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CENTRO DE BIOCIÊNCIAS- CCBIO
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA VEGETAL-PPGBV

THATIANY TEIXEIRA BEZERRA

RELAÇÃO DA DINÂMICA DA FLORAÇÃO E DO FLUXO POLÍNICO ENTRE ESPÉCIES SINCRONOPÁTRICAS E ENANTIOSTÍLICAS DE *CHAMAECRISTA* MOENCH. (FABACEAE)

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Orientadora: Profª. Drª. Isabel Cristina Machado

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Ao meu povo.

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Eu sou aquela mulher
a quem o tempo
muito ensinou.
Ensinou a amar a vida.
Não desistir da luta.
Recomeçar na derrota.
Renunciar a palavras e pensamentos negativos.
Acreditar nos valores humanos.
Ser otimista.

Cora Coralina

RESUMO

A diversificação sexual em plantas é um tema central na ecologia da polinização, especialmente em espécies com necessidade de cruzamento entre morfos, como na enantiostilia. Investigamos as influências da dinâmica de produção de morfos e da sincronopatria na fenologia da floração e na reprodução de espécies enantiostílicas, além de verificar a presença e os efeitos da interferência do pólen heteroespecífico (HP) na formação de frutos e sementes em quatro espécies de *Chamaecrista* (Fabaceae) ocorrentes na Reserva do Patrimônio Natural de Maracaípe, PE. No primeiro capítulo, acompanhamos a fenologia e a produção de morfos florais ao longo de dezoito meses e verificamos a ocorrência de isopletia em indivíduos e populações de *Chamaecrista diphylla*, *C. flexuosa*, *C. ramosa* e *C. rotundifolia*. Observamos proporções gerais de morfos semelhantes às descritas em estudos prévios. No entanto, em nível individual, encontramos variações funcionais nos morfos, relacionadas ao número de flores, sugerindo adaptações ainda pouco compreendidas. No segundo capítulo, testamos a influência de pólen heteroespecífico no sucesso reprodutivo das mesmas espécies de *Chamaecrista*. Confirmamos que o HP reduz o fitness reprodutivo, variando conforme as espécies e os morfos envolvidos. Cruzamentos coespecíficos favorecem a frutificação, reforçando o papel da enantiostilia como barreira reprodutiva. Além disso, espécies coflorescentes influenciam a deposição de pólen de forma complexa, dependendo da proporção de mistura de pólen e características florais. Os resultados destacam os mecanismos de isolamento reprodutivo e estratégias adaptativas em ambientes biodiversos, avançando no entendimento da interação planta-polinizador.

Palavras-chave: Hercogamia recíproca, funcionalidade morfológica, fenologia da floração, polinização interespecífica.

ABSTRACT

Sexual diversification in plants is a central theme in pollination ecology, especially in species that require crossbreeding between morphs, such as enantiostyly. We investigated the influences of morph production dynamics and synchronopatry on flowering phenology and reproduction of enantiostylous species, in addition to verifying the presence and effects of heterospecific pollen (HP) interference in fruit and seed formation in four species of Chamaecrista (Fabaceae) occurring in the Maracaípe Natural Heritage Reserve, PE. In the first chapter, we monitored the phenology and production of floral morphs over eighteen months and verified the occurrence of isoplety in individuals and populations of *Chamaecrista diphylla, C. flexuosa, C. ramosa* and *C. rotundifolia*. We observed general proportions of morphs similar to those described in previous studies. However, at the individual level, we found functional variations in morphs related to the number of flowers, suggesting adaptations that are still poorly understood. In the second chapter, we tested the influence of heterospecific pollen on the reproductive success of the same *Chamaecrista* species. We confirmed that HP reduces reproductive fitness, varying according to the species and morphs involved. Conspecific crosses favor fruit set, reinforcing the role of enantiostyly as a reproductive barrier. In addition, co-flowering species influence pollen deposition in a complex way, depending on the pollen mixing ratio and floral characteristics. The results highlight the mechanisms of reproductive isolation and adaptive strategies in biodiverse environments, advancing the understanding of plant-pollinator interactions.

Keywords: Reciprocal herkogamy, morphological functionality, flowering phenology, interspecificpollination.

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values are shown above each plot and asterisks indicate significant differences between groups (***P < 0.001, **P < 0.01, *P < 0.05).

Figure S1 – Daily morph ratios in forty individuals (n=54 days) studied in the period 2019-2020 for the species *C. diphylla*, in a Restinga area in Northeastern Brazil. The gray band on the x-axis indicates the range of variation that corresponds to the isoplethy of the floral morphs (values between 0.25-0.75; cyan points), with the left of it (values between 0-0.24; salmon pink points) indicating the highest frequency of left flowers and the right of it (values between 0.76-1; salmon pink points) indicating the highest frequency of right flowers.

Figure S2– Daily morph ratios in forty individuals (n=54 days) studied in the period 2019-2020 for the species *C. rotundifolia*, in a Restinga area in Northeastern Brazil. The gray band on the x-axis indicates the range of variation that corresponds to the isoplethy of the floral morphs (values between 0.25-0.75; cyan points), with the left of it (values between 0-0.24; salmon pink points) indicating the highest frequency of left flowers and the right of it (values between 0.76-1; salmon pink points) indicating the highest frequency of right flowers.

Figure S3- Daily morph ratios in forty individuals (n=54 days) studied in the period 2019-2020 for the species *C. flexuosa*, in a Restinga area in Northeastern Brazil. The gray band on the x-axis indicates the range of variation that corresponds to the isoplethy of the floral morphs (values between 0.25-0.75; cyan points), with the left of it (values between 0-0.24; salmon pink points) indicating the highest frequency of left flowers and the right of it (values between 0.76-1; salmon pink points) indicating the highest frequency of right flowers.

Figure S4- Daily morph ratios in forty individuals (n=54 days) studied in the period 2019-2020 for the species *C. ramosa*, in a Restinga area in Northeastern Brazil. The gray band on the x-axis indicates the range of variation that corresponds to the isoplethy of the floral morphs (values between 0.25-0.75; cyan points), with the left of it (values between 0-0.24; salmon pink points) indicating the highest frequency of left flowers and the right of it (values between 0.76-1; salmon pink points) indicating the highest frequency of right flowers.

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SUMÁRIO

1. APRESENTAÇÃO

A diversificação sexual nas plantas é um tema central e crescente na ecologia da polinização, especialmente em espécies que necessitam de cruzamentos entre morfos florais, como é o caso da enantiostilia. A enantiostilia é um tipo de polimorfismo floral caracterizado pela presença de flores que possuem estiletes que se curvam para a direita ou para a esquerda (flores direitas x flores esquerdas).

A funcionalidade deste polimorfismo nas flores D e E está naturalmente relacionada com o fluxo de pólen nas plantas da mesma espécie. Esse arranjo morfológico permite que polinizadores, como insetos, transportem pólen de uma flor para outra de morfotipos diferentes, promovendo a polinização cruzada e reduzindo a autopolinização. Assim, a enantiostilia é relatada como uma estratégia adaptativa que aumenta a variabilidade genética nas populações. Neste contexto, esse estudo visou investigar os efeitos da cofloração e a dinâmica do fluxo de pólen intra e interespecífico no sucesso reprodutivo em espécies de *Chamaecrista* Moench. (Caesalpinioideae–Fabaceae).

Realizamos nossa investigação na vegetação de restinga da Reserva Particular do Patrimônio Natural de Nossa Senhora do Oiteiro de Maracaípe, no município de Ipojuca, Pernambuco, Brasil. Nessa região ocorrem naturalmente as espécies *Chamaecrista diphylla, C. flexuosa, C. ramosa* e *C. rotundifolia*. A escolha dessas quatro espécies permite uma comparação abrangente dos padrões morfológicos florais locais e das adaptações da enantiostilia monomórfica, permitindo entender como diferenças, como tamanho e número de flores, podem influenciar os mecanismos relacionados a interações de polinização.

No capítulo 1, acompanhamos a dinâmica da floração e produção de morfos em espécies enantiostílicas monomórficas ao longo do tempo. Os resultados mostraram que, quando considerado o número total de flores, foi observado um padrão semelhante de proporção de morfos florais nas populações, similar ao encontrado em estudos anteriores com espécies enantiostílicas monomórficas. Entretanto, em nível individual, algumas plantas apresentaram variações funcionais de morfos, sem padrão consistente. Notamos ainda que o número de flores produzidas por indivíduos afeta diretamente a proporção de morfos, apontando para possíveis adaptações ainda não totalmente compreendidas.

No capítulo 2, investigamos os efeitos da presença e do acúmulo de pólen heteroespecífico (HP) em flores de *Chamaecrista* com enantiostilia, testando a hipótese de que a deposição de HP afeta negativamente o sucesso reprodutivo. Confirmamos que o pólen heteroespecífico reduz o fitness reprodutivo, com variações dependendo das espécies envolvidas e dos morfos receptores. Os resultados indicam que cruzamentos coespecíficos favorecem a frutificação, indicando que a enantiostilia funciona como barreira reprodutiva importante no isolamento do fluxo polínico entre as espécies.

A presença de espécies coflorescentes influencia de forma complexa a deposição de pólen coespecífico, revelando respostas dinâmicas na interação interespecífica que dependem da proporção de mistura de pólen e das características florais. Esses achados encontrados são importantes para a compreensão dos mecanismos de isolamento e das estratégias adaptativas em plantas com enantiostilia monomórfica. Portanto, esta tese avança na compreensão da perspectiva das plantas nas interações com polinizadores em ambientes com alta diversidade floral e de sobreposição de nicho.

2. FUNDAMENTAÇÃO TEÓRICA

2.1. Diversidade de Chamaecrista (L.) Moench em ecossistemas ameaçados

O gênero *Chamaecrista* (L.) Moench, com mais de 330 espécies distribuídas de forma Pantropical (Irwin and Barneby, 1982), ocupa um lugar de destaque sendo o oitavo gênero mais diverso de angiospermas e o segundo da família Fabaceae no país, com 269 espécies e 95 variedades, das quais 225 são endêmicas (Flora do Brasil, 2024). Segundo Rando et al. (2013) a grande diversidade do gênero está concentrada em ambientes de vegetação de campos rupestres e cerrado, com muitas espécies endêmicas. Algumas das espécies, principalmente as arborescentes, ocorrem em florestas úmidas, com registros de endemismos na Amazônia e na Mata Atlântica (Rando et al., 2016). Nesse contexto, as restingas do Brasil, que abrigam 25 espécies de *Chamaecrista* (Flora do Brasil, 2024), destacam-se como ecossistemas litorâneos de alta diversidade biológica no país.

A Reserva Particular do Patrimônio Natural (RPPN) de Nossa Senhora do Oiteiro de Maracaípe, localizada no litoral de Pernambuco, é um remanescente importante da vegetação de restinga, possui uma área de 130 hectares, dos quais 70 hectares são cobertos por restinga com uma rica diversidade de flora (Almeida et al., 2009; Almeida et al., 2024). São encontradas atualmente na reserva cinco espécies representantes *Chamaecrista diphylla* (L.) Greene, *C. flexuosa* (L.) Greene, *C. ramosa* (Vogel) H.S.Irwin ; Barneby, *C. rotundifolia* (Pers.) Greene e *C. nictitans* (L.) Moench (não enantiostílica; ver Almeida et al., 2024). A composição da restinga na RPPN é influenciada por uma combinação única de fatores ambientais, como o regime hídrico dos solos, a fertilidade e o teor de alumínio, que contribuem para a formação de distintas fisionomias de vegetação, refletindo diretamente na diversidade de espécies encontradas (Almeida et al., 2009). Apesar das semelhanças florísticas entre as restingas do Nordeste, a vegetação da RPPN Maracaípe é caracterizada por algumas peculiaridades ecológicas, como a composição florística das famílias Orchidaceae, Cyperaceae, Poaceae e Myrtaceae (Almeida et al., 2009).

O gênero *Chamaecrista*, pertencente à tribo Cassieae da subfamília Caesalpinioideae, é reconhecido pela sua diversidade e complexidade taxonômica, incluindo árvores, arbustos, subarbustos ou ervas, anuais, bianuais ou perenes (Correia e Conceição, 2017). A divisão do gênero *Cassia* em três gêneros distintos por Irwin e Barneby (1982) e as análises filogenéticas moleculares recentes (Bruneau et al., 2008; Conceição et al., 2009; LPWG, 2017; Silva et al., 2022) indicam que *Chamaecrista* é um grupo monofilético. As espécies desse gênero apresentam variações morfológicas notáveis, como inflorescências distintas e a presença de nectários extraflorais, quando presentes, localizados no pecíolo, menos frequentemente na raque entre os pares de folíolos ou no eixo da inflorescência. Estas características são amplamente descritas em diversos estudos taxonômicos (Irwin e Barneby, 1982; Souza et al., 2019; 2020). A filogenia de *Chamaecrista* proposta por Conceição et al. (2009) identifica clados com diferentes características morfológicas, incluindo espécies arborescentes e herbáceas com inflorescências ramifloras, axilares ou terminais, e presença ou ausência de nectários extraflorais. Além disso, algumas espécies de *Chamaecrista* são utilizadas como plantas ornamentais, forrageiras, daninhas (Souza e Lorenzi, 2000) mas também são fixadoras de nitrogênio no solo, portanto, muito indicadas para a recuperação de solos degradados (Sprent e Pearsons, 2000).

As restingas, como a de Maracaípe, integram uma complexa rede ecológica com alta diversidade, em grande parte devido ao seu contexto geográfico e ambiental (Sacramento et al., 2007). Apesar dos intensos impactos sofridos por essa área costeira nas últimas décadas, principalmente desmatamentos, recorrentes queimadas e poluição as restingas dessa região continuam a ser ecossistemas de grande importância para a conservação da biodiversidade. Estudos florísticos, como os de Zickel et al. (2007) e Almeida et al. (2009), são fundamentais para a preservação das populações vegetais e a compreensão da dinâmica ecológica desses ambientes. A conservação da RPPN Maracaípe é essencial para a proteção de várias espécies, incluindo as do gênero *Chamaecrista*, que representam um componente importante da flora endêmica da região litorânea.

Atualmente, as restingas correspondem a cerca de 5% do litoral do estado de Pernambuco (Zickel et al., 2004), o que torna ainda mais relevante a preservação e o estudo dessa vegetação. Por esse motivo usamos como modelo espécies de *Chamaecrista*, em uma área de zona costeira em Pernambuco, afim de contribuir para entendimento da morfologia floral como base para o estabelecimento de padrões de transferência do pólen nestas espécies, e ainda, sobre as influências da sincronopatria e da dinâmica de floração, indicando os processos relacionados aos efeitos das interações planta-polinizador que direcionam evolutivamente a especiação e a coexistência dessas plantas.

2.2.Flores com enantiostilia e sua especialização na polinização cruzada

Nas angiospermas, a diversidade de tipos florais e suas interações com agentes polinizadores são um tema central na botânica e na biologia evolutiva, estudado desde os tempos de Darwin, Lineu e Fisher (Barrett, 2002; 2010). As adaptações florais fornecem uma base para compreender os mecanismos que promovem a polinização cruzada e as transições evolutivas nos sistemas reprodutivos (Barrett, 2010). A interação dos visitantes florais com as estruturas reprodutivas sugere que a enantiostilia possa aumentar a precisão da polinização (Richman e Venable, 2018). Acredita-se que a diversificação dos insetos visitantes tenha ocorrido em paralelo à das flores das angiospermas (Endress, 1994), o que mantém o interesse por plantas com polimorfismos florais e suas funções ecológicas. Plantas polimórficas apresentam diferentes morfotipos florais dentro da mesma espécie, adaptados para promover a transferência de gametas masculinos entre indivíduos, favorecendo assim o sucesso reprodutivo (Jesson e Barrett, 2002; 2003; Barrett e Fairnie, 2024).

Essas adaptações florais desempenham um papel importante nas interações planta-polinizador, moldando o sucesso reprodutivo e a eficiência da polinização (Lloyd e Barrett, 1996; Harder e Barrett, 2006; Waser e Ollerton, 2006). Segundo Opendal et al. (2023), a variação nas características florais afeta diretamente a deposição de pólen e o fitness reprodutivo, podendo refletir respostas a pressões seletivas, como disponibilidade de polinizadores e competição interespecífica. Tais interações ecológicas despertam sucessivamente o fascínio pelo estudo da diversidade floral e sua evolução, como destacou Darwin (1877). Um ponto recorrente em comum nos estudos de espécies polimórficas é o papel da especialização floral como adaptação evolutiva para favorecer a polinização cruzada em detrimento da autopolinização. Em flores hermafroditas, a separação espacial (hercogamia) das estruturas sexuais reduz a interferência entre estigmas e anteras, limitando a geitonogamia (fecundação entre flores do mesmo indivíduo) (Webb e Lloyd, 1986; Jesson e Barrett, 2003; Richman e Venable, 2018).

A hercogamia encontrada nas flores com polimorfismos possui importância relacionada à eficiência da polinização cruzada, pois promove a separação espacial entre as partes masculinas e femininas em flores hermafroditas (Webb e Lloyd, 1986; Barrett, 2010). Essa separação pode ocorrer por diferenças de altura entre estigmas e anteras ou pela disposição lateral dos estigmas, o que também contribui para evitar a autopolinização em espécies especialistas. Plantas com hercogamia geralmente apresentam dois morfotipos florais, e a hercogamia recíproca desempenha um papel essencial nas espécies enantiostílicas, facilitando a troca de pólen entre morfotipos distintos (Barrett, 2002; 2010; Richman e Venable, 2018). Este polimorfismo floral é representado por assimetrias no desenvolvimento dos elementos sexuais em flores zigomorfas, resultando no posicionamento diferente de estames e estiletes curvados em direções opostas em flores de uma mesma planta ou de plantas diferentes (Oliveira e Maruyama, 2014).

A reciprocidade entre antera e estigma em diferentes morfos caracteriza a hercogamia lateral ou recíproca, encontrada na enantiostilia, também chamada de "flores em espelho" (Webb e Lloyd, 1986). Esse arranjo resulta em flores zigomorfas e assimétricas, mas com flores com estiletes orientados em direções opostas, formando imagens especulares (Todd, 1882; Knuth, 1906; Jesson e Barrett, 2003). Além disso, na enantiostilia, essa correspondência entre órgãos sexuais complementares garante padrões mais precisos de deposição e fluxo de pólen entre indivíduos (Barrett, 2024). Por essas características, as flores enantiostílicas refletem sua importância evolutiva na maximização do fluxo de pólen coespecífico e no sucesso reprodutivo das plantas (Barrett, 2024). Darwin (1877) foi pioneiro ao interpretar na heterostilia, outro polimorfismo com essa característica, que possui em comum com a enantiostilia uma adaptação destinada à promoção da maior variabilidade genética que reflete na capacidade adaptativa das plantas em diversos ambientes. Contudo, até hoje os mecanismos que apoiam a evolução desse polimorfismo continuam pouco compreendidos, demandando mais estudos integrativos (Dellinger et al., 2014; 2019; Opendal et al., 2023).

A enantiostilia, um tipo de polimorfismo floral caracterizado por flores com estiletes orientados à esquerda (morfo E) ou à direita (morfo D) do eixo floral, surge de forma polifilética nas angiospermas, independentemente, em pelo menos 16 eventos evolutivos, distribuídos em 11 famílias e 42 gêneros, frequentemente em espécies polinizadas por insetos (Morais et al., 2020; Barrett e Fairnie, 2024). As evidências existentes são que esse polimorfismo parece coevoluir com polinizadores, maximizando o fluxo de pólen coespecífico (Barrett e Richards, 1990; Jesson e Barrett, 2005). Outras características florais que definem as flores enantiostílicas incluem a ausência de néctar, heteranteria, anteras poricidas, e um estilete curvo com um pequeno estigma (Almeida e Castro, 2019).

Essas flores especializadas possuem uma conformação tal que dificulta ou impede a autopolinização, reforçam a proteção do gineceu, evitam a ocorrência da geitonogamia e aumentam a eficiência da captura e deposição de pólen nas flores (Fenster, 1995; Barrett e Richards, 19990; Jesson e Barrett, 2003; Jesson e Barrett, 2005). Em algumas espécies da família Fabaceae, da subtribo Cassiinae, observa-se um mecanismo de polinização denominado ricochete, no qual uma pétala que envolve os verticilos florais se move com a chegada do polinizador, expondo anteras e estigmas (Westerkamp, 2004). A deposição de pólen de forma indireta exercida por pétalas e aqui observada nas espécies do gênero *Chamaecrista* é bastante semelhante ao descrito para outras espécies do gênero e para espécies de *Cassia* (Westerkamp, 2004; Dutra et al., 2009; Costa et al., 2013). No entanto, em *C. ramosa,* o movimento dos grãos é semelhante a um looping, conforme descrito por Almeida e colaboradores (2013a). Nesse processo, com a vibração subsequente, realizada por abelhas, deslocando o pólen em direção à parte adaxial das pétalas, que atua como uma superfície de reflexão, antes de serem depositados no dorso dos polinizadores.

No gênero *Senna,* no entanto, tem sido observado o contrário: a deposição de pólen feita de forma direta, o que foi verificado neste estudo para *Senna macranthera* e *Senna. macranthera* var. *pudibunda*. (Carvalho e Oliveira, 2003; Laporta, 2005). *Senna martiana* aparece como exceção, já que apresenta deposição indireta de pólen (Almeida et al., 2015a; b; Amorim et al., 2017). Durante as visitas, as anteras chegam a tocar nas pétalas, causando uma pequena depressão no local de contato, a qual direciona os grãos de pólen ao dorso dos visitantes. Sendo assim, o que pode ser entendido como padrão para espécies enantiostílicas e que a morfologia floral é essencial para garantir o sucesso reprodutivo nessas plantas, elevando suas taxas de polinização cruzada intermorfo e reduzindo a autogamia.

Este polimorfismo estilar pode ocorrer nas formas monomórficas ou dimórficas, dependendo da expressão genética da morfologia dos morfos florais (Dulberger, 1981; Jesson e Barrett, 2003). Embora os fatores que implicam nessa expressão morfológica nesse polimorfismo ainda não sejam totalmente compreendidos (Bezerra et al., 2024). A forma enantiostílica monomórfica apresenta diferentes padrões de distribuição de flores com orientação do estilete para a esquerda - E e para a direita – D, dentro de inflorescências individuais. Isso pode incluir produção aleatória de flores E e D, posicionamento fixo (assimetria pendular) ou uniformidade de um morfo por inflorescência, mas com variação entre inflorescências de uma mesma planta (Barrett, 2002; Jesson et al., 2003). Nesse último caso, as flores são distribuídas de forma randômica ou seguindo algum padrão de distribuição não randômico (Barrett, 2002). Ocorrências conhecidas de enantiostilia monomórfica foram registradas nas famílias Fabaceae, Gesneriaceae, Haemodoraceae, Pontederiaceae, Solanaceae, Tecophilaeaceae e Vochysiaceae. Sendo, portanto, a forma monomórfica predominante em diversidade (Jesson et al. 2003b).

Pesquisas recentes (Almeida et al., 2013a,b; 2015a,b; 2018; Amorim et al., 2017) destacam a necessidade de investigações adicionais sobre a função adaptativa da enantiostilia monomórfica e os mecanismos seletivos que regem sua evolução. Estes estudos têm corroborado que a produção de flores de espécies enantiostílicas monomórficas frequentemente apresenta proporções equilibradas de flores E e D diariamente ao nível de populações, o que contribui para o sucesso reprodutivo (Tang e Huang, 2005; Ren et al., 2013; Almeida et al., 2018). Em outros estudos, destaca-se que a segregação de pólen em lados distintos dos corpos dos polinizadores é mais eficaz em abelhas maiores, reforçando a função adaptativa da hercogamia recíproca na enantiostilia (Amorim et al., 2017; Minnaar e Anderson, 2022).

A enantiostilia dimórfica, por outro lado, é caracterizada por um polimorfismo genético no qual alguns indivíduos produzem flores com o estilete curvado para a direita, enquanto outros produzem flores com o estilete curvado para a esquerda. Logo, representando proporções de plantas com razão 1:1, conhecida como isopletia (Jesson e Barrett, 2002), o que gera uma taxa de visitação igual entre morfos. Em espécies enantiostílicas, monomórficas ou dimórficas, não há indícios de anisopletia (ausência de isopletia) (Almeida et al. 2015b). Estudos sugerem que essa forma dimórfica evoluiu a partir da enantiostilia monomórfica, sendo justificada pela funcionalidade do próprio sistema reprodutivo (Jesson e Barrett, 2003; Morais et al., 2020). A distribuição da enantiostilia dimórfica é relativamente rara, ocorrendo exclusivamente em plantas monocotiledôneas e restrita a apenas sete espécies, distribuídas em três famílias: Haemodoraceae, Pontederiaceae e Tecophilaeaceae (Dulberger e Ornduff, 1980; Barrett, 2002; Jesson et al., 2003; Ness et al., 2011; Palmer, 2016).

Estudos teóricos e genéticos realizados por Jesson e Barrett (2003) e por Morais et al. (2020) indicam que espécies enantiostílicas possuem como ancestrais representantes com estilete em uma única posição, passando do tipo monomórfico e posteriormente à forma dimórfica. Os autores argumentam que esta última forma é reprodutivamente vantajosa, especialmente em períodos de limitação de pólen, por elevar as taxas de xenogamia. Em espécies com enantiostilia, a isopletia é mais frequentemente observada em populações dimórficas, enquanto em populações monomórficas, a razão pode variar dependendo da produção de flores e da organização ao nível de população ou a nível de indivíduo (Bezerra et al., 2024).

Flores enantiostílicas são frequentemente associadas à polinização por abelhas grandes (Hymenoptera: Apoidea), que são capazes de liberar pólen das anteras poricidas por meio de vibrações de seus músculos (Vallejo-Marín, 2019). Esse processo é conhecido como "*buzz-*

pollination" ou “polinização por zumbido” (Buchmann, 1983; Cardinal et al., 2018). Durante esse processo, as vibrações ressoam nas anteras, fazendo com que os grãos de pólen ganhem energia e sejam expelidos pelos poros apicais das anteras. O pólen expelido se deposita no corpo do polinizador, possivelmente atraído por forças eletrostáticas (De Luca et al., 2013). Destaca-se que apenas visitantes florais que emitem vibrações de alta frequência promovem a fertilização efetiva (Fenster, 1995; Dellinger et al., 2019). Flores de espécies *Chamaecrista* evidenciam uma morfologia altamente especializada, com adaptações nos morfotipos que favorecem a polinização mediada por vibração (Arceo-Gómez et al., 2011). Estudos atuais buscam relacionar abelhas, seu tamanho e forma corporal e comportamento, os quais influenciam as propriedades das vibrações (Raymundez-Urrutia e Hokche, 2008; De Luca et al., 2013; Arroyo-Correa et al., 2018; Vallejo-Marín, 2019; Morais e al., 2020).

Outro aspecto associado à enantiostilia é a heteranteria, outras características são compartilhadas, como anteras poricidas e polén seco com paredes lisas (Barrett, 2003; Almeida et al., 2013a,b; 2015a,b). Müller (1883) propôs a "hipótese da divisão do trabalho", na qual estames alimentadores atraem polinizadores, enquanto estames polinizadores direcionam pólen para locais específicos no corpo do visitante (Vallejo-Marín et al., 2009; Vallejo-Marín, 2022). Esse mecanismo é comum em espécies enantiostílicas, especialmente aquelas com polinização por vibração, um processo especializado que envolve a liberação de pólen devido às vibrações das abelhas (Vallejo-Marín, 2022). Além disso, a hercogamia também contribui para reduzir os possíveis danos que as flores podem sofrer durante a vibração das estruturas exercida pelas abelhas na polinização (Dulberger, 1981; Jesson e Barrett, 2002).

Por outro lado, nem todas as espécies enantiostílicas possuem as características típicas dessa síndrome de polinização. Algumas secretam néctar e não apresentam heteranteria, como as espécies de *Wachendorfia* (Haemodoraceae) e *Barberetta* (Haemodoraceae) (Jesson e Barrett, 2003; Johnson et al., 2023). Também é importante notar que, embora a enantiostilia monomórfica esteja frequentemente associada à redução da geitonogamia e ao aumento da polinização cruzada (Fenster, 1995), os mecanismos precisos que sustentam sua evolução continuam sendo um campo promissor para investigações futuras.

Dessa forma, compreender totalmente a funcionalidade floral ainda é uma provocação atual, assim como as pressões seletivas que podem originar e manter este tipo de polimorfismo, demandando mais evidências e estudos integrativos (Dellinger et al., 2014; 2019; Opendal et al., 2023). As perspectivas para estudos futuros sobre enantiostilia, conforme apresentadas aqui, abrangem diversas frentes de pesquisa que destacam a complexidade e a relevância desse polimorfismo floral. Primeiramente, sugere-se a investigação da variabilidade morfológica em táxons que frequentemente exibem enantiostilia, tais como a tribo Cassiinae, um grupo com notável diversidade morfológica potencialmente associada à enantiostilia. Além disso, os autores ressaltam a importância de explorar táxons ainda sem registros de enantiostilia, uma abordagem que pode revelar formas inéditas de polimorfismo floral e ampliar o entendimento das adaptações morfológicas nas interações planta-polinizador. Também é destacada a necessidade de estudos sobre os aspectos funcionais da enantiostilia, incluindo como as características morfológicas influenciam a eficiência da polinização e o fluxo de pólen. Por fim, há uma lacuna significativa no conhecimento genético e evolutivo desse polimorfismo, sendo necessários estudos sobre os mecanismos genéticos subjacentes e a expressão dos morfos florais, especialmente em espécies monomórficas, para compreender melhor sua origem e manutenção ao longo do tempo (Almeida et al. 2013a,b; 2015a,b; 2018).

2.3 Fenologia e Dinâmica da floração em espécies enantiostílicas

As espécies enantiostílicas apresentam morfotipos florais distintos que dependem da presença do morfo recíproco oposto para garantir o sucesso reprodutivo. Além disso, essas espécies frequentemente dependem de polinizadores especializados para garantir o fluxo eficiente de pólen entre os morfos, sendo mais vulneráveis à perda de polinizadores do que aquelas que são polinizadas por visitantes generalistas (Washitani, 1999). Nesse contexto, torna-se essencial a produção equilibrada de morfos nas populações naturais, o que é denominado isopletia (proporção 1:1). Esta proporção favorece a polinização legítima e promove uma frequência proporcional de visitantes para ambos os morfos florais (Ganders, 1979; Barrett, 2002).

A enantiostilia pode ser classificada em duas formas principais. A enantiostilia monomórfica, que é predominante em diversidade, subdivide-se em recíproca, na qual o estilete e a antera se desviam reciprocamente, e não recíproca, em que apenas o estilete apresenta desvio (Barrett et al., 2000; Jesson et al., 2003; Amorim et al., 2017). Por outro lado, a enantiostilia dimórfica, menos frequente, ocorre exclusivamente em monocotiledôneas e é restrita a três famílias: Haemodoraceae, Pontederiaceae e Tecophilaeaceae (Morais et al., 2020).

Em espécies com enantiostilia monomórfica, estudos mostraram que a produção de flores com estiletes desviados para a esquerda (E) e direita (D) é geralmente equilibrada, tanto em nível de planta quanto de população. Isso foi evidenciado em várias espécies, incluindo duas de *Monochoria* (Pontederiaceae) (Tang e Huang, 2005), *Hiptage benghalensis* (Malphigiaceae) (Ren et al., 2013) e em espécies dos gêneros *Senna* e *Chamaecrista* (Almeida et al., 2018). Já em espécies com enantiostilia dimórfica, a proporção de morfos E e D em ambientes é frequentemente de 1:1, como observado em *Wachendorfia paniculata* (Haemodoraceae) (Jesson e Barrett, 2002) e *Barberetta aurea* (Haemodoraceae) (Johnson et al., 2023). No entanto, existem relatos sobre a quebra desse polimorfismo floral isso pode levar a proporções desequilibradas de morfos ou até à fixação de uma morfologia em situação atípica (Almeida et al, 2016).

Nas espécies enantiostílicas dimórficas, cada indivíduo produz exclusivamente um dos morfos florais (D ou E) e, geralmente, abre poucas flores por dia. Em contraste, nas espécies monomórficas, ambos os morfos podem ser produzidos pelo mesmo indivíduo, com dinâmicas de produção variando entre padrões randômicos, alternados ou concentrados em inflorescências específicas (Almeida et al., 2018). Estratégias como a separação temporal (produzindo flores de morfos diferentes em dias distintos) e a redução do número de flores por dia são observadas para minimizar a geitonogamia e promover o cruzamento (Barrett et al., 2002; Almeida et al., 2013a, 2018).

Entre as principais estratégias conhecidas está a separação temporal, na qual os indivíduos produzem flores dos morfos E e D em dias diferentes, tornando-se funcionalmente dimórficos, e, desta forma, as chances de geitonogamia tornam-se mínimas (Barrett et al., 2002). Outra estratégia é a redução de flores por dia, geralmente de um mesmo morfo, para promover o cruzamento de forma mais eficaz do que a enantiostilia monomórfica (Barrett et al., 2002; Almeida et al., 2013a; 2018; Bezerra et al., 2024).

Estudos evolutivos sugerem que a enantiostilia evoluiu a partir de ancestrais com estiletes posicionados em uma única orientação, passando da monomorfia para a dimorfia. Embora a enantiostilia dimórfica seja mais eficaz no transporte de pólen entre indivíduos, a forma monomórfica reduz a geitonogamia em até 50% (Jesson e Barrett, 2003; Morais et al., 2020). Independentemente da forma, as populações enantiostílicas geralmente mantêm uma proporção equilibrada de morfos (isopletia), e relatos de anisopletia (desequilíbrio na proporção de morfos) são raros (Almeida et al., 2018).

A interação entre plantas seus polinizadores dependem de uma sincronia entre a floração e a atividade de forrageio. Essa relação é particularmente sensível em contextos onde flores de diferentes espécies compartilham os mesmos polinizadores, o que pode levar à deposição de pólen interespecífico, obstruindo os estigmas e dificultando o crescimento de tubos polínicos legítimos (Trevizan et al., 2021; 2024b). Uma estratégia observada para minimizar essa competição é a dessincronização dos períodos de floração ao longo do ano ou do dia, reduzindo a sobreposição temporal de recursos florais entre espécies sincronopátricas (Machado e Semir, 2006).

Informações acerca da fenologia de flores e da dinâmica da produção de morfos fornecem evidencias sobre separação temporal e morfo-funcional, e levantam hipóteses sobre a relação entre espécies próximas mais relacionadas e as diferentes estratégias de floração para cada táxon, devido a uma possível relação genética que favoreceria a hibridação (Costa et al., 2013; Bezerra, 2019). Para Ollerton e Lack (1992), espécies relacionadas taxonomicamente, apresentam padrões distintos de floração e frutificação ao longo do ano, e as interações interespecíficas determinam os padrões fenológicos que têm um importante papel na dinâmica da comunidade.

Nesse contexto, as características adaptativas das espécies enantiostílicas, especialmente da tribo Cassiinae (Fabaceae), somada ao elevado grau de sobreposição de nicho, tornam essas plantas modelos valiosos para estudos evolutivos e ecológicos. A compreensão das estratégias reprodutivas em ambientes naturais oferece insights fundamentais sobre os efeitos de mudanças ambientais nos padrões de reprodução e na dinâmica da floração nas comunidades vegetais.

2.4 Relações reprodutivas e reciprocidade floral na enantiostilia

A reciprocidade entre flores, caracterizada pelo posicionamento recíproco dos elementos florais (estigmas e anteras) em morfotipos distintos, é um fenômeno comum que facilita o fluxo direcional e contínuo de pólen durante a polinização cruzada legítima (Barrett e Richards, 1990). Estudos com flores enantiostílicas demonstram que a deposição e captura de pólen ocorrem em áreas opostas do corpo dos polinizadores em cada morfotipo floral. Além disso, em alguns casos, essa reciprocidade é acompanhada pela localização das pétalas (Jesson e Barrett, 2005; Almeida et al., 2013a,b; 2015b). No entanto, é importante notar que os morfotipos florais não diferem em atração visual para as abelhas. Logo, a reciprocidade é o principal fator que intermedeia a troca de pólen coespecífico via interação com os polinizadores.

Pesquisas sugerem que a reciprocidade nas flores enantiostílicas aumenta a eficiência do uso de pólen na reprodução, otimizando a função masculina (Oliveira e Maruyama, 2014). Embora espécies enantiostílicas formem frutos predominantemente por cruzamentos intermorfos, há evidências de compatibilidade intramorfo (Barrett, 2002; Almeida et al., 2013a, b; 2015a; Almeida e Castro, 2019). Contudo, até o momento, não foram observadas alterações no alongamento do tubo polínico entre polinizações intra e intermorfo (Almeida et al., 2015a). A incompatibilidade auto e intramorfo é rara, o que reforça o papel dos caracteres morfológicos florais em maximizar cruzamentos intermorfos e reduzir a autopolinização ou geitonogamia (Barrett, 2002; Almeida et al., 2015a).

A maioria dos estudos sobre reciprocidade floral concentra-se em análises intraespecíficas, mas abordagens que investigam reciprocidade entre flores de um mesmo indivíduo (Morais et al., 2020; Braga et al., 2022) ou entre morfos de espécies (Bezerra, 2019) são raras. O estudo de Bezerra (2019) analisou a reciprocidade intra e interespecífica em flores, constatando que, embora as amplitudes de deposição de pólen sejam compatíveis entre espécies, a sobreposição de pólen no corpo dos polinizadores parece ser incomum.

Variações no sistema hercogâmico recíproco podem impactar o sucesso reprodutivo das plantas, influenciando a funcionalidade dos morfos (Barrett, 2002). Algumas espécies não apresentam hercogamia recíproca precisa, tanto no comprimento dos estiletes (mais comum na heterostilia) quanto na amplitude de ocorrência (mais comum na enantiostilia) (Morais et al, 2020). Desvios no padrão de reciprocidade podem reduzir a deposição de pólen legítimo e aumentar a contaminação com pólen ilegítimo, dificultando a germinação de grãos legítimos e comprometendo a fertilização de óvulos (Trevizan et al., 2024; Jiang, et al., 2018). Em espécies heterostílicas, tais desvios podem estar associados a perturbações ambientais, como a ausência ou ineficiência dos polinizadores ou a perda de um dos morfos na população (Barrett, 2002). Por outro lado, desvios no sistema enantiostílico podem reduzir a competição por pólen no corpo dos polinizadores e aumentar a especificidade dos cruzamentos, promovendo o fluxo interespecífico sem inviabilizar a polinização legítima (Favre e McDade, 2001; Castro e Araújo, 2004; Bezerra, 2019).

Além disso, estudos sugerem a possibilidade do sistema enantiostílico apresentar desvios “atípicos” em relação ao padrão hercogâmico recíproco (Almeida et al., 2013a; 2016). Estes autores atribuem a possibilidade de reduzir a competição por pólen no corpo do polinizador, já que, diferindo nos principais posicionamentos do estigma, aumenta-se a área de captação dos grãos e aumenta as chances de fluxo interespecífico. Neste sentido, a consequente redução da reciprocidade pode apresentar-se como promotora de aumento da área de deposição e captação de pólen no corpo do polinizador, aumentando a especificidade dos cruzamentos, logo, não inviabilizando o fluxo de pólen legitimo (Favre e McDade 2001; Castro e Araújo, 2004; Teixeira e Machado 2004; Sampson e Krebs, 2012; Jiang, et al., 2018).

Estudos sobre reciprocidade com espécies polimórficas são amplos e bem esclarecidos para a heterostilia. Além disso, propõem índices quantitativos para calcular a hercogamia recíproca, verificados a partir da altura das anteras e dos estigmas em populações naturais (Trevizan et al., 2021). Isso significa, que o pólen de um respectivo morfo poderá ser depositado sobre o estigma do morfo oposto se o posicionamento de ambos estiver no mesmo nível ou próximos, sendo o valor de inacurácia (reciprocidade) baseado a partir da posição dos estigmas e anteras de toda a população em comparação com a posição do ótimo (Trevizan et al., 2024a, b). Por sua vez, estruturas tais como altura e disposição das anteras e dos estigmas, podem ser afetados por fatores ecológicos e morfológicos, tais como, polinizadores, dimensão das peças florais e a produção de proporção dos morfos diária (Hodgins e Barrett, 2008, Keller et al., 2012, Novo et al., 2018, Wu et al., 2018).

De fato, a condição monomórfica ocorre em outros táxons polinizados por animais, presumivelmente para limitar a interferência sexual e a autopolinização (Braga et al., 2022). A pesquisa recente sobre enantiostilia tem revelado uma impressionante diversidade de sistemas enantiostílicos, com muitos exemplos encontrados em espécies que ocorrem em regiões tropicais e subtropicais. Como destacado por Almeida e Castro (2019), a enantiostilia é um fenômeno complexo que ainda requer mais estudos para entender suas implicações evolutivas.

Embora a enantiostilia seja um polimorfismo floral amplamente estudado, ainda há desafios na medição da reciprocidade efetiva, especialmente ao conectar características morfológicas com fatores ecológicos interespecíficos. Espécies de *Chamaecrista,* caracterizadas por tipos enantiostílicos (Almeida et al., 2015b), possuem muitas semelhanças, inclusive de locais de captação e deposição de pólen, o que pode eventualmente ocasionar cruzamentos interespecíficos. Nesse contexto, o grupo Cassiinae tem despertado o interesse devido às características adequadas para estudos evolutivos e ecológicos referentes à biologia da polinização, ao sistema reprodutivo e as variações de respostas adaptativas das populações naturais a processos de mudanças ambientais (Bezerra, 2019).

2.5 Isolamento do fluxo de pólen

A funcionalidade da enantiostilia está intimamente relacionada com o fluxo de pólen. Portanto, estudos que investigam a existência de carga de pólen interespecífico (coespecífico e interespecífico) são fundamentais para entender espécies simpátricas que compartilham polinizadores. É esperado que espécies polimórficas tendem a apresentar diferenças no diâmetro dos grãos de pólen entre os morfos (Teixeira e Machado, 2004). No entanto, são escassos os estudos que investigam a funcionalidade da enantiostilia em espécies que estão sob efeito de sobreposição de floração e partilha de polinizadores. Além disso, são raros os estudos que consideram o fluxo potencial coespecífico e o fluxo interespecífico de pólen depositado de acordo com Bergamo et al. (2024), plantas hermafroditas alcançam sucesso reprodutivo tanto pela produção de sementes (função feminina) quanto pela disseminação de pólen (função masculina), ambas dependentes de uma polinização eficiente, na qual o pólen é transferido das anteras para os estigmas. O estudo destaca que, embora esses componentes de sucesso reprodutivo estejam interligados, ainda há lacunas sobre como eles se correlacionam e quais fatores em nível comunitário influenciam cada um em contextos de cofloração.

O isolamento reprodutivo das plantas se refere à ausência de fluxo gênico entre populações naturais. No grupo taxonômico *Chamaecrista*, existem espécies relacionadas que compartilham períodos de floração sobrepostos. Quando essas espécies coexistem em simpatria, elas podem compartilhar ou competir por polinizadores devido à sua morfologia semelhante e à possibilidade de formação de híbridos (Costa et al., 2013; Correia e Conceição, 2017; Bezerra, 2019). Porém, este potencial pode ser limitado por mecanismos pré e pós-zigóticos que atuam como barreiras reprodutivas entre os táxons. É a superfície do estigma que reconhece ou rejeita o grão de pólen, através do mecanismo de autoincompatibilidade, no entanto, espécies enantiostílicas monomórficas são autocompatíveis (Almeida et al., 2015a; Almeida e Castro, 2019). Nessas espécies o conhecimento sobre fenologia, dinâmica da floração, ecologia da polinização, formação de tubo polínico, são ferramentas indispensáveis para caracterizar diversas barreiras entre as plantas relacionadas, possibilitando o entendimento da estrutura genética de suas populações e o padrão de sua evolução (Opendal et al., 2023).

Além disso, em *Chamaecrista*, existem relatos da ocorrência da formação de híbridos naturais (Conceição et al., 2009; Costa et al., 2013). Estudos recentes com este gênero demostram no cenário de simpatria e eventuais intercruzamentos, o que poderia estender-se para os outros gêneros do grupo Cassiinae (Bezerra, 2019; Costa et al., 2013). Entretanto, os autores supracitados destacam a barreira reprodutiva morfológico-funcional como principal mecanismo de obstáculo para formação de híbridos naturais, corroborando com teorias clássicas descritas para sua funcionalidade morfológica. Segundo Westerkamp (2007), a barreira morfológica é eficaz no direcionamento da deposição dos grãos de pólen no momento da visita, no corpo das abelhas; deste modo, minimiza a perda de pólen por pilhadores e maximiza a dispersão dos mesmos.

Dessa forma, a separação de barreiras físicas e morfológicas pode ser elucidada através da identificação das estratégias e direcionamento da deposição de pólen intra e interespecífico nos estigmas das flores recíprocas (Westerkamp, 2004; Costa et al., 2013). Além disso, a contaminação por pólen ilegítimo pode causar a oclusão do estigma, impedindo que grãos de pólen viáveis cheguem ao estigma e germinem, o que impede a fertilização (Bergamo et al., 2024). As diferenças morfológicas nas flores, além de aumentarem as taxas de xenogamia, podem ser cruciais para evitar a hibridização entre espécies (Barrett, 2002). Ainda, em sistemas onde a dispersão do pólen é mediada por animais, o tamanho efetivo da população e a amplitude de fluxo gênico atual podem ser estimados através de medidas do movimento dos polinizadores entre flores e plantas e da distância de dispersão das sementes (Vallejo-Marin M. 2019).

Experimentos anteriores demonstraram as consequências da competição de pólen heteroespecífico nos estigmas no sistema de polinização legítimo. Esses eventos incluem a produção de aleloquímicos que limitam a germinação de pólen em Caprifoliaceae (Thomson et al., 2006), o entupimento da região estigmática em Turneraceae (Shore e Barrett, 2011), e o fechamento do estigma em Polemoniaceae e Ranunculaceae (Waser e Fugate, 1986). Também é conhecido que a competição de pólen heteroespecífico também pode causar o entupimento do estilete por pólen próprio incompatível em Liliaceae (Palmer et al., 1989), interferir no crescimento do tubo polínico no estilete (Arceo-Gómez e Ashman, 2011), e germinar óvulos, especialmente entre espécies de plantas intimamente relacionadas (Burgess et al., 2008). No entanto, é importante notar que Waser e Fugate (1986) observaram que a formação de sementes não foi reduzida quando o pólen de ambas as espécies foi aplicado simultaneamente nos estigmas. Isso sugere que um efeito competitivo pode exigir a chegada de pólen estrangeiro antes do pólen da mesma espécie, e que o fechamento do estigma foi influenciado pela quantidade de carga polínica e não pela identidade da planta polinizada.

O estudo de Thomson et al. (1981) avaliou o impacto do pólen heteroespecífico ao longo de uma faixa contínua de proporções de pólen heteroespecífico, que manteve constante a quantidade de pólen conspecífico, usando uma única espécie doadora de pólen heteroespecífica.

Um estudo realizado por Moraes e Traveset (2005) investigou a possibilidade de que as flores de *Carpobrotu* spp. (uma espécie exótica da família Aizoaceae) competissem com o pólen de quatro outras espécies nativas que compartilham o mesmo habitat e época de floração. A hipótese era que a presença das flores exóticas poderia influenciar a visitação dos polinizadores e, consequentemente, afetar a reprodução das espécies nativas. No entanto, os resultados mostraram que a presença de pólen de *Carpobrotus* nos estigmas das espécies nativas era muito baixa. Além disso, experimentos de polinização manual revelaram que o pólen exótico não interferia significativamente com o pólen nativo, não afetando a produção de frutos naturalmente.

A competição pela polinização é uma interação importante na reprodução de plantas taxonomicamente relacionadas. Estudos anteriores relataram a identidade de morfologias polínicas em espécies de *Chamaecrista*, incluindo *C. flexuosa, C. diphilla, C. rotundifolia* e *C. ramosa* (Buril et al., 2011; Luz et al., 2013; Matos et al., 2014; Rede de catálogos polínicos, 2020). Segundo esses autores, o gênero *Chamaecrista* é caracterizado por apresentar grãos de pólen subprolatos ou prolatos, sincolpados, que podem ser considerados esternopolínicos. Com base nesse conhecimento, é possível analisar o fluxo de pólen nos estigmas entre os morfos e determinar a porcentagem de fluxo de pólen coespecífico e heteroespecífico. Essa abordagem traz perspectivas de avanços para espécies enantiostílicas monomórficas (Almeida e Castro, 2019).

Em um estudo realizado por Pereira (2007), foi investigado o fluxo intraespecífico genético em quatro subpopulações de *Chamaecrista*, em ambiente de Campo Rupestre. Este trabalho permitiu considerar que a dinâmica de floração pode contribuir para a ocorrência de cruzamentos preferenciais entre agrupamentos vizinhos. Destaca-se que, apesar de haver alternância de plantas vizinhas ao longo da fenologia e entre subpopulações, o estudo mostrou que há na verdade o favorecimento no padrão misto de fluxo gênico gamético entre grupos variados de plantas nesta população. Nesse contexto alguns autores investem em experimentos de polinização mista, isto é, incluem tratamentos, tais como: pólen coespecífico legítimo, e uma mistura de pólen coespecífico e pólens impróprios (heteroespecífico) (Brown et al., 2001; Streher et al., 2020).

Estudos que investigaram a ocorrência de transporte de pólen interespecífico em diferentes grupos de plantas, como por exemplo o de Fonseca et al. (2016) que coletou estigmas de duas espécies de plantas, *Canistropsis seidelii* (Bromeliaceae) e *Psychotria nuda* (Rubiaceae), e verificou que houve deposição de grãos interespecíficos de pólen nos estigmas das duas espécies. No entanto, o número de grãos interespecíficos foi baixo, embora seja frequente. Isso sugere que o transporte de pólen interespecífico pode ter pouco impacto no sucesso reprodutivo das plantas que participam dessa interação. Em outro estudo, Barral (2013) investigou a deposição de pólen impróprio no estigma de três espécies simpátricas do gênero *Senna* (Fabaceae), que são classificadas como enantiostílicas monomórficas. Embora tenha sido detectada a deposição de pólen ilegítimo, a proporção foi baixa e não gerou o entupimento/congestionamento do estigma.

Assim, a fundamentação teórica apresentada evidencia a complexidade das relações reprodutivas em espécies polimórficas, com destaque para as adaptações morfológicas e funcionais do na família Fabaceae e no gênero *Chamaecrista*. A dinâmica entre enantiostilia, o fluxo polínico e os mecanismos de isolamento reprodutivo oferecem uma visão aprofundada das estratégias evolutivas que promovem a polinização cruzada, a diversidade genética e a coexistência de espécies em ecossistemas biodiversos, como as restingas. Este arcabouço teórico fundamenta a relevância de investigar as interações planta-polinizadora, considerando os desafios ecológicos e evolutivos associados às mudanças ambientais e à conservação da biodiversidade.

CAPÍTULO 1

Flowering phenology in species of *Chamaecrista* Moench.: temporal generation of functionally enantiostylous dimorphic individuals

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Flowering phenology in species of *Chamaecrista* Moench.: temporal generation of functionally enantiostylous dimorphic individuals

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- **Background and Aims:** The dynamics of floral morph production in enantiostylous species has a direct influence on reproductive success. Here, we investigated the variation in morph production during flowering in four species of *Chamaecrista* with monomorphic enantiostyly: *C. diphylla, C. flexuosa, C. ramosa* and *C. rotundifolia.*
- **Methods**: We verified morph production over two blooms in 40 individuals per species. We counted the number of flowers with style facing right and left in the individuals to detect variations in morph proportions at individual and population levels. We recorded the proportion of morphs, assessed whether individuals tend to be more anisoplethic or isoplethic based on daily production and total (accumulated) production and whether the maintenance of a functionality (right, left or isoplethic) is constant between days.
- **Key Results:** Species generally exhibited isoplethy, regardless of the flowering period. Most individuals were isoplethic at the end of the sampling period, but they generally changed their morph ratios daily, showing a dynamism of functionality. Individuals of *C. diphylla, C. rotundifolia* and C. *flexuosa* often show anisoplethy. However, *C. ramosa*, whose individuals had more open flowers per day, did not show any difference in the proportion of days in which it presented isoplethy or anisoplethy.
- **Conclusions:** Although populations with monomorphic enantiostyly exhibit isoplethy, they may strategically alternate in the production of morphs generating functionally dimorphic individuals. The occurrence of dimorphic individuals hinders geitonogamy and promotes intermorph cross-flow between plants of the same species. Our results, therefore, add a temporal factor in discussing the existence of dimorphic functionality in monomorphic enantiostylous individuals.

- **Key words:** anisoplethy, mirror-image flowers, morphological functionality, reproductive strategies, temporal variation.

INTRODUCTION

Enantiostyly is a polymorphism that expresses two floral forms that differ in the curvature of the style in relation to the floral axis (Jesson and Barrett, 2002; Jesson et al., 2003b). Flowers with right-directed styles are classified as the "right morph" (R), and those with left-directed styles are classified as the "left morph" (L) (Barrett, 2002). The production of these morphs in individuals characterizes two basic types: monomorphic enantiostyly, where both right and left morphs are produced on the same plant, and dimorphic enantiostyly, where each individual produces only one morph (Barrett, 2000).

Studies indicate that dimorphic enantiostyly evolved from monomorphic ancestor (Jesson and Barrett, 2003). Monomorphic enantiostyly is often associated with reproductively advantageous, especially in periods of pollen limitation, and typically leads to greater reproductive success than dimorphic enantiostyly (Mora-Carrera et al., 2019). According to Morais et al. (2020), monomorphic enantiostyly is more prevalent than dimorphic, occurring in 17 angiosperm families, while dimorphic enantiostyly is limited to three monocot families. However, the microevolutionary factos restricting the extent of dimorphic enantiostyly remanin complex (Jesson et al., 2003b).

The biological function of enantiostyly is strongly linked to the promotion of successful male fitness at the individual level. Enantiostyly facilitates the deposition of pollen from each morph onto specific regions of the pollinators' bodies (Almeida and Castro, 2019). This spatial separation reduces pollen competition and increases the likelihood of effective cross-pollination between R and L morphs. The adaptive value of monomorphic species seems to have evolved as a strategy to avoid self-pollination (autogamy) and intramorph competition (Jesson and Barrett, 2003). In monomorphic populations, differences are observed in the level of intensity of the flowering and in the expression of the production of distinct floral morphs per individual.

It is observed that the production of morphs is dynamic in time and environment, a fact that provides monomorphic plants with a variable physical condition between flowering days, within and between populations (Tang and Huang, 2005; Almeida et al. 2013b; 2018). This condition is reflected by the alternation of morphs R and L in the individual (regardless of the number of flowers), since with the absence of one of the morphs the plant overcomes the conflict with geitonogamy (Barrett et al., 2000), or with low flower production, which then allows the population to have predominantly right and left individuals every day (Tang and Huang, 2005; Almeida et al. 2013b; 2018).

Plants can adjust their resource allocation patterns between male and female functions in response to ecological interactions. This adjustment can lead to variations in sex ratios or proportions of floral morphs over time, a phenomenon influenced by frequency-dependent selection (Charnov, 1982; Lopez and Dominguez, 2003). In enantiostylous populations, the daily balance of floral morphs represents an adaptive strategy to promote intermorph cross-pollination, a crucial process for maintaining genetic diversity (Freeman et al., 1980; Gao et al., 2006). Moreover, variations in the proportion of morphs within individuals can act as a preventive mechanism against self-fertilization through geitonogamy, contributing to an increase in effective population size and resilience to environmental pressure (Lopez and Dominguez, 2003).

In the context of monomorphic enantiostyly, it is possible to make an analogy with the theory of sex allocation in dioecious species (Charnov, 1982). Although morphs R and L do not represent distinct sexes, the ratio between them plays a role similar to that of a sex ratio. Thus, the ability to adjust the proportion of morphs within a population of hermaphrodite flowers acts as a functional adaptation analogous to sex allocation in a dioecious population, maximizing the efficiency of cross-pollination throughout the flowering season. Almeida et al. (2018) separate this individual tendency to produce only one floral morph (or a predominance of one floral morph) between the: functionally right (RF) and left (LF) categories, and the tendency to produce both morphs in a balanced ratio of functionally reciprocal (REC). The authors indicated that the same category can, in some cases, be maintained for several days or even have patterns of functional alternation, consequently reflecting in the frequency of balance in the populations. So, there is an expectation that the expression of the morphs reveals the differences and similarities of reproductive strategies in the species, even in periods of overlapping flowering.

The simultaneous presence of different floral morphs in the population enables intermorph crosses (Almeida et al., 2015), in which L and R flowers deposit and receive pollen from the opposite morph, on the opposite reciprocal side of the pollinator's body, favoring the transfer between morphs and contributing to the co-specific deposition of pollen (Gao et al., 2006; Braga et al., 2022). Given the importance for pollen dispersal and reproductive success, it is known that the general rule is to find a balanced daily frequency (1:1) in the production of floral morphs within populations of both monomorphic and dimorphic species, and this condition in heterostylous species is known as isoplethy (Ganders, 1979; Lima et al., 2024). In turn, anisoplethy signals disproportionality in this production which for enantiostyly is somewhat unknown (Almeida et al., 2018). The balanced proportion of morphs for dimorphic enantiostyly has a clearer functionality, promoting intermorph pollination and increasing pollen transfer between individuals (Mora-Carrera et al., 2019). In turn, monomorphic enantiostyly is responsible for preventing at least half of geitonogamy (Jesson and Barrett, 2005).

Throughout the life cycle of plants, the supply of flowers is discontinuous, which impacts reproduction and survival, especially in relation to the occurrence of isoplethy or anisoplethy in populations (Ramirez-Parada et al., 2020). We believe that monomorphic enantiostylous species with high flower production are more likely to achieve isoplethy, since the greater number of flowers facilitates balanced pollination between different morphs. In contrast, species with low flower production may exhibit anisoplethy, resulting in less efficient pollination and the possible predominance of one morph. This is supported by studies showing that flower density can positively influence pollination efficiency and fruit production through density-dependent selection (Castillo et al., 2002). Alternating morphs can be an adaptive response in environments where pollination is limited, maximizing the likelihood of reproductive success by diversifying the morphotype produced. This alternation strategy promotes greater morphological variability and reduces competition for pollination, similar to what occurs in dimorphic enantiostyly. This adaptability is consistent with previous findings of plant can alter their morphologic state in response to environmental changes (Freeman et al., 1980). In addition, fundamental aspects of reproduction, such as the relationship between phenological behavior and the dynamics of floral morph production, are still little explored. Thus, enantiostylous species represent interesting models for investigating strategies related to the temporal dynamics of floral morph production, from the individual to the population level.

In the same plant community, taxonomically related species within the same family or genus may exhibit distinct reproductive strategies throughout the year (Ollerton and Lack, 1992). Monomorphic enantiostylous species of *Chamaecrista* Moench. (Fabaceae) have similarities in their floral biology, interact through specialized pollination services mediated by bees, and the main reproductive barrier in mediating pollen flow is related to floral morphology (Almeida et al., 2015). Many species of this genus are sympatric and bloom at the same time of day and year, being considered synchronopatric (Madeira and Fernandes, 1999; Costa et al., 2013). Therefore, seasonal and synchronized events in tropical regions and in flowering phenology can generate adaptive advantages related to sexual strategies for geographically close species (Pedroni et al., 2002; Barret, 2002). In general, for polymorphic species it is still unknown what the implications of interspecific co-flowering are on the dynamics of morph production, especially in evolutionarily related species.

In our study, we analyze these issues in a scenario of overlapping blooms of four sympatric *Chamaecrista* species that have genetic and ecological similarities, including, in some cases, possibilities of interspecific pollinator sharing, based on preliminary observations (Bezerra, 2019). This study aims to analyze the variation in the production of floral morphs, throughout the flowering period, within individuals and populations. Three hypotheses were tested: H1) There is balanced distribution of floral morphs in populations and individuals throughout flowering; H2) The daily proportion of L and R flowers in monomorphic enantiostylous species with more flowers per individual tends towards isoplethy, while species with fewer flowers are more likely to exhibit anisoplethy; H3) Individuals that produce a smaller number of flowers daily have a greater chance of alternating the morph produced, therefore, repeat the same feature less for more consecutive days.

MATERIALS AND METHODS

Study site

The study was carried out in the Private Natural Heritage Reserve, RPPN Nossa Senhora do Oiteiro de Maracaípe, regulated by 58-DOU 187-E, 27/09/2000, section/pg. 1/98, owned by the Parish of São Miguel, in the municipality of Ipojuca, Pernambuco, Brazil (08º 831' 132 48"S; 35º 801' 05"W). The area is part of the Ipojuca River basin, and the territory is made up of 80% restinga (sand dune habitat) and 20% mangrove, totaling 76.21 hectares. The climate is tropical, with average annual temperatures ranging from 23 to 28ºC and average variations of 2,000 mm/year, with the greatest intensity between May and August (Inmet, 2021). The soil is classified as Neossolo Quartzarênico, with three main plant physiognomies: forest, non-flooded field and flooded field. The most representative families are Poaceae, Cyperaceae, Myrtaceae, Orchidaceae, Rubiaceae, Bromeliaceae and Fabaceae (Almeida et al., 2009). In forest physiognomy, phanerophyte life forms predominate, while in the flooded and non-flooded field physiognomies, camephytes, terophytes and cryptophytes predominate. The soils of these physiognomies differ in their chemical composition and the water table only rises in the flooded field (Almeida et al. (2009).

Species studied

Four sympatric species with subherbaceous and herbaceous habit (Bezerra, 2019) of the genus *Chamaecrista* Moench. (Fabaceae) were studied: *i) C. diphylla* (L.) Greene; *ii) C. flexuosa* (L.) Greene*; iii) C. ramosa* (Vogel) H.S.Irwin ; Barneby and *iv) C. rotundifolia* (Pers.) Greene (Fig. 1). The plants are monomorphic enantiostylous, with yellow, asymmetrical flowers with heteromorphic petals. The flowers last from four to eleven hours. All are pollinated by solitary bees, able to vibrate their poricidal anthers for the release of pollen grains, the only available floral resource (Almeida et al., 2015; Bezerra, 2019). The inclusion of these four species was based on local diversity of enantiostylous species, which allows for a broader comparison of possible variations in floral morphology. Each species represents a unique case that contributes to a more comprehensive understanding of the adaptive mechanisms involved in enantiostyly. In addition, there are differences between the species studied, especially when compared to the size of flowers, or the type and number of flowers produced per day.

Phenology monitoring

Flowering phenology was investigated by counting the number of open flowers of each floral morph on each individual (n= 40 individuals per species). The individuals of each species were part of four natural subpopulations, located in close geographical proximity within the reserve area. The individuals were monthly monitored for three consecutive days over a period of 18 months (July 2019 to December 2020). During this period, monitoring was carried out for each individual and species. The proportions of flowers produced from different floral morphs were recorded, distinguishing between the right (R) and left (L) styled, and counting both daily production and the total accumulated over the days monitored, as indicated by Almeida et al. (2018).

Variation of the ratio of floral morphs and functional categories

From the phenological data, the ratio of floral morphs within each individual studied was established. The ratio of morphs was calculated by dividing the number of right flowers by the total number of flowers produced on the day sampled. Thus, the ratio ranges from 0-1, with values closer to 0 (zero) indicating greater production of left flowers (LF) and values closer to 1 (one) indicating greater production of right flowers (RF). We established that the range between 0.25-0.75 indicates isoplethy, and the value 0.5 is considered fully isoplethy, i.e., morph ratios equal to 1:1 (adapted from Almeida et al., 2018). From these data, we investigated whether the ratio of floral morphs, at the population level, varied throughout flowering and whether the ratio of floral morphs varied among individuals of the same species. Data were analyzed for each species using generalized linear models (GLMs) to investigate the effects of flowering date and individual on morph ratio. Morph ratio was modeled with a binomial distribution using the "logit" function. The analyses were conducted using the "lmer" function in the "lme4" package in R (Bates et al., 2015). To obtain the significance of each factor, a type II analysis of variance was conducted using the "Anova" function in the "car" package (Fox, 2016). Effects of subpopulations and year sampled were not considered, as in a previous analysis they were not significant for any of the species.

Using the ratio of floral morphs, individuals were classified as showing isoplethy (ratio between 0.25-0.75) or anisoplethy (ratio between 0-0.24, functionally left, and 0.76-1, functionally right) on each day of their flowering. Subsequently, generalized linear mixed-effects models (GLMMs) were performed to analyze whether individuals of each species had a higher proportion of recording in isoplethy or anisoplethy. Morph ratio was modeled with a binomial distribution using the "logit" function. Individual plants were included as a random factor. The analyses were conducted using the "glmer" function in the "lme4" package (Bates et al., 2015). The significance was tested with type II analysis of variance as described above.

Temporal variation in repetition of functional categories

Individuals can be, for example, functionally isoplethic or functionally right-left for several days or throughout the flowering period, or they can be dynamic, changing their functionality every day. To analyze the dynamic nature, individuals were analyzed for the maximum level of repetition within the same functional category (right, left, or isoplethy). For this, the maximum number of days sampled on which an individual consecutively exhibited the same functional category was considered. GLMMs were used to investigate the effects of functional category on the maximum number of repetitions. Morph ratio was modeled with a binomial distribution using the "logit" function. The analyses were conducted using the "glmer" function in the "lme4" package (Bates et al., 2015) and significance was tested with type II analysis of variance. All analyses were performed using the statistical tool R version 3.6.3 (R Development Core Team, 2020).

RESULTS

Reproductive phenology

The flowering of *Chamaecrista diphylla* and *C. rotundifolia* occurred over three months of the year (Fig. 2), with both species reaching their peak flowering between July and September. On the other hand, *C. flexuosa* and *C. ramosa* are perennials that exhibit continuous flowering throughout the year (Fig. 2). In an exception, no plants were flowering in January 2020. *Chamaecrista flexuosa* exhibited the highest flower production from July to September, while for *C. ramosa* the peak occurred between April and May of each year. The average daily flower production in the population was 12 (mean per individual plant= 0.30) for *C. diphylla*, 18 (mean= 0.45) for *C. rotundifolia*, 29 (mean= 0.72) for *C. flexuosa* and 131 (mean = 3.3) for *C. ramosa*.

Variation in morph ratios and functional categories

The morph ratios in the populations of all four species did not change significantly (P > 0.346) during the flowering period (upper plots of Fig. 2). Thus, regardless of the sampled period, these populations tended to exhibit isoplethy, although some exceptions were recorded (e.g. August 2000 in *C. rotundifolia*; November 2000 in *C. flexuosa*; October 2000 in *C. ramosa*; Fig. 1). There were no significant (P > 0.481) variations in the accumulated morph ratios within the studied individuals of the four species (lower plots of Fig. 2). The morph ratios of the individuals generally remained within the range of 0.25 to 0.75, which is considered isoplethic (Fig. 2). Only a few individuals displayed average morph ratios outside the isoplethy range, limited to *C. diphylla* (n=4 individuals) and *C. rotundifolia* (n=5 individuals).

Although most individuals of the four species exhibited isoplethy during the study period (Fig. 2), most of these individuals displayed the ability to alter the morph proportions daily (Fig. S1, S2, S3 and S4). Only one individual of *C. diphylla* (individual #16; Fig. S1) and one of *C. rotundifolia* (individual #32; Fig. S2) consistently maintained isoplethy, regardless of the day investigated. Five individuals were always anisoplethic (functionally right or left) for *C. diphylla* (individuals #8, 21, 23, 30 and 32; Fig. S1), eight for *C. rotundifolia* (individuals #7, 20, 22, 27, 31, 33, 36 and 38; Fig. S2) and just one for *C. flexuosa* (individual #31; Fig. S3). Most individuals alternated the morph proportions throughout the flowering period, with some days exhibiting isoplethy while other showed anisoplethy. In the case of *C. diphylla*, *C. rotundifolia* and *C. flexuosa*, individuals had a significantly higher proportion of days with anisoplethy compared to isoplethy (upper plots of Fig. 3). However, for *C. ramosa*, the proportion of days on which individuals exhibited isoplethy and anisoplethy was not statistically different (Fig. 3). Individuals that represented the pattern of variation found for each species are shown in figure 3 (lower plots).

Temporal variation in the repetition of functional categories

The four species studied showed some level of repetition of the functional categories (right, left, or isoplethy). We observed that some individuals were able to maintain the same functional category for up to seven consecutive days of sampling. However, we also found plants that never repeated the same category, indicating a high degree of dynamism (Fig. 4). For *C. diphylla*, *C. rotundifolia* and *C. flexuosa*, the maximum number of repetitions did not vary significantly among functional categories (Fig. 4). The only species that displayed significant differences among categories was *C. ramosa*. In this case, the individuals studied exhibited a greater repetition of days with isoplethy compared to functionally right and left (Fig. 4).

DISCUSSION

We evaluated the dynamics of production of floral morphs in populations of four monomorphic enantiostylous species of *Chamaecrista* and tested hypotheses related to number of flowers produced daily, influencing the total, daily and individual production of right and left flowers. The hypotheses involving the influence of the number of flowers on the variation in the production of floral morphs and on the alteration of the functionality of the individuals were corroborated. During flowering, the number of anisoplethic individuals was higher than that of isoplethic individuals in most species, as was the number of variants in functionalities (LF, RF, or isoplethy). Below, we discuss these data and the possible impacts of the strategies observed in the flower production of the studied species on the functionality of enantiostyly and the reproductive success of the plants.

The absence of disproportionality in the production of morphs in different populations, regardless of the occurrence of overlapping flowerings, indicates that the dynamics of morph production is not altered in co-flowering events, supporting our first hypothesis. To formulate this hypothesis, we based ourselves on the understanding of-Almeida and Castro (2019) about the possible interference of interspecific pollen in the production of flowers of different morphs in monomorphic enantiostylous species. We believe that the test of this hypothesis must be repeated with other enantiostylous species, since the genetic or environmental mechanisms of expression of the morphs are not known (Jesson et al. 2003; Tang and Huang, 2005; Almeida et al. 2018; Almeida and Castro, 2019; Barrett and Fairnier, 2024). We highlight that, in our model, the only species that overlap flowering and share pollinators (*C. ramosa* and *C. flexuosa*) show, in most cases, distinct pollen deposition and uptake sites on the pollinator's body (Almeida et al. 2013a; 2013b; 2015a; 2015b), reducing the interference that interspecific pollen can cause in flower production.

A greater number of flowers increased the tendency for isoplety in the proportion of morphs in individuals, corroborating our hypothesis. This becomes clear when we look at the daily averages of flowers produced by plants of the four species. *Chamaecrista ramosa*, the only species to produce on average more than one flower per individual, showed a similar proportion of isoplethic and anisoplethic individuals, differing from the other species, which presented anisoplethic individuals in a significantly greater number. This is due to the greater probability of expression of a single floral morph, when daily flower production is low. This strategy was observed in other Cassiinae species by Almeida et al. (2018) and by Tang and Huang (2005) in two species of *Monochoria* (Pontederiaceae). The presence of only one floral morph per individual in monomorphic enantiostylic species is analogous to the situation in dimorphic enantiostylic individuals, resulting in a dimorphic functional condition for these plants, making intermorph crossings within the individual impossible and, consequently, reducing geitonogamy rates (Barrett, 2002; Jesson and Barrett, 2003; Tang and Huang, 2005; Almeida et al., 2018).

The impact of flower number on geitonogamy rates was also reported in the study by Mora-Carrera et al. (2019). The authors simulated contexts of high and low pollinator density, by manipulating the number of flowers of individuals of the monomorphic enantiostylous species *Solanum rostratum* (Solanaceae). Furthermore, they simulated the occurrence of dimorphic individuals (isoplethic; LF and RF) in a monomorphic population. The results demonstrated that under high pollinator densities (low number of flowers), dimorphic individuals had higher rates of xenogamy. In the experiment with low pollinator density (high number of flowers), the rates were equal between monomorphic and dimorphic plants. In our study, we propose that the reduced number of flowers would decrease the chances of geitonogamy, which contradicts, in a way, what was observed here. However, the monomorphic individuals, manipulated by the authors, were isoplethic (2 right and 2 left flowers), differing from what we found, as the reduction of flowers generated a majority of anisoplethic individuals. Therefore, we understand that monomorphic enantiostylous species can present xenogamy rates like the dimorphic ones in different contexts of flower and pollinator densities, which can vary at different stages of flowering, similar to what was found by Wang et al (1998).

Monomorphic species that produce inflorescences show different ways of displaying floral morphs, in some cases with a certain predictability of the positioning of the morphs (Jesson et al., 2003a; Johnson et al., 2023). However, in plants that produce isolated flowers, such as the species in our study, there is no known pattern of production of these flowers (Tang and Huang, 2005; Almeida et al. 2013a; 2013b; 2018) nor the factors that are involved, as previously mentioned. We saw in our study that, even in populations and individuals presenting isoplethy in terms of the total number of flowers, the majority of individuals were variant anisoplethic (LF and RF), when we consider daily floral production, similar to results obtained in other studies (Tang and Huang, 2005; Almeida et al. 2018). The occurrence of variant and non-variant individuals may demonstrate the existence of genetically predetermined factors. However, the absence of variation in the pattern of these individuals raises the possibility of environmental factors acting, such as pollen limitation, due to preferential pollen deposition in one of the morphs (Almeida and Castro, 2019). This could signal an excess of flowers from one of the morphs, which would lead to an increase in the flower production of the opposite morph.

The variation in functional categories observed in individuals allowed us to evaluate the repetition of these categories throughout the observation period. Our results indicate that individuals with fewer flowers had fewer days with repetition of functional categories, corroborating our third hypothesis. In general, the number of consecutive days in the same functional category was low (usually two to four days, depending on the species), when we consider anisoplety, similar to what was observed by Tang and Huang (2005) and Almeida et al. (2018). The exception was *C. ramosa*, which presented a greater repetition of isoplethic days, due to the greater number of flowers produced, when compared to the other species in our study, which makes it difficult to maintain anisoplety (Almeida et al., 2018; Tang and Huang, 2005). The low repetition of anisoplethic functional categories reinforces the hypothesis of the interference of environmental factors in the production of floral morphs, in monomorphic enantiostylic species, due to the great variation of individuals with an apparent absence of patterns.

Our study is one of the few that has followed flowering and morph production dynamics in enantiostylic species over an extended period. However, we corroborate what has been found in research with monomorphic enantiostylous species, with the same or shorter duration, which observed a similar proportion of morphs as a standard in populations, when considering the total number of flowers. However, when the evaluation considers the dynamics of morph production in individuals, we identify the existence of individuals with functional categories capable of alternating them, despite apparently not following a standard. The occurrence of these individuals and the lack of patterns in the production sequence of these flowers imposes the need for further investigation into the factors that mediate these strategies. Although we do not understand other possible factors with the power to interfere in this important process, our study provides relevant information regarding the dynamics of morph production in monomorphic enantiostylic species, with the unprecedented indication that the number of flowers produced has a direct effect on the proportion of morphs in individuals.

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FIGURES AND CAPTIONS

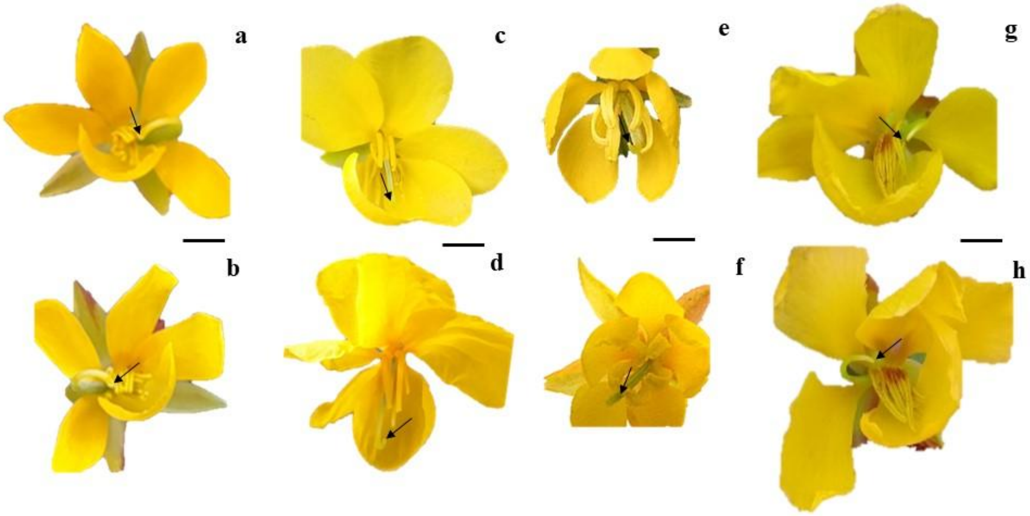

Figure 1. Flowers (right (R) and left (L) morphs) of the species of *Chamaecrista* (Fabaceae) studied: (a-b) *C. diphylla* (L.) Greene; (c-d) *C. flexuosa* (L.) Greene; (e-f) *C. rotundifolia* (Pers.) Greene; and (g-h) *C. ramosa* (Vogel) H.S.Irwin ; Barneby. The arrows indicate the stigma position. Scale bar = 10 mm.

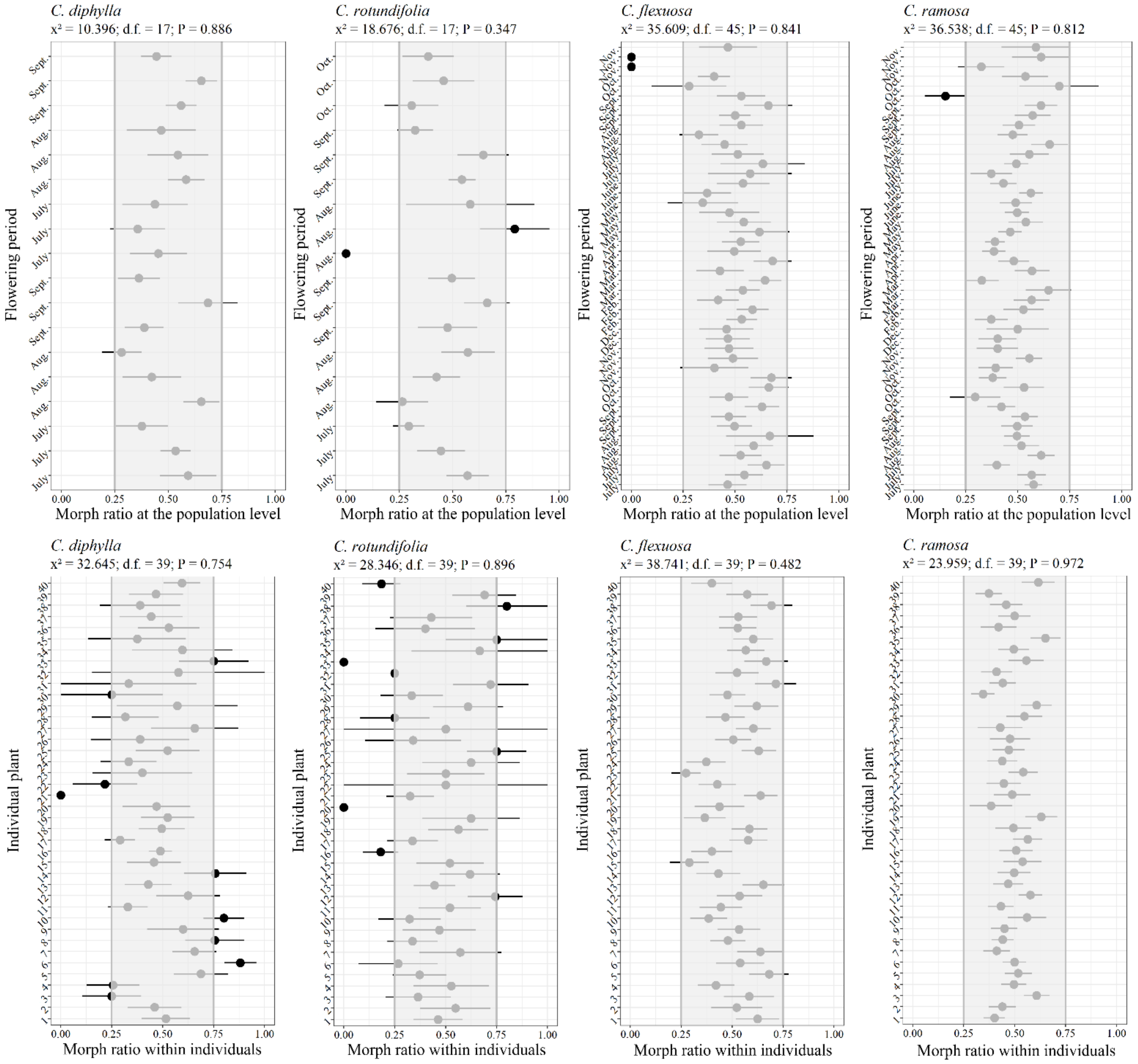

Figure 2. Range of variation in morph ratios at the population level (upper column plots), throughout the flowering of each species, and variation in morph ratios within the forty individuals studied of each species (lower column plots) during the sampled period (2019-2020) in a Restinga area in Northeastern Brazil. The morph ratios can vary from 0-1, with the central gray band on the x-axis indicating the range of isoplethy (0.25-0.75). Morph ratio values between 0-0.24 indicate greater left functionality and between 0.76-1 greater right functionality. The mean of this range is indicated in each sample as well as the standard error. The statistical values are shown above each plot.

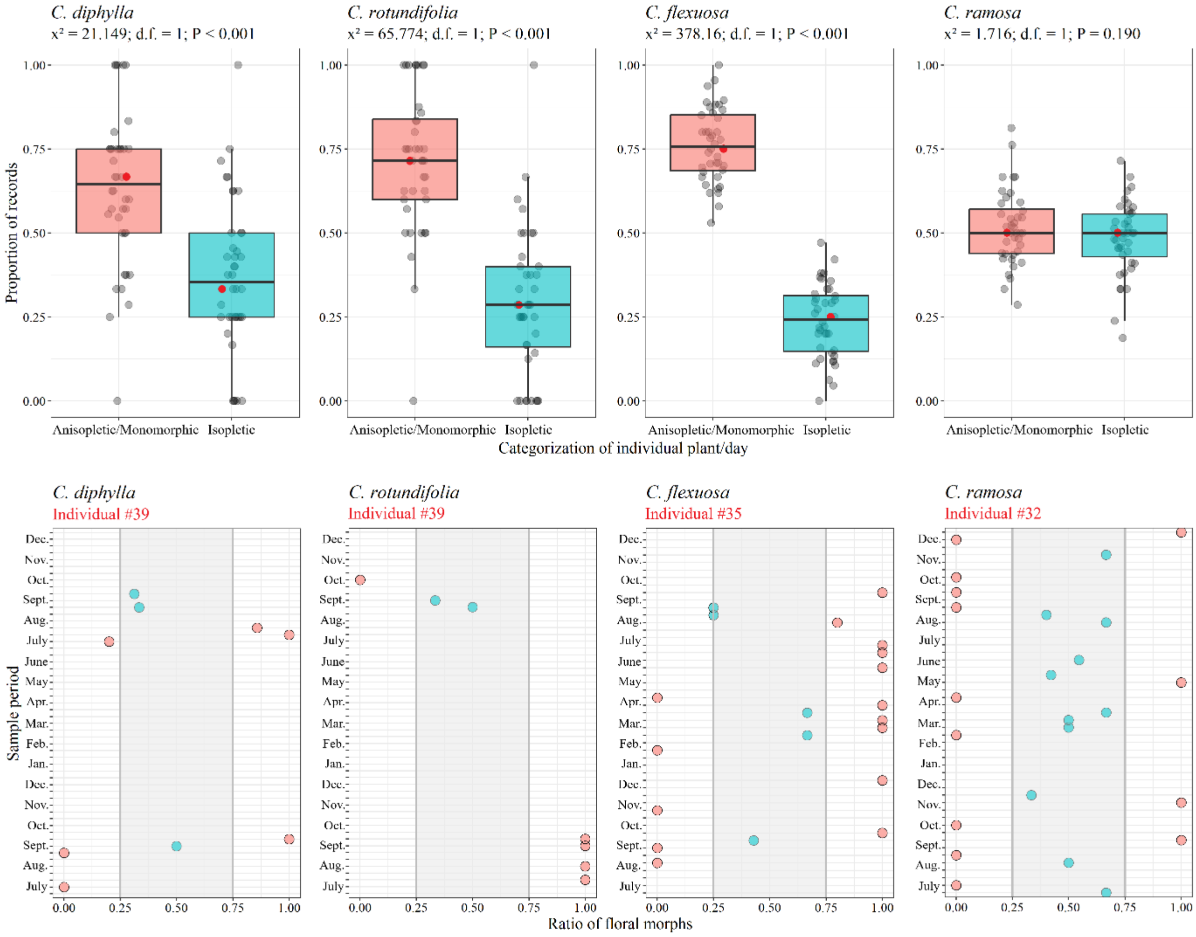

Figure 3. The upper boxplots show the daily proportion of isoplethic and anisoplethic individuals for each species studied, in a Restinga area in Northeastern Brazil. The gray points show the data for each individual plant during the days recorded in flowering. The statistical values comparing the two groups are shown above each plot. The lower plots show the variation in morph ratio within an individual plant of each species during the sampled period (2019-2020). The individuals plotted are those that are closest to the population median (red points in upper boxplots) for each species. Cyan points that are within the gray band (values between 0.25-0.75) indicate isoplethy on that particular sampling date, for the individual considered. Salmon pink dots outside the gray band (values between 0-0.24 and 0.76-1) indicate anisoplethic for that sampling date.

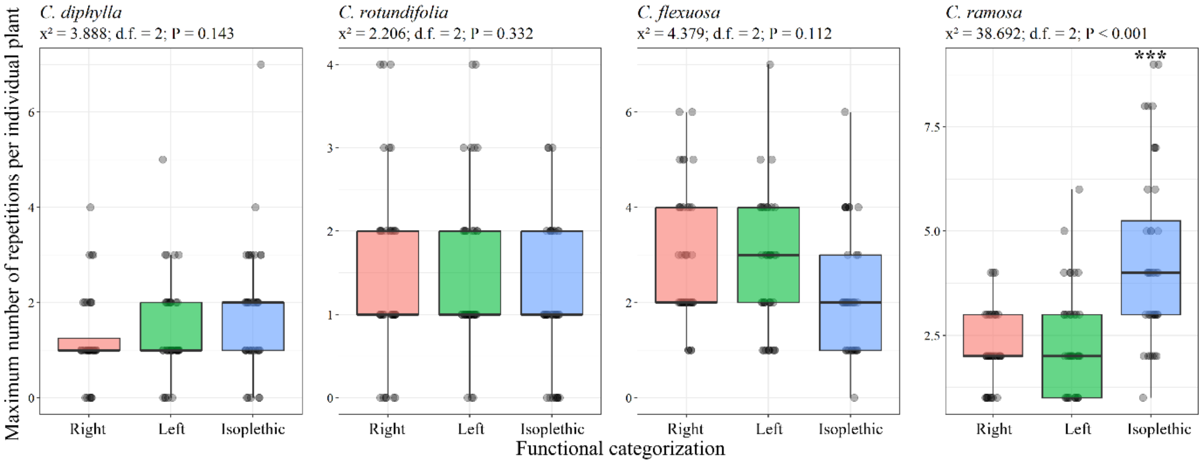

Figure 4. Boxplots of the maximum number of days sampled on which individuals repeatedly exhibited more right, left, or isoplethic flowers, for each species studied, in a Restinga area in Northeastern Brazil. The gray points show the maximum number of repetitions of each individual plant for each functional categorization. General statistical values are shown above each plot and asterisks indicate significant differences between groups (***P < 0.001, **P < 0.01, *P < 0.05).

4. APÊNDICE -MATERIAL SUPLEMENTAR

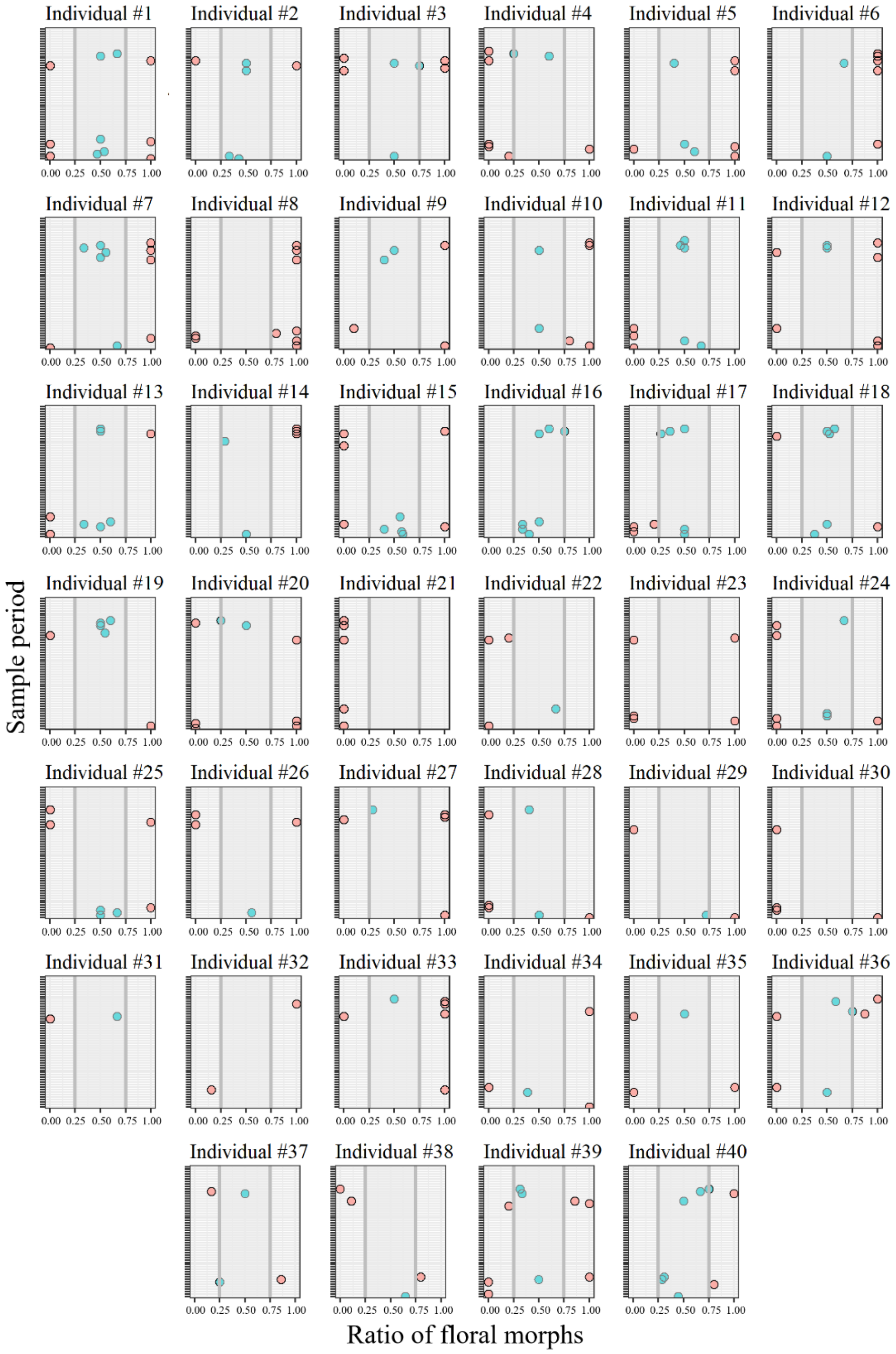

Figure S1. Daily morph ratios in forty individuals (n=54 days) studied in the period 2019-2020 for the species *Chamaecrista diphylla*, in a Restinga area in Northeastern Brazil. The gray band on the x-axis indicates the range of variation that corresponds to the isoplethy of the floral morphs (values between 0.25-0.75; cyan points), with the left of it (values between 0-0.24; salmon pink points) indicating the highest frequency of left flowers and the right of it (values between 0.76-1; salmon pink points) indicating the highest frequency of right flowers.

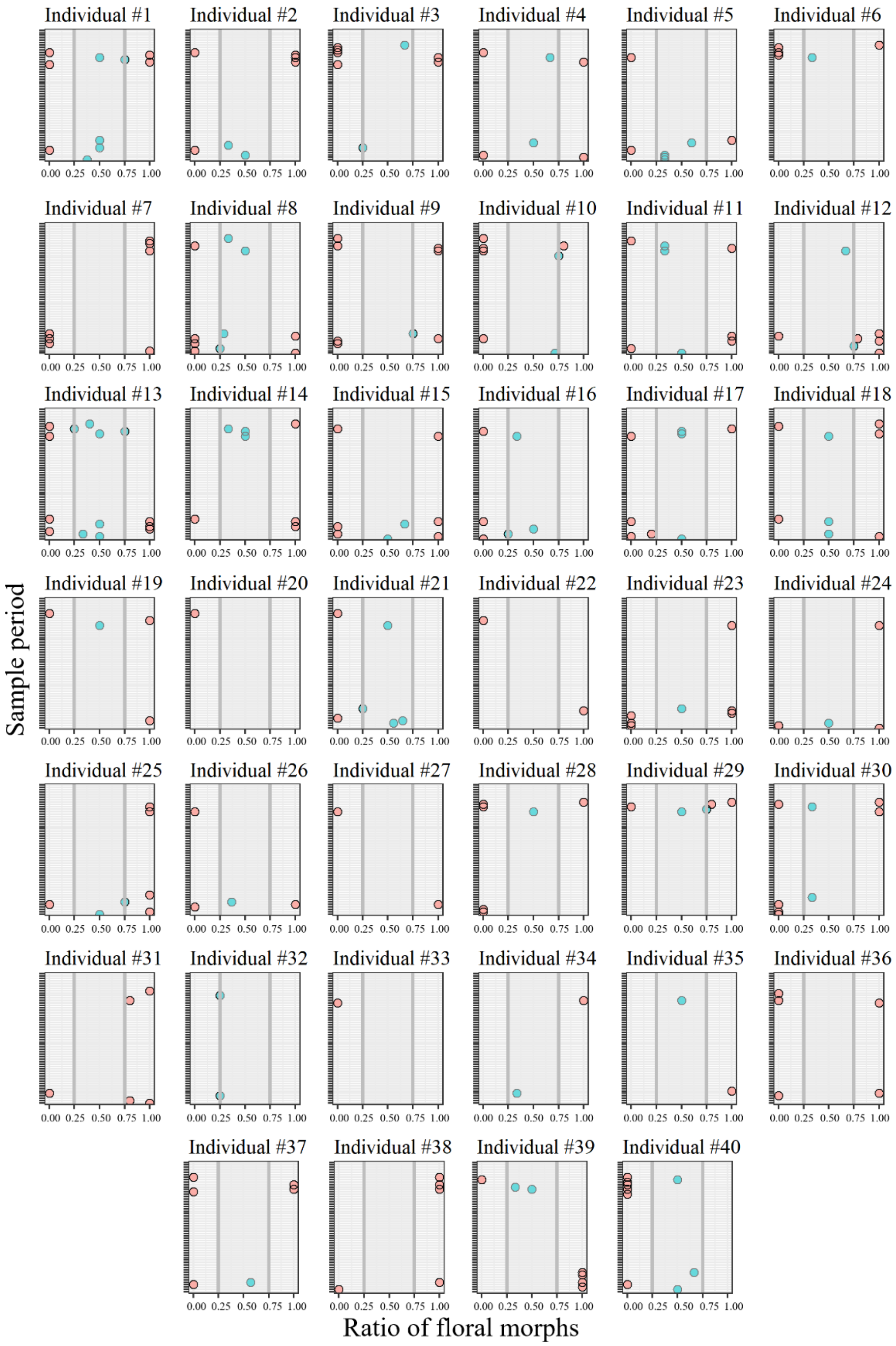

Figure S2. Daily morph ratios in forty individuals (n=54 days) studied in the period 2019-2020 for the species *Chamaecrista rotundifolia*, in a Restinga area in Northeastern Brazil. The gray band on the x-axis indicates the range of variation that corresponds to the isoplethy of the floral morphs (values between 0.25-0.75; cyan points), with the left of it (values between 0-0.24; salmon pink points) indicating the highest frequency of left flowers and the right of it (values between 0.76-1; salmon pink points) indicating the highest frequency of right flowers.

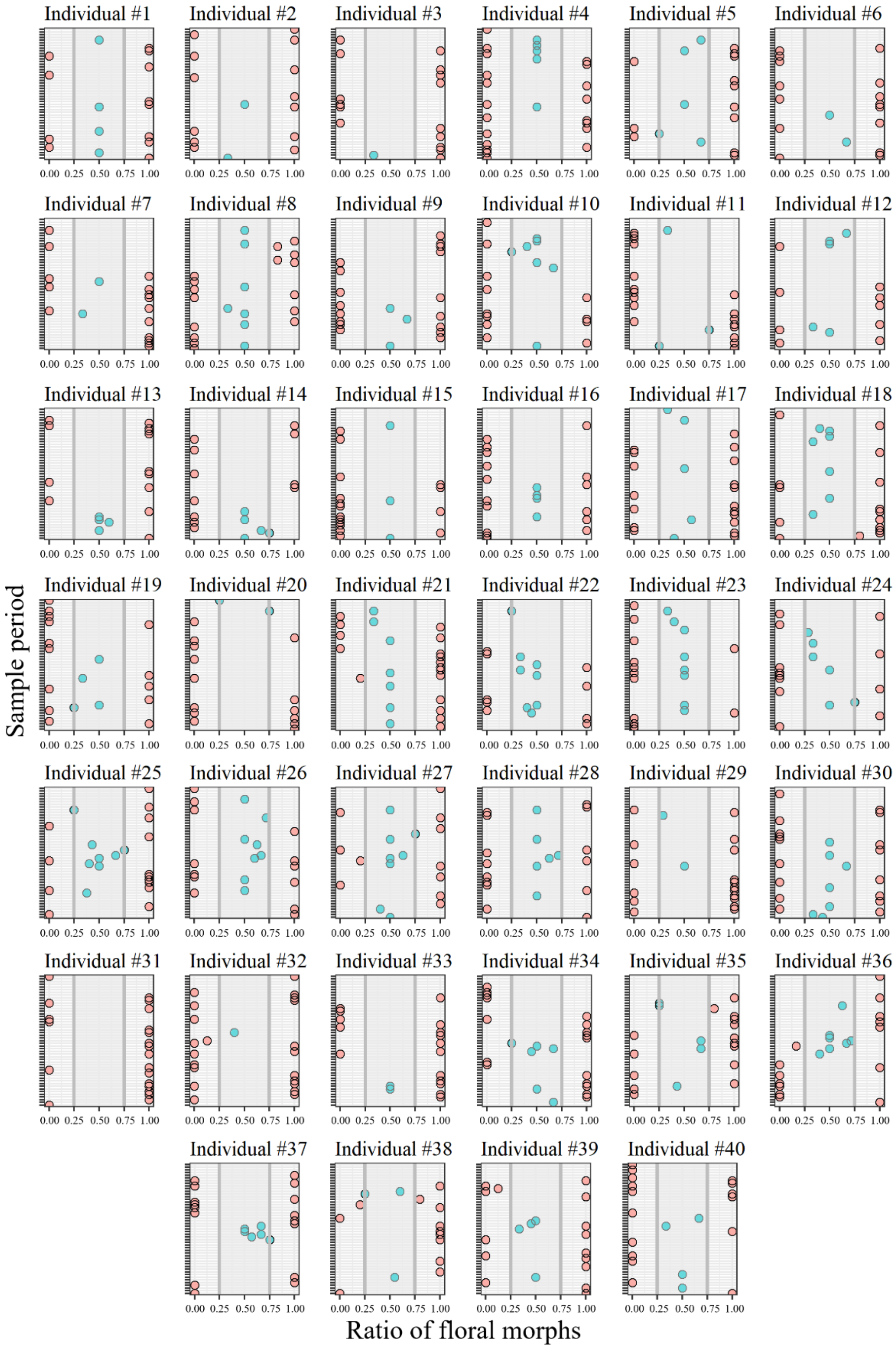

Figure S3. Daily morph ratios in forty individuals (n=54 days) studied in the period 2019-2020 for the species *Chamaecrista flexuosa*, in a Restinga area in Northeastern Brazil. The gray band on the x-axis indicates the range of variation that corresponds to the isoplethy of the floral morphs (values between 0.25-0.75; cyan points), with the left of it (values between 0-0.24; salmon pink points) indicating the highest frequency of left flowers and the right of it (values between 0.76-1; salmon pink points) indicating the highest frequency of right flowers.

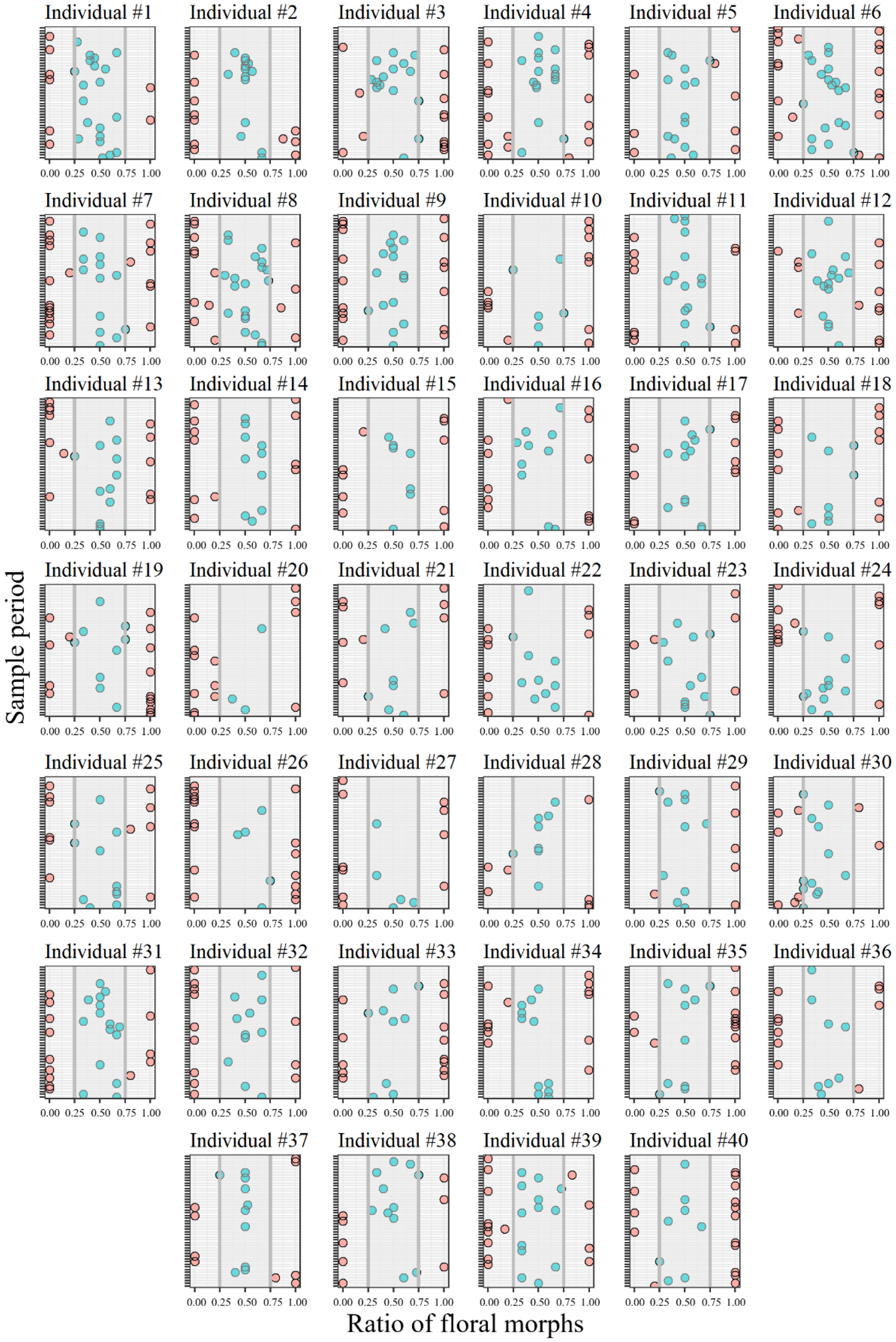

Figure S4. Daily morph ratios in forty individuals (n=54 days) studied in the period 2019-2020 for the species *Chamaecrista ramosa*, in a Restinga area in Northeastern Brazil. The gray band on the x-axis indicates the range of variation that corresponds to the isoplethy of the floral morphs (values between 0.25-0.75; cyan points), with the left of it (values between 0-0.24; salmon pink points) indicating the highest frequency of left flowers and the right of it (values between 0.76-1; salmon pink points) indicating the highest frequency of right flowers.

CAPÍTULO 2

Drivers of heterospecific pollen interference: The case of four co-flowering enantiostylous congeners

Manuscript to be submitted to Annals of Botany

Drivers of heterospecific pollen interference: The case of four co-flowering enantiostylous congeners

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Article type: Original.

Short title: Pollen flow in synchronopatric species.

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Background and Aims: This study investigated the effect of heterospecific pollen (HP) deposition on the reproductive success of four enantiostylous *Chamaecrista* species in an area of restinga vegetation in Ipojuca, PE, Brazil. The aim was to understand how the presence of HP affects the reproduction of these species.

Methods: In the field, we carried out manual crosses with controlled exposure of flowers to HP in different proportions (0%, 30%, 70%, and 100%) between species with overlapping flowering. Fruit formation was monitored to evaluate reproductive success.

Key Results: The results showed that reproductive success was negatively affected by the presence of HP. The response of reproductive success was analyzed considering the recipient species, the floral morphotype (right/left) and the proportion of HP applied, using Generalized Linear Mixed Models (GLMM). The results indicated that HP may have variable effects on female reproductive success. For example, *C. diphylla* proved to be more tolerant to the presence of HP, while *C. ramosa* and *C. rotundifolia* showed specific effects in response to different donors and *C. flexuosa* was the most negatively impacted. Increasing the proportion of HP did not alter reproductive success in *C. diphylla* and *C. rotundifolia*, but negatively affected *C. flexuosa* and *C. ramosa* which showed an increase in reproductive success in the presence of HP from the following donor species: *C. diphylla* and *C. rotundifolia*.

Conclusions: These observations suggest interspecific complexity and indicate that the coexistence of sympatric species may be mediated by differential tolerance to HP, with implications for adaptation and genetic diversity for *Chamaecrista*. We believe we contribute to the understanding of reproductive strategies in shared pollination systems and reinforce the importance of pre and post-pollination barriers in the maintenance of sympatric species with monomorphic enantiostyly.

Keywords: Reproductive success; Monomorphic enantiostyly; Interspecific compatibility; Manual crossings; Reproductive ecology; Restinga.

INTRODUCTION

The interaction among flowers in the context of pollination plays an essential role in plant reproduction, influencing species abundance and diversity (Gamba and Muchhala, 2020). When visitors alternate between flowers of two or more species, heterospecific pollen transfer (HP; Janovský and Štenc, 2023) occurs, potentially impacting plant reproductive success due to competition for pollinators (Arceo-Gómez et al., 2016). The effects of HP on reproductive fitness are mediated by the relationships between donor and recipient species and by specific interactions between floral characteristics (Arceo-Gómez et al., 2019).

Although the sharing of pollinators between plant species can be advantageous in some situations, it often entails losses, such as the loss of conspecific pollen (CP) and the deposition of HP (Moreira-Hernández and Muchhala, 2019). The main negative effects include interference with pollen tube growth, clogging or closure of the stigma, and inhibition of CP germination (Thomson et al., 2006; Arceo-Gómez and Ashman, 2011; Shore and Barrett, 2011). These consequences result in significant energy costs for plants, as they compromise the formation of viable seeds and the fertilization of ovules, representing an evolutionary challenge for plants (Pacini and Franchi, 2020). The effects can vary according to abiotic conditions, with reports of negative effects from intraspecific competition (Bergamo et al., 2020). Furthermore, Arceo-Gómez et al. (2011) indicate that the diversity of heterospecific donors does not necessarily lead to a greater decrease in reproductive success, since the effects depend on the specific interactions between the plant species involved.

On the other hand, some studies suggest that HP deposition can generate positive or neutral effects. Under certain conditions, species such as *Sisyrinchium wettsteinii* (Iridaceae) have shown tolerance to HP, especially when it comes from phylogenetically distant species, and may even optimize reproduction in environments with low pollinator availability (Streher et al., 2020). Related benefits include increased pollinator attraction and greater efficiency in CP deposition, especially in communities with high floral diversity (Lopes et al., 2021; Hao, Fang and Huang, 2023). In certain circumstances, reports of a neutral effect specifically demonstrate that the presence of HP does not necessarily impair the performance of co-specific pollen, suggesting that many species are tolerant of heterospecific pollen to a certain extent, and benefit from pollinator diversity, although these cases are less common in the literature (Hao et al., 2023; Caruso and Alfaro; 2000; and Montgomery, 2009). In this way, the presence of pollen from other species in some cases can help or facilitate plants in optimizing their reproduction. These implications highlight the complexity of the interactions between heterospecific and co-specific pollen, showing that, in some scenarios, the presence of HP can be advantageous. This also suggests that plants can develop adaptive mechanisms that favor the tolerance and even the use of HP for the benefit of their reproduction.

In this context, heterospecific pollen interference has important implications for speciation, ecological diversification and the evolution of flowering plants (Moreira-Hernández and Muchhala, 2019). This phenomenon is especially relevant in species with floral polymorphism, such as enantiostylous ones, in which flowers of different morphs (right/left) promote cross-pollination and reduce interference between co-specific pollen (from the same morph) and heterospecific pollen (Webb and Lloyd, 1986; Barrett, 2002; Almeida and Cardoso, 2019). This, in turn, contributes to increasing the individual reproductive success of plants (Ghazoul, 2006; Moeller, 2005). Enantiostyly is of great importance for maximizing male reproductive efficiency, as it regulates effective pollen dispersal. Studies such as those by Barrett (2002), Jesson and Barrett (2003) and Almeida et al. (2019) show that its functionality is closely linked to legitimate pollen flow, thus ensuring reproductive success.

The interaction between heterospecific (HP) and conspecific (CP) pollen is a promising field for research into reproductive dynamics, especially regarding the influence of HP deposition on stigmas on female fitness (Moreira-Hernández and Muchhala, 2019; Rowan and Casper, 2023). The reproductive system in species with enantiostyly may be self-incompatible, avoiding self-fertilization and reducing the possibility of inbreeding (Rodríguez and Sanoja, 2008). However, other species with monomorphic enantiostyly (when two floral morphs - right and left - occur on the same plant) do not have a self-incompatibility system, as in the case of *Chamaecrista* spp. (Fabaceae) (Arceo-Gómez et al., 2012; Sólis-Monteiro and Vallejo-Marín, 2017; Almeida et al., 2013a,b; 2015b; 2024).

This understanding is particularly relevant in species with enantiostyly, such as those of the genus *Chamaecrista*, which often share pollinators due to the geographical and temporal overlap of their flowers, which can result in the formation of natural hybrids and indicate partial reproductive compatibility (Conceição et al., 2008; Costa et al., 2013; Bezerra, 2019; Rando et al., 2024). Therefore, competition for pollen in these species, especially among those that are phylogenetically related, offers an ideal scenario for investigating the effects of HP under natural conditions. Thus, understanding the impacts of pollen flow and determining intra- and interspecific effects in *Chamaecrista* can bring significant advances in knowledge about plants with enantiostyly.

The coexistence of coflowering species subject to heterospecific (HP) and co-specific (CP) pollen transfer presents remarkable complexity, with a wide range of variations in the consequences of these interactions (Opedal et al., 2023). This study aimed to fill a gap in the direct investigation of the incidences and presence of HP in plants, especially in the context of enantiostyly, by evaluating patterns and correlations of variation in HP reception and its influence on the coexistence of related species.

In recent decades, studies on HP deposition have highlighted its importance for reproductive success, especially in communities where phylogenetically close species co-flourish (Harder and Barclay, 1994; Pacini and Franchi, 2020). Understanding the factors that contribute to HP interference is essential, as this interaction can impact cross-pollination rates and the formation of viable seeds. Closely related species that share pollinators are particularly susceptible to these effects, due to the greater similarity between pollen grains and similarities in floral morphology. However, despite its relevance, this topic is still little explored under natural conditions.

Classical studies such as those by Heslop-Harrison and Heslop-Harrison (1991) have addressed how morphological and functional variations in pollen influence pollination efficiency in co-flowering environments, highlighting the complexity of pollen interactions. Morales and Traveset (2008) demonstrate that HP deposition can have positive or negative effects on co-specific pollen deposition, depending on patterns of phenology and floral morphology. In a complementary way, Hao et al. (2023) showed that HP can paradoxically favor co-specific pollen deposition, resulting in greater reproductive success. In addition, Moreira-Hernández et al. (2023) pointed out that exposure to HP can induce greater tolerance in plants, promoting adaptations that benefit reproductive success in sympatric species. In this same context, Pérez-Barrales and Armbruster (2023) emphasized the need to integrate HP deposition into analyses of pollination fitness, arguing that the interaction between co-specific and heterospecific pollen is essential for understanding reproductive success in plant communities.

Therefore, this work sought to advance this understanding by investigating specific factors that contribute to HP interference in phylogenetically close, co-flowering species. The factors evaluated include: the identity of the HP donor, the intensity of HP deposition and the distinct floral morphs linked to enantiostyly. Enantiostyly, characterized by variation in the positioning of the stigma and anthers, is a floral strategy that can directly influence pollinator-plant interactions, promoting greater fundamental outcrossing (outcrossing between individuals), particularly in monomorphic enantiostyly. Therefore, this condition is a promising model for understanding how plants can mitigate or respond to HP interference.

In this scenario, our central hypothesis is that heterospecific HP pollen deposition reduces reproductive fitness among related *Chamaecrista* species. To test this hypothesis, we investigated the effects of HP on interspecific interactions and L/R (left/right) floral morphotypes by manually applying varying pollen loads to the stigmas. Thus, our aim was to contribute to a deeper understanding of adaptive strategies, such as tolerance or benefits to HP deposition in enantiostylous species.

MATERIAL AND METHODS

Study location

The study was carried out at Nossa Senhora do Oiteiro de Maracaípe Private Natural Heritage Reserve (RPPN), in the municipality of Ipojuca, Pernambuco, Brazil (08º 831' 48"S; 35º 801' 05"W), regulated by Order 58-DOU 187-E- 27/09/2000 - section/pg. 1/98, owned by the Parish of São Miguel. The area covers 76.21 hectares, with vegetation made up of remnant fragments of Atlantic Forest, predominantly “Restinga”. The local climate is tropical, hot and humid, with average annual temperatures ranging from 24 to 28ºC and average annual rainfall of 2000 mm/year (Inmet, 2021). The region is characterized by two periods throughout the year, a wetter one from April to June and a drier one from December to February.

Species studied and phenological analysis

This study covers four species with a sub-shrubby and herbaceous habit belonging to the genus *Chamaecrista* Moench (Fabaceae): i) *C. diphylla* (L.) Greene; ii) *C. flexuosa* (L.)

Greene; iii) *C. ramosa* (Vogel) H.S.Irwin; Barneby and iv) *C. rotundifolia* (Pers.) Greene. All four species have monomorphic enantiostyly (i.e. with flowers from both morphs on the same plant), pollen flowers, yellow, asymmetrical, with heteromorphic petals (Fig. 1) and diurnal anthesis, occurring between four and eleven o'clock in the morning. They all interact with solitary bees, sharing pollinators, which vibrate the anthers during visitation (Saltini et al., 2023). This mechanism triggers the release of pollen grains and plays a decisive role in pollination efficiency (Almeida et al. 2013a,b; 2015b; Nogueira et al., 2018).

To assess the overlap in flowering between species, 40 individuals per species were monitored monthly (from July 2019 to December 2020) in four natural populations distributed throughout the reserve. At each sampling, the number of flowers was counted, distinguishing right (R) and left (L) morph flowers. To test the occurrence of flowering overlap between species, we used the Rosario null model approach (Castro-Arellano et al., 2010) using Czekanowski's paired overlap indices ($\bar{\alpha}$) (Feinsinger et al., 1981). The values of ($\bar{\alpha}$) were calculated from the monthly sum of the average number of flowers accumulated in 40 individuals/species. This index indicates the overlap in flowering between two species and ranges from 0 to 1, with values close to 1 indicating high co-flowering between species.

The Rosario approach uses the average value of overlap indices ($\bar{\alpha}$) derived from all possible overlaps between the species considered and compares it with the average value of simulated overlaps ($\bar{\alpha}$ sim), generated by a null distribution containing 10.000 iterations. If there is consistent synchrony between species, the value of $\bar{\alpha}$ should be among the highest of the simulated values ($p < 0.05$), *i.e.* the value of $\bar{\alpha}$ should be higher than expected by chance. We used R version 3.6.3 (R Development Core Team, 2020) to calculate the overlap indices between species, the niche.overlap function from the spaa package (Culp, 2011) and the TIMEOVERLAP v.1.0 software for the null model test (Castro-Arellano et al., 2010).

Pollen mix treatments on factors influencing HP interference in species

An experiment involving manual crosses between plants of the four *Chamaecrista* species studied was carried out in the field. The donor flowers were collected from groups other than the recipient flowers. We carried out bidirectional and intermorphic crosses (RxL and LxR flowers) between the species with variable pollen load to assess the influence of related species, floral morphotype and HP receiving intensity on reproductive success. In the different treatments, the pollen load was transferred manually from dehiscent anthers using fine-tipped metal tweezers. To ensure the exact proportion of each type of pollen, we used a methodology based on counting anthers, considering that 10 anthers correspond to 100%, regardless of the species. For each treatment, the proportions were calculated according to the experimental objective. For example, in the treatment with 30% co-specific pollen (CP) and 70% heterospecific pollen (HP), we collected pollen from three CP anthers and seven HP anthers. After collection, the pollen was homogenized internally in a Petri dish to ensure proper mixing before being applied to the stigmas of flowers in anthesis. To avoid contamination, the tweezers were replaced after each mixture and properly sanitized with 70% ethyl alcohol.

We evaluated the effect of heterospecific pollen intensity (HP) using four treatments with different pollen loads: a) 0% HP (100% CP); b) 30% HP + 70% CP; c) 70% HP + 30% CP, and d) 100% HP (0% CP). The day before the treatments began, the flower buds of all the species were isolated and protected with voil bags to prevent unwanted visits. Subsequently, in each individual, two flowers in anthesis per treatment were selected daily and identified with specific markings: a line to distinguish the morph and a colored ribbon to indicate the corresponding treatment. After the crosses were made, the flowers were again covered with the bags for the entire functional period. To monitor natural pollination, the flowers were left exposed to the action of floral visitors and then bagged until the fruit was formed and ripened. Successful fruit formation was recorded for all treatments.

To test the influence of related species, floral morphotype and HP receiving intensity on the reproductive success of co-flowering species, Generalized Linear Mixed Models (glmer function from the lme4 package, GLMM) with binomial error distribution were used, considering fruit formation (success or failure) from each crossing (n = 1876) as the response variable. The proportion of HP (0%, 30%, 70% and 100%), the recipient species (*C. diphylla, C. flexuosa, C. ramosa* and *C. rotundifolia*) and the recipient morphotype (right and left) were included as fixed effects, while the pollen donor species and the identity of the plant used in the cross were inserted as random effects.

The interactions “proportion of HP × species”, “proportion of HP × floral morphotype” and “species × floral morphotype” were also considered. To test whether the effect of HP on the species depends on the donor species, Generalized Linear Models (GLM) were used for each recipient species separately, including proportion of HP and donor species, as well as the interaction between the two, as predictor variables. Likelihood-ratio tests were used to test the significance of the predictor variables and their interactions using the Anova function (car package). Post-hoc tests were carried out using the emmeans package to test the significance of comparisons between factors of the predictor variables. The analyses in this study were carried out in R v.4.2.3 (R Core Team, 2020).

RESULTS

Flowering overlap between enantiostylous congener species

We found an indication of high flowering overlap between the four species studied (Table 1, Fig. 1). Flowering overlap was more intense than expected by chance ($\bar{\alpha}$=0.52, $\bar{\alpha}_{\mathrm{sim}}$=0.36, p=0.0005). We observed that the highest overlap indices occurred between *C. flexuosa* and *C. ramosa* (α =0.74) and between *C. rotundifolia* and *C. diphylla* (α= 0.74). The index showed intermediate values of overlap between *C. diphylla* and *C. flexuosa* (α= 0.44) and *C. ramosa* (α= 0.45). Finally, the lowest intensity of co-flowering synchronicity occurred between *C. flexuosa* and *C. rotundifolia* and also between *C. rotundifolia* and *C. ramosa* (α= 0.39).

Factors influencing HP interference in species

The results of the pollen mixing experiments indicate that the effect of interspecific flow on female reproductive success can be quite variable (positive, negative or neutral) and are shown here to depend on: the recipient morph (Fig. 2), the intensity of HP mixing (Fig. 3), and also the donor species (Fig. 4).

The magnitude of their effect varied differently between the recipient species and the two floral morphotypes (R/L). We observed that the reproductive success of the recipient morphotypes did not vary differently between the recipient species (Table 2). In figure 2, on the other hand, we can see that, regardless of the species, when subjected to higher HP values, close to 100%, the right morph had its reproductive fitness more negatively impacted compared to the left morph, which performed better in the fruit set.

All the species tested were negatively affected by the presence of HP in relation to reproductive fitness (Fig. 3a-d). However, *C. diphylla* proved to be more tolerant to the presence of HP than the donors of the other species, while *C. flexuosa* was the most negatively affected (Fig. 3a, b). On the other hand, the gradual increase in the proportion of HP (≥30%) did not alter reproductive success in *C. diphylla* (Fig. 3a) and *C. rotundifolia* (Fig. 3d), while *C. flexuosa* suffered a negative impact. Surprisingly, with the same increase to which the other species were subjected, *C. ramosa* achieved an increase in reproductive success (Fig. 3c), but not equivalent to that found by co-specific pollen deposition (Fig. 3d).

In relation to the donor species, the effect of HP on *C. diphylla* and *C. flexuosa* was not dependent on the species that donated the pollen, unlike what we observed in relation to the species *C. ramosa* and *C. rotundifolia* (Tab. 3). In *C. ramosa*, pollen deposition from *C. flexuosa* had a neutral impact on its reproductive success and an increase in the proportion of pollen deposition from *C. diphylla* and *C. rotundifolia* increased reproductive fitness (Fig. 4a-c). In *C. rotundifolia*, reproductive success increased with the proportion of *C. ramosa* pollen deposition, indicating high compatibility between the two species (Fig. 4f). Intermediate proportions of HP (70%) from *C. diphylla* and *C. flexuosa* pollen had different effects on the reproductive success of *C. rotundifolia* (Fig. 4d-e). When *C. diphylla* was the donor, there was a decrease in fruit set, while with *C. flexuosa* as the donor, there was an increase in fruit set. However, when there was 100% HP from both donor species, *C. rotundifolia* achieved similar reproductive success at lower levels of HP (30%) (Fig. 4d-e).

DISCUSSION

This study shows that co-flowering enantiostylous species can exert both negative and positive effects on the reproductive success of congeneric species. The results confirm our main hypothesis by demonstrating non-linear responses influenced by: *i)* the intensity of the interaction between them (*i.e.* amount of heterospecific HP pollen received), *ii)* the identity of the donor, and *iii)* the floral morphotype (R/L). Thus, we found that the HP deposition reduces reproductive fitness among related *Chamaecrista* species. These findings highlight that the effects of plant-plant interactions and the tolerance levels of HP receipt in phylogenetically related species are highly variable in nature, indicating a complex interplay between competition and facilitation that influences coexistence between plants (Arceo-Gómez and Ashman, 2016). This observation directly validates the premise that co-flowering and synchronopatry between *Chamaecrista* species is associated with a wide variation in the effects of pollen interactions. These results help to understand both the dynamics of pollen transfer between coflowering enantiostylous species and the adaptive strategies of these plants in contexts of high ecological diversity.

We observed that the overlapping of the flowering phenophase between the species studied was associated with the rainfall regime. On the other hand, the four species show variations in peak flowering (Bezerra et al. 2024, *under review*). Bearing in mind that co-flowering does not restrict pollen flow between species, the levels of flowering overlap observed show patterns of interaction and the potential for natural interference in receiving HP. Thus, the results obtained experimentally probably reflect pollinator-mediated interactions between the species studied. The potential for hybrids is often reported for sympatric species with overlapping flowering periods (Jorgensen and Olesen, 2001; Seehausen, 2004; Souza et al., 2017), and for *Chamaecrista* species (Conceição et al., 2008; Costa et al., 2013). The study by Conceição et al. (2008) also pointed out that the taxonomic history of *Chamaecrista* is complex due to the occurrence of hybrid zones. However, the negative effects of co-flowering can be reduced if each plant is pollinated by different pollinator species; the activity of shared pollinators is structured over time (Adler et al., 2018); or differences in floral morphology result in the deposition of pollen in different regions of the bodies of shared pollinators (Solís-Montero and Vallejo-Marín, 2017). Even the Vallejo-Marín (2017) study found greater co-specific pollen deposition when the bees were of the same size or larger than the separation between anther and stigma within a flower.

However, it is common for moments of intense flowering to reciprocally favor the attraction of specialized bees, which are essential to increase the chances of dispersing conspecific pollen, improving both the quality of the pollination service and the density of flowers visited (Gross et al., 2000; Souza et al., 2017; Scaccabarozzi et al., 2020). Despite the presence of heterospecific pollen, the species studied demonstrated tolerance to receiving this type of pollen, maintaining high rates of fruit set even under conditions of interspecific interaction. This tolerance suggests that the impact of HP is minimized at low levels, allowing plants to continue to reproduce continuously. According to Morales and Traveset (2008), many studies on co-flowering species that share pollinators report reproductive depression in periods of overlapping flowering peaks. However, this does not appear to be the case for the species analyzed, in which high rates of natural fruiting indicate that competition is attenuated by the facilitation promoted by the abundance of pollinators (Bezerra, 2019). These authors suggest that competitive and temporally variable environments allow conditions that favor the coexistence of species. Thus, the coexistence observed among *Chamaecrista* species can be attributed to the dynamic interaction between tolerance to HP and the benefits of shared pollinator attraction at times of simultaneous flowering.

In other studies with *Chamaecrista*, it was observed that the pattern of simultaneous flowering among sympatric plants is frequently found (Madeira and Fernandes, 1999; Arceo-Gómez et al., 2012; Almeida et al., 2015b). Wadgymar et al. (2015) mention that the floral display period in *C. fasciculada* is influenced by several factors, including environmental conditions that affect floral longevity and influence pollinator activity, which, in turn, affects the quality of the pollination service. In addition, the variation in stigmatic receptivity, pollen release and nectar/pollen variation throughout the day is mentioned by Stone et al. (1998), which can be understood as a way to prevent pollen transmission between species that include pollinators. They cited an example with sympatric *Acacia* species, which showed high intraspecific synchrony and segregated pollen release patterns between species throughout the day. The temporal pattern of pollen release of *Acacia* species is consistent with the activity of shared pollinators, and this pattern was attributed to pollinator-mediated competition.

The variability in the effects of manual deposition of HP on morphotypes R and L revealed important implications for the reproductive success of the studied species. Data analysis showed that fruit set was generally higher in conspecific than in heterospecific crosses, indicating that enantiostyly plays a significant role in reproductive isolation between sympatric species. This dynamic, enhanced by the floral morphology specialized in pollen deposition and removal, favors cross-pollination between morphs of the same species (Jesson and Barrett, 2003; Almeida et al., 2024). This mechanism restricts pollen deposition to specific locations in pollinators (Lin and Tan, 2007; Almeida et al., 2015ab), acting as a barrier to gene flow and increasing the purity of conspecific pollen loads (Montgomery and Rathcke, 2012). These results reinforce the idea that tolerance to HP varies between species and contributes to selectivity in the pollination system, protecting sympatric species from negative effects associated with interspecific pollen flow.

Our results show that morphotype L is more tolerant to HP than morphotype R, especially at maximum HP increment. Interestingly, the female function of the flower is different between morphs and this contributes to the complexity of interactions and heterospecific pollen flow, suggesting adaptations that favor one morphotype over the other in specific ecological contexts (Werren and Charnov, 1978; Castillo et al., 2002; Almeida et al., 2024). This evidence supports previous theoretical studies (Lin and Tan, 2007; Cardona et al., 2020), highlighting the influence of floral morphological traits, such as enantiostyly, on pollination interactions and reproductive success. Studies, such as that of Arceo-Gómez et al. (2012) demonstrated that, even among morphologically similar flowers of *Chamaecrista*, there are functional variations, such as differences in position, size of structures and pollen production between floral morphs that can lead to changes in reproductive efficiency, favoring certain morphs in different environmental conditions and pollination contexts.

This functional differentiation between floral morphs in enantiostyly may reflect adaptations related to the reproductive characteristics of plants. In our study, morphotype L had a lower impact on HP deposition, regardless of the species, suggesting that differences between morphotypes may modulate the effects of interspecific pollen flow. The balanced coexistence of both morphs within the same population in monomorphic enantiostyly indicates a possible maintenance of genetic and functional diversity, advantageous in contexts of environmental variability (Almeida et al., 2015b).

Studies on floral morphotypes in other species, such as *Penstemon* (Plantaginaceae), show that variation between morphotypes influences plant reproduction (Cardona et al., 2020). For example, fuchsia flowers produce more resources and are visited more frequently due to pollinator preferences. Although our previous studies with the genus *Chamaecrista* (Almeida et al., 2013ab) have not yet identified behavioral preferences of pollinators between morphotypes, the current results indicate that functional differences, particularly in relation to HP deposition, play a relevant role in the reproductive dynamics of the analyzed species. Additionally, observations by Nogueira et al. (2018) on *Chamaecrista desvauxii* var. *latistipula* reveal that bees exhibit specific behaviors in the anthers at different stages of floral development, often touching the stigmatic region during pollen collection, even before the flowers are fully opened. These results reinforce that functional differences between morphotypes, associated with mutualistic interactions with pollinators, can expand the window of opportunity for reproductive success under varied conditions.

We can relate the effects of HP on floral morphs to the findings of Werren and Charnov (1978). Although the Operational Sex Ratio (OSR) deals with the relationship between males and females, the dynamics between R and L morphs in enantiostylous plants suggests an analogy. The OSR is an important concept in ecology and evolutionary biology, as it can influence the reproductive strategies of individuals within a population. An unbalanced OSR (with more males than females or vice versa) can lead to different selective pressures and resource allocation strategies between the sexes. As discussed by Bezerra et al. (2024), changes in the proportion of morphs throughout the reproductive season can affect pollination efficiency and, consequently, reproductive fitness, discussed here in this work. We believe that this variation can also act to avoid or minimize the negative effects of the HP position. This comparative perspective expands our understanding of how selective pressures influence the evolution of pollination strategies in contexts of species competition, highlighting the importance of functional diversity in ecological interactions.

Our results regarding *C. flexuosa* reveal significant susceptibility to the effects of heterospecific pollen (HP) at low levels (see Fig. 3b), especially when compared to the other co-flowering species. Despite the phylogenetic proximity between the species studied, there is a notable variation in tolerance to HP, which demonstrates that the evolutionary relationship is not the only determining factor for this compatibility. This complexity may be associated with other aspects not yet observed, such as morphological, physiological or ecological differences, which modulate reproductive responses. We suggest that the other species studied, therefore, may present distinct adaptations that allow greater tolerance to HP, such as differences in female function or stigma physiology, which mitigate the negative impacts of interspecific pollen flow. In the case of *C. flexuosa*, the deficiency of these mechanisms may explain its greater susceptibility, while the other sympatric species appear more resilient. This variation highlights the complexity of reproductive interactions between phylogenetically close species and suggests that coexistence in diverse environments is related to multiple factors in addition to evolutionary proximity. It was observed that crosses of *C. flexuosa* with 100% heterospecific pollen and 0% conspecific pollen resulted in low fruit formation. Furthermore, a high rate of fruit abortion was recorded, and seed germination inviability was observed in 100% of the cases of seeds from these crosses. These results indicate that inviability in the germination stage constitutes the main post-zygotic isolation barrier (Bezerra, 2019).

The literature corroborates that some plants are more sensitive to HP deposition, directly affecting their reproductive success. For example, studies with *Sisyrinchium wettsteinii* (Iridaceae) and *Fuchsia* (Onagraceae) (Streher et al., 2020) indicated that HP competition can impair pollen tube growth in some species, resulting in lower seed set. Similarly, *C. flexuosa* demonstrates a negative response to HP deposition, suggesting that this factor limits reproductive success, similarly to that observed in *Fuchsia*. Other species, such as *Silene chungtienensis* and *S. gracilicaulis* (Hao et al., 2023), have also been described as highly susceptible to the negative effects of HP, with seed production being significantly reduced in cross-pollination contexts. Likewise, *C. flexuosa* appears to share this sensitivity pattern, with competition between conspecific and heterospecific pollen affecting reproductive fitness.

Furthermore, the study by Morales and Traveset (2008) reinforces that, in environments with high floristic diversity, they may be more vulnerable to pollen deposition from competing species, which in the case of *C. flexuosa* further limits reproductive success. This pollen deposition from competing species may limit the reproductive success of *C. flexuosa*, since competition for pollinators and the loss of conspecific pollen may negatively impact seed set and the reproductive fitness of the species. This vulnerability may be intensified depending on the morphological similarity between some species that flower concomitantly with other species of the same genus. For Gavini et al. (2021), plants that suffer from HP deposition are in environments with high diversity of co-flowering plants. This context, which is similar to the environment where the plants studied here are inserted, suggests that interspecies competition for reproductive resources may be widely present in ecosystems with high diversity. These examples highlight how plants that share morphological and phylogenetic characteristics tend to suffer similar losses from HP deposition. This fact reinforces the importance of better understanding the effects of interspecific pollen flow on the reproductive success of plants in diverse ecosystems and in coexistence with phylogenetically close species.

Exceptionally, *C. ramosa* showed an increase in reproductive success in the presence of HP, although this increase did not reach the levels observed with conspecific pollen. This result suggests a “herd effect” (Streher et al., 2020; Suárez-Mariño et al., 2019), in which the presence of HP, by generating competition on the stigmas, can stimulate the germination of CP and the growth of the pollen tube. This mechanism, observed in interactions between coflowering plants, can manifest itself as both facilitation and competition. In the context of facilitation, the clustering of flowers attracts more pollinators, increasing pollination efficiency and favoring reproductive success. On the other hand, competition arises when the mixing of pollen on the stigmas reduces fruit formation, especially in species less tolerant to HP. In the case of *C. ramosa*, it is argued that the proportion of HP appears to have triggered a facilitating effect on female function, possibly associated with adaptations that allow greater tolerance to interspecific competition in stigmas. In contrast, in *C. flexuosa*, competition in stigmas and the negative effects of HP prevail, showing that the impact of the “herd effect” depends on the level of HP and the specific adaptations of each species. These reports corroborate our findings, confirming that specific characteristics of each species are fundamental in interactions. In addition, they highlight the need for further studies on how the intensity of HP and stigma characteristics modulate the dynamics of facilitation and competition between species in co-flowering communities. Such an approach can clarify the mechanisms that shape coexistence and reproductive success in biodiversity environments, expanding the understanding of the “herd effect” in the context of enantiostyly and its ecological implications.

Interspecific pollen flow can increase genetic variability, and in some cases it can confer greater adaptive flexibility to species, allowing them to tolerate the presence of heterospecific pollen (Conceição et al., 2008; Palma-Silva et al., 2011). However, the long-term impact of HP transfer still needs to be better understood, especially in terms of the genetic integrity of parental populations (Bello et al., 2018). Natural hybrids can occur in Fabaceae and include examples such as *Acacia* (Sedgely et al., 1992), *Baptisia* (Leebens-Mack and Milligan, 1998), *Sindora* (Choo et al., 2022), *Psoralea* (Bello et al., 2015) and *Vachellia* (Binks et al., 2015), among many others, highlighting that hybridization in this taxonomic group is a recurrent phenomenon. This diversity of responses to HP in the species studied highlights the importance of the interaction between genetic and ecological factors, suggesting that hybridization and tolerance to HP may be a relevant adaptation in biodiverse and disturbed environments (Palma-Silva et al., 2011), as the Atlantic Forest and associated ecosystems, such as mangroves and restinga vegetation.

The results obtained indicate that the influence of heterospecific pollen on reproductive success varied among recipient species, depending on the donor species (cf. Tab. 3). This reflects the complexity of interactions between sympatric species in shared ecosystems. The observed variation suggests that reproductive barriers between species may be more permeable in some species than in others, as discussed by Souza et al. (2017), who demonstrated the existence of genetic barriers in self-compatible species that depend on pollinators for fertilization.

In contrast, *C. ramosa* and *C. rotundifolia* showed distinct effects depending on the identity of the donor species. For example, the deposition of HP from *C. flexuosa* on *C. ramosa* suggests limited compatibility between the two species, or that *C. flexuosa* pollen is not relevant in the reproductive process of *C. ramosa*. However, the increase in the proportion of pollen from *C. diphylla* and *C. rotundifolia* increased the reproductive fitness of *C. ramosa*, indicating that these species have greater genetic compatibility, possibly facilitating pollen transfer between them. Cardona et al. (2020) demonstrated that differentiation in pollination traits can promote hybrid zones in sympatric plants, which could explain the increased fitness in species such as *C. ramosa* and *C. rotundifolia.*

In *C. rotundifolia*, we observed a positive effect on reproductive success with the deposition of *C. ramosa* pollen, indicating high compatibility between the two species. This result is consistent with other studies showing that overlapping morphological and ecological traits can facilitate pollen flow. Mesquita-Neto et al. (2018) pointed out that the attraction of different pollinators can result in cross-fidelity and increased gene flow, favoring compatibility between cogeneric species. However, in our study, intermediate proportions of HP (70%) from *C. diphylla* and *C. flexuosa* pollen resulted in distinct effects on *C. rotundifolia*. While *C. diphylla* pollen decreased fruit set, *C. flexuosa* pollen increased reproductive success. This suggests variation in compatibility between pollen types, as discussed in Jesson et al. (2005), in which different morphological interactions between species may result in variations in reproductive efficiency.

The influence of the HP donor species on fitness can also be explained by the phylogenetic relationship between species. Streher et al. (2020) found that HP from closely related species can have a significant negative impact on pollen tube growth, while HP from distantly related species can even benefit the performance of conspecific pollen. Costa et al. (2013) mention that interincompatibility between *Chamaecrista* taxa is driven by different mechanisms, depending on their degrees of phylogenetic proximity. This is corroborated by our results, which indicate that closely related species, such as *C. diphylla* and *C. ramosa*, showed greater compatibility, while other interactions, such as with *C. flexuosa,* were less consistent.

Hao et al. (2023) corroborate that the effectiveness of HP depends on the compatibility between species, suggesting that the phylogenetic relationship between donor and recipient may determine the impact on reproductive success. According to the authors, species with greater tolerance to HP tend to maintain fitness, while others suffer more pronounced negative impacts.

When observing the HP proportions, the return of the reproductive fitness of *C. rotundifolia* to initial levels with 100% HP of *C. diphylla* and *C. flexuosa* suggests that, in large quantities, pollen from both species does not significantly affect the reproductive success of *C. rotundifolia*. This result can be compared to the more precise and selective pollen deposition mechanisms in species with specialist pollinators, as discussed by Souza et al. (2017), in which precision in pollen deposition can minimize the negative impact of unwanted pollen in certain species. Furthermore, this result corroborates the view of Opedal et al. (2023) that some species can develop tolerance to HP accumulation.

In summary, the relationship between HP donors and the reproductive success of recipient species is complex and depends on several factors, such as pollen-stigma compatibility, the phylogeny of the species involved, and the functionality of the pollination ecology. Studies such as those by Morales and Traveset (2008) and Palma-Silva et al. (2011) reinforce the importance of understanding how heterospecific pollen influences reproductive dynamics in sympatric communities. Morales and Traveset (2008) highlight that interactions between different combinations of donor and recipient species can result in a wide range of effects, from negative, such as reduced fecundity, to positive, such as reproductive facilitation in some circumstances. Similarly, Palma-Silva et al. (2011) argue that although heterospecific pollen can increase the genetic diversity of recipient populations, it can also introduce challenges, such as the dilution of specific adaptive traits. Therefore, the interaction between HP and recipient species must be evaluated within a broader ecological context, considering both evolutionary opportunities and potential risks to the genetic integrity of the species.

This study investigated the impact of heterospecific pollen (HP) deposition on the reproductive success of enantiostylous *Chamaecrista* species, considering variations in pollen loads, interspecific interactions and interactions between floral morphotypes. The results confirmed the hypothesis that HP reduces reproductive fitness, with varying effects depending on the donor and recipient species, in addition to the HP proportions. We observed that conspecific crosses presented higher reproductive success, suggesting that enantiostyly plays a fundamental role in the reproductive isolation between sympatric species. The results show that *C. diphylla* and *C. flexuosa* are resilient to the HP donor species, while *C. ramosa* and *C. rotundifolia* present varied responses. Notably, *C. rotundifolia* presented an increase in reproductive success with *C. ramosa* pollen, indicating high compatibility between them. In contrast, pollen deposition by *C. diphylla* impaired the fruit set of *C. rotundifolia*, while *C. flexuosa* had a positive effect, revealing a complex dynamic of interspecific compatibility.

The differential tolerance of the L morphotype especially in 100% HP collection suggests adaptations that favor one morphotype over the other in specific ecological contexts. This evidence supports previous studies highlighting the influence of enantiostyly on pollination interactions and reproductive success. More broadly, this study contributes significantly to understanding how sympatric *Chamaecrista* plants employ adaptive strategies in the face of HP deposition, either by tolerance or by exploiting specific effects. Taken together, these results highlight the complexity of these adaptive strategies, combined with varying levels of ecological specialization, and reinforce the importance of considering the variability of reproductive responses in shared pollination scenarios and interspecific interactions. These data may provide a basis for future research on the evolution and maintenance of reproductive barriers in floral systems with enantiostyly.

We suggest that, although HP may be detrimental to some species, it may, in certain contexts, be integrated into evolutionary strategies that favor genetic diversity and adaptation, especially in environments with high flowering synchrony. Our results, therefore, provide insights into reproductive dynamics in polymorphic plants, highlighting ecological implications of pollen interactions on species coexistence and adaptation in natural coastal environments.

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Table 1. Synchronopatry matrix with species x mean observed flowering overlap indices (α) (Czekanowski) obtained for eighteen months of observation at the RPPN Nossa Senhora do Oiteiro de Maracaípe, Ipojuca, Pernambuco, on the coast of northeastern Brazil.

Species/ Index (α)	*C. flexuosa*	*C. rotundifolia*	*C. ramosa*
C. rotundifolia	0.39	-	-
C. ramosa	0.74	0.39	-
C. diphylla	0.44	0.71	0.45

Table 2. Effect of increasing heterospecific pollen deposition (HP) and its relationship with the recipient species and floral morphotypes.

Predictors	χ^2	df	*p*-value
HP proportion	**132.9659**	**3**	**<0.001**
Receptor species	**101.0562**	**3**	**<0.001**
Floral morphotype	**8.2207**	**1**	**0.004**
HP proportion × Receptor species	**35.6729**	**9**	**<0.001**
HP proportion × Floral morphotype	**12.7056**	**3**	**0.005**
Receptor species × Floral morphotype	2.0335	3	0.565

*bold: significant values

Table 3. Effect of increased heterospecific pollen (HP) deposition on species of *Chamaecrista* and its relationship with donor species.

Receptor species	(LR) χ^2	df	p-value
C. diphylla	0.9503	4	0.917
C. flexuosa	3.1635	4	0.531
C. ramosa	**26.136**	**4**	**<0.001**
C. rotundifolia	**31.4348**	**4**	**<0.001**

*bold: significant values

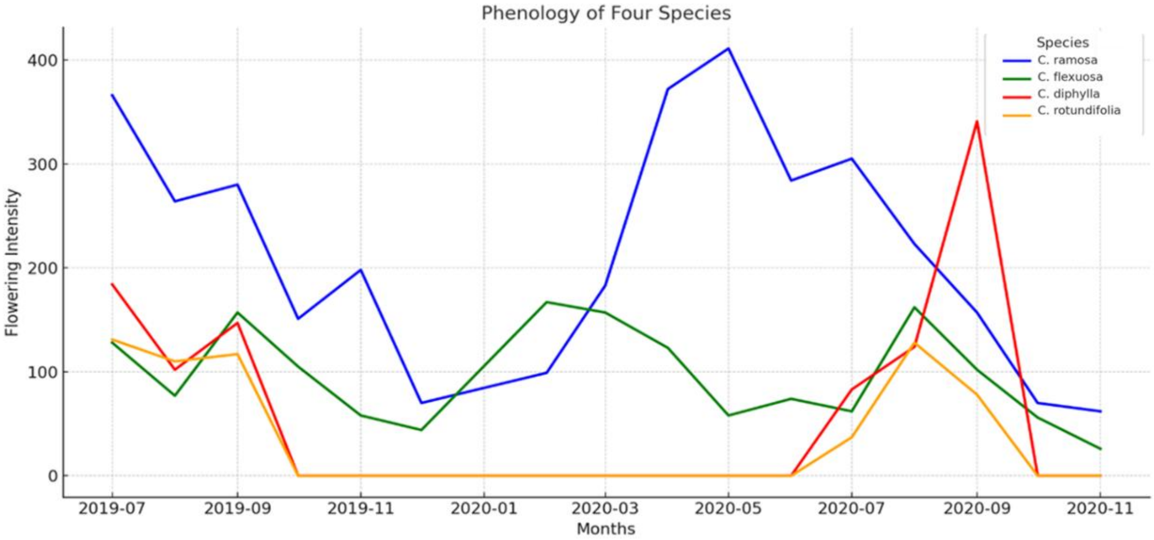

Figure 1. Flowering overlap of the four species of *Chamaecrista* monitored in RPPN Nossa Senhora do Oiteiro de Maracaípe, PE, Brazil, showing the monthly intensity (number of flowers per day analyzed) of the phenophase over time (2019-2020).

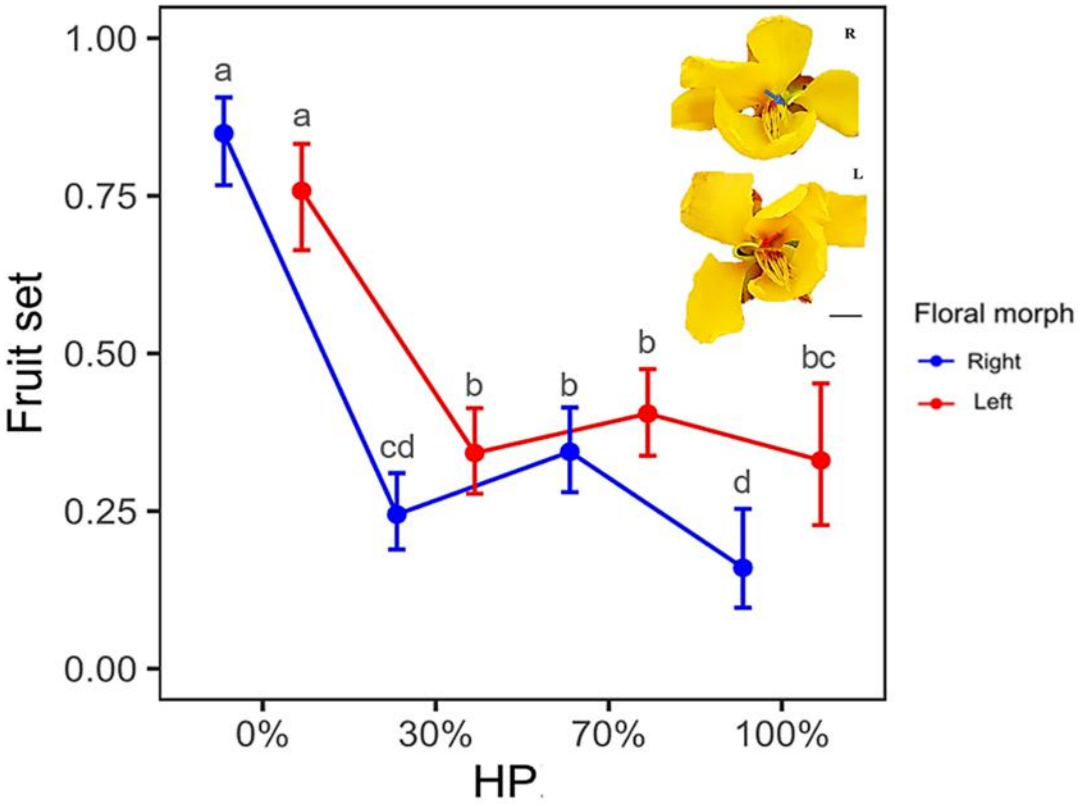

Figure 2. Effect on reproductive fitness of different HP ratios (%) on right x left morphs in four enantiostylous *Chamaecrista* species in a coastal vegetation area in Pernambuco, Brazil. HP= heterospecific pollen deposited. In the upper right corner, representation of the morphs of the right (R) and left (L) flowers of *C. ramosa* (Vogel) H.S.Irwin ; Barneby. The arrows indicate the position of the stigma. Scale bar = 10 mm.

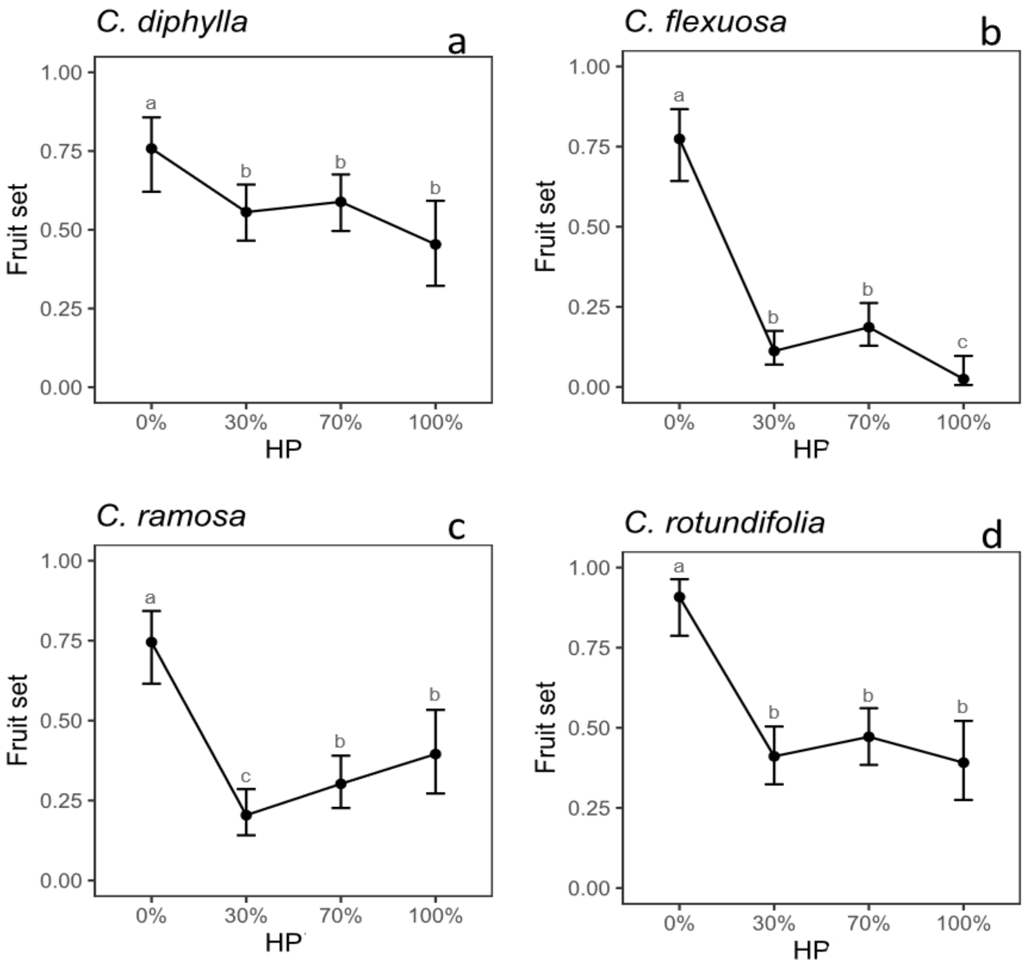

Figure 3 - Fruit formation rate from pollen mix treatments carried out on four *Chamaecrista* species in a coastal vegetation area in Pernambuco, Brazil. HP= heterospecific pollen deposited.

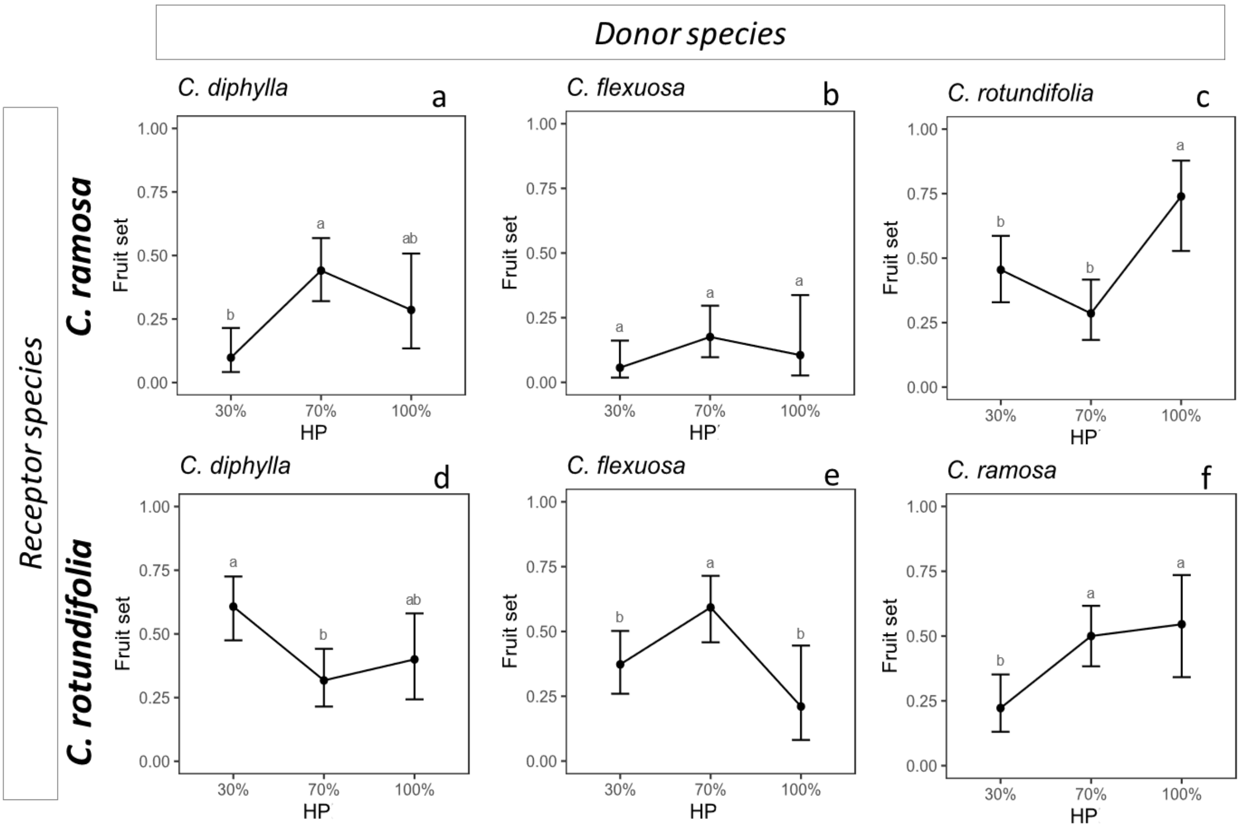

Figure 4. Effect of donor pollen from the species *Chamaecrista diphylla*, *C. flexuosa* and *C. rotundifolia* on the reproductive success of *C. ramosa* (a-c) and *C. rotundifolia* (d-g). From pollen mix treatments carried out in a coastal vegetation area in Pernambuco, Brazil. HP= heterospecific pollen deposited.

6. CONSIDERAÇÕES FINAIS

As espécies com enantiostilia monomórfica possuem uma dinâmica de floração complexa e seu entendimento é essencial no estudo da ecologia da polinização. Este estudo contribuiu significativamente para a compreensão das dinâmicas reprodutivas dessas plantas ao investigar os padrões de produção de morfos florais, a interferência de pólen heteroespecífico (HP) e as interações interespecíficas em populações sincronopátricas de quatro espécies de *Chamaecrista*. Os resultados obtidos revelaram que, apesar da predominância de isopletia em nível populacional, os indivíduos podem alternar a produção funcional de morfos florais ao longo do tempo, gerando um dinamismo estratégico que favorece o fluxo cruzado entre morfos DxE (direito x esquerdo). Essa flexibilidade funcional reflete uma adaptação importante ao contexto ecológico, promovendo maior eficiência reprodutiva. Além disso, as variações na tolerância ao HP entre espécies e morfotipos e doadores revelaram a complexidade das barreiras reprodutivas pré e pós-polinização, destacando o papel do HP como um modulador do sucesso reprodutivo e da coexistência em ambientes de alta diversidade floral, como a restinga. De forma geral, este trabalho reforça a importância da enantiostilia monomórfica como um sistema chave para o estudo de estratégias reprodutivas em plantas, oferecendo “insights” valiosos sobre como características morfológicas e funcionais de interação moldam as respostas adaptativas a condições ambientais e interações ecológicas. Tais descobertas não apenas ampliam a compreensão dos mecanismos de isolamento reprodutivo, mas também são importantes para a compreensão das forças evolutivas que mantêm a diversidade genética e promovem a coexistência de espécies simpátricas. Assim, este estudo destaca a relevância da exploração de aspectos temporais e funcionais na reprodução das plantas enantiostílicas, fornecendo uma base para investigações futuras sobre a relação entre morfologia floral, dinâmica de polinização e adaptação em ecossistemas biodiversos.

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8.1 ANEXO – GLOSSÁRIO

i. **Anisopletia**: condição em que a proporção de diferentes morfos florais em uma população ou indivíduo é desequilibrada, levando a uma predominância de um morfo sobre o outro, o que pode comprometer a eficiência reprodutiva da espécie devido à redução da polinização cruzada.

ii. **Barreira reprodutiva**: qualquer fator que impede a reprodução entre diferentes espécies, contribuindo para a manutenção da integridade genética das populações.

iii. **Buzz pollination:** (ou polinização por vibração) Processo em que abelhas grandes liberam pólen de flores com anteras poricidas por meio de vibrações musculares, produzindo um zumbido que expulsa o pólen, permitindo a polinização.

iv. **Cofloração**: fenômeno em que diferentes espécies de plantas florescem simultaneamente, potencialmente influenciando a polinização entre elas.

v. **Compatibilidade intermorfos:** capacidade que apresentam duas flores de diferentes tipos morfológicos de trocarem pólen, ou seja, polinização entre morfo distintos (*i.e.* na enantiostilia com flores direitas x flores esquerdas).

vi. **Compatibilidade intramorfo:** capacidade que apresentam duas flores do mesmo tipo morfológico de trocarem pólen, ou seja, de morfo igual (*i.e.* na enantiostilia com flores direitas x flores direitas).

vii. **Dimorfismo sexual**: O dimorfismo sexual é um fenômeno biológico caracterizado pela presença de duas formas distintas dentro de uma mesma espécie, que se diferenciam por características morfológicas, fisiológicas, funcionais e de história de vida, resultantes da seleção natural, seleção sexual.

viii. **Dinâmica da floração**: padrões e variações na produção de flores ao longo do tempo, influenciados por fatores ambientais e interações biológicas.

ix. **Enantiostilia**: polimorfismo floral em que as flores de uma planta apresentam estilos que se curvam para a direita ou para a esquerda, resultando em uma simetria espelhada entre os morfotipos florais. Em flores enantiostílicas, um morfo de flor terá o estilo curvado para um lado e a antera posicionada no lado oposto, o que facilita a transferência de pólen entre flores de diferentes morfotipos quando polinizadores, como abelhas, visitam as flores.

x. **Enantiostilia dimórfica:** é uma condição em que uma população de plantas apresenta dois morfos florais distintos, com estiletes orientados em direções opostas (para a esquerda e para a direita) em proporções aproximadamente iguais. Essa variação morfológica favorece a polinização cruzada, aumentando a eficiência na transferência de pólen entre flores coespecíficas.

xi. **Enantiostilia monomórfica:** refere-se a uma condição em que uma população de plantas possui flores com estiletes orientados em direções opostas, mas a proporção de morfos pode variar dentro da mesma planta. Essa condição pode incluir padrões de distribuição aleatória ou fixa dos morfos, mas não necessariamente mantém a proporção 1:1, o que pode impactar a eficiência da polinização.

xii. **Fitness reprodutivo**: medida do sucesso reprodutivo de um organismo, geralmente avaliada pela quantidade de descendentes que ele produz, contribuindo para a próxima geração.

xiii. **Fluxo polínico:** refere-se ao movimento e transferência de pólen entre flores, que pode ocorrer dentro de uma mesma planta (autopolinização) ou entre plantas diferentes (polinização cruzada). Esse fluxo é crucial para a fertilização e a produção de sementes.

xiv. **Funcionalidade morfológica:** diz respeito à relação entre a forma e a estrutura das partes de uma planta (como flores e órgãos reprodutivos) e suas funções biológicas, especialmente em relação à polinização e à reprodução. Essa funcionalidade é importante para a eficiência na atração de polinizadores e na transferência de pólen.

xv. **Geitonogamia:** é um tipo de polinização que ocorre quando o pólen é transferido de uma flor para outra flor da mesma planta. Embora possa resultar em fertilização, a geitonogamia pode reduzir a variabilidade genética, pois envolve a autopolinização.

xvi. **Hercogamia:** adaptação floral que impede ou reduz a autopolinização em plantas hermafroditas, promovendo a polinização cruzada. Estratégia envolve a separação espacial entre os órgãos reprodutivos masculinos (anteras) e femininos (estigmas) na mesma flor.

xvii. **Hercogamia recíproca:** é uma forma específica de hercogamia em que os estames e os estigmas de diferentes morfos florais estão dispostos de maneira que favorece a troca de pólen entre eles. Isso aumenta a eficiência da polinização cruzada, pois os polinizadores transferem pólen de um morfo para o outro.

xviii. **Heteranteria:** é um mecanismo floral que envolve a presença de diferentes tipos de anteras em uma mesma flor ou em flores de uma mesma planta. Estas anteras podem ter funções distintas, como anteras alimentadoras que fornecem pólen para atrair polinizadores e anteras polinizadoras que direcionam o pólen para locais específicos do corpo do polinizador. Frequentemente ocorre em espécies com enantiostilia e outras adaptações florais especializadas.

xix. **Isolamento reprodutivo**: mecanismos que impedem a reprodução entre diferentes espécies, contribuindo para a especiação. O isolamento reprodutivo pode ser pré-zigótico, quando ocorre antes da formação do zigoto, ou pós-zigótico, quando ocorre depois. Alguns exemplos são: separação mecânica, separação espacial, separação temporal, separação fisiológica, entre outros.

xx. **Isopletia**: termo utilizado para indicar uma proporção ou razão equilibrada de morfos florais em uma população ou indivíduo, geralmente relatada na proporção 1:1.

xxi. **Morfos florais:** diferentes apresentações de formas ou tipos de flores dentro de uma mesma espécie, que podem ter características distintas, como tamanho e forma ou posicionamento de estruturas florais. Estas características podem ocorrer na mesma planta ou em plantas distintas na população.

xxii. **Pólen heteroespecífico**: refere-se ao pólen que provém de uma espécie diferente daquela que está recebendo o pólen. O termo "heteroespecífico" enfatiza a diferença entre as espécies envolvidas no processo de polinização.

xxiii. **Polimorfismo floral:** a presença de diferentes formas ou morfos de flores dentro de uma mesma espécie, que podem ter adaptações específicas para polinização.

xxiv. **Polinização Cruzada**: processo em que o pólen de uma flor fertiliza os óvulos de outra planta com flor, promovendo a diversidade genética.

xxv. **Polinização especializada**: tipo de polinização que depende de polinizadores específicos, que são adaptados para interagir com determinadas espécies de plantas.

xxvi. **Reciprocidade floral**: refere-se à disposição dos órgãos sexuais (antera e estigma) de tal forma que, ao visitar flores de diferentes morfotipos, os polinizadores transferem pólen de uma flor para outra, aumentando a probabilidade de fertilização cruzada entre diferentes morfotipos de flores.

xxvii. **Sincronopatria:** refere-se à ocorrência de diferentes espécies que habitam a mesma área geográfica e que apresentam períodos de floração ou atividade reprodutiva coincidentes. Essa sobreposição temporal pode levar à competição por nicho (polinizadores e recursos), influenciando as interações ecológicas.

8.2 ANEXO - NORMAS PARA PUBLICAÇÃO NOS PERÍODICOS

NORMAS PARA PUBLICAÇÃO NO PERIÓDICO PLANT BIOLOGY

Title page: The first page of each paper should indicate: The title, the authors' names and affiliations, a short title for use as running head, the name, address, e-mail address, phone and fax number of the corresponding author, 3 to 7 keywords, and a one-sentence summary of the key message of the paper. Abbreviation: List with abbreviations where appropriate.

Abstract: The abstract must be usable as a stand-alone document and must not exceed 250 words. For all papers submitted from 2016 onwards, a structured abstract is required using four bullet points to indicate (1) the research conducted including its rationale, (2) central methods applied, (3) key results, and (4) main conclusions including key points of discussion. It should not contain citations of other papers. For reviews, bullet points do not apply.

The following sections cover the usual contents: Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References (see below), Tables (see below), Figure legends (see below), Illustrations (see below).

In Materials and Methods, Results and Discussion subheadings are possible. If an author chooses to combine sections Results and Discussion, an additional section Conclusions can be added, but this must be brief.

Tables must be serially numbered in Arabic numerals and each must carry a brief descriptive heading. Tables reproduced from other publications must state their precise source. Only signs that can be typeset should be used in the tables and legends. Please provide Tables in Word and include them at the end of the manuscript after the References, each on a separate page. Refrain from using both tables and graphs to demonstrate the same results.

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