UNIVERSIDADE FEDERAL DE UBERLÂNDIA
INSTITUTO DE BIOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA, CONSERVAÇÃO E BIODIVERSIDADE

THE EFFECT OF GROOMING ON PLANT REPRODUCTION: A THEORETICAL APPROACH

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UBERLÂNDIA, MG

AGOSTO DE 2023

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Tese apresentada ao Programa de Pós-graduação em Ecologia, Conservação e Biodiversidade da Universidade Federal de Uberlândia, como requisito final para obtenção do título de Doutora em Ecologia, Conservação e Biodiversidade.

UBERLÂNDIA, MG

AGOSTO DE 2023

Dados Internacionais de Catalogação na Publicação (CIP)
Sistema de Bibliotecas da UFU, MG, Brasil.

M314e
2023

Marcelo, Vanessa Gonzaga, 1994-
The effect of grooming on plant reproduction [recurso eletrônico] :a theoretical approach / Vanessa Gonzaga Marcelo. - 2023.

Orientador: Vinícius Lourenço Garcia de Brito.
Coorientadora: Flávia Maria Darcie Marquitti.
Tese (Doutorado) - Universidade Federal de Uberlândia, Programa de Pós-Graduação em Ecologia, Conservação e Biodiversidade.
Modo de acesso: Internet.
Disponível em: http://doi.org/10.14393/ufu.te.2023.8049
Inclui bibliografia.
Inclui ilustrações.

1. Ecologia. I. Brito, Vinícius Lourenço Garcia de, 1985-, (Orient.). II. Marquitti, Flávia Maria Darcie, 1985-, (Coorient.). III. Universidade Federal de Uberlândia. Programa de Pós-Graduação em Ecologia, Conservação e Biodiversidade. IV. Título.

CDU: 574

André Carlos Francisco
Bibliotecário - CRB-6/3408

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Coordenação do Programa de Pós-Graduação em Ecologia, Conservação e Biodiversidade
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ATA DE DEFESA - PÓS-GRADUAÇÃO

Programa de Pós-Graduação em:	Ecologia, Conservação e Biodiversidade				
Defesa de:	Tese, número 88, PPGECB				
Data:	trinta de agosto de dois mil e vinte e três	Hora de início:	14:05	Hora de encerramento:	18:30
Matrícula do Discente:	11913ECR006				
Nome do Discente:	Vanessa Gonzaga Marcelo				
Título do Trabalho:	THE EFFECT OF GROOMING ON PLANT REPRODUCTION: A THEORETICAL APPROACH				
Área de concentração:	Ecologia				
Linha de pesquisa:	Ecologia de comunidades e biodiversidade				
Projeto de Pesquisa de vinculação:	Alternativas reprodutivas e diversidade funcional de plantas no Cerrado				

Reuniu-se por videoconferência a Banca Examinadora designada pelo Colegiado do Programa de Pós-graduação em Ecologia, Conservação e Biodiversidade assim composta pelos doutores: Nathália Susin Streher - University of Pittsburgh; Camila Silveira de Souza - Unimontes; Pamela Cristina Santana - Lund University; Raphael Matias da Silva - UFJ e Vinicius Lourenço Garcia de Brito - INBIO/UFU, orientador(a) do(a) candidato(a).

Iniciando os trabalhos o(a) presidente da mesa, Dr(a). Vinicius Lourenço Garcia de Brito, apresentou a Comissão Examinadora e o(a) candidato(a), agradeceu a presença do público e concedeu ao(à) Discente a palavra para a exposição do seu trabalho. A duração da apresentação do(a) Discente e o tempo de arguição e resposta foram conforme as normas do Programa.

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Documento assinado eletronicamente por **Raphael Matias da Silva**, **Usuário Externo**, em 04/09/2023, às 09:16, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

Documento assinado eletronicamente por **Camila Silveira de Souza**, **Usuário Externo**, em 04/09/2023, às 09:18, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

Documento assinado eletronicamente por **Nathália Susin Streher**, **Usuário Externo**, em 04/09/2023, às 09:58, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

Documento assinado eletronicamente por **Pamela Cristina Santana**, **Usuário Externo**, em 07/09/2023, às 04:06, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

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Referência: Processo nº 23117.057539/2023-23 SEI nº 4723108

Dedico essa tese a todos os pesquisadores brasileiros que persistem em fazer ciência no nosso país!

AGRADECIMENTOS

Eu acredito que nós não conseguimos realizar nada sozinhos. Dessa forma, agradeço a todos que me ajudaram de alguma forma a chegar até aqui e realizar meu sonho de ser doutora. Em especial, agradeço:

A Deus, por abençoar meu caminho, guiar meus pensamentos e decisões e por me fortalecer nessa caminhada da vida!

Ao meu orientador Vinícius Brito, por ter aceitado trabalhar em colaboração comigo. Agradeço a confiança, acreditando mais em mim do que eu mesma em inúmeras vezes. Obrigada por ser tão empático, compreensivo e por todas as oportunidades que me ofereceu ao longo do meu doutorado. Hoje, uma etapa deste desafio que foi meu projeto de doutorado está sendo vencida e não seria possível sem o nosso trabalho em equipe. Gratidão pela parceria!

A Flávia Marquitti, minha coorientadora, que sempre foi uma inspiração como pesquisadora e que foi essencial para o desenvolvimento do meu projeto. Nada seria possível sem sua expertise e inteligência!

Ao nosso colaborador, Mario Vallejo-Marín, por todas as ideias e discussões que tivemos no desenvolvimento do modelo. Gratidão por sempre estar disposto e empolgado para trabalharmos juntos. Agradeço ainda pelas suas vindas sempre produtivas ao Brasil e por nos receber no Vallejo-Marin Lab.

Ao meu esposo Eloidis Mendonça, meu amor e companheiro de vida, obrigada por compartilhar sua força comigo e por ser um exemplo de ser humano. Obrigada por ser minha motivação diária e por apoiar o meu crescimento e escolhas. Agradeço também a sua/nossa família e aos nossos amigos por comemorarem conosco as nossas conquistas!

A minha mãe Ângela Gonzaga e ao meu pai Joaquim Marcelo, pelo exemplo de esforço e dedicação. Agradeço por sempre me apoiarem e por tudo que fizeram por mim até hoje. Estendo aqui o agradecimento a toda minha família, em especial, as minhas irmãs (Dayane e Patrícia), meus tios, meus avós e meus afilhados. Vocês são fonte de inspiração e meu orgulho!

A minhas amigas Jessyca Santana, Lilian Mello e Monize Altomare, meus exemplos de mulheres e pesquisadoras. Obrigada por serem tão presentes em minha vida, mesmo na distância. Gratidão por todas as mensagens de conforto e apoio e por fazerem o meu doutorado muito mais leve!

As minhas amigas e colegas de quarto Paola Raupp e Jaqueline Vaz, obrigada por todo apoio quando mudamos para Uberlândia e nas aulas de estatística. Gratidão pelos surtos coletivos e festas apocalípticas na nossa casinha.

A todos os membros do NERP por estarem sempre prontos para ajudar. A todos, agradeço o convívio diário na salinha do NERP, e pelas reuniões semanais de muito conhecimento e que muitas vezes foi uma sessão terapêutica. Obrigada por compartilhar momentos e me auxiliar de alguma forma.

Aos membros do Vallejo-Marín Lab pelas válidas contribuições e discussões sobre o primeiro capítulo da minha tese.

Aos colegas da biologia da polinização, Pedro Bérgamo e Pamela Santana, pelas discussões que tivemos no início do doutorado que ajudaram muito a desenvolver novas ideias desta tese.

Aos membros da banca da qualificação e aos da defesa, por todas as contribuições no trabalho e pela disponibilidade em participar dessas etapas tão importante na minha vida acadêmica.

À Rede de Serviços Ambientais (RESA) e a Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

À Universidade Federal de Uberlândia e ao Programa de Pós-Graduação em Ecologia, Conservação e Biodiversidade.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), pela concessão da bolsa doutorado.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

A todos minha imensa gratidão!

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ABSTRACT

Plants that offer pollen as a resource face a dilemma, as the same pollen serves both as the carrier of male gametes and as a resource exploited by floral visitors. Some visitors, like bees, have structures specialized in pollen collection and are capable of grooming. Pollinator grooming behavior has been referred to as one of the biggest barriers to male success, affecting pollen fates. Another factor that can affect the fate of pollen is variation in the position and/or morphology of reproductive organs, as it can affect pollen placement by anthers on the pollinator body and pollen deposition on the stigma. To date, only a few theoretical investigations have attempted to model the effect of grooming on pollen transfer, and empirical studies have been limited to species in which pollen movement can be tracked. Thus, due to challenges in tracking individual pollen grains, we do not know the effect of grooming and variations in reproductive organs on pollen transfer and mating between individuals of a flower population. We use a spatially explicit agent-based modeling (ABMs) approach to determine the effect of pollen redistribution and removal in the "pollen landscape" on the vector body, affecting plant reproductive success and mating between floral individuals, associated with variation in reproductive organs. For this, we simulate visits of pollen vectors to a sequence of different flowers in a population. In our first chapter, we showed that pollen redistribution and removal restructure the pollen landscape in the body of the bee, in a way that increases the pollen delivery in consecutive visits and the number of pollen donors deposited on the stigmas. Therefore, unexpectedly, grooming can have a positive effect on male and female reproductive success during plant reproduction. In the second chapter, the mating networks were affected both by the grooming behavior and by the morphological variation of the reproductive organs, with the grooming making populations less connected and more specialized. The absence of grooming, together with the variation in the positioning of the stigma, makes populations more connected and generalist. The

grooming behavior breaks the dominance of some individuals observed in the absence of grooming, making the relationships between individuals better distributed. Floral individuals visited by vectors without grooming and that have variation in stigma position are reproductively more generalists. Our model predicts that species pollinated by non-grooming vectors are more variable in stigma position than species pollinated by grooming vector. However, empirical studies will be necessary to prove the hypotheses we generated with our model, allowing greater knowledge of the effects suffered by the male component that can affect pollen transfer.

Keywords: floral traits; morphological variation; network metrics; pollen carryover; pollen delivery; pollen landscape; pollen layering; pollen movement; pollen placement; pollen transfer; spatially explicit agent-based model.

RESUMO

Plantas que oferecem pólen como recurso enfrentam um dilema, pois o mesmo pólen serve tanto como portador de gametas masculinos quanto como recurso explorado por visitantes florais. Alguns visitantes, como as abelhas, possuem estruturas especializadas na coleta de pólen e são capazes de realizar o *grooming*. O comportamento de *grooming* dos polinizadores tem sido referido como uma das maiores barreiras para o sucesso masculino, afetando o destino do pólen. Outro fator que pode afetar o destino do pólen é a variação na posição e/ou morfologia dos órgãos reprodutivos, pois pode afetar a colocação do pólen pelas anteras no corpo do polinizador e a deposição do pólen no estigma. Até o momento, apenas algumas investigações teóricas tentaram modelar o efeito do *grooming* na transferência de pólen, e os estudos empíricos foram limitados a espécies nas quais o movimento do pólen pode ser rastreado. Assim, devido aos desafios no rastreamento de grãos de pólen individuais, não sabemos o efeito do *grooming* e das variações nos órgãos reprodutivos na transferência de pólen e no acasalamento entre indivíduos de uma população de flores. Usamos uma abordagem de modelo baseado em agente espacialmente explícito (ABMs) para determinar o efeito da redistribuição e remoção do pólen na "paisagem do pólen" no corpo do vetor, afetando o sucesso reprodutivo da planta e o acasalamento entre indivíduos florais, associado à variação nos órgãos reprodutivos. Para isso, simulamos visitas de vetores de pólen a uma sequência de diferentes flores em uma população. Em nosso primeiro capítulo, mostramos que a redistribuição e a remoção do pólen reestruturam a paisagem polínica no corpo da abelha, de forma a aumentar a entrega de pólen em visitas consecutivas e o número de doadores de pólen depositados nos estigmas. Portanto, inesperadamente, o *grooming* pode ter um efeito positivo no sucesso reprodutivo masculino e feminino durante a reprodução da planta. No segundo capítulo, as redes de acasalamento foram afetadas tanto pelo comportamento de *grooming* quanto pela variação morfológica dos órgãos reprodutivos, com

o *grooming* tornando as populações menos conectadas e mais especializadas. A ausência de *grooming*, aliada à variação no posicionamento do estigma, torna as populações mais conectadas e generalistas. O comportamento de *grooming* quebra a dominância de alguns indivíduos observada na ausência de *grooming*, tornando as relações entre os indivíduos mais bem distribuídas. Indivíduos florais visitados por vetores sem *grooming* e que apresentam variação na posição do estigma são reprodutivamente mais generalistas. Nosso modelo prevê que as espécies polinizadas por vetores que não fazem *grooming* são mais variáveis na posição do estigma do que as espécies polinizadas por vetores que fazem *grooming*. No entanto, estudos empíricos serão necessários para comprovar as hipóteses que geramos com nosso modelo, permitindo maior conhecimento dos efeitos sofridos pelo componente masculino que podem afetar a transferência de pólen.

Palavras-chave: características florais; variação morfológica; métricas de rede; transporte de pólen; entrega de pólen; paisagem de pólen; camadas de pólen; movimento do pólen; colocação de pólen; transferência de pólen; modelo baseado em agente espacialmente explícito.

GENERAL INTRODUCTION

The term sexual selection, a process firstly proposed by Darwin (1871), may occur when there is competition among individuals of one sex for mates, and some advantageous traits of one individual is chosen by the other sex, resulting in differential reproductive success. Darwin proposed two mechanisms for the evolution of such traits: intra-sexual competition, which generally involves males competing to secure a female mate, and inter-sexual choice, whereby often females selectively choose males with specific traits. This concept was initially applied only to animals, particularly those with strong secondary sexual dimorphism (reviews in Andersson, 1994; Wiens and Tuschhoff, 2020).

Bateman (1948) was likely the first to suggest the occurrence of sexual selection in plants (Toledo et al., 2020). Since most flowering plants are hermaphrodites (Bawa, 1980), it provides opportunities to enhance mating success through one or both sexual functions (Campbell, 1989; Moore and Pannell, 2011). The Bateman's principle provided insights into the degree to which sexual selection could impact both functions of hermaphrodites. The reproductive success of males is often limited by their ability to secure access to receptive females and successfully compete with other males for mating opportunities. Although female reproductive success is limited primarily by its ability to allocate the necessary resources to produce offspring (Bateman, 1948), female function can also be limited by the availability of partners to fertilize ovules (see Burd, 1994). Given that many pollen grains often compete for a limited number of ovules (Stephenson and Bertin, 1983), consequently the sexual selection should primarily operate on the male function rather than the female function (Bateman, 1948).

In the context of pollination, distinguishing between male-male competition and female choice can be challenging (Stanton, 1994). However, scientists typically use the term "male-male competition" to refer to the competition for access to female gametes, while

"female choice" is based on the female's ability to select and secure male gametes quality after pollination (Eberhard, 1996). Previous studies have shown the effects of pollen traits such as pollen size (McCallum and Chang, 2016) and pollen-tube growth rate (Harder et al., 2016) that increase male competition and mating success in the post-pollination stage. Although we have evidence of the impact of reproductive traits on post-pollination success, how male reproductive traits influence pollination success remains much less studied.

Male success can be influenced by events that occur prior to stigma arrival (Minnaar et al., 2019), even before pollen transfer. In plants, male function may possess differential advantages between their rivals in terms of increases in pollen production (Stanton et al., 1991), presence of mechanisms associated with pollen presentation (Harder and Thomson, 1989; Harder and Wilson, 1994) and presence of the floral traits that increases the attraction of the pollinator (Thomson, 1986; Conner and Rush, 1996; de Jager et al., 2016). However, these advantages are only impactful if they are accompanied by an amplified rate of visits, a greater deposition of pollen on the visitor's body compared to their rivals or without increasing of geitonogamy (Minnaar et al., 2019).

Most of flowering plants rely on vectors to transfer pollen (Tong et al., 2023), and different vectors present different foraging behaviors that affect pollen fates (Wessinger, 2021). Also, the simultaneous presence of co- and heterospecific flowers can introduce more variability in the fate of pollen grains (Barrett, 2003). In fact, most pollen grains are lost during their pathway to stigmas (Harder and Thomson, 1989). Among other factors that influence pollen transfer and consequently male success, we can mention the mechanical fit between flowers and pollen vectors, the placement and capture of pollen grains, transfer of geitonogamous pollen, grooming and passive pollen loss as well as interspecific interactions (Minnaar et al., 2019). In this thesis, we will focus on the effects pollen vector behavior on male reproductive success.

One previous study proposed a theoretical framework that explores the concept of the pollen landscape, which is a pollen landscape formed horizontally and vertically on the vector body (3-dimensional), generated from sequential pollen placement by anthers, sequential capture by stigmas, and grooming behavior by the pollen vector (Harder and Wilson, 1998). The horizontally structured landscape is modified by the positioning of pollen by anthers, subsequent capture by the stigmas and the grooming behavior (Harder and Wilson, 1998). In contrast, the vertically structured landscape is influenced by the sequential placement and capture of pollen by different individuals, leading to the formation of distinct layers of pollen (Lertzman, 1981; Harder and Wilson, 1998, Morris et al., 1995). The vertically and horizontally structured landscapes also suffer different effects according to the variations of the female and male floral traits (Harder and Wilson, 1998). This study showed that males determine the horizontal structure by redistributing pollen in safe or exposed places, whereas females would have little influence on this structure. In the vertical structure, males govern the rate of buried pollen and females determine the rate of exposure of buried pollen. More recently, a study provided the first empirical evidence of pollen layering, showing that the pollen from the initial flower visited can hinder the potential placement of pollen from subsequent individuals, indicating pollen preclusion (Moir and Anderson, 2023). Regarding the effect of grooming, in addition to theoretical studies (Harder and Wilson, 1998), studies have observed pollen dispersion comparing grooming or non-grooming pollinators, relating grooming to a decrease in pollen dispersal or siring success (Castellano et al., 2003; Holmquist et al., 2012; Pearson et al., 2023). But, in general, the removal or redistribution of pollen by the vector (grooming) has always been treated as the major factor leading to pollen loss (Thomson, 1986).

To assess how the structure of pollen layers on the vector's pollen landscape, the variation in floral traits and the vector´s grooming behavior affect plant reproductive success,

it is necessary to track individual pollen grains. Floral traits or other factors influencing female reproductive success can be easily identified, as it is measured by the number of pollen deposited on the stigma, fertilized ovules and seed production. On the other hand, male success is measured by the number of pollen removed, distance from the pollen movement and pollen success in conspecific stigmas (Young, 2002). However, these measures of male success do not reflect overall success since few pollen that leave the anther manage to reach the stigma, and those that arrive are only a proportion of the number that were produced (Harder and Thomson, 1989; Gong and Huang, 2014). The difficulty of measuring male success compared to female success generated a huge disparity of studies in the literature (Pearson et al., 2023). Improving the tracking of pollen grains in studies has been a challenge due to the limitations in differentiating pollen between individuals in most species. Some studies have utilized species with distinct pollen colors or sizes (Thomson, 1986; Harder and Thomson, 1989; Luo et al., 2008; Wang et al., 2018), or those that naturally form pollen groups (pollinaria) (Harder et al., 2021) to distinguish and track pollen grains and assess their dispersal. In cases where distinguishing individual pollen grains is not possible, researchers have used fluorescent powders as an analogue for pollen (Price and Waser, 1982; Waser and Price, 1984). Although this method can provide insights into pollen movement, it is important to note that fluorescent powders may not fully replicate the natural behavior and transportation of pollen, because they can be more sensitive and may not accurately show the interactions between pollen and pollinators (Adler and Irwin, 2006).

In recent years, the use of quantum dots has emerged as a promising technique for pollen tracking (Minnaar and Anderson, 2019; Moir and Anderson, 2023; Kern et al., 2023). Quantum dots are nanocrystals with distinct optical properties that emit different colors based on their size (Minnaar and Anderson, 2019). However, the use of quantum dots in pollen tracking is still limited, both in terms of their application techniques and the number of

available colors (Opedal et al., 2023). Alternatively, paternity tests can be employed to assess reproductive success. However, conducting these tests can be costly and time-consuming, especially when dealing with many potential fathers (Van Rossum et al., 2011).

Combined with the technical difficulties in tracking pollen movement, manipulating pollen vectors to simulate visits and grooming behavior experimentally would not accurately show the natural patterns of pollen movement. Therefore, the utilization of theoretical models to track pollen grains from individuals can provide valuable insights into pollen transfer dynamics and enhance our understanding of male and female reproductive success. By accurately tracking pollen grains, such models can offer valuable information about their structuring in the pollen landscape, including their origin from specific individual flowers and their destination upon arrival. These models can also help elucidate the effects of pollen removal or redistribution on the vector's body through grooming behaviors. By considering variations in floral traits, researchers can assess how these traits influence pollen dispersal patterns and understand their potential impact on the evolution of such traits. In this case, theoretical models can be useful to give new insights into factors that affect pollen transfer, generating new hypotheses that can later be tested.

Agents based model (ABM) is a suitable tool for ecology and consists of individual agents and an environment, such that agents inhabit a spatial environment and interact with the environment and each other (Murphy et al., 2020). Our study aimed to utilize ABM to investigate the influence of vector grooming behavior on the distribution of pollen grains in the vector body and on pollen transfer dynamics. This doctoral thesis consists of two chapters, each addressing different aspects of the research. The first chapter focuses on understanding how grooming behavior can alter the composition of pollen on the body of the pollen vector, consequently affecting the reproductive success of plants. In the second chapter, we investigate the grooming effects on pollen donation and reception, specifically observing the

mating interactions between female and male individuals within the flower conspecific population, associated with floral morphological variations. This chapter employs the approach of interaction networks to study the intricate dynamics of pollen transfer. Combining ABM simulations and interaction network analysis, our study aims to shed light on the relationships between grooming behavior, pollen transfer, and reproductive success. These findings have the potential to contribute to our understanding of male and female reproductive interactions mediated by pollinators and provide valuable insights into the mechanisms that shape plant reproductive strategies.

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CAPÍTULO 1

POLLINATOR GROOMING BEHAVIOR ALTERS POLLEN LANDSCAPES ON BEES' BODIES AND INCREASES POLLEN CARRYOVER TO OTHER FLOWERS

ABSTRACT

Premise of the study: Pollen participates both as the carrier of male gametes in the reproduction of flowering plants and as a key resource exploited by floral visitors, especially bees. Pollinator behavior significantly alters the patterns of pollen removal and deposition, often called pollen fates. To date, few theoretical investigations have attempted to jointly model pollen movement and pollinator behavior, and empirical studies on this matter are restricted to species in which pollen movement can be tracked.

Methods: We used a spatially explicit agent-based modeling approach to determine how bee grooming behavior may alter pollen fates and affect plant reproduction. Specifically, we asked whether pollen redistribution and removal during pollen grooming may change the "pollen landscape" on a bee´s body and, consequently, affect both pollen delivered by the anthers and pollen deposition onto stigmas.

Key results: Our model shows that both pollen redistribution and removal restructure the "pollen landscape" on the bee´s body, increasing pollen carryover (delivery in consecutive visits), and increasing pollen diversity (number of pollen donors deposited) on stigmas in sequential flower visits.

Conclusions: Our results counterintuitively show that pollen grooming may have a positive effect on both male and female reproductive success during plant reproduction.

Keywords: pollen carryover; pollen delivery; pollen fates; pollen landscape; pollen layering; pollen placement; pollen movement; spatially explicit agent-based model.

INTRODUCTION

Most flowering plants (ca. of 90%) depend on animal vectors for pollen transport and delivery (Ollerton et al., 2011; Ollerton, 2017) and this pollen transfer by animals often causes pollen loss to pollination (Harder and Barrett, 1996). During pollen transfer, pollen can be covered by conspecific or heterospecific pollen (pollen layering), and/or they can be lost or displaced for instance by the behavior of the pollen vector (pollen grooming) (Michener et al., 1978; Harder and Barrett, 1996; Barrett, 1998; Thorp, 2000). Pollen layering occurs when pollen grains are buried by layers of pollen placed on vectors' bodies in sequential flower visits (Price and Waser, 1982; Lertzman and Gass, 1983; Moir and Anderson, 2023). Pollen grooming occurs when pollen vectors displace pollen grains on their bodies, from the original sites of pollen placement by anthers to new sites and this action has mostly been understood as a reproductive cost for plants (Thomson, 1986; Harder, 1990; Holmquist et al., 2012). Both pollen layering and grooming potentially alter the 3-D distribution and donor composition of pollen on the bodies of pollen vectors during sequential floral visits, generating a dynamic pollen landscape (Lertzman and Gass, 1983; Morris et al., 1995; Harder and Wilson, 1998). Pollen landscapes were first theorized 40 years ago (Lertzman, 1981), but how they may be altered by successive layering and grooming and their potential impact on male and female components of plant reproductive success has been rarely studied from both empirical and theoretical perspectives.

During sequential floral visits, different pollen vector groups may vary in their interaction with pollen grains placed on their bodies. Some vectors are not expected to move pollen grains on their bodies, while other ones may perform grooming. Specifically in bees, grooming can cause pollen loss due to removal to the scopae, where pollen is no longer viable for pollination (Thorp, 1979) or due to pollen falling from the bee's body (Minnaar et al., 2019). In contrast, there is empirical evidence that groomed pollen grains that are not

removed can be redistributed and reach body areas different from where they were initially placed (Harder and Barrett, 1996; Natalis and Wesselingh, 2012; Tong and Huang, 2018). Ultimately, sequential pollen placement by different flowers followed by both effects of grooming, either removal or redistribution, have the potential to alter the pollen landscape on the vectors´ bodies.

Although early pollen landscape models considered pollen transport only by non-grooming vectors, like hummingbirds, vector grooming behavior was later incorporated (Harder and Barrett, 1996; Harder and Wilson, 1998). These later models analyzed pollen dispersal by comparing the vertical structure formed by sequential placement that results in overlapping pollen in layers and the horizontal structure formed by different intensity of contact with anthers and stigmas, that can be disrupted by grooming behavior, leading to change of location between safe and exposed locations (Harder and Wilson, 1998). This spatial arrangement of pollen grains from different donors on vector bodies is complex and dynamic and may generate hotspots and cold spots with greater diversity of pollen donors (Minnaar and Anderson, 2021). Moreover, theoretical multi-donor pollen landscapes models predicts that even deeply buried pollen grains might resurface on vector's body and still be deposited on stigmas (Lertzman, 1981; Lertzman and Gass, 1983; Morris et al., 1995). If this is the case, we might expect two main effects of pollen removal and redistribution by grooming vectors on male and female components of plant reproductive success. Firstly, pollen redistribution may constantly bring out deep buried pollen grains to upper and more exposed layers, increasing pollen delivery by a given donor in sequential visits and consequently its pollen carryover, *i.e.*, the number of pollinated flowers by a given donor (Fig. 1A). Also, by altering pollen landscape on vector´s body, pollen redistribution may also keep the diversity of pollen deposited on stigmas high in sequential visits as compared vectors that only remove pollen to scopae or out of their bodies (Fig. 1B).

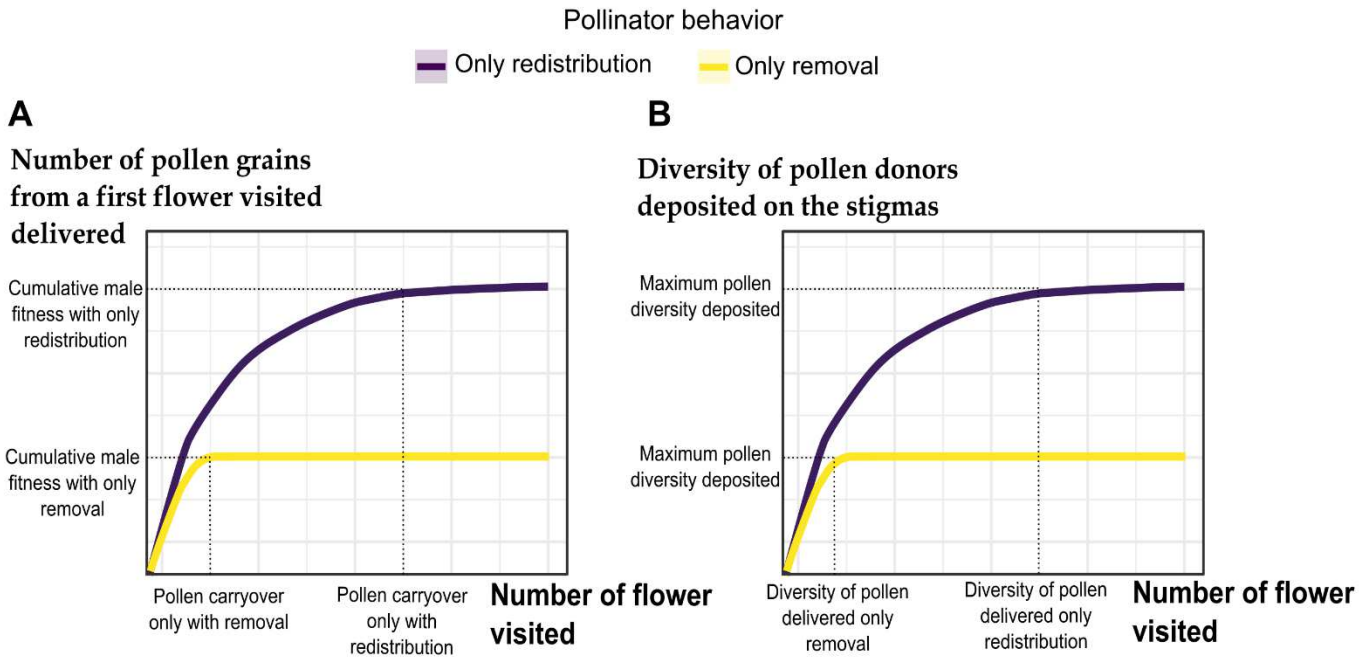

Figure 1. Expected effects of pollen redistribution and removal by grooming vectors on male and female components of plant reproductive success. Hypothetical pollen vectors that only redistribute pollen grains on their bodies during sequential visits may bring out deeply buried pollen grains to the most exposed layers, increasing both the total number of pollen delivered and pollen carryover, *i.e.,* the number of flowers pollinated by a given donor as compared to vectors that only removes pollen grains from their bodies (A). By altering the pollen landscape, pollen vectors performing only redistribution may also keep the diversity of pollen deposited on the stigmas high as compared to vectors that only remove pollen to their scopae or outside their bodies (B).

Empirical studies that assess pollen transfer and delivery require tracking the movement of individual pollen grains (Minnaar et al., 2019). Given the difficulty to achieve this pollen-level resolution in practice, empirical studies are often restricted to species that present trackable pollen grains either because they are aggregated in pollinaria (Johnson and Harder, 2018; Harder et al., 2021), or because they can be distinguished by size and color polymorphisms (Thomson, 1986; Harder and Thomson, 1989; Luo et al., 2008; Wang et al.,

2018). Other studies have observed pollen carryover using fluorescent powders as pollen analog (Lertzman, 1981; Price and Waser, 1982; Waser and Price, 1984) or pollen transfer by staining techniques using quantum dots (Minnaar and Anderson, 2019). However, these techniques are still hard to apply in more detailed studies about pollen landscape structured after sequential visits of several individual flowers, mainly because material analogous to pollen may influence the results or due to the low number of colors available (Minnaar and Anderson, 2019). Furthermore, even if we were able to track the pollen grains of individuals, we would hardly be able to simulate visits and grooming behavior experimentally. In this sense, theoretical studies may help to understand the influence of grooming behavior and layering in the structure of pollen landscape on the vector´s body and their effect on plant reproductive success, establishing hypotheses to be tested based on theoretical expectations.

Agent-based models are computational models that explore how individuals interact with each other and with their environment (Railsback and Grimm, 2019). In our model, the agents are individual pollen grains, and the space corresponds to the vector´s body where the pollen grains are placed, redistributed, removed, or deposited onto stigmas. In this sense, we developed a spatially explicit agent-based model (ABM) to understand the influence of pollen layering and grooming behavior in the structure of pollen landscape in the vector´s body and their effect on plant reproductive success. We address two questions: 1) How does the pollen landscape on a vector´s body changes after several flower visits in different grooming behavior? 2) What is the potential effect of pollen grooming and layering in pollen delivery to conspecific stigmas and in the diversity of the pollen grains deposited? We simulated different numbers of visits and different grooming behaviors and quantified pollen donor diversity on the pollinator´s body. We also evaluated the effect of grooming intensity and pollen removal and redistribution on both pollen delivery and on the diversity of pollen donors deposited onto stigmas.

MODEL AND SIMULATIONS

Model — We built ABMs to simulate the placement of individual pollen grains on the pollen vector's body and the grooming behavior after sequential visits to evaluate effect of grooming on the quantity and quality of pollen grains delivered by a pollinator. Since ABMs are tools that allow exploring how agents interact with each other and with the environment, these components and process involved in our model are described below.

The ABM has two main components: flowered plants and one pollen vector. Each plant bears a single bisexual flower with one anther and one stigma with fixed positions relatively to the vectors body. The population has a total of *f* single flowered plants each flower with different anther and stigma positions. Further floral traits are the following: mean number *r* of pollen grains released by anthers per visit, size α of the pollen patch, which affects the distribution of released pollen grains on the bee´s body, and maximum number σ of pollen grains that fit on the stigma (Fig. S1). The vector´s traits used in our model describe a 3-dimensional space where pollen grains can be placed. Therefore, such traits are width (w), height (h) and depth (d), and in our case w = h (Fig. S2). Considering that pollen grains can overlap due to the sequential placement by different flowers in the same region on the vector body (Lertzman, 1981; Lertzman and Gass, 1983, Minnaar et al., 2019), depth defines the maximum number of layers that can be occupied by pollen grains.

In our study, an individual bee can touch specific regions of its body, performing grooming behavior. We simulated a constant grooming between floral visits to assess the effect of intensity on the reproductive success of plants, although the intensity of grooming behavior may vary between floral visits performed by bees (Holmquist et al., 2012). During grooming behavior, the bee either displaces pollen grains from their original placement site

and redistributes them to new sites in its body (redistribution) (Harder and Barrett, 1996, Natalis and Wesselingh, 2012; Tong and Huang, 2018) or removes pollen grains from its body (removal) (Thomson, 1986). We created two possible grooming behaviors: random (gr), in which the bee redistributes and/or removes pollen randomly on its entire body; and ventral (gv), in which the bee redistributes and/or removes pollen grains on the ventral thorax and abdomen.

The bee interacts with the flower following sequential processes during its visit: (I) pollen deposition onto stigmas, (II) pollen placement on the bee´s body by anthers and (III) grooming (Fig. 2, see below). To parameterize the model, such as the size of the bee's body, amount of pollen released and amount of pollen received by the stigma, we used *Solanum lycocarpum* A. St. Hil (Solanaceae) and *Epicharis* Klug, 1807 (Apidae, Centridini). In this system, the anther places pollen in the center of the bee's body and the stigma removes pollen from the same bee body´s region. The values used in the parameterization are based on previous investigation of this plant species floral traits and its pollinators (Marcelo et al., 2021) (Table 1).

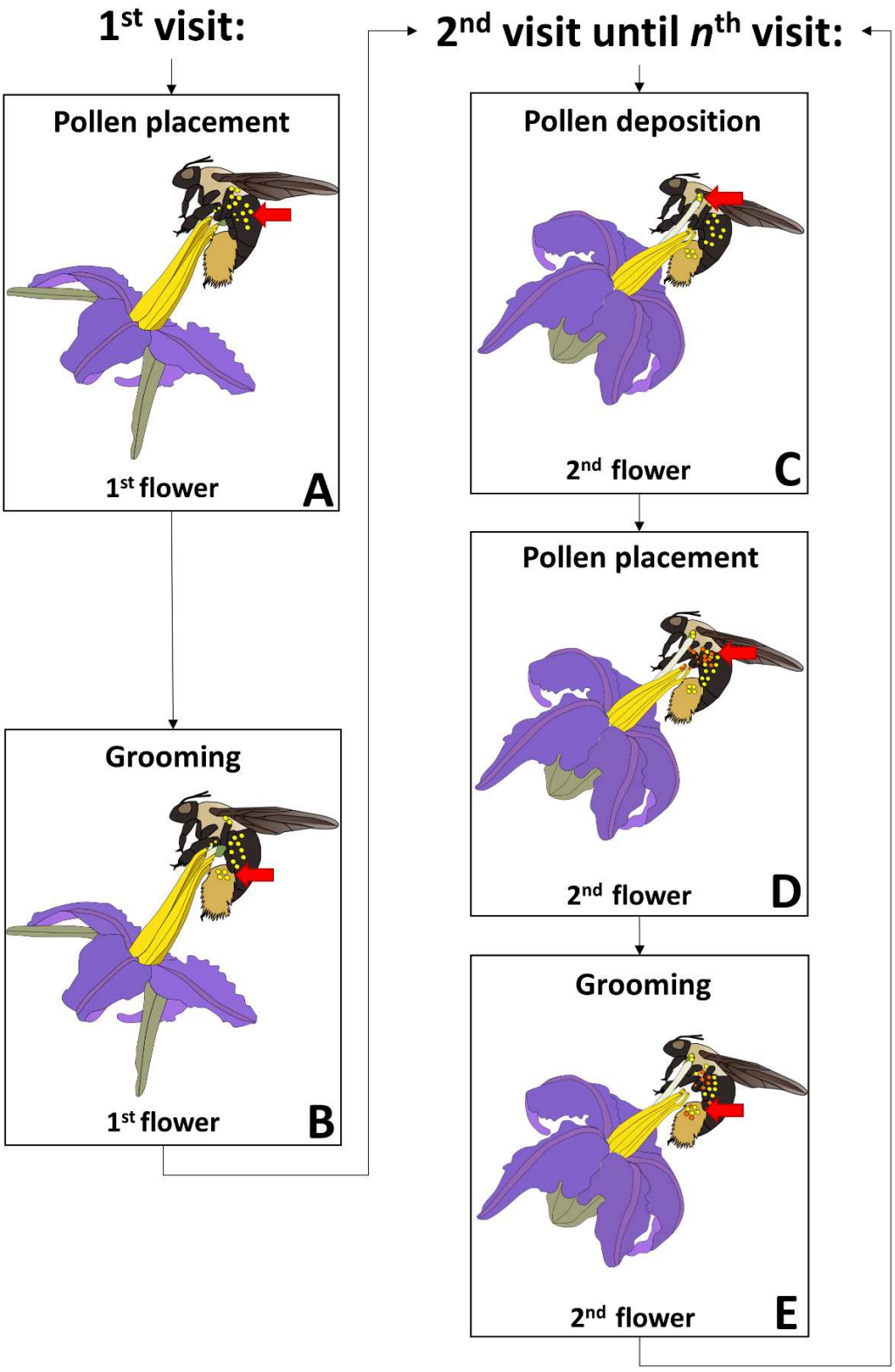

Figure 2. The rationale of the spatially explicit agent-based model used in this study to understand how pollen layering and grooming behavior in sequential visits may alter pollen landscape as well delivery and receipt of pollen. In the first flower visit, the bee´s body is clean since there was no previous pollen placement. Therefore, pollen deposition does not

occur on the stigma of the first flower. Thus, in this first visit, only pollen placement by the anthers on the bee's body can occur (A – see the red arrow). Subsequently, the bee does the grooming behavior, by moving its legs and collecting the pollen grains. In our model, it means that randomly (or spatially) the pollen with be redistributed or removed (B – red arrow). In the second visited flower of the sequence, the bee already has pollen grains from the first flower visited. Therefore, the first action is pollen deposition, that is, the stigma of the second flower receives pollen from the bee's body (C – red arrow). Now, pollen grains from the anther of the second visited flower are placed on the bee (D – red arrow), and, afterwards, the bee removes and redistributes pollen in the grooming behavior (E – red arrow). Such actions are repeated in the third visit and so on until the total number of visits – *v*, is achieved.

Table 1. Description of standard values used in the model parameterization based on data from *Solanum lycocarpum* A. St. Hil (Solanaceae) and *Epicharis* sp. Klug, 1807 (Apidae, Centridini) (Marcelo et al., 2021). Some parameters are fixed: dimension of the bee's body, which is width x height (which defines the horizontal structure of pollen grains in different regions of the bee's body) x depth (which defines the vertical structure of overlapping pollen grains, defining the number of layers) and totaling 1,600,000 locations available for placing pollen grains; pollen patch (average position where pollen can be placed on the bee's body); mean number of pollen grains released by anthers per visit; mean number of pollen grains delivered to stigmas; number of flowers created for floral visits and number of visits performed by bees. Other values were varied during the simulations: grooming intensity (proportion of sites on the body where the bee touches) and redistribution intensity (proportion of sites the bee touches in which pollen grains will be redistributed to the vector's body).

Description	Parameter	Value
Number of simulations	N	100
Fixed		
Number of positions that defines the dimension of the bee's body	w x h x d	400 x 400 x 10
Number of positions delimiting the pollen patch in w and h dimensions	α	30
Number of pollen grains released per visit	r	200.000

Total number of pollen grains that fit on the stigma	σ	400
Number of flowers	*f*	100
Number of visits	*v*	100
Variables		
Grooming intensity	Γ	{0.2, 0.4, 0.6, 0.8}
Redistribution intensity	μ	{0, 0.5, 0.99}

Variables — To understand the effect of grooming behavior on pollen landscape and plant reproduction, we performed 100 simulations combining different values of grooming intensities and redistribution and/or removal in bees with different grooming behaviors. Grooming intensity (Γ) represents the fraction of sites on the body that the bee touches, and from where pollen grains may be redistributed or removed. Redistribution (μ) is the fraction of pollen grains that are relocated to another place on the bee's body. Touched pollen grains that were not redistributed are then removed to scopae. Therefore, both redistribution and removal are complementary values. For example, in a scenario in which a fraction of 0.99 of pollen grains are redistributed, the remaining 0.01 are removed. We analyzed bees with grooming intensity values of Γ = {0.2, 0.4, 0.6 or 0.8}, and redistribution values of μ = {0, 0.5, or 0.99}, which respectively correspond to bees that performs "only removal", "removal and redistribution" in the same proportion or "only redistribution" of touched pollen grains in their bodies in different intensities.

Simulations — The simulation starts with the creation of a sequence of different flowers (Fig. S3). The order of floral visit is randomly defined (without replacement) and, in our simulations, each flower receives a single visit. The bee performs a total of *v* visits according to this sequence. The positions of anthers and stigmas relatively to the bee´s body are defined from a normal distribution with a mean equivalent to the center of the bee's body with a variance of fifteen positions (in w and h).

Then, the following processes happen (see Fig. 2):

I – Pollen deposition: previous pollen placed on bee's body is deposited on the stigma of the current visited flower. The pollen grains deposited are those from the upper pollen layer of the bee's body. The number and the identity of deposited pollen grains depend on both the region where the stigma touches and on the stigma's availability to receive pollen.

II – Pollen placement: pollen grains from the anthers are placed on the bee's body according to the position of the anther, the pollen patch (Fig. S4) and the amount of pollen released by anthers (Fig. S5). Pollen grains are placed in the deepest layers available, making it possible for pollen grains to overlap (layering). Each pollen placed has a position and layer on the bee's body, which are labeled by their identity. If all layers of a specific position of the bee´s body is already fully occupied by pollen grains, pollen falls off during pollen placement.

III – Grooming: bee grooming behavior is defined by the grooming intensity Γ and the redistribution/removal values. The positions which the bee touches and redistributes or removes pollen grains are determined according to the grooming behavior (random grooming or thorax-ventral grooming). For both pollen redistribution and removal, the bee touches only the upper layers occupied by pollen grains. For redistribution, the bee moves pollen from its original place to a new position and the deepest available layer. If all defined layers and positions are already covered by pollen grains, the pollen that would be redistributed is removed.

Analyses — We evaluated the diversity of pollen grains (Shannon-index) on the bee's body simulating 5, 10, 50 and 100 visits by bees without grooming, and with random and ventral grooming. The diversity of pollen donors (Shannon-index) in each layer of the bee's body was also quantified after 100 visits, according to different intensities of grooming and redistribution/removal values considering bees with random or ventral grooming. Finally, we

evaluated pollen delivered by the first visited flower on the stigmas of the next 99 flowers (pollen carryover) as well the diversity (Shannon index) of pollen donors deposited onto stigmas after 100 visits considering the same intensities of grooming and redistribution/removal values, as well as each type of grooming behavior (random and ventral grooming). We performed 100 iterations for each simulated bee.

All simulations were done in R version 4.0.2 (R Development Core Team 2020). The code is available at Zenodo (10.5281/zenodo.6863683). We used the following packages: vegan (Oksanen et al., 2020); Rcpp (Eddelbuettel et al., 2022); dplyr (Wickham et al., 2019); plyr (Wickham, 2020); purr (Henry and Wickham, 2020); reshape2 (Wickham, 2007); gsubfn (Grothendieck, 2018); ggpubr (Kassambara, 2020); plotrix (Lemon, 2006); plot.matrix (Klinke and Chevalier, 2022); ggplot2 (Wickham, 2016); viridis (Garnier et al., 2021) and wesanderson (Karthik et al., 2018).

RESULTS

Distribution of pollen on the bee's body — The sequential number of visits and the grooming behavior affected the spatial distribution and the diversity of pollen grains on the bee´s body (Fig. 3). The diversity of pollen grains increased with the number of visits especially in bees without grooming behavior and bees performing ventral grooming behavior (Fig. 3). However, the spatial pattern of pollen donor diversity is different between these bees. In the non-grooming bee, the central regions of the pollen patch placed on the bee were less diversified than its periphery. Interestingly, pollen donor diversity accumulated on the lateral sides of the body in the bee with ventral grooming.

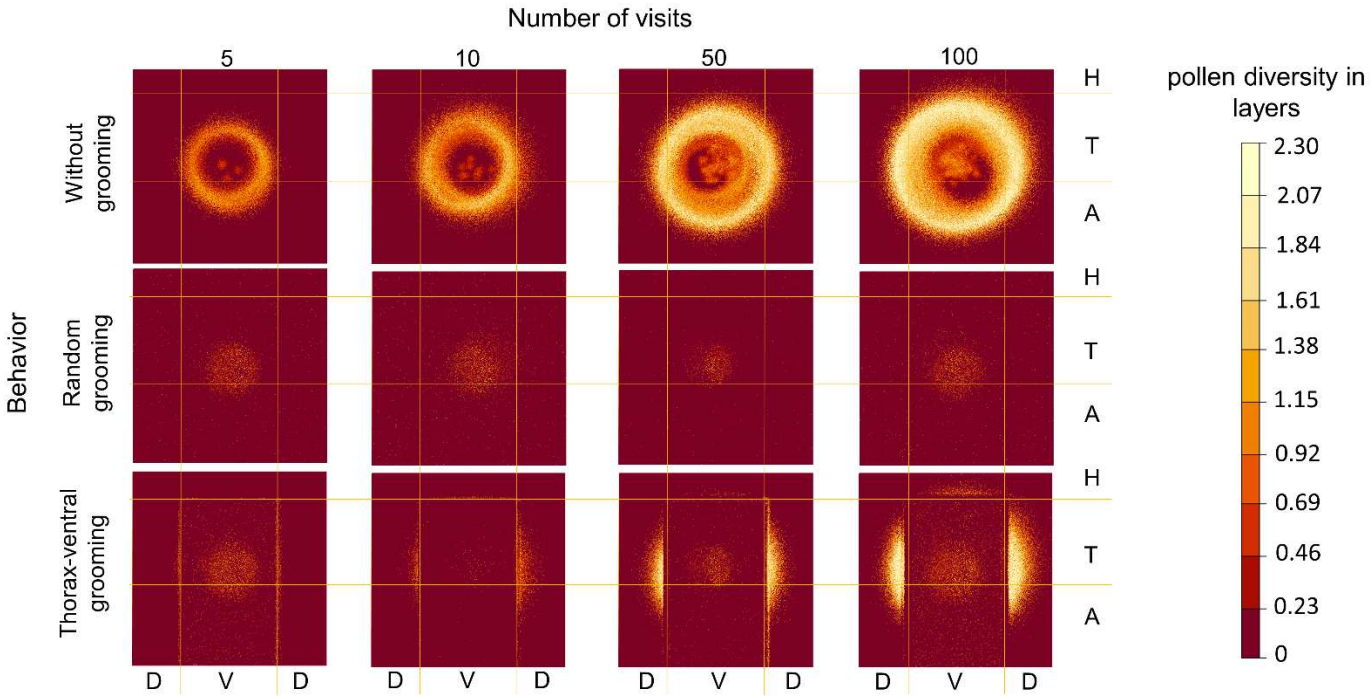

Figure 3. Diversity of pollen grain donors on the bee´s body after different numbers of visits (5, 10, 50, and 100) and according to different grooming behaviors (without grooming, random grooming, and ventral grooming) in a single iteration. Yellow lines indicate the boundaries of the bee's body regions: horizontal lines delimit the head (H), thorax (T), and abdomen (A) regions while vertical lines delimit the ventral (V) and dorsal (D) regions. The light ivory color indicates the highest diversity.

These patterns of pollen diversity distribution are related to both abundance and richness of pollen grains placed in bees with or without grooming behavior (Fig. S6 and S7). Pollen grains accumulated on the ventral regions of the thorax and abdomen in bees without grooming behavior, quickly filling the available sites for pollen placing. In such bees, the highest richness occurs in the periphery of the pollen patch. On the other hand, pollen grains were redistributed on the bee´s body never saturating the available sites on the bee´s venter when bees performed ventral grooming behavior. In such bees, the highest richness of pollen donors occurs on the lateral sides of their body.

Pollen layers — The diversity of pollen donors represented in different layers of pollen on the body of bees is affected by both the intensity of grooming behavior and redistribution/removal (Fig. 4). In general, bees that redistribute all or half of the touched pollen grains in their venter carry higher pollen diversity than bees that only remove pollen to their scopae. However, the diversity of pollen grains is not evenly distributed across pollen layers. After 100 visits, the highest values of pollen diversity are found in the outermost layers in bees that only redistribute pollen grains (Fig. 4A – D). In bees performing both redistribution and removal, the greatest diversity occurs in the intermediate layers (Fig. 4A – C). In the high intensity grooming scenarios, bees that only remove pollen grains show the highest pollen diversity in the deepest layer (Fig. 4C – D). The diversity of pollen grains across layers is similar for bees with random grooming behavior (Fig. S8).

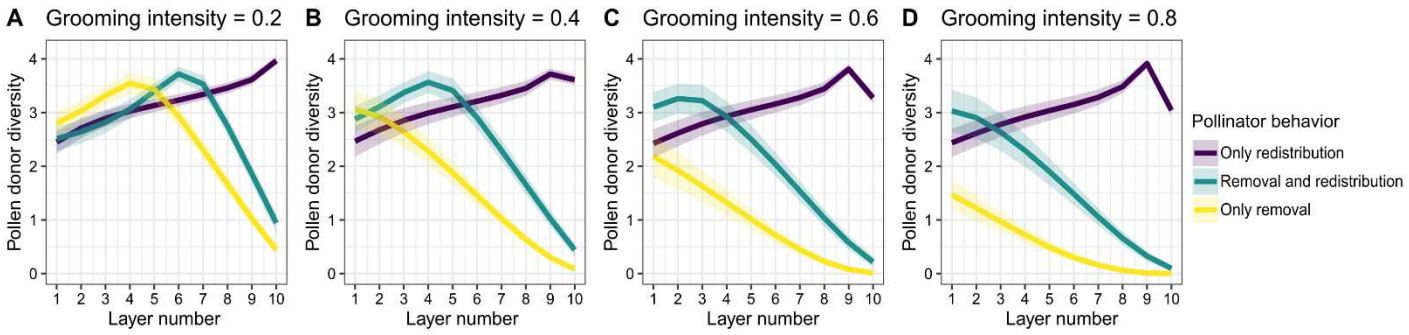

Figure 4. Diversity (Shannon index values) of pollen grains on the layers of the bee's body according to different intensities of grooming and redistribution/removal values in ventral grooming bees after 100 floral visits. On the x-axis, the higher the layer number, the outermost the layer. The pollinator behavior is represented by different colors, in which purple is "only redistribution" ($\mu = 0.99$), navy-blue is "removal and redistribution" ($\mu = 0.5$) and yellow is "only removal" ($\mu = 0$). Number of iterations = 100.

Grooming effect on plant reproduction — Although the cumulative curve of pollen delivered by the first visited flower increased in sequential visits, total pollen delivered decreases as the

grooming intensity increased in all bees (Fig. 5A - D). However, the cumulative curve of pollen delivered behaves differently in bees with different grooming behaviors. The cumulative curves of pollen delivery saturate faster for bees that only remove pollen grains as compared with bees that only redistribute and bees that redistributes and remove pollen grains from their bodies, especially in higher grooming intensity. This result indicates that pollen carryover is higher in bees performing some level of pollen redistribution. Interestingly, there is no clear difference in the cumulative curve of pollen delivery between bees that only redistributes and bees that redistributes and removes pollen grains from their bodies indicating that redistribution, rather than absence of pollen removal, is the major factor affecting the increase of pollen carryover by these bees. Similar results for the cumulative curve of pollen delivered were found in simulated bees with random grooming, however, unlike bees with ventral grooming, the highest values were found for bees that remove and redistribute pollen. (Fig. S9).

The diversity of pollen deposited onto stigmas along sequential floral visits increased in all grooming intensities and in all simulated bees (Fig. 5E – H). However, in higher values of grooming intensity, stigmas receive greater diversity of pollen grains from bees performing only redistribution and bees performing redistribution and removal as compared to bees that only remove pollen grains from their bodies. Similar results for diversity of pollen deposited onto stigmas in sequential visits were found in simulated bees with random grooming (Fig. S9). In general, the results are not drastically affected by the setting of the parameter values (see the parameter space analysis in Fig. S10 – S13).

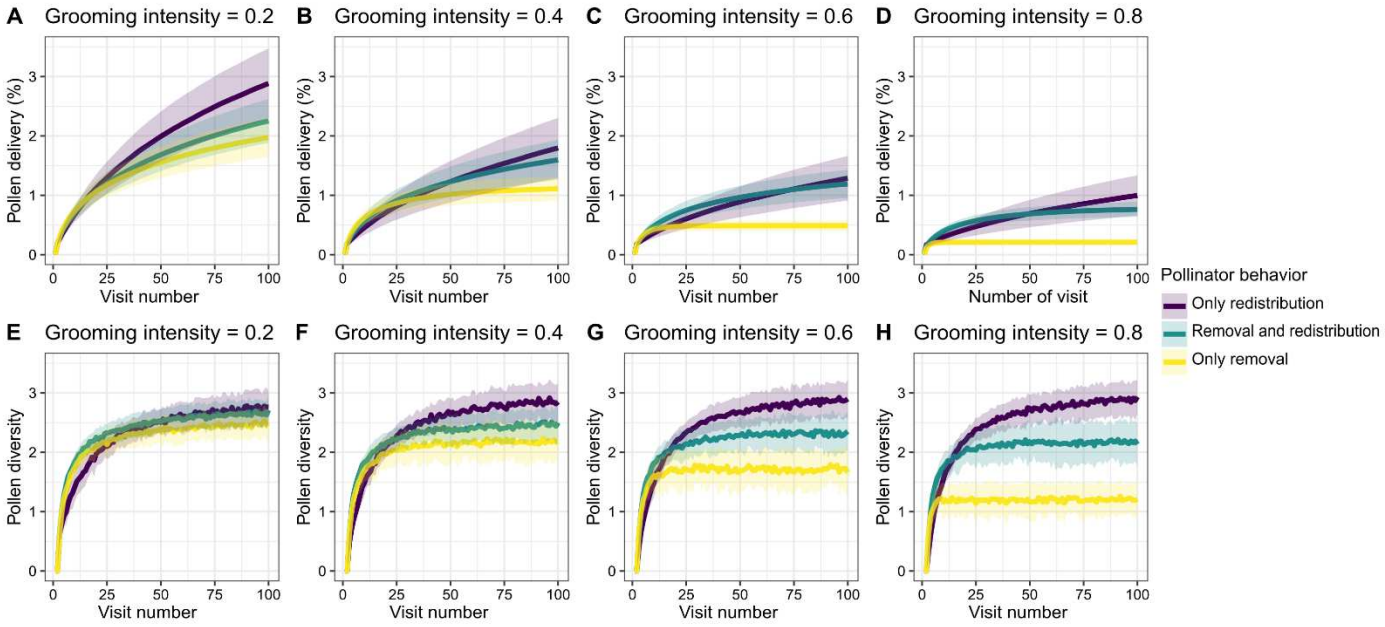

Figure 5. Effect of grooming intensity and removal/redistribution by ventral grooming bees on reproductive success. Male success was measured as the proportion of released pollen grains from the first flower that is delivered to stigmas of conspecific flowers. Female reproductive success was measured as the diversity (Shannon index) of pollen grains received by stigmas in sequential visits (E-H). Pollinator behavior is represented by different colors, in which purple is "only redistribution" ($\mu = 0.99$), navy-blue is "removal and redistribution" ($\mu = 0.5$) and yellow is "only removal" ($\mu = 0$).

DISCUSSION

Our simulations illustrate that grooming behavior has the potential to significantly restructure the spatial distribution of the pollen grains on the bee's body, altering both the horizontal and vertical pollen landscape. A high diversity of pollen grains accumulated on the unreachable lateral regions of the ventral grooming bee´s body. Also, the intermediate pollen layers on the bee´s body showed a peak of maximum pollen diversity as result of pollen redistribution due to bee grooming behavior. By altering the pollen landscape, grooming behavior has the potential to influence the number of pollen grains delivered to other flowers as well as the diversity of pollen grains deposited onto stigmas.

The effect of grooming on pollen landscape — During their foraging bout, bees sequentially visit flowers in search of pollen and/or nectar (Westerkamp, 1996). The sequential pollen placement from different flower donors may result in a pollen landscape where pollen grains from different donors and in different amounts are placed on the bee's body (Minnaar et al., 2019). Our study shows that, by removing or redistributing pollen grains, heterogeneous grooming behavior has the potential to restructure this landscape. With the constant removal of pollen from the ventral thorax-abdominal regions of the bee´s body, the available sites in this grooming region never saturate, allowing the placement of pollen grains from new donors in each floral visit. Consequently, such central-ventral region, where anthers and stigmas interact with pollen grains in our model, is less diverse in pollen donors while there is an accumulation of pollen grains from different donors in the lateral regions of the bee's body. Sites where bees do not touch pollen grains are called safe sites (Koch et al., 2017; Tong and Huang, 2018). Such sites have been reported for natural bees and may create hot and cold spots of outcrossed pollen (Minnaar and Anderson, 2021). Although we did not directly address the impact of hot and cold spots on the evolution of anthers and stigmas position on the bee´s body in our study, it is expected that both would evolve to positions that favor the contact with such safe sites, especially in flowers which pollen grains are a major feeding resource for bees. In fact, during the evolutionary history of pollen flowers, the position of anthers and stigmas has recurrently evolved to such positions, in some cases resulting in enantiostyly (Vallejo-Marín et al., 2010; Barrett, 2021; Melo et al., 2021).

The sequential placement of pollen grains in similar regions of the bee´s body potentially creates pollen layers (Lertzman and Gass, 1983). However, the diversity distribution along the vertical dimension of the pollen landscape can be drastically altered by bee grooming behavior. In bees performing both pollen redistribution and removal, the constant interaction with pollen grains from superficial layers generate a pattern of high

pollen diversity in intermediate layers, which correspond to the layers with neither so rare nor so frequent disturbances. The development of techniques to track the identity of individual pollen grains from several and different plants in different pollen layers might allow an empirical test of this prediction and a better comprehension of pollen landscapes on vectors bodies.

The effect of grooming on the reproductive success of plants — The sequential placement of pollen by different anthers in sequential visits potentially facilitate the formation of multiple pollen donor layers. As our theoretical model assumes that the vertical dimension of the bee´s body is not infinite, and that pollen layering does not increase indefinitely with sequential visits, the first placed pollen grains may have a spatial competitive advantage as compared to pollen placed in further visits. However, this competitive advantage would decrease when vectors perform intense grooming with some level of redistribution. As pollen placed by the anthers in some regions were found in distant and safe regions after some visits in real bees, it is assumed that they can redistribute pollen grains on their bodies while performing grooming behavior (Harder and Barrett, 1996, Natalis and Wesselingh, 2012, Tong and Huang, 2018). By altering the pollen landscape, pollen removal and redistribution may allow deep buried pollen grains to resurface explaining the increase in male success of first visited flowers after intense grooming in long sequential visits (Holmquist et al., 2012).

In natural systems (Harder and Thomson, 1989, Gong and Huang, 2014), as well as in our simulations, approximately 5% of the pollen grains placed on the vector's body reach conspecifics stigmas. Although we cannot clearly distinguish the isolated redistribution effect from the removal effect on pollen delivery in our simulations, bees with some level of redistribution during grooming behavior deposited a higher number of pollen grains from the first flower and delivered them to a higher number of different flowers (i.e., higher pollen carryover), no matter if bees removed none or some pollen grains from their bodies. Pollen

carryover is an important component of male fitness and affects the extent of gene flow (Morris et al., 1994). If the success of crosses between near neighbors were lower than crosses between moderately spaced plants (Levin, 1984), the increase in the pollen carryover would increase the reproductive success of pollen donors (Morris et al., 1995) and reduce the effects of geitonogamy in mass-flowering plants (Morris et al., 1994). Pollen carryover is reduced by the sequential pollen placement in non-grooming vectors (Price and Waser, 1982). Previous computer simulations suggested that such a decrease may be explained by the effect of pollen dilution or layering (Lertzman, 1981; Lertzman and Grass, 1982). Our simulations suggest that, if there is some degree of pollen redistribution during grooming behavior, pollen carryover may be increased by grooming vectors, possibly by restructuring the pollen layered landscape (but see Thomson, 1986). As pollen grains remain viable for a long period (Pacini and Franchi, 2020), the positive effect of grooming on pollen delivery may even be higher than what has been predicted in our theoretical model, since a single bee can perform more than 300 visits in a day (Raine and Chittka, 2007).

Finally, pollen grains are not necessarily successful when they are deposited on stigmas. As the available space for pollen tube growth within styles and the total number of ovules are limited, post-pollination processes such as pollen tube competition and female choice should have direct reproductive implications (Moore and Pannel, 2011). Our model predicted that pollen vectors with high grooming intensity may deliver pollen as diverse as pollen vectors with low grooming intensity when there is some level of redistribution. Some non-grooming pollen vectors, such as birds, deliver a greater amount and richness of pollen donors to conspecific stigmas when compared to other vectors due to a combination of high mobility and limited grooming (Krauss et al. 2017). However, our simulations predicted that the diversity of pollen deposited on stigmas by vectors performing some level of pollen redistribution when grooming in high intensities can reach values twice as high as the

diversity of pollen delivered by vectors that only removes pollen grains from their bodies with the same grooming intensity. High diverse pollen loads enhance the chances of receiving pollen from a high compatible donor, increasing the number of fertilized ovules, reducing seed and fruit abortion (Janzen, 1977; Stephenson, 1981; Sutherland, 1987). The diversity of pollen donors on stigmas may also enhance the quantity and quality of the progeny through interactions among pollen donors because pollen tubes may grow faster in the presence of competitors (Lankinen and Skogsmyr, 2002; Kron and Husband, 2006). Therefore, if grooming behavior promotes some pollen redistribution on the vector´s body, it may ultimately enhance reproductive performance via seed quantity and quality. Given the ubiquity of bee pollinators, we expect that grooming behavior could often directly drive the evolution of floral traits that affect pollen placement on the pollinator's body.

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CAPÍTULO 2

GROOMING BEHAVIOR AND STIGMA POSITION ALTER THE MATING NETWORK AMONG PLANT INDIVIDUALS

ABSTRACT

Premise of the study: The pathway to male reproductive success in plants is marked by events that drastically decrease the chances of individual pollen grains reaching the stigma, being grooming one of the greatest effects. Another factor that can alter pollen fate is the morphology of the reproductive organs, as it can affect both pollen placement by the anthers on the pollinator's body and the pollen deposition onto stigma. However, due to difficulties in tracking individual pollen grains, we do not know how grooming behavior and position of reproductive organs can alter mating among individuals in a flower population.

Methods: To determine the effect of grooming and variation in the position of reproductive organs on mating among flowering individuals, we simulate visits of pollen vectors to a sequence of different flowers in a population, using spatially explicit agent-based modeling. After the simulations, we accessed the mating matrices between individuals, and used network metrics to assess the effect of grooming and the variation in the position of reproductive organs in populations and individuals within populations.

Key results: Grooming behavior makes populations reproductively less connected and more specialized. On the other hand, variation in the positioning of the stigma, associated with the absence of grooming, makes populations more connected and generalist. The order of visits has a great effect in the absence of grooming, so that some few individuals are reproductively dominant. However, with grooming, this dominance is broken, making the relationships among individuals more evenly distributed. Flower individuals visited by vectors without grooming are reproductively more generalists than when the vector has the behavior, mainly with the variation in stigma position.

Conclusions: Grooming behavior affects the structure of mating interactions within a population, reducing the effect of variation in the reproductive structures of flowers,

mainly the stigmas. Our model predicts that intrapopulation stigma variability should be favored in non-grooming animal-pollinated species.

Keywords: floral traits; morphological variation; networks metrics; pollen placement; pollen deposition; pollen transfer; spatially explicit agent-based model.

INTRODUCTION

The pathway to male success in plant reproduction is marked by several events of pollen loss (Inouye et al., 1994). In this pathway, each avenue can result in male fitness decreases consequently acting as selective forces for plant reproductive traits (Minnaar et al., 2019). In fact, only a small portion of the pollen produced by flowers reaches stigmas of the same species (Thomson and Thomson, 1989; Gong and Huang, 2014). Among these multiple possible fates, individual pollen grains on the pollinator's body may be covered by pollen grains from other individuals (Moir and Anderson, 2023), may be removed by grooming behavior (Thomson, 1986; Harder and Wilson, 1998) or simply falling off pollinator's bodies (Minnaar et al., 2019).

Pollinator grooming causes substantial pollen loss (Flanagan et al., 2009), particularly when performed by pollen eating and pollen collecting animals like bees (Thomson, 1986; Rademaker et al., 1997). Bees are efficient in collecting, grooming, packing and transporting pollen grains because they have evolved various structural and behavioral adaptations for this (Thorp, 2000). Bees can actively collect pollen using their forelegs or mouthparts, or they passively accumulate pollen on their hairy bodies through grooming behavior (Westerkamp, 1996). Bees also have several structures such as brushes, combs, paw scrapers to collect and groom pollen, to later transfer pollen to the scopae or corbiculae to take and transport to the nest (Jander, 1976, Thorp, 2000). In this grooming process, bees can also redistribute pollen on their bodies (Castellanos et al., 2003), moving pollen to areas of the body different from where they were initially placed (Harder and Barrett, 1996; Tong and Huang, 2018). Among other pollinators, hummingbirds seem to groom themselves occasionally just to groom their bills (Castellanos et al., 2003) and bats may take intermittent pauses during foraging to groom their fur and consume pollen (Muchhala and Thomson, 2010).

Such differences in grooming as well as foraging behavior and the variation in their spatial movements performed by different pollinators can influence unique patterns of pollen dispersal (Wright, 1946; Wessinger, 2021). Some pollinators can be inefficient at dispersing pollen among individuals of the same species, limiting the diversity of potential mates (Harder and Barrett, 1996). Longer distances of pollen dispersal decrease the likelihood of biparental inbreeding, such as mating between closely related individuals (Karron et al., 2021), decrease the amount of geitonogamous transfer (Morris et al., 1994; Karron et al., 2009) and may affect the quality of offspring produced (Campbell, 1985). Furthermore, the dispersal of pollen in a natural plant population should have multiple evolutionary consequences (Campbell and Waser, 1989) often resulting in correlated evolution of floral characters and mating systems (Harder and Barrett, 1996). Although some studies have already compared pollen dispersal between pollinators (Young, 2002; Castellanos et al., 2003; Muchhala and Thomson, 2010; Holmquist et al., 2012; Krauss et al., 2017; Schmidt-Lebuhn et al., 2019; Pearson et al., 2023), many have compared only the distance at which pollen arrived on the stigma in different populations. So, it is still necessary to understand how pollinator behavior can affect pollen movement and the direction of pollen flow among different individuals.

Besides grooming, the morphology of floral reproductive organs also potentially affects mating among conspecific plant individuals. The positioning of anthers and stigmas relatively to pollinators body may affect the pollen placement on the pollinators body as well as their deposition onto stigmas (Harder and Wilson, 1998). In this sense, the pollinators body may be understood as a dynamic landscape, where each pollen grain occupies a given locus and are more or less subject to pollinators grooming given the position of the anthers they originated from and the stigmas where they are

supposed to be deposited. In fact, safe and unsafe sites on pollinators have long been recognized (Harder and Barrett, 1996; Koch et al., 2017, Tong and Huang, 2018). In addition, the positions of the reproductive organs determine the susceptibility of transported pollen to burial by pollen from subsequently visited plants (Harder and Barrett, 1996). However, given the empirical difficulties in tracking individual pollen grains during pollination (Minnaar and Anderson, 2019), the joint effect of grooming and flower morphology in pollen transfer has virtually never been studied. In this sense, theoretical models associated to interaction networks techniques can bring us information about the mating patterns at the populational levels as well as at the level of individuals within their population.

In this study, we used a spatially explicit agent-based model (ABM) to understand how grooming behavior, associated with variations in the positions of reproductive organs, can affect mating among individuals of a plant population. We simulated the visits of pollen vectors with different grooming behaviors in a sequence of flowers with different floral traits. As pollinators carry pollen grains from one plant to another, they connect these plant individuals, allowing the construction of a mating network. After that, we rescue mating matrices between male and female functions of individuals and subsequently create the mating networks from where we extract the metrics at the populational and individual level. Our aim was to answer the following main questions: 1) How can grooming behavior change the structures of mating networks in the population? 2) How can grooming behavior along with the visit order affect the interactions of each sexual function of flowering plants? 3) Does variation in reproductive organs (anther and stigma) alter mating networks? We hypothesize that the grooming behavior has the potential to alter the structure of the mating network, changing the network metrics, which indicate differences in the number of interactions

and relationships between individuals in the mating network. We also hypothesize that the interactions are affected by grooming and the order of visits, so that in the absence of grooming, mating occurs between closer individuals in the order of visits. But in the presence of grooming, due to redistribution, the pollen of the first visited individuals can stay longer on the vectors body and reach flowers further down the sequence of visits. Finally, we hypothesize that variation in the position of reproductive organs can affect interactions in the mating network, such that greater variation increases the probability of the anther and stigma reaching a greater number of partners and avoiding sites exposed to grooming.

MATERIAL AND METHODS

Model — We use an ABM to simulate pollen transfer between male and female functions in plant individuals. The model used for the analyzes in this chapter was the same one developed in Chapter 1, so any further information about the model is detailed in Chapter 1.

Basically, our model has two components, flowering plants and pollen vector. In the context of our model, a flowering plant individual has a single bisexual flower, and the population is composed of f flowers. Each flower created has the following traits: mean number r of pollen grains released by anthers per visit, size α of the pollen patch on the vector body, and maximum number σ of pollen grains that fit on the stigma (Fig. S1). Additionally, each flower has different stigma and anther positions where they touch the vector body. In our model, these positions where reproductive organs can place and remove pollen were defined by a normal distribution with a mean value in the center of the vector body and variable standard deviation value. We created four types of populations considering the variation of anther and stigma positions: *without*

variation, in which the anther and stigma will always touch similar regions in the vector's body (SD=1 for both anther and stigma positions); *male variation*, with the variation only occurring in the position where the anther touches (SD=15 for anther positions and SD=1 for stigma positions); *female variation*, so that only the position where the stigma touches is varied (SD=15 for stigma positions and SD=1 for anther positions); and *both variations*, that is, both the stigma and the anther vary the positions where they touch the vector's body (SD=15 for both anther and stigma positions) (Table 2). The variation in 15 positions was defined through tests carried out in which this value would clearly show a variation, without the floral organs touching outside the ventral region of the bee's body.

Table 2. Description of standard values used in the model to generate the pollen transfer matrices. We performed for each variable the *N* of simulations. Some parameters are fixed in all simulations, being: width x height x depth (which defines the size of the vector body); pollen patch (average position where pollen can be placed on the bee's body); mean number of pollen grains released by anthers per visit; mean number of pollen grains delivered to stigmas; number of flowers created for floral visits (*f*) and number of visits performed by pollen vectors (*v*). To analyze the effect of grooming and variations in the positions of reproductive organs, some parameters were variable. The type of grooming is the combination between the intensity of grooming (Γ = proportion of sites on the body where the pollen vector touches) and the intensity of redistribution (μ = proportion of sites the pollen vector touches in which pollen grains will be redistributed in the vector's body). The type of variation is the number of positions in which the organs will vary to touch the body of the vector, so that *A* indicates the number of positions that the anther varies, and *S* indicates the number of positions that the stigma varies.

Description	Value
Number of simulations	N=10

Fixed	
Number of positions that defines the dimension of the bee's body	w x h x d = 400 x 400 x 10
Number of positions delimiting the pollen patch in w and h dimensions	$\alpha = 30$
Number of pollen grains released per visit	$r = 200.000$
Total number of pollen grains that fit on the stigma	$\sigma = 400$
Number of flowers	f =50
Number of visits	$v = 50$
Variables	
Types of grooming behavior	
No grooming	$\{\Gamma = 0.01, \mu = 0\}$
Partial grooming	$\{\Gamma = 1, \mu = 0.05\}$
Total grooming	$\{\Gamma = 1, \mu = 0\}$
Types of variation in the positions of reproductive organs	
Both variation	A= 15, S= 15
Female variation	A= 1, S= 15
Male variation	A= 15, S= 1
Without variation	A= 1, S= 1

The second component is the pollen vector, which is a three-dimensional space, with height (h), width (w) and depth (d) measurements that determine the characteristics of the pollen vector body (Fig. S2). This pollen vector transports pollen between flowers in the population, and may have grooming behavior, touching body regions where they remove and redistribute pollen grains. Grooming behavior has two variables in our model. The first variable, grooming intensity (Γ), indicates the proportion of locations that the pollen vector can touch to displace pollen. The second variable, redistribution (μ), represents the proportion of pollen grains that will be displaced from their original

position and relocated within the vector's body. Pollen grains that are not redistributed within the vector's body will be displaced to the scopa.

Based on these considerations, we have defined three types of possible grooming behaviors performed by the pollen vector. The first behavior corresponds to no grooming, where the minimal grooming intensity is 0.01 and the redistribution is 0. In the second behavior, the pollen vector performs partial grooming by removing and redistributing in the same proportion, due to a grooming intensity of 1 and a redistribution of 0.5. Lastly, the pollen vector can perform total grooming, removing all pollen grains from its body. In this case, the grooming intensity is 1, and the redistribution is 0 (Table 2).

Simulations **—** Initially, in each simulation, we created a sequence of *f* flowers to be visited, with different floral traits (Fig. S3). But even though the flowers are different, we always identify them in order (from 1 to *f*) so that we can track pollen transfers within the flower sequence. Each flower receives only one visit, and it does not receive pollen from itself. The pollen vector performs *v* visits to the vector of flowers we created. During each visit to a flower, the vector engages in a series of interactions that follow a specific order. Firstly, **pollen deposition** occurs as the current flower receives pollen grains that were previously placed on the vector's body from visited flowers. The deposition of pollen on the stigma is influenced by the contact position and the stigma's ability to receive pollen. Subsequently, **pollen placement** occurs, with pollen grains being placed from the anthers to the vector's body. The positioning of these pollen grains on the vector is determined by factors such as the contact position of the anther, the amount of pollen released, and the pollen patch. Finally, **grooming** behavior occurs, which the vector displaces pollen grains from their original placement site and

redistributes them to new sites in its body (redistribution) or removes pollen grains from its body (removal).

Analyses — To assess the impact of grooming behavior and the influence of variation in reproductive organs positions on pollen transfer among flowers, we conducted 10 simulations for each grooming type and variation.

Through our model, it was possible to track the pollen grains that left the anther of an individual and arrived at the stigma of another. Then, we built the quantitative matrices of interaction between the individuals of the population considering their male and female functions. These matrices were used to build bipartite pollen transfer networks in which the nodes are the male and female functions of the individual flowers, and the links are the pollen grains that have been transferred from anthers to stigmas during a sequence of visits. These networks were created considering different grooming behaviors and with different values of variation in anther and stigma positions. We draw the networks with the *plotweb* function in the *bipartite* package (Dormann et al., 2009).

To compare networks generated from different behaviors and positions of reproductive organs, we calculated metrics at the population level: connectance, nestedness, modularity and specialization. In ecological network theory, connectance is the realized proportion of possible connections (Dunne et al., 2002), that is, the ratio between the number of observed interactions and the number of possible interactions. Connectance was calculated using the *networklevel* function. In our case, connectance reflects the realized mating potential of the population. The nestedness of the population was estimated by the nesting metric NODF (Almeida-Neto et al., 2008), calculated using the *nested* function (NODF2 method). Values of 0 indicate non-nestedness, values of 100 indicate perfect nesting. A perfectly nestedness network is one in which

individuals with fewer interactions interact with a subset of individuals with more interactions (Bascompte and Jordano, 2007). In our study, nestedness reflects the level of mating dominance exerted by male or female function within population. Modularity was calculated using the *computeModules* function (Beckett method). Higher modularity values indicate the existence of modules in which individuals preferentially interact within a subgroup of individuals (Pinheiro et al., 2019). Networks that present values closer to 0 are fully connected and those with values closer to 0.9 are networks with a greater number of modules. In our case, modularity measures the formation of mating groups of plant that interact more with each other, according to the moment they are visited, so that mating occurs between individuals closer in the sequence. Specialization at the network level was measured using the H2' index, which is a two-dimensional measure derived from the index of Shannon and is very useful for comparisons between different networks (Blüthgen et al., 2006). To calculate the H2' index we use the *networklevel* function. This metric ranges from 0 (no specialization) to 1 (complete specialization) and thus reflects the degree of mating specialization among individuals, meaning at least one of the following: (I) the more specialized they are, the more the location of their reproductive organs is an important defining factor for reproduction (II) the more the visiting order limits the pollen options.

To analyze metrics related to individuals within the network, we calculated metrics of degree and specialization. The degree represents the sum of links per individual, i.e. the total number of ovules sired (male function) or the total number of fertilized ovules (female function). Lower individual degree values indicate a greater number of partners. Specialization, on the other hand, was calculated by the d' index, which can be used to analyze variation within networks (Blüthgen et al., 2006). The index d' refers to the specialization of each individual based on its discrimination from

the random selection of partners and indicates how much a given individual is selective. For the d' metric, higher values indicate that individuals are more specialists. Both metrics were calculated using the *specieslevel* function.

All simulations and analyses were done in R version 4.3.1 (R Development Core Team, 2023). All functions used in the analysis of metrics of networks and individuals are in the *bipartite* package (Dormann et al., 2009) in the R program. Other packages used in the development of the model were mentioned in Chapter 1.

RESULTS

Influence of grooming behavior and variation in the positions of reproductive organs at the network level –

Grooming and variation on reproductive organs position alter the structure of mating interaction network (Fig. S14, S15, S16, S17). The networks obtained from pollen vectors that do not perform grooming behavior had higher connectance and nestedness values and lower values of modularity and specialization (Fig. 6). However, the network metrics in absence of grooming behavior were affected by the variation in the positioning of reproductive organs, especially the female variation. Connectance and nestedness were higher in populations with female variation as compared to populations without female variation when grooming was absent (Fig. 6 A-B). On the other hand, female variation decreased the values of modularity and specialization on the same populations (Fig. 6 C-D).

In the presence of grooming behavior, networks presented lower connectance and nestedness values while they increased in the modularity index and specialization level in the mating network. Interestingly, partial, or total grooming produced almost

the same values of network metrics no matter if there was variation in floral organs, indicating that grooming tends to extinguish the mating consequences of floral organs variation.

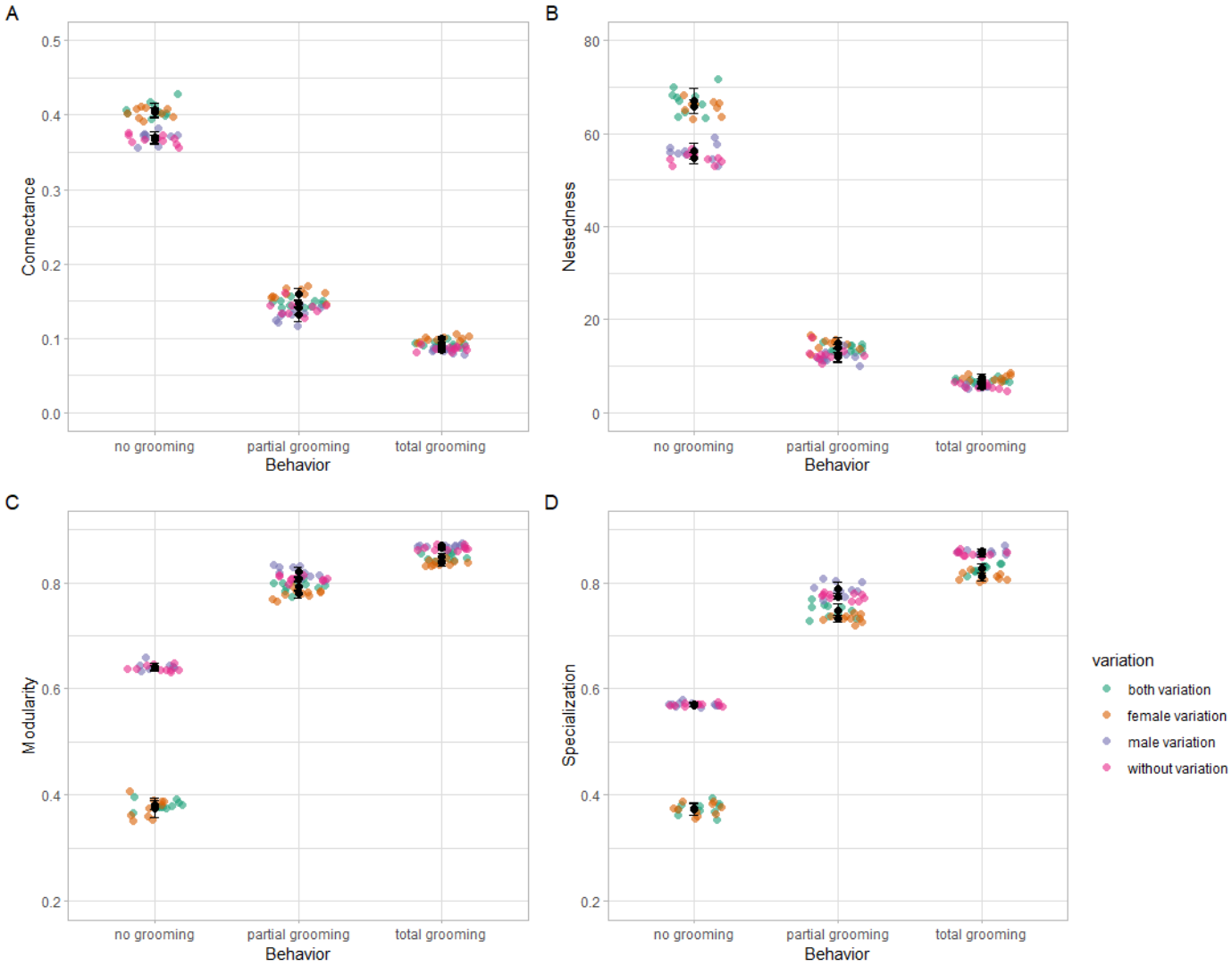

Figure 6. Network metrics according to different grooming behaviors and different variations in the positions of reproductive organs. The calculated metrics are A=connectance; B= nestedness, measured by the NODF metric; C=modularity and D= network specialization, measured by H2'. Metrics were calculated for each network obtained from different grooming behaviors: no grooming, in which both grooming is 0.01 and redistribution intensity is 0; partial grooming, in which the grooming intensity is 1 and redistribution is 0.5 and total grooming, in which grooming intensity is 1 and redistribution is 0. The metrics were also calculated according to different variations in the positions of the reproductive organs, as follows: both variation, the variation occurs

in both the anther and stigma positions (green dots); female variation, the variation occurs only in the position of the stigma (brown dots); male variation, in which the variation occurs only in the position of the anther (lilac dots) and without variation that the flowers do not have any variation in the positions of the reproductive organs (pink dots). Each colored dot in the figure indicates a value for each network created in each simulation (total of 10 simulations per treatment). The black dot indicates the average value for each behavior and type of variation and the black bars indicate the standard deviation (SD) values.

Influence of grooming behavior and variation in the positions of reproductive organs in individuals according to the order of visits –

The grooming behavior and the variation in the position of floral reproductive organs also affect the mating success of the individuals through both male and female functions. In general, in the absence of grooming, the degree of female function increases along the sequence of visiting flowers whereas the male component shows the inverse pattern (Fig. 7). This means that the first visited flower interacts more with other flowers through the male function while the last visited flower interacts more through the female function. Despite the variation on floral reproductive organs seems to have little impact on the interaction degree of individuals, the grooming behavior clearly diminishes and make it are more uniformly distributed along the sequence of visited individuals. Also, when the pollen vector performs total grooming, the degree values of the female and male functions are smaller and the partial one has intermediate values.

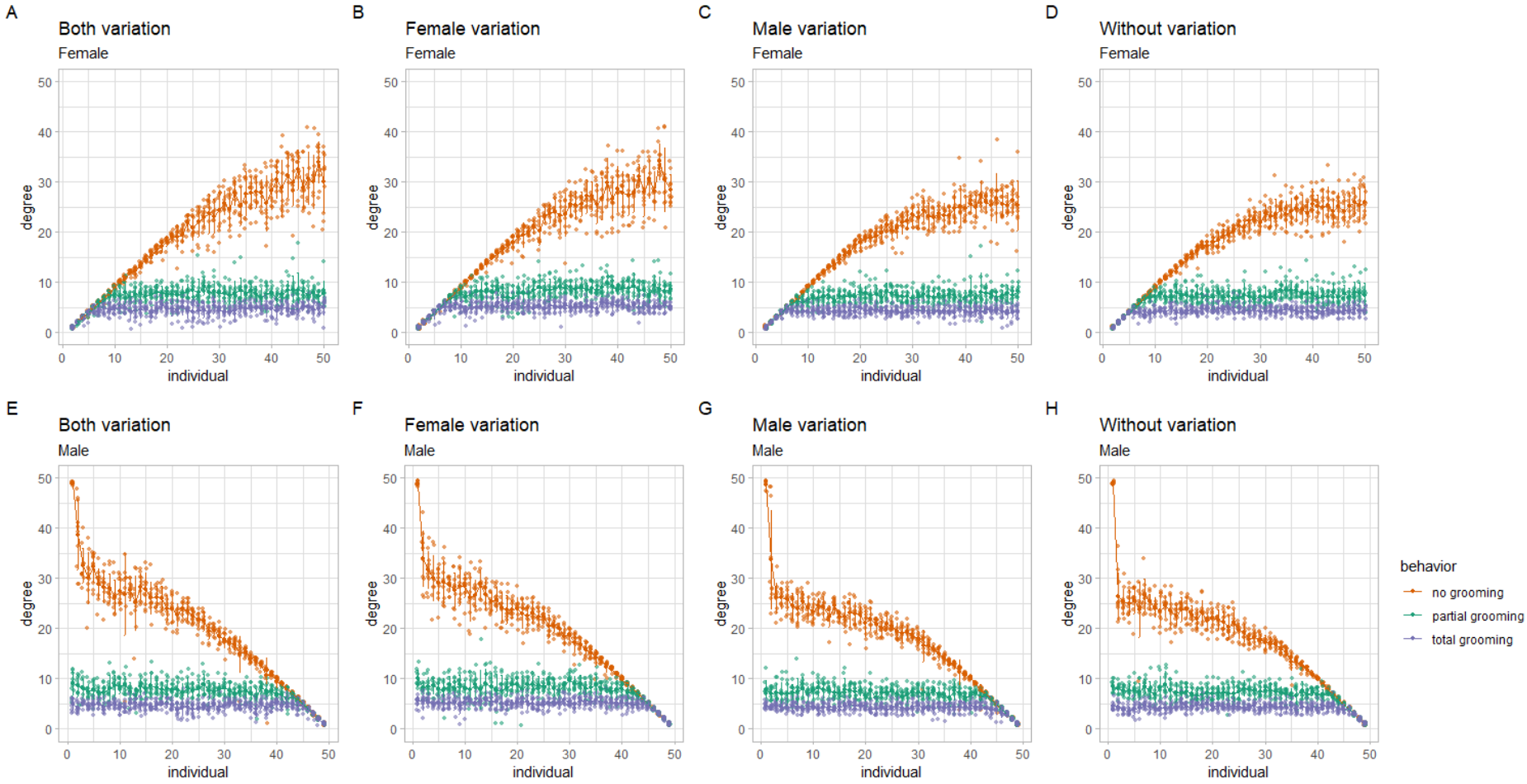

Figure 7. Metric degree at the level of male and female individuals according to types of behavior and variations in the positions of reproductive organs. Graphs A to D show the degree values for female individuals and graphs E to H show the degree values for male individuals along the order of visits (1 to 50). In A and E the flowers visited vary in the position of anther and stigma; in B and F the flowers vary only in the position of the stigma, in C and G the variation occurs only in the anther and in D and H the flowers do not vary in the positions of the anther and stigma.

Still the degrees differ according to the grooming behavior, being the networks of vectors without grooming (orange dots), partial grooming (green dots) and total grooming (blue dots). Each colored dot in the figure indicates a value for each network created in each simulation (total of 10 simulations per treatment). The darkest dot indicates the average value for each individual and type of grooming and the bars indicate the standard deviation (SD) values.

Grooming increases reproductive specialization in both male and female components, regardless of the position of the reproductive organs. In the absence of grooming, female and male individuals are less specialists, especially when they show variation in the position of the stigma and in both reproductive organs. Even without grooming, the order of visits influenced the specialization in male function, so that values increased along the sequences of flowers visited, with males visited last being more specialists (Fig. 8).

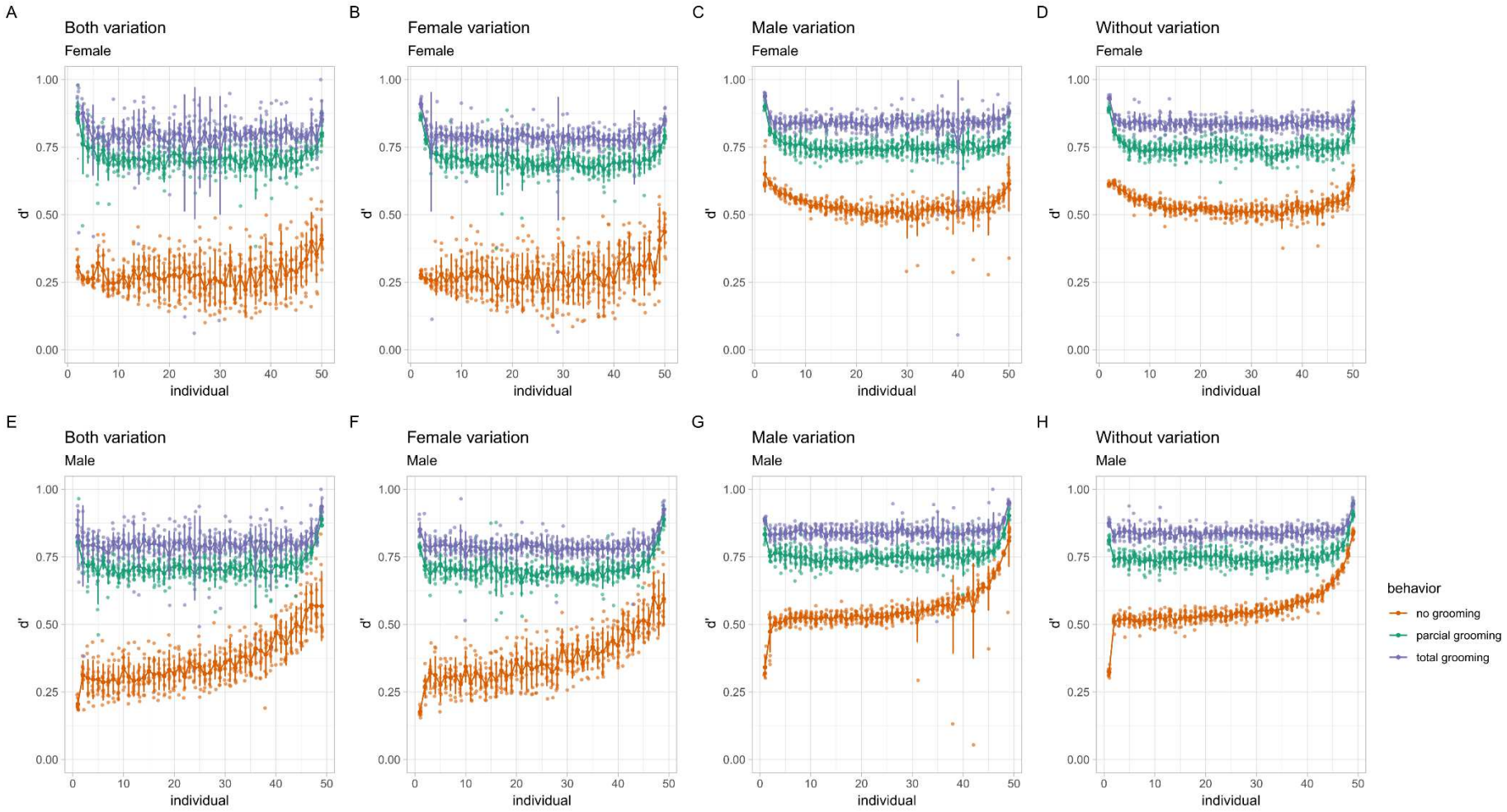

Figure 8. Metric specialization (d') at the level of male and female individuals according to types of behavior and variations in the positions of reproductive organs. Graphs A to D show the specialization values for female individuals and graphs E to H show the specialization values for male individuals along the order of visits (1 to 50). In A and E the flowers visited vary in the position of anther and stigma; in B and F the

flowers vary only in the position of the stigma, in C and G the variation occurs only in the anther and in D and H the flowers do not vary in the positions of the anther and stigma. Still the specializations differ according to the grooming behavior, being the networks of vectors without grooming (orange dots), partial grooming (green dots) and total grooming (blue dots). Each colored dot in the figure indicates a value for each network created in each simulation (total of 10 simulations per treatment). The darkest dot indicates the average value for each individual and type of grooming and the bars indicate the standard deviation (SD) values.

DISCUSSION

Based on simulations, we observed that both grooming behavior and the morphological variation has an impact on mating networks. In the absence of grooming behavior, mating networks are more connected and nested but less modular and specialized. When pollen vectors exhibited total grooming behavior, the mating networks became less connected and nested, but the network structure became more modular and specialized. Furthermore, in absence of grooming, we found that variation in stigma position had an impact on the network structure, increasing the interactions in population and making it more generalist. Additionally, in the absence of grooming behavior, the values of female and male degrees were strongly related to the sequence of visits. However, this relationship was disrupted when vectors exhibited grooming behavior. Partial grooming was found to have an intermediate value, indicating a more equitable distribution of degrees among individuals. On the other hand, individuals visited by non-grooming vectors are reproductively more generalist than those visited by vectors with some level of grooming behavior.

Effect of grooming on mating network - Grooming behavior is one of the biggest factors leading to pollen loss (Flanagan et al., 2009). Bees collect pollen and perform grooming after each floral visit, but the intensity varies (Holmquist et al., 2012), being greater as the pollen load in the vector increases (Harder, 1990). In general, studies have shown that pollen removal in grooming behavior causes a reduction in pollen carryover (Thomson, 1986; Rademaker et al., 1997; Holmquist et al., 2012) and even a decrease in pollen diversity arriving in the stigma (Marcelo et al., *in press*). Other pollinators also perform grooming but at lower intensities, for example, bats perform grooming occasionally during some breaks in foraging (Muchhala and Thomson, 2010) and hummingbirds perform it rarely, only remove pollen from the beak (Castellano et al., 2003). Non-grooming vectors do not remove pollen grains from their collection structures, resulting in a minimal loss of pollen during transfer

and enabling a greater amount of pollen grains to reach the stigmas. Because pollen grains remain on the vector's body, exactly where they were initially placed, they have the potential to be transferred to multiple stigmas during consecutive visits to different flowers. In this case, the first visited male function may has an advantage compared to the others, since it quickly fills the spaces in the vector's body, managing to stay longer and reach further in the sequence of visits. Thus, in this case, variation in pollen capture positions by the stigma improves the exploitation of the vector's body by the stigmas, increasing the chance of stigma reaching regions where there may be more diverse pollen (Waser and Price, 1984; Marcelo et al., *in press*). The variation of the anthers has little influence on the interactions, since the pollen grains probably form a wide stain on the pollinator's body, not mattering much the variation in the positions where they place pollen.

In network theory, nestedness is often interpreted as a metric of specialization asymmetry, where specialists (individuals with few links) interact with generalists (individuals with many links) (Blüthgen et al., 2008). So, in our study, when nestedness is high, the mating network exhibits a structure where individuals with few links have a higher probability of interacting with the same individuals that generalists interact with. We have observed that when vectors exhibit grooming behavior, we notice a less nested structure in the network. In this case, there is no clear segregation between specialist and generalist in male and female functions.

In our study, contrary to our initial expectations, mating networks in grooming vectors were more modular than those in non-grooming vectors. When the vector performs grooming, pollen transfer occurs only between the closest flowers in the order of visits. We hypothesize that this can be explained by the way in which the model was created. In the grooming vector, both removal and redistribution of pollen grains occur in body regions and layers. In redistribution, the pollen touched by the vector are displaced to new positions and layers.

Thus, the order of redistribution of pollen in the layers follows the same sequence as the order of visits, so that the pollen in the most external layers, where the stigmas possibly touch, are mainly the most recently visited pollen. In a highly modular network, where individuals within each module interact more frequently among themselves there is an increased likelihood of specialized interactions and the presence of specialist individuals (Alcalá-Corona et al., 2021). The mating networks with more modules and high specializations were the ones with vector grooming behavior. By observing the specialization of individuals, we observed that with the grooming behavior, individuals become similarly specialized.

Effect of order of visits on pollen transfer - We observed that in the absence of grooming behavior, the degree of individuals reflects the effect of the order of visits. The degree of female individuals increases as the visits progress, while the male degree follows the opposite pattern, decreasing along the sequence of visited flowers. However, when grooming behavior is present, the degrees are more evenly distributed among individuals no matter the visiting sequency.

If the sequency of visits plays a significant role in the success of pollen transfer, then it could be advantageous for male plants to be the first to be visited. Consequently, flowers that bloom early or have more attractive features may have higher male advantage if the pollen vector doesn't disturb pollen transport. In polygamous animals, it is common that first copulating males have a greater advantage than the later ones (Weldingh et al., 2011; Rodrigues et al., 2019). In some plants, individuals that opened their flowers relatively early in the flowering season showed the highest selection through total fitness (O'Connell and Johnston, 1998). However, in our study we observed that female individuals, in the absence of grooming by the visitor, have a greater number of connections when visited last, due to the greater number of previous visits. By increasing the diversity of pollen deposited on stigmas, multiple mating in plants promotes pollen competition, which allows for the selection of

diverse male gametophytes (Pannell and Labouche, 2013). When the vector exhibits grooming behavior, it becomes evident, both through network structure and metrics, that the interactions between individuals are more alike. In general, we have observed that with grooming behavior the position of the flowers in the visit order does not affect the number of interactions and decrease the dominance of the first male flower visited. The grooming behavior, in this case, may not favor plants with synchronous anthesis time because the moment in which they are visited does not matter much. Our model predicts that populations visited by non-grooming pollinators, such as hummingbirds, should be more reproductively connected when compared to populations visited by grooming vectors, such as bees. So, this does not necessarily happen due to flight distance and pollen dispersion, but also due to this grooming behavior. Previous studies have compared pollen transport and pollen dispersal distance by different flower visitors with different grooming behavior (Castellanos et al., 2003; Muchhala and Thomson, 2010; Holmquist et al., 2012; Krauss et al., 2017; Pearson et al., 2023). In general, nectar-seeking pollinators and non-grooming such as bats and traplining hummingbirds were more effective at transferring pollen compared to pollen-collecting and grooming pollinators such as bees (Wessinger, 2021). Thus, bees have shorter pollen dispersion due to the combination of shorter flight distances, ideal foraging behavior and, as we confirmed in this study, also due to regular pollen grooming (Krauss et al., 2017).

Effect of varying positions of reproductive organs on pollen transfer – We observed that when there was variation in anther position, the resulting network structure was similar to the absence of variation in reproductive organs. However, the variation in stigma position had a stronger impact on pollen transfer, increasing the number of interactions and decreasing the specialization. In the case where the positions of the anthers vary and those of the stigmas do not, this led to variations in the placement of pollen grains, perhaps placing in the peripheral areas of the pollen pool (Waser and Price, 1984) and the stigma position remains the same,

there may be greater failure of stigmas to contact the available pollen pool (Waser and Price, 1984). If there is no variation in the positions of the anther the pre-existing loads from the first flowers visited can prevent the deposition of new pollen ("pollen preclusion", Moir and Anderson 2023). The absence of variation in stigmas can still increase the chance of capture more pollen from the previous few males (Minnaar et al., 2019). On the other hand, if there is variation in stigma position, regardless of whether there is variation in anther position or not, the stigmas may capture pollen grains from different deposition regions of the vector´s body, increasing the chances of receiving pollen from multiple donors. Studies have observed that with some level of variation in the positions of anthers and stigmas there was a greater probability of successful pollen transfer and greater carryover distance (Lertzman, 1981; Lertzman and Gass, 1983; Waser, 1983; Waser and Price, 1984; Campbell and Waser, 1989). This finding helps to explain why in our study, when flowers exhibited variation in stigma position or both reproductive organs, the resulting networks were less specialized, as they likely interacted with a greater number of different flowers. Recipient flowers by controlling the position of the pollinator, can optimize the contact between the stigma and the visiting pollinator, increasing the chances of successful pollination (Galen and Plowright, 1985).

The greater effect of the position of the stigma than the anther was observed when vectors do not groom themselves, because when they do, there is pollen loss and redistribution between each visit, making pollen transfers between individuals more similar. According to another theoretical model, plants visited by grooming pollinators, the male components determine the pollen distribution around the vector's body on exposed or safe sites, while the female components have little opportunity to modify the landscape (Harder and Thomson, 1989). When visited by non-grooming visitors, male components govern pollen preclusion rates and female characteristics determine the rate at which precluded pollen layers were subsequently exposed (Harder and Thomson, 1989; Minnaar et al., 2019).

CONCLUSIONS

Any trait that exhibits variation and has an impact on an individual's mating success has the potential to be subject to sexual selection (Delph and Ashman, 2006). In other study, researchers discovered that there is a higher likelihood of selection acting on floral traits through female reproductive function, indicating that selection is more prominent in terms of pollen receipt rather than pollen donation (Morgan and Schoen, 1997). By integrating morphological analyses with pollen tracking, empirical and theorical studies contribute to our understanding of the evolutionary processes that drive trait variation in plants and how selection acts on these traits in the context of pollination biology. This comprehensive approach enhances our knowledge of the intricate interactions between plants and their pollinators and provides valuable insights into the mechanisms underlying floral adaptation and evolution. In our study, we observed that plant individuals had a greater number of interactions being more generalists in the scenario with non-grooming pollen vectors and variations in stigma positions. Thus, our study proposes that grooming affects the structure of mating interactions within a population and that it can reduce the effect of variation in the reproductive organs of flowers, especially the stigmas. Therefore, our model predicts that species pollinated by non-grooming vectors, like hummingbirds, should be more variable in the position of stigmas than species pollinated by grooming vectors, like bees.

The evidence indicating that a higher number of pollinator visits positively impacts pollen tube growth, as well as fruit and seed production, highlights the importance of multiple visits for successful plant reproduction (Stavert et al., 2020; Furtado et al., 2023). In your model, where we currently evaluate a single visit from a single vector and it could be worth exploring the possibility of increasing the number of visits is received by each flower and the number of vectors visiting simultaneously. This modification, assessing the deficit of pollinators, could simulate the effect of multiple visits which could potentially alter pollen

transfer. Expanding your model to include a population of vectors visiting the flowers would be a valuable future direction. This would allow us to explore the dynamics of multiple pollinators interacting with the flower population, potentially leading to a more realistic representation of the pollination process. Additionally, evaluating the effect of pollen dosing, where pollen is released gradually over multiple visits (Castellano et al., 2006), would provide insights into the significance of pollen availability over time. The accuracy of the positioning of reproductive organs in flowers is another important aspect to consider. Analyzing the accuracy of the placement of reproductive organs, affecting the efficiency of pollen deposition and reception, helps to elucidate the evolutionary dynamics of traits that may prevent pollen loss through grooming behaviors. By incorporating these aspects into your model, we can improve knowledge about the mechanisms that drive the evolution of floral traits involved in successful pollination and reproductive success.

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GENERAL CONCLUSIONS

In this thesis, we were able to create an individual-based model that analyzed several variables that may somehow influence pollen movement. We found that the grooming behavior can alter the pollen landscape in the body of the pollen vector. In those with the grooming behavior, in the horizontal landscape, the greatest diversity of pollen grains is found on the sides of the vector's body, where the vector can hardly groom itself. In the landscape, however, the greatest diversity is found in the intermediate layers that undergo intermediate disturbance. Also, separating the effect of removal and redistribution, we show that when the bee has some level of redistribution there is an increase in pollen carryover, possibly due to the restructuring of the pollen layers. In addition, we observed that when there is redistribution, bees deliver more diverse pollen grains, which can increase the chances of receiving compatible pollen, increasing ovules fertilization.

We also observed that grooming also affects the structure of mating networks. With some grooming behavior, relationships happen between a few individuals and relationships are more specialized and without grooming, individuals interact more with each other and in a more general way, especially if there is variation in the position of the stigmas. Our model predicts that a species visited by non-grooming pollinators is likely to have the most connected mating networks, when compared to mating networks by those who groom, not only because of dispersal distance, but because they do not groom. The order of visits has a great effect when visited by a vector without grooming, so that the first male and last female visited have more interactions with the others. On the other hand, when the vector has a partial behavior, this grooming breaks the mating dominance of such individuals, increasing specialization in both functions. On the other hand, individuals when visited by vectors without grooming, become more generalist, donating, and receiving pollen from more individuals, when compared to those visited by vectors with some grooming behavior,

because in this case there is some pollen loss, and the relationships happen more strongly with closer flowers. Regarding the variation in the position of the reproductive organs, we saw that the variation in the position of the stigma has an impact on the structuring of networks, especially when there is no grooming behavior, as it increases the generalization of individuals both by the female component and by the male component. This shows that variation in stigma position should be greater in species that are visited by non-grooming vectors.

By incorporating data on pollinator behavior and floral characteristics, theoretical models can simulate realistic scenarios and generate predictions about pollen movement and reproductive outcomes. This approach may provide new avenues for studying the complex dynamics of plant-pollinator interactions and contribute to our understanding of the factors that shape reproductive success in flowering plants. Overall, the integration of theoretical models with empirical data on pollination can increase our knowledge of pollen transfer processes and their ecological and evolutionary implications. In the next steps, we would like to carry out empirical studies, using new technologies such as quantum dots, to test the new hypotheses generated with the model.

SUPPLEMENTARY MATERIAL

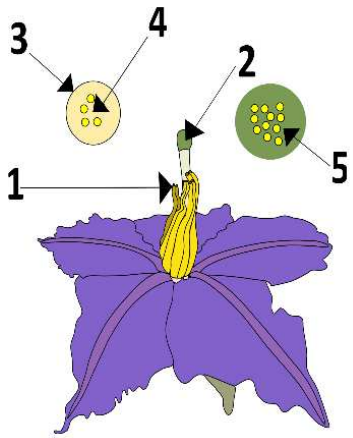

Figure S1. The flower component of the agent-based model. Each flower created has 1- anther position and 2-stigma position, 3- pollen patch (α); 4- mean number of pollen grains released by anthers per visit (r) and 5-maximum number of pollen grains that fit on the stigma (σ).

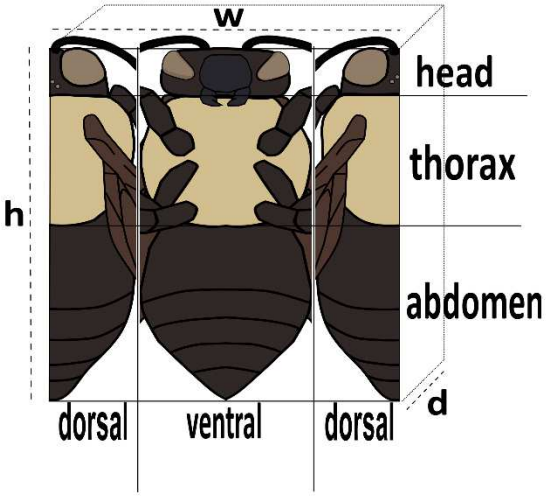

Figure S2. The vector component of the agent-based model. The modeled bee has a size defined by its width (w), height (h) (in our case w = h) and depth (d = number of layers). We also defined the bee´s body regions. Lines indicate the boundaries of the bee's body regions: the horizontal lines delimit the head, thorax, and abdomen regions and the vertical lines delimit the ventral and dorsal regions.

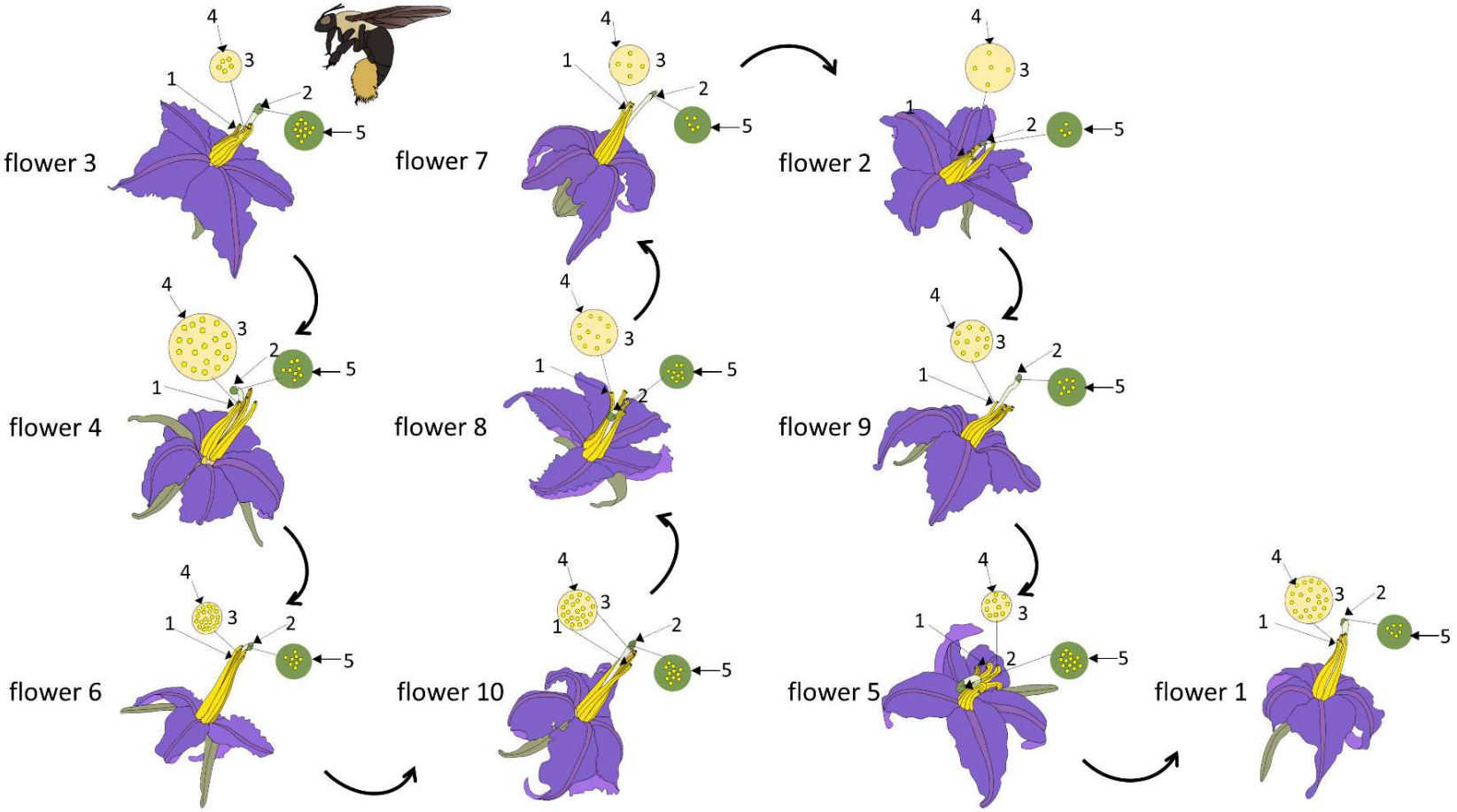

Figure S3. Example of a random sequence of flowers created in which the bee will deliver and collect pollen. In this example, the population has a total of ten flowers each one with different anther (1) and stigma (2) positions. Each flower in possess the following traits: size α of the pollen patch placed on the bee´s body (3) and mean number *r* of pollen grains released by anthers per visit (4), which affects the distribution of released pollen grains on bee´s body. Also, each flower has a maximum number σ of pollen grains that fit on the stigma (5).

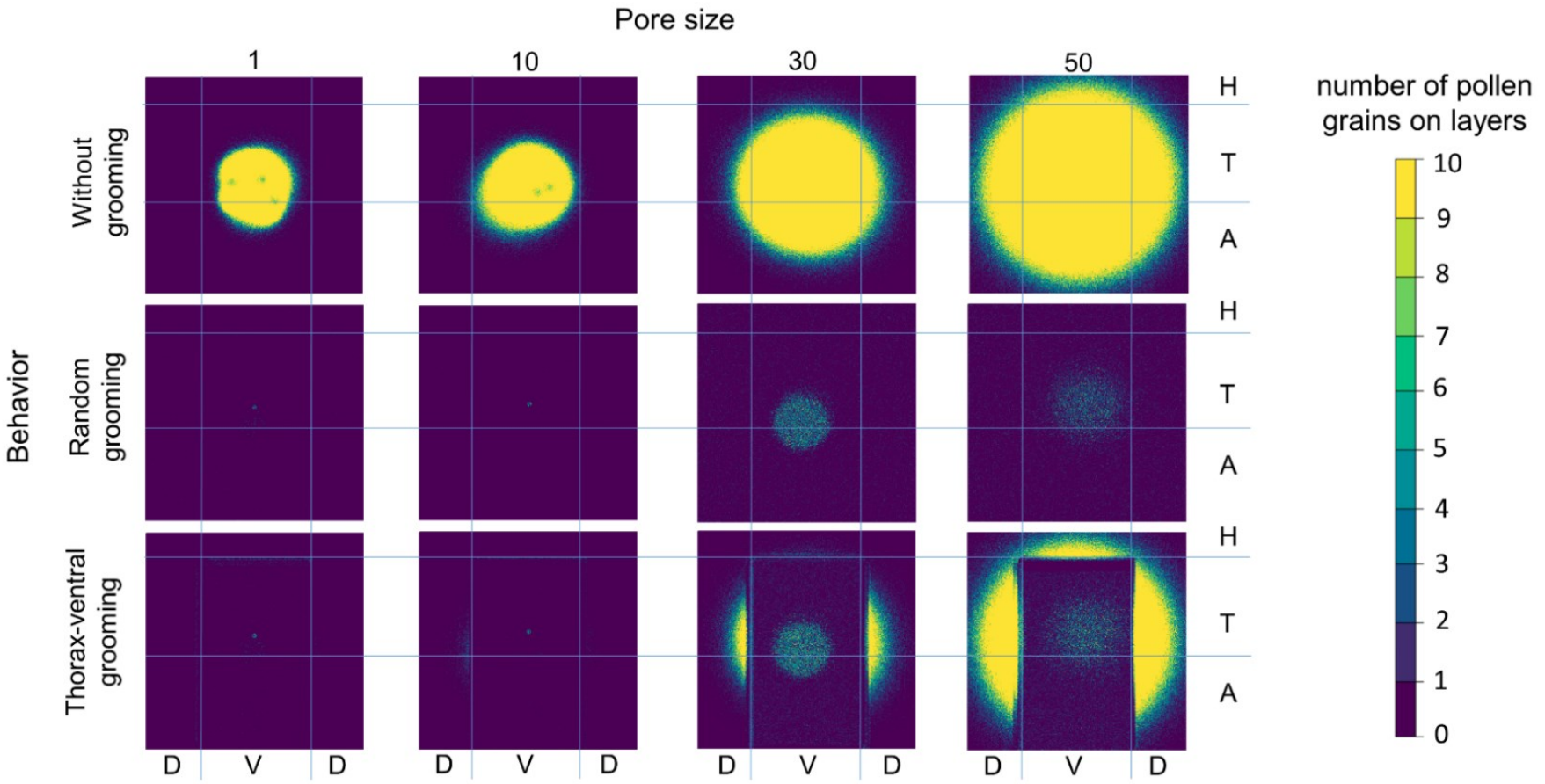

Figure S4. The total number of pollen grains accumulated on the bee´s body after one hundred visits in flowers with different pollen patch (1, 10, 30 and 50) and according to different grooming behaviors (without grooming, random grooming, and ventral grooming). Blue lines indicate the boundaries of the bee's body regions: horizontal lines delimit the head (H), thorax (T), and abdomen (A) regions while vertical lines delimit the ventral (V) and dorsal (D) regions. The yellow color indicates the highest number of pollen grains. The bees in this figure are the result of a single iteration, after 100 visits, on 100 different flowers, with flowers having pollen patch values of 30 positions, releasing 200,000 pollen per visit and their ovules supporting 400 pollen grains. The bees had 400 positions in height and width and 10 in depth. Furthermore, the bees were able to touch 80% of the body regions ($\Gamma = 0,8$) and 30% of this pollen was redistributed around their body ($\mu = 0,3$).

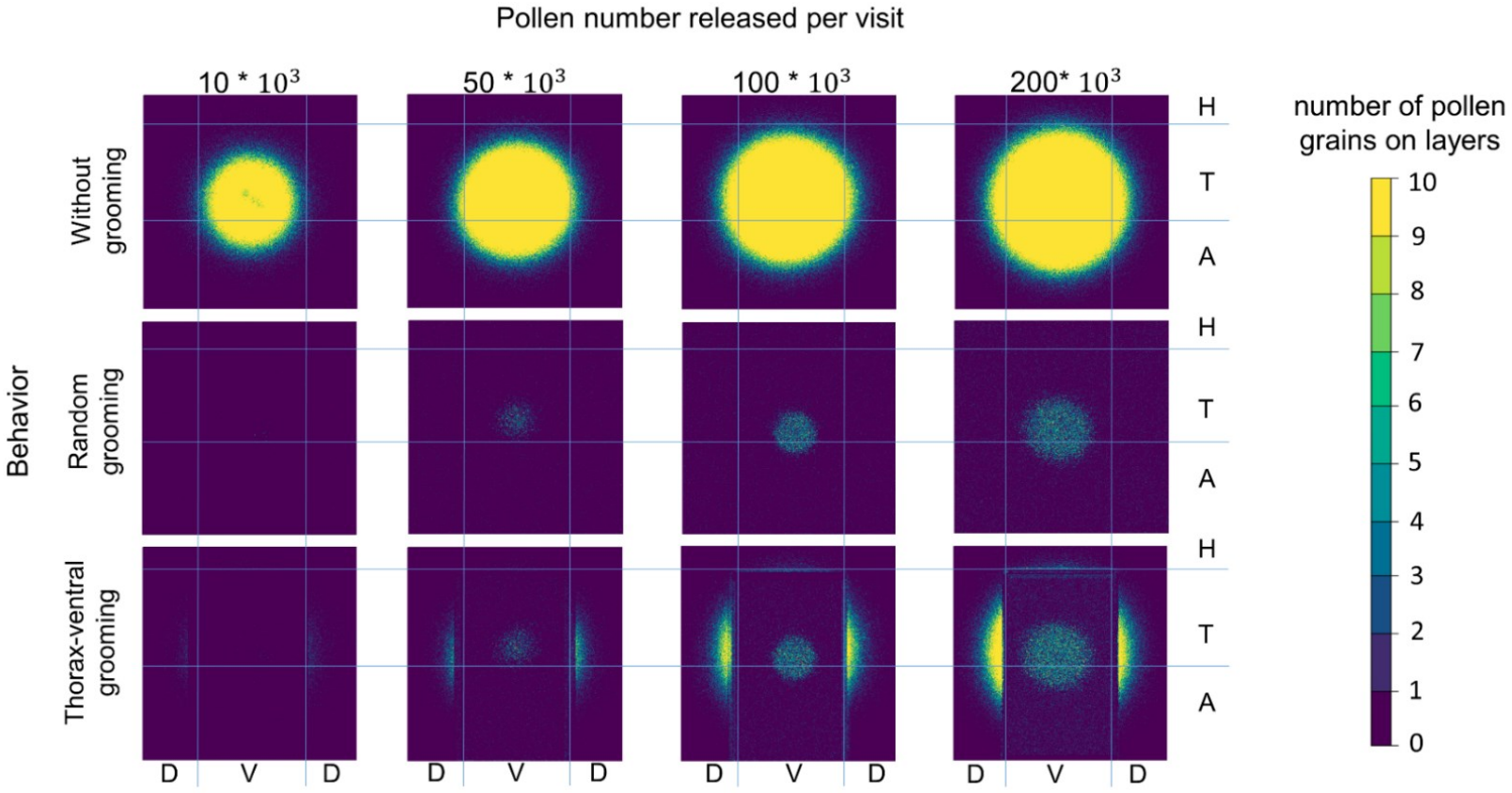

Figure S5. The total number of pollen grains accumulated on the bee´s body after a hundred visits in flowers with different pollen numbers released per visit (10 x 10^3, 50 x 10^3, 100 x 10^3 and 200 x 10^3), and according to different grooming behaviors (without grooming, random grooming, and ventral grooming). Blue lines indicate the boundaries of the bee's body regions: horizontal lines delimit the head (H), thorax (T), and abdomen (A) regions while vertical lines delimit the ventral (V) and dorsal (D) regions. The yellow color indicates the highest number of pollen grains. The bees in this figure are the result of a single iteration, after 100 visits, on 100 different flowers, with flowers having pollen patch values of 30 positions, releasing 200,000 pollen per visit and their ovules supporting 400 pollen grains. The bees had 400 positions in height and width and 10 in depth. Furthermore, the bees were able to touch 80% of the body regions ($\Gamma = 0,8$) and 30% of this pollen was redistributed around their body ($\mu = 0,3$).

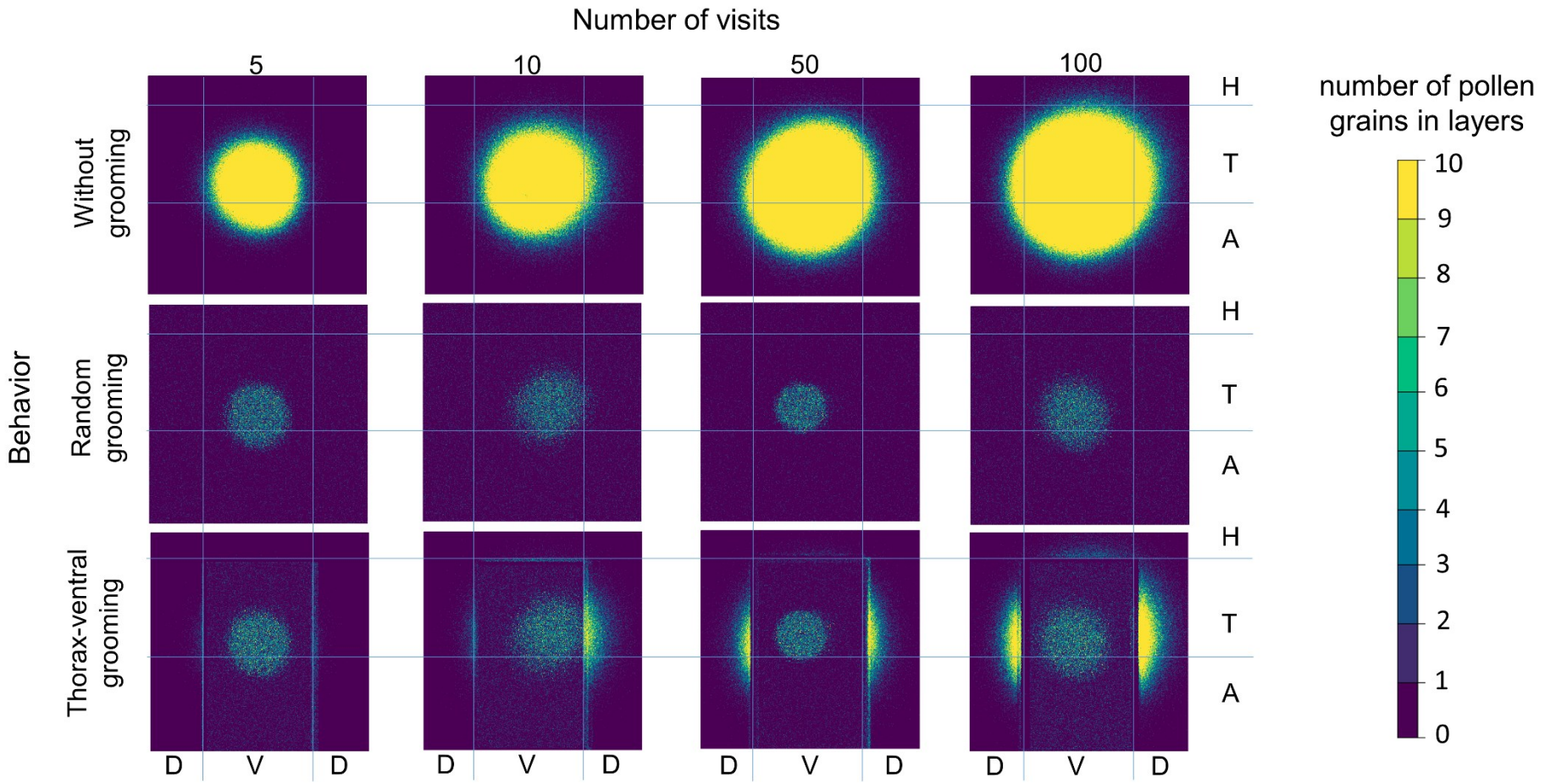

Figure S6. The total number of pollen grains accumulated on the bee´s body after different numbers of visits (1, 10, 50, and 100) and according to different grooming behaviors (without grooming, random grooming, and ventral grooming). Blue lines indicate the boundaries of the bee's body regions: horizontal lines delimit the head (H), thorax (T), and abdomen (A) regions while vertical lines delimit the ventral (V) and dorsal (D) regions. The yellow color indicates the highest number of pollen grains. The bees in this figure are the result of a single iteration, after 100 visits, on 100 different flowers, with flowers having pollen patch values of 30 positions, releasing 200,000 pollen per visit and their ovules supporting 400 pollen grains. The bees had 400 positions in height and width and 10 in depth. Furthermore, the bees were able to touch 80% of the body regions ($\Gamma = 0,8$) and 30% of this pollen was redistributed around their body ($\mu = 0,3$).

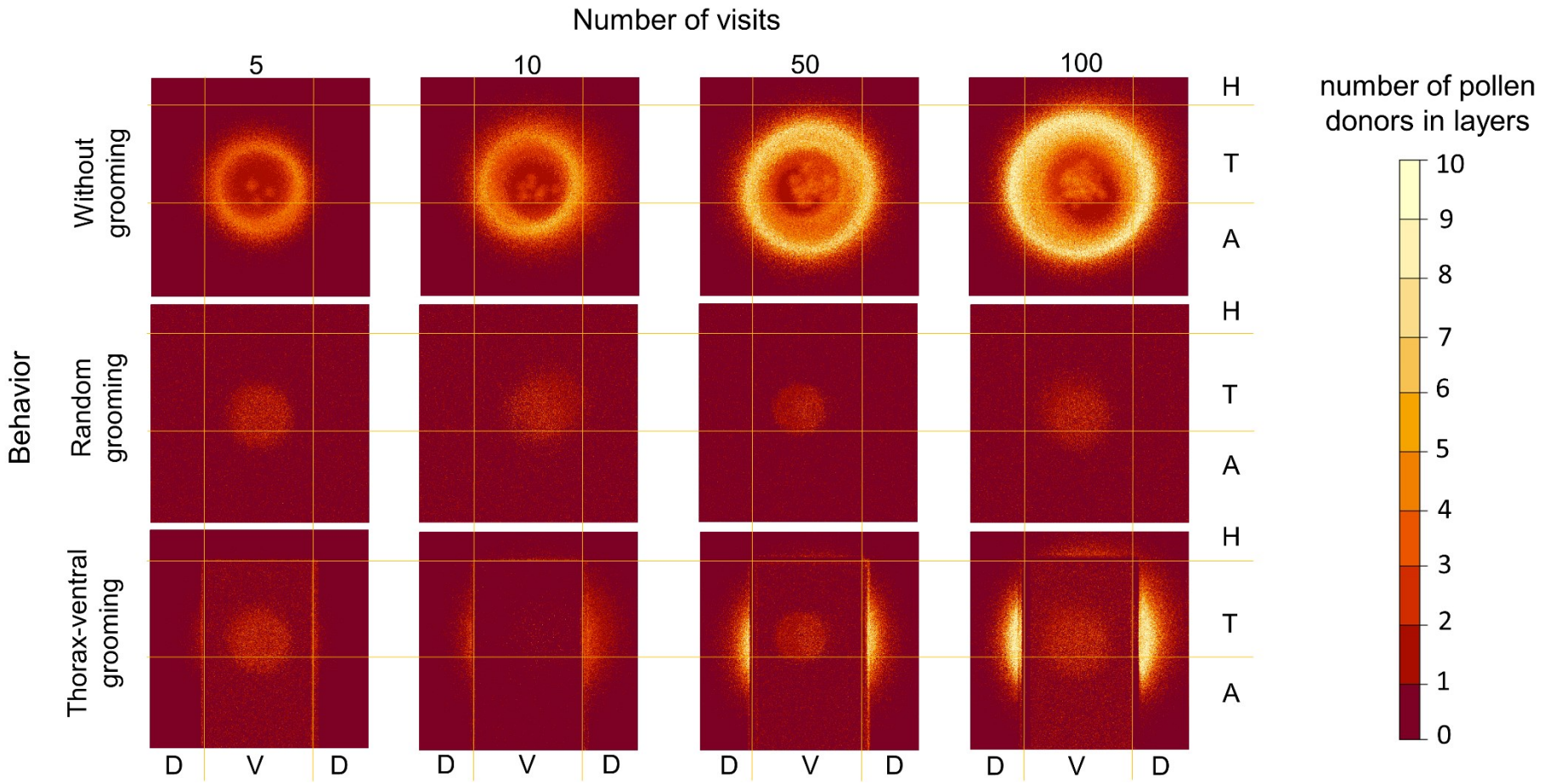

Figure S7. Number of different pollen grain donors on the bee´s body after different numbers of visits (1, 10, 50, and 100) and according to different grooming behaviors (without grooming, random grooming, and ventral grooming). Yellow lines indicate the boundaries of the bee's body regions: horizontal lines delimit the head (H), thorax (T), and abdomen (A) regions while vertical lines delimit the ventral (V) and dorsal (D) regions. The light ivory color indicates the highest number of different pollen donors. The bees in this figure are the result of a single iteration, after 100 visits, on 100 different flowers, with flowers having pollen patch values of 30 positions, releasing 200,000 pollen per visit and their ovules supporting 400 pollen grains. The bees had 400 positions in height and width and 10 in depth. Furthermore, the bees were able to touch 80% of the body regions ($\Gamma = 0,8$) and 30% of this pollen was redistributed around their body ($\mu = 0,3$).

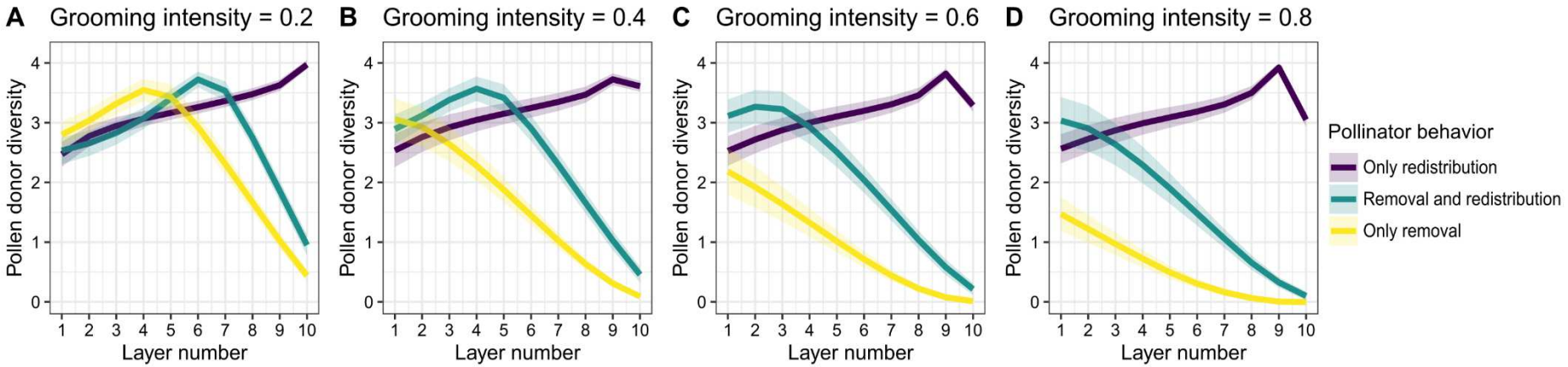

Figure S8. Diversity (Shannon index values) of pollen grains on the layers of the bee's body according to different intensities of grooming and removal/redistribution values in random grooming bees after 100 floral visits. On the x-axis, the higher the layer number, the outermost the layer. The pollinator behavior is represented by different colors, in which purple is "only redistribution" ($\mu = 0.99$), navy-blue is "removal and redistribution" ($\mu = 0.5$) and yellow is "only removal" ($\mu = 0$). Number of iterations = 100.

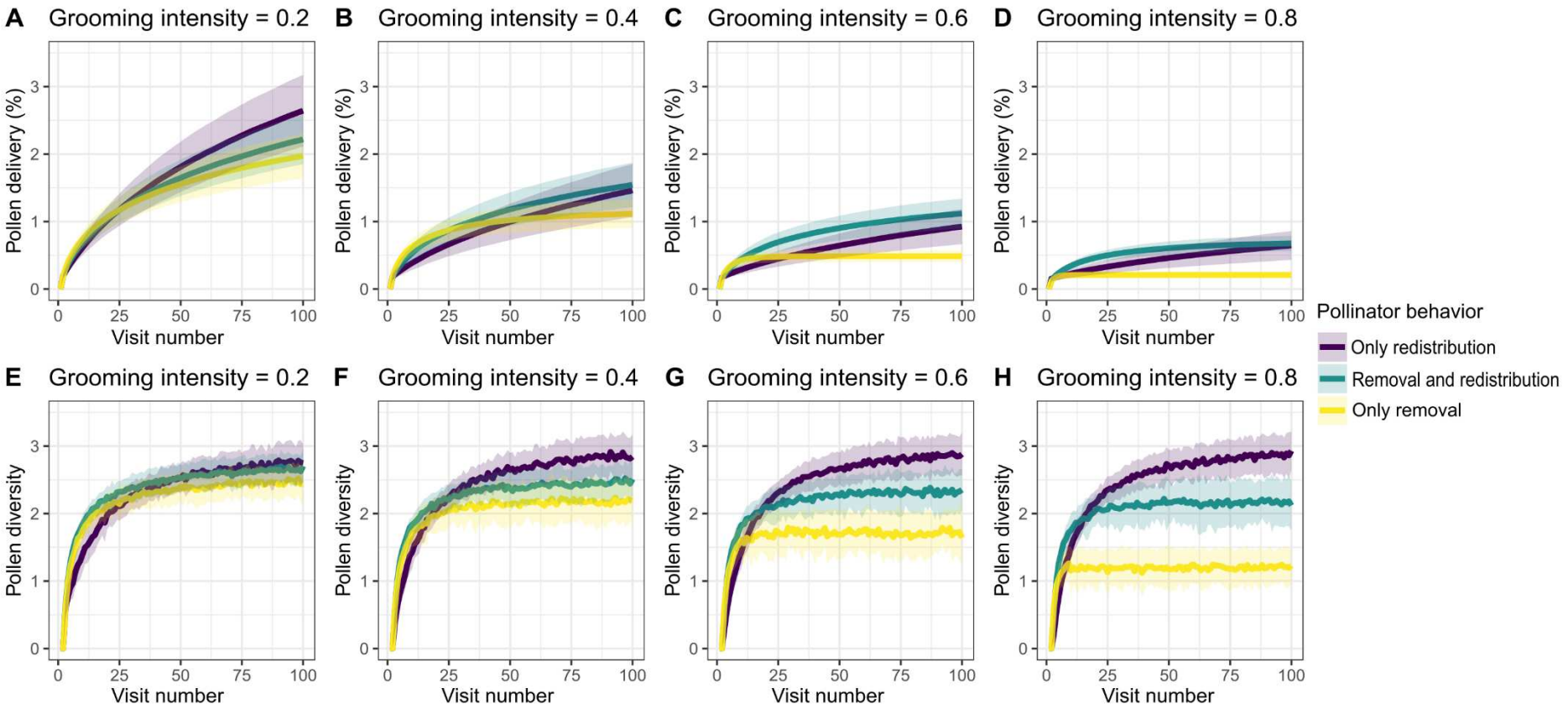

Figure S9. Effect of grooming intensity and redistribution in random grooming bees on the fitness of the first visited flower, measured by the proportion of released pollen grains from the first flower that is delivered to stigmas of conspecific flowers (A-D). And effect of grooming intensity and redistribution on the diversity (Shannon index) of pollen grains received by stigmas in sequential visits (E-H). The pollinator behavior is represented by different colors, in which purple is "only redistribution" ($\mu = 0.99$), navy-blue is "removal and redistribution" ($\mu = 0.5$) and yellow is "only removal" ($\mu = 0$). Number of iterations = 100.

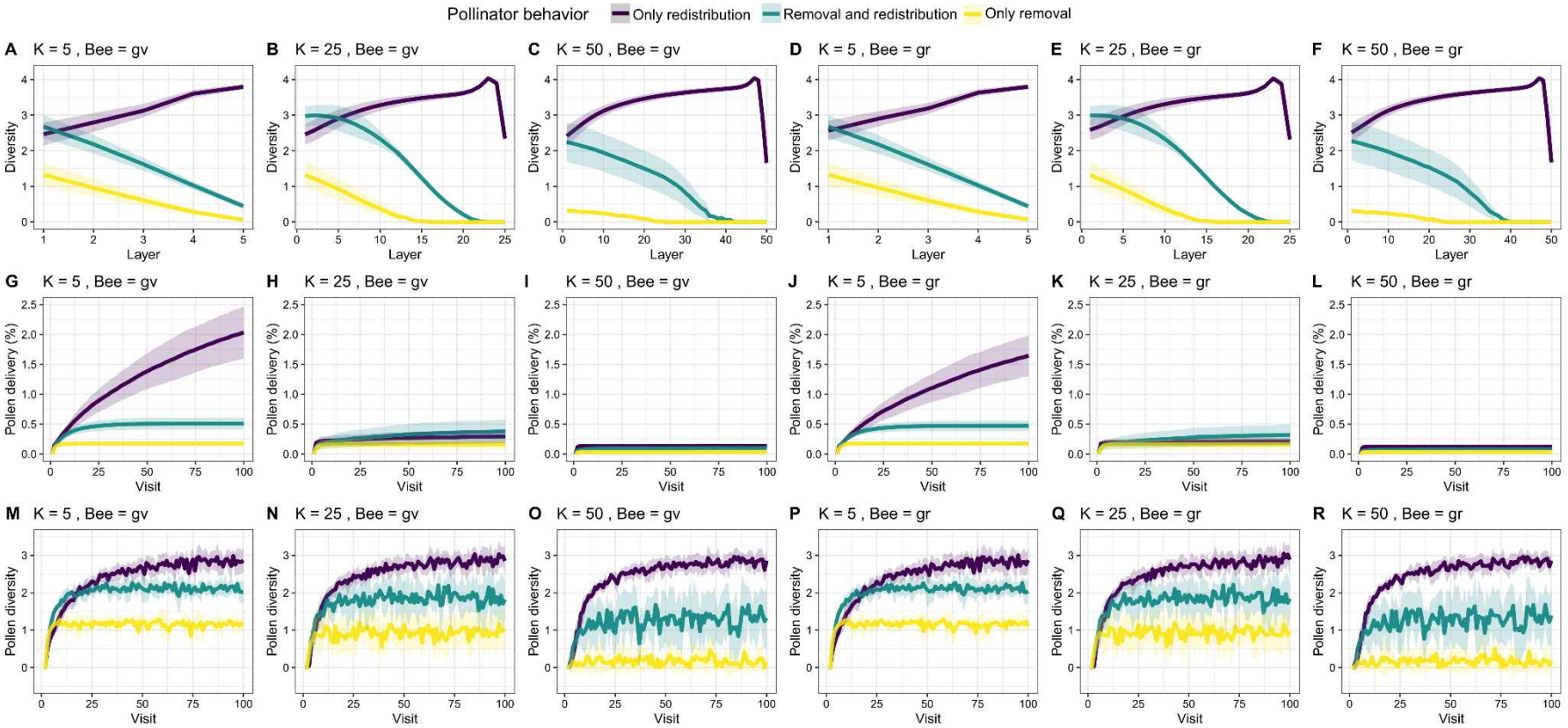

Figure S10. K parameter space analysis. k indicates the number of layers in the bee's body. Plots show (A-F) pollen diversity in bee body layers, (G-L) percentage of pollen from the first flower delivered to conspecific stigma, and (M-R) pollen diversity on stigmas in sequential visits, when k is 5 (A, G ,M, D, J ,P), 25 (B, H, N, E, K, Q) and 50 (C, I, O, F, L, R) and when behavior is thorax-ventral grooming (A, B, C, G, H, I, M, N, O) and random grooming (D, E, F, J, K, L, P, Q, R). The pollinator behavior is represented by different colors, in which purple is “only redistribution” ($\mu = 0.99$), navy-blue is “removal and redistribution” ($\mu = 0.5$) and yellow is “only removal” ($\mu = 0$). Number of iterations = 10.

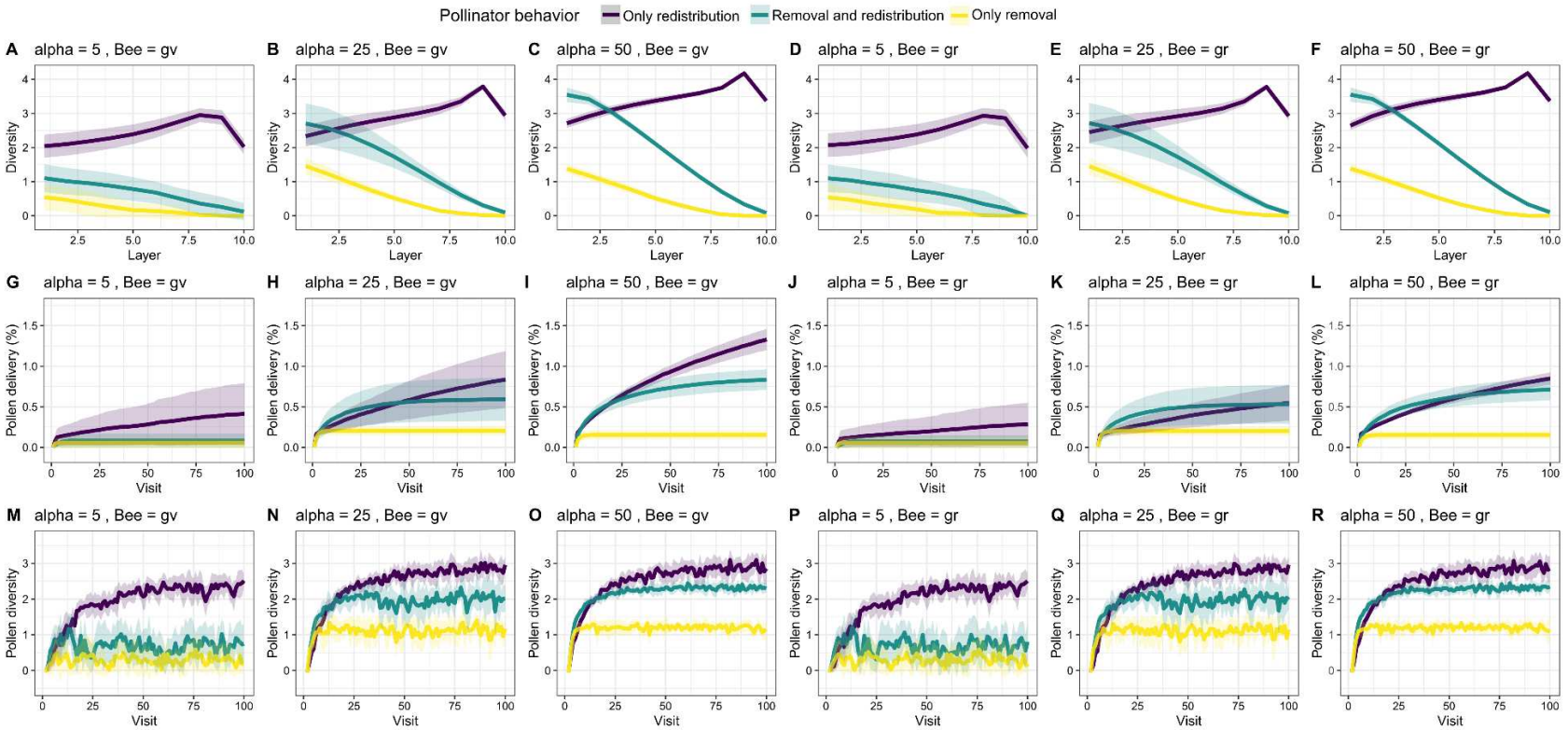

Figure S11. Alpha parameter space analysis (α). Alpha indicates the number of positions that delimit the pollen patch. Plots show (A-F) pollen diversity in bee body layers, (G-L) percentage of pollen from the first flower delivered to conspecific stigma, and (M-R) pollen diversity on stigmas in sequential visits, when alpha is 5 (A, G ,M, D, J ,P), 25 (B, H, N, E, K, Q) and 50 (C, I, O, F, L, R) and when behavior is thorax-ventral grooming (A, B, C, G, H, I, M, N, O) and random grooming (D, E, F, J, K, L, P, Q, R). The pollinator behavior is represented by different colors, in which purple is "only redistribution" (μ = 0.99), navy-blue is "removal and redistribution" (μ = 0.5) and yellow is "only removal" (μ = 0). Number of iterations = 10.

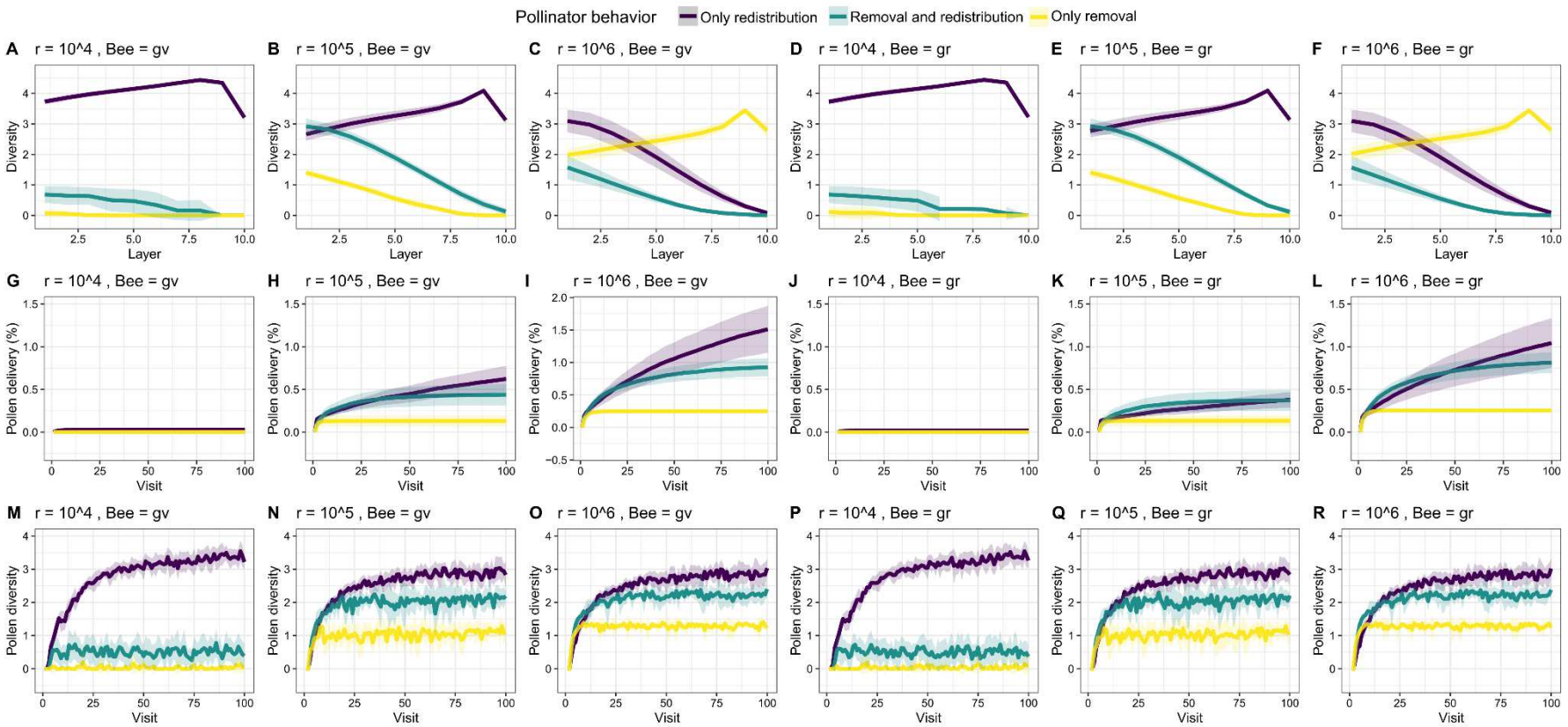

Figure S12. R parameter space analysis. r indicates the number of pollen released per visit. Plots show (A-F) pollen diversity in bee body layers, (G-L) percentage of pollen from the first flower delivered to conspecific stigma, and (M-R) pollen diversity on stigmas in sequential visits, when r is 10^4 (A, G ,M, D, J ,P), 10^5 (B, H, N, E, K, Q) and 10^6 (C, I, O, F, L, R) and when behavior is thorax-ventral grooming (A, B, C, G, H, I, M, N, O) and random grooming (D, E, F, J, K, L, P, Q, R). The pollinator behavior is represented by different colors, in which purple is "only redistribution" ($\mu = 0.99$), navy-blue is "removal and redistribution" ($\mu = 0.5$) and yellow is "only removal" ($\mu = 0$). Number of iterations = 10.

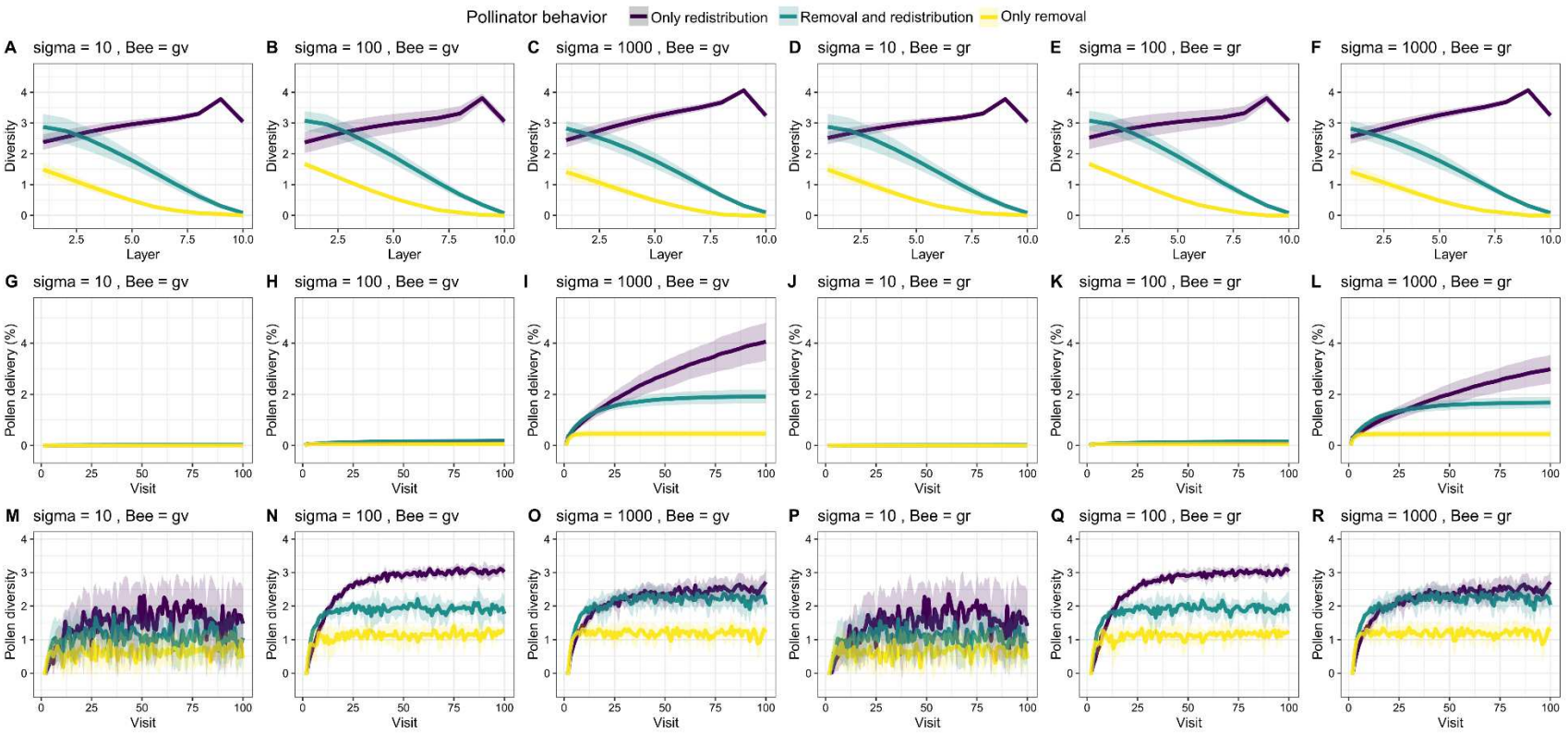

Figure S13. Sigma parameter space analysis (σ). Sigma indicates the number of pollen received by stigma. Plots show (A-F) pollen diversity in bee body layers, (G-L) percentage of pollen from the first flower delivered to conspecific stigma, and (M-R) pollen diversity on stigmas in sequential visits, when sigma is 10 (A, G ,M, D, J ,P), 100 (B, H, N, E, K, Q) and 1000 (C, I, O, F, L, R) and when behavior is thorax-ventral grooming (A, B, C, G, H, I, M, N, O) and random grooming (D, E, F, J, K, L, P, Q, R). The pollinator behavior is represented by different colors, in which purple is "only redistribution" (μ = 0.99), navy-blue is "removal and redistribution" (μ = 0.5) and yellow is "only removal" (μ = 0). Number of iterations = 10.

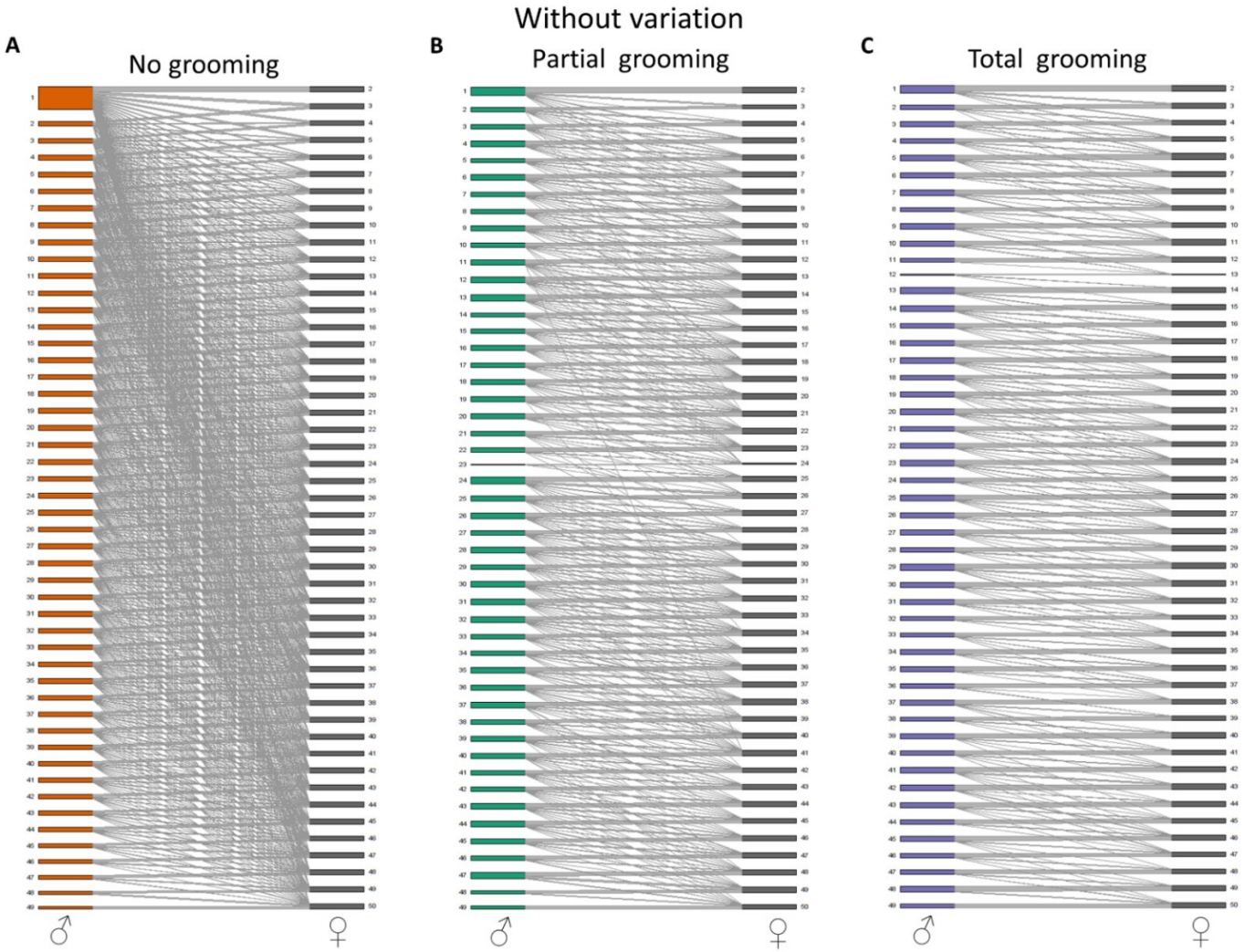

Figure S14. Interaction networks generated from the interaction of male and female individuals without variation in anther and stigma positions with bees with different behaviors: grooming, partial grooming and total grooming.

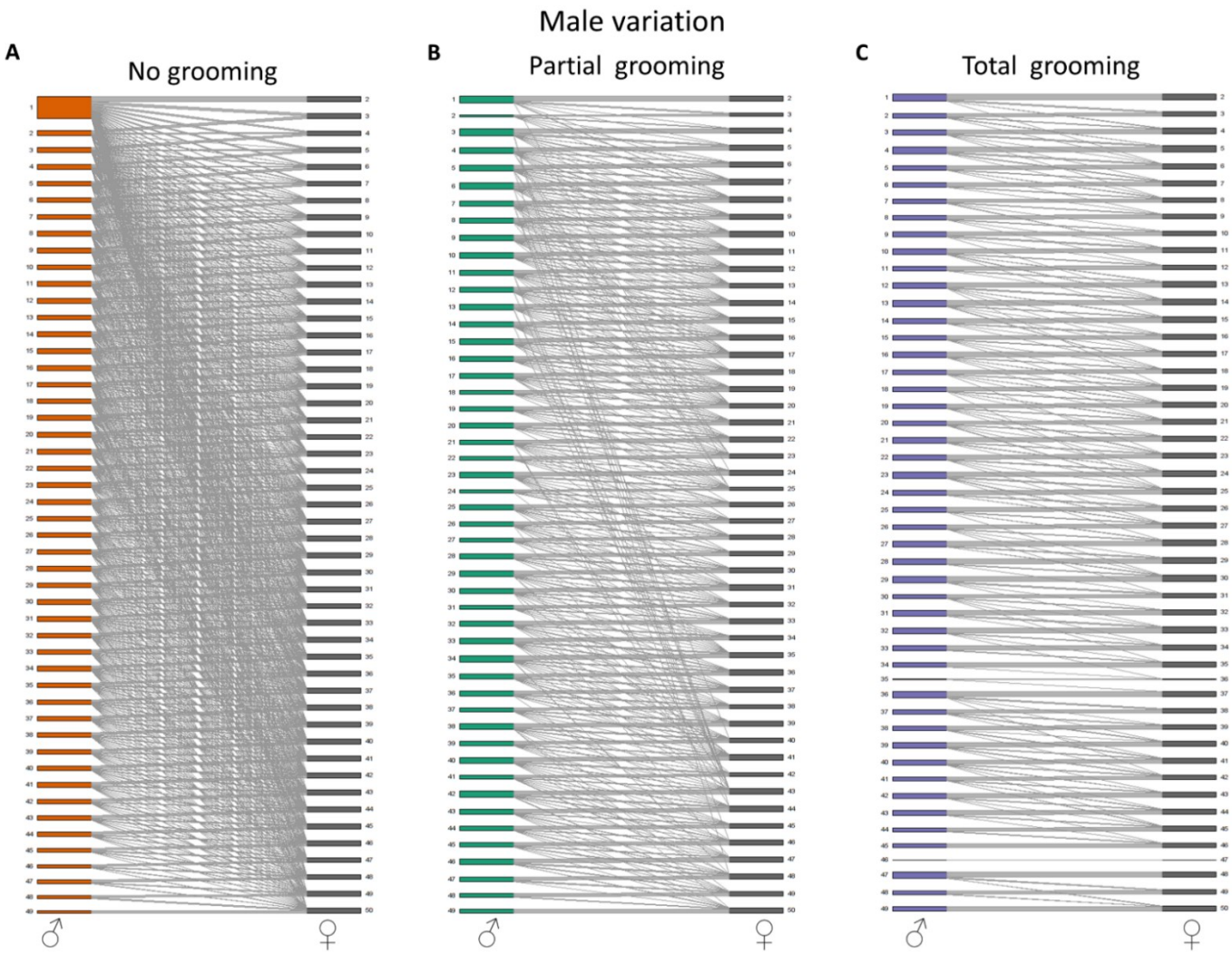

Figure S15. Interaction networks generated from the interaction of male individuals with variation in anther positions and female individuals without variation in stigma with bees with different behaviors: grooming, partial grooming and total grooming.

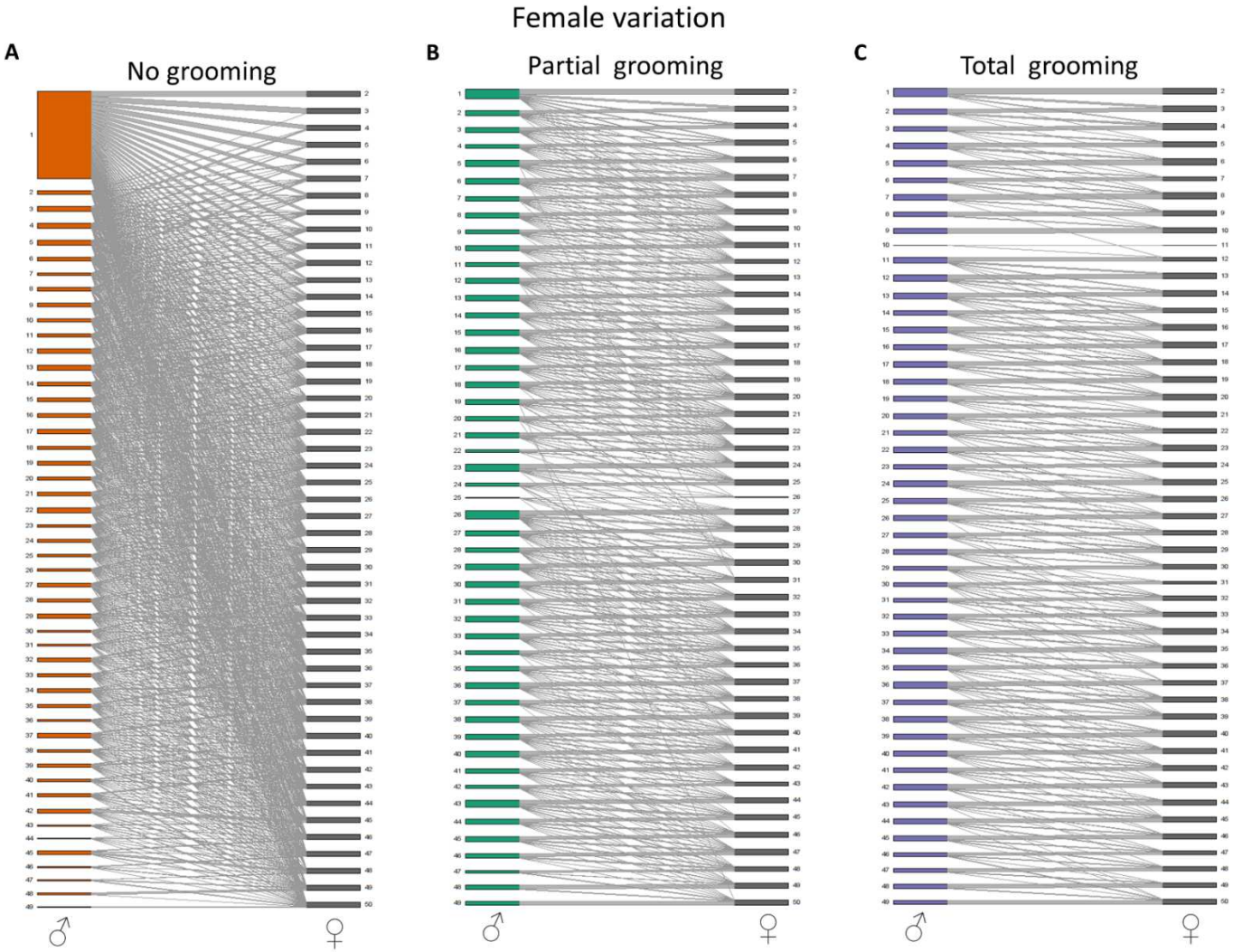

Figure S16. Interaction networks generated from the interaction of male individuals without variation in anther positions and females with variation in stigma position with bees with different behaviors: grooming, partial grooming and total grooming.

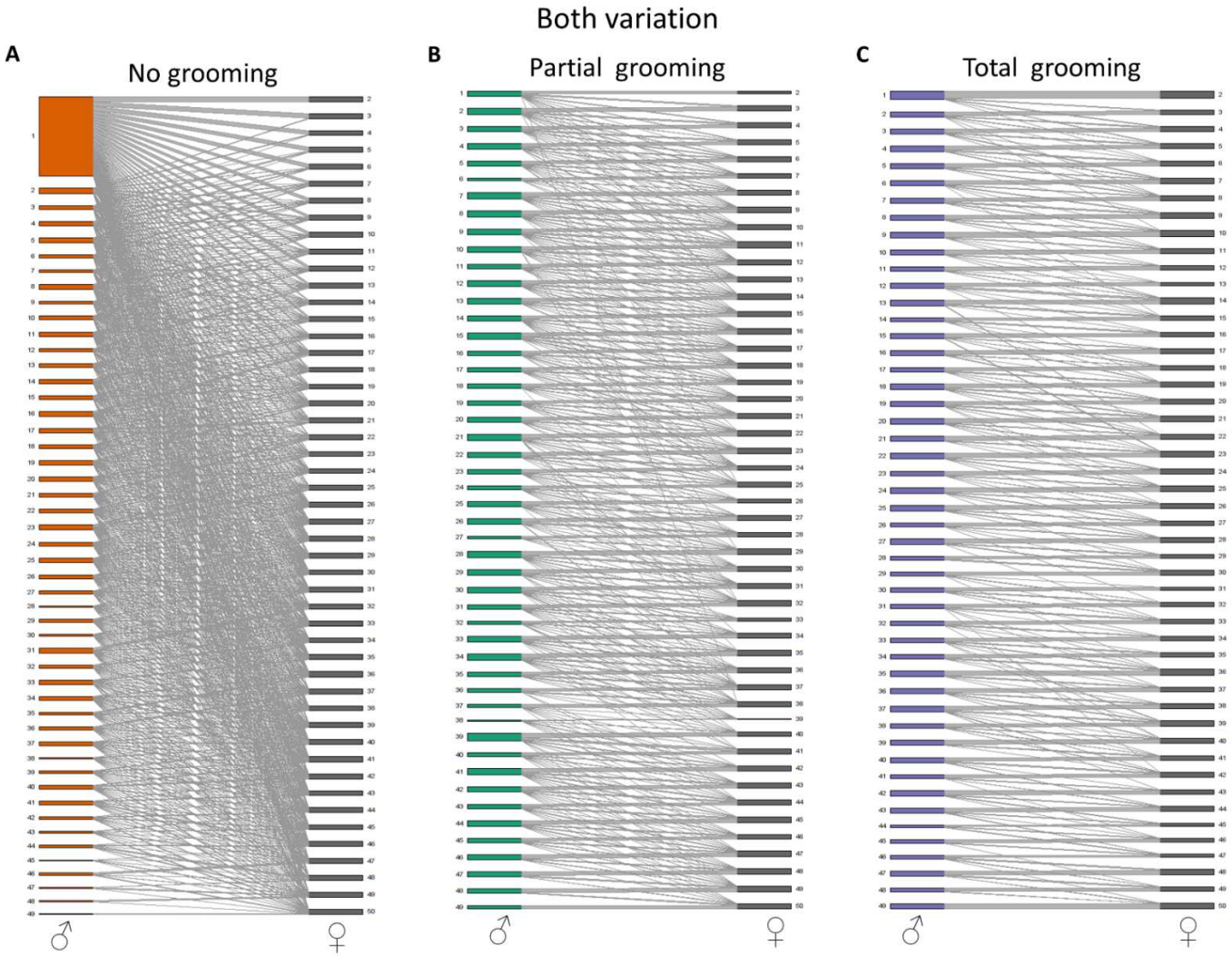

Figure S17. Interaction networks generated from the interaction of male and female individuals with variation in anther position and stigma with bees with different behaviors: grooming, partial grooming and total grooming.