). *Statistical methods for meta-analysis*. Academic Press, San Diego, USA.

Hegland, S.J. (2014). Floral neighbourhood effects on pollination success in red clover are scale-dependent. *Funct. Ecol.*, 28, 561-568.

Hegland, S.J., Grytnes, J.-A. & Totland, O. (2009). The relative importance of positive and negative interactions for pollinator attraction in a plant community. *Ecol. Res.*, 24, 929-936.

Heystek, A. & Pauw, A. (2014). Does competition for pollinators contribute to structuring *Erica* communities? *J. Veg. Sci.*, 25, 648-656.

Inouye, D., Larson, B.M.H., Ssymank, A. & Kevan, P.G. (2015). Flies and flowers III: ecology of foraging and pollination. *J. Poll. Ecol.*, 16, 115-133.

Johnson, C.A. & Bronstein, J.L. (2019). Coexistence and competitive exclusion in mutualism. *Ecology*, doi:10.1002/ecy.2708.

Johnson, S.D., Hollens, H. & Kuhlmann, M. (2012). Competition versus facilitation: conspecific effects on pollinator visitation and seed set in the iris *Lapeirousia oreogena*. *Oikos*, 121, 545-550.

Klinkhamer, P.G.L. & de Jong, T.J. (1993). Attractiveness to pollinators: a plant's dilemma. 66, 180-184.

Kunin, W.E. (1997). Population size and density effects in pollination: pollinator foraging and plant reproductive success in experimental arrays of *Brassica kaber*. *J. Ecol.*, 85, 225-234.

Lachmuth, S., Henrichmann, C., Horn, J., Pagel, J. & Schurr, F.M. (2018). Neighbourhood effects on plant reproduction: an experimental-analytical framework and its application to the invasive *Senecio inaequidens*. *J. Ecol*, 106, 761-773.

Lanuza, J.B., Bartomeus, I. & Godoy, O. (2018). Opposing effects of floral visitors and soil conditions on the determinants of competitive outcomes maintain species diversity in heterogeneous landscapes. *Ecol. Lett.*, 21, 865-874.

Lázaro, A., Lundgren, R. & Totland, Ø. (2009). Co-flowering neighbors influence the diversity and identity of pollinator groups visiting plant species. *Oikos*, 118, 691-702.

Lázaro, A., Jakobosson, A. & Totland, Ø. (2013). How do pollinator visitation rate and seed set related to species' floral traits and community context? *Oecologia*, 173, 881-893.

Lázaro, A., Lundgren, R. & Totland, Ø. (2014). Experimental reduction of pollinator visitation modifies plant-plant interactions for pollination. *Oikos*, 123, 1037-1048.

Martin, F.W. (1959). Staining and observing pollen tubes in the style by means of fluorescence. *Stain Technol.*, 34, 125-128.

Makino, T.T., Ohashi, K. & Sakai, S. (2007). How do floral display size and the density of surrounding flowers influence the likelihood of bumble bee revisitation to a plant? *Funct. Ecol.*, 21, 87-95.

Mitchell, R.J., Flanagan, R.J., Brown, B.J., Waser, N.M. & Karron, J.D. (2009). New frontiers in competition for pollination. *Ann. Bot.*, 103, 1403-1413.

Moeller, D.A. (2004). Facilitative interactions among plants via shared pollinators. *Ecology*, 85, 3289-3301.

Murphy, S.J., Wiegand, T. & Comita, L.S. (2017). Distance-dependent seedling mortality and long-term spacing dynamics in a neotropical forest community. *Ecol. Lett*., 20, 1469-1578.

Nottebrock, H., Schmid, B., Mayer, K., Devaux, C., Esler, K., Böhning-Gaese, K. et al. (2017). Sugar landscapes and pollinator-mediated interactions in plant communities. *Ecography*, 40, 1129-1138.

Ohashi, K. & Yahara, T. (2001). Behavioural responses of pollinators to variation in floral display size and their influences on the evolution of floral traits. In: Cognitive Pollination Ecology {eds. Chittka, L. & Thomson, J.D.}. Cambridge University Press, Cambridge, UK, pp 274-296.

Ollerton, J., Killick, A., Lamborn, E., Watts, S. & Whiston, M. (2007). Multiple meanings and modes: on the many ways to be a generalist flower. *Taxon*, 56, 717-728.

Pauw, A. (2013). Can pollination niches facilitate plant coexistence? *Trends Ecol. Evol.*, 28, 30-37.

Rathcke, B. (1983). Competition and facilitation among plants for pollination. In: Pollination Biology {ed. Real, L.}. Academic Press, Orlando, FL, pp 305-325.

Ribeiro, K.T., Medina, B.M.O. & Scarano, F.B. (2007). Species composition and biogeographic relations of the rock outcrop floral on the high plateau of Itatiaia, SE-Brazil. *Braz. J. Bot.*, 30, 623-639.

Robertson, A.W. (1992). The relationship between floral display size, pollen carryover and geitonogamy in *Myosotis colensoi* (Kirk) Macbride (Boraginaceae). *Biol. J. Linn. Soc.*, 46, 333-349.

Seifan, M., Hoch, E.-M., Hanoteaux, S. & Tielboerger, K. (2014). The outcome of shared pollination services is affected by the density and spatial pattern of an attractive neighbour. *J. Ecol.*, 102, 953-962.

Soliveres, S., Maestre, F.T., Berdugo, M. & Allan, E. (2015). A missing link between facilitation and plant species coexistence: nurses benefit generally rare species more than common ones. *J. Ecol.*, 103, 1183-1189.

Thomson, J.D., Fung, H.F. & Ogilvie, J.E. (2019). Effects of spatial patterning of co-flowering plant species on pollination quantity and purity. *Ann. Bot.*, 123, 303-310.

Totland, Ø. (1993). Pollination in alpine Norway: flowering phenology, insect visitors, and visitation rates in two plant-communities. *Can. J. Bot.*, 71, 1072-1079.

Tur, C., Saez, A., Traveset, A. & Aizen, M.A. (2016). Evaluating the effects of pollinator-mediated interactions using pollen transfer networks: evidence of widespread facilitation in south Andean plant communities. *Ecol. Lett.*, 19, 576–586.

Vázquez, D.P., Blüthgen, N., Cagnolo, L. & Chacoff, N.P. (2009). Uniting pattern and process in plant-animal mutualist networks: a review. *Ann. Bot.*, 103, 1445-1457.

Viechtbauer, W. (2010). Conducting meta-analyses in R with the metafor package. *Journal of Statistical Software*, 36, 1-4.

Ward, M., Johnson, S.D. & Zalucki, M.P. (2013). When bigger is not better: intraspecific competition for pollination increases with population size in invasive milkweeds. *Oecologia*, 171, 883-891.

Wolowski, M., Carvalheiro, L.G. & Freitas, L. (2017). Influence of plant-pollinator interactions on the assembly of plant and hummingbird communities. *J. Ecol.*, 105, 332-334.

Ye, Z-M., Dai, W-K., Jin, X-F., Gituru, R.W., Wang, Q-F. & Yang, C-F. (2014). Competition and facilitation among plants for pollination: can pollinator abundance shift the plant-plant interactions? *Plant Ecol.*, 215, 3-13.

Zuur, A.F., Ieno, E.N. & Elphick, C.S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods Ecol. Evol.*, 1, 3-14.

Supplementary material

Appendix 1. Details on sampling effort and plant classification.

We accumulated several hours of observation per species included in the attribute classification (4.3 hours on average per species included in the attribute classification). However, we had to employ observation sessions of 30min-1hr in order to register pollinators due to the natural low visitation rates in the area. Therefore, we ended up with few visitation rate samples for several species, and could not to include this response variable in the "Determinants of density-dependence" analyses. We registered 19.78 individual visits per observation session on average, and we thus are confident that this dataset is sufficient to classify the plant species in broad categories. We acknowledge that a detailed classification (e.g. specialization based on number of pollinator species, or using pollinator groups such as large bees, small bees, small flies, etc) would demand more sampling effort.

1) Functional specialization. Plants were classified based on Ollerton et al. (2007) concept of functional specialization: specialized if pollinated by one pollinator group (e.g. by bees) and generalized if pollinated by more than one group. We used this broad classification to avoid underrepresenting the generalization due to sampling effort issues. We included only plant species pollinated solely by hummingbirds, by large bees, or by few fly pollinators in the specialized category. Generalized plants were pollinated by several insect groups, by both hummingbirds and large bees or by both bees and flies. Therefore, it is unlikely that the specialized plants would exhibit more pollinator partners than the generalized species.

2) Floral display size. Floral display size category was determined by a histogram showing the distribution of floral display size per species in the community (Figure S1). The figure shows that several plant species have < 25 flowers, while the rest of the species are evenly distributed in categories of > 25, > 50, > 75. To restrict to only two categories, we used 25 flowers as the cutoff as it rendered in two categories with a similar number of species.

3) Pollinator group. Each species was assigned to one pollinator group category, corresponding to the pollinator group that performed most of the visits (> 70%). However, some species had two pollinator groups visiting in similar proportions (e.g. ~60% by bees and ~40% by flies). These species were then assigned to both pollinator group categories.

Table S1. Plant species included in this study of the highland grassland of the National Itatiaia Park, Southeastern Brazil. Sampling effort of visitation rate (hours) and number of pistils analyzed. Pollinator group indicates the main pollinator groups of each species. The category “Various insects” represents species pollinated by a diverse array of groups (bees, flies, wasps, beetles and ants). Functional specialization indicates if it is pollinated by one pollinator group (specialized) or more than one (generalized, Ollerton et al. 2007). Flower display size categories were based on the distribution of open flowers per species (Fig. S1), with small indicating species < 25 open flowers per individual and large > 25 flowers. Species in bold were included in the slope extraction analyses.

Family/Species	**Hours observed**	**Pollination group**	**Functional specialization**	**Flower display**	**Pistils**
Alstroemeriaceae					
***Alstroemeria foliosa* Mart. Ex Schult. & Schult.f.**	2h	Bees and hummingbirds	Generalized	Small	11
***Alstroemeria isabelleana* Herb.**	2h	Hummingbirds	Specialized	Small	15
Amaryllidaceae					
Hippeastrum morelianum Lem.	2h	Hummingbirds	Specialized	Small	2
Apiaceae					
Eryngium glaziovianum Urb.	1h	Various insects	Generalized	Large	-
Eryngium paniculatum Cav. & Dombey ex F.Delaroche	2h	Various insects	Generalized	Large	-
Apocynaceae					
Oxypetalum glaziovii (E.Fourn.) Fontella & Marquete	1h	Butterflies	Specialized	Small	2
Asteraceae					
Achyrocline satureioides (Lam.) DC.	5h30m	Various insects	Generalized	Large	-
Baccharis altimontana G. Heiden et al.	2h	Various insects	Generalized	Small	-
Baccharis itatiaiae Wawra	3h	Various insects	Generalized	Large	-
Baccharis platypoda DC.	2h	Various insects	Generalized	Large	-
Baccharis megapotamica Spreng.	7h	Various insects	Generalized	Large	-
Leptostelma maximum D.Don	1h	Various insects	Generalized	Small	-
Grazielia intermedia (DC.) R.M.King H H.Rob.	4h	Various insects	Generalized	Large	-
Symphyopappus compressus (Gardner) B.L.Rob.	3h	Various insects	Generalized	Large	-
Mikania parodii Cabrera	1h	Flies	Specialized	Large	-
***Stevia* cf. *decussata* Baker**	5h30m	Various insects	Generalized	Large	96

Senecio oleosus Vell.	3h	Bees and flies	Generalized	Small	-
Senecio nemoralis Dusén	4h	Various insects	Generalized	Small	-
Hypochaeris radicata L.	1h	Various insects	Generalized	Large	-
Verbesina glabrata Hook. & Arn.	6h	Various insects	Generalized	Small	-
Mikania cf. *cordifolia* (L.f) Willd.	1h	Various insects	Generalized	Large	-
Trixis glaziovii Baker	2h	Various insects	Generalized	Small	-
Berberidaceae					
***Berberis glazioviana* Brade**	7h	Bees	Specialized	Large	20
Bromeliaceae					
***Fernseea itatiaiae* (Wawra) Baker**	6h	Hummingbirds	Specialized	Small	24
Vriesea itatiaiae Wawra	1h	Hummingbirds	Specialized	Small	2
Campanulaceae					
***Lobelia camporum* Pohl**	2h	Bees[1]	Specialized	Small	8
***Siphocampylus westinianus* (Thunb.) Pohl**	4h	Hummingbirds	Specialized	Small	10
Caprifoliaceae					
Valeriana glaziovii Taub.	2h	Flies	Specialized	Large	-
Caryophyllaceae					
***Cerastium dicrotrichum* Fenzl ex. Rohrb.**	2h	Flies	Specialized	Small	10
Eriocaulaceae					
Paepalanthus itatiaiensis Ruhland	6h	Various insects	Generalized	Large	-
Ericaceae					

Agarista hispidula **(DC.) Hook. Ex Nied.**	1h	Hummingbirds	Specialized	Small	8
Agarista oleifolia (Cham.) G.Don	-	-	-	Large	2
Gaultheria eriophylla **(Pers.) Sleumer ex Burtt**	2h	Hummingbirds	Specialized	Small	20
Gaultheria serrata **(Vell.) Sleumer ex Kin.-Gouv.**	10h	Hummingbirds	Specialized	Large	79
Gaylussacia amoena **Cham.**	5h	Bees and flies	Generalized	Large	45
Gaylussacia fasciculata Gardner	-	-	-	Large	-
Escalloniaceae					
Escallonia bifida **Link & Otto**	4h	Various insects	Generalized	Large	17
Escallonia laevis **(Vell.) Sleumer**	2h30m	Bees	Specialized	Small	11
Euphorbiaceae					
Croton dichrous Müll.Arg.	4h30m	Various insects	Generalized	Large	-
Fabaceae					
Lupinus gibertianus **C.P.Sm.**	4h30m	Bees	Specialized	Large	17
Mimosa itatiaiensis Dusén	6h	Bees and flies	Generalized	Large	-
Mimosa monticola Dusén	4h	Bees and flies	Generalized	Large	-
Geraniaceae					
Geranium brasiliense **Progel**	1h30m	Flies	Specialized	Small	19
Iridaceae					
Alophia sellowiana **Klatt**	4h	Bees and flies	Generalized	Small	18
Sisyrinchium wettsteinii **Hand.-Mazz.**	14h	Bees and flies	Generalized	Small	75
Sisyrinchium glaziovii **Baker**	3h	Bees and flies	Generalized	Small	47
Lamiaceae					
Cunila galioides **Benth.**	6h30m	Bees and flies	Generalized	Large	52

***Hesperozygis myrtoides* (A.St.-Hil. Ex Benth.) Epling**	1h30m	Bees and flies	Generalized	Large	43
***Lepechinia speciosa* (A.St.-Hil. Ex Benth.) Epling**	6h30m	Hummingbirds	Specialized	Large	31
***Salvia itatiaensis* Dusén**	7h	Bees and hummingbirds	Generalized	Large	29
Lentibulariaceae					
***Utricularia reniformis* A.St.-Hil.**	3h	Bees[2]	Specialized	Small	8
Melastomataceae					
***Chaetostoma glaziovii* Cogn.**	2h	Bees	Specialized	Large	12
***Leandra eichleri* Cogn.**	6h30m	Bees	Specialized	Large	34
***Pleroma hospita* (Schrank et Mart. ex DC.) Triana**	3h30m	Various insects	Generalized	Large	21
Myrtaceae					
***Myrceugenia alpigena* (DC.) Landrum**	2h	Various insects	Generalized	Large	18
Onagraceae					
***Fuchsia campos-portoi* Pilg. & Schulze-Menz**	5h	Hummingbirds	Specialized	Large	20
***Fuchsia regia* (Vell.) Munz**	1h	Hummingbirds	Specialized	Small	10
Orchidaceae					
Cyclopogon apricus (Lindl.) Schltr.	1h	-	-	-	-
Orobanchaceae					
***Buddleja speciosissima* Taub.**	5h	Hummingbirds	Specialized	Large	10
***Esterhazya eitenorum* Barringer**	9h	Bees and hummingbirds	Generalized	Small	67

Oxalidaceae					
***Oxalis confertissima* A.St.-Hil**	9h	Bees and flies	Generalized	Large	90
***Oxalis rupestris* A.St.-Hil.**	2h	Bees and flies	Generalized	Small	8
Primulaceae					
***Lysimachia filiformis* (Cham. & Schltdl.) U. Manns & Anderb.**	3h	Various insects	Generalized	Large	9
Proteaceae					
Roupala montana Aubl.	-	-	-		1
Rubiaceae					
***Coccocypselum lyman-smithii* Standl.**	2h30m	Bees	Specialized	Small	15
Solanaceae					
Solanum enantiophyllantum Bitter	1h	Bees	Specialized	Large	2
Velloziaceae					
Barbacenia gounelleana Beauerd	3h	Hummingbirds	Specialized	Small	2
Xyridaceae					
***Xyris fusca* L.A.Nilsson**	2h	Bees and flies	Generalized	Small	10

[1] Freitas, L. & Sazima, M. (2006). Pollination in a tropical high-altitude grassland in Brazil: interactions at the community level. *Ann. Mo. Bot. Gard.*, 93, 465-516.

[2] Clivati, D., Cordeiro, G. D., Plachno, B. J. & Miranda, V. F. O. (2014). Reproductive biology and pollination of *Utricularia reniformis* A.St.-Hil. (Lentibulariaceae). *Plant Biol.*, 16, 677-682.

Table S2. GLMM coefficients of the visitation rate (Poisson error structure), pollen receipt and pollen tubes (Gaussian error structure) models using total heterospecific density as fixed effect. Conspecific frequency means the sum of conspecific flowers at community-level. Conspecific density was measured as number of conspecific flowers at plot level. Heterospecific density was measured as total number of heterospecific flowers at plot level (regardless of potential pollinator sharing). β = effect estimate, SE = standard error, χ_2 = equivalent F statistic. Bold values indicate significative effects at p <0.05.

Fixed effects	Visitation rate		Pollen receipt		Pollen tubes	
	β ± SE	χ_2 (p-value)	β ± SE	χ_2 (p-value)	β ± SE	χ_2 (p-value)
Conspecific frequency	**-0.162 ± 0.197**	**3.96 (0.037)**	-0.064 ± 1.800	0.04 (0.950)	-0.077 ± 0.041	3.05 (0.081)
Conspecific density	**-0.062 ± 0.198**	**5.83 (0.017)**	**5.549 ± 1.635**	**12.08 (<0.001)**	**0.170 ± 0.041**	**18.32 (<0.001)**
Heterospecific density	0.126 ± 0.133	0.63 (0.453)	0.360 ± 1.177	0.04 (0.850)	0.059 ± 0.031	3.10 (0.079)
Conspecific frequency * Heterospecific density	-0.219 ± 0.189	0.15 (0.701)	-2.415 ± 1.424	2.88 (0.090)	-0.075 ± 0.040	6.09 (0.013)
Conspecific density * Heterospecific density	0.218 ± 0.206	0.16 (0.686)	2.275 ± 1.387	2.69 (0.101)	0.098 ± 0.039	3.52 (0.061)
Observation time	**-0.093 ± 0.087**	**4.05 (0.038)**	-	-	-	-

Table S3. GLMM coefficients of the visitation rate (Poisson error structure), pollen receipt and pollen tube germination (Gaussian error structure) models using quadratic terms for all fixed effects. Landscape conspecific density means the sum of conspecific flowers at landscape-level. Plot conspecific density was measured as number of conspecific flowers at plot-level. Heterospecific density was measured as total number of heterospecific flowers at plot-level with potential pollinator sharing. β = model estimate, SE = standard error, χ^2 = equivalent F statistic. Bold values indicate significative effects at $p < 0.05$.

Fixed effects	Visitation rate		Pollen receipt		Pollen tubes	
	β ± SE	χ^2 (p-value)	β ± SE	χ^2 (p-value)	β ± SE	χ^2 (p-value)
Landscape conspecific density^2	-0.213 ± 0.103	0.34 (0.559)	-0.004 ± 0.046	0.09 (0.766)	-0.029 ± 0.045	0.01 (0.910)
Plot conspecific density^2	0.302 ± 0.208	1.02 (0.313)	**-0.009 ± 0.043**	**4.58 (0.032)**	-0.035 ± 0.043	2.45 (0.117)
Heterospecific density^2	-0.117 ± 0.220	0.02 (0.899)	-0.091 ± 0.056	0.25 (0.620)	-0.137 ± 0.056	0.27 (0.601)
Landscape conspecific density^2 * Heterospecific density^2	0.173 ± 0.157	2.19 (0.139)	0.032 ± 0.035	0.83 (0.360)	0.025 ± 0.035	0.52 (0.470)
Plot conspecific density^2 * Heterospecific density^2	-0.103 ± 0.186	1.04 (0.309)	**0.058 ± 0.029**	**4.17 ± (0.041)**	**0.067 ± 0.029**	**5.51 (0.019)**
Observation time^2	**-0.093 ± 0.087**	**3.38 (0.046)**	-	-	-	-

Table S4. AIC values of the models with fixed linear terms and models with quadratic fixed terms. Each model is indicated by its response variable: visitation rate (Poisson error structure), pollen receipt and pollen tubes (Gaussian error structure).

Model	AIC (Linear)	AIC (Quadratic)
Visitation rate	929.04	936.30
Pollen receipt	2802.62	2823.73
Pollen tubes	2783.88	2810.01

Table S5. Correlation matrix between the fixed effects of the GLMM for visitation rates. Landscape conspecific density means the sum of conspecific flowers at landscape-level. Plot conspecific density was measured as number of conspecific flowers at plot-level. Heterospecific density was measured as number of heterospecific flowers at plot level with potential pollinator sharing. Correlation coefficient expressed as Pearson' r and associated p-value in parenthesis.

	Landscape conspecific density	Plot conspecific density	Heterospecific density
Landscape conspecific density	-	0.30 (< 0.001)	0.06 (0.392)
Plot conspecific density	-	-	0.10 (0.132)

Table S6. Correlation matrix between the fixed effects of the GLMM for pollen receipt and pollen tubes. Landscape conspecific density means the sum of conspecific flowers at landscape-level. Plot conspecific density was measured as number of conspecific flowers at plot-level. Heterospecific density was measured as number of heterospecific flowers at plot-level with potential pollinator sharing. Correlation coefficient expressed as Pearson' r and associated p-value in parenthesis.

	Landscape conspecific density	Plot conspecific density	Heterospecific density
Landscape conspecific density	-	0.29 (< 0.001)	0.06 (0.075)
Plot conspecific density	-	-	0.14 (<0.001)

Table S7. GLMM coefficients of the effect of landscape conspecific density on pollen receipt and pollen tubes per species. β_j = slope per species, SE = standard error. We considered the effect positive (evidence of landscape-level positive density-dependence, PDD) when $\beta_j \pm 2SE > 0$, neutral when $\beta_j \pm 2SE$ overlapped 0, and negative (evidence of landscape-level negative density-dependence, NDD) when $\beta_j \pm 2SE < 0$.

	Pollen receipt			Pollen tubes		
Plant species (Abbreviation)	Slope (βj)	SE	Effect	Slope (βj)	SE	Effect
Agarista hispidula (Aghi)	-	-	-	-	-	-
Alstroemeria foliosa (Alfo)	-	-	-	-	-	-
Alstroemeria isabelleana (Alis)	-0.447	0.144	Negative	-0.696	0.207	Negative
Alophia sellowiana (Alse)	-0.041	0.091	Neutral	-0.062	0.100	Neutral
Berberis glaziovii (Begl)	-	-	-	-	-	-
Buddleja speciosissima (Busp)	-0.399	0.052	Negative	-0.764	0.063	Negative
Cerastium dicrotricum (Cedi)	-	-	-	-	-	-
Chaetostoma glaziovii (Chgl)	1.433	0.269	Positive	1.871	0.263	Positive
Cococcypselum lyman-smithii (Coly)	-	-	-	-	-	-
Cunila galioides (Cuga)	0.522	0.263	Neutral	0.414	0.272	Neutral
Escallonia laevis (Esla)	0.601	0.086	Positive	0.117	1.097	Positive
Escallonia bifida (Esbi)	0.620	0.046	Positive	0.280	0.058	Positive
Esterhazya eitenorum (Esei)	-0.772	0.057	Negative	-0.192	0.081	Negative
Fernseea itatiaiae (Feit)	-1.898	0.086	Negative	-1.400	0.150	Negative
Fuchsia campos-portoi (Fuca)	0.662	0.048	Positive	0.459	0.080	Positive
Fuchsia regia (Fure)	-	-	-	-	-	-
Gaylussacia amoena (Gaam)	-0.294	0.062	Negative	-0.365	0.061	Negative
Gaultheria eriophylla (Gaer)	0.992	0.149	Positive	1.011	0.184	Positive

Gaultheria serrata (Gase)	-0.267	0.038	Negative	-0.170	0.042	Negative
Geranium brasiliense (Gebr)	-0.122	0.281	Neutral	-0.167	0.284	Neutral
Hesperozygis myrtoides (Hemy)	0.790	0.254	Positive	0.645	0.232	Positive
Leandra sulfurea (Lesu)	0.888	0.252	Positive	1.120	0.347	Positive
Lepechinia speciosa (Lesp)	-0.338	0.288	Neutral	0.068	0.288	Neutral
Lobelia camporum (Loca)	-	-	-	-	-	-
Lupinus gilbertianus (Lugi)	-0.064	0.219	Neutral	-0.176	0.219	Neutral
Lysimachia filiformis (Lyfi)	-	-	-	-	-	-
Myrceugenia alpigena (Myal)	-0.299	0.138	Negative	-0.874	0.141	Negative
Oxalis confertissima (Oxco)	0.596	0.058	Positive	0.075	0.575	Positive
Oxalis rupestris (Oxru)	-3.345	0.535	Negative	-1.061	0.484	Negative
Pleroma hospita (Plho)	-0.725	0.068	Negative	0.780	0.188	Positive
Salvia itatiaiensis (Sait)	1.161	0.413	Positive	1.358	0.501	Positive
Sisyrinchium wettsteinii (Siwt)	1.228	0.132	Positive	1,225	0.137	Positive
Sisyrinchium glaziovii (Sigl)	-0.358	0.068	Negative	-0.901	0.088	Negative
Siphocampylus westinianus (Siwe)	-1.223	0.438	Negative	-1.221	0.470	Negative
Stevia cf. *decussata* (Stde)	-0.146	0.109	Neutral	-0.032	0.121	Neutral
Utricularia reniformis (Utre)	-7.206	1.080	Negative	-5.079	0.949	Negative
Xyris fusca (Xyfu)	-	-	-	-	-	-
OVERALL	-0.05	0.02	Negative	-0.07	0.02	Negative

Table S8. GLMM coefficients of the effect of plot conspecific density on pollen receipt and pollen tubes per species. β_j = slope per species, SE = standard error. We considered the effect positive (evidence of plot-level positive density-dependence, PDD) when $\beta_j \pm 2SE > 0$, neutral when $\beta_j \pm 2SE$ overlapped 0, and negative (evidence of plot-level negative density-dependence, NDD) when $\beta_j \pm 2SE < 0$.

	Pollen receipt			Pollen tubes		
Plant species (Abbreviation)	Slope (βj)	SE	Effect	Slope (βj)	SE	Effect
Agarista hispidula (Aghi)	1.096	0.266	Positive	1.175	0.264	Positive
Alstroemeria foliosa (Alfo)	4.741	0.902	Positive	4.339	1.030	Positive
Alstroemeria isabelleana (Alis)	-2.840	0.400	Negative	-1.928	0.472	Negative
Alophia sellowiana (Alse)	-0.221	0.283	Neutral	-0.684	0.335	Negative
Berberis glaziovii (Begl)	0.218	0.060	Positive	0.247	0.061	Positive
Buddleja speciosissima (Busp)	0.032	0.068	Neutral	-0.355	0.088	Negative
Cerastium dicrotricum (Cedi)	-1.029	0.211	Negative	0.942	0.447	Positive
Chaetostoma glaziovii (Chgl)	1.315	0.140	Positive	1.505	0.144	Positive
Cococcypselum lyman-smithii (Coly)	-0.052	0.105	Neutral	0.370	0.151	Positive
Cunila galioides (Cuga)	1.022	0.214	Positive	0.914	0.214	Positive
Escallonia laevis (Esla)	1.517	0.088	Positive	0.962	0.127	Positive
Escallonia bifida (Esbi)	0.929	0.053	Positive	0.401	0.072	Positive
Esterhazya eitenorum (Esei)	0.397	0.082	Positive	0.869	0.102	Positive
Fernseea itatiaiae (Feit)	-0.775	0.123	Negative	-0.465	0.185	Negative
Fuchsia campos-portoi (Fuca)	0.382	0.032	Positive	0.239	0.057	Positive
Fuchsia regia (Fure)	-0.780	0.345	Negative	0.229	0.383	Neutral
Gaylussacia amoena (Gaam)	0.096	0.085	Neutral	-0.031	0.086	Neutral
Gaultheria eriophylla (Gaer)	1.672	0.121	Positive	2.232	0.152	Positive

Gaultheria serrata (Gase)	0.282	0.049	Positive	0.355	0.051	Positive
Geranium brasiliense (Gebr)	-0.103	0.355	Neutral	-0.285	0.362	Neutral
Hesperozygis myrtoides (Hemy)	1.134	0.183	Positive	0.872	0.185	Positive
Leandra sulfurea (Lesu)	0.745	0.174	Positive	0.587	0.194	Positive
Lepechinia speciosa (Lesp)	0.340	0.200	Neutral	0.282	0.213	Neutral
Lobelia camporum (Loca)	2.376	0.977	Positive	1.610	0.913	Neutral
Lupinus gilbertianus (Lugi)	-0.008	0.221	Neutral	0.007	0.225	Neutral
Lysimachia filiformis (Lyfi)	-1.310	0.578	Negative	-0.757	0.576	Neutral
Myrceugenia alpigena (Myal)	-0.059	0.162	Neutral	-0.083	0.168	Neutral
Oxalis confertissima (Oxco)	0.611	0.044	Positive	0.655	0.054	Positive
Oxalis rupestris (Oxru)	-0.796	0.441	Neutral	-0.079	0.460	Neutral
Pleroma hospita (Plho)	-0.144	0.094	Neutral	-0.053	0.098	Neutral
Salvia itatiaiensis (Sait)	1.614	0.423	Positive	1.149	0.391	Positive
Sisyrinchium wettsteinii (Siwt)	1.148	0.098	Positive	1.237	0.126	Positive
Sisyrinchium glaziovii (Sigl)	0.829	0.095	Positive	0.245	0.137	Neutral
Siphocampylus westinianus (Siwe)	0.029	0.309	Neutral	0.166	0.323	Neutral
Stevia cf. *decussata* (Stde)	0.364	0.132	Positive	0.108	0.145	Neutral
Utricularia reniformis (Utre)	6.382	0.942	Positive	5.698	0.863	Positive
Xyris fusca (Xyfu)	4.697	0.874	Positive	4.336	0.790	Positive
OVERALL	0.46	0.02	Positive	0.40	0.02	Positive

Table S9. GLMM coefficients of the effect of plot heterospecific density on pollen receipt and pollen tubes per species. β_j = slope per species, SE = standard error. We considered the effect positive (evidence of interspecific facilitation) when $\beta_j \pm 2SE > 0$, neutral when $\beta_j \pm 2SE$ overlapped 0, and negative (evidence of interspecific competition) when $\beta_j \pm 2SE < 0$ (following Tur et al. 2016).

	Pollen receipt			Pollen tubes		
Plant species (Abbreviation)	Slope (βj)	SE	Effect	Slope (βj)	SE	Effect
Agarista hispidula (Aghi)	0.459	0.114	Positive	0.475	0.113	Positive
Alstroemeria foliosa (Alfo)	0.350	0.090	Positive	0.252	0.111	Positive
Alstroemeria isabelleana (Alis)	-0.463	0.204	Negative	-0.244	0.098	Negative
Alophia sellowiana (Alse)	-0.035	0.027	Neutral	-0.063	0.030	Negative
Berberis glaziovii (Begl)	-0.816	0.078	Negative	-0.849	0.077	Negative
Buddleja speciosissima (Busp)	0.122	0.037	Positive	-0.007	0.049	Neutral
Cerastium dicrotricum (Cedi)	-0.831	0.082	Negative	-0.103	0.108	Neutral
Chaetostoma glaziovii (Chgl)	-0.601	0.108	Negative	-0.586	0.107	Negative
Cococcypselum lyman-smithii (Coly)	-0.197	0.046	Negative	0.134	0.066	Positive
Cunila galioides (Cuga)	0.621	0.080	Positive	0.591	0.081	Positive
Escallonia laevis (Esla)	1.04	0.055	Positive	0.656	0.075	Positive
Escallonia bifida (Esbi)	0.200	0.033	Positive	-0.035	0.036	Neutral
Esterhazya eitenorum (Esei)	0.272	0.030	Positive	0.213	0.035	Positive
Fernseea itatiaiae (Feit)	-0.663	0.051	Negative	-0.251	0.062	Negative
Fuchsia campos-portoi (Fuca)	0.128	0.024	Positive	0.048	0.044	Neutral
Fuchsia regia (Fure)	-0.142	0.187	Negative	-0.593	0.202	Negative
Gaylussacia amoena (Gaam)	0.338	0.055	Positive	0.433	0.059	Positive
Gaultheria eriophylla (Gaer)	0.176	0.043	Positive	0.185	0.051	Positive

Gaultheria serrata (Gase)	-0.051	0.022	Negative	0.070	0.023	Positive
Geranium brasiliense (Gebr)	0.073	0.102	Neutral	0.068	0.102	Neutral
Hesperozygis myrtoides (Hemy)	0.303	0.094	Positive	0.299	0.098	Positive
Leandra sulfurea (Lesu)	0.386	0.083	Positive	0.565	0.101	Positive
Lepechinia speciosa (Lesp)	0.045	0.089	Neutral	0.125	0.095	Neutral
Lobelia camporum (Loca)	0.820	0.343	Positive	0.801	0.315	Positive
Lupinus gilbertianus (Lugi)	-0.205	0.106	Neutral	0.200	0.109	Neutral
Lysimachia filiformis (Lyfi)	-0.235	0.418	Neutral	0.061	0.413	Neutral
Myrceugenia alpigena (Myal)	1.265	0.168	Positive	1.522	0.196	Positive
Oxalis confertissima (Oxco)	0.001	0.020	Neutral	0.034	0.024	Neutral
Oxalis rupestris (Oxru)	0.289	0.090	Positive	0.046	0.114	Neutral
Pleroma hospita (Plho)	0.204	0.068	Positive	0.094	0.067	Neutral
Salvia itatiaiensis (Sait)	0.268	0.173	Neutral	0.213	0.174	Neutral
Sisyrinchium wettsteinii (Siwt)	0.379	0.033	Positive	0.328	0.036	Positive
Sisyrinchium glaziovii (Sigl)	0.009	0.026	Neutral	0.019	0.038	Neutral
Siphocampylus westinianus (Siwe)	-0.794	0.180	Negative	-0.554	0.177	Negative
Stevia cf. *decussata* (Stde)	0.108	0.052	Positive	-0.022	0.059	Neutral
Utricularia reniformis (Utre)	0.198	0.175	Neutral	0.210	0.171	Neutral
Xyris fusca (Xyfu)	1.764	0.298	Positive	1.740	0.286	Positive
OVERALL	0.08	0.02	Positive	0.08	0.02	Positive

Tur, C., Saez, A., Traveset, A. & Aizen, M.A. (2016). Evaluating the effects of pollinator-mediated interactions using pollen transfer networks: evidence of widespread facilitation in south Andean plant communities. *Ecol. Lett.*, 19, 576–586.

Table S10. Proportion of each landscape-level density-dependence sign (positive density-dependence, neutral or negative density-dependence) in the community and per plant attribute category. The effect was considered positive (evidence of positive density-dependence, PDD) when $\beta_j \pm 2SE > 0$, neutral when $\beta_j \pm 2SE$ overlapped 0, and negative (evidence of negative density-dependence, NDD) when $\beta_j \pm 2SE < 0$. Specialization indicates pollination by one pollinator group (specialized) or generalization by more than one group (Ollerton et al. 2007). Floral display size categories were based on the distribution of open flowers per species in the community (Fig. S1), with small indicating species < 25 open flowers per individual and large > 25 flowers. Number of species (proportion within the category). Pollinator group indicates the main pollinator group visiting the species. Some species had more than two groups visiting in similar proportions and were represented in both categories (e.g. bees and flies).

	Pollen receipt			**Pollen tubes**		
Attribute/Category	**PDD**	**Neutral**	**NDD**	**PDD**	**Neutral**	**NDD**
Specialization						
Specialized	4 (30.8%)	3 (23.1%)	6 (46.2%)	4 (30.8%)	3 (23.1%)	6 (46.2%)
Generalized	6 (40.0%)	3 (20.0%)	6 (40.0%)	7 (46.67%)	3 (20.0%)	5 (33.3%)
Floral display size						
Small	4 (26.7%)	3 (20.0%)	8 (53.3%)	4 (26.7%)	3 (20.0%)	8 (53.3%)
Large	6 (46.2%)	3 (23.1%)	4 (30.8%)	7 (53.8%)	3 (23.1%)	3 (23.1%)
Pollinator group						
Bees	7 (50.0%)	3 (21.4%)	4 (28.6%)	7 (50.0%)	3 (21.4%)	4 (28.6%)
Flies	3 (27.2%)	4 (36.4%)	4 (36.4%)	4 (36.4%)	4 (36.4%)	3 (27.3%)
Hummingbirds	1 (10.0%)	3 (30.0%)	6 (60.0%)	1 (10.0%)	3 (30.0%)	6 (60.0%)
TOTAL	10 (35.7%)	6 (21.4%)	12 (42.9%)	11 (39.3%)	6 (21.4%)	11 (39.3%)

Table S11. Proportion of each plot-level conspecific density-dependence (positive density-dependence, neutral or negative density-dependence) in the community and per plant attribute category. The effect was considered positive (evidence of positive density-dependence, PDD) when $\beta_j \pm 2SE > 0$, neutral when $\beta_j \pm 2SE$ overlapped 0, and negative (evidence of negative density-dependence, NDD) when $\beta_j \pm 2SE < 0$. Specialization indicates pollination by one pollinator group (specialized) or generalization by more than one group (Ollerton et al. 2007). Floral display size categories were based on the distribution of open flowers per species in the community (Fig. S1), with small indicating species < 25 open flowers per individual and large > 25 flowers. Number of species (proportion within the category). Pollinator group indicates the main pollinator group visiting the species. Some species had more than two groups visiting in similar proportions and were represented in both categories (e.g. bees and flies).

	Pollen receipt			**Pollen tubes**		
Attribute/Category	**PDD**	**Neutral**	**NDD**	**PDD**	**Neutral**	**NDD**
Specialization						
Specialized	9 (47.4%)	6 (31.6%)	4 (21.1%)	10 (52.6%)	6 (31.6%)	3 (15.8%)
Generalized	12 (66.7%)	5 (27.8%)	1 (05.56%)	10 (55.6%)	7 (38.9%)	1 (05.6%)
Floral display size						
Small	11 (50.0%)	6 (27.3%)	5 (22.8%)	11 (50.0%)	8 (36.4%)	3 (13.6%)
Large	10 (66.7%)	5 (33.3%)	0	9 (60.0%)	5 (33.3%)	1 (06.7%)
Pollinator group						
Bees	14 (77.8%)	4 (33.3%)	0	13 (72.2%)	4 (22.2%)	1 (05.6%)
Flies	7 (53.8%)	5 (38.5%)	1 (07.7%)	6 (46.2%)	6 (46.2%)	1 (07.7%)
Hummingbirds	7 (53.8%)	3 (23.1%)	3 (23.1%)	7 (53.8%)	3 (23.1%)	3 (23.1%)
TOTAL	21 (56.8%)	11 (29.7%)	5 (13.5%)	20 (54.1%)	13 (35.1%)	4 (10.8%)

Table S12. Proportion of each interspecific interaction sign (interspecific facilitation, neutral or interspecific competition) in the community and per plant trait category. The effect was considered positive (evidence of interspecific facilitation) when $\beta_j \pm 2SE > 0$, neutral when $\beta_j \pm 2SE$ overlapped 0, and negative (evidence of interspecific competition) when $\beta_j \pm 2SE < 0$ (following Tur et al. 2016). Specialization indicates pollination by one pollinator group (specialized) or generalization by more than one group (Ollerton et al. 2007). Floral display size categories were based on the distribution of open flowers per species in the community (Fig. S1), with small indicating species < 25 open flowers per individual and large > 25 flowers. Number of species (proportion within the category). Pollinator group indicates the main pollinator group visiting the species. Some species had more than two groups visiting in similar proportions and were represented in both categories (e.g. bees and flies).

	Pollen receipt			**Pollen tubes**		
Attribute/Category	**Positive**	**Neutral**	**Negative**	**Positive**	**Neutral**	**Negative**
Specialization						
Specialized	6 (31.5%)	4 (21.1%)	9 (47.4%)	6 (31.6%)	7 (36.8%)	6 (31.6%)
Generalized	13 (72.2%)	5 (27.8%)	0	9 (50%)	8 (44.4%)	1 (05.6%)
Floral display size						
Small	9 (47.4%)	4 (21.1%)	6 (31.5%)	9 (47.4%)	5 (26.3%)	5 (26.3%)
Large	10 (55.6%)	5 (27.8%)	3 (16.7%)	6 (33.3%)	10 (55.6%)	2 (11.1%)
Pollinator group						
Bees	10 (55.6%)	6 (33.3%)	2 (11.1%)	10 (55.6%)	5 (27.8%)	3 (16.6%)
Flies	8 (61.5%)	4 (30.8%)	1 (07.7%)	6 (46.2%)	6 (46.2%)	1 (07.7%)
Hummingbirds	6 (46.2%)	2 (15.4%)	5 (38.5%)	5 (38.5%)	4 (30.8%)	4 (30.8%)
TOTAL	19 (51.4%)	9 (24.3%)	9 (24.3%)	15 (40.5%)	15 (40.5%)	7 (18.9%)

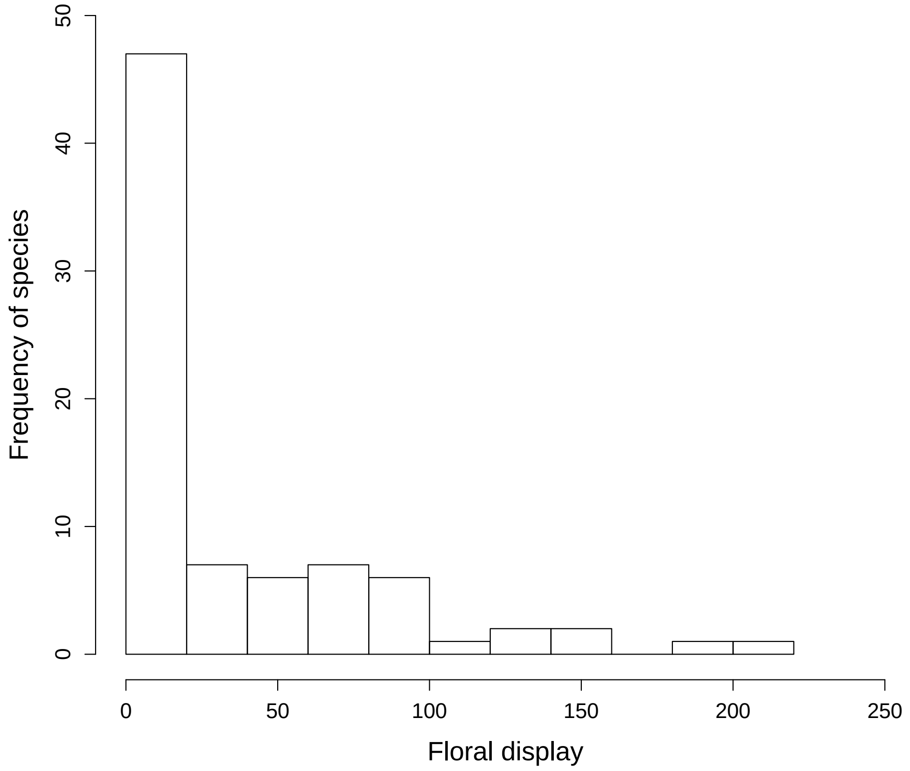

Figure S1. Distribution of the floral display size (measured as number of open flowers) per species in the highland grasslands of the Itatiaia National Park, RJ, Brazil.

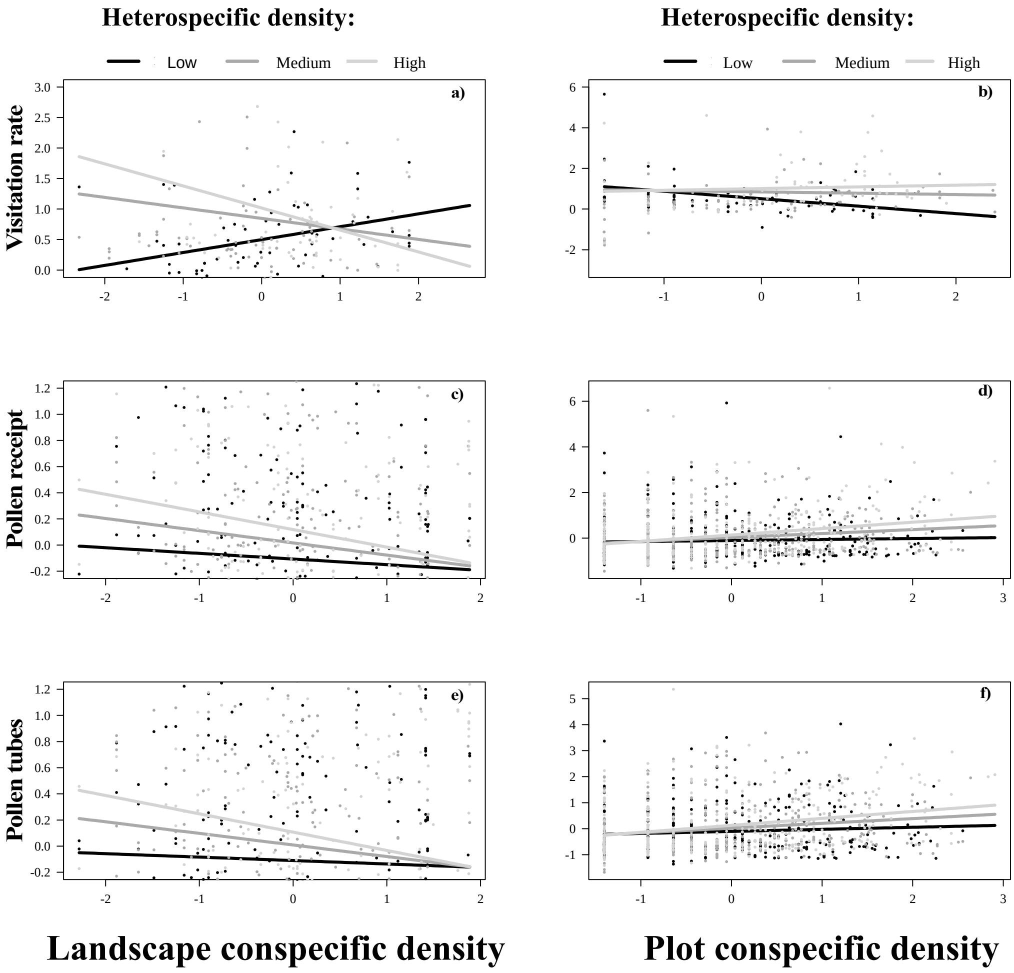

Figure S2. Interactive effects between landscape conspecific density with heterospecific density (left panels) and between plot conspecific density with heterospecific density (right panels) on visitation rates and pollination outcomes. Each dot represents the partial residuals (after removing variation explained by other variables in each model) for each focal plant species-date combination (panels a-b) and individual stigma samples (panels c-f). Black dots and lines represent the landscape or plot conspecific density effect for species co-occurring with low heterospecific density (below the first quartile), dark grey for species co-occurring with intermediate heterospecific density (between the first and third quartile), and light grey for species co-occurring with high heterospecific density (above the third quartile). Visitation rates are on visits.flower^{-1}.hour^{-1}. The variables ‘pollen receipt’ and ‘pollen tubes’ were z-transformed (within plant species) to achieve comparable values among different species.

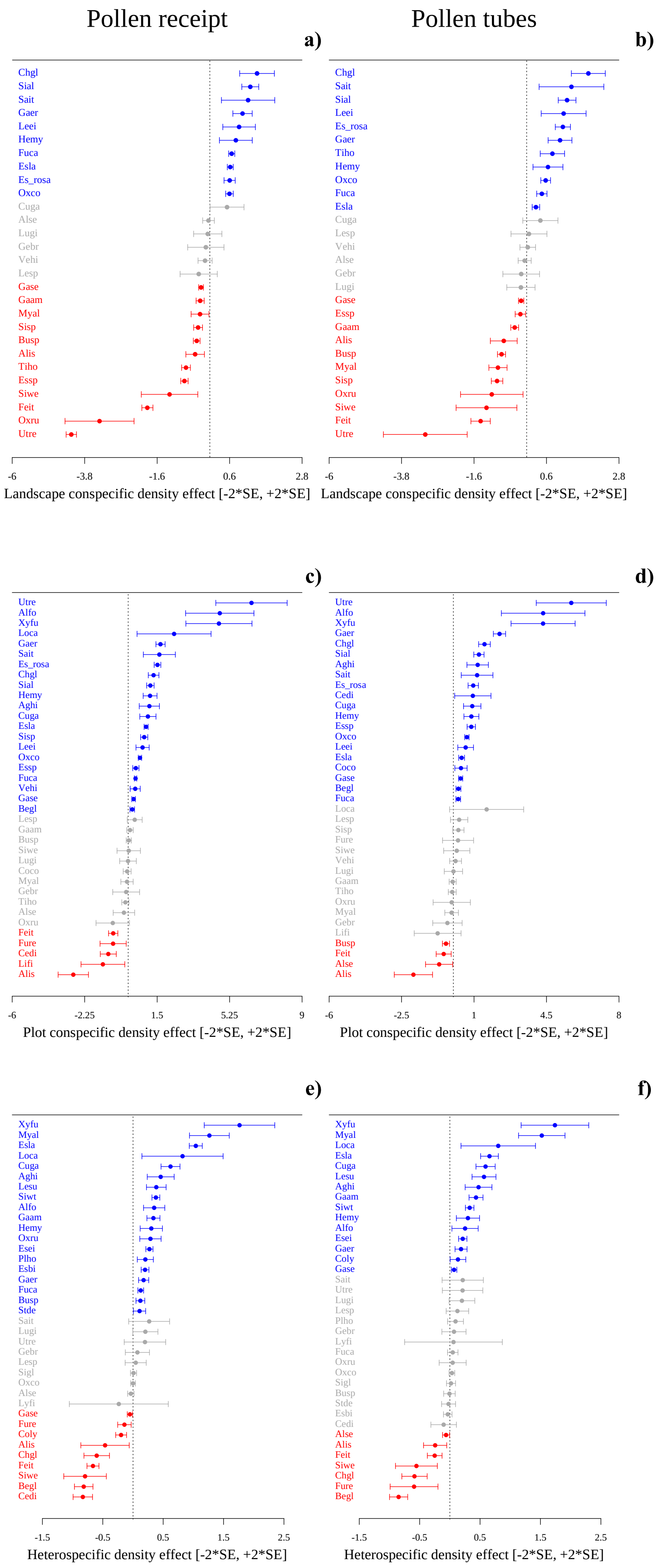
Pollen receipt
Pollen tubes
a)
Chgl
Sial
Sait
Gaer
Leei
Hemy
Fuca
Esla
Es_rosa
Oxco
Cuga
Alse
Lugi
Gebr
Vehi
Lesp
Gase
Gaam
Myal
Sisp
Busp
Alis
Tiho
Essp
Siwe
Feit
Oxru
Utre
-6
-3.8
-1.6
0.6
2.8
Landscape conspecific density effect [-2*SE, +2*SE]
b)
Chgl
Sait
Sial
Leei
Es_rosa
Gaer
Tiho
Hemy
Oxco
Fuca
Esla
Cuga
Lesp
Vehi
Alse
Gebr
Lugi
Gase
Essp
Gaam
Alis
Busp
Myal
Sisp
Oxru
Siwe
Feit
Utre
-6
-3.8
-1.6
0.6
2.8
Landscape conspecific density effect [-2*SE, +2*SE]
c)
Utre
Alfo
Xyfu
Loca
Gaer
Sait
Es_rosa
Chgl
Sial
Hemy
Aghi
Cuga
Esla
Sisp
Leei
Oxco
Essp
Fuca
Vehi
Gase
Begl
Lesp
Gaam
Busp
Siwe
Lugi
Coco
Myal
Gebr
Tiho
Alse
Oxru
Feit
Fure
Cedi
Lifi
Alis
-6
-2.25
1.5
5.25
9
Plot conspecific density effect [-2*SE, +2*SE]
d)
Utre
Alfo
Xyfu
Gaer
Chgl
Sial
Aghi
Sait
Es_rosa
Cedi
Cuga
Hemy
Essp
Oxco
Leei
Esla
Coco
Gase
Begl
Fuca
Loca
Lesp
Sisp
Fure
Siwe
Vehi
Lugi
Gaam
Tiho
Oxru
Myal
Gebr
Lifi
Busp
Feit
Alse
Alis
-6
-2.5
1
4.5
8
Plot conspecific density effect [-2*SE, +2*SE]
e)
Xyfu
Myal
Esla
Loca
Cuga
Aghi
Lesu
Siwt
Alfo
Gaam
Hemy
Oxru
Esei
Plho
Esbi
Gaer
Fuca
Busp
Stde
Sait
Lugi
Utre
Gebr
Lesp
Sigl
Oxco
Alse
Lyfi
Gase
Fure
Coly
Alis
Chgl
Feit
Siwe
Begl
Cedi
-1.5
-0.5
0.5
1.5
2.5
Heterospecific density effect [-2*SE, +2*SE]
f)
Xyfu
Myal
Loca
Esla
Cuga
Lesu
Aghi
Gaam
Siwt
Hemy
Alfo
Esei
Gaer
Coly
Gase
Sait
Utre
Lugi
Lesp
Plho
Gebr
Lyfi
Fuca
Oxru
Oxco
Sigl
Busp
Stde
Esbi
Cedi
Alse
Alis
Feit
Siwe
Chgl
Fure
Begl
-1.5
-0.5
0.5
1.5
2.5
Heterospecific density effect [-2*SE, +2*SE]

Figure S3. Estimated slopes (βj ± 2 SE) for the effect of landscape conspecific density, plot conspecific density and plot heterospecific flower density (with potential pollinator sharing at the plot level) on a, c, and e) pollen receipt (quantitative female component) and b, d, and f) pollen tubes (qualitative female component) for each focal plant species of the highland grassland of the Itatiaia National Park, Southeastern Brazil. Colored species represent each interaction sign: blue indicate positive effects (landscape- and plot-level positive density-dependence and interspecific facilitation), grey neutral effects and red negative effects (landscape- and plot-level negative density-dependence and interspecific competition). Species abbreviations follow Tables S7-S9.

CAPÍTULO 2.

Pollinator-mediated facilitation is associated to floral abundance, trait similarity and enhanced community-level fitness

Pedro Joaquim Bergamo[1,2*], Nathália Susin Streher[3], Marina Wolowski[4], Marlies Sazima[5]

[1]Graduate Program in Ecology, University of Campinas, Campinas, Brazil

[2]Mediterranean Institute for Advanced Studies, CSIC-UIB, Mallorca, Spain

[3]Graduate Program in Plant Biology, University of Campinas, Campinas, Brazil

[4]Institute of Natural Sciences, Federal University of Alfenas, Alfenas, Brazil

[5]Plant Biology Department, Institute of Biology, University of Campinas, Campinas, Brazil

Published in *Journal of Ecology*: https://doi.org/10.1111/1365-2745.13348

Abstract

1. Pollinator-mediated processes (biotic filtering, facilitation or competition) are often inferred by patterns of plant reproductive trait diversity (clustering or evenness of reproductive traits within the community). However, one single pattern can be generated by distinct processes, making difficult to predict the main process of community assembly. Incorporating fitness estimates should improve the link between pattern and process.

2. We investigated patterns of flowering phenology and reproductive traits (floral color, floral size and anther height) along the season of a pollinator-depauperated and generalized community. We used data on fitness (pollen receipt and number of pollen tubes) to provide a functional link between trait patterns and assembly mechanisms. We also investigated if the degree of co-flowering depended on the floral abundance and pollination functional group (fly-, bee-, hummingbird-pollinated and generalist species) of the plant species.

3. High floral abundance in the flowering season was associated with low trait diversity in the community. Both features increased fitness at the community-level. This indicates that similar

species are benefited at periods of high floral abundance, probably due to the joint attraction of generalist pollinators in this pollinator-depauperated community. In general, rare species flowered more synchronously with the community than abundant ones, although distinct patterns emerged depending on the floral trait and pollination functional group. Furthermore, species highly synchronous and possessing similar floral color in relation to the community had higher fitness indicating that facilitative mechanisms act favoring flowering synchrony and trait similarity.

Synthesis

Patterns of flowering synchrony and floral trait similarity indicate pollination facilitation in the studied community. Plants benefited from co-flowering with species possessing similar floral color via shared pollinator attraction. Thus, we empirically demonstrated some of the predictions of community assembly theory.

Keywords: *campos de altitude*, community assembly rules, competition, highland grasslands, floral traits, pollination ecology, pollinator sharing, reproductive success

Introduction

Plant-animal interactions, such as pollination, may influence plant community assembly (Sargent & Ackerly, 2008). The evaluation of reproductive trait patterns has been used to infer which pollinator-mediated processes act on community assembly (Pellissier, Alvarez, & Guisan 2012; Eisen & Geber, 2018). For instance, high trait diversity and phylogenetic evenness are often associated to competition, indicating pressures that led to divergent uses of pollinators (Muchhala, Johnsen, & Smith 2014). On the other hand, low trait diversity and phylogenetic clustering may indicate biotic filtering, when the plants resemble each other due to ecological sorting and adaptation towards the dominant pollinator (Kemp, Bergh, Soares, & Ellis 2019) or facilitation, when phenotypically similar species benefit from sharing its pollination niche (de Jager, Dreyer, & Ellis 2011). To date, most studies reported static trait patterns including all co-occurring species. However, there is wide variation in flowering phenology within the community. Thus, how much species overlap its flowering and potentially compete or facilitate with each other through shared pollinators should vary within the community (Arceo-Gómez, Kaczorowski, & Ashman 2018). This is especially important in systems characterized by a long flowering season, on which the co-flowering community composition changes along time.

Incorporating the phenological dimension revealed important fine-scale processes structuring co-flowering communities. For instance, it has been shown that floral trait composition in the community often changes over time (Filella et al., 2013), patterns of co-flowering are associated with floral trait similarity (Makino & Yokoyama, 2015; Bergamo, Wolowski, Maruyama, Vizentin-Bugoni, & Sazima 2018; Fantinato, Del Vecchio, Giovanetti, Acosta, & Buffa 2018) and floral traits mediate the temporal variation in pollination networks (Kantsa et al., 2018). Moreover, not only trait composition, but overall floral abundance also changes during the flowering season. Plants flowering in contrasting phenological environments (high vs. low overall floral abundance) deal with distinct contexts of pollinator-mediated interactions. High floral abundance may lead to strong competition for pollinators and, in this context, it is expected high trait diversity (implying divergent pollinator use), whereas high floral availability associated with low trait diversity (phenotypically similar co-flowering species) may lead to benefits of the joint attraction of pollinators (Moeller, 2004). Similarly, low floral abundance should foster competition and divergent pollinator use if the flower-pollinator ratio is high (Lázaro, Jakobsson, & Totland 2013), while if pollinators are scarce, facilitation is fostered due to benefits of joint attraction (Tur, Sáez, Traveset, & Aizen 2016). Nevertheless, other processes such as convergent evolution and biotic filtering would also generate low trait similarity (Briscoe Runquist, Grossenbacher, Porter, Kay, & Smith 2016). Therefore, studies should consider multiple processes generating the observed structure patterns.

Most studies inferring processes from floral trait patterns do not associate them with fitness estimates. This is problematic because floral trait diversity in the community was shown to influence the fitness of focal species (Albor, García-Franco, Parra-Tabla, Díaz-Castelazo, & Arceo-Gómez 2019). However, we lack fitness assessments at the community-level, which are necessary to link trait patterns to assembly processes. In this context, trait and phylogenetic diversity linked to competition or facilitation are predicted to enhance the overall fitness of the co-flowering community (“mean community fitness”, Wolowski, Carvalheiro, & Freitas 2017). However, studies addressing how trait patterns determine overall fitness patterns have not been conducted within co-flowering communities.

The co-flowering patterns should also depend on the abundance and pollination system of each plant species. First, rare species are generally more subjected to competition for pollination (Ghazoul, 2005). In this context, rare species are expected to be less synchronous than abundant ones to reduce competition and ensure conspecific pollination (Gumbert, Kunze, & Chittka 1999). When facilitation is prevalent, synchrony would benefit rare species due to enhanced attraction of shared pollinators (Feldman, Morris, & Wilson 2004). Second, floral traits (e.g. floral color, size and anther height) that mediate competition and facilitation by pollinators differ among plants belonging to distinct pollinator systems (Carvalheiro et al., 2014, Bergamo et al., 2017). Therefore, the association between flowering synchrony and trait similarity should also differ among pollination functional groups within a community.

Two main mechanisms are discussed to structure co-flowering communities: flowering synchrony and trait similarity among species (Feinsinger, 1987). Reduced flowering synchrony is expected under competition, leading species with low synchrony with the community to present higher fitness (Waser, 1978; Aizen & Rovere, 2010), while aggregated flowering is favored under facilitation, with highly synchronous species presenting higher fitness due to benefits from joint attraction of pollinators (Moeller, 2004; Wolowski et al., 2017; Eisen & Geber, 2018). Trait similarity of each species within the community can also influence such outcomes on fitness (Sargent, Kembel, Emery, Forrestel, & Ackerly 2011). Therefore, low or high floral trait overlap of the plant species within the community is expected to be associated with competition or facilitation, respectively, both leading to higher fitness. However, it has rarely been tested the contribution of both mechanisms structuring whole co-flowering communities (but see Wolowski et al., 2017 for a test with hummingbird-pollinated plants).

Here, we investigated flowering phenology and trait patterns in a plant community of a tropical highland grassland (Figure 1). Previous evidence showed that heterospecific floral density is positively related to visitation rates and pollination for several plant species in this community, indicating a prevalence of facilitation over competition (Bergamo, Streher, Traveset, Wolowski, & Sazima, 2019). This allowed us to test if facilitation is also predicted by reproductive trait similarity as proposed by the community assembly theory applied to plant-pollinator interactions (Sargent & Ackerly, 2008; Figure 1). Specifically, we explored if community-level trait similarity (low trait diversity) was associated with floral abundance (hypothesis 1). Then, we expected community-level fitness to increase with trait similarity (low trait diversity) and high floral abundance (hypothesis 2). We also investigated if flowering synchrony depended on the species abundance, trait similarity and pollination functional groups (hummingbird-, bee-, fly- or generalist-pollination, i.e., visited by three or more groups) (hypothesis 3). Finally, we tested if species with high flowering synchrony and trait similarity had higher fitness (hypothesis 4).

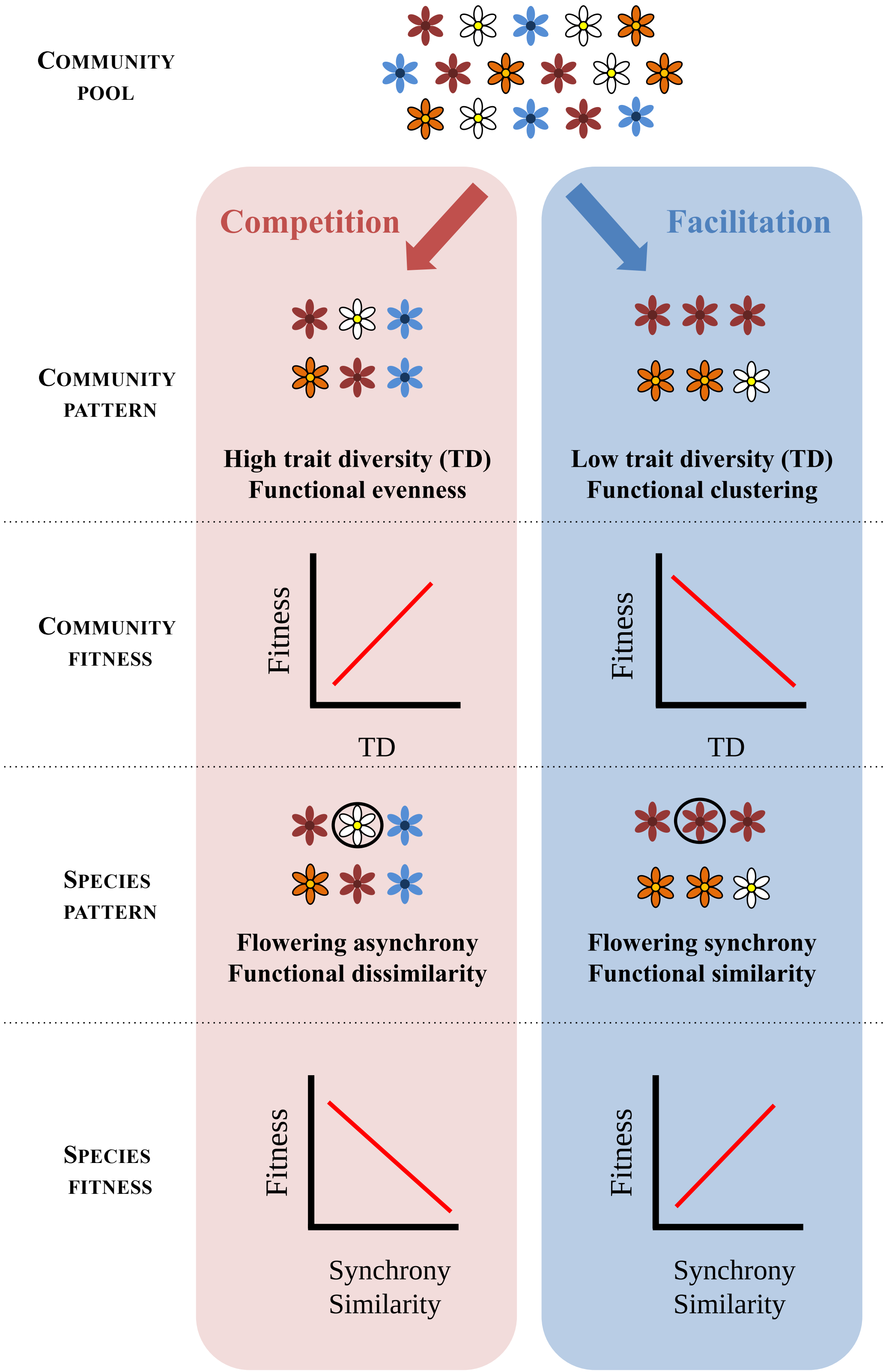
COMMUNITY POOL
Competition
Facilitation
COMMUNITY PATTERN
High trait diversity (TD)
Functional evenness
Low trait diversity (TD)
Functional clustering
COMMUNITY FITNESS
Fitness
TD
Fitness
TD
SPECIES PATTERN
Flowering asynchrony
Functional dissimilarity
Flowering synchrony
Functional similarity
SPECIES FITNESS
Fitness
Synchrony
Similarity
Fitness
Synchrony
Similarity

Figure 1. Framework of the expected functional patterns (based on plant reproductive traits) and responses on fitness related to the structuring processes of plant-pollinator interactions on plant community assembly. Plant species can be sorted from the community pool into each co-flowering community under two scenarios: competition for pollination or facilitation for pollination as the main structuring process. **Competition scenario:** at the community-level, it is expected high trait diversity and functional evenness, indicating divergent pollinator use. Therefore, high trait diversity would enhance community-level fitness. At the species-level, it is expected plant species to flower asynchronously or to possess distinct reproductive traits when flowering synchronously with the community. These plant species would benefit due to divergent pollinator use and diminished competition. **Facilitation scenario:** at the community-level, it is expected low trait diversity and functional clustering, indicating similar pollinator use. Thus, low trait diversity would enhance community-level fitness. At the species-level, it is expected plant species to flower synchronously and to show similar reproductive traits with the community. These plant species would benefit due to pollinator sharing and facilitation.

Material and Methods

Study system

The study was conducted in the Itatiaia National Park, southeastern Brazil, in a community consisting of highland grassland vegetation (~ 2300 m a.s.l., 22º21' S, 44º40' W). This vegetation is known as *campos de altitude*, which is characterized by extensive grasslands and small herbs growing in rocky outcrops and shallow soils at mountaintops surrounded by Atlantic Forest at lower altitude. There is a marked seasonality, with a warm and humid season in spring/summer and a cold and dry season in the winter. The mean annual temperature is of 14.4 ºC with minimum temperatures reaching -10 ºC and mean annual precipitation of 2400 mm (Ribeiro, Medina, & Scarano, 2007). More than 150 plant species were previously registered in the study area (Ribeiro et al., 2007). Our study included flower counts for 76 plant species, with a detailed register of the flowering phenology and measurement of the floral traits for a subset of 63 species (Table S1).

Flowering phenology

Flowering phenology was registered on 17 monthly censuses (once per month, every four weeks) during two consecutive seasons (October/2016 to May/2017 season and September/2017 to May/2018 season). We did not sample in the peak of the dry season (June-August) because almost no plant species were flowering. Flower counts were conducted on 101 plots with 2 m_2 established on three transects. We established a minimum distance of 50 m between plots, totaling 0,34ha of area sampled. The number of flowers on each plant individual was counted monthly in all plots. For species with many flowers (e.g. the ones with large inflorescences), we estimated the number of flowers per inflorescence and extrapolated by counting the number inflorescences. For species with flowers arranged in capitula (e.g. Asteraceae) or other similar arrangements (e.g. Apiaceae), and therefore hard to individualize each flower, we used the number of inflorescences as a surrogate of abundance since each inflorescence functions as a pollination blossom (Bergamo et al., 2017). With the flower counts, we obtained a flowering phenology matrix with the number of flowers of each species on each month. Then, we calculated the overall floral abundance of the community and richness of species flowering per month. The floral abundance of each plant species was estimated as the sum number of flowers on each of the two seasons. We calculated flowering overlap among all pairwise species combinations (including only the 63 focal species) using the Czechanowski index (Feinsinger, Spears, & Poole 1981). This is an adequate index for intensity of phenological measurements, since it is based on the overlap of the histograms representing the distribution of flowers along the months of two species.

Community-level fitness

Fitness data were gathered from Bergamo et al. (2019). It was estimated as number of conspecific pollen grains in the stigma (hereafter, pollen receipt) and number of pollen tubes growing in the style (hereafter, pollen tubes). These two measurements are frequently used as quantitative and qualitative components, respectively, of the contribution of pollinators to the plant female fitness (Alonso, Herrera, & Ashman 2012; Tur et al., 2016). This was measured to a subset of 44 species in every monthly census (except October/2016 and September/2017 due to low floral availability). We selected species on which were possible to manipulate the flowers without moving pollen from the anthers to the stigmas (Table S1). The pistils of flowers at the end of the anthesis were collected and stored in 70% alcohol. Pollen grains and pollen tubes were counted in a fluorescence microscope. Since plant species vary in pollen receipt due to intrinsic differences in pollen production and pistil traits, we calculated z-scores (scaled to a mean of 0 and standard deviation of 1) within species for pollen receipt and pollen tubes. Then, mean community fitness was calculated as the mean pollen receipt and pollen tubes (after z-transformation) across all samples on each month (Table S2).

Pollination functional groups

We assigned each plant species in a pollination functional group using the plant-pollinator interaction data of Bergamo et al. (2019). For each month, a random subset of the plots with flowering individuals was observed and all visits to flowers registered. Observation sessions lasted 1 h due to the low visitation rates in the area. Visitors were classified in major groups (bees, flies, wasps, beetles, ants and hummingbirds) and we accounted only visits with potential for pollination (i.e. when the visitor touched anthers and stigmas). Visits by *Apis mellifera* were not considered, since it is a supergeneralist invasive bee and would not indicate if that plant species evolved towards bee-pollination. Species visited in similar frequencies by two groups (e.g. ~50% bees and ~ 50% flies) were assigned to both pollination functional groups. Species visited by several groups (three or more) in similar frequencies were assigned to the "generalist" category. We included 15 species in the hummingbird-pollinated category, 22 species in the bee-pollinated category, 11 species in the fly-pollinated category and 21 species in the generalist category (Table S1).

Floral traits

We selected floral traits previously known to be relevant for plant-pollinator interactions, namely: floral reflectance (pollinator attraction), floral size (pollinator attraction and morphological fit between flower and pollinator), anther and stigma height (related to the site of pollen placement and pickup in the pollinator body). Since anther and stigma height were highly correlated, we removed the latter from subsequent analyses. Floral reflectance was measured as the petal spectral reflectance using a USB4000 spectrophotometer (OceanOptics, Inc., Dunedin, FL, USA) coupled with a deuterium-halogen light source (DH-2000; OceanOptics, Inc., Ostfildern, Germany), with a light emission range between 215 nm and 1700 nm. All reflectance measurements were taken at a 45º angle, using barium sulphate and a black chamber as white and black standards, respectively. Morphological features were measured directly in the field using a digital caliper. Floral size was measured as total petal length for tubular flowers and as maximum floral diameter for radial flowers. For species with capitula or similar arrangements, we measured the capitula/inflorescence diameter. We measured anther height from the base of the flower to the top of the anther. We measured floral traits for all 63 focal species, with an average of 9.4 flowers per species and each sample from a distinct plant individual (Table S1).

We calculated the trait diversity in the community using the Rao's quadratic entropy index (Botta-Dukát, 2005), which is more related to trait convergence and divergence indexes commonly used to infer community assembly processes (Botta-Dukát & Czúcz, 2016). In this context, low trait diversity indicates trait clustering and high trait diversity indicates trait evenness. We calculated the same index weighted by each species abundance in the month (number of flowers in the month) and without abundance weighting (Table S2). Abundance-weighted indices give a better approximation of total appearance of the community and thus, how strong is trait convergence and divergence (Kantsa et al., 2018).

Floral reflectance and color

We first reduced the number of reflectance variables by applying a PCA in the 300-700nm range of the reflectance of all 63 species. Reflectances were standardized (by the maximum value per reflectance) prior to PCA procedures. We selected the first four PCA axes, which included 96.6% of the variation (see Appendix 1 for details) and used them with the floral size and anther height measurements to calculate the trait diversity per month (hypotheses 1 and 2).

For the analysis restricted to a single pollinator group (hypothesis 3), we used pollinator visual models to achieve meaningful color variables. We used the photoreceptor sensitivities of *Sephanoides sephaniodes* as hummingbird visual system (Herrera et al., 2008). For bees, we used sensitivities of the native stingless bee *Melipona quadrifasciata* (Menzel, Ventura, Werner, Joaquim, & Backhaus 1989) since Meliponini bees were the most common pollinators. For flies, we used sensitivities of the hoverfly *Eristalis tenax* (Lunau, 2014) since syrphids were the most common fly pollinators in the study system. Generalist species were mainly pollinated by hoverflies, muscoid flies, bees and wasps. For the generalist species, first we used fly vision models and then repeated the same analysis using bee vision models. We calculated the quantum catches (Qi's) for each plant species using its floral reflectance, corresponding photoreceptor sensitivities of its main pollinator group, a standard daylight function (D65) and a common green leaves reflectance function (AV400). To use them in color space models, we calculated relative quantum catches (qi's) with von Kries correction. We obtained perceptual distances as Euclidian distances in the tetrahedron model for hummingbirds (Vorobyev, Osorio, Bennett, Marshall, & Cuthill 1998), hexagon model for bees (Chittka, 1992) and categorical model for flies (Troje, 1993).

Phylogeny

Flowering phenology may be constrained by shared ancestry, leading to a phylogenetic signal in flowering overlap (i.e. closely related plants with similar flowering periods, Brito et al., 2017). Similarly, close related plants may share the same pollination niche, leading to phylogenetic signal in floral traits (Staggemeier, Diniz-Filho, & Morellato 2010). To account potential influences of evolutionary relatedness, we built a plant phylogenetic hypothesis for the 63 focal species based on the calibrated seed plant supertree of Zanne et al. (2014) (Figure S1). We used the updated version of this supertree implemented in the R-function S.Phylo.Maker (Qian & Jin, 2016). Flowering phenology, floral traits and floral abundance were all poorly correlated with phylogenetic relatedness in the studied community, therefore, we assumed that all models were robust to constraints of shared evolutionary history (Appendix S2).

Statistical analyses

Is trait similarity (low trait diversity) associated with floral abundance?

We used null models and linear models to investigate how floral abundance and trait diversity are associated. First, we calculated the trait diversity (abundance-weighted Rao's Q) per month. Then, the observed Rao's Q value of each month was compared with a null distribution generated by 10,000 random monthly communities created from the species pool (abundances across all months lumped together, Table S3). We used the independent-swap algorithm to create the null distribution (Gotelli 2000). To investigate if convergent evolution also contributed to the observed patterns, we used the same null model approach with the equivalent index for phylogenetic diversity, which is based on the phylogenetic distances between plant species (Table S4). Convergent evolution should lead high phylogenetic diversity to be associated with months of low trait diversity. Secondly, we fitted a model using monthly trait diversity (abundance-weighted) as the response variable and monthly floral abundance, phylogenetic diversity and season (2016/2017 or 2017/2018) as predictor variables. We repeated the same procedure with the Rao's Q index without abundance-weight to check if extremely abundant species were influencing the results. We also correlated floral abundance with richness of species flowering to check if floral abundant months were also richer, which may indicate that abundant species had not a strong influence in the results.

Does community-level fitness increase with floral abundance and trait similarity (low trait diversity)?

We fitted linear models to investigate the relationship between community structure and fitness. We used the mean pollen receipt and pollen tubes (mean community fitness, both z-transformed) per month as response variables. Since trait diversity and floral abundance were associated (see Results), we fitted separate models: one with trait diversity and season and the other with floral abundance and season as predictors.

Does flowering synchrony depend on the species floral abundance, trait similarity and pollination functional group?

We tested if flowering synchrony was associated with floral abundance. Moreover, we also tested if such abundance relationships and traits associated with flowering synchrony differed among the pollination functional groups: hummingbirds (n = 15 plant species), bees (n = 22), flies (n = 11) and generalists (n = 21). For this, we fitted linear mixed models using the flowering synchrony of the 63 focal species as the response variable. We calculated the synchrony considering the flowering overlap within the species of each pollination functional group. Similarly, we used only the second season of flowering phenology to avoid overrepresentation of the pairwise flowering overlap. We also recalculated the floral reflectance, size and anther height similarity considering only the species within each pollination functional group. Then, floral abundance per species (total number of flowers counted per species, log transformed), all trait similarity variables and their interaction with pollination functional group (i.e. hummingbird-, bee-, fly- or generalist-pollination) were used as fixed predictors. Since some species were assigned to both bee- and fly-pollinated, we used plant species identity as random predictor. VIF values for all fixed terms were low (all < 3), indicating no collinearity (Zuur, Ieno, & Elphick 2010).

Does species fitness increase with flowering synchrony and trait similarity?

We fitted linear models to test how flowering synchrony and trait overlap per species influence fitness. Fitness was estimated as mean relative pollen receipt and relative pollen tubes per species, since relative measurements are necessary to achieve comparable fitness values among species (Alonso et al., 2012). We relativized by dividing each sample with the maximum fitness value observed for each plant species. We did not use the z-scores since they were scaled to a mean of zero within species. This was restricted to a subset of 37 species that had more than eight sampled individuals for fitness data and all traits measured (Table S5). We fitted two linear models: one for pollen receipt and one for pollen tubes as response variables. As predictors, we used flowering synchrony and trait similarity of the species in relation to the community (Table S5). We had a single value per species and thus no random effects were needed. Flowering synchrony was calculated using the index proposed by Freitas and Bolmgren (2008), which also considers the flowering length of each species. We calculated the synchrony of the 37 species in relation to the flowering of all species (76) recorded in the study. To avoid inflating flowering synchrony, we used only the second season of flowering phenology. We took this decision based on the positive correlation of matrices of flowering overlap of both seasons (assessed by Mantel test), showing that interannual variation in flowering phenology was small ($r = 0.660$, $p = 0.001$). And because averaging across years would lead to overestimate flowering length of some species, also an important parameter of the synchrony index. We chose the second season because we sampled one month more (September) than in the first season. Trait similarity of the 37 species was calculated by generating trait distance matrices based on Euclidian distances of floral reflectance (PCA axes), size and anther height of the 63 species for which we had trait data. Then, trait similarity of each species in relation to its co-flowering community was calculated as abundance-weighted functional distinctiveness as proposed by Violle et al. (2017). High values of functional distinctiveness indicate less trait similarity of the species in relation to the co-flowering community.

All analyses were done in R environment (R Core Team, 2019) and specifically packages were used for each analyses: visual models were done in *pavo* package (Maia, Eliason, Bitton, Doucet, & Shawkey 2013), phylogenetic analyses were conducted in *phytools* package (Revell, 2012), and generalized linear models were fitted with *lme4* package (Bates, Maechler, Bolker, & Walker 2015).

Results

Trait similarity (low trait diversity) is associated with floral abundance

We found significant trait similarity (low trait diversity) in the months with high floral abundance (January/2017 to April/2017 and December/2017 to March/2018) and random trait structure in the other months (Figure 2A, Table S2-S3). Random phylogenetic structure predominated, except for November/2016 (evenness) and January/2018 (clustering, Figure 2B, Table S4). Accordingly, linear models showed that months with high floral abundance exhibited low trait diversity ($\beta = -0.014 \pm 0.001$, $R_2 = 0.44$, $F_{1,14} = 17.71$, $p = 0.001$, Figure 2C). Phylogenetic diversity ($\beta = 0.024 \pm 0.012$, $R_2 = 0.18$, $F_{1,14} = 4.27$, $p = 0.076$, Figure 2D) and season had no effect on trait diversity ($\beta = 0.594 \pm 0.324$, $R_2 = 0.03$, $F_{1,14} = 0.01$, $p = 0.964$). However, the removal of an outlier showed that phylogenetic diversity was positively related to trait diversity ($\beta = 0.042 \pm 0.091$, $R_2 = 0.49$, $F_{1,13} = 23.70$, $p < 0.001$). The relationship with floral abundance remained when using trait diversity without abundance-weight ($\beta = -0.013 \pm 0.001$, $R_2 = 0.28$, $F_{1,14} = 5.64$, $p = 0.034$). Similarly, phylogenetic diversity (without abundance-weight; $\beta = 0.003 \pm 0.022$, $R_2 = 0.08$, $F_{1,14} = 1.16$, $p = 0.301$) and season had no effect on trait diversity ($\beta = 0.212 \pm 0.550$ $R_2 = 0.04$, $F_{1,14} = 0.15$, $p = 0.705$). Phylogenetic diversity (without abundance-weight) had no effect on trait diversity after removal an outlier ($\beta = 0.005 \pm 0.022$, $R_2 = 0.06$, $F_{1,13} = 1.16$, $p = 0.809$). Floral abundance was positively correlated with richness of species flowering (Pearson' $r = 0.809$, $t = 5.33$, $p < 0.001$). Therefore, we assumed that such relationship was not driven by few dominant species.

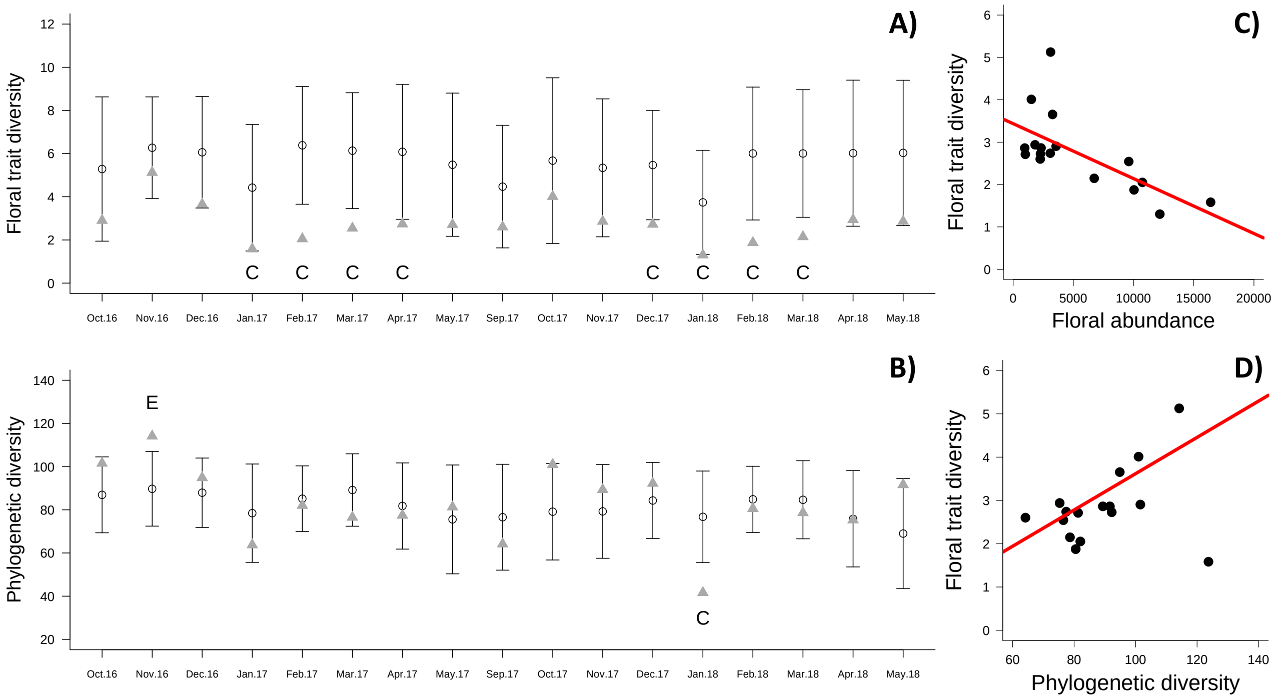

Figure 2. Assembly patterns of the co-flowering community in highland grasslands in Itatiaia National Park, Brazil. Trait and phylogenetic diversity were calculated as the Rao's Q index (Botta-Dukát, 2005) and floral abundance as the sum of flowers counted. A) Floral trait diversity per month. B) Phylogenetic diversity per month. Grey triangles represent the observed diversity value per month while white circles represent average diversity values across a null distribution (Gotelli, 2000). "C" letter indicates significant clustering and "E" letter significant evenness structure. C. Relationship of trait diversity and abundance. D. Relationship of trait diversity and phylogenetic diversity. Each black dot represents a monthly census. The red line represents the regression coefficient.

Community-level fitness increased with floral abundance and trait similarity

Months with high floral abundance ($\beta = 0.018 \pm 0.001$, $R_2 = 0.53$, $F_{1,12} = 13.65$, $p = 0.003$, Figure 3A) and with trait similarity ($\beta = -0.072 \pm 0.029$, low trait diversity, $R_2 = 0.36$, $F_{1,12} = 5.36$, $p = 0.039$, Figure 3B) had higher community-level pollen receipt. Months with high floral abundance also exhibited higher mean community-level pollen tubes ($\beta = 0.016 \pm 0.001$, $R_2 = 0.36$, $F_{1,12} = 6.39$, $p = 0.026$, Figure 3C) whereas trait diversity had no effect ($\beta = -0.049 \pm 0.035$, $R_2 = 0.14$, $F_{1,12} = 1.90$, $p = 0.194$, Figure 3D).

Flowering synchrony is dependent on floral abundance, trait similarity and pollination functional groups

Flowering synchrony depended on plant species abundance and pollination functional groups (Table 1, Figure 4). Rare species flowered more synchronically with the rest of the community than abundant ones ($\chi_2 = 5.98$, $p = 0.014$). The interaction between abundance and pollination functional group revealed that this relationship was mainly driven by bee- and fly-pollinated species ($\chi_2 = 7.57$, $p = 0.052$, Figure 4A). Species flowered synchronically if exhibiting similar floral color in relation to the community regardless of pollination functional group ($\chi_2 = 6.56$, $p < 0.001$, Figure 4B). Pollination functional group interacted with floral size similarity in determining flowering synchrony ($\chi_2 = 8.49$, $p = 0.039$, Figure 4C). In general, fly- and hummingbird-pollinated species flowered synchronously if exhibiting similar floral size in relation to the community. On the other hand, bee- and generalist-pollinated species flowered synchronously if exhibiting distinct floral size in relation to the community. Although we found an effect of anther height similarity alone ($\chi_2 = 4.17$, $p = 0.041$), pollination functional group also interacted with this variable in determining flowering synchrony ($\chi_2 = 8.31$, $p = 0.040$, Figure 4D). Bee-pollinated species flowered synchronously if exhibiting similar anther height while fly- and generalist-pollinated species if exhibiting distinct anther height in relation to the community. Results were qualitatively similar when using bee-visual models for generalist species (Table S6).

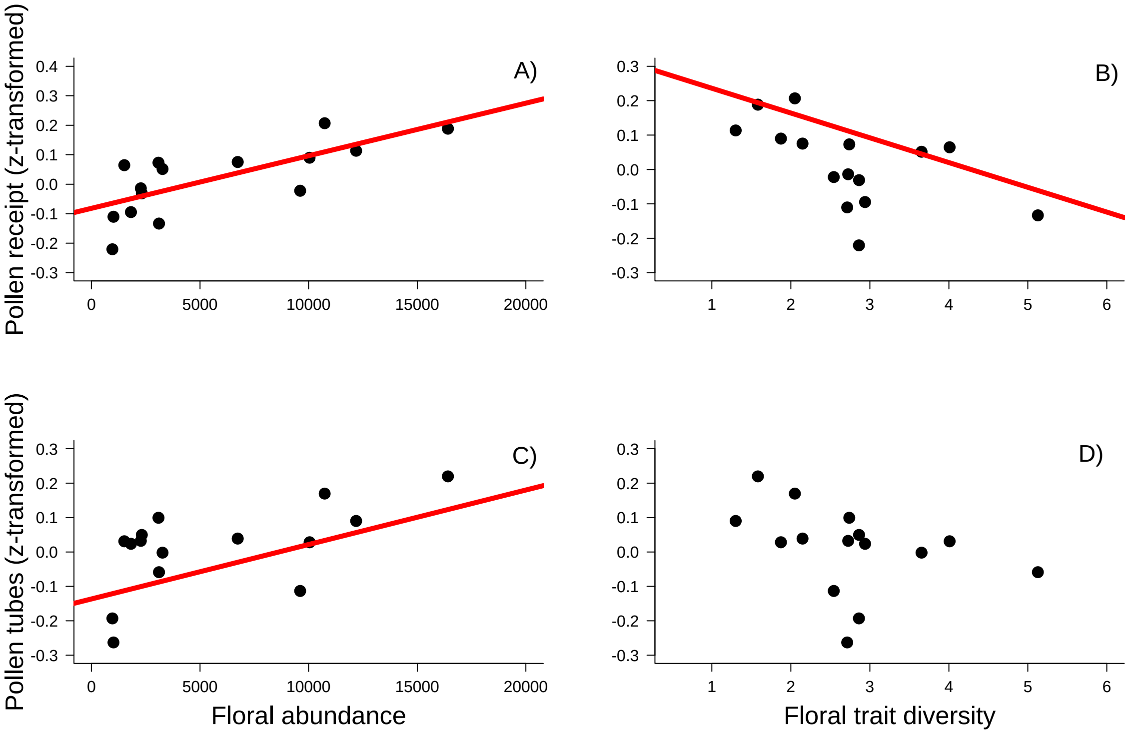

Figure 3. Relationships between mean community fitness with floral abundance and floral trait diversity of the co-flowering community in highland grasslands in Itatiaia National Park, Brazil. Community fitness was estimated as mean pollen receipt (after z-transformation), or as mean pollen tubes (after z-transformation). Floral abundance was estimated as total number of flowers counted in the monthly census and floral trait diversity was calculated as the Rao's Q index (Botta-Dukát, 2005). Black dots represent each monthly census. The red line represents the regression coefficient of the model including the predictor variable + season of census. A) Effect of flower abundance and B) Effect of floral trait diversity on mean community-level pollen receipt. C) Effect of flower abundance and D) Effect of floral trait diversity on mean community-level pollen tubes.

Table 1. Parameters of the models explaining flowering synchrony of the plant species of highland grasslands in Itatiaia National Park, Brazil. Floral abundance, floral trait similarity and pollination functional group, i.e. bee-, fly-, hummingbird- and generalist-pollination, were used as predictors. Generalist pollination represent species pollinated by three or more pollinator groups. Floral color distances were calculated with fly-vision models for the generalist species. Flowering synchrony index followed Freitas and Bolmgren, (2008) and trait similarity calculated as proposed by Violle et al. (2017). β = estimate of the effect, χ_2 = linear mixed effect value. Bold values indicate significant effects at p <0.05.

Predictor variables	**β**	**χ_2**	**p**
Pollination functional group	**-**	**22.80**	**< 0.001**
Floral abundance	**-0.04 ± 0.01**	**5.98**	**0.014**
Floral color similarity	**-0.26 ± 0.10**	**6.56**	**0.010**
Floral size similarity	0.04 ± 0.08	0.05	0.826
Anther height similarity	**-0.07 ± 0.07**	**4.17**	**0.041**
Pollination functional group * Abundance	**-0.35 ± 0.04**	**7.57**	**0.052**
Pollination functional group * Color	-	5.60	0.133
Pollination functional group * Size	**-**	**8.49**	**0.039**
Pollination functional group * Anther height	**-**	**8.31**	**0.040**

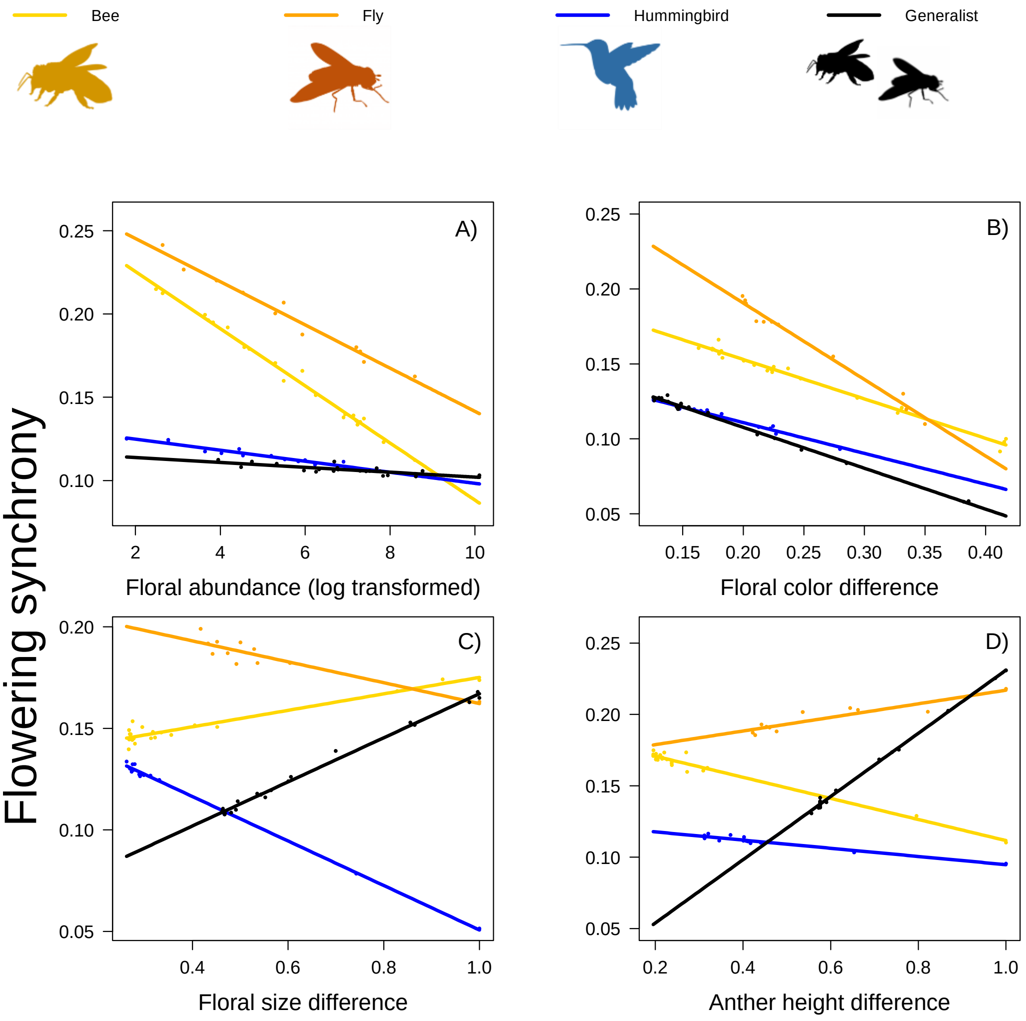

Figure 4. Relationships between flowering synchrony with species abundance, floral trait similarity and pollination functional group in highland grasslands in Itatiaia National Park, Brazil. Flowering synchrony was estimated as proposed by Freitas & Bolmgren, (2008) and trait similarity followed Violle et al., (2017). A) Effect of floral abundance (log transformed). B) Effect of floral color similarity. C) Effect of floral size similarity. D) Effect of Anther height similarity. Grey dots represent the partial residuals (after eliminating the contribution of the other predictor variables) and the colored lines are the regression coefficients. Each color represents a pollination functional group: bee-, fly-, hummingbird- and generalist-pollination. Generalist pollination represents species pollinated by three or more pollinator groups.

Species-level fitness increased with flowering synchrony and trait similarity

Species highly synchronous with the community had higher pollen receipt ($\beta = 0.612 \pm 0.232$, $R_2 = 0.21$, $F_{1,33} = 10.70$, $p = 0.003$, Figure 5A) and pollen tubes ($\beta = 0.527 \pm 0.226$, $R_2 = 0.19$, $F_{1,33} = 9.44$, $p = 0.004$, Figure 5C). Species with similar floral reflectance in relation to the community had higher pollen receipt ($\beta = -1.315 \pm 0.519$, $R_2 = 0.24$, $F_{1,33} = 4.33$, $p = 0.045$, Figure 5B) and pollen tubes ($\beta = -1.301 \pm 0.510$, $R_2 = 0.24$, $F_{1,33} = 4.74$, $p = 0.037$, Figure 5D). Relative fitness was not explained by floral size (pollen receipt: $\beta = 0.001 \pm 0.008$, $R_2 = 0.08$, $F_{1,33} = 1.49$, $p = 0.231$; pollen tubes: $\beta = -0.002 \pm 0.007$, $R_2 = 0.09$, $F_{1,33} = 1.82$, $p = 0.187$) nor anther size similarity (pollen receipt: $\beta = -0.053 \pm 0.090$, $R_2 = 0.08$, $F_{1,33} = 0.34$, $p = 0.561$; pollen tubes: $\beta = -0.016 \pm 0.089$, $R_2 = 0.08$, $F_{1,33} = 0.03$, $p = 0.861$).

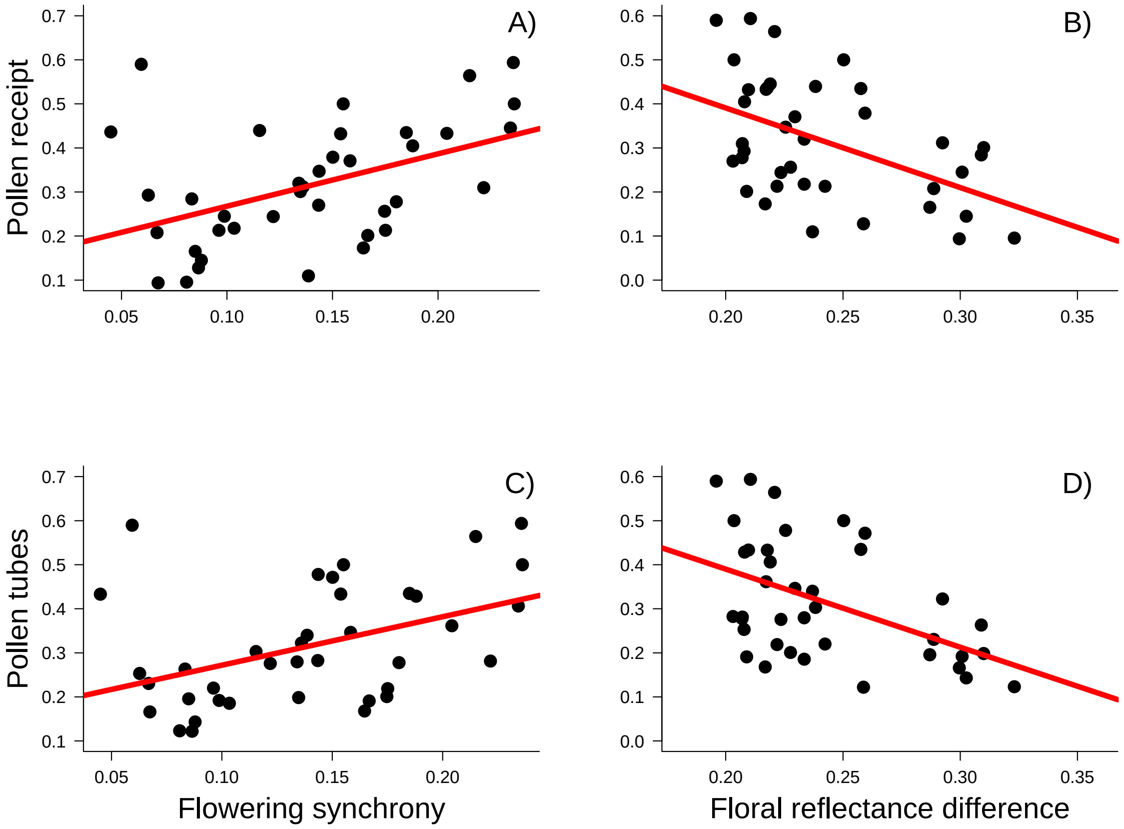

Figure 5. Relationships between species fitness with flowering synchrony and floral trait similarity in highland grasslands in Itatiaia National Park, Brazil. To achieve comparable values among species, fitness measurements were relativized by dividing the corresponding fitness value with the maximum observed within each species. Flowering synchrony index followed Freitas and Bolmgren, (2008) and trait similarity calculated as proposed Violle et al., (2017). Black dots represent each plant species and the red line is the regression coefficient. A) Effect of flowering synchrony and B) Effect of floral reflectance similarity on pollen receipt. C) Effect of flowering synchrony and D) Effect of floral reflectance similarity on pollen tubes.

Discussion

Trait similarity (low trait diversity) is associated with floral abundance

We found significant low trait diversity (trait clustering) in floral abundant months. Moreover, floral trait diversity linearly decreased with floral abundance in the community, which indicates an overall pattern of increasing floral similarity between co-flowering species. These patterns are consistent with facilitation. The linear models also revealed high trait diversity in months of low floral abundance. However, we found no significant trait structure in months of low floral abundance, indicating a minor role of competition in the community assembly. Phylogenetic diversity was predominantly random and the removal of an outlier showed a positive relationship with trait diversity, making convergent evolution unlikely. The relationship between phylogenetic and trait diversity vanished when using phylogenetic diversity without abundance-weight, indicating that some abundant plant lineages generated this association. Overdominance of few plant species leading to functionally homogenous communities was also unlikely since the relationship remained when using functional diversity indexes with no abundance weight and plant species richness increased with floral abundance. Finally, we argue that such trait clustering was not generated due to a dominant pollinator group selecting or sorting similar plant species (biotic filtering), since this community is marked by high levels of generalization, from the perspective of both plants and pollinators (Freitas & Sazima, 2006; Danieli-Silva et al., 2012). Therefore, we suggest pollinator facilitation as the major process driving the co-flowering of similar species assuming that increased trait similarity leads to high pollinator sharing (Junker, Blüthgen, & Keller 2015).

Community-level fitness increased with floral abundance and trait similarity

Community-level fitness was higher in floral abundant periods with functional similarity, as predicted when pollination facilitation is the major process (Wolowski et al., 2017). The studied community is a grassland surrounded by tropical forests and periods of high floral abundance probably attracted more pollinators from adjacent areas and maintained them in the area. Such increase on fitness was widespread in the community probably due to the benefits of sharing generalist pollinators. Pollination networks of other generalized tropical communities showed less interaction partitioning (and thus, high pollinator sharing) when floral abundance is high (Souza et al., 2018). Our results also suggest a similar mechanism in the studied community. Furthermore, it was shown that high floral trait diversity in the community decreases pollinator visitation frequency to focal plant species (Fornoff et al., 2017; Albor et al., 2019), probably due to stronger searching costs for generalist pollinators in a functionally diverse array (Cakmak et al., 2009). Facilitation between similar flowers in the studied community may operate similarly, with effects also on pollen receipt.

The effect of trait similarity disappeared when evaluating pollen tubes at the community-level. Accordingly, the prevalence of facilitation in the community diminished when evaluating pollen tubes (Bergamo et al., 2019). Benefits of pollinator sharing may be offset by costs due to heterospecific pollen transfer (Thomson, Fung, & Ogilvie 2019). This may also indicate a "diffuse facilitation" scenario (*sensu* Feldman et al., 2004), on which some species strongly benefit from shared attraction while others have weak benefits or remain neutral. Other possibility is that that density-dependent processes are more important than trait-mediated ones in the studied community. In fact, intraspecific negative density-dependence in pollination coupled with low occurrence of density-dependent interspecific competition was shown to stabilize plant coexistence in the studied community (Bergamo et al., 2019). Instead of the community-level patterns used here, trait-mediated processes may be observed only between plant species pairs (Lanuza, Bartomeus, & Godoy 2018). Future studies that assess pairwise fitness differences would be important to determine the relative importance of density-dependence vs. trait-mediated processes mediating plant coexistence.

Flowering synchrony is dependent on floral abundance, trait similarity and pollination functional groups

We found that bee- and fly-pollinated rare species flowered more synchronically with the community than abundant ones. Rare species are often in disadvantage due to pollinator-mediated Allee effects (Evans, Cavers, Ennos, Vanbergen, & Heard 2017). Our abundance measurements may also reflect species-level differences on floral display, with the rare species also bearing few flowers per plant. Species with small displays also suffer disadvantages due to pollinator-mediated Allee effects (Ghazoul, 2005). In high-altitude grasslands, Allee effects were shown to be weak (Bergamo et al., 2019) and our findings indicate that these rare and/or small display plants may not experience such effects due to the benefits of co-flowering. However, the effect of abundance was weak for hummingbird- and generalist-species. This may be related to hummingbird preferences for patches possessing a high number of flowers (Justino, Maruyama, & Oliveira 2012), and thus, rare and small display species would benefit less from co-flowering. On the other hand, generalist species shares a distinct fraction of their pollinators making benefits of co-flowering largely diffuse and not dependent on species abundance. Overall, we showed that relationships between co-flowering and species floral abundance differ for each pollination functional group within the community.

Species with similar floral color were the most synchronous with the community regardless of their pollination functional group. This general effect of color similarity agrees with the community-level patterns of trait similarity and fitness. As expected, the relation between co-flowering and the other floral traits differed among pollination functional groups. Floral color similarity had a clear relationship with synchrony for fly-pollinated species. This concurs with other fly-pollinated communities also marked by high color similarity (Bergamo, Telles, Arnold, & Brito 2018; Ishii, Kubota, Tsujimoto, & Kudo 2019). Moreover, the most synchronous fly-pollinated species had distinct anther height, which may reduce reproductive interference when sharing pollinators (Stewart & Dudash, 2017; Bergamo et al., 2018a, Fantinato et al., 2018). For hummingbird-pollinated plants, the most synchronous species had similar floral color and size. These patterns indicate facilitative interactions mediated by trait similarity, in contrast with the weak relationship with abundance for this pollination functional group. These mixed patterns suggest a balance between competition and facilitation, which concur with other assembly studies on hummingbird-pollinated plants from the Atlantic forest (Wolowski et al., 2017; Bergamo et al., 2018a).

The importance of high color similarity for bee-pollinated species found here was also reported as a mechanism of facilitation in other bee-pollinated plant communities (de Jager et al., 2011). Moreover, we found the counter-intuitive pattern of synchrony associated with anther height similarity for this pollination functional group. This could reflect the restricted safe sites to deposit pollen on the bee’s body without the risk of grooming (Koch, Lunau, & Wester 2017). Interestingly, bee-pollinated species flowered more synchronically when distinct in floral size. This could be associated to divergent use of pollinators (and thus, competition). However, the prevalence of facilitation among bee-pollinated species (Bergamo et al., 2019) suggests that such pattern may be driven by the frequently unnoticed mechanism of facilitation through floral diversity (Ghazoul, 2006). In this context, plant species with distinct traits attract and maintain a diverse pollinator community (in this case, a diverse bee community), which ultimately leads to benefits through pollinator sharing (Ghazoul, 2006). Such a scenario is even more likely between generalist species, on which flowering synchrony was associated with distinct floral size and anther height. For generalist species, the benefits of attracting a diverse pollinator community is more likely since all of them could share most pollinators.

Species-level fitness increased with flowering synchrony and trait similarity

Synchronous species and with similar colors in relation to the community were the most fit, consistent with facilitation (Wolowksi et al., 2017). This suggests that joint attraction may favor high flowering synchrony and color similarity (Geber & Moeller, 2006; Eisen & Geber, 2018), leading to the observed high floral abundance coupled with low trait diversity at the community-level. In this community, flowers are exposed to a bright environment since it is an open habitat and vegetation is composed mainly by shrubs and herbs. It is known that bright habitats enhance color conspicuousness (Endler, 1993), which may increase the importance of floral color as a reliable signal for pollinators in this community. Interestingly, floral color similarity explained relative fitness per species for both pollen receipt and pollen tubes, although trait diversity was not related to the latter at the community-level. This discordance indicates that even if several species benefit by being similar, these trait-mediated relationships should be context-dependent with some neutral and competitive outcomes also operating at the community-level (Tur et al., 2016). Floral size did not have an effect, which may indicate that this trait is more related to specific pollinator groups, as found for some pollination functional groups in the flowering synchrony analysis. Nevertheless, our findings support a structuring role of pollinator-mediated interactions via both flowering phenology and floral traits.

Conclusion

We found flowering and floral trait patterns consistent with pollination facilitation. Since previous assessments in the studied community showed that facilitation prevailed over competition for pollination, we could empirically demonstrate some of the community assembly theoretical predictions. Moreover, the link between patterns and process is likely due to reproductive advantages of species highly synchronous and similar in floral color in relation to the community, especially for rare plant species. Nevertheless, distinct patterns emerged depending on the floral trait and pollination functional group being analyzed. Although most plant species benefited from facilitation, some presented functional patterns consistent with competition. Therefore, distinct pollinator-mediated processes may act on the same community. We stimulate measurements of fitness at the community-level to help disentangling by which mechanisms pollinator-mediated assemblage processes generated the observed flowering and trait patterns.

Acknowledgments

We thank the Instituto Chico Mendes de Biodiversidade and the Itatiaia National Park for research permit in protected areas (ICMBio/SISBIO nº 58349-1). P. Medina and V. Zambon for help in the field. N. Escobar, P. Medina and R. Romanini for help with pollen counting. J. Amaral, L. Bacci, J. A. M. Carmo, J. Dutilh, M. Egea, C. D. Inácio, L. Meireles, A. Scatigna, J. Semir and G. Shimizu for plant identification. Two anonymous reviewers and the associate editor made criticism that greatly improved earlier versions of the manuscript. This work was supported by FAPESP (grants 2016/06434-0 and 2018/02996-0 to P.J.B.), CNPq (grants 436335/2018-2 to M.W. and 302781/2016-1 to M.S.) and CAPES (Ph.D. scholarship to N.S.S., Finance Code 001).

Data Availability Statement: Datasets supporting the analyses are available at FigShare: doi.org/10.6084/m9.figshare.11371089.v1

References

Aizen, M. A., & Rovere, A. E. (2010). Reproductive interactions mediated by flowering overlap in a temperate hummingbird-plant assemblage. Oikos, 119, 696-706.

Albor, C., García-Franco, J. G., Parra-Tabla, V., Díaz-Castelazo, & Arceo-Gómez, G. (2019). Taxonomic and functional diversity of the co-flowering community differentially affect *Cakile edentula* pollination at different spatial scales. Journal of Ecology, doi:10.1111/1365-2745.13183.

Alonso, C., Herrera, C. M., & Ashman, T-L. (2012). A piece of the puzzle: a method for comparing pollination quality and quantity across multiple species and reproductive events. New Phytologist, 193, 532-542.

Arceo-Gómez, G., Kaczorowski, R. L., & Ashman, T-L. (2018). A network approach to understanding patterns of coflowering in diverse communities. International Journal of Plant Sciences, 179, 569-582.

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. Journal of Statistical Software, 67, 1–48.

Bergamo, P. J., Wolowski, M., Maruyama, P. K., Vizentin-Bugoni, J., Carvalheiro, L., & Sazima, M. (2017). The potential indirect effects among plants via shared hummingbird pollinators are structured by phenotypic similarity. Ecology, 98, 1849-1858.

Bergamo, P. J., Wolowski, M., Maruyama, P. K., Vizentin-Bugoni, J., & Sazima, M. (2018)a. Trait patterns across space and time suggest an interplay of facilitation and competition acting on Neotropical hummingbird-pollinated plant communities. Oikos, 127, 1690-1700.

Bergamo, P. J., Telles, F. J., Arnold, S. E. J., & Brito, V. L. G. (2018)b. Flower colour within communities shifts from overdispersed to clustered along an alpine altitudinal gradient. Oecologia, 188, 223-235.

Bergamo, P. J., Streher, N. S., Wolowski, M., Traveset, A., & Sazima, M. (2019). Pollination outcomes reveal negative frequency-dependence coupled with interspecific facilitation among plants. *Ecology Letters,* 2nd round of review.

Botta-Dukát, Z. (2005). Rao’s quadratic entropy as a measure of functional diversity based on multiple traits. Journal of Vegetation Science, 16, 533-540.

Botta-Dukát, Z., & Czúcz, B. (2016). Testing the ability of functional diversity indices to detect trait convergence and divergence using individual-based simulation. 7, 114-126.

Briscoe Runquist, R., Grossenbacher, D., Porter, S., Kay, K., & Smith, J. (2016). Pollinator-mediated assemblage processes in California wildflowers. Journal of Evolutionary Biology, 29, 1045-1058.

Brito, V. L. G., Maia, F. R., Silveira, F. A. O., Fracasso, C. M., Lemos-Filho, J. P., Fernandes, G. W., ..., Staggemeier, V. G. (2017). Reproductive phenology of Melastomataceae species with contrasting reproductive systems: contemporary and historical drivers. Plant Biology, 19, 806-817.

Cakmak, I., Sanderson, C., Blocker, T. D., Pham, L. L., Checotah, S., Norman, A. A., …, & Wells, H. (2009). Different solutions by bees to a foraging problem. Animal Behaviour, 77, 1273-1280.

Carvalheiro, L. G., Biesmeijer, J. C., Benadi, G., Fründ, J., Stang, M., Batomeus, I., …, & Kunin, W. E. (2014). The potential for indirect effects between co-flowering plants via shared pollinators depends on resource abundance, accessibility and relatedness. Ecology Letters, 17, 1389-1399.

Chittka, L. (1992). The colour hexagon: a chromaticity diagram based on photoreceptor excitations as a generalized representation of colour opponency. Journal of Comparative Physiology A, 170, 533-543.

Danieli-Silva, A., Souza, J.M.T., Donatti, A.J., Campos, R.P., Vicente-Silva, J., Freitas, L., & Varassin, I. G. (2012). Do pollination syndromes cause modularity and predict interactions in a pollination network in tropical high-altitude grasslands? Oikos, 121, 35-43.

de Jager, M. L., Dreyer, L. L., & Ellis, A. G. (2011). Do pollinators influence the assembly of flower colours within plant communities? Oecologia, 166, 543-553.

Eisen, K. E., & Geber, M. A. (2018). Ecological sorting and character displacement contribute to the structure of communities of *Clarkia* species. Journal of Evolutionary Biology, 31, 1440-1458.

Endler, J. A. (1993). The color of light in forests and its implications. Ecological Monographs, 63, 1-27.

Evans, T. M., Cavers, S., Ennos, R., Vanbergen, A. J., & Heard, M. S. (2017). Florally rich habitats reduce insect pollination and the reproductive success of isolated plants. Ecology and Evolution, 7, 6507-6518.

Fantinato, E., Del Vecchio, S., Giovanetti, M., Acosta, A. T. R., & Buffa, G. (2018). New insights into plants co-existence in species-rich communities: The pollination interaction perspective. Journal of Vegetation Science, 29, 6-14.

Feinsinger, P. (1987). Effects of plant species on each other’s pollination: Is community structure influenced? Trends in Ecology and Evolution, 2, 123-126.

Feinsinger, P., Spears, E. E., & Poole, R. W. (1981). A simple measure of niche breadth. Ecology, 62, 27-32.

Feldman, T. S., Morris, W. F., & Wilson, W. G. (2004). When can two plant species facilitate each other's pollination? Oikos, 105, 197-207.

Filella, I., Primante, C., Llusià, J., Martín González, A. N., Seco, R., Farré-Armengol, G., ..., Peñuelas, J. (2013). Floral advertisement scent in a changing plant-pollinators market. Scientific Reports, 3, 3434.

Fornoff, F., Klein, A-M., Hartig, F., Benadi, G., Venjakob, C., Martin Schaefer, H., & Ebeling, A. (2017). Functional flower traits and their diversity drive pollinator visitation. Oikos, 126, 1020-1030.

Freitas, L., & Sazima, M. (2006). Pollination biology in a tropical high-altitude grassland in Brazil: interactions at the community-level. Annals of the Missouri Botanical Garden, 93, 465-516.

Freitas, L., & Bolmgren, K. (2008). Synchrony is more than overlap: measuring phenological synchronization considering time length and intensity. Brazilian Journal of Botany, 31, 721-724.

Geber, M. A., & Moeller, D. A. (2006). Pollinator responses to plant communities and implications for reproductive character evolution. In L. D. Harder & S. C. H. Barrett (Eds.), *Ecology and evolution of flowers* (pp. 102-119), Oxford, UK: Oxford University Press.

Ghazoul, J. (2005). Pollen and seed dispersal among dispersed plants. Biological Reviews, 80, 413-443.

Ghazoul, J. (2006). Floral diversity and the facilitation of pollination. Journal of Ecology, 94, 295-304.

Gotelli, NJ. (2000). Null model analysis of species co-occurrence patterns. Ecology, 81, 2606-2621.

Gumbert, A., Kunze, J., & Chittka, L. (1999). Floral colour diversity in plant communities, bee colour space and a null model. Proceedings of the Royal Society B: Biological Sciences, 266, 1711-1716.

Herrera, G., Zagal, J. C., Diaz, M., Fernández, M. J., Vielma, A., Cure, M., ..., Palacios, A. G. (2008). Spectral sensitivities of photoreceptors and their role in colour discrimination in the green-backed firecrown hummingbird (*Sephanoides sephaniodes*). Journal of Comparative Physiology A, 194, 785-794.

Ishii, H. S., Kubota, M. X., Tsujimoto, S. G., & Kudo, G. (2019). Association between community assemblage of flower colours and pollinator fauna: a comparison between Japanese and New Zealand alpine plant communities. Annals of Botany, 123, 533-541.

Junker, R. R., Blüthgen, N., & Keller, A. (2015). Functional and phylogenetic diversity of plant communities differently affect the structure of flower-visitor interactions and reveal convergences in floral traits. Evolutionary Ecology, 29, 437-450.

Justino, D. G., Maruyama, P. K., & Oliveira, P. E. (2012). Floral resource availability and hummingbird territorial behavior on a Neotropical savanna shrub. Journal of Ornithology, 153, 189-197.

Kantsa, A., Raguso, R. A., Dyer, A. G., Olesen, J. M., Tscheulin, T., & Petanidou, T. (2018). Disentangling the role of floral sensory stimuli in pollination networks. Nature Communications, 9, 1041.

Kemp, J. E., Bergh, N. G., Soares, M., & Ellis, A. G. (2019). Dominant pollinators drive non-random community assembly and shared flower colour patterns in daisy communities. Annals of Botany, 123, 277-288.

Koch, L., Lunau, K., & Wester, P. (2017). To be on the safe site – Ungroomed spots on the bee's body and their importance for pollination. PLoS One, 12, e0182522.

Lanuza, J. B., Bartomeus, I., & Godoy, O. (2018). Opposing effects of floral visitors and soil conditions on the determinants of competitive outcomes maintain species diversity in heterogenous landscapes. Ecology Letters, 21, 865-874.

Lázaro, A., Jakobsson, A., & Totland, Ø. (2013). How do pollinator visitation rate and seed set relate to species' floral traits and community context? Oecologia, 173, 881-893.

Lunau, K. (2014). Visual ecology of flies with particular reference to colour vision and colour preferences. Journal of Comparative Physiology A, 200, 497-512.

Maia, R., Eliason, C. M., Bitton, P-P., Doucet, S. M., Shawkey, M. D. (2013). Pavo: an R package for the analysis, visualization and organization of spectral data. Methods in Ecology and Evolution, 4, 906-913.

Makino, T. T., & Yokoyama, J. (2015). Nonrandom composition of flower colors in a plant community: mutually different co-flowering natives and disturbance by aliens. PLoS One, doi:10.1371/journal.pone.0143443.

Menzel, R., Ventura, D. F., Werner, A., Joaquim, L. C. M., & Backhaus, W. (1989). Spectral sensitivity of single photoreceptors and color vision in the stingless bee, *Melipona quadrifasciata*. Journal of Comparative Physiology A, 166, 151-164.

Moeller, D. A. (2004). Facilitative interactions among plants via shared pollinators. Ecology, 85, 3289-3301.

Muchhala, N., Johnsen, S., & Smith, S. D. (2014). Competition for hummingbird pollination shapes flower color variation among in Andean Solanaceae. Evolution, 68, 2275-2286.

Pellissier, L., Alvarez, N., & Guisan, A. (2012). Pollinators as drivers of plant distribution and assemblage into communities. In S. Patiny (Ed.), *Evolution of plant-pollinator relationships* (pp. 392-413). Cambridge, UK: Cambridge University Press.

Qian, H., & Jin, Y. (2016). An updated megaphylogeny of plants, a tool for generating plant phylogenies and an analysis of phylogenetic community structure. Journal of Plant Ecology, 9, 233-239.

R Development Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Revell, L. J. (2014). phytools: an R package for phylogenetic comparative biology (and other things). Methods in Ecology and Evolution, 3, 217-223.

Ribeiro, K. T., Medina, B. M. O., & Scarano, F. R. (2007). Species composition and biogeographic relations of the rock outcrop flora on the high plateau of Itatiaia, SE-Brazil. Brazilian Journal of Botany, 30, 623-639.

Sargent, R. D., & Ackerly, D. D. (2008). Plant-pollinator interactions and the assembly of plant communities. Trends in Ecology and Evolution, 23, 123-130.

Sargent, R. D., Kembel, S. W., Emery, N. C., Forrestel, E. J., & Ackerly, D. D. (2011). Effect of local community phylogenetic structure on pollen limitation in an obligately insect-pollinated plant. American Journal of Botany, 98, 283-289.

Souza, C. S., Maruyama, P. K., Aoki, C., Sigrist, M. R., Raizer, J., Gross, C. L., & Araujo, A. C. (2018). Temporal variation in plant-pollinator networks from season tropical environments: higher specialization when resources are scarce. Journal of Ecology, 106, 2409-2420.

Staggemeier, V. G., Diniz-Filho, J. A. F., & Morellato, L. P. C. (2010). The shared influence of phylogeny and ecology on the reproductive patterns of Myrtae (Myrtaceae). Journal of Ecology, 98, 1409-1421.

Stewart, A. B., & Dudash, M. R. (2017). Field evidence of strong differential pollen placement by Old World bat-pollinated plants. Annals of Botany, 119, 73-79.

Thomson, J. D., Fung, H. F., & Ogilvie, J. E. (2019). Effects of spatial patterning of co-flowering plant species on pollination quantity and purity. Annals of Botany, 123, 303-310.

Troje, N. (1993). Spectral categories in the learning behavior of blowflies. Zeitschrift für Naturforschung C, 48, 96-104.

Tur, C., Sáez, A., Traveset, A., & Aizen, M. A. (2016). Evaluating the effects of pollinator-mediated interactions using pollen transfer networks: evidence of widespread facilitation in south Andean plant communities. Ecology Letters, 19, 576-586.

Violle, C., Thuiller, W., Mouquet, N., Munoz, F., Kraft, N. J. B., Cadotte, M. W., …, & Mouillot, D. (2017). Functional rarity: the ecology of outliers. Trends in Ecology & Evolution, 32, 356-367.

Vorobyev, M., Osorio, D., Bennett, T. D., Marshall, N. J., & Cuthill, I. C. (1998). Tetrachromacy, oil droplets and bird plumage colours. Journal of Comparative Physiology A, 183, 621-633.

Waser, N. M. (1978). Competition for hummingbird pollination and sequential flowering in two Colorado wildflowers. Ecology, 59, 934-944.

Wolowski, M., Carvalheiro, L., & Freitas, L. (2017). Influence of hummingbird-plant interactions on the structuring of plant and hummingbird communities. Journal of Ecology, 105, 332-344.

Zanne, A. E., Tank, D. C., Cornwell, W. K., Eastman, J. M., Smith, S. A., FritzJohn, R. G., …, Beaulieu, J. M. (2014). Three keys to the radiation of angiosperms into freezing environments. Nature, 506, 89-92.

Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution, 1, 3-14.

Supplementary material

Appendix S1 – Colour analyses

We plotted the correspondent spectral wavelengths (from 300 to 700nm) against the PCA axes (Figure S1). This showed that PC1 corresponds to UV waveband, PC2 to blue waveband, PC3 to green waveband and PC4 to red waveband. Moreover, we calculated two distance matrices: one based on the original reflectance data per species and a second based on the PCA axes per species. A Mantel test revealed a high correlation between these two matrices ($r = 0.80$, $p = 0.001$), and thus, we are confident that our PCA approach had captured the reflectance properties of each plant species.

Appendix S2 – Phylogenetic signal

To assess the phylogenetic signal in flowering phenology, we calculated the phylogenetic signal as the correlation between flowering overlap and phylogenetic distance matrices using a Mantel test (Staggemeier, Diniz-Filho, & Morellato, 2010). We used the same approach for floral reflectance, using the Euclidian distance matrix based on the three PCA axes for the whole community and based on the visual models within each pollinator group (see below). For morphological traits (floral size and anther height) and floral abundance, we used the *K* statistic (Blomberg, Garland, & Ives 2003). It assesses the correlation between trait variation and phylogenetic relationships. When $K > 1$, closely related species are more similar than expected by Brownian motion, while $K < 1$ indicates that closely related species are less similar than expected. The significance of *K* values was assessed by comparing the observed value for each trait with a null distribution generated by 10,000 random trees. Random trees were obtained by reshuffling species into null phylogenies. We tested if the observed *K* differs from 0, indicating some degree of phylogenetic signal.

Flowering phenology, floral traits and floral abundance were all poorly correlated with phylogenetic relatedness in the studied community. Flowering phenology distances were not correlated with phylogenetic distances (Mantel's r = -0.04, p = 0.735). Similarly, floral reflectance distances were not correlated with phylogenetic distances (Mantel's r = 0.02, p =0.2733. Within the pollination functional groups, floral color distances as perceived by bees (r = -0.11, p = 0.886), flies (r = 0.05, p = 0.284) or hummingbirds (r = 0.15, p = 0.139) were not correlated with phylogenetic distances. We found a low phylogenetic signal for the generalist species when evaluating colors in the bee-vision (r = 0.37, p = 0.010) and fly-vision (r = 0.37, p = 0.009). *K* values for all floral traits and floral abundance were low and not different from 0, indicating no phylogenetic signal (Floral size: *K* = 0.48, p = 0.058; Anther height: *K* = 0.48, p = 0.065; Abundance: *K* = 0.22, p = 0.768). Thus, we assumed that all models were robust to constraints of shared evolutionary history.

Table S1. Plant species included in this study from highland grassland in Itatiaia National Park, Brazil. Pollination system indicates the pollinator groups visiting the flowers. The category “Generalist” represents species visited by three or more groups (generally visited by bees, flies, wasps, beetles and ants). Abundance refers to total floral abundance of the species. Morphological traits are expressed on mean ± standard deviation. For hummingbird-pollinated plants, corolla effective length was used as floral size (Wolf, Stiles, & Hainsworth 1976). Sampling effort expressed as number of flowers on which all floral traits were collected (floral colour, floral size and anther height). Abbreviations are given for the 63 species from which we collected floral traits and were included in the phylogenetic tree built for this study (Figure S1).

Family/Species (Abbreviation)	**Pollination system**	**Floral abundance (n)**	**Floral size (mm)**	**Anther height (mm)**	**Sample size (n)**
Alstroemeriaceae					
Alstroemeria foliosa Mart. Ex Schult. & Schult.f. (Alfo)	Bees and hummingbirds	38	27.55 ± 5.80	32.61± 3.09	9
Alstroemeria isabelleana Herb. (Alis)	Hummingbirds	85	24.18 ± 3.53	29.07 ± 2.62	8
Amaryllidaceae					
Hippeastrum morelianum Lem. (Himo)	Hummingbirds	16	64.90 ± 7.23	80.31 ± 6.86	8
Apiaceae					
Eryngium glaziovianum Urb. (Ergl)	Generalist	206	15.98 ± 1.70	0	8
Eryngium paniculatum Cav. & Dombey ex F.Delaroche (Erpa)	Generalist	801	11.90 ± 0.49	0	8
Apocynaceae					
Oxypetalum glaziovii (E.Fourn.) Fontella & Marquete (Oxgl)	Generalist	39	4.88 ± 0.82	2.98 ± 0.12	4
Asteraceae					
Achyrocline satureioides (Lam.) DC. (Acsa)	Generalist	2170	4.16 ± 0.15	0	5
Baccharis altimontana G. Heiden et al. (Baal)	Generalist	520	4.48 ± 0.08	0	6
Baccharis itatiaiae Wawra (Bait)	Generalist	2523	3.31 ± 0.77	0	11
Baccharis platypoda DC. (Bapl)	Generalist	2358	2.81 ± 0.91	0	6
Baccharis megapotamica Spreng. (Bame)	Generalist	24428	3.52 ± 0.54	0	10
Baccharis sp. (Basp)	Generalist	876	3.42 ± 0.44	0	10
Chionolaena capitata (Baker) Freire	-	-	-	-	-

Chionolaena isabellae Baker	-	-	-	-	-
Leptostelma maximum D.Don (Lema)	Generalist	89	16.01 ± 1.32	0	8
Grazielia intermedia (DC.) R.M.King H H.Rob. (Grin)	Generalist	6401	2.77 ± 0.66	0	7
Grazielia serrata (Spreng.) Rm.King & H.Rob.	-	-	-	-	-
Symphyopappus compressus (Gardner) B.L.Rob.	-	-	-	-	-
Mikania parodii Cabrera	-	-	-	-	-
Stevia cf. *decussata* Baker (Stde)	Generalist	5469	4.97 ± 1.03	5.59 ± 0.69	10
Senecio oleosus Vell. (Seol)	Generalist	391	15.80 ± 4.00	3.92 ± 2.58	10
Senecio nemoralis Dusén (Sene)	Generalist	1841	8.79 ± 1.59	5.48 ± 0.82	10
Hypochaeris radicata L. (Hyra)	Generalist	52	14.36 ± 5.71	5.23 ± 1.29	8
Verbesina glabrata Hook. & Arn. (Vegl)	Generalist	559	14.48 ± 0.25	3.90 ± 0.20	9
Mikania cf. *cordifolia* (L.f) Willd.	-	-	-	-	-
Trixis glaziovii Baker	-	-	-	-	-
Berberidaceae					
Berberis glazioviana Brade (Begl)	Bees	2557	7.49 ± 1.07	4.31 ± 0.43	10
Bromeliaceae					
Fernseea itatiaiae (Wawra) Baker (Feit)	Hummingbirds	249	9.43 ± 0.59	8.62 ± 0.84	12
Vriesea itatiaiae Wawra	Hummingbirds	-	-	-	-
Campanulaceae					
Lobelia camporum Pohl (Loca)	Bees[1]	46	7.34 ± 1.22	7.78 ± 0.90	8

Siphocampylus westinianus (Thunb.) Pohl (Siwe)	Hummingbirds	93	39.00 ± 3.98	54.51 ± 4.65	10
Caprifoliaceae					
Valeriana glaziovii Taub.	Flies	-	-	-	-
Caryophyllaceae					
Cerastium dicrotrichum Fenzl ex. Rohrb. (Cedi)	Flies	50	6.03 ± 0.11	4.11 ± 0.33	10
Eriocaulaceae					
Paepalanthus itatiaiensis Ruhland (Pait)	Generalist	789	4.93 ± 0.59	0	16
Ericaceae					
Agarista hispidula (DC.) Hook. Ex Nied. (Aghi)	Hummingbirds	56	9.92 ± 1.10	7.23 ± 0.51	12
Agarista oleifolia (Cham.) G.Don	-	-	-	-	-
Gaultheria eriophylla (Pers.) Sleumer ex Burtt (Gaer)	Hummingbirds	181	6.35 ± 0.42	4.85 ± 0.45	7
Gaultheria serrata (Vell.) Sleumer ex Kin.-Gouv. (Gase)	Hummingbirds	1584	8.28 ± 0.55	5.73 ± 0.44	19
Gaylussacia amoena Cham. (Gaam)	Bees	1398	9.22 ± 0.86	5.32 ± 0.91	20
Gaylussacia fasciculata Gardner (Gafa)	-	45	5.99 ± 1.03	3.22 ± 0.45	5
Escalloniaceae					
Escallonia laevis (Vell.) Sleumer (Esla)	Bees	96	14.21 ± 3.94	9.84 ± 1.30	7
Escallonia bifida Link & Otto (Esbi)	Generalist	861	5.79 ± 2.74	6.29 ± 0.84	9

Euphorbiaceae					
Croton dichrous Müll.Arg. (Crdi)	Generalist	2823	4.23 ± 1.52	3.38 ± 0.53	10
Fabaceae					
Lupinus gibertianus C.P.Sm. (Lugi)	Bees	1037	13.54 ± 1.29	12.79 ± 0.82	8
Mimosa itatiaiensis Dusén (Miit)	Bees and flies	1344	13.22 ± 1.40	13.22 ± 1.40	9
Mimosa monticola Dusén (Mimo)	Bees and flies	377	14.25 ± 1.39	14.25 ± 1.39	9
Geraniceae					
Geranium brasiliense Progel (Gebr)	Flies	93	8.58 ± 0.82	4.25 ± 0.81	10
Griseliniaceae					
Griselinia ruscifolia (Clos) Taub.	-	-	-	-	-
Iridaceae					
Alophia sellowiana Klatt (Alse)	Bees	41	33.52 ± 3.91	11.32 ± 0.84	10
Sisyrinchium wettsteinii Hand.-Mazz. (Siwt)	Bees and flies	199	13.91 ± 1.18	5.59 ± 0.91	15
Sisyrinchium glaziovii Baker (Sigl)	Bees and flies	243	10.01 ± 0.68	3.09 ± 0.30	12
Lamiaceae					
Cunila galioides Benth. (Cuga)	Bees and flies	5335	6.81 ± 1.14	7.48 ± 1.28	10
Hesperozygis myrtoides (A.St.-Hil. Ex Benth.) Epling (Hemy)	Bees and flies	1472	19.66 ± 1.34	16.13 ± 1.70	10
Lepechinia speciosa (A.St.-Hil. Ex Benth.) Epling (Lesp)	Hummingbirds	402	29.37 ± 1.91	27.03 ± 1.63	10
Salvia itatiaensis Dusén (Sait)	Bees and hummingbirds	995	14.51 ± 0.42	12.80 ± 0.61	7
Lentibulariaceae					
Utricularia reniformis A.St.-Hil. (Utre)	Bees[2]	12	17.82 ± 4.54	6.47 ± 2.46	10

Melastomataceae					
Chaetostoma glaziovii Cogn. (Chgl)	Bees	1256	9.75 ± 0.86	9.75 ± 0.55	8
Leandra sulfurea (Naudin) Cogn. (Lesu)	Bees	1383	3.75 ± 1.02	6.03 ± 0.72	10
Pleroma hospita (Schrank et Mart. ex DC.) Triana (Plho)	Generalist	1690	9.30 ± 2.62	6.88 ± 2.33	10
Myrtaceae					
Myrceugenia alpigena (DC.) Landrum (Myal)	Generalist	1470	6.04 ± 1.68	7.00 ± 0.99	10
Onagraceae					
Fuchsia campos-portoi Pilg. & Schulze-Menz (Fuca)	Hummingbirds	343	10.71 ± 2.36	26.98 ± 4.95	8
Fuchsia regia (Vell.) Munz (Fure)	Hummingbirds	16	11.59 ± 1.35	39.24 ± 4.94	10
Orchidaceae					
Cyclopogon apricus (Lindl.) Schltr. (Cyap)	-	117	6.80 ± 0.83	0	5
Habenaria parviflora Lindl. (Hapa)	-	340	6.76 ± 1.10	0	6
Habenaria rolfeana Schltr.	-	-	-	-	-
Orobanchaceae					
Buddleja speciosissima Taub. (Busp)	Hummingbirds	508	27.28 ± 2.26	26.66 ± 2.39	10
Esterhazya eitenorum Barringer (Esei)	Bees and hummingbirds	516	28.32 ± 3.29	38.96 ± 4.03	24
Oxalidaceae					
Oxalis confertissima A.St.-Hil (Oxco)	Bees and flies	1606	8.28 ± 0.92	5.06 ± 0.76	8
Oxalis rupestris A.St.-Hil. (Oxru)	Flies	23	14.84 ± 1.67	8.57 ± 0.98	7

Primulaceae					
Lysimachia filiformis (Cham. & Schltdl.) U. Manns & Anderb. (Lyfi)	Generalist	115	6.01 ± 0.65	4.21 ± 0.61	9
Myrsine gardneriana A.DC.	-	1470	-	-	-
Proteaceae					
Roupala montana Aubl. (Romo)	-	170	8.85 ± 0.40	6.00 ± 0.71	5
Rubiaceae					
Coccocypselum lyman-smithii Standl. (Coly)	Bees	108	9.96 ± 2.96	4.29 ± 1.23	8
Solanaceae					
Solanum enantiophyllantum Bitter (Soen)	Bees	65	9.89 ± 1.99	7.32 ± 0.88	6
Velloziaceae					
Barbacenia gounelleana Beauerd (Bago)	Hummingbirds	6	19.45 ± 1.98	8.01 ± 1.49	8
Xyridaceae					
Xyris fusca L.A.Nilsson (Xyfu)	Bees and flies	14	7.19 ± 2.17	1.95 ± 0.34	10

---

[1] Freitas and Sazima (2006).

[2] Clivati, Corderio, Plachno, and Miranda (2014).

Table S2. Floral richness and abundance, floral trait diversity and community reproductive success per month in highland grasslands in Itatiaia National Park, Brazil. Trait diversity$_1$ is the abundance-weighted Rao's Q index while $_2$ had no abundance weight (Botta-Dukát, 2005). PR = pollen receipt (z-transformed) and PT = pollen tubes (z-transformed). CV is the coefficient of variation of the same fitness variables.

Month Year	Species flowering	Flower Abundance	Trait diversity$_1$	Trait diversity$_2$	Mean PR	Mean PT
October 2016	17	3571	2.90	8.25	-	-
November 2016	28	3112	5.13	5.02	-0.133	-0.059
December 2016	40	3275	3.65	6.36	0.052	-0.002
January 2017	45	16412	1.58	5.27	0.188	0.220
February 2017	47	10739	2.05	5.11	0.207	0.170
March 2017	39	9611	2.54	5.30	-0.022	-0.113
April 2017	30	3089	2.74	6.28	0.073	0.099
May 2017	15	1018	2.71	6.12	-0.110	-0.263
September 2017	17	2253	2.60	8.03	-	-
October 2017	17	1515	4.01	8.24	0.064	0.031
November 2017	18	2321	2.86	7.94	-0.031	0.050
December 2017	38	2276	2.73	5.09	-0.014	0.032
January 2018	47	12187	1.30	5.98	0.113	0.090
February 2018	46	10044	1.87	4.52	0.090	0.028
March 2018	37	6735	2.15	5.28	0.075	0.039
April 2018	24	1821	2.94	6.18	-0.095	0.024
May 2018	13	967	2.86	6.63	-0.220	-0.193

Table S3. Null model analyses of trait diversity (TD) per month in highland grasslands in Itatiaia National Park, Brazil. We obtained null distributions of the abundance-weighted Rao's Q index (Botta-Dukát, 2005) using the independent swap-algorithm (Gotelli, 2000).

Month Year	Null TD (mean ± SD)	Pattern	p
October 2016	5.27 ± 3.52	Random	0.167
November 2016	6.27 ± 2.48	Random	0.378
December 2016	6.06 ± 2.72	Random	0.099
January 2017	4.42 ± 3.08	Clustered	0.002
February 2017	6.38 ± 2.87	Clustered	0.001
March 2017	6.14 ± 2.82	Clustered	0.010
April 2017	6.08 ± 3.29	Clustered	0.041
May 2017	5.48 ± 3.49	Random	0.115
September 2017	4.47 ± 2.99	Random	0.210
October 2017	5.56 ± 4.04	Random	0.386
November 2017	5.34 ± 3.36	Random	0.174
December 2017	5.47 ± 2.67	Clustered	0.038
January 2018	3.74 ± 2.54	Clustered	0.014
February 2018	6.00 ± 3.24	Clustered	0.002
March 2018	6.01 ± 3.11	Clustered	0.007
April 2018	6.02 ± 3.56	Random	0.096
May 2018	6.03 ± 3.54	Random	0.097

Table S4. Null model analyses of phylogenetic diversity per month in highland grasslands in Itatiaia National Park, Brazil. We obtained the phylogenetic diversity (PD) using the equivalent abundance-weighted Rao's Q index for phylogenetic distances (Botta-Dukát, 2005). Then, we obtained null distributions using the independent swap-algorithm (Gotelli, 2000).

Month Year	PD	Null PD (mean ± SD)	Pattern	p
October 2016	101.56	86.94 ± 18.48	Random	0.862
November 2016	114.16	89.74 ± 18.18	Even	0.017
December 2016	94.85	87.93 ± 16.90	Random	0.624
January 2017	123.72	108.45 ± 23.94	Random	0.356
February 2017	82.02	85.12 ± 16.01	Random	0.361
March 2017	76.51	89.18 ± 17.64	Random	0.182
April 2017	77.43	81.77 ± 20.98	Random	0.322
May 2017	81.29	75.57 ± 26.51	Random	0.507
September 2017	64.16	76.56 ± 25.81	Random	0.272
October 2017	100.98	79.12 ± 23.50	Random	0.852
November 2017	89.34	79.26 ± 22.82	Random	0.620
December 2017	92.24	84.33 ± 18.51	Random	0.631
January 2018	41.63	76.77 ± 22.32	Clustered	0.048
February 2018	80.53	84.84 ± 16.15	Random	0.345
March 2018	78.65	84.65 ± 19.03	Random	0.299
April 2018	75.31	75.85 ± 23.45	Random	0.436
May 2018	91.62	69.00 ± 26.84	Random	0.819

Table S5. Relative fitness, flowering synchrony and trait similarity of co-flowering species of highland grasslands in Itatiaia National Park, Brazil. Relativization was done by dividing all samples the maximum value of that fitness variable per species. Flowering synchrony was calculated based on Freitas and Bolmgrem (2008) index and trait similarity based on Violle et al. (2017) index. PR = pollen receipt. PT = pollen tubes.

Plant species	PR	PT	Synchrony	Colur similarity	Floral size similarity	Anther height similarity
Agarista hispidula	0.590	0.590	0.059	0.196	9.312	0.669
Alstroemeria foliosa	0.256	0.201	0.175	0.228	24.797	1.862
Alstroemeria isabelleana	0.213	0.219	0.175	0.222	23.082	1.645
Alophia sellowiana	0.312	0.322	0.136	0.292	22.213	0.645
Barbacenia gounelleana	0.436	0.433	0.045	0.218	10.761	0.585
Berberis glaziovii	0.293	0.253	0.063	0.208	21.301	0.733
Buddleja speciosissima	0.440	0.303	0.115	0.238	8.026	1.608
Cerastium dicrotricum	0.278	0.278	0.180	0.207	7.258	0.505
Chaetostoma glaziovii	0.379	0.471	0.150	0.259	6.886	0.585
Cococcypselum lyman-smithii	0.201	0.191	0.167	0.209	7.672	0.487
Cunila galioides	0.433	0.361	0.204	0.217	8.017	0.519
Escallonia bifida	0.270	0.282	0.143	0.203	7.702	0.488
Escallonia laevis	0.095	0.123	0.081	0.323	27.597	0.591
Esterhazya eitenorum	0.208	0.230	0.067	0.289	8.641	2.332
Fernseea itatiaiae	0.347	0.478	0.144	0.226	11.612	0.670
Fuchsia campos-portoi	0.577	0.628	0.179	0.240	19.978	1.501
Fuchsia regia	0.128	0.122	0.086	0.259	8.639	2.255
Gaylussacia amoena	0.145	0.143	0.088	0.303	9.344	0.595
Gaultheria eriophylla	0.094	0.166	0.067	0.300	8.942	0.589
Gaultheria serrata	0.173	0.168	0.165	0.217	6.976	0.615
Geranium brasiliense	0.371	0.346	0.158	0.230	11.231	0.487

Hesperozygis myrtoides	0.218	0.186	0.103	0.233	10.547	0.894
Leandra eichleri	0.320	0.280	0.134	0.233	18.853	0.583
Lepechinia speciosa	0.564	0.564	0.215	0.221	6.963	1.516
Lobelia camporum	0.284	0.263	0.083	0.309	9.496	0.479
Lupinus gilbertianus	0.405	0.429	0.188	0.208	7.276	0.826
Lysimachia filiformis	0.310	0.281	0.222	0.207	8.062	0.436
Myrceugenia alpigena	0.165	0.196	0.085	0.287	8.958	0.509
Oxalis confertissima	0.432	0.433	0.154	0.210	8.152	0.616
Oxalis rupestris	0.445	0.406	0.234	0.219	7.079	0.598
Pleroma hospita	0.244	0.276	0.122	0.224	9.070	0.507
Salvia itatiaiensis	0.245	0.192	0.099	0.301	9.243	0.786
Sisyrinchium wettsteinii	0.301	0.198	0.135	0.310	7.340	0.586
Sisyrinchium glaziovii	0.213	0.220	0.096	0.242	40.869	0.562
Siphocampylus westinianus	0.110	0.340	0.139	0.237	9.771	3.361
Stevia cf. *decussata*	0.435	0.435	0.185	0.258	9.554	0.581
Utricularia reniformis	0.500	0.500	0.236	0.204	7.347	0.486
Xyris fusca	0.590	0.590	0.059	0.196	9.312	0.583

Table S6. Parameters of the models explaining flowering synchrony of the plant species of highland grasslands in Itatiaia National Park, Brazil. Floral abundance, floral trait similarity and pollination guild, i.e. bee-, fly-, hummingbird- and generalist-pollination, were used as predictors. Generalist pollination represents species pollinated by three or more pollinator groups. Floral color distances were calculated with bee-vision models for the generalist species. Flowering synchrony index followed Freitas and Bolmgren, (2008) and trait similarity calculated as proposed Violle et al., (2017). β = estimate of the effect, χ_2 = linear mixed effect value. Bold values indicate significant effects at p <0.05.

Predictor variables	**β**	**χ_2**	**p**
Pollination guild	**-**	**23.37**	**< 0.001**
Floral abundance	**-0.04 ± 0.01**	**5.64**	**0.018**
Floral color similarity	**-0.26 ± 0.10**	**4.67**	**0.031**
Floral size similarity	0.04 ± 0.08	0.02	0.882
Anther height similarity	**-0.07 ± 0.08**	**4.14**	**0.042**
Pollination guild * Abundance	**-**	**7.40**	**0.054**
Pollination guild * Color	-	5.01	0.171
Pollination guild * Size	**-**	**8.01**	**0.043**
Pollination guild * Anther height	**-**	**8.26**	**0.041**

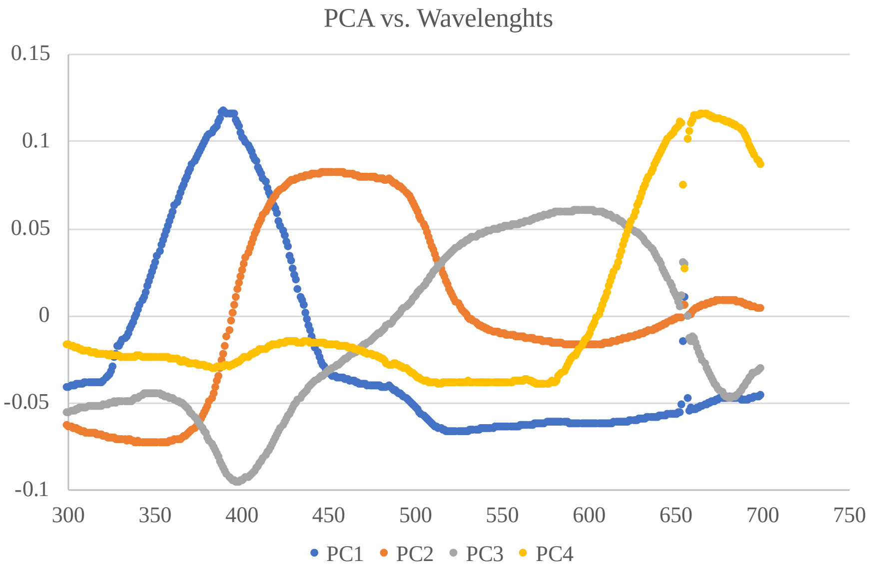

Figure S1. PCA axes from the spectral reflectance of each plant species in highland grasslands in Itatiaia National Park, Brazil against the spectral wavelengths (from 300 to 700nm).

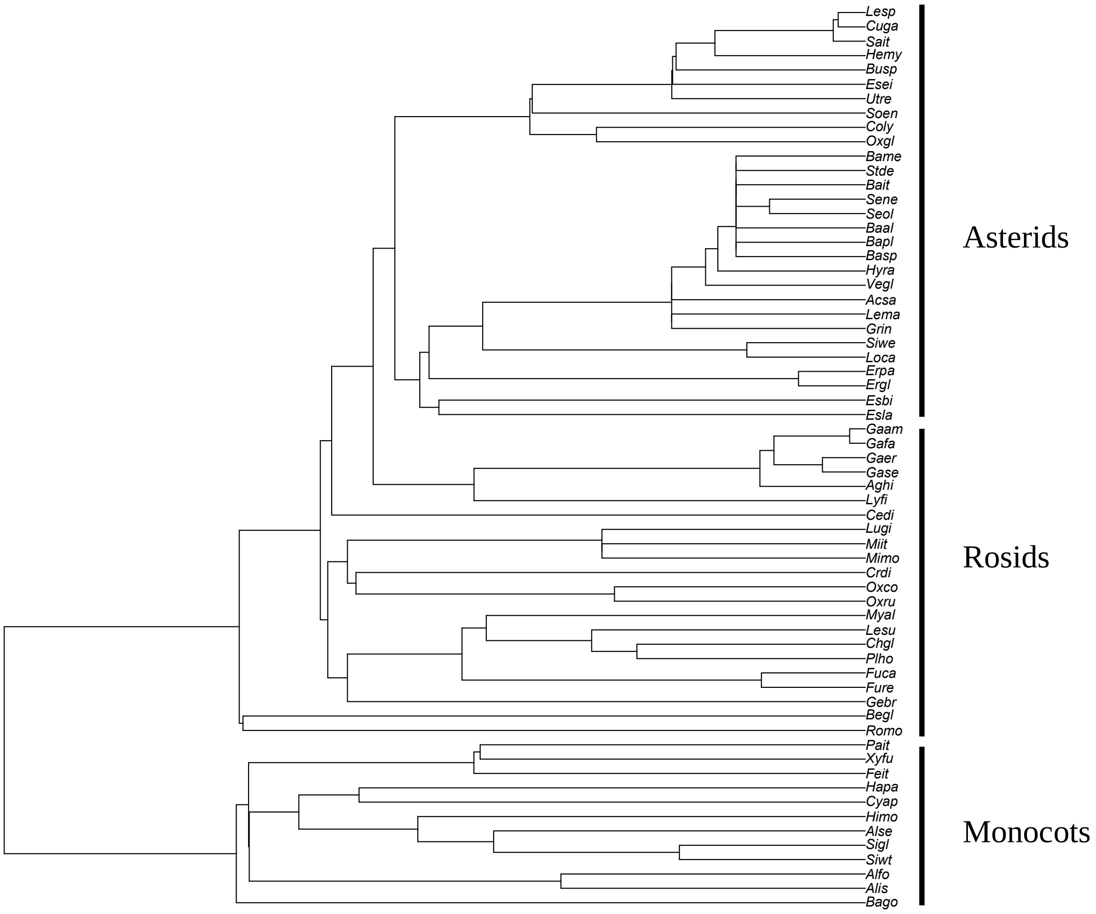

Figure S2. Phylogenetic hypothesis built for the co-flowering plant species in highland grasslands in Itatiaia National Park, Brazil. Tree topology was based on the calibrated supertree of Zanne et al. (2014) implemented in the S.Phylo.Maker function (Qian & Huang, 2016). Tip labels indicate species abbreviations given in Table S1.

References

Blomberg, S. P., Garland Jr., T., Ives, A. R. (2003). Testing for phylogenetic signal in comparative data: behavioral traits are more labile. Evolution, 57, 717-745.

Botta-Dukát, Z. (2005). Rao's quadratic entropy as a measure of functional diversity based on multiple traits. Journal of Vegetation Science, 16, 533-540.

Freitas, L., & Bolmgren, K. (2008). Synchrony is more than overlap: measuring phenological synchronization considering time length and intensity. Brazilian Journal of Botany, 31, 721-724.

Gotelli, N. (2000). Null models analysis of species co-occurrences patterns. Ecology, 81, 2606-2621

Qian, H., & Jin, Y. (2016). An updated megaphylogeny of plants, a tool for generating plant phylogenies and an analysis of phylogenetic community structure. Journal of Plant Ecology, 9, 233-239.

Staggemeier, V. G., Diniz-Filho, J. A. F., & Morellato, L. P. C. (2010). The shared influence of phylogeny and ecology on the reproductive patterns of Myrtae (Myrtaceae). Journal of Ecology, 98, 1409-1421.

Violle, C., Thuiller, W., Mouquet, N., Munoz, F., Kraft, N. J. B., Cadotte, M. W., …, & Mouillot, D. (2017). Functional rarity: the ecology of outliers. Trends in Ecology & Evolution, 32, 356-367.

Wolf, L. L., Stiles, F. G., & Hainsworth, F. R. (1976). Ecological organization of a tropical, highland hummingbird community. Journal of Animal Ecology, 45, 349-379.

Zanne, A. E., Tank, D. C., Cornwell, W. K., Eastman, J. M., Smith, S. A., FritzJohn, R. G., …, Beaulieu, J. M. (2014). Three keys to the radiation of angiosperms into freezing environments. Nature, 506, 89-92.

CAPÍTULO 3.

Pollinator-mediated indirect effects on plant fitness revealed by network indices

***Formatted under *The American Naturalist* guidelines**

Pedro Joaquim Bergamo[1*], Anna Traveset[2] and Amparo Lázaro[2]

[1]Graduate Program in Ecology, University of Campinas. Monteiro Lobato St. 255, 13083-862, Campinas, São Paulo, Brazil.

[2]Mediterranean Institute for Advanced Studies. Global Change Research Group. Miquel Marqués 21, 07190-Esporles, Mallorca, Balearic Islands, Spain.

*Author for correspondence: pjbergamo@gmail.com; +5535216170

Running head: Pollinator-mediated indirect effects

Summary

Pollinator-mediated indirect effects are often not restricted to plant species pairs, but are widespread in the plant community. A framework is needed that allows evaluating indirect effects through shared pollinators in a community context, as well as their consequences on plant fitness. We used indices describing apparent competition to assess the extent to which plant species affect and are affected by others through shared pollinators in a pollination network from a species-rich dune community. For 23 plant species, we related those indices to fitness (seeds/flower) over two years. We further linked plant traits and indices to uncover functional aspects of pollinator-mediated effects. Species with a large effect on others showed higher fitness and traits that increase pollinator attraction and generalization (large flowers and long flowering length). By contrast, species more affected by others were less fit and showed less attractive or specialized traits (few and zygomorphic flowers). The framework developed here showed that attractive species indirectly reduce the fitness of less attractive ones by sharing pollinators, providing support for a pattern of interspecific competition for pollination at the community-level.

Key-words

Competition; facilitation; floral traits; island ecology; Mediterranean dune; Müller’s index; mutualistic networks; plant fitness; pollinator-mediated indirect interactions; seed set

Introduction

In ecological communities, indirect interactions occur when one species influences how another species interacts with a third one. Plant species can indirectly interact with each other through shared pollinators (Carvalheiro et al. 2014). Pollinator-mediated indirect interactions occur when pollinator visitation rates and fitness of a focal plant species are influenced by the presence of another plant species with which it shares pollinators. There are two main types of pollinator-mediated indirect interactions: competition for pollinator attraction, when visitation rates and fitness of the plant species sharing pollinators decrease (Mitchell et al. 2009), or facilitation for pollinator attraction, when its visitation rates and fitness increase (Braun and Lortie 2019). Furthermore, a plant species may donate or receive pollen from a second one, which can ultimately lead to interference competition between them and reduced fitness (interspecific pollen transfer, Morales and Traveset 2008). Therefore, indirect interactions between plant species emerge as a consequence of their overlap in pollinator use. These indirect effects can determine community assembly (Wolowski et al. 2017) and influence reproductive trait evolution (Eisen and Geber 2018). When such indirect effects involve alien invasive species, they further raise conservation issues, as they can be detrimental to the pollination service of native species (Traveset and Richardson 2014). Pollinator-mediated effects are often not restricted to plant species pairs, but widespread in the plant community (Rathcke 1983; Bergamo et al. 2020). Thus, it is of timely importance to evaluate the mechanisms underlying the indirect effects through shared pollinators in a community context, as well as their consequences for plant fitness.

Our understanding of ecological interactions in a community context has largely benefited from network theory (Dátillo and Rico-Gray 2018). Surprisingly, however, the use of network approaches to understand indirect effects has remained mostly restricted to antagonistic interactions (e.g. apparent competition in host-parasitoid, Müller et al. 1999 or plant-herbivore networks, Morris et al. 2004). In plant-pollinator mutualistic networks, plant species often share animal partners that are essential for plant reproduction (Vázquez et al. 2009). Therefore, indirect interactions are an important component structuring pollination networks (Guimarães et al. 2017). The construction of plant-plant pollen transfer networks, in particular, has been useful to predict both competitive and facilitative interactions between plant species sharing pollinators (Tur et al. 2016). However, we still know relatively little about the fitness consequences of such indirect effects at the community-level. Most network studies focused at investigating how pollination affects plant fitness have been conducted at the intraspecific level (e.g. Gómez and Perfectti 2011; Maldonado et al. 2013; Hoffmeister et al. 2016) while few studies have been carried out at the interspecific level (Wolowski et al. 2013; Lázaro et al. 2020). To our knowledge, no study has yet linked indirect effects in pollination networks and plant fitness at the interspecific level, a necessary step to identify the direction (competition vs. facilitation) of pollinator-mediated indirect effects.

Müller et al. (1999) developed network indices to describe potential indirect effects by measuring pairwise apparent competition between two species sharing natural enemies. The Müller’s index captures how one species affects other of the same trophic level through their shared partners (i.e. second order effects, Strauss 1991), while the most commonly used network indices (degree, strength, specialization) are based on the interaction patterns between the different trophic levels (i.e. first order effects). We developed a framework to apply the Müller’s index to pollination networks, quantifying how much a plant species (1) influences others (i.e. how much it contributes to all visits made by shared pollinators – hereafter “influential degree”) and (2) is influenced by others (i.e. how much other plants contribute to the visits of its shared pollinators – hereafter “influenced degree”). We focused on pre-pollination processes, namely competition and facilitation for pollinator attraction, which can be inferred in pollination networks that use the visitation frequency of each animal species to each plant species as the link weight (Carvalheiro et al. 2014). The Müller’s index was decomposed to capture pre-pollination processes by measuring how a plant species influences or is influenced depending on the visitation frequency of shared pollinators to other plant species in the community (Figure 1A). The novelty here is the use of single values describing the indirect effect per species (influential and influenced degree) and the incorporation of specific predictions on plant fitness: under competition for pollinator visitation, influential species (high value of influential degree) would benefit by dominating shared pollinators and reduce the fitness of influenced ones (high value of influenced degree, Figure 1B). Competition may also lead both influential and influenced species to suffer fitness costs due to reduced visitation rates of shared pollinators (Figure 1B). Under facilitation, influenced species would benefit from the shared pollinator attraction with influential ones, and both would increase their fitness (Figure 1B). Facilitation also occurs when influenced species benefit from shared pollinator attraction at no cost for the influential species (Figure 1B).

Functional traits often mediate indirect interactions between plants through shared pollinators (Underwood et al. 2020). Floral trait similarity (mostly in floral tube length), differences on floral abundance, and evolutionary relatedness between the plant pair sharing pollinators have emerged as important factors regulating potential indirect effects (Carvalheiro et al. 2014; Bergamo et al. 2017). Moreover, it is well known how floral traits (e.g. flower morphology) and floral availability (e.g. flowering length and overlap) mediate interaction patterns in plant-pollinator networks (Vizentin-Bugoni et al. 2014; CaraDonna et al. 2017). Besides the traits commonly used to determine interaction patterns, several other traits are known to influence pollinator attraction and generalization levels, such as floral size and display (Conner and Rush 1996), flower shape (Lázaro et al. 2008), plant dependence on pollinators (Tur et al. 2013; Lázaro et al. 2020) and nectar properties (Klumpers et al. 2019). However, it is unknown whether such floral traits can also determine species' indirect effects through shared pollinators within pollination networks. Plant species with traits that enhance its attractiveness and generalization are expected to be important for the pollinators of several other species (Carvalheiro et al. 2014), and thus, should exhibit high influential degree.

In this study, we investigated how indirect effects within pollination networks are related to plant fitness estimates. For this, we analyzed a dataset of a pollination network (over two years), plant fitness (seeds per flower) and plant traits (flower abundance, flowering length, flower shape, flower size, corolla tube length, nectar volume, pollinator dependence) of a species-rich dune community. In species-rich communities, plant competition may be prevalent over facilitation when attractive species that interact with several pollinators are co-flowering with less attractive ones (Mitchell et al. 2009). In contrast, prevalence of facilitation is expected when pollinators are scarce (Bergamo et al. 2020). Thus, we expected that species influencing others the most (high influential degree) showed higher fitness than the species influenced by others the most (high influenced degree) through shared pollinators (hypothesis 1). Finally, highly influential species might possess population and flower traits that increase pollinator attraction (e.g. high floral abundance in the community, long flowering periods, large flowers and high nectar production) compared to highly influenced ones, and also traits related to ecological generalization, i.e. actinomorphic flowers and strong dependence on pollinators (hypothesis 2).

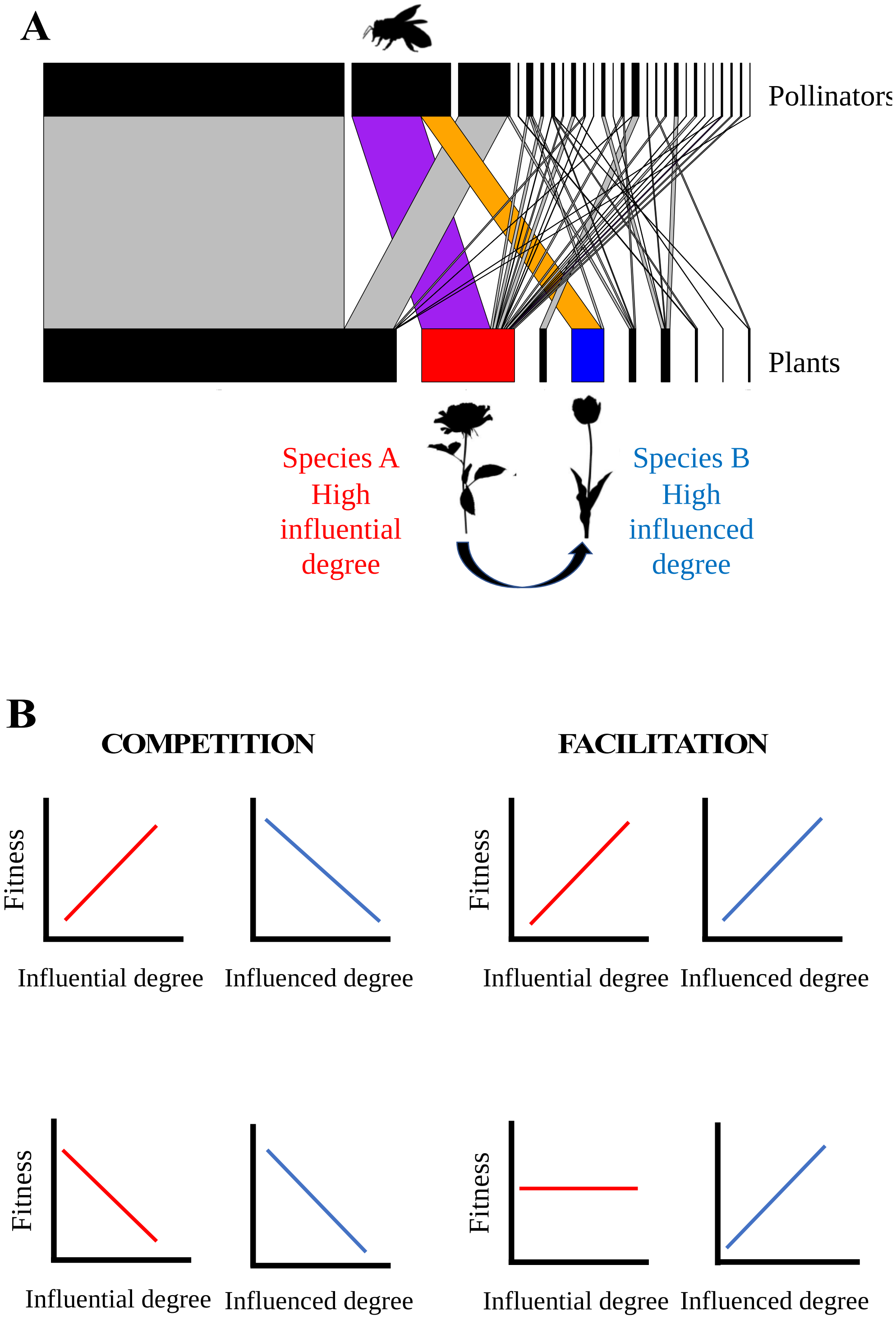

Figure 1. Conceptual framework relating indirect effects and fitness estimates to unveil pollinator-mediated processes within plant communities. A. The figure illustrates the effect of a plant species A (colored in red in the network) through a shared pollinator with a plant

species B (colored in blue). Species A receives a high proportion of the total visits made by the shared pollinator (interactions colored in purple). This shared pollinator makes a high proportion of the visits to species B (colored in orange). Thus, the Müller's index predicts a high indirect effect of species A on species B (Müller et al. 1999). In this study, the Müller's index was decomposed into two components: 1) influential degree, i.e., how much a plant species influences other species in the network through shared pollinators and 2) influenced degree, i.e., how much a plant species is influenced by others through shared pollinators. The species A shares pollinators with a large fraction of the community and is expected to have a high influential degree, while species B strongly shares its few pollinators with the community and is expected to have a high influenced degree. Plant-pollinator interaction data to draw the network from Vázquez and Simberloff (2003). B. Relationship between plant fitness and influential (red line) / influenced degree (blue line) under distinct scenarios. Interspecific competition for pollinator visitation (left panels) is predicted when influential species benefit by dominating shared pollinators and reduce the fitness of influenced ones, or when both influential and influenced species, suffer from reduced visitation rates of shared pollinators (Mitchell et al. 1999). Under facilitation, influenced species would benefit from the shared pollinator attraction with influential ones, and both would increase their fitness or when the influenced species benefit at no cost for the rest of the community (Feldman et al. 2004). This framework is intended to identify only the direction of the effects on fitness, while the strength of the cost or the benefit is not included.

Materials and Methods

Study site

We carried out fieldwork in Son Bosc within the protected area inside the s'Albufera Natural Park (39º46'28.1", N, 3º07'45.34"E). The sampling area consisted of a dune ecosystem at sea level in the northern region of Mallorca. Previous studies registered ca. 80 flowering species representing a species-rich plant community (Castro-Urgal et al. 2014; Traveset et al. 2018; Lázaro et al. 2020). Particular characteristics of this area favour the establishment of insect populations, such as nesting sites in the sandy soils, leading to the registration of ca. 125 flower-visitor species (Traveset et al. 2018). In this context, there is extensive ecological opportunity for plants to specialize on or share pollinator species and, thus, this community represents a good model to investigate variation in patterns of indirect effects among plants sharing pollinators. We used the same 23 focal species selected in Lázaro et al. (2020) for which data on plant-pollinator interactions, plant fitness and population and flower traits were available. These species were the most abundant species in the community and displayed a large variety of floral traits. More details about these species can be found in Lázaro et al. (2020).

Plant- pollinator network

We used the observation data of flower visitors on plants in 2016 (38 plant and 119 pollinator species) and 2017 (38 plant and 174 pollinator species) carried out by Lázaro et al. (2020), in the bloom period of the plant species included in this study (April-July). The network consisted of herbaceous plant species and insect pollinators of several groups (bees, wasps, flies, beetles and butterflies). Insect censuses on focal plants were conducted once per week, in the period of high pollinator activity (10:00am-17:00pm), each census lasting 5 min. We considered only visits on which the insect touched reproductive organs of the flower. We pooled all visits of an insect species to a plant species across all observation periods. Then, we built one quantitative network for each year based on visitation frequency (number of visits) of each pollinator to each plant. We consider this link weight to be the most adequate to estimate the contribution of a plant species to the diet of a pollinator species, and thus, also to define indirect effects in pollination networks (see also Carvalheiro et al. 2014; Bergamo et al. 2017). For each network, we estimated the sampling completeness of the interactions by contrasting the observed number of unique plant-pollinator interactions with the number estimated through the Chao1 method (Chacoff et al. 2012). We estimated 54.95% of sampling completeness in 2016 and 63.40% in 2017 (see Appendix 1: Fig. S1 for rarefaction curves).

Plant fitness

Plant fitness was estimated in 20 and 30 individuals per species in 2016 and 2017 (each year using different individuals), respectively (Lázaro et al. 2020). For each species, we obtained two estimates of plant reproduction: (1) fruit set, i.e. number of fruits/infructescences of each individual divided by the number of flowers/inflorescences, and (2) seed set, estimated as number of seeds per fruit (from one fruit/infructescence per individual); for this, we collected one fruit per individual to maximize the number of individuals sampled for each of the 23 study plant species each year. We used seeds per flower as the final fitness component, expressed as fruit set x seeds per fruit/infructescence. Since this measure is a ratio, it allows comparison among distinct plant species. We averaged data across individuals within species and year.

Population and flower traits

We used the trait data measured for each plant species in the field by Lázaro et al. (2020) due to their importance in determining interaction patterns of pollination networks

(Tur et al. 2013; Vizentin-Bugoni et al. 2014; CaraDonna et al. 2017; Klumpers et al. 2019). Population traits: (1) Flower abundance (flowers/m$_2$); average number of open flowers (flower units: flowers or inflorescences depending on the species) of each species counted in five transects of 50x2m sampled once every two weeks each year; (2) Flowering length; number of days the focal species was observed in bloom across all temporal surveys each year. Flower traits: (3) Flower shape; zygomorphic vs. actinomorphic flower or inflorescences (depending on the reproductive unit used for each species); (4) Flower size; average largest diameter (width, height or tube length depending on the flower/inflorescence morphology of each species), measured with digital calliper in 30 individuals per species (one flower unit per species); (5) Corolla tube length, to estimate resource accessibility (Vizentin-Bugoni et al. 2014; Klumpers et al. 2019); (6) Nectar volume, measured as the nectar standing crop of 10 individuals per species (one flower unit per individual) by means of microcapillars; and (7) Dependence on pollinators, calculated as 1 – B/C, where B are the seeds per flower produced by 10 branches/individuals prevented from insect visitation (i.e. bagged before flower anthesis) and C are the seeds per flower of 10 control branches/individuals, open to natural pollination. Values range from 0 (no pollinator dependence) to 1 (total pollinator dependence). This variable has been shown to be very relevant in determining plant specialization and structural positions within plant-pollinator networks in the studied community (Tur et al. 2013; Lázaro et al. 2020). All traits presented large interspecific variation (Table S1). Therefore, we are confident that we chose adequate traits to investigate interspecific variation.

Indirect effects within pollination networks

To apply the apparent competition indices to pollination networks, we calculated potential indirect effects in the two networks (one per study year) between all plant species pairs using the index proposed by Müller et al. (1999), using the PAC function in the *bipartite* R-package (Dormann et al. 2009):

$$d_{ij} = \sum_n \left[\frac{\propto_{ik} \propto_{jk}}{\sum_l \propto_{il} \sum_m \propto_{mk}} \right] \text{(Eq. 1)}$$

Where: d_{ij} = indirect effect of plant species *j* on plant species *i*; $\sum_n$ = summation across all n pollinators shared between plant species *i* and *j*; α_{ik} = visitation frequency of shared pollinator *k* on plant *i*; α_{ik} = visitation frequency of shared pollinator *k* on plant *j;* $\sum_l \alpha_{il}$ = total number of visits that plant *i* received in the network; $\sum_m \alpha_{mk}$ = total number of visits performed by the shared pollinator *m* in the network.

The Müller's index is calculated for all pairwise combinations in a network and has been used across different insect- and bird-pollinated plant networks from both temperate and tropical zones, helping to reveal factors related to the degree of potential indirect effects between plant species sharing pollinators (Carvalheiro et al. 2014; Bergamo et al. 2017). It is a quantitative index based on visitation data and varies from 0 (no effect of plant species *i* on plant species *j*) to 1 (maximum effect of plant species *i* on plant species *j*). This index represents how much species A contributes to the diet of all pollinators shared with species B. The Müller's index produces asymmetric effects between the plant species pair. It is weighted by the importance of the shared pollinator to the influenced species, measured as the proportion of visits that such shared pollinator contributes to the total visitation registered to the influenced species.

We calculated the Müller's index for all plant species pairs that overlapped flowering in the pollination networks (range: 0 – 0.69, Appendix S2: Fig. S2). Then, we built two plant-plant matrices (one for each year) with such index, with column values representing how much a plant species influences other species, and rows representing how much the plant species is influenced by other species. To obtain a single value per species and year, we summed all column values per species (separately for each year), representing the total effect of the species on all other plant species in the network (the sum of the effects of plant *j* on all other plant species in the network, see Eq. 1, hereafter "Influential degree"). Similarly, we summed all row values per species, representing the total effect of all species in the network on a particular one (the sum of the effects plant *i* received from all other plant species in the network, see Eq. 1, hereafter "Influenced degree"). This is the first study to use single values per species describing how much they influence other plant species and are influenced by other plant species in a community (influential and influenced degree, respectively) and relate them to plant fitness. We excluded the diagonal, setting these values to zero, as they are estimates of the potential for intraspecific competition.

Statistical analyses

To test whether those species influencing others the most showed higher fitness than the species being influenced the most through shared pollinators (hypothesis 1), we fitted one generalized linear mixed model (GLMM), using a Poisson distribution and log link function. In this model, the number of seeds per flower was the response variable, influential and influenced degree were included as fixed continuous predictors, and plant species as a random effect to avoid pseudoreplication (Zuur et al. 2010). Year and its interaction with the other predictive variables were also included as fixed effects. Previous VIF analyses revealed no collinearity between influential and influenced degrees (VIF values < 3). The correlation between influential and influenced degrees was also tested in order to confirm that the interpretation of their effects could be separated; there was not such a significant correlation neither of the two years (2016: Spearman's rho = -0.31, p = 0.086 and 2017: rho = -0.30, p = 0.103). Nevertheless, there was a general trend showing that highly influential species are poorly influenced by others in the community, while highly influenced species can be both moderately or poorly influential (Apprendix S3: Fig. S3).

Lastly, we investigated whether plant species influencing others the most were related to traits that increase pollinator attraction and generalization, whereas plant species being influenced by others the most were less attractive to pollinators and specialized (hypothesis 2). For this, we fitted two separate GLMMs: one with Gaussian distribution and log link for the model with influential degree as response variable, and one with Gaussian distribution and logit link for the model with influenced degree as response variable. Plant species identity was included as a random effect, and all seven population and flower traits (flower abundance, flowering length, floral shape and size, corolla tube length, nectar volume and pollinator dependence) and their interactions with year were included as fixed predictor variables; previous VIF analyses revealed no collinearity (VIFs < 3 for all variables, Zuur et al. 2010). Since the full model had a high number of predictors, we selected the best models with up to four variables (due to sample size restrictions), based on their AICc values using the function dredge of the *MuMIn* R-package (Barton 2018). All GLMMs were fitted using the *lme4* R-package (Bates et al. 2015). All analyses were performed in R 5.3.5 (R Core Team 2019). R codes available as Supporting information (Appendix 3: Supporting text S1).

Results

Indirect effects on plant fitness

The number of seeds per flower increased with influential degree consistently the two years ($\chi_2 = 41.98$, df =1, $p < 0.001$), although the relationship was stronger in 2017 than in 2016 ($\chi_2 = 8.39$, df = 1, $p < 0.001$, Figure 2A). Conversely, seeds per flower decreased with influenced degree both years ($\chi_2 = 54.15$, df = 1, $p < 0.001$), the relationship being stronger in 2016 than in 2017 ($\chi_2 = 13.27$, df = 1, $p < 0.001$, Figure 2B). Overall, more seeds were produced in 2016 than 2017, as shown in Lázaro et al. (2020).

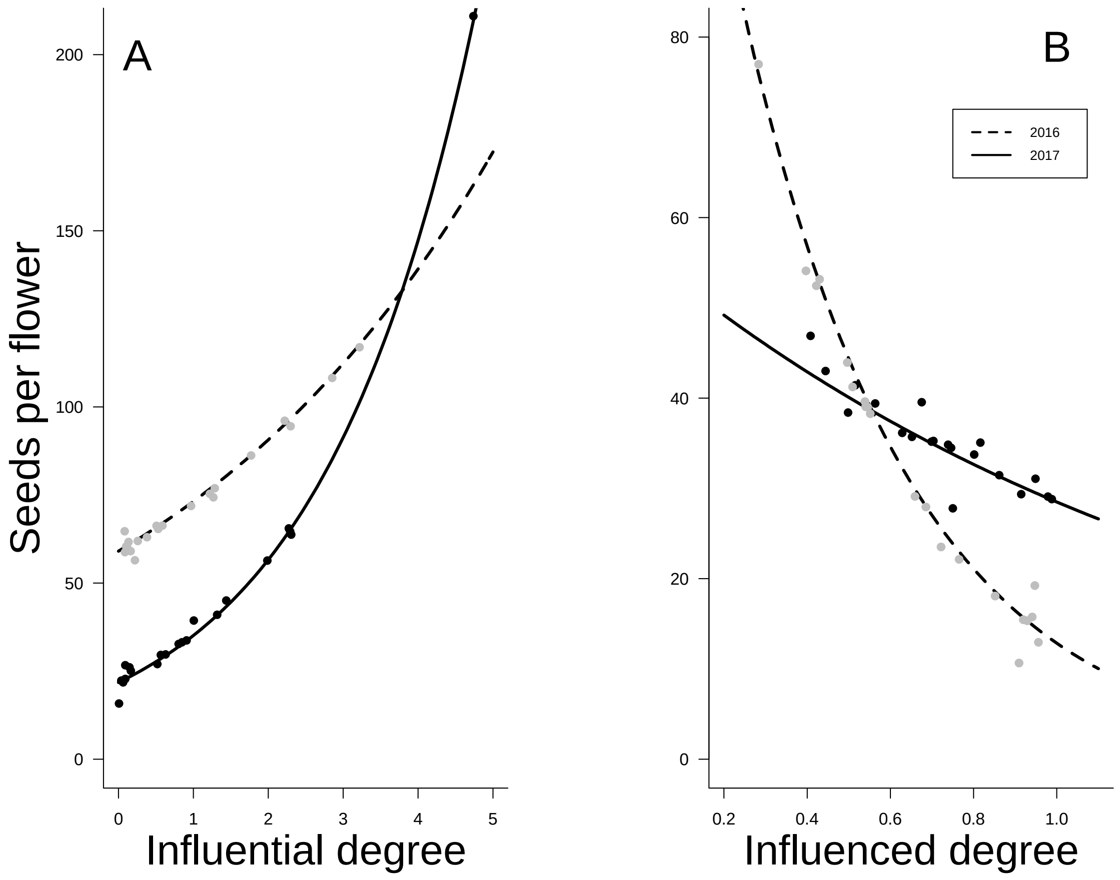

Figure 2. Partial residual plots showing the relationships between fitness (seeds per flower) and A) influential and B) influenced degree (Müller et al. 1999). A. Effect of influential degree, i.e. how much a plant species influences others through shared pollinators in the community. B. Effect of influenced degree, i.e. how much a plant species is influenced by others through shared pollinators in the community. Dots represent the partial residuals of the model (after fixing the contribution of the other predictors in the model). Whenever the interaction with year was significant, the effect was plotted separately for each year. Grey dots and dashed line represent the relationship for 2016 while black dots and filled line the relationship for 2017.

Population and flower traits associated with indirect effects

The best model explaining influential degree included floral size, flowering length, year and the interaction between flowering length and year. Plant species with large flowers influenced others the most via indirect effects ($\chi_2 = 6.40$, df = 1, $p = 0.009$, Figure 3A). Likewise, species flowering for longer periods affected others the most via indirect effects, but only in 2016, as revealed by the interaction with year ($\chi_2 = 15.45$, df = 1, $p < 0.001$; Figure 3B). Nevertheless, influential degree did not differ between years ($\chi_2 = 1.74$, df = 1, $p = 0.187$). For this variable, alternative models (ΔAICc < 2) did not include floral size and/or flowering length, but included pollinator dependence, nectar volume and its interaction with year, flower abundance and its interaction with year (Table 1).

The best model explaining influenced degree encompassed flower shape, flower abundance, year, and the interaction between shape and year. Zygomorphic species were more indirectly affected than actinomorphic species ($\chi_2 = 4.24$, df = 1, $p = 0.039$), with stronger effects in 2016 than 2017 as revealed by the interaction with year ($\chi_2 = 9.97$, df = 1, $p < 0.001$, Figure 3C). Additionally, plant species producing fewer flowers were more indirectly affected by other species than those producing many flowers ($\chi_2 = 7.40$, df = 1, $p = 0.007$, Figure 3D). Overall, influenced degree was higher in 2017 (Mean ± SE: 0.71 ± 0.05) than in 2016 (Mean ± SE: 0.66 ± 0.04), although differences were only marginally significant ($\chi_2 = 375$, df = 1, $p = 0.053$). For influenced degree, no other alternative model performed similarly (all ΔAICc > 2).

Table 1. The best and alternative models (ΔAICc < 2) explaining influential degree, i.e. how much a plant species influences other species in the networks through shared pollinators (Müller et al. 1999). All models included plant species identity as random effect. Bold values represent the best model and weight represents the explanatory power of the model.

Fixed effects	df	ΔAICc	weight
Floral size + Flowering length * Year	**7**	**0**	**0.19**
Pollinator dependence + Flowering length * Year	7	1.27	0.11
Flowering length + Flower abundance * Year	7	1.36	0.10
Pollinator dependence + Nectar volume * Year	7	1.91	0.08

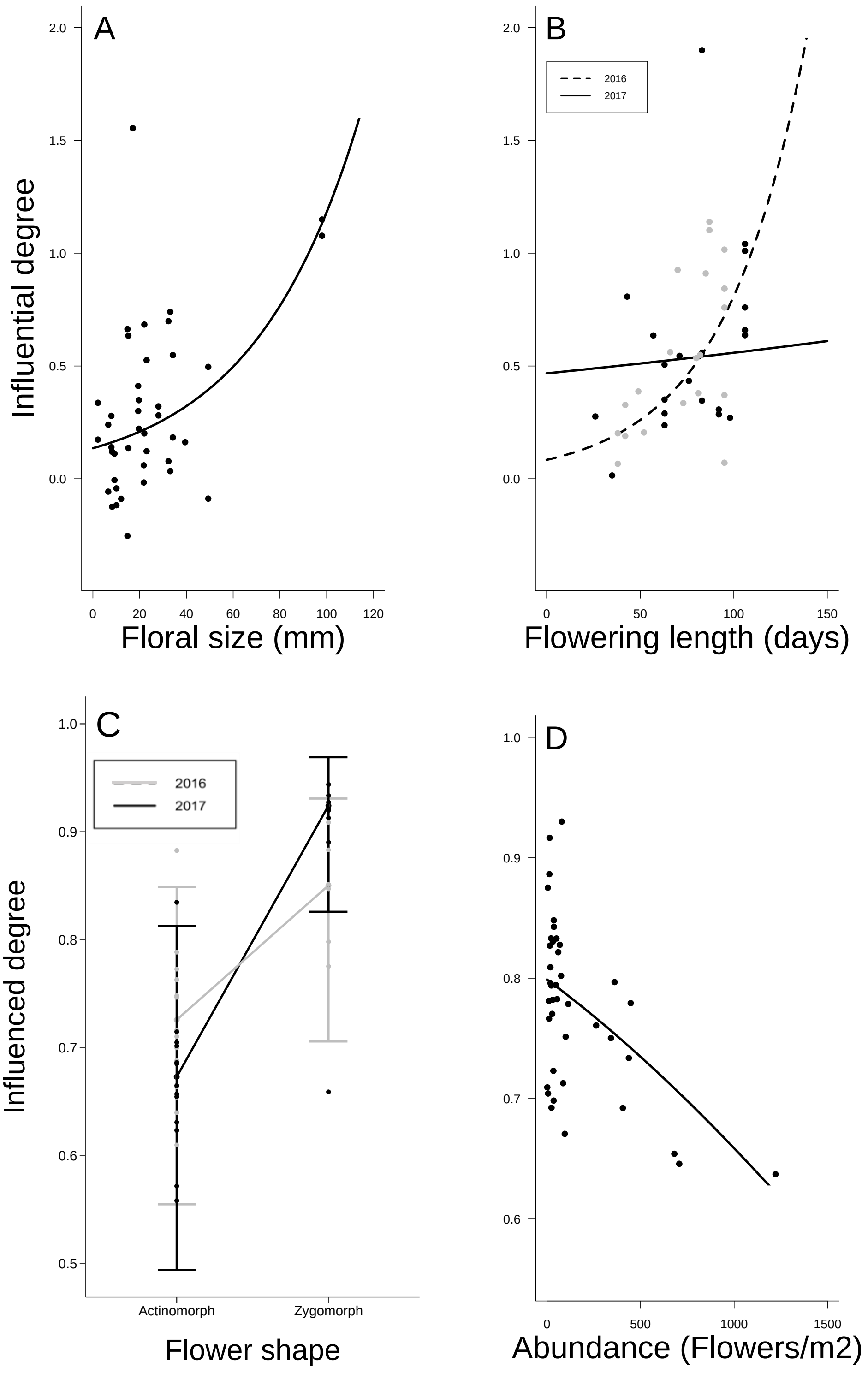

Figure 3. Partial residual plots showing the relationships between influential / influenced degree (Müller et al. 1999) and the population and flower traits. A. Relationship between floral size (in mm) and influential degree, i.e. how much a plant species influences others in the community. B. Relationship between flowering length (in days) and influential degree. C.

Relationship between flower shape (actinomorphic or zygomorphic) and influenced degree, i.e. how much a plant species is influenced by other species in the community. Error bars are the standard error and diagonal lines connect the mean values per year for each shape category. D. Relationship between flower abundance (number of flowers per m_2) and influenced degree. Dots represent the partial residuals of the models (after fixing the contribution of the other predictors in the model). Whenever the interaction with year was significant, the effect was plotted separately for each year. Grey dots and dashed line represent the relationship for 2016 while black dots and filled line the relationship for 2017.

Discussion

Overall, we showed that indirect interactions within pollination networks have effects on plant fitness. The trait analyses contributed to uncover the pathways by which such indirect effects occurred in the studied community: species with population and flower traits that increase pollinator attraction and generalization affected specialized species displaying less attractive traits. Recent meta-analyses have shown that co-flowering species have no consistent fitness effects through shared pollinators on plant neighbors (Charlebois and Sargent 2017) and that community context has a minor role in the phenotypic selection on floral traits (Caruso et al. 2019). However, in contrast to those meta-analyses focused on pairwise studies, our network approach considered several co-flowering species. Plant species are usually embedded within large networks on which multiple indirect pathways are possible to propagate effects on fitness (Guimarães et al. 2017). Moreover, studies considering the conjunct effect of several co-flowering species often report impacts of pollinator-mediated indirect interactions on visitation rates and plant fecundity (Hegland et al. 2009; Lázaro et al. 2014; Nottebrock et al. 2017; Albor et al. 2019). One first conclusion of our network analysis, therefore, is that it is a useful tool to predict the effect of pollinator-mediated indirect interactions in a community context.

The advantage of combining network analyses with fitness estimates is that it allows distinguishing between competitive and facilitative processes through shared pollinators (Figure 1). In general, the inclusion of fitness estimates has improved our understanding on how pollination networks predict ecological function (Gómez and Perffecti 2011; Maldonado et al. 2013; Hoffmeister et al. 2016; Lázaro et al. 2020). Nevertheless, pollinator-mediated competition and facilitation may simultaneously operate in plant communities (Lázaro et al. 2014; Bergamo et al. 2018). The framework developed here is intended to identify the prevalent of competition vs. facilitation instead of implying that distinct processes cannot simultaneously operate in a given community. Future studies can use the analyses outlined here to identify which groups of species within the network are more likely to indirectly affect others through competitive or facilitative processes. We believe that such framework is applicable to other plant communities and has the potential to unveil pollinator-mediated indirect effects across distinct networks.

Indirect effects on plant fitness

Consistent with predictions of competitive effects, we found that the species influencing others the most produced more seeds per flower while the species mostly influenced by others were less fit. The former benefited from higher visitation while usurping pollinators from the latter (Caruso 2000; Mitchell et al. 2009). Pollination networks of species-rich communities from continental islands, including the one studied here (Castro-Urgal & Traveset 2014; Traveset et al. 2018), often exhibit relatively high specialization relative to mainland areas (Traveset et al. 2016). High specialization in pollination networks is often attributed to interspecific competition for pollinators (Souza et al. 2018), and our results provide evidence linking such network specialization to competitive processes. Although interspecific competition emerged as the most prevalent structuring process of the study community as revealed by the relationship of the network indices and plant fitness, it cannot be discarded that some species may experience pollinator-mediated facilitation. In this context, fitness components determined by visit quality such as seed and/or seedling quality, may change the indirect effect caused by pre-pollination ones (Ashman et al. 2020), however, they are outside the scope of this study. Regarding the species that outcompete others, previous evidence demonstrated that plant species with high weighted centrality in this plant-pollinator network were more fit (Lázaro et al. 2020), indicating the fitness benefits of being highly connected within a network. Our results add up to this pattern by showing that fitness differences among the plant species were also driven by how much a species is important to the shared pollinators of others and how much such shared pollinators are important to the influenced species.

Population and flower traits associated with indirect effects

Plant species with larger flowers and populations flowering for longer periods were those affecting other plant species in the studied community. Large-flowered species dominated the visitation of several pollinator species, which resulted in their high influential degree. This was actually expected, as large flowers are often associated to high pollinator attraction (Conner and Rush 1996) and higher fitness in this community (Lázaro et al. 2020). Plants flowering for longer periods have also higher chances to interact with a high number of pollinators (Vizentin-Bugoni et al. 2014; Maruyama et al. 2014), are thus more likely to affect other plants through more indirect pathways and, consequently, exhibit a high influential degree. Previous evidence indicated that these plant traits were important determining high generalization in this plant-pollinator network (Lázaro et al. 2020). Our alternative models also showed that other attractive traits (species offering large amounts of nectar or producing many flowers at the population level) or those associated with generalization level (e.g. high dependence on pollinators, Tur et al. 2013; Lázaro et al. 2020) were related to how much a plant species affects others. Thus, traits determining overall generalization in the plant-pollinator network also may help to predict which plant species will affect more other species through shared pollinators.

Rare species (producing fewer flowers at the population level) and with zygomorphic flowers were influenced by other plant species. Flower abundance also was positively related to generalization in the plant-pollinator network, with rare species having fewer links (Lázaro et al. 2020). Thus, our results indicate that the few pollinators of these plant species strongly interact with other plant species, leading to their high influenced degree. This is also concordant with previous studies that have indicated a relationship between flower abundance and indirect effects (Carvalheiro et al. 2014; Bergamo et al. 2017). Flower shape may have influenced degree in a similar fashion, given that zygomorphic flowers are often more specialized than actinomorphic ones (Sargent 2004; Lázaro et al. 2008). An important implication of these findings is that while some traits were associated to how much a plant species affect others, different ones determined how much a species is affected by other species. Hence, our findings indicate that different functional mechanisms regulate these two aspects inherent to indirect interactions.

Conclusions

Indirect effects within the pollination network were related to plant fitness. Highly influential species (those that highly influence other plant species though shared pollinators) produced more seeds per flower while highly influenced ones (those very influenced by other plant species though shared pollinators) were less fit, consistent with overall interspecific competition for pollination at the community-level. Plant traits helped to uncover functional mechanisms regulating indirect effects in pollination networks. In the studied community, plant species with traits that indicate high pollinator attraction and generalization influenced species displaying less attractive traits and specialization. Our study highlights how network tools can improve our understanding of the mechanisms by which pollinator-mediated indirect effects influence plant fitness at the community-level.

Acknowledgments

We thank the taxonomists for insect identifications: Miguel Ángel González-Estévez (all the insects in general), David Baldock (Hymenoptera in general), Lech Borowiec (Bruchidae), José Vicent Falcó (Braconidae), Hans-Peter Tschorsnig (Tachinidae), Adrian Pont (Muscidae) and Martin J. Ebejer (Syrphidae). David Alomar and Javier Ruiz for their help in the field; and David Alomar and Carmelo Gómez-Martínez for their help in the lab. PJB was supported by FAPESP (2016/06434-0 and 2018/02996-0). AL was supported by a postdoctoral contract co-funded by the Regional Government of the Balearic Islands and the European Social Fund 2014 – 2020, and by a Ramón y Cajal contract from the Spanish Ministry of Economy and Competitiveness. AL was also supported by project CGL2017-89254-R and AT by project CGL2017-88122-P, both financed by the Spanish Ministry of Economy and Competitiveness and Feder founds (Call 2017). The Servei de Protecció d’ Espècies, Espais de Natura Balear (Conselleria d’ Agricultura, Medi Ambient i Territori) provided permissions to work at the study site.

Author Contribution

PJB, AT and AL conceived the study. AL collected the data. PJB analyzed the data and wrote the first draft. All authors contributed to the final manuscript and gave final approval for publication.

Literature cited

Albor, C., J. G. García-Franco, V. Parra-Tabla, C. Díaz-Castelazo, and G. Arceo-Gómez. 2019. Taxonomic and functional diversity of the co-flowering community differentially affect *Cakile edentula* pollination at different spatial scales. Journal of Ecology 107:2167-2181.

Ashman, T.-L., C. Alonso, V. Parra-Tabla, and G. Arceo-Gómez. 2020. Pollen on stigmas as proxies of pollinator competition and facilitation: complexities, caveats and future directions. Annals of Botany doi:10.1093/aob/mcaa012.

Barton, K. 2018. MuMIn: Multi-Model Inference. http://CRAN.R-project.org/package=MuMIn.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1-48.

Bergamo, P. J., M. Wolowski, P. K. Maruyama, J. Vizentin-Bugoni, L. G. Carvalheiro, and M. Sazima. 2017. The potential indirect effects among plants via shared hummingbird pollinators are structured by phenotypic similarity. Ecology 98:1849-1858.

Bergamo, P. J., M. Wolowski, P. K. Maruyama, J. Vizentin-Bugoni, and M. Sazima. 2018. Trait patterns across space and time suggest an interplay of facilitation and competition acting on Neotropical hummingbird-pollinated plant communities. Oikos 127:1690-1700.

Bergamo, P. J., N. S. Streher, A. Traveset, M. Wolowski, and M. Sazima. 2020. Pollination outcomes reveal negative density-dependence coupled with interspecific facilitation. Ecology Letters 23:129-139.

Braun, J., and C. J. Lortie. 2019. Finding the bees knees: a conceptual framework and systematic review of the mechanisms of pollinator-mediated facilitation. Perspectives in Plant Ecology, Evolution and Systematics 36:33-40.

CaraDonna, P. J., W. K. Petry, R. M. Brennan, J. L. Cunningham, J. L. Bronstein, N. M. Waser, and N. J. Sanders. 2017. Interaction rewiring and the rapid turnover of plant-pollinator networks. Ecology Letters 20:385-394.

Carvalheiro, L. G., J. C. Biesmeijer, G. Benadi, J. Fründ, M. Stang, I. Bartomeus, C. N. Kaiser-Bunbury, M. Baude, S. I. F. Gomes, V. Merckx, K. C. R. Baldock, A. T. D. Bennett, R. Boada, R. Bommarco, R. Cartar, N. Chacoff, J. Dänhardt, L. V. Dycks, C. F. Dormann, J. Ekroos, K. S. E. Henson, A. Holzschuh, R. R. Junker, M. Lopezaraiza-Mikel, J. Memmott, A. Montero-Castaño, I. L. Nelson, T. Petanidou, E. F. Power, M. Rundlöf, H. G. Smith, J. C. Stout, K. Temitope, T. Tscharntke, T. Tscheulin, M. Vilà, and W. E. Kunin. 2014. The potential for indirect effects between co-flowering plants via shared pollinators depends on resource abundance, accessibility and relatedness. Ecology Letters 17:1389-1399.

Caruso, C. M. 2000. Competition for pollination influences selection on floral traits of *Ipomopsis aggregata*. Evolution 54:1546-1557.

Caruso, C. M., K. E. Eisen, R. A. Martin, and N. Sletvold. 2019. A meta-analysis of the agents of selection on floral traits. Evolution 73:4-14.

Castro-Urgal, R., and A. Traveset. 2014. Differences in flower visitation networks between an oceanic and a continental island. Botanical Journal of the Linnean Society 174:478-488.

Chacoff, N. P., D. P. Vázquez, S. B. Lomáscolo, E. L. Stevani, J. Dorado, and B. Padrón. 2012. Evaluating sampling completeness in a desert plant-pollinator network. Journal of Animal Ecology 81:190-200.

Charlebois, J. A., and R. D. Sargent. 2017. No consistent pollinator-mediated impacts of alien plants on natives. Ecology Letters 20:1479-1490.

Conner, J. K., and S. Rush. 1996. Effects of flower size and number on pollinator visitation to wild radish, Raphanus raphanistrum. Oecologia 105:509-516.

Dáttilo, W., and V. Rico-Gray. 2018. *Ecological networks in the tropics*. Springer Nature, Cham, Switzerland.

Dormann, C. F., J. Fründ, N. Blüthgen, and B. Gruber. 2009. Indices, graphs and null models: analyzing bipartite ecological networks. The Open Ecology Journal 2:7-24.

Eisen, K. E., and M. A. Geber. 2018. Ecological sorting and character displacement contribute to the structure of communities of *Clarkia* species. Journal of Evolutionary Biology 31:1440-1458.

Feldman, T. S., W. F. Morris, and W. G. Wilson. 2004. When can two plant species facilitate each other's pollination? Oikos 105:197-207.

Gómez, J. M., and F. Perfectti. 2011. Fitness consequences of centrality in mutualistic individual-based networks. Proceedings of the Royal Society B: Biological Sciences 279:rspb.2011.2244.

Guimarães Jr, P. R., M. M. Pires, P. Jordano, J. Bascompte, and J. N. Thompson. 2017. Indirect effects drive coevolution in mutualistic networks. Nature 550:511-514.

Hegland, S. J., J. A. Grytnes, and Ø. Totland. 2009. The relative importance of positive and negative interactions for pollinator attraction in a plant community. Ecological Research 29:929-936.

Hoffmeister, M., N. Wittköpper, and R. R. Junker. 2016. Herbivore-induced changes in flower scent and morphology affect the structure of flower-visitor networks but not plant reproduction. Oikos 125:1241-1249.

Klumpers, S. G. T., M. Stang, P. G. L. Klinkhamer. 2019. Foraging efficiency and size matching in a plant-pollinator community: the importance of sugar content and tongue length. Ecology Letters 22:469-479.

Lázaro, A., S. J. Hegland, and Ø. Totland. 2008. The relationships between floral traits and specificity of pollination systems in three Scandinavian plant communities. Oecologia 157:249-257.

Lázaro, A., R. Lundgren, and Ø. Totland. 2014. Experimental reduction of pollinator visitation modifies plant-plant interactions for pollination. Oikos 123:1037-1048.

Lázaro, A., C. Gómez-Martínez, D. Alomar, M. A. González-Estévez, and A. Traveset. 2020. Linking species-level network metrics to flower traits and plant fitness. Journal of Ecology doi:10.1111/1365.2745.13334.

Maldonado, M. B., S. B. Lomáscolo, and D. P. Vázquez. 2013. The importance of pollinator generalization and abundance for the reproductive success of a generalist plant. PLoS One e:0075482.

Maruyama, P. K. , J. Vizentin-Bugoni, G. M. Oliveira, P. E. Oliveira, and B. Dalsgaard. 2014. Morphological and spatio-temporal mismatches shape a Neotropical savanna plant-hummingbird network. Biotropica 46:740-747.

Mitchell, R. J., R. J. Flanagan, B. J. Brown, N. M. Waser, and J. D. Karron. 2009. New frontiers in competition for pollination. Annals of Botany 103:1403-1413.

Morales, C. L., and A. Traveset. 2008. Interspecific pollen transfer: magnitude, prevalence and consequences for plant fitness. Critical Reviews in Plant Sciences 27:221-238.

Morris, R. J., O. T. Lewis, and H. C. J. Godfray. 2004. Experimental evidence for apparent competition in a tropical forest food web. Nature 428:310-313.

Müller, C. B., I. C. T. Adriaanse, R. Belshaw, and H. C. J. Godfray. 1999. The structure of an aphid-parasitoid community. Journal of Animal Ecology 68:346-370.

Nottebrock, H., B. Schmid, K. Mayer, C. Devaux, K. J. Esler, K. Böhning-Gaese, M. Schleuning, J. Pagel, and F. M. Schurr. 2017. Sugar landscapes and pollinator-mediated interactions in plant communities. Ecography 40:1129-1138.

R Core Team. 2019. R: A language and environment for statistical computing. Vienna, Austria.

Rathcke, B. 1983. Competition and facilitation among plants for pollination. Pages 305-329 *in* L. Real, editor. *Pollination biology*. Academic Press, New York, USA.

Sargent, R. D. 2004. Floral symmetry affects speciation rates in angiosperms. Proceedings of the Royal Society B: Biological Sciences 271:603-608.

Souza, C. S., P. K. Maruyama, C. Aoki, M. R. Sigrist, J. Raizer, C. L. Gross, and A. C. Araujo. 2018. Temporal variation in plant-pollinator networks from seasonal tropical environments: higher specialization when resources are scarce. Journal of Ecology 106:2409-2420.

Strauss, S. Y. 1991. Indirect effects in community ecology: their definition, study and importance. Trends in Ecology & Evolution 6:206-210.

Traveset, A., and D. M. Richardson. 2014. Mutualistic interactions and biological invasions. Annual Review of Ecology, Evolution, and Systematics 45:89-113.

Traveset, A., C. Tur, K. Trøjelsgaard, R. Heleno, R. Castro-Urgal, and J. M. Olesen. 2016. Global patterns of mainland and insular pollination networks. Global Ecology and Biogeography 25:880-890

Traveset, A., R. Castro-Urgal, X. Rotllàn-Puig, and A. Lázaro. 2018. Effects of habitat loss on the plant-flower visitor network structure of a dune community. Oikos 127:45-55.

Tur, C., R. Castro-Urgal, and A. Traveset. 2013. Linking plant specialization to dependence in interactions for seed set in pollination networks. PLoS One e:0078294.

Tur, C., A. Saéz, A. Traveset, and M. Aizen. 2016. Evaluating the effects of pollinator-mediated interactions using pollen transfer networks: evidence of widespread facilitation in south Andean plant communities. Ecology Letters 19:576-586.

Underwood, N., P. A. Hambäck, and B. D. Inouye. 2020. Pollinators, herbivores, and plant neighborhood effects. The Quarterly Review of Biology 95:37-57.

Vázquez, D. P., and D. Simberloff. 2003. Changes in interaction biodiversity induced by an introduced ungulate. Ecology Letters 6:1077–1083.

Vázquez, D. P., N. Blüthgen, L. Cagnolo, and N. P. Chacoff. 2009. Uniting pattern and process in plant-animal mutualistic networks: a review. Annals of Botany 103:1445-1457.

Vizentin-Bugoni, J., P. K. Maruyama, and M. Sazima. 2014. Processes entangling interactions in communities: forbidden links are more important than abundance in a hummingbird-plant network. Proceedings of the Royal Society B: Biological Sciences 281 rspb.2013.2397.

Wolowski, M., T.-L. Ashman, and L. Freitas. 2013. Community-wide assessment of pollen limitation in hummingbird-pollinated plants of a tropical montane rain forest. Annals of Botany 112:903-910.

Wolowski, M., L. G. Carvalheiro, and L. Freitas. 2017. Influence of plant-pollinator interactions on the assembly of plant and hummingbird communities. Journal of Ecology 105:332-344.

Zuur, A. F., E. N. Ieno, and C. S. Elphic. 2010. A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution 1:3-14.

Supporting Information of “Pollinator-mediated indirect effects on plant fitness revealed by network indices”

Pedro Joaquim Bergamo, Anna Traveset and Amparo Lázaro

Table S1. Coefficients of variation (CV multiplied by 100) describing interspecific variation for all traits included in this study.

Trait	**CV (multiplied by 100)**
Flower abundance	235.78
Flowering length	30.67
Flower size	86.64
Corolla tube length	178.18
Nectar volume	161.57
Pollinator dependence	37.52

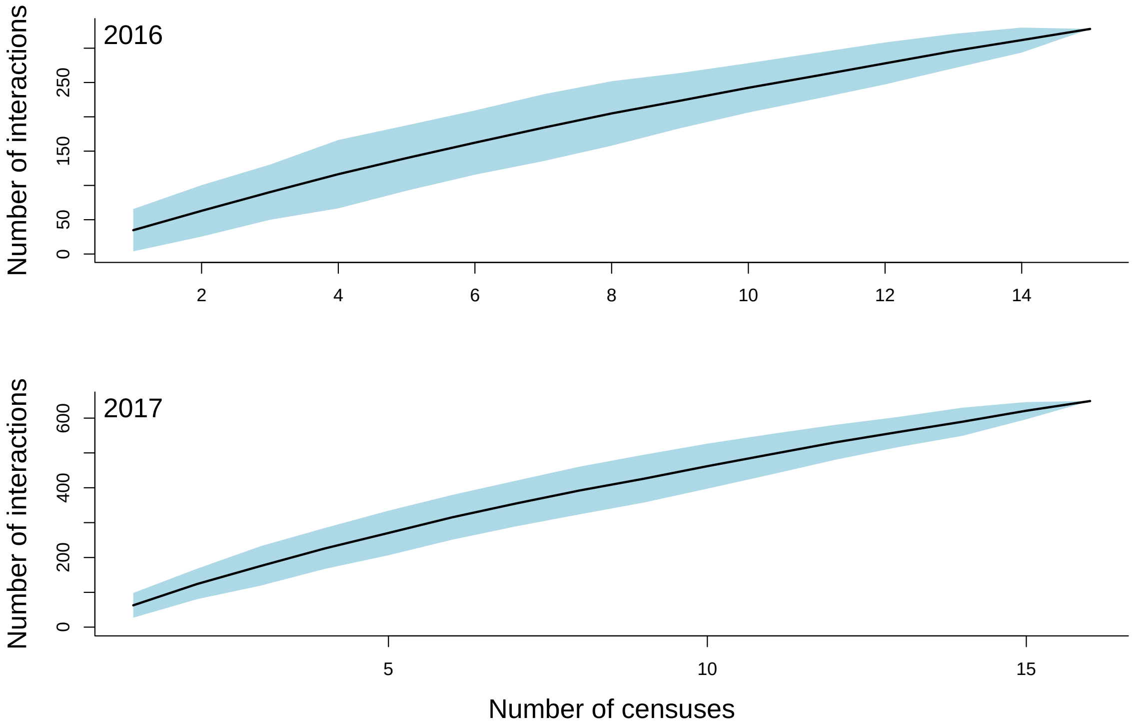

Figure S1. Rarefaction curves showing the number of unique plant-pollinator interactions in the pollination networks accumulated per census (day of observation) for each year. The blue area represents 95% confidence intervals.

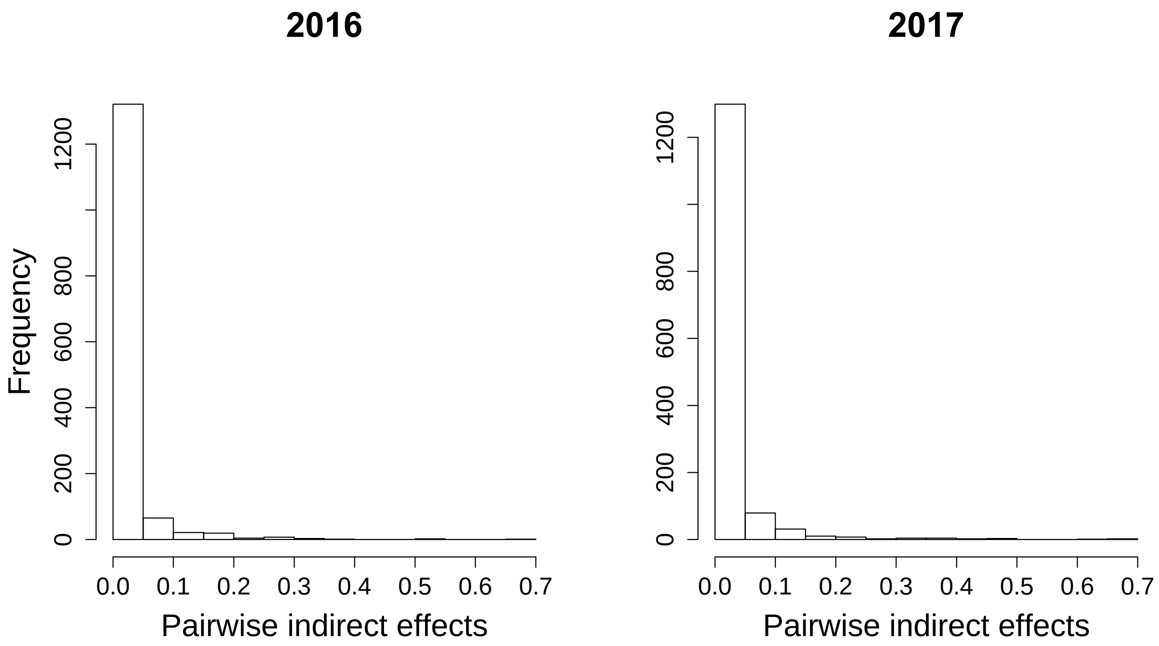

Figure S2. Distribution of the values of the Müller's index describing the indirect effect of one plant species on another through shared pollinators (Müller *et al.*, 1999).

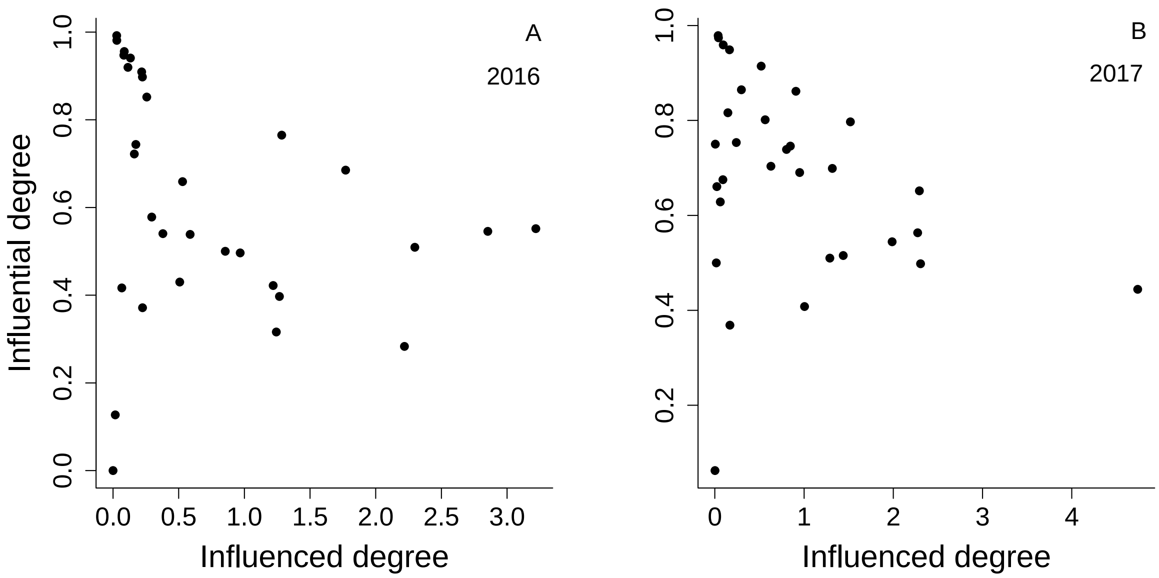

Figure S3. Dispersion diagram showing the relationship between influential degree (how much a plant species influences other species in the networks through shared pollinators) and influenced degree (how much a plant species is influenced by others through shared pollinators in the community) in the plant-pollinator network of a dune community in Mallorca in 2016 (A) and 2017 (B).

Literature cited

Müller, C. B., I. C. T. Adriaanse, R. Belshaw, and H. C. J. Godfray. 1999. The structure of an aphid-parasitoid community. Journal of Animal Ecology 68:346-370.

Appendix 3 – Supporting text S1

R code of the statistical analyses performed in this study

```
require(bipartite)
##preparing matrices of pairwise indirect effects
data<-read.csv2("SB_DataBase_ME_24_08_2018.csv", header=T)

network2016_freq<-read.csv2("2016_IntMatrix.csv", header=T)
network2016_freq2<-network2016_freq[,-1]
rownames(network2016_freq2)<-network2016_freq$PlantSpecies
network2016_freq2<-as.matrix(network2016_freq2)
network2016_freq2<-PAC(network2016_freq2)
diag(network2016_freq2)<-0

network2017_freq<-read.csv2("2017_IntMatrix.csv", header=T)
network2017_freq2<-network2017_freq[,-1]
rownames(network2017_freq2)<-network2017_freq$PlantSpecies
network2017_freq2<-as.matrix(network2017_freq2)
network2017_freq2<-PAC(network2017_freq2)
diag(network2017_freq2)<-0

hist(network2016_freq2, xlab= "Pairwise indirect effects", main="2016", cex.lab=1.8, cex.axis=1.4, cex.main=2)
hist(network2017_freq2, xlab= "Pairwise indirect effects", main="2017", ylab="", cex.lab=1.8, cex.axis=1.4, cex.main=2)
```

```
##saving matrix of indirect effects
network2017_freq3<-as.data.frame(network2017_freq2)
write.csv(network2017_freq3, "matrix_effects2017.csv")
network2016_freq3<-as.data.frame(network2016_freq2)
write.csv(network2016_freq3, "matrix_effects2016.csv")

##obtaining influential and influenced degree
effects2016<-data.frame(act_2016_freq=colSums(network2016_freq2),
rec_2016_freq=rowSums(network2016_freq2))
effects2017<-data.frame(act_2017_freq=colSums(network2017_freq2),
rec_2017_freq=rowSums(network2017_freq2))

##dataset with influential and influenced degree
data2<-read.csv2("SB_DataBase_ME_24_08_2018_effects copy.csv", header=T, dec=".")
data2$Year<-factor(data2$Year)
data3<-na.omit(data2)

##1) Effect of influential and influenced degree on plant fitness
Freq<-glmer(SeedsFlowerR~Rec_Freq*Year+Act_Freq*Year + (1 | Species), data=data2,
family=poisson)
summary(Freq)
drop1(Freq,.~., test="Chisq")

##correlations between years
effectscorr1<-subset(effects2016, rownames(effects2016)%in%rownames(effects2017))
```

```
effectscorr2<-subset(effects2017, rownames(effects2017)%in%rownames(effects2016))

cor1<-cor.test(effectscorr1$rec_2016_freq, effectscorr1$act_2016_freq,
alternative=c("two.sided"), method="spearman")
cor2<-cor.test(effectscorr2$rec_2017_freq, effectscorr2$act_2017_freq,
alternative=c("two.sided"), method="spearman")

##2) Effect of plant traits on influential and influenced degree
#Influenced degree
Trait_Rec <-
glmer(Rec_Freq~FlowerShape*Year+TubeLenght*Year+MeanAbunTotal*Year+phenology*
Year+DP*Year+FloralUnitSize*Year+NectarVol*Year + (1 | Species), data=data3,
family=gaussian(link = "logit"), na.action="na.fail")
Anova(Trait_Rec)
Trait_Rec_Sel<-dredge(Trait_Rec, m.lim=c(1,4))
summary(model.avg(Trait_Rec_Sel))

##Shape, Abund, Year, Shape*Year
Trait_Rec_best <- glmer(Rec_Freq~FlowerShape*Year+MeanAbunTotal + (1 | Species),
data=data3, family=gaussian(link = "logit"), na.action="na.fail")
Anova(Trait_Rec_best)

#Influential degree
Trait_Act <-
glmer(Act_Freq~FlowerShape*Year+TubeLenght*Year+MeanAbunTotal*Year+phenology*
```

```
Year+FloralUnitSize*Year+NectarVol*Year+DP*Year + (1 | Species), data=data3, family=gaussian(link = "log"), na.action="na.fail")
Anova(Trait_Act)
Trait_Act_Sel<-dredge(Trait_Act, m.lim=c(1,4))
summary(model.avg(Trait_Act_Sel))

##Flower size, Year, phenology, year*phenology
Trait_Act_best <- glmer(Act_Freq~phenology*Year+FloralUnitSize + (1 | Species), data=data3, family=gaussian(link = "log"), na.action="na.fail")
Anova(Trait_Act_best)
```

CAPÍTULO 4.

Pollinator-mediated facilitation alleviates pollen limitation through effects on the quality of plant reproduction

Formatted under *Proceedings of the Royal Society B: Biological Sciences* guidelines

Pedro Joaquim Bergamo[1,2*], Leandro Freitas[2], Marlies Sazima[3], Marina Wolowski[4]

[1]Graduate Program in Ecology, University of Campinas, Campinas, Brazil

[2]Rio de Janeiro Botanical Garden, Rio de Janeiro, Brazil

[3]Plant Biology Department, Institute of Biology, University of Campinas, Campinas, Brazil

[4]Institute of Natural Sciences, Federal University of Alfenas, Alfenas, Brazil

*Author for correspondence: pjbergamo@gmail.com

Abstract

Facilitation and competition among plant species are extremes of a gradient that have variable consequences for each species' fitness. However, it is unclear if pollinator-mediated indirect interactions affect pollen limitation, i.e. the contribution of pollination to plant fitness. Here, we investigated pollinator-mediated indirect effects on pollen limitation of a tropical hummingbird-pollinated community structured by facilitation. We employed indices describing how much a plant species influences (‘influential degree’) and is influenced (‘influenced degree’) through shared pollinators within the plant-hummingbird network. Since facilitation often increases pollination quantity but not quality, we expected both indices to be associated with reductions in pollen limitation estimates that depend on pollination quantity and quality (fruit set and seed number) rather than estimates more strictly related to quality (seed weight and germination). Additionally, we related both indices with plant traits. Influential and influenced degree were associated to decreases in pollen limitation, but only for seed weight and germination. Thus, facilitation may also increase the delivery of high-quality pollination. Generalized with high quality nectar species facilitated specialized and with low quality nectar ones. Pollinator sharing patterns were important drivers of pollen limitation. Given the global pollinator declines, future studies should consider how competition and facilitation affect pollination deficits.

Key-words

Competition; facilitation; hummingbirds; plant fitness; pollination quality; reproductive traits

Introduction

Pollen limitation occurs when plant reproductive success is below its full potential due to poor pollination quantity or quality. Pollen limitation has been reported in plant species across all angiosperms clades and of all biomes worldwide[1,2]. It is timely to understand the drivers of pollen limitation because of the global pollinator declines and their associated ecosystem services[3]. However, most studies have measured pollen limitation for only one or a few species within a community[2]. Community-level studies may highlight the importance of interaction patterns and plant traits associated with pollen limitation since habitat characteristics and climatic conditions are relatively similar across the plant species. Nevertheless, no general patterns arose from community-level studies, with some of them reporting relationships between pollen limitation and mating system[4,5,6,7], pollination generalization[8] or pollinator dependence[7,9]. Such lack of general predictors across studies suggests that pollen limitation is context-dependent at the community-level.

Pollen limitation may be context-dependent because the reproduction of a given plant species is also influenced by the composition of its co-flowering community. For instance, pollen limitation was stronger for plant species occurring at species-rich communities[10] or when co-flowering with phylogenetically close species[11]. Ecological networks have become a powerful tool to describe plant-pollinator interactions at the community-level[12], and thus, could be used to assess how the co-flowering community affects pollen limitation. The only study employing a network metric (describing plant specialization) did not found an association with pollen limitation in a hummingbird-pollinated plant community[13]. This could be because the plant specialization metric describes the interaction patterns between the different trophic levels in a network (i.e., related to plant-pollinator interactions) and does not assess how one species affects other of the same trophic level (i.e., related to co-flowering interactions). Therefore, there is a need to move to a community-level perspective on how plants sharing pollinators may affect each other's reproduction.

For co-flowering interactions, network indices characterizing apparent competition were recently applied in pollination networks to describe how much a plant species influences others or is influenced by others through shared pollinators[14,15]. In this context, these indices quantify (1) the proportion of flower visits a plant species receives relative to all visits made by shared pollinators in the network (influential degree) and (2) the proportion of flower visits other plants receive relative to all the visits made by its shared pollinators in the network (influenced degree). The influential and influenced degree express how much plant species may influence the visitation frequency of shared pollinators and thus, are related to processes such as competition or facilitation for pollinator attraction[16,17]. We applied such network indices to the aforementioned hummingbird-pollinated plant community. This hummingbird-pollinated plant community is marked by a prevalence of pollinator-mediated facilitation[18]. Facilitation through shared pollinators leads to overall fitness benefits for the co-flowering plants interacting[19]. Within our framework, plant species with high influence through shared pollinators (high influential degree) would reflect a high attractive role. Therefore, highly influential species would benefit and show reduced pollen limitation. Under facilitation, species that are strongly influenced by others through shared pollinators (high influenced degree) would benefit from the shared pollinator attraction with influential species and show reduced pollen limitation. Under competition, the influential species would concentrate the shared pollinators visits, leading to fitness costs and enhanced pollen limitation to the influenced ones. However, it is unclear how pollinator-mediated effects (from competition to facilitation) influence pollen limitation at the community-level.

Quantity and quality of pollination should be taken into account when evaluating pollen limitation. In this context, facilitative effects are expected to increase the pollination quantity while decrease the pollination quality[17]. This because the shared pollinator attraction between plants facilitating each other often results in heterospecific pollen transfer[20]. Moreover, the self-pollen fraction of conspecific pollen loads is an important component of pollen quality, with consequences for pollen limitation[7]. However, it is unclear how competition and facilitation affect the self-pollen fraction and its consequences for pollen limitation. While plant reproductive estimates such as fruit set and seed number per fruit are related with both the quantity and quality of pollination[21], some aspects of the plant reproduction such as seed weight and germination are more strictly related to the pollination quality[22]. Thus, we expect that fitness benefits from facilitation would be associated with reductions in fruit set and seed number, rather than on the seed weight and germination estimates of pollen limitation. Indeed, the magnitude of pollen limitation varied when evaluated by fruit set, seed number, seed weight and germination in this hummingbird-pollinated community[13]. Nevertheless, common predictors of pollen limitation (phylogenetic relatedness, specialization in pollination, self-incompatibility and plant density) were not related to the degree of pollen limitation in this community[13]. To the best of our knowledge, no study has investigated if pollinator-mediated indirect effects differ between reproductive estimates of pollen limitation.

Several reproductive traits influence the magnitude of pollen limitation across the Angiosperms[23,24,25]. Similarly, pollinator-mediated indirect effects described with pollination network indices are regulated by plant reproductive functional traits such as corolla length, reproductive parts’ position, floral color, nectar production and floral abundance[14,15]. Therefore, reproductive traits may provide evidence about functional mechanisms regulating the relationship between pollinator-mediated effects and pollen limitation. When facilitation is prevalent, species that strongly influence others would be the ones highly attractive to pollinators, while species that are strongly influenced would benefit from such shared attraction[26]. In this context, species with high influential degree (facilitator species) should exhibit attractive reproductive traits (e.g., abundant species possessing flowers with generalized morphologies and high-rewarding resources) while species with high influenced degree (facilitated species) would show less attractive traits.

Mating system and pollinator dependence are also important drivers of pollen limitation[23,25]. In the context of shared pollinator attraction, highly facilitated plant species (species showing high influenced degree) may also receive more heterospecific pollen[20]. The fitness consequences of heterospecific pollen depend on plant mating system and pollinator dependence. Self-incompatible (therefore, highly pollinator-dependent) species are predicted to be more protected from detrimental heterospecific pollen effects than self-compatible ones[27]. Therefore, we expect that highly influenced (facilitated) species to be more associated with self-incompatibility and pollinator-dependency than highly influential (facilitator) ones, and by consequence, more protected from potential harmful effects of heterospecific pollen deposition.

Here, we investigated how pollinator-mediated indirect effects are related to pollen limitation. We used a dataset of pollen limitation (based on supplemental hand pollination and evaluated as fruit set, seed number, seed weight and germination), pollination networks and reproductive traits of a hummingbird-pollinated plant community[13,28,29,30,31]. Plant species with high flowering synchrony with the others in this community exhibited low pollen limitation and similar floral traits, suggesting a prevalence of facilitation over competition[18,30]. Therefore, we expected patterns associated with facilitation: highly influential species (facilitator species that strongly influence others) and highly influenced ones (facilitated species that are strongly influenced by others) through shared pollinators would exhibit fitness benefits and therefore, reduced pollen limitation (hypothesis 1). Then, facilitation should be stronger in pollen limitation measured as fruit set and seed number than measured as seed weight and germination (hypothesis 2). Regarding reproductive traits, highly influential species should play the major attractive role and possess more attractive traits, while highly influenced ones would be associated to less attractive traits (hypothesis 3). Finally, we expected highly influenced species to be more associated to self-incompatibility and pollinator-dependency than highly influential ones (hypothesis 4).

Material and Methods

Study system

The study area was in the Itatiaia National Park, in the Atlantic forest, southeastern Brazil (22º27′ S, 44º36′ W). It is located between 900 and 1200m a.s.l. and classified as tropical montane rainforest[32]. The climate is characteristic of sub-tropical highlands (‘Cwb’ in Köppen classification). We here included data for 21 species, representing 58% of the hummingbird-pollinated flora at this site[13]. We selected the species which pollen limitation (PL) data were available[13], representing ~90% of the floral resources[18] and ~76% of the plant-hummingbird interactions[28] in this community. Therefore, these species are a representative portion of the community and provide sufficient data to estimate pollinator sharing patterns. The most representative plant families were Bromeliaceae (10 species), Gesneriaceae (4) and Acanthaceae (3) (Supplementary material Table S1). These plant species were pollinated by five hummingbird species[28]: *Phaethornis eurynome* (Lesson, 1832), *Phaethornis squalidus* (Temminck, 822), *Clytolaema rubricauda* (Boddaert, 1783), *Leucochloris albicollis* (Vieillot, 1818) and *Thalurania glaucopis* (Gmelin, 1788).

Pollen limitation

We used previously collected pollen limitation data in this community[13]. Briefly, experimental and natural pollination were compared to estimate PL. For this, experimental manual cross-pollination (Cp) was conducted on recently-opened flowers (previously prevented from visitation with bags) with pollen from another individuals. Consequently, it was guaranteed that Cp flowers received only outcross pollen and thus, would express the full reproductive potential[13]. Natural pollination (Np) was monitored in flowers exposed to pollinators and bagged after flower senescence to protect fruits against seed predators. Cp and Np were conducted within the same plant individuals due to limited number of individuals per species, but there was no evidence of resource allocation effects[13]. Number of individuals and flowers used for the treatments varied between species due to flower availability and display size differences[13]. Then, fruits were followed until maturation for both treatments. These four reproductive estimates were obtained for each treatment (Np and Cp): fruit set (number of developed fruits / number of flowers); seed number (number of seeds per fruit); average seed weight (weight of seeds / number of seeds weighted) and germination (percentage of germinated seeds up to 60 days). PL was estimated per species as the effect size (standardized mean difference between Np and Cp treatments) for each reproductive estimate[13] (Table S1). We found moderate correlations only between fruit set and seed number (Pearsons' r = 0.62, p = 0.004) and between seed weight and germination estimates of PL (Pearsons' r = 0.66, p = 0.004, Table S2). Therefore, fruit set and seed number PL estimates reflect distinct aspects of the pollination service in comparison with seed weight and germination estimates, as predicted.

Pollinator-mediated effects in pollination networks

We gathered previously collected plant-hummingbird interaction data in this community[13,28]. Observations were conducted to patches containing individuals of each plant species, registering the number of visits that each hummingbird species made to the focal plant. We considered only visits with potential pollination (i.e. when the hummingbird touched anthers and stigmas of the flower). Each observation session lasted from one to six hours, conducted during the morning and the afternoon (from 0600h up to 1800h). At least 15 hours of pollinator observation was conducted for each plant species. A sampling effort of 15 hours per plant species was shown to be sufficient for plant-hummingbird networks of the Atlantic forest[33]. The final interaction network was composed by the interaction frequencies (number of visits) each hummingbird species made to each plant species. Although PL and interaction data were not collected simultaneously, plant-hummingbird interactions in the studied community are strongly constrained by corolla-bill length match[33]. Therefore, the plant-hummingbird network reflects pollinator sharing patterns relevant to understand the dynamics of pollinator-mediated indirect interactions and its effects on plant fitness.

We applied the apparent competition metrics developed for ecological networks[34] (hereafter “Müller’s index”) to all plant species pairs in the plant-hummingbird network:

$$d_{ij} = \sum_n \left[\frac{\propto_{ik}}{\sum_l \propto_{il}} \frac{\propto_{jk}}{\sum_m \propto_{mk}} \right] \text{(Eq. 1)}$$

Where: d_{ij} = indirect effect of plant species *j* on plant species *i*; $\sum_n$ = summation across all n pollinators shared between plant species *i* and *j*; α_{ik} = visitation frequency of shared pollinator *k* on plant *i*; α_{jk} = visitation frequency of shared pollinator *k* on plant *j*; $\sum_l \alpha_{il}$ = total number of visits that plant *i* received in the network; $\sum_m \alpha_{mk}$ = total number of visits performed by the shared pollinator *m* in the network.

The Müller’s index has been used across insect- and hummingbird-pollinated plant networks from both temperate and tropical zones to describe the degree of potential indirect effects between plant species sharing pollinators[14,15]. The Müller’s index represents how much species A contributes to the visits of all pollinators shared with species B. It varies from 0 (no effect of plant species A on plant species B) to 1 (maximum effect of plant species A on plant B species). Moreover, effects are asymmetric, i.e., the influence of A on B is different from the influence of B on A. This occurs because the index is weighted by the importance of the shared pollinator to the influenced species. The importance is measured as the proportion of visits that the shared pollinator species has in relation to the total number of visits the influenced species received. We obtained a plant-plant matrix with column values representing how much the plant species affects others and rows representing how much the plant species is affected by others. To obtain a single value per species, we summed all column values per species (Table S1), representing the total effect of the species in the community (hereafter, "Influential degree"). Similarly, we summed all row values per species, representing the total effect one species receives from all other plant species in the community (hereafter, "Influenced degree"). We did not consider the diagonal (effect of the plant species on itself), which would represent potential intraspecific competition. Influential and influenced degree per species were negatively correlated (Spearman's rho = -0.77, p < 0.001, Figure S1). This correlation confirms that each plant species a had one major role in the context of indirect effects within the network, i.e. a highly influential species were poorly influenced by others and vice-versa. Our study differs from others which used the Müller's index as the pairwise effect among all species in a network[14,15]. We did all calculations using the function PAC, in the *bipartite* R-package[35].

Population and reproductive traits

We used population and reproductive trait data previously collected in this community (Table S1)[18,29,30,31]. (1) Flower abundance: total number of flowers by species counted during monthly flowering phenology surveys conducted along one year. (2) Effective corolla length: total length (in mm) from the base of the flower to the corolla tube opening[36]. (3) Anther height: total length (in mm) from the base of the flower to the tip of the anther, representing reproductive parts' position[15]. Floral color traits were assessed by measuring the spectral reflectance of the petals. Then, to achieve relevant traits in the hummingbird subjective view, the spectral reflectance was analyzed with hummingbird visual models to obtain the (4) chromatic contrast and (5) achromatic contrast[15]. Nectar traits were obtained by bagging floral buds to prevent visitation and letting nectar accumulate in recently opened flowers for 12 hours. Then, it was measured (6) nectar volume (in μl), (7) nectar concentration (in %Brix) and calculated (8) nectar sugar content (in mg) following[37]. Controlled pollination experiments were conducted to obtain the (9) ISI (index of self-incompatibility)38 and (10) IA (index of autogamy). We considered a plant species as self-compatible (SC) if ISI ≥ 0.2[38] and pollinator independent (capable of autonomous self-pollination) if IA ≥ 0.3[39]. We chose those traits since they were shown to be important determining plant-hummingbird interaction patterns[15] and pollen limitation[23,24,25].

Statistical analyses

We tested if both influential and influenced degree were associated to increases in fitness (negative effect on PL, hypothesis 1). For this, we fitted meta-analytical models with random effects (one for each reproductive estimate) to calculate the overall effect size on PL[13,40]. We used the inverse of the variance as weight in all models[40]. Meta-analytical models including phylogenetic relatedness were already shown to provide a worse fit to our PL data[13]. The novelty here was to include the influencing and influenced degree as moderators in the meta-analytical models and calculate effect sizes for each index. Positive effect sizes (with 95% confidence intervals not overlapping zero) of each moderator indicate a proportional enhance in PL (fitness cost), while negative ones (with 95% confidence intervals not overlapping zero) indicate a proportional reduction in PL (fitness benefit)[41]. When confidence intervals overlapped zero, we interpreted this as no overall effect on PL[40]. We fitted *post hoc* contrasts to inspect the differences between influential and influenced degree within each meta-analytical model. We fitted one model per reproductive estimate to check if pollinator-mediated indirect effects were stronger on fruit set and seed number than on seed weight and germination (hypothesis 2).

Then, we investigated relationships with population and reproductive traits. Specifically, we tested if influential degree was associated to more attractive traits whereas influenced degree with less attractive ones (hypothesis 3). We also tested if influenced degree was more associated with self-incompatibility and pollinator-dependency than influential degree (hypothesis 4). We fitted two GLMs: one for influential degree as response variable using Gaussian distribution and log link to meet model assumptions. For the second model, we used influenced degree as response variable with Gaussian distribution and logit link. This second model followed a logit-normal distribution which is adequate for variables that are proportions bounded by zero and one such as the influenced degree[14,15,42]. First, we computed VIF (variance inflation factor) for all ten plant traits (flower abundance, effective corolla length, anther height, chromatic contrast, achromatic contrast, nectar volume, nectar concentration, nectar sugar content, ISI and IA) as predictor variables. VIF analyses revealed high collinearity for anther height (with corolla length) and nectar volume (with nectar sugar content), so we dropped them from the models[43]. Since sample size (n = 21) did not allow us to include all predictors in one model, we selected the best models with up to two variables based on its AICc values with the dredge function of the *MuMIn* R-package[44]. Network indices and all traits exhibited weak phylogenetic signal (Fig. S2, Table S3). The only exception was nectar volume, which was not retained in the GLMs (Table S3). Therefore, we assume that results were robust to constraints of phylogenetic relatedness. We used the *metafor* R-package[41] for the meta-analytical procedures and the *lme4* R-package[45] to fit the GLMs.

Results

How much a species affects (influential degree) and is affected (influenced degree) by others were associated to reductions in pollen limitation for seed weight and germination, consistent with overall facilitation (Figure 1). We found negative effect sizes not overlapping with zero for both indices for the seed weight (-2.85 ± 0.56; -22.83 ± 6.88, respectively) and germination models (-3.15 ± 0.65; -33.51 ± 8.03, respectively). Moreover, the negative effect on pollen limitation was stronger for influenced degree when comparing with influential degree for seed weight ($\chi_2 = 9.87$, df = 17, $p = 0.002$) and germination ($\chi_2 = 16.81$, df = 14, $p < 0.001$). On the other hand, influential and influenced degree effect sizes overlapped zero for fruit set (-0.17 ± 0.43; -1.66 ± 5.29, respectively) and seed number models (-0.30 ± 0.40; -3.67 ± 5.08, respectively). Accordingly, the effect on pollen limitation did not differ between influential and influenced degree for fruit set ($\chi_2 = 0.09$, df = 18, $p = 0.761$) nor seed number ($\chi_2 = 0.52$, df = 16, $p = 0.469$). Therefore, pollinator-mediated indirect effects were stronger in seed weight and germination (more strictly related to pollination quality) than fruit and seed set (related to both pollination quantity and quality), contrary to our expectations.

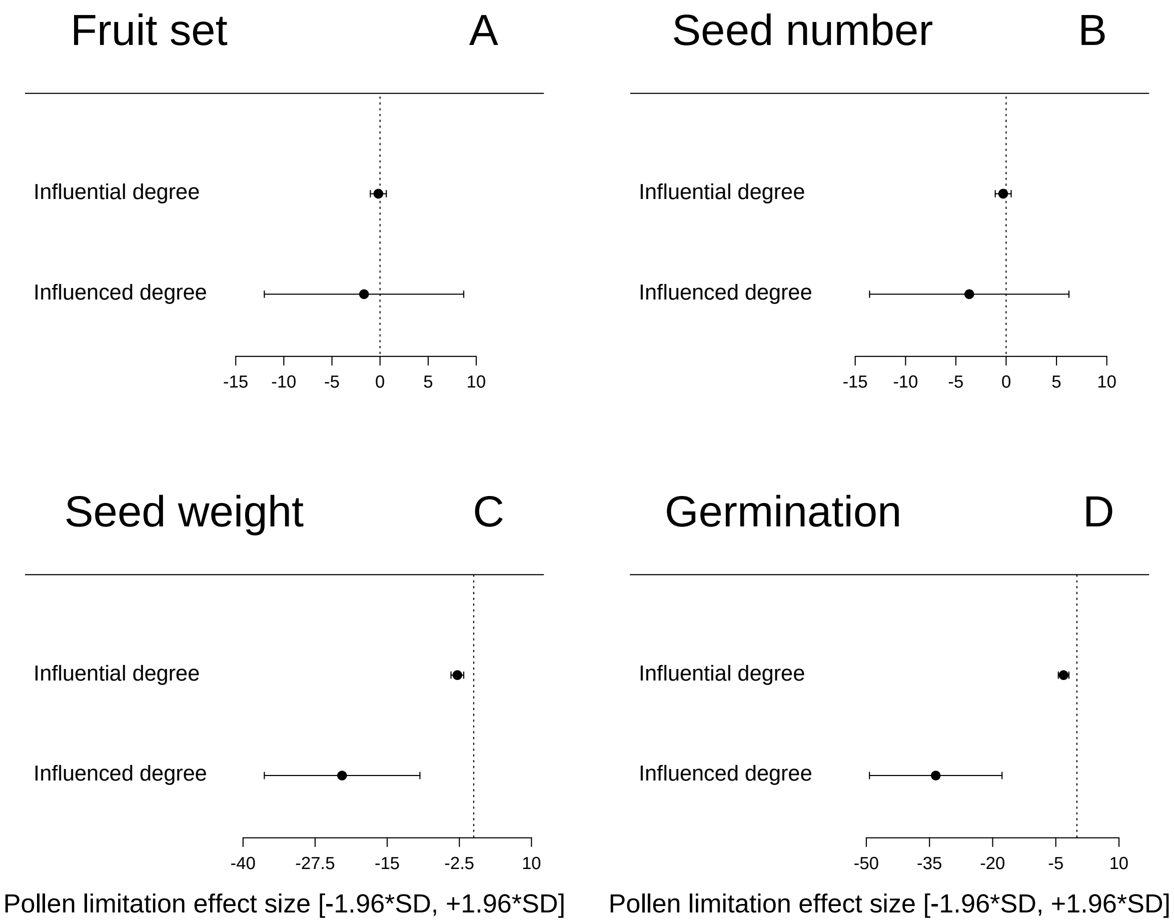

Figure 1. Effect sizes for influential degree (how much a plant species affects others in the community through shared pollinators) and influenced degree (how much a plant species is affected by others in the community through shared pollinators) as moderators of pollen limitation of hummingbird-pollinated plant species of a tropical montane rainforest, Brazil. Positive effect sizes (with 95% confidence intervals not overlapping zero) would indicate a proportional increase in pollen limitation, while negative ones (with 95% confidence intervals not overlapping zero) would indicate a proportional decrease in pollen limitation[41]. When confidence intervals overlapped zero, we interpreted as no overall effect on pollen limitation[40]. Effect sizes were calculated on four meta-analytical models, one for each reproductive estimate: A) fruit set, B) seed number, C) seed weight and D) germination.

Reproductive traits determined how much a species affects (influential degree) or is affected (influenced degree) by others through shared pollinators (Figure 2). Two models were equivalent in explaining influential degree: both included effective corolla length, the first in combination with self-compatibility and the other with nectar sugar content (Table 2). Plant species affecting other the most (high influential degree) had short flowers ($\beta = -0.05 \pm 0.02$, $\chi_2 = 7.89$, df = 1, p = 0.005), in addition to marginal significance towards high sugar content ($\beta = 0.04 \pm 0.02$, $\chi_2 = 3.40$, df = 1, p = 0.055). Contrary to expectations, self-incompatible plants had higher influential degree than self-compatible ones (1.32 ± 1.13 vs. 0.56 ± 0.35 on average ± standard-deviation, respectively, $\beta = 0.77 \pm 0.39$, $\chi_2 = 6.00$, df = 1, p = 0.014). For influenced degree, two models were equivalent: both included effective corolla length, the first in combination with nectar sugar content and the second with self-compatibility (Table 2). Plant species affected by others the most (high influenced degree) had long flowers ($\beta = 0.09 \pm 0.02$, $\chi_2 = 17.81$, df = 1, p < 0.001) and low sugar content ($\beta = -0.08 \pm 0.02$, $\chi_2 = 8.68$, df = 1, p = 0.003). Contrary to expectations, self-compatible plants had higher influenced degree than self-incompatible ones ($\beta = -0.66 \pm 0.38$, 0.96 ± 0.03 vs. 0.90 ± 0.09 on average ± standard-deviation, respectively, $\chi_2 = 4.40$, df = 1, p = 0.045).

Table 1. Best equivalent models (ΔAICc < 2.0) for the relationship between influential degree (how much a plant species affects others trough shared pollinators) and influenced degree (how much a plant species is influenced by others through shared pollinators) with reproductive traits of hummingbird-pollinated plant species of a tropical montane rainforest, Brazil.

Response variable	**Fixed effects**	**ΔAICc**	**df**	**weight**
Influential degree	Effective corolla length + Self-compatibility	0.00	4	0.36
	Effective corolla length + Nectar sugar content	1.60	4	0.10
Influenced degree	Effective corolla length + Nectar sugar content	0.00	4	0.73
	Effective corolla length + Self-compatibility	1.71	4	0.07

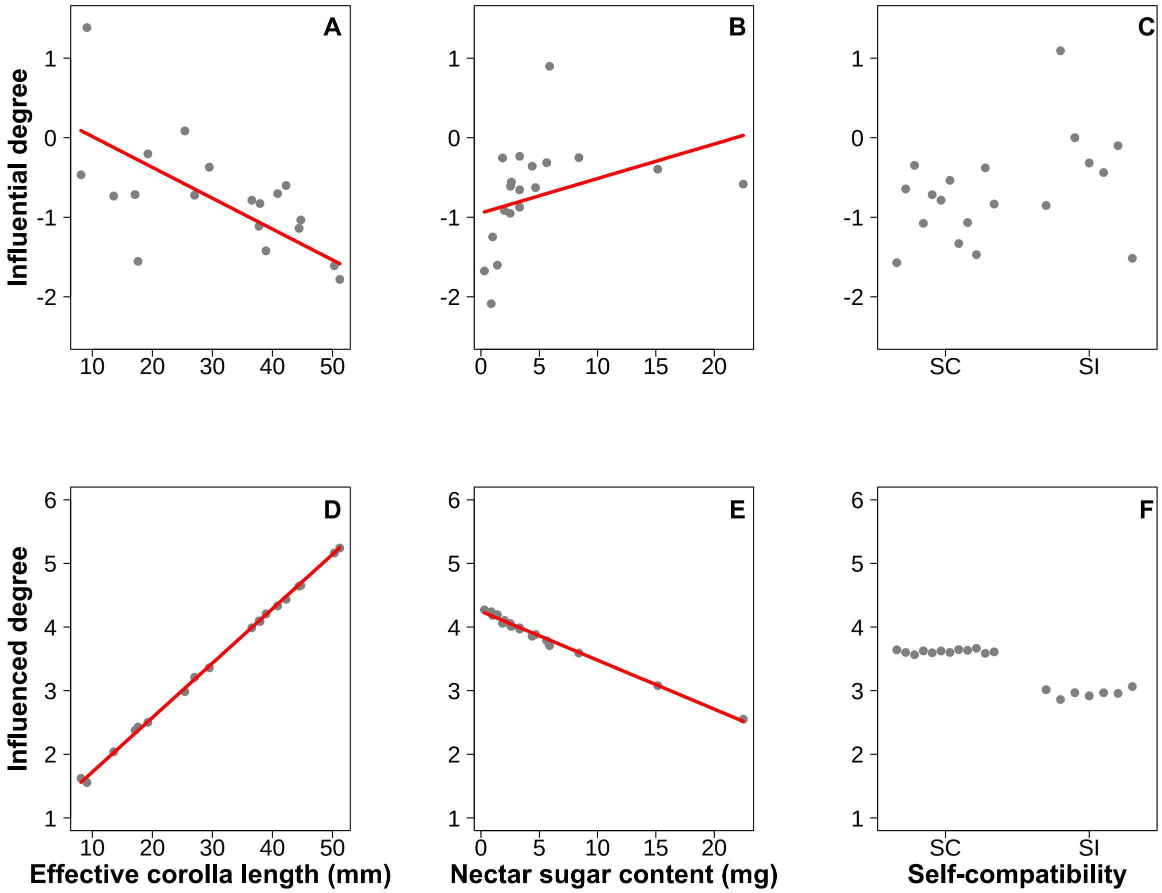

Figure 2. Relationship between pollinator-mediated indirect effects and reproductive traits among hummingbird-pollinated plant species of a tropical montane rainforest, Brazil. Association between effective corolla length (in mm) with A) influential degree (how much a plant species affects others in the community through shared pollinators) and on D) influenced degree (how much a plant species is affected by others in the community though shared pollinators. Association between nectar sugar content (in mg) with B) influential degree and E) influenced degree. Association between mating system (SI – self-incompatible and SC – self-compatible) with C) influential degree and F) influenced degree. Dots represent the partial residuals of the models (after fixing the contribution of the other predictors in the model) and the red lines represent the regression line.

Discussion

Pollinator-mediated facilitation alleviated PL

We here showed that pollinator-mediated facilitation alleviated PL for seed weight and germination at the community-level. These results build on recent previous studies showing how network metrics explain plant fitness[46,47,48], strengthening the relationship between community structure and ecosystem function. Influential plant species (facilitator species that strongly affects others) and influenced ones (facilitated species that are strongly affected by others) had reduced pollen limitation, consistent with overall facilitative effects through shared hummingbird attraction. The importance of competition between hummingbird-pollinated species through interspecific pollen transfer or differences in pollinator attraction has long been stressed[49,50,51,52,53]. However, our results suggest that shared hummingbird attraction can compensate these negative effects. Indeed, evidence from floral trait patterns and fitness benefits among co-flowering plants indicated facilitation as an important process for Atlantic forest hummingbird-pollinated plant communities[18,30]. Additionally, we here demonstrate that such facilitative effects are also embedded in pollination networks.

Reduced PL for seed weight and germination but not for fruit and seed set

Contrary to our expectations, facilitative effects were perceived only on seed weight and germination. Most studies have measured pollen limitation only for fruit and seed set[2], and therefore, may potentially missed important drivers determining pollination decays. A possibility is that the relationship between pollen receipt with fruit and seed set saturates more steeply than with seed weight and germination[54]. Additionally, features of bird-flower interactions may explain why facilitative effects were not detected for fruit set and seed number. First, pollen carryover studies have shown that only a few hummingbird visits are necessary to deposit enough pollen to set fruit and seeds[55]. If the amount of pollen necessary to set fruits and seeds saturates with a few visits, the enhanced hummingbird attraction due to facilitation would have little effect on these reproductive estimates. Secondly, it was shown that the quality of the pollen received (outcross vs. self-pollen fraction) saturates less steeply than the pollen quantity in a hummingbird-pollinated species[21]. Consequently, enhanced attraction of hummingbirds should increase the chances of receiving high-quality pollination. Indeed, facilitative effects had positive consequences for the reproductive estimates more strictly related to pollination quality in the studied system. In accordance with our results, pollinator exclusion experiments have revealed that bird pollinators positively impact reproductive estimates such as seed weight and germination, while insect pollinators had no effect[56]. Future studies associating indirect effects and pollen limitation in other pollination systems will render thoughtful comparisons.

Facilitation was linked to positive effects on reproduction quality of reproduction in the studied community, despite previous evidence from the literature showing that facilitation incurs in costs to the quality of pollination[20]. In natural communities, facilitation is particularly strong when resources are scarce, including reproductive resources such as pollinators[19,20,57]. Facilitation when pollinators are scarce may be especially important because reduced pollinator activity may diminish the seed germination rate of several plant species[58]. Moreover, we found a strong positive effect on the fitness of highly influenced (facilitated) plant species than on highly influential (facilitator) ones, even though the latter species are presumed to have the dominant pollinator attraction role in the community. These dominant and attractive species are expected to have higher relative fitness than the less attractive ones[48]. In this context, large relative fitness differences between attractive vs. less attractive plant species could destabilize coexistence[46,59]. Therefore, an implication of our results is that facilitation may foster plant coexistence by reducing the presumed fitness differences between attractive vs. less attractive plant species. Our results suggest that facilitation is an equalizing mechanism by strongly alleviating pollen limitation of less attractive species in comparison with attractive ones.

Generalized, high-rewarding and self-incompatible species facilitated specialized, low-rewarding and self-compatible ones

Effective corolla length was consistently selected in the best models, confirming the importance of this trait in determining indirect effects among hummingbird-pollinated species[15]. Moreover, corolla length was highly correlated with anther height, indicating that species that strongly impact others through shared pollinators also place pollen in similar parts of the hummingbird body[15]. Additionally, we demonstrated that species with short corollas were highly influential (facilitator species affecting others the most), which are accessible to several hummingbird species. This probably reflects the importance of some very attractive short-tubed species such as *Aechmea vanhoutteana*, which are generalized and strongly interacts with several hummingbird species in the studied community[28]. Moreover, species with long corollas were influenced the most (facilitated species affected by others the most), which are specialized in hermit (long-billed hummingbirds) pollination[60]. This concurs with previous evidence that suggested facilitation among hermit-pollinated species[49,61].

Nectar sugar content also mediated the indirect effects. In this case, influential (facilitator) species were more rewarding by producing sugar-rich nectar while influenced (facilitated) ones were less rewarding, with low sugar content. This concurs with evidence showing that co-flowering interactions within bird-pollinated *Protea* communities are mediated by nectar sugar content[62]. Additionally, nectar sugar content was highly correlated with nectar volume, indicating that sugar-rich species also offered copious nectar. The relationship between indirect effects and nectar sugar content (and consequently, nectar volume) fits the ‘magnet effect’ scenario, on which highly-rewarding plant species enhance pollinator attraction of deceptive (no nectar producing) ones[63]. In this hummingbird-pollinated plant community, the highly-rewarding species acted as ‘magnet species’ for poorly-rewarding ones. Overall, these results pointed that influential and influenced plant species exhibit opposite traits.

Our results also showed a link between indirect effects and mating system. Facilitative effects through shared pollinator attraction generally enhance interspecific pollination and heterospecific pollen deposition, with consequences for mating system evolution[27]. We expected influenced (facilitated) species to be self-incompatible as a mechanism against fitness costs of receiving heterospecific pollen deposition. However, contrary to expectations, influential (facilitator) species were self-incompatible. This may indicate that heterospecific pollen deposition is infrequent or have no detrimental effects in the studied community. Thus, the association between being a facilitator and self-incompatibility may reflect the common association between attractive floral traits and outcrossing[64]. On the other hand, self-compatible species were the most influenced (facilitated) ones. Processes that increase outcrossing are important for self-compatible species, which may suffer from inbreeding depression when selfing levels are high[65]. In this context, the possibility of facilitation to increase the pollination quality (and thus, the chance of outcrossing) is especially important for the influenced and self-compatible species. Therefore, the benefits on the quality of the reproduction found here for the influenced species may be associated with self-compatibility.

Conclusions

We found that indirect effects embedded in the pollination network alleviated pollen limitation, consistent with facilitation. These positive effects were detected only for reproductive estimates more strictly related to the quality of pollination, namely seed weight and germination. Facilitation was also related to trait complementarity. Plant species with short-tubed flowers, sugar-rich nectar and self-incompatibility facilitated plant species with long-tubed flowers, low sugar nectar and self-compatibility. Linking pollination networks and plant fitness contributed to understand the dynamics of co-flowering interactions. To understand how interaction patterns and reproductive traits mediate pollination deficits is urgent to predict how plant communities will respond to pollinator declines, climate change and plant invasions.

Data availability

All data, data sources and codes are available in the main text and supplementary material and will be deposited in an appropriate repository.

Acknowledgments

We thank the Instituto Chico Mendes de Biodiversidade and the Itatiaia National Park for research permit in protected areas (ICMBio/SISBIO nº 58349-1). We also thank MBF Canela for kindly sharing data of plant-hummingbird interactions.

Funding

This work was supported by FAPESP (grants 2016/06434-0 and 2018/02996-0 to PJB), CNPq (grants 445750/2014-6 and 304794/2018-0 to LF, 436335/2018-2 to MW and 302781/2016-1 to MS), FAPERJ (grant 202.775/2018 to LF).

Authors’ contributions

PJB and MW conceived the ideas. PJB analysed the data and wrote the first draft. All authors contributed critically to the drafts and gave final approval for publication.

References

1. Ashman TL et al. 2004. Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology* **85**, 2408-2421. (doi:10.1890/03-8024)

2. Bennet JM et al. 2018. GloPL, a global data base on pollen limitation of plant reproduction. *Sci. Data* **5**, 180249. (doi:10.1038/sdata.2018.249)

3. Knight TM, Ashman TL, Bennett JM, Burns JH, Passonneau S, Steets JA. 2018. Reflections on, and visions for, the changing field of pollination ecology. *Ecol. Lett.* **21**, 1282-1295. (doi:10.1111/ele.13094)

4. Motten AF. 1986. Pollination ecology of the spring wildflower community of a temperate deciduous forest. *Ecol. Monogr.* **56**, 21-42. (doi:10.2307/2937269)

5. Merrett MF, Robertson AW, Peterson PG. 2007. Pollination performance and vulnerability to pollination breakdown of sixteen native shrub species from New Zealand. *New Zeal. J. Bot.* **45**, 579-591. (doi:10.1080/00288250709509740)

6. González AV, Pérez F. 2010. Pollen limitation and reproductive assurance in the flora of the coastal Atacama Desert. *Int. J. Plant Sci.* **171**, 607-614. (doi:10.1086/653153)

7. Rodger JG, Ellis AG. 2016. Distinct effects of pollinator dependence and self-incompatibility on pollen limitation in South African biodiversity hotspots. *Biol. Lett.* **12**, 20160253. (doi:10.1098/rsbl.2016.0253)

8. Hegland SJ, Totland Ø. 2008. Is the magnitude of pollen limitation in a plant community affected by pollinator visitation and plant species specialisation levels? *Oikos* **117**, 883-891. (doi:10.1111/j.0030-1299.2008.16561.x)

9. Lázaro A, Lundgren R, Totland Ø. 2015. Pollen limitation, species' floral traits and pollinator visitation: different relationships in contrasting communities. *Oikos* **124**, 174–186. (doi:10.1111/oik.01525)

10. Vamosi JC, Knight TM, Steets JA, Mazer SJ, Burd M, Ashman TL. 2006. Pollination decays in biodiversity hotspots. *Proc. Natl. Acad. Sci. USA*, **103** 956-961. (doi:10.1073/pnas.0507165103)

11. Sargent RD, Kembel SW, Emery NC, Forrestel EJ, Ackerly DD. 2011. Effect of local community phylogenetic structure on pollen limitation in an obligately insect-pollinated plant. *Am. J Bot.* **98**, 283-289. (doi:10.3732/ajb.1000329)

12. Vizentin-Bugoni, J, Maruyama PK, Souza CS, Ollerton J, Rech AR, Sazima M. 2018. Plant-pollinator networks in the tropics: a review. In *Ecological networks in the tropics* (eds Dáttilo W, Rico-Gray V), pp. 73-91. Cham, Switzerland: Springer Nature.

13. Wolowski M, Ashman TL, Freitas L. 2013. Community-wide assessment of pollen limitation in hummingbird-pollinated plants of a tropical montane rain forest. *Ann. Bot.* **112**, 903-910. (doi:10.1093/aob/mct140)

14. Carvalheiro LG et al. 2014. The potential for indirect effects between co-flowering plants via shared pollinators depends on resource abundance, accessibility and relatedness. *Ecol. Lett.* **17**, 1389-1399. (doi:10.1111/ele.12342)

15. Bergamo PJ, Wolowski M, Maruyama PK, Vizentin-Bugoni J, Carvalheiro LG, Sazima M. 2017. The potential indirect effects among plants via shared hummingbird pollinators are structured by phenotypic similarity. *Ecology* **98**, 1849-1858. (doi:10.1002/ecy.1859)

16. Mitchell RJ, Flanagan RJ, Brown BJ, Waser NM, Karron JD. 2009. New frontiers in competition for pollination. *Ann. Bot.* **103**, 1403-1413. (doi:10.1093/aob/mcp062)

17. Braun J, Lortie CJ. 2019. Finding the bees knees: a conceptual framework and systematic review of the mechanisms of pollinator-mediated facilitation. *Perspect. Plant Ecol. Evol. Syst.* **36**, 33-40. (doi:10.106/j.ppees.2018.12.003)

18. Wolowski M, Carvalheiro LG, Freitas L. 2017. Influence of plant-pollinator interactions on the assembly of plant and hummingbird communities. *J Ecol*. **105**, 332-344. (doi:10.1111/1365-2745.12684)

19. Bergamo PJ, Streher NS, Wolowski M, Traveset A, Sazima M. 2020. Pollination outcomes reveal negative-density dependence coupled with interspecific facilitation among plants. *Ecol. Lett.* **23**, 129-139. (doi:10.1111/ele.13415)

20. Tur C, Saéz A, Traveset A, Aizen MA. 2016. Evaluating the effects of pollinator-mediated interactions using pollen transfer networks: evidence of widespread facilitation in south Andean plant communities. *Ecol. Lett.* **19**, 576-586. (doi:10.1111/ele.12594)

21. Aizen MA, Harder LD. 2007. Expanding the limits of the pollen-limitation concept: effects of pollen quantity and quality. *Ecology* **88**, 271-281. (doi:10.1890/06-1017)

22. Schupp EW, Jordano P, Gómez JM. 2017. A general framework for effectiveness concepts in mutualisms. *Ecol. Lett.* **20**, 577-590. (doi:10.1111/ele.12764)

23. Knight TM, Steets JA, Vamosi JC, Mazer SJ, Burd M, Campbell DR, Dudash MR, Johnton MO, Mitchell RJ, Ashman, T-L. 2005. Pollen limitation of plant reproduction: pattern and process. *Ann. Rev. Ecol. Evol. Syst.* **36**, 467-497. (doi:10.1146/annurev.ecolsys.36.102403.115320)

24. Wolowski M, Ashman TL, Freitas L. 2014. Meta-analysis of pollen limitation reveals the relevance of pollination generalization in the Atlantic Forest of Brazil. *PLoS One* **9**, e89498. (doi:10.1371/journal.pone0089498)

25. Burns JH et al. 2019. Plant traits moderate pollen limitation of introduced and native plants: a phylogenetic meta-analysis of global scale. *New Phytol.* **223**, 2053-2075. (doi:10.1111/nph.15935)

26. Moeller DA. 2004. Facilitative interactions among plants via shared pollinators. *Ecology* **85**, 3289-3301. (doi:10.1890/03-0810)

27. Ashman TL, Arceo-Gómez G. 2013. Toward a predictive understanding of the fitness costs of heterospecific pollen receipt and its importance in co-flowering communities. *Am. J. Bot.* **100**, 1061-1070. (doi:10.3732/ajb.1200496)

28. Canela MBF. 2006. Interações entre plantas e beija-flores numa comunidade de Floresta Atlântica Montana em Itatiaia, RJ. PhD Thesis, Universidade Estadual de Campinas, Campinas, SP, Brasil.

29. Wolowski M, Saad CF, Ashman, TL, Freitas L. 2013. Predominance of self-compatibility in hummingbird-pollinated plants in the Neotropics. *Sci. Nat.* **100**, 69-79. (doi:10.1007/s00114-012-0995-0)

30. Bergamo PJ, Wolowski M, Maruyama PK, Vizentin-Bugoni J, Sazima M. 2018. Trait patterns across space and time suggest an interplay of facilitation and competition acting on Neotropical hummingbird-pollinated plant communities. *Oikos* **127**, 1690-1700. (doi:10.1111/oik.05571)

31. Bergamo PJ, Wolowski M, Telles FJ, Brito VLG, Varassin IG, Sazima M. 2019. Bracts and long-tube flowers of hummingbird-pollinated plants are conspicuous to hummingbirds but not to bees. *Biol. J. Linn. Soc.* **126**, 533-544. (doi:10.1093/biolinnean/bl217)

32. Veloso HP, Rangel-Filho ALR, Lima JCA. 1991. *Classificação da vegetação brasileira, adaptada a um sistema universal*. Brasília, Brasil: IBGE.

33. Vizentin-Bugoni J, Maruyama PK, Debastiani VJ, Duarte LS, Dalsgaard B, Sazima M. 2016. Influences of sampling effort on detected patterns and structuring processes of a Neotropical plant-hummingbird network. *J. Anim. Ecol.* **85**, 262-272. (doi:10.1111/1365-2656.12459)

34. Müller CB, Adriaanse ICT, Belshaw R, Godfray HCJ. 1999. The structure of an aphid-parasitoid community. *J. Anim. Ecol.* **68**, 346-370. (doi:10.1046/j.1365-2656.1999.00288.x)

35. Dormann CF, Fründ J, Blüthgen N, Gruber B. 2009. Indices, graphs and null models: analyzing bipartite ecological networks. *Open Ecol. J.* **2**, 7-24. (doi:10.2174/1874213000902010007)

36. Wolf LL, Stiles FG, Hainsworth FR. 1976. Ecological organization of a tropical, highland hummingbird community. *J. Anim. Ecol.* **45**, 349-379. (doi:10.2307/3879)

37. Galetto L, Bernardello G. 2005. Rewards in flowers: Nectar. In *Practical Pollination* Biology (eds Dafni A, Kevan PG, Husband BC), pp 261-313. Ontario, Canada: Enviroquest.

38. Zapata TR, Arroyo MTK. 1978. Plant reproductive ecology of a secondary deciduous tropical forest in Venezuela. *Biotropica* **10**: 221-230 (doi:10.2307/2387907)

39. Ramírez N, Brito Y. 1990. Reproductive biology of a tropical palm swamp community in the Venezuelan Llanos. *Am. J. Bot.* **77**, 1260-1271. (doi:10.1002/j.1537-2197.1990.tb11378x)

40. Hedges LV, Olkin I. 1985. Statistical methods for meta-analysis. San Diego, USA: Academic Press.

41. Viechtbauer W. 2010. Conducting meta-analyses in R with the metafor package. *J. Stat. Soft.* **36**, 1-4. (doi:10.18637/jss.v036.i03)

42. Mead R. 1965. A generalised logit-normal distribution. *Biometrics* **21**: 721-723. (doi:10.2307/2528553)

43. Zuur AF, Ieno EN, Elphic CS. 2010. A protocol for data exploration to avoid common statistical problems. *Methods Ecol. Evol.* **1**, 3-14. (doi:10.1111/j.2041-210X.2009.000)

44. Barton K. 2018. MuMIn: Multi-model inference. R package version 1.40.4. https://CRAN.R-project.org/package=MuMIn [last accessed on 10/12/2019].

45. Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Soft.* **67**, 1-48. (doi:10.18637/jss.v067.i01)

46. Magrach A, Molina FP, Bartomeus I. 2019. Niche complementarity among pollinators increases community-level plant reproductive success. *bioRxiv*. (doi:10.1101/629931)

47. Arceo-Gómez G, Barker D, Stanley A, Watson T, Daniels J. 2020. Plant-pollinator network structural properties differentially affect pollen transfer dynamics and pollination success. *Oecologia* **192**: 1037-1045. (doi:10.1007/s00442-020-04637-5)

48. Lázaro A, Gómez-Martínez C, Alomar D, González-Estévez MA, Traveset A. 2020. Linking species-level network metrics to flower traits and plant fitness. *J. Ecol.* (doi: 10.1111/1365.2745.13334)

49. Stiles FG. 1977. Co-adapted competitors: the flowering seasons of hummingbird-pollinated plants in a tropical forest. *Science* **198**, 1177-1178. (doi:10.1126/science.198.4322.1177)

50. Waser NM. 1978. Competition for hummingbird pollination and sequential flowering in two Colorado wildflowers. *Ecology* **59**, 934-944. (doi:10.2307/1938545)

51. Murcia C, Feinsinger P. 1996. Interspecific pollen loss by hummingbirds visiting flower mixtures: effects of floral architecture. *Ecology* **77**, 550-560. (doi:10.2307/2265629)

52. Caruso CM. 2000. Competition for pollination influences selection on floral traits of *Ipomopsis aggregata*. *Evolution* **54**, 1546-1557. (doi:10.1111/j.0014-3820.2000.tb99799.x)

53. Aizen MA, Rovere AE. 2010. Reproductive interactions mediated by flowering overlap in a temperate hummingbird-plant assemblage. *Oikos* **119**, 696-706. (doi:10.1111/j.1600-0706.2009.17762.x)

54. Hildesheim LS, Opedal HØ, Armbruster WS, Pélabon C. 2019. Quantitative and qualitative consequences of reduced pollen loads in a mixed-mating plant. *Ecol. Evol.*, **9**, 14253-14260. (doi:10.1002/ece3.5858)

55. Price MV, Waser NM. 1982. Experimental studies of pollen carryover: hummingbirds and *Ipomopsis aggregata*. *Oecologia* **54**, 353-358. (doi:10.1007/BF00380004)

56. Hervías-Parejo S, Traveset A. 2018. Pollination effectiveness of opportunistic Galápagos birds compared to that of insects: from fruit set to seedling emergence. *Am. J. Bot.* **105**, 1142-1153. (doi:10.1002/ajb2.1122)

57. Lázaro A, Lundgren R, Totland Ø. 2014. Experimental reduction of pollinator visitation modifies plant-plant interactions for pollination. *Oikos* **123**, 1037-1048. (doi:10.1111/oik.01268)

58. Lundgren R, Lázaro A, Totland Ø. 2016. Experimental simulation of pollinator decline causes community-wide reductions in seedling diversity and abundance. *Ecology* **97**, 1420-1430. (doi:10.1890/15-0787.1)

59. Lanuza JB, Bartomeus I, Godoy O. 2018. Opposing effects of floral visitors and soil conditions on the determinants of competitive outcomes maintain species diversity in heterogeneous landscapes. *Ecol. Lett.* **21**, 865-874. (doi:10.1111/ele.12954)

60. Buzato S, Sazima M, Sazima I. 2000. Hummingbird-pollinated floras at three Atlantic forest sites. *Biotropica*, **32** 824-841. (doi:10.1111/j.1744.7429.2000.tb.00621.x)

61. Araújo FP, Hoffmann D, Sazima M. 2018. The planalto hermit, *Phaethornis pretrei* – a key species in a Neotropical savanna community in Central Brazil. *J. Nat. Hist.* **52**, 37-38. (doi:10.1080/00222933.2018.1536767)

62. Nottebrock H, Schmid B, Mayer K, Devaux C, Esler KJ, Böhning-Gaese K, Schleuning M, Pagel J, Schurr FM. 2017. Sugar landscapes and pollinator-mediated interactions in plant communities. *Ecography* **40**, 1129-1138. (doi:10.1111/ecog.02441)

63. Johnson SD, Peter CI, Nilsson LA, Ågren J. 2003. Pollination success in a deceptive orchid is enhanced by co-occurring rewarding magnet plants. *Ecology* **84**, 2919-2927. (doi:10.1890/02-0471)

64. Barrett SCH. 2013. The evolution of plant reproductive systems: how often are transitions irreversible? *Proc. R. Soc. B* **280**, 20130913. (doi:10.1098/rspb.2013.0913)

65. Barrett SCH, Harder LD. 2017. The ecology of mating and its evolutionary consequences in seed plants. *Ann. Rev. Ecol. Evol. Syst.* **48**, 135-157. (doi:10.1146/annurev-ecolsys-110316-023021**)**

Supplementary material

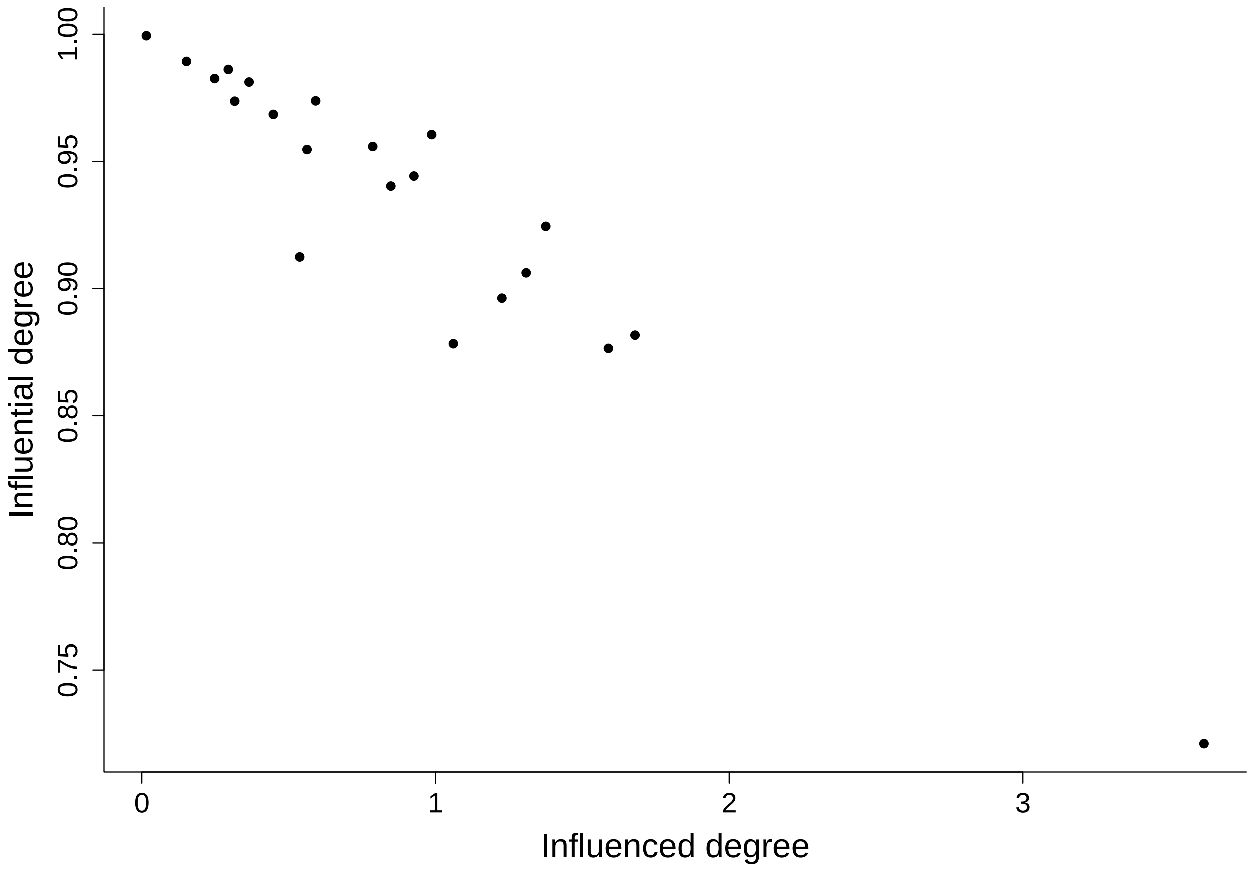

Figure S1. Dispersion diagram showing the relationship between influential degree (how much a plant species influences other species in the networks through shared pollinators) and influenced degree (how much a plant species is influenced by others through shared pollinators in the community) in the plant-hummingbird network of a tropical montane forest, Brazil.

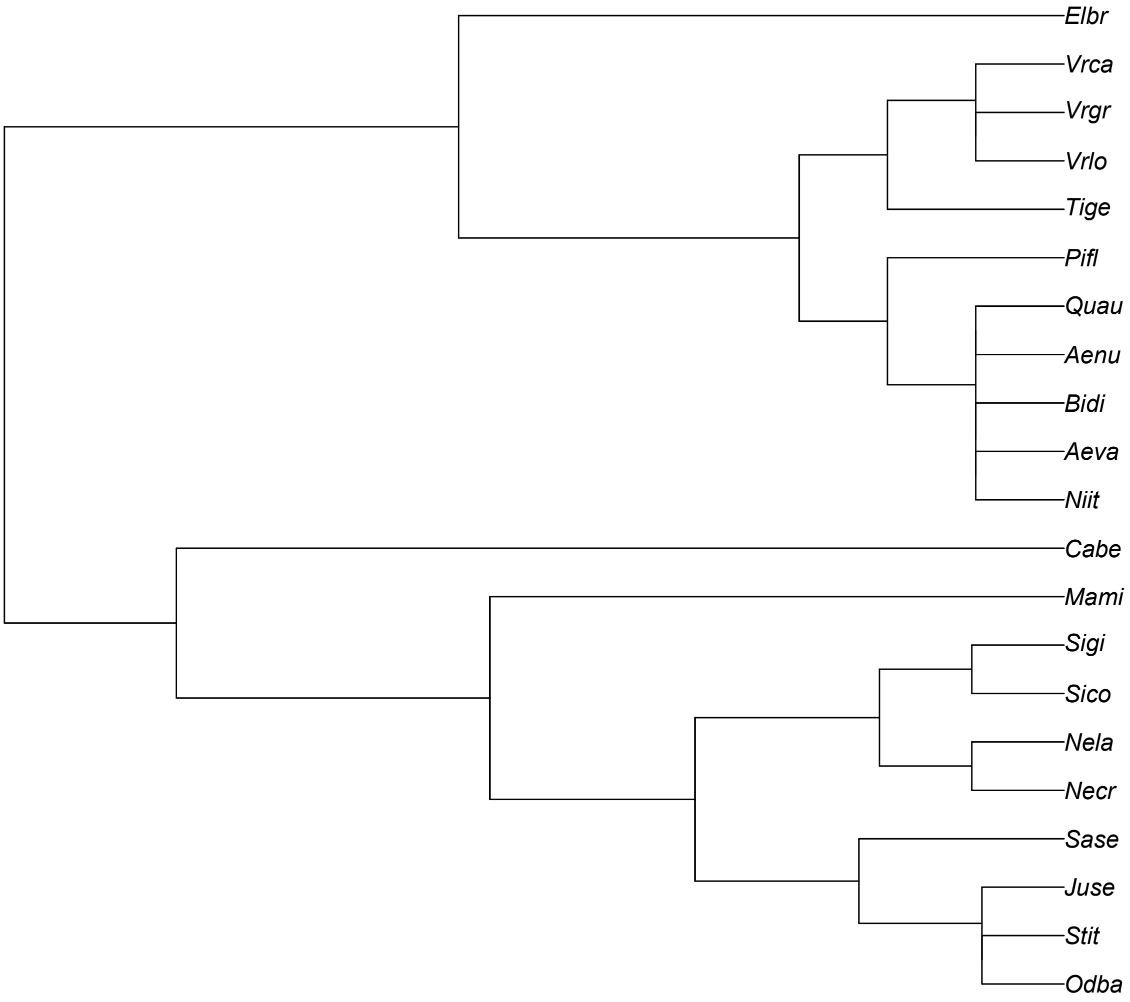

Figure S2. Phylogenetic hypothesis for the hummingbird-pollinated plant species of a tropical montane forest, Brazil. Tree topology was based on an Angiosperm supertree[1] with further taxonomical corrections[2] and modifications to resolve relationships among subfamilies of Bromeliaceae[3]. Tip labels indicate species abbreviations given in Table S1.

Table S1. Pollen limitation, network indices and traits of the hummingbird-pollinated plant species of a tropical montane rainforest, Brazil. Pollen limitation (effect size ± variance) data were previously collected in the studied community[4]. Influential degree (how much a plant species affect others) and influenced degree (how much a plant species is affected by others) calculations were based on the Müller's index[5] and plant-hummingbird interaction data[6]. Trait data were previously collected in the studied community[6,7,8,9,10]. CCB = Chromatic contrast against the background. ACB = Achromatic contrast against the background. We classified mating system in SI (self-incompatible) or SC (self-compatible) and pollinator dependence (PD) in D (dependent) or I (independent).

	Pollen limitation				Network indices		Population and reproductive traits									
Plant species	Fruit set	Seed number	Seed weight	Germination	Influential degree	Influenced degree	Flower abundance	Corolla length (mm)	Anther heigh (mm)	Nectar volume (µ l)	Nectar conc. (%Brix)	Sugar content (mg)	CCB	ACB	Mating system	PD
Aechmea nudicaulis	0.95 ± 0.29	0.51 ± 0.11	2.56 ± 0.40	1.98 ± 0.32	1.31	0.91	78	13.56	12.28	10.90	17.75	02.02	12.96	12.32	SI	D
Aechmea vanhoutteana	-0.12 ± 0.29	0.28 ± 0.02	-4.01 ± 0.69	0 ± 0.33	3.62	0.72	98	09.10	1.96	26.21	22.79	05.88	10.65	07.22	SI	D
Billbergia distachia	0.65 ± 0.15	0.35 ± 0.08	0.12 ± 0.20	-1.67 ± 0.29	0.99	0.96	145	37.92	50.41	20.10	16.50	03.32	10.31	05.51	SI	D
Callianthe bedfordiana	1.01 ± 0.12	0.99 ± 0.11	1.12 ± 0.24	1.93 ± 0.31	1.06	0.88	1606	27.03	24.82	150.00	15.00	22.50	18.90	11.36	SI	D
Elleanthus brasiliensis	0.60 ± 0.54	NA	NA	NA	0.54	0.91	146	08.12	6.45	04.15	21.11	0.88	03.85	12.72	SC	D

Staurogyne itatiaiae	*Sinningia gigantifolia*	*Sinningia cooperi*	*Salvia sellowiana*	*Quesnelia augusto-coburgii*	*Pitcairnia flammea*	*Odontonema barlerioides*	*Nidularium itatiaiae*	*Nematanthus lanceolatus*	*Nematanthus crassifolius*	*Manettia mitis*	*Justicia sebastianopolitana*
-0.37 ± 0.14	3.28 ± 0.98	1.04 ± 0.62	0.23 ± 0.18	0.07 ± 0.10	0.46 ± 0.15	0.72 ± 0.31	0.26 ± 0.11	0.14 ± 0.25	0.31 ± 0.14	0.17 ± 0.20	-0.19 ± 0.09
0.33 ± 0.68	1.23 ± 1.38	0.40 ± 0.24	-0.04 ± 0.12	0.46 ± 0.05	1.15 ± 0.21	NA	0.28 ± 0.03	0.30 ± 0.30	-0.60 ± 0.27	-0.03 ± 0.09	-0.96 ± 0.30
-3.93 ± 1.04	-4.51 ± 2.02	4.54 ± 2.04	0.20 ± 0.09	-2.13 ± 0.34	-0.69 ± 0.22	0.67 ± 0.09	0.80 ± 0.22	0.26 ± 0.20	2.54 ± 0.40	0.85 ± 0.22	-0.03 ± 0.05
-1.19 ± 0.42	0.78 ± 0.37	5.21 ± 1.03	NA	-2.10 ± 0.33	1.48 ± 0.47	NA	1.19 ± 0.42	0.29 ± 0.34	2.32 ± 0.37	0.47 ± 0.21	NA
0.32	0.29	0.02	0.79	1.68	0.59	0.56	0.25	1.59	0.15	1.23	0.85
0.97	0.99	1.00	0.96	0.88	0.97	0.95	0.98	0.88	0.99	0.90	0.94
184	76	54	905	27	442	219	249	121	521	420	1032
17.12	37.76	38.92	40.86	19.26	36.56	44.72	51.21	26.10	50.31	25.41	29.49
14.58	32.22	59.68	50.11	17.21	58.72	43.06	47.25	22.38	48.84	31.29	38.94
02.00	10.10	05.00	20.50	35.00	34.00	15.00	42.25	NA	12.50	25.50	11.50
15.00	22.40	20.80	27.50	24.00	13.80	17.40	28.90	NA	26.50	17.20	22.00
0.30	02.50	01.00	05.64	08.40	04.69	02.61	15.17	NA	03.31	04.39	02.53
10.75	13.44	20.93	11.47	11.27	20.23	10.04	11.36	17.02	19.44	21.84	18.17
06.35	08.52	10.31	08.88	01.47	11.11	08.37	11.92	10.70	10.80	11.25	09.66
SC	SC	SC	SC	SI	SC	SC	SI	SC	SC	SC	SC
D	D	D	D	D	I	D	D	D	D	D	D

Vriesea longicaulis	*Vriesea gradata*	*Vriesea carinata*	*Tillandsia geminiflora*
-0.21 ± 0.22	0.85 ± 0.12	0.90 ± 0.59	1.93 ± 0.93
0.08 ± 0.37	0.89 ± 0.70	0.82 ± 0.68	0.82 ± 0.38
-0.51 ± 0.21	1.08 ± 0.24	-1.38 ± 0.26	0.92 ± 0.23
0.54 ± 0.21	4.00 ± 0.69	-0.27 ± 0.20	-0.08 ± 0.33
1.38	0.45	0.93	0.37
0.92	0.97	0.94	0.98
13	20	8	91
35.63	44.43	42.26	17.61
40.39	56.28	54.41	13.93
NA	12.13	08.53	07.60
NA	25.50	19.80	18.85
NA	03.32	01.85	01.40
29.94	33.83	26.71	15.25
13.62	13.23	13.03	09.22
SC	SC	SC	SI
I	I	I	D

Table S2. Correlation matrix (Pearson's r) between the estimates of pollen limitation (fruit set, seed number, seed weight and germination) of the hummingbird-pollinated plant species of a tropical montane forest, Brazil. Bold values represent significative correlations at $p < 0.05$ level.

	Fruit set	Seed number	Seed weight
Fruit set	-	**0.62**	-0.02
Seed number	-	-	-0.23
Seed weight	-	-	-
Germination	-	-	-

Table S3. Phylogenetic signal (Blomberg's *K*[11]) of the network indices and traits of the hummingbird-pollinated species of a tropical montane forest, Brazil. The *K* statistic estimates the correlation trait variation with the corresponding phylogenetic distance per species. *K* values > than 1 indicate species are more similar than expected given their phylogenetic relatedness under a Brownian-motion model of evolution. *K* values < than 1 indicate that c species are less similar than expected given their phylogenetic relatedness by the same model[11]. We randomly reshuffled species in 10,000 null phylogenies under a Brownian-motion model of branching and calculated the *K* statistic for each random tree. To assess significance, we estimated if the observed *K* value for each trait departed from the null distribution of 10,000 random values of *K*. We could assess if the observed *K* differed from 1, indicating departures from the expected evolution under Brownian motion; and if the observed *K* differed from 0, indicating some degree of phylogenetic signal. Phylogenetic signal procedures were done with the *multiPhylosignal* function of the *picante* package[12] and *phylosig* function of the *phytools* package[13] in the R environment. Bold values indicate significant departures of *K* values from 1 or from 0.

Variable	***K***	**p (*K* different from 1)**	**p (*K* different from 0)**
Influential degree	0.15	< **0.001**	0.602
Influenced degree	0.16	**<0.001**	0.641
Flower abundance	0.59	**0.031**	**0.021**
Effective length	0.19	< **0.001**	0.694
Anther height	0.19	< **0.001**	0.564
Nectar volume	1.31	0.077	**0.005**
Nectar concentration	0.21	< **0.001**	0.521
Nectar sugar content	0.47	**0.015**	0.067
CCB	0.47	**0.015**	**0.002**
ACB	0.21	< **0.001**	0.472
Mating system	0.30	**0.003**	**0.042**
Pollinator dependence	0.36	**0.003**	**0.030**

References

1. Zanne et al. 2014. Three keys to the radiation of angiosperms into freezing environments. *Nature* **506**, 89-92. (doi:10.1038/nature12872)

2. Qian H, Jin Y. 2016. An updated megaphylogeny of plants, a tool for generating plant phylogenies and an analysis of phylogenetic community structure. *J. Plant. Ecol.* **9**, 233-239. (doi:10.1093/jpe/rtv047)

3. Givnish TJ et al. 2011. Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae: insights from an eight-locus plastid phylogeny. *Am. J. Bot.* **98**, 872-895. (doi:10.3732/ajb.1000059)

4 . Wolowski M, Ashman TL, Freitas L. 2013. Community-wide assessment of pollen limitation in hummingbird-pollinated plants of a tropical montane rain forest. *Ann. Bot.* **112**, 903-910. (doi:10.1093/aob/mct140)

5. Müller CB, Adriaanse ICT, Belshaw R, Godfray HCJ. 1999. The structure of an aphid-parasitoid community. *J. Anim. Ecol.* **68**, 346-370. (doi:10.1046/j.1365-2656.1999.00288.x)

6. Canela MBF. 2006. Interações entre plantas e beija-flores numa comunidade de Floresta Atlântica Montana em Itatiaia, RJ. PhD Thesis, Universidade Estadual de Campinas, Campinas, SP, Brasil.

7. Bergamo PJ, Wolowski M, Maruyama PK, Vizentin-Bugoni J, Sazima M. 2018. Trait patterns across space and time suggest an interplay of facilitation and competition acting on Neotropical hummingbird-pollinated plant communities. *Oikos* **127**, 1690-1700. (doi:10.1111/oik.05571)

8. Bergamo PJ, Wolowski M, Telles FJ, Brito VLG, Varassin IG, Sazima M. 2019. Bracts and long-tube flowers of hummingbird-pollinated plants are conspicuous to hummingbirds but not to bees. Biol. J. Linn. Soc. **126**, 533-544. (doi:10.1093/biolinnean/bl217)

9. Wolowski M, Saad CF, Ashman TL, Freitas L. 2013. Predominance of self-compatibility in hummingbird-pollinated plants in the Neotropics. *Sci. Nat.* **100**, 69-79. (doi:10.1007/s00114-012-0995-0)

10. Wolowski M, Carvalheiro LG, Freitas L. 2017. Influence of plant-pollinator interactions on the assembly of plant and hummingbird communities. *J. Ecol.* **105**, 332-344. (doi:10.1111/1365-2745.12684)

11. Blomberg SP, Garland Jr T, Ives AR. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**, 717-745. (doi:10.1111/j/0014-3820.2003.tb00285.x)

12. Kembel SW, Cowan PD, Helmus MR, Cornwll WK, Morlon H, Ackerly DD, Blomber SP, Webb CO. 2010. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* **26**, 1463-1464. (doi:10.1093/bioinformatics/btq166)

13. Revell LJ. 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* **3**, 217-223. (doi:10.1111/j.2041-210X.2011.00169.x)

CONCLUSÃO GERAL

Utilizamos uma abordagem ao nível da comunidade para entender quais fatores determinam os efeitos indiretos mediados por polinizadores e as consequências destes efeitos para a estrutura e dinâmica de comunidade de plantas. Realizamos a amostragem nos campos de altitude tropicais, um ecossistema no qual esta abordagem nunca havia sido realizada. Desenvolvemos e aplicamos a abordagem de redes para identificar efeitos indiretos a uma comunidade de plantas de dunas mediterrâneas e à flora ornitófila de uma Floresta tropical montana. A alta variação na polinização (de taxas de visitação à taxa de germinação das sementes) entre as espécies de planta mediou processos ecológicos em todas as comunidades estudadas, indicando a polinização como um eixo importante do nicho das plantas. Esta variação se refletiu em componentes do sucesso reprodutivo das plantas, o que permite inferir a polinização como um possível processo estruturador destas comunidades. A polinização atuou como processo estabilizador da coexistência ao favorecer espécies de plantas raras em comparação a abundantes nos Campos de altitude. Além disso, as diferenças no sucesso reprodutivo, devido à polinização, se refletiram em padrões funcionais da comunidade de plantas. Por fim, ressaltamos como características ecológicas e atributos das plantas mediam as interações indiretas entre plantas mediadas por polinizadores.

Aumentar a quantidade de estudos e variedade de ecossistemas nos quais se elucida a natureza dos efeitos indiretos mediados por polinizadores possibilitaria responder perguntas ao nível biogeográfico e macroecológico. Podemos mudar a escala de análise e hipotetizar que a prevalência de competição *vs*. facilitação na polinização ao nível da comunidade também esteja estruturada no espaço. Por exemplo, existe uma tendência latitudinal de maior competição *vs*. facilitação em latitudes menores, refletindo o efeito da riqueza na limitação polínica? Ou então, fatores climáticos e geográficos podem explicar a prevalência de competição *vs*. facilitação ao redor do globo? Fatores relacionados a histórias biogeográficas e evolutivas distintas também podem ter efeito, como por exemplo, comunidades com climas mais estáveis ao longo do tempo geológico exibirem maior especialização e por consequência, maior importância da competição em relação a facilitação. Espera-se que os trabalhos desta tese incentivem a aplicação de abordagens ao nível da comunidade em outros locais.

Processos denso-dependentes negativos na polinização favoreceram a coexistência entre plantas raras e abundantes nos campos de altitude. Este resultado divergiu do encontrado em comunidades de *Protea*, nas quais a denso-dependência positiva aumentaria as vantagens de espécies abundantes sobre raras (Nottebrock et al. 2017). Apenas um estudo em uma comunidade de *fynbos* também encontrou evidências de denso-dependência negativa na polinização ao nível da comunidade (Benadi & Pauw 2018). Os *fynbos* e os campos de altitude possuem histórias biogeográficas e composição de linhagens distintas de plantas. Além disso, a grande quantidade de sistemas de polinização especializados reportados para os *fynbos* indica que este ecossistema deve ser relativamente mais especializado que a flora altamente generalizada dos campos de altitude. Porém, as taxas de visitação são baixas nas duas comunidades (0.85.visitas.flor$_{-1}$.hora$_{-1}$ em média nos campos de altitude *vs*. 0.76 em média nos fynbos). Isto leva a especular que a denso-dependência negativa na polinização pode ocorrer em comunidades bastante distintas entre si, desde que a abundância/atividade de polinizadores seja baixa.

Espera-se denso-dependência negativa quando há especialização, pois a especialização intensifica a competição intraspecífica em relação a interspecífica. Como consequência, espécies abundantes estariam sob maior intensidade de competição intraspecífica que espécies raras em comunidades especializadas (Pauw 2013). De fato, as plantas especialistas dos campos de altitude estavam sob mais denso-dependência negativa que as generalistas, indo de acordo com a teoria geral. No entanto, como explicar a denso-dependência negativa geral em uma comunidade generalizada? Demonstramos que facilitação na polinização promove a coexistência pois espécies raras se beneficiaram mais que abundantes e assim, contribuindo para gerar denso-dependência negativa nesta comunidade generalizada como um todo. Portanto, postulamos que a facilitação deve ser especialmente importante em comunidades generalizadas. Uma evidência neste sentido é que espécies generalistas foram mais facilitadas que especialistas nos campos de altitude. Ainda, as baixas taxas de visitação podem ser um fator adicional, pois espera-se maior risco de extinção local para plantas raras em comparação a abundantes neste cenário (Ghazoul 2005). Portanto, a facilitação mediando a coexistência deve se tornar mais importante em cenários que as espécies raras estão sob maior risco de extinção (Feldman et al. 2004). Desta maneira, abre-se caminho para expandir a teoria de coexistência em polinização e incluir fatores como a relação entre generalização e facilitação nesta equação.

O padrão funcional das plantas em cofloração nos campos de altitude estava de acordo com o previsto pela teoria de montagem de comunidades (Sargent & Ackerly 2008): maior similaridade floral entre espécies no pico de floração da comunidade. Devido a prevalência de facilitação na polinização desta comunidade, pudemos associar de maneira inequívoca padrão e processo. Ainda, espécies sincrônicas e com cores de flor similares entre si exibiram maior sucesso reprodutivo, resultando no padrão geral da comunidade. Desta maneira, a incorporação de medidas de sucesso reprodutivo elucidou por quais vias a facilitação levou ao padrão de similaridade floral. Também fizemos uma ligação com padrões denso-dependentes: os sinais da facilitação na estrutura da comunidade foram mais evidentes para plantas raras que abundantes. Portanto, um mesmo processo (facilitação) influenciou a coexistência e determinou a estrutura da comunidade. Isto demonstra que os efeitos da polinização na coexistência podem ser reforçados através da estrutura da comunidade (Benadi 2015).

Inferir a polinização como um processo estruturador de comunidades têm sido cada vez mais comum e atribuímos isto a difusão dessas ideias devido ao arcabouço teórico proposto por Sargent e Ackerly (2008). Porém, ainda é incerto se a polinização pode estruturar outros aspectos das comunidades de plantas. Uma expectativa teórica é de que a facilitação resulte em agregações espaciais entre as plantas que se beneficiam (Brooker et al. 2008). No entanto, ainda não foi testado se efeitos indiretos mediados por polinizadores influenciam a estrutura espacial na escala da comunidade. Neste sentido, facilitação geraria agregados heterospecíficos enquanto competição e filtragem ambiental gerariam agregados coespecíficos. Em outra frente, fornecemos evidências de processos denso-dependentes na polinização, mas não se estes processos determinam a distribuição de abundâncias na comunidade. A facilitação pode promover a manutenção de espécies raras se estas se beneficiam mais que abundantes. Já a competição pode causar exclusão de espécies raras se estas competem com as abundantes, intensificando efeitos Allee. Desta maneira, facilitação geraria distribuições de abundâncias distintas da competição. Portanto, é necessário desenvolver um arcabouço teórico e analítico que inclua aspectos demográficos e permita prever o efeito de competição *vs*. facilitação na polinização em outros aspectos da estrutura e dinâmica da comunidade de plantas.

Nas dunas mediterrâneas, competição na polinização era esperada devido a relativa alta especialização da rede planta-polinizador (Castro-Urgal & Traveset 2014). Já para a flora ornitófila de floresta montana, a facilitação na polinização era esperada devido a evidências da relação entre a sincronia de floração com menor limitação polínica e a partir da estrutura funcional desta comunidade (Wolowski et al. 2017, Bergamo et al. 2018). Desta maneira, pudemos validar o arcabouço teórico e analítico proposto e incentivar o seu uso em outras comunidades planta-polinizador. Uma implicação destes resultados é que as redes planta-polinizador também contém informações sobre as interações indiretas entre as plantas. Porém, pouco sabemos sobre como os efeitos indiretos mediados por polinizadores podem determinar a estrutura de redes de interação.

Esforços recentes têm tentado conectar a teoria de montagem de comunidades e a estrutura de redes de interação (Ponisio et al. 2019). Devido a associação entre generalização e facilitação na polinização encontrada nos campos de altitude, postulamos que comunidades marcadas por facilitação devem exibir alta generalização das redes e aninhamento. Por outro lado, a associação entre especialização e competição na polinização sugere que comunidades com prevalência de competição devem exibir alta especialização das redes e modularidade. Ainda, a denso-dependência na polinização também pode influenciar a estrutura das redes de interação. Denso-dependência negativa intensa indica forte competição intraspecífica para plantas abundantes. Estas plantas podem então exibir alta generalização como resposta a diminuir a competição intraspecífica e gerar aninhamento. Já a denso-dependência positiva pode levar a efeitos Allee e reforçar as desvantagens de plantas raras. Neste caso, as plantas raras podem se beneficiar ao segregar seu nicho e contribuir para gerar modularidade. Portanto, estudos futuros podem associar efeitos indiretos mediados por polinizadores e a estrutura das redes de interação planta-polinizador.

Com a abordagem de redes ecológicas, identificamos os atributos das espécies de planta que causam os efeitos indiretos e das que recebem estes efeitos. Desta maneira, caracterizamos a assimetria inerente aos efeitos indiretos mediados por polinizadores (Carvalheiro et al. 2014, Bergamo et al. 2017). Apesar de serem sistemas completamente distintos, plantas generalistas e atrativas afetaram plantas especialistas e menos atrativas nas duas comunidades. Ainda, identificamos este mesmo padrão em uma comunidade marcada por competição e em outra marcada por facilitação. As espécies generalistas e atrativas concentram a atividade dos polinizadores. Por outro lado, as especialistas e menos atrativas que compartilham estes polinizadores podem se prejudicar ou se beneficiar do papel de atração que as generalistas desempenham na comunidade. Mesmo sendo apenas duas comunidades, estes resultados sugerem um interessante padrão de invariância ecológica na assimetria dos efeitos indiretos.

A abordagem de comunidades contribuiu para identificar fatores ecológicos que determinam a direção da denso-dependência na polinização e dos efeitos indiretos mediados por polinizadores (competição *vs*. facilitação). Ainda, descrevemos as assimetrias dos efeitos indiretos, ou seja, quais espécies causam e quais recebem estes efeitos. Destacamos o papel de padrões de interação (especialização *vs*. generalização), o grupo funcional de polinizador e atributos reprodutivos (*display* floral, cor das pétalas, morfologia floral, atributos do néctar e sistema de autoincompatibilidade) mediando os efeitos indiretos. A abordagem de comunidades também contribuiu para entender como os efeitos indiretos influenciam a coexistência, estrutura e dinâmica das comunidades de plantas. A polinização pode gerar denso-dependência negativa (desvantagens para espécies abundantes), benefícios para espécies raras devido a facilitação, estrutura funcional agregada nas comunidades e determinar diferenças interespecíficas no sucesso reprodutivo. A incorporação da teoria de coexistência, medidas de sucesso reprodutivo e redes ecológicas são promissoras para elucidar a ecologia dos efeitos indiretos mediados por polinizadores. Entender melhor a ecologia dos efeitos indiretos mediados por polinizadores também tem impactos na sociedade devido às possíveis retroalimentações entre estes efeitos e agentes de mudança global, como os declínios de polinizadores e invasões biológicas.

REFERÊNCIAS BIBLIOGRÁFICAS

Abrams, P. A., & Matsuda, H. (1996). Positive indirect effects between prey species that share predators. *Ecology*, 77, 610-616.

Arceo-Gómez, G., & Ashman, T-L. (2014)a. Heterospecific pollen receipt affects self pollen more than outcross pollen: implications for mixed-mating plants. *Ecology*, 95, 2946-2952.

Arceo-Gómez, G., & Ashman, T-L. (2014)b. Coflowering community context influences female fitness and alters the adaptive value of flower longevity in *Mimulus guttatus*. *The American Naturalist*, 183, E50-E63.

Arceo-Gómez, G., & Ashman, T-L. (2016). Invasion status and phylogenetic relatedness predict cost of heterospecific pollen receipt: implications for native biodiversity decline. *Journal of Ecology*, 104, 1003-1008.

Armbruster, W.S., Edwards, M.R., Debevec, E.M. (1994). Floral character displacement generates assemblage structure of Western Australian triggerplants (*Stylidium*). *Ecology*, 75, 315-329.

Albor, C., García-Franco, J.G., Parra-Tabla, V., Díaz-Castelazo, C., Arceo-Gómez, G. (2019). Taxonomic and functional diversity of the co-flowering community differentially affect *Cakile edentula* pollination at different spatial scales. *Journal of Ecology*, 107, 2167-2181.

Albrecht, M., Ramis, M.R. & Traveset, A. (2016). Pollinator-mediated impacts of invasive plants on the pollination of native plants: the role of spatial scale and distinct behaviour among pollinator guilds. *Biological Invasions*, 18, 1801-1812.

Ashman, T-L., Knight, T.M., Steets, J.A., Amarasekare, P., Burd, M., Campbell, D.R., Dudash, M.R. et al. (2004). Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology*, 85, 2408-2421.

Ashman, T-L., & Arceo-Gómez, G. (2013). Toward a predictive understanding of the fitness costs of heterospecific pollen receipt and its importance in co-flowering communities. *American Journal of Botany*, 100, 1061-1070.

Badillo-Montaño, R., Aguirre, A., Munguía-Rosas, M.A. (2019). Pollinator-mediated interactions between cultivated papaya and co-flowering plant species. *Ecology and Evolution*, 9, 587-597.

Baker, H.G. (1967). Support for Baker's law – as a rule. *Evolution*, 21, 853-856.

Bartomeus, I., Vilà, M., Santamaría, L. (2008). Contrasting effects of invasive plants in plant-pollinator networks. *Oecologia*, 155, 761-770.

Bell, J.M., Karron, J.D., Mitchell, R.J. (2005). Interspecific competition for pollination lowers seed production and outcrossing in *Mimulus ringens*. *Ecology*, 86, 762-771.

Benadi, G. (2015). Requirements for plant coexistence through pollination niche partitioning. *Proceedings of the Royal Society B - Biological Sciences*, 282, rspb.2015.0117.

Benadi, G. & Pauw, A. (2018). Frequency dependence of pollinator visitation rates suggests that pollination niches can allow plant species coexistence. *Journal of Ecology*, 106, 1982-1901.

Bergamo, P.J., Wolowski, M., Maruyama, P.K., Vizentin-Bugoni, J., Carvalheiro, L.G. & Sazima, M. (2017). The potential indirect effects among plants via shared hummingbird pollinators are structured by phenotypic similarity. *Ecology*, 98, 1849-1858.

Bergamo, P.J., Wolowski, M., Maruyama, P.K., Vizentin-Bugoni, J. & Sazima, M. (2018). Trait patterns across space and time suggest and interplay of facilitation and competition acting on Neotropical hummingbird-pollinated plant communities. *Oikos*, 127, 1690-1700.

Bergamo, P.J., Wolowski, M., Telles, F.J., Brito, V.L.G., Varassin, I.G., & Sazima, M. (2019). Bracts and long-tube flowers of hummingbird-pollinated plants are conspicuous to hummingbirds but not to bees. *Biological Journal of the Linnean Society*, 126, 533-544.

Braun, J., & Lortie, C.J. (2019). Finding the bees knees: a conceptual framework and systematic review of the mechanisms of pollinator-mediated facilitation. *Perspectives in Plant Ecology, Evolution and Systematics*, 36, 33-40.

Briscoe Runquist, R., & Stanton, M.L. (2013). Asymmetric and frequency-dependent pollinator-mediated interactions may influence competitive displacement in two vernal pool plants. *Ecology Letters*, 16, 183-190.

Brooker, R.W., Maestre, F.T., Callaway, R.M., Lortie, C.L., Cavieres, L.A., Kunstler, G., Liancourt, P. et al. (2008). Facilitation in plant communities: the past, the present, and the future. *Journal of Ecology*, 96, 18-34.

Brown, B.J., Mitchell, R.J., Graham, S.A. (2002). Competition for pollination between an invasive species (purple loosestrife) and a native congener. *Ecology*, 83, 2328-2336.

Bruckman, D., & Campbell, D.R. (2016). Pollination of a native plant changes with distance and density of invasive plants in a simulated biological invasion. *American Journal of Botany*, 103, 1-8.

Buzato, S., Sazima, M., & Sazima, I. (2000). Hummingbird-pollinated floras at three Atlantic forest sites. *Biotropica*, 32, 824-841.

Callaway, R. M., & Walker, L. R. (1997). Competition and facilitation: a synthetic approach to interactions in plant communities. *Ecology*, 78, 1958-1965.

Campbell, D.R. (1985). Pollen and gene dispersal: the influences of competition for pollination. *Evolution*, 39, 418-431.

Campbell, D.R., & Motten, A.F. (1985). The mechanism of competition for pollination between two forest herbs. *Ecology*, 66, 554-563.

Canela, M.B.F. (2006). Interações entre plantas e beija-flores numa comunidade de Floresta Atlântica Montana em Itatiaia, RJ. PhD Thesis, Universidade Estadual de Campinas, Campinas, SP, Brasil.

Carvalheiro, L.G., Biesmeijer, J.C., Benadi, G., Fründ, J., Stang, M., Bartomeus, I. et al. (2014). The potential for indirect effects between co-flowering plants via shared pollinators depends on resource abundance, accessibility and relatedness. *Ecology Letters*, 17, 1389-1399.

Caruso, C.M. (1999). Pollination of *Ipomopsis aggregata* (Polemoniaceae): effects of intra- vs. interspecific competition. *American Journal of Botany*, 86, 663-668.

Castro-Urgal, R., & Traveset, A. (2014). Differences in flower visitation networks between an oceanic and a continental island. *Botanical Journal of the Linnean Society*, 174, 478-488.

Charlebois, J.A., & Sargent, R.D. (2017). No consistent pollinator-mediated impacts of alien plants on natives. *Ecology Letters*, 20, 1479-1490.

Danieli-Silva, A., Souza, J.M.T., Donatti, A.J., Campos, R.P., Vicente-Silva, J., Freitas, L. et al. (2012). Do pollination syndromes cause modularity and predict interactions in a pollination network in tropical high-altitude grasslands? *Oikos*, 121, 35-43.

Dormann, C.F., Fründ, J., Martin Schaefer, H. (2017). Identifying causes of patterns in ecological networks: opportunities and limitations. *Annual Review of Ecology, Evolution, and Systematics*, 48, 559-584.

Eisen, K.E., & Geber, M.A. (2018). Ecological sorting and character displacement contribute to the structure of communities of *Clarkia* species. *Journal of Evolutionary Biology*, 31, 1440-1458.

Eisen K.E., Wruck, A.C., Geber, M.A. (2020). Floral density and co-occurring congeners alter patterns of selection in annual plant communities. *Evolution*, doi:10.1111/evo.13960.

Essenberg, C.J. (2012). Explaining variation in the effect of floral density on pollinator visitation. *American Naturalist*, 180, 153-166.

Evans, T.M., Cavers, S., Ennos, R., Vanbergen, A.J., & Heard, M.S. (2017). Florally rich habitats reduce insect pollination and the reproductive success of isolated plants. *Ecology and Evolution*, 7, 6507-6518.

Feldman, T.S., Morris, W.F. & Wilson, W.G. (2004). When can two plant species facilitate each other's pollination? *Oikos*, 105, 197-207.

Fishman, L. & Wyatt, R. (1999). Pollinator-mediated competition, reproductive character displacement, and the evolution of selfing in *Arenaria uniflora* (Caryophyllaceae). *Evolution*, 53, 1723-1733.

Fornoff, F., Klein, A-M., Hartig, F., Benadi, G., Venjakob, C., Martin Schaefer, H., & Ebeling, A. (2017). Functional flower traits and their diversity drive pollinator visitation. *Oikos*, 126, 1020-1030.

Freitas, L. & Sazima, M. (2006). Pollination in a tropical high-altitude grassland in Brazil: interactions at the community level. *Annals of the Missouri Botanical Garden*, 93, 465-516.

Frost, C.M., Peralta, G., Rand, T.A., Didham, R.K., Varsani, A., Tylianakis, J.M. (2016). Apparent competition drives community-wide parasitism rates and changes in host abundance across ecosystem boundaries. *Nature Communications*, 7, 12644.

Gerhold, P., Cahill Jr, J.F., Winter, M., Bartish, I.G., Prinzing, A. (2015). Phylogenetic patterns are not proxies of community assembly mechanisms (they are far better). *Functional Ecology*, 29, 600-614.

Ghazoul, J. (2005). Pollen and seed dispersal among dispersed plants. *Biological Reviews*, 80, 413-443.

Ghazoul, J. (2006). Floral diversity and the facilitation of pollination. *Journal of Ecology*, 94, 295-304.

Gibson, M.R., Richardson, D.M., Pauw, A. (2012). Can floral traits predict an invasive plant's impact on native plant-pollinator communities? *Journal of Ecology*, 100, 1216-1223.

Goodell, K., & Parker, I.M. (2017). Invasion of a dominant floral resource: effects on the floral community and pollination of native plants. *Ecology*, 98, 57-69.

Gumbert, A., Kunze, J., & Chittka, L. (1999). Floral colour diversity in plant communities, bee colour space and a null model. *Proceedings of the Royal Society B: Biological Sciences*, 266, 1711-1716.

Hegland, S.J., & Totland, Ø. (2012). Interactions for pollinator visitation and their consequences for reproduction in a plant community. *Acta Oecologica*, 43, 95-103.

Hegland, S.J. (2014). Floral neighbourhood effects on pollination success in red clover are scale-dependent. *Functional Ecology*, 28, 561-568.

Hegland, S.J., Grytnes, J.-A. & Totland, O. (2009). The relative importance of positive and negative interactions for pollinator attraction in a plant community. *Ecological Research*, 24, 929-936.

Herrera, C.M. (2020). Flower traits, habitat, and phylogeny as predictors of pollinator service: a plant community perspective. *Ecological Monographs*, doi:10.1002/ecm.1402.

Heystek, A. & Pauw, A. (2014). Does competition for pollinators contribute to structuring *Erica* communities? *Journal of Vegetation Science*, 25, 648-656.

Holt, R. D. (1977). Predation, apparent competition, and the structure of prey communities. *Theoretical Population Biology*, 12, 197-229.

Ishii, H. S., Kubota, M. X., Tsujimoto, S. G., & Kudo, G. (2019). Association between community assemblage of flower colours and pollinator fauna: a comparison between Japanese and New Zealand alpine plant communities. *Annals of Botany*, 123, 533-541.

Johnson, C.A. & Bronstein, J.L. (2019). Coexistence and competitive exclusion in mutualism. *Ecology*, 100, e02708.

Johnson, S.D., Peter, C.I., Nilsson, L.A., & Ågren, J. (2003). Pollination success in a deceptive orchid is enhanced by co-occurring rewarding magnet plants. *Ecology*, 84, 2919-2927.

Johnson, C. A., & Bronstein, J. L. (2019). Coexistence and competitive exclusion in mutualism. *Ecology*, 100, e02708.

Kay, K.M. (2006). Reproductive isolation between two closely related hummingbird pollinated neotropical gingers. *Evolution*, 60, 538-552.

Knight, T.M. (2003). Floral density, pollen limitation, and reproductive success in *Trillium grandiflorum*. *Oecologia*, 137, 557-563.

Kunin, W.E. (1993). Sex and the single mustard: population density and pollinator behavior effects on seed-set. *Ecology*, 74, 2145-2160.

Kunin, W.E. (1997). Population size and density effects in pollination: pollinator foraging and plant reproductive success in experimental arrays of *Brassica kaber*. *Journal of Ecology*, 85, 225-234.

Lachmuth, S., Henrichmann, C., Horn, J., Pagel, J. & Schurr, F.M. (2018). Neighbourhood effects on plant reproduction: an experimental-analytical framework and its application to the invasive *Senecio inaequidens*. *Journal of Ecology*, 106, 761-773.

Lanuza, J.B., Bartomeus, I. & Godoy, O. (2018). Opposing effects of floral visitors and soil conditions on the determinants of competitive outcomes maintain species diversity in heterogeneous landscapes. *Ecology Letters*, 21, 865-874.

Lázaro, A., Lundgren, R. & Totland, Ø. (2009). Co-flowering neighbors influence the diversity and identity of pollinator groups visiting plant species. *Oikos*, 118, 691-702.

Lázaro, A., Jakobosson, A. & Totland, Ø. (2013). How do pollinator visitation rate and seed set related to species' floral traits and community context? *Oecologia*, 173, 881-893.

Lázaro, A., Lundgren, R. & Totland, Ø. (2014). Experimental reduction of pollinator visitation modifies plant-plant interactions for pollination. *Oikos*, 123, 1037-1048.

Lázaro, A., Lundgren, R., & Totland Ø. (2015). Pollen limitation, species' floral traits and pollinator visitation: different relationships in contrasting communities. *Oikos*, 124, 174–186.

Lázaro, A., Gómez-Martínez, C., Alomar, D., González-Estévez, M.A., & Traveset, A. (2020). Linking species-level network metrics to flower traits and plant fitness. *Journal of Ecology*, doi: 10.1111/1365.2745.13334.

Lopezaraiza-Mikel, M., Hayes, R.B., Whalley, M.R., Memmott, J. (2007). The impact of an alien plant on a native plant-pollinator network: an experimental approach. *Ecology Letters*, 10, 539-550.

Lundgren, R., Lázaro, A., & Totland, Ø. (2016). Experimental simulation of pollinator decline causes community-wide reductions in seedling diversity and abundance. *Ecology*, 97, 1420-1430.

Luttbeg, B., Rowe, L., & Mangel, M. (2003). Prey state and experimental design affect relative size of trait-and density-mediated indirect effects. *Ecology*, 84, 1140-1150.

MacArthur, R., & Levins, R. (1967). The limiting similarity, convergence, and divergence of coexisting species. *The American Naturalist*, 101, 377-385.

McEwen, J.R., & Vamosi, J.C. (2010). Floral colour versus phylogeny in structuring subalpine flowering communities. *Proceedings of the Royal Society B – Biological Sciences*, 277, rspb.2010.0501.

Mitchell, R.J., Flanagan, R.J., Brown, B.J., Waser, N.M. & Karron, J.D. (2009). New frontiers in competition for pollination. *Annals of Botany*, 103, 1403-1413.

Moeller, D.A. (2004). Facilitative interactions among plants via shared pollinators. *Ecology*, 85, 3289-3301.

Morales, C.L., & Traveset, A. (2008). Interspecific pollen transfer: magnitude, prevalence and consequences for plant fitness. *Critical Reviews in Plant Sciences*, 27, 221-238.

Morales, C.L., & Traveset, A. (2009). A meta-analysis of impacts of alien vs. native plants on pollinator visitation and reproductive success of co-flowering native plants. *Ecology Letters*, 12, 716-728.

Moreira-Hernandéz, J.I., & Muchhala, N. (2019). Importance of pollinator-mediated interspecific pollen transfer for Angiosperm evolution. *Annual Review of Ecology, Evolution and Systematics*, 50, 191-217.

Morris, R.J., Lewis, O.T., Godfray, H.C.J. (2004). Experimental evidence for apparent competition in a tropical forest food web. *Nature*, 428, 310-313.

Muchhala, N., Brown, Z., Armbruster, W.S., Potts, M.D. (2010). Competition drives specialization in pollination systems through costs to male fitness. *The American Naturalist*, 176, 732-743.

Muchhala, N., Johnsen, S., & Smith, S. D. (2014). Competition for hummingbird pollination shapes flower color variation among in Andean Solanaceae. *Evolution*, 68, 2275-2286.

Muchhala, N., & Potts, M.D. (2006). Character displacement among bat-pollinated flowers of the genus *Burmeistera*: analysis of mechanis, process and pattern. *Proceedings of the Royal Socienty B - Biological Sciences*, 274, rsbp.2007.0670

Müller, C.B., Adriaanse, I.C.T., Belshaw, R., & Godfray, H.C.J. (1999). The structure of an aphid-parasitoid community. *Journal of Animal Ecology*, 68, 346-370.

Murcia, C., & Feinsinger, P. (1996). Interspecific pollen loss by hummingbirds visiting flower mixtures: effects of floral architecture. *Ecology*, 77, 550-560.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., Fonseca, G.A.B., Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, 403, 853-858.

Nottebrock, H., Schmid, B., Mayer, K., Devaux, C., Esler, K., Böhning-Gaese, K. et al. (2017). Sugar landscapes and pollinator-mediated interactions in plant communities. *Ecography*, 40, 1129-1138.

Ogilvie, J.E., & Thomson, J.D. (2016). Site fidelity by bees drives pollination facilitation in sequentially blooming plant species. *Ecology*, 97, 1442-1451.

Ohgushi, T., Schmitz, O., & Holt, R. D. (Eds.). (2012). *Trait-mediated indirect interactions: ecological and evolutionary perspectives*. Cambridge University Press, Cambridge, UK.

Ollerton, J., Winfree, R., Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, 120, 321-326.

Pace, M. L., Cole, J. J., Carpenter, S. R., & Kitchell, J. F. (1999). Trophic cascades revealed in diverse ecosystems. *Trends in Ecology & Evolution*, 14, 483-488.

Pauw, A. (2013). Can pollination niches facilitate plant coexistence? *Trends in Ecology and Evolution*, 28, 30-37.

Pauw, A., & Johnson, C.M. (2018). Mutualism between co-occurring plant species in South Africa’s Mediterranean climate heathland is meadiated by birds. *Plant Biology*, 20, 224-230.

Pauw, A. (2019). A bird’s-eye view of pollination: biotic interactions as drivers of adaptation and community change. *Annual Review of Ecology, Evolution and Systematics*, 50, 477-502.

Ponisio, L.C., Valdovinos, F.S., Allhof, K.T., Gaiarsa, M.P., Barner, A., Guimarães Jr., P.R., Hembry, D.H. et al. (2019). A network perspective for community assembly. *Frontiers in Ecology and Evolution*, 7, 103.

Rathcke, B. (1983). Competition and facilitation among plants for pollination. In: Pollination Biology {ed. Real, L.}. Academic Press, Orlando, FL, pp 305-325.

Ribeiro, K.T., Medina, B.M.O. & Scarano, F.B. (2007). Species composition and biogeographic relations of the rock outcrop floral on the high plateau of Itatiaia, SE-Brazil. *Brazilian Journal of Botany*, 30, 623-639.

Sargent, R.D., & Ackerly, D.D. (2008). Plant-pollinator interactions and the assembly of plant communities. *Trends in Ecology and Evolution*, 23, 123-130.

Sargent, R.D., Kembel, S.W., Emery, N.C., Forrestel, E.J., & Ackerly, D.D. (2011). Effect of local community phylogenetic structure on pollen limitation in an obligately insect-pollinated plant. *American Journal of Botany*, 98, 283-289.

Schluter, D. (2000). Ecological character displacement in adaptive radiation. *The American Naturalist*, 156, S4-S16.

Seifan, M., Hoch, E.-M., Hanoteaux, S. & Tielboerger, K. (2014). The outcome of shared pollination services is affected by the density and spatial pattern of an attractive neighbour. *Journal of Ecology*, 102, 953-962.

Soliveres, S., Maestre, F.T., Ulrich, W., Manning, P., Boch, S., Bowker, M.A., Prati, D., Delgado-Baquerizo, M., Quero, J.L., Schöning, I. & Gallardo, A. 2015. Intransitive competition is widespread in plant communities and maintains their species richness. *Ecology letters*, 18, 790-798.

Strauss, S. Y. (1991). Indirect effects in community ecology: their definition, study and importance. *Trends in Ecology & Evolution*, 6, 206-210.

Strauss, S. Y., & Irwin, R. E. (2004). Ecological and evolutionary consequences of multispecies plant-animal interactions. *Annual Review of Ecology, Evolution and Systematics*, 35, 435-466.

Thomson, J.D., Fung, H.F. & Ogilvie, J.E. (2019). Effects of spatial patterning of co-flowering plant species on pollination quantity and purity. *Annals of Botany*, 123, 303-310.

Tilman, D. (1982). *Resource competition and community structure*. Princeton university press, New Jersey, USA.

Traveset, A., Castro-Urgal, R., Rotllàn-Puig, X., Lázaro, A. (2018). Effects of habitat loss on the plant-flower visitor network structure of a dune community. *Oikos*, 127, 45-55.

Traveset, A., & Navarro, L. (2018). Plant reproductive ecology and evolution in the Mediterranean islands: state of the art. *Plant Biology*, 20, 63-77.

Tur, C., Saez, A., Traveset, A. & Aizen, M.A. (2016). Evaluating the effects of pollinator-mediated interactions using pollen transfer networks: evidence of widespread facilitation in south Andean plant communities. *Ecology Letters,* 19, 576–586.

Vamosi, J.C., Knight, T.M., Steets, J.A., Mazer, S.J., Burd, M., & Ashman, T-L. (2006). Pollination decays in biodiversity hotspots. *Proceedings of the National Academy of Sciences,* 103, 956-961.

Vandermeer, J., Hazlett, B., & Rathcke, B. (1985). Indirect facilitation and mutualism. In: The biology of mutualism {ed., Boucher, D.B.}. Oxford University Press, New York, USA.

Veloso, H.P., Rangel-Filho, A.L.R., & Lima, J.C.A. (1991). Classificação da vegetação brasileira, adaptada a um sistema universal (1st ed). Brasília, Brasil: IBGE.

Votjkó-E, A., de Bello, F., Durka, W., Kühn, I., & Götzenberger, L. (2020). The neglected importance of floral traits in trait-based plant community assembly. *Journal of Vegetation Science,* doi:10.1111/jvs.12877.

Ward, M., Johnson, S.D. & Zalucki, M.P. (2013). When bigger is not better: intraspecific competition for pollination increases with population size in invasive milkweeds. *Oecologia*, 171, 883-891.

Waser, N.M. (1978). Competition for hummingbird pollination and sequential flowering in two Colorado wildflowers. *Ecology*, 59, 934-944.

Waser, N.M., & Real, L.A. (1979). Effective mutualism between sequentially flowering plant species. *Nature*, 281, 670-672.

Waser, N.M., Chittka, L., Price, M.V., Williams, N.M., Ollerton, J. (1996). Generalization in pollination systems, and why it matters. *Ecology*, 77, 1043-1060.

Webb, C.O., Ackerly, D.D., McPeek, M.A., Donoghue, M.J. (2002). Phylogenies and community ecology. *Annual Review of Ecology and Systematics*, 33, 475-505.

Westphal, C., Bommarco, R., Carré, G., Lamborn, E., Morison, N., Petanidou, T., Potts, S.G. et al. (2007). Measuring bee diversity in different European habitats and biogeographical regions. *Ecological Monographs*, 78, 653-671.

Wolowski, M., Saad, C.F., Ashman, T-L., & Freitas, L. (2013)a. Predominance of self-compatibility in hummingbird-pollinated plants in the Neotropics. *The Science of Nature*, 100, 69-79.

Wolowski, M., Ashman, T-L., & Freitas, L. (2013)b. Community-wide assessment of pollen limitation in hummingbird-pollinated plants of a tropical montane rain forest. *Annals of Botany*, 112, 903-910.

Wolowski, M., Carvalheiro, L.G. & Freitas, L. (2017). Influence of plant-pollinator interactions on the assembly of plant and hummingbird communities. *Journal of Ecology,* 105, 332-334.

Wootton, J. T. (1994). The nature and consequences of indirect effects in ecological communities. *Annual review of ecology and systematics*, 25, 443-466.

Ye, Z-M., Dai, W-K., Jin, X-F., Gituru, R.W., Wang, Q-F. & Yang, C-F. (2014). Competition and facilitation among plants for pollination: can pollinator abundance shift the plant-plant interactions? *Plant Ecology*, 215, 3-13.

ANEXOS

I – Declaração referente à Bioética e Biossegurança

UNICAMP
COORDENADORIA DE PÓS-GRADUAÇÃO
INSTITUTO DE BIOLOGIA
Universidade Estadual de Campinas
Caixa Postal 6109. 13083-970, Campinas, SP, Brasil
Fone (19) 3521-6378. email: cpgib@unicamp.br
IB

DECLARAÇÃO

Em observância ao **§5º do Artigo 1º da Informação CCPG-UNICAMP/001/15**, referente a Bioética e Biossegurança, declaro que o conteúdo de minha Tese de Doutorado, intitulada "***POLLINATOR-MEDIATED INDIRECT EFFECTS ON PLANT COMMUNITIES***", desenvolvida no Programa de Pós-Graduação em Ecologia do Instituto de Biologia da Unicamp, não versa sobre pesquisa envolvendo seres humanos, animais ou temas afetos a Biossegurança.

Assinatura: Pedro J. Bergamo
Nome do(a) aluno(a): Pedro Joaquim Bergamo

Assinatura: Marlies Sazima
Nome do(a) orientador(a): Marlies Sazima

Data: 26/04/2020

II – Declaração referente aos direitos autorais

Declaração

As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Dissertação/Tese de Mestrado/Doutorado, intitulada **POLLINATOR-MEDIATED INDIRECT EFFECTS ON PLANT COMMUNITIES**, não infringem os dispositivos da Lei n.° 9.610/98, nem o direito autoral de qualquer editora.

Campinas, 26/04/2020

Assinatura : Pedro J. Bergamo
Nome do(a) autor(a): **Pedro Joaquim Bergamo**
RG n.° 48.573.183-6

Assinatura : Marlies Sazima
Nome do(a) orientador(a): **Marlies Sazima**
RG n.° 3985551