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Écologie évolutive de la reproduction chez une plante dioïque : effets du paysage, des pollinisateurs et de la compétition pollinique

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I. LA FLEUR, PRODUIT DE L'EVOLUTION ADAPTATIVE

Les fleurs, dont la fonction principale est d'assurer la reproduction sexuée chez les angiospermes, présentent une remarquable diversité de traits, reflétant la variété des stratégies de reproduction au sein de ce groupe et ayant probablement contribué à son succès évolutif. Comprendre comment une telle diversité a évolué est une question centrale en biologie évolutive.

I-1. Diversité des stratégies de reproduction et variation des traits floraux

I-1-a. Systèmes sexuels

Le système sexuel ancestral et le plus répandu chez les angiospermes est l'hermaphrodisme, présent chez environ 72% des espèces (Takhtajan, 1969 ; Renner, 2014). Chez les espèces hermaphrodites, chaque fleur possède à la fois les fonctions sexuelles mâle et femelle. Cependant, il existe d'autre systèmes sexuels présentant une grande variété dans la répartition des fonctions sexuelles entre les fleurs et les individus au sein des populations. Par exemple, une espèce est dite monoïque lorsque chaque individu porte à la fois des fleurs mâles et femelles séparées (7% des espèces d'angiospermes, Cronk, 2022), et dioïque lorsque chaque individu ne possède qu'une seule fonction sexuelle (6 à 7% des espèces d'angiospermes, Renner, 2014). Il existe également des formes intermédiaires entre l'hermaphrodisme et la dioécie, comme la gynodioécie, où une population comprend à la fois des individus hermaphrodites et des individus femelles.

I-1-b. Systèmes de reproduction

Chez les angiospermes chez lesquelles les deux fonctions sexuelles sont présentes au sein d'un seul et même individu, la reproduction sexuée peut avoir lieu selon deux stratégies opposées. Tout d'abord, elle peut s'effectuer au sein d'une même plante ; les grains de pollen, qui contiennent les gamètes mâles, sont alors déposés sur les stigmates du même individu : c'est ce qu'on appelle l'auto-pollinisation. Lorsque ces grains de pollen atteignent les ovules, qui contiennent quant à eux les gamètes femelles, la fécondation a lieu, pouvant conduire à la formation de graines. Dans ce cas, on parle d'autogamie (ou auto-fécondation). Environ 40% sont au moins partiellement autogames, et cette autogamie est obligatoire chez 10 à 15% d'entre elles (Goodwillie et al., 2005). Alternativement, la pollinisation peut avoir lieu entre deux individus distincts : c'est ce que l'on appelle l'allo-pollinisation, pouvant mener à l'allogamie (ou allo-fécondation). Ce système de reproduction, considéré comme ancestral (Stebbins, 1974 ; Barrett, 2010), est retrouvé chez environ 50% des espèces d'angiospermes (Igic & Kohn, 2006). Différentes adaptations peuvent favoriser l'allogamie chez les espèces hermaphrodites, y compris l'auto-incompatibilité (i.e., mécanisme empêchant la fécondation des ovules d'un individu par les grains de pollen du même individu), la dichogamie (i.e., séparation temporelle des organes sexuels mâle et femelle) et l'herkogamie (i.e., séparation spatiale des organes sexuels mâle et femelle). Certaines espèces

combinent autogamie et allogamie, on parle alors de système de reproduction mixtes. Chez les espèces dioïques et les espèces auto-incompatibles, seule l'allogamie est possible.

I-1-c. Modes de dispersion du pollen

Les plantes à fleurs sont des organismes sessiles et non sentients et ne peuvent donc pas rechercher activement leurs partenaires sexuels. Leur reproduction sexuée repose généralement sur un agent externe, communément appelé vecteur de pollen. Ce vecteur peut être abiotique (vent, eau, Ackerman, 2000 ; Culley et al., 2002) ou biotique (animaux, Ollerton et al., 2011), ce dernier correspondant à l'état ancestral (Linder, 1998 ; Barrett, 2010). La pollinisation par les animaux, prépondérante chez les angiospermes concerne au moins 80% des espèces (Ollerton et al., 2011 ; Tong et al., 2023). Au total, environ 350 000 espèces d'animaux sont reportées comme étant des pollinisateurs (**Figure 1**, Ollerton, 2017), incluant à la fois des vertébrés (Regan et al., 2015) et des invertébrés (Wardhaugh, 2015).

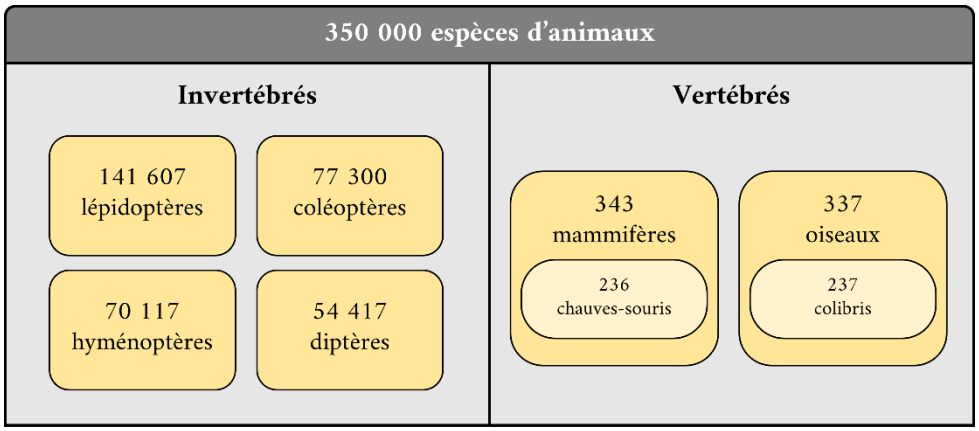

Figure 1. Nombre d'espèces animales impliquées dans la pollinisation, organisées par grand groupe taxonomique (Regan et al., 2015 ; Wardhaugh, 2015 ; Ollerton, 2017).

I-1-d. Traits floraux et syndromes de pollinisation

Les traits floraux regroupent l'ensemble des caractéristiques liées à la structure des fleurs, telles que le nombre, la taille, la forme, la couleur ou encore l'odeur des fleurs, mais également la position relative des organes reproducteurs, la quantité de gamètes produite ou encore le ratio entre le nombre de grains de pollen produit et celui des ovules. Dans le paragraphe suivant, nous décrirons les suites de traits typiquement associées à diverses stratégies de reproduction, et verrons en quoi ces suites de traits constituent des syndromes de pollinisation, ayant évolué de manière conjointe en réponse aux pressions de sélection liées à la pollinisation.

Contrairement aux espèces allogames, les espèces autogames sont souvent caractérisées par des fleurs de petite taille, peu colorées et peu odorantes, produisant peu de pollen et de nectar, traits reflétant leur plus faible dépendance aux vecteurs de pollen pour la reproduction (Barrett, 2010 ; Slotte et al., 2012). Les fleurs des espèces autogames peuvent également être moins ouvertes, ce qui peut mener dans les cas extrêmes à la cléistogamie (i.e., lorsque la fécondation a lieu sans que la fleur ne s'ouvre, **Figure 2**). De

plus, les organes mâle et femelle sont peu séparés spatialement et temporellement, favorisant le contact entre ceux-ci (i.e., réduction de la dichogamie et de l'herkogamie, Brys et al., 2013 ; Torang et al., 2017). Enfin, le ratio pollen/ovule est plutôt faible car la fécondation n'implique pas de pertes importantes en pollen durant le transport (Yashiro et al., 1999).

Chez les espèces d'angiospermes à pollinisation anémophile (i.e., pollinisées par le vent), on observe souvent une forte production de fleurs, ce qui favorise la production de grains de pollen et maximise les chances que ces derniers atteignent les stigmates de fleurs réceptives. Les grains de pollen étant dispersés de façon aléatoire, ils sont également produit en plus grande quantité au sein de chaque fleur par rapport aux espèces à pollinisation biotique (Welsford et al., 2016), et le ratio pollen/ovule est également plus élevé afin de maximiser les chances de rencontre avec un ovule. Cet investissement important dans la fonction mâle est cependant modulé par certains traits, notamment la hauteur des plantes (Burd & Allen, 1988). De plus, les stigmates des espèces anémophiles sont souvent longs et plumeux, facilitant ainsi la capture de pollen (Sullivan, 1975). Enfin, les fleurs sont de petites tailles, souvent peu colorées et inodores, reflétant une absence de dépendance aux vecteurs de pollen animaux (**Figure 2**, Culley et al., 2002 ; Friedman & Barrett, 2009). Chez les espèces à pollinisation biotique, les grains de pollen sont souvent ornés de reliefs, ce qui facilite leur adhésion au corps des pollinisateurs (Lu et al., 2022). Les stigmates sont généralement plus petits, puisqu'ils ne doivent pas intercepter le pollen transporté par le vent, celui-ci étant directement déposé sur la fleur (Sullivan, 1975). Chez les espèces à pollinisation biotique, la reproduction repose en grande partie sur la capacité des individus à attirer de façon efficace les pollinisateurs, qui dépend notamment de leurs traits floraux dits "attractifs". Il est possible de classer ces traits en deux grandes catégories : les signaux visuels et les signaux olfactifs, ces signaux pouvant être en outre associés à des "récompenses" pour le pollinisateur (e.g., ressources nutritives, sites de ponte) (Wiebes, 1979 ; Simpson & Neff, 1981 ; Raguso, 2008 ; Hossaert-McKey et al., 2010 ; Nicolson, 2011 ; Vaudo et al., 2015).

Les signaux visuels, tels que le nombre et la taille des fleurs facilitent la détection de ces dernières, et donc potentiellement la fréquence des visites de la part des pollinisateurs (Harder & Barrett, 1995 ; Ohashi & Yahara, 1998 ; Totland, 2001 ; Gómez et al., 2008 ; Bauer et al., 2017). Ces traits peuvent également constituer un signal de la quantité de pollen et/ou de nectar disponible (Stanton & Preston, 1988 ; Makino & Sakai, 2007 ; Brunet et al., 2015). La couleur florale peut également faciliter la détection des fleurs par les pollinisateurs (Lunau, 1991 ; Giurfa et al., 1995 ; Briscoe & Chittka, 2001 ; Gegear & Burns, 2007 ; Lunau et al., 2021), mais elle peut aussi guider leurs choix alimentaires. Les pollinisateurs présentent souvent des préférences innées pour certaines couleurs, qui varient selon leurs capacités sensorielles (par exemple, hyménoptères : fleurs violettes ou jaune, Fenster et al., 2004 ; oiseaux : rouges ou oranges, Raven, 1972) mais peuvent être modulées par l'apprentissage (Riveros & Gronenberg, 2012). De même, les signaux olfactifs tels que les bouquets de composés organiques volatiles (COVs) émis par les fleurs peuvent favoriser l'attraction des pollinisateurs à longue distance,

mais aussi guider leurs choix alimentaires (Chittka & Raine, 2006 ; Raguso, 2008 ; Wright & Schiestl, 2009).

Après l'attraction des pollinisateurs, le pollen doit être efficacement prélevé depuis les anthères (fonction mâle) ou déposé sur le stigmate (fonction femelle), cette efficacité dépendant étroitement de l'ajustement entre la fleur et le corps des pollinisateurs. Divers traits peuvent contribuer à cet ajustement, notamment la largeur et la profondeur du tube floral et la position des organes reproducteurs (Campbell et al., 1996 ; Alexandersson & Johnson, 2002). La correspondance étroite entre la profondeur des tubes floraux et la longueur du bec des colibris (Poblete Palacios et al., 2019), ou de la trompe des papillons (Bloch & Erhardt, 2008) observée dans plusieurs systèmes est souvent citée comme un exemple classique de coadaptation et illustre l'importance de l'ajustement aussi bien pour la plante que pour le pollinisateur.

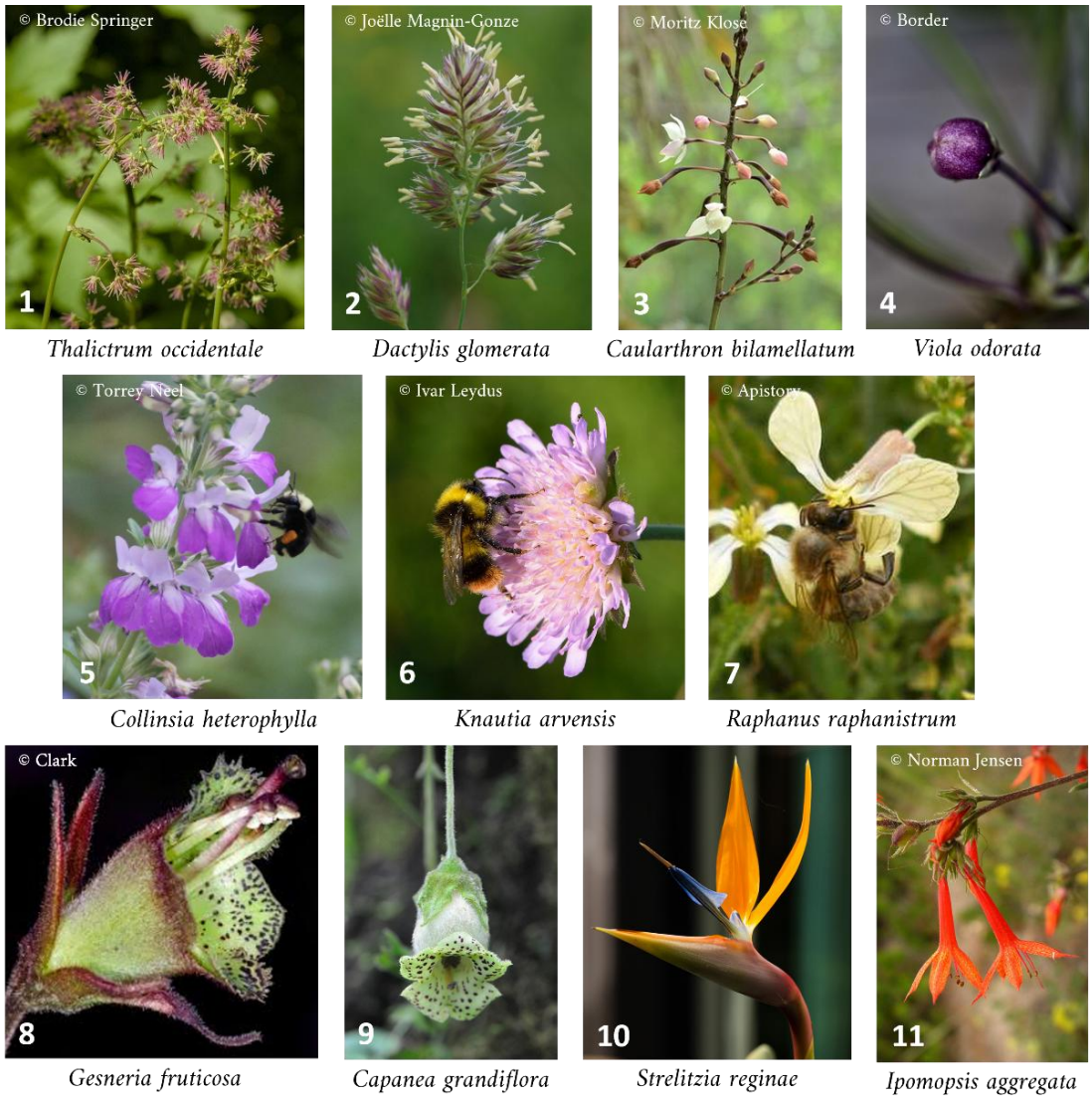

Figure 2. Exemples représentant la variation des traits floraux chez les angiospermes, illustrant différents syndromes de pollinisation. Les espèces 1 et 2 correspondent à des espèces anémophiles. Les espèces 3 et 4 correspondent à des espèces cléistogames. Les espèces 5, 6 et 7 sont pollinisées par des hyménoptères. Les espèces 8 et 9 sont pollinisées par des chauves-souris. Les espèces 10 et 11 sont pollinisées par des oiseaux.

Tout comme certaines combinaisons de traits caractéristiques ont évolué de manière récurrente chez les espèces autogames ou anémophiles, divers syndromes de pollinisation ont évolué en association avec les différents types d'animaux pouvant assurer la pollinisation biotique (**Figure 2**, Fenster et al., 2004 ; Junker & Parachnowitsch, 2015). Par exemple, les plantes pollinisées par des oiseaux présentent généralement des fleurs rouges, peu odorantes, produisant de grande quantités de nectar (Cronk & Ojeda, 2008). Les plantes pollinisées par des chauves-souris possèdent souvent des fleurs vertes ou blanches, en forme de cloche, sécrétant du nectar durant la nuit (Martén-Rodríguez et al., 2009). Enfin, les espèces pollinisées par des hyménoptères ont des fleurs souvent violettes ou jaunes dotées d'un périanthe jouant le rôle de plateforme d'atterrissage et produisant du nectar en quantité modérée (Fenster et al., 2004). Les études contrôlant pour l'effet de la phylogénie confirment l'importance des pollinisateurs dans l'évolution des traits floraux à l'échelle macro-évolutive, et mettent en lumière des évolutions convergentes et des transitions de traits floraux faisant suite à des transitions de pollinisateurs (van der Niet & Johnson, 2012). Par exemple, l'étude de Darragh (2025) sur des espèces ancestralement pollinisées par des hyménoptères montre que la transition vers la pollinisation par des oiseaux entraîne systématiquement une perte du bouquet floral, tandis que chez les espèces restant pollinisées par les hyménoptères, ce trait est conservé, voire renforcé.

Au-delà de la large diversité interspécifique des traits floraux, il est également fréquent d'observer de la variation phénotypique au sein d'une même espèce, notamment entre différentes populations. Des variations phénotypiques entre populations ont ainsi été documentées dans de nombreux systèmes pour différents traits attractifs tels que la couleur (Newman et al., 2012), l'odeur (Mant et al., 2005 ; Joffard et al., 2020), la forme de la corolle (Gómez et al., 2008) ou encore le nombre de fleurs par inflorescence (Gilbert et al., 1996). Si ces variations phénotypiques peuvent résulter de multiples processus—tels que la dérive génétique[1] ou la plasticité phénotypique[2]—les études indiquent qu'elles sont souvent adaptatives, notamment vis-à-vis des variations dans le contexte de pollinisation (Brys & Jacquemyn, 2012 ; Acoca-Pidolle et al., 2024), bien que les pollinisateurs ne soient pas les seuls agents de sélection potentiellement impliqués dans l'évolution des phénotypes floraux (Caruso et al., 2019). Dans les paragraphes qui suivent, nous verrons de quelle manière cette sélection opère et quelles méthodes permettent de la mesurer.

[1] Processus stochastique qui induit des changements aléatoires de fréquences alléliques à cause des effets d'échantillonnage dans la transmission des allèles d'une génération à la suivante. Sa contribution aux variations phénotypiques peut être importante, surtout dans les petites populations (Lande, 1976 ; Lynch, 2007).

[2] Processus d'acclimatation, potentiellement rapide et parfois réversible, par lequel le phénotype d'un individu dépend en partie de l'environnement (Merilä & Hendry, 2014).

I-2. Processus de sélection des traits phénotypiques

Afin de prédire l'évolution d'un trait sous sélection d'une génération à l'autre, il est possible d'utiliser la *Breeder's equation*, un outil central de la génétique quantitative. Cette équation relie la réponse attendue à la sélection à deux paramètres fondamentaux : l'intensité de la sélection exercée sur la population et l'héritabilité du trait considéré. En d'autres termes, elle permet d'estimer dans quelle mesure un trait phénotypique peut évoluer lorsque certains individus contribuent davantage à la génération suivante, offrant ainsi un cadre théorique pour comprendre l'évolution et l'adaptation des populations. Cette équation se présente sous la forme suivante :

$$R = h^2 S,$$

R correspondant à la réponse à la sélection (changement moyen du trait dans la génération suivante), h^2 à l'héritabilité (fraction de la variance phénotypique due à la variance génétique additive) et S au différentiel de sélection (différence moyenne du trait entre les individus sélectionnés et la population initiale).

La première condition pour qu'un trait évolue est donc que ce trait soit héritable. La revue d'Ashman & Majetic (2006) montre qu'en moyenne, l'héritabilité des traits floraux est de 39% (moyenne sur 68 études). En particulier, les traits liés à la production de récompenses pour les pollinisateurs ont une héritabilité plus faible (21%) que ceux liés aux signaux visuels (e.g., 34% pour le nombre de fleurs, 46% pour la taille de la corolle). La deuxième condition pour qu'un trait évolue est que ce trait soit soumis à une pression de sélection, ce qui signifie que certains phénotypes doivent contribuer davantage à la reproduction que d'autres. Dans la *Breeder's equation*, le différentiel de sélection S correspond à l'écart moyen du trait entre les individus qui contribuent à la reproduction et l'ensemble de la population. Lande & Arnold (1983) ont montré que ce différentiel de sélection peut s'interpréter comme la pente de la régression de la *fitness* (i.e., valeur sélective) sur le trait, établissant un lien direct entre la force de la sélection observée et la réponse évolutive attendue. La *fitness* correspond au succès reproducteur des individus mesuré sur l'ensemble de leur vie et peut varier en fonction de la probabilité de survie (i.e., probabilité de multiplier les épisodes de reproduction), de la fertilité (i.e., investissement dans la quantité et/ou la qualité des gamètes) ou du succès d'accouplement (i.e., accès aux partenaires sexuels, **Figure 3**).

Les trois composantes de la *fitness* peuvent être influencées par différents traits floraux, qui peuvent avoir un effet sur une ou plusieurs composantes, parfois dans la même direction et parfois dans des directions opposées. Par exemple, le nombre de fleurs produites peut jouer un rôle à la fois dans la production totale de gamètes, donc être sous sélection de fertilité, et dans l'accès aux partenaires sexuels, en influençant l'attraction des pollinisateurs (Barbot et al., 2022 ; Moquet et al., 2022), et donc être sous sélection sexuelle. Par ailleurs, il peut exister des compromis entre les différentes composantes de la *fitness*, la floraison étant par exemple susceptible d'impacter la croissance l'année suivante (Jacquemyn et al., 2010).

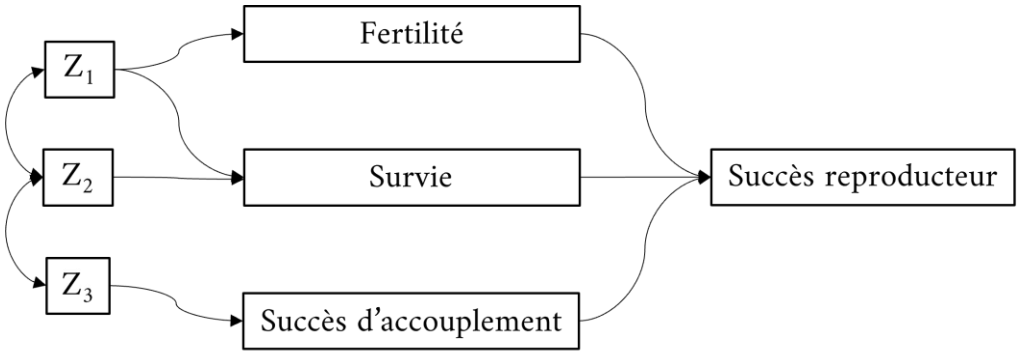

Figure 3. Représentation graphique des types de sélection pouvant influencer le succès reproducteur, où les traits phénotypiques correspondent à Z_i (inspiré de Arnold, 1994). Les flèches entre les traits phénotypiques représentent les covariances génétiques et environnementales possibles entre les traits. Dans cet exemple, tous les traits influencent le succès reproducteur, ils sont donc tous sous sélection. Le trait Z_1 est sous sélection de fertilité et de viabilité, le trait Z_2 sous sélection de viabilité et le trait Z_3 est sous sélection sexuelle.

I-3. Focus sur la sélection sexuelle

La sélection sexuelle, définie par Darwin (Darwin, 1859 ; Darwin, 1871) comme le processus par lequel certains caractères augmentent en fréquence dans les populations parce qu'ils confèrent aux individus qui les portent un avantage dans l'accès aux partenaires sexuels, s'applique aussi bien aux plantes qu'aux animaux (Moore & Pannell, 2011). Chez les plantes, l'accès aux partenaires sexuels dépend de processus ayant lieu à la fois avant et après la pollinisation. La compétition entre individus du même sexe pour l'accès aux partenaires sexuels (i.e., compétition intrasexuelle) peut en effet s'exprimer au stade pré-pollinisation. Dans ce cas, les traits sous sélection sexuelle devraient varier en fonction du mode de dispersion du pollen. Chez les espèces anémophiles, la sélection sexuelle agit par exemple sur les traits liés à l'architecture de l'inflorescence (Tonnabel et al., 2021), tandis que chez les espèces pollinisées par les animaux, elle agit sur les traits impliqués dans l'interaction avec les pollinisateurs, c'est-à-dire ceux ayant un rôle dans l'attraction ou dans l'ajustement morphologique entre la fleur et le pollinisateur, mentionnés précédemment. Pour cette raison, la sélection médiée par les pollinisateurs peut être considérée comme une composante de la sélection sexuelle pré-pollinisation. En effet, la quantité de pollen exportée (pour la fonction mâle) ou reçue (pour la fonction femelle), de même que le nombre de plantes avec lesquelles la plante focale échange du pollen, dépendent directement de la fréquence des visites de la part pollinisateurs, et de l'efficacité de ces visites. Ainsi, les traits qui maximisent l'attraction des pollinisateurs (e.g., nombre de fleurs, Moquet et al., 2020) et/ou l'efficacité du transfert de pollen devraient être sous sélection sexuelle (Barbot et al., 2022), et cette sélection devrait être médiée par les pollinisateurs (Moore & Pannell, 2011 ; Barbot et al., 2023).

La compétition intra-sexuelle peut s'exprimer à nouveau après la pollinisation, cette fois ci entre grains de pollen, pour l'accès aux ovules. On parle alors de compétition pollinique. Une telle compétition

est notamment susceptible d'avoir lieu si le nombre de grains de pollen viables déposés sur les stigmates est supérieur au nombre d'ovules disponibles pour la fertilisation, et si ces grains de pollen proviennent d'individus différents (McCallum & Chang, 2016). Dans ce contexte, si les traits polliniques varient entre donneurs et s'ils ont un effet sur le succès de fertilisation, les grains de pollen de ces donneurs devraient fertiliser un nombre disproportionné d'ovules. Les traits polliniques tels que la taille des grains de pollen (McCallum & Chang, 2016), leur taux de germination ou encore la vitesse de croissance des tubes polliniques (Snow & Spira, 1991 ; Skogsmyr & Lankinen, 2002), devraient alors être sous sélection. A noter que des études montrent que la polyandrie est très commune chez les plantes à l'échelle du fruit, indiquant que les fleurs reçoivent fréquemment du pollen de plusieurs donneurs (Pannell & Labouche, 2013 ; Joffard et al., 2025).

I-4. Méthodes de mesures de la sélection

I-4-a. Différentiels et gradients de sélection

Pour estimer la force, la direction et la forme de la sélection, il est possible d'utiliser des régressions simples entre la *fitness* (**Encadré 1**) et un trait phénotypique quantitatif. La pente de cette régression correspond au différentiel de sélection mentionné précédemment (S). Un trait associé à un différentiel de sélection significatif peut être considéré comme étant sous sélection, et cette sélection peut être directe (i.e., il existe une relation de causalité entre le phénotype et le succès reproducteur) et/ou indirecte (i.e., le trait examiné est corrélé à un autre trait qui affecte le succès reproducteur, sans avoir lui-même d'effet direct sur ce dernier). De telles corrélations entre traits peuvent s'expliquer par des contraintes liées à l'allocation des ressources ou par l'existence de covariances génétiques, pouvant résulter d'une pléiotropie (i.e., un gène influence plusieurs traits simultanément), ou d'un déséquilibre de liaison (i.e., différents gènes sont transmis ensemble plus fréquemment que ne le prédirait une ségrégation indépendante). Afin de dissocier la sélection directe et indirecte, une possibilité est d'avoir recours à des régressions multiples entre le succès reproducteur et plusieurs traits phénotypiques, une méthode proposée par Lande & Arnold (1983). Dans ce cas, chaque pente de la régression multiple est nommée gradient de sélection. De plus, afin de pouvoir comparer l'intensité de la sélection entre différentes études, différentes espèces et différents traits phénotypiques, il est conseillé de relativiser la *fitness* (*fitness* individuelle divisée par la *fitness* moyenne) et de standardiser les traits phénotypiques par leur variance (moyenne = 0 et variance = 1, Lande & Arnold, 1983).

Encadré 1 : Estimation de la *fitness*

La *fitness* est définie par le succès reproducteur des individus mesuré sur l'ensemble de leur vie. Cependant, cette variable est en pratique difficile à quantifier. Ainsi, les études se focalisent généralement sur une composante de la *fitness* seulement. Par exemple, le succès reproducteur peut être estimé sur un seul épisode de reproduction chez des espèces itéropares (Barbot et al., 2023), voire sur une phase de cet épisode de reproduction (Zhou et al., 2020). De plus, le succès reproducteur est le plus souvent mesuré chez une seule fonction sexuelle, généralement la fonction femelle. Ce succès reproducteur peut être alors être quantifié par le nombre de graines produites, le taux de mise à graines (i.e., nombre de graines / nombre d'ovules) ou encore le taux de mise à fruits (i.e., nombre de fruits / nombre de fleurs) (Caruso et al., 2019). Le succès reproducteur mâle peut quant à lui être estimé à partir de mesures de l'export de pollen (Campbell, 1989 ; Campbell et al., 1991). Toutefois, plusieurs études ont montré que ces mesures ne sont pas toujours représentatives du nombre de descendants produits (Harder & Barrett, 1996 ; Snow & Lewis, 1993). La seule méthode permettant d'estimer directement ce nombre repose sur l'utilisation d'analyses de paternité (Meagher, 1986 ; Hodgins & Barrett, 2008 ; Chen & Pannell, 2023), qui nécessitent le génotypage desdits descendants et de leurs parents potentiels à l'aide de marqueurs moléculaires polymorphes. L'assignation de la paternité s'effectue ensuite à l'aide de méthodes statistiques spécifiques (Marshall et al., 1998 ; Jones & Ardren, 2003 ; Oddou-Muratorio et al., 2018).

I-4-b. Sélection médiée par les pollinisateurs

La sélection médiée par les pollinisateurs est une composante de la sélection qui agit sur les traits maximisant l'attraction des pollinisateurs et/ou l'efficacité des visites, favorisant ainsi le succès reproducteur des individus, y compris par le biais du succès d'accouplement. Cependant, il n'est pas toujours évident de déterminer, lorsqu'un trait est sous sélection, s'il l'est parce qu'il joue un rôle dans l'interaction plante-pollinisateur, ou parce qu'il a un effet sur d'autres composantes de la *fitness*. Par exemple, comme mentionné précédemment, le nombre de fleurs produites peut influencer simultanément la production totale de gamètes et l'attraction des pollinisateurs, et donc être à la fois sous sélection de fertilité et sous sélection médiée par les pollinisateurs.

Afin d'estimer la part de la sélection médiée par les pollinisateurs chez les plantes femelles, il est possible de polliniser manuellement un groupe de plantes afin de saturer leurs stigmates en pollen (notées HP), et de comparer la sélection exercée chez ces dernières à celle exercée chez des plantes pollinisées naturellement (notées OP) (Galen, 1996 ; Sandring & Ågren, 2009 ; Jiang & Li, 2017 ; Lu et al., 2021 ; Qiu et al., 2023 ; Wu et al., 2023). L'écart entre les gradients estimés chez les plantes pollinisées naturellement et chez celles supplémentées en pollen correspond à la part de sélection médiée par les pollinisateurs ($\Delta\beta = \beta_{OP} - \beta_{HP}$, **Figure 4**). Si l'on considère que la sélection médiée par les pollinisateurs et la sélection sexuelle se chevauchent, c'est-à-dire que les traits impliqués dans l'interaction plante-pollinisateur influencent le succès reproducteur notamment par le biais du succès d'accouplement

(Moore & Pannell, 2011 ; Barbot et al., 2023), cette approche permet d'estimer l'intensité de la sélection sexuelle, mais uniquement via la voie femelle.

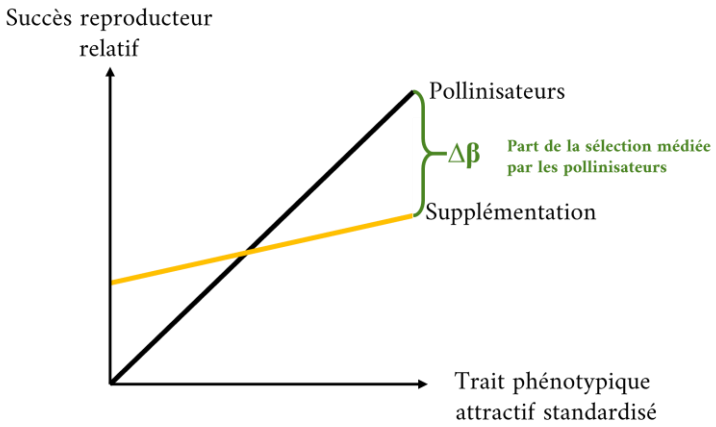

Figure 4. Représentation schématique du lien entre le succès reproducteur et un trait phénotypique attractif, chez des plantes pollinisées naturellement (noir) et des plantes pollinisées à la main (jaune). La différence de pente entre ces deux groupes correspond à la part de la sélection médiée par les pollinisateurs. Le succès reproducteur est relativisé et le trait phénotypique attractif est standardisé séparément dans chaque groupe.

I-4-c. Gradients de Bateman

Pour rappel, la sélection sexuelle est définie comme la composante de la sélection agissant sur les traits qui influencent l'accès aux partenaires sexuels. Or, la notion même de partenaires sexuels est difficile à définir. Certains auteurs distinguent le nombre de partenaires sexuels "copulatoires" (Anthes et al., 2017), c'est-à-dire le nombre d'individus avec lesquels un individu s'accouple (e.g., nombre de femelles vers lesquelles un mâle exporte du pollen, ou nombre de mâles dont une femelle reçoit du pollen), et le nombre de partenaires sexuels "génétiques", qui reflète le nombre de partenaires sexuels avec lesquels un individu s'est effectivement reproduit. Dans ce manuscrit, c'est le nombre de partenaires sexuels génétiques qui sera utilisé et estimé à l'aide d'analyses de paternité (cf. **Encadré 1**). En mesurant à la fois les traits phénotypiques et le nombre de partenaires sexuels, il est possible d'estimer des gradients de sélection sexuelle, selon une approche similaire à celle utilisée pour estimer les gradients de sélection totale, en remplaçant le succès reproducteur par le nombre de partenaires sexuels (Henshaw et al., 2018).

Néanmoins, une des principales limites de cette approche réside dans le fait qu'on ne connaît pas toujours les traits phénotypiques impliqués dans la sélection sexuelle, et que certains d'entre eux sont difficiles à mesurer (e.g., traits polliniques). Il est toutefois possible d'évaluer l'intensité de la sélection sexuelle sans faire d'hypothèse préalable au sujet des traits concernés, en estimant le gradient de Bateman, correspondant à la pente de la régression du succès reproducteur sur le succès d'accouplement (Bateman, 1948). Si la pente de cette régression est nulle, aucun bénéfice n'est associé à l'acquisition de partenaires supplémentaires. À l'inverse, une pente significativement positive suggère que les individus augmentent

leur succès reproducteur lorsqu'ils acquièrent des partenaires supplémentaires. Dans ce cas, la sélection sexuelle peut avoir lieu, et les traits qui favorisent l'acquisition desdits partenaires devraient être sous sélection.

Cette méthode présente cependant certaines limites. En effet, certains traits peuvent simultanément influencer le succès d'accouplement et le succès reproducteur, créant ainsi une covariance entre ces deux variables sans lien direct avec la sélection sexuelle (e.g., nombre de gamètes par fleur, Sanghvi et al., 2025). L'inclusion de ces traits comme covariables dans la régression susmentionnée permet d'atténuer ces effets : on parle alors de gradients de Bateman partiels (Henshaw et al., 2018).

II. PARAMETRES MODULANT LES PATRONS DE SELECTION SUR LES TRAITS FLORAUX

Après avoir décrit les différentes composantes de la sélection et les approches permettant de les quantifier, il s'agit maintenant de comprendre ce qui façonne leur intensité et leur direction. Nous examinerons plus particulièrement comment la fonction sexuelle (mâle vs. femelle), la disponibilité en gamètes du sexe opposé (limitante ou non) et l'identité des pollinisateurs peuvent influencer ces dynamiques sélectives.

II-1. Effets de la fonction sexuelle / du sexe sur les patrons de sélection sexuelle

En théorie, il est attendu que les patrons de sélection sexuelle varient entre fonctions sexuelles. En effet, les traits liés à l'accès aux partenaires sexuels devraient être sous sélection plus forte chez la fonction mâle que chez la fonction femelle (Bateman, 1948). Cette prédiction repose sur l'anisogamie, c'est-à-dire la différence de taille des gamètes entre fonctions sexuelles, qui résulte en une différence de nombre de gamètes produits. Le nombre de grains de pollen par fleur étant largement supérieur au nombre d'ovules (Kodric-Brown & Brown, 1987), le succès reproducteur de la fonction mâle devrait en effet être principalement limité par l'accès aux partenaires sexuels et/ou à leurs gamètes, contrairement à la fonction femelle, dont le succès reproducteur devrait dépendre davantage des ressources et du nombre d'ovules disponibles (**Figure 5**). Comme mentionné précédemment, chez les espèces d'angiospermes entomophiles, l'accès aux partenaires sexuels est dans un premier temps médié par les pollinisateurs. Si certains traits confèrent un avantage en termes d'attraction des pollinisateurs, et si cet avantage se traduit par un export accru du pollen sur les pistils de fleurs réceptives, alors ces traits devraient être sous sélection via la fonction mâle. Ainsi, dans les étapes précédant la pollinisation, il est attendu que les traits floraux sélectionnés via la fonction mâle soient principalement liés à l'attraction avec les pollinisateurs. Plusieurs études montrent par exemple une sélection sur la taille des fleurs plus forte chez la fonction mâle que la fonction femelle (Hodgins & Barrett, 2008 ; Zhou et al., 2020 ; Barbot et al., 2023). La compétition pour l'accès aux gamètes femelles peut ensuite se poursuivre après la pollinisation, les traits avantageux dans une situation de compétition pollinique pouvant également être soumis à la sélection (e.g., vitesse de croissance des tubes polliniques, Skogsmyr & Lankinen, 1999). Au contraire, le succès

reproducteur femelle est souvent considéré comme étant essentiellement limité par les ressources pouvant être allouées à la reproduction, tout du moins lorsque le service de pollinisation ne limite pas la production de graines. En effet, la production d'ovules constitue un investissement énergétique important par rapport à la production de pollen, et la maturation des graines nécessite également des ressources substantielles en termes de nutriments et d'énergie. Il est donc attendu que les traits sélectionnés via la fonction femelle soient liés à la fertilité plutôt qu'à l'attraction des pollinisateurs (e.g., nombre d'ovules par fleur, Hodgins & Barrett, 2008 ; Zhou et al., 2020 ; Barbot et al., 2023).

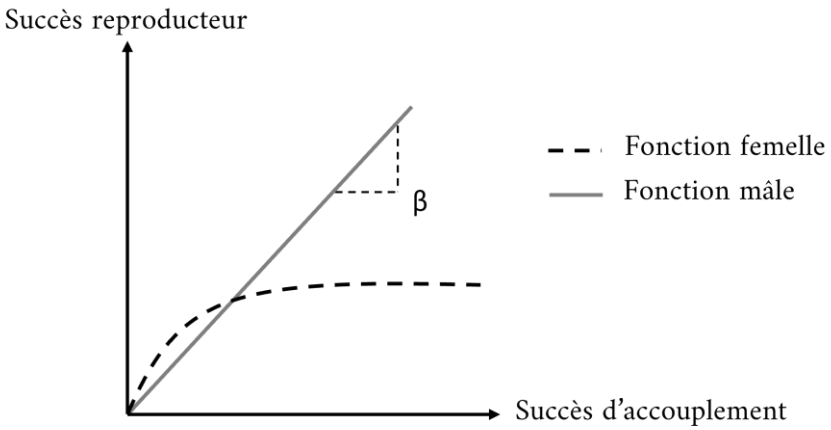

Figure 5. Relation entre le succès reproducteur (nombre de descendants) et le succès d'accouplement (nombre de partenaires reproducteurs), pour chaque fonction sexuelle. La pente de cette relation (β) obtenue par la régression entre le succès reproducteur et le succès d'accouplement correspond au gradient de Bateman. Ici, les mâles sont soumis à une plus forte sélection sexuelle que les femelles.

Les gradients de Bateman ont été majoritairement estimés chez les animaux. La méta-analyse de Janicke et al. (2016) réalisée sur 66 espèces animales montre que le gradient de Bateman est généralement plus fort chez les mâles, suggérant un avantage reproductif à multiplier les partenaires (**Figure 5**). Chez les femelles, le succès reproducteur augmente avec le nombre de partenaires, mais sature une fois tous les ovules fécondés (**Figure 5**, Bateman, 1948 ; Burd, 1994). Chez les plantes, les gradients de Bateman n'ont été mesurés que chez un petit nombre d'espèces, mais les résultats obtenus correspondent aux attendus théoriques, ces gradients étant systématiquement plus forts chez les mâles que chez les femelles (e.g., chez une mousse, Johnson & Shaw, 2016 ; chez une algue, Lavaut et al., 2023 ; chez trois angiospermes, Tonnabel et al., 2019b ; Barbot et al., 2022 ; Kwok & Dorken, 2022).

Si de telles mesures restent rares chez les plantes, des preuves indirectes suggèrent que la sélection sexuelle opère chez ces organismes, notamment chez les espèces à sexes séparés, où des différences de phénotypes floraux entre mâles et femelles ont été largement documentées. Ces différences concernent par exemple le nombre et la taille des fleurs (Delph et al., 1996 ; Moquet et al., 2020) ainsi que la production de nectar (Bawa & Opler, 1975), les mâles présentant le plus souvent des phénotypes plus attractifs pour les pollinisateurs. Ce dimorphisme sexuel est généralement interprété comme le résultat

de la sélection sexuelle, sur la base de deux hypothèses : (i) les traits floraux confèrent un avantage reproductif en facilitant l'accès aux partenaires et en augmentant le nombre de descendants, et (ii) l'effet de ces traits sur le succès reproducteur dépend du sexe (**Figure 6**, Arnold, 1994 ; Barrett & Hough, 2013).

II-2. Effets de la disponibilité en partenaires sexuels : limitation pollinique et densité en pollinisateurs

Il existe une situation dans laquelle le succès reproducteur femelle est, comme le succès reproducteur mâle, limité par l'accès aux partenaires sexuels. Cette situation est décrite sous le terme de "limitation pollinique" et désigne un cas où la quantité de pollen viable déposé sur les stigmates n'est pas suffisante pour fertiliser l'ensemble des ovules disponibles (Harder & Aizen, 2010). La limitation pollinique est souvent quantifiée en comparant la production de graines d'un groupe d'individus pollinisés naturellement à celle d'un groupe d'individus sur lesquels l'ensemble des fleurs sont saturées en pollen manuellement (Burd, 1994). Si la production de graines du premier groupe est significativement inférieure à celle du deuxième, on en conclut que le premier groupe n'a pas reçu assez de grains de pollen viables pour fertiliser tous les ovules et qu'il y a donc de la limitation pollinique. La limitation pollinique semble être un phénomène courant chez les plantes, les revues sur ce sujet mentionnant qu'environ deux tiers des espèces d'angiospermes sont impactées (Burd, 1994 ; Ashman et al., 2004 ; Knight et al., 2005 ; Bennett et al., 2018). Dans cette situation, la sélection sur les traits favorisant l'accès aux partenaires sexuels devrait émerger chez la fonction femelle, et s'intensifier chez la fonction mâle.

En conditions naturelles, la quantité de pollen reçue par les fleurs varie entre populations, mais aussi au sein d'une seule et même population, en fonction de plusieurs facteurs tels que la taille des populations (Agren, 1996 ; Zhang & Lou, 2015), la configuration spatiale des individus au sein de ces populations (Labouche et al., 2017), le sex-ratio (De Cauwer et al., 2010), ou encore la disponibilité en partenaires compatibles (Leducq et al., 2010). Chez les espèces entomophiles, la quantité de pollen reçue par les fleurs peut devenir insuffisante pour fertiliser l'ensemble des ovules disponibles suite à une réduction de la densité en pollinisateurs dans l'environnement (Delmas et al., 2016), elle-même causée par diverses perturbations anthropiques. Celles-ci incluent la modification et la fragmentation des paysages (Ricketts et al., 2008 ; Winfree et al., 2009), entraînant une diminution des ressources florales disponibles (Potts et al., 2010 ; Winfree et al., 2011 ; Woodard & Jha, 2017), l'utilisation intensive de pesticides et d'herbicides (Botina et al., 2023), la propagation de parasites et de pathogènes (Potts et al., 2010), ainsi que la pollution de l'air, du sol et de l'eau, qui compromettent la survie des pollinisateurs (LeBuhn & Vargas, 2021). Depuis les années 1980, un déclin des pollinisateurs a notamment été observé en Europe (Biesmeijer et al., 2006 ; Goulson et al., 2008 ; Potts et al., 2010) et aux États-Unis (vanEngelsdorp et al., 2008). Les effets négatifs de la modification de l'habitat sur les pollinisateurs sont généralisés,

touchant la plupart des groupes taxonomiques, incluant les bourdons, les papillons et les syrphes (Moquet et al., 2018 ; Rhodes, 2018 ; Habel et al., 2019).

Face à ce déclin et à l'augmentation de la limitation pollinique qui en résulte, deux trajectoires évolutives mutuellement exclusives peuvent être attendues (Thomann et al., 2015). La première est l'évolution de traits permettant aux plantes de réduire leur dépendance vis-à-vis des pollinisateurs en favorisant l'autogamie, par exemple via la diminution de l'herkogamie et de la dichogamie (e.g., Barrett, 2003 ; Carvallo & Medel, 2010 ; Brys & Jacquemyn, 2012 ; Kalisz et al., 2012). Chez les espèces suivant cette trajectoire évolutive, on peut également s'attendre à une contre-sélection des traits liés à l'attraction des pollinisateurs. L'étude de Acoca-Pidolle et al. (2024) montre, par exemple, que le taux d'autogamie de plantes évoluant dans environnement pauvre en pollinisateurs a augmenté, cette augmentation s'accompagnant d'une réduction de la taille des fleurs et de la production de nectar. La seconde trajectoire évolutive, à l'inverse, implique une sélection renforcée sur les traits favorisant l'attraction des pollinisateurs. Une étude menée par Sletvold & Ågren (2016) sur *Gymnadenia conopsea* a ainsi montré que la sélection médiée par les pollinisateurs sur la hauteur des plantes, la taille de la corolle et la longueur de l'éperon s'intensifiait chez des plantes chez lesquelles l'accès aux pollinisateurs était expérimentalement réduit. D'autres études empiriques ont confirmé que la force de la sélection sur les traits liés à l'attraction des pollinisateurs augmentait en cas de limitation pollinique (Caruso, 2000 ; Dai & Galloway, 2013 ; Bartkowska & Johnston, 2015 ; Emel et al., 2017). Chez les espèces dioïques ou auto-incompatibles, seule cette deuxième trajectoire évolutive est possible.

La limitation pollinique peut ainsi moduler les prédictions énoncées précédemment concernant les différences de patrons de sélection sexuelle entre fonctions sexuelle. Comme mentionné précédemment, en cas de forte densité en pollinisateurs, le succès reproducteur mâle, mais pas femelle devrait être limité par l'accès aux partenaires sexuels. La sélection exercée sur les traits floraux attractifs devrait alors être forte via la fonction mâle mais pas via la fonction femelle (**Figure 6**-A). En revanche, si la densité en pollinisateurs est suffisamment faible pour induire de la limitation pollinique, le succès reproducteur mâle comme femelle devrait être limité par l'accès aux partenaires sexuels. La sélection exercée sur les traits attractifs devrait alors être forte via les deux fonctions sexuelles (**Figure 6**-B).

A ce jour, l'effet de la densité en pollinisateurs sur l'intensité de la sélection a été étudiée quasiment exclusivement via la fonction femelle, et sur les traits liés à l'attraction des pollinisateurs. Or, pour comprendre pleinement les conséquences du déclin des pollinisateurs sur l'évolution des traits floraux, il est essentiel de considérer la sélection agissant via les deux fonctions sexuelles mais également sur l'ensemble des traits pertinents—ceux intervenant avant comme après la pollinisation.

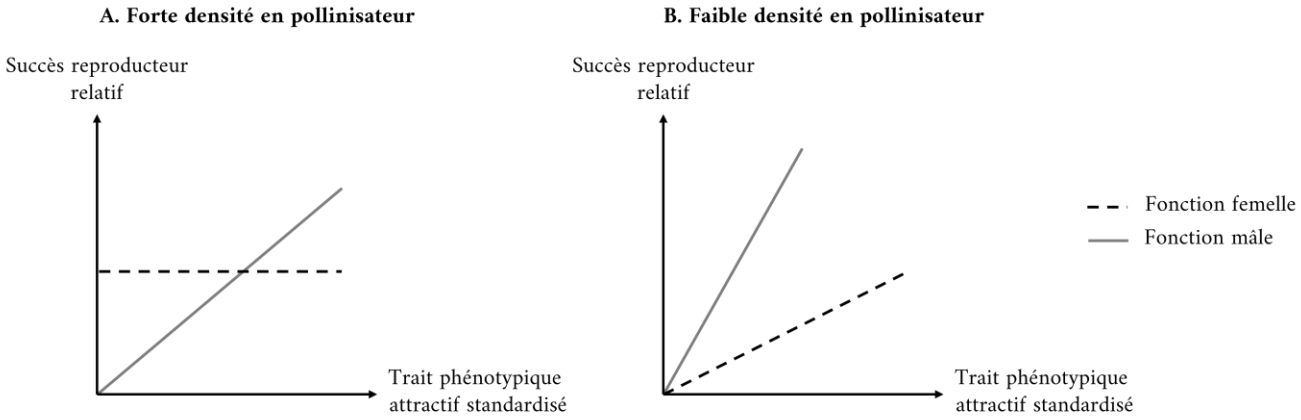

Figure 6. Différentiels de sélection attendus, caractérisant la relation entre le succès reproducteur et un trait phénotypique attractif en fonction de la densité en pollinisateurs et des fonctions sexuelles. Le succès reproducteur est relativisé au sein du contexte de pollinisation et des fonctions sexuelles. Le trait phénotypique attractif est standardisé de la même manière.

Tout comme l'impact du déclin des pollinisateurs sur la sélection dépend de la fonction sexuelle considérée, il devrait aussi varier selon les traits floraux étudiés. En effet, une réduction de la densité en pollinisateurs ne devrait pas influencer de la même façon la sélection sur les traits impliqués dans les processus pré- et post-pollinisation. Plus la limitation pollinique est forte, plus il y a d'ovules disponibles pour un nombre restreint de grains de pollen. Ainsi, l'intensité de la compétition pollinique devrait décroître avec l'augmentation de la limitation pollinique (**Figure 7**, Christopher et al., 2020). Les traits associés aux performances des grains de pollen dans un contexte de compétition pour l'accès aux ovules (e.g., vitesse de croissance du tube pollinique) ne devraient alors plus être soumis à la sélection.

II-3. Effets de la composition des communautés de pollinisateurs

Chez les espèces d'angiospermes pollinisées par des animaux, on distingue les espèces dites "spécialistes", pollinisées par un seul groupe fonctionnel de pollinisateurs, et les espèces dites "généralistes", qui sont pollinisées par plusieurs groupes fonctionnels de pollinisateurs. La majorité des espèces appartiennent à cette dernière catégorie (Waser et al., 1996).

Chez ces espèces, la composition des cortèges de pollinisateurs varie souvent dans l'espace (Herrera, 1988), créant ainsi une "mosaïque de sélection" (Thompson, 2005). Chez *Erysimum mediohispanicum*, par exemple, différents pollinisateurs exercent des préférences distinctes pour la forme des corolles, générant ainsi une variation spatiale de la sélection sur ce trait, les abeilles privilégiant les pétales étroits et les bombyles les pétales arrondis (Gómez et al., 2008). De même, chez *Digitalis purpurea*, la coexistence de bourdons et de colibris dans une population est associée à une augmentation de la hauteur et de la largeur du tube floral par rapport à une population visitée uniquement par des bourdons (Mackin et al., 2021).

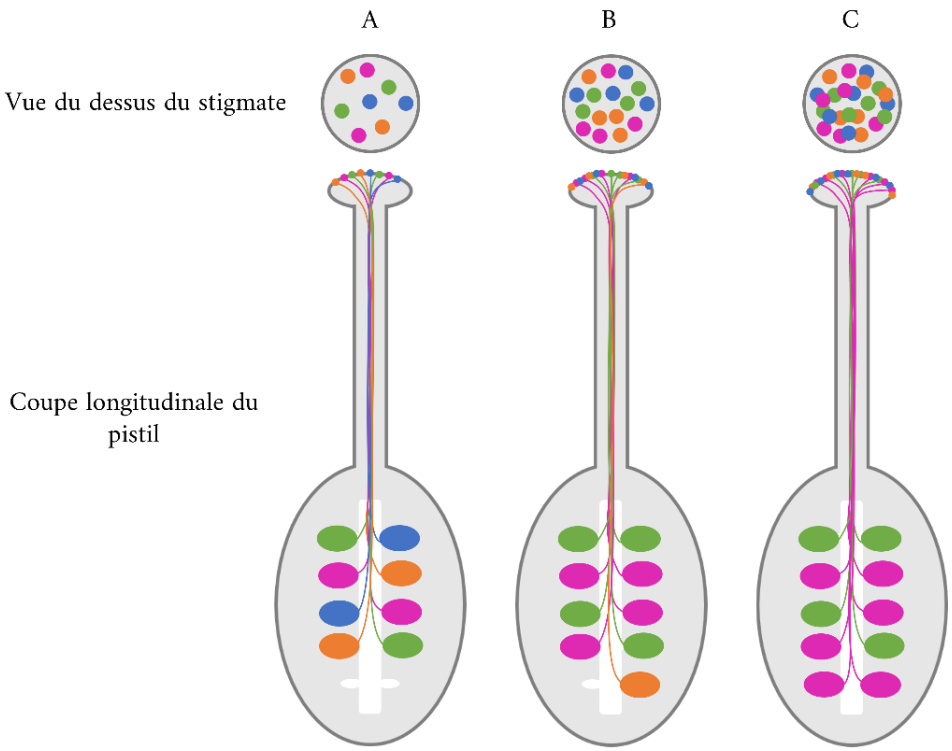

Figure 7. Schéma inspiré de Christopher et al. (2020) illustrant l'effet de la quantité de pollen déposée sur un stigmate sur la compétition pollinique. Les points colorés représentent les grains de pollen issus de quatre donneurs distincts, différenciés par leurs traits polliniques. Les ovules non fécondés apparaissent petits et blancs, tandis que les ovules fécondés sont plus grands et colorés selon l'origine du pollen qui les a fertilisés. Le dépôt de pollen augmente entre les cas A, B et C. Le cas A reflète une situation de limitation pollinique : il y a moins de grains de pollen déposé sur le stigmate que d'ovules disponibles. Chaque grain de pollen peut alors féconder un ovule, indépendamment de ses capacités compétitives. En revanche, à mesure que le dépôt de pollen augmente, les grains se retrouvent en compétition pour l'accès aux ovules : seuls les plus performants accèderont alors à un ovule.

Si le concept de mosaïque de sélection souligne la variabilité spatiale des pressions de sélection, il est également important de considérer que, localement, une même population peut être visitée par plusieurs types de pollinisateurs, générant des dynamiques de sélection potentiellement complexes. Dans ce cas, plusieurs scénarios évolutifs peuvent être envisagés quant à l'évolution des traits floraux. Tout d'abord, la sélection est dite conflictuelle si les différents pollinisateurs sélectionnent un même trait dans des directions différentes (**Figure 8**-A, Muchhala, 2007 ; Gervasi & Schiestl, 2017). A l'inverse, ces différents pollinisateurs peuvent exercer des pressions de sélection convergentes sur un même trait (**Figure 8**-B, Wu et al., 2018), ou bien sélectionner des traits distincts (**Figure 8**-C, Wu et al., 2018).

Dans chacun de ces scénarios, la sélection est dite additive si la sélection totale correspond à la somme des sélections exercées par chaque pollinisateur de manière indépendante (**Figure 8**-D, terHorst et al., 2015 ; terHorst et al., 2017). Dans le cas contraire, on parlera en revanche de sélection non-additive (**Figure 8**-E), pouvant résulter (i) d'effets écologiques indirects, lorsque la sélection exercée par un pollinisateur est modifiée par la présence d'autres pollinisateurs, par exemple du fait de modifications comportementales (e.g., évitement de certaines fleurs, Rashed & Sherratt, 2007), ou (ii) d'effet dits "de

fitness", liés à une modification de la distribution de la valeur sélective. Ce type d'effet est notamment attendu si le succès reproducteur des individus est homogénéisé en présence de multiples pollinisateurs, causant ainsi une diminution de l'opportunité pour la sélection (terHorst et al., 2015 ; terHorst et al., 2017). En outre, il est important de mentionner que les pollinisateurs contribuent rarement de manière strictement équivalente au succès reproducteur des plantes, certains étant localement plus fréquents, ou plus efficaces que d'autres. De telles variations devraient là encore résulter en une sélection non-additive, puisque l'intensité des pressions de sélection exercée par chaque pollinisateur devrait alors être pondérée par leur contribution relative au succès reproducteur.

II-4. Compétition intra-sexuelle et échelle des flux de pollen

L'échelle spatiale à laquelle se produit la compétition intra-sexuelle pré-pollinisation dépend des caractéristiques propres à chaque espèce et n'est souvent pas connue, alors qu'elle peut avoir des effets importants sur la disponibilité en partenaires sexuels et sur le nombre de compétiteurs directs pour y accéder. En effet, lorsque les flux de pollen se font sur de courtes distances, le nombre de compétiteurs est réduit par rapport à une situation où ces flux s'étendent sur de plus longues distances. Plusieurs approches permettent de déterminer l'échelle des flux de pollen. Une approche indirecte consiste à analyser la structure génétique spatiale, afin d'inférer les flux de gènes, en admettant que la dispersion des graines s'effectue sur de plus courtes distances que celle du pollen (Heuertz et al., 2003 ; Ismail et al., 2017 ; Hardy et al., 2019). Ces analyses reposent sur la corrélation entre les distances génétiques et les distances spatiales entre chaque paire d'individus d'une population. Elles permettent de déterminer si des individus proches géographiquement sont génétiquement plus apparentés qu'attendu sous l'hypothèse d'une distribution aléatoire des génotypes, et de préciser à quelle échelle cette tendance s'observe. Les approches directes, quant à elles, reposent sur des analyses de paternité permettant d'observer directement les flux de pollen contemporains. Une fois les accouplements reconstitués, il convient de mesurer la distance géographique séparant chaque paire d'individus impliqués dans une reproduction effective afin d'estimer la distance de dispersion du pollen.

Les distances de dispersion moyenne du pollen varient fortement selon les espèces. Notamment, de fortes variations sont observées entre les arbres (souvent de l'ordre de centaines de mètres, García et al., 2007 ; Oddou-Muratorio et al., 2018) et les plantes herbacées (plutôt de l'ordre de quelques dizaines de mètres, parfois bien moins, Llaurens et al., 2008 ; Van Rossum et al., 2011). Chez ces dernières, le mode de dispersion du pollen semble avoir en revanche un effet limité sur les distances de dispersion du pollen : de faibles valeurs sont rapportées aussi bien pour les espèces anémophiles (De Cauwer et al., 2012 ; Tonnabel et al., 2019a) que pour les espèces entomophiles (DiLeo et al., 2018 ; Barbot et al., 2025).

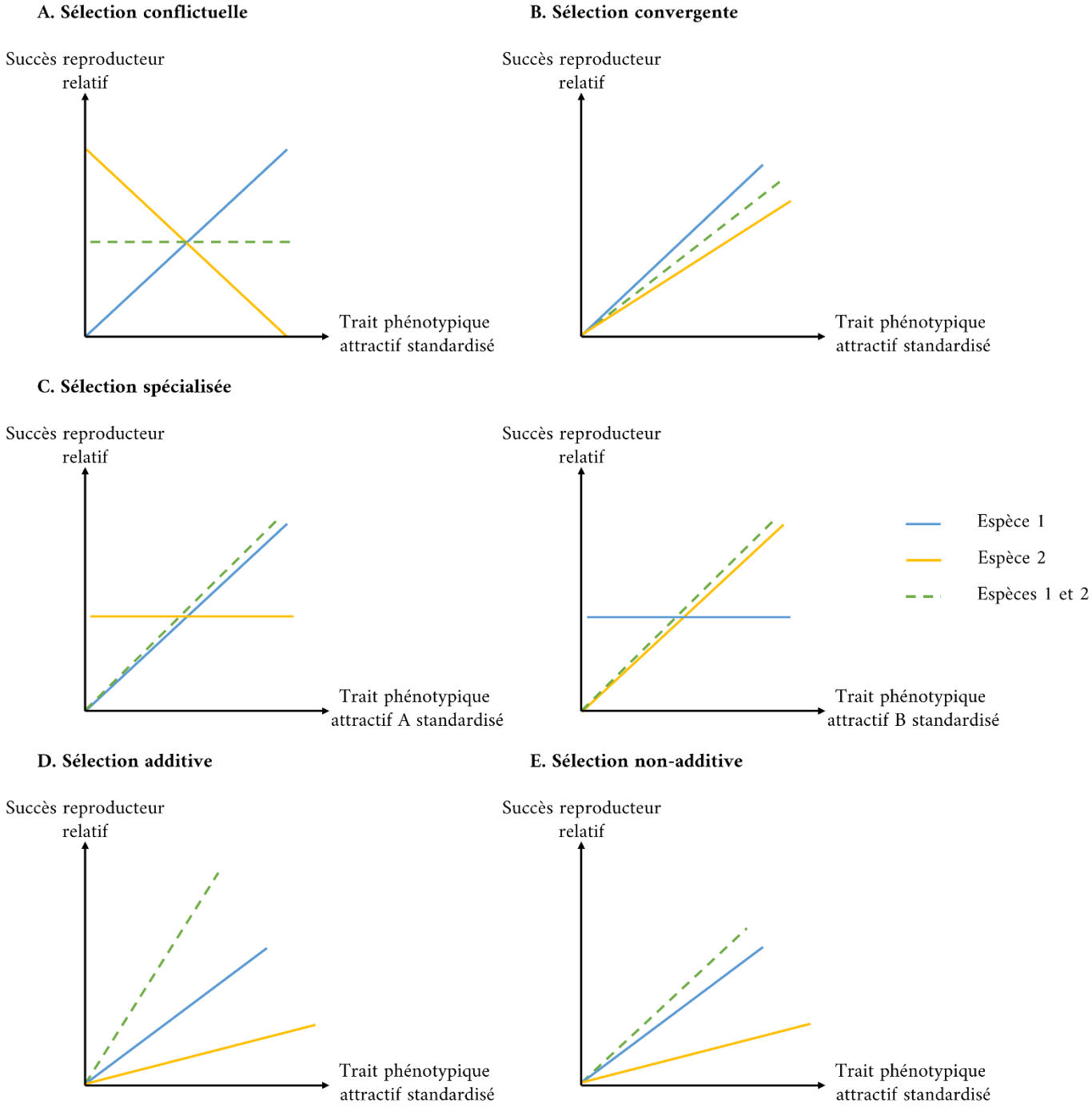

Figure 8. Exemples de cas de figures où la sélection est dite A. conflictuelle, B. convergente et C. spécialisée. Dans ces trois cas de figures, la sélection peut être D. additive ou E. non-additive. Les droites en lignes pleines représentent la relation entre le succès reproducteur et un trait phénotypique attractif, lorsque les pollinisateurs sont seuls (bleu : espèce 1, jaune : espèce 2). La droite verte en ligne pointillée représente la relation entre le succès reproducteur et un trait phénotypique attractif lorsque les pollinisateurs sont en contacts. Dans le cas de la sélection additive, le différentiel de sélection correspond à la somme des différentiels de sélection observés chez les deux insectes. Le succès reproducteur est relativisé et le trait phénotypique attractif est standardisé. Schémas adaptés de terHorst et al. (2015) et de Sahli & Conner (2011).

Chez les espèces entomophiles, la dispersion du pollen dépend en premier lieu des mouvements de pollinisateurs. La "théorie de l'approvisionnement optimal" (MacArthur & Pianka, 1966) permet de comprendre ces mouvements en les resituant dans un contexte de comportement de recherche alimentaire. Cette théorie stipule que les pollinisateurs adoptent des stratégies visant à optimiser l'acquisition de ressources, en minimisant le temps et l'énergie alloués à leur recherche. Chez les insectes, les ressources recherchées sont principalement nutritives, comme le pollen et le nectar, et les coûts énergétiques associés dépendent des déplacements entre les fleurs ou les plantes, ainsi que du temps passé sur chaque fleur. Comme mentionné précédemment, les insectes utilisent les signaux attractifs des plantes pour guider leurs choix alimentaires, optimisant ainsi l'acquisition de ressources, mais leurs visites tendent également à s'effectuer de proche en proche, afin de limiter les coûts liés aux déplacements (Marden & Waddington, 1981 ; Hill et al., 2001), un comportement qui pourrait contribuer à créer de la structure génétique spatiale au sein des populations. La dégradation et la fragmentation des habitats étant susceptibles d'affecter non seulement la densité et la composition des communautés de pollinisateurs, mais aussi les mouvements de ces derniers (Goverde et al., 2002), elles pourraient modifier substantiellement les flux de pollen, avec un impact potentiel sur la compétition pour l'accès aux partenaires sexuels, les patrons de sélection, et la réponse évolutive des populations.

III. LE COMPAGNON ROUGE COMME MODELE D'ETUDE

Silene dioica (L.) Clairv. est une espèce angiosperme herbacée et pérenne de la famille des Caryophyllaceae. C'est une espèce dioïque possédant des chromosomes sexuels de type XY. Cette espèce est largement répartie dans le centre et le nord de l'Europe et peut être rencontrée le long d'un gradient allant d'habitats semi-naturels tels que les clairières forestières ou les lisières de bois à des milieux plus anthropisés comme les bords de routes, à condition que ces milieux soient suffisamment humides et partiellement ombragés. Sa durée de vie varie de 5 à 10 ans (Giles et al., 1998). Dans le nord de la France, sa période de floraison s'étend de la fin avril à la mi-juillet, avec un pic de floraison fin mai (Barbot et al., 2022). Les graines sont dispersées par gravité et germent généralement au printemps suivant (Giles & Goudet, 1997). Les corolles de *S. dioica* sont roses avec cinq pétales bifides et les calices sont vert à pourpre et oblong (**Figure 9**). Les fleurs mâles possèdent dix étamines et les fleurs femelles présentent cinq lobes stigmatiques. Le fruit forme une capsule sans cloisons. Plusieurs traits floraux (e.g., nombre de fleurs, largeur de corolle) sont variables entre et au sein des populations (Barbot, 2021) et l'étude de Giles et al. (2006) suggère que ces variations pourraient être en partie héritables. Cette espèce présente par ailleurs un dimorphisme sexuel pour plusieurs traits floraux. En particulier, les mâles ont plus de fleurs ouvertes, ces fleurs étant en outre plus grandes (**Figure 9**, Kay et al., 1984 ; Moquet et al., 2020). Les mâles fleurissent également plus longtemps que les femelles. Ces traits étant liés à l'attraction des pollinisateurs (Barbot et al., 2022 ; Moquet et al., 2022), les mâles tendent à attirer davantage ces derniers, bien que les femelles produisent plus de nectar par fleur (Barbot et al., 2023).

En cohérence avec ces observations, plusieurs études menées sur des populations expérimentales de *S. dioica* ont montré que les patrons de sélection différaient entre les sexes. Ces études ont montré que chez les femelles, la sélection s'exerçait sur le nombre de gamètes par fleur et le nombre de fleurs ouvertes, tandis que chez les mâles, elle concernait la largeur de la corolle, la durée de floraison et le nombre de fleurs ouvertes (Barbot et al., 2022 ; Barbot et al., 2023). De plus, ces études ont mis en évidence un gradient de Bateman plus fort chez les mâles que chez les femelles, indiquant une asymétrie dans l'intensité de la sélection sexuelle entre les sexes (Barbot et al., 2023). Ces résultats sont conformes aux attendus théoriques lorsque la réception de pollen ne limite pas le succès reproducteur femelle (i.e., pas de limitation pollinique), ce qui était le cas dans les populations expérimentales étudiées. Cependant, ceci pourrait varier en fonction de l'environnement et notamment du contexte de pollinisation, par exemple si la densité en pollinisateurs est réduite du fait de l'anthropisation de l'habitat. Il serait donc intéressant d'examiner ces dynamiques *in natura*, dans plusieurs populations potentiellement soumises à différents niveaux de limitation pollinique.

Par ailleurs, *S. dioica* présente un système de pollinisation généraliste, étant principalement pollinisée par des bourdons (*Bombus*) et des syrphes (*Syrphidae*) (Kay et al., 1984 ; Barbot et al., 2022). Or, de précédentes études ont montré que ces deux groupes exerçaient des pressions de sélection contrastées sur certains traits floraux chez différentes espèces. Chez *Brassica rapa*, il a par exemple été démontré que la hauteur des plantes avait un effet positif sur l'attraction des bourdons mais négatif sur celle des syrphes (Gervasi & Schiestl, 2017). De la même manière, chez *Erysimum mediohispanicum*, les bourdons privilégiaient les corolles larges tandis que les syrphes préféraient les corolles étroites et les petits calices (Gómez et al., 2008). Ainsi, *S. dioica* est un modèle idéal pour explorer comment les interactions plantes-pollinisateurs généralistes peuvent façonner l'évolution des traits floraux.

Parmi les zones d'ombres qui persistent, très peu d'informations sont disponibles concernant la dynamique de transfert du pollen et les processus post-pollinisation chez *S. dioica*. Les travaux de Barbot et al. (2022, 2025) montrent que les flux de pollen sont spatialement restreints chez cette espèce (distance moyenne de dispersion entre 1.50 m et 2 m). Cependant, ces études ont été menées en populations expérimentales sur des surfaces réduites, pouvant causer une sous-estimation des distances de dispersion. En populations naturelles, l'échelle des flux de gènes reste largement méconnue. Des études suggèrent l'existence d'une forte structure génétique spatiale à fine échelle (Giles & Goudet, 1997 ; Ingvarsson & Giles, 1999) suggérant des flux de gènes spatialement restreints, mais il s'agit là d'estimations indirectes. Il serait donc pertinent d'aborder cette question en s'appuyant sur des analyses de paternité, permettant une estimation directe des distances de dispersion, et en échantillonnant et comparant plusieurs populations naturelles, afin de mettre en évidence d'éventuelles variations dans les patrons de dispersion du pollen liées aux conditions environnementales. L'échelle spatiale à laquelle ont lieu les flux de pollen devrait influencer le nombre de donneurs de pollen entrant en compétition pour un même pool d'ovules, or les processus post-pollinisation sont également des aspects peu explorés chez

S. dioica (mais voir Örjes, 2021 ; voir Brothers & Delph, 2017 chez *S. latifolia* ; voir Montgomery et al., 2010 chez *S. dioica* et *S. latifolia*). L'intensité de ces processus post-pollinisation devrait également être modulée par la quantité de pollen reçue par les stigmates, qui pourrait elle-même dépendre de facteurs démographiques à l'échelle populationnelle, mais aussi des traits floraux individuels.

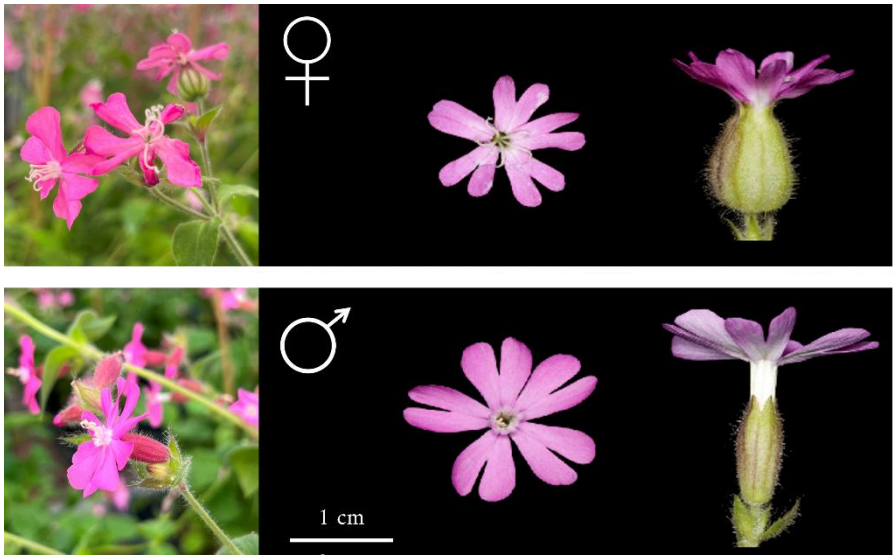

Figure 9. Photos de *Silene dioica*, représentant le dimorphisme sexuel pour la taille des fleurs. Les fleurs femelles (haut) ont des corolles moins larges et des calices moins hauts que les fleurs mâles (bas). Crédit photo : I. De Cauwer, P. Goujon & M. Vanderplanck.

IV. OBJECTIFS DE LA THESE

Au cours de cette thèse, nous allons étudier l'écologie évolutive de la reproduction de *S. dioica*, en explorant l'effet des variations de communautés de pollinisateurs liées aux variations paysagères, les différences entre sexes et les processus post-pollinisation.

Dans le chapitre I, intitulé "Structure génétique des populations chez le compagnon rouge (*Silene dioica*): effets des composantes du paysage", la diversité génétique et la structure génétique spatiale ont été caractérisées dans le nord de la France, entre et au sein de 29 populations naturelles, en utilisant cinq marqueurs microsatellites (manuscrit A). Grâce à des méthodes visant à mettre en lumière la structure génétique des populations ainsi qu'à des analyses de paternité au sein des populations, nous avons identifié les échelles spatiales auxquelles s'exercent les flux de gènes. Ces résultats ont fourni des éléments essentiels pour définir l'échelle à laquelle se situe la compétition entre individus du même sexe pour l'accès aux partenaires sexuels, et, par conséquent, déterminer la bonne échelle à laquelle appréhender les processus de sélection. En parallèle, l'intégration de la structure de l'habitat (milieu forestier *vs* milieu agricole) a permis d'évaluer l'influence de la matrice paysagère sur la diversité génétique des populations.

Dans le chapitre II, intitulé "Les différences d'habitat n'altèrent pas les patrons de sélection sexuelle chez *Silene dioica*", une étude a été réalisée au sein de six populations naturelles, afin de comparer la sélection phénotypique exercée sur les traits floraux entre des populations forestières et anthropisées (manuscrit B). Dans chaque population, la diversité et la densité en pollinisateurs ont été quantifiées, et la qualité du service de pollinisation a été estimée afin de déterminer si les facteurs limitant le succès reproducteur femelle différaient entre types d'habitat. Enfin, les patrons de sélection ainsi que les gradients de Bateman ont été estimés pour les deux sexes. Ce travail se distingue par le fait qu'il prend en compte simultanément les deux sexes dans deux environnements contrastés, ce qui en fait une contribution originale à l'étude des mécanismes de sélection liés à l'habitat et à l'interaction avec les pollinisateurs.

Dans le chapitre III, intitulé "Différents pollinisateurs imposent-ils une sélection divergente sur les traits floraux ?", une manipulation expérimentale a été réalisée afin de comparer l'activité et la sélection phénotypique exercée par deux pollinisateurs communs de *S. dioica* : les bourdons et les syrphes (manuscrit C). Notre étude précédente, conduite en populations naturelles, a montré que les fréquences relatives de ces deux groupes de pollinisateurs variaient entre populations. Nous avons donc cherché à évaluer le rôle respectif de ces deux groupes dans la sélection exercée sur les traits floraux. Pour ce faire, nous avons mis plusieurs populations expérimentales de *S. dioica* en présence de bourdons, de syrphes ou de ces deux groupes à la fois. Dans chaque population, nous avons mesuré la fréquence des visites de la part des pollinisateurs, l'intensité de la limitation pollinique et la sélection exercée sur les traits floraux. Cette étude est ainsi l'une des premières à estimer et comparer la sélection exercée sur les traits floraux par différents groupes de pollinisateurs chez les deux sexes.

Enfin, le chapitre IV, intitulé "De la pollinisation à la paternité : déterminants et effet de la charge pollinique sur le succès de fertilisation" porte sur les facteurs faisant varier la charge pollinique stigmatique au sein de 24 populations naturelles, ainsi que sur l'impact de cette variation sur les processus post-pollinisation (manuscrit D). La première partie du manuscrit vise ainsi à déterminer les facteurs environnementaux, démographiques et phénotypiques responsables de la variation de la charge pollinique observée en milieu naturel, tandis que la seconde explore les effets d'une telle variation sur la variance du succès reproducteur mâle à l'aide de pollinisations manuelles visant à générer trois niveaux de compétition pollinique (i.e., faible, moyen, fort). Cette étude se distingue par son approche intégrative, reliant les variations écologiques observées sur le terrain aux différences de charge pollinique, puis aux dynamiques de compétition pollinique qui en résultent. Elle permet ainsi de mieux comprendre comment l'environnement peut façonner, par l'intermédiaire de la pollinisation, le processus de sélection sexuelle chez les plantes.

V. RÉFÉRENCES

Ackerman, J. D. (2000). Abiotic pollen and pollination : Ecological, functional, and evolutionary perspectives. *Plant Systematics and Evolution*, 222, 167-185. https://doi.org/10.1007/BF00984101

Acoca-Pidolle, S., Gauthier, P., Devresse, L., Deverge, A., Pons, V., & Cheptou, P.-O. (2024). Ongoing convergent evolution of a selfing syndrome threatens plant-pollinator interactions. *New Phytologist*, 242(2), 717-726. https://doi.org/10.1111/nph.19422

Agren, J. (1996). Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. *Ecology*, 77(6), 1779-1790. https://doi.org/10.2307/2265783

Alexandersson, R., & Johnson, S. D. (2002). Pollinator–mediated selection on flower–tube length in a hawkmoth–pollinated *Gladiolus* (Iridaceae). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 269(1491), 631-636. https://doi.org/10.1098/rspb.2001.1928

Anthes, N., Häderer, I. K., Michiels, N. K., & Janicke, T. (2017). Measuring and interpreting sexual selection metrics : Evaluation and guidelines. *Methods in Ecology and Evolution*, 8(8), 918-931. https://doi.org/10.1111/2041-210X.12707

Arnold, S. J. (1994). Bateman's principles and the measurement of sexual selection in plants and animals. *The American Naturalist*, 144, S126-S149. https://doi.org/10.1086/285656

Ashman, T.-L., Knight, T. M., Steets, J. A., Amarasekare, P., Burd, M., Campbell, D. R., Dudash, M. R., Johnston, M. O., Mazer, S. J., Mitchell, R. J., Morgan, M. T., & Wilson, W. G. (2004). Pollen limitation of plant reproduction : Ecological and evolutionary causes and consequences. *Ecology*, 85(9), 2408-2421. https://doi.org/10.1890/03-8024

Ashman, T.-L., & Majetic, C. J. (2006). Genetic constraints on floral evolution : A review and evaluation of patterns. *Heredity*, 96(5), 343-352. https://doi.org/10.1038/sj.hdy.6800815

Barbot, E. (2021). *Accès à la reproduction chez* Silene dioica : *Une espèce dioïque entomophile*. http://www.theses.fr/2021LILUR018/document

Barbot, E., Dufaÿ, M., & De Cauwer, I. (2023). Sex-specific selection patterns in a dioecious insect-pollinated plant. *Evolution*, 77(7), 1578-1590. https://doi.org/10.1093/evolut/qpad069

Barbot, E., Dufaÿ, M., Godé, C., & De Cauwer, I. (2025). Investigating the effects of diurnal and nocturnal pollinators on male and female reproductive success and on floral trait selection in *Silene dioica*. *Peer Community Journal*, 5, e11. https://doi.org/10.24072/pcjournal.508

Barbot, E., Dufaÿ, M., Tonnabel, J., Godé, C., & De Cauwer, I. (2022). On the function of flower number : Disentangling fertility from pollinator-mediated selection. *Proceedings of the Royal Society B: Biological Sciences*, 289(1987), 20221987. https://doi.org/10.1098/rspb.2022.1987

Barrett, S. C. H. (2003). Mating strategies in flowering plants : The outcrossing–selfing paradigm and beyond. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 358(1434), 991-1004. https://doi.org/10.1098/rstb.2003.1301

Barrett, S. C. H. (2010). Understanding plant reproductive diversity. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1537), 99-109. https://doi.org/10.1098/rstb.2009.0199

Barrett, S. C. H., & Hough, J. (2013). Sexual dimorphism in flowering plants. *Journal of Experimental Botany*, 64(1), 67-82. https://doi.org/10.1093/jxb/ers308

Bartkowska, M. P., & Johnston, M. O. (2015). Pollen limitation and its influence on natural selection through seed set. *Journal of Evolutionary Biology*, 28(11), 2097-2105. https://doi.org/10.1111/jeb.12741

Bateman, A. J. (1948). Intra-sexual selection in *Drosophila*. *Heredity*, 2(3), 349-368. https://doi.org/10.1038/hdy.1948.21

Bauer, A. A., Clayton, M. K., & Brunet, J. (2017). Floral traits influencing plant attractiveness to three bee species : Consequences for plant reproductive success. *American Journal of Botany*, 104(5), 772-781. https://doi.org/10.3732/ajb.1600405

Bawa, K. S., & Opler, P. A. (1975). Dioecism in tropical forest trees. *Evolution*, 29(1), 167-179. https://doi.org/10.1111/j.1558-5646.1975.tb00824.x

Bennett, J. M., Steets, J. A., Burns, J. H., Durka, W., Vamosi, J. C., Arceo-Gómez, G., Burd, M., Burkle, L. A., Ellis, A. G., Freitas, L., Li, J., Rodger, J. G., Wolowski, M., Xia, J., Ashman, T.-L., & Knight, T. M. (2018). GloPL, a global data base on pollen limitation of plant reproduction. *Scientific Data*, 5(1), 180249. https://doi.org/10.1038/sdata.2018.249

Biesmeijer, J. C., Roberts, S. P. M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., Schaffers, A. P., Potts, S. G., Kleukers, R., Thomas, C. D., Settele, J., & Kunin, W. E. (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science*, 313(5785), 351-354. https://doi.org/10.1126/science.1127863

Bloch, D., & Erhardt, A. (2008). Selection toward shorter flowers by butterflies whose probosces are shorter than floral tubes. *Ecology*, 89(9), 2453-2460. https://doi.org/10.1890/06-2023.1

Botina, L. L., Barbosa, W. F., Acosta, J. P. L., Bernardes, R. C., Cortes, J. E. Q., Pylro, V. S., Mendonça, A. C., Barbosa, R. C., Lima, M. A. P., & Martins, G. F. (2023). The impact of early-life exposure to three agrochemicals on survival, behavior, and gut microbiota of stingless bees (*Partamona helleri*). *Environmental Science and Pollution Research*, 30(27), 70143-70158. https://doi.org/10.1007/s11356-023-27385-4

Briscoe, A. D., & Chittka, L. (2001). The evolution of color vision in insects. *Annual Review of Entomology*, 46(1), 471-510. https://doi.org/10.1146/annurev.ento.46.1.471

Brothers, A. N., & Delph, L. F. (2017). Divergence in style length and pollen size leads to a postmating-prezygotic reproductive barrier among populations of *Silene latifolia*. *Evolution*, 71(6), 1532-1540. https://doi.org/10.1111/evo.13248

Brunet, J., Thairu, M. W., Henss, J. M., Link, R. I., & Kluever, J. A. (2015). The effects of flower, floral display, and reward sizes on bumblebee foraging behavior when pollen is the reward and plants are dichogamous. *International Journal of Plant Sciences*, 176(9), 811-819. https://doi.org/10.1086/683339

Brys, R., Geens, B., Beeckman, T., & Jacquemyn, H. (2013). Differences in dichogamy and herkogamy contribute to higher selfing in contrasting environments in the annual *Blackstonia perfoliata* (Gentianaceae). *Annals of Botany*, 111(4), 651-661. https://doi.org/10.1093/aob/mct031

Brys, R., & Jacquemyn, H. (2012). Effects of human-mediated pollinator impoverishment on floral traits and mating patterns in a short-lived herb : An experimental approach. *Functional Ecology*, 26(1), 189-197. https://doi.org/10.1111/j.1365-2435.2011.01923.x

Burd, M. (1994). Bateman's principle and plant reproduction : The role of pollen limitation in fruit and seed set. *The Botanical Review*, 60(1), 83-139. https://doi.org/10.1007/BF02856594

Burd, M., & Allen, T. F. H. (1988). Sexual allocation strategy in wind-pollinated plants. *Evolution*, 42(2), 403-407. https://doi.org/10.1111/j.1558-5646.1988.tb04145.x

Campbell, D. R. (1989). Measurements of selection in a hermaphroditic plant : Variation in male and female pollination success. *Evolution*, 43(2), 318-334. https://doi.org/10.1111/j.1558-5646.1989.tb04230.x

Campbell, D. R., Waser, N. M., & Price, M. V. (1996). Mechanisms of hummingbird-mediated selection for flower width in *Ipomopsis aggregata*. *Ecology*, 77(5), 1463-1472. https://doi.org/10.2307/2265543

Campbell, D. R., Waser, N. M., Price, M. V., Lynch, E. A., & Mitchell, R. J. (1991). Components of phenotypic selection : Pollen export and flower corolla width in *Ipomopsis aggregata*. *Evolution*, 45(6), 1458-1467. https://doi.org/10.1111/j.1558-5646.1991.tb02648.x

Caruso, C. M. (2000). Competition for pollination influences selection on floral traits of *Ipomopsis aggregata*. *Evolution*, 54(5), 1546-1557. https://doi.org/10.1111/j.0014-3820.2000.tb00700.x

Caruso, C. M., Eisen, K. E., Martin, R. A., & Sletvold, N. (2019). A meta-analysis of the agents of selection on floral traits. *Evolution*, 73(1), 4-14. https://doi.org/10.1111/evo.13639

Carvallo, G. O., & Medel, R. (2010). Effects of herkogamy and inbreeding on the mating system of *Mimulus luteus* in the absence of pollinators. *Evolutionary Ecology*, 24(2), 509-522. https://doi.org/10.1007/s10682-009-9322-4

Chen, K.-H., & Pannell, J. R. (2023). Unisexual flowers as a resolution to intralocus sexual conflict in hermaphrodites. *Proceedings of the Royal Society B: Biological Sciences*, 290(2011), 20232137. https://doi.org/10.1098/rspb.2023.2137

Chittka, L., & Raine, N. E. (2006). Recognition of flowers by pollinators. *Current Opinion in Plant Biology*, 9(4), 428-435. https://doi.org/10.1016/j.pbi.2006.05.002

Christopher, D. A., Mitchell, R. J., & Karron, J. D. (2020). Pollination intensity and paternity in flowering plants. *Annals of Botany*, 125(1), 1-9. https://doi.org/10.1093/aob/mcz159

Cronk, Q. (2022). The distribution of sexual function in the flowering plant : From monoecy to dioecy. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 377(1850), 20210486. https://doi.org/10.1098/rstb.2021.0486

Cronk, Q., & Ojeda, I. (2008). Bird-pollinated flowers in an evolutionary and molecular context. *Journal of Experimental Botany*, 59(4), 715-727. https://doi.org/10.1093/jxb/ern009

Culley, T. M., Weller, S. G., & Sakai, A. K. (2002). The evolution of wind pollination in angiosperms. *Trends in Ecology & Evolution*, 17(8), 361-369. https://doi.org/10.1016/S0169-5347(02)02540-5

Dai, C., & Galloway, L. F. (2013). Sexual selection in a hermaphroditic plant through female reproductive success. *Journal of Evolutionary Biology*, 26(12), 2622-2632. https://doi.org/10.1111/jeb.12254

Darragh, K. (2025). The convergent evolution of hummingbird pollination results in repeated floral scent loss through gene downregulation. *Molecular Biology and Evolution*, 42(2), msaf027. https://doi.org/10.1093/molbev/msaf027

Darwin, C. (1859). *On the origin of species*. John Murray, London.

Darwin, C. (1871). *The descent of man, and selection in relation to sex*. John Murray, London.

De Cauwer, I., Arnaud, J. -F., Schmitt, E., & Dufay, M. (2010). Pollen limitation of female reproductive success at fine spatial scale in a gynodioecious and wind-pollinated species, *Beta vulgaris* ssp. *Maritima*. *Journal of Evolutionary Biology*, 23(12), 2636-2647. https://doi.org/10.1111/j.1420-9101.2010.02119.x

De Cauwer, I., Arnaud, J., Klein, E. K., & Dufaÿ, M. (2012). Disentangling the causes of heterogeneity in male fecundity in gynodioecious *Beta vulgaris* ssp. *Maritima*. *New Phytologist*, 195(3), 676-687. https://doi.org/10.1111/j.1469-8137.2012.04191.x

Delmas, C. E. L., Fort, T. L. C., Escaravage, N., & Pornon, A. (2016). Pollen transfer in fragmented plant populations : Insight from the pollen loads of pollinators and stigmas in a mass-flowering species. *Ecology and Evolution*, 6(16), 5663-5673. https://doi.org/10.1002/ece3.2280

Delph, L. F., Galloway, L. F., & Stanton, M. L. (1996). Sexual dimorphism in flower size. *The American Naturalist*, 148(2), 299-320. https://doi.org/10.1086/285926

DiLeo, M. F., Holderegger, R., & Wagner, H. H. (2018). Contemporary pollen flow as a multiscale process : Evidence from the insect-pollinated herb, *Pulsatilla vulgaris*. *Journal of Ecology*, 106(6), 2242-2255. https://doi.org/10.1111/1365-2745.12992

Emel, S. L., Franks, S. J., & Spigler, R. B. (2017). Phenotypic selection varies with pollination intensity across populations of *Sabatia angularis*. *New Phytologist*, 215(2), 813-824. https://doi.org/10.1111/nph.14608

Fenster, C. B., Armbruster, W. S., Wilson, P., Dudash, M. R., & Thomson, J. D. (2004). Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics*, 35(1), 375-403. https://doi.org/10.1146/annurev.ecolsys.34.011802.132347

Friedman, J., & Barrett, S. C. H. (2009). Wind of change : New insights on the ecology and evolution of pollination and mating in wind-pollinated plants. *Annals of Botany*, 103(9), 1515-1527. https://doi.org/10.1093/aob/mcp035

Galen, C. (1996). Rates of floral evolution : Adaptation to bumblebee pollination in an alpine wildflower, *Polemonium viscosum*. *Evolution*, 50(1), 120-125. https://doi.org/10.1111/j.1558-5646.1996.tb04478.x

García, C., Jordano, P., & Godoy, J. A. (2007). Contemporary pollen and seed dispersal in a *Prunus mahaleb* population : Patterns in distance and direction. *Molecular Ecology*, 16(9), 1947-1955. https://doi.org/10.1111/j.1365-294x.2006.03126.x

Gegear, R. J., & Burns, J. G. (2007). The birds, the bees, and the virtual flowers : Can pollinator behavior drive ecological speciation in flowering plants? *The American Naturalist*, 170(4), 551-566. https://doi.org/10.1086/521230

Gervasi, D. D. L., & Schiestl, F. P. (2017). Real-time divergent evolution in plants driven by pollinators. *Nature Communications*, 8(1), 14691. https://doi.org/10.1038/ncomms14691

Gilbert, F., Willmer, P., Semida, F., Ghazoul, J., & Zalat, S. (1996). Spatial variation in selection in a plant-pollinator system in the wadis of Sinai, Egypt. *Oecologia*, 108(3), 479-487. https://doi.org/10.1007/BF00333724

Giles, B. E., & Goudet, J. (1997). Genetic differentiation in *Silene dioica* metapopulations : Estimation of spatiotemporal effects in a successional plant species. *The American Naturalist*, 149(3), 507-526. https://doi.org/10.1086/286002

Giles, B. E., Lundqvist, E., & Goudet, J. M. (1998). Restricted gene flow and subpopulation differentiation in *Silene dioica*. *Heredity*, 80(6), 715-723. https://doi.org/10.1046/j.1365-2540.1998.00348.x

Giles, B. E., Pettersson, T. M., Carlsson-Granér, U., & Ingvarsson, P. K. (2006). Natural selection on floral traits of female *Silene dioica* by a sexually transmitted disease. *New Phytologist*, 169(4), 729-739. https://doi.org/10.1111/j.1469-8137.2005.01544.x

Giurfa, M., Nunez, J., Chittka, L., & Menzel, R. (1995). Colour preferences of flower-naive honeybees. *Journal of Comparative Physiology A*, 177(3), 247-259. https://doi.org/10.1007/BF00192415

Gómez, J. M., Bosch, J., Perfectti, F., Fernández, J. D., Abdelaziz, M., & Camacho, J. P. M. (2008). Spatial variation in selection on corolla shape in a generalist plant is promoted by the preference patterns of its local pollinators. *Proceedings of the Royal Society B*: *Biological Sciences*, 275(1648), 2241-2249. https://doi.org/10.1098/rspb.2008.0512

Goodwillie, C., Kalisz, S., & Eckert, C. G. (2005). The evolutionary enigma of mixed mating systems in plants : Occurrence, theoretical explanations, and empirical evidence. *Annual Review of Ecology, Evolution, and Systematics*, 36(1), 47-79. https://doi.org/10.1146/annurev.ecolsys.36.091704.175539

Goulson, D., Lye, G. C., & Darvill, B. (2008). The decline and conservation of bumblebees. *Annual Review of Entomology*, 53(1), 191-208. https://doi.org/10.1146/annurev.ento.53.103106.093454

Goverde, M., Schweizer, K., Baur, B., & Erhardt, A. (2002). Small-scale habitat fragmentation effects on pollinator behaviour : Experimental evidence from the bumblebee *Bombus veteranus* on calcareous grasslands. Biological Conservation, 104(3), 293-299. https://doi.org/10.1016/s0006-3207(01)00194-x

Habel, J. C., Ulrich, W., Biburger, N., Seibold, S., & Schmitt, T. (2019). Agricultural intensification drives butterfly decline. *Insect Conservation and Diversity*, 12(4), 289-295. https://doi.org/10.1111/icad.12343

Harder, L. D., & Aizen, M. A. (2010). Floral adaptation and diversification under pollen limitation. *Philosophical Transactions of the Royal Society B*: *Biological Sciences*, 365(1539), 529-543. https://doi.org/10.1098/rstb.2009.0226

Harder, L. D., & Barrett, S. C. H. (1995). Mating cost of large floral displays in hermaphrodite plants. *Nature*, 373(6514), 512-515. https://doi.org/10.1038/373512a0

Harder, L. D., & Barrett, S. C. H. (1996). Pollen dispersal and mating patterns in animal-pollinated plants. In *Floral biology* : *Studies on floral evolution in animal-pollinated plants* (p. 140-190). Springer, Boston, MA.

Hardy, O. J., Delaide, B., Hainaut, H., Gillet, J., Gillet, P., Kaymak, E., Vankerckhove, N., Duminil, J., & Doucet, J. (2019). Seed and pollen dispersal distances in two African legume timber trees and their reproductive potential under selective logging. *Molecular Ecology*, 28(12), 3119-3134. https://doi.org/10.1111/mec.15138

Henshaw, J. M., Jennions, M. D., & Kruuk, L. E. B. (2018). How to quantify (the response to) sexual selection on traits. *Evolution*, 72(9), 1904-1917. https://doi.org/10.1111/evo.13554

Herrera, C. M. (1988). Variation in mutualisms : The spatiotemporal mosaic of a pollinator assemblage. *Biological Journal of the Linnean Society*, 35(2), 95-125. https://doi.org/10.1111/j.1095-8312.1988.tb00461.x

Heuertz, M., Vekemans, X., Hausman, J. -F., Palada, M., & Hardy, O. J. (2003). Estimating seed vs. Pollen dispersal from spatial genetic structure in the common ash. *Molecular Ecology*, 12(9), 2483-2495. https://doi.org/10.1046/j.1365-294X.2003.01923.x

Hill, P. S. M., Hollis, J., & Wells, H. (2001). Foraging decisions in nectarivores : Unexpected interactions between flower constancy and energetic rewards. *Animal Behaviour*, 62(4), 729-737. https://doi.org/10.1006/anbe.2001.1775

Hodgins, K. A., & Barrett, S. C. H. (2008). Natural selection on floral traits through male and female function in wild populations of the heterostylous daffodil *Narcissus triandrus*. *Evolution*, 62(7), 1751-1763. https://doi.org/10.1111/j.1558-5646.2008.00404.x

Hossaert-McKey, M., Soler, C., Schatz, B., & Proffit, M. (2010). Floral scents : Their roles in nursery pollination mutualisms. *Chemoecology*, 20(2), 75-88. https://doi.org/10.1007/s00049-010-0043-5

Igic, B., & Kohn, J. R. (2006). The distribution of plant mating systems : Study bias against obligately outcrossing species. *Evolution*, 60(5), 1098-1103. https://doi.org/10.1111/j.0014-3820.2006.tb01186.x

Ingvarsson, P. K., & Giles, B. E. (1999). Kin-structured colonization and small-scale genetic differentiation in *Silene dioica*. *Evolution*, 53(2), 605-611. https://doi.org/10.1111/j.1558-5646.1999.tb03795.x

Ismail, S. A., Ghazoul, J., Ravikanth, G., Kushalappa, C. G., Uma Shaanker, R., & Kettle, C. J. (2017). Evaluating realized seed dispersal across fragmented tropical landscapes : A two-fold approach using parentage analysis and the neighbourhood model. *New Phytologist*, 214(3), 1307-1316. https://doi.org/10.1111/nph.14427

Jacquemyn, H., Brys, R., & Jongejans, E. (2010). Size-dependent flowering and costs of reproduction affect population dynamics in a tuberous perennial woodland orchid. *Journal of Ecology*, 98(5), 1204-1215. https://doi.org/10.1111/j.1365-2745.2010.01697.x

Janicke, T., Häderer, I. K., Lajeunesse, M. J., & Anthes, N. (2016). Darwinian sex roles confirmed across the animal kingdom. *Science Advances*, 2(2), e1500983. https://doi.org/10.1126/sciadv.1500983

Jiang, X., & Li, Q. (2017). Self- and intra-morph incompatibility and selection analysis of an inconspicuous distylous herb growing on the Tibetan plateau (*Primula tibetica*). *Ecology and Evolution*, 7(15), 5746-5753. https://doi.org/10.1002/ece3.3151

Joffard, N., Le Roncé, I., Langlois, A., Renoult, J., Buatois, B., Dormont, L., & Schatz, B. (2020). Floral trait differentiation in *Anacamptis coriophora* : Phenotypic selection on scents, but not on colour. *Journal of Evolutionary Biology*, 33(8), 1028-1038. https://doi.org/10.1111/jeb.13657

Joffard, N., Lecat, I., Ollier, K., Jolivel, C., Schmitt, E., Godé, C., Barbot, E., Dufaÿ, M., & De Cauwer, I. (2025). Floral display size does not affect the distribution of paternal diversity in *Silene dioica*. *bioRxiv*. https://doi.org/10.1101/2025.07.19.665651

Johnson, M. G., & Shaw, A. J. (2016). The effects of quantitative fecundity in the haploid stage on reproductive success and diploid fitness in the aquatic peat moss *Sphagnum macrophyllum*. *Heredity*, 116(6), 523-530. https://doi.org/10.1038/hdy.2016.13

Jones, A. G., & Ardren, W. R. (2003). Methods of parentage analysis in natural populations. *Molecular Ecology*, 12(10), 2511-2523. https://doi.org/10.1046/j.1365-294X.2003.01928.x

Junker, R. R., & Parachnowitsch, A. L. (2015). Working towards a holistic view on flower traits—How floral scents mediate plant–animal interactions in concert with other floral characters. *Journal of the Indian Institute of Science*, 95(1), 43-68. https://www.cabidigitallibrary.org/doi/full/10.5555/20153065813

Kalisz, S., Randle, A., Chaiffetz, D., Faigeles, M., Butera, A., & Beight, C. (2012). Dichogamy correlates with outcrossing rate and defines the selfing syndrome in the mixed-mating genus *Collinsia*. *Annals of Botany*, 109(3), 571-582. https://doi.org/10.1093/aob/mcr237

Kay, Q. O. N., Lack, A. J., Bamber, F. C., & Davies, C. R. (1984). Differences between sexes in floral morphology, nectar production and insect visits in a dioecious species, *Silene dioica*. *New Phytologist*, 98(3), 515-529. https://doi.org/10.1111/j.1469-8137.1984.tb04145.x

Knight, T. M., Steets, J. A., Vamosi, J. C., Mazer, S. J., Burd, M., Campbell, D. R., Dudash, M. R., Johnston, M. O., Mitchell, R. J., & Ashman, T.-L. (2005). Pollen limitation of plant reproduction : Pattern and process. *Annual Review of Ecology, Evolution, and Systematics*, 36(1), 467-497. https://doi.org/10.1146/annurev.ecolsys.36.102403.115320

Kodric-Brown, A., & Brown, J. H. (1987). Anisogamy, sexual selection, and the evolution and maintenance of sex. *Evolutionary Ecology*, 1(2), 95-105. https://doi.org/10.1007/BF02067393

Kwok, A., & Dorken, M. E. (2022). Sexual selection on male but not female function in monoecious and dioecious populations of broadleaf arrowhead (*Sagittaria latifolia*). *Proceedings of the Royal Society B*: *Biological Sciences*, 289(1986), 20220919. https://doi.org/10.1098/rspb.2022.0919

Labouche, A., Richards, S. A., & Pannell, J. R. (2017). Effects of pollination intensity on offspring number and quality in a wind-pollinated herb. *Journal of Ecology*, 105(1), 197-208. https://doi.org/10.1111/1365-2745.12659

Lande, R. (1976). Natural selection and random genetic drift in phenotypic evolution. *Evolution*, 30(2), 314-334. https://doi.org/10.1111/j.1558-5646.1976.tb00911.x

Lande, R., & Arnold, S. (1983). The measurement of selection on correlated characters. *Evolution*, 37, 1210-1226. http://dx.doi.org/10.2307/2408842

Lavaut, E., Valero, M., Mauger, S., Guillemin, M. L., Destombe, C., & Dufaÿ, M. (2023). Sexual selection in seaweed? Testing Bateman's principles in the red alga *Gracilaria gracilis*. *Proceedings of the Royal Society B*: *Biological Sciences*, 290(2006), 20231668. https://doi.org/10.1098/rspb.2023.1668

LeBuhn, G., & Vargas, L. J. (2021). Pollinator decline : What do we know about the drivers of solitary bee declines? *Current Opinion in Insect Science*, 46, 106-111. https://doi.org/10.1016/j.cois.2021.05.004

Leducq, J.-B., Gosset, C. C., Poiret, M., Hendoux, F., Vekemans, X., & Billiard, S. (2010). An experimental study of the S-Allee effect in the self-incompatible plant *Biscutella neustriaca*. *Conservation Genetics*, 11(2), 497-508. https://doi.org/10.1007/s10592-010-0055-2

Linder, H. P. (1998). Morphology and the evolution of wind pollination. In *Reproductive biology in systematics, conservation and economic botany* (S. J. Owens&P. J. Rudall, p. 123-135). Kew, UK: Royal Botanic Gardens.

Llaurens, V., Castric, V., Austerlitz, F., & Vekemans, X. (2008). High paternal diversity in the self-incompatible herb *Arabidopsis halleri* despite clonal reproduction and spatially restricted pollen dispersal. *Molecular Ecology*, 17(6), 1577-1588. https://doi.org/10.1111/j.1365-294X.2007.03683.x

Lu, N. -N., Ma, Y., Hou, M., & Zhao, Z. -G. (2021). The function of floral traits and phenotypic selection in *Aconitum gymnandrum* (Ranunculaceae). *Plant Biology*, 23(6), 931-938. https://doi.org/10.1111/plb.13305

Lu, X., Ye, X., & Liu, J. (2022). Morphological differences between anemophilous and entomophilous pollen. *Microscopy Research and Technique*, 85(3), 1056-1064. https://doi.org/10.1002/jemt.23975

Lunau, K. (1991). Innate flower recognition in bumblebees (*Bombus terrestris, B. lucorum*; Apidae) : Optical signals from stamens as landing reaction releasers. *Ethology*, 88(3), 203-214. https://doi.org/10.1111/j.1439-0310.1991.tb00275.x

Lunau, K., Scaccabarozzi, D., Willing, L., & Dixon, K. (2021). A bee's eye view of remarkable floral colour patterns in the south-west Australian biodiversity hotspot revealed by false colour photography. *Annals of Botany*, 128(7), 821-824. https://doi.org/10.1093/aob/mcab088

Lynch, M. (2007). The frailty of adaptive hypotheses for the origins of organismal complexity. *Proceedings of the National Academy of Sciences*, 104(suppl_1), 8597-8604. https://doi.org/10.1073/pnas.0702207104

MacArthur, R. H., & Pianka, E. R. (1966). On optimal use of a patchy environment. *The American Naturalist*, 100(916), 603-609. https://doi.org/10.1086/282454

Mackin, C. R., Peña, J. F., Blanco, M. A., Balfour, N. J., & Castellanos, M. C. (2021). Rapid evolution of a floral trait following acquisition of novel pollinators. *Journal of Ecology*, 109(5), 2234-2246. https://doi.org/10.1111/1365-2745.13636

Makino, T. T., & Sakai, S. (2007). Experience changes pollinator responses to floral display size : From size-based to reward-based foraging. *Functional Ecology*, 21(5), 854-863. https://doi.org/10.1111/j.1365-2435.2007.01293.x

Mant, J., Peakall, R., & Schiestl, F. P. (2005). Does selection on floral odor promote differentiation among populations and species of the sexually deceptive orchid genus *Ophrys*? *Evolution*, 59(7), 1449. https://doi.org/10.1554/04-547

Marden, J. H., & Waddington, K. D. (1981). Floral choices by honeybees in relation to the relative distances to flowers. *Physiological Entomology*, 6(4), 431-435. https://doi.org/10.1111/j.1365-3032.1981.tb00658.x

Marshall, T. C., Slate, J., Kruuk, L. E. B., & Pemberton, J. M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, 7(5), 639-655. https://doi.org/10.1046/j.1365-294x.1998.00374.x

Martén-Rodríguez, S., Almarales-Castro, A., & Fenster, C. B. (2009). Evaluation of pollination syndromes in Antillean Gesneriaceae : Evidence for bat, hummingbird and generalized flowers. *Journal of Ecology*, 97, 348-359. https://doi.org/doi: 10.1111/j.1365-2745.2008.01465.x

McCallum, B., & Chang, S. (2016). Pollen competition in style : Effects of pollen size on siring success in the hermaphroditic common morning glory, *Ipomoea purpurea*. *American Journal of Botany*, 103(3), 460-470. https://doi.org/10.3732/ajb.1500211

Meagher, T. R. (1986). Analysis of paternity within a natural population of *Chamaelirium luteum*. 1. Identification of most-likely male parents. *The American Naturalist*, 128(2), 199-215. https://doi.org/10.1086/284554

Merilä, J., & Hendry, A. P. (2014). Climate change, adaptation, and phenotypic plasticity : The problem and the evidence. *Evolutionary Applications*, 7(1), 1-14. https://doi.org/10.1111/eva.12137

Montgomery, B. R., Soper, D. M., & Delph, L. F. (2010). Asymmetrical conspecific seed-siring advantage between *Silene latifolia* and *S. dioica*. *Annals of Botany*, 105(4), 595-605. https://doi.org/10.1093/aob/mcq013

Moore, J. C., & Pannell, J. R. (2011). Sexual selection in plants. *Current Biology*, 21(5), R176-R182. https://doi.org/10.1016/j.cub.2010.12.035

Moquet, L., Jacquemart, A.-L., Dufaÿ, M., & De Cauwer, I. (2022). Effects of sexual dimorphism on pollinator behaviour in a dioecious species. *Oikos*, 2022(3), e08662. https://doi.org/10.1111/oik.08662

Moquet, L., Lateur, L., Jacquemart, A.-L., De Cauwer, I., & Dufaÿ, M. (2020). Temporal dynamics of sexual dimorphism in a dioecious species. *Annals of Botany*, 126(3), 471-480. https://doi.org/10.1093/aob/mcaa088

Moquet, L., Laurent, E., Bacchetta, R., & Jacquemart, A. (2018). Conservation of hoverflies (Diptera, Syrphidae) requires complementary resources at the landscape and local scales. *Insect Conservation and Diversity*, 11(1), 72-87. https://doi.org/10.1111/icad.12245

Muchhala, N. (2007). Adaptive trade-off in floral morphology mediates specialization for flowers pollinated by bats and hummingbirds. *The American Naturalist*, 169(4), 494-504. https://doi.org/10.1086/512047

Newman, E., Anderson, B., & Johnson, S. D. (2012). Flower colour adaptation in a mimetic orchid. *Proceedings of the Royal Society B: Biological Sciences*, 279(1737), 2309-2313. https://doi.org/10.1098/rspb.2011.2375

Nicolson, S. W. (2011). Bee food : The chemistry and nutritional value of nectar, pollen and mixtures of the two. *African Zoology*, 46(2), 197-204. https://doi.org/10.1080/15627020.2011.11407495

Oddou-Muratorio, S., Gauzere, J., Bontemps, A., Rey, J.-F., & Klein, E. K. (2018). Tree, sex and size : Ecological determinants of male vs. Female fecundity in three *Fagus sylvatica* stands. *Molecular Ecology*, 27(15), 3131-3145. https://doi.org/10.1111/mec.14770

Ohashi, K., & Yahara, T. (1998). Effects of variation in flower number on pollinator visits in *Cirsium purpuratum* (Asteraceae). *American Journal of Botany*, 85(2), 219-224. https://doi.org/10.2307/2446309

Ollerton, J. (2017). Pollinator diversity : Distribution, ecological function, and conservation. *Annual Review of Ecology, Evolution, and Systematics*, 48(1), 353-376. https://doi.org/10.1146/annurev-ecolsys-110316-022919

Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, 120(3), 321-326. https://doi.org/10.1111/j.1600-0706.2010.18644.x

Örjes, E. (2021). Does size matter? : A study on pollen size distribution and pollen viability in *Silene latifolia* and *S. dioica* (Dissertation). http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-435339

Pannell, J. R., & Labouche, A.-M. (2013). The incidence and selection of multiple mating in plants. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368(1613), 20120051. https://doi.org/10.1098/rstb.2012.0051

Poblete Palacios, J. A., Soteras, F., & Cocucci, A. A. (2019). Mechanical fit between flower and pollinators in relation to realized precision and accuracy in the hummingbird-pollinated *Dolichandra cynanchoides*. *Biological Journal of the Linnean Society*, 126(4), 655-665. https://doi.org/10.1093/biolinnean/bly219

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010). Global pollinator declines : Trends, impacts and drivers. *Trends in Ecology & Evolution*, 25(6), 345-353. https://doi.org/10.1016/j.tree.2010.01.007

Qiu, Y., Yan, X., Ma, H., Wang, Y., Yang, R., Opedal, Ø. H., & Zhao, Z. (2023). Proximity to oilseed rape fields affects plant pollination and pollinator-mediated selection on a co-flowering plant on the Tibetan Plateau. *Evolutionary Applications*, 16(4), 814-823. https://doi.org/10.1111/eva.13538

Raguso, R. A. (2008). Wake up and smell the roses : The ecology and evolution of floral scent. *Annual Review of Ecology, Evolution, and Systematics*, 39(1), 549-569. https://doi.org/10.1146/annurev.ecolsys.38.091206.095601

Rashed, A., & Sherratt, T. N. (2007). Mimicry in hoverflies (Diptera : Syrphidae): a field test of the competitive mimicry hypothesis. *Behavioral Ecology*, 18(2), 337-344. https://doi.org/10.1093/beheco/arl089

Raven, P. H. (1972). Why are bird-visited flowers predominantly red? Evolution, 26(4), 674-674. https://doi.org/10.1111/j.1558-5646.1972.tb01975.x

Regan, E. C., Santini, L., Ingwall-King, L., Hoffmann, M., Rondinini, C., Symes, A., Taylor, J., & Butchart, S. H. M. (2015). Global trends in the status of bird and mammal pollinators. *Conservation Letters*, 8(6), 397-403. https://doi.org/10.1111/conl.12162

Renner, S. S. (2014). The relative and absolute frequencies of angiosperm sexual systems : Dioecy, monoecy, gynodioecy, and an updated online database. *American Journal of Botany*, 101(10), 1588-1596. https://doi.org/10.3732/ajb.1400196

Rhodes, C. J. (2018). Pollinator decline – an ecological calamity in the making? *Science Progress*, 101(2), 121-160. https://doi.org/10.3184/003685018x15202512854527

Ricketts, T. H., Regetz, J., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., Bogdanski, A., Gemmill-Herren, B., Greenleaf, S. S., Klein, A. M., Mayfield, M. M., Morandin, L. A., Ochieng', A., & Viana, B. F. (2008). Landscape effects on crop pollination services : Are there general patterns? *Ecology Letters*, 11(5), 499-515. https://doi.org/10.1111/j.1461-0248.2008.01157.x

Riveros, A. J., & Gronenberg, W. (2012). Decision-making and associative color learning in harnessed bumblebees (*Bombus impatiens*). *Animal Cognition*, 15(6), 1183-1193. https://doi.org/10.1007/s10071-012-0542-6

Sandring, S., & Ågren, J. (2009). Pollinator-mediated selection on floral display and flowering time in the perennial herb *Arabidopsis lyrata*. *Evolution*, 63(5), 1292-1300. https://doi.org/10.1111/j.1558-5646.2009.00624.x

Sanghvi, K., Henshaw, J. M., Kacelnik, A., Janicke, T., & Sepil, I. (2025). Diagnosing confounded Bateman gradients. *Evolution*, qpaf127. https://doi.org/10.1093/evolut/qpaf127

Simpson, B. B., & Neff, J. L. (1981). Floral rewards : Alternatives to pollen and nectar. *Annals of the Missouri Botanical Garden*, 68(2), 301. https://doi.org/10.2307/2398800

Skogsmyr, I., & Lankinen, Å. (1999). Selection on pollen competitive ability in relation to stochastic factors influencing pollen deposition. *Evolutionary Ecology Research*, 1(8), 971-985. https://www.cabidigitallibrary.org/doi/full/10.5555/20001611764

Skogsmyr, I., & Lankinen, A. (2002). Sexual selection : An evolutionary force in plants? *Biological Reviews of the Cambridge Philosophical Society*, 77(4), 537-562. https://doi.org/10.1017/S1464793102005973

Sletvold, N., & Ågren, J. (2016). Experimental reduction in interaction intensity strongly affects biotic selection. *Ecology*, 97(11), 3091-3098. https://doi.org/10.1002/ecy.1554

Slotte, T., Hazzouri, K. M., Stern, D., Andolfatto, P., & Wright, S. I. (2012). Genetic architecture and adaptive significance of the selfing syndrome in *Capsella*. *Evolution*, 66(5), 1360-1374. https://doi.org/10.1111/j.1558-5646.2011.01540.x

Snow, A. A., & Lewis, P. O. (1993). Reproductive traits and male fertility in plants : Empirical approaches. *Annual Review of Ecology and Systematics*, 24(1), 331-351. https://doi.org/10.1146/annurev.es.24.110193.001555

Snow, A. A., & Spira, T. P. (1991). Differential pollen-tube growth rates and nonrandom fertilization in *Hibiscus moscheutos* (Malvaceae). *American Journal of Botany*, 78(10), 1419-1426. https://doi.org/10.1002/j.1537-2197.1991.tb12608.x

Stanton, M. L., & Preston, R. E. (1988). Ecological consequences and phenotypic correlates of petal size variation in wild radish, *Raphanus sativus* (Brassicaceae). *American Journal of Botany*, 75(4), 528-539. https://doi.org/10.1002/j.1537-2197.1988.tb13471.x

Stebbins, G. L. (1974). *Flowering plants : Evolution above the species level*. Cambridge, MA: Belknap.

Sullivan, V. I. (1975). Pollen and pollination in the genus *Eupatorium* (Compositae). *Canadian Journal of Botany*, 53(6), 582-589. https://doi.org/10.1139/b75-072

Takhtajan, A. (1969). *Flowering plants : Origin and disposal*. Oliver and Boyd.

terHorst, C. P., Lau, J. A., & Conner, J. K. (2017). Quantifying nonadditive selection caused by indirect ecological effects : Reply. *Ecology*, 98(4), 1171-1175. https://doi.org/10.1002/ecy.1744

terHorst, C. P., Lau, J. A., Cooper, I. A., Keller, K. R., Rosa, R. J. L., Royer, A. M., Schultheis, E. H., Suwa, T., & Conner, J. K. (2015). Quantifying nonadditive selection caused by indirect ecological effects. *Ecology*, 96(9), 2360-2369. https://doi.org/10.1890/14-0619.1

Thomann, M., Imbert, E., Engstrand, R. C., & Cheptou, P.-O. (2015). Contemporary evolution of plant reproductive strategies under global change is revealed by stored seeds. *Journal of Evolutionary Biology*, 28(4), 766-778. https://doi.org/10.1111/jeb.12603

Thompson, J. N. (2005). *The geographic mosaic of coevolution.* University of Chicago Press, Chicago, Illinois, USA.

Tong, Z.-Y., Wu, L.-Y., Feng, H.-H., Zhang, M., Armbruster, W. S., Renner, S. S., & Huang, S.-Q. (2023). New calculations indicate that 90% of flowering plant species are animal-pollinated. *National Science Review*, 10(10), nwad219. https://doi.org/10.1093/nsr/nwad219

Tonnabel, J., David, P., Janicke, T., Lehner, A., Mollet, J.-C., Pannell, J. R., & Dufaÿ, M. (2021). The scope for postmating sexual selection in plants. *Trends in Ecology & Evolution*, 36(6), 556-567. https://doi.org/10.1016/j.tree.2021.02.013

Tonnabel, J., David, P., Klein, E. K., & Pannell, J. R. (2019a). Sex-specific selection on plant architecture through "budget" and "direct" effects in experimental populations of the wind-pollinated herb, *Mercurialis annua*. *Evolution*, 73(5), 897-912. https://doi.org/10.1111/evo.13714

Tonnabel, J., David, P., & Pannell, J. R. (2019b). Do metrics of sexual selection conform to Bateman's principles in a wind-pollinated plant? *Proceedings of the Royal Society B: Biological Sciences*, 286(1905), 20190532. https://doi.org/10.1098/rspb.2019.0532

Toräng, P., Vikström, L., Wunder, J., Wötzel, S., Coupland, G., & Ågren, J. (2017). Evolution of the selfing syndrome : Anther orientation and herkogamy together determine reproductive assurance in a self-compatible plant. *Evolution*, 71(9), 2206-2218. https://doi.org/10.1111/evo.13308

Totland, Ø. (2001). Environment-dependent pollen limitation and selection on floral traits in an alpine species. *Ecology*, 82(8), 2233-2244. http://dx.doi.org/10.1890/0012-9658(2001)082[2233:EDPLAS]2.0.CO;2

van der Niet, T., & Johnson, S. D. (2012). Phylogenetic evidence for pollinator-driven diversification of angiosperms. *Trends in Ecology & Evolution*, 27(6), 353-361. https://doi.org/10.1016/j.tree.2012.02.002

Van Rossum, F., Stiers, I., Van Geert, A., Triest, L., & Hardy, O. J. (2011). Fluorescent dye particles as pollen analogues for measuring pollen dispersal in an insect-pollinated forest herb. *Oecologia*, 165(3), 663-674. https://doi.org/10.1007/s00442-010-1745-7

vanEngelsdorp, D., Hayes, J., Underwood, R. M., & Pettis, J. (2008). A survey of honey bee colony losses in the U.S., fall 2007 to spring 2008. *PLoS ONE*, 3(12), e4071. https://doi.org/10.1371/journal.pone.0004071

Vaudo, A. D., Tooker, J. F., Grozinger, C. M., & Patch, H. M. (2015). Bee nutrition and floral resource restoration. *Current Opinion in Insect Science*, 10, 133-141. https://doi.org/10.1016/j.cois.2015.05.008

Wardhaugh, C. W. (2015). How many species of arthropods visit flowers? *Arthropod-Plant Interactions*, 9(6), 547-565. https://doi.org/10.1007/s11829-015-9398-4

Waser, N. M., Chittka, L., Price, M. V., Williams, N. M., & Ollerton, J. (1996). Generalization in pollination systems, and why it matters. *Ecology*, 77(4), 1043-1060. https://doi.org/10.2307/2265575

Welsford, M. R., Hobbhahn, N., Midgley, J. J., & Johnson, S. D. (2016). Floral trait evolution associated with shifts between insect and wind pollination in the dioecious genus *Leucadendron* (Proteaceae). *Evolution*, 70(1), 126-139. https://doi.org/10.1111/evo.12821

Wiebes, J. T. (1979). Co-evolution of figs and their insect pollinators. *Annual Review of Ecology and Systematics*, 10(1), 1-12. https://doi.org/10.1146/annurev.es.10.110179.000245

Winfree, R., Aguilar, R., Vázquez, D. P., LeBuhn, G., & Aizen, M. A. (2009). A meta-analysis of bees' responses to anthropogenic disturbance. *Ecology*, 90(8), 2068-2076. https://doi.org/10.1890/08-1245.1

Winfree, R., Bartomeus, I., & Cariveau, D. P. (2011). Native pollinators in anthropogenic habitats. *Annual Review of Ecology, Evolution, and Systematics*, 42(1), 1-22. https://doi.org/10.1146/annurev-ecolsys-102710-145042

Woodard, S. H., & Jha, S. (2017). Wild bee nutritional ecology : Predicting pollinator population dynamics, movement, and services from floral resources. *Current Opinion in Insect Science*, 21, 83-90. https://doi.org/10.1016/j.cois.2017.05.011

Wright, G. A., & Schiestl, F. P. (2009). The evolution of floral scent : The influence of olfactory learning by insect pollinators on the honest signalling of floral rewards. *Functional Ecology*, 23(5), 841-851. https://doi.org/10.1111/j.1365-2435.2009.01627.x

Wu, Y., Liu, G., Sletvold, N., Duan, X., Tong, Z., & Li, Q. (2023). Soil water and nutrient availability interactively modify pollinator-mediated directional and correlational selection on floral display. *New Phytologist*, 237(2), 672-683. https://doi.org/10.1111/nph.18537

Wu, Y., Zhong, T., Zhang, Z.-Q., & Li, Q.-J. (2018). Pollinator-mediated selection on floral traits varies in space and between morphs in *Primula secundiflora*. *AoB PLANTS*, 10(5), ply059. https://doi.org/10.1093/aobpla/ply059

Yashiro, K., Sakai, Y., & Namai, H. (1999). Relationships between pollen-ovule ratio and autofertility, self-compatibility, automatic self-pollination ability in heterogeneous incomplete autogamous plants, Thai mustard. *Breeding science*, 49(1), 39-42. https://doi.org/10.1270/jsbbs.49.39

Zhang, L.-J., & Lou, A.-R. (2015). Pollen limitation in invasive populations of *Solanum rostratum* and its relationship to population size. *Journal of Plant Ecology*, 8(2), 154-158. https://doi.org/10.1093/jpe/rtv013

Zhou, J., Reynolds, R. J., Zimmer, E. A., Dudash, M. R., & Fenster, C. B. (2020). Variable and sexually conflicting selection on *Silene stellata* floral traits by a putative moth pollinator selective agent. *Evolution*, 74(7), 1321-1334. https://doi.org/10.1111/evo.13965

CHAPITRE I

Structure génétique des populations chez le compagnon rouge (*Silene dioica*) : effets des composantes du paysage

Ce premier chapitre a pour objectif de déterminer l'échelle spatiale à laquelle s'exerce la compétition intra-sexuelle pré-pollinisation en conditions naturelles, et à déterminer si cette échelle varie en fonction du type d'habitat. Chez une espèce de plante pollinisée par les insectes, cette question revient à déterminer l'échelle à laquelle les individus sont en compétition pour l'attraction des pollinisateurs, c'est-à-dire l'échelle à laquelle ont lieu les flux de pollen. Cette question sera abordée en combinant une approche indirecte, basée sur l'analyse de la structure génétique spatiale des populations afin d'inférer les flux de gènes historiques, et une approche directe, visant à estimer les flux de pollen contemporains à partir d'analyses de paternité réalisées dans plusieurs populations naturelles de *S. dioica*.

Manuscript A: Population genetic structure in the red campion (*Silene dioica*): effects of landscape components

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Abstract

In the plant kingdom, gene flow occurs through pollen and seed dispersal, shaping both within-population spatial genetic structure and among-population genetic differentiation. Dispersal-related processes, such as pollination and seed dispersal, modulate patterns of genetic connectivity among subdivided populations. Intensive agriculture and associated land-use changes can strongly affect gene flow by reducing pollinator abundance and altering seed dispersal pathways. Landscape effects on levels of gene flow are increasingly recognized, yet their impact on fine-scale genetic structure in common herbaceous plants—a key component of many ecosystems—remain poorly understood. This calls for combining historical and contemporary gene flow estimates using extensive sampling and paternity analyses. Here, we examined the spatial patterns of neutral genetic diversity at both regional and local scales in the widespread insect pollinated red campion (*Silene dioica*). Using 1005 individuals from 29 sampled populations spanning habitats from semi-natural to strongly human-modified, we showed moderate levels of genetic differentiation that did not deviate from an isolation-by-distance pattern of population structure, with high levels of individual admixture. We found no significant effect of habitat features on within-population levels of genetic diversity. At finer spatial scales, five out of six populations exhibited strong spatial genetic structuring suggestive of distinct neighbourhoods of individuals occurring at scales not exceeding 10 m. Progeny analyses further revealed predominantly short-distance pollen dispersal alongside substantial immigration. These genetic patterns were unaffected by the degree of anthropogenic modification, highlighting an interesting mix of local and long-distance pollen flow. Altogether, our results highlighted the remarkable resilience of this widespread herbaceous species to anthropogenic habitat modification, maintaining substantial genetic connectivity despite insect-mediated pollen dispersal and gravity-driven seed dispersal.

Key words

Gene flow, genetic diversity, pollen dispersal, isolation-by-distance, habitat quality

Contents

INTRODUCTION

In plants, pollen or seed dispersal followed by successful reproduction lead to differential levels of gene flow that shape the spatial organisation of genetic diversity within populations and modulate the levels of genetic divergence among populations (Slatkin, 1985 ; Petit et al., 2005 ; Ellegren & Galtier, 2016 ; Wang et al., 2023 ; Yang et al., 2025). Over recent decades, agricultural intensification has fragmented habitats (Mullu, 2016), which negatively affects pollen and seed dispersal, and consequently alters population genetic structure (Sebbenn et al., 2011 ; Gómez-Fernández et al., 2016). Habitat fragmentation can reduce migration because populations become more isolated, and can also decrease population size, both processes thereby increasing the effects of genetic drift (Van Rossum et al., 2004 ; Honnay & Jacquemyn, 2007 ; Aavik et al., 2013 ; Ellegren & Galtier, 2016 ; Guiller et al., 2023). In short-lived plant species, the time elapsed since the onset of modern agricultural intensification represents a substantial number of generations, potentially generating detectable genetic signatures, manifesting as correlations between land-use and spatial genetic structure (Emel et al., 2021 ; Guiller et al., 2023 ; Huang et al., 2025), even in species that are common and locally abundant.

Various species-specific life-history traits influence pollen and seed flow, and can therefore shape the spatial organization of genetic diversity among populations, at the regional level. In at least 80% of angiosperm species (Ollerton et al., 2011 ; Tong et al., 2023) reproduction depends on interactions between plants and animal pollinators, primarily insects. In generalist plant species, which are pollinated by multiple types of pollinators, the composition of pollinator communities can vary across space (Herrera, 1988), with the potential to influence patterns of spatial genetic structure. For example, flight distances vary among pollinator species, with larger-bodied species generally foraging over greater distances than smaller ones (Zurbuchen et al., 2010), thereby promoting efficient gene flow among distant plant populations (Gamba & Muchhala, 2020). Seed dispersal also contributes to gene flow dynamics but also to the colonization of suitable environments, allowing for the successful establishment of new populations (Cain et al., 2000 ; Petit et al., 2005 ; Fievet et al., 2007 ; Dellafiore et al., 2010). Similar to pollination mode, seed dispersal mode can therefore influence the spatial organization of genetic diversity (Howe & Smallwood, 1982 ; Petit et al., 2005 ; Beckman & Sullivan, 2023). For instance, species with wind-dispersed seeds exhibit lower levels of among-population genetic differentiation than those with gravity-dispersed seeds (Hamrick & Godt, 1996). Nonetheless, dispersal patterns, both of pollen and seeds, can be strongly influenced by anthropogenic factors. Agricultural intensification commonly induce habitat fragmentation that negatively affect pollinator communities through reductions in floral resources and the intensive use of pesticides and herbicides (Goverde et al., 2002 ; Ricketts et al., 2008 ; Winfree et al., 2009 ; Woodard & Jha, 2017). Pollinator decline can also directly reduce plant population sizes (Biesmeijer et al., 2006), potentially increasing genetic differentiation among populations due to genetic drift (Sullivan et al., 2019). Habitat fragmentation

further limits pollen flow by decreasing matrix permeability (Aguilar et al., 2008 ; Aavik et al., 2013 ; Aavik et al., 2014). Conversely, agricultural activities may occasionally facilitate long-distance seed dispersal via machinery, promoting the establishment of new populations and the introduction of novel genetic variants (Strykstra et al., 1997 ; Vitalos & Karrer, 2009 ; Klinger et al., 2021 ; Taylor et al., 2012 ; Latron et al., 2023). Overall, agricultural intensification is expected to strongly affect spatial patterns of pollen and seed flow, though its effects remain difficult to predict and depend on species-specific traits (Aavik et al., 2013 ; Aavik et al., 2014 ; Favre-Bac et al., 2016 ; DiLeo et al., 2018).

Shifting the focus from regional among-population processes to local within-population dynamics highlights the role of short-distance movements in shaping genetic structure. Even in plant species pollinated by highly mobile insects—which could be expected to promote gene flow among distant populations—once insects arrive within a geographical patch of conspecific individuals, they tend to move sequentially between nearby individuals over short distances to maximize energy intake (nectar, pollen) while minimizing costs, in line with optimal foraging theory (Schmitt, 1980 ; Marden & Waddington, 1981 ; Hill et al., 2001), thereby shaping the fine-scale genetic structure of plant populations (MacArthur & Pianka, 1966 ; DiLeo et al., 2018). This may result in fine-scale isolation-by-distance, whereby genetic relatedness decreases with increasing distance among individuals, especially in species where seed dispersal is spatially restricted (e.g., in species with gravity-dispersed seeds). However, human activity may also alter such fine-scale genetic structure in multiple ways. In a fragmented habitat with isolated plant populations, insects' tendency to visit neighbouring plants may be strengthened, as they must optimize their foraging flight to a greater extent (MacArthur & Pianka, 1966 ; Goverde et al., 2002), potentially enhancing fine-scale isolation-by-distance. Conversely, some anthropogenic practices can facilitate local seed dispersal, thereby homogenizing genetic variation within populations. For example, grazing, when carried out after flowering, may promote seed dispersal at the local scale, reducing fine-scale genetic structure (Smith et al., 2009 ; Rico & Wagner, 2016). Consistent with these processes, empirical studies report contrasting outcomes, with some showing that habitat degradation can modify fine-scale spatial genetic structure within plant populations, either intensifying it (Van Rossum & Triest, 2007 ; De-Lucas et al., 2009) or decreasing it (Born et al., 2008), while others find little or no detectable effect (Smith et al., 2018). Complementary to fine-scale population genetic structure analyses, which reveal the cumulative effects of habitat on patterns of gene flow over multiple generations, paternity analyses provide direct insights into contemporary pollen movements (Jones & Ardren, 2003) and are powerful tools for understanding the immediate effects of anthropogenic changes on gene flow. Studies using such approaches have shown that in herbaceous plant species, pollen dispersal is often spatially restricted (typically under 25 meters, De Cauwer et al., 2012 ; DiLeo et al., 2018 ; Tonnabel, et al., 2019 ; Barbot et al., 2022). However, how habitat degradation can affect pollen dispersal within populations remains unclear.

In this study, we examined spatial patterns of neutral genetic diversity both at the regional and local scales in a common herbaceous species, the red campion (*Silene dioica*). Although typically described as a forest-associated plant, this species is able to colonise a wide range of habitats—including field edges, roadsides, and other anthropogenic environments—provided that conditions remain partially shaded and soils moist (Kay et al., 1984 ; Giles & Goudet, 1997a,b ; Barbot et al., 2022). We sampled a set of populations representative of the red campion's range in population size and habitat types, and genotyped individuals using microsatellite loci. At the regional scale, we tested for the effect of landscape features on within-population genetic diversity, predicting that populations surrounded by large areas of forest and semi-natural habitats would display higher levels of genetic diversity than those embedded in predominantly agricultural landscapes, where genetic diversity may be reduced due to genetic drift. We also quantified the extent of genetic differentiation among populations. Given that the red campion is insect-pollinated with gravity-dispersed seeds, we predicted substantial spatial genetic structure and patterns of isolation-by-distance. At the local scale, we examined patterns of spatial genetic structure within populations, focusing on a subset of populations representing the most contrasting environments: three forest populations and three populations from anthropogenic habitats characterized by a high proportion of cultivated fields. Using progeny genotyping and paternity analyses, we also estimated pollen dispersal distances in these six populations. By integrating genetic analyses across different scales and diverse ecological contexts, this study offers new insights into the complex interplay of spatial configuration of landscape components and species specific life-history traits that may shape patterns of gene flow.

MATERIALS AND METHODS

1. Study species

The red campion (*Silene dioica* (L) Clairv.) (Caryophyllaceae) is a herbaceous, perennial, and dioecious angiosperm widely distributed in central and northern Europe, often inhabiting semi-natural or anthropogenic habitats (Kay et al., 1984 ; Giles & Goudet, 1997a). It has a generalist pollination system, primarily mediated by two major taxonomic groups: Hymenoptera, including large insects such as bumblebees (*Bombus*) and smaller ones such as halictid bees, and Diptera, mainly hoverflies (Kay et al., 1984 ; Barbot et al., 2022). Other contributing groups include Lepidoptera (both butterflies and moths, Barbot et al., 2022 ; Barbot et al., 2025) and Coleoptera (Jolivel et al., in prep.). Its lifespan ranges from 5 to 10 years (Giles et al., 1998). In northern France, the species' flowering period spans from late April to mid-July, peaking in late May (Barbot et al., 2022). The seeds are dispersed by gravity, and typically germinate the following spring (Giles & Goudet, 1997b).

2. Habitat characterisation and sampling design

We sampled 29 natural populations across northern France and Belgium (**Figure 1**). Populations were separated by a mean distance of 30 km (range: 0.27–72.14 km). Our sampling was not exhaustive and aimed to capture a representative subset of populations in the region. Land use in the area surrounding each population was characterized and quantified using the QGIS software (version 3.30.0) and GroupStats plugin (version 2.2.7). Land use was assessed within a one-kilometer radius around each population to align with the typical foraging range of bumblebees (Osborne et al., 2008), which are common pollinators of *S. dioica* and among the most mobile insect visitors. Four mapping data sources were used depending on the location of the population and the sampling year: the ARCH project, the OS Picardie project, the COSW project, and the THEIA project. Habitat types included forest habitats (coniferous and deciduous forests), semi-natural habitats (meadows and pastures), crop habitats (fields and orchards), and urbanized habitats (cities and industrial sites) (supplementary material – Habitat characterization). We also measured the distance to the nearest wooded area, considering only fragments larger than 300 m². A Principal Component Analysis (PCA) was then performed on habitat characteristics to identify which variables to include in the statistical analyses presented below (supplementary material – Habitat characterization). PC1, which explained 49.9% of the total variance, indicated that distance to forest, crop habitat surface, and semi-natural habitat surface were negatively correlated with forest habitat surface. PC2, which explained 22.1% of the variance, was primarily associated with urban habitat surface. Forest habitat surface (ranging from 0% to 98%) and urban habitat surface (ranging from 0% to 53%), which best represented PC1 and PC2 respectively, were therefore retained as explanatory variables in the subsequent statistical analyses.

Leaf samples from 1,005 individuals were collected across the 29 sampled populations. To investigate the spatial genetic structure both among and within populations, we implemented two complementary sampling strategies. Between 2018 and 2019, we carried out broad regional sampling by randomly collecting an average of 17.61 ± 0.70 SE individuals from 23 natural populations (supplementary material – Population description). This regional sampling was designed to represent the diversity of habitats where the species occurs. In 2023, we conducted a more intensive sampling within six carefully selected populations with comparable surface areas, representing contrasting habitat types: three forest-dominated populations ("F") with at least 90% forest and semi-natural cover surrounding each population, and three anthropogenic populations ("A") characterized by more than 40% agricultural and urbanized land. In each of these six populations, 50 males and 50 females were randomly sampled to enable a fine-scale investigation of spatial genetic structure within populations. For all populations, census size was estimated by counting the total number of flowering individuals (supplementary material – Population description).

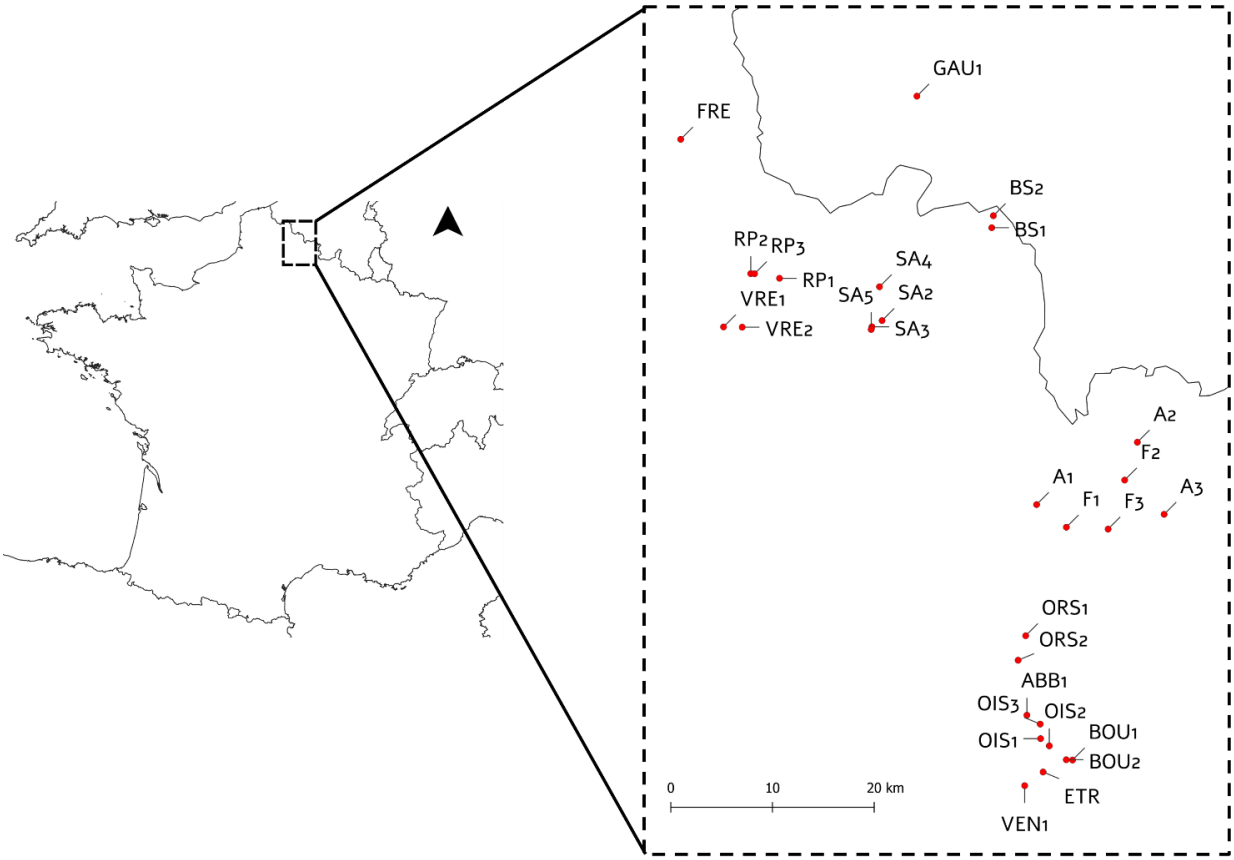

Figure 1. Location and labels of sampled populations of red campion (*Silene dioica*) in northern France and Belgium. The grey line corresponds to the border between countries.

Additional manipulations were carried out in the six intensively sampled populations to standardize demographic conditions and to collect seeds for paternity analyses. During peak flowering in 2023, all inflorescences of non-sampled individuals within these populations and within a 100 meters radius around them were removed to ensure comparable population sizes and densities across study sites (50 flowering males and 50 flowering females per population). Besides, paternity analyses were performed on a subsample of the seeds produced during a ten-day flowering window. For each female (50 per population, 300 in total), fruits were harvested at maturity and we randomly selected eight fruits and genotyped two seeds per fruit, resulting in 16 offspring per female for a total of 4,800 offspring genotyped across all populations.

3. Molecular genotyping

Total genomic DNA was extracted from 1,005 adult individuals and 4,800 offspring using the NucleoMag 96 Plant Kit (Macherey-Nagel, Oesingen, Switzerland) and an automated DNA purification station (KingFisher®, Thermo Fisher Scientific, Waltham, USA). Seed DNA extraction protocols are described in Joffard et al. (2025). PCR amplifications were performed using five nuclear microsatellites isolated and described in Barbot et al. (2022). Electrophoresis of DNA products was performed using an automated capillary sequencer (3130 Genetic Analyzer, Applied Biosystems, USA) and multilocus

genotypes were scored using GeneMapper (Applied Biosystems, USA). Individuals showing ambiguous genotypes were subjected to a second PCR to confirm allele calls. Missing genotypes accounted for less than 1% of the data.

4. Levels of genetic diversity

Three measures of genetic diversity were estimated within each population: the allelic richness (A_r, the number of alleles standardized to the size of the smallest sampled population and averaged across the five loci), the intra-population fixation index (F_{IS}, a measure of deviation from panmixia), and the observed heterozygosity (H_o). These indices were calculated using SPAGeDi (version 1.5, Hardy & Vekemans, 2002) and FSTAT (version 2.9.4, Goudet, 2003). The statistical significance of F_{IS} values was assessed through 1,000 random permutations of alleles among individuals within populations.

The relationships between genetic diversity, departures from Hardy-Weinberg equilibrium and landscape features were tested using linear regressions (Gaussian distribution) with habitat characteristics (forest and urban habitat surfaces within a one-kilometer radius around each population) and census population size as explanatory variables. Explanatory variables were standardized (with a mean of 0 and a variance of 1).

For the six populations sampled in 2023, the observed levels of A_r, F_{IS} and H_o were compared between habitat types (forest vs. anthropogenic habitats). The statistical significance of differences between habitat types was assessed using 1,000 permutations of individuals among populations using FSTAT (version 2.9.4, Goudet, 2003).

5. Spatial genetic structure and levels of genetic differentiation among populations

We investigated spatial genetic structure among populations, excluding one population where fewer than 10 individuals could be sampled. First, the global genetic differentiation was estimated by calculating the mean multilocus F_{ST} (Weir & Cockerham, 1984) across all populations using FSTAT (version 2.9.4, Goudet, 2003). Statistical significance was evaluated using 1,000 random permutations of multilocus genotypes among populations. Second, pairwise F_{ST} values were computed and their statistical significance was tested using the same permutation procedure and a Bonferroni correction for multiple tests. Third, population-specific F_{ST} (Weir & Goudet, 2017) values were calculated to evaluate the degree of genetic differentiation of each population relative to the others. This fixation index provides insight into the contribution of each population to the overall genetic structure (Weir & Goudet, 2017).

The relationship between population-specific F_{ST} and landscape features was then tested using linear regressions (Gaussian distribution) including forest and urban habitat surfaces and census population sizes as explanatory variables.

Finally, we tested the correlation between genetic differentiation (pairwise F_{ST}) and geographical distances using Mantel tests (1,000 permutations, Smouse et al., 1986). We considered three types of

distances between populations: (i) straight-line Euclidean distance, (ii) distances recalculated through a Delaunay triangulation network using the software PASSAGE (Rosenberg & Anderson, 2011) which connects nearby populations stepwise and could better reflect limited gene dispersal in this insect-pollinated and gravity dispersed species, and (iii) distances measured along road networks, since *S. dioica* often occurs in road ditches where human activities may facilitate gene flow.

6. Population genetic delineations

We used two complementary approaches to investigate the population genetic affiliations at the regional scale. First, a neighbour-joining tree of populations was built from D_A genetic distances (Nei et al., 1983 ; Saitou & Nei, 1987) and node-support was assessed by 1,000 bootstrap replicates using POPTREE2 (Takezaki et al., 2010).

Second, we performed a Bayesian assignment analysis using STRUCTURE (version 2.3.4, Pritchard et al., 2000 ; Hubisz et al., 2009), without prior information on the sampling locations. This method assigns individuals to K clusters based on multilocus genotypes, minimizing deviations from Hardy-Weinberg equilibrium and linkage disequilibrium between loci. Ten independent runs of values of K ranging from 1 to 29 (the number of sampled populations) with 1,000,000 Markov Chain Monte Carlo (MCMC) iterations and a burn-in period of 100,000 iterations were performed. The most likely value of K was inferred by using the ΔK statistic, which evaluates the rate of change in log-likelihood between two successive K values (Evanno et al., 2005).

7. Within-population spatial genetic structure

Fine-scale analyses focused on six intensively sampled populations previously described and selected to represent contrasting habitat types: three in forest-dominated and three in predominantly agricultural landscapes. We evaluated the patterns of spatial genetic structure by testing the relationship between pairwise kinship coefficients (F_{ij}, Loiselle et al., 1995) and pairwise geographical distances between individuals using SPAGeDi (version 1.5, Hardy & Vekemans, 2002). Mean F_{ij} values were computed for eight geographical distance classes ($[0–5[$; $[5–10[$; $[10–15[$; $[15–20[$; $[20–30[$; $[30–40[$; $[40–50[$; $[60–\infty[$), allowing visual comparisons between the different populations. The significance of mean F_{ij} values in each distance class was assessed through 1,000 permutations of individual locations.

To compare the strength of the spatial genetic structure among populations displaying heterogeneity in the spatial distribution of individuals, we calculated the Sp statistic, defined as $-b/(1-\hat{F}_{ij})$, where b is the slope of the regression of pairwise F_{ij} against log-transformed pairwise geographical distances, and $\hat{F}_{ij}$ is the mean kinship between neighbouring individuals ($d_{ij} < 2$ m) (Vekemans & Hardy, 2004).

8. Paternity analyses

From the six intensively sampled populations, multilocus genotypes of georeferenced maternal plants and their progeny were used to perform fractional paternity assignments, allowing us to estimate pollen dispersal distances, and to quantify the proportion of pollen originating from outside the population.

We applied a spatially explicit mating model that integrates females, male and seed genotypes with spatial coordinates of individuals to estimate, for each seed, the probability of siring of all potential fathers (Oddou-Muratorio et al., 2005 ; Klein et al., 2008). This approach allowed us to infer the mean reproductive success (MRS) of each male and the pollen dispersal distance for each male (δ), as well as the parameters of the population-level pollen dispersal kernel. The kernel, modelled with the negative exponential power function (see Klein et al., 2006), describes the likelihood of fertilization as a function of distance. Its parameters include b, a shape parameter describing how sharply siring probability declines with distance ($b > 1$ corresponding to a thin-tailed kernel, $b < 1$ to a fat-tailed kernel, $b = 1$ to an exponential kernel), and μ_δ, a scale parameter that reflects the average dispersal distance. Model parameters were estimated with three MCMC (simulations of 500 000 steps and a burn-in of 100 000 steps each), with MRS and δ treated as two latent random variables following Gamma distributions, with means and variances of $(1, \sigma_{RS})$ and $(\mu_\delta, \sigma_\delta)$ respectively. The migration rate (m, proportion of seeds for which paternity could not be assigned to a male from the focal population) was also estimated. Uniform priors were used for σ_{RS}, μ_δ, σ_δ, b and m within the values intervals range of $[0.01–100]$, $[1–100]$, $[0–100]$, $[0.1–10]$, $[0–1]$ respectively.

We then compared the patterns of pollen flow between the two forest and agricultural habitats using individual dispersal estimates in a Gaussian generalized linear mixed model with population as a random effect.

RESULTS

1. Levels of genetic diversity

Mean allelic richness (A_r) ranged from 3.0 to 5.2 (**Figure 2**, supplementary material – Population description) and observed heterozygosity (H_o) ranged from 0.373 to 0.644. The mean multilocus intra-population fixation index (F_{IS}) was 0.057 (range: -0.281–0.235), and 12 of the 29 populations exhibited significantly positive F_{IS} values, indicating departures from random mating ($p < 0.05$). Mapping of A_r, H_o and F_{IS} values revealed no obvious geographical gradients or spatial clustering (**Figure 2**).

Neither population size nor habitat characteristics (i.e., forest and urban habitat surfaces in the one-kilometer radius around each population) affected within-population genetic diversity indices (A_r, F_{IS}, H_o, supplementary material – Genetic diversity and differentiation, all at $p > 0.05$). Similarly, the six intensively sampled populations representing contrasting habitat types (forest vs. anthropogenic),

showed no significant differences in mean levels of genetic diversity or departures from Hardy-Weinberg expectations between habitat types (all at $p > 0.05$).

2. Spatial genetic structure and levels of genetic differentiation among populations

Populations exhibited a gradient of genetic differentiation ranging from moderate to strong, with pairwise multilocus F_{ST} values spanning 0.002 to 0.230 and an overall multilocus F_{ST} estimate of 0.069 ($p < 0.001$, 95% confidence interval [0.055–0.088]). Out of the 378 pairwise F_{ST} values, 96.6% were significant after Bonferroni correction (supplementary material – Pairwise F_{ST}). All but one non-significant pairwise F_{ST} values occurred between populations separated by less than 5 km (**Figure 3**). Population-specific F_{ST} values varied among populations but showed no clear geographic pattern (**Figure 2**) and were unaffected by either population census size or habitat-derived variables (supplementary material – Genetic diversity and differentiation, $p > 0.05$).

Finally, a significant pattern of isolation-by-distance was detected (**Figure 3**), with continuous increasing genetic differentiation between pairwise populations with Euclidean geographical distance ($rz = 0.210$, $p < 0.001$). Correlations using Delaunay triangulation ($rz = 0.208$, $p < 0.01$) or road-based distances ($rz = 0.201$, $p < 0.01$) were comparable, indicating that alternative distance measures did not improve the fit to an isolation-by-distance model of population structure.

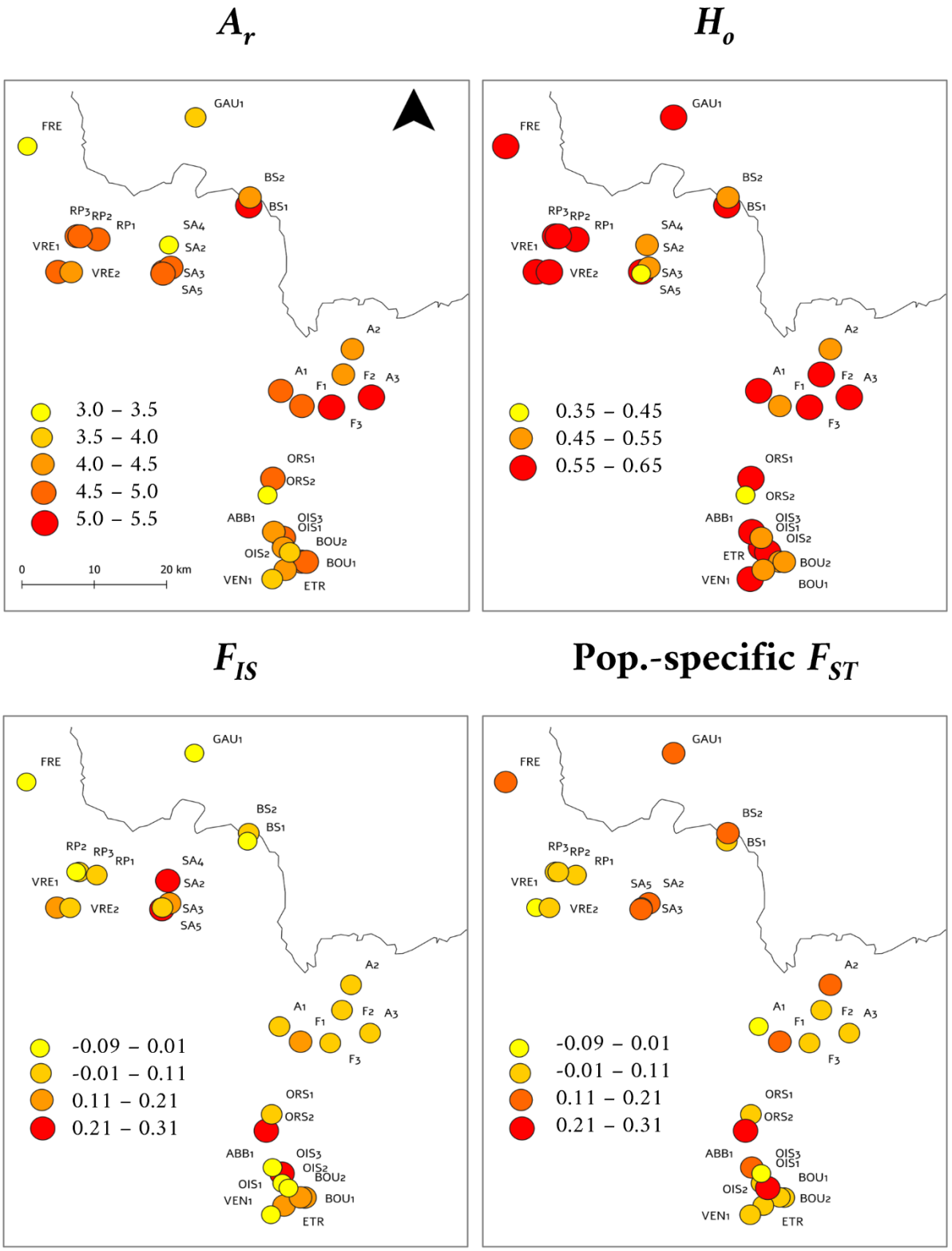

Figure 2. Geographical representation of population genetic features in red campion (*S. dioica*) populations sampled in northern France and in Belgium. The grey line corresponds to the border between countries. A_r: allelic richness, H_o: observed heterozygosity, F_{IS}: intra-population fixation index, Pop.-specific F_{ST}: population-specific F_{ST} as defined by Weir & Goudet (2017).

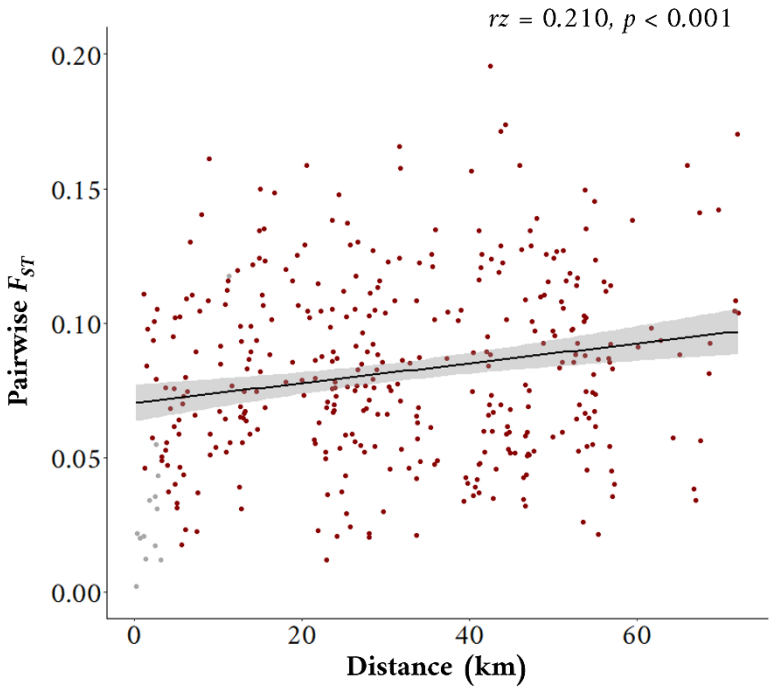

Figure 3. Scatterplot of pairwise F_{ST} estimates against Euclidean geographical distance in *S. dioica*. Significant pairwise F_{ST} values are shown in red, and non-significant values in grey.

3. Population genetic delineations

Genetic affinities among populations were first visualized using a neighbour-joining tree, which revealed a geographically coherent grouping into four distinct population clusters broadly reflecting the spatial distribution of the sampled populations (**Figure 4**).

Bayesian assignment analyses also identified four genetic clusters (supplementary material – Population genetic delineations), largely consistent with the structure observed in the neighbour-joining tree. Notably, however, all sampled populations exhibited extremely high levels of admixture, with many individuals showing mixed assignment across clusters, revealing pervasive gene flow throughout the study region (**Figure 4**).

4. Within-population spatial genetic structure

A significant pattern of isolation-by-distance was detected within 5 of the 6 populations (**Figure 5**). Kinship coefficients at short distances (below ten meters) were significantly higher than expected under the null hypothesis of no significant spatial genetic structure in all populations (**Figure 5**). The *Sp* statistic varied among populations (range: 0.004–0.022), indicating variations in the strength of spatial genetic structure, but showed no apparent relationship with habitat type (supplementary material – Genetic diversity and differentiation, $p > 0.05$).

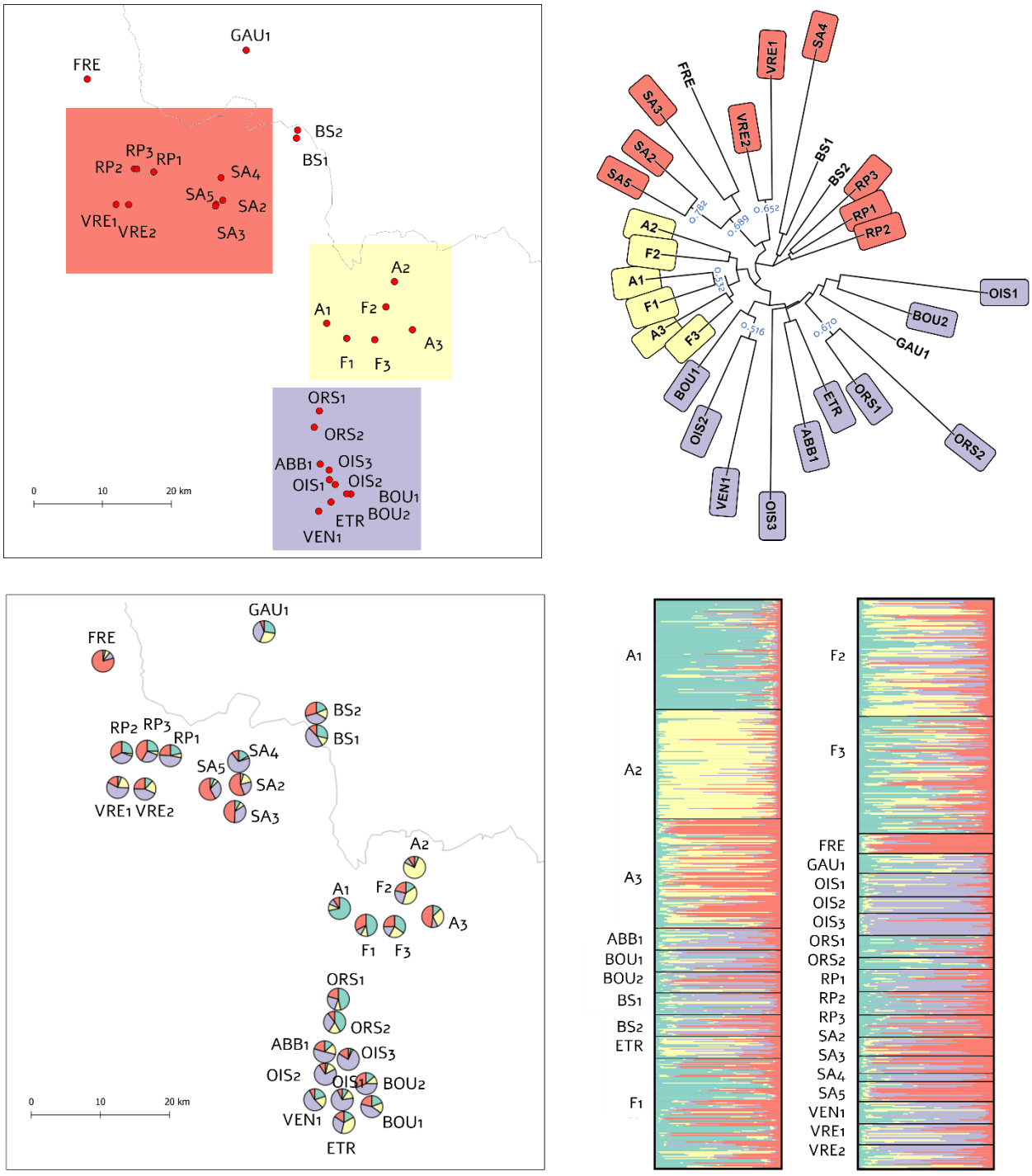

Figure 4. The top panel shows a neighbour-joining tree of red campion populations based on Nei's DA genetic distance (Nei et al., 1983). Blue numbers indicate bootstrap values greater than 0.5. Colors correspond to the geographical distribution of sampled populations. The lower panel displays the results of Bayesian clustering for the most likely number of clusters, $K = 4$. Pie charts represent the average probability of each population belonging to one of the four clusters. Barplots show individual assignment probabilities to each of the four genetically distinct clusters (each group is represented by a color, each line represents an individual).

5. Paternity analyses

Pollen dispersal patterns varied among populations, with differences in both the scale and shape of the dispersal kernels, ranging from sharply peaked to more gradual distributions, but without any qualitative apparent relationship to habitat type (**Figure 6**). Despite this variation, mean pollen dispersal distances remained very restricted, with values well below 10 m in five of the six populations and reaching 25 m only in the last one (supplementary material – Pollen dispersal kernel). Analyses of male-specific pollen dispersal also revealed no significant differences between forested and agricultural habitats ($F_{1,294}$ = 0.603, p = 0.437), indicating similar dispersal ranges across environments. Notably, these short pollen dispersal distances were coupled with substantial external pollen immigration, with migration rates ranging from 0.35 to 0.53: at least one-third of the pollen cloud in each population originated from external donors, irrespective of habitat context (supplementary material – Pollen dispersal kernel).

DISCUSSION

Anthropogenic habitat degradation is classically expected to reduce local genetic variation and increase differentiation among populations (De-Lucas et al., 2009 ; Sebbenn et al., 2011 ; Gómez-Fernández et al., 2016 ; Almeida-Rocha et al., 2020). Even in common plant species, agricultural intensification is expected to reshape fine-scale spatial genetic structure, particularly in short-lived species, with effects amplified in those with limited dispersal, such as species dispersing seeds by gravity and pollinated by insects (Van Rossum et al., 2004 ; Aavik et al., 2013 ; DiLeo et al., 2018 ; Gamba & Muchhala, 2020). Here, we investigated the drivers of neutral genetic diversity in populations of the widespread red campion (*Silene dioica*) across a land-use gradient. At the regional scale, we observed (i) no detectable effect of habitat type or population size on genetic diversity, (ii) significant population differentiation despite high levels of admixture, and (iii) clear isolation-by-distance patterns. Focusing on a subset of populations representing the extremes of the agricultural-forest spectrum, we found (i) pronounced intra-population spatial genetic structure, consistent with (ii) extremely limited pollen dispersal distances revealed by paternity analyses and coupled with (iii) substantial pollen immigration, explaining the high levels of population admixture. Importantly and contrary to our expectations, these fine-scale patterns were not influenced by the type of habitat in which the populations occurred. Together, our results suggest that gene flow in this species is multimodal, combining very short-distance pollen flow with long-distance dispersal of pollen and/or seeds, which likely underpins its remarkable resilience to anthropogenic landscape modification.

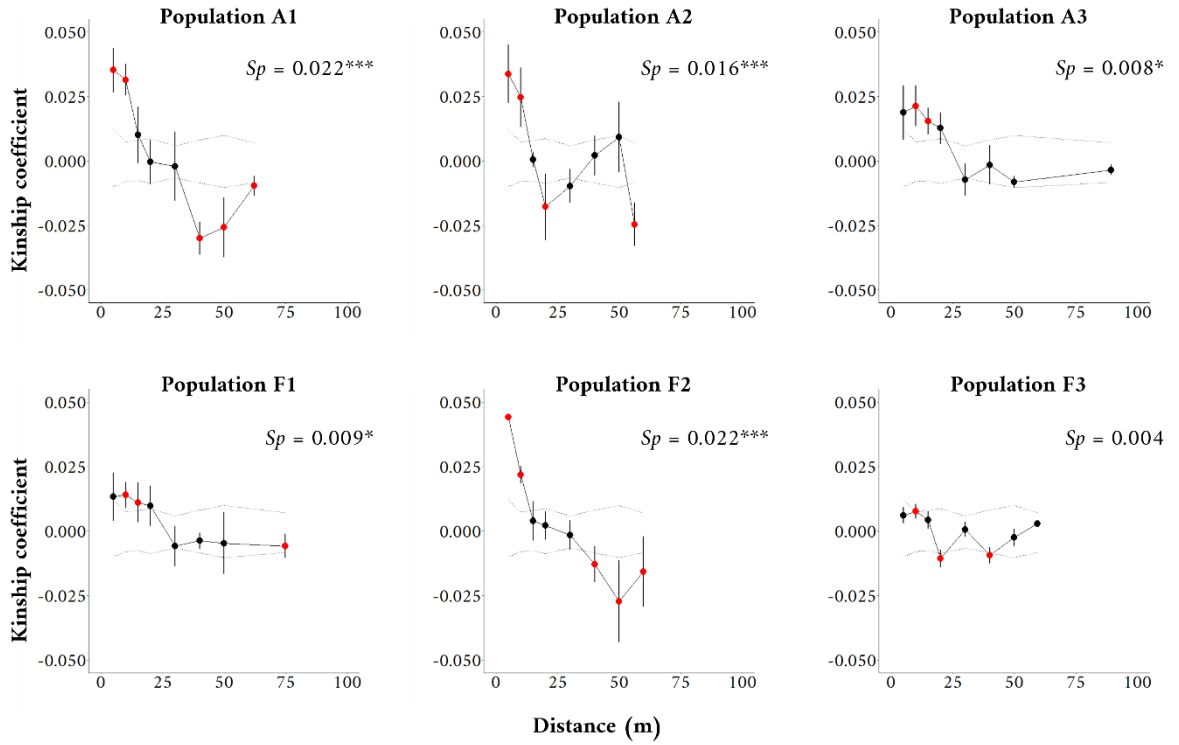

Figure 5. Spatial autocorrelograms showing the relationship between pairwise geographical distances among individuals (m) and pairwise kinship coefficient (± standard error) calculated following Loiselle et al. (1995), for six red campion populations. Standard deviations for each F_{ij} value per distance class were obtained using jackknifing over loci. Significant mean F_{ij} values are indicated in red ($p < 0.05$) and significant estimates of *Sp* statistics by stars (***: $p < 0.001$, *: $p < 0.05$).

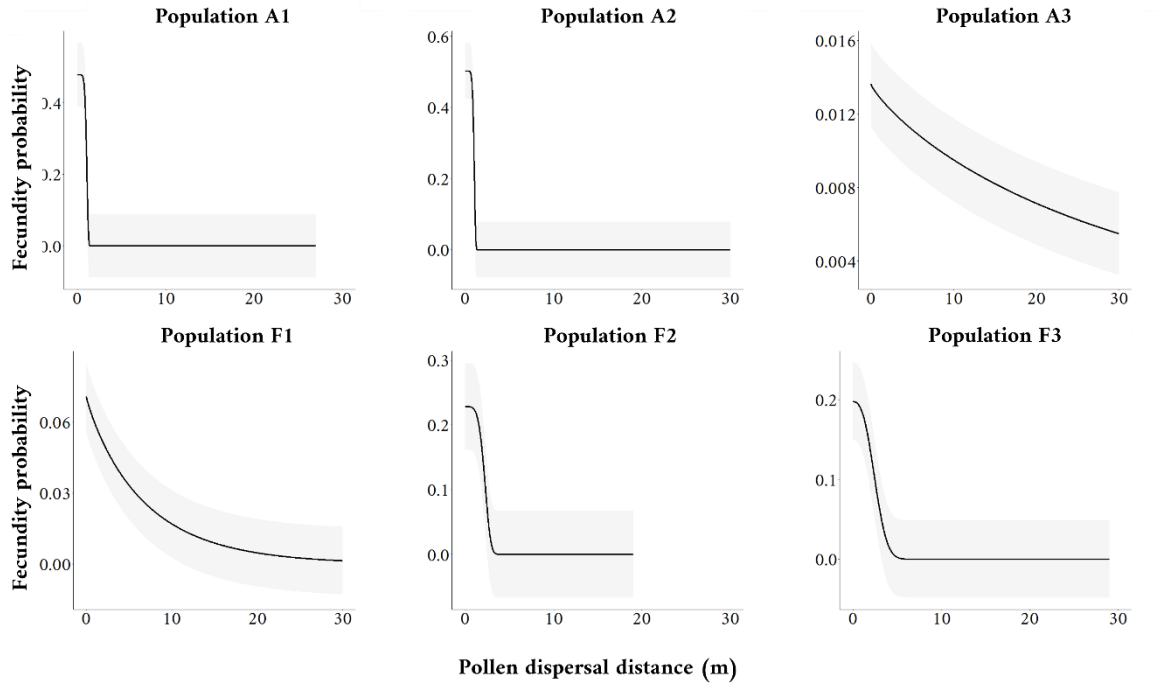

Figure 6. Plot representing the estimated pollen dispersal kernels for six populations of the red campion (*S. dioica*). Populations with names starting with "F" are located in predominantly forested areas, while populations starting with "A" are found in mainly agricultural areas. The grey area represents the standard deviation around the mean.

Moderate level of genetic differentiation and isolation-by-distance

Several lines of evidence point to substantial long-distance gene flow in *Silene dioica*. First, populations grouped in the neighbour-joining tree were geographically widespread. Second, Bayesian clustering analyses revealed high levels of genetic admixture among the detected clusters. Third, pairwise F_{ST} values were generally significant but moderate (overall F_{ST} = 0.069). Finally, paternity analyses in a subset of populations indicated high pollen immigration rates, further supporting frequent long-distance pollen dispersal events.

Moderate levels of genetic structure appear to be characteristic of *S. dioica* and have also been detected in populations from very different environments, such as insular archipelagos (Giles & Goudet, 1997b). More broadly, these patterns are consistent with what is observed in species pollinated by large insects, which typically exhibit reduced spatial genetic structure (reviewed in Wessinger, 2021 ; Gamba & Muchhala, 2023). As a generalist, *S. dioica* is visited by multiple functional pollinator groups, including bumblebees, which are known for their extensive dispersal capacities (Hagen et al., 2011). In addition to pollen-mediated gene flow, occasional seed dispersal may also contribute to population connectivity, as observed in other plant species (Fievet et al., 2007 ; Von Der Lippe & Kowarik, 2007 ; Auffret, 2011 ; Favre-Bac et al., 2016), and could be further investigated through parentage analyses of naturally emerging seedlings in wild populations.

Although moderate levels of genetic differentiation occurred, gene flow events are nonetheless spatially restricted. At the regional scale, we detected a classic isolation-by-distance pattern with Euclidean distances. This isolation-by-distance pattern reflected the balance between gene flow and genetic drift (Hutchison & Templeton, 1999 ; Phillipsen et al., 2015): gene flow homogenizes nearby populations, while increasing distance reduces dispersal and allows drift to amplify the genetic differentiation. The high variance observed in pairwise F_{ST} further suggested that this equilibrium is not yet fully established in our study region, with drift exerting a stronger influence than gene flow at this stage (Slatkin, 1993 ; Hutchison & Templeton, 1999).

Interestingly, the variance in differentiation levels was unrelated to population size and to habitat characteristics, as shown by the non-significant effect of these variables on population-specific F_{ST} values. We also detected no effect of census population size on allelic richness or on observed heterozygosity. The absence of a population size effect could be explained by generally large census sizes in our study region (mean census size well above 200 individuals, see supplementary material – Population description), limiting the effects of genetic drift and buffering against the loss of genetic diversity. This contrasts with many studies showing that larger populations harbour greater genetic diversity, produce more propagules, attract more pollinators, and display higher levels of gene flow among populations (Van Rossum et al., 2004 ; Aavik et al., 2013 ; Aavik et al., 2014 ; Crichton et al., 2016 ; Sullivan et al., 2019). In *S. dioica*, however, which is abundant and widespread in the study region, pollen flow among

populations seems sufficiently high to buffer against potential effects of population size on genetic structure.

The absence of a habitat effect on population-specific F_{ST} estimates suggested that forest cover did not hinder pollinator movements—as previously suggested in another part of the species' range (Westerbergh & Saura, 1994)—and that agricultural intensification did not contribute to the isolation of populations embedded within highly cultivated landscapes. Accordingly, land use did not affect the levels of genetic diversity in our data set, in contrast to the negative effects reported for other species (Aguilar et al., 2008 ; Vranckx et al., 2012 ; Favre-Bac et al., 2016 ; but see Odat et al., 2004 for positive effects).

Several mechanisms may explain why no habitat effect was detected here. First, pollinator communities appear to be relatively unaffected by habitat type (Jolivel et al., in prep.). The high diversity of floral resources (botanical survey, pers. obs.) and the presence of mixed landscape features (e.g., meadows, agricultural hedgerows) around each studied population may help maintaining high pollinator densities (Jolivel et al., in prep. ; Winfree et al., 2011) and substantial within and among population gene flow (DiLeo et al., 2018). One other plausible explanation for the observed patterns is that agricultural machinery or grazing contributes to seed dispersal (Strykstra et al., 1997 ; Vitalos & Karrer, 2009 ; DiLeo et al., 2017). This anthropogenic dispersal mechanism could enhance dispersal events between otherwise isolated populations. Then, the spatial scales considered may not have been optimal to capture habitat effects. *S. dioica* is pollinated by various types of pollinators (Barbot et al., 2022 ; Barbot et al., 2025), it is possible that some of these pollinators disperse pollen over longer distances beyond the scale considered in this study (i.e., 1 km). Temporal scales could also play a role if the genetic signature of recent land-use changes is not yet detectable (Huang et al., 2025). In a metapopulation context, processes such as colonization-extinction dynamics may also have a greater influence on genetic structure than habitat itself. As a consequence, local populations fluctuate over time, preventing them from reaching demographic or genetic equilibrium (Giles & Goudet, 1997a,b).

Fine-scale genetic structure and local pollen dispersal

At finer spatial scales, local patterns of gene flow were investigated within a subset of populations using two complementary approaches. First, we quantified fine-scale spatial genetic structure through the *Sp* statistic, which integrates the cumulative effects of pollen and seed dispersal over multiple generations, thus providing a historical signature of gene flow (Loiselle et al., 1995 ; Vekemans & Hardy, 2004). Second, contemporary pollen dispersal was inferred from paternity analyses. The observed levels of fine-scale spatial genetic structure were congruent with those reported for other insect-pollinated species (reviewed in Gamba & Muchhala, 2023 ; Miguel-Peñaloza et al., 2023). Because *S. dioica* is dioecious, significant patterns of spatial genetic structure and local levels of inbreeding cannot be attributed to self-fertilization. Instead, it likely reflects the foraging behavior of pollinators, which tend to move between

neighbouring plants in order to optimize their foraging routes (MacArthur & Pianka, 1966 ; Marden & Waddington, 1981 ; Hill et al., 2001), thereby often generating high levels of correlated paternity (Hardy et al., 2004). This tendency for correlated paternity has indeed been documented in our focal species (Joffard et al., 2025). Combined with the limited seed dispersal expected in a barochorous species, it provides a parsimonious explanation for the detected fine-scale spatial genetic structure patterns and, more broadly, for the generally positive F_{IS} values across our whole dataset, with statistical significance reached in approximately one third of the populations.

The patterns of contemporary pollen flow revealed by paternity analyses reinforce this interpretation. We detected highly leptokurtic distributions of pollination events, with fertilization probability declining steeply as the distance between pollen donors and females increased. Mean pollination distances within populations were less than 10 m in five out of six populations, and reached only 25 m in the remaining one. Importantly, these distances did not differ between populations located in forest *versus* agricultural habitats, suggesting that habitat context did not constrain local pollen movements in the focal species. In the literature, paternity analyses in natural populations are disproportionately focused on trees, yet the strong restriction of pollen flow appears to be a common pattern in entomophilous herbaceous plants, based on the few studies available (Hardy et al., 2004 ; DiLeo et al., 2018 ; Barbot et al., 2022).

Beyond their role in shaping the fine-scale spatial genetic structure described above, such restricted dispersal patterns are also expected to influence the strength and scale of selection on floral traits. Indeed, our results suggested that male-male competition for access to pollinators—and consequently to females—occurs primarily among close neighbours, within very localized spatial scales.

CONCLUSION

In the context of land-use change, gene flow within and among plant populations may be altered. By combining landscape characterization with analyses of spatial patterns of neutral genetic diversity, our results provide strong evidence for substantial long-distance gene flow in *S. dioica*, despite its reliance on insect-mediated pollen dispersal and predominantly gravity-driven seed dispersal. This pattern may be linked to the species' high abundance across a wide range of habitats, including agricultural landscapes, which probably facilitated exchanges among populations. Moreover, its generalist pollination system, which includes pollinators with extensive dispersal capacities, likely enhances connectivity among subdivided populations. Overall, *S. dioica* appeared remarkably resilient to anthropogenic habitat modification, maintaining substantial genetic connectivity preventing genetic erosion even in strongly altered landscapes. Furthermore, isolation-by-distance was detected both among and within populations, likely reflecting a combination of near-neighbour and long-distance pollinator movements. This combination of patterns suggested a specific mechanism of pollen movement: pollinators may enter in a population carrying pollen from previously visited populations and then move among neighbouring plants, introducing non-local alleles while maintaining fine-scale spatial genetic structure. Overall, these

findings highlighted how pollinator behaviour shape the population genetic structure, allowing dioecious plants with limited intrinsic dispersal capacities to preserve genetic diversity even in fragmented landscapes.

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REFERENCES

Aavik, T., Holderegger, R., & Bolliger, J. (2014). The structural and functional connectivity of the grassland plant *Lychnis flos-cuculi*. *Heredity*, 112(5), 471-478. https://doi.org/10.1038/hdy.2013.120

Aavik, T., Holderegger, R., Edwards, P. J., & Billeter, R. (2013). Patterns of contemporary gene flow suggest low functional connectivity of grasslands in a fragmented agricultural landscape. *Journal of Applied Ecology*, 50(2), 395-403. https://doi.org/10.1111/1365-2664.12053

Aguilar, R., Quesada, M., Ashworth, L., Herrerias-Diego, Y., & Lobo, J. (2008). Genetic consequences of habitat fragmentation in plant populations : Susceptible signals in plant traits and methodological approaches. *Molecular Ecology*, 17(24), 5177-5188. https://doi.org/10.1111/j.1365-294x.2008.03971.x

Almeida-Rocha, J. M., Soares, L. A., Andrade, E. R., Gaiotto, F. A., & Cazetta, E. (2020). The impact of anthropogenic disturbances on the genetic diversity of terrestrial species : A global meta-analysis. *Molecular Ecology*, 29(24), 4812-4822. https://doi.org/10.1111/mec.15688

Auffret, A. G. (2011). Can seed dispersal by human activity play a useful role for the conservation of European grasslands? : HMD for conservation. *Applied Vegetation Science*, 14(3), 291-303. https://doi.org/10.1111/j.1654-109x.2011.01124.x

Barbot, E., Dufaÿ, M., Godé, C., & De Cauwer, I. (2025). Investigating the effects of diurnal and nocturnal pollinators on male and female reproductive success and on floral trait selection in *Silene dioica*. *Peer Community Journal*, 5, e11. https://doi.org/10.24072/pcjournal.508

Barbot, E., Dufaÿ, M., Tonnabel, J., Godé, C., & De Cauwer, I. (2022). On the function of flower number : Disentangling fertility from pollinator-mediated selection. *Proceedings of the Royal Society B: Biological Sciences*, 289(1987), 20221987. https://doi.org/10.1098/rspb.2022.1987

Beckman, N. G., & Sullivan, L. L. (2023). The causes and consequences of seed dispersal. *Annual Review of Ecology, Evolution, and Systematics*, 54(1), 403-427. https://doi.org/10.1146/annurev-ecolsys-102320-104739

Biesmeijer, J. C., Roberts, S. P. M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., Schaffers, A. P., Potts, S. G., Kleukers, R., Thomas, C. D., Settele, J., & Kunin, W. E. (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science*, 313(5785), 351-354. https://doi.org/10.1126/science.1127863

Born, C., Hardy, O. J., Chevallier, M., Ossari, S., Attéké, C., Wickings, E. J., & Hossaert-Mckey, M. (2008). Small-scale spatial genetic structure in the Central African rainforest tree species *Aucoumea klaineana* : A stepwise approach to infer the impact of limited gene dispersal, population history and habitat fragmentation. *Molecular Ecology*, 17(8), 2041-2050. https://doi.org/10.1111/j.1365-294X.2007.03685.x

Cain, M. L., Milligan, B. G., & Strand, A. E. (2000). Long-distance seed dispersal in plant populations. *American Journal of Botany*, 87(9), 1217-1227. https://doi.org/10.2307/2656714

Crichton, R. J., Dalrymple, S. E., Woodin, S. J., & Hollingsworth, P. M. (2016). Conservation genetics of the annual hemiparasitic plant *Melampyrum sylvaticum* (Orobanchaceae) in the UK and Scandinavia. *Conservation Genetics*, 17(3), 547-556. https://doi.org/10.1007/s10592-015-0803-4

De Cauwer, I., Arnaud, J., Klein, E. K., & Dufaÿ, M. (2012). Disentangling the causes of heterogeneity in male fecundity in gynodioecious *Beta vulgaris* ssp. *Maritima*. *New Phytologist*, 195(3), 676-687. https://doi.org/10.1111/j.1469-8137.2012.04191.x

Dellafiore, C. M., Gallego Fernández, J. B., & Muñoz Vallés, S. (2010). The rabbit (*Oryctolagus cuniculus*) as a seed disperser in a coastal dune system. *Plant Ecology*, 206(2), 251-261. https://doi.org/10.1007/s11258-009-9639-7

De-Lucas, A., Gonzalez-Martinez, S., Vendramin, G., Hidalgo, E., & Heuertz, M. (2009). Spatial genetic structure in continuous and fragmented populations of *Pinus pinaster* Aiton. *Molecular Ecology*, 18(22), 4564-4576. https://doi.org/10.1111/j.1365-294X.2009.04372.x

DiLeo, M. F., Holderegger, R., & Wagner, H. H. (2018). Contemporary pollen flow as a multiscale process : Evidence from the insect-pollinated herb, *Pulsatilla vulgaris*. *Journal of Ecology*, 106(6), 2242-2255. https://doi.org/10.1111/1365-2745.12992

DiLeo, M. F., Rico, Y., Boehmer, H. J., & Wagner, H. H. (2017). An ecological connectivity network maintains genetic diversity of a flagship wildflower, *Pulsatilla vulgaris*. *Biological Conservation*, 212, 12-21. https://doi.org/10.1016/j.biocon.2017.05.026

Ellegren, H., & Galtier, N. (2016). Determinants of genetic diversity. *Nature Reviews Genetics*, 17(7), 422-433. https://doi.org/10.1038/nrg.2016.58

Emel, S. L., Wang, S., Metz, R. P., & Spigler, R. B. (2021). Type and intensity of surrounding human land use, not local environment, shape genetic structure of a native grassland plant. *Molecular Ecology*, 30(3), 639-655. https://doi.org/10.1111/mec.15753

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure : A simulation study. *Molecular Ecology*, 14(8), 2611-2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Favre-Bac, L., Mony, C., Ernoult, A., Burel, F., & Arnaud, J.-F. (2016). Ditch network sustains functional connectivity and influences patterns of gene flow in an intensive agricultural landscape. *Heredity*, 116(2), 200-212. https://doi.org/10.1038/hdy.2015.90

Fievet, V., Touzet, P., Arnaud, J., & Cuguen, J. (2007). Spatial analysis of nuclear and cytoplasmic DNA diversity in wild sea beet (*Beta vulgaris* ssp. *Maritima*) populations : Do marine currents shape the genetic structure? *Molecular Ecology*, 16(9), 1847-1864. https://doi.org/10.1111/j.1365-294X.2006.03208.x

Gamba, D., & Muchhala, N. (2020). Global patterns of population genetic differentiation in seed plants. *Molecular Ecology*, 29(18), 3413-3428. https://doi.org/10.1111/mec.15575

Gamba, D., & Muchhala, N. (2023). Pollinator type strongly impacts gene flow within and among plant populations for six Neotropical species. *Ecology*, 104(1), e3845. https://doi.org/10.1002/ecy.3845

Giles, B. E., & Goudet, J. (1997a). A case study of genetic structure in a plant metapopulation. In *Metapopulation Biology* (p. 429-454). Elsevier. https://doi.org/10.1016/B978-012323445-2/50023-7

Giles, B. E., & Goudet, J. (1997b). Genetic differentiation in *Silene dioica* metapopulations : Estimation of spatiotemporal effects in a successional plant species. *The American Naturalist*, 149(3), 507-526. https://doi.org/10.1086/286002

Giles, B. E., Lundqvist, E., & Goudet, J. M. (1998). Restricted gene flow and subpopulation differentiation in *Silene dioica*. *Heredity*, 80(6), 715-723. https://doi.org/10.1046/j.1365-2540.1998.00348.x

Gómez-Fernández, A., Alcocer, I., & Matesanz, S. (2016). Does higher connectivity lead to higher genetic diversity? Effects of habitat fragmentation on genetic variation and population structure in a gypsophile. *Conservation Genetics*, 17(3), 631-641. https://doi.org/10.1007/s10592-016-0811-z

Goudet, J. (2003). *FSTAT version 2. 9. 4 : A program to estimate and test population genetics parameters. Updated from Goudet* [1995]. *Available from http://www.unil.ch/izea/softwares/fstat.html*.

Goverde, M., Schweizer, K., Baur, B., & Erhardt, A. (2002). Small-scale habitat fragmentation effects on pollinator behaviour : Experimental evidence from the bumblebee *Bombus veteranus* on calcareous grasslands. *Biological Conservation*, 104(3), 293-299. https://doi.org/10.1016/s0006-3207(01)00194-x

Guiller, A., Decocq, G., Kichey, T., Poli, P., Vandepitte, K., Dubois, F., Honnay, O., & Closset-Kopp, D. (2023). Spatial genetic structure of two forest plant metapopulations in dynamic agricultural landscapes. *Landscape and Urban Planning*, 231, 104648. https://doi.org/10.1016/j.landurbplan.2022.104648

Hagen, M., Wikelski, M., & Kissling, W. D. (2011). Space use of bumblebees (*Bombus spp.*) revealed by radio-tracking. *PLoS ONE*, 6(5), e19997. https://doi.org/10.1371/journal.pone.0019997

Hamrick, J. L., & Godt, M. J. W. (1996). Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 351(1345), 1291-1298. https://doi.org/10.1098/rstb.1996.0112

Hardy, O. J., González-Martínez, S. C., Colas, B., Fréville, H., Mignot, A., & Olivieri, I. (2004). Fine-scale genetic structure and gene dispersal in *Centaurea corymbosa* (Asteraceae). II. Correlated paternity within and among sibships. *Genetics*, 168(3), 1601-1614. https://doi.org/10.1534/genetics.104.027714

Hardy, O. J., & Vekemans, X. (2002). SPAGeDi : A versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, 2(4), 618-620. https://doi.org/10.1046/j.1471-8286.2002.00305.x

Herrera, C. M. (1988). Variation in mutualisms : The spatiotemporal mosaic of a pollinator assemblage. *Biological Journal of the Linnean Society*, 35(2), 95-125. https://doi.org/10.1111/j.1095-8312.1988.tb00461.x

Hill, P. S. M., Hollis, J., & Wells, H. (2001). Foraging decisions in nectarivores : Unexpected interactions between flower constancy and energetic rewards. *Animal Behaviour*, 62(4), 729-737. https://doi.org/10.1006/anbe.2001.1775

Honnay, O., & Jacquemyn, H. (2007). Susceptibility of common and rare plant species to the genetic consequences of habitat fragmentation. *Conservation Biology*, 21(3), 823-831. https://doi.org/10.1111/j.1523-1739.2006.00646.x

Howe, H. F., & Smallwood, J. (1982). Ecology of seed dispersal. *Annual Review of Ecology and Systematics*, 13(1), 201-228. https://doi.org/10.1146/annurev.es.13.110182.001221

Huang, S., Feigs, J. T., Brunet, J., Cousins, S. A. O., Diekmann, M., Hedwall, P.-O., Holzhauer, S. I. J., Liira, J., & Naaf, T. (2025). Variable time lags in genetic response of three temperate forest herbs to 70 years of agricultural landscape change. *Landscape Ecology*, 40(4), 1-16. https://doi.org/10.1007/s10980-025-02091-3

Hubisz, M. J., Falush, D., Stephens, M., & Pritchard, J. K. (2009). Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources*, 9(5), 1322-1332. https://doi.org/10.1111/j.1755-0998.2009.02591.x

Hutchison, D. W., & Templeton, A. R. (1999). Correlation of pairwise genetic and geographic distance measures : Inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution*, 53(6), 1898-1914. https://doi.org/10.1111/j.1558-5646.1999.tb04571.x

Joffard, N., Lecat, I., Ollier, K., Jolivel, C., Schmitt, E., Godé, C., Barbot, E., Dufaÿ, M., & De Cauwer, I. (2025). Floral display size does not affect the distribution of paternal diversity in *Silene dioica*. *bioRxiv*. https://doi.org/10.1101/2025.07.19.665651

Jolivel, C., Joffard, N., Godé, C., Schmitt, E., & De Cauwer, I. (in prep.). Habitat differences do not disrupt sexual selection patterns in *Silene dioica*.

Jones, A. G., & Ardren, W. R. (2003). Methods of parentage analysis in natural populations. *Molecular Ecology*, 12(10), 2511-2523. https://doi.org/10.1046/j.1365-294X.2003.01928.x

Kay, Q. O. N., Lack, A. J., Bamber, F. C., & Davies, C. R. (1984). Differences between sexes in floral morphology, nectar production and insect visits in a dioecious species, *Silene dioica*. *New Phytologist*, 98(3), 515-529. https://doi.org/10.1111/j.1469-8137.1984.tb04145.x

Klein, E. K., Desassis, N., & Oddou-Muratorio, S. (2008). Pollen flow in the wildservice tree, *Sorbus torminalis* (L.) Crantz. IV. Whole interindividual variance of male fecundity estimated jointly with the dispersal kernel. *Molecular Ecology*, 17(14), 3323-3336. https://doi.org/10.1111/j.1365-294x.2008.03809.x

Klein, E. K., Lavigne, C., & Gouyon, P.-H. (2006). Mixing of propagules from discrete sources at long distance : Comparing a dispersal tail to an exponential. *BMC Ecology*, 6(1), 3. https://doi.org/10.1186/1472-6785-6-3

Klinger, Y. P., Eckstein, R. L., Hansen, W., Kleinebecker, T., Otte, A., & Ludewig, K. (2021). Mowing machinery and migratory sheep herds are complementary dispersal vectors for grassland species. *Applied Vegetation Science*, 24(2). https://doi.org/10.1111/avsc.12579

Latron, M., Arnaud, J., Schmitt, E., & Duputié, A. (2023). Idiosyncratic shifts in life-history traits at species' geographic range edges. *Oikos*, 2023(2), e09098. https://doi.org/10.1111/oik.09098

Loiselle, B. A., Sork, V. L., Nason, J., & Graham, C. (1995). Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany*, 82(11), 1420-1425. https://doi.org/10.1002/j.1537-2197.1995.tb12679.x

MacArthur, R. H., & Pianka, E. R. (1966). On optimal use of a patchy environment. *The American Naturalist*, 100(916), 603-609. https://doi.org/10.1086/282454

Marden, J. H., & Waddington, K. D. (1981). Floral choices by honeybees in relation to the relative distances to flowers. *Physiological Entomology*, 6(4), 431-435. https://doi.org/10.1111/j.1365-3032.1981.tb00658.x

Miguel-Peñaloza, A., Cultid-Medina, C. A., Pérez-Alquicira, J., & Rico, Y. (2023). Do habitat fragmentation and degradation influence the strength of fine-scale spatial genetic structure in plants? A global meta-analysis. *AoB PLANTS*, 15(3), plad019. https://doi.org/10.1093/aobpla/plad019

Mullu, D. (2016). A review on the effect of habitat fragmentation on ecosystem. *Journal of Natural Sciences Research*, 6(15), 1-15. https://www.researchgate.net/publication/322235107

Nei, M., Tajima, F., & Tateno, Y. (1983). Accuracy of estimated phylogenetic trees from molecular data. *Journal of Molecular Evolution*, 19(2), 153-170. https://doi.org/10.1007/BF02300753

Odat, N., Jetschke, G., & Hellwig, F. H. (2004). Genetic diversity of *Ranunculus acris* L. (Ranunculaceae) populations in relation to species diversity and habitat type in grassland communities. *Molecular Ecology*, 13(5), 1251-1257. https://doi.org/10.1111/j.1365-294x.2004.02115.x

Oddou-Muratorio, S., Klein, E. K., & Austerlitz, F. (2005). Pollen flow in the wildservice tree, *Sorbus torminalis* (L.) Crantz. II. Pollen dispersal and heterogeneity in mating success inferred from parent–offspring analysis. *Molecular Ecology*, 14(14), 4441-4452. https://doi.org/10.1111/j.1365-294x.2005.02720.x

Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, 120(3), 321-326. https://doi.org/10.1111/j.1600-0706.2010.18644.x

Osborne, J. L., Martin, A. P., Carreck, N. L., Swain, J. L., Knight, M. E., Goulson, D., Hale, R. J., & Sanderson, R. A. (2008). Bumblebee flight distances in relation to the forage landscape. *Journal of Animal Ecology*, 77(2), 406-415. https://doi.org/10.1111/j.1365-2656.2007.01333.x

Petit, R. J., Duminil, J., Fineschi, S., Hampe, A., Salvini, D., & Vendramin, G. G. (2005). Comparative organization of chloroplast, mitochondrial and nuclear diversity in plant populations. *Molecular Ecology*, 14(3), 689-701. https://doi.org/10.1111/j.1365-294X.2004.02410.x

Phillipsen, I. C., Kirk, E. H., Bogan, M. T., Mims, M. C., Olden, J. D., & Lytle, D. A. (2015). Dispersal ability and habitat requirements determine landscape-level genetic patterns in desert aquatic insects. *Molecular Ecology*, 24(1), 54-69. https://doi.org/10.1111/mec.13003

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155(2), 945-959. https://doi.org/10.1093/genetics/155.2.945

Ricketts, T. H., Regetz, J., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., Bogdanski, A., Gemmill-Herren, B., Greenleaf, S. S., Klein, A. M., Mayfield, M. M., Morandin, L. A., Ochieng', A., & Viana, B. F. (2008). Landscape effects on crop pollination services : Are there general patterns? *Ecology Letters*, 11(5), 499-515. https://doi.org/10.1111/j.1461-0248.2008.01157.x

Rico, Y., & Wagner, H. H. (2016). Reduced fine-scale spatial genetic structure in grazed populations of *Dianthus carthusianorum*. *Heredity*, 117(5), 367-374. https://doi.org/10.1038/hdy.2016.45

Rosenberg, M. S., & Anderson, C. D. (2011). PASSaGE: Pattern Analysis, Spatial Statistics, and Geographic Exegesis. Version 2. *Methods in Ecology and Evolution*, 2(3), 229-232. https://doi.org/10.1111/j.2041-210X.2010.00081.x

Saitou, N., & Nei, M. (1987). The neighbor-joining method : A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4(4), 406-425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Schmitt, J. (1980). Pollinator foraging behavior and gene dispersal in *Senecio* (Compositae). *Evolution*, 934-943. https://doi.org/10.1111/j.1558-5646.1980.tb04031.x

Sebbenn, A. M., Carvalho, A. C. M., Freitas, M. L. M., Moraes, S. M. B., Gaino, A. P. S. C., Da Silva, J. M., Jolivet, C., & Moraes, M. L. T. (2011). Low levels of realized seed and pollen gene flow and strong spatial genetic structure in a small, isolated and fragmented population of the tropical tree *Copaifera langsdorffii* Desf. *Heredity*, 106(1), 134-145. https://doi.org/10.1038/hdy.2010.33

Slatkin, M. (1985). Gene flow in natural populations. *Annual Review of Ecology & Systematics*, 393-430. https://doi.org/10.1146/annurev.es.16.110185.002141

Slatkin, M. (1993). Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*, 47(1), 264-279. https://doi.org/10.1111/j.1558-5646.1993.tb01215.x

Smith, J. R., Ghazoul, J., Burslem, D. F. R. P., Itoh, A., Khoo, E., Lee, S. L., Maycock, C. R., Nanami, S., Ng, K. K. S., & Kettle, C. J. (2018). Are patterns of fine-scale spatial genetic structure consistent between sites within tropical tree species? *PLOS ONE*, 13(3), e0193501. https://doi.org/10.1371/journal.pone.0193501

Smith, S. E., Arredondo, T., Aguiar, M., Huber-Sannwald, E., Alpuche, A., Aguado, A., Grageda, O. A., Halbrook, K., & Bottini, C. (2009). Fine-scale spatial genetic structure in perennial grasses in three environments. *Rangeland Ecology & Management*, 62(4), 356-363. https://doi.org/10.2111/08-159.1

Smouse, P. E., Long, J. C., & Sokal, R. R. (1986). Multiple regression and correlation extensions of the mantel test of matrix correspondence. *Systematic Zoology*, 35(4), 627-632. https://doi.org/10.2307/2413122

Strykstra, R. J., Verweij, G. L., & Bakker, J. P. (1997). Seed dispersal by mowing machinery in a Dutch brook valley system. *Acta Botanica Neerlandica*, 46(4), 387-401. https://doi.org/10.1111/plb.1997.46.4.387

Sullivan, E. R., Barker, C., Powell, I., & Ashton, P. A. (2019). Genetic diversity and connectivity in fragmented populations of *Rhinanthus minor* in two regions with contrasting land-use. *Biodiversity and Conservation*, 28(12), 3159-3181. https://doi.org/10.1007/s10531-019-01811-x

Takezaki, N., Nei, M., & Tamura, K. (2010). POPTREE2 : Software for constructing population trees from allele frequency data and computing other population statistics with Windows interface. *Molecular Biology and Evolution*, 27(4), 747-752. https://doi.org/10.1093/molbev/msp312

Taylor, K., Brummer, T., Taper, M. L., Wing, A., & Rew, L. J. (2012). Human-mediated long-distance dispersal : An empirical evaluation of seed dispersal by vehicles. *Diversity and Distributions*, 18(9), 942-951. https://doi.org/10.1111/j.1472-4642.2012.00926.x

Tong, Z.-Y., Wu, L.-Y., Feng, H.-H., Zhang, M., Armbruster, W. S., Renner, S. S., & Huang, S.-Q. (2023). New calculations indicate that 90% of flowering plant species are animal-pollinated. *National Science Review*, 10(10), nwad219. https://doi.org/10.1093/nsr/nwad219

Tonnabel, J., David, P., Klein, E. K., & Pannell, J. R. (2019). Sex-specific selection on plant architecture through "budget" and "direct" effects in experimental populations of the wind-pollinated herb, *Mercurialis annua*. *Evolution*, 73(5), 897-912. https://doi.org/10.1111/evo.13714

Van Rossum, F., Campos De Sousa, S., & Triest, L. (2004). Genetic consequences of habitat fragmentation in an agricultural landscape on the common *Primula veris*, and comparison with its rare congener, *P. vulgaris*. *Conservation Genetics*, 5(2), 231-245. https://doi.org/10.1023/B:COGE.0000030007.85492.70

Van Rossum, F., & Triest, L. (2007). Fine-scale spatial genetic structure of the distylous *Primula veris* in fragmented habitats. *Plant Biology*, 9(3), 374-382. https://doi.org/10.1055/s-2006-924632

Vekemans, X., & Hardy, O. J. (2004). New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology*, 13(4), 921-935. https://doi.org/10.1046/j.1365-294X.2004.02076.x

Vitalos, M., & Karrer, G. (2009). Dispersal of *Ambrosia artemisiifolia* seeds along roads : The contribution of traffic and mowing machines. *Neobiota*, 8, 53-60.

Von Der Lippe, M., & Kowarik, I. (2007). Long-distance dispersal of plants by vehicles as a driver of plant invasions. *Conservation Biology*, 21(4), 986-996. https://doi.org/10.1111/j.1523-1739.2007.00722.x

Vranckx, G., Jacquemyn, H., Muys, B., & Honnay, O. (2012). Meta-analysis of susceptibility of woody plants to loss of genetic diversity through habitat fragmentation. *Conservation Biology*, 26(2), 228-237. https://doi.org/10.1111/j.1523-1739.2011.01778.x

Wang, Y., Tian, X., Wang, H., Castañé, C., Arnó, J., Wu, S., Xian, X., Desneux, N., Liu, W., Zhang, Y., Zhang, G., & Wan, F. (2023). Genetic diversity and genetic differentiation pattern of *Tuta absoluta* across China. *Entomologia Generalis*, 43(6), 1171-1181. https://doi.org/10.1127/entomologia/2023/2026

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-Statistics for the analysis of population structure. *Evolution*, 38(6), 1358-1370. https://doi.org/10.2307/2408641

Weir, B. S., & Goudet, J. (2017). A unified characterization of population structure and relatedness. *Genetics*, 206(4), 2085-2103. https://doi.org/10.1534/genetics.116.198424

Wessinger, C. A. (2021). From pollen dispersal to plant diversification : Genetic consequences of pollination mode. *New Phytologist*, 229(6), 3125-3132. https://doi.org/10.1111/nph.17073

Westerbergh, A., & Saura, A. (1994). Gene flow and pollinator behaviour in *Silene dioica* populations. *Oikos*, 71(2), 215-224. https://doi.org/10.2307/3546269

Winfree, R., Aguilar, R., Vázquez, D. P., LeBuhn, G., & Aizen, M. A. (2009). A meta-analysis of bees' responses to anthropogenic disturbance. *Ecology*, 90(8), 2068-2076. https://doi.org/10.1890/08-1245.1

Winfree, R., Bartomeus, I., & Cariveau, D. P. (2011). Native pollinators in anthropogenic habitats. *Annual Review of Ecology, Evolution, and Systematics*, 42(1), 1-22. https://doi.org/10.1146/annurev-ecolsys-102710-145042

Woodard, S. H., & Jha, S. (2017). Wild bee nutritional ecology : Predicting pollinator population dynamics, movement, and services from floral resources. *Current Opinion in Insect Science*, 21, 83-90. https://doi.org/10.1016/j.cois.2017.05.011

Yang, Y., Sang, J., He, G., Zou, R., Gao, L., Chai, S., Li, D., Jiang, Y., Tang, J., & Wei, X. (2025). Isolation effects and soil properties drive genetic differentiation in *Ormosia microphylla* populations. *Scientific Reports*, 15(1), 25824. https://doi.org/10.1038/s41598-025-09432-x

Zurbuchen, A., Landert, L., Klaiber, J., Müller, A., Hein, S., & Dorn, S. (2010). Maximum foraging ranges in solitary bees : Only few individuals have the capability to cover long foraging distances. *Biological Conservation*, 143(3), 669-676. https://doi.org/10.1016/j.biocon.2009.12.003

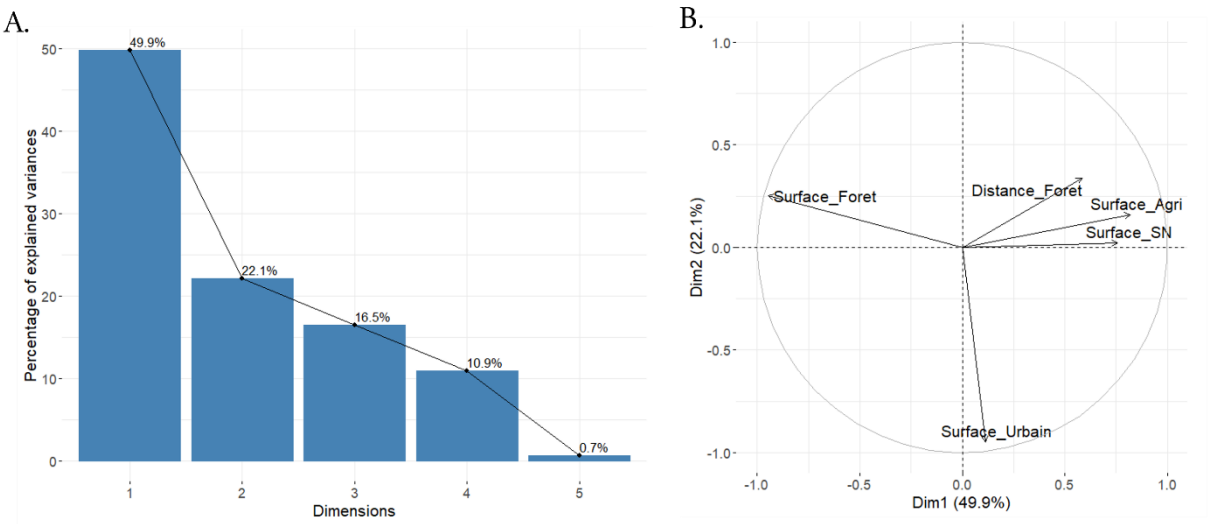
SUPPLEMENTARY MATERIAL

1. Habitat characterization

Table S1. Classification of the different types of land use, according to the four mapping data sources: data from the ARCH project, the OS Picardie project, the COSW project and the THEIA project.

	ARCH	OS Picardie	COSW	THEIA
Forest	Deciduous forests Conifers plantations Polderian forests Riparian forests	Valley floor deciduous Deciduous forests Conifers forests Mixed forests Harvested forests	Deciduous forests Conifers forests Mixed forests Forests	Deciduous forests Coniferous forests
Semi-natural	Wet meadows Mesophilic meadows Mesophilic pastures Forage meadows Grassy strips Bushes Brownfields Improved meadows Wet tall grass Vegetation belts Submerged	Natural laws and Meadows Valley floor meadows Moors and bushes	Abandoned lands Brownfields Fallow lands Temporary Permanent	Meadows Grass
Crop	Orchards Young plantations Poplars plantations Indeterminate Crops	Plots and crops Familial gardens Orchards Vineyards Nurseries, market Arable lands	Market gardening Arable lands Crops	Rapeseed Straw cereals Proteaginous Soybeans Sunflower Corn Rice Tubers / Roots Orchards Vineyards
Urban	Roadsides Urban parks Road networks Towns, villages Industrial sites	Railway infrastructures Roadway Urban green spaces Landfills Encampment Cemeteries Airports Building sites Commercial zones Industrial zones Sport equipments Urban fabric Rural habitats Historic habitats Large building	Industrial activities Landfills Active quarries Farm buildings Sport equipments Stores Urban fabric	Dense urban area Sparse urban area Industrial and commercial Road

Figure S1. Results of the PCA on habitat characteristics within a one-kilometer radius. A. Percentage of variance explained by each axis, B. Correlation circle of the variables.



2. Population description

Table S2. Population description of 29 sampled populations of the red campion (*S. dioica*). Year: sampling year, Location: neighbouring city close to the sampled population, Longitude & Latitude: GPS coordinates, N_{census}: census population size, $N_{sampled}$: number of individuals sampled, DistForest: Distance to the forest (m), Forest: surface of forest habitat (%), SN: surface of semi-natural habitat (%), Crop: surface of crop habitat (%), Urban: surface of urban habitat (%), A_r: allelic richness, H_o: observed heterozygosity, F_{IS}: intra-population fixation index, Pop.-specific F_{ST}: population-specific F_{ST} according to Weir & Goudet (2017). Significant values of F_{IS} fixation index are indicated by stars (***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$).

Weir, B. S., & Goudet, J. (2017). A unified characterization of population structure and relatedness. Genetics, 206(4), 2085-2103. https://doi.org/10.1534/genetics.116.198424

ID	Year	Location	Longitude	Latitude	N_{census}	$N_{sampled}$	DistForet	Forest	SN	Crop	Urban	A_r	H_o	F_{IS}	*Pop.-specific* F_{ST}
A1	2023	Potelle	3.6598483	50.2326281	800	100	0	0.07	0.44	0.37	0.11	4.7	0.610	**0.102*****	-0.035
A2	2023	Bavay	3.7996608	50.2868433	2000	100	0	0.15	0.31	0.41	0.13	4.2	0.504	**0.098*****	0.148
A3	2023	Pont-sur-Sambre	3.8355342	50.2229467	300	100	71	0.09	0.47	0.31	0.12	5.1	0.644	0.018	0.000
ABB1	2019	Rejet-de-Beaulieu	3.6441633	50.0466608	138	20	0	0.05	0.57	0.31	0.04	4.5	0.560	-0.068	0.199
BOU1	2019	Boué	3.7062825	50.0066967	625	20	0	0.60	0.15	0.05	0.17	4.8	0.490	**0.201*****	0.070
BOU2	2019	Boué	3.6976417	50.007015	39	19	0	0.39	0.23	0.05	0.31	4.9	0.546	**0.123***	0.054
BS1	2019	Condé-sur-l'Escaut	3.6008908	50.477405	93	20	0	0.65	0.16	0.09	0.11	5.2	0.630	-0.014	0.052
BS2	2019	Condé-sur-l'Escaut	3.6033158	50.4880025	46	20	0	0.72	0.02	0.11	0.14	4.4	0.540	0.071	0.115
ETR	2019	Etreux	3.665685	49.996355	54	20	226.53	0.03	0.23	0.48	0.23	4.4	0.486	**0.193****	0.087
F1	2023	Locquignol	3.7006172	50.2124031	150	100	0	0.98	0.02	0.00	0.00	4.8	0.493	**0.114*****	0.151
F2	2023	Locquignol	3.7815922	50.2535217	250	100	0	0.80	0.18	0.00	0.02	4.5	0.562	0.037	0.110
F3	2023	Locquignol	3.7580894	50.2103733	400	100	0	0.77	0.17	0.04	0.01	5.2	0.640	0.017	0.008
FRE	2018	Templeuve-en-Pévèle	3.1701685	50.5567963	42	17	0	0.13	0.34	0.35	0.15	3.1	0.588	-0.046	0.142
GAU1	2019	Tournai (Belgium)	3.498345	50.5940175	178	17	0	0.16	0.19	0.11	0.53	3.8	0.588	-0.051	0.145
OIS1	2019	Oisy	3.6626792	50.02587	148	20	1,268.89	0.00	0.34	0.56	0.10	4.2	0.642	0.005	0.017
OIS2	2019	Oisy	3.674575	50.0194143	25	14	412.23	0.03	0.55	0.27	0.11	3.8	0.557	-0.087	0.216
OIS3	2019	Fesmy-le-Sart	3.6623211	50.0387	59	19	173.11	0.03	0.66	0.22	0.05	5.0	0.526	**0.233*****	-0.037
ORS1	2019	Ors	3.6433133	50.1168417	618	19	0	0.44	0.31	0.20	0.04	4.7	0.600	0.035	0.053
ORS2	2019	Ors	3.63263	50.0952633	10	10	0	0.02	0.60	0.15	0.19	3.0	0.373	**0.233***	0.266
RP1	2019	Marchiennes	3.3065456	50.4338289	121	19	0	0.53	0.08	0.32	0.07	4.7	0.589	0.035	0.069
RP2	2018	Beuvry-la-Forêt	3.2667383	50.438065	176	20	0	0.51	0.10	0.27	0.12	4.6	0.628	-0.021	0.062
RP3	2019	Beuvry-la-Forêt	3.2720429	50.437919	179	19	0	0.51	0.10	0.27	0.12	4.6	0.554	0.064	0.099
SA2	2019	Raismes	3.4482052	50.3960708	31	16	0	0.53	0.08	0.10	0.12	4.7	0.482	**0.174****	0.114
SA3	2018	Raismes	3.4341156	50.3906056	21	15	0	0.36	0.09	0.16	0.11	4.8	0.568	0.066	0.076
SA4	2019	Saint-Amand-les-Eaux	3.4449333	50.4259333	7	7	0	0.71	0.12	0.03	0.14	3.2	0.543	**0.281***	*NA*
SA5	2019	Raismes	3.4330632	50.38825	113	17	0	0.30	0.09	0.20	0.16	4.6	0.412	**0.212*****	0.209
VEN1	2019	Vénérolles	3.6402917	49.9844033	69	19	0	0.02	0.26	0.60	0.10	3.7	0.635	0.003	0.028
VRE1	2018	Vred	3.2288759	50.3910685	29	18	0	0.14	0.30	0.26	0.26	4.7	0.604	**0.160***	-0.090
VRE2	2018	Rieulay	3.2546317	50.3907883	88	20	0	0.20	0.26	0.36	0.06	4.2	0.600	0.013	0.073

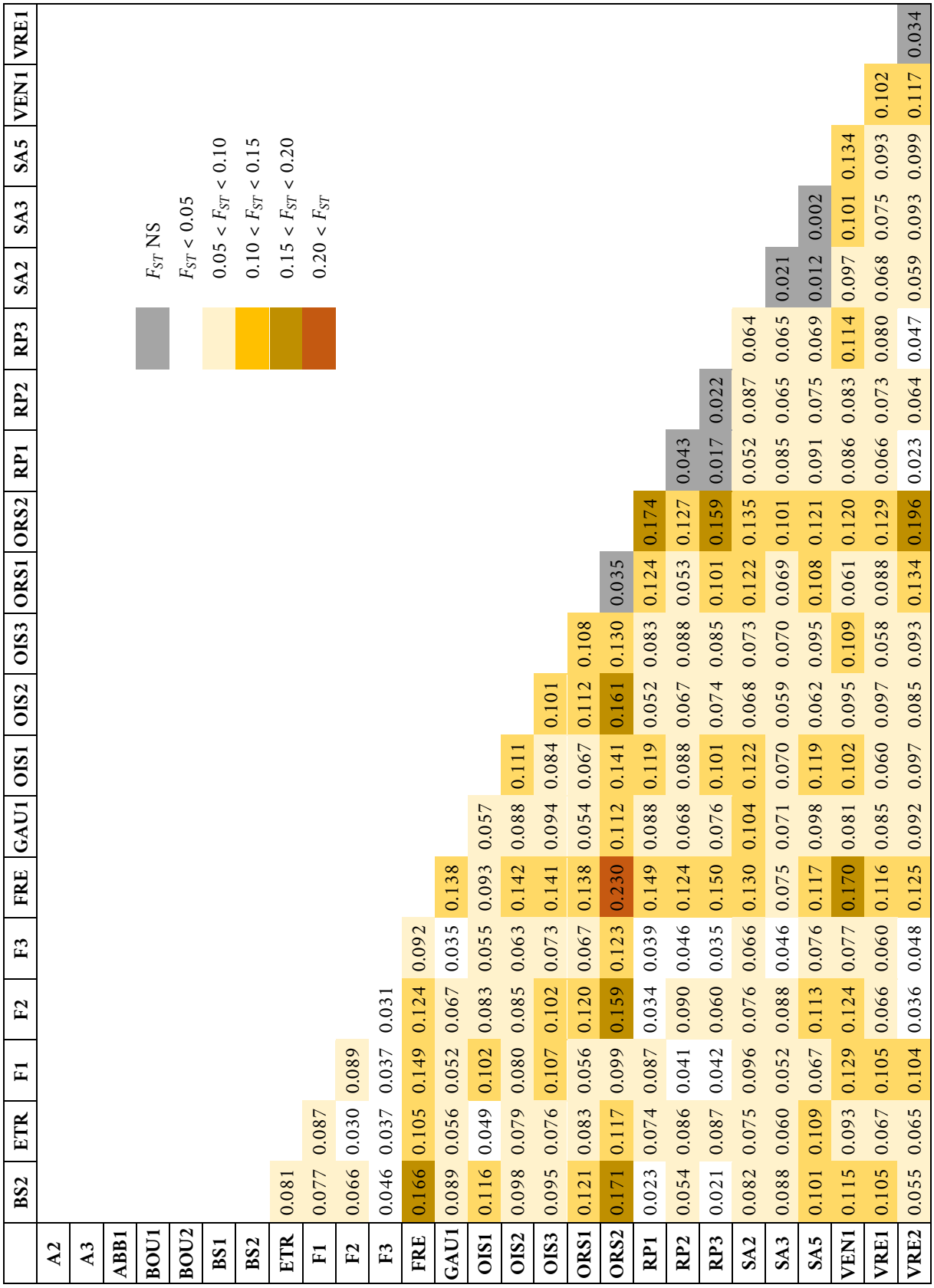
3. Genetic diversity and differentiation

Table S3. Linear model estimates of the relationships between levels of genetic diversity (A_r and H_o) or genotypic structure (F_{IS}) and population size and habitat descriptors within a one-kilometer radius. Values represent the estimated slopes of the models (all non-significant, $p > 0.05$). Explanatory variables were standardized (with a mean of 0 and a variance of 1) to facilitate comparison of slopes. A_r: allelic richness, H_o: observed heterozygosity, F_{IS}: inbreeding coefficient, F_{ST} spe: population-specific F_{ST}.

	A_r	H_o	F_{IS}	F_{ST} *spe*
Forest habitat	0.174	-0.005	-0.012	0.004
Urban habitat	-0.098	-0.010	0.005	0.007
Population size	0.070	-0.002	0.012	-0.002

4. Pairwise F_{ST}

Table S4. Pairwise population F_{ST}. Non significative values after Bonferroni correction are indicated in grey.

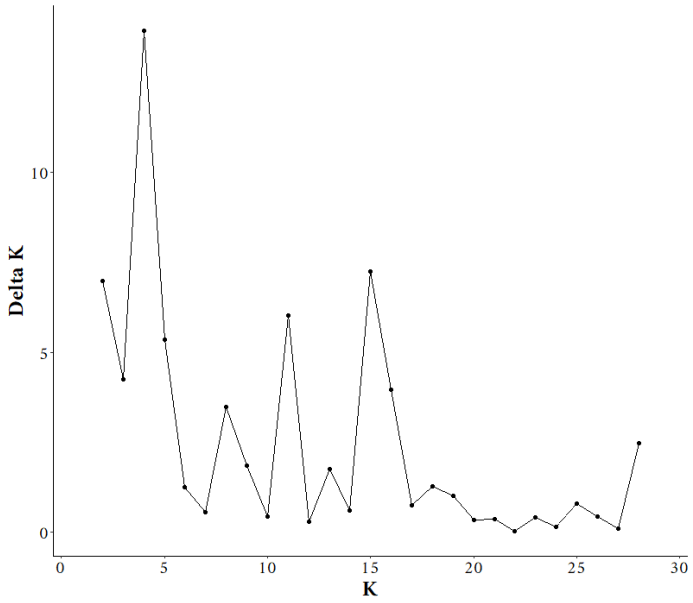


	BS2	ETR	F1	F2	F3	FRE	GAU1	OIS1	OIS2	OIS3	ORS1	ORS2	RP1	RP2	RP3	SA2	SA3	SA5	VEN1	VRE1
A2																				
A3																				
ABB1																				
BOU1																				
BOU2																				
BS1																				
BS2																				
ETR	0.081																			
F1	0.077	0.087																		
F2	0.066	0.030	0.089																	
F3	0.046	0.037	0.037	0.031																
FRE	0.166	0.105	0.149	0.124	0.092															
GAU1	0.089	0.056	0.052	0.067	0.035	0.138														
OIS1	0.116	0.049	0.102	0.083	0.055	0.093	0.057													
OIS2	0.098	0.079	0.080	0.085	0.063	0.142	0.088	0.111												
OIS3	0.095	0.076	0.107	0.102	0.073	0.141	0.094	0.084	0.101											
ORS1	0.121	0.083	0.056	0.120	0.067	0.138	0.054	0.067	0.112	0.108										
ORS2	0.171	0.117	0.099	0.159	0.123	0.230	0.112	0.141	0.161	0.130	0.035									
RP1	0.023	0.074	0.087	0.034	0.039	0.149	0.088	0.119	0.052	0.083	0.124	0.174								
RP2	0.054	0.086	0.041	0.090	0.046	0.124	0.068	0.088	0.067	0.088	0.053	0.127	0.043							
RP3	0.021	0.087	0.042	0.060	0.035	0.150	0.076	0.101	0.074	0.085	0.101	0.159	0.017	0.022						
SA2	0.082	0.075	0.096	0.076	0.066	0.130	0.104	0.122	0.068	0.073	0.122	0.135	0.052	0.087	0.064					
SA3	0.088	0.060	0.052	0.088	0.046	0.075	0.071	0.070	0.059	0.070	0.069	0.101	0.085	0.065	0.065	0.021				
SA5	0.101	0.109	0.067	0.113	0.076	0.117	0.098	0.119	0.062	0.095	0.108	0.121	0.091	0.075	0.069	0.012	0.002			
VEN1	0.115	0.093	0.129	0.124	0.077	0.170	0.081	0.102	0.095	0.109	0.061	0.120	0.086	0.083	0.114	0.097	0.101	0.134		
VRE1	0.105	0.067	0.105	0.066	0.060	0.116	0.085	0.060	0.097	0.058	0.088	0.129	0.066	0.073	0.080	0.068	0.075	0.093	0.102	
VRE2	0.055	0.065	0.104	0.036	0.048	0.125	0.092	0.097	0.085	0.093	0.134	0.196	0.023	0.064	0.047	0.059	0.093	0.099	0.117	0.034

	A1	A2	A3	ABB1	BOU1	BOU2	BS1
A2	0.077						
A3	0.039	0.037					
ABB1	0.104	0.083	0.078				
BOU1	0.029	0.053	0.024	0.074			
BOU2	0.043	0.086	0.059	0.044	0.020		
BS1	0.077	0.137	0.085	0.139	0.093	0.114	0.046
BS2	0.054	0.105	0.063	0.126	0.045	0.075	0.103
ETR	0.056	0.021	0.022	0.070	0.012	0.055	0.123
F1	0.053	0.107	0.054	0.086	0.052	0.050	0.103
F2	0.051	0.047	0.033	0.112	0.021	0.071	0.071
F3	0.022	0.059	0.018	0.079	0.012	0.036	0.158
FRE	0.124	0.135	0.091	0.159	0.104	0.108	0.134
GAU1	0.052	0.075	0.052	0.098	0.034	0.038	0.127
OIS1	0.069	0.075	0.058	0.105	0.056	0.050	0.127
OIS2	0.089	0.108	0.076	0.076	0.031	0.057	0.111
OIS3	0.086	0.116	0.076	0.098	0.062	0.068	0.157
ORS1	0.055	0.115	0.078	0.104	0.067	0.031	0.217
ORS2	0.107	0.148	0.129	0.102	0.116	0.064	0.057
RP1	0.042	0.101	0.059	0.116	0.021	0.061	0.073
RP2	0.049	0.125	0.069	0.105	0.040	0.045	0.067
RP3	0.047	0.116	0.058	0.108	0.036	0.054	0.122
SA2	0.086	0.090	0.065	0.048	0.051	0.044	0.111
SA3	0.070	0.079	0.049	0.037	0.051	0.032	0.135
SA5	0.105	0.127	0.087	0.042	0.077	0.043	0.145
VEN1	0.068	0.126	0.104	0.110	0.059	0.040	0.111
VRE1	0.061	0.084	0.051	0.110	0.055	0.065	0.091
VRE2	0.057	0.089	0.052	0.129	0.026	0.070	

5. Population genetic delineations

Figure S2. Variation of the *ΔK* statistic across successive tested values of *K* ranging from one to 29 (mean over 10 replicates).



6. Pollen dispersal kernel

Table S5. Spatially explicit mating model parameters estimates for six populations of red campion (*S. dioica*). σ_{RS}: male reproductive success variance, μ_δ: mean pollen dispersal distance (m), σ_δ: variance of pollen dispersal distance, b: shape of the pollen dispersal kernel, m: migration rate. Populations with names starting with "F" are located in predominantly forested areas, while populations starting with "A" are found in mainly agricultural areas.

Population	σ_{RS}	μ_δ	σ_δ	b	m
A1	0.96	2.31	4.39	7.63	0.35
A2	1.55	2.19	4.64	8.20	0.41
A3	1.64	25.43	2.06	0.84	0.48
F1	2.16	6.02	10.38	0.92	0.53
F2	1.30	4.95	2.56	4.64	0.40
F3	1.41	5.50	3.28	2.64	0.37

CHAPITRE II

Les différences d'habitat n'altèrent pas les patrons de sélection sexuelle chez *Silene dioica*

Les résultats du premier chapitre ont montré que les flux de pollen étaient leptokurtiques, c'est-à-dire majoritairement restreints à de courtes distances, mais avec quelques événements occasionnels de dispersion à plus longue distance. Cette distribution suggère qu'au sein de ce que l'on considère comme une population naturelle, les individus peuvent effectivement être en compétition pour l'attraction des pollinisateurs et donc pour l'accès aux partenaires sexuels. Dans ce contexte, les traits floraux impliqués dans cette attraction—mais aussi dans l'efficacité du transfert de pollen—pourraient être sous sélection. Dans ce chapitre, nous explorerons dans quelle mesure le phénotype floral influence le succès reproducteur individuel, notamment via son effet sur l'accès aux partenaires sexuels. Nous examinerons également si cette sélection varie selon le sexe des individus, afin de tester les prédictions classiques de la théorie de la sélection sexuelle, mais aussi selon le type d'habitat, en comparant les patrons de sélection observés dans des sites forestiers et anthropisés, ces derniers étant potentiellement caractérisés par une densité en pollinisateurs réduite.

Manuscript B: Habitat differences do not disrupt sexual selection patterns in *Silene dioica*

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Abstract

In most angiosperms, pollinators mediate access to sexual partners, making attractive floral traits key targets of selection. Sexual selection theory predicts stronger selection through the male function, due to greater dependence on mate acquisition. However, habitat degradation may alter plant-pollinator interactions and disrupt sex-specific selection. In particular, reduced pollinator density may increase the dependency of female reproductive success on mate acquisition and strengthen selection on floral traits in both sexes. We tested this hypothesis in the dioecious *Silene dioica* by measuring pollinator visitation, pollination service, and phenotypic selection on floral traits in both sexes across six populations located in either forest or anthropogenic habitats. Despite lower visitation in anthropogenic sites, pollination service was comparable between habitats with consistent selection on fertility-related traits in females. In contrast, we found stronger selection on a male trait likely promoting efficient pollen transfer in anthropogenic habitats, indicating a sex-specific response to land use change. Finally, male—but not female—reproductive success increased with the number of sexual partners in both habitats, supporting stronger sexual selection in males. These findings highlight the importance of distinguishing between sexes when studying floral trait evolution and suggest that male-biased sexual selection can persist despite environmental change.

Key words

Pollinator density, floral traits, pollinator-mediated selection, fertility selection, dioecy

Contents

INTRODUCTION

A large majority of flowering plant species rely on insects for pollen transfer (Ollerton et al., 2011). In these plants, reproductive success partly depends on complex interactions between plants and their pollinators, including the ability of plants to attract pollinators, and of pollinators to effectively remove, transport and deposit pollen onto receptive stigmas. Floral traits—such as flower number, floral colour, scent, morphology, or nectar production—can influence the frequency of pollinator visits (Giurfa et al., 1995 ; Totland, 2001 ; Bauer et al., 2017 ; Barbot et al., 2022) and/or the mechanical fit between flowers and pollinators (De Jager & Peakall, 2019 ; Trunschke et al., 2020 ; Newman et al., 2025), thereby affecting both pollen export (i.e., male function) and pollen receipt (i.e., female function). Over the last decades, pollinator-mediated selection has been reported across a wide range of floral traits and species (reviewed in Caruso et al., 2019), and is viewed as a major evolutionary force underlying the remarkable diversification of angiosperms (Fenster et al., 2004 ; Harder & Johnson, 2009 ; Schiestl & Johnson, 2013). Importantly, most studies documenting pollinator-mediated selection on floral traits have focused on female reproductive success, typically quantified through individual seed production (e.g., Chapurlat et al., 2015 ; Brown & Caruso, 2023 ; Cha et al., 2023). While this approach has yielded valuable insights, it provides only a partial view of the selective forces acting on floral traits, as it overlooks a substantial proportion of total reproductive output—either within hermaphroditic individuals or, in dioecious species, across the unmeasured sex. Yet selection on floral traits is unlikely to be symmetric: it should differ between sexual functions both in strength and the traits targeted. Specifically, if pollinator-mediated selection arises from variance in mate acquisition caused by differences in pollinator attraction, then pollinator-mediated selection can be regarded as a component of sexual selection (Moore & Pannell, 2011 ; Barbot et al., 2023). The sex typically most constrained in mate acquisition is expected to be males, and sexual selection is thus predicted to act more strongly through the male function (Bateman, 1948 ; Stephenson & Bertin, 1983 ; Burd, 1994). Conversely, because most plants produce fewer ovules than pollen (Kodric-Brown & Brown, 1987), relatively few pollinator visits may be sufficient to maximize seed set. Female reproductive success is therefore expected to be primarily resource-limited, at least when pollinators are abundant. From this logic follows a clear prediction: selection through the male function is expected to act predominantly on traits related to pollinator attraction (e.g., flower size), whereas selection via the female function should primarily target traits related to fertility, that is traits affecting the number and quality of gametes produced (e.g., the number of flowers or number of ovules per flower). Few studies have measured selection exerted through both sexual functions, but available evidence supports such a prediction (Hodgins & Barrett, 2008 ; Zhou et al., 2020 ; Barbot et al., 2023).

One limitation in our understanding of sexual selection and of potential differences between sexual functions is that our knowledge of traits actually influencing reproductive success is often incomplete.

However, sex differences in the dependency of reproductive success on mate acquisition can be quantified without any prior assumption about relevant traits, by estimating Bateman gradients (i.e., slopes of the regression of reproductive success on number of sexual partners, Bateman, 1948). Positive Bateman gradients are expected for the male function, reflecting the reproductive advantage of acquiring additional mates. In contrast, a flat gradient is expected for the female function, indicating that acquiring more mates does not provide any reproductive advantage. This expectation appears to be broadly supported in animals (Janicke et al., 2016) and has been explored more recently in plants, with consistent support across all species tested (e.g. in a moss, Johnson & Shaw, 2016 ; in a seaweed, Lavaut et al., 2023 ; in a wind-pollinated species, Tonnabel et al., 2019 ; and in insect-pollinated species, Barbot et al., 2022 ; Kwok & Dorken, 2022).

The prediction that both selection on attractive floral traits and Bateman gradients should be stronger through the male than through the female function is valid only under optimal pollination conditions, when receptive stigmas receive sufficient pollen to fertilize all available ovules. However, pollen limitation—whereby pollen receipt limits seed production—is common in nature (e.g., reviewed in Burd, 1994 ; Ashman et al., 2004 ; Knight et al., 2005 ; Bennett et al., 2018) and can be exacerbated by anthropogenic disturbances, such as habitat fragmentation, changes in floral resource availability, or increased pesticide and herbicide use (Winfree et al., 2011 ; Crispim et al., 2018), which reduce pollinator abundance and diversity (Ricketts et al., 2008 ; Winfree et al., 2009). Under such conditions, female reproductive success, like male reproductive success, may become limited by pollinator availability and mate acquisition. Consequently, both sexual functions (or sexes, in dioecious species) should benefit from additional pollinator visits, and selection on attractive floral traits is expected to intensify in both males and females. This should be associated with steeper Bateman gradients in both sexes, reflecting stronger reproductive gains with increased access to mates. Such effects are likely to be particularly strong through the female function, so that pollen limitation should make selection patterns more similar between sexes. A few empirical studies have examined the relationship between pollinator density and selection strength and confirmed that selection on floral traits becomes stronger under pollen limitation (Dai & Galloway, 2013 ; Bartkowska & Johnston, 2015 ; Sletvold & Ågren, 2016 ; Emel et al., 2017 ; Brown & Caruso, 2023). However, such studies have almost exclusively focused on the female function (but see Wu et al., 2023a), while a comprehensive understanding of floral trait evolution under pollinator decline requires considering both sexual functions together.

In this study, we addressed this gap by examining whether land use change alters plant–pollinator interactions and selection patterns in natural populations of *Silene dioica*, a dioecious species. Specifically, we quantified and compared pollinator visitation rates, pollination service, selection exerted on floral traits and Bateman gradients in both sexes between two contrasting habitats, by sampling three populations in minimally disturbed forest environments and three in intensively managed agricultural landscapes. We expected lower pollinator visitation rates and reduced pollination service in the

agricultural context. Consequently, we predicted stronger selection on floral traits in both females—where pollinator-mediated selection may arise—and males—where it may intensify—in the anthropogenic habitat, as well an increase in the strength of sexual selection, as indicated by steeper Bateman gradients in both sexes.

MATERIALS AND METHODS

1. Study species

Silene dioica (L) Clairv. (Caryophyllaceae) is a herbaceous, perennial and dioecious angiosperm widely distributed in central and northern Europe. The species is described as predominantly forest-associated but can be found along a gradient from semi-natural habitats such as forest clearings or woodland edges to more anthropogenic environments like road verges and hedgerows, provided that conditions remain sufficiently moist and partially shaded (Kay et al., 1984). It has a generalist pollination system and is primarily pollinated by bumblebees, honeybees, butterflies and hoverflies, the first two being the most frequent (Kay et al., 1984 ; Barbot et al., 2022).

This species exhibits sexual dimorphism in several floral traits linked to pollinator attraction, such as flower size and number, with males displaying more conspicuous inflorescences (Kay et al., 1984 ; Moquet et al., 2020 ; Barbot et al., 2023). In northern France, the species' flowering period spans from late April to mid-July, peaking in late May.

2. Study populations

Based on the land use in the area surrounding each population in a one kilometer radius (i.e., corresponding to the typical foraging range of bumblebees, one of the most common *S. dioica* pollinators, Osborne et al., 2008), three populations from forest habitats (labelled "F"), containing at least 90% forest and semi-natural habitats, and three populations from anthropogenic habitats (labelled "A"), containing more than 40% agricultural and urbanized habitats, were selected (see supplementary material – Habitat characterization). Study sites were selected to minimize spatial clustering of populations within each habitat type (**Figure 1**).

In each population, 50 male and 50 female plants were randomly selected, ensuring individuals were at a comparable phenological stage (at or just before peak flowering) and aiming for a comparable density between the six monitored populations (supplementary material – Spatial configuration). These one hundred plants were individually tagged, and their geographical position was recorded (GPS 60 - Garmin). Outside of the tagged individuals, all *S. dioica* plants within the populations and within a 100-meter radius around the population had their inflorescences clipped before flowering, to ensure that the pollination events occurring during the study were primarily carried out by the fifty selected males.

In each of the six populations, flowering was monitored over ten days. On the first day, all pre-existing open flowers were marked with one colour of paint. During the experiment, newly opened flowers were marked with a different colour, allowing us to distinguish between flowers produced before and during the experiment when assessing both female and male reproductive success. The experiment was stopped after ten days because flower production became negligible, particularly in females.

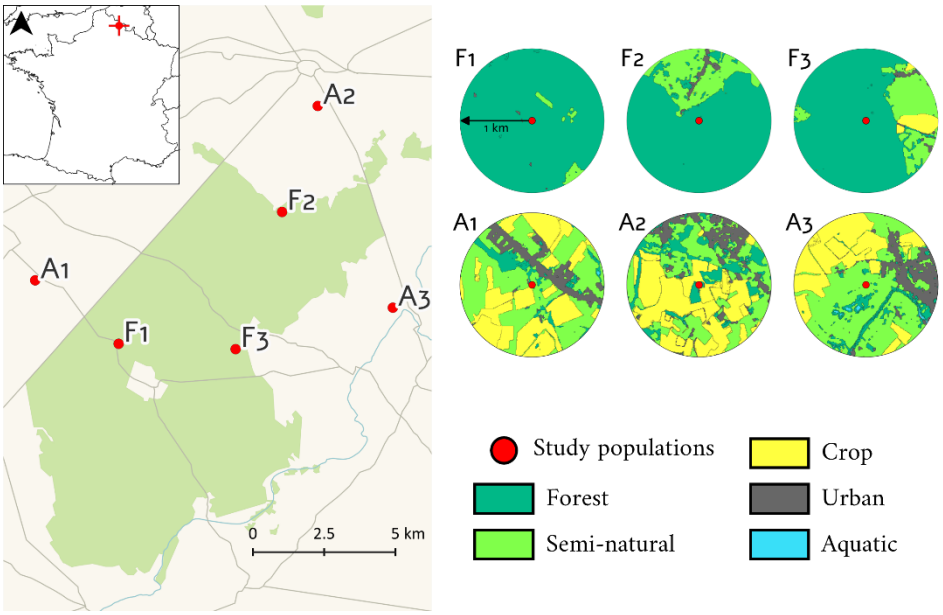

Figure 1. Location of the 6 populations (F: populations from forest habitats, A: populations from anthropogenic habitats) of *Silene dioica* sampled in the Mormal forest (Northern France) (left panel) and land use within a radius of 1 km around the center of each population (right panel).

3. Floral trait measurements

On the first day of the experiment, for each individual, the number of open flowers was counted, and calyx height (mm) and corolla width (mm) were measured on two randomly selected flowers using calipers (precision: 0.01 mm). These two measurements were averaged before the analyses described below.

For female plants, the number of ovules per flower was estimated from three randomly selected fruits produced during the flowering survey, using an image analysis method developed by Barbot et al. (2022). This method involves dissecting the content of a fruit and digitizing it using a high-resolution scanner. Seeds and undeveloped ovules are differentiated based on their size and counted using an R script for image analysis (EBImage package in R, Pau et al., 2010). The number of ovules per flower corresponds to the sum of the seeds and undeveloped ovules, and was averaged over the three dissected fruits. For male plants, the number of pollen grains was estimated from two anthers collected from a nearly opened flower bud, using a particle counter (CASY Cell Counter & Analyzer, OLS) and following the protocol of Dufaÿ et al. (2008). This number was then multiplied by five to obtain the number of pollen grains per flower.

This set of floral traits was selected to include: (i) a trait whose role in pollinator attraction has already been established in the model species (corolla width, Barbot et al., 2025); (ii) a trait likely involved in pollinator fit (calyx height, which probably influences how deeply insects must enter the corolla to access nectar); and (iii) a trait directly related to fertility (number of gametes per flower). The last trait, number of open flowers, could affect individual reproductive success through its documented effect on pollinator attraction (Barbot et al., 2022 ; Moquet et al., 2022) and/or through its effect on gamete production, given the clear correlation between daily floral display and total flower production in both sexes (Barbot et al., 2023).

4. Pollinator diversity and density characterization

To assess whether habitat type (F vs. A) affected pollinator density, pollinator observation sessions were conducted every other day during the duration of the experiment in each population (four observation days). Each session lasted 20 minutes, always between 10:00 AM and 4:30 PM, and involved three independent observers, positioned at three distinct locations within the population. In total, 12 sessions were conducted in each population (three observers × four observation days). Each observer was responsible for a randomly chosen patch of focal plants (containing between 2 and 9 individuals, depending on their size and spatial arrangement) and recorded the identity of all pollinators visiting the patch (Orders: Coleoptera, Diptera, Hymenoptera, Lepidoptera, others), as well as the total number of flowers visited by each pollinator during its visit to the focal patch. A visit was defined as a contact between a pollinator and the reproductive structures of a flower. The total number of open flowers was recorded within each patch. Two estimators of pollinator density were derived from these observations: (i) the total number of visits and (ii) the flower visitation rate, calculated as the total number of visits divided by the total number of flowers open in the patch.

5. Estimation of pollination service quality

We performed a hand pollination experiment using plants kept in pots, in order to reveal potential differences in pollination service among populations independently of the quantity of resources available to the plants. Twenty-four female plants from a greenhouse collection were positioned within each study population and watered regularly during the experiment. These plants originated from seeds sampled in previous years in wild populations from the same area (in and around the Mormal State Forest) and grown in standardized conditions in a greenhouse at Lille University. Plants were randomly assigned to one of the following treatments: open pollination (OP, 12 plants) and hand pollination (HP, 12 plants). Plants were arranged in groups of four along the length of the population, alternating between groups of four OP plants and groups of four HP plants (supplementary material – Spatial configuration). Hand pollinations were carried out every other day, using pollen collected from fresh male flowers harvested from non-surveyed male plants located in neighbouring populations, to avoid removing flowers of the

surveyed males. Hand pollinations involved rubbing the anthers of male flowers onto the stigmas of all open female flowers, ensuring that each stigma was fully saturated with pollen from at least two donors. At the end of the flowering survey, potted plants were returned to the greenhouse and monitored until fruit collection.

6. Reproductive success estimation

Females – For both sets of females (females from the six natural populations and potted females used to estimate the quality of pollination service), the number of fruits produced during the experiment was recorded. We estimated the number of seeds and ovules in three randomly selected fruits per female as described above. Then, for females from natural populations, we estimated total seed production—as a proxy of female reproductive success—by multiplying the average number of seeds from the three fruits by the total number of fruits produced by each female. This estimation of total reproductive success was used for selection analyses. For each potted female, we calculated fruit set (number of fruits / number of flowers produced during the experiment) and seed set (number of seeds / number of ovules per fruit, in the three digitized fruits), as these indices allow us to assess whether pollen receipt was sufficient to maximize fruit initiation and seed production.

Males – Reproductive success of male plants was estimated using paternity analyses on a subsample of the seeds produced during the experiment. Total genomic DNA was extracted from dried leaf tissue for all adults (600 plants) and directly from seeds for 16 offspring sampled in eight randomly selected fruits per surveyed female, (4,800 offspring in total, see Joffard et al., 2025 for seed DNA extractions) using the NucleoMag 96 Plant kit (Macherey Nagel, Oesingen, Switzerland) and an automated DNA purification station (KingFisher®, Thermo Fisher Scientific, Waltham, USA). PCR amplifications were performed on five nuclear microsatellites (Barbot et al., 2022).

A spatially explicit mating model, developed by Oddou-Muratorio et al. (2018) and modified by Tonnabel et al. (2019) was used to estimate male reproductive success. This model uses information about the genotypes of the mothers, potential fathers and seeds, as well as the spatial distribution of individuals, to estimate male reproductive success (MRS) and mean pollen dispersal distance per male (δ). This fractional paternity assignment method estimates the siring probability across all potential fathers for each genotyped seed. An average siring probability is calculated for all mother-potential father couples based on the 16 genotyped seeds. The number of offspring shared between a male and a female is then estimated as the product of the male's siring probability and the female's reproductive success (e.g., total number of seeds produced during the experiment, see previous section). The reproductive success of each male then corresponds to the sum of the seeds sired across all females in the population. Model parameters estimations are described in the manuscript A, section Paternity analyses.

7. Mating success estimation

Mating success (i.e., number of sexual partners) was estimated using the categorical paternity assignment method implemented in CERVUS 3.0.7 (Marshall et al., 1998 ; Kalinowski et al., 2007). For each seed and each candidate male, a logarithm of odds (LOD) score was calculated, representing the likelihood ratio that the candidate is the true sire *versus* an unrelated male. To determine confidence in assignments, 10,000 simulations of reproduction events were performed to generate critical thresholds for delta LOD scores—the difference between the highest and second highest LOD scores—accounting for a 2% genotyping error rate and using an 80% confidence threshold. These thresholds were then applied to assign the most likely sire for each seed by comparing offspring genotypes to candidate males. Because the number of unambiguously assigned seeds varied among females, a bootstrapping procedure was used: for each iteration, 8 seeds were randomly sampled for each female, a process that was repeated 100 times. The number of sexual partners for each male and female individual was then averaged across iterations.

8. Statistical analyses

Floral trait variation – Variation in floral traits between sexes and habitat types (F vs. A) was analysed using generalized linear mixed models (GLMMs), with a negative binomial distribution for the number of open flowers and a Gaussian distribution for calyx height and corolla width. Sex, habitat type and their interaction were treated as fixed effects. We also compared the number of ovules per flower and the number of pollen grains per flower between habitat types, using GLMMs with a Gaussian distribution, with habitat type treated as a fixed effect. Population was included as a random effect in all models.

Pollinator density – The total number of pollinator visits and the flower visitation rate were compared between habitat types (F vs. A) using a GLMM with a negative binomial distribution, including population and observation session as random effects.

Quality of pollination service – The quality of pollination service was compared between habitat types by assessing fruit set and seed set variation between OP and HP plants. To this end, we built GLMMs with a binomial distribution including pollination treatment (OP vs. HP), habitat type (F vs. A) and their interaction as fixed effects, and population as a random effect. For the model focusing on seed set variation, female identity was also included as a random effect since seed set was measured on three fruits per plant. A significant interaction between pollination treatment and habitat type would indicate variation in the quality of pollination service between populations inhabiting forest and anthropogenic habitats.

Selection gradients – Selection gradients (β) were estimated following Lande & Arnold (Lande & Arnold, 1983), using multiple regression analyses with relative reproductive success (individual

reproductive success divided by mean reproductive success) as the response variable and standardized trait values (with a mean of 0 and a variance of 1) as explanatory variables (i.e., number of open flowers, number of gametes per flower, corolla width and calyx height). Selection gradients were estimated separately for males and females because reproductive success was estimated using different methods in the two sexes. Reproductive success was relativized and traits standardized separately for each population and sex.

To assess whether selection gradients varied between habitats, we used an ANCOVA including all traits, habitat type and interactions between each trait and habitat type as fixed effects and population as a random effect. A significant interaction between the effects of any trait and habitat type would indicate that the selection exerted on this trait differs between populations inhabiting forest and anthropogenic habitats.

Finally, since inbreeding depression can generate spurious correlations between traits and reproductive success—if both are simultaneously affected by inbreeding (Willis, 1996)—we also estimated selection gradients while including a proxy for individual inbreeding as a covariate (internal relatedness, i.e., frequency of homozygous alleles corrected for allele frequency, Amos et al., 2001). This allowed us to account for the fine-scale spatial genetic structure documented in *S. dioica* (Giles et al., 1998 ; Ingvarsson & Giles, 1999 ; Jolivel, et al., in prep.b), which likely reflects family structure arising from limited gene flow (Barbot et al., 2022). Such structure can lead to biparental inbreeding and thus needs to be controlled for when estimating selection gradients.

Relationship between traits, reproductive success and mating success – As a final step, structural equation modelling (SEM, Kline, 2011) was used to simultaneously quantify the direct effect of traits on reproductive success, the direct effect of traits on mating success (i.e., number of sexual partners), and the relationship between mating success and reproductive success, which here serves as a proxy for the Bateman gradient. If a trait influences reproductive success primarily through its effect on mating success, then this trait can be considered to be under sexual selection. One model was built for each population and sex, using the *piecewiseSEM* package in R. Reproductive success and mating success were relativized and traits standardized separately for each population and sex.

All statistical analyses were performed using R (version 4.2.3). Normality of residuals and homogeneity of variance were verified for all linear models. Inspection of variance inflation factors (VIFs) in models estimating selection gradients indicated no problem of multicollinearity (all VIFs < 2, Quinn & Keough, 2002). Overdispersion in generalized linear models with a negative binomial distribution was checked and found to be less than 2.

RESULTS

1. Floral trait variation

The number of open flowers, corolla width and calyx height exhibited sexual dimorphism, with males having both more open flowers, and larger flowers (open flowers: $F_{1,593} = 674.57$, $p < 0.001$, corolla width: $F_{1,582} = 228.08$, $p < 0.001$, calyx height: $F_{1,593} = 470.16$, $p < 0.001$, supplementary material – Floral trait variation). Additionally, the number of open flowers was significantly higher in the anthropogenic habitat compared to the forest habitat ($F_{1,593} = 11.83$, $p < 0.001$). The interaction between sex and habitat type was significant for calyx height only, with stronger sexual dimorphism in the anthropogenic habitat ($F_{1,593} = 4.30$, $p < 0.05$). The number of gametes per flower (i.e., number of ovules for females and number of pollen grains for males) did not differ between habitat types (Ovules: $F_{1,287} = 2.51$, $p = 0.113$, Pollen: $F_{1,294} = 1.03$, $p = 0.301$).

2. Pollinator diversity and density

Across the whole dataset, the two most abundant pollinator orders were Hymenoptera (66% of all recorded visits), represented mainly by *Bombus* species, along with less frequent visitors such as honeybees and halictids, and Diptera (27% of visits), predominantly hoverflies (Syrphidae), with occasional occurrences of other families such as bee flies (Bombyliidae). Other observed orders included Coleoptera and Lepidoptera, but these were only recorded in populations F1 and A3, respectively. Apart from population A3, where hoverflies were the most frequent pollinators, the representation of the different pollinator groups appeared qualitatively similar across populations, with Hymenoptera accounting for the majority of visits, and no consistent differences observed between forest and anthropogenic sites (**Figure 2**).

The total number of visits per hour did not differ between populations located in forest and anthropogenic habitats (Forest: 51.00 ± 57.80 visits/hour, Anthropogenic: 75.25 ± 80.56 visits/hour, $F_{1,67} = 0.54$, $p = 0.478$). However, flower visitation rates (i.e., number of visits per flower per hour) were lower in populations located in anthropogenic habitats than in those located in the forest habitat ($F_{1,67} = 4.34$, $p < 0.05$), owing to the larger floral displays of plants in the former. Flowers in the forest habitat received, on average, 1.4 times more visits than those in anthropogenic habitat (Forest: 0.660 ± 0.527 visits/flower/hour; Anthropogenic: 0.476 ± 0.594 visits/flower/hour).

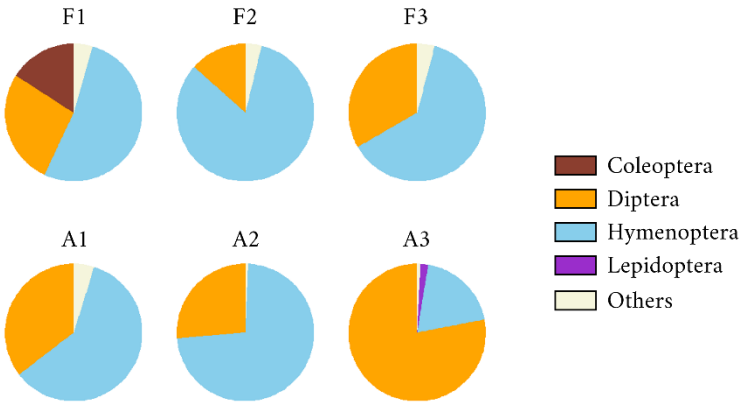

Figure 2. Relative frequencies of visits by different insect orders across the six natural populations (F: populations from forest habitats, A: populations from anthropogenic habitats).

3. Quality of pollination service

Fruit set was not affected by pollination treatment ($F_{1,135}$ = 0.36, p = 0.544), habitat type ($F_{1,135}$ = 1.55, p = 0.243), or their interaction ($F_{1,135}$ = 1.04, p = 0.303) (supplementary material – Pollination service quality). Similarly, seed set was unaffected by pollination treatment ($F_{1,391}$ = 3.64, p = 0.060), habitat type ($F_{1,391}$ = 0.21, p = 0.590), or their interaction ($F_{1,391}$ = 0.29, p = 0.594) (supplementary material – Pollination service quality).

4. Selection gradients

Females – Selection gradients were significantly positive in all populations for the number of gametes per flower, and in all populations but one for the number of open flowers (F3, see **Figure 3**). Significant positive selection was also observed on calyx height in populations A1 and A3. As revealed by the significant interaction between trait and habitat type, selection on the number of open flowers differed between habitat types and was stronger in the anthropogenic habitat than in the forest one ($\gamma_{habitat \times flowers}$: 0.270 ± 0.089, $p < 0.05$). No effect of habitat was detected for the selection exerted on other traits ($\gamma_{habitat \times gametes}$: 0.006 ± 0.083, p = 0.942; $\gamma_{habitat \times corolla}$: -0.044 ± 0.087, p = 0.611; $\gamma_{habitat \times calyx}$: 0.138 ± 0.085, p = 0.104). Including individual internal relatedness in the model did not alter these conclusions (supplementary material – Selection gradients with IR).

Males - Positive selection was observed on the number of open flowers in populations F3 and A2, on the number of gametes per flower in population F2, and on calyx height in population A3 (**Figure 3**). Selection on calyx height differed between habitat types, with stronger selection in the anthropogenic habitat than in the forest one ($\gamma_{habitat \times calyx}$: 0.283 ± 0.141, $p < 0.05$). No effect of habitat was detected for the selection exerted on other traits ($\gamma_{habitat \times flowers}$: -0.032 ± 0.119, p = 0.787; $\gamma_{habitat \times gametes}$: -0.075 ± 0.121, p = 0.533; $\gamma_{habitat \times corolla}$: -0.112 ± 0.142, p = 0.427). Including internal relatedness in the

analyses did not qualitatively change the patterns observed in selection gradients. However, internal relatedness had a significant negative effect on male reproductive success in one population (F2, supplementary material – Selection gradients with IR).

5. Relationship between traits, reproductive success and mating success

SEM allowed us to jointly estimate the effect of traits on reproductive and mating success, as well as the link between the two (i.e., proxy of the Bateman gradient), thereby disentangling direct and indirect effects of traits on reproductive success.

Females – Results obtained using SEM mirrored those obtained using the Lande & Arnold method in all populations (**Figure 4**, supplementary material – Structural equation modelling). A positive effect of the number of open flowers and the number of gametes per flower was detected in almost all populations, and a positive effect of calyx height was detected in populations A1 and A3. SEM analyses showed that these traits affected reproductive success in a direct way, not through an effect on mating success. Number of open flowers in population F1 and corolla width in populations F3 and A2 both had a positive effect on mating success. In these three cases, however, this positive effect on mating success did not translate into a positive effect on reproductive success, as there was no positive relationship between mating success and reproductive success. A negative relationship between the two was even found in populations F1 and A1.

Males – Overall, results obtained using SEM were largely congruent with those obtained using the Lande & Arnold method (**Figure 4**, supplementary material – Structural equation modelling). A positive, direct effect of number of open flowers and calyx height on reproductive success was detected in populations F3 and A3, respectively, while the previously observed positive effect of number of open flowers in population A2 appeared to be mainly indirect, as this trait had a positive effect on mating success, but no direct effect on reproductive success in this population. A higher number of flowers was also associated with a higher number of sexual partners in populations A3 and F3. In contrast to the pattern observed in females, in males, a positive relationship between mating success and reproductive success was detected in all but one population (F2), with no consistent differences in intensity between habitats.

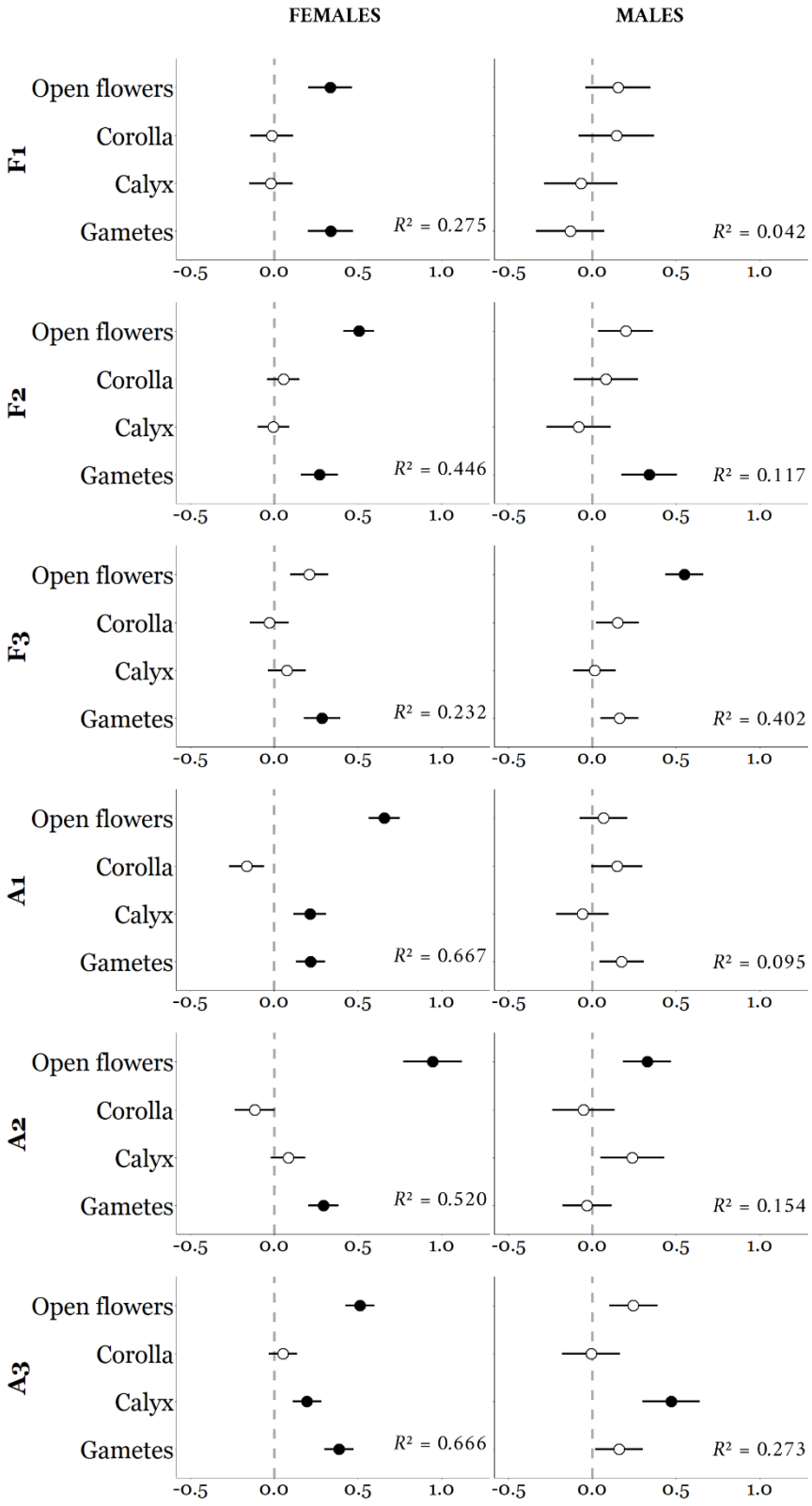

Figure 3. Selection gradients (± standard error) on floral traits for females (left panel) and males (right panel) in each natural population (F: populations from forest habitats; A: populations from anthropogenic habitats). Open flowers: number of open flowers; Corolla: corolla width; Calyx: calyx height; Gametes: number of gametes (ovules for females, pollen for males) per flower. Significant values are shown in black ($p < 0.05$).

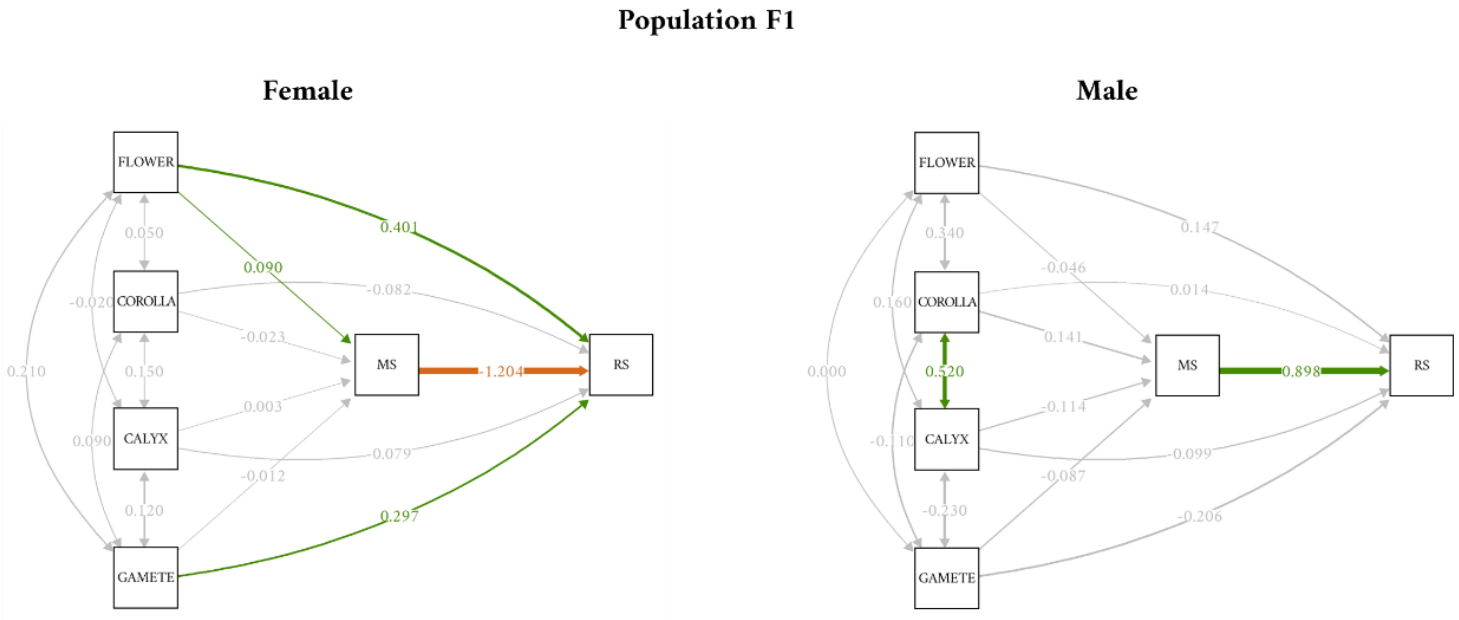

Figure 4. Path diagram of the structural equation model for population F1. Positives estimates are in green, negatives estimates are in orange, non-significant estimates in grey ($p > 0.05$). Line thickness represents the strength of the relationship between variables. Flower: number of open flowers; Corolla: corolla width; Calyx: calyx height; Gamete: number of ovules per flower; MS: mating success (i.e., number of sexual partners); RS: reproductive success (i.e., total seed production in females, total seeds sired in males).

DISCUSSION

In most angiosperms, access to sexual partners is largely mediated by pollinators, making floral traits key targets of selection. Sexual selection theory predicts stronger selection through the male function, yet environmental changes may alter this pattern. In particular, pollinator decline may increase intrasexual competition for mate acquisition and pollinator attraction, potentially leading to stronger selection on floral traits and steeper Bateman gradients in both sexes. In this study, we examined how habitat variation influences sex-specific selection on floral traits in the dioecious species *Silene dioica* by comparing populations located in forest and anthropogenic sites. Despite limited evidence for reduced pollinator activity and pollination service in anthropogenic sites, selection on a trait enhancing pollen transfer varied between habitats in males, whereas selection on female traits, primarily related to fertility, remained stable across sites.

Habitat affected pollinator visitation rates but not pollination service

Bumblebees and hoverflies were confirmed to be the main diurnal pollinators of *S. dioica* (Kay et al., 1984 ; Barbot et al., 2022), together accounting for 59-84% of floral visits across all sites. Although the relative frequencies of pollinator functional groups varied among populations, no systematic differences were observed between forest and anthropogenic habitats.

As expected, visitation rates were significantly higher in the forest habitat, with flowers receiving, on average, 1.4 times more visits than flowers from anthropogenic habitat, whereas total visit number did not differ between habitats. This suggests that pollinator density may not vary markedly between habitats, and that the reduced flower visitation rate observed in anthropogenic habitats may be due to variation in floral display size among plants rather than to differences in pollinator density *per se*. Indeed, plants in anthropogenic habitats had significantly larger floral displays, perhaps due to both greater light availability in more open environments (Kilkenny & Galloway, 2008) and to the diffusion of fertilisers in that habitat (e.g., Vaudo et al., 2022 in control condition).

Given the observed differences in visitation rates, we expected reduced pollination service quality in anthropogenic habitats—reflected by a stronger difference in reproductive success between open- and hand-pollinated plants (Ashman et al., 2004 ; Lázaro et al., 2015 ; Koski et al., 2018). In contrast to this prediction, we found no significant difference in the fruit set and seed set of open- and hand-pollinated plants in either habitat, suggesting that seed production in potted plants was not limited by pollen receipt. Since potted plants have access to fewer resources than wild individuals due to limited soil volume, we cannot extrapolate these results to naturally growing plants, which may experience greater pollen limitation. Nonetheless, our findings indicate that if differences in pollination service quality exist between habitats, they are not detectable in plants with small floral displays and are therefore likely to be modest.

In light of what is known about the effects of agriculture on pollinator communities (Ricketts et al., 2008 ; Winfree et al., 2009 ; Potts et al., 2010 ; Winfree et al., 2011 ; Woodard & Jha, 2017 ; Crispim et al., 2018) and on pollen limitation (Siopa et al., 2023), the results of the present study are surprising and suggest that common, generalist-pollinated plants may exhibit a certain resilience to pollinator decline. Moreover, the presence of diverse floral resources (botanical survey, pers. obs.), mixed landscape features (e.g., meadows, agricultural hedgerows, supplementary material – Habitat characterization), as well as the moderate distances to the forest (A1: 2.2 km, A2: 3.2 km, A3: 1.8 km, see **Figure 1**) in each of the three studied anthropogenic sites, may contribute to maintaining high pollinator densities despite the surrounding agricultural pressure (Winfree et al., 2011).

Selection on fertility-related traits in females

In females, the main selection patterns detected are consistent with our observation that variation in pollinator visitation rates between habitats is insufficient to substantially affect pollination service. Our analyses instead reveal selection acting on traits directly linked to fertility: number of ovules per flower (in all populations studied) and number of open flowers (in all but one population), both of which directly determine the number of seeds a female can produce. These selection patterns align with those previously found in experimental populations with no pollen limitation (Barbot et al., 2022 ; Barbot et al., 2023 ; Barbot et al., 2025), suggesting that under sufficient pollen receipt, female reproductive

success is largely determined by fertility traits. Interestingly, we detected a difference in the strength of selection on flower number between habitats, whereby selection was significantly stronger in anthropogenic populations. Although flower number is known to be a major determinant of pollinator attraction in *S. dioica* (Barbot et al., 2022 ; Moquet et al., 2022) and other species (Harder & Barrett, 1995 ; Ohashi & Yahara, 1998 ; Bauer et al., 2017), we argue that the documented difference in visitation rates between habitats is unlikely to be the source of strengthened selection on flower number in anthropogenic sites. First, as noted above, the quality of pollination service did not differ between habitats, suggesting that the reduced visitation rate was not sufficient to impact female reproductive output in potted plants. Second, SEM analyses showed that the effect of flower number on reproductive success was predominantly direct, that is not mediated by enhanced access to mates. Moreover, in cases where flower number apparently facilitated mate acquisition, we did not detect any positive association between mating success and reproductive success. We therefore argue that the stronger selection observed in the anthropogenic habitat does not result from reduced pollinator visitation, but from differences in trait distribution between habitats. Specifically, the number of open flowers exhibited higher variance in anthropogenic than in forest habitats (supplementary material – Floral trait variation). Because standardized selection gradients are calculated on variance-standardized traits (Lande & Arnold, 1983), enabling meaningful comparisons of selection strength across traits and studies, differences in trait variance between habitats can influence the estimated strength of selection even when the underlying fitness function remains unchanged (De Lisle & Svensson, 2017).

Several hypotheses may explain why the number of open flowers is more variable in anthropogenic habitat than in forest one. Earlier in this discussion, the higher mean value of this variable in anthropogenic habitat was attributed to greater light availability (Kilkenny & Galloway, 2008) and to diffusion of fertilizer in this habitat (Vaudo et al., 2022). However, these factors may vary locally, for instance due to the presence of hedgerows or proximity to crop fields. In addition, agricultural disturbances such as machinery traffic, mowing or trampling can artificially lead to smaller plants along field margins. Abiotic factors therefore play an important role in shaping selection patterns on floral traits (Caruso et al., 2019). For example, Wu et al. (2023b) showed, in a nutrient manipulation experiment, that the strength of selection varied across treatments for both the flower number and size.

Our results therefore suggest an absence of competition for mate acquisition in females, as indicated by the lack of a relationship between mating success and reproductive success in most populations. Unexpectedly, we even detected a negative effect of mating success on reproductive success in two populations, implying that females producing more seeds also exhibited lower paternal diversity. Such a negative relationship may stem from variation in the intensity of post-pollination processes among females, due to variation in either pollen receipt or resource availability. Specifically, high pollen receipt may simultaneously promote pollen competition and increase seed production, potentially biasing paternity toward the most competitive males (Christopher et al., 2020). Likewise, high-quality females,

or females with better access to resources, may both produce more seeds and exhibit greater potential for female choice (Bertin, 1985 ; Marshall & Ellstrand, 1988 ; Marshall & Diggle, 2001 ; Marshall & Oliveras, 2001). Clearly, more studies are needed to disentangle the respective roles of pollinator visitation patterns and post-pollination processes in shaping the relationship between mating success and reproductive success.

Mate acquisition is the strongest predictor of male reproductive success

Because females typically produce a limited number of gametes, even a relatively low number of pollinator visits may be sufficient to maximize female reproductive success. In contrast, males produce more flowers and more gametes per flower (Kodric-Brown & Brown, 1987 ; Eckhart, 1999), including in *S. dioica* (Kay et al., 1984 ; Barbot et al., 2023 ; this study), so that male reproductive success is expected to saturate for much higher visitation rates. Such a difference in the factors potentially limiting male and female reproductive success may affect selection patterns. Specifically, we expect selection in males to primarily target traits enhancing mate acquisition rather than fertility-related traits.

In this study, we found a positive and significant relationship between mating success and reproductive success (i.e., positive Bateman gradient) in five populations out of six, indicating that siring success increases with mate acquisition, in line with theoretical expectations (Bateman, 1948) and previous findings (Johnson & Shaw, 2016 ; Lavaut et al., 2023 ; Tonnabel et al., 2019 ; Barbot et al., 2022 ; Kwok & Dorken, 2022). Moreover, unlike in females, selection in males did not systematically act on fertility-related traits. We detected positive selection on gamete number in one population only (vs. six populations in females), and positive selection on flower number in two (vs. five in females). Altogether, these results underscore the high potential for sexual selection in males.

Yet, the traits included in this study proved to be poor predictors of male mating success. Specifically, the only trait found to be under sexual selection was flower number, which was previously shown to enhance mate acquisition in *S. dioica*. However, such an effect was not attributed to increased pollinator attraction but rather to altered pollinator movements and pollen export dynamics (Barbot et al., 2022). Moreover, no correlation was found between corolla width—a trait known to be involved in pollinator attraction (Barbot et al., 2025)—and male mating success across the six sampled populations. These results suggest that differential pollinator attraction may not be the primary source of variation in male mating success, and consequently not the main driver of variation in siring success in *S. dioica*. Instead, variation in siring success may depend on differential performances at subsequent stages of the male fitness pathway. In line with this hypothesis, we detected positive selection on calyx height in one population, a trait promoting efficient pollen removal in other species (Kuriya et al., 2015). Interestingly, selection on this trait differed between habitats, being stronger in the anthropogenic than in the forest one. Because the distribution of this trait was the same in the two habitat types, we suspect that such a difference result from variation in the selective environment experienced by plants in each type of sites.

Specifically, reduced pollinator visitation in anthropogenic sites may intensify male–male competition for pollinator service (e.g., access to space on pollinators, Moir & Anderson, 2023 ; Santana et al., 2025), and increase selection on traits promoting pollen export.

Despite the inclusion of calyx height, the explanatory power of the male traits considered in this study remained limited, as reflected by the markedly lower R^2 values for selection models run on males compared to females. While such low R^2 values could partly result from more precise estimations of reproductive success in females than in males (Snow & Lewis, 1993), they may also indicate that we did not consider the male traits most important for shaping variation in siring success, particularly traits affecting pollen competition (e.g., pollen size, McCallum & Chang, 2016 ; Tonnabel et al., 2021). In *S. dioica*, pollinators can transport substantial amounts of pollen (Joffard et al., 2025), and stigmas in natural populations often receive pollen loads that are significantly larger than needed to fertilize all ovules (Jolivel et al., in prep.a), suggesting ample opportunities for post-pollination processes to occur (Christopher et al., 2020 ; Tonnabel et al., 2021). In line with this hypothesis, including internal relatedness improved model fit in males—but not females—and revealed a significant effect of relatedness on siring success in one population, but no effect on the traits considered in this study. This suggests that inbreeding depression affects other traits, perhaps associated with higher competitiveness at the post-pollination stage (Losdat et al., 2014). Overall, our results indicate a strong potential for sexual selection in males, yet this selection does not primarily target the attractive traits included in this study. Because pollination service was generally high across our study populations, including in anthropogenic sites, male-male competition may instead be expressed at later stages of the male fitness pathway.

CONCLUSION

In the context of ongoing pollinator decline and habitat degradation, understanding how floral traits evolve under varying pollination environments is crucial. By combining field observations, experimental assessment of pollination service quality and selection gradient estimations across contrasting habitats, our study revealed that the effect of habitat on selection on floral traits differed between the sexes. While female reproductive success was governed by fertility-related traits across all study populations, males showed a consistent positive relationship between mating success and reproductive success, indicating strong potential for sexual selection, as well as more variable selection patterns, with differential selection on one trait likely related to pollen removal efficiency between habitats. These findings emphasize the need to consider both sexual functions and local pollination environments to fully understand how selection shapes floral trait evolution.

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REFERENCES

Amos, W., Worthington Wilmer, J., Fullard, K., Burg, T. M., Croxall, J. P., Bloch, D., & Coulson, T. (2001). The influence of parental relatedness on reproductive success. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 268(1480), 2021-2027. https://doi.org/10.1098/rspb.2001.1751

Ashman, T.-L., Knight, T. M., Steets, J. A., Amarasekare, P., Burd, M., Campbell, D. R., Dudash, M. R., Johnston, M. O., Mazer, S. J., Mitchell, R. J., Morgan, M. T., & Wilson, W. G. (2004). Pollen limitation of plant reproduction : Ecological and evolutionary causes and consequences. *Ecology*, 85(9), 2408-2421. https://doi.org/10.1890/03-8024

Barbot, E., Dufaÿ, M., & De Cauwer, I. (2023). Sex-specific selection patterns in a dioecious insect-pollinated plant. *Evolution*, 77(7), 1578-1590. https://doi.org/10.1093/evolut/qpad069

Barbot, E., Dufaÿ, M., Godé, C., & De Cauwer, I. (2025). Investigating the effects of diurnal and nocturnal pollinators on male and female reproductive success and on floral trait selection in *Silene dioica*. *Peer Community Journal*, 5, e11. https://doi.org/10.24072/pcjournal.508

Barbot, E., Dufaÿ, M., Tonnabel, J., Godé, C., & De Cauwer, I. (2022). On the function of flower number : Disentangling fertility from pollinator-mediated selection. *Proceedings of the Royal Society B: Biological Sciences*, 289(1987), 20221987. https://doi.org/10.1098/rspb.2022.1987

Bartkowska, M. P., & Johnston, M. O. (2015). Pollen limitation and its influence on natural selection through seed set. *Journal of Evolutionary Biology*, 28(11), 2097-2105. https://doi.org/10.1111/jeb.12741

Bateman, A. J. (1948). Intra-sexual selection in *Drosophila*. *Heredity*, 2(3), 349-368. https://doi.org/10.1038/hdy.1948.21

Bauer, A. A., Clayton, M. K., & Brunet, J. (2017). Floral traits influencing plant attractiveness to three bee species : Consequences for plant reproductive success. *American Journal of Botany*, 104(5), 772-781. https://doi.org/10.3732/ajb.1600405

Bennett, J. M., Steets, J. A., Burns, J. H., Durka, W., Vamosi, J. C., Arceo-Gómez, G., Burd, M., Burkle, L. A., Ellis, A. G., Freitas, L., Li, J., Rodger, J. G., Wolowski, M., Xia, J., Ashman, T.-L., & Knight, T. M. (2018). GloPL, a global data base on pollen limitation of plant reproduction. *Scientific Data*, 5(1), 180249. https://doi.org/10.1038/sdata.2018.249

Bertin, R. I. (1985). Nonrandom fruit production in *Campsis radicans* : Between-year consistency and effects of prior pollination. *The American Naturalist*, 126(6), 750-759. https://doi.org/10.1086/284451

Brown, K. S., & Caruso, C. M. (2023). The effect of experimental pollinator decline on pollinator-mediated selection on floral traits. *Ecology and Evolution*, 13(11), e10706. https://doi.org/10.1002/ece3.10706

Burd, M. (1994). Bateman's principle and plant reproduction : The role of pollen limitation in fruit and seed set. *The Botanical Review*, 60(1), 83-139. https://doi.org/10.1007/BF02856594

Caruso, C. M., Eisen, K. E., Martin, R. A., & Sletvold, N. (2019). A meta-analysis of the agents of selection on floral traits. *Evolution*, 73(1), 4-14. https://doi.org/10.1111/evo.13639

Cha, Y.-P., Zhang, J., Ma, Y.-M., Tong, Z.-L., Wu, Y., Luo, L., & Li, Q.-J. (2023). Variations in pollinator-mediated selection of floral traits across flowering times. *Journal of Plant Ecology*, 16(6), rtad024. https://doi.org/10.1093/jpe/rtad024

Chapurlat, E., Ågren, J., & Sletvold, N. (2015). Spatial variation in pollinator-mediated selection on phenology, floral display and spur length in the orchid *Gymnadenia conopsea*. *New Phytologist*, 208(4), 1264-1275. https://doi.org/10.1111/nph.13555

Christopher, D. A., Mitchell, R. J., & Karron, J. D. (2020). Pollination intensity and paternity in flowering plants. *Annals of Botany*, 125(1), 1-9. https://doi.org/10.1093/aob/mcz159

Crispim, B., Bajay, M., de Vasconcelos, A., Deo, T., Braga, R., Telles, M., Vieira, M., Carnevali, T., Solórzano, J., & Grisolia, A. (2018). Relationship between genetic variability and land use and land cover in populations of *Campomanesia adamantium* (Myrtaceae). *Diversity*, 10(4), 106. https://doi.org/10.3390/d10040106

Dai, C., & Galloway, L. F. (2013). Sexual selection in a hermaphroditic plant through female reproductive success. *Journal of Evolutionary Biology*, 26(12), 2622-2632. https://doi.org/10.1111/jeb.12254

De Jager, M. L., & Peakall, R. (2019). Experimental examination of pollinator-mediated selection in a sexually deceptive orchid. *Annals of Botany*, 123(2), 347-354. https://doi.org/10.1093/aob/mcy083

De Lisle, S. P., & Svensson, E. I. (2017). On the standardization of fitness and traits in comparative studies of phenotypic selection. *Evolution*, 71(10), 2313-2326. https://doi.org/10.1111/evo.13325

Dufaÿ, M., Vaudey, V., De Cauwer, I., Touzet, P., Cuguen, J., & Arnaud, J. -F. (2008). Variation in pollen production and pollen viability in natural populations of gynodioecious *Beta vulgaris* ssp. *maritima* : Evidence for a cost of restoration of male function? *Journal of Evolutionary Biology*, 21(1), 202-212. https://doi.org/10.1111/j.1420-9101.2007.01454.x

Eckhart, V. M. (1999). Sexual dimorphism in flowers and inflorescences. In *Gender and sexual dimorphism in flowering plants*. Springer Berlin Heidelberg, Berlin.

Emel, S. L., Franks, S. J., & Spigler, R. B. (2017). Phenotypic selection varies with pollination intensity across populations of *Sabatia angularis*. *New Phytologist*, 215(2), 813-824. https://doi.org/10.1111/nph.14608

Fenster, C. B., Armbruster, W. S., Wilson, P., Dudash, M. R., & Thomson, J. D. (2004). Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics*, 35(1), 375-403. https://doi.org/10.1146/annurev.ecolsys.34.011802.132347

Giles, B. E., Lundqvist, E., & Goudet, J. M. (1998). Restricted gene flow and subpopulation differentiation in *Silene dioica*. *Heredity*, 80(6), 715-723. https://doi.org/10.1046/j.1365-2540.1998.00348.x

Giurfa, M., Nunez, J., Chittka, L., & Menzel, R. (1995). Colour preferences of flower-naive honeybees. *Journal of Comparative Physiology A*, 177(3), 247-259. https://doi.org/10.1007/BF00192415

Gleiser, G., Internicola, A. I., Austerlitz, F., & Bernasconi, G. (2014). Stabilizing selection on nectar concentration in wild *Petunia axillaris*, as revealed by genetic analysis of pollen dispersal. *Evolutionary Ecology*, 28(5), 869-884. https://doi.org/10.1007/s10682-014-9714-y

Harder, L. D., & Barrett, S. C. H. (1995). Mating cost of large floral displays in hermaphrodite plants. *Nature*, 373(6514), 512-515. https://doi.org/10.1038/373512a0

Harder, L. D., & Johnson, S. D. (2009). Darwin's beautiful contrivances : Evolutionary and functional evidence for floral adaptation. *New Phytologist*, 183(3), 530-545. https://doi.org/10.1111/j.1469-8137.2009.02914.x

Hodgins, K. A., & Barrett, S. C. H. (2008). Natural selection on floral traits through male and female function in wild populations of the heterostylous daffodil *Narcissus triandrus*. *Evolution*, 62(7), 1751-1763. https://doi.org/10.1111/j.1558-5646.2008.00404.x

Ingvarsson, P. K., & Giles, B. E. (1999). Kin-structured colonization and small-scale genetic differentiation in *Silene dioica*. *Evolution*, 53(2), 605-611. https://doi.org/10.1111/j.1558-5646.1999.tb03795.x

Janicke, T., Häderer, I. K., Lajeunesse, M. J., & Anthes, N. (2016). Darwinian sex roles confirmed across the animal kingdom. *Science Advances*, 2(2), e1500983. https://doi.org/10.1126/sciadv.1500983

Joffard, N., Lecat, I., Ollier, K., Jolivel, C., Schmitt, E., Godé, C., Barbot, E., Dufaÿ, M., & De Cauwer, I. (2025). Floral display size does not affect the distribution of paternal diversity in *Silene dioica*. *bioRxiv*. https://doi.org/10.1101/2025.07.19.665651

Johnson, M. G., & Shaw, A. J. (2016). The effects of quantitative fecundity in the haploid stage on reproductive success and diploid fitness in the aquatic peat moss *Sphagnum macrophyllum*. *Heredity*, 116(6), 523-530. https://doi.org/10.1038/hdy.2016.13

Jolivel, C., Barbot, E., Schmitt, E., Godé, C., Dufaÿ, M., Joffard, N., & De Cauwer, I. (in prep.a). From pollination to paternity: determinants and effect of pollen load on fertilization success.

Jolivel, C., Arnaud, J., Barbot, E., Godé, C., Joffard, N., & De Cauwer, I. (in prep.b). Population genetic structure in the red campion (*Silene dioica*): effects of landscape components.

Kalinowski, S. T., Taper, M. L., & Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, 16(5), 1099-1106. https://doi.org/10.1111/j.1365-294X.2007.03089.x

Kay, Q. O. N., Lack, A. J., Bamber, F. C., & Davies, C. R. (1984). Differences between sexes in floral morphology, nectar production and insect visits in a dioecious species, *Silene dioica*. *New Phytologist*, 98(3), 515-529. https://doi.org/10.1111/j.1469-8137.1984.tb04145.x

Kilkenny, F. F., & Galloway, L. F. (2008). Reproductive success in varying light environments : Direct and indirect effects of light on plants and pollinators. *Oecologia*, 155(2), 247-255. https://doi.org/10.1007/s00442-007-0903-z

Kline, R. B. (2011). *Principles and practice of structural equation modeling*. NY: Guilford Publications.

Knight, T. M., Steets, J. A., Vamosi, J. C., Mazer, S. J., Burd, M., Campbell, D. R., Dudash, M. R., Johnston, M. O., Mitchell, R. J., & Ashman, T.-L. (2005). Pollen limitation of plant reproduction : Pattern and process. *Annual Review of Ecology, Evolution, and Systematics*, 36(1), 467-497. https://doi.org/10.1146/annurev.ecolsys.36.102403.115320

Kodric-Brown, A., & Brown, J. H. (1987). Anisogamy, sexual selection, and the evolution and maintenance of sex. *Evolutionary Ecology*, 1(2), 95-105. https://doi.org/10.1007/BF02067393

Koski, M. H., Ison, J. L., Padilla, A., Pham, A. Q., & Galloway, L. F. (2018). Linking pollinator efficiency to patterns of pollen limitation : Small bees exploit the plant–pollinator mutualism. *Proceedings of the Royal Society B*: *Biological Sciences*, 285(1880), 20180635. https://doi.org/10.1098/rspb.2018.0635

Kuriya, S., Hattori, M., Nagano, Y., & Itino, T. (2015). Altitudinal flower size variation correlates with local pollinator size in a bumblebee-pollinated herb, *Prunella vulgaris* L. (Lamiaceae). *Journal of Evolutionary Biology*, 28(10), 1761-1769. https://doi.org/10.1111/jeb.12693

Kwok, A., & Dorken, M. E. (2022). Sexual selection on male but not female function in monoecious and dioecious populations of broadleaf arrowhead (*Sagittaria latifolia*). *Proceedings of the Royal Society B*: *Biological Sciences*, 289(1986), 20220919. https://doi.org/10.1098/rspb.2022.0919

Lande, R., & Arnold, S. (1983). The measurement of selection on correlated characters. *Evolution*, 37, 1210-1226. http://dx.doi.org/10.2307/2408842

Lavaut, E., Valero, M., Mauger, S., Guillemin, M. L., Destombe, C., & Dufaÿ, M. (2023). Sexual selection in seaweed? Testing Bateman's principles in the red alga *Gracilaria gracilis*. *Proceedings of the Royal Society B*: *Biological Sciences*, 290(2006), 20231668. https://doi.org/10.1098/rspb.2023.1668

Lázaro, A., Lundgren, R., & Totland, Ø. (2015). Pollen limitation, species' floral traits and pollinator visitation : Different relationships in contrasting communities. *Oikos*, 124(2), 174-186. https://doi.org/10.1111/oik.01525

Losdat, S., Chang, S. -M., & Reid, J. M. (2014). Inbreeding depression in male gametic performance. *Journal of Evolutionary Biology*, 27(6), 992-1011. https://doi.org/10.1111/jeb.12403

Marshall, D. L., & Diggle, P. K. (2001). Mechanisms of differential pollen donor performance in wild radish, *Raphanus sativus* (Brassicaceae). *American Journal of Botany*, 88(2), 242-257. https://doi.org/10.2307/2657015

Marshall, D. L., & Ellstrand, N. C. (1988). Effective mate choice in wild radish : Evidence for selective seed abortion and its mechanism. *The American Naturalist*, 131(5), 739-756. https://doi.org/10.1086/284816

Marshall, D. L., & Oliveras, D. M. (2001). Does differential seed siring success change over time or with pollination history in wild radish, *Raphanus sativus* (Brassicaceae)? *American Journal of Botany*, 88(12), 2232-2242. https://doi.org/10.2307/3558385

Marshall, T. C., Slate, J., Kruuk, L. E. B., & Pemberton, J. M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, 7(5), 639-655. https://doi.org/10.1046/j.1365-294x.1998.00374.x

McCallum, B., & Chang, S. (2016). Pollen competition in style : Effects of pollen size on siring success in the hermaphroditic common morning glory, *Ipomoea purpurea*. *American Journal of Botany*, 103(3), 460-470. https://doi.org/10.3732/ajb.1500211

Moir, M., & Anderson, B. (2023). Pollen layering and male–male competition : Quantum dots demonstrate that pollen grains compete for space on pollinators. *American Journal of Botany*, 110(6), e16184. https://doi.org/10.1002/ajb2.16184

Moore, J. C., & Pannell, J. R. (2011). Sexual selection in plants. *Current Biology*, 21(5), R176-R182. https://doi.org/10.1016/j.cub.2010.12.035

Moquet, L., Jacquemart, A.-L., Dufaÿ, M., & De Cauwer, I. (2022). Effects of sexual dimorphism on pollinator behaviour in a dioecious species. *Oikos*, 2022(3), e08662. https://doi.org/10.1111/oik.08662

Moquet, L., Lateur, L., Jacquemart, A.-L., De Cauwer, I., & Dufaÿ, M. (2020). Temporal dynamics of sexual dimorphism in a dioecious species. *Annals of Botany*, 126(3), 471-480. https://doi.org/10.1093/aob/mcaa088

Newman, E. L., Ellis, A., & Anderson, B. (2025). Pollinator and flower morphology interact to influence pollen receipt. *bioRxiv*, 2025-05. http://dx.doi.org/10.1101/2025.05.16.654420

Oddou-Muratorio, S., Gauzere, J., Bontemps, A., Rey, J.-F., & Klein, E. K. (2018). Tree, sex and size : Ecological determinants of male vs. Female fecundity in three *Fagus sylvatica* stands. *Molecular Ecology*, 27(15), 3131-3145. https://doi.org/10.1111/mec.14770

Ohashi, K., & Yahara, T. (1998). Effects of variation in flower number on pollinator visits in *Cirsium purpuratum* (Asteraceae). *American Journal of Botany*, 85(2), 219-224. https://doi.org/10.2307/2446309

Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, 120(3), 321-326. https://doi.org/10.1111/j.1600-0706.2010.18644.x

Osborne, J. L., Martin, A. P., Carreck, N. L., Swain, J. L., Knight, M. E., Goulson, D., Hale, R. J., & Sanderson, R. A. (2008). Bumblebee flight distances in relation to the forage landscape. *Journal of Animal Ecology*, 77(2), 406-415. https://doi.org/10.1111/j.1365-2656.2007.01333.x

Pau, G., Fuchs, F., Sklyar, O., Boutros, M., & Huber, W. (2010). EBImage—An R package for image processing with applications to cellular phenotypes. *Bioinformatics*, 26(7), 979-981. https://doi.org/10.1093/bioinformatics/btq046

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010). Global pollinator declines : Trends, impacts and drivers. *Trends in Ecology & Evolution*, 25(6), 345-353. https://doi.org/10.1016/j.tree.2010.01.007

Quinn, G. P., & Keough, M. J. (2002). Experimental design and data analysis for biologists. *Cambridge University Press*.

Ricketts, T. H., Regetz, J., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., Bogdanski, A., Gemmill-Herren, B., Greenleaf, S. S., Klein, A. M., Mayfield, M. M., Morandin, L. A., Ochieng', A., & Viana, B. F. (2008). Landscape effects on crop pollination services : Are there general patterns? *Ecology Letters*, 11(5), 499-515. https://doi.org/10.1111/j.1461-0248.2008.01157.x

Santana, P. C., Mulvaney, J., Santana, E. M., Moir, M., & Anderson, B. (2025). Competition for pollen deposition space on pollinators generates last-male advantage. *Functional Ecology*, 39(2), 555-566. https://doi.org/10.1111/1365-2435.14736

Schiestl, F. P., & Johnson, S. D. (2013). Pollinator-mediated evolution of floral signals. *Trends in Ecology & Evolution*, 28(5), 307-315. https://doi.org/10.1016/j.tree.2013.01.019

Siopa, C., Castro, H., Loureiro, J., & Castro, S. (2023). PolLimCrop, a global dataset of pollen limitation in crops. *Scientific Data*, 10(1), 905. https://doi.org/10.1038/s41597-023-02797-6

Sletvold, N., & Ågren, J. (2016). Experimental reduction in interaction intensity strongly affects biotic selection. *Ecology*, 97(11), 3091-3098. https://doi.org/10.1002/ecy.1554

Snow, A. A., & Lewis, P. O. (1993). Reproductive traits and male fertility in plants : Empirical approaches. *Annual Review of Ecology and Systematics*, 24(1), 331-351. https://doi.org/10.1146/annurev.es.24.110193.001555

Stephenson, A. G., & Bertin, R. I. (1983). Male competition, female choice and sexual selection in plants. In *Pollination biology*. Academic Press, Orlando.

Tonnabel, J., David, P., Janicke, T., Lehner, A., Mollet, J.-C., Pannell, J. R., & Dufaÿ, M. (2021). The scope for postmating sexual selection in plants. *Trends in Ecology & Evolution*, 36(6), 556-567. https://doi.org/10.1016/j.tree.2021.02.013

Tonnabel, J., David, P., Klein, E. K., & Pannell, J. R. (2019). Sex-specific selection on plant architecture through "budget" and "direct" effects in experimental populations of the wind-pollinated herb, *Mercurialis annua*. *Evolution*, 73(5), 897-912. https://doi.org/10.1111/evo.13714

Totland, Ø. (2001). Environment-dependent pollen limitation and selection on floral traits in an alpine species. *Ecology*, 82(8), 2233-2244. http://dx.doi.org/10.1890/0012-9658(2001)082[2233:EDPLAS]2.0.CO;2

Trunschke, J., Sletvold, N., & Ågren, J. (2020). Manipulation of trait expression and pollination regime reveals the adaptive significance of spur length. *Evolution*, 74(3), 597-609. https://doi.org/10.1111/evo.13927

Vaudo, A. D., Erickson, E., Patch, H. M., Grozinger, C. M., & Mu, J. (2022). Impacts of soil nutrition on floral traits, pollinator attraction, and fitness in cucumbers (*Cucumis sativus* L.). *Scientific Reports*, 12(1), 21802. https://doi.org/10.1038/s41598-022-26164-4

Willis, J. H. (1996). Measures of phenotypic selection are biased by partial inbreeding. *Evolution*, 50(4), 1501-1511. https://doi.org/10.1111/j.1558-5646.1996.tb03923.x

Winfree, R., Aguilar, R., Vázquez, D. P., LeBuhn, G., & Aizen, M. A. (2009). A meta-analysis of bees' responses to anthropogenic disturbance. *Ecology*, 90(8), 2068-2076. https://doi.org/10.1890/08-1245.1

Winfree, R., Bartomeus, I., & Cariveau, D. P. (2011). Native pollinators in anthropogenic habitats. *Annual Review of Ecology, Evolution, and Systematics*, 42(1), 1-22. https://doi.org/10.1146/annurev-ecolsys-102710-145042

Woodard, S. H., & Jha, S. (2017). Wild bee nutritional ecology : Predicting pollinator population dynamics, movement, and services from floral resources. *Current Opinion in Insect Science*, 21, 83-90. https://doi.org/10.1016/j.cois.2017.05.011

Wu, Y., Duan, X., Shu, B., & Li, Q. (2023a). Pollination intensity and nutrient availability interactively shape directional selection on flower size in an orchid herb. *Evolutionary Ecology*, 37(4), 721-734. https://doi.org/10.1007/s10682-023-10242-8

Wu, Y., Liu, G., Sletvold, N., Duan, X., Tong, Z., & Li, Q. (2023b). Soil water and nutrient availability interactively modify pollinator-mediated directional and correlational selection on floral display. *New Phytologist*, 237(2), 672-683. https://doi.org/10.1111/nph.18537

Zhou, J., Reynolds, R. J., Zimmer, E. A., Dudash, M. R., & Fenster, C. B. (2020). Variable and sexually conflicting selection on *Silene stellata* floral traits by a putative moth pollinator selective agent. *Evolution*, 74(7), 1321-1334. https://doi.org/10.1111/evo.13965

SUPPLEMENTARY MATERIAL

1. Habitat characterization

Text S1. Populations of *S. dioica* containing at least 100 plants were identified in the study region, in and around the Mormal State forest, a large forest area located in northern France (50°12'24.76"N, 3°44'14.84"E). The land use in the area surrounding each population (one-kilometer radius) was characterized and quantified using the QGIS software (version 3.30.0) and the GroupStats plugin (version 2.2.7), using cartographic data processed by CNES for the Theia data center (www.theia.land.fr) from Copernicus satellite imagery. The one-kilometre radius was chosen because it aligns with the typical foraging range of bumblebees (Emel et al., 2021), which are the most common *S. dioica* pollinators. Habitat types are detailed in the supplementary material of the manuscript A, section Habitat characterization and were grouped from different subcategories to calculate the percentage of each habitat type around the six populations (Table S1).

Table S1. Percentage of each habitat type within a one kilometer radius around the center of each of the six studied populations. Populations whose names start with "F" are located in largely forested areas, while populations starting with "A" are found in more anthropogenic areas.

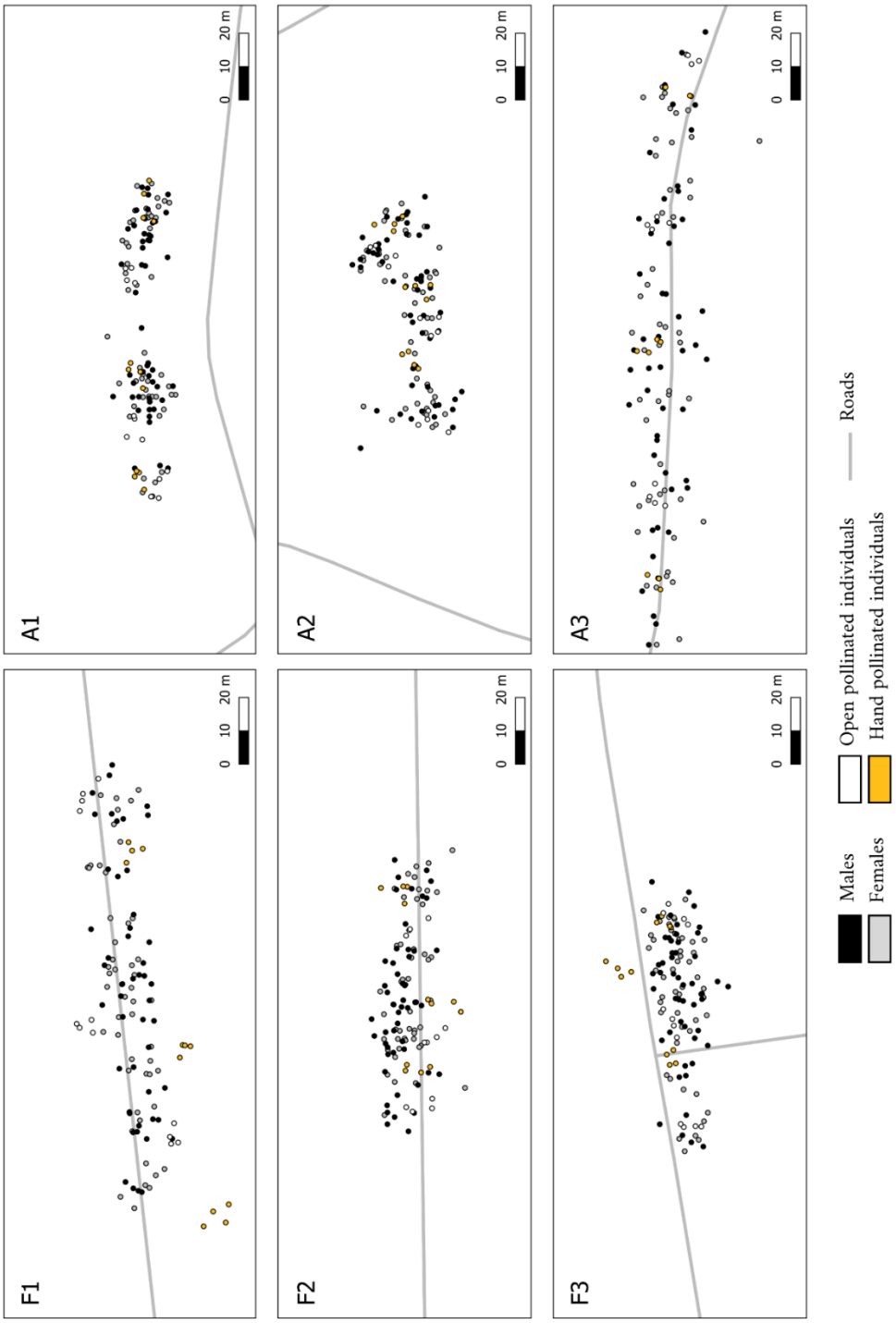
Population	Forest	Semi-natural	Crop	Urban	Aquatic
F1	0.976	0.023	0	0.002	0
F2	0.799	0.184	0	0.016	0
F3	0.772	0.175	0.041	0.012	0
A1	0.072	0.443	0.372	0.113	0.001
A2	0.151	0.311	0.412	0.126	0
A3	0.086	0.474	0.312	0.122	0.005

Reference

Emel, S. L., Wang, S., Metz, R. P., & Spigler, R. B. (2021). Type and intensity of surrounding human land use, not local environment, shape genetic structure of a native grassland plant. Molecular Ecology, 30(3), 639-655. https://doi.org/10.1111/mec.15753

2. Spatial configuration

Figure S1. Spatial distribution of each individual, in each population (F: populations from forest habitats, A: populations from anthropogenic habitats). In the figure, males from natural populations are shown in black and females in grey. Individuals positioned in each population to assess pollination service quality are indicated in yellow (hand-pollinated) and white (open-pollinated).



3. Floral trait variation

Table S2. Trait mean ± SD for each sex and habitat. Flowers: number of open flowers; Corolla: average corolla width (mm); Calyx: average calyx height (mm); Ovules: number of ovules per flower; Pollen: number of pollen grains per flower; Fruit set: number of fruits divided by the number of flowers, Seed set: number of seeds divided by the number of ovules per fruit, average in three digitized fruits, Seed: number of seed produced.

Traits	**Forest**		**Anthropogenic**		F_{sex}	p_{sex}	$F_{hab.}$	$p_{hab.}$	$F_{sex*hab.}$	$p_{sex*hab.}$
	Females	**Males**	**Females**	**Males**						
Flowers	2.63 ± 2.28	16.05 ± 15.93	6.04 ± 5.40	41.81 ± 47.04	674.57	**< 0.001**	11.83	**< 0.001**	1.29	0.257
Corolla	17.53 ± 2.94	20.61 ± 3.05	19.13 ± 3.19	22.78 ± 3.29	228.08	**< 0.001**	1.40	0.236	1.59	0.207
Calyx	12.78 ± 1.30	14.84 ± 1.17	13.08 ± 1.30	15.57 ± 1.71	470.16	**< 0.001**	0.83	0.361	4.30	**< 0.05**
Ovules	256.86 ± 65.96	-	275.94 ± 68.70	-	-	-	2.51	0.113	-	-
Pollen	-	70,426.06 ± 18,032.02	-	87,162.95 ± 55,031.75	-	-	1.03	0.301	-	-
Fruit	0.966 ± 0.080	-	0.980 ± 0.056	-	-	-	0.63	0.429	-	-
Seed set	0.563 ± 0.219	-	0.598 ± 0.209	-	-	-	2.35	0.125	-	-
Seed	2,376.29 ± 2,061.87	-	4,984.21 ± 4,984.18	-	-	-	3.52	0.060	-	-

Table S3. Trait variance for each sex and population. Flowers: number of open flowers; Corolla: average corolla width (mm); Calyx: average calyx height (mm); Ovules: number of ovules per flower; Pollen: number of pollen grains per flower.

	Population	Flowers	Corolla	Calyx	Gametes
Females	F1	2.91	6.57	1.59	2,748.61
	F2	8.16	7.41	1.28	4,350.01
	F3	4.09	8.27	1.22	6,290.66
	A1	31.07	8.50	1.96	3,737.99
	A2	33.56	5.51	1.62	4,379.84
	A3	7.93	4.74	1.34	6,492.11
Males	F1	77.09	5.28	1.59	5×10^8
	F2	407.59	6.76	0.90	3×10^8
	F3	212.78	9.21	1.45	2×10^8
	A1	1,077.57	7.60	1.48	6×10^9
	A2	4,044.50	7.48	1.16	4×10^8
	A3	934.22	5.61	2.07	2×10^8

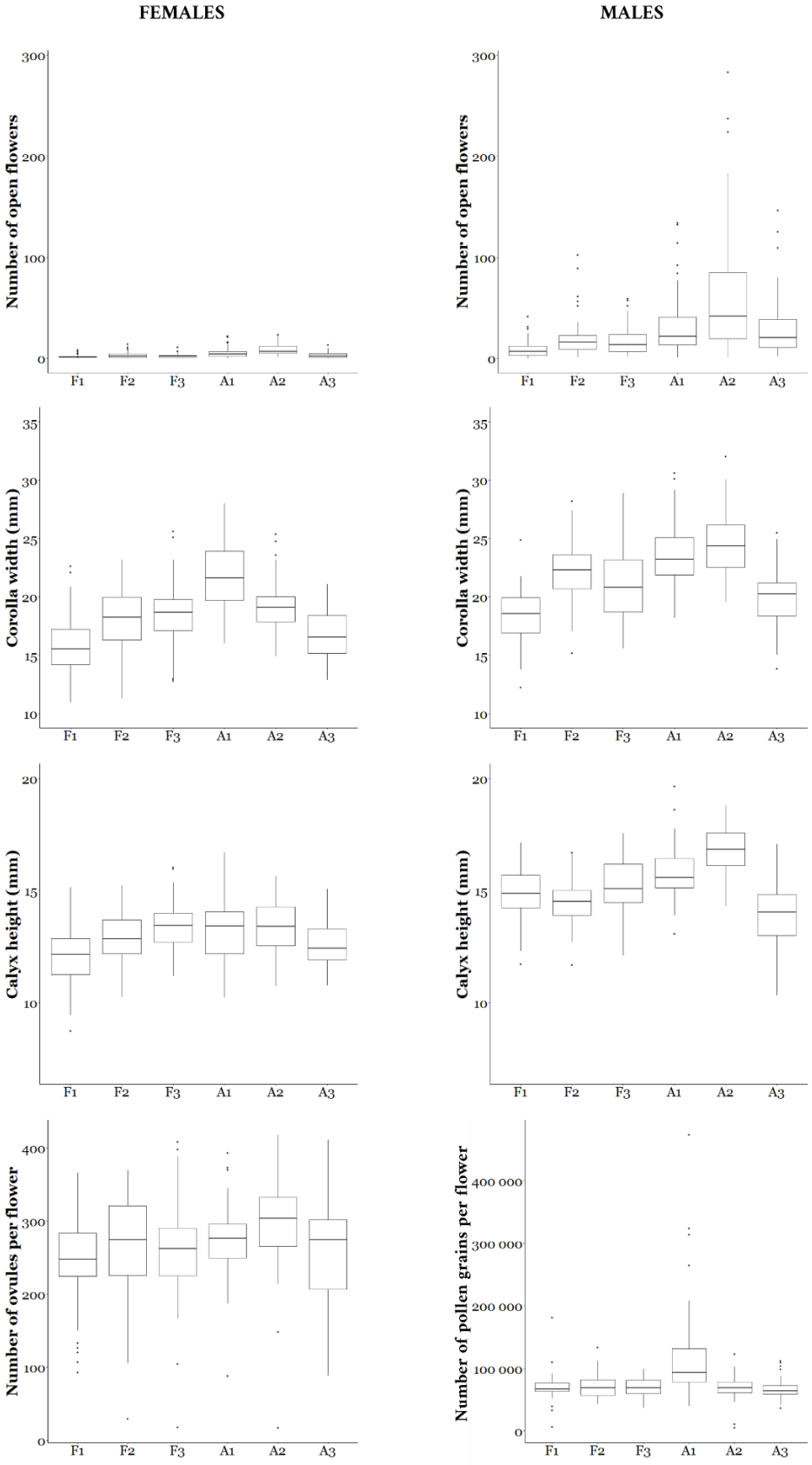
Table S4. Result for Levene tests for homogeneity of variance. Significant values after Bonferroni correction are indicated in gold (*: $p < 0.0055$). Flowers: number of open flowers; Corolla: average corolla width (mm); Calyx: average calyx height (mm); Ovules: number of ovules per flower; Pollen: number of pollen grains per flower.

	Females			Males		
Flowers	A1	A2	A3	A1	A2	A3
F1	**2.4×10^{-5}**	**5.8×10^{-8}**	0.045	**1.1×10^{-4}**	**1.2×10^{-6}**	**2.3×10^{-4}**
F2	**5.0×10^{-3}**	**1.0×10^{-4}**	0.900	0.037	**7.9×10^{-5}**	0.074
F3	**1.0×10^{-4}**	**3.9×10^{-7}**	0.147	**9.1×10^{-3}**	**2.4×10^{-5}**	0.020
Corolla	A1	A2	A3	A1	A2	A3
F1	0.204	0.713	0.751	0.285	0.352	0.931
F2	0.598	0.218	0.206	0.655	0.760	0.595
F3	0.474	0.402	0.409	0.254	0.192	0.025
Calyx	A1	A2	A3	A1	A2	A3
F1	0.568	0.832	0.581	0.756	0.433	0.329
F2	0.252	0.404	0.992	0.189	0.320	**8.2×10^{-3}**
F3	0.101	0.162	0.536	0.511	0.215	0.442
Gametes	A1	A2	A3	A1	A2	A3
F1	0.302	0.827	0.083	**1.6×10^{-3}**	0.955	0.613
F2	0.093	0.424	0.252	**1.6×10^{-3}**	0.855	0.333
F3	0.251	0.689	0.157	**7.0×10^{-4}**	0.380	0.929

Table S5. Correlations among floral traits, and between floral traits and internal relatedness, for males (upper triangle) and females (lower triangle) in each population (F: populations from forest habitats, A: populations from anthropogenic habitats), described using Pearson's correlation coefficient. Significant values after Bonferroni correction are indicated by stars (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$). Open flowers: number of open flowers; Corolla: corolla width; Calyx: calyx height; Gametes: number of gametes (ovules for females, pollen grains for males) per flower; IR: Internal Relatedness.

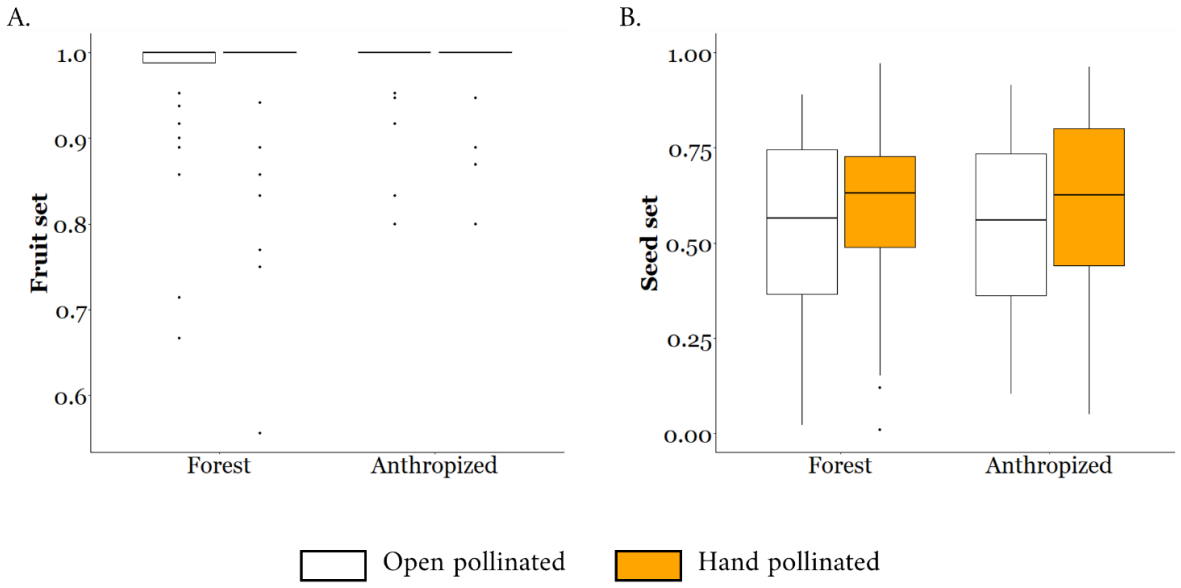
		Open flowers	Corolla	Calyx	Gametes	IR
F1	Open flowers		0.34	0.16	0.00	-0.23
	Corolla	0.05		**0.52*** **	-0.11	0.00
	Calyx	-0.02	0.15		-0.23	-0.06
	Gametes	0.21	0.09	0.12		0.08
	IR	-0.08	0.06	-0.19	-0.34	
F2	Open flowers		0.05	-0.03	-0.07	0.08
	Corolla	0.04		**0.51*** **	-0.02	-0.05
	Calyx	-0.10	0.37		-0.11	0.09
	Gametes	-0.24	0.27	0.07		0.15
	IR	-0.04	-0.08	0.11	0.18	
F3	Open flowers		0.10	-0.08	0.04	-0.09
	Corolla	0.18		**0.45** **	0.12	0.34
	Calyx	0.09	0.28		0.06	0.32
	Gametes	0.16	0.17	0.07		-0.01
	IR	0.16	0.01	0.13	-0.06	
A1	Open flowers		0.15	-0.24	**0.37***	-0.09
	Corolla	0.34		**0.55*** **	0.11	0.06
	Calyx	0.30	**0.48** **		0.08	0.20
	Gametes	0.14	0.33	0.11		0.07
	IR	0.12	0.15	0.29	-0.13	
A2	Open flowers		0.02	0.09	-0.10	0.01
	Corolla	**0.50*** **		**0.64*** **	0.11	-0.21
	Calyx	0.17	**0.44** **		0.22	-0.21
	Gametes	0.04	0.15	0.22		-0.09
	IR	0.01	-0.10	0.15	-0.15	
A3	Open flowers		-0.09	0.09	-0.02	-0.20
	Corolla	0.16		**0.55*** **	0.11	0.02
	Calyx	0.21	0.14		0.08	0.09
	Gametes	0.14	0.11	0.23		-0.15
	IR	0.01	0.08	-0.13	0.30	

Figure S2. Boxplots of floral traits measured in each population, with females (left panel) and males (right panel) shown separately (F: populations from forest habitats, A: populations from anthropogenic habitats).



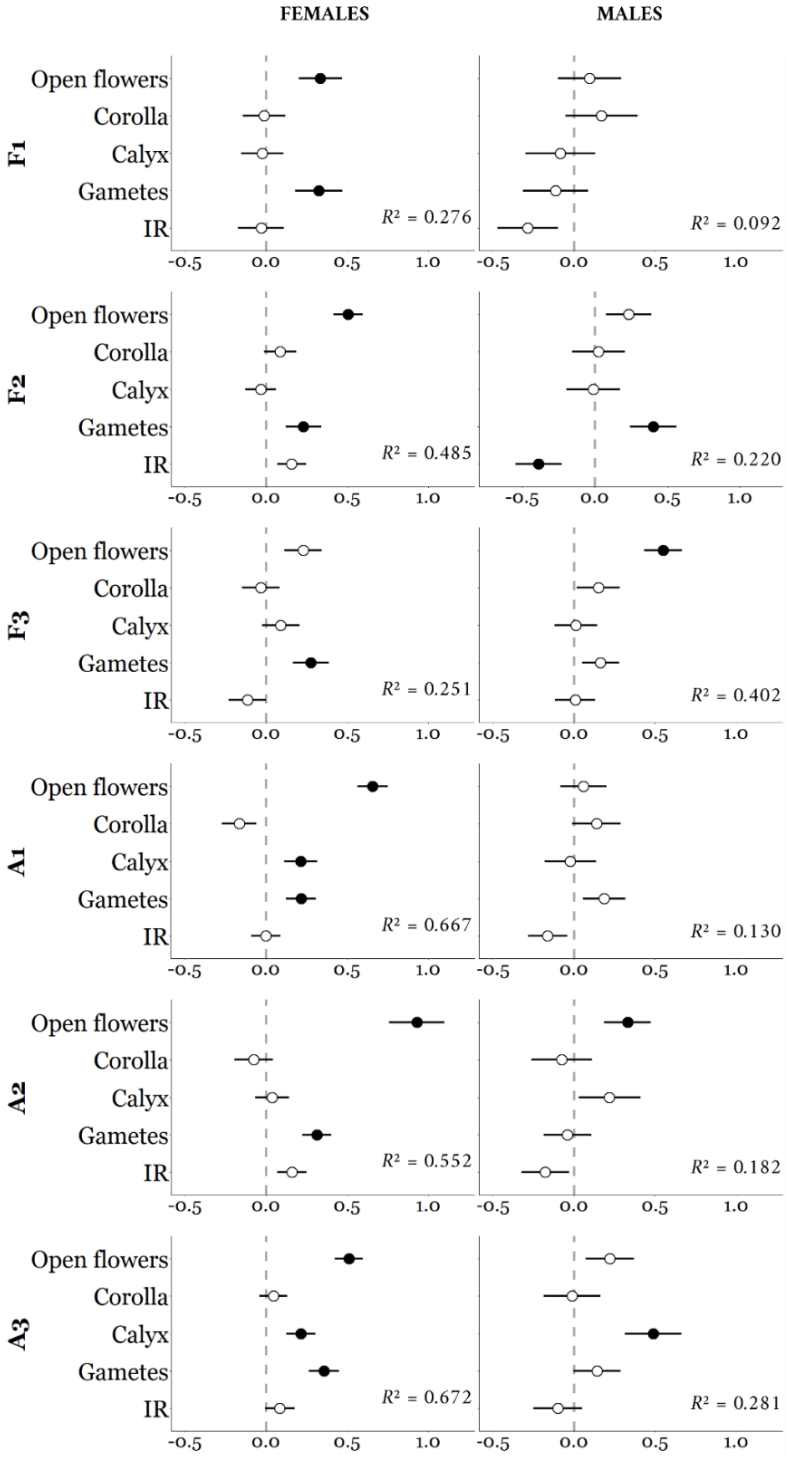
4. Pollination service quality

Figure S3. Boxplots of fruit set (panel A) and seed set (panel B) for open pollinated (white) and hand-pollinated (orange) plants in each habitat.



5. Selection gradients with IR

Figure S4. Selection gradients (± standard error) on floral traits including internal relatedness (i.e., a proxy for individual inbreeding) as a covariate for females (left panel) and males (right panel) in each population (F: populations from forest habitats, A: populations from anthropogenic habitats). The slope of the relationship between reproductive success and internal relatedness is shown in the figure. Open flowers: number of open flowers; Corolla: corolla width; Calyx: calyx height; Gametes: number of gametes (ovules for females, pollen grains for males) per flower; IR: Internal Relatedness. Significant values are indicated in black ($p < 0.05$).



6. Structural equation modelling

Figure S5. Path diagram of the structural equation model in females in each population (F: populations from forest habitats, A: populations from anthropogenic habitats). Positives estimates are in green, negatives estimates are in orange, non-significant estimates in grey ($p > 0.05$). Line thickness represents the strength of the relationship between variables. Flower: number of open flowers; Corolla: corolla width; Calyx: calyx height; Gamete: number of ovules per flower; MS: mating success (i.e., number of sexual partners); RS: reproductive success (i.e., total seed production).

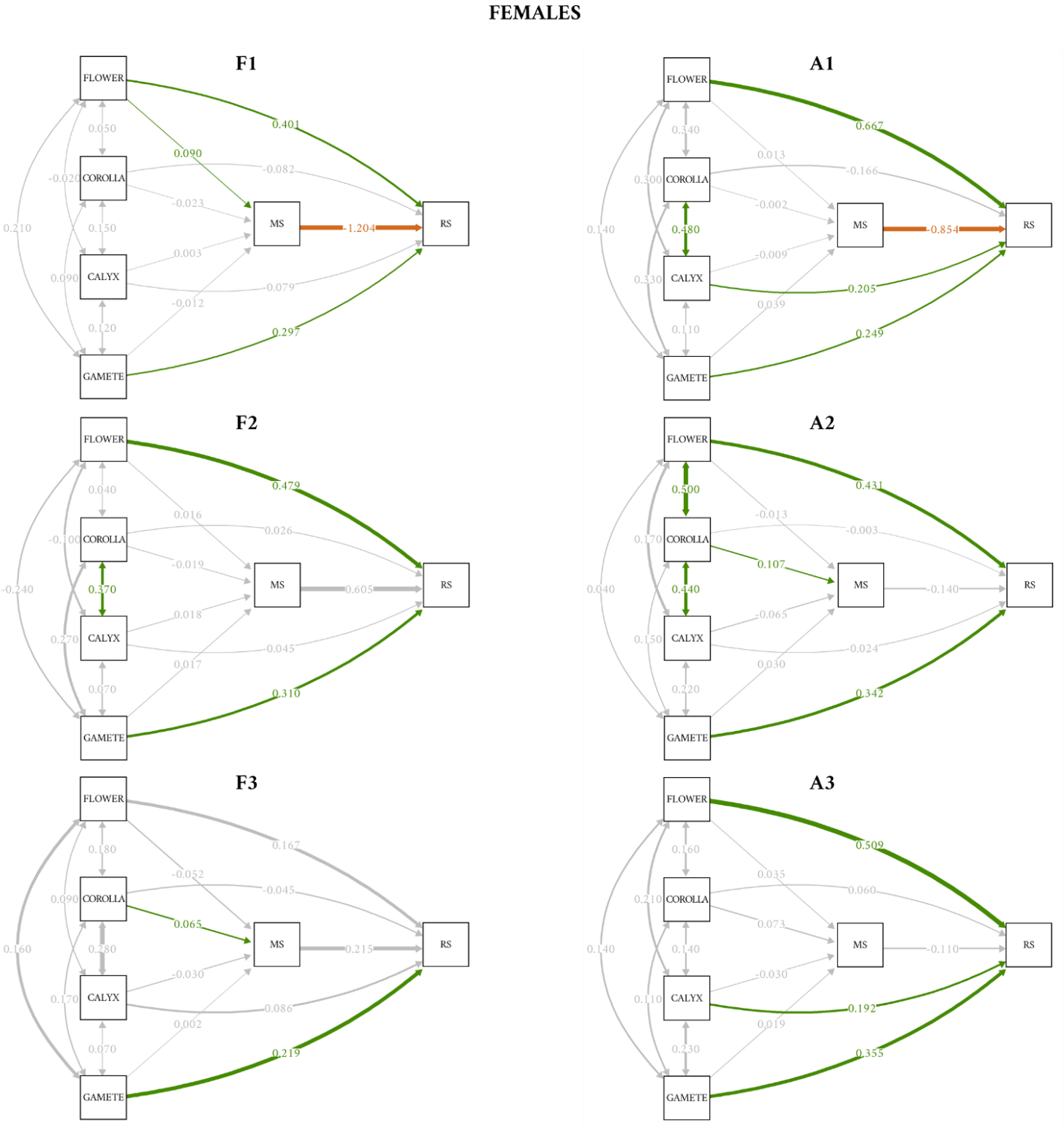
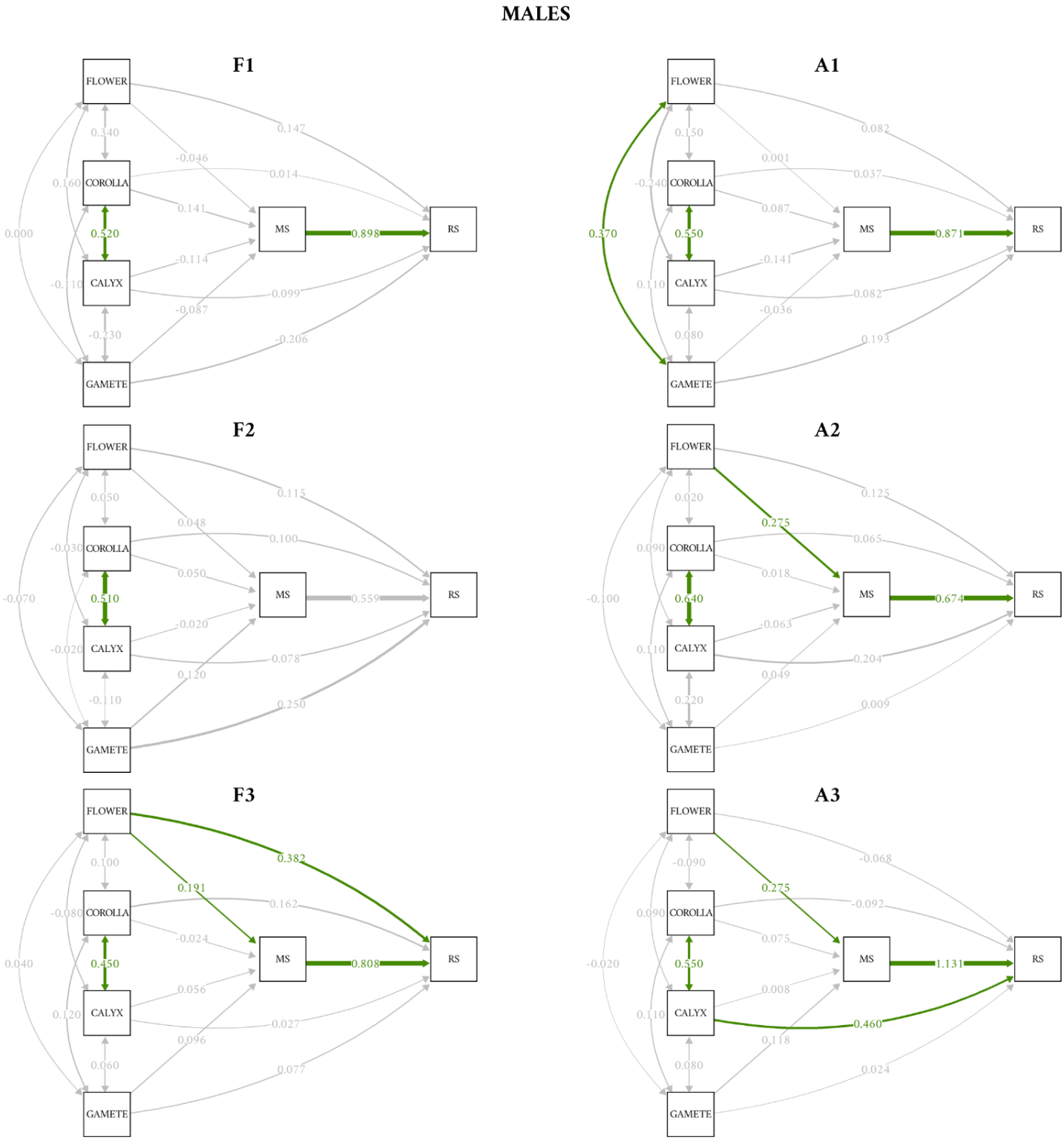


Figure S6. Path diagram of the structural equation model in males in each population (F: populations from forest habitats, A: populations from anthropogenic habitats). Positives estimates are in green, negatives estimates are in orange, non-significant estimates in grey ($p > 0.05$). Line thickness represents the strength of the relationship between variables. Flower: number of open flowers; Corolla: corolla width; Calyx: calyx height; Gamete: number of pollen grains per flower; MS: mating success (i.e., number of sexual partners); RS: reproductive success (i.e., total seeds sired).



Chapitre III

Différents pollinisateurs imposent-ils une sélection divergente sur les traits floraux ?

Le deuxième chapitre nous a permis de caractériser les communautés de pollinisateurs de *S. dioica*. Dans l'ensemble des populations étudiées, les bourdons et les syrphes étaient les pollinisateurs les plus fréquents. Cependant, leurs fréquences relatives variaient selon les populations. Ces deux groupes d'insectes diffèrent par leurs préférences sensorielles, leur morphologie et leur comportement, impactant potentiellement leur efficacité de pollinisation et la sélection qu'ils exercent sur les traits floraux. C'est cette hypothèse que nous allons examiner dans ce troisième chapitre. De plus, les gradients de sélection exercées sur des plantes mises en présence de bourdons et de syrphes à la fois ont été mesurés et comparés à la somme des gradients mesurés lorsque les plantes étaient pollinisées par l'un ou l'autre de ces pollinisateurs, afin de tester l'additivité des pressions de sélection imposées par les bourdons et par les syrphes.

Manuscript C: Do different pollinators impose divergent selection on floral traits?

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Abstract

Many angiosperm species are visited by multiple functional groups of pollinators, which differ in their sensory capacities, as well as in their morphological and behavioural characteristics. Floral traits associated with pollinator attraction and the fit between flowers and their pollinators may therefore be subject to different selective pressures (divergent selection or variation on selection intensity), and the effect of this selection may—or not—be additive in the presence of different pollinators (i.e., sum of selection mediated by each pollinator independently). In this study, the visitation behaviour of two common pollinators of *Silene dioica*—bumblebees and hoverflies—and the phenotypic selection exerted on floral traits in both male and female plants were explored using an experimental approach. We further study whether the simultaneous presence of both pollinators leads to non-additive selection. Our results showed that bumblebees were more effective pollinators than hoverflies, leading to pollen limitation in the latter treatment. Consequently, in females, pollinator-mediated selection on a floral trait related to mechanical fit was observed under the hoverfly treatment, whereas under the bumblebee treatment, where pollination was optimal, fertility selection was detected. In males, bumblebees positively selected for the number of open flowers, whereas hoverflies negatively selected for plant height. Additive selection was detected in each case studied.

Key words

Generalist pollination, pollinator-mediated selection, non-additive selection, dioecy

Contents

INTRODUCTION

Angiosperms display a remarkable diversity of floral traits (Harder & Johnson, 2009), which is often believed to reflect interactions with pollen vectors (Fenster et al., 2004). More than 80% of angiosperm species are animal-pollinated (Ollerton et al., 2011), and numerous studies have shown that floral traits are frequently subject to pollinator-mediated selection (Fenster et al., 2004 ; Schiestl & Johnson, 2013 ; Caruso et al., 2019). In plants pollinated by a single functional group of pollinators (i.e., species that share similar sensory capacities, preferences, morphology, and foraging behaviour), the optimal values of floral traits are expected to reflect the characteristics of that group. Such specialization often gives rise to a *pollination syndrome*, defined as a set of floral traits that promote the attraction and use of a specific functional group of pollinators (Fenster et al., 2004 ; Junker & Parachnowitsch, 2015). In line with this concept, bird-pollinated plants tend to have red, weakly scented flowers that produce abundant nectar (Cronk & Ojeda, 2008); bat-pollinated plants usually have green or white, bell-sharped flowers that secrete large amounts of nectar during the night (Martén-Rodríguez et al., 2009) and bee-pollinated plants are described as having yellow or blue flowers with a landing platform and producing a moderate amount of nectar (Fenster et al., 2004). Yet, in many cases, plants are pollinated by multiple functional groups of animals (i.e., generalist pollination, Waser et al., 1996), raising the question of how floral phenotypes can evolve and diversify under such diffuse interactions (Ohashi et al., 2021 ; Kay, 2025).

Different functional groups of pollinators may select for different traits (Brunet et al., 2021) or impose either convergent or divergent selective pressures on the same traits (Muchhala, 2007 ; Gervasi & Schiestl, 2017). While such divergent selective pressures may, in some cases, result in disruptive selection favouring specialization, other evolutionary outcomes are possible, including adaptive generalization through temporal switch (e.g., change in scent profiles between day and night), intra-individual polymorphism or modulation of pollinator preferences *via* multi-trait combinations (Ohashi et al., 2021). For each of these scenarios, selection may be additive (i.e., when total selection equals the sum of selection mediated by each pollinator alone) or non-additive (terHorst et al., 2015). Non-additive selection can occur through indirect ecological effects, whereby selection exerted by one pollinator is altered in the presence of another (e.g., due to changes in its foraging behaviour), or through changes in the distribution of fitness that alters the opportunity for selection (terHorst et al., 2015). In addition, pollinators may vary in their efficiency, which depends on their visitation frequency and capacity to remove, transport and deposit pollen. Such variation in efficiency can produce non-additive selection: each pollinator alone can exert significant selection on floral traits, but when combined, the most effective pollinators dominate fitness variation, thereby "masking" the weaker contribution of less effective pollinators.

Because plants typically produce much more pollen grains than ovules (Kodric-Brown & Brown, 1987), male reproductive success is expected to be limited by access to ovules, whereas female reproductive success should be mainly resource-limited, at least when pollinators are abundant (Johnson & Shaw, 2016 ; Tonnabel et al., 2019 ; Barbot et al., 2022 ; Kwok & Dorken, 2022 ; Lavaut et al., 2023). Consequently, selection is expected to differ between the male and female functions. Specifically, no pollinator-selection is expected in

females, unless pollinator efficiency is low, thereby causing pollen limitation of seed set. In contrast, selection patterns in males should be primarily shaped by pollinator preferences and morphology, and selective pressures should only be additive if both pollinators are equally effective and do not alter each other's behaviour.

In this study, we used an experimental approach to measure selection exerted on floral traits by the two most common pollinators of the dioecious species *Silene dioica* (i.e., bumblebees and hoverflies, Kay et al., 1984 ; Barbot et al., 2022 ; Jolivel et al., in prep.) to examine how pollinator identity shapes selection patterns. Previous studies have shown that these two pollinators can exert contrasting selection pressures on floral traits due to differences in body size and foraging behaviour (Gómez et al., 2008 ; Wu & Li, 2017 ; Gervasi & Schiestl, 2017). For example, plant height was positively selected by bumblebees but negatively selected by hoverflies in *Brassica rapa* (Gervasi & Schiestl, 2017). Likewise, bumblebees preferred narrow corollas, whereas hoverflies favored wider ones in *Erysimum mediohispanicum* (Gómez et al., 2008). In *Primula secundiflora*, the number of open flowers was positively selected by bumblebees but not by hoverflies (Wu & Li, 2017). Differences in efficiency are also expected between these two groups, bumblebees are expected to visit more flowers than hoverflies (Ishii & Sakai, 2001 ; Miyake & Sakai, 2005), and to transfer pollen more efficiently (Morse, 1981 ; Gyan & Woodell, 1987 ; Ollerton et al., 2025). In our experiment, plants were exposed either to bumblebees alone, hoverflies alone, or both pollinators simultaneously. For each treatment, we recorded pollinator visits and estimated selection on floral traits in both sexes. Because of the previously mentioned behavioural and morphological differences between these two groups, we expected variation in the target of selection, but also in the direction and strength of such selection, with stronger selection in hoverfly-pollinated plants due to stronger pollen limitation, and non-additive selection due to unbalanced fitness contributions.

MATERIALS AND METHODS

1. Study species

Silene dioica (L) Clairv. (Caryophyllaceae) is a herbaceous, perennial, and dioecious angiosperm widely distributed in central and northern Europe (Kay et al., 1984). It has a generalist pollination system and is pollinated by various functional groups of insects, including bumblebees (*Apidae*) and hoverflies (*Syrphidae*) (Kay et al., 1984 ; Barbot et al., 2022, Jolivel et al., in prep.), with *Bombus terrestris* and *Episyrphus balteatus*, the two taxa considered in our study, being regular visitors. Together, both pollinator groups account for 70% of the visits observed in the study region, but their relative frequencies can vary markedly even at a fine spatial scale (Jolivel et al., in prep.). When *S. dioica* plants are exposed to the full pollinator assemblage, females tend to experience positive selection on traits directly related to fertility—such as flower number and number of gametes per flower—whereas in males, selection tend to favor traits associated with pollinator attraction or morphological fit, including calyx height and corolla width (Barbot et al., 2022 ; Barbot et al., 2023 ; Jolivel et al., in prep.).

2. Design experimental

Plants used in the experiment originated from seeds collected in wild populations of *S. dioica* in the Mormal forest (northern France), sown in 2022 and grown under controlled greenhouse conditions at the University of Lille. In spring 2024, experimental populations were established in tents with four treatments: bumblebee pollination (B), hoverfly pollination (H), bumblebee and hoverfly pollination (BH), and hand pollination (HP). Each tent assigned to an insect-pollination treatment (B, H, BH) contained 20 male and 20 female plants, arranged in a grid with alternating sexes, and was exposed for 16 days to ten insects (ten bumblebees, ten hoverflies, or five of each, depending on the treatment). Each of these three insect-pollination treatments was replicated twice (two tents per treatment), with each plant and insect used in only one replicate.

Bumblebees (*B. terrestris*) were provided by Biobest® (one hive per tent), and their number in the tent was maintained at five (BH) or ten (B) by preventing additional bees from leaving the hives. Hoverflies (*E. balteatus*) were collected on the University of Lille campus with butterfly nets, and exactly five (BH) or ten (H) individuals were introduced per tent, with dead individuals replaced as needed to maintain a constant population. Prior to the experiment, both pollinator species were trained to forage on *S. dioica* plants for two days.

The HP treatment consisted of a single tent with 20 female plants. Hand pollinations were carried out daily, using pollen collected from fresh male flowers harvested from plants outside the experimental setup. For each hand pollination, the anthers of male flowers were rubbed onto the stigmas of all open female flowers, ensuring that each stigma was saturated with pollen.

3. Pollinator observations

Pollinator observation sessions were conducted two times a day throughout the experiment (26 observations per experimental population in total). Each observation involved surveying a group of 8 plants (4 males and 4 females) for 20 minutes between 9:00 AM and 5:00 PM to count the number of visits per plant. In the case of mixed tents, the identity of the insects was recorded (bumblebee or hoverfly). A visit was defined as a contact between a pollinator and the reproductive structures of the flower. From these sessions, the visitation rate was defined as the total number of visits per plant, standardized by the number of pollinators present during the session (ten bumblebees, ten hoverflies, or five of each, depending on the treatment).

4. Floral traits measurements

For each plant, the number of open flowers was counted, and plant height (cm), calyx height (mm) and corolla width (mm) were measured. Calyx height and corolla width were measured using calipers (with a precision of 0.01 mm) on two randomly selected flowers from each plant and averaged. The number of gametes per flower was estimated in both sexes. In females, fruits produced during the experiment were harvested at maturity. The number of ovules per flower was estimated from three randomly selected fruits per plant, using an image analysis method developed by Barbot et al. (2022). This method involves dissecting a fruit and digitizing its content using a high-resolution scanner. Seeds and undeveloped ovules are differentiated based

on their size and counted using an R script for image analysis (EBImage package in R, Pau et al., 2010). The total number of ovules per flower was calculated as the sum of seeds and undeveloped ovules and averaged over the three fruits. For male plants, the number of pollen grains per flower was estimated from two anthers collected from a nearly opened flower bud, using a particle counter (CASY Cell Counter & Analyzer, OLS) following the protocol of Dufaÿ et al. (2008).

Floral traits did not vary among experimental populations (supplementary material – Floral trait variation).

5. Reproductive success estimation

Females - For each female plant, the number of fruits produced during the experiment was recorded to calculate fruit set (number of fruits / number of flowers). For three randomly selected fruits per female, the number of seeds and ovules was estimated as described above to calculate seed set (number of seeds / number of ovules). These two fitness proxies provide information on whether pollen receipt was sufficient to maximize fruit initiation and ovule fertilisation. Finally, the contents of all fruits were pooled, and the total number of seeds produced during the experiment was determined using a seed counter (elmor C3, version 1.7.0.490) and used as a proxy for total female reproductive success, used for selection analyses.

Males – Reproductive success of male plants was estimated using paternity analysis on a subsample of the seeds produced during the experiment. Total genomic DNA from all insect-pollinated adult plants (120 females and 120 males) and 1,920 offspring (16 seeds per female) was extracted using the NucleoMag 96 Plant kit (Macherey Nagel, Oesingen, Switzerland) and an automated DNA purification station (KingFisher®, Thermo Fisher Scientific, Waltham, USA). PCR amplifications were performed on five nuclear microsatellites (Barbot et al., 2022). Paternity was then assigned using the categorical method implemented in CERVUS (version 3.0.7, Marshall et al., 1998 ; Kalinowski et al., 2007), with an 80% confidence threshold. This method estimates the most likely sire by comparing the genotype of each offspring to that of each candidate male, accounting for a 2% genotyping error rate. The average proportion of seeds sired by each male was then estimated for each female and multiplied by the total number of seeds produced by that female. Male reproductive success was finally calculated as the sum of the seeds sired across all females in the population.

6. Statistical analysis

Pollinator density – Pollinator observations were analyzed in two complementary ways. First, we aimed at comparing experimental populations pollinated by bumblebees *versus* hoverflies alone, to compare visitation frequency between these two groups. To this end, we compared the visitation rate (modelled with a negative binomial distribution) between H and B tents using a generalized linear mixed model (GLMM), with treatment, plant sex and interaction between these two factors as fixed effects, including tent and observation session as random effects.

Second, we aimed at testing whether visitation frequency differed between single and mixed tents for each pollinator group. To this end, two GLMMs (one for bumblebees and one for hoverflies) were used to test the

effects of treatment type (single vs. mixed), plant sex, and their interaction on the visitation rate with tent and observation session included as random factors.

Pollen limitation – Pollen limitation was quantified within each experimental population, by comparing fruit set, seed set, and total number of seeds between each insect-pollinated tent and the hand-pollinated (HP) tent. GLMMs were used with a binomial distribution for fruit set and seed set, and a Gaussian distribution for total seed production. Experimental population was treated as fixed effect. For the seed set model, female identity was added as a random effect, since seed set was measured on three fruits per female. For all three models, post-hoc Tukey tests were applied to perform pairwise comparisons between tents.

Selection gradients – Selection gradients (β) were estimated using multiple regression analyses following Lande & Arnold (1983). Reproductive success, defined as individual reproductive success divided by the mean reproductive success, was used as the response variable and standardized trait values (mean = 0 and variance = 1) as explanatory variables, including plant height, number of open flowers, corolla width, calyx height and number of gametes per flower. Reproductive success was relativized and trait values standardized separately for each experimental population and sex. In females, reproductive success corresponded to the number of seeds produced, whereas in males, reproductive success corresponded to the number of seeds sired, estimated through paternity analyses. Selection analyses were performed independently for males and females, due to the different methods of reproductive success estimation in the two sexes.

Selection gradients were also estimated in hand-pollinated plants (HP) and subtracted from those obtained in insect-pollinated females (OP) to quantify pollinator-mediated selection ($\Delta\beta_{poll} = \beta_{OP} - \beta_{HP}$). The significance of pollinator-mediated selection was tested using an ANCOVA for each experimental population, including traits, pollination treatment (insect vs. hand) and their interaction as fixed effects. A significant interaction between a trait and pollination treatment would indicate significant pollinator-mediated selection on this trait in females.

To assess whether selection patterns varied depending on pollinator identity, we used separate ANCOVAs for each sex. Only the four single-pollinator populations were considered. Each model included all traits, pollinator identity (bumblebees vs. hoverflies) and their interactions as fixed effects, with experimental population as a random effect. A significant interaction between a trait and pollinator identity would indicate that selection exerted on this trait differs between bumblebees and hoverflies. Unlike in females, pollinator-mediated selection in males cannot be measured experimentally. However, if selection on a floral trait differs between pollinator groups, this implies that the trait is under pollinator-mediated selection.

Additive selection – Additive selection was compared to selection mediated by both groups of pollinators simultaneously (i.e., mixed treatment). This analysis was performed on floral traits for which selection was found to differ between bumblebee- and hoverfly-pollinated plants. Additive selection was simulated as the sum of the selection mediated by each pollinator separately, as described in terHorst et al. (2015). For each floral trait, a simulated distribution of additive selection was generated. This distribution corresponds to the sum of two gradient values, each randomly sampled from one "bumblebee" tent (B1 or B2) and one "hoverfly" tent (H1 or H2). At each iteration, the identity of the two tents (B1 or B2; H1 or H2) was chosen at random,

and for each tent, a gradient value was sampled from a Gaussian distribution whose mean corresponded to the observed selection gradient and whose standard deviation corresponded to its standard error. This random sampling procedure was repeated 10,000 times. The observed selection gradients for each floral trait in the mixed tents were then compared to the simulated distribution under the additivity scenario. Observed values falling outside the central 95% of the simulated distribution were considered as evidence of non-additive selection.

All statistical analyses were performed using R (version 4.2.3). Normality of residuals and homogeneity of variance were verified for all linear models. Inspection of variance inflation factors (VIFs) in models estimating selection gradients indicated no problem of multicollinearity (all VIFs < 2, Quinn & Keough, 2002 ; supplementary material – Floral trait variation). Overdispersion in generalized linear models with a negative binomial distribution was checked and found to be less than 2.

RESULTS

1. Pollinator density

In single tents, bumblebee-pollinated plants had a higher visitation rate than hoverfly-pollinated ones (**Table 1**). There was a significant interaction between pollinator identity and plant sex, with male having a higher visitation rate than females in bumblebee-pollinated plants but not in hoverfly-pollinated ones ($F_{1,419}$ = 43.61, p < 0.001). In the analyses comparing single and mixed tents, bumblebees were once again found to visit male plants more often than female ones, while visitation rate did not differ between treatment types (**Table 2**). No difference between treatment types or sexes was detected for hoverflies.

Table 1. Results of the GLMMs testing for the effect of pollinator identity, plant sex, and their interaction on visitation rate in single tents.

Predictor	Estimate	*F*	*p*
Pollinator identity	-1.516	33.97	< **0.001**
Plant sex	1.361	38.95	< **0.001**
Pollinator identity * Plant sex	-1.942	5.05	< **0.01**

Table 2. Results of the GLMMs testing for the effect of treatment type, plant sex, and their interaction on visitation rate for each pollinator group.

	Bumblebee			Hoverfly		
Predictor	**Estimate**	***F***	***p***	**Estimate**	***F***	***p***
Treatment type	-0.428	0.40	0.436	0.750	0.597	0.437
Plant sex	1.137	74.47	< **0.001**	0.256	0.185	0.709
Treatment type * Plant sex	0.203	0.50	0.474	-0.837	0.633	0.366

2. Pollen limitation

Fruit set ($F_{6,133}$ = 664.16, p < 0.001), seed set ($F_{6,412}$ = 11.69, p < 0.001) and number of seeds ($F_{6,133}$ = 11.79, p < 0.001) were found to vary among experimental populations, being lower in hoverfly-pollinated than in hand-pollinated plants (**Figure 1**). Plants from mixed tents also showed a lower seed set than hand-pollinated plants, whereas bumblebee-pollinated plants did not differ from hand-pollinated ones.

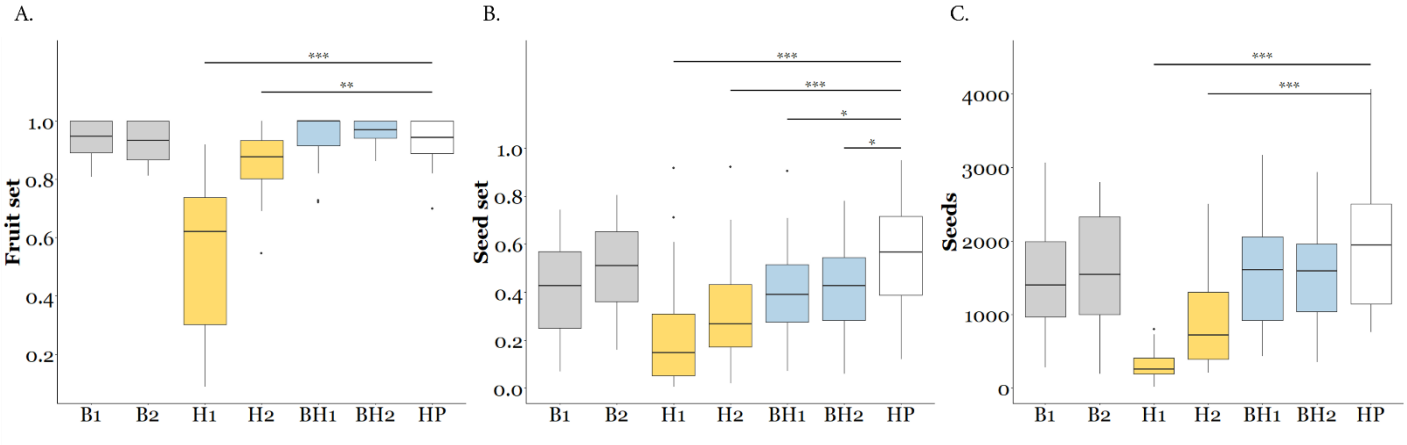

Figure 1. Boxplots of A. fruit set, B. seed set and C. number of seeds produced for insect-pollinated (coloured) and hand-pollinated (white) females in each experimental populations (B: bumblebees in grey, H: hoverflies in yellow, BH: mixed in blue, HP: hand-pollinated in white). Significant differences from the hand-pollinated (HP) tent are indicated by stars (*: p < 0.05, **: p < 0.01, ***: p < 0.001).

3. Selection gradients

In females, selection gradients were significantly positive for the number of gametes per flower in three experimental populations (B1, B2, BH2), and significantly negative for calyx height in one population (H1) (**Figure 2**). Only selection on calyx height in H1 was found to differ between insect- and hand-pollinated plants, as indicated by the ANCOVA ($\Delta\beta_{poll}$ = -0.556 ± 0.251, p < 0.05), suggesting that counter-selection on this trait in this experimental population is hoverfly-mediated. No significant effect of pollinator identity was detected on the selection exerted on any floral traits in females ($\gamma_{pollinator \times height}$: 0.082 ± 0.144, p = 0.567; $\gamma_{pollinator \times flower}$: -0.037 ± 0.144, p = 0.800; $\gamma_{pollinator \times corolla}$: 0.035 ± 0.158, p = 0.823; $\gamma_{pollinator \times calyx}$: -0.309 ± 0.160, p = 0.053; $\gamma_{habitat \times gamete}$: 0.009 ± 0.139, p = 0.951).

In males, positive selection was observed on number of open flowers in both bumblebees-only experimental populations, and on plant height in both hoverflies-only experimental populations (**Figure 2**). Accordingly, selection exerted on these traits differed between pollinator identity, selection on number of open flowers being stronger under bumblebees than hoverflies pollination ($\gamma_{pollinator \times flower}$: -0.330 ± 0.159, p < 0.05) and selection on plant height being stronger under hoverflies than bumblebees ($\gamma_{pollinator \times height}$: 0.466 ± 0.164, p < 0.01). However, for both traits, the observed selection gradients in the mixed tents did not significantly deviate from the simulated distribution under the additivity scenario, indicating no evidence of non-additive selection (**Figure 3**). No effect of pollinator identity was detected for the selection exerted on other traits ($\gamma_{pollinator \times corolla}$: -0.256 ± 0.168, p = 0.127; $\gamma_{pollinator \times calyx}$: -0.023 ± 0.163, p = 0.889; $\gamma_{habitat \times gamete}$: 0.108 ± 0.151, p = 0.473).

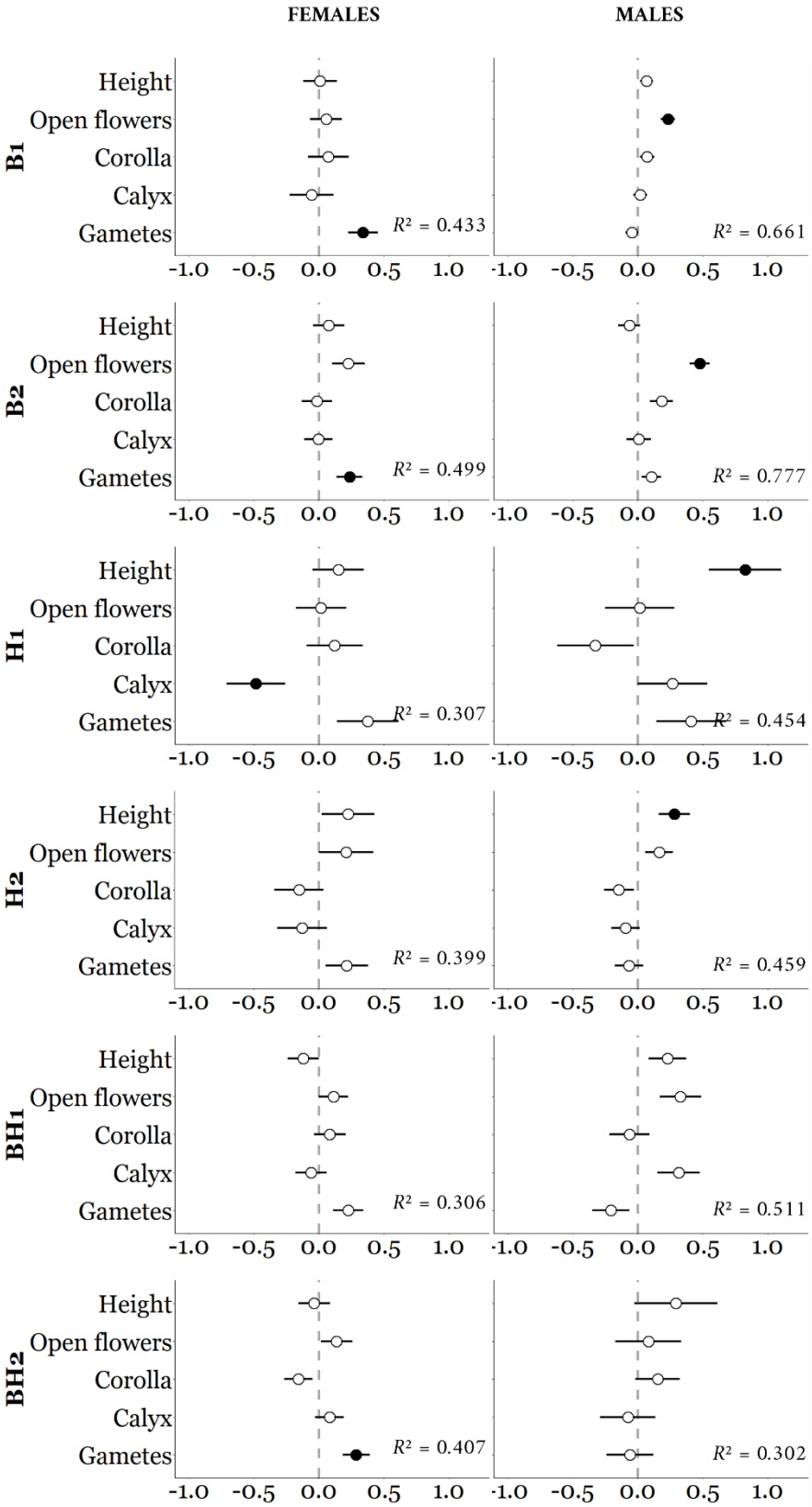

Figure 2. Selection gradients (± standard error) on floral traits for females (left panel) and males (right panel) in each experimental population (B: bumblebees, H: hoverflies, BH: mixed). Height: plant height, Open flowers: number of open flowers, Corolla: corolla width, Calyx: calyx height, Gametes: number of gametes (ovules for females, pollen for males) per flower. Significant values are indicated in black ($p < 0.05$).

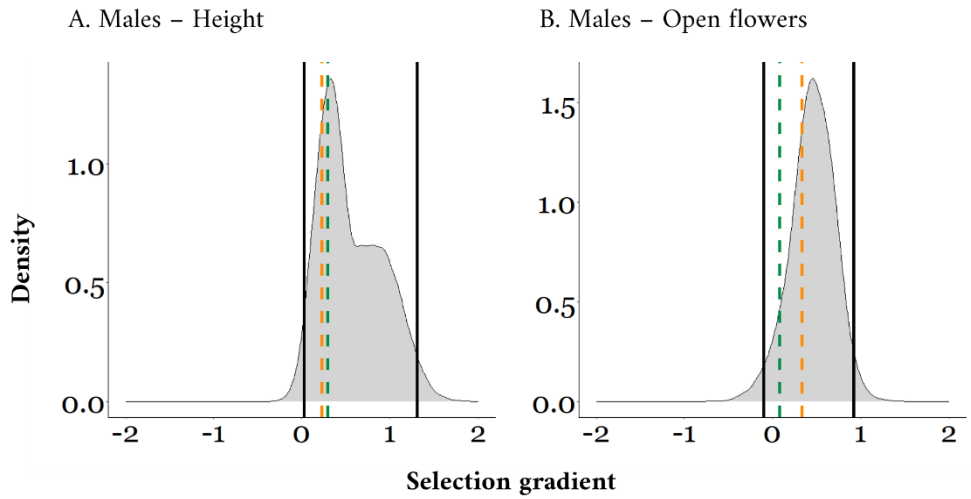

Figure 3. Simulated distribution of selection gradients under the additivity scenario (grey area). Black vertical bars indicate the 2.5% and 97.5% quantiles of the simulated distribution. Dashed vertical bars represent the observed selection gradients in the mixed tents (BH1: orange; BH2: green). An observed selection gradient falling outside the central 95% of the simulated distribution would indicate a case of non-additive selection. This analysis was performed on floral traits for which selection was found to differ between bumblebee- and hoverfly-pollinated plants.

DISCUSSION

Most angiosperm species rely on animal vectors for pollination, and numerous studies have demonstrated that pollinators frequently exert selection on floral traits (Fenster et al., 2004 ; Schiestl & Johnson, 2013 ; Caruso et al., 2019). While some species are involved in specialized interactions with pollinators, others are visited by multiple pollinator functional groups, which may impose convergent or conflicting selective pressures on floral traits, or target different traits. Characterizing these selective pressures, and understanding how they combine when multiple pollinator groups—potentially differing in effectiveness—are present is key to predicting how shifts in pollinator communities may shape floral evolution. In this study, we experimentally manipulated the presence of bumblebees and hoverflies to examine how pollinator identity affects visitation frequency, pollen limitation and selection on floral traits in both sexes in *S. dioica*.

Pollen limitation variation is driven by pollinator behaviour

Pollen limitation estimates based on the three measures of female reproductive success (fruit set, seed set and total number of seed) indicated that reproduction of hoverfly-pollinated females was limited by pollen receipt, in line with the low visitation rate observed for this pollinator group. In contrast, reproduction in bumblebee-pollinated females was not pollen-limited, consistent with the high foraging activity observed for this group and high pollen transfer efficiency reported in previous studies (Gyan & Woodell, 1987 ; Ollerton et al., 2025 ; Joffard et al., 2025). The lower seed set observed in the mixed treatment compared to the hand pollination one suggests reduced pollinator service in the mixed tents. Because the visitation rate did not differ between single and mixed tents, neither for bumblebees nor for hoverflies, suggesting no change in their foraging behaviour due to the presence of the other group (e.g. due to aggressive behaviour, Benelli, 2015), this reduced

seed set probably indicates that the five hoverflies present in the mixed tents did not compensate for the loss of five bumblebees compared to bumblebee-only tents. However, total seed production did not differ between the mixed and hand pollination treatments, indicating that this reduced seed set was compensated by increased flower production and suggesting that in *S. dioica*, suboptimal pollination can be partially offset by plasticity in flower production.

Selection patterns depend on the strength of pollen limitation in females

In females, positive selection on number of gametes per flower was detected across all experimental populations and was significant in those where plants were pollinated by bumblebees. This pattern likely reflects fertility selection in bumblebee-pollinated plants and is consistent with previous studies showing a positive effect of the number of gametes per flower on seed production, both in *S. dioica* (Barbot et al., 2023 ; Jolivel et al., in prep.) and in other species (Cilas et al., 2010 ; Wang et al., 2011). Our results did not reveal any other significant selection gradients for plants pollinated by bumblebees. Note that this does not imply that bumblebees lack phenotypic preferences (e.g., number of open flower was shown to affect pollinator attraction in *S. dioica*, Barbot et al., 2022 ; Moquet et al., 2022), but rather that these preferences were masked by their high efficiency, as the reproduction of all females was saturated by pollen receipt.

Moreover, negative selection on calyx height was detected in both hoverfly-pollinated experimental populations, and this selection was significant and mediated by pollinators in one of them. Calyx height, just as floral tube length, is a classic "fit" trait that is often expected to be under positive selection, because tall calices should promote deep probing, thereby maximizing contact between the pollinator's body and the plant's reproductive structures. However, some studies have reported negative selection on this trait (Li et al., 2016), suggesting that the relationship between calyx height and pollen transfer efficiency is context-dependant. In our case, we suggest that this pattern is due to the short proboscis of *E. balteatus* (approximately 2.9 mm, Doyle et al., 2020), which cannot reach nectar in flowers with tall calices and, as a result, tend to avoid visiting such flowers or spend less time on them. Although calyx height also had a negative effect on seed production in the other hoverfly-pollinated experimental population, this effect was not significant, perhaps due to weaker pollen limitation in this population (supplementary material – Pollen limitation). In the bumblebee-pollinated populations, selection gradients on calyx height were close to zero. Such contrasting selection patterns between bumblebee- and hoverfly-pollinated plants highlight that pollinator identity could shape selection on calyx height in females, although this effect was too limited to be detected when comparing selection gradients between treatments (no significant interaction between calyx height and pollinator group).

Selection on floral display size in bumblebee-pollinated males and on plant height in hoverfly-pollinated ones

In males, selection patterns differed between the two groups of pollinators, whereby different floral traits were targeted by each of them. In plants pollinated by bumblebees, the number of open flowers was under positive selection, which may be explained by both fertility and pollinator-mediated selection, as this trait is directly

linked to the amount of pollen produced but also influences pollinator attraction (Moquet et al., 2022). Indeed, Barbot et al. (2022) showed, using trait manipulation, that the number of open flowers increased the frequency of pollinator visits but also the amount of pollen carried by pollinators, thereby allowing male with large floral displays to export their pollen towards more females. Previous studies report inconsistent evidence regarding selection on the number of open flowers in hoverfly-pollinated plants, with some reporting increased visitation for larger floral displays (Ishii, 2004) while others found no such relationship (Miller et al., 2013). Here, we did not detect any selection on this trait in hoverfly-pollinated plants, indicating that producing more flowers did not confer any reproductive advantage in these plants, despite the associated increase in pollen production. Instead, plant height was found to be under positive selection in these plants, in line with the positive effect of this trait on hoverfly visitation reported in previous studies (Klecka et al., 2018). Our results therefore show that selection targets distinct traits in bumblebee- and hoverfly-pollinated plants. Although the foraging behaviour of each pollinator was found to be unaffected by the presence of the other (i.e., no indirect ecological effects, terHorst et al., 2015), the two groups were not equally efficient, with bumblebees contributing more to plant reproductive success. Based on these observations, we hypothetized that the selection mediated by hoverflies would be masked by that mediated by bumblebees, resulting in non-additive selection. Nevertheless, the observed selection gradients in mixed tents did not deviate from the simulated distribution under the additivity scenario, with positive gradients for both the number of open flowers and plant height, although none of them were found to be significant. This result may be explained by the differences in gradients observed among tents within the same treatment, as well as by the high variance associated with these gradients in one tent where pollination was carried out by hoverflies. These observations therefore do not allow us to exclude the hypothesis of non-additive selection.

CONCLUSION

By combining experimental manipulation of pollinator identity, quantification of visitation rates, hand pollinations and estimation of selection gradients in *S. dioica*, our study shows that bumblebees were more active than hoverflies, and that pollinator identity influences selection on floral traits. In both sexes, bumblebees and hoverflies targeted different traits. In females, this difference was related to the strength of pollen limitation: when pollination was optimal (i.e., in the bumblebee treatment), fertility-related traits were under positive selection, but when reproduction was pollen limited (i.e., in the hoverfly treatment), a floral trait related to the mechanical fit between the plant and the pollinator was selected. In males, selection targeted the number of open flowers in bumblebee-pollinated plants and plant height in hoverfly-pollinated plants. Because pollinator community composition often varies among natural populations, such distinct selective pressures could promote local adaptation to the most frequent pollinators—provided that variation in local pollinator assemblages remain stable across generations. Overall, our results underscore that both sexual functions and pollinator identity must be considered to understand how selection drives floral evolution in generalist species.

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REFERENCES

Barbot, E., Dufaÿ, M., & De Cauwer, I. (2023). Sex-specific selection patterns in a dioecious insect-pollinated plant. *Evolution*, 77(7), 1578-1590. https://doi.org/10.1093/evolut/qpad069

Barbot, E., Dufaÿ, M., Tonnabel, J., Godé, C., & De Cauwer, I. (2022). On the function of flower number : Disentangling fertility from pollinator-mediated selection. *Proceedings of the Royal Society B: Biological Sciences*, 289(1987), 20221987. https://doi.org/10.1098/rspb.2022.1987

Benelli, G. (2015). Should I fight or should I flight? How studying insect aggression can help integrated pest management. *Pest Management Science*, 71(7), 885-892. https://doi.org/10.1002/ps.3974

Brunet, J., Flick, A. J., & Bauer, A. A. (2021). Phenotypic selection on flower color and floral display size by three bee species. *Frontiers in Plant Science*, 11, 587528. https://doi.org/10.3389/fpls.2020.587528

Caruso, C. M., Eisen, K. E., Martin, R. A., & Sletvold, N. (2019). A meta-analysis of the agents of selection on floral traits. *Evolution*, 73(1), 4-14. https://doi.org/10.1111/evo.13639

Cilas, C., Machado, R., & Motamayor, J.-C. (2010). Relations between several traits linked to sexual plant reproduction in *Theobroma cacao* L. : Number of ovules per ovary, number of seeds per pod, and seed weight. *Tree Genetics & Genomes*, 6(2), 219-226. https://doi.org/10.1007/s11295-009-0242-9

Cronk, Q., & Ojeda, I. (2008). Bird-pollinated flowers in an evolutionary and molecular context. *Journal of Experimental Botany*, 59(4), 715-727. https://doi.org/10.1093/jxb/ern009

Doyle, T., Hawkes, W. L. S., Massy, R., Powney, G. D., Menz, M. H. M., & Wotton, K. R. (2020). Pollination by hoverflies in the Anthropocene. *Proceedings of the Royal Society B: Biological Sciences*, 287(1927), 20200508. https://doi.org/10.1098/rspb.2020.0508

Dufaÿ, M., Vaudey, V., De Cauwer, I., Touzet, P., Cuguen, J., & Arnaud, J. -F. (2008). Variation in pollen production and pollen viability in natural populations of gynodioecious *Beta vulgaris* ssp. *maritima* : Evidence for a cost of restoration of male function? *Journal of Evolutionary Biology*, 21(1), 202-212. https://doi.org/10.1111/j.1420-9101.2007.01454.x

Fenster, C. B., Armbruster, W. S., Wilson, P., Dudash, M. R., & Thomson, J. D. (2004). Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics*, 35(1), 375-403. https://doi.org/10.1146/annurev.ecolsys.34.011802.132347

Gervasi, D. D. L., & Schiestl, F. P. (2017). Real-time divergent evolution in plants driven by pollinators. *Nature Communications*, 8(1), 14691. https://doi.org/10.1038/ncomms14691

Gómez, J. M., Bosch, J., Perfectti, F., Fernández, J. D., Abdelaziz, M., & Camacho, J. P. M. (2008). Spatial variation in selection on corolla shape in a generalist plant is promoted by the preference patterns of its local pollinators. *Proceedings of the Royal Society B: Biological Sciences*, 275(1648), 2241-2249. https://doi.org/10.1098/rspb.2008.0512

Gyan, K. Y., & Woodell, S. R. J. (1987). Analysis of insect pollen loads and pollination efficiency of some common insect visitors of four species of woody Rosaceae. *Functional Ecology*, 1(3), 269-274. https://doi.org/10.2307/2389430

Harder, L. D., & Johnson, S. D. (2009). Darwin's beautiful contrivances : Evolutionary and functional evidence for floral adaptation. *New Phytologist*, 183(3), 530-545. https://doi.org/10.1111/j.1469-8137.2009.02914.x

Ishii, H. S. (2004). Increase of male reproductive components with size in an animal-pollinated hermaphrodite, *Narthecium asiaticum* (Liliaceae). *Functional Ecology*, 18, 130-137. https://doi.org/10.1111/j.1365-2435.2004.00826.x

Ishii, H. S., & Sakai, S. (2001). Effects of display size and position on individual floral longevity in racemes of *Narthecium asiaticum* (Liliaceae). *Functional Ecology*, 15(3), 396-405. https://doi.org/10.1046/j.1365-2435.2001.00536.x

Joffard, N., Lecat, I., Ollier, K., Jolivel, C., Schmitt, E., Godé, C., Barbot, E., Dufaÿ, M., & De Cauwer, I. (2025). Floral display size does not affect the distribution of paternal diversity in *Silene dioica*. *bioRxiv*. https://doi.org/10.1101/2025.07.19.665651

Johnson, M. G., & Shaw, A. J. (2016). The effects of quantitative fecundity in the haploid stage on reproductive success and diploid fitness in the aquatic peat moss *Sphagnum macrophyllum*. *Heredity*, 116(6), 523-530. https://doi.org/10.1038/hdy.2016.13

Jolivel, C., Joffard, N., Godé, C., Schmitt, E., & De Cauwer, I. (in prep.). Habitat differences do not disrupt sexual selection patterns in *Silene dioica*.

Junker, R. R., & Parachnowitsch, A. L. (2015). Working towards a holistic view on flower traits—How floral scents mediate plant–animal interactions in concert with other floral characters. *Journal of the Indian Institute of Science*, 95(1), 43-68. https://www.cabidigitallibrary.org/doi/full/10.5555/20153065813

Kalinowski, S. T., Taper, M. L., & Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, 16(5), 1099-1106. https://doi.org/10.1111/j.1365-294X.2007.03089.x

Kay, K. M. (2025). Beyond the Grant–Stebbins model : Floral adaptive landscapes and plant speciation. *Annals of Botany*, 136(4), 699-720. https://doi.org/10.1093/aob/mcaf096

Kay, Q. O. N., Lack, A. J., Bamber, F. C., & Davies, C. R. (1984). Differences between sexes in floral morphology, nectar production and insect visits in a dioecious species, *Silene dioica*. *New Phytologist*, 98(3), 515-529. https://doi.org/10.1111/j.1469-8137.1984.tb04145.x

Klecka, J., Hadrava, J., Biella, P., & Akter, A. (2018). Flower visitation by hoverflies (Diptera : Syrphidae) in a temperate plant-pollinator network. *PeerJ*, 6, e6025. https://doi.org/10.7717/peerj.6025

Kodric-Brown, A., & Brown, J. H. (1987). Anisogamy, sexual selection, and the evolution and maintenance of sex. *Evolutionary Ecology*, 1(2), 95-105. https://doi.org/10.1007/BF02067393

Kwok, A., & Dorken, M. E. (2022). Sexual selection on male but not female function in monoecious and dioecious populations of broadleaf arrowhead (*Sagittaria latifolia*). *Proceedings of the Royal Society B*: *Biological Sciences*, 289(1986), 20220919. https://doi.org/10.1098/rspb.2022.0919

Lande, R., & Arnold, S. (1983). The measurement of selection on correlated characters. *Evolution*, 37, 1210-1226. http://dx.doi.org/10.2307/2408842

Lavaut, E., Valero, M., Mauger, S., Guillemin, M. L., Destombe, C., & Dufaÿ, M. (2023). Sexual selection in seaweed? Testing Bateman's principles in the red alga *Gracilaria gracilis*. *Proceedings of the Royal Society B*: *Biological Sciences*, 290(2006), 20231668. https://doi.org/10.1098/rspb.2023.1668

Li, H.-D., Zhao, Y.-H., Ren, Z.-X., Wu, Z.-K., Wolfe, L. M., Bernhardt, P., & Wang, H. (2016). Fitness-related selection on floral traits in the distylous plant *Primula poissonii* (Primulaceae). *Journal of Plant Ecology*, 10(3), 559-568. https://doi.org/10.1093/jpe/rtw055

Marshall, T. C., Slate, J., Kruuk, L. E. B., & Pemberton, J. M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, 7(5), 639-655. https://doi.org/10.1046/j.1365-294x.1998.00374.x

Martén-Rodríguez, S., Almarales-Castro, A., & Fenster, C. B. (2009). Evaluation of pollination syndromes in Antillean Gesneriaceae : Evidence for bat, hummingbird and generalized flowers. *Journal of Ecology*, 97, 348-359. https://doi.org/doi: 10.1111/j.1365-2745.2008.01465.x

Miller, N., Al-Dobai, S., Legaspi, J., & Sivinski, J. (2013). Estimating attraction of Syrphidae (Diptera) to flowering plants with interception traps. *Biocontrol Science and Technology*, 23(9), 1040-1052. https://doi.org/10.1080/09583157.2013.813907

Miyake, Y. C., & Sakai, S. (2005). Effects of number of flowers per raceme and number of racemes per plant on bumblebee visits and female reproductive success in *Salvia nipponica* (Labiatae). *Ecological Research*, 20(4), 395-403. https://doi.org/10.1007/s11284-004-0035-4

Moquet, L., Jacquemart, A.-L., Dufaÿ, M., & De Cauwer, I. (2022). Effects of sexual dimorphism on pollinator behaviour in a dioecious species. *Oikos*, 2022(3), e08662. https://doi.org/10.1111/oik.08662

Morse, D. H. (1981). Interactions among syrphid flies and bumblebees on flowers. *Ecology*, 62(1), 81-88. https://doi.org/10.2307/1936671

Muchhala, N. (2007). Adaptive trade-off in floral morphology mediates specialization for flowers pollinated by bats and hummingbirds. *The American Naturalist*, 169(4), 494-504. https://doi.org/10.1086/512047

Ohashi, K., Jürgens, A., & Thomson, J. D. (2021). Trade-off mitigation : A conceptual framework for understanding floral adaptation in multispecies interactions. *Biological Reviews*, 96(5), 2258-2280. https://doi.org/10.1111/brv.12754

Ollerton, J., Coulthard, E., Tarrant, S., Woolford, J., Jorge, L. R., & Rech, A. R. (2025). Butterflies, bumblebees and hoverflies are equally effective pollinators of *Knautia arvensis* (Caprifoliaceae), a generalist plant species with compound inflorescences. *Journal of Applied Entomology*, 149(5), 685-696. https://doi.org/10.1111/jen.13345

Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, 120(3), 321-326. https://doi.org/10.1111/j.1600-0706.2010.18644.x

Pau, G., Fuchs, F., Sklyar, O., Boutros, M., & Huber, W. (2010). EBImage—An R package for image processing with applications to cellular phenotypes. *Bioinformatics*, 26(7), 979-981. https://doi.org/10.1093/bioinformatics/btq046

Quinn, G. P., & Keough, M. J. (2002). Experimental design and data analysis for biologists. *Cambridge University Press*.

Schiestl, F. P., & Johnson, S. D. (2013). Pollinator-mediated evolution of floral signals. *Trends in Ecology & Evolution*, 28(5), 307-315. https://doi.org/10.1016/j.tree.2013.01.019

terHorst, C. P., Lau, J. A., Cooper, I. A., Keller, K. R., Rosa, R. J. L., Royer, A. M., Schultheis, E. H., Suwa, T., & Conner, J. K. (2015). Quantifying nonadditive selection caused by indirect ecological effects. *Ecology*, 96(9), 2360-2369. https://doi.org/10.1890/14-0619.1

Tonnabel, J., David, P., Klein, E. K., & Pannell, J. R. (2019). Sex-specific selection on plant architecture through "budget" and "direct" effects in experimental populations of the wind-pollinated herb, *Mercurialis annua*. *Evolution*, 73(5), 897-912. https://doi.org/10.1111/evo.13714

Wang, X., Li, X., Zhang, J., Feng, G., Zhang, S., Huang, L., Zhuo, R., & Jin, L. (2011). Characterization of nine alfalfa varieties for differences in ovule numbers and ovule sterility. *Australian Journal of Crop Science*, 5(4), 447-452. https://search.informit.org/doi/10.3316/informit.281539491135288

Waser, N. M., Chittka, L., Price, M. V., Williams, N. M., & Ollerton, J. (1996). Generalization in pollination systems, and why it matters. *Ecology*, 77(4), 1043-1060. https://doi.org/10.2307/2265575

Wu, Y., & Li, Q.-J. (2017). Phenotypic selection on flowering phenology and pollination efficiency traits between Primula populations with different pollinator assemblages. *Ecology and Evolution*, 7(19), 7599-7608. https://doi.org/10.1002/ece3.3258

SUPPLEMENTARY MATERIAL

1. Floral trait variation

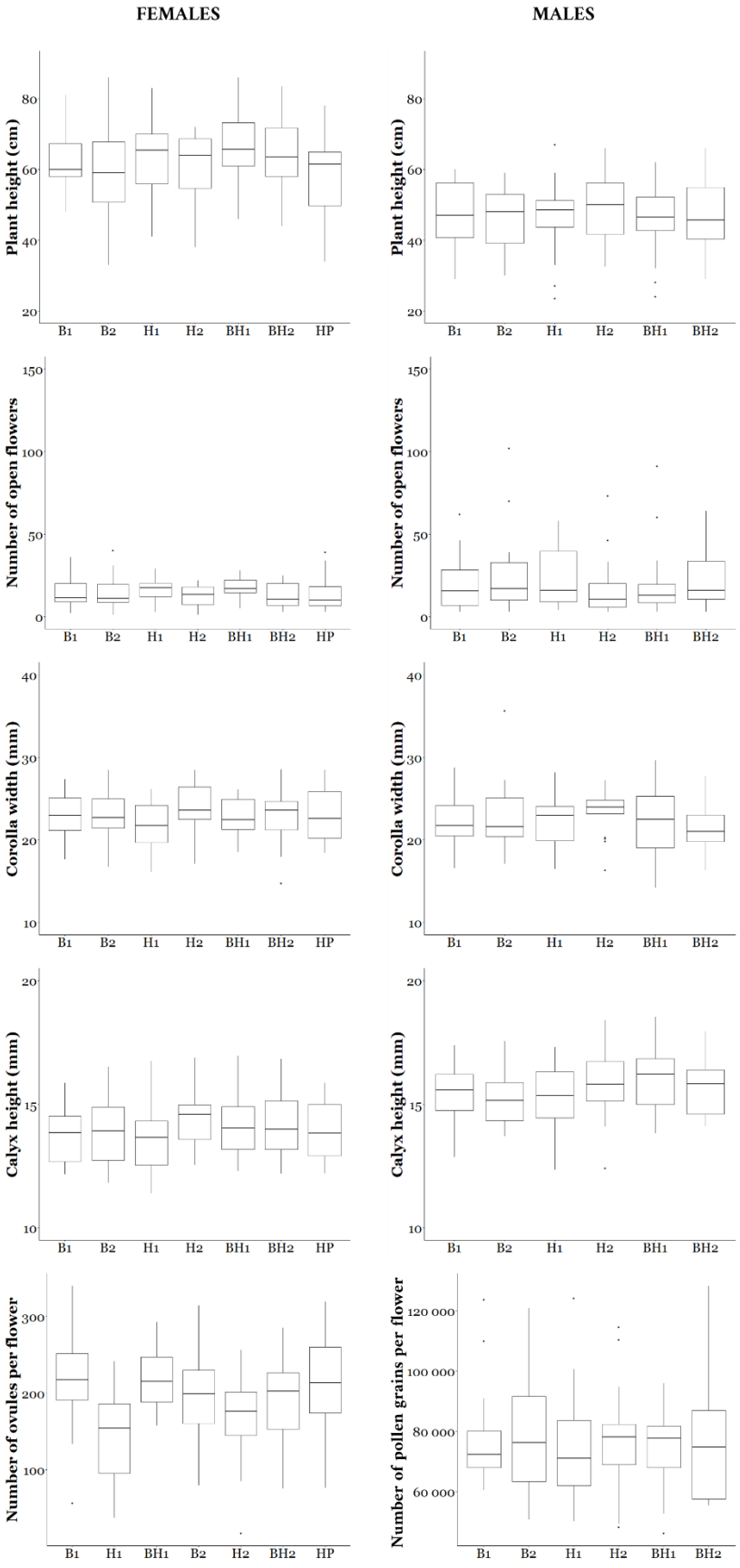
Table S1. Floral trait variation between sexes and experimental populations. Variation in floral traits between sexes and experimental populations was analysed using generalized linear models (GLM), with a negative binomial distribution for the number of open, and a Gaussian distribution for the height plant, the calyx height and the corolla width. Sex, experimental population and their interaction were treated as fixed effects. Height: plant height, Open flowers: number of open flowers, Corolla: corolla width, Calyx: calyx height, Ovules: number of ovules per flower in females, Pollen: number of pollen grains per flower in males.

Traits	F_{sex}	p_{sex}	F_{pop}	P_{pop}	$F_{sex*pop}$	$p_{sex*pop}$
Height	143.61	**< 0.001**	1.48	0.187	1.18	0.319
Open flowers	16.62	**< 0.001**	6.23	0.398	3.31	0.652
Corolla	1.34	0.249	1.37	0.228	0.52	0.760
Calyx	94.10	**< 0.001**	2.13	0.051	0.40	0.846
Ovules	-	-	4.14	**< 0.001**	-	-
Pollen	-	-	0.26	0.933	-	-

Table S2. Correlations among floral traits, for males (upper triangle) and females (lower triangle) in each experimental population (B: bumblebees, H: hoverflies, BH: mixed, HP: hand-pollinated), described using Pearson's correlation coefficient. Significant values after Bonferroni correction are indicated by stars (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$). Height: plant height, Open flowers: number of open flowers, Corolla: corolla width, Calyx: calyx height, Gametes: number of gametes (ovules for females, pollen grains for males) per flower.

		Height	Open flowers	Corolla	Calyx	Gametes
B1	Height		0.27	-0.07	0.01	-0.16
	Open flowers	-0.37		-0.46	-0.32	-0.08
	Corolla	0.08	0.10		0.39	0.09
	Calyx	0.21	0.27	**0.73****		-0.16
	Gametes	0.43	-0.21	0.05	0.06	
B2	Height		0.34	0.30	0.42	-0.01
	Open flowers	0.51		-0.10	-0.07	0.14
	Corolla	0.11	-0.29		0.57	-0.15
	Calyx	0.05	-0.17	0.44		0.12
	Gametes	-0.03	0.04	0.09	0.04	
H1	Height		0.11	0.44	0.05	-0.11
	Open flowers	0.43		0.12	0.10	-0.37
	Corolla	-0.06	-0.01		0.35	0.02
	Calyx	0.29	0.26	0.47		0.11
	Gametes	-0.18	-0.19	**0.60***	0.48	
H2	Height		0.22	0.34	0.30	0.23
	Open flowers	**0.65***		-0.19	-0.10	0.08
	Corolla	0.08	-0.03		0.10	-0.18
	Calyx	0.11	-0.01	**0.61***		0.26
	Gametes	-0.03	-0.30	0.11	0.18	
BH1	Height		0.39	-0.23	-0.17	0.17
	Open flowers	-0.15		-0.40	-0.47	0.16
	Corolla	0.26	-0.11		0.47	-0.30
	Calyx	0.11	-0.14	0.33		-0.38
	Gametes	0.22	0.02	0.00	-0.12	
BH2	Height		**0.67****	0.28	0.57	0.50
	Open flowers	0.48		-0.10	0.07	0.48
	Corolla	0.26	-0.04		0.16	0.30
	Calyx	0.12	-0.23	0.36		0.02
	Gametes	0.07	-0.20	0.16	0.16	
HP	Height					
	Open flowers	0.51				
	Corolla	-0.09	-0.03			
	Calyx	-0.31	-0.25	**0.61***		
	Gametes	-0.11	-0.38	-0.01	0.01	

Figure S1. Boxplots of floral traits measured in each experimental population, with females (left panel) and males (right panel) shown separately (B: bumblebees, H: hoverflies, BH: mixed, HP: hand-pollinated).



2. Pollen limitation

Table S3. Mean pollen limitation estimators (fruit set, seed set and number of seeds produced) in each experimental populations (B: bumblebees, H: hoverflies, BH: mixed, HP: hand-pollinated) and results of post-hoc Tukey tests comparing each insect-pollinated populations to the hand-pollinated population.

Population	Fruit set	$F_{fruit\ set}$	$p_{fruit\ set}$	*Seed set*	$F_{seed\ set}$	$p_{seed\ set}$	*Seed*	F_{seed}	p_{seed}
B1	0.937	0.211	0.999	0.418	2.89	0.060	1483.30	2.24	0.283
B2	0.930	0.220	0.999	0.504	1.24	0.880	1569.95	1.87	0.507
H1	0.554	13.05	**< 0.001**	0.213	7.47	**< 0.001**	311.00	7.27	**< 0.001**
H2	0.858	3.44	**< 0.01**	0.314	4.82	**< 0.001**	863.80	4.90	**< 0.001**
BH1	0.944	-0.855	0.977	0.402	3.093	**< 0.05**	1611.45	1.69	0.626
BH2	0.965	-2.28	0.242	0.409	2.99	**< 0.05**	1549.45	1.95	0.449
HP	0.926	-	-	0.562	-	-	2004.30	-	-

CHAPITRE IV

De la pollinisation à la paternité : déterminants et effet de la charge pollinique sur le succès de fertilisation

Les résultats du deuxième chapitre indiquent que la sélection sexuelle peut opérer chez les mâles, comme le montrent les gradients de Bateman positifs. Pourtant, nous n'avons pas détecté de sélection systématique sur les traits floraux connus pour attirer les pollinisateurs chez ces mâles. Ces observations pourraient suggérer que ce ne sont pas les processus pré-pollinisation, mais plutôt les processus post-pollinisation qui sont déterminants pour le succès reproducteur mâle. Jusqu'à présent, une catégorie de traits potentiellement impliqués dans la compétition intra-sexuelle n'avait pas été considérée : les traits polliniques. Ce dernier chapitre vise donc à évaluer leur rôle, en lien avec l'effet de la quantité de pollen déposée sur les stigmates. Après avoir exploré les facteurs déterminant les variations de charge pollinique stigmatique en conditions naturelles, nous réalisons dans ce dernier chapitre des croisements contrôlés afin de tester l'hypothèse selon laquelle la compétition pollinique devrait s'intensifier avec la quantité de pollen déposée sur les stigmates, engendrant ainsi une plus grande variance dans le succès de fertilisation profitant aux mâles produisant les grains de pollen les plus performants.

Manuscript D: From pollination to paternity: determinants and effect of pollen load on fertilization success

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Abstract

In plants, male-male competition can occur both before and after pollen deposition on the stigma of a receptive flower, when the stigmatic pollen load (i.e., the number of pollen grains deposited on the stigma) exceeds the number of ovules available for fertilization, and when these pollen grains originate from different donors. Pollen load is expected to affect the intensity of pollen competition, and the outcome of such competition likely depends on pollen traits. The objectives of this study were to identify the factors underlying variation in pollen load among female plants in natural populations of the dioecious species *Silene dioica*, and to evaluate the consequences of such variation for mating patterns. Field observations revealed substantial variation in pollen load, which was positively associated with both the proportion of males in the population and corolla width of the recipient plants. A hand pollination experiment was then performed to test the hypothesis that variance in male reproductive success would increase with pollen load, with males producing the best-germinating pollen grains or the fastest-growing pollen tubes pre-empting most ovules. Our results indicated non-random mating in more than 25% of the fruits. The proportion of seeds sired by each male was repeatable across fruits and depended on several pollen traits, but contrary to our expectations, paternity skew was not higher in the high pollen load treatment, indicating a limited effect of pollen load variation on male reproductive success variance. On the other hand, we detected a significant interaction between pollen tube growth speed and treatment, suggesting that faster pollen tube growth becomes relatively more advantageous under stronger pollen competition.

Key words

Pollen receipt, mating patterns, pollen competition, sexual selection

Contents

INTRODUCTION

Sexual selection, a powerful evolutionary force, arises from differences among individuals in their access to sexual partners and acts through two complementary processes: competition within a sex (intrasexual selection) and mate choice (intersexual selection) (Darwin, 1871 ; Trivers, 1972). Male gametes are typically produced in markedly larger quantities than female gametes, an asymmetry that should promote competition among males for access to females, while the latter are more likely to exert mate choice (Bateman, 1948 ; Trivers, 1972). In plants, competition among pollen grains for access to ovules can occur at two stages: before and after pollen deposition on the stigmas of receptive flowers. During the pre-pollination phase, any trait that enhances effective pollen transfer to sexual partners may represent a potential target of sexual selection. For example, in wind-pollinated species, selection can favour morphological traits that promote pollen dispersal (e.g., branches length, Tonnabel et al., 2019), while in animal-pollinated species, selection often targets traits involved in pollinator attraction, such as flower size or scent (Hodgins & Barrett, 2008 ; Kulbaba & Worley, 2012 ; Briscoe Runquist et al., 2017 ; Barbot et al., 2023). After pollen deposition on the stigmas, a subsequent phase of male-male competition may occur, characterized by competition among pollen grains for access to ovules. This second phase of competition is more likely to takes place if two conditions are fulfilled: (i) the number of pollen grains deposited on the stigma (i.e., stigmatic pollen load) must exceed that of ovules available for fertilization, and (ii) pollen grains from different males must reach the same stigma.

Regarding the first condition, studies examining variation in stigmatic pollen load under natural conditions often report substantial variation among populations and individuals (Emberlin & Norris-Hill, 1991 ; Németh & Smith-Huerta, 2003 ; Kulbaba & Worley, 2014), both in wind-pollinated and insect-pollinated species, with pollen loads sometimes exceeding the number of ovules available (Molano-Flores et al., 1999 ; Waites & Ågren, 2004 ; Friedman & Barrett, 2009). Demographic factors such as population size and density can influence pollen loads (Delmas et al., 2016 ; Labouche et al., 2017). Indeed, in insect-pollinated species—which represent more than 80% of all angiosperm species (Ollerton et al., 2011)—larger and more dense populations tend to attract more pollinators (i.e., "magnet effect"), resulting in larger stigmatic pollen loads (Sáez et al., 2014). Moreover, the quantity of pollen available should increase with the proportion of males (i.e., in dioecious species) within a population, while population density may affect the likelihood of pollen transfer (Delmas et al., 2016). Beyond demographic factors, pollinator abundance may also be influenced by environmental factors, particularly those related to habitat quality and landscape structure. In particular, habitat degradation and fragmentation caused by human activities may negatively impact pollen receipt by altering pollinator communities (Knight et al., 2005). For instance, a decline in pollinator abundance has been documented when comparing natural and agricultural habitats, or with increasing distance from forested areas (Ricketts et al., 2008 ; Winfree et al., 2009 ; Bailey et al., 2014), which might in turn affect average stigmatic pollen loads in plant populations. In generalist species, stigmatic pollen loads may further be influenced by the composition of local pollinator assemblages, provided that different pollinator taxa vary in their foraging behaviour and pollen transfer effectiveness (King et al., 2013). Within populations, the spatial distribution of plants and their ability to attract floral visitors can influence pollinator visitation rates and thus pollen deposition. Because pollen

dispersal is often spatially limited, especially in herbaceous species (Van Rossum et al., 2011 ; Barbot et al., 2022), isolated individuals may receive less pollen than those surrounded by many potential pollen donors (Stehlik & Barrett, 2006 ; Brys & Jacquemyn, 2009). In addition, floral traits, such as flower number, morphology, color, scent or nectar production are known to affect the frequency of pollinator visits (Giurfa et al., 1995 ; Totland, 2001 ; Bauer et al., 2017 ; Barbot et al., 2022), and thus pollen receipt (Koski & Ashman, 2015 ; Temeles et al., 2016).

Regarding the second condition, studies show that multiple paternity within fruit is common in flowering plants, indicating that stigmas often receive pollen from multiple donors, either sequentially or simultaneously (Campbell, 1998 ; Mitchell et al., 2005 ; Pannell & Labouche, 2013 ; Joffard et al., 2025). The extent of within-flower polyandry, usually inferred from the number of sires per fruit, may be influenced by various factors, including pollinator density and identity, as well as pollen carryover (i.e., proportion of pollen transferred from one flower to the next, Mitchell et al., 2013). In addition, demographic factors, such as density and distribution of individuals within populations, may also shape mating patterns and affect the likelihood that stigmas receive pollen from multiple donors (Rhodes et al., 2017).

When both conditions are fulfilled, pollen donors may compete for access to ovules. This should result in some males siring more seeds than others, provided that they differ in pollen performance, thereby increasing the variance in male reproductive success relative to a scenario where all males contribute equally to the seed pool. In other words, mating should become non-random (Snow & Spira, 1991b ; Björkman et al., 1995 ; Snow & Spira, 1996) and selection may target pollen traits that affect fertilization success, such as pollen tube growth speed (Snow & Spira, 1991a ; Skogsmyr & Lankinen, 2002). The intensity of pollen competition and selection on pollen traits should depend on the size of the pollen load, which should determine the opportunity for selective sorting (Christopher et al., 2020). As a result, variance in male reproductive success—which can be explored through the level of paternity correlation—is expected to increase with pollen load (Christopher et al., 2020).

In this study, we first assessed whether pollen load varied among female plants and among populations under natural conditions in the dioecious species *Silene dioica*, and we investigated the factors governing such variation, including demographic and ecological factors, as well as floral traits. Specifically, we tested the hypothesis that pollen load should increase with population size and density, proportion of males, forest cover and plant attractiveness. We then investigated the consequences of such variation in pollen load on mating patterns. Specifically, we tested the hypothesis that pollen competition should intensify with pollen load, resulting in an increase in male reproductive success variance. To this end, we conducted a hand pollination experiment using a mixture of pollen from five donors, which was applied under three pollen load treatments, and we performed paternity analyses to estimate each male's siring success. We also measured several pollen traits to assess their relationship with siring success. By jointly examining natural variation in pollen load in wild populations and its consequences for male reproductive success, this study provides insights into how pollen load and its determinants influence mating patterns and sexual selection in plants.

MATERIALS AND METHODS

1. Study species

Silene dioica (L.) Clairv. (Caryophyllaceae) is a herbaceous, perennial, and dioecious angiosperm widely distributed in central and northern Europe. The species is typically described as forest-associated, but it is also found across a range of semi-natural and anthropogenic habitats. It has a generalist pollination system and is primarily pollinated by bumblebees and hoverflies, bumblebees being its most frequent pollinator (Kay et al., 1984 ; Jolivel et al., in prep.). In northern France, the species' flowering period spans from late April to mid-July, peaking in late May (Barbot et al., 2022). This species exhibits sexual dimorphism in several floral traits such as flower size and number, with males displaying more conspicuous inflorescences (Kay et al., 1984 ; Moquet et al., 2020 ; Barbot et al., 2023). Individuals with both larger floral displays and larger flowers are known to attract more pollinators (Barbot et al., 2022 ; Moquet et al., 2022). Reproduction is typically promiscuous (Joffard et al., 2025). Male flowers have ten anthers, which together contain an average of 80,000 pollen grains, while female flowers have five stigmatic lobes, exserted beyond the corolla and leading to an ovary that contains on average 270 ovules (Jolivel et al., in prep.).

2. Variation in pollen load under natural conditions

a. Sampling design

Individual stigmas were sampled on 2 to 11 female plants per population (on average 8.67 ± 2.43 SD, 209 females in total)—after at least 24 hours of sunshine and with daily temperatures above 13°C—during the flowering peak in 2018 and 2019, across 24 natural populations distributed in northern France and Belgium (**Figure 1**). Sampled individuals were randomly selected and evenly distributed throughout each population. Population boundary coordinates were recorded to estimate the area occupied by the plants (m^2) (GPS 60 – Garmin, supplementary material – Habitat characterization).

b. Habitat characterization, population demography and individual floral traits

Habitat characterization was conducted using the QGIS software (version 3.30.0) and the GroupStats plugin (version 2.2.7), by calculating the proportion of different habitat types within a one-kilometer radius around the sampled populations. The one-kilometer radius was chosen because it aligns with the typical foraging range of bumblebees (Osborne et al., 2008). Three mapping data sources were used depending on the location of the population: data from the ARCH project, from the OS Picardie project and from the COSW project. The habitat types included forests, semi-natural areas, cropland, and urban areas (see manuscript A, supplementary material – Habitat characterization). A Principal Component Analysis (PCA) was performed to select variables to include in subsequent analyses. The first two axes accounted for 54.0% and 26.9% of the total variance. Forest and urban habitat surfaces were retained, as they best explained PC1 and PC2, respectively.

The size of each population was estimated based on the number of flowering individuals during sampling, distinguishing between males and females to estimate the proportion of males. Population density was estimated by dividing the total number of individuals in the population by the area occupied by plants. For each sampled female, the number of open flowers was counted and corolla width (mm) was measured on two randomly selected flowers using a caliper (precision: 0.01 mm) and averaged before subsequent analyses. Then, the whole stigma of one flower per sampled female was collected and stored in FAA (H2O, ethanol, formaldehyde, acetic acid; 2:6:1:1) until pollen counts.

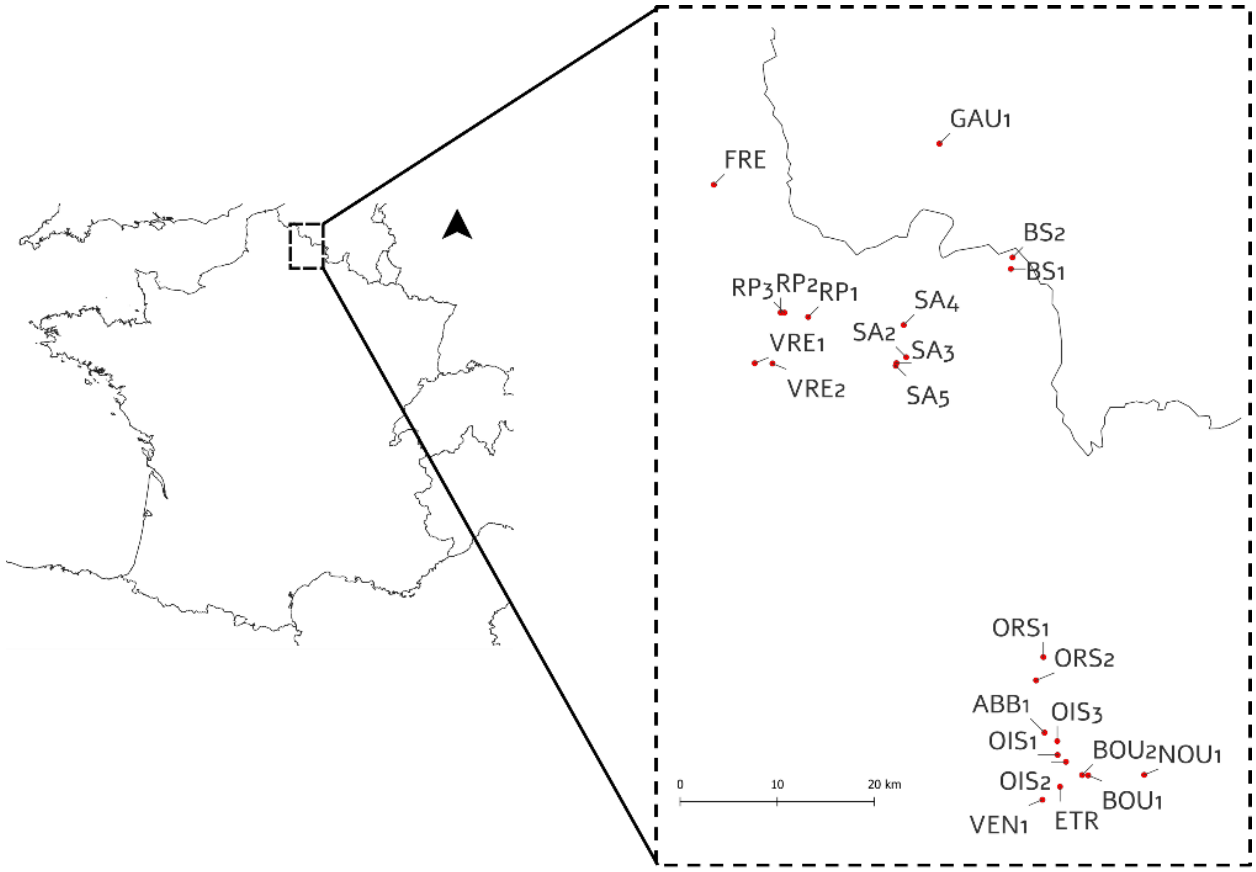

Figure 1. Location of the 24 populations of *S. dioica* sampled in this study. The grey line corresponds to the border between France and Belgium.

c. Pollen load estimation

To quantify the stigmatic pollen load, a single lobe was sampled per flower. Each stigmatic lobe was then mounted on microscope slides and observed under a fluorescence microscope at x50 magnification, where UV illumination made pollen grains visible without the need for staining (AxioImager2; ZEISS, Oberkochen, Germany). Pictures were taken all along each stigmatic lobe using ZEN software (ZEISS, Oberkochen, Germany) and a manual count was performed. Pollen counts were repeatable among stigmatic lobes of the same flower, as verified on eight flowers ($F_{1,7}$ = 0.52, p = 0.47). An estimate of overall pollen loads was obtained by multiplying the number of pollen grains counted on one stigmatic lobe by five.

d. Statistical analysis

A multiple regression analysis was performed with pollen load as the response variable and population size, proportion of males, density, forest and urban habitat surface, number of open flowers and corolla width as explanatory variables. Correlations among explanatory variables were either non-significant or weak (supplementary material – Variable correlations). To facilitate the comparison of effect sizes across predictors measured in different units, all explanatory variables were standardized (mean = 0, variance = 1) prior to analysis. Population and sample year were included as random factors.

3. Hand pollination experiment

a. Experimental design

Plant material originated from seeds sampled in wild populations in the Mormal Forest (northern France) and grown under controlled greenhouse conditions at the University of Lille.

Hand pollinations were conducted using a mixture of pollen from five donors, applied to the stigmas of 60 flowers from 10 female plants, under three pollen load treatments (small, medium and large). This protocol was replicated twice, using two different sets of five pollen donors and 10 female plants. Males were selected to ensure minimal variation in pollen viability (>96% for each male), as estimated using Peterson stain (Peterson et al., 2010).

For each replicate, pollen from all five males was collected from four dehiscent flowers per male, pooled together in a single Petri dish, and thoroughly mixed to create a homogeneous pollen mixture. The pollen mixture was then applied to the stigmas of six flowers per female plant, with two flowers per pollen load treatment (2 flowers × 3 treatments × 10 females = 60 flowers). To this end, a horsehair was dipped in the pollen mix and gently brushed onto each stigmatic lobe. This process was repeated two times (small pollen load treatment), 10 times (medium pollen load treatment) or 20 times (large pollen load treatment). Preliminary tests conducted on 30 flowers confirmed that this protocol generated significant different pollen load across treatments (366 ± 171 pollen grains for the small pollen load treatment, 1,272 ± 648 for the medium pollen load treatment and 2,830 ± 1,281 for the large pollen load treatment, $F_{2,25} = 156.42$, $p < 0.001$; supplementary material – Pollen deposition). A single pollen mixture was prepared for each replicate and applied on the same day by a single operator to minimize operator-related variation.

Fruits resulting from these crosses were harvested at maturity. The number of ovules per flower was estimated by dissecting each fruit and digitizing its content using a high-resolution scanner. Seeds and undeveloped ovules were distinguished based on size and counted using an R script (EBImage package in R, Pau et al., 2010). The number of ovules per flower was calculated as the sum of the seeds and undeveloped ovules. Then, for each fruit, seed set was calculated by dividing the number of seeds by the number of ovules.

b. Male reproductive success estimation

The total genomic DNA of all adults (30 plants: five males and 10 females × two replicates) and 1,920 offspring (16 seeds per fruit for 60 fruits × two replicates) was extracted using the NucleoMag 96 Plant kit (Macherey Nagel, Oesingen, Switzerland) and PCR amplifications were performed on five nuclear microsatellites following

Joffard et al. (2025) and Barbot et al. (2022). Then, the number of seeds sired by each male, in each fruit, was estimated using the categorical paternity assignment method implemented in CERVUS (version 3.0.7, Marshall et al., 1998 ; Kalinowski et al., 2007), with an 80% confidence criterion. This method identifies the most likely sire by comparing offspring genotypes to candidate males, accounting for a 2% genotyping error rate.

The proportion of seeds sired by each male in each fruit was then calculated, and the coefficient of correlated paternity r_p was calculated for each fruit as the sum of p_i^2, where p_i represents the proportion of seeds sired by the i-th pollen donor (Ritland, 1989). The r_p coefficient thus corresponds to the probability that two seeds from the same fruit were sired by the same male.

c. Pollen trait characterization

The number of pollen grains produced per flower and their size were estimated from two anthers collected from a nearly opened flower bud using a particle counter (CASY Cell Counter & Analyzer, OLS) and following the protocol of Dufaÿ et al. (2008). As pollen grain size distribution in *S. dioica* is multimodal (Jürgens et al., 2012), with two clear modes in our plant collection (25 µm vs. 30 µm, pers. obs.), the proportion of pollen grains in each category and their respective mean size were estimated. A two-component normal mixture model was fitted to estimate both the proportion of small and large pollen grains and the mean size of pollen grains in each category (*flexmix* package in R, Leisch, 2004).

In addition, three pollen traits were measured *in vitro*: pollen tube growth speed, maximum pollen tube length and pollen germination rate. To monitor pollen tube growth, for each male, pollen collected from three flowers with dehiscent anthers was deposited on a Petri dish containing a Brewbaker & Kwack solution (1963), sucrose (11%), agar (0.8%) and water. Petri dishes were kept in an incubator at 25°C during the whole experiment. Between 6 and 17 germinated pollen grains per male were photographed 2 hours and 4 hours after pollen deposition to monitor pollen tube elongation, at x50 magnification, using ZEN software (ZEISS, Oberkochen, Germany). Pollen tube length was measured using the ImageJ software (version 1.53). Then, pollen tube growth speed was calculated for each photographed pollen grains, as follow: (Length a T_1 – Length at T_0) / (T_1 – T_0), with T_0 corresponding to pollen deposition, and T_1 correspond to either 2 or 4 hours after pollen deposition. Note that because T_0 corresponds to pollen deposition and not to the actual start of germination, the calculated growth rate also reflects variation in germination speed. These speed measurements were then averaged across pollen tubes for each male. The length of the longest pollen tube (hereafter "maximum pollen tube length") was also recorded. Finally, 24 hours after pollen deposition, between 74 and 239 pollen grains per male were photographed and their germination was scored to calculate pollen germination rate for each male (i.e., number of germinated pollen grains / total number of pollen grains).

d. Statistical analysis

Effect of pollen load treatment on seed set – Seed set was compared among pollen load treatments using a GLMM with a binomial distribution including replicate and female identity nested within replicate as random effects. Post-hoc Tukey's tests were then performed for pairwise comparisons.

Mating patterns – To determine whether the contributions of the different males were equal or not and whether the variance in male reproductive success was affected by the treatment, observed values of r_p were compared to a theoretical distribution generated under a random mating scenario, assuming that the pollen mixture contained equal amounts of pollen from each male. To generate this distribution, the identities of five males were randomly drawn 16 times (corresponding to the 16 genotyped offspring per fruit) with replacement, and the coefficient of correlated paternity r_p was calculated based on these 16 draws. This procedure was repeated 100,000 times. An observed coefficient was considered as significantly deviating from the random expectation if it exceeded the 95th quantile of this theoretical distribution.

To assess the effect of pollen load treatment on mating patterns, we compared (i) the coefficient of correlated paternity r_p (modelled as a Gaussian response) and (ii) the probability that r_p significantly deviated from the random expectation of equal paternal contributions (modelled as a binomial response) across treatments. For both models, we included treatment as a fixed effect and female identity and replicate as random effects.

Next, we aimed to assess whether certain males consistently outperformed others, either due to higher competitive abilities or to an overrepresentation in the pollen mixture. To this end, we used the *rptR* package (Stoffel et al., 2017) to estimate the repeatability of male identity with respect to the proportion of seeds sired (modelled as a Gaussian response), including treatment as a fixed effect, as well as female and fruit identity as random effects. This analysis quantifies the consistency of male siring success across fruits, with repeatability defined as the ratio of among-fruit to total variance. This analysis was performed separately for each replicate (because different males were used in replicate 1 and 2). The significance of repeatability estimates was estimated using 1,000 permutations of male identities within each fruit, resulting in 1,000 repeatability estimates against which our observed values were compared. Then, we aimed at comparing repeatability among pollen load treatments, to test the hypothesis that the consistency of male reproductive success across fruits increased with pollen load, due to stronger pollen competition and higher opportunity for selective sorting. To this end, we calculated the repeatability of male identity with respect to the proportion of seeds sired separately for each treatment, including female and fruit identity as random effects and using 1,000 bootstrap iterations. For each iteration, we computed R_{dif}, the difference in repeatability between treatments for the following pairs: large *versus* medium pollen loads, medium *versus* small pollen loads, and large *versus* small pollen loads. To test for significant differences in repeatability between treatments, we compared the proportion of R_{dif} values inferior to 0 against a 0.05 threshold.

Finally, to assess whether male reproductive success depended on pollen traits, and whether this relationship varied among pollen load treatments, a multiple regression analysis was performed with proportion of seeds sired by each male as the response variable and pollen traits, treatment and interactions between each pollen traits and treatment as explanatory variables, including fruit identity and male-female couple as random effects. For this analysis, only fruits for which the coefficient of correlated paternity significantly deviated from the random expectation were considered. Prior to analysis, a Principal Component Analysis (PCA) was conducted on pollen traits in order to determine which ones to include (supplementary material – Plant traits). In line

with the results of this PCA, we included five pollen traits, putatively reflecting different functional aspects of male gametophyte performance: number of pollen grains per flower, proportion of small pollen grains, size of small pollen grains, pollen germination rate and pollen tube growth speed at 4h. Pollen traits were standardized (mean = 0, variance = 1) separately for each replicate.

All statistical analyses were performed using R (version 4.2.3). Normality of residuals and homogeneity of variance were verified for all linear models. Overdispersion in generalized linear models with a negative binomial distribution was checked and found to be less than 2. For the hand pollination experiment, data from one female were not included in the analyses because only 3 fruits out of 6 developed, and the number of seeds inside those fruits was very low (under 20 seeds, vs. 128 seeds on average in the rest of the dataset). Two additional flowers did not develop into fruits and were thus excluded from the analysis.

RESULTS

1. Variation in pollen load under natural conditions

The average pollen load across the whole data set was 552.50 ± 757.85 (SD) pollen grains, with values ranging from 0 to 3,530. Pollen load varied substantially among populations, with population means ranging from 48.00 ± 38.25 (SD) to 1,252.00 ± 339.77 (SD) pollen grains (**Figure 2**). Average pollen loads were found to exceed the number of ovules per flower in 67% of the sampled populations, and in 46% of the sampled flowers. Multiple regression analysis revealed a positive effect of proportion of males and corolla width on pollen load (proportion of males: $F_{1,197} = 4.234$; corolla width: $F_{1,197} = 5.835$; $p < 0.05$ in both cases). No other covariate showed a significant effect (**Table 1**).

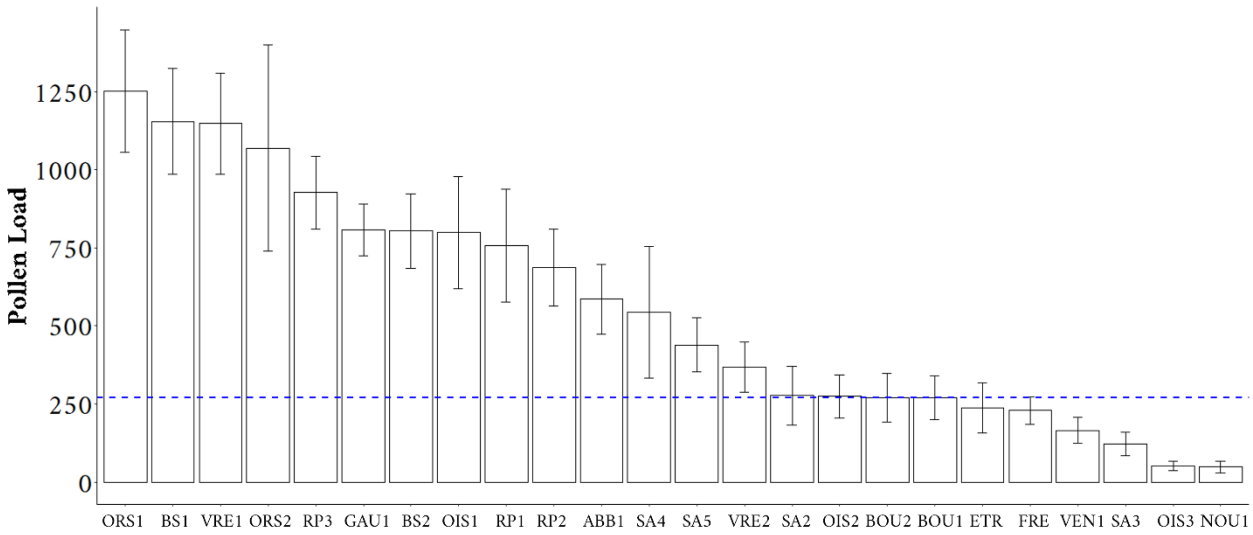

Figure 2. Average pollen load (± SE) per population, ordered from highest to lowest. The horizontal dashed line indicates the mean number of ovules per flower measured in six natural populations within the same area.

Table 1. Results of the multiple regression analysis, with pollen load as the response variable and population size, proportion of males, population density, proportion of forest and urban habitat surface, number of open flowers and corolla width of the recipient female as explanatory variables. Population of origin and sampling year were included as random variables in the model. All explanatory variables were centered and scaled prior to analysis. Variables indicated in bold had a significant effect on pollen load.

Predictor	Estimate	*F*	*p*
Population size	21.23	1.730	0.200
Proportion of males	44.66	4.234	**< 0.05**
Population density	8.00	0.094	0.569
Forest habitat surface	-11.77	0.254	0.583
Urban habitat surface	7.08	0.210	0.647
Number of flowers	-13.80	0.886	0.195
Corolla width	25.61	5.835	**< 0.05**

2. Hand pollination experiment

Effect of pollination treatment on seed set – Seed set varied among pollen load treatments ($F_{2,107}$ = 8.49, p < 0.001, **Figure 3**). Pairwise comparisons revealed that the large pollen load treatment differed from the other two (Small-Large: $F_{2,108}$ = -3.36, p < 0.01; Medium-Large: $F_{2,108}$ = 3.741, p < 0.001), with seed set being 6.30% and 5.95% lower than in the small and medium pollen load treatments, respectively. Seed set did not differ between the small and medium pollen load treatments (Small-Medium: $F_{2,108}$ = 0.38, p = 0.924).

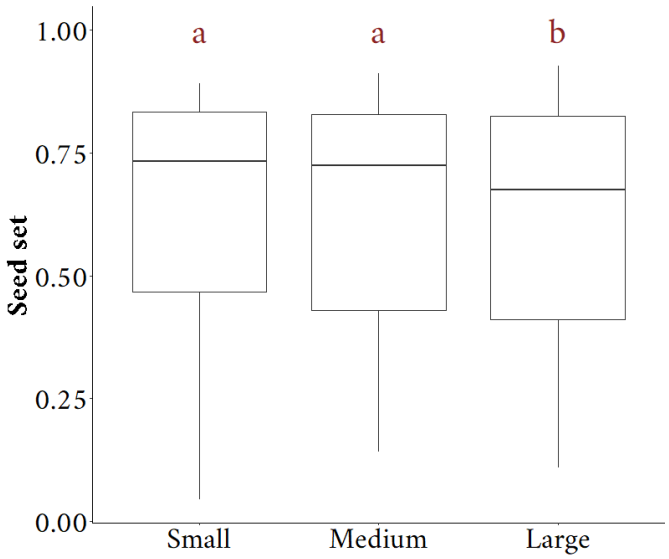

Figure 3. Seed set per fruit for each pollen load treatment. Different letters indicate statistically significant differences between treatments according to pairwise comparisons (p < 0.05).

Mating patterns – Paternity analyses resulted in 87% of genotyped seeds being successfully assigned to a male. The coefficient of correlated paternity (r_p) averaged 0.286 (± 0.065 SD) across the whole dataset, which is higher than the theoretical expectation of 0.200 under the random mating scenario assuming equal

representation of each male in the pollen mixture. This coefficient was not significantly different among pollen load treatments ($F_{2,108}$ = 0.835, p = 0.434), with mean r_p values of 0.279 ± 0.041 (SD), 0.281 ± 0.075 (SD) and 0.296 ± 0.074 (SD) for the small, medium and large pollen load treatments, respectively.

The coefficient of correlated paternity was higher than expected under the random mating scenario for 27% of the fruits across the whole dataset (**Figure 4**). Moreover, 18%, 24% and 38% of the fruits deviated from the random expectation in the small, medium and large pollen load treatments, respectively. However, this difference was not statistically significant ($F_{2,109}$ = 1.72, p = 0.179).

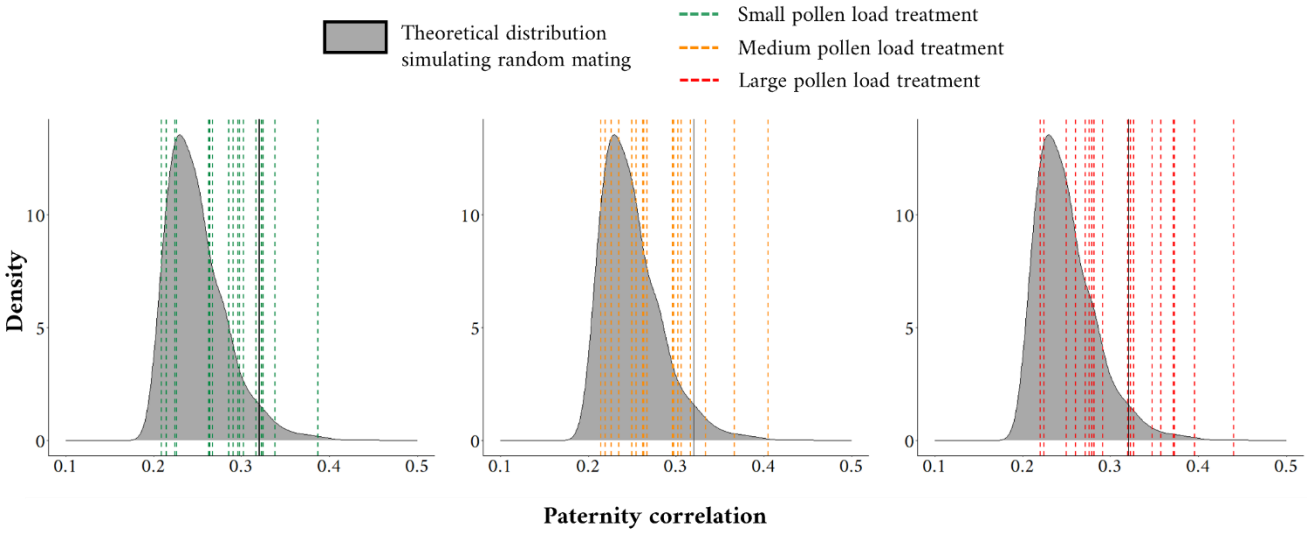

Figure 4. Observed values of r_p (coloured dashed lines) and theoretical distributions under the random mating scenario (grey shading), shown for all three pollen load treatments. The vertical grey line corresponds to the 95th quantile of the theoretical distribution.

Paternal contributions were repeatable across all fruits in both replicates, although repeatability was higher in replicate 2 than in replicate 1 (R = 0.125 for replicate 1 and R = 0.450 for replicate 2; $p < 0.05$ in both cases). To test whether repeatability estimates differed among pollen load treatments, we computed pairwise differences (R_{dif}) and tested their significance using bootstrap resampling. Pairwise comparisons revealed no significant difference in repeatability estimates among treatments in either replicate ($p > 0.05$).

Multiple regressions testing for the effect of pollen traits on paternal contributions indicated a positive effect of proportion of small pollen grains and size of small pollen grains, as well as a negative effect of germination rate and pollen tube growth speed at 4h (**Table 2**). A significant interaction between pollen tube growth speed at 4h and treatment was detected, where the negative relationship between this trait and siring success was more pronounced in the small and medium treatments than in the high treatment (**Figure 5**).

Table 2. Effect of pollen traits and treatments on paternal contributions. Pollen number: number of pollen grains per flower, % Small: percentage of small pollen grains, Size small: average pollen grain size in the first size class (15 to 25 µm), Germination rate: pollen germination rate, PTG 4h: pollen tube growth speed (µm/h) at 4h. Variables indicated in bold had a significant effect on siring success.

Variable	Estimate	*F*	*p*
Treatment	-	0.00	0.999
Pollen number	0.004	1.89	0.180
% Small	0.088	5.50	**< 0.001**
Size small	0.122	0.03	**< 0.001**
Germination rate	-0.032	5.85	**< 0.05**
PTG 4h	-0.122	17.01	**< 0.01**
Pollen number*Treatment	-	2.60	0.074
% Small*Treatment	-	0.03	0.673
Size small*Treatment	-	0.01	0.999
Germination rate*Treatment	-	0.32	0.664
PTG 4h* Treatment	-	2.05	**< 0.05**

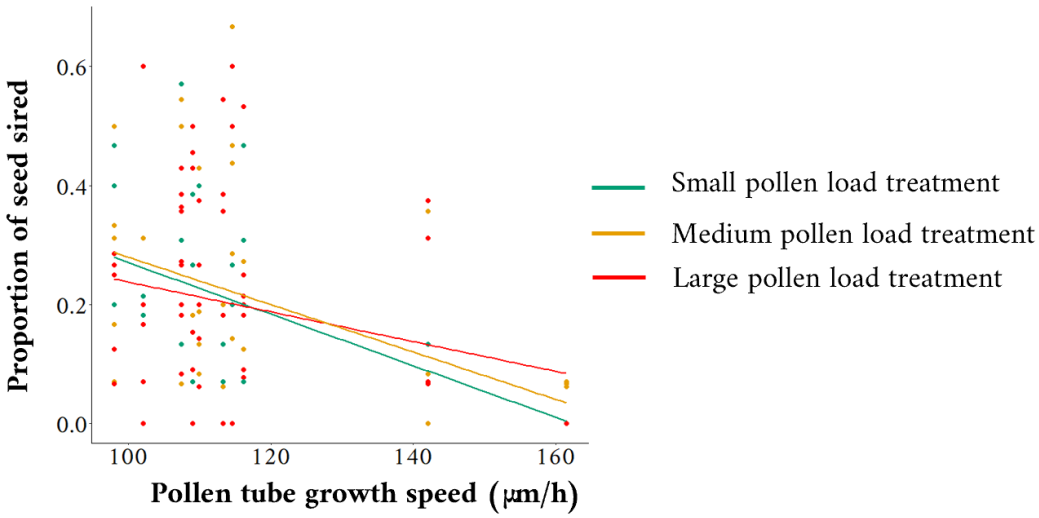

Figure 5. Effect of pollen tube growth speed (µm/h) at 4h on the proportion of seed sired by each male, for each pollen load treatment.

DISCUSSION

Variation in stigmatic pollen load among individuals and populations may lead to variation in the intensity of pollen competition, which may occur when pollen grains are deposited in greater amount than the number of ovules available for fertilization and originate from multiple donors. The aim of this study was, first, to investigate the determinants of pollen load variation among 24 populations of a dioecious species, *Silene dioica*, and secondly, to assess the impact of such variation on mating patterns. Field observations in natural populations showed that pollen load varied considerably among both individuals and populations, and was positively affected by the proportion of males in the population, as well as corolla width of recipient females. Moreover, in half of the cases, the number of pollen grains deposited on the stigma was higher than the typical number of ovules per flowers in this species, potentially creating opportunities for pollen competition to occur. Secondly, a hand pollination experiment was conducted by applying a mix of pollen from five males, with three different pollen loads. Paternal contributions deviated from the random expectation in 27% of the fruits across the whole dataset, and these contributions were repeatable across fruits (R = 0.125 and R = 0.450 for replicates 1 and 2, respectively). Paternity skew did not differ among treatments, but variation in siring success was associated with variation in several pollen traits, and a significant interaction between pollen tube growth speed and treatment was detected. Hereafter, we discuss these results in the light of the ecological and evolutionary consequences of pollen load variation and pollen competition in natural populations.

Sex-ratio and flower size determine pollen receipt

Substantial variation in pollen load was observed across the 24 populations sampled in this study, with population means ranging from 48 to 1,252 pollen grains per stigma. Our results show that the more male-biased the sex-ratio is, the larger the mean pollen load tends to be, suggesting that pollen availability is an important factor governing pollen receipt in natural populations of *S. dioica*. Male plants are known to produce more flowers than female plants in *S. dioica* (Moquet et al., 2020), leading to a substantially male-biased floral sex-ratio. Consequently, pollinators are more likely to visit multiple male flowers successively, thereby collecting more pollen before depositing it on a female flower (Aizen, 2001). Female plants are also more likely to be located near male plants, which further increases the amount of pollen deposited on their stigmas (Aizen, 1997). Contrary to our expectations, population size did not affect mean pollen load, suggesting that pollen availability relative to the number of receptive female flowers is more important than absolute pollen production for pollen receipt. This could be explained by the limited amount of pollen that pollinators can carry on their bodies (i.e., pollinators may visit multiple male flowers, but the maximum amount of pollen adhering to their bodies may be quickly reached, O'Neill & O'Neill, 2011). Likewise, stigmas may have a limited capacity for pollen receipt, meaning that only a finite number of pollen grains may successfully adhere (Henselek et al., 2018). Our findings also suggests that neither population size nor density strongly affect pollinator density (i.e., no magnet effect), or that increased pollinator density does not translate into larger pollen loads. Beyond these demographic parameters, habitat characteristics could also influence pollinator communities and, in turn, pollen receipt. Here, we hypothesized that habitat degradation could negatively

influence pollinator density, thereby reducing pollinator visitation rates and ultimately limiting pollen receipt, an effect that has been reported in other systems. For example, Irwin et al. (2018) found reduced pollen loads in urban compared to non-urban habitats in *Gelsemium sempervirens*. However, pollinator response to habitat quality may be highly context-dependant, depending on pollinator identity, resource availability or climatic conditions (Koski et al., 2018 ; Shaw et al., 2020). In our study, no effect of habitat was observed on pollen load. Because pollination in *S. dioica* is highly generalist, pollination may be resilient to variation in habitat quality because a wide range of insect visitors are capable of pollen transfer. Moreover, pollinator decline may not necessarily translate into reduced pollen receipt, for example due to compensatory mechanisms such as increased insect foraging effort in degraded environments (Goverde et al., 2002).

Variation in pollen load was also apparent among individuals within populations. Such variation was partly explained by variation in corolla width among recipient females, in line with the previously reported positive effect of this trait on pollinator attraction in various plant species (Stanton & Preston, 1988 ; Conner & Rush, 1996 ; Delgado et al., 2023), including *S. dioica* (Barbot et al., 2022). In contrast, we found no effect of floral display size (i.e., number of open flowers) on pollen load, unlike previous studies (Harder & Johnson, 2005 ; Brys & Jacquemyn, 2009) and despite its well-established role in pollinator attraction (Ohashi & Yahara, 1998 ; Harder & Barrett, 1995 ; Bauer et al., 2017), including in *S. dioica* (Barbot et al., 2022 ; Moquet et al., 2022). A potential explanation for this result could be that although plants with larger floral displays tend to attract more pollinators, the increased number of flowers available for visitation may equalize the number of visits received per flower. Alternatively, stigmatic pollen load may not increase with flower visitation rate if stigmas experience pollen saturation, as hypothesized earlier.

High pollen loads were observed under natural conditions, suggesting opportunities for post-pollination processes to occur. However, note that even small pollen loads are not necessarily indicative of insufficient pollen receipt, as our pollen load data represent a snapshot taken at a single point during the lifespan of female flowers (i.e., we cannot exclude that additional pollen grains would have been received later).

Unequal paternal contributions, independently of pollen load

To explore the potential consequences of variation in pollen load on mating patterns, a hand pollination experiment was conducted, involving three pollen load treatments. Unequal paternal contributions were observed in more than 25% of the fruits. Since the same pollen mix was used to pollinate all flowers within a replicate, a systematic overrepresentation of one or several males in the mixture should have resulted in consistent dominance across fruits. Yet, we observed equal paternal contribution in about 75% of them. This suggests that the observed deviations are unlikely to stem from variation in pollen proportions within the mix, and may instead reflect differences in male competitive abilities or male-female compatibilities.

Paternal contributions were found to be repeatable across fruits (R = 0.125 and R = 0.450 in replicate 1 and 2, respectively). However, it is worth noting that repeatability was lower in replicate 1 compared to replicate 2. In replicate 1, males that contributed the most to the seed pool were not consistently the same across females, suggesting that no male was intrinsically superior or had consistently higher competitive

abilities. Here, siring success may depend on male-female compatibilities (Lankinen et al., 2016). Such compatibility effects may explain why one of the males (M03) did not sire any seed in one of the female (F04) in replicate 1. In contrast, in replicate 2, the high repeatability values observed for the proportion of seeds sired by each male suggests that some—in this case, one in particular—perform well on all maternal plants, while others consistently sire few seeds, indicating variation in pollen performance among males.

On the other hand, our results indicate no statistically significant effect of pollen load size treatment on paternity skew, despite the higher potential for selective sorting with increasing pollen load. One explanation for this finding could be that the traits influencing fertilization success are density-independent, so that males with higher intrinsic pollen performances will sire more seeds, regardless of whether ovules are limiting or not. However, because in all three treatments, the number of pollen grains exceeded the number of ovules available for fertilization (as indicated by the fact that seed set did not increase with pollen load), we cannot exclude a density-dependant, but asymptotic advantage, whereby competitive males would sire more seeds than their rivals when ovules are limiting, but not more so when pollen competition is strong than when it is weak. Interestingly, our results show that seed set was lower for the high pollen load treatment than for the two others, which may be explained by stigma clogging, whereby large amounts of pollen deposited simultaneously on the stigma can form clumps that prevent additional pollen grains from adhering to the stigmatic surface, as observed in *Brassica napus* (Lankinen et al., 2018).

Pollen traits determining male reproductive success

Our results show a positive effect of both the proportion and size of small pollen grains, and a negative effect of germination rate and pollen tube growth speed on the proportion of seeds sired by each male. Due to a higher surface-to-volume ratio in smaller pollen grains, the latter tend to rehydrate faster, which may give them an advantage in the race for ovule fertilization (Cruzan, 1990). On the other hand, pollen grain size is known to be correlated with nutrient reserves, particularly starch (Baker & Baker, 1979), which could explain why this trait often correlates with pollen tube growth speed and length (Kumar & Sarkar, 1980 ; Fratini et al., 2006), thereby influencing fertilization rate (Delph et al., 1998). However, in our case, we suspect that the observed positive effect of pollen grain size on siring success arises from a correlation between this trait and pollen viability. Indeed, the small pollen grains category includes both viable and non-viable pollen grains, the latter being the smallest within this category (pers. obs.). Therefore, the positive effect of pollen size likely reflects a positive effect of pollen viability rather than size *per se*.

Pollen tube growth speed was shown to be an important pollen trait promoting fertilization success in various species (e.g., in *Hibiscus moscheutos,* Snow & Spira, 1991a ; in *Silene vulgaris,* Delph et al., 1998 ; in *Violor tricolor,* Skogsmyr & Lankinen, 1999), while in this study, we found a negative correlation between this trait and siring success. Although we have no obvious explanation for such this finding, it is worth noting that *in vitro* may not always reflect *in vivo* pollen performances (Stone et al., 1995 ; Biewer-Heisler et al., 2024). Nevertheless, a significant interaction between pollen tube growth speed and treatment was detected, whereby the negative effect of this trait on siring success was reduced when increasing pollen deposition. This pattern

could suggest a negative correlation between pollen tube growth speed and another, unmeasured trait that promotes fertilization success. The relative advantage of these traits may vary with the intensity of pollen competition, with pollen tube growth becoming increasingly beneficial as pollen competition intensifies.

CONCLUSION

In this study, we investigated the causes and consequences of variation in pollen load in the dioecious species *Silene dioica* using two complementary approaches. Natural variation in pollen load was found to be governed by the proportion of males in the population and corolla width of recipient females. In half of the cases, flowers received more pollen grains than needed to fertilize all of their ovules, enabling pollen competition. The consequences of pollen load variation on mating patterns were further studied through experimental crosses. Both paternity correlation and number of fruits with unequal paternal contributions increased with pollen load, but this difference was not statistically significant, pointing to a low effect size. As pollen competition intensifies, the effect of pollen tube growth speed on the proportion of seeds sired by males was shown to become less negative. These findings suggest that while increasing pollen deposition does not alter variance in male reproductive success, the traits driving fertilization success may depend on the competitive context.

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REFERENCES

Aizen, M. A. (1997). Influence of local floral density and sex ratio on pollen receipt and seed output : Empirical and experimental results in dichogamous *Alstroemeria aurea* (Alstroemeriaceae). *Oecologia*, 113(3), 404-412. https://doi.org/10.1007/s004420050252

Aizen, M. A. (2001). Flower sex ratio, pollinator abundance, and the seasonal pollination dynamics of a protandrous plant. *Ecology*, 82(1), 127-144. https://doi.org/10.1890/0012-9658(2001)082[0127:FSRPAA]2.0.CO;2

Bailey, S., Requier, F., Nusillard, B., Roberts, S. P. M., Potts, S. G., & Bouget, C. (2014). Distance from forest edge affects bee pollinators in oilseed rape fields. *Ecology and Evolution*, 4(4), 370-380. https://doi.org/10.1002/ece3.924

Baker, H. G., & Baker, I. (1979). Starch in angiosperm pollen grains and its evolutionary significance. *American Journal of Botany*, 66(5), 591-600. https://doi.org/10.1002/j.1537-2197.1979.tb06262.x

Barbot, E., Dufaÿ, M., & De Cauwer, I. (2023). Sex-specific selection patterns in a dioecious insect-pollinated plant. *Evolution*, 77(7), 1578-1590. https://doi.org/10.1093/evolut/qpad069

Barbot, E., Dufaÿ, M., Tonnabel, J., Godé, C., & De Cauwer, I. (2022). On the function of flower number : Disentangling fertility from pollinator-mediated selection. *Proceedings of the Royal Society B: Biological Sciences*, 289(1987), 20221987. https://doi.org/10.1098/rspb.2022.1987

Bateman, A. J. (1948). Intra-sexual selection in *Drosophila*. *Heredity*, 2(3), 349-368. https://doi.org/10.1038/hdy.1948.21

Bauer, A. A., Clayton, M. K., & Brunet, J. (2017). Floral traits influencing plant attractiveness to three bee species : Consequences for plant reproductive success. *American Journal of Botany*, 104(5), 772-781. https://doi.org/10.3732/ajb.1600405

Biewer-Heisler, T. J., Gibson, M. J., Sornay, E., & Moyle, L. C. (2024). *Gene expression and pollen performance indicate altered postmating selection between* Solanum *species with different mating systems*. https://doi.org/10.1101/2024.10.31.621143

Björkman, T., Samimy, C., & Pearson, K. J. (1995). Variation in pollen performance among plants of *Fagopyrum esculentum*. *Euphytica*, 82(3), 235-240. https://doi.org/10.1007/BF00029566

Brewbaker, J. L., & Kwack, B. H. (1963). The essential role of calcium ion in pollen germination and pollen tube growth. *American Journal of Botany*, 50(9), 859-865. https://doi.org/10.2307/2439772

Briscoe Runquist, R. D., Geber, M. A., Pickett-Leonard, M., & Moeller, D. A. (2017). Mating system evolution under strong pollen limitation : Evidence of disruptive selection through male and female fitness in *Clarkia xantiana*. *The American Naturalist*, 189(5), 549-563. https://doi.org/10.1086/691192

Brys, R., & Jacquemyn, H. (2009). Floral display size and spatial distribution of potential mates affect pollen deposition and female reproductive success in distylous *Pulmonaria officinalis* (Boraginaceae). *Plant Biology*. https://doi.org/10.1111/j.1438-8677.2009.00259.x

Campbell, D. R. (1998). Multiple paternity in fruits of *Ipomopsis Aggregata* (Polemoniaceae). *American Journal of Botany*, 85(7), 1022-1027. https://doi.org/10.2307/2446369

Christopher, D. A., Mitchell, R. J., & Karron, J. D. (2020). Pollination intensity and paternity in flowering plants. *Annals of Botany*, 125(1), 1-9. https://doi.org/10.1093/aob/mcz159

Conner, J. K., & Rush, S. (1996). Effects of flower size and number on pollinator visitation to wild radish, *Raphanus raphanistrum*. *Oecologia*, 105(4), 509-516. https://doi.org/10.1007/BF00330014

Cruzan, M. B. (1990). Variation in pollen size, fertilization ability, and postfertilization siring ability in *Erythronium grandiflorum*. *Evolution*, 44(4), 843-856. https://doi.org/10.1111/j.1558-5646.1990.tb03809.x

Darwin, C. (1871). *The descent of man, and selection in relation to sex*. John Murray, London.

Delgado, T., Leal, L. C., El Ottra, J. H. L., Brito, V. L. G., & Nogueira, A. (2023). Flower size affects bee species visitation pattern on flowers with poricidal anthers across pollination studies. *Flora*, 299, 152198. https://doi.org/10.1016/j.flora.2022.152198

Delmas, C. E. L., Fort, T. L. C., Escaravage, N., & Pornon, A. (2016). Pollen transfer in fragmented plant populations : Insight from the pollen loads of pollinators and stigmas in a mass-flowering species. *Ecology and Evolution*, 6(16), 5663-5673. https://doi.org/10.1002/ece3.2280

Delph, L. F., Weinig, C., & Sullivan, K. (1998). Why fast-growing pollen tubes give rise to vigorous progeny : The test of a new mechanism. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 265(1399), 935-939. https://doi.org/10.1098/rspb.1998.0381

Dufaÿ, M., Vaudey, V., De Cauwer, I., Touzet, P., Cuguen, J., & Arnaud, J. -F. (2008). Variation in pollen production and pollen viability in natural populations of gynodioecious *Beta vulgaris* ssp. *maritima* : Evidence for a cost of restoration of male function? *Journal of Evolutionary Biology*, 21(1), 202-212. https://doi.org/10.1111/j.1420-9101.2007.01454.x

Emberlin, J., & Norris-Hill, J. (1991). Spatial variation of pollen deposition in north london. *Grana*, 30(1), 190-195. https://doi.org/10.1080/00173139109427798

Fratini, R., García, P., & Ruiz, M. L. (2006). Pollen and pistil morphology, *in vitro* pollen grain germination and crossing success of *Lens* cultivars and species. *Plant Breeding*, 125(5), 501-505. https://doi.org/10.1111/j.1439-0523.2006.01277.x

Friedman, J., & Barrett, S. C. H. (2009). The consequences of monoecy and protogyny for mating in wind-pollinated *Carex*. *New Phytologist*, 181(2), 489-497. https://doi.org/10.1111/j.1469-8137.2008.02664.x

Giurfa, M., Nunez, J., Chittka, L., & Menzel, R. (1995). Colour preferences of flower-naive honeybees. *Journal of Comparative Physiology A*, 177(3), 247-259. https://doi.org/10.1007/BF00192415

Goverde, M., Schweizer, K., Baur, B., & Erhardt, A. (2002). Small-scale habitat fragmentation effects on pollinator behaviour : Experimental evidence from the bumblebee *Bombus veteranus* on calcareous grasslands. *Biological Conservation*, 104(3), 293-299. https://doi.org/10.1016/s0006-3207(01)00194-x

Harder, L. D., & Barrett, S. C. H. (1995). Mating cost of large floral displays in hermaphrodite plants. *Nature*, 373(6514), 512-515. https://doi.org/10.1038/373512a0

Harder, L. D., & Johnson, S. D. (2005). Adaptive plasticity of floral display size in animal-pollinated plants. *Proceedings of the Royal Society B*: *Biological Sciences*, 272(1581), 2651-2657. https://doi.org/10.1098/rspb.2005.3268

Henselek, Y., Eilers, E. J., Kremen, C., Hendrix, S. D., & Klein, A.-M. (2018). Pollination requirements of almond (*Prunus dulcis*) : Combining laboratory and field experiments. *Journal of Economic Entomology*, 111(3), 1006-1013. https://doi.org/10.1093/jee/toy053

Hodgins, K. A., & Barrett, S. C. H. (2008). Natural selection on floral traits through male and female function in wild populations of the heterostylous daffodil *Narcissus triandrus*. *Evolution*, 62(7), 1751-1763. https://doi.org/10.1111/j.1558-5646.2008.00404.x

Irwin, R. E., Warren, P. S., & Adler, L. S. (2018). Phenotypic selection on floral traits in an urban landscape. *Proceedings of the Royal Society B*: *Biological Sciences*, 285(1884), 20181239. https://doi.org/10.1098/rspb.2018.1239

Joffard, N., Lecat, I., Ollier, K., Jolivel, C., Schmitt, E., Godé, C., Barbot, E., Dufaÿ, M., & De Cauwer, I. (2025). Floral display size does not affect the distribution of paternal diversity in *Silene dioica*. *bioRxiv*. https://doi.org/10.1101/2025.07.19.665651

Jolivel, C., Joffard, N., Godé, C., Schmitt, E., & De Cauwer, I. (in prep.). Habitat differences do not disrupt sexual selection patterns in *Silene dioica*.

Jürgens, A., Witt, T., & Gottsberger, G. (2012). Pollen grain size variation in Caryophylloideae : A mixed strategy for pollen deposition along styles with long stigmatic areas? *Plant Systematics and Evolution*, 298(1), 9-24. https://doi.org/10.1007/s00606-011-0518-z

Kalinowski, S. T., Taper, M. L., & Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, 16(5), 1099-1106. https://doi.org/10.1111/j.1365-294X.2007.03089.x

Kay, Q. O. N., Lack, A. J., Bamber, F. C., & Davies, C. R. (1984). Differences between sexes in floral morphology, nectar production and insect visits in a dioecious species, *Silene dioica*. *New Phytologist*, 98(3), 515-529. https://doi.org/10.1111/j.1469-8137.1984.tb04145.x

King, C., Ballantyne, G., & Willmer, P. G. (2013). Why flower visitation is a poor proxy for pollination : Measuring single-visit pollen deposition, with implications for pollination networks and conservation. *Methods in Ecology and Evolution*, 4(9), 811-818. https://doi.org/10.1111/2041-210X.12074

Knight, T. M., Steets, J. A., Vamosi, J. C., Mazer, S. J., Burd, M., Campbell, D. R., Dudash, M. R., Johnston, M. O., Mitchell, R. J., & Ashman, T.-L. (2005). Pollen limitation of plant reproduction : Pattern and process. *Annual Review of Ecology, Evolution, and Systematics*, 36(1), 467-497. https://doi.org/10.1146/annurev.ecolsys.36.102403.115320

Koski, M. H., & Ashman, T.-L. (2015). An altitudinal cline in UV floral pattern corresponds with a behavioral change of a generalist pollinator assemblage. *Ecology*, 96(12), 3343-3353. https://doi.org/10.1890/15-0242.1

Koski, M. H., Ison, J. L., Padilla, A., Pham, A. Q., & Galloway, L. F. (2018). Linking pollinator efficiency to patterns of pollen limitation : Small bees exploit the plant–pollinator mutualism. *Proceedings of the Royal Society B*: *Biological Sciences*, 285(1880), 20180635. https://doi.org/10.1098/rspb.2018.0635

Kulbaba, M. W., & Worley, A. C. (2012). Selection on floral design in *Polemonium brandegeei* (Polemoniaceae) : Female and male fitness under hawkmoth pollination. *Evolution*, 66(5), 1344-1359. https://doi.org/10.1111/j.1558-5646.2011.01536.x

Kulbaba, M. W., & Worley, A. C. (2014). Patterns of pollen removal and deposition in *Polemonium brandegeei* (Polemoniaceae) : The role of floral visitors, floral design and sexual interference. *Plant Biology*, 16(6), 1087-1095. https://doi.org/10.1111/plb.12163

Kumar, D., & Sarkar, K. (1980). Correlations between pollen diameter and rate of pollen tube growth in maize (*Zea mays* L.). *Indian Journal of Experimental Biology*, 18, 1242-1244. https://www.cabidigitallibrary.org/doi/full/10.5555/19831618252

Labouche, A., Richards, S. A., & Pannell, J. R. (2017). Effects of pollination intensity on offspring number and quality in a wind-pollinated herb. *Journal of Ecology*, 105(1), 197-208. https://doi.org/10.1111/1365-2745.12659

Lankinen, Å., Lindström, S. A. M., & D'Hertefeldt, T. (2018). Variable pollen viability and effects of pollen load size on components of seed set in cultivars and feral populations of oilseed rape. *PLoS ONE*, 13(9), e0204407. https://doi.org/10.1371/journal.pone.0204407

Lankinen, Å., Smith, H. G., Andersson, S., & Madjidian, J. A. (2016). Selection on pollen and pistil traits during pollen competition is affected by both sexual conflict and mixed mating in a self-compatible herb. *American Journal of Botany*, 103(3), 541-552. https://doi.org/10.3732/ajb.1500148

Leisch, F. (2004). FlexMix : A general framework for finite mixture models and latent class regression in *R*. *Journal of Statistical Software*, 11(8). https://doi.org/10.18637/jss.v011.i08

Marshall, T. C., Slate, J., Kruuk, L. E. B., & Pemberton, J. M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, 7(5), 639-655. https://doi.org/10.1046/j.1365-294x.1998.00374.x

Mitchell, R. J., Karron, J. D., Holmquist, K. G., & Bell, J. M. (2005). Patterns of multiple paternity in fruits of *Mimulus ringens* (Phrymaceae). *American Journal of Botany*, 92(5), 885-890. https://doi.org/10.3732/ajb.92.5.885

Mitchell, R. J., Wilson, W. G., Holmquist, K. G., & Karron, J. D. (2013). Influence of pollen transport dynamics on sire profiles and multiple paternity in flowering plants. *PLoS ONE*, 8(10), e76312. https://doi.org/10.1371/journal.pone.0076312

Molano-Flores, B., Hendrix, S. D., & Heard, S. B. (1999). The effect of population size on stigma pollen load, fruit set, and seed set in *Allium stellatum* Ker. (Liliaceae). *International Journal of Plant Sciences*, 160(4), 753-757. https://doi.org/10.1086/314160

Moquet, L., Jacquemart, A.-L., Dufaÿ, M., & De Cauwer, I. (2022). Effects of sexual dimorphism on pollinator behaviour in a dioecious species. *Oikos*, 2022(3), e08662. https://doi.org/10.1111/oik.08662

Moquet, L., Lateur, L., Jacquemart, A.-L., De Cauwer, I., & Dufaÿ, M. (2020). Temporal dynamics of sexual dimorphism in a dioecious species. *Annals of Botany*, 126(3), 471-480. https://doi.org/10.1093/aob/mcaa088

Németh, M. B., & Smith-Huerta, N. L. (2003). Pollen deposition, pollen tube growth, seed production, and seedling performance in natural populations of *Clarkia unguiculata* (Onagraceae). *International Journal of Plant Sciences*, 164(1), 153-164. https://doi.org/10.1086/344549

Ohashi, K., & Yahara, T. (1998). Effects of variation in flower number on pollinator visits in *Cirsium purpuratum* (Asteraceae). *American Journal of Botany*, 85(2), 219-224. https://doi.org/10.2307/2446309

Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, 120(3), 321-326. https://doi.org/10.1111/j.1600-0706.2010.18644.x

O'Neill, R. P., & O'Neill, K. M. (2011). Pollen load composition and size in the leafcutting bee *Megachile rotundata* (Hymenoptera : Megachilidae). *Apidologie*, 42(2), 223-233. https://doi.org/10.1051/apido/2010057

Osborne, J. L., Martin, A. P., Carreck, N. L., Swain, J. L., Knight, M. E., Goulson, D., Hale, R. J., & Sanderson, R. A. (2008). Bumblebee flight distances in relation to the forage landscape. *Journal of Animal Ecology*, 77(2), 406-415. https://doi.org/10.1111/j.1365-2656.2007.01333.x

Pannell, J. R., & Labouche, A.-M. (2013). The incidence and selection of multiple mating in plants. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368(1613), 20120051. https://doi.org/10.1098/rstb.2012.0051

Pau, G., Fuchs, F., Sklyar, O., Boutros, M., & Huber, W. (2010). EBImage—An R package for image processing with applications to cellular phenotypes. *Bioinformatics*, 26(7), 979-981. https://doi.org/10.1093/bioinformatics/btq046

Peterson, R., Slovin, J. P., & Chen, C. (2010). A simplified method for differential staining of aborted and non-aborted pollen grains. *International Journal of Plant Biology*, 1(2), e13. https://doi.org/10.4081/pb.2010.e13

Rhodes, M. K., Fant, J. B., & Skogen, K. A. (2017). Pollinator identity and spatial isolation influence multiple paternity in an annual plant. *Molecular Ecology*, 26(16), 4296-4308. https://doi.org/10.1111/mec.14115

Ricketts, T. H., Regetz, J., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., Bogdanski, A., Gemmill-Herren, B., Greenleaf, S. S., Klein, A. M., Mayfield, M. M., Morandin, L. A., Ochieng', A., & Viana, B. F. (2008). Landscape effects on crop pollination services : Are there general patterns? *Ecology Letters*, 11(5), 499-515. https://doi.org/10.1111/j.1461-0248.2008.01157.x

Ritland, K. (1989). Correlated matings in the partial selfer *Mimulus guttatus*. *Evolution*, 43(4), 848-859. https://doi.org/10.1111/j.1558-5646.1989.tb05182.x

Sáez, A., Morales, C. L., Ramos, L. Y., & Aizen, M. A. (2014). Extremely frequent bee visits increase pollen deposition but reduce drupelet set in raspberry. *Journal of Applied Ecology*, 51(6), 1603-1612. https://doi.org/10.1111/1365-2664.12325

Shaw, R. F., Phillips, B. B., Doyle, T., Pell, J. K., Redhead, J. W., Savage, J., Woodcock, B. A., Bullock, J. M., & Osborne, J. L. (2020). Mass-flowering crops have a greater impact than semi-natural habitat on crop pollinators and pollen deposition. *Landscape Ecology*, 35(2), 513-527. https://doi.org/10.1007/s10980-019-00962-0

Skogsmyr, I., & Lankinen, Å. (1999). Selection on pollen competitive ability in relation to stochastic factors influencing pollen deposition. *Evolutionary Ecology Research*, 1(8), 971-985. https://www.cabidigitallibrary.org/doi/full/10.5555/20001611764

Skogsmyr, I., & Lankinen, A. (2002). Sexual selection : An evolutionary force in plants? *Biological Reviews of the Cambridge Philosophical Society*, 77(4), 537-562. https://doi.org/10.1017/S1464793102005973

Snow, A. A., & Spira, T. P. (1991a). Differential pollen-tube growth rates and nonrandom fertilization in *Hibiscus moscheutos* (Malvaceae). *American Journal of Botany*, 78(10), 1419-1426. https://doi.org/10.1002/j.1537-2197.1991.tb12608.x

Snow, A. A., & Spira, T. P. (1991b). Pollen vigour and the potential for sexual selection in plants. *Nature*, 352(6338), 796-797. https://doi.org/10.1038/352796a0

Snow, A. A., & Spira, T. P. (1996). Pollen-tube competition and male fitness in *Hibiscus moscheutos*. *Evolution*, 50(5), 1866-1870. https://doi.org/10.1111/j.1558-5646.1996.tb03573.x

Stanton, M. L., & Preston, R. E. (1988). Ecological consequences and phenotypic correlates of petal size variation in wild radish, *Raphanus sativus* (Brassicaceae). *American Journal of Botany*, 75(4), 528-539. https://doi.org/10.1002/j.1537-2197.1988.tb13471.x

Stehlik, I., & Barrett, S. C. H. (2006). Pollination intensity influences sex ratios in dioecious *Rumez nivalis*, a wind-pollinated plant. *Evolution*, 60(6), 1207-1214. https://doi.org/10.1111/j.0014-3820.2006.tb01199.x

Stoffel, M. A., Nakagawa, S., & Schielzeth, H. (2017). rptR : Repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 8(11), 1639-1644. https://doi.org/10.1111/2041-210X.12797

Stone, J. L., Thomson, J. D., & Dent-Acosta, S. J. (1995). Assessment of pollen viability in hand-pollination experiments : A review. *American Journal of Botany*, 82(9), 1186-1197. https://doi.org/10.1002/j.1537-2197.1995.tb11591.x

Temeles, E. J., Newman, J. T., Newman, J. H., Cho, S. Y., Mazzotta, A. R., & Kress, W. J. (2016). Pollinator competition as a driver of floral divergence : An experimental test. *PLOS ONE*, 11(1), e0146431. https://doi.org/10.1371/journal.pone.0146431

Tonnabel, J., David, P., & Pannell, J. R. (2019). Do metrics of sexual selection conform to Bateman's principles in a wind-pollinated plant? *Proceedings of the Royal Society B: Biological Sciences*, 286(1905), 20190532. https://doi.org/10.1098/rspb.2019.0532

Totland, Ø. (2001). Environment-dependent pollen limitation and selection on floral traits in an alpine species. *Ecology*, 82(8), 2233-2244. http://dx.doi.org/10.1890/0012-9658(2001)082[2233:EDPLAS]2.0.CO;2

Trivers, R. (1972). Parental investment and sexual selection. In *Sexual selection and the descent of man, pages* (p. 136-179). Routledge.

Van Rossum, F., Stiers, I., Van Geert, A., Triest, L., & Hardy, O. J. (2011). Fluorescent dye particles as pollen analogues for measuring pollen dispersal in an insect-pollinated forest herb. *Oecologia*, 165(3), 663-674. https://doi.org/10.1007/s00442-010-1745-7

Waites, A. R., & Ågren, J. (2004). Pollinator visitation, stigmatic pollen loads and among-population variation in seed set in *Lythrum salicaria*. *Journal of Ecology*, 92(3), 512-526. https://doi.org/10.1111/j.0022-0477.2004.00893.x

Winfree, R., Aguilar, R., Vázquez, D. P., LeBuhn, G., & Aizen, M. A. (2009). A meta-analysis of bees' responses to anthropogenic disturbance. *Ecology*, 90(8), 2068-2076. https://doi.org/10.1890/08-1245.1

SUPPLEMENTARY MATERIAL

A - Variation in pollen load in natural populations

1. Habitat characterization

Table S1. Sampling year, GPS coordinates, population size (N), number of female individuals sampled (F), sex-ratio (proportion of males), and density (individuals/m²), surface of forest and urban habitat, number of open flowers and corolla width (mm) of the 24 sampled populations of *S. dioica*.

ID	Year	Location	Longitude	Latitude	N	F	Sex-ratio	Density	Forest	Urban	Flower	Corolla
ABB1	2019	Rejet-de-Beaulieu	3.6441633	50.0466608	138	10	0.63	0.14	0.05	0.04	1.7 ± 1.2	18.1 ± 2.5
BOU1	2019	Boué	3.7062825	50.0066967	625	10	0.67	0.25	0.60	0.17	3.8 ± 3.8	21.6 ± 6.7
BOU2	2019	Boué	3.6976417	50.007015	39	10	0.72	0.02	0.39	0.31	2.4 ± 1.6	20.5 ± 2.4
BS1	2019	Condé-sur-l'Escaut	3.6008908	50.477405	93	10	0.81	0.16	0.65	0.11	1.2 ± 0.4	18.3 ± 3.2
BS2	2019	Condé-sur-l'Escaut	3.6033158	50.4880025	46	10	0.72	0.55	0.72	0.14	3.2 ± 3.7	18.9 ± 4.9
ETR	2019	Etreux	3.665685	49.996355	54	10	0.48	0.05	0.03	0.23	5.7 ± 4.3	22.5 ± 2.4
FRE	2018	Templeuve-en-Pévèle	3.1701685	50.5567963	42	9	0.64	0.52	0.13	0.15	4.3 ± 3.1	18.0 ± 1.9
GAU1	2019	Tournai (Belgium)	3.498345	50.5940175	178	10	0.57	0.15	0.16	0.53	4.4 ± 2.1	22.6 ± 1.5
NOU1	2019	Le Nouvion-en-Thiérache	3.7868666	50.0066167	139	10	0.55	0.56	0.56	0.2	3.1 ± 3.2	19.9 ± 2.3
OIS1	2019	Oisy	3.6626792	50.02587	148	10	0.59	0.03	0.00	0.1	5.7 ± 3.6	22.4 ± 1.8
OIS2	2019	Oisy	3.674575	50.0194143	25	6	0.52	0.01	0.03	0.11	3.3 ± 3.4	20.2 ± 5.1
OIS3	2019	Fesmy-le-Sart	3.6623211	50.0387	59	10	0.51	0.13	0.03	0.05	2.9 ± 4.1	23.8 ± 3.3
ORS1	2019	Ors	3.6433133	50.1168417	618	10	0.64	0.37	0.44	0.04	2.3 ± 1.4	21.7 ± 2.6
ORS2	2019	Ors	3.63263	50.0952633	10	2	0.80	0.00	0.02	0.19	4.5 ± 4.9	18.5 ± 0.5
RP1	2019	Marchiennes	3.3065456	50.4338289	121	9	0.78	0.02	0.53	0.07	1.0 ± 0.0	18.1 ± 2.3
RP2	2018	Beuvry-la-Forêt	3.2667383	50.438065	176	10	0.83	0.08	0.51	0.12	2.8 ± 2.6	20.2 ± 3.3
RP3	2019	Beuvry-la-Forêt	3.2720429	50.437919	179	11	0.66	0.11	0.51	0.12	1.9 ± 0.8	19.3 ± 2.9
SA2	2019	Raismes	3.4482052	50.3960708	31	6	0.81	0.05	0.53	0.12	1.7 ± 1.0	20.1 ± 2.4
SA3	2018	Raismes	3.4341156	50.3906056	21	5	0.76	0.00	0.36	0.11	3.4 ± 4.3	18.1 ± 4.1
SA4	2019	Saint-Amand-les-Eaux	3.4449333	50.4259333	7	3	0.57	7.00	0.71	0.14	3.3 ± 2.3	22.0 ± 1.7
SA5	2019	Raismes	3.4330632	50.38825	113	9	0.74	0.02	0.30	0.16	3 ± 4.6	16.0 ± 2.2
VEN1	2019	Vénérolles	3.6402917	49.9844033	69	10	0.49	0.58	0.02	0.1	6.5 ± 5.0	22.4 ± 2.8
VRE1	2018	Vred	3.2288759	50.3910685	34	8	0.76	0.06	0.14	0.26	2.3 ± 1.0	26.9 ± 6.6
VRE2	2018	Rieulay	3.2546317	50.3907883	88	10	0.84	0.02	0.20	0.06	2.6 ± 1.6	20.0 ± 2.3
Mean	-	-	-	-	127.2	9	0.67	0.454	0.32	0.15	3.2 ± 3.1	20.5 ± 3.9

2. Variable correlations

Table S2. Correlations among explanatory variables included in the multiple regression, described using Pearson's correlation coefficient. Significant values after Bonferroni correction are indicated by stars (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$). N: population size, Sex-ratio: proportion of males, Density: individuals/m², Forest: surface of forest habitat, Urban: surface of urban habitat, Flower: number of open flowers, Corolla: corolla width.

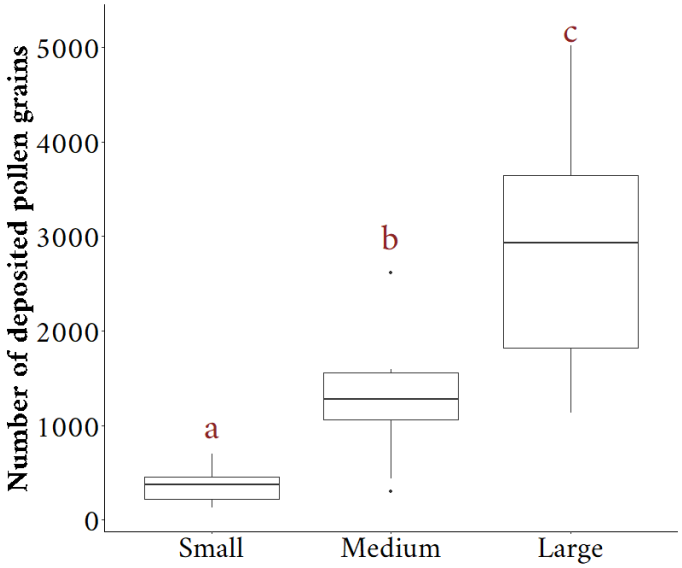
	N	Sex-ratio	Density	Foret	Urban	Flower
Sex-ratio	-0.018					
Density	-0.058	-0.181				
Forest	**0.289*** **	**0.507*** **	**0.238** **			
Urban	-0.100	-0.158	-0.029	-0.058		
Flower	-0.028	**-0.291** **	0.037	**-0.247** **	0.119	
Corolla	0.066	**-0.239** **	0.043	**-0.220** **	0.172	**0.199***

B - Hand pollination experiment

1. Pollen deposition

Text S1. The number of deposited pollen grains per flower was compared between treatment (i.e., small, medium, large pollen load) using a generalized linear mixed model (GLMM), with a negative binomial distribution, including the female identity on which the pollen grains were deposited as a random effect. Post-hoc Tukey's tests were then performed to compare treatments between them.

Figure S1. Number of deposited pollen grains per flower, according to each intensity treatments (small, medium, large pollen load). Different letters indicate significative differences between treatments ($p < 0.05$).



2. Plants traits

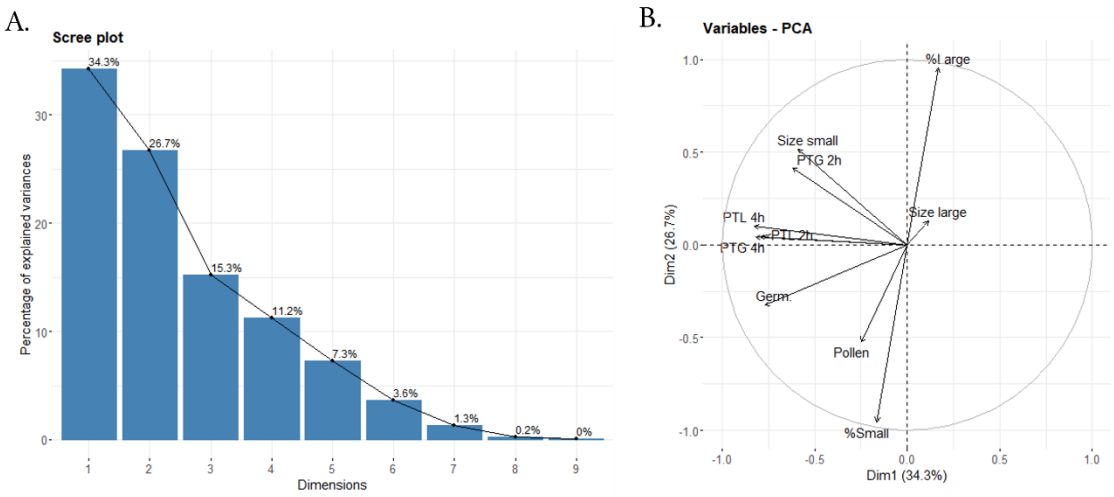
Table S3. Floral traits measurements for each male. Pollen: number of pollen grains per flower, %Small: percentage of small pollen grains, Size small: average pollen grain size in the first size class (15 to 25 µm), Size large: average pollen grain size in the second size class (25 to 35 µm), Viab.: pollen viability rate determined via Petersen staining, Germ.: pollen germination rate, PTG: pollen tube growth speed (µm/h) at 2h and 4h after pollen grains were deposited in the Petri dish, PTL: maximum pollen tube length (µm) at 2h and 4h after pollen grains were deposited in the Petri dish.

	Male	Pollen	%Small	Size small	Size large	Viab.	Ger	PTG	PTG	PTL	PTL
Replicate 1	M01	41,275.0	0.27	24.69 ± 6.89	30.71 ± 1.51	0.99	0.41	115.40	116.17	473.0	698.9
	M02	60,775.0	0.21	23.03 ± 6.32	27.93 ± 1.32	0.98	0.07	141.12	102.11	417.0	661.5
	M03	55,791.7	0.23	23.59 ± 6.25	28.86 ± 1.55	0.97	0.07	101.83	113.30	323.0	587.3
	M04	57,200.0	0.16	25.03 ± 8.30	29.38 ± 1.40	0.98	0.04	98.42	107.46	325.5	697.6
	M05	60,991.7	0.19	25.21 ± 6.63	29.70 ± 1.44	0.97	0.57	159.81	109.09	451.9	654.8
	Mean	55,206.6	0.21	24.31 ± 6.88	29.32 ± 1.44	0.98	0.23	123.32	109.63	398.1	660.0
Replicate 2	M06	80,491.7	0.29	22.62 ± 5.44	30.17 ± 1.52	0.96	0.46	102.84	109.92	437.3	657.8
	M07	70,633.3	0.32	23.73 ± 5.86	28.50 ± 1.55	0.97	0.36	60.55	114.57	317.6	733.0
	M08	80,708.3	0.29	24.09 ± 6.30	28.34 ± 1.40	0.96	0.81	130.77	98.00	414.5	531.0
	M09	86,233.3	0.25	24.82 ± 6.30	28.88 ± 1.99	0.97	0.64	177.31	142.05	640.2	997.7
	M10	52,216.7	0.26	25.86 ± 6.48	28.18 ± 1.63	0.96	0.99	122.89	161.50	420.9	875.4
	Mean	74,056.7	0.28	24.22 ± 6.08	28.82 ± 1.62	0.97	0.65	118.87	125.21	446.1	758.9

Table S4. Correlations between pollen traits for males, using Pearson's correlation coefficient. Significant values after Bonferroni correction are indicated by stars (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$). Pollen: number of pollen grains per flower, %Small: percentage of small pollen grains, Size small: average pollen grain size in the first size class (15 to 25 µm), Size large: average pollen grain size in the second size class (25 to 35 µm), Germ.: pollen germination rate, PTG: pollen tube growth speed (µm/h) at 2h and 4h after pollen grains were deposited in the Petri dish, PTL: maximum pollen tube length (µm) at 2h and 4h after pollen grains were deposited in the Petri dish.

	% Small	Size small	Size large	Germ.	PTG 2h	PTG 4h	PTL 2h	PTL 4h
Pollen	0.36	-0.36	-0.23	0.27	0.22	-0.09	0.37	0.14
% Small		-0.32	.0.04	0.45	-0.36	0.14	0.07	0.04
Size small			0.02	0.48	0.32	0.59	0.23	0.48
Size large				-0.12	-0.06	-0.18	0.18	-0.11
Germ.					0.35	0.57	0.46	0.36
PTG 2h						0.22	**0.81***	0.34
PTG 4h							0.41	**0.84***
PTL 2h								0.62

Figure S2. Results of the PCA on pollen traits. A. Percentage of variance explained by each axis, B. Correlation circle of the variables. The first two axes of this PCA explained 34.3% and 26.7% of the total variance, respectively. PC1 revealed that proportion of large pollen grains and average size of large pollen grains were negatively correlated with number of pollen grains per flower and proportion of small pollen grains, while PC2 revealed that pollen tube growth speed at 2h, and maximum pollen tube length at 2h and 4h were positively correlated, as well as pollen tube growth speed at 4h and average pollen grain size in the first size class. Pollen: number of pollen grains per flower, %Small: percentage of small pollen grains, %Large: percentage of large pollen grains, Size small: average pollen grain size in the first size class (15 to 25 µm), Size large: average pollen grain size in the second size class (25 to 35 µm), Germ.: pollen germination rate, PTG: pollen tube growth speed (µm/h) at 2h and 4h after pollen grains were deposited in the Petri dish, PTL: maximum pollen tube length (µm) at 2h and 4h after pollen grains were deposited in the Petri dish.



3. Paternity correlation

Table S5. Value of the paternity correlation, according to each replicate and each pollen load treatment. Significative values are indicated by star $(p < 0.05)$ and the name of the male correspond to the male that sired the most seeds in the fruit.

	Females	Small-1	Small -2	Medium-1	Medium-2	Large-1	Large-2
Replicate 1	F01	0.209	0.226	**0.367* (M04)**	0.250	0.276	**0.327* (M04)**
	F02	0.224	0.226	0.226	0.262	**0.440* (M02)**	0.281
	F03	**0.387* (M04)**	0.264	0.263	0.306	**0.326* (M03, M04)**	0.260
	F04	0.291	**0.338* (M05)**	0.306	**0.405* (M04)**	**0.357* (M05)**	**0.373* (M05)**
	F05	0.317	0.262	0.235	0.317	**0.372* (M03)**	0.291
	F06	**0.325* (M01)**	0.298	**0.334* (M01)**	0.214	0.271	0.250
	F07	0.291	0.267	0.296	0.302	0.279	0.281
	F08	0.267	**0.323* (M05)**	0.298	*NA*	0.320	0.220
	F09	0.285	0.214	0.214	0.267	0.224	**0.347* (M05)**
	F10	0.302	0.296	0.255	0.219	**0.323* (M05)**	**0.395* (M01)**
Replicate 2	F11	0.260	0.240	0.302	0.236	**0.344* (M06, M09)**	0.224
	F12	0.242	0.258	**0.337* (M06)**	**0.334* (M08)**	0.298	0.224
	F13	0.280	0.279	0.250	**0.487* (M07)**	0.271	**0.413* (M07)**
	F14	**0.334* (M07)**	0.245	0.317	**0.357* (M08)**	**0.367* (M07)**	0.298
	F15	0.258	**0.325* (M08)**	0.211	0.226	0.251	0.289
	F16	0.245	0.317	**0.351* (M07)**	0.235	**0.361* (M07)**	0.302
	F17	0.258	0.291	0.220	0.296	0.242	*NA*
	F18	**0.360* (M06, M08)**	0.298	0.275	0.298	0.296	0.255
	F19	0.279	0.240	**0.328* (M07)**	0.271	**0.357* (M07)**	0.260

Figure S3. Pie chart indicating for each fruit produced by each female and in each pollen load treatment (small, medium, large), the proportion of seeds sired by each male in replicate 1 (blue: M01, yellow: M02, purple: M03, pink: M04, green: M05). Significant values are indicated by a star and a red box ($p < 0.05$).

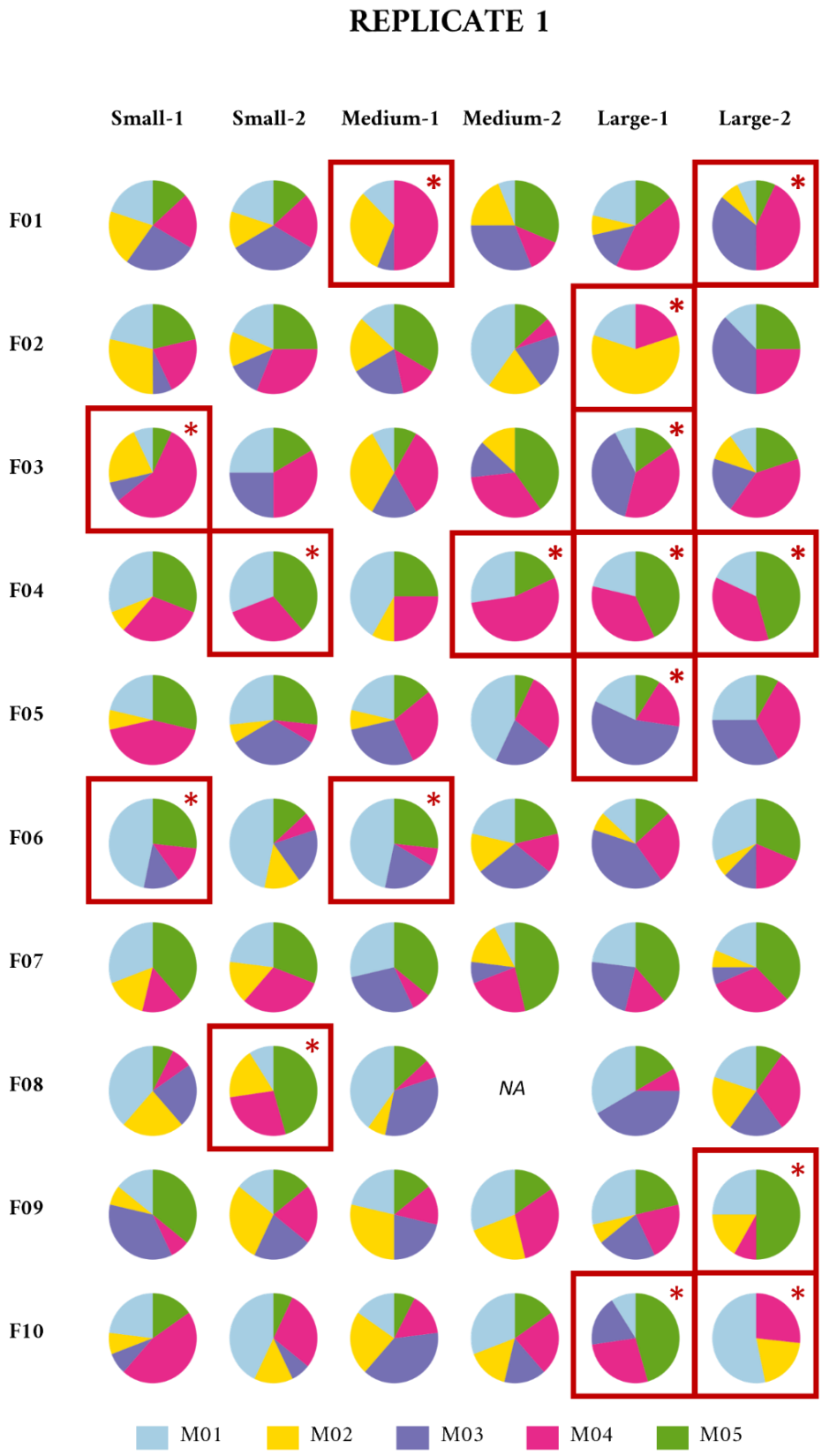
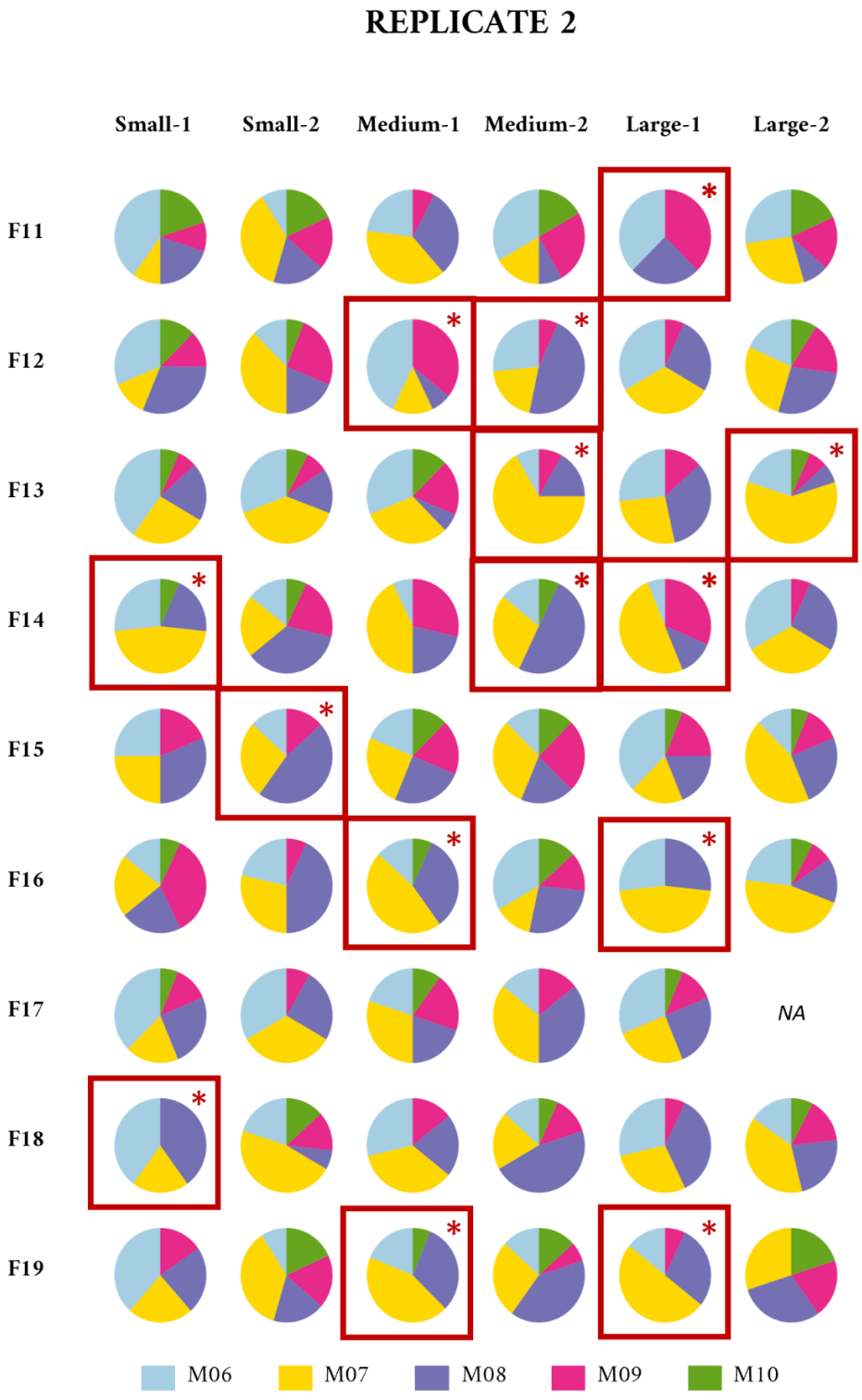


Figure S4. Pie chart indicating for each fruit produced by each female and in each pollen load treatment (small, medium, large), the proportion of seeds sired by each male in replicate 2 (blue: M06, yellow: M07, purple: M08, pink: M09, green: M10). Significant values are indicated by a star and a red box ($p < 0.05$).



DISCUSSION

Sommaire

Chez les plantes pollinisées par les insectes, la sélection agissant sur les traits floraux résulte d'interactions multiples entre fertilité, attractivité, succès d'accouplement, succès reproducteur et environnement. Elle est souvent médiée—du moins en partie—par les interactions entre les plantes et leurs pollinisateurs (Barrett, 2002 ; Harder & Johnson, 2009 ; Caruso et al., 2019), agissant par l'intermédiaire de la composante d'accès aux partenaires, et recoupant de ce fait le processus de sélection sexuelle (Moore & Pannell, 2011). Ces pressions de sélection ne sont pas uniformes : leur intensité et leur direction varient entre fonctions sexuelles et en fonction du contexte écologique. Des études antérieures menées sur des populations expérimentales de *S. dioica* ont montré que les patrons de sélection chez cette espèce différaient entre les sexes : chez les femelles, la sélection s'exerçait principalement sur des traits liés à la fertilité, tandis que chez les mâles, elle portait davantage sur des traits liés à l'attraction des pollinisateurs (Barbot et al., 2022 ; Barbot et al., 2023). Ces résultats étaient par ailleurs confortés par des gradients de Bateman plus forts chez les mâles que chez les femelles, indiquant une asymétrie dans l'intensité de la sélection sexuelle entre les sexes (Barbot et al., 2023). Dans ce travail de thèse, nous avons cherché à identifier l'échelle spatiale à laquelle pourrait s'exercer la compétition entre individus du même sexe pour l'accès aux individus du sexe opposé et à leurs gamètes, et les facteurs susceptibles d'influencer les patrons de sélection, à la fois au stade pré- et post-pollinisation. Dans cette discussion, nous explorons dans un premier temps comment l'habitat façonne les communautés de pollinisateurs, le service de pollinisation, les flux de gènes ainsi que la diversité et la différenciation génétiques des populations. Nous abordons ensuite les effets conjoints du sexe, du type d'habitat et de l'identité des pollinisateurs sur les patrons de sélection opérant sur les traits floraux impliqués dans l'interaction plante-pollinisateur. Enfin, nous examinons les facteurs qui modulent la compétition pollinique et les traits associés à cette compétition, permettant ainsi de relier les interactions écologiques aux pressions de sélection s'exerçant au cours des différentes phases de la pollinisation. En articulant ces différents niveaux d'analyse, ce travail fournit de nouveaux éléments nécessaires pour comprendre comment les interactions écologiques et la structure spatiale des populations façonnent le processus de sélection sexuelle chez les plantes.

I. Role de l'habitat sur la biologie des populations et sur les communautes de pollinisateurs

L'une des hypothèses de cette thèse était que, chez les espèces entomophiles, les modifications de l'habitat devraient influencer plusieurs composantes clés de la biologie des populations : les flux de gènes entre et au sein des populations, la diversité génétique de ces dernières, ainsi que le dépôt de pollen et le degré de limitation pollinique. Cette hypothèse reposait en grande partie sur la prédiction selon laquelle de telles modifications d'habitat devraient impacter les communautés de pollinisateurs, causant une diminution de leur densité et de leur diversité en milieu anthropisé, avec des répercussions en cascade sur la reproduction et la structure génétique des populations.

I-1. Abondance et composition des communautés de pollinisateurs

Silene dioica, bien que généralement considérée comme inféodée aux milieux forestiers, est suffisamment généraliste pour se rencontrer également dans d'autres environnements, notamment dans les agrosystèmes, où elle profite des haies et des structures semi-naturelles. Dans cette thèse, une étude des communautés des pollinisateurs a été réalisée dans six populations naturelles, réparties entre deux types d'habitat contrastés : un habitat forestier et un habitat anthropisé (90% de surfaces forestières et semi-naturelles vs. 40% de surfaces agricoles et urbaines, manuscrit 2). Aucune différence n'a été mise en évidence en termes d'abondance ou de diversité de pollinisateurs entre ces deux types d'habitat. Ce résultat contraste avec de nombreuses études et revues qui rapportent généralement un effet substantiel des modifications d'habitat sur ces variables (Goulson et al., 2008 ; vanEngelsdorp et al., 2008 ; Potts et al., 2010). Par exemple, Ricketts et al. (2008) rapportent que, chez les hyménoptères, le taux de visite des pollinisateurs chute d'environ 50% à 0,6 km d'un habitat naturel, tandis que la richesse spécifique diminue au-delà de 1,5 km. Toutefois, ces effets sont souvent observés dans des contextes où les habitats sont fortement différenciés : Winfree et al. (2009) reportent un effet négatif sur les communautés des pollinisateurs lorsque la perte d'habitat naturel est extrême (i.e., lorsque 95% de l'habitat est converti au profit des activités humaines). De plus, ces effets peuvent varier selon les taxons étudiés (Ricketts et al., 2008). Les tendances observées aux Royaume-Uni et aux Pays-Bas illustrent bien cette variabilité : les abeilles connaissent un déclin général depuis les années 80, tandis que les réponses des syrphes sont plus contrastées, avec des diminutions dans certaines régions et des augmentations dans d'autres (Biesmeijer et al., 2006).

L'hétérogénéité spatiale dans la nature des habitats retrouvés autour de nos populations anthropisées pourrait expliquer l'absence d'effet détecté sur les communautés de pollinisateurs (Bukovinszky et al., 2017 ; Neira et al., 2024). Bien que certaines de ces populations se soient trouvées au sein d'une matrice fortement agricole, certains aménagements paysagers caractéristiques de notre région d'étude, tels que les haies, ont pu favoriser la connectivité entre habitats et ainsi atténuer les effets délétères de l'anthropisation sur les communautés de pollinisateurs (Cranmer et al., 2012 ; Garratt et al., 2017 ; Von Königslöw et al., 2022). La présence de prairies aux abords de nos populations anthropisées (31 à 47%) a potentiellement un effet comparable : en offrant une forte densité et une importante diversité de plantes nourricières, ces prairies pourraient constituer un habitat tampon rendant les environnements anthropisés moins contraignants pour les pollinisateurs (Buri et al., 2014). Au contraire, nos populations forestières se trouvaient dans une matrice paysagère plus uniforme, étant entourées de 77% à 98% d'habitat forestier, or la diversité et l'abondance des pollinisateurs est souvent plus élevée en bordure de forêts qu'à l'intérieur de celles-ci (Ren et al., 2023).

Au-delà de l'abondance des pollinisateurs, une variable importante lorsqu'on s'intéresse à la sélection médiée par les pollinisateurs est le taux de visite par fleur. Dans notre étude, bien qu'aucune différence significative n'ait été observée dans l'abondance totale des pollinisateurs entre habitats forestier et anthropisé, le taux de visite par fleur était significativement plus élevé dans l'habitat anthropisé. Cette différence ne peut être expliquée par une variation dans la composition des communautés locales de pollinisateurs, globalement similaires entre habitats. Elle semble résulter principalement d'un effet de l'habitat sur la taille des plantes : les individus situés

dans l'habitat anthropisé produisaient davantage de fleurs, peut-être en lien avec un meilleur accès à la lumière et à des nutriments d'origine agricole (Kilkenny & Galloway, 2008 ; Vaudo et al., 2022). Ainsi, même si l'activité agricole ne modifie pas directement les communautés de pollinisateurs, elle semble ici influencer le taux de visite par fleur en agissant sur la disponibilité des ressources florales. Ce résultat souligne l'importance de considérer simultanément l'abondance et le taux de visite pour évaluer l'intensité des interactions plantes-pollinisateurs.

Concernant l'identité des pollinisateurs, les principaux pollinisateurs diurnes de *S. dioica* étaient, dans les six populations étudiées, les bourdons et les syrphes (59 à 84% de l'ensemble des visites observées dans chaque population), en accord avec des observations antérieures (Kay et al., 1984 ; Barbot et al., 2022). Ces deux groupes d'insectes incluent de nombreuses espèces généralistes, capables de se nourrir sur un large éventail d'espèces végétales (Lucas et al., 2018 ; Lanterman Novotny et al., 2023), et devraient donc être assez résilients face aux changements environnementaux. D'autres groupes de pollinisateurs, tels que des coléoptères et des bombyles, ont également été recensés lors de nos observations. Cependant, la fréquence des bombyles était variable entre les populations (0% à 25%) et les coléoptères n'ont été observés que dans l'une d'entre elles. Leur efficacité de pollinisation n'est en outre pas connue chez notre espèce d'étude, les capacités de transfert de pollen de ces insectes étant peu documentées au sein des Caryophyllacées (Kephart, 2006). Le rôle des coléoptères dans la pollinisation est extrêmement variable, allant de systèmes hautement spécialisés où ils constituent les principaux, voire les seuls, pollinisateurs efficaces (Haran et al., 2023) à d'autres où ils interviennent surtout comme florivores ou herbivores (Bernhardt, 2000). Quant aux bombyles, une efficacité moindre par rapport à celle des bourdons et des syrphes a été documentée chez quelques espèces de plantes (Cuautle & Thompson, 2010). Enfin, notre caractérisation des communautés de pollinisateurs présente deux limites qu'il convient de souligner. Premièrement, bien que la pollinisation nocturne soit connue pour être efficace chez *S. dioica* (Barbot et al., 2025), nous n'avons pas collecté de données sur ce groupe fonctionnel dans nos populations. Deuxièmement, la diversité des pollinisateurs a été évaluée à un niveau taxonomique élevé (i.e., à l'échelle de l'ordre), masquant ainsi des variations potentielles à des niveaux taxonomiques plus fins.

Si le type d'habitat ne semble pas impacter la composition des communautés de pollinisateurs, nous avons toutefois observé des différences marquées entre populations : dans cinq populations, les bourdons dominaient suivis des syrphes, tandis que dans une population ce schéma était inversé. Cette différence pourrait s'expliquer par la présence de ressources spécifiques nécessaires à ce groupe dans cette dernière population. Ces différences de compositions ont motivé l'étude de la sélection médiée par ces deux pollinisateurs (manuscrit 3), car les différences connues de perception et de préférences de ces derniers pourraient avoir des implications importantes en termes d'évolution florale (Gervasi & Schiestl, 2017).

I-2. Charge pollinique stigmatique et service de pollinisation

Comme mentionné précédemment, nous avons observé une différence en termes de taux de visite entre habitats, ce taux de visite s'étant révélé plus fort au sein des populations forestières qu'au sein des populations

anthropisées. Sur la base de ce résultat, nous aurions pu nous attendre à ce que la charge pollinique stigmatique varie également entre habitats. Nous n'avons cependant détecté aucune corrélation entre cette charge et la composition de l'habitat au voisinage des 24 populations étudiées (surfaces forestières et agricoles). Cette absence d'effet pourrait s'expliquer par des écarts de taux de visite trop faibles pour entraîner une variation significative dans le dépôt de pollen. Par ailleurs, d'autres facteurs peuvent influencer ces dépôts, tels que le temps de vols entre les visites successives, la probabilité de visiter plusieurs fois la même fleur, la durée de visite par fleur, ou encore les comportements de toilettage des insectes (Thomson, 1986 ; Harder, 1990). Des variations de la charge pollinique stigmatique ont cependant été observées entre populations et entre individus au sein des populations, comme rapporté chez d'autre espèces (Németh & Smith-Huerta, 2003 ; Waites & Ågren, 2004). Nos résultats ont ainsi démontré que cette charge augmentait avec la proportion de mâles dans la population et la taille de la corolle des plantes femelles. L'effet positif de la proportion de mâles dans la population peut s'expliquer par le fait qu'une augmentation de cette proportion implique (i) une augmentation de la quantité de pollen totale circulant dans la population, (ii) une probabilité accrue qu'un pollinisateur se charge en pollen avant de visiter une fleur femelle réceptive (Aizen, 2001) et (iii) une probabilité accrue de proximité avec une plante mâle, favorisant le dépôt de pollen (Aizen, 1997). Des variations de charge pollinique stigmatique ont également été observées entre individus, variations en partie expliquées par la taille des fleurs. Ce trait est impliqué dans l'attraction des pollinisateurs chez de nombreuses espèces (Totland, 2001 ; Gómez et al., 2008), y compris chez *S. dioica* (Barbot et al., 2022 ; Barbot et al., 2025) et peut donc favoriser le dépôt de pollen (Temeles et al., 2019). D'autres facteurs auraient pu avoir un effet sur le dépôt de pollen mais n'ont pas été considérés dans cette étude, notamment la composition des communautés des pollinisateurs, susceptible d'affecter la quantité de pollen transférée vers les stigmates selon de précédentes études (e.g., bourdons > syrphes, Gyan & Woodell, 1987 ; Ollerton et al., 2025).

En accord avec nos résultats ne montrant pas d'effet de la composition de l'habitat sur la charge pollinique stigmatique, nous n'avons pas observé de différence dans la qualité du service de pollinisation entre populations forestières et anthropisées. La qualité du service de pollinisation a, dans cette étude, été estimée en supplémentant en pollen les fleurs de plantes issues de notre collection de *S. dioica* cultivée en serre, afin de comparer le succès reproducteur femelle de ces plantes avec celui de plantes provenant elles aussi de notre collection, mais ayant été pollinisées naturellement. Ces plantes, maintenus dans des pots placés dans les populations étudiées, ne présentaient toutefois pas le même phénotype que celui des plantes des populations naturelles. En particulier, le nombre de fleurs ouvertes était plus élevé chez ces dernières. Cette différence limite l'extrapolation de nos résultats concernant le degré de limitation pollinique aux plantes naturellement présentes dans ces populations. En effet, si les plantes en pots pollinisées naturellement ont reçu suffisamment de pollen pour maximiser leur reproduction compte tenu des ressources dont elles disposaient, les plantes naturellement présentes dans les populations étudiées, avec un nombre de fleurs plus important et un accès accru aux ressources du sol, pourraient ne pas avoir reçu une quantité de pollen suffisante pour maximiser leur succès reproducteur. Malgré cela, les valeurs élevées des taux de mise à fruits et de mise à graines chez ces

plantes ci (manuscrit B, supplementary material – Floral trait variation) suggèrent que, si limitation pollinique il y a, cette dernière est vraisemblablement faible.

I-3. Flux de gènes, diversité et différenciation génétique des populations

La dégradation et la fragmentation des habitats peuvent réduire la taille des populations, amplifiant ainsi les effets de la dérive génétique et causant une érosion de la diversité génétique au sein des populations (Harris & Johnson, 2004 ; Lienert, 2004 ; Honnay & Jacquemyn, 2007). La fragmentation peut en outre entraîner l'isolement de ces populations, limitant ainsi les flux de gènes et érodant là encore leur diversité génétique. Chez les espèces entomophiles, la connectivité des populations peut notamment être réduite si les insectes se déplacent moins fréquemment entre populations (Murren, 2002 ; Hadley & Betts, 2012), renforçant ainsi la structure génétique spatiale. En accord avec ces prédictions, plusieurs études ont mis en évidence un effet négatif de la dégradation et de la fragmentation des habitats sur la diversité génétique des populations (Gong et al., 2010 ; Vranckx et al., 2012 ; Gómez-Fernández et al., 2016 ; Almeida-Rocha et al., 2020), tandis que d'autres ont détecté un effet positif (Odat et al., 2004) ou encore une absence d'effet significatif (Ægisdóttir et al., 2009). Dans notre étude, nous n'avons observé aucun effet de l'habitat, ni sur la taille des populations, ni sur la diversité génétique neutre. De plus, les populations situées dans des milieux anthropisés ne semblaient pas plus différenciées génétiquement que celles situées en milieu forestier (pas de différence de *population-specific* F_{ST} entre habitats). Ces résultats pourraient s'expliquer par le fait que notre espèce d'étude est écologiquement assez généraliste : elle occupe une large gamme d'habitats et est impliquée dans des interactions de pollinisation elles aussi assez généralistes. Par ailleurs, notre échantillonnage n'était pas exhaustif, des populations non échantillonnées pouvant se trouver entre les populations échantillonnées, maintenant ainsi la connectivité et les flux de gènes entre ces populations. Ceci pourrait expliquer la capacité de cette espèce à se maintenir dans des matrices paysagères fragmentées, et à échapper, du moins pour le moment, aux effets de la fragmentation.

La fragmentation de l'habitat pourrait par ailleurs renforcer la structure génétique spatiale à fine échelle. En effet, en règle générale, il est attendu que les pollinisateurs visitent des fleurs de proche en proche (Marden & Waddington, 1981 ; Hill et al., 2001), en accord avec la "théorie de l'approvisionnement optimal" (i.e., *optimal foraging theory*, MacArthur & Pianka, 1966). Selon cette théorie, pour maximiser l'acquisition des ressources, ces derniers cherchent à réduire le temps et l'énergie consacrés à leur recherche. Or, la diminution de la taille et de la connectivité des populations pourrait amener les pollinisateurs, dans les habitats anthropisés, à augmenter davantage leur taux de visite (Goverde et al., 2002) et, suivant ce même schéma, à visiter d'autant plus les individus proches spatialement. L'étude de Miguel-Peñaloza et al. (2023) portant sur 73 espèces n'a cependant révélé aucun effet significatif du degré d'anthropisation sur l'indice *Sp*, indicateur du degré de structure génétique spatiale à fine échelle au sein des populations. Cependant, il est à noter que seulement 18% des études inclues dans cette revue portaient sur des espèces herbacées entomophiles. Dans notre étude, nous avons observé de la structure génétique spatiale à fine échelle au sein de nos populations d'études, suggérant que les flux de gènes sont restreints spatialement. Ces résultats n'étaient cependant pas impactés par le type

d'habitat, en accord avec le fait que l'habitat n'impactait pas ni la taille, ni l'isolement des populations. Les flux de pollen, qui sont une composante majeure des flux de gènes, ont par ailleurs été estimé directement par le biais d'analyses de paternité. Les résultats de ces analyses ont montré que les distances de dispersion du pollen étaient limitées (entre 2 et 25 m, en moyenne 7,73 m) et ne différaient pas entre habitats. Ces faibles distances sont comparables à celles observées chez d'autre herbacées (De Cauwer et al., 2012 ; DiLeo et al., 2018 ; Tonnabel, et al., 2019), y compris dans des populations expérimentales de *S. dioica* (Barbot et al., 2022), et peuvent s'expliquer par le comportement de recherche de nourriture des pollinisateurs, mentionnés précédemment. En contradiction apparente avec ces flux de pollen à courtes distances, nous avons mis en évidence des taux de migration élevés, suggérant que les pollinisateurs peuvent à la fois se déplacer sur de courtes distances au sein des populations, et parcourir de plus longues distances entre populations, ce qui suggère une dispersion du pollen multimodale.

Ces résultats sont particulièrement précieux pour estimer l'intensité de la sélection *in natura*. Il est en effet crucial de savoir à quelle distance les gamètes mâles sont transportés pour pouvoir déterminer la taille du voisinage compétitif (i.e., quels individus sont en compétition pour l'attraction des pollinisateurs ?), une question souvent négligée dans la littérature empirique. Dans notre cas, la distance moyenne de dispersion est de quelques mètres, avec des flux possibles au-delà, sur quelques dizaines de mètres. Nos sites d'études constituent donc bien des ensembles d'individus pouvant se reproduire les uns avec les autres.

II. QUELS SONT LES FACTEURS IMPACTANT LES PATRONS DE SELECTION SUR LES TRAITS FLORAUX IMPLIQUES DANS L'INTERACTION PLANTE-POLLINISATION ?

Nous allons à présent aborder les patrons de sélection sur les traits floraux, en nous concentrant sur ceux liés à l'interaction entre les plantes et les pollinisateurs, déterminants dans l'acquisition des partenaires sexuels lors de la phase pré-pollinisation. Nous testons ici l'hypothèse que les mâles, théoriquement plus dépendants des pollinisateurs pour leur succès reproducteur, subissent une sélection plus forte sur les traits attractifs (Hodgins & Barrett, 2008 ; Zhou et al., 2020 ; Barbot et al., 2023) et présentent une relation plus étroite entre le succès reproducteur et le succès d'accouplement que les femelles (Tonnabel et al., 2019 ; Barbot et al., 2022 ; Kwok & Dorken, 2022). Nous examinons ensuite dans quelle mesure ces patrons varient selon l'environnement de pollinisation. D'une part, un taux de visite plus faible dans les habitats anthropisés, sans limitation pollinique marquée chez les femelles (voir partie I), pourrait rester sans effet sur la sélection observée chez les femelles mais modifier la sélection sur les traits attractifs chez les mâles. D'autre part, l'identité des pollinisateurs pourrait façonner différemment la sélection selon le sexe, les mâles étant potentiellement plus sensibles aux variations de comportement et d'efficacité entre types de pollinisateurs.

II-1. Effet du sexe

Selon la théorie de la sélection sexuelle, le succès reproducteur des mâles est principalement limité par l'accès aux partenaires sexuels, tandis que chez les femelles ce sont les ressources disponibles pour la reproduction qui devraient être limitantes (Bateman, 1948). Ces prédictions ont été confirmées chez quelques espèces de plantes

(e.g., chez une mousse, Johnson & Shaw, 2016 ; chez une algue, Lavaut et al., 2023 ; chez trois angiospermes, Tonnabel et al., 2019 ; Barbot et al., 2022 ; Kwok & Dorken, 2022). Dans cette situation, la sélection devrait essentiellement cibler des traits liés à la fertilité chez les femelles, tandis que chez les mâles, les traits liés à l'accès aux partenaires sexuels devraient également être sous sélection (Hodgins & Barrett, 2008 ; Zhou et al., 2020 ; Barbot et al., 2023).

Les patrons de sélection ne peuvent pas être directement comparés entre mâles et femelles, car le succès reproducteur est estimé de manière différente selon le sexe. Chez les femelles, il est mesuré de façon directe en récoltant les fruits et en quantifiant le nombre de graines produites, ce qui fournit une estimation relativement précise du succès reproducteur absolu. Chez les mâles, en revanche, il repose sur des analyses de paternité : toutes les graines ne peuvent pas être génotypées, ce qui fournit une estimation relative du succès reproducteur mâle (i.e., contribution relative de chaque mâle au pool de graines produit par chaque femelle), susceptible d'être affectée à la fois par la variance d'échantillonnage et par les erreurs de génotypage. Ces différences méthodologiques empêchent la construction de modèles statistiques intégrant simultanément les deux sexes. Néanmoins, il reste possible d'effectuer des comparaisons qualitatives, que l'on peut interpréter à la lumière des prédictions théoriques concernant les patrons de sélection sexuelle.

En gardant à l'esprit les limites de notre estimation de la limitation pollinique discutées précédemment, nos résultats suggèrent que le service de pollinisation est globalement efficace au sein des populations étudiées. De ce fait, nous nous attendions à une relation saturante entre le succès d'accouplement et le succès reproducteur chez les femelles (qui produisent moins de fleurs et de gamètes que les mâles, ce qui implique qu'un petit nombre de visites peuvent suffire à les saturer en pollen), et une relation positive entre ces deux variables chez les mâles (qui produisent davantage de fleurs, chacune contenant une grande quantité de gamètes, et qui bénéficient donc systématiquement d'une attraction accrue des pollinisateurs). En accord avec ces prédictions, nos résultats indiquent une relation significativement positive chez les mâles dans cinq des six populations étudiées, relation qui n'a pas été observée chez les femelles. Ces résultats concordent avec ceux de précédentes études (e.g., chez une mousse, Johnson & Shaw, 2016 ; chez une algue, Lavaut et al., 2023 ; chez trois angiospermes, Tonnabel et al., 2019 ; Barbot et al., 2022 ; Kwok & Dorken, 2022).

Chez les femelles, les patrons de sélection semblaient relativement constants d'une population à l'autre (sélection positive sur le nombre de fleurs et de gamètes par fleur), contrairement à ce qui a été observé chez les mâles où ces patrons apparaissaient beaucoup plus variables, aussi bien en termes de traits ciblés que d'intensité. Ce résultat contredit notre hypothèse d'une sélection systématique sur des traits liés à l'attraction, comme la taille de la corolle, chez les mâles. Deux explications peuvent être envisagées pour ces résultats : (i) l'attraction des pollinisateurs n'est pas un facteur clé pour le succès reproducteur mâle (soit parce que les mâles qui sont les plus attractifs ne sont pas ceux qui exportent le plus de pollen, soit parce qu'ils exportent certes plus de pollen mais ne fertilisent pas plus d'ovules), (ii) l'attraction des pollinisateurs est un facteur clé pour le succès reproducteur mâle, mais elle n'est pas régie par les traits inclus dans cette étude. Pour tester ces hypothèses, il faudrait être capable d'estimer le succès de chaque individu aux différentes étapes de la voie mâle (i.e., taux de visite, export du pollen et fertilisation des ovules), pour comparer leur variance (indiquant

l'intensité de la compétition intrasexuelle à chacune de ces étapes), et pour les corréler aux traits des plantes. Le service de pollinisation semblant efficace dans les six populations étudiées, il est possible que les traits les plus importants pour le succès reproducteur mâle soient effectivement ceux qui confèrent un avantage dans la compétition pollinique, un aspect qui sera discuté dans la dernière partie de cette discussion.

II-2. Effet de l'habitat

Chez les femelles comme chez les mâles, nous avons mis en évidence une interaction significative entre un trait floral et le type d'habitat. Plus précisément, la sélection sur le nombre de fleurs ouvertes était plus forte dans les populations anthropisées que forestières chez les femelles. Chez les mâles, c'est en revanche la sélection sur la hauteur du calice qui était plus forte dans les habitats anthropisés que forestiers. Toutefois, les mécanismes expliquant ces variations diffèrent probablement selon le sexe.

Chez les mâles, cette variation pourrait être liée aux différences de taux de visite observées entre habitats. En effet, le succès reproducteur mâle étant principalement limité par l'accès aux partenaires sexuels, une diminution de ce taux de visites pourrait restreindre d'autant plus cet accès. Ainsi, la réduction de ce taux en milieu anthropisé pourrait renforcer la sélection sur un trait lié à l'ajustement entre la fleur et le pollinisateur (Thomann et al., 2015). La diminution de l'activité des pollinisateurs n'ayant pas impacté la qualité du service de pollinisation, cette hypothèse ne peut en revanche pas expliquer l'intensification de la sélection sur le nombre de fleurs ouvertes observée chez les femelles dans les populations anthropisées.

Dans notre étude, les femelles des populations forestières avaient moins de fleurs ouvertes et ce trait était moins variable, comparées aux femelles des populations anthropisées. Ces différences phénotypiques pourraient être attribuées à des conditions abiotiques contrastées, notamment en termes d'ensoleillement ou de disponibilité en nutriments liée à la présence de fertilisants dans les sols agricoles (Kilkenny & Galloway, 2008 ; Vaudo et al., 2022). La plus grande variance observée en milieu anthropisé pourrait également résulter d'une hétérogénéité micro-environnementale plus élevée. Des études complémentaires en conditions contrôlées sont nécessaires pour évaluer la part de la plasticité phénotypique dans cette variation, mais quelle que soit son origine, elle est susceptible d'impacter l'estimation des gradients de sélection. Les différences de distribution des traits entre les habitats peuvent entraîner des variations dans les patrons de sélection. En effet, même si les gradients de sélection sont calculés à partir de traits dont la variance a été standardisée (Lande & Arnold, 1983), des écarts de variance entre habitats peuvent tout de même influencer l'estimation de l'intensité de la sélection (De Lisle & Svensson, 2017).

Au-delà des différences entre habitats forestiers et anthropisés, une partie des variations observées dans les patrons de sélection entre populations pourrait s'expliquer par les différences dans la composition des communautés de pollinisateurs présentes localement dans chacune des populations étudiées. En effet, si les pollinisateurs qui dominent ces communautés varient d'une population à l'autre, et si ces pollinisateurs diffèrent dans leurs préférences et dans leurs caractéristiques morphologiques et comportementales, alors la sélection sur les traits floraux pourrait varier elle aussi. Cependant, il n'est pas possible de tirer de conclusions définitives

sur la base d'une étude observationnelle seulement, ce qui souligne l'intérêt d'expérimentations manipulant directement l'identité des pollinisateurs pour tester ces hypothèses.

II-3. Effet de l'identité des pollinisateurs

En milieu naturel, les bourdons et les syrphes étaient les pollinisateurs les plus abondants de *S. dioica* et leurs fréquences relatives variaient entre populations (manuscrit 2). Afin de mieux comprendre leur rôle dans la reproduction de *S. dioica*, une expérimentation en conditions contrôlées a été réalisée (manuscrit 3). Celle-ci nous a permis (i) d'évaluer leur activité de pollinisation respective, (ii) de quantifier l'intensité de la limitation pollinique lorsque les plantes étaient pollinisées par des bourdons et/ou des syrphes, (iii) d'estimer et comparer la sélection médiée par ces pollinisateurs, et (iv) d'étudier le caractère additif de cette sélection lorsque les plantes étaient mises en présence des deux pollinisateurs à la fois.

Le rôle des pollinisateurs en tant qu'agents de sélection peut être estimé selon deux approches. Chez les femelles, il est possible de comparer les patrons de sélection entre des plantes supplémentées en pollen et des plantes pollinisées naturellement. Toute différence entre ces deux groupes reflète la part de la sélection médiée par les pollinisateurs. Une seconde approche, applicable aux deux sexes, consiste à comparer la sélection médiée par différents pollinisateurs. En conditions contrôlées où seule l'identité du pollinisateur varie, toute différence de sélection peut être attribuée à l'effet des pollinisateurs. D'une manière générale, des différences sensorielles, comportementales et morphologiques peuvent induire des différences dans la direction et l'intensité de la sélection, ainsi que dans la nature des traits ciblés. Notamment, plusieurs études portant sur différentes espèces d'angiospermes ont montré que les bourdons exerçaient une sélection positive sur la hauteur des plantes et sur la taille des fleurs, contrairement aux syrphes qui exerçaient une sélection négative sur ces deux traits (Gómez et al., 2008 ; Gervasi & Schiestl, 2017). De plus, seuls les bourdons exerçaient une sélection positive sur le nombre de fleurs ouvertes dans une autre étude (Wu & Li, 2017). Chez d'autres espèces de plantes, des différences dans la quantité de pollen transférée entre fleurs ont été reportées entre ces insectes (bourdons > syrphes, Morse, 1981 ; Gyan & Woodell, 1987 ; Ollerton et al., 2025), indiquant que ces derniers pourraient en outre induire différents niveaux de limitation pollinique, ce qui devrait là encore affecter l'intensité de la sélection.

Dans notre étude, nous avons observé que les bourdons étaient plus actifs que les syrphes, causant ainsi une différence de niveaux de limitation pollinique, le succès reproducteur femelle étant limitant par l'apport en pollen chez les plantes pollinisées par des syrphes, mais pas chez celles pollinisées par des bourdons. Les différences de patrons de sélection observées chez les femelles entre populations pollinisées par les bourdons et par les syrphes reflètent ces différences de niveaux de limitation pollinique, avec de la sélection sur les traits liés à la fertilité chez les populations pollinisées par les bourdons, et de la sélection sur un trait putativement lié à l'ajustement entre la fleur et le pollinisateur (i.e., la hauteur du calice) dans une des populations pollinisées par les syrphes—celle dans laquelle la limitation pollinique était la plus intense. Cette sélection était négative, indiquant que les femelles ayant des calices courts produisaient plus de graines, un résultat surprenant au vu de la relation positive attendue entre la profondeur du tube floral et l'efficacité du transfert de pollen

(Alexandersson & Johnson, 2002 ; Muchhala, 2007). Des études explorant la relation entre hauteur du calice, fréquence et durée des visites de la part des syrphes et dépôt de pollen seraient nécessaires pour mieux comprendre l'effet de ce trait sur le succès reproducteur femelle.

Chez les mâles, les traits ciblés par la sélection différaient entre les populations pollinisées par les bourdons et par les syrphes, avec une sélection positive sur le nombre de fleurs chez les premières et une sélection négative sur la hauteur de la plante chez les secondes, des résultats concordants avec ceux de précédentes études (Gómez et al., 2008 ; Gervasi & Schiestl, 2017 ; Wu & Li, 2017) et révélant peut-être une différence dans les traits guidant les choix alimentaires de ces deux insectes. Nos résultats ont en outre montré qu'en dépit de l'importante différence d'activité observée entre ces derniers, la sélection exercée sur ces deux traits—nombre de fleurs ouvertes et hauteur de la plante—chez les mâles pollinisées par les bourdons et par les syrphes à la fois ne pouvait pas être considérée comme non-additive. Un tel résultat pourrait s'expliquer par les différences de gradients observées entre populations exposées aux syrphes, et par la forte incertitude autour de l'estimation de ces gradients dans l'une d'entre elles, induisant une distribution théorique couvrant un large intervalle et donc un manque de puissance statistique.

D'autres approches ont été proposées pour étudier le caractère additif ou non de la sélection. En particulier, terHorst et al. (2015) utilisent une ANCOVA à trois facteurs (i.e., trait × présence de l'insecte 1 × présence de l'insecte 2), une interaction significative de ceux-ci indiquant une sélection non-additive sur le trait en question. Cette approche, qui nécessite une combinaison où les deux pollinisateurs sont absents, ne semble cependant pas s'appliquer aux espèces purement allofécondantes telles que *S. dioica*.

III. COMPETITION POLLINIQUE

Chez les mâles, la compétition pour l'accès aux partenaires sexuels peut intervenir à deux moments au cours de la reproduction : avant et après l'arrivée du pollen sur le stigmate. Le fait que l'on observe une relation positive entre le succès reproducteur et le succès d'accouplement dans cinq populations naturelles sur six indique que cette compétition a bien lieu chez *S. dioica*, suggérant que certains traits devraient être sous sélection sexuelle. L'absence de lien systématique entre les traits floraux connus pour être impliqués dans l'attraction des pollinisateurs et le succès reproducteur mâle suggère en outre que les processus post-pollinisation pourraient jouer un rôle majeur. En effet, certains stigmates reçoivent une quantité de pollen bien supérieure au nombre d'ovules disponibles pour la fertilisation (manuscrit 4) et ce pollen provient généralement de plusieurs donneurs (Joffard et al., 2025), créant ainsi des conditions favorables à l'expression de la compétition pollinique. Dans la dernière expérience décrite dans cette thèse, différents mâles ont été mis en compétition sur plusieurs femelles à trois intensités de dépôt de pollen différentes, reflétant potentiellement trois niveaux d'intensités de la compétition pollinique. Différents traits polliniques ont également été mesurés sur ces mâles. Nous avions pour objectif de tester l'hypothèse selon laquelle l'intensité de la compétition pollinique augmente avec le dépôt de pollen, induisant une plus grande variance dans le succès de fertilisation. Dans ce cas, les mâles ayant les traits polliniques les plus avantageux devraient engendrer plus de graines que leurs rivaux.

Nos résultats ne montrent pas de différence entre traitements dans les valeurs de corrélation de paternité et dans le nombre de fruits pour lesquels les contributions des mâles sont inégales. Ceci s'explique peut-être par le fait que nos trois traitements correspondent potentiellement à des situations de forte compétition pollinique, le nombre de grains de pollen déposés dans chacun de ces trois traitements étant respectivement 1,4 fois, 5 fois et 10 fois supérieur au nombre moyen d'ovules par fleur. Ces trois traitements devaient à l'origine représenter trois situations contrastées : (1) un dépôt de pollen inférieur au nombre d'ovule, (2) un dépôt de pollen légèrement supérieur au nombre d'ovule, (3) un dépôt de pollen largement supérieur au nombre d'ovule. En l'absence de données sur les taux de germination du pollen *in vivo* chez *S. dioica*, nous avons fait l'hypothèse que ces taux de germination seraient modérés (comme c'est le cas *in vitro*) et avons ajusté la quantité de pollen déposée dans chaque traitement en fonction de cette hypothèse. Les taux de germination de pollen semblent cependant avoir été plus élevés que prévu.

Dans notre expérimentation, les deux réplicas mettent en évidence des situations contrastées. Le succès de fertilisation est moins répétable dans le premier réplica que dans le deuxième. Dans le réplica 1, les mâles qui engendrent le plus de graines au sein des fruits ne sont pas systématiquement les mêmes mâles entre chaque femelle. Ceci pourrait indiquer que dans ce réplica, l'issue de la compétition pollinique dépend en partie de la compatibilité entre plantes mâles et femelles. En revanche, dans le second réplica, un mâle domine chez toutes les femelles, ce qui pourrait s'expliquer par un avantage lié à de meilleurs performances intrinsèques (e.g., meilleure viabilité) ou à de meilleurs capacités compétitives (e.g., croissance du tube plus rapide), mais aussi par un simple effet d'une contribution disproportionnée au mix de pollen déposé sur les stigmates.

Pour finir, nous avons détecté un effet significatif de la plupart des traits polliniques mesurés sur la proportion de graines engendrées. Les effets négatifs du taux de germination et de la vitesse de croissance du tube pollinique retiennent cependant notre attention. Ces relations négatives, surprenantes au vu de la littérature sur le sujet (Snow & Spira, 1991 ; Delph et al., 1998 ; Skogsmyr & Lankinen, 2002), pourraient s'expliquer par une corrélation avec un autre trait non mesuré ou par le fait que les performances *in vitro* ne reflètent pas nécessairement les performances *in vivo* (Stone et al., 1995 ; Biewer-Heisler et al., 2024). Il n'en demeure pas moins que nous avons détecté une interaction significative entre la vitesse de croissance des tubes polliniques et le traitement, indiquant un effet négatif de ce trait sur la proportion de graines engendrées dans les traitements "faible" et "moyen", mais moins négatif dans le traitement "fort", indiquant peut-être que ce trait devient comparativement avantageux lorsque la compétition pollinique s'intensifie. Des études complémentaires sont néanmoins nécessaires pour mieux comprendre l'effet des différents traits—et combinaisons de traits—polliniques sur le succès de fertilisation chez *S. dioica*, espèce chez laquelle la production de graines est rarement limitée par l'apport en pollen et chez laquelle la compétition pollinique est donc probablement fréquente.

Dans notre étude, le rôle potentiel d'autres facteurs tels que le moment et l'endroit du dépôt de pollen sur la fleur n'ont pas été considérés et pourraient influencer le succès de fertilisation. Des études montrent par exemple que le premier mâle arrivé sur le stigmate peut bénéficier d'un avantage en termes de nombre de graines engendrées (Snow et al., 2000 ; Burkhardt et al., 2009 ; mais voir Lankinen & Madjidian, 2011). Dans la même idée, l'endroit du dépôt sur le stigmate (i.e., position basale ou apicale) pourrait également influencer

le succès de fertilisation, en modulant l'accès des tubes polliniques aux ovules (Delph et al., 1998 ; Brothers & Delph, 2017) : les grains de pollen déposés au plus proche de ces derniers auraient alors un succès plus important.

IV. CONCLUSION

Dans cette thèse, à travers plusieurs études aux approches complémentaires, nous avons analysé l'influence de facteurs écologiques sur les processus de sélection sexuelle chez *Silene dioica*. Nous avons d'abord mis en évidence une distribution bimodale des flux de pollen : d'une part, des taux élevés de migration entre populations, et d'autre part, des transferts de pollen à très courte distance au sein des populations. Ces flux intra-populations indiquent que la compétition pour l'accès aux partenaires sexuels a lieu sur une échelle spatiale restreinte. Dans ces mêmes populations, les taux de visite des pollinisateurs étaient plus faibles dans les populations anthropisées que dans les populations forestières, tandis que la qualité du service de pollinisation était similaire entre types d'habitat. Chez les plantes femelles, la sélection s'exerçait principalement sur des traits liés à la fertilité, tandis que chez les plantes mâles, les patrons de sélection étaient moins consistants entre populations, bien qu'une relation positive entre le succès reproducteur et le succès d'accouplement soit systématiquement observée, en accord avec les prédictions théoriques. En populations naturelles, les principaux pollinisateurs de *S. dioica* étaient les bourdons et les syrphes. Une expérimentation en conditions contrôlées a montré que la pollinisation par les syrphes engendrait de la limitation pollinique, contrairement à celle des bourdons. Dans ce contexte, de la sélection sur un trait lié à l'ajustement entre la fleur et le pollinisateur a été détectée chez les femelles pollinisées par les syrphes, tandis que de la sélection de fertilité a été observée chez celles pollinisées par les bourdons. Chez les mâles, nous avons détecté de la sélection sur le nombre de fleurs ouvertes chez les plantes pollinisées par les bourdons, et de la sélection sur la hauteur de la plante chez celles pollinisées par les syrphes. Enfin, chez les mâles, la compétition intra-sexuelle peut s'exprimer au-delà de la pollinisation, après le dépôt du pollen sur les stigmates. Nous avons ainsi montré un effet de plusieurs traits polliniques sur le succès de fertilisation des mâles, et l'effet de l'un d'entre eux (i.e., vitesse de croissance des tubes polliniques) variait en fonction de l'intensité du dépôt pollinique. Nos résultats soulignent la nécessité de prendre en compte à la fois les processus pré- et post-pollinisation, les deux fonctions sexuelles et l'identité des pollinisateurs pour comprendre pleinement la sélection exercée sur les traits floraux et leur évolution.

V. RÉFÉRENCES

Ægisdóttir, H. H., Kuss, P., & Stöcklin, J. (2009). Isolated populations of a rare alpine plant show high genetic diversity and considerable population differentiation. *Annals of Botany*, 104(7), 1313-1322. https://doi.org/10.1093/aob/mcp242

Aizen, M. A. (1997). Influence of local floral density and sex ratio on pollen receipt and seed output : Empirical and experimental results in dichogamous *Alstroemeria aurea* (Alstroemeriaceae). *Oecologia*, 113(3), 404-412. https://doi.org/10.1007/s004420050252

Aizen, M. A. (2001). Flower sex ratio, pollinator abundance, and the seasonal pollination dynamics of a protandrous plant. *Ecology*, 82(1), 127-144. https://doi.org/10.1890/0012-9658(2001)082[0127:FSRPAA]2.0.CO;2

Alexandersson, R., & Johnson, S. D. (2002). Pollinator–mediated selection on flower–tube length in a hawkmoth–pollinated *Gladiolus* (Iridaceae). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 269(1491), 631-636. https://doi.org/10.1098/rspb.2001.1928

Almeida-Rocha, J. M., Soares, L. A., Andrade, E. R., Gaiotto, F. A., & Cazetta, E. (2020). The impact of anthropogenic disturbances on the genetic diversity of terrestrial species : A global meta-analysis. *Molecular Ecology*, 29(24), 4812-4822. https://doi.org/10.1111/mec.15688

Barbot, E., Dufaÿ, M., & De Cauwer, I. (2023). Sex-specific selection patterns in a dioecious insect-pollinated plant. *Evolution*, 77(7), 1578-1590. https://doi.org/10.1093/evolut/qpad069

Barbot, E., Dufaÿ, M., Godé, C., & De Cauwer, I. (2025). Investigating the effects of diurnal and nocturnal pollinators on male and female reproductive success and on floral trait selection in *Silene dioica*. *Peer Community Journal*, 5, e11. https://doi.org/10.24072/pcjournal.508

Barbot, E., Dufaÿ, M., Tonnabel, J., Godé, C., & De Cauwer, I. (2022). On the function of flower number : Disentangling fertility from pollinator-mediated selection. *Proceedings of the Royal Society B: Biological Sciences*, 289(1987), 20221987. https://doi.org/10.1098/rspb.2022.1987

Barrett, S. C. H. (2002). The evolution of plant sexual diversity. *Nature Reviews Genetics*, 3(4), 274-284. https://doi.org/10.1038/nrg776

Bateman, A. J. (1948). Intra-sexual selection in *Drosophila*. *Heredity*, 2(3), 349-368. https://doi.org/10.1038/hdy.1948.21

Bernhardt, P. (2000). Convergent evolution and adaptive radiation of beetle-pollinated angiosperms. *Plant Systematics and Evolution*, 222(1), 293-320. https://doi.org/10.1007/BF00984108

Biesmeijer, J. C., Roberts, S. P. M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., Schaffers, A. P., Potts, S. G., Kleukers, R., Thomas, C. D., Settele, J., & Kunin, W. E. (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science*, 313(5785), 351-354. https://doi.org/10.1126/science.1127863

Biewer-Heisler, T. J., Gibson, M. J., Sornay, E., & Moyle, L. C. (2024). *Gene expression and pollen performance indicate altered postmating selection between* Solanum *species with different mating systems*. https://doi.org/10.1101/2024.10.31.621143

Brothers, A. N., & Delph, L. F. (2017). Divergence in style length and pollen size leads to a postmating-prezygotic reproductive barrier among populations of *Silene latifolia*. *Evolution*, 71(6), 1532-1540. https://doi.org/10.1111/evo.13248

Bukovinszky, T., Verheijen, J., Zwerver, S., Klop, E., Biesmeijer, J. C., Wäckers, F. L., Prins, H. H. T., & Kleijn, D. (2017). Exploring the relationships between landscape complexity, wild bee species richness and reproduction, and pollination services along a complexity gradient in the Netherlands. *Biological Conservation*, 214, 312-319. https://doi.org/10.1016/j.biocon.2017.08.027

Buri, P., Humbert, J.-Y., & Arlettaz, R. (2014). Promoting pollinating insects in intensive agricultural matrices : Field-scale experimental manipulation of hay-meadow mowing regimes and its effects on bees. *PLoS ONE*, 9(1), e85635. https://doi.org/10.1371/journal.pone.0085635

Burkhardt, A., Internicola, A., & Bernasconi, G. (2009). Effects of pollination timing on seed paternity and seed mass in *Silene latifolia* (Caryophyllaceae). *Annals of Botany*, 104(4), 767-773. https://doi.org/10.1093/aob/mcp154

Caruso, C. M., Eisen, K. E., Martin, R. A., & Sletvold, N. (2019). A meta-analysis of the agents of selection on floral traits. *Evolution*, 73(1), 4-14. https://doi.org/10.1111/evo.13639

Cranmer, L., McCollin, D., & Ollerton, J. (2012). Landscape structure influences pollinator movements and directly affects plant reproductive success. *Oikos*, 121(4), 562-568. https://doi.org/10.1111/j.1600-0706.2011.19704.x

Cuautle, M., & Thompson, J. N. (2010). Diversity of floral visitors to sympatric *Lithophragma* species differing in floral morphology. *Oecologia*, 162(1), 71-80. https://doi.org/10.1007/s00442-009-1424-8

De Cauwer, I., Arnaud, J., Klein, E. K., & Dufaÿ, M. (2012). Disentangling the causes of heterogeneity in male fecundity in gynodioecious *Beta vulgaris* ssp. *Maritima*. *New Phytologist*, 195(3), 676-687. https://doi.org/10.1111/j.1469-8137.2012.04191.x

De Lisle, S. P., & Svensson, E. I. (2017). On the standardization of fitness and traits in comparative studies of phenotypic selection. *Evolution*, 71(10), 2313-2326. https://doi.org/10.1111/evo.13325

Delph, L. F., Weinig, C., & Sullivan, K. (1998). Why fast-growing pollen tubes give rise to vigorous progeny : The test of a new mechanism. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 265(1399), 935-939. https://doi.org/10.1098/rspb.1998.0381

DiLeo, M. F., Holderegger, R., & Wagner, H. H. (2018). Contemporary pollen flow as a multiscale process : Evidence from the insect-pollinated herb, *Pulsatilla vulgaris*. *Journal of Ecology*, 106(6), 2242-2255. https://doi.org/10.1111/1365-2745.12992

Garratt, M. P. D., Senapathi, D., Coston, D. J., Mortimer, S. R., & Potts, S. G. (2017). The benefits of hedgerows for pollinators and natural enemies depends on hedge quality and landscape context. *Agriculture, Ecosystems & Environment*, 247, 363-370. https://doi.org/10.1016/j.agee.2017.06.048

Gervasi, D. D. L., & Schiestl, F. P. (2017). Real-time divergent evolution in plants driven by pollinators. *Nature Communications*, 8(1), 14691. https://doi.org/10.1038/ncomms14691

Gómez, J. M., Bosch, J., Perfectti, F., Fernández, J. D., Abdelaziz, M., & Camacho, J. P. M. (2008). Spatial variation in selection on corolla shape in a generalist plant is promoted by the preference patterns of its local pollinators. *Proceedings of the Royal Society B: Biological Sciences*, 275(1648), 2241-2249. https://doi.org/10.1098/rspb.2008.0512

Gómez-Fernández, A., Alcocer, I., & Matesanz, S. (2016). Does higher connectivity lead to higher genetic diversity? Effects of habitat fragmentation on genetic variation and population structure in a gypsophile. *Conservation Genetics*, 17(3), 631-641. https://doi.org/10.1007/s10592-016-0811-z

Gong, W., Gu, L., & Zhang, D. (2010). Low genetic diversity and high genetic divergence caused by inbreeding and geographical isolation in the populations of endangered species *Loropetalum subcordatum* (Hamamelidaceae) endemic to China. *Conservation Genetics*, 11(6), 2281-2288. https://doi.org/10.1007/s10592-010-0113-9

Goulson, D., Lye, G. C., & Darvill, B. (2008). The decline and conservation of bumblebees. *Annual Review of Entomology*, 53(1), 191-208. https://doi.org/10.1146/annurev.ento.53.103106.093454

Goverde, M., Schweizer, K., Baur, B., & Erhardt, A. (2002). Small-scale habitat fragmentation effects on pollinator behaviour : Experimental evidence from the bumblebee *Bombus veteranus* on calcareous grasslands. *Biological Conservation*, 104(3), 293-299. https://doi.org/10.1016/s0006-3207(01)00194-x

Gyan, K. Y., & Woodell, S. R. J. (1987). Analysis of insect pollen loads and pollination efficiency of some common insect visitors of four species of woody Rosaceae. *Functional Ecology*, 1(3), 269-274. https://doi.org/10.2307/2389430

Hadley, A. S., & Betts, M. G. (2012). The effects of landscape fragmentation on pollination dynamics : Absence of evidence not evidence of absence. *Biological Reviews*, 87(3), 526-544. https://doi.org/10.1111/j.1469-185X.2011.00205.x

Haran, J., Li, X., Allio, R., Shin, S., Benoit, L., Oberprieler, R. G., Farrell, B. D., Brown, S. D. J., Leschen, R. A. B., Kergoat, G. J., & McKenna, D. D. (2023). Phylogenomics illuminates the phylogeny of flower weevils (Curculioninae) and reveals ten independent origins of brood-site pollination mutualism in true weevils. *Proceedings of the Royal Society B: Biological Sciences*, 290(2008), 20230889. https://doi.org/10.1098/rspb.2023.0889

Harder, L. D. (1990). Behavioral responses by bumble bees to variation in pollen availability. *Oecologia*, 85(1), 41-47. https://doi.org/10.1007/BF00317341

Harder, L. D., & Johnson, S. D. (2009). Darwin's beautiful contrivances : Evolutionary and functional evidence for floral adaptation. *New Phytologist*, 183(3), 530-545. https://doi.org/10.1111/j.1469-8137.2009.02914.x

Harris, L. F., & Johnson, S. D. (2004). The consequences of habitat fragmentation for plant-pollinator mutualisms. *International Journal of Tropical Insect Science*, 24(1), 29-43. https://doi.org/10.1079/IJT20049

Hill, P. S. M., Hollis, J., & Wells, H. (2001). Foraging decisions in nectarivores : Unexpected interactions between flower constancy and energetic rewards. *Animal Behaviour*, 62(4), 729-737. https://doi.org/10.1006/anbe.2001.1775

Hodgins, K. A., & Barrett, S. C. H. (2008). Natural selection on floral traits through male and female function in wild populations of the heterostylous daffodil *Narcissus triandrus*. *Evolution*, 62(7), 1751-1763. https://doi.org/10.1111/j.1558-5646.2008.00404.x

Honnay, O., & Jacquemyn, H. (2007). Susceptibility of common and rare plant species to the genetic consequences of habitat fragmentation. *Conservation Biology*, 21(3), 823-831. https://doi.org/10.1111/j.1523-1739.2006.00646.x

Joffard, N., Lecat, I., Ollier, K., Jolivel, C., Schmitt, E., Godé, C., Barbot, E., Dufaÿ, M., & De Cauwer, I. (2025). Floral display size does not affect the distribution of paternal diversity in *Silene dioica*. *bioRxiv*. https://doi.org/10.1101/2025.07.19.665651

Johnson, M. G., & Shaw, A. J. (2016). The effects of quantitative fecundity in the haploid stage on reproductive success and diploid fitness in the aquatic peat moss *Sphagnum macrophyllum*. *Heredity*, 116(6), 523-530. https://doi.org/10.1038/hdy.2016.13

Kay, Q. O. N., Lack, A. J., Bamber, F. C., & Davies, C. R. (1984). Differences between sexes in floral morphology, nectar production and insect visits in a dioecious species, *Silene dioica*. *New Phytologist*, 98(3), 515-529. https://doi.org/10.1111/j.1469-8137.1984.tb04145.x

Kephart, S. (2006). Pollination mutualisms in Caryophyllaceae. *New Phytologist*, 169(4), 637-640. https://doi.org/10.1111/j.1469-8137.2006.01656.x

Kilkenny, F. F., & Galloway, L. F. (2008). Reproductive success in varying light environments : Direct and indirect effects of light on plants and pollinators. *Oecologia*, 155(2), 247-255. https://doi.org/10.1007/s00442-007-0903-z

Kwok, A., & Dorken, M. E. (2022). Sexual selection on male but not female function in monoecious and dioecious populations of broadleaf arrowhead (*Sagittaria latifolia*). *Proceedings of the Royal Society B*: *Biological Sciences*, 289(1986), 20220919. https://doi.org/10.1098/rspb.2022.0919

Lande, R., & Arnold, S. (1983). The measurement of selection on correlated characters. *Evolution*, 37, 1210-1226. http://dx.doi.org/10.2307/2408842

Lankinen, Å., & Madjidian, J. A. (2011). Enhancing pollen competition by delaying stigma receptivity : Pollen deposition schedules affect siring ability, paternal diversity, and seed production in *Collinsia heterophylla* (Plantaginaceae). *American Journal of Botany*, 98(7), 1191-1200. https://doi.org/10.3732/ajb.1000510

Lanterman Novotny, J., Lybbert, A., Reeher, P., Mitchell, R. J., & Goodell, K. (2023). Bumble bee banquet : Genus- and species-level floral selection by Midwestern *Bombus*. *Ecosphere*, 14(2), e4425. https://doi.org/10.1002/ecs2.4425

Lavaut, E., Valero, M., Mauger, S., Guillemin, M. L., Destombe, C., & Dufaÿ, M. (2023). Sexual selection in seaweed? Testing Bateman's principles in the red alga *Gracilaria gracilis*. *Proceedings of the Royal Society B*: *Biological Sciences*, 290(2006), 20231668. https://doi.org/10.1098/rspb.2023.1668

Lienert, J. (2004). Habitat fragmentation effects on fitness of plant populations – a review. *Journal for Nature Conservation*, 12(1), 53-72. https://doi.org/10.1016/j.jnc.2003.07.002

Lucas, A., Bodger, O., Brosi, B. J., Ford, C. R., Forman, D. W., Greig, C., Hegarty, M., Neyland, P. J., & De Vere, N. (2018). Generalisation and specialisation in hoverfly (Syrphidae) grassland pollen transport networks revealed by DNA metabarcoding. *Journal of Animal Ecology*, 87(4), 1008-1021. https://doi.org/10.1111/1365-2656.12828

MacArthur, R. H., & Pianka, E. R. (1966). On optimal use of a patchy environment. *The American Naturalist*, 100(916), 603-609. https://doi.org/10.1086/282454

Marden, J. H., & Waddington, K. D. (1981). Floral choices by honeybees in relation to the relative distances to flowers. *Physiological Entomology*, 6(4), 431-435. https://doi.org/10.1111/j.1365-3032.1981.tb00658.x

Miguel-Peñaloza, A., Cultid-Medina, C. A., Pérez-Alquicira, J., & Rico, Y. (2023). Do habitat fragmentation and degradation influence the strength of fine-scale spatial genetic structure in plants? A global meta-analysis. *AoB PLANTS*, 15(3), plad019. https://doi.org/10.1093/aobpla/plad019

Moore, J. C., & Pannell, J. R. (2011). Sexual selection in plants. *Current Biology*, 21(5), R176-R182. https://doi.org/10.1016/j.cub.2010.12.035

Morse, D. H. (1981). Interactions among syrphid flies and bumblebees on flowers. *Ecology*, 62(1), 81-88. https://doi.org/10.2307/1936671

Muchhala, N. (2007). Adaptive trade-off in floral morphology mediates specialization for flowers pollinated by bats and hummingbirds. *The American Naturalist*, 169(4), 494-504. https://doi.org/10.1086/512047

Murren, C. J. (2002). Effects of habitat fragmentation on pollination : Pollinators, pollinia viability and reproductive success. *Journal of Ecology*, 90(1), 100-107. https://doi.org/10.1046/j.0022-0477.2001.00638.x

Neira, P., Blanco-Moreno, J. M., Olave, M., Caballero-López, B., & Sans, F. X. (2024). Effects of agricultural landscape heterogeneity on pollinator visitation rates in Mediterranean oilseed rape. *Agriculture, Ecosystems & Environment*, 363, 108869. https://doi.org/10.1016/j.agee.2023.108869

Németh, M. B., & Smith-Huerta, N. L. (2003). Pollen deposition, pollen tube growth, seed production, and seedling performance in natural populations of *Clarkia unguiculata* (Onagraceae). *International Journal of Plant Sciences*, 164(1), 153-164. https://doi.org/10.1086/344549

Odat, N., Jetschke, G., & Hellwig, F. H. (2004). Genetic diversity of *Ranunculus acris* L. (Ranunculaceae) populations in relation to species diversity and habitat type in grassland communities. *Molecular Ecology*, 13(5), 1251-1257. https://doi.org/10.1111/j.1365-294x.2004.02115.x

Ollerton, J., Coulthard, E., Tarrant, S., Woolford, J., Jorge, L. R., & Rech, A. R. (2025). Butterflies, bumblebees and hoverflies are equally effective pollinators of *Knautia arvensis* (Caprifoliaceae), a generalist plant species with compound inflorescences. *Journal of Applied Entomology*, 149(5), 685-696. https://doi.org/10.1111/jen.13345

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010). Global pollinator declines : Trends, impacts and drivers. *Trends in Ecology & Evolution*, 25(6), 345-353. https://doi.org/10.1016/j.tree.2010.01.007

Ren, P., Didham, R. K., Murphy, M. V., Zeng, D., Si, X., & Ding, P. (2023). Forest edges increase pollinator network robustness to extinction with declining area. *Nature Ecology & Evolution*. https://doi.org/10.1038/s41559-022-01973-y

Ricketts, T. H., Regetz, J., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., Bogdanski, A., Gemmill-Herren, B., Greenleaf, S. S., Klein, A. M., Mayfield, M. M., Morandin, L. A., Ochieng', A., & Viana, B. F. (2008). Landscape effects on crop pollination services : Are there general patterns? *Ecology Letters*, 11(5), 499-515. https://doi.org/10.1111/j.1461-0248.2008.01157.x

Skogsmyr, I., & Lankinen, A. (2002). Sexual selection : An evolutionary force in plants? *Biological Reviews of the Cambridge Philosophical Society*, 77(4), 537-562. https://doi.org/10.1017/S1464793102005973

Snow, A. A., & Spira, T. P. (1991). Differential pollen-tube growth rates and nonrandom fertilization in *Hibiscus moscheutos* (Malvaceae). *American Journal of Botany*, 78(10), 1419-1426. https://doi.org/10.1002/j.1537-2197.1991.tb12608.x

Snow, A. A., Spira, T. P., & Liu, H. (2000). Effects of sequential pollination on the success of "fast" and "slow" pollen donors in *Hibiscus moscheutos* (Malvaceae). *American Journal of Botany*, 87(11), 1656-1659. https://doi.org/10.2307/2656742

Stone, J. L., Thomson, J. D., & Dent-Acosta, S. J. (1995). Assessment of pollen viability in hand-pollination experiments : A review. *American Journal of Botany*, 82(9), 1186-1197. https://doi.org/10.1002/j.1537-2197.1995.tb11591.x

Temeles, E. J., Liang, J., Levy, M. C., & Fan, Y.-L. (2019). Floral isolation and pollination in two hummingbird-pollinated plants : The roles of exploitation barriers and pollinator competition. *Evolutionary Ecology*, 33(4), 481-497. https://doi.org/10.1007/s10682-019-09992-1

terHorst, C. P., Lau, J. A., Cooper, I. A., Keller, K. R., Rosa, R. J. L., Royer, A. M., Schultheis, E. H., Suwa, T., & Conner, J. K. (2015). Quantifying nonadditive selection caused by indirect ecological effects. *Ecology*, 96(9), 2360-2369. https://doi.org/10.1890/14-0619.1

Thomann, M., Imbert, E., Engstrand, R. C., & Cheptou, P.-O. (2015). Contemporary evolution of plant reproductive strategies under global change is revealed by stored seeds. *Journal of Evolutionary Biology*, 28(4), 766-778. https://doi.org/10.1111/jeb.12603

Thomson, J. D. (1986). Pollen transport and deposition by bumble bees in *Erythronium* : Influences of floral nectar and bee grooming. *The Journal of Ecology*, 74(2), 329. https://doi.org/10.2307/2260258

Tonnabel, J., David, P., Klein, E. K., & Pannell, J. R. (2019). Sex-specific selection on plant architecture through "budget" and "direct" effects in experimental populations of the wind-pollinated herb, *Mercurialis annua*. *Evolution*, 73(5), 897-912. https://doi.org/10.1111/evo.13714

Tonnabel, J., David, P., & Pannell, J. R. (2019). Do metrics of sexual selection conform to Bateman's principles in a wind-pollinated plant? *Proceedings of the Royal Society B: Biological Sciences*, 286(1905), 20190532. https://doi.org/10.1098/rspb.2019.0532

Totland, Ø. (2001). Environment-dependent pollen limitation and selection on floral traits in an alpine species. *Ecology*, 82(8), 2233-2244. http://dx.doi.org/10.1890/0012-9658(2001)082[2233:EDPLAS]2.0.CO;2

vanEngelsdorp, D., Hayes, J., Underwood, R. M., & Pettis, J. (2008). A survey of honey bee colony losses in the U.S., fall 2007 to spring 2008. *PLoS ONE*, 3(12), e4071. https://doi.org/10.1371/journal.pone.0004071

Vaudo, A. D., Erickson, E., Patch, H. M., Grozinger, C. M., & Mu, J. (2022). Impacts of soil nutrition on floral traits, pollinator attraction, and fitness in cucumbers (*Cucumis sativus* L.). *Scientific Reports*, 12(1), 21802. https://doi.org/10.1038/s41598-022-26164-4

Von Königslöw, V., Fornoff, F., & Klein, A.-M. (2022). Pollinator enhancement in agriculture : Comparing sown flower strips, hedges and sown hedge herb layers in apple orchards. *Biodiversity and Conservation*, 31(2), 433-451. https://doi.org/10.1007/s10531-021-02338-w

Vranckx, G., Jacquemyn, H., Muys, B., & Honnay, O. (2012). Meta-analysis of susceptibility of woody plants to loss of genetic diversity through habitat fragmentation. *Conservation Biology*, 26(2), 228-237. https://doi.org/10.1111/j.1523-1739.2011.01778.x

Waites, A. R., & Ågren, J. (2004). Pollinator visitation, stigmatic pollen loads and among-population variation in seed set in *Lythrum salicaria*. *Journal of Ecology*, 92(3), 512-526. https://doi.org/10.1111/j.0022-0477.2004.00893.x

Winfree, R., Aguilar, R., Vázquez, D. P., LeBuhn, G., & Aizen, M. A. (2009). A meta-analysis of bees' responses to anthropogenic disturbance. *Ecology*, 90(8), 2068-2076. https://doi.org/10.1890/08-1245.1

Wu, Y., & Li, Q.-J. (2017). Phenotypic selection on flowering phenology and pollination efficiency traits between Primula populations with different pollinator assemblages. *Ecology and Evolution*, 7(19), 7599-7608. https://doi.org/10.1002/ece3.3258

Zhou, J., Reynolds, R. J., Zimmer, E. A., Dudash, M. R., & Fenster, C. B. (2020). Variable and sexually conflicting selection on *Silene stellata* floral traits by a putative moth pollinator selective agent. *Evolution*, 74(7), 1321-1334. https://doi.org/10.1111/evo.13965

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Ecologie évolutive de la reproduction chez une plante dioïque :
effets du paysage, des pollinisateurs et de la compétition pollinique

Résumé : Les angiospermes présentent une diversité remarquable de traits floraux, la sélection sexuelle étant considérée comme l'un des moteurs clés de leur évolution. La théorie prédit qu'en raison de l'anisogamie, le succès reproducteur des mâles devrait être principalement limité par l'accès aux partenaires sexuels et à leurs gamètes, entraînant une compétition intra-sexuelle pouvant s'exprimer à la fois avant et après la pollinisation, mobilisant principalement des traits floraux impliqués dans l'interaction plantes-pollinisateurs—chez les espèces à pollinisation biotique—et des traits polliniques. A l'inverse, le succès reproducteur des femelles devrait être principalement limité par les ressources disponibles pour la production d'ovules, entraînant une sélection sur les traits liés à la fertilité. Toutefois, lorsque les pollinisateurs sont rares, l'accès aux partenaires sexuels pourrait devenir limitant pour la reproduction des femelles, la sélection ciblant alors des traits floraux impliqués dans les interactions plantes-pollinisateurs. L'abondance des pollinisateurs pourrait également moduler la force relative de la compétition intra-sexuelle aux stades pré- et post-pollinisation. En particulier, la compétition pollinique devrait s'intensifier avec la quantité de pollen déposée sur les stigmates. Par ailleurs, la plupart des espèces d'angiospermes sont généralistes dans leurs interactions avec les pollinisateurs, or différents groupes de pollinisateurs peuvent exercer des pressions de sélection distinctes selon leurs capacités sensorielles, leur morphologie et leur comportement. Cette thèse explore ces différentes hypothèses chez l'espèce dioïque *Silene dioica*, à travers plusieurs questions : (i) A quelle échelle spatiale la compétition pour l'accès aux pollinisateurs a-t-elle lieu ? (ii) Les patrons de sélection diffèrent-ils entre individus mâles et femelles ? (iii) Sont-ils affectés par l'anthropisation de l'habitat, potentiellement associée à un déclin des pollinisateurs ? (iv) Sont-ils influencés par l'identité de ces pollinisateurs ? Et enfin, (v) la compétition pollinique contribue-t-elle à façonner le succès reproducteur mâle ? Nos résultats montrent des flux de pollen spatialement restreints en populations naturelles, suggérant que les individus d'une même population peuvent être en compétition pour l'accès aux pollinisateurs. Par ailleurs, les patrons de sélection mis en évidence différaient entre sexes : chez les mâles, une relation positive a été observée entre le succès d'accouplement et le succès reproducteur, suggérant que la sélection sexuelle pourrait opérer. Chez les femelles, cette relation n'a pas été observée et nous avons détecté une sélection directionnelle positive sur plusieurs traits liés à la fertilité. Nos observations ont montré un taux de visite plus faible dans les sites anthropisés que forestiers, causant potentiellement une différence de sélection sur la hauteur du calice chez les mâles. La qualité du service de pollinisation n'était cependant pas impactée, de même que les patrons de sélection chez les femelles. Nous avons par ailleurs observé que les bourdons et les syrphes sélectionnaient des traits floraux distincts chez les mâles. Enfin, nous avons pu mettre en évidence un effet significatif de la majorité des traits polliniques mesurés sur le succès de fertilisation des mâles, avec un effet modulé par la quantité de pollen déposée sur les stigmates pour l'un d'entre eux. Dans l'ensemble, ces résultats indiquent que la densité et l'identité des pollinisateurs influencent les patrons de sélection de manière différenciée entre mâles et femelles, et entre les stades pré- et post-pollinisation.

Mots-clés : sélection sexuelle, sélection phénotypique, flux de gènes, pollinisateurs, traits floraux, compétition pollinique

Evolutionary ecology of reproduction in dioecious plant:
effects of landscape, pollinators and pollen competition

Abstract: Angiosperms display a remarkable diversity of floral traits, with sexual selection considered one of the key drivers of their evolution. Theory predicts that, due to anisogamy, male reproductive success should be primarily limited by access to mates and their gametes, leading to intra-sexual competition both before and after pollination. This competition involves mainly floral traits associated with plant-pollinator interactions—in animal-pollinated species—as well as pollen traits. In contrast, female reproductive success is expected to be primarily limited by the resources available for ovule production, resulting in selection on traits related to fertility. However, when pollinators are scarce, access to mates may become limiting for female reproduction, leading to selection on floral traits involved in plant-pollinator interactions. Pollinator abundance may also modulate the relative strength of intra-sexual competition at pre- and post-pollination stages. In particular, pollen competition should intensify with increasing pollen loads on stigmas. Finally, most angiosperms are generalists in their interactions with pollinators, and different pollinator groups may exert distinct selective pressures depending on their sensory capacities, morphology, and behavior. This thesis explores these hypotheses in the dioecious species *Silene dioica*, addressing several questions: (i) At what spatial scale does competition for access to pollinators occur? (ii) Do selection patterns differ between males and females? (iii) Are these patterns affected by habitat anthropization, potentially associated with pollinator decline? (iv) Are they influenced by pollinator identity? And finally, (v) does pollen competition contribute to shaping male reproductive success? Our results revealed spatially restricted pollen flow within natural populations, suggesting that individuals from the same population may compete for access to pollinators. Moreover, selection patterns differed between sexes: in males, a positive relationship was observed between mating success and reproductive success, suggesting that sexual selection may operate. In females, this relationship was not detected, and we observed positive directional selection on several traits related to fertility. Pollinator visitation rates were lower in anthropized sites than in forested ones, potentially leading to differential selection on calyx height in males. However, pollination service quality and female selection patterns remained unaffected. We also found that bumblebees and hoverflies exerted selection on distinct male floral traits. Finally, most measured pollen traits had a significant effect on male fertilization success, with one trait showing an effect modulated by the amount of pollen deposited on stigmas. Overall, these findings indicate that pollinator density and identity influence selection patterns differently between sexes and across the pre- and post-pollination stages.

Keywords: sexual selection, phenotypic selection, gene flow, pollinators, floral traits, pollen competition