UNIVERSIDADE ESTADUAL DE CAMPINAS
INSTITUTO DE BIOLOGIA

BEATRIZ LUCAS ARIDA

AS CONSEQUÊNCIAS DO POLIMORFISMO DE COLORAÇÃO FLORAL NO SUCESSO REPRODUTIVO DE UMA ORQUÍDEA NEOTROPICAL POLINIZADA POR ENGODO

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Dissertação apresentada ao Instituto de Biologia da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do Título de Mestra em Biologia Vegetal.

Dissertation presented to the Institute of Biology of the University of Campinas in partial fulfillment of the requirements for the degree of Master in Plant Biology.

Orientador: Prof. Dr. Fábio Pinheiro
Coorientador: Profª. Drª. Maria Gabriela Gutierrez Camargo

ESTE TRABALHO CORRESPONDE À VERSÃO FINAL DA DISSERTAÇÃO DEFENDIDA PELA ALUNA BEATRIZ LUCAS ARIDA, E ORIENTADA PELO PROF. DR. FÁBIO PINHEIRO.

CAMPINAS

2025

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

Arida, Beatriz Lucas, 1998-
Ar42c The consequences of flower colour polymorphism on the reproductive success of a Neotropical deceptive orchid / Beatriz Lucas Arida. – Campinas, SP : [s.n.], 2025.

Orientador: Fábio Pinheiro.
Coorientador: Maria Gabriela Gutierrez Camargo.
Dissertação (mestrado) – Universidade Estadual de Campinas (UNICAMP), Instituto de Biologia.

1. Seleção natural. 2. *Epidendrum*. 3. Orquídea. 4. Polinização. 5. Polimorfismo (Genética). 6. Atributos florais. 7. Flores - Cor. 8. Polinizadores. I. Pinheiro, Fábio, 1978-. II. Camargo, Maria Gabriela Gutierrez. III. Universidade Estadual de Campinas (UNICAMP). Instituto de Biologia. IV. Título.

Informações complementares

Título em outro idioma: As consequências do polimorfismo de coloração floral no sucesso reprodutivo de uma orquídea Neotropical polinizada por engodo
Palavras-chave em inglês:
Natural selection
Epidendrum
Orchids
Pollination
Genetic polymorphisms
Floral traits
Flowers - Color
Pollinators
Área de concentração: Biologia Vegetal
Titulação: Mestra em Biologia Vegetal
Banca examinadora:
Fábio Pinheiro [Orientador]
Edlley Max Pessoa da Silva
Natalia Costa Soares
Data de defesa: 22-04-2025
Programa de Pós-Graduação: Biologia Vegetal

Objetivos de Desenvolvimento Sustentável (ODS)
Não se aplica

Identificação e informações acadêmicas do(a) aluno(a)
- ORCID do autor: https://orcid.org/0000-0003-1798-5558
- Currículo Lattes do autor: http://lattes.cnpq.br/5695874256194694

BANCA DA APROVAÇÃO

Prof. Dr. Fábio Pinheiro

Prof. Dr. Edlley Max Pessoa da Silva

Profª. Drª. Natalia Costa Soares

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

AGRADECIMENTOS

Agradeço ao Prof. Dr. Fabio Pinheiro pelos sete anos em que esteve ao meu lado, durante todo o decorrer da minha vida acadêmica, como meu orientador, mas também conselheiro, incentivador e amigo, por me dar apoio emocional, por ser extremamente respeitoso, paciente e compreensivo, por me fornecer aulas, textos e discussões que expandiram meus conhecimentos, da sala de aula aos bares. Por me apoiar sempre, inclusive nos perrengues mais impensáveis, de acidente de carro até caderno comido por cabras. Sou grata ao seu brilhantismo e humanidade.

Agradeço aos meus co-orientadores, Profª. Drª. Maria Gabriela Gutierrez Camargo e Prof. Dr. Giovanni Scopece pela orientação e dedicação para com este projeto. Agradeço especialmente ao Prof. Dr. Giovanni Scopece por me receber durante o estágio no exterior, por ter me feito sentir segura e acolhida.

Agradeço aos colegas do Laboratório de Ecologia Evolutiva e Genômica de Plantas pelas discussões esclarecedoras e também pelas que fizeram surgir mais dúvidas do que respostas, pois são as dúvidas que mais impulsionam um projeto científico. E também aos colegas do Laboratório do Prof. Dr. Giovanni Scopece pelo acolhimento e amizade, especialmente à Lucrezia Laccetti, pela ajuda com as análises e dedicação para com o meu aprendizado.

Agradeço a todos que participaram deste projeto por meio do Comitê de Acompanhamento e da Banca da Qualificação, pelas sugestões realizadas com dedicação e cuidado: Prof. Dr. André Lucci Freitas, Profª. Drª. Bárbara Simões Santos Leal, Prof. Dr. Cleber Juliano Neves Chaves, Prof. Dr. Fabio Pinheiro, Profª. Drª. Ingrid Koch, Profª. Drª. Juliana Lischka Sampaio Mayer e Profª. Drª. Maria Gabriela Gutierrez Camargo. Agradeço especialmente ao Prof. Dr. André Lucci Freitas pela participação ativa no desenvolvimento deste projeto.

Agradeço a todos os que me acompanharam e me ajudaram em trabalhos de campo, Celso Perez dos Santos, Edlley Max Pessoa da Silva, Gabriel Pavan Sabino, Raphael da Silva. Em especial àqueles que me auxiliaram na observação de polinizadores, por ficarem ao meu lado durante cansativas horas na procura por borboletas: Giovana Narezi Trotta, Iris Dechiare Passos Ribeiro, Lucas Oliveira Mello e Welington Luis Sachetti Júnior.

Agradeço a todos que participaram da identificação de espécies de Lepidoptera, Prof. Dr. André Lucci Freitas, Dr. Simeão de Souza Moraes e Dr. Gustavo Mattos Accacio.

Agradeço à minha mãe, Catarina Elisabeth Lucas, por estar ao meu lado em todos os altos e baixos de uma vida inteira e por me apoiar em tudo. Agradeço ao meu pai, Samir Arida, por sempre ter sido um grande

incentivador à minha curiosidade pela natureza, poética e fisicamente. Agradeço ao meu irmão, Felipe Lucas Arida, por ter sido o primeiro a me mostrar que a escolha de um curso deve ser por amor. Agradeço ao meu grande amigo Cássio Shamy, pelos conselhos e por ser meu ídolo na arte de viver. Agradeço a todos os meus amigos que moram ou moraram comigo, por me fazerem rir e me apoiarem no nosso cotidiano singular, em especial a Lucas Oliveira Mello, por todo o apoio e carinho de anos.

Agradeço à Università degli Studi di Napoli Federico II, que me recebeu e me forneceu todo o suporte necessário para o desenvolvimento deste projeto de pesquisa durante meu estágio no exterior, e à Universidade Estadual de Campinas, que me forneceu suporte, assistência à saúde, atividades culturais e esportivas desde o meu ingresso na graduação até aqui. Sou grata a toda a comunidade universitária, estudantes, professores, técnicos e funcionários, por manterem o funcionamento da universidade e assim permitirem a minha formação acadêmica e profissional.

Agradeço a todas as instituições que forneceram apoio financeiro. O apoio para este trabalho foi fornecido pela Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; processos nº 2020/02150-3, 2021/03868-8 e 2021/10639-5). Fundos adicionais foram fornecidos por: Auxílio do Fundo de Apoio ao Ensino, Pesquisa e Extensão (FAEPEX – Funcamp) a Fabio Pinheiro, Bolsas de Iniciação Científica e de Mestrado a Beatriz Lucas Arida (FAPESP; processos nº 2021/10798-6 e 2023/01736-2), Bolsas de Produtividade em Pesquisa a André Victor Lucci Freitas (CNPq; processo nº 304291/2020-0) e Fabio Pinheiro (CNPq; processo nº 302849/2021-1). Este estudo também foi financiado em parte pela Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; Código Financeiro 001). Giovanni Scopece foi financiado no âmbito do *National Recovery and Resilience Plan* (NRRP; Edital nº 3138, retificado pelo Decreto nº 3175 do Ministério Italiano da Universidade e Pesquisa). Lucrezia Laccetti foi financiada no âmbito da Bolsa de Doutorado na área de Desenvolvimento Sustentável pelo *Italian National Social Security Institute* (INPS).

RESUMO

Plantas com estratégia de polinização por engodo geralmente exibem níveis elevados de polimorfismo. As razões que estão na base da associação entre polimorfismo floral e estratégias de engodo, entretanto, permanecem desconhecidas. *Epidendrum fulgens* Brongn. é uma orquídea Neotropical polinizada por engodo alimentar por borboletas, que possui um polimorfismo de coloração floral intrapopulacional, com flores de coloração vermelha, amarela e laranja, até agora inexplorado. Aqui, investigamos as consequências desse polimorfismo no seu sucesso reprodutivo. Realizamos experimentos de campo e em jardim experimental, com o objetivo de detectar a força e direção da seleção mediada por polinizadores ao longo do tempo, e testar se a presença de múltiplos morfotipos de coloração floral aumenta o sucesso reprodutivo da espécie. Em campo, monitoramos o sucesso reprodutivo e a morfologia floral de indivíduos de *E. fulgens* em duas populações ao longo de duas épocas de floração, e com esses dados realizamos análises de gradiente de seleção natural. Em jardim experimental, organizamos parcelas de indivíduos cultivados contendo plantas de coloração floral iguais e diferentes (isto é, mono e polimórficas), as expusemos aos polinizadores locais e monitoramos seu sucesso reprodutivo. Em ambas as atividades, também monitoramos a abundância e composição da comunidade local de polinizadores. Em campo, os morfotipos de coloração floral tiveram sucessos reprodutivos similares, mas encontramos coerências entre a diferenciação morfológica e a direção da seleção natural mediada por polinizadores, que foi muito dinâmica. Em jardim experimental, as parcelas mono e polimórficas também tiveram desempenho igual, com sucesso reprodutivo altamente variável ao longo do tempo. Também encontramos uma comunidade de polinizadores altamente diversificada tanto em campo como em jardim experimental. Nossos resultados sugerem que o polimorfismo floral em *E. fulgens* é mantido por uma combinação de fatores, incluindo seleção flutuante mediada por polinizadores, acasalamento preferencial positivo devido a diferentes preferências de polinizadores e herdabilidades de diferentes fenótipos. A seleção natural variou ao longo do tempo e do espaço, indicando uma interação dinâmica entre polinizadores e morfotipos florais.

ABSTRACT

Deceptive plants often exhibit elevated levels of polymorphism. The reasons laying at the basis of the association between flower polymorphism and deceptive strategies, however, remain unclear. *Epidendrum fulgens* Brongn. is a Neotropical deceptive orchid pollinated by butterflies which has an unexplored intrapopulational flower colour polymorphism, with red, yellow and orange flowers. Here, we investigate the consequences of this polymorphism on its reproductive success. We performed field and common garden experiments, aiming to detect pollinator-mediated natural selection strength and direction over time, and test whether the presence of multiple colour morphs increases species reproductive success. In the field, we monitored plants' reproductive success and floral morphology on two populations over two flowering seasons and with this data we performed selection gradient analyses. In the common garden we assembled plots of cultivated plants with same and different flower colour individuals (i.e., mono and polymorphic plots), exposed them to pollinators and monitored their reproductive success. In both activities, we also monitored the local pollinator community. In the field, colour morphs performed equally, but we found coherences between morphological differentiation and the direction of natural selection, which was very dynamic. In common garden, mono and polymorphic plots also performed equally, with highly variable reproductive success over time. We also found a highly diverse pollinator community both in the field and in the common garden. Our results suggest that flower polymorphism in *E. fulgens* is maintained by combining factors, including varying pollinator-mediated selection, assortative mating due to differential pollinator preferences and different phenotypes' inheritabilities. Natural selection varied across time and space, indicating a dynamic interplay between pollinators and flower morphs.

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1. APRESENTAÇÃO

A presente dissertação de mestrado versa sobre as consequências do polimorfismo de coloração floral no sucesso reprodutivo de uma orquídea Neotropical polinizada por engodo, *Epidendrum fulgens* Brongn. Está dividida nas seções indicadas no Sumário (Página 10), de forma que a seção 3 (Manuscrito) corresponde ao manuscrito final resultante deste projeto, dentro das normas de formatação e idioma requisitados pela revista Plant Biology, na qual foi aceito para publicação no dia 24 de fevereiro de 2025.

2. INTRODUÇÃO GERAL

A região Neotropical detém a maior biodiversidade vegetal da Terra, sendo que somente a América Latina abriga pelo menos um terço da biodiversidade global (Raven *et al*. 2020). Porém, a biodiversidade é um conceito que se expande em diversas categorias além da riqueza de espécies, conhecida como biodiversidade *lato sensu*, incluindo também as diversidades de interações, genética, morfológica ou fenotípica, etc., sendo que a diversidade fenotípica intraespecífica é frequentemente esquecida em relação à biodiversidade de espécies. A variação fenotípica intraespecífica em plantas molda interações bióticas e abióticas, por isso, é de suma importância que este tema continue sendo investigado pela biologia evolutiva. Apesar disso, somente na última década o interesse na variação fenotípica intraespecífica em plantas se intensificou dentro da ecologia evolutiva (Westerband *et al*. 2021).

Um caso extremo de variação fenotípica intraespecífica ocorre quando indivíduos de uma mesma espécie se enquadram em categorias morfológicas distintas, uma condição conhecida como polimorfismo fenotípico (Westerband *et al*. 2021). As Angiospermas são conhecidas por sua grande diversidade morfológica de flores, apresentando uma ampla gama de variabilidade em características florais, como formato, tamanho e coloração, com altas taxas de polimorfismos fenotípicos (Sapir *et al*. 2021). Por serem particularmente variáveis dentro das populações, flores são um modelo ideal para investigar a origem e a manutenção destes polimorfismos.

Atributos florais, como coloração e forma, são comumente usados por polinizadores como um sinal para identificação de fontes de alimento como néctar ou pólen (van der Kooi *et al*. 2021). Assim, a seleção natural mediada por polinizadores mantém a coesão destes atributos dentro do nível de espécie, fenômeno conhecido em espécies monomórficas como constância floral (Waser 1986, Waser & Price 1981). Existem, porém, espécies vegetais que utilizam deste fenômeno de modo a burlar a seleção natural e conseguirem ser polinizadas por animais sem a oferta de nenhuma recompensa floral a eles, chamadas de plantas com polinização por engodo (Sprengel 1793). Nestes casos, o polimorfismo floral é esperado, uma vez que o reconhecimento de uma flor sem recompensa é desvantajoso para o sucesso reprodutivo da espécie. Assim, polimorfismos fenotípicos florais são esperados em plantas com polinização por engodo, e de fato existem altas taxas de polimorfismos associadas a estas plantas (Heinrich 1975; Salzmann *et al*. 2007; Ackerman *et al*. 2011). Porém, apesar de um intenso esforço de pesquisa, os mecanismos que criam e mantêm polimorfismos florais intraespecíficos ainda não são completamente compreendidos (Sapir *et al*. 2021).

Apesar da falta de consenso, diversas hipóteses já foram propostas e testadas. A hipótese do aprendizado de evitação de polinizadores (Dukas & Real 1993; Smithson & Macnair 1997; Raine & Chittka 2007) diz que os polinizadores tendem a evitar o fenótipo sem recompensa, ao passo que são capazes de discriminar as flores, potencialmente levando às elevadas taxas de polimorfismos identificadas em plantas polinizadas por engodo. Uma hipótese alternativa, proposta por Gigord *et al*. (2001), enfatiza a seleção dependente de frequência negativa, que diz que fenótipos mais raros teriam uma vantagem sobre os mais frequentes por serem menos reconhecidos, causando a manutenção do polimorfismo. Kagawa & Takimoto (2016) associam a origem do polimorfismo de coloração floral em espécies de engodo à discriminação de cores imprecisa por polinizadores. Outra hipótese, por Scopece *et al*. (2017), enfatiza que uma seleção direcional, mas que flutua sobre os morfotipos em diferentes estações de floração, pode surtir as mesmas consequências, chamada de seleção flutuante. Por fim, Jacquemyn & Brys (2020) relatam que uma seleção fraca sobre os atributos florais também pode levar ao polimorfismo.

Estratégias de polinização por engodo de polinizadores evoluíram em diversas famílias de plantas, mas são particularmente frequentes em Orchidaceae, na qual há uma proporção atipicamente alta de espécies que empregam essas estratégias. Estima-se que cerca de um terço das espécies de orquídeas não oferecem nenhuma recompensa floral e, portanto, atraem polinizadores por meio de alguma estratégia de engodo (Shrestha *et al*. 2020). Além disso, taxas mais altas de variabilidade fenotípica intraespecífica em espécies de orquídeas de polinização por engodo foram relatadas em comparação com as que oferecem alguma recompensa ao polinizador (Heinrich 1975; Salzmann *et al*. 2007; Ackerman *et al*. 2011).

Na presente dissertação de mestrado, nós acrescentamos evidências para a compreensão da relação entre o polimorfismo fenotípico floral e as estratégias de engodo de polinizadores. Para isto, utilizamos como modelo *Epidendrum fulgens* Brongn. (Orchidaceae), uma espécie Neotropical que possui um polimorfismo fenotípico de coloração floral, além de uma variabilidade em forma e tamanho de flores, e que emprega a estratégia de engodo alimentar (simulação de recurso alimentar aos polinizadores), sendo sua polinização realizada por borboletas. Investigamos a intensidade e a direção da seleção natural mediada por polinizadores sobre diversos atributos florais (como coloração floral e atributos morfológicos), ao longo de duas épocas de floração consecutivas, em duas populações naturais (Bertioga e Ilha do Cardoso – SP), e a relação entre o polimorfismo de coloração floral e componentes do sucesso reprodutivo das plantas, tanto em condições planejadas em jardim experimental, quanto nas duas populações naturais. Fizemos especificamente as seguintes perguntas e hipóteses:

i. A variação da coloração floral é discreta em *E. fulgens*? Hipótese i: *E. fulgens* possui indivíduos com categorias discretas de cor, caracterizando um polimorfismo fenotípico de coloração floral.

ii. Se sim, diferentes morfotipos de coloração floral experimentam diferentes sucessos reprodutivos ao longo das épocas de floração em populações naturais? Hipótese ii: diferentes morfotipos de coloração floral de *E. fulgens* experimentam diferentes sucessos reprodutivos ao longo do tempo.

iii. Diferentes morfotipos de coloração floral são expostos a diferentes seleções mediadas por polinizadores? Hipótese iii: diferentes morfotipos de coloração floral de *E. fulgens* estão sob diferentes regimes de seleção natural mediada pelos polinizadores ao longo do tempo, corroborando a hipótese de seleção flutuante proposta por Scopece *et al*. (2017).

iv. Se sim, isso reflete uma diferenciação morfológica entre os morfotipos? Hipótese iv: os diferentes regimes de seleção mediada por polinizadores reflete-se em diferenciação morfológica direcional de forma e tamanho de flores entre os morfotipos de coloração floral.

v. A presença de diferentes morfotipos de coloração floral tem efeito no sucesso reprodutivo da espécie? Hipótese v: a presença de diferentes morfotipos de coloração floral possui um impacto positivo no sucesso reprodutivo de *E. fulgens*, ocasionando a manutenção do polimorfismo fenotípico.

3. MANUSCRITO

Title: The consequences of flower colour polymorphism on the reproductive success of a Neotropical deceptive orchid

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Short title: Colour polymorphism in *Epidendrum*

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Keywords: Fluctuating selection; *Epidendrum*; Orchidaceae; food deception; phenotypic polymorphism; floral traits; pollinator-mediated selection.

Key message: Intrapopulation flower colour polymorphism is maintained by varying pollinator-mediated selection, assortative mating and different phenotype's inheritability in a Neotropical orchid species.

ABSTRACT

- Deceptive plants often exhibit elevated levels of polymorphism. The reasons laying at the basis of the association between flower polymorphism and deceptive strategies, however, remain unclear. *Epidendrum fulgens* is a Neotropical deceptive orchid pollinated by butterflies which has an unexplored intrapopulational flower colour polymorphism. Here, we investigate the consequences of this polymorphism on its reproductive success.
- We performed field and common garden experiments, aiming to detect pollinator-mediated selection strength and direction over time, and test whether the presence of multiple colour morphs increases species reproductive success. In the field, we monitored plants' reproductive success and floral morphology on two populations over two flowering seasons, and with this data we performed selection gradient analyses. In the common garden we assembled plots of cultivated plants with same and different flower colour individuals (i.e., mono- and polymorphic plots), exposed them to pollinators and monitored their reproductive success. In both activities, we also monitored the local pollinator community.
- In the field, colour morphs performed equally, but we found coherences between morphological differentiation and the direction of selection, which was very dynamic. In common garden, mono- and polymorphic plots also performed equally, with highly variable reproductive success over time. We also found a highly diverse pollinator community.
- Our results suggest that flower polymorphism in *E. fulgens* is maintained by combining factors, including varying pollinator-mediated selection, assortative mating due to differential pollinator preferences and different phenotypes' inheritabilities. Natural selection varied across time and space, indicating a dynamic interplay between pollinators and flower morphs.

INTRODUCTION

Intraspecific variation in phenotypic traits is widespread in nature. Darwin emphasised the general presence of this intraspecific variation considering it as the raw material upon which natural selection might act (Darwin 1859). In general, phenotypic variation is fuelled by genetic recombination and mutation and is eroded instead by natural selection that eliminates all but the most favourable genetic combinations (Stebbins 1950). Therefore, intraspecific variation found in natural populations should result from an equilibrium between the genetic processes generating variation and the selecting forces leading to trait homogeneity (Ellegren & Galtier 2016). An extreme case of phenotypic variation occurs when individuals of a species fall into two or more discrete categories, a condition referred to as phenotypic polymorphism (Westerband *et al.* 2021).

Flowers, i.e. the sexual organs of Angiosperms, are an ideal model to investigate the origin and the maintenance of phenotypic polymorphisms, as they are particularly variable within populations (e.g. Darwin's book *The different forms of flowers on plants of the same species*, Darwin 1877). Among floral traits, colour is one of the most polymorphic (Sapir *et al.* 2021). This trait is typically used by pollinators as a signal to identify sources as nectar or pollen (van der Kooi *et al.* 2021). In most Angiosperms, flowers within populations have weakly variable colours (i.e. monomorphic species), because plant individuals that pollinators are able to recognize as conspecific are more easily visited (e.g. *flower constancy*; Waser 1986). In these plant–pollinator relationships, plants are continuously exposed to the choice of pollinators, which imposes a directional selection on flower traits (Schiestl & Johnson 2013), hence eroding intraspecific variation. This flower constancy is expected to be advantageous for both involved parts, as it increases plant reproductive success, by more efficient pollen transfer between conspecific individuals, and it increases pollinators foraging efficiency, as searching and learning to handle new floral types is costly (Free 1963; Chittka *et al.* 1999; Goulson 2000). This is the most common mechanism by which pollinator-mediated natural selection on floral traits maintains intraspecific cohesion and reduces floral variation (Waser & Price 1981).

Despite this general situation, however, in many plant species, flowers exhibit strong discrete or continuous colour variation (i.e., polymorphic species). Discrete polymorphism in flower colour is likely to result from genetic variation at a single or a few loci (Wu *et al.* 2013), whilst continuous variation is likely to result from a multigenic architecture with additive effects or from differential expression of genes involved in pigment biosynthesis (Davies *et al.* 2012; Scopece *et al.* 2020). Despite an intense research effort, the mechanisms that create and maintain flower colour polymorphisms within plant species are still not completely understood and highly debated (Sapir *et al.* 2021). Competing hypotheses are that colour variation can be maintained due to balancing selection exerted by multiple selection regimes, fluctuating selection over time and space, heterozygote advantage and frequency-dependent selection (Kellenberger *et al.* 2019; Sapir *et al.* 2021 and references therein). A lack of selection may also maintain variation, although this remains so far almost untested (but see Jacquemyn & Brys 2020).

A peculiar situation regards a category of plant species that offer no reward to their pollinators, known as deceptive plant species (Sprengel 1793). This pollination strategy evolved in several plant families but is particularly frequent in Orchidaceae, where an unusually high proportion of species employs deceptive pollination strategies (Shrestha *et al.* 2020). Differently from rewarding species, in deceptive species floral constancy is not expected, because, after a few unrewarded visits pollinators tend to avoid the rewardless phenotype (pollinator avoidance learning; Dukas & Real 1993; Smithson & Macnair 1997; Raine & Chittka 2007). In these pollination strategies, thus, pollinator-mediated natural selection is not expected to eliminate floral variation, potentially leading to high intraspecific flower polymorphisms. Accordingly, higher rates of intraspecific phenotypic variability in deceptive orchid species compared to rewarding ones have been reported (Heinrich 1975; Salzmann *et al.* 2007; Ackerman *et al.* 2011). The traditional hypothesis to explain this elevated phenotypic polymorphism in deceptive species is that it would slow down pollinator avoidance learning ability thus increasing plant reproductive success (Heinrich 1975; Nilsson 1992; Smithson & Macnair 1997; Ferdy *et al.* 1998). However, in a meta-analysis of studies conducted to test this hypothesis, Juillet & Scopece (2010) showed that a relationship between flower polymorphism and increased reproductive success has not been found. Alternative explanations raised to justify this elevated polymorphism in deceptive orchids emphasise a negative frequency-dependent selection (Gigord *et al.* 2001), a weak selection on floral traits (Jacquemyn & Brys 2020) or a directional but fluctuating selection on floral traits in different flowering seasons (Scopece *et al.* 2017). The origin of flower colour polymorphism in deceptive species has also been associated with inaccurate colour discrimination by pollinators (Kagawa & Takimoto 2016).

The lack of concordance in studies attempting to explain the reasons that lay at the basis of the elevated phenotypic polymorphism in deceptive orchids calls for additional case studies, in particular in under-investigated regions and groups. Here, using the colour polymorphic Neotropical orchid species *Epidendrum fulgens* Brongn., we investigated the intensity and direction of pollinator-mediated natural selection over two consecutive flowering seasons in natural populations, and the relation between flower colour polymorphism and reproductive success components in common garden conditions and in natural populations. Using these data, we specifically asked the following questions:

i. Is flower colour variation discrete in *E. fulgens*?

ii. If yes, do different flower colour morphs experience different reproductive success along their flowering season in natural populations?

iii. Are different colour morphs exposed to different pollinator-mediated selection?

iv. If yes, does it reflect an among-morph morphological differentiation?

v. Does the presence of different colour morphs have an effect on reproductive success?

MATERIAL AND METHODS

Study species, experimental design and characterization of colour polymorphism

Epidendrum fulgens is a Neotropical orchid species belonging to the subtribe Laeliinae. The species is found on open sand dune vegetation, growing directly on sandy soils along the South and Southeastern Brazilian coast (de Mattos *et al.* 2023). It has a long flowering season in the time of the year with the highest rainfall, with the peak of flowering between October and April. This species does not have floral nectaries and employs a generalised food-deceptive strategy to attract pollinators (mainly butterflies, Fuhro *et al.* 2010), which are necessary for pollen transfer (Pinheiro *et al.* 2015) and fruit development, with no evidence of spontaneous autogamy (Fuhro *et al.* 2010). *E. fulgens* has an evident intrapopulation variation in flower colour, with petals and sepals ranging from yellow to red with orange intermediates (Fig. 1).

With the aims of understanding the mechanisms that maintain intrapopulation polymorphism and their consequences on reproductive success, we performed field observations in natural populations of *E. fulgens* (hereafter, natural populations survey). Then, in order to understand whether the presence of different flower colour morphs has an effect on reproductive success, we settled up an experiment manipulating the presence of different colour morphs in a common garden setup (hereafter, plot experiment). In both activities, reproductive success was then estimated. Following Scopece *et al*. (2015), pollinia removal was used as a proxy for male reproductive success (hereafter MRS) and fruit formation was used as a proxy for female reproductive success (hereafter FRS).

To characterise flower colour polymorphism, we collected reflectance data of the labellum, petal, lateral sepal and dorsal sepal of 21 flowers from different individuals, 7 of each extreme colour (yellow and red) and 7 intermediates (orange), with a Ocean Optics USB4000 Fiber Optic Spectrometer (Jaz Modular Optical Sensing Suite), allowing to detect visible and ultraviolet wavelengths and covering the sensitivity of the photoreceptors of different species of butterflies (van der Kooi *et al.* 2021). Using these data, we validated the three colour morph categories of *E. fulgens*: yellow, orange and red (hereafter, Y, O and R) (e.g. Dalrymple *et al.* 2015).

Natural populations survey

Field activities were carried out in two natural populations with similar climate conditions, Bertioga (BE) and Ilha do Cardoso (CA), both in the coastal region of São Paulo State in Brazil. To estimate the frequency of the three flower colour morphs, we used the transect method proposed by Cottam & Curtis (1956), and took note of the number of individuals of each morph in an area of 400 m^2. This estimation was carried out a total of six times along the flowering phenology of the investigated species (four times in BE and two in CA). In both populations, we labelled 15 individuals from each colour morph. In the period from December 2021 to April 2023, a total of 14 field expeditions in the natural populations were carried out (ten to BE and four to CA). In the different expeditions, we followed the same labelled individuals. Over time, however, when some of the labelled individuals were lost, we added new individuals in order to keep the original sample size. By doing so, a total of 70 individuals were monitored in BE and a total of 68 in CA.

For each labelled individual, we recorded eight morphological traits potentially involved in pollinator attractiveness: petal length (PetL), petal width (PetW), labellum area (LabA), labellum shape (LabS), lateral sepal length (LatSepL), lateral sepal width (LatSepW), dorsal sepal length (DorSepL), dorsal sepal width (DorSepW). For these measurements, we collected one flower from each labelled individual in each flowering season (276 flowers in total), dissected floral pieces and distended them between transparent sheets to avoid 3D deformation. Then, we took a digital scan of each flower using a table scanner (HP Scanjet 4670), with a reference scale on the back. Calibrated images were thus used to extract flower traits measurements using ImageJ 1.33 (https://imagej.nih.gov/ij/). LabS was obtained instead by performing a landmark-based geometric morphometric analysis. To do this, we selected a set of 23 landmarks and semilandmarks (e.g. Bookstein 1997; Tyteca 2000) using TpsUtil and TpsDig 2 (Rohlf 2015). The coordinates of the landmarks and semilandmarks of the labellum were superimposed with a Generalised Procrustes Analysis (GPA) (Rohlf & Slice 1990). Then, we performed a Principal Component Analysis (PCA) and PC values were mapped back onto labellum morphology using *eigenvectors* (i.e., a set of vectors associated with the set of landmarks and semilandmarks and their variation in direction and strength). Finally, in each field expedition, data on male and female reproductive success were recorded on each labelled individual.

To have an overview of Lepidoptera characterising the *E. fulgens* flower visitors and to assess possible changes in its composition and abundance over time, we performed direct observations (for a total of 80 hours) using the approach described in Fuhro *et al.* (2010). Observations were conducted during day time, from 6 a.m. to 4 p.m., since *E. fulgens* is pollinated only in daylight (Fuhro *et al.* 2010). All butterflies found were recorded and classified into one of the three following categories: a. pollinators (butterflies that were seen removing a pollinia of *E. fulgens* or with it attached to the proboscis), b. potential pollinators (were not seen removing pollinia of *E. fulgens* but have the potential of being pollinators due to their characteristics of behaviour, size, vision system and feeding habits) and c. not pollinators (were not seen removing pollinia of *E. fulgens* and have no potential of being pollinators due to incompatible characteristics). Some of the butterflies interacted with individuals of *E. fulgens* but didn’t remove pollinia (e.g. oviposited), in that case they were not classified as flower visitors, but we indicate the presence of interaction on the Fig. S5).

Plot experiment

The plot experiment was conducted using 60 *E. fulgens* individuals cultivated in common garden conditions at the experimental area of the Department of Plant Biology at the Campus of the Universidade

Estadual de Campinas (Campinas – SP, Brazil). To test whether the presence of different colour morphs affect the reproductive success, we assembled different plots of cultivated individuals, and exposed them to local wild pollinators. Each plot encompassed around 20 inflorescences. Mono- and polymorphic plots were placed far from each other (more than 20 metres) in an open area, where naturally occurring butterflies can be found, including those species already described as pollinators of *E. fulgens*. We repeated the experiment twelve times, randomising individuals and plot position. Each round of exposure lasted five days, with nine days of break between them. After each round, MRS and FRS were estimated as explained above. This experiment lasted from October 2022 to March 2023.

In these plots, we estimated percent MRS (related to the pollinia removal; PR) and FRS (related to the fruit formation; FF) over time. We assembled three monomorphic plots (i.e. plots with only Y, only O and only R colour morphs; hereafter, MY, MO and MR) and one polymorphic plot (with Y, O and R morphs in the same proportion; hereafter, P, and the morphs inside them PY, PO and PR). Percent MRS and FRS were calculated for each of the four plots ($MRS_{MY/MO/MR/P}$ and $FRS_{MY/MO/MR/P}$) and for each of the three colour morphs within the polymorphic plot ($MRS_{PY/PO/PR}$ and $FRS_{PY/PO/PR}$) using the following formulas (here referred to the Y morph, but similarly applied for all of the three morphs):

$$MRS_{MY} = PR_{MY} / (PR_{MY} + PR_{MO} + PR_{MR} + PR_{P}) * 100;$$

$$FRS_{MY} = FF_{MY} / (FF_{MY} + FF_{MO} + FF_{MR} + FF_{P}) * 100;$$

$$MRS_{PY} = PR_{PY} / (PR_{PY} + PR_{PO} + PR_{PR}) * 100;$$

$$FRS_{PY} = FF_{PY} / (FF_{PY} + FF_{PO} + FF_{PR}) * 100$$

To understand which species of Lepidoptera characterise *E. fulgens* pollinator community at the experimental suburban area, we performed direct observations (for a total of ten hours) using the approach detailed for natural populations survey.

Pollen limitation

To test for pollen limitation, in the BE population, we performed manual crosses in the field, saturating 142 flowers from 16 individuals with pollinia collected from other individuals of the same population. On these individuals, we thus estimated the number of flowers pollinated and the number of fruits produced. We then compared these data with data collected in all of the ten field expeditions, on open-pollinated individuals from the same BE population. We calculated a pollen limitation index for fruit formation using the following equation (PL; Larson & Barrett 2000):

$$PL = 1 - (P_o / P_h)$$

where P_o is the proportion of fruits by flowers in open-pollinated individuals, and P_h is the proportion of fruits by flowers in pollen-saturated plants. The index ranges from 0 (no pollen limitation) to 1 (high pollen limitation).

Statistical analysis

To explore the level of phenotypic variability in *E. fulgens*, we calculated a coefficient of variation (CV) as the ratio between standard deviation and mean for each of the eight investigated floral traits in the two flowering seasons and then averaged them to obtain a single value representative for the species.

Relative frequency and phenotypic differences across the three flower colour morphs were tested in the two populations by performing a Dunn Kruskal-Wallis multiple comparisons (with Bonferroni correction) for pairwise comparisons. The same approach was used to detect differences across the three colour morphs MRS and FRS both in the natural populations survey and in the plot experiment. To detect a putative temporal variation in reproductive success, these parameters were also investigated separately in the two flowering seasons and in three different periods within one flowering season. The three periods were identified according to average precipitation data from 1990 to 2022 for Campinas, provided by the Center for Meteorological and Climatic Research Applied to Agriculture of the Universidade Estadual de Campinas (https://www.cpa.unicamp.br/), as follows: increasing rainfall (October to early December, Period 1), elevated rainfall (mid December to early February, Period 2) and decreasing rainfall (mid February to end of March, Period 3).

To infer the strength and direction of selection in both populations, in each colour morph, we estimated selection gradients in the two flowering seasons. In BE we also assessed differences in selection separately in each of the three periods within the flowering season. To do this, first we tested for the presence of intercorrelated floral traits using a Spearman's rank correlation test in order to remove highly correlated traits ($\varrho > 0.8$). Then, following Lande & Arnold (1983), we performed multiple single linear regressions using MRS and FRS as response variables and standardised floral traits (z-scores) as predictors. All the regression models were performed using the *lme4* R package (Bates *et al.* 2015).

RESULTS

Flower phenotype polymorphism

Reflectance data showed different colour polymorphism patterns on different floral pieces: labellum showed a continuous variation, petal and lateral sepal showed a discrete polymorphism, and dorsal sepal showed an overlap between orange and yellow (Fig. 1B). Morphometric data revealed a moderately low average CV in *E. fulgens* (average CV = 9,38), however CVs of different morphological traits showed some differences (PetL = 6.59%, PetW = 13.76%, LabA = 14.46%, LatSepL = 7.38%, LatSepW = 7.50%, DorSepL = 7.30%, DorSepW = 8.68%). LabS was mainly explained by a variation along PC1 (23.48% variance explained) which represents the distance between the upper lobes of the labellum (see Fig. S2).

The three colour morphs showed significant differences in PetL, LatSepL and DorSepL and PetW in the BE population (Fig. 2; Fig. S3), while in CA we found significant differences in LabS (Fig. 2; Fig. S3).

Natural populations survey and pollen limitation

The frequency of the three flower colour morphs on natural populations was different between BE and CA (Fig. S1). In BE population, we found that Y and R have the same frequency, and both are different from O, which is the most frequent (Dunn's $\text{test}_{Y\mathit{vs}O} = 4.56$, $P < 0.001$; Dunn's $\text{test}_{R\mathit{vs}O} = 4.52$, $P < 0.001$), while in CA, we found that the three colour morphs differ from each other, with R being the less frequent, followed by Y, and O being the most frequent (Dunn's $\text{test}_{R\mathit{vs}Y} = -1.92$, $P = 0.02$; Dunn's $\text{test}_{R\mathit{vs}O} = 3.90$, $P < 0.001$; Dunn's $\text{test}_{Y\mathit{vs}O} = 1.98$, $P = 0.03$) (see Fig. S1).

In both populations, the three morphs showed no significant differences in MRS and FRS in each of the two flowering seasons (Fig. 3). The same result was observed in the BE population by dividing the flowering seasons in three different periods (see Fig. S4).

Overall, the results obtained in the selection gradients analysis suggest few marginally significant values indicating a weak directional selection with floral traits being under different selection regimes in the two populations and in the two seasons (Table 1 and 2; Fig. 4). Particularly, in the first season, in BE, we detected a significant negative selection on PetW for the MRS in the Y morph, a significant negative selection on LabS for the MRS in the O morph, and a significant positive selection on PetL for the FRS in the R morph. In the same population, in the second season, we detected a significant positive selection on PetW for the FRS, both in the O and R morph (Table 1; Fig. 4A). In CA, instead, in the first season, we detected a significant positive selection on LabS for the FRS in the R morph (Fig. 4). In the second season, we detected a significant positive selection on PetW for the MRS in the Y morph, a significant positive selection both for the MRS and FRS on PetW in the O morph, and a significant positive selection on PetW for the FRS in the R morph (Table 2; Fig. 4B). In BE, by dividing each season in different periods, we found different selective patterns in the different periods (Table 3).

In the BE and CA populations, we identified 35 species of Lepidoptera in total (see Fig. S5). 13 of these species were observed pollinating *E. fulgens* or with its pollinia attached to their proboscis and 16 were identified as potential pollinators. In total, we observed 16 events of pollination (average of 0.2 pollination event per hour of observation). Of the 35 observed species, eight could only be seen during the first flowering season, 23 in the second and four in both. In the BE population, pollen limitation was 0.82, with the average proportion of fruits in open-pollinated individuals of 0.16 and in pollen-saturated plants of 0.91.

Plot experiment

In the plot experiment, we found no significant differences for any comparisons of FRS, this can be due to a very low fruiting rate on this species (average of 0.013 fruits developed per flower on the overall plot experiment), which is a common pattern in food-deceptive species. Over the whole experiment the average MRS of the monomorphic plots (M) was also not significantly different from the polymorphic plot (P) (Fig. 5A). However, when considering the monomorphic plots (MY, MO, MR) and the morphs within the polymorphic (PY, PO, PR) separately, plants in the PY plot showed a higher reproductive success than R morph plants on both treatments ($MRS_{PY} > MRS_{MR}$, MRS_{PR}) and than O morph in monomorphic plot ($MRS_{PY} > MRS_{MO}$) (Fig. 5B). By dividing the plot experiment in three different periods according to rainfall regimes, we also found significant differences in MRS in the first two periods, while in Period 3 we detected no differences for any of the comparisons (Fig. 6). In Period 1, PY is higher than R and O in both treatments ($MRS_{PY} > MRS_{MO}$, MRS_{MR}, MRS_{PO}, MRS_{PR}), and in Period 2, PY is higher than R in both treatments ($MRS_{PY} > MRS_{MR}$, MRS_{PR}) and than O morph in monomorphic plot ($MRS_{PY} > MRS_{MO}$) such as the overall data (without considering periods) (Fig. 6).

We identified 12 species of Lepidoptera in the experimental suburban area, seven of which are also present in the natural population of BE (Fig. S5). Of these, four taxa were identified as pollinators of *E. fulgens*, and in total, we observed four events of pollination (average of 0.4 pollination event per hour of observation). Despite habitat differences, the potentially pollinating species in natural populations and in the experimental area are extremely similar, or equivalent species in terms of habitat preference (open and sunny areas).

DISCUSSION

The origin of flower colour polymorphism is highly debated (Sapir *et. al* 2021), ranging from weak selection or neutral processes (Wang *et al.* 2016, Jacquemyn & Brys 2020) to active divergent selection (Eckhart *et al.* 2006; Newman *et al.* 2012; Bergamo *et al.* 2016). Whatever it is the mechanism for its origin, the presence of polymorphism can have important consequences on pollinator behaviour, hence influencing plant reproductive success and selection patterns. Here, by surveying natural populations of the colour polymorphic deceptive orchid species *Epidendrum fulgens*, and by conducting a manipulative experiment, we demonstrated that pollinator preferences for different morphs change in space, in flowering seasons and in rainfall periods inside a season, that selection on floral traits involved in pollinator attraction is weak but follows different trajectories in different colour morphs and that pollinators prefer a colour morph over the others. Taken together these results suggest that pollinators are able to detect different flower colour morphs, that a different composition of the pollinator community changes pollination patterns and that this ability has consequences on plant reproductive performances and on the evolution of floral polymorphic traits.

The presence of a polymorphism in flower colour of *E. fulgens* is known since the first botanical explorations in the Neotropical region (Reichenbach 1878). Whether it was a continuous or a discrete variation was however untested. Here, to resolve this issue, we analysed the reflectance *spectrum* of different flower pieces of the extreme colour morphs (R and Y) and of intermediate O morphs. Our results show a clear discrete polymorphism in petals and lateral sepals but not in labellum (Fig. 1). Overall, a discrete polymorphism in a colour trait is often considered to result from genetic variation at a single or a few loci involved in pigment biosynthesis (e.g. Wu *et al.* 2013). Although the determination of the genetic architecture of this phenotypic trait is beyond the scopes of our study, different phenotypic patterns in different floral pieces might point to a role of expression rather than inherited genetic mutations. Alternatively, the presence of a modifier gene expressed in petal and lateral sepals (and not in labellum) might also alter the expression of flower colour genes through its action (e.g. Scopece *et al.* 2020).

Discrete flower colour polymorphism is an infrequent but phylogenetically widespread in plants and is particularly common in deceptive orchids (e.g. Gigord *et al.* 2001; Juillet *et al.* 2010; Jersáková *et al.* 2015; Narbona *et al.* 2018; Jiménez-López *et al.* 2020). This association between colour polymorphism and deceptive orchids has been considered a consequence of negative frequency-dependent selection, because the rarest morph should have an advantage in being less recognizable by pollinators (e.g. Gigord *et al.* 2001). However, by comparing MRS and FRS in the three colour morphs in natural populations we found no significant differences (Fig. 3), thus suggesting that none of the morphs is advantaged in natural populations and speaking against negative frequency-dependent selection hypothesis (Gigord *et al.* 2001). This general finding was also confirmed when dividing the flowering season in three different periods defined by rainfall dynamics.

Different colour morphs can be subjected to different selection if the pollinator set involved in their pollination are partially different and show different preferences. In natural populations, selection might be due to different factors, but in severely pollen-limited species the strength of selection is considered to be mainly due to the action of pollinators (Sletvold *et al.* 2010; Sletvold & Ågren 2014). In our study we found that reproductive success of *E. fulgens* is severely limited and that overall selection on floral traits is weak but varies in space, in seasons and in rainfall periods. This result, already reported for Mediterranean deceptive orchids (Scopece *et al.* 2017), is likely linked to variation in the pollinator community, a trend common in generalist plant species where local differences in prevalent pollinators might lead to local adaptation (Frachon *et al.* 2023). Accordingly, in our pollinator observations we found that the pollination strategy of *E. fulgens* is generalist, with at least 12 species of Lepidoptera (i.e. 35.3% of the 34 reported Lepidoptera species; see Fig. S5) contributing to pollen transfer in the studied populations. This situation is typical of generalised food-deceptive species (reviewed by Fantinato *et al.* 2017), including several *Epidendrum* species (reviewed by Pinheiro & Cozzolino 2013).

If selective pressures are continuous and maintain the same direction, they might lead to morphological differentiation (Harder & Johnson 2009). Here, we found coherence between morphological differentiation and the direction of selection in some of the studied traits. In the population of BE, for instance, the trait PetL was positively selected (for FRS, Fig. 4A) only in R morph, which has also significantly longer petals than the other two morphs (Fig. 2A); PetW was negatively selected (for MRS, Fig. 4A) only in Y morph, which has significantly thinner petals than the other two morphs (Fig. 2D). These results suggest the presence of a partially different pollinator set for the different morphs that might lead to the observed morphological differentiation of colour morphs. Spatial and temporal habitat heterogeneity is one of the mechanisms explaining the maintenance of polymorphisms within populations (Delph & Kelly 2014) and, in this context, the diversity of pollinator assemblage might be interpreted as an important community feature enhancing the species variation. In fact, de Mattos *et al.* (2023) showed that pollinators' diversity and abundance not only contribute to the morphological variation of flowers but also improve the habitat suitability of *E. fulgens*, partially shaping its geographic distribution.

We found evidence of a variable direction of natural selection, with variation among-years, among-populations and acting over different floral traits (Tables 1 and 2). Despite this variation, we detect no evidence of any trait being under positive selection on one flowering season and negative on the next one, which suggests that different pollinators indeed have different preferences and their long-term consistency of choices affect the reproduction pattern of the colour morphs causing assortative mating. Assortative mating is a known mechanism of maintenance of phenotypic variation (Kondrashov & Shpak

1998) and could explain the coherence between morphological trait variation on opposite directions among colour morphs. This could contribute to explain the maintenance of the observed polymorphism on *E. fulgens*, regarding its highly rich and dynamic pollinator's community (see Fig. S5). Also, we could only detect this pattern in a multi-year study, thus we emphasise the importance of long-term studies on evolutionary questions, and we call for more investigative studies on seasonal variation on selection patterns in deceptive orchid species.

In deceptive orchids, phenotypic polymorphism has been considered to be involved in a decrease of pollinator avoidance learning, thus leading to the prediction of a higher reproductive success in polymorphic populations (Heinrich 1975). To test this prediction, we performed a plot experiment manipulating the presence of different colour morphs. Our experiment showed that contrary to the prediction, monomorphic and polymorphic plots experienced similar levels of MRS and FRS. This finding confirms previous findings (reviewed by Juillet & Scopece 2010) and challenges the role of polymorphism in decreasing pollinator avoidance learning.

Our plot experiment also showed that Y morph in the polymorphic plot (PY) is more attractive than the R both in monomorphic and within polymorphic plot (MR and PR) (MRS in Fig. 5B). This can be linked to the perception ability of Neotropical butterflies, as it has been documented a higher attractiveness for the yellow spectrum for several butterfly species (Weiss & Papaj 2003; Ômura & Honda 2005 and references therein). Recent studies have shown that the yellow colour may be interpreted by butterflies as a positive sign of the presence of nectar, being chosen among other colours (Maharaj & Bourne 2017, Santana *et al.* 2022). This higher attractiveness of the Y morph was not observed in natural populations (likely because of different variables such as relative abundance, heterogeneous distribution, sharing pollinators with other species of local flora, proximity to other *E. fulgens* populations, elevated richness of pollinator community), but it might contribute to explain why the Y morph is more abundant than the R morph in CA population. However, why the higher attractiveness of the Y morph does not translate into an overall higher frequency of plants carrying this phenotype in the natural populations is still an open question.

The three morphs show different abundances in natural populations with a clear prevalence of the O morph over the R and Y (see Fig. S1). The prevalence of the O colour morph in natural populations is likely linked to the inheritability of the intermediate phenotype. Indeed, Pinheiro *et al*. (2010; 2016) report hybrids in the *Epidendrum* genus showing orange flowers, which were generated by crosses between species with yellow and red/pink flowers. Alternatively, flower colour morphs can also show differential tolerance to abiotic factors (Warren & Mackenzie 2001; Arista *et al.* 2013) due to the association between some floral pigments and protective flavonoids and anthocyanins, and be selected either for or against in different habitats (Jiménez-López *et al.* 2020). Indeed, O and R morphs show red pigments also along their stems and leaves, contrasting with the pure green leaves and stems present in the Y morph (F Pinheiro personal observation). Future studies should investigate how flower pigments may influence physiological responses on vegetative organs related to soil properties (Schemske & Bierzychudek 2007; Wu *et al.* 2022), drought, light and heat stress (Arista *et al.* 2013; Costa *et al.* 2023).

Flower colour polymorphism is a common phenomenon in nature, and its maintenance has been a topic of much debate (Sapir *et al.* 2021). Taken together, our results suggest that flower colour polymorphism is maintained by a combination of factors, including weak but varying pollinator-mediated selection, assortative mating due to differential pollinator preferences, and the intermediate phenotype's inheritability. We detected variations in pollinator preferences and selection intensities across space, seasons and rainfall periods, indicating a dynamic interplay between pollinators and flower morphs through space and time (Scopece *et al.* 2017; Frachon *et al.* 2023). Our manipulative experiment further demonstrated that the presence of different colour morphs did not significantly affect overall reproductive success, suggesting that the observed polymorphism is likely maintained by other mechanisms, excluding the occurrence of deterring pollinator avoidance learning. While the different colour morphs did not show differences in their overall reproductive success in the field, our plot experiment revealed that the Y morph was more attractive to butterflies than the red morph. The fact that the higher attractiveness of the Y morph does not translate into an overall higher frequency of plants carrying this phenotype in the field deserves future studies, aiming to investigate the role of floral pigments in other plant physiological responses to abiotic variables such as water, light and heat stress. Furthermore, other biotic interactions, such as mycorrhizal (Burkle & Zabinski 2023) and ant-plant associations (Ibarra-Isassi & Oliveira 2018) are essential data in studies seeking to understand patterns of adaptation at small geographic scales. Considering all of these contributing factors together, a more comprehensive understanding of the selective pressures shaping floral variation in hyperdiverse regions (such as the Neotropics) can be achieved.

ACKNOWLEDGEMENTS

We are grateful to the efforts of many co-workers and friends who helped us in the fieldwork: Celso Perez dos Santos, Edlley Max Pessoa da Silva, Gabriel Pavan Sabino, Giovana Narezi Trotta, Iris Dechiare Passos Ribeiro, Lucas Oliveira Mello, Raphael da Silva, Welington Luis Sachetti Júnior. We thank the help of Simeão de Souza Moraes and Gustavo Mattos Accacio in identifying butterfly species. We thank members of the Laboratory of Evolutionary Ecology and Genomics (LEEG) at Universidade Estadual de Campinas (UNICAMP) for helpful discussions and comments regarding our results about the origins of flower colour polymorphisms. Support for this work was provided by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; grants nº 2020/02150-3, 2021/03868–8, and 2021/10639-5). Additional funds were provided by grants from Fundo de Apoio ao Ensino, Pesquisa e Extensão (FAEPEX – FUNCAMP) to FP, and fellowships to BLA (FAPESP grants nº 2021/10798-6 and 2023/01736-2), AVLF (CNPq productivity grant nº 304291/2020–0) and FP (CNPq productivity grant nº 302849/2021-1). This study was also financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. GS was also funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n. 3175 of 18 December 2021 of the Italian Ministry of University and Research funded by the European Union – NextGenerationEU. LL was funded in the frame of the Ph.D. scholarship in the field of sustainable development financially supported by INPS (National Institute of Social Insurance).

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FIGURES

Fig. 1. Inflorescences of *Epidendrum fulgens* of the three colour morphs: yellow, orange and red (A). Reflectance average (line) and standard deviation (line shade) spectra of each measured floral piece (from left to right: labellum, petal, lateral sepal and dorsal sepal) of the three morphs (represented by their respective colour) (B).

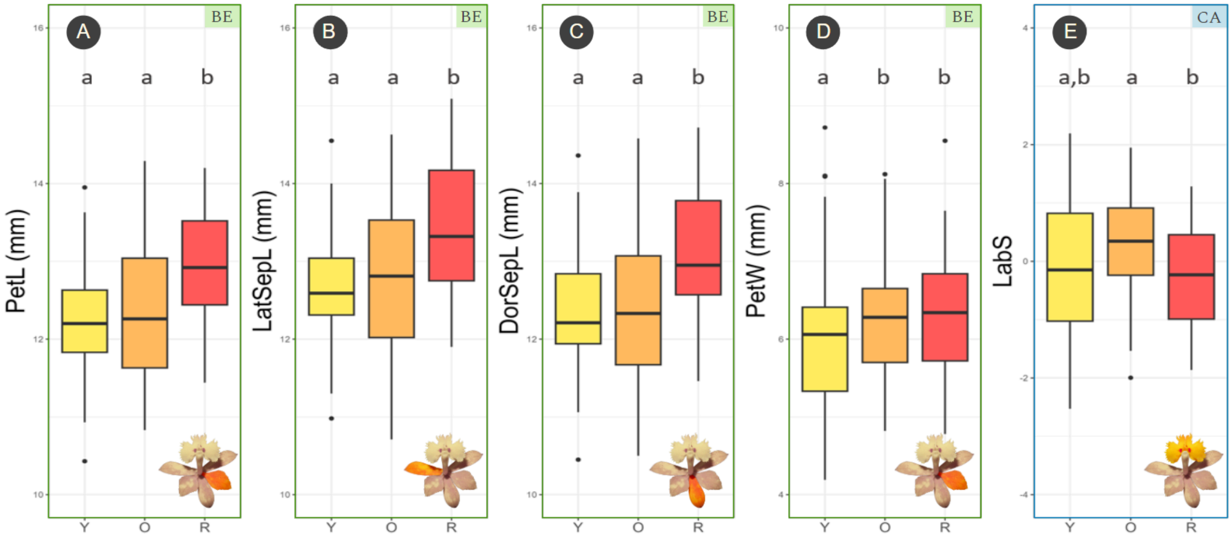

Fig. 2. Phenotypic traits showing significant differences among colour morphs of *Epidendrum fulgens* in Bertioga (A to D, green charts) and Cardoso (E, blue chart) populations: petal length (A), lateral sepal length (B), dorsal sepal length (C), petal width (D) and labellum shape (E). Different letters indicate significant differences ($p < 0.05$).

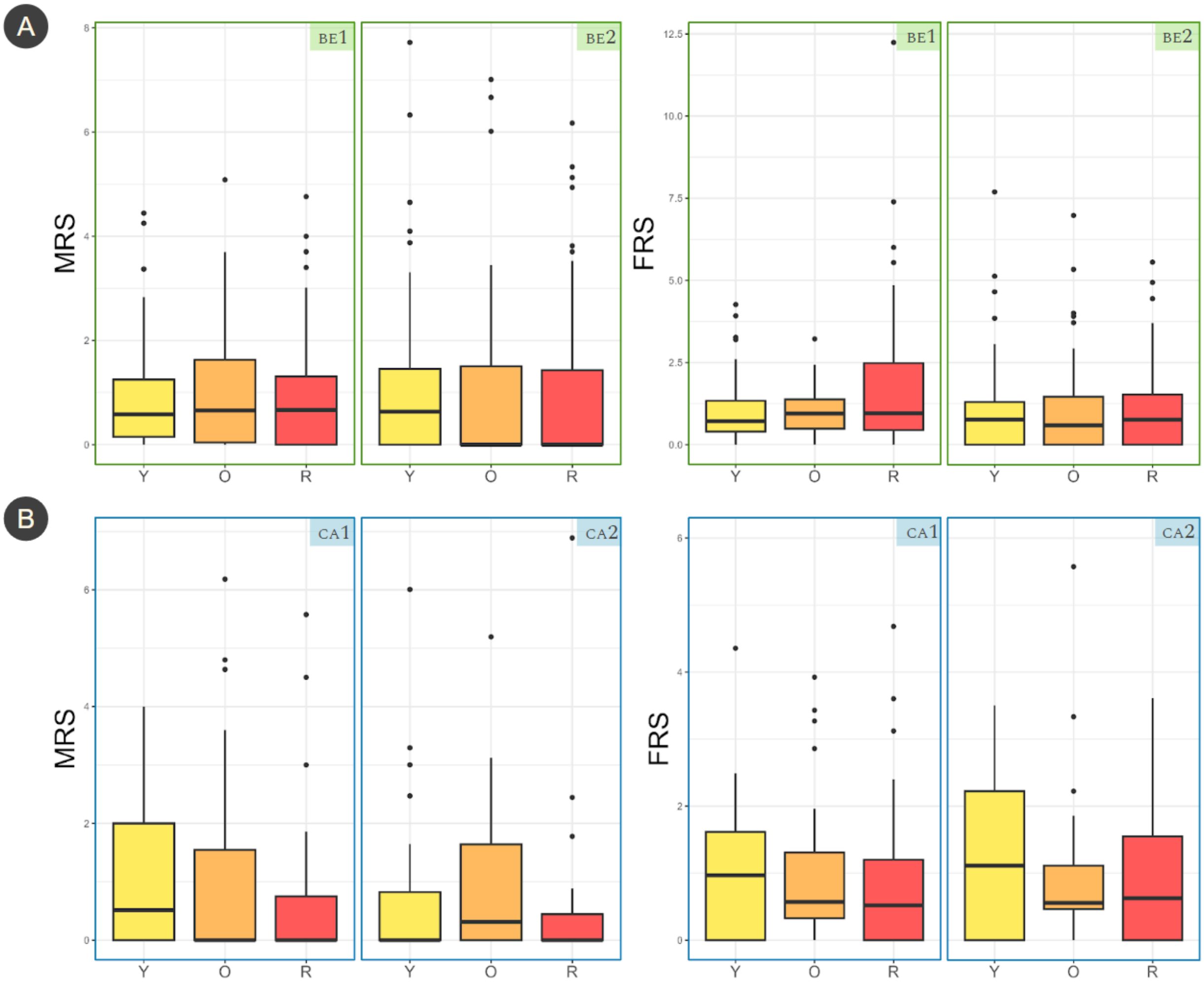

Fig. 3. Male reproductive success (MRS) and female reproductive success (FRS) of the three colour morphs (Y, O, R) in the two flowering seasons (1 and 2) on the two natural populations: Bertioga (A, green charts) and Ilha do Cardoso (B, blue charts). No significant differences ($p < 0.05$) were detected.

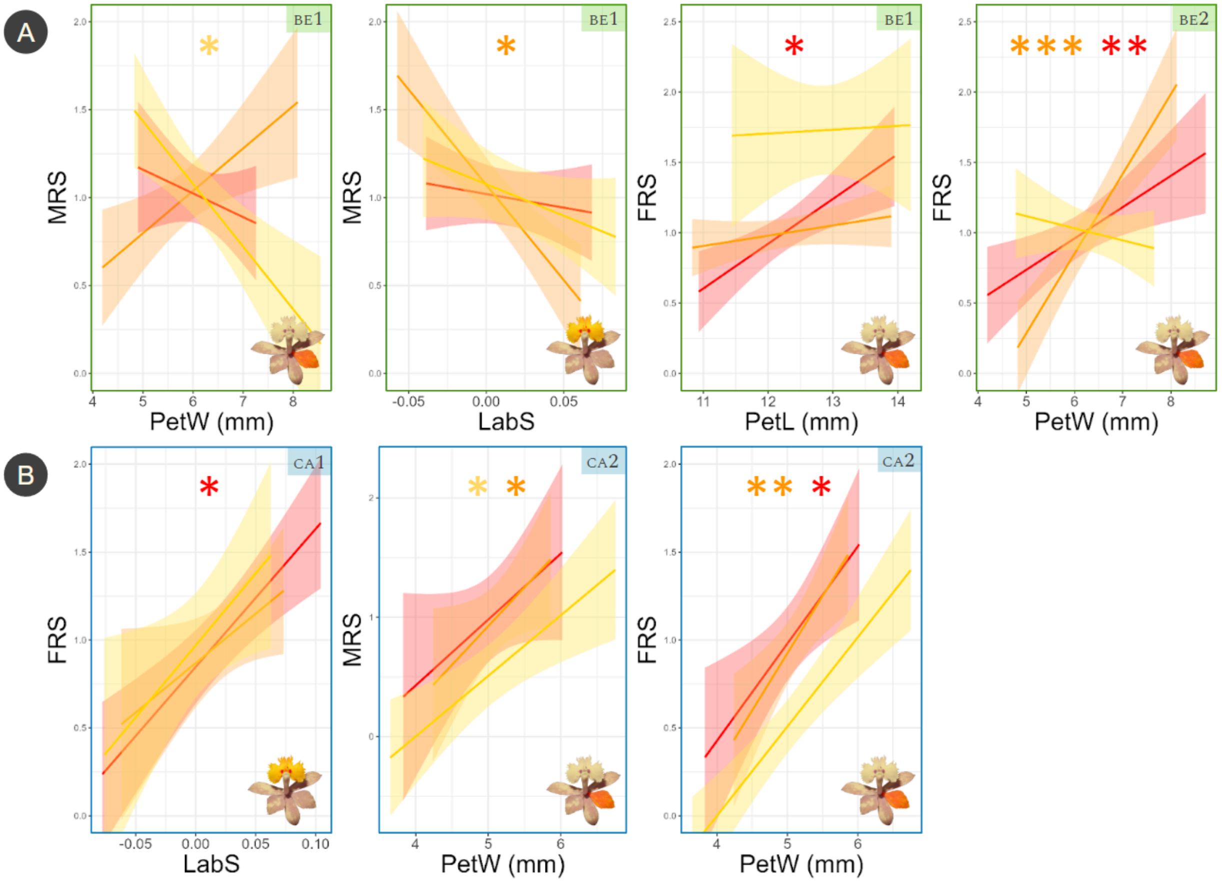

Fig. 4. Significant selection gradients, with the mean of the predicted values (line) and its 95% confidence interval (line shade), of the morphological traits on each colour morph (Y, O, R, represented by their respective colour): petal width (PetW), labellum shape (LabS) and petal length (PetL) on both populations, Bertioga (A, green charts) and Ilha do Cardoso (B, blue charts) on the two flowering seasons (1 and 2). Asterisks and their respective colours indicate that there is a significant difference in the selection gradient of the correspondant flower colour morph. '***' $P \leq 0.001$; '**' $0.001 < P \leq 0.01$, '*' $0.01 < P \leq 0.05$.

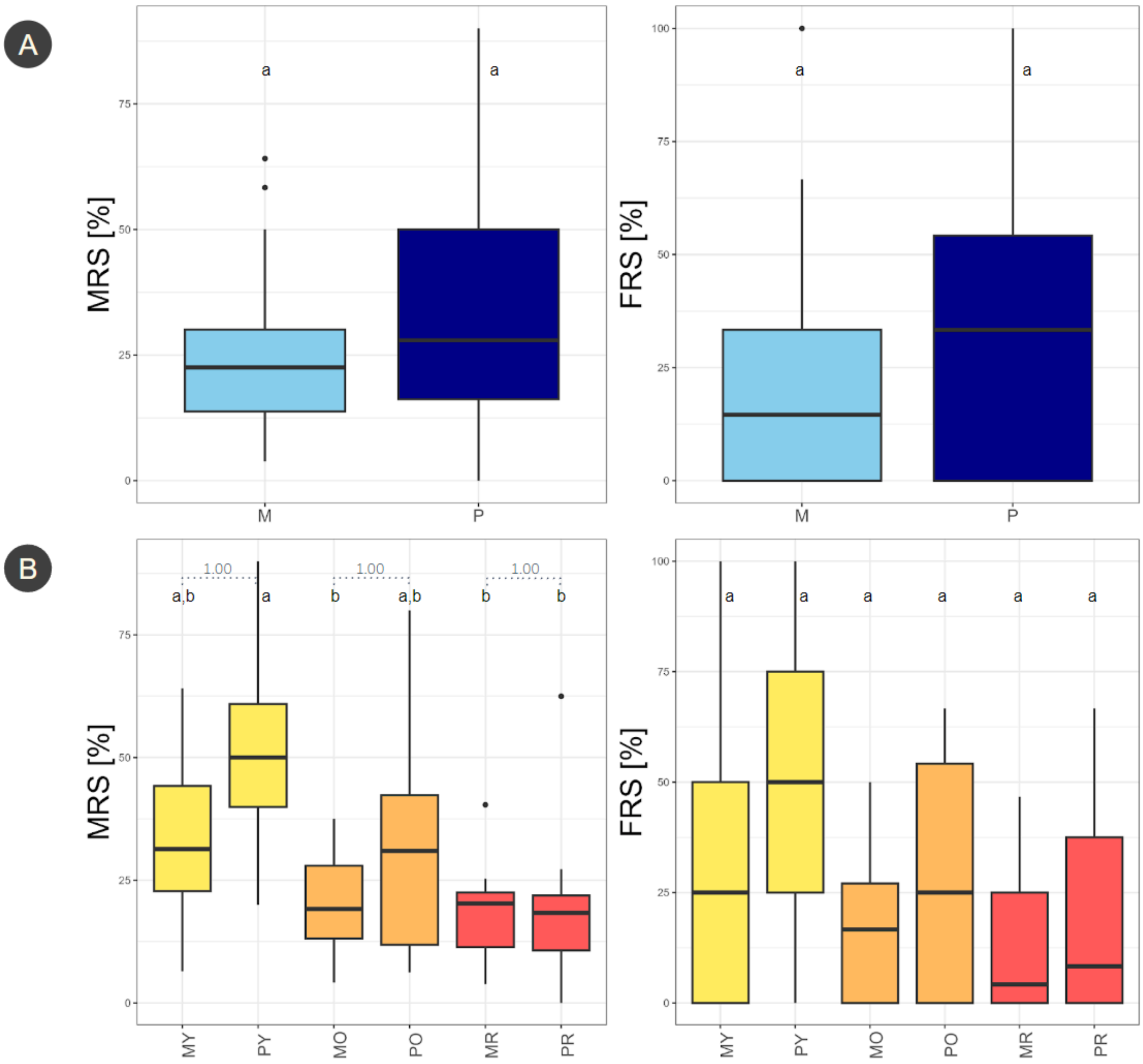

Fig. 5. Male reproductive success (MRS) and female reproductive success (FRS) of monomorphic and polymorphic plots (A); MRS and FRS of each monomorphic plot (MY, MO and MR) and each morph within the polymorphic plot (PY, PO and PR) (B). Different letters indicate significant differences ($p < 0.05$).

Fig. 6. Male reproductive success (MRS) and female reproductive success (FRS) of each monomorphic plot (MY, MO and MR) and each morph within the polymorphic plot (PY, PO and PR) in the three periods. Different letters indicate significant differences ($p < 0.05$).

SUPPORTING INFORMATION

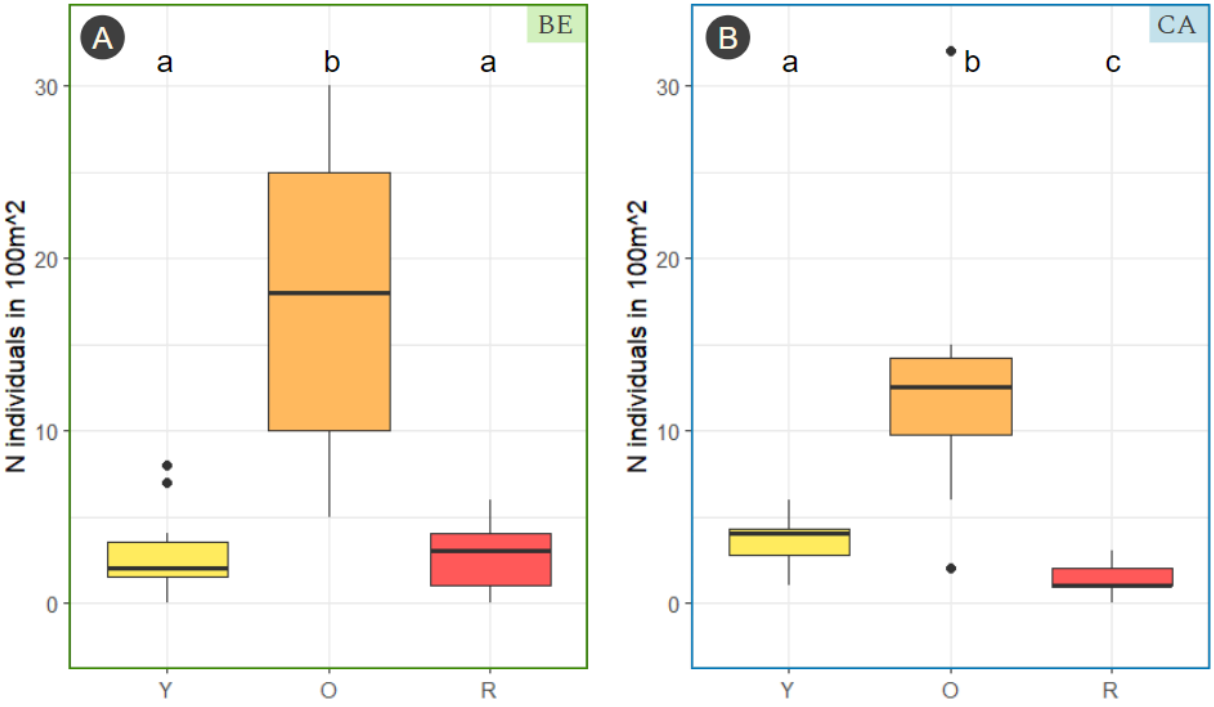

Fig. S1. Boxplots of the overall number of individuals of each flower colour morph (Y, O and R) found in an area of 100 m^2 on both natural populations: Bertioga (A, green chart) and Ilha do Cardoso (B, blue chart). Different letters indicate significant differences ($p < 0.05$).

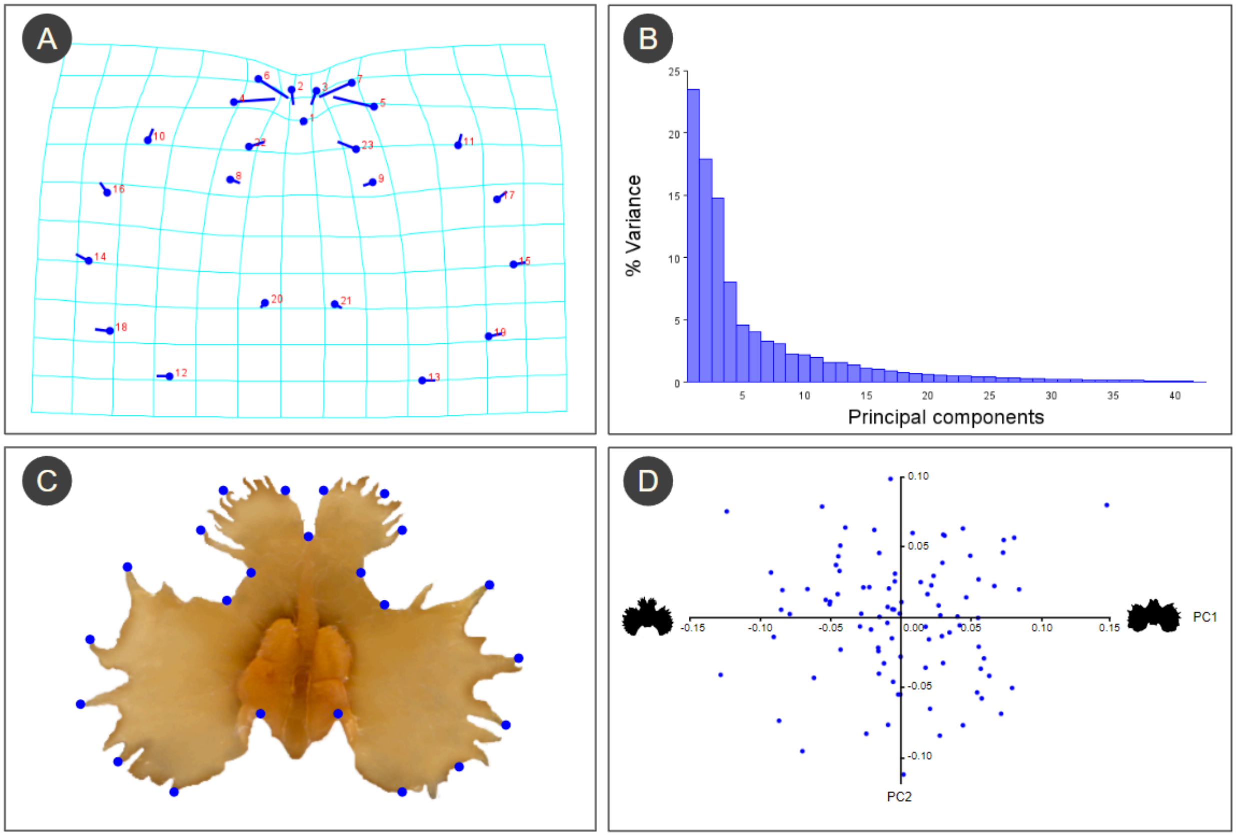

Fig. S2. A. PC1 eigenvectors associated with each landmark or semilandmark and their distortion. B. Percentage of variance of labellum shape that each principal component explains (from PC1 to PC42). C. Image of a labellum with positioned landmarks and semilandmarks. D. PCA plot of labellum shape of all flowers collected from both populations (BE and CA) (y axis is PC2 and x axis is PC1; by the sides of x axis there is a representative image of the shape variation on each direction of PC1, according to its eigenvectors).

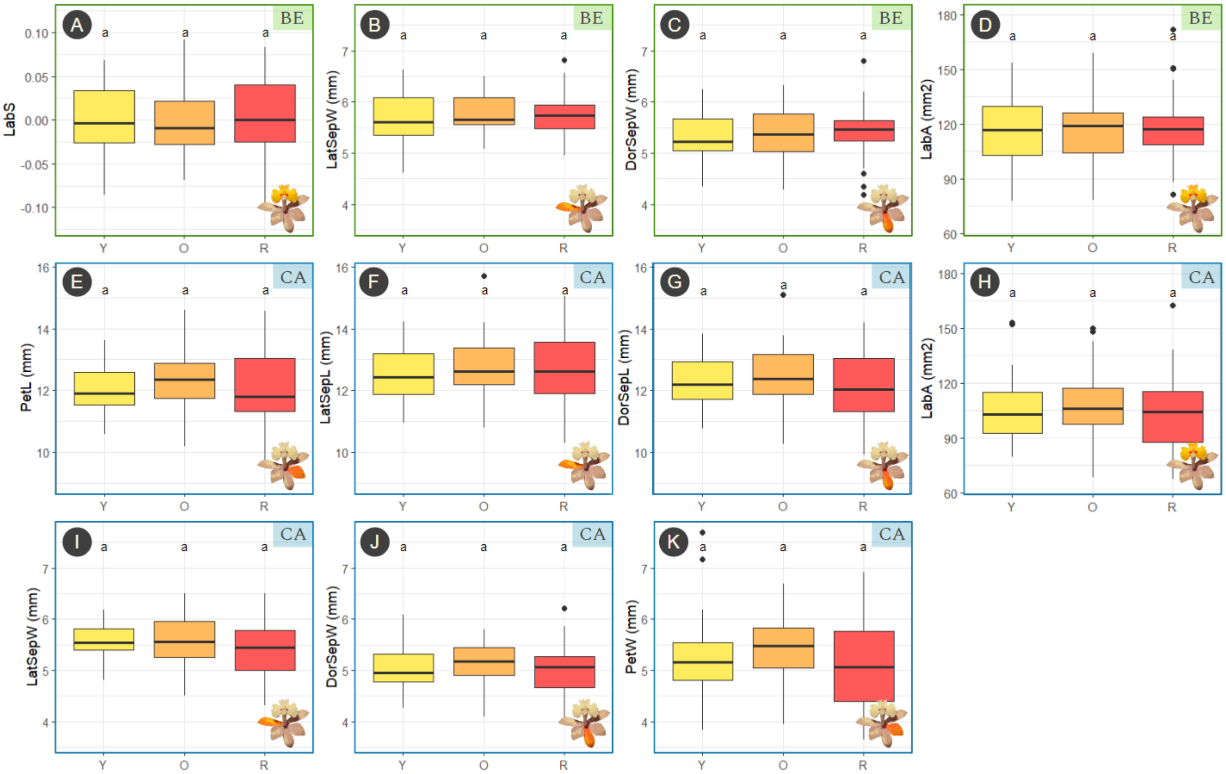

Fig. S3. Phenotypic traits with no significant differences among colour morphs of *E. fulgens* in Bertioga (A to D, green charts) and Ilha do Cardoso (E to K, blue charts) populations: labellum shape (A), lateral sepal width (B and I), dorsal sepal width (C and J), labellum area (D and H), petal length (E), lateral sepal length (F), dorsal sepal length (G) and petal width (K). Different letters indicate significant differences ($p < 0.05$).

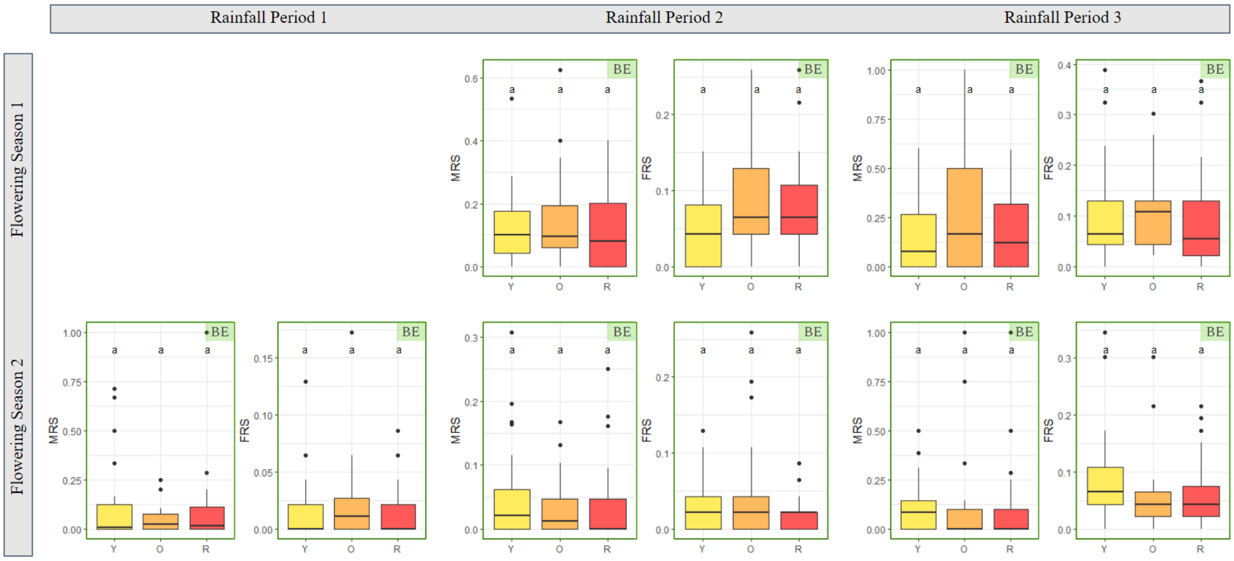

Fig. S4. Male reproductive success (MRS) and female reproductive success (FRS) of the three colour morphs (Y, O, R) in the BE population by dividing the flowering seasons in three rainfall periods.

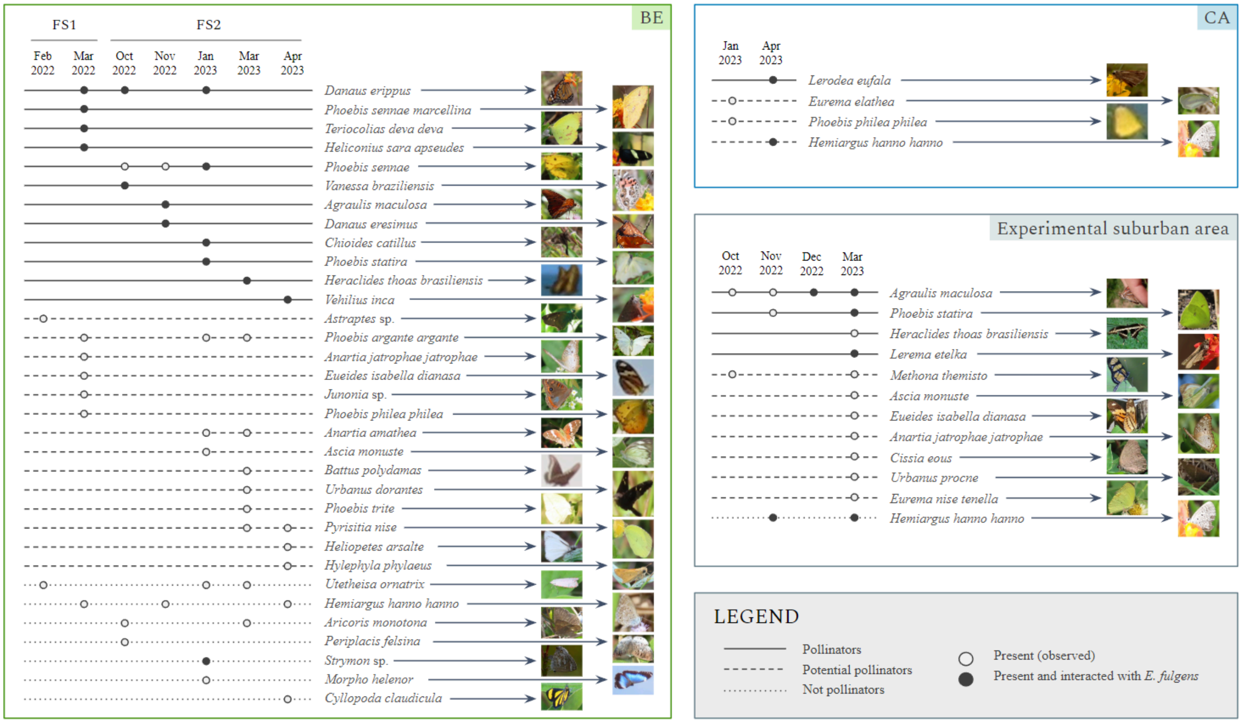

Fig. S5. Lepidoptera's community on the two natural populations, Bertioga (BE) and Ilha do Cardoso (CA), and in the experimental suburban area. Dots represent the presence of the respective butterfly species (empty dots for presence without interaction and filled dots for presence with interaction with *Epidendrum fulgens*), and line types represent their category: pollinators (full line), potential pollinators (dashed line), not pollinators (dotted line).

TABLES

Table 1. Selection gradients in the Bertioga population across the two flowering seasons. '***' $P \leq 0.001$; '**' $0.001 < P \leq 0.01$, '*' $0.01 < P \leq 0.05$.

		Yellow		Orange		Red	
		1st flowering season	2nd flowering season	1st flowering season	2nd flowering season	1st flowering season	2nd flowering season
MRS	Petal length (mm)	-0.019	0.006	0.014	-0.016	0.008	0.049
	Petal width (mm)	**-0.149***	0.036	0.153	0.108	-0.094	0.181
	PC1	-0.023	0.012	**-0.185***	0.002	-0.066	0.034
	Labellum area (mm^2)	-0.005	-0.005	-0.010	-0.005	0.006	-0.009
FRS	Petal length (mm)	0.099	0.064	0.064	0.023	**0.226***	0.015
	Petal width (mm)	-0.103	0.175	0.175	**0.348*****	-0.051	**0.270****
	PC1	0.024	0.127	0.127	0.091	0.064	0.070
	Labellum area (mm^2)	0.003	-0.006	-0.006	-0.010	0.003	-0.006

Table 2. Selection gradients in the Cardoso population across the two flowering seasons. '**' $0.001 < P \leq 0.01$, '*' $0.01 < P \leq 0.05$.

		Yellow		Orange		Red	
		1st flowering season	2nd flowering season	1st flowering season	2nd flowering season	1st flowering season	2nd flowering season
MRS	Petal length (mm)	-0.132	-0.092	-0.084	-0.104	-0.239	-0.151
	Petal width (mm)	0.435	**0.680***	0.429	**0.437***	0.517	0.542
	PC1	-0.005	-0.021	-0.091	-0.035	0.117	-0.005
	Labellum area (mm^2)	-0.021	-0.018	-0.020	-0.003	-0.007	-0.004
FRS	Petal length (mm)	-0.220	-0.128	-0.178	-0.084	-0.209	-0.097
	Petal width (mm)	0.234	0.379	0.243	**0.912****	0.109	**0.421***
	PC1	0.155	0.033	0.130	-0.023	**0.253***	-0.006
	Labellum area (mm^2)	-0.005	-0.005	-0.004	-0.017	0.009	-0.008

Table 3. Selection gradients in the Bertioga population across the three periods of each of the two flowering seasons. ‘**’ $0.001 < P \leq 0.01$, ‘*’ $0.01 < P \leq 0.05$.

	1st Flowering season					2nd Flowering season			
			Yellow					**Yellow**	
		Period 1	**Period 2**	**Period 3**			**Period 1**	**Period 2**	**Period 3**
MRS	Petal length (mm)	-0.284	-0.795	0.282	MRS	Petal length (mm)	-0.411	-0.306	0.187
	Petal width (mm)	-0.110	-0.600	-0.455		Petal width (mm)	-0.120	-0.107	-0.312
	PC1	-0.245	0.480	-0.358		PC1	-0.286	0.249	0.562
	Labellum area (mm^2)	0.000	-0.031	0.009		Labellum area (mm^2)	-0.002	-0.019	0.006
FRS	Petal length (mm)	0.179	-0.091	0.073	FRS	Petal length (mm)	-0.156	-0.173	0.031
	Petal width (mm)	-0.320	-0.260	-0.074		Petal width (mm)	-0.252	0.220	-0.34
	PC1	0.145	-0.192	0.145		PC1	0.136	-0.047	0.243
	Labellum area (mm^2)	0.018	0.013	-0.008		Labellum area (mm^2)	0.004	-0.002	-0.006
			Orange					**Orange**	
		Period 1	**Period 2**	**Period 3**			**Period 1**	**Period 2**	**Period 3**
MRS	Petal length (mm)	-0.530	-0.025	-0.351	MRS	Petal length (mm)	0.919	-0.341	-0.346
	Petal width (mm)	-0.481	-0.221	0.065		Petal width (mm)	**0.738***	**0.942****	0.171
	PC1	-0.502	-0.395	-0.356		PC1	0.374	-0.060	-0.315
	Labellum area (mm^2)	0.021	0.043	0.012		Labellum area (mm^2)	-0.038	**-0.066***	-0.079
FRS	Petal length (mm)	0.039	0.345	-0.160	FRS	Petal length (mm)	-0.065	-0.364	0.059
	Petal width (mm)	0.684	-0.042	0.243		Petal width (mm)	**0.981****	**0.920****	**0.455***
	PC1	0.217	-0.039	**-0.345***		PC1	-0.362	-0.225	-0.052
	Labellum area (mm^2)	-0.026	-0.011	-0.003		Labellum area (mm^2)	-0.012	**-0.069***	-0.009
			Red					**Red**	
		Period 1	**Period 2**	**Period 3**			**Period 1**	**Period 2**	**Period 3**
MRS	Petal length (mm)	0.318	-0.209	0.492	MRS	Petal length (mm)	0.672	-0.063	-0.261
	Petal width (mm)	0.324	-0.508	**0.442***		Petal width (mm)	0.556	-0.104	0.063
	PC1	-0.171	-0.349	0.000		PC1	-0.081	0.025	0.290
	Labellum area (mm^2)	0.002	-0.015	0.023		Labellum area (mm^2)	0.025	0.000	0.000
FRS	Petal length (mm)	**0.982****	0.306	-0.032	FRS	Petal length (mm)	0.845	0.089	0.011
	Petal width (mm)	0.601	0.200	-0.223		Petal width (mm)	**0.932****	0.041	0.145
	PC1	0.23	-0.093	**-0.370***		PC1	0.202	0.165	0.240
	Labellum area (mm^2)	0.019	0.002	0.015		Labellum area (mm2)	0.018	0.003	0.006

4. CONSIDERAÇÕES FINAIS

Polimorfismos florais são um fenômeno comum na natureza e sua manutenção tem ganhado importância dentro da ecologia evolutiva (Sapir et al. 2021). Aqui, ao pesquisar populações naturais e ao conduzir um experimento manipulativo com *E. fulgens*, demonstramos que as preferências dos polinizadores por diferentes morfotipos florais mudam ao longo do espaço e tempo, e que a seleção em atributos florais envolvida na atração do polinizador é fraca, mas segue trajetórias diferentes em diferentes morfotipos de coloração floral. Juntos, esses resultados sugerem que os polinizadores são capazes de detectar diferentes morfologias de coloração floral e que essa capacidade tem consequências no desempenho reprodutivo das plantas e na evolução do polimorfismo floral.

Em nosso estudo, confirmamos a natureza discreta do polimorfismo de coloração floral de *E. fulgens*, descobrimos que o sucesso reprodutivo de *E. fulgens* é severamente limitado e confirmamos que sua estratégia de polinização de fato é altamente generalista. Encontramos evidências de uma direção variável da seleção natural, com variação entre anos, entre populações e atuando sobre diferentes atributos florais. Apesar dessa variação, nossos resultados sugerem coerência na diferenciação morfológica em direções opostas entre morfotipos de coloração floral. Esses resultados sugerem a presença de um conjunto de polinizadores parcialmente diferente para as diferentes morfologias florais e com consistência de escolhas a longo prazo, causando acasalamento preferencial negativo. Além disso, nosso experimento controlado mostrou que, ao contrário de nossa previsão, a presença de diferentes morfotipos de coloração floral não tem efeito no sucesso reprodutivo da espécie, desafiando o papel do polimorfismo na diminuição do aprendizado de polinizadores.

Tomados em conjunto, nossos resultados sugerem que o polimorfismo de coloração floral é mantido por uma combinação de fatores, incluindo seleção fraca, mas variável, mediada por polinizadores, acasalamento preferencial negativo devido a preferências diferenciais de polinizadores e a provável maior herdabilidade do fenótipo de coloração intermediário. Considerando os nossos resultados, este trabalho contribuiu para uma compreensão mais abrangente das pressões seletivas que moldam e mantêm a variação floral em regiões hiperdiversas como os Neotrópicos e em plantas altamente polimórficas como a família Orchidaceae.

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6. APÊNDICE I: “DECLARAÇÃO DE DIREITOS AUTORAIS”

Declaro para os devidos fins legais que as cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Dissertação de Mestrado, intitulada “As consequências do polimorfismo de coloração floral no sucesso reprodutivo de uma orquídea Neotropical polinizada por engodo”, não infringem os dispositivos da Lei n.º 9.610/98, nem o direito autoral de qualquer editora.

Campinas, 02 de março de 2025

Beatriz Lucas Arida
Autora da dissertação
Aluna de Mestrado do PPG-BV/UNICAMP

Fabio Pinheiro
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7. APÊNDICE II: “DECLARAÇÃO DE BIOÉTICA E BIOSSEGURANÇA”

Em observância ao §5º do Artigo 1º da Informação CCPG – UNICAMP/001/15, referente a Bioética e Biossegurança, declaro que o conteúdo de minha Dissertação de Mestrado, intitulada “As consequências do polimorfismo de coloração floral no sucesso reprodutivo de uma orquídea Neotropical polinizada por engodo”, desenvolvida no Programa de Pós-Graduação em Biologia Vegetal do Instituto de Biologia da Universidade Estadual de Campinas, não versa sobre pesquisa envolvendo seres humanos, animais ou temas afetos à Biossegurança.

Campinas, 02 de março de 2025

Beatriz Lucas Arida	Fabio Pinheiro
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