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**Jaakko Soininen**

# Floral Mutualists, Antagonists, and Within-Species Diversity

The Significance of Within-Species Diversity of the  
Plant *Geranium sylvaticum* to its Interactions with  
Pollinators and the *Zaenagrus geranii* -Weevil

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UNIVERSITY OF JYVÄSKYLÄ  
FACULTY OF MATHEMATICS  
AND SCIENCE

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## ABSTRACT

Soininen, Jaakko

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Diss.

Natural communities are built on a multitude of interactions. Pollination is an interaction between flower visiting insects and flowering plants. Most studies on pollination indeed come to be onto the premise of protecting this vulnerable interaction. Pollinator insects compete with each other for flower rewards, and plants themselves gain varying returns for the cost of floral resources they present the visitors with. Floral antagonists may utilize flower tissues (florivores) or developing seeds (pre-dispersal seed predators) and cause harm to plant reproduction. Due to the similar preferences of pollinators and florivores to similarly showy and colourful flowers, the florivores not only damage plant reproduction directly but can affect the behaviour of pollinator insects. In this work, the aim is to investigate florivory and pollination in the context of plant within-species diversity, a road less travelled in the general literature of pollination ecology. Within-species diversity is regarded essential to the preservation of species since it enables adaptation in changing environment and evolution itself. I aim to explore the aspect of within-species diversity by presenting three studies where field observations and manipulative treatments are used to examine the relations of pollinators, florivores and plants. The study system of the original studies is based on the plant *Geranium sylvaticum*, its obligate florivore, *Zaenaga geranii* weevil, and the associated pollinator insects. In the first study the role of variation between plant sexes in pollinator behaviour and pollinator efficiency are assessed and sexual dimorphism is explained as an adaptation to insect behaviour. Second study assesses the effects of florivory and seed predation on the reproduction of *G. sylvaticum*, finding that *Z. geranii* affects plant reproduction preferring certain traits and genotypes. Inter-specific pollinator competition and potential for resource partitioning within plant species is the focus of the third study. Introduced honeybees affect native pollinator visitations, and this effect differs between plant genotypes. In synthesis, within-species diversity is a pivotal factor in plant-insect interactions.

Keywords: Florivory; inter-specific pollinator competition; plant reproduction; pollination; pre-dispersal seed predation; within-species diversity.

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# TIIVISTELMÄ

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Kasvinsyöjät, pölyttäjät ja lajinsisäinen monimuotoisuus

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Diss.

Ekologiset yhteisöt syntyvät eliöiden välisten vuorovaikutusten varaan. Esimerkiksi pölytys syntyy kasvien ja niiden kukissa vierailevien hyönteisten vuorovaikutuksesta. Pölyttäjähönteiset kilpailevat keskenään, ja kasvit itse hyötyvät vaihtelevissa määrin eri pölyttäjälajien vierailuista. Kasvit vuorovaikuttavat myös kukkia ja siemeniä syövien hyönteisten kanssa. Kukkien- ja siemensyöjät vaurioittavat lisääntymissolukoita ja vaikuttavat näin kasvien lisääntymiseen. Koska kukkien ulkoasu vaikuttaa pölyttäjien käyttäytymiseen, voi kukkiensyönti vaikuttaa myös pölytykseen. Tässä työssä tutkin kasvien ja hyönteisten vuorovaikutuksia lajinsisäisen monimuotoisuuden näkökulmasta. Lajinsisäistä monimuotoisuutta pidetään välttämättömänä eliöiden yhteisöjen selviytymiselle, sillä se mahdollistaa sekä sopeutumisen ympäristömuutoksiin että evoluution. Tämä työ pohjautuu kolmeen maastossa manipulaatioin ja tarkkailuin toteutettuun tutkimukseen, jotka nivoutuvat metsäkurjenpolven, sen kukkia syövän kurjenpolvikärsäkkään sekä pölyttäjien ympärille. Osatutkimuksessa I selvitettiin lajinsisäisen monimuotoisuuden merkitystä pölyttäjien käyttäytymiselle ja selitettiin metsäkurjenpolven sukupuolierot sopeumina pölyttäjien käyttäytymiseen. Osatyössä II tutkittiin kukkiensyönnin vaikutusta kasvin lisääntymiselle ja havaittiin kukkien ja siementen syönnin vaikuttavan kasvin lisääntymiseen. Lisäksi havaittiin näiden vaikutusten eroavan genotyyppien välillä. Osatutkimuksessa III arvioitiin tarhamehiläisen ja luonnonpölyttäjien kilpailua, ja tutkimuksessa havaittiin mehiläisen vaikuttavan negatiivisesti kimalaisten vierailuihin, ja nämä vaikutukset erosivat kasvigenotyypeittäin. Yhteenvedona voidaan todeta kasvin lajinsisäisen monimuotoisuuden tärkeä rooli kasvien ja hyönteisten välisissä vuorovaikutuksissa.

Avainsanat: Kasvien lisääntyminen; kukkiensyönti; lajinsisäinen monimuotoisuus; pölyttäjienvälinen kilpailu; pölytys; siemensyönti.

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## LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original studies which will be referred to in the text by their Roman numerals I-III.

- I Soininen J.O.S. & Kytöviita M.-M. 2022. *Geranium sylvaticum* increases pollination probability by sexually dimorphic flowers. *Ecology and Evolution* 12, e9670.
- II Soininen J.O.S. & Kytöviita M.-M. 2023. Plant sex and parental genotype affect florivory and seed predation by *Zaenaidus geranii* in *Geranium sylvaticum*. Manuscript.
- III Soininen J.O.S., Paukkunen J. & Kytöviita M.-M. 2023. Managed honeybees affect the foraging behaviour of bumblebees in *Geranium sylvaticum*. Manuscript.

## AUTHOR CONTRIBUTIONS

Contributor names are abbreviated as follows and ordered alphabetically in the table according to surname: JS = Jaakko Soininen, MMK = Minna-Maarit Kytöviita, JP = Juho Paukkunen.

Role	I	II	III
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Experimental setup conceptualization	MMK, JS	MMK	MMK, JP
Study site and system	MMK	MMK	MMK
Constructing the experiment	JS	MMK, JS	MMK, JS
Data collection	JS	MMK, JS	MMK, JP, JS
Data analysis	JS	JS	JS
First draft	JS	JS	JS
Writing	MMK, JS	MMK, JS	MMK, JP, JS



*To my Mother, Brother, my irreplaceable friends and this world we live in*

# 1 INTRODUCTION

## 1.1 The importance of pollinators

Mundane as it may seem at a first glance to watch a bumblebee land on and take off from a flower, species of many ecosystems owe their existence to the simple act of pollination. At a closer inspection, the multitude of interactions around pollination constitute a complex web. On the petals of even the simplest of flowers intersect the paths of various insects, both pollinators as well as *florivores* and *pre-dispersal seed predators* who all utilize the flowers and developing seeds to their ends. The interaction of the plant and the floral antagonists and mutualists is complex, but even more so considering the three-way interactions since the parties may interact with each other as well as plant species and plant genotypes. Alarming, however, the diversity of insect species is quickly waning, with drops in insect biomass and species diversity reported around the world (Hallmann *et al.* 2017, Wagner 2020, Zattara and Aizen 2021). With globalization and crises like the climate change contributing to introduction of new pathogens, pests and invasive species, the many interactions that constitute our natural communities of species are threatened. Thus, the need arises to understand these interactions to be better able to protect them. Perhaps this is possible by widening our perspective from simply viewing species as species and approaching plants, pollinators and florivores from a novel viewpoint, through their *within-species diversity*.

## 1.2 Pollination

### 1.2.1 Of bees and flowers

Zoophilous pollination comprises the mutualistic interaction between plants and insects, where the mobile, typically flying, insect transports pollen from a flower with male sexual organs to a receptive stigma. This service however does not arise from the good-will of the insects, but rather comes at the price of nectar or the consumption of some of the pollen. Many plants and pollinators are so immersed in their mutualistic relationship, that the pollinators cannot survive without a constant supply of flowers for forage (Ebeling *et al.* 2008), and the reproduction of plants is reduced or precluded by the lack of pollination for a variety of species (Bierzuchudek 1981 and references therein, Rodger *et al.* 2021). Earliest evidence of entomophilous, insect mediated pollination dates back to 110–105 MYA (Peñalver *et al.* 2012). Arthropod visitors to plant reproductive displays became the first pollinators. Though at this point no mutualistic interactions had evolved, pollen and spores were transported between plant individuals and locations while feeding on flowers (Lunau 2000, and the citations therein). Coevolution has moulded the interactions so that it is now beneficial for the plant to go as far as to attract and reward visitors with nectar, a solution easily digested by insects and with a high caloric content. The quality of nectar itself is affected via adaptations regarding the preferred pollinators, further attesting to coevolution (Abrahamczyk *et al.* 2017). Furthermore, evolution has since produced adaptations making pollen more transportable and visually attractive (Hu *et al.* 2008) and flowers more attractive as well (Darwin 1862, Sauquet and Magallón 2018). All floral visitors however are not pollinators, since some are either incompatible with the flower to an extent that they merely eat the reward but do not pick up or transport pollen (Kandori 2002). In addition to these so-called *nectar thieves*, some floral visitors are *nectar robbers* who damage the flowers in order to have access to the nectar and this has various impacts to plants in terms of adaptations (Maloof and Inoue 2000).

Between plants, competition arises as each plant has the goal of attracting a pollinator that is the most efficient in transporting pollen both in ample amounts, over large distances and between as many flowers as possible. This has been coined pollinator competition, or competition for pollinators between plants (Thomson and Page 2020). It further benefits the plant if a pollinator has a high degree of floral constancy (Waser 1986). Floral constancy is a behaviour where, despite various flowers are present, the pollinator visits similar flowers (Waser 1986). Bees and bumblebees are typically credited as having high levels of floral constancy (Osborne *et al.* 1999). Floral constancy was noted already in 1900s (Plateau 1901) and is proposed as per present understanding to arise for example from pollinator memory constraints, efficiency and the acquisition of handling skills (Woodward and Laverty 1992, Alcorn *et al.* 2012, Ishii and Kadoya 2016). Floral constancy is beneficial to the target plant since it guarantees the transport

of con-specific pollen between flowers (Waser 1986). Although, the flip side of the coin is being passed over by pollinators that forage by a different search image.

The choice between a certain flower or plant individual for a foraging pollinator is an important one and based mainly on both olfactory and visual cues (Goulson *et al.* 1998, Chittka and Raine 2006, Barragán-Fonseca *et al.* 2020). Chittka and Raine (2006) note that due to optical constraints in the bee eye, a flower must be perceived in an angle between 5-15 degrees in the visual field of a bee, for its colour to register. In other words, a flower 1 cm in diameter must be seen at a distance no more than 11.5 cm away. From a distance further than this, the bees rather identify flowers by their contrast to the (usually) green background (Chittka and Raine 2006). Olfactory cues are utilized at a distance shorter than the visual cues. Olfactory cues are typically detected across some meters but may affect the floral choice itself at a relatively short range and are typically ruled out by visual cues (Barragán-Fonseca *et al.* 2020). It must be noted that the use of different cues depends on various factors like the pollinator species, distance, and status of the flower (Barragán-Fonseca *et al.* 2020). Bees also identify the scents of pathogens, herbivores, and previous floral visitors (Goulson *et al.* 1998) which further affects their choice. Overall, it can be supposed that visual cues including flower shape and contrast against background can be viewed from further away than colour and olfactory cues, since individual flowers cannot be distinguished from each other at a distance. Visual cues, which can be detected from further away and are often the strongest cues affecting pollinator choice (Chittka and Raine 2006, Barragán-Fonseca *et al.* 2020), are also the principal factor affected by florivores. The shape of a flower is among the first signals identified by the pollinator, which potentially causes florivory to have an important effect on the pollinator behaviour, since pollinator behaviour is tied to appearance and symmetry of flowers (Møller and Eriksson 1995, Rodríguez *et al.* 2004). While analysing pollinator behaviour in terms of signal preference, it must be borne in mind that there are varying levels of specificity and signal preferences even among different genera of pollinators. For example, many hoverfly genera have distinct floral source preferences (Klecka *et al.* 2018).

Though floral constancy benefits the plants, the degree of specialization and generalism within the interaction between the plant and insect also matters (Bloch *et al.* 2006, Classen *et al.* 2020) and the idea was originally proposed by Vogel (1954) in form of pollination syndromes. Species of plants ensure that their pollen is efficiently transported and ends up on a conspecific stigma and also eliminate the matter of pollinator competition by specialist co-evolution between a plant and a certain pollinator (Thøstesen and Olesen 1996). Specialistic pollinators are typically well adapted to the phenology and morphology of the plant and visit other plants much less often (Herre 1989, Thøstesen and Olesen 1996). Furthermore, as specialist flowers are usually inaccessible for other pollinators, the pollinator insect also experiences less competition as a result, as in the classical examples of specialization and coevolution such as the fig wasps (Herre 1989) and Darwin's moth (Case detailed in Arditti *et al.* 2012). General flowers then again can be pollinated by various types of insects and are

exemplified by open and accessible morphologies (Olesen *et al.* 2007) and are therefore credited as having focal role in ecological communities as interaction hubs (Martín González *et al.* 2010). For generalist flowers, some pollinator might always be present, but it is not as adapted, as efficient as a specialist pollinator might be (Thøstesen and Olesen 1996) and will risk receiving heterospecific pollen by sharing pollinators with other plants (Wei *et al.* 2021). For a pollinator too the inter-specific competition becomes by definition more pronounced when visiting generalist flowers. On the other hand, the pollinator will have a steadier supply of flowers the higher the levels of generalism it is capable of (Willmer 2011).

A pronounced interaction between the species diversity of pollinators and plants becomes apparent when viewing the requirements of the two groups. Since pollinator insects are typically obligate floral resource feeders, they depend their survival on the presence of flowers during their active period. Some pollinators are furthermore obligate nectar feeders (like butterflies and moths), and others may utilize a mixed diet. Bees forage in terms of the needs of the adults (mainly nectar) and the needs of the larvae (mainly pollen). Since a species of plant generally does not bloom equally abundantly constantly, different species are required to create a flowering continuation throughout the growth season (Ebeling *et al.* 2008). Furthermore, pollinators require differential qualities of nutrition (Knox *et al.* 1971, Zimmerman and Pleasants 1982) and some might focus their foraging efforts on nectar, while others on pollen. For example, some hoverflies mainly forage on nectar, but there are also species that include or focus on pollen feeding as part of their diet (Gilbert 1981). Eusocial bees would forage for nectar and pollen depending on the current need of their hive (Free 1967). Differential nutritional needs aggravate on the matter that bees are healthier and possess a stronger immunity system when their food sources are diverse (Pasquale *et al.* 2013).

Plants equally benefit from a diversity of pollinator insects, since their abundance ensures that pollination takes place at different weather conditions, different times of the year (Albrecht *et al.* 2012). A diverse pollinator community will also provide resilience if certain species of pollinators are present at lesser numbers due to year-to-year fluctuations. Strikingly, studies have found that pollination services are enhanced by pollinator diversity rather than pollinator abundance (Klein *et al.* 2003), with emphasis on the native pollinator species (Kremen *et al.* 2002, Button and Elle 2014). Decreased pollinator diversity in turn leads to decreased plant diversity (Ramos-Jiliberto 2020).

### **1.2.2 Competition for pollinators**

In order to understand the different interactions that are woven between plants and their mutualists and antagonists, it must be accounted for that plants also interact competitively with other plants through their pollinators and antagonists. One basis for these interactions is competition for pollinators. Pollinator competition generally leads to diminished rates of pollinator visitations received for the plants that are less preferred by the pollinators

(Temeles *et al.* 2016). In some cases, however, plants may facilitate each other's pollination, as observed by, for example, Ghazoul (2006) and Watanabe and Maesako (2021). This denotes that a shared pollinator community does not necessarily entail detriment to the pollination received.

Pollinator competition affects plants in many ways. For example, pollen limited plants suffer reproductive constraints when facing intense competition for pollinators, and as a result, reduced rates of visitations (Janečková *et al.* 2019). Flowers may also become clogged with pollen from other plants (Kunin 1993), reducing the chances of successful pollination, and the pollen from a specific plant may be wasted if it is foraged to excess (Parker *et al.* 2016) or transported to incompatible flowers.

Pollinator competition drives plant morphological traits like floral attractiveness, coloration, and nectar production (Hirota *et al.* 2013) towards the goal of attracting the most preferred and efficient pollinators. Since in many ecosystems plant reproduction has been found to be pollinator limited (Bierzychudek 1981, Kohama *et al.* 2021), the ability to attract and retain pollinators becomes critical for the reproductive success of plants. Therefore, pollinator competition also plays a crucial role in shaping plant species and genetic diversity as well as the evolution of floral traits (Temeles *et al.* 2016). Competition for pollinators is furthermore aggravated by floral constancy (Waser 1986) (Discussed earlier) which reduces the stochasticity of pollinator visitations between flowers.

Pollinator competition can affect community-level processes. Some studies suggest that high levels of pollinator competition can lead to increased plant diversity due to the pressure to differentiate and adapt (Temeles *et al.* 2016, Classen *et al.* 2020). Specialization between plants and pollinators can enable coexistence and the efficiency of pollination, while as a by-product generating more niches within the community (Nicholls and Altieri 2013). It should be noted that the degree of competition among plants or among pollinators is mutually exclusive to an extent. Whereas pollinator competition between plants generally reduces plant reproductive fitness, competition between pollinators is beneficial to the plants, and vice versa. Therefore, plants should be expected to strive towards inter-specific pollinator competition by diversification. For example, Classen *et al.* (2020) note that competition among pollinators can have positive effects on plant diversity. It was demonstrated in their study that as pollinators compete for limited resources, such as nectar and pollen, plants are more likely to receive visits from a diverse range of pollinators. The resulting increased visitation rates by different pollinator species can enhance cross-pollination and gene flow, ultimately contributing to higher plant diversity within the community.

### **1.2.3 Inter-specific pollinator competition**

Competition between different species of pollinators is an important factor in determining the pollinator community of an area or a certain plant species. Inter-specific pollinator competition can be expressed as a span of different kinds of

interactions between the pollinator insects (Thomson and Page 2020). In some cases, pollinators may engage in territorial or aggressive behaviour, interference. Large and aggressive species drive out or interfere with the foraging activities of other pollinators (Strange *et al.* 2011, Henry and Rodet 2018, Taggar *et al.* 2021). This kind of competition is, however, relatively rare across pollinator-pollinator interactions. Most often pollinators compete indirectly by pre-empting floral resources and in some cases nesting sites or other resources (Henry and Rodet 2018, Thomson and Page 2020, Taggar *et al.* 2021). Flowers that are already emptied by a more efficient pollinator spend wasted time and potentially longer flight distances for other competitors in order to receive the same rewards. In addition to resource competition, horizontal pathogen – parasite and disease – spillover when visiting the same flowers between different species of pollinators can also reduce fitness of the pathogen recipient (Durrer and Schmid-Hempel 1994, Fürst *et al.* 2014).

Inter-specific competition between pollinators usually aggravates when a foreign species is introduced into the community (Dohzono and Yokoyama 2010, Nishikawa and Shimamura 2016, Ghisbain *et al.* 2021). Ghisbain *et al.* (2021) do note that there are surprisingly low levels of competition observed between native species of pollinators. Native pollinators usually have pre-established levels of niche differentiation and partitioning which co-evolved over a length of time enabling co-existence (Ranta and Vepsäläinen 1981, Nishikawa and Shimamura 2016), but foreign species do not share a similar degree of differentiation (Nishikawa and Shimamura 2016). Therefore, we can deduce effects to both the pollinator community and the pollination services since competition arises at the overlap of niches. Native species might be affected by both resource and interference competition, but introduced species also come with novel parasites and pathogens that the native species may not have encountered before (e.g. Fürst *et al.* 2014) or are at least present in higher abundance in association with the introduced species, like “MIMS”.

MIMS stands for Massively Introduced Managed Species, which are species managed and introduced by humans usually for economic gain (Geslin *et al.* 2017). Albeit they may interact with the native wildlife just the same as the alien invasive species, which is sanctioned and frowned upon, the introduction of MIMS is often seen in a much more positive light. Geslin *et al.* (2017) state that the principal consequence imposed by MIMS is exploitative competition. One such MIMS, an introduced pollinator species whose foreign origin is often overlooked is the honeybee, which is farmed in nearly every country for the honey and wax it produces. Honeybee is highly generalist and will visit a hefty proportion of flower species in any given area (Huryñ 1997). In Finland alone there are 70000 beehives (Anon 2023a), and a single colony may contain up to 65000 individual bees (Beekman and Ratnieks 2000) that usually forage within 6 kilometres from their hives (Visscher and Seeley 1982). There is evidence of horizontal pathogen spillover from honeybees to native pollinators (Fürst *et al.* 2014, Nanetti *et al.* 2021), but exploitative competition also stems from the sheer numbers. Many studies have raised concern about the negative effects of farmed

honeybees on native pollinators (Huryñ 1997, Goulson and Sparrow 2009, Fürst *et al.* 2014). For example, it has been found that honeybees reduce the colony size and production of queens in bumblebees (Elbgami *et al.* 2014). Bumblebees also exhibit smaller body and colony sizes in the proximity of beehives (Goulson and Sparrow 2009). Many competing species are prone to competitive exclusion (Hardin 1960, Henry and Rodet 2018). Honeybee hybrids are as well a threat to native pollinators and for example Roubik (1978) noted decreases in the abundance of various native insects in response to Africanized honeybees. Despite the body of evidence, worldwide very few constraints or restrictions to beekeeping are posed. Henry and Rodet (2018) note that where hives cannot be sustained all year round, they are often moved to protected areas. Permitting cattle to a nature preserve would feel absurd, but introducing a beehive to such locations unlikely has restrictions or sanctions administered upon it.

Theory states that the number of available niches is equal to the number of different resources present, and the overlap of these niches creates competition (Feinsinger *et al.* 1981, Montero-Castaño and Vila 2016). The utilization of these resources in space, time and temperature also matters for competition or for resource partitioning. Competition between pollinators can be alleviated via niche partitioning or niche differentiation (Roubik *et al.* 1986, Scriven *et al.* 2016, Mizunaga and Kudo 2017, Jeavons *et al.* 2020). In other words, pollinator species might reallocate their behaviour to different plant species (Roubik *et al.* 1986), phenologies and temperatures (Mizunaga and Kudo 2017) in accordance with competition. For example, different bumblebee species of different tongue lengths have been found to coexist by utilizing different species of plants in accordance with the flower morphology compatible with their respective tongue length (Heinrich 1976, Ranta and Lundberg 1980, Pyke 1982). While resource partitioning in time and temperature exists, a greater plant diversity can sustain a higher number of different pollinator species and act to alleviate competition between pollinator species (Ebeling *et al.* 2008, Ramos-Jiliberto 2020).

### **1.3 Florivory and pre-dispersal seed predation**

#### **1.3.1 Plants and floral antagonists**

Plants are attacked by various herbivores that suck on the sap or feed on tissues of leaves, stems, or roots (Strauss *et al.* 2002). Herbivory has been a principal effector on the course of plant evolution (Arimura *et al.* 2011, Althoff *et al.* 2013, Snoeck *et al.* 2022). The loss of tissues impedes further nutrient accumulation and may have long lasting effects on the growth and health of the plant (Kosola *et al.* 2001). Additional risk of pathogen infections and other harmful insects (Wallin and Raffa 2001) and decreased water potential as well as increased evaporation (Senock *et al.* 1991, McCall and Irwin 2006) arise from the wounded or damaged tissues. The loss of resources is furthermore aggravated by the loss of photosynthetic surface area (Nabity *et al.* 2009). Over their long evolutionary



history, plants have developed various defences such as mechanical obstacles that include hardness and toughness (Lucas *et al.* 2000) or spikes and spines (Belovsky *et al.* 1991). In addition to mechanical defences, chemical ones such as toxic or inedible compounds against herbivores may be utilized (e.g. Feeny 1970). In many cases plants do not need to fend off herbivores or seed predators to survive but can rather compensate with growth (McNaughton 1983, Järemo and Palmqvist 2001), which is usually cheaper than defensive chemicals or structures.

Boaventura *et al.* (2022) classify flower enemies as florivores, nectar robbers and thieves, pre-dispersal seed predators, flower parasites and saproflorivores, which denotes that flowers are in fact attacked by various means by various groups of enemies. At the intersection of pollination and herbivory lies florivory, which is herbivory directed to the primary and secondary reproductive structures of the plants (McCall and Irwin 2006). Although folivory (i.e. feeding on leaves) may reduce plant reproduction and in rare cases the pollination received (Barber and Adler 2011), florivory has potential for greater effects to plant reproduction (McCall and Irwin 2006). Florivores, in comparison to their folivore counterparts may encounter fewer mechanical defences and receive more energy and nutrients than folivores (Bandeili and Müller 2010). In the study by Bandeili and Müller (2010) this difference manifested within species as flower feeding larvae of the sawfly *Athalia rosae* had a higher fitness than their leaf feeding conspecifics. Florivores may also consume pollen and nectar while on the flowers, in a similar fashion with pollinators (McCall and Irwin 2006, Söber *et al.* 2010), and may even carry out some measure of pollination (Etl *et al.* 2022). Some florivore insects also act as pre-dispersal seed predators that feed on developing seeds, and thus cause multiple harm to plant reproduction (Cawthra 1957, McArt *et al.* 2013)

Concerning florivory and pre-dispersal seed predation, although much less overall tissue may be fed on, the effects to plant reproduction are still pronounced due to the high reproductive value of flowers and the pollen and ovules they contain (Rose *et al.* 2005, McCall and Irwin 2006). Therefore, insects that damage reproductive structures are cited as influencing plant populations and reproductive systems (Rose *et al.* 2005). However, literature for florivory is conflicting as some studies have found pronounced effects on plant reproduction (e.g. many studies detailed in the review by McCall and Irwin 2006), while in some systems no effects are found (Carper *et al.* 2016, Vega-Polanco *et al.* 2020).

### 1.3.2 Direct damage

Direct damage to reproductive structures physically impairs seed and embryo development (McCall and Irwin 2006). Seeds that are damaged in their early developmental stages before seedcoat hardening are also more likely to be aborted (Stephenson 1981). In addition to the damage to the female reproductive structures responsible for seed production, florivores that eat the parts responsible for the production of pollen also reduce the reproductive success of the male function (Krupnick and Weis 1999, Ashman 2002). It is notable that in

some cases damaged seeds might leave more resources for the whole seed set, resulting in lower abortion rates than otherwise expected (McNaughton 1983).

In the case of florivory, whether the effects of florivory are mainly attested to direct or indirect effects or both are discussed in several studies (Mothershead and Marquis 2000, McCall and Irwin 2006, Haas and Lortie 2020). Damage to flowers may prompt the plant to take preventive action or compensate for the damage, which in turn contributes to the level of overall stress experienced by the plant (Savatin *et al.* 2014). Flowers can function either as resource sinks or resource “faucets” (Galen *et al.* 1999), making them expensive to produce and upkeep. Many factors, however, may contribute to flowers being aborted (Marshall and Ellstrand 1988), which means that the developing seeds and the plant itself have less resources to source from the damaged structures should they act as sinks. When flowers act as faucets, florivory would result in wasted reproductive investment. Usually, the countermeasures to damage by florivory, or the response to damage in seeds in case of flowers leads to increased abortions (Stephenson 1981, Zhang *et al.* 2011). Therefore, it is reasonable to state that viewing only the effects of florivory as a function of the seed set in certain fruits may reveal only part of the truth.

### 1.3.3 Pre-dispersal seed predation

Production of seeds poses a formidable cost to the parent plant. Though herbivores may damage plant tissues to some extent, seed predation is by definition lethal to the plant individual contained in the seed. Therefore, pre-dispersal seed predation can have pronounced evolutionary consequences (Brody 1992, Kon *et al.* 2005, Xu *et al.* 2015). The nutritional content makes seeds attractive targets to species of granivores that feed on them during the various developmental stages of the seeds. Seeds are especially vulnerable in their pre-dispersal state, as they are displayed, aggravated, and the seed coat is yet to harden. Developing seeds are also more likely to be aborted than developed ones (Stephenson 1981). The developing seeds with undeveloped seedcoat may also be easier to feed on as the soft living walls, although generally abundant in protective chemicals, have yet to harden (Janzen 1976). Pre-dispersal seed predation affects the reproductive output of plants (Xu *et al.* 2015), though it may often be difficult and arbitrary to distinguish florivory directed to primary reproductive structures from pre-dispersal seed predation. Some insects that are primarily considered florivores may also act as pre-dispersal seed predators, eating both flowers and seeds/developing fruits of the plants. These multi-functioning florivores and granivores include species like *Zaenobius geranii* (Cawthra 1957), *Popillia japonica* (Scarabaeidae) on the plant *Oenothera biennis* (McArt *et al.* 2013), *Anthonomus grandis* (Curculidae) on cotton (Grigolli *et al.* 2017), *Anthonomus eugenii* on Capsicum -species (Chabaane *et al.* 2021) and *Lygus lineoralis* (Lygaeidae) on cotton (George *et al.* 2021). Acting this way on many stages and functions of the reproductive cycle, the damage to flowers by various floral enemies (Boaventura *et al.* 2022) accumulates. In the case of many of the

pre-dispersal seed predators, the insects feed on developing seeds as larvae. The larvae are of limited mobility and cannot utilize already dispersed seeds.

### 1.3.4 Florivory and pollination services

In addition to direct damage to gametes, effects of florivory may also be attested to the result in changes in the phenotype of the plant, resulting in indirect effects to reproductive success (Tsuji and Ohgushi 2018). When comparing flowers damaged by florivory and flowers that were undamaged, seed production was lower in flowers experiencing florivory only in the case of naturally pollinated flowers, while there was no difference between hand pollinated, damaged or undamaged flowers (Leavitt and Robertson 2006). This experiment serves as an example of how florivore induced pollen limitation may reduce the seed set of the flowers indirectly via pollen limitation rather than direct damage to gametes. Feeding damage to the flowers not only alters the flower size and symmetry, but also affects floral odour and volatile emissions (Zangerl and Berenbaum 2009) and floral rewards (Krupnick and Weis 1999), all of which are crucial to attracting pollinator insects (Cresswell and Robertson 1994, Leavitt and Robertson 2006, McCall and Irwin 2006). Reduced attractiveness to pollinators may result in reduced pollinator visitation rates and reduced time spent in the flowers (Møller 1995, Krupnick and Weis 1999, McCall and Irwin 2006, Cardel and Koptur 2010). Mothershead and Marquis (2000) noted that reduction in seed set caused by florivory was due to changes in flower morphology that reduced visitation rates. Many flowers require more than a single pollinator visit to maximize their seed production, and different pollinator insects have differential pollination efficiencies (Motten *et al.* 1981, Kamo *et al.* 2022). Therefore, in such cases florivory, despite having indirect effects, might not altogether preclude seed production, but reduce it like in the study by Tsuji and Ohgushi (2018).

### 1.3.5 Florivore preferences

Flowers vary between plant individuals as different plant genotypes may differ in various aspects of morphology and phenology. Furthermore, gynodioecious or dioecious populations of plants that contain individuals of different sexes often show distinct floral sexual dimorphism (Ågren and Willson 1991). Florivores have preferences as to different genetic clones, morphologies, sexes and floral phases of the plants (Marquis 1984, McCall and Barr 2012, Alves-Silva *et al.* 2013). These preferences stem from nutritional requirements as well as the potential differences in defensive chemicals in flowers of different colours, or colour intensities (McCall and Barr 2012, McCall *et al.* 2013). For example, Tsuchimatsu *et al.* (2014) noted that the flower feeding weevil *Z. geranii* showed preferences towards colour traits of the *Geranium thunbergii* flowers. Tsuchimatsu *et al.* (2014) state that pink flowers rather than white ones received more florivory damage as well as damage to seeds by the weevils. The authors hypothesized this difference is associated with differences in chemical composition between the colour morphs. In a study by McCall and Barr (2012), it was stated that in

*Nemophila menienzii*, the florivore preference is mainly due to flower diameter, and hermaphrodites are preferred by the florivores over females. Flowers with the male function are typically larger and showier, offer more rewards (Delph and Lively 1992, Klinkhamer and De Jong 1993, Varga *et al.* 2013), and by definition offer pollen which female flowers do not possess. In some cases, the defence chemical ratios may also be different between male and female plants due to the different goals of the flowers in terms of their sexual functions, and this has been cited to be typical especially in gynodioecious species (Rabska *et al.* 2020).

### **1.3.6 Similar preferences with pollinators**

While the plant's floral display is a means to attract pollinators, in doing so they may also inadvertently attract the unwanted attention of herbivores, resulting in a trade-off situation (Galen 1999, Adler and Bronstein 2004, Theis 2006, McCall and Irwin 2006, Cardel and Koptur 2010). The most intensive damage is often observed in large, showy and rewarding flowers (Leege and Wolfe 2002), the flowers that are expected to receive most pollinator visitations as well (Møller 1995, Bond and Maze 1999, Martin 2004). Due to the same flowers hypothetically receiving most florivores and most pollinators, the paths of the plant's mutualists and antagonists are very likely to cross here. Pollinators are in many cases found to be affected by the presence and floral damage by florivores. Florivores on the other hand do not receive any negative effects from pollinators. Rather, those florivores that are also pre-dispersal seed predators should benefit from the flowers being pollinated and fertilized.

## **1.4 Within-species diversity**

### **1.4.1 What is within-species diversity?**

When Charles Darwin wrote the words "Endless forms most beautiful" (Darwin 1859), he principally meant the diversity of various species found on earth, and how they had come to be as a result of evolution. However, evolution itself would not happen without within-species variation (Hughes *et al.* 2008). The division of the tree of life into species hardly does justice to the great variation found in nature. Within-species diversity is the within-species and within-population component of diversity, composed of the genotypic and phenotypic variation (Hughes *et al.* 2008). In terms of a plant species, this may be denoted by differing leaf and flower morphologies, their varying shapes and colours and differential colour patterns (Burkle *et al.* 2013). Nowadays the value of within-species diversity has become acknowledged more widely (Hughes *et al.* 2008). So much so, that it is regarded as of integral value in nature conservation biology (Hughes *et al.* 2008). The variation within species acts as a storage of genetic material of traits and enables a population to adapt especially in changing conditions

(Hughes *et al.* 2008). This makes it an especially important trait of natural communities facing changing environment in our modern times.

#### 1.4.2 Within-species diversity and species interactions

With the ongoing global pollination crisis, the pollinator species are threatened, for example, by the lack of nutritional diversity due to the loss of plant diversity. Therefore, the diversity in pollination systems should not only be viewed in the context of a species. Instead, a larger context of species interactions should be employed. It is true that for pollinator insects, plant species diversity is crucial since it increases their diversity (Nicholls and Altieri 2013, Isbell *et al.* 2017) and offers various benefits like increased potential for resource partitioning (Spiesman and Gratton 2016), flowering continuation (Ebeling *et al.* 2008) and healthier diet (Pasquale *et al.* 2013). In terms of the significance of plant species diversity to the pollinator insects, within-species diversity of plants may yet function similarly. Furthermore, the overyielding phenomena, where niche partitioning between species reduces competition and improves overall resource exploitation (Trenbath 1974, Isbell *et al.* 2017) is well known in terms of biomass production. The idea could theoretically reflect floral resource yield too, though the matter has not been scientifically addressed. The fact that plant diversity contributes to the available niche partitions for pollinators (Nicholls and Altieri 2013), and plants may even facilitate each other's pollination (Ghazoul 2006) represent unexpected aspects of diversity.

Previous studies draw many links between plant genetic, or within-species diversity and pollinator behaviour in flower choice (Hoballah *et al.* 2007, Genung *et al.* 2010). Since pollinators have different preferences, abilities and requirements (Kandori 2002, Layek *et al.* 2022, Sagwe *et al.* 2022), diversity at within-species level could function similarly to that of species level. For example, different plant genotypes within the same species could also provide a healthier, more complex diet to the insects much in the same way as in the case of species diversity (Pasquale *et al.* 2013) as well as vary in their flowering phenology over short and long periods of time (Fogelström and Ehrlén 2019). Florivores and pollinators generally prefer similar traits in plants indicative of high-quality food yield, but the literature on the similarity of preference to within-species traits between the florivores and pollinators is scarce.

An especially conspicuous and therefore significant aspect of within-species diversity is the variability in sex expression among various plant species exhibiting dioecy. Dioecious plant populations consist of two sexes; males and females, as opposed to monoecy, where both sexual functions are found within same plant individuals (Ågren and Willson 1991, Eckhart and Chapin 1997, Miller and Venable 2003). A sub-category of dioecy is gynodioecy, where mutations and cytoplasmic determinants have rendered a loss of male function in the hermaphrodites creating all-female plants, so that the populations consist of females and hermaphrodites (Schnable and Wise 1998, Budar *et al.* 2003). Maintenance of gynodioecy in populations is evolutionarily problematic since the females, along with their male function, effectively forfeit half of their

reproductive fitness too. The loss of pollen needs to be compensated (Lewis 1941, Lloyd 1976), and increased offspring fitness and viability via avoidance of inbreeding depression (Puterbaugh *et al.* 1997, Dufay and Billard 2012) as well as resource allocation (Eckhart 1992, Ashman 1994, Chang 2006) have been suggested as means for the said compensation. Furthermore, the stepping-stone hypothesis states that gynodioecy is an intermediary from monoecy evolving towards dioecy. Therefore, gynodioecy would be caused by hermaphrodites, biased towards their male function in the proportion of their sexual fitness losing their female function as a result (Lloyd 1976, Spigler and Ashman 2012). Because plants of different sexes realize their reproductive fitness via different means, the reproductive evolutionarily stable strategies and therefore morphologies between the sexes also differ (Thomson and Brunet 1990). Female flowers naturally do not offer any pollen and therefore do not interest the pollen foragers, they also have a less showy floral morphology and offer lesser rewards than males or hermaphrodites, causing females to experience reduced visitation rates (Bond and Maze 1999, Asikainen and Mutikainen 2005, Cuevas *et al.* 2008, Varga and Kytöviita 2010, Van Etten and Chang 2014). Female plants source all of their reproductive fitness from the female function, which means that they should mainly allocate to seed production and to receiving sufficient amounts of pollen for pollination. Hermaphrodite and male plants however rely greatly on their ability to pollinate females, since the frequency of pollinated flowers can be much higher than the frequency of the plants own seed production (Lloyd 1976, Charlesworth 1981, Vaughton and Ramsey 1998, Varga and Kytöviita 2010, Van Etten and Chang 2014). Female flowers do need successful visits to produce seeds, and a single visit might be enough for pollination. However, hermaphrodite- and male flower are expected to benefit equally of all of the subsequent visitations where pollen is picked up. Therefore, trends where male plants allocate to their floral displays even at the cost of their own survival and abortion of ovules become apparent (Bond and Maze 1999). Female plants then again, produce seeds of higher quality and quantity (Ashman 1994, Chang 2006).

For florivores as well a pattern where plants with a higher degree of maleness exhibit higher rates of florivore damage and visitation rates becomes apparent (Marshall and Ganders 2001, Theis *et al.* 2007, McCall and Barr 2012). This is natural since maleness results in increased allocation to floral display, and the display itself is what the florivores feed on. There are some theories as to what factors instil the florivore preference. For example, some genotypes may be defended better, reducing florivore fitness and thus affecting their preference (Mody *et al.* 2015). Higher concentrations of toxins in the female flowers could be expected in order to protect the valuable ovules. On the other hand, pollen is relatively dispensable, and can direct visitors, pollinators and florivores to male flowers (Collin *et al.* 2002, Ibanez *et al.* 2012). The main body of literature however tends to explain florivore preference to male flowers with similar preferences of pollinators and florivores to floral characteristics like corolla width (Leege and Wolfe 2002, McCall and Barr 2012). Florivores might also prefer flowers with pollen with less preference to nectar than pollinator insects, like many bees, that

tend to prefer flowers with high concentrations of nectar (Soper Gorden and Adler 2016).

Plant species diversity and within-species diversity could both be important components to the survival of native pollinator species. Diversity can alleviate the competition between pollinators (Kettle *et al.* 2011, Theodorou *et al.* 2020). Although in this context within-species diversity has not received much attention, I speculate that within-species diversity could add a further level of diversity that enhances the adaptability and stability of native pollinator species populations. Various aspects of diversity enable species of pollinators to niche partition between different species to alleviate competition and coexist (Kettle *et al.* 2011, Wang *et al.* 2019, Theodorou *et al.* 2020, Hodge *et al.* 2022). However, considering a plant that is a pollinator generalist, abundant within the community and widely preferred by pollinators, competition is likely to ensue regardless. Competition is furthermore aggravated when competition takes place between a native species and an introduced species with no similar resource partitioning patterns (Nishikawa and Shimamura 2016, Ghisbain *et al.* 2021). Therefore, if within-species diversity could act in the same way as species diversity, it could further reduce inter-specific pollinator competition and overlap in flower visitations. Consequentially horizontal pathogen transfer, as well as exploitative competition, should be expected to subside. By enabling more niche partitioning and complexity into the environment, within-species diversity could sustain a higher variation and stability of pollinator species. Even in the case of reduced plant species diversity, by ensuring the persistence of within-species diversity or genetically diverse species, we could potentially contribute to the preservation of diversity at the higher trophic levels. Therefore, the interest arises to evaluate the significance of the diversity within species in the context of florivores, pollinators and plants.

## 1.5 *Geranium sylvaticum* and its associate insects

*Geranium sylvaticum* (L.) is a dicot, perennial, herbaceous flowering plant (Stroh *et al.* 2014). It is part of the Geraniaceae family which consists of 824 described species (Anon 2023b, Anon 2023c). *G. sylvaticum* is commonly found throughout Finland (Anon 2023c). *G. sylvaticum* is gynodioecious, meaning that the populations contain both female and hermaphrodite plants and often individuals that fall between the two (Varga and Kytöviita 2010). Gynodioecious reproductive system is present in 0.5 % of dicot species (Godin and Demyanova 2013). Throughout this thesis and the included original papers, the sex of *G. sylvaticum* is referred to via the mean count of stamen in a flower. The flowers contain 0–10 stamens, and a typical hermaphrodite plant has 10, whereas a female has none, though the plants with intermediate sex typically have stamen counts somewhere in between. Female flowers are characterized as being very small in diameter and offer less rewards (Asikainen and Mutikainen 2005, Varga and Kytöviita 2010) and naturally no pollen to pollinators. *G. sylvaticum* is

morphologically strikingly diverse, as in addition to its various sexes, the flowers show colour variation from nearly white to deep purple and pink, and pollen can be purple, green-greyish or yellow. Furthermore, the nectar guides and the general shape and size of the flowers and petals greatly varies.

Pollinator community of *G. sylvaticum* is diverse. Natural plants usually have 10–100 flowers (Asikainen and Mutikainen 2003), and the flower of the plant represents a dish-bowl shaped open flower accessible to various insects (Kozuharova 2002). The primary pollinators of *G. sylvaticum* are the bumblebees, though various other insects visit the flowers too (Varga and Kytöviita 2014). During the data collection of this thesis and the original studies (I, III), solitary bees and other Hymenoptera, bumblebees, Diptera, especially hoverflies and different Nematocera, as well as beetles and butterflies were seen visiting the flowers. Visitations from a variety of insects are also noted by the literature (Asikainen and Mutikainen 2005, Varga and Kytöviita 2010).

Most intriguing herbivore that utilizes *G. sylvaticum* for food is the obligate florivore of Geraniales, *Zacladus geranii* (See Cawthra 1957 for a species account). *Z. geranii*, or cranesbill weevil is a curculid beetle, which as an adult, feeds on the flowers, principally the petals of *G. sylvaticum*. The emergence of adults coincides with *G. sylvaticum* flowering (Davis 1973), during which they mate and oviposit in the ovaries of *G. sylvaticum* flowers (Cawthra 1957, Davis 1973). The larvae reside within the developing seeds and emerge through the seed coat leaving only the husk of the seed behind. The larvae drop on the ground and pupate over a span of approximately two weeks (Soininen and Kytöviita, unpublished data), and then overwinter as adults. This adaptation to complete the larval phase in synchrony with the *G. sylvaticum* seed development leaves *Z. geranii* with a very short time to complete its larval stage. *Z. geranii* is both a pre-dispersal seed predator and a florivore, which makes it an interesting study species as an antagonist of *G. sylvaticum*.

Various interactions take place between plants, their pollinators and florivores. As florivores are attracted to similar traits in plants as pollinators are (Theis 2006), and in doing so affect the attractiveness of flowers to pollinators, these three interacting parties are inseparably interwoven. Therefore, *G. sylvaticum*, its pollinator community and *Z. geranii* together bring about an intriguing three-way system in which to study the interactions of the herbivores, pollinators, and the within-species diversity of plants.



## 2 AIMS OF THIS STUDY

Pollinators worldwide are threatened. Various global challenges like climate change, introduced species, novel pests and parasites coupled with the loss of habitat and the by flow of foreign chemicals like neonicotinoids all contribute to the direness of the situation (Goulson *et al.* 2015, Singla *et al.* 2021). The value of pollinators is immeasurable to both natural ecosystems and the livelihood of humans as well. 85 % of commercially produced crop species depend on pollination and overall, the portion of 80 % of vascular plants worldwide require zoophilous pollination (Buchmann and Nabhan 1996). Native pollinators cannot be replaced with commercially farmed honeybees alone, since at larger orchards or plantations the number of honeybees from a handful of nests may already be insufficient, and honeybees are incapable of buzz pollination demanded by various crops (De Luca and Vallejo-Marín 2013, Rocha *et al.* 2023). Even when honeybees are used, their pollination services are more efficient in the presence of native pollinator species (Greenleaf and Kremen 2006). Abundant number of native pollinator species ensures the pollination services of a variety of crops, and in a variety of weather conditions and points of time (Albrecht *et al.* 2012). Though within-species diversity has received ever increasing attention (Hajjar *et al.* 2008, Hughes *et al.* 2008) there still remains much to understand about this characteristic of populations of organisms.

In this thesis and the original studies contained, the goal is to address the significance of within-species diversity of a plant to its network of interactions from different perspectives. Firstly, (I) within-species diversity is viewed from the perspective of the plant in relation to pollinator behaviour, and goals of the morphologically different sexes of *Geranium sylvaticum*. Then, (II) the focus is shifted from pollinator behaviour to florivore behaviour as effects of florivory and pre-dispersal seed predation by *Zacladus geranii* are weighed. Rather than just view the effects of flower eating insects, the aim is to clarify the significance of within-species variation of plants to the florivory and pre-dispersal seed predation of *Z. geranii*. Furthermore, the potential of *Z. geranii* to induce evolutionary pressure on *G. sylvaticum* needs to be evaluated to better understand the interactions of the species. Finally, (III) the aspect of plant within-

species variation is expanded to inter-specific pollinator species competition. Here the role of plant within-species diversity to pollinator insects is investigated. Furthermore, it is evaluated whether plant within-species diversity could enable niche partitioning between native and introduced pollinators, and how exactly do these insects interact with each other in the landscape of morphologically differing *G. sylvaticum*. These questions aim to provide evolutionarily and ecologically interesting insight, but when answered they also contribute to the body of literature needed to ensure protection of our native pollinator species.

In this thesis and the original studies, the aim is to address the following questions around florivory, pollination and plant within-species diversity:

- I. What is the significance of plant within-species diversity to the pollinator insect behaviour, and thus, to the plant itself?
- II. How does *Z. geranii* florivory and pre-dispersal seed predation affect *G. sylvaticum* reproduction, and what aspects of within-species diversity determine florivore behaviour?
- III. Does plant within-species diversity have significance to the competition between introduced and native pollinator species?

## 3 METHODS

### 3.1 The Geranium study field (I, II, III)

The studies were conducted at the Geranium study field (Fig. 1 and 3) in Konnevesi (Coordinates: 62°35'N 26°14'E). The site has been established in 2008 on a former agricultural field by Minna-Maarit Kytöviita. In the field, 531 plants, original parent clone replicates (n = 108) and their offspring (n = 423) are growing in natural conditions. The parent plant genotypes (n = 14) are originally collected from nature and then cloned in laboratory conditions. After these plants have been cross-pollinated to produce offspring with each other in replicates of all possible combinations with different mother and father plants and sexes. The plants are tagged with an individual specific ID which can be used to access all the information on the genotype, life history and previously measured traits of the plants.



FIGURE 1 *G. sylvaticum* growing in the study field photographed at the emergence of the study plants in May 2021. ©Jaakko Soininen

### 3.2 Plant sexual dimorphism and insect behaviour (I)

In the study I, a novel approach was used to measure the pollination probability of the insects. It was hypothesized based on the field observations of insect behaviour, that the female flower size is an adaptation to enable the flowers more efficient receipt of pollen by various pollinator insects. Instead of traditional real-time eye-ball observations, the study plants in the *Geranium* study field were filmed with a video camera. *Geranium sylvaticum* flowers only for a short time, and video recording enabled us to maximize the amount of data. Furthermore, enabled by the zoom, replay and frame-by-frame viewing, it was possible to carefully assess the movements of the insects in the flower. From computer screen, each flower visit, the identity of the visitor, sex of the plant and flower as well as the contacts made to reproductive organs were recorded to the data.

### 3.3 Effect of florivory on plant reproduction (II)

Due to the extensive nature of the study II, it is best understood via the three phases it consisted of: the flower tagging phase, the measurement phase and the counting phase.

During flower tagging phase, plastic labels were attached to the perianth of a flower with a black cotton sewing thread. The experiment consisted of three treatments, control (C), florivory (F) and manipulation (M) (Fig. 2). For each set of marking three flowers representing the three treatments were selected. During the time of marking, a flower's stigma phase whether receptive or unreceptive, sex, castration by *Zacladus geranii*, presence of pollen on the stigma and the treatment were recorded according to the code in the label. For the control treatment, average flowers with no signs of florivory were selected and marked. For the natural florivory treatment, any flowers that had distinguishable markings of florivory were selected. For the manipulation treatment, *Z. geranii* florivory was simulated in average flowers that were punctured with a steel punch so that a single flower had a total of 5 holes across three petals. For a single repeat, flowers as similar as possible in age, form and sex were selected. *G. sylvaticum* pollen is visible to the eye, and it was ensured that a flower tagged for the florivory- or manipulation treatment was not already pollinated.

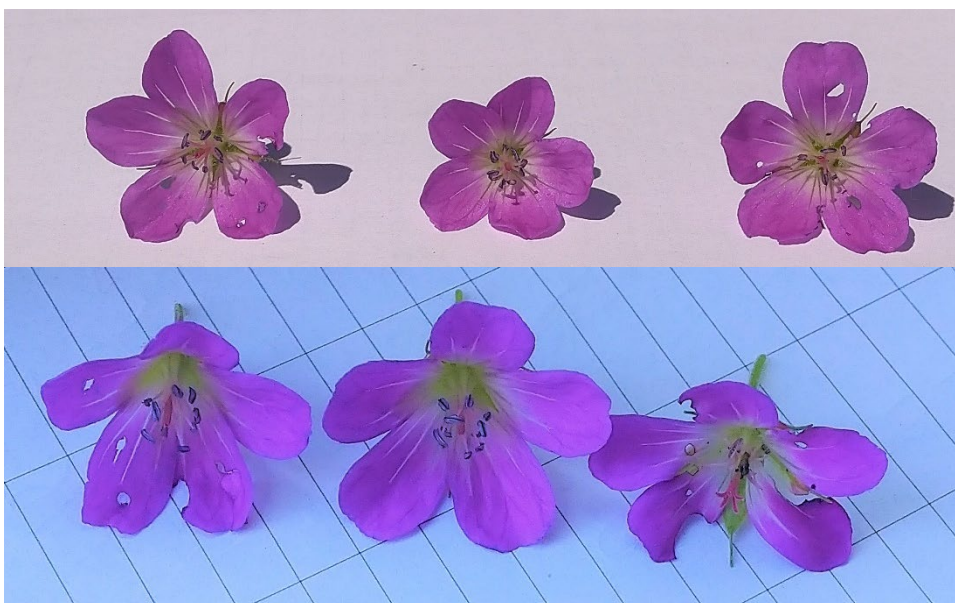


FIGURE 2 Two sets of flowers reflecting the three treatments in the study II. Left = manipulation, artificial florivory, centre = control, natural healthy flower, right = florivory, flower damaged by *Z. geranii*. Flower in the lower right corner has had most of its stamen incised by *Z. geranii*. ©Jaakko Soininen



The measurement phase was carried out semi-daily alongside the tagging phase and consisted of measuring the plant parameters relating to flowering and florivory. The parameters measured in field conditions were the number of flowers, number of flowers damaged by *Z. geranii*, ratio of receptive stigma to the unreceptive ones (among minimum of 30 flowers count), and the receptiveness of the stigma damaged by *Z. geranii*. Furthermore, the phase of flowering whether beginning, middle or end phase was determined by the ratio of buds, flowers, and fruits/wilted flowers (early phase = more buds or flowers than fruits, and late phase = more fruits than flowers and buds). Parameters like the number of floral shoots, plant sex (number of stamens divided by the number of flowers count among min. 30 flowers), colour of the flowers and colour of the pollen were also measured, but it was only necessary to calculate these once per plant. The data gathered during measurements phase was combined with the pre-existing data on the genotype and life history trait of the individuals.

The last phase, counting phase consisted of measuring the seed production of the individual tagged flowers as well as the overall seed production of the whole plant. The fruit of a *G. sylvaticum* is a schizocarp with loci to produce up to five seeds. The developed seeds are flung away by the side of the elongated style which builds pressure as it dries. The seed scars, undeveloped embryo and missing sides of the style can be used to count the number of seeds produced out of the maximum seed production per schizocarp which is five. After the labels were counted, each plant was collected in a paper bag, and dried in a drying oven for 24 hours to preserve the material. After drying, the seeds were counted as described above. The tagged flowers were removed from the plant and out of the developed schizocarps, the number of seeds was counted. If a flower had wilted before starting to develop seeds (seen as style elongation) it was counted as wilted, aborted flower. If the style had elongated and the ovules visibly started to develop but the flower had not developed any seeds, it was treated as a developed fruit with a seed production of zero. Any damage by *Z. geranii* was recorded and was often visible as the mericarp, the cup covering the ovule/seed had been punctured as the developed *Z. geranii* larvae had made their way out of the seed.



FIGURE 3 Study plants growing in the Geranium study field in full bloom in June 2021.  
©Jaakko Soininen

### 3.4 Competition between honeybees and native pollinators (III)

It was hypothesized that the competition with honeybees affects native pollinators such as bumblebees, solitary bees and hoverflies (III). Furthermore, the aim was to find whether the effects of competition were uniform across all genotypes, i.e. would the bumblebee foraging behaviour in different plant genotypes change in response. To test this, a honeybee hive was placed next to the Geranium study site. Since there were very few honeybees observed without the hive, it was possible to remove honeybee presence for the most part by transporting the nest 20 km away. This was done so, that the control and beehive treatments took place by alternating between two consecutive days of each treatment during the summers 2021 and 2022. During this time, the plants were observed during the active hours of pollinator insects (6:00AM–9:00PM). Visitation parameters like the species of insect and the number of flowers visited were recorded, and the plant genotype as well as other plant parameters like sex and the number of open flowers was noted. Environmental variables like amount of sun, wind and the temperature were noted as they affect the behaviour of pollinators.

## 4 RESULTS AND DISCUSSION

### 4.1 Significance of within-species diversity to the plant (I)

Study I addresses the significance of within-species diversity through pollinator insect behaviour. The aim was to firstly understand whether within-species diversity could arise and become pronounced in response to pollinator behaviour. Secondly, effort was made to determine what is the significance of within-species diversity to the plants. Insects visiting species of *Geranium*, despite their efficiency to pollinate, all receive rewards due to the open-to-all morphology of flowers (Kandori 2002). The efficiency to pollinate may however be drastically altered by floral traits (I). In dioecious and gynodioecious species, the most notable component of within species diversity is the plant sex, which affects many aspects of individual plant traits but is mostly expressed via the inflorescence (Ashman 2005). Although in many cases, pollinator visitations and preferences are determined by the floral morphology and required handling skills (Woodward and Lavery 1992, Alcorn *et al.* 2012, Ishii and Kadoya 2016), morphology can have differential effects between the genotypes of plants. In the study I visitor behaviour was found to be an important factor in determining whether a visitor could pollinate the flower.

It was found that in the case of gynodioecious *Geranium sylvaticum*, whose different sexes dictate differential optima for the pollinator behaviour (I), the sex of the plant presupposes, that female plants are dependent on the receipt of pollen. Pollen is needed for the female sexual fitness, which is realized exclusively through seed production. Hermaphrodites then again are credited to source their reproductive fitness from both their male and female functions, and primarily rely on their male function as they gain a higher fitness benefit for fertilizing the females in the population (Lloyd 1976, Charlesworth 1981, Thomson and Brunet 1990, Barrett 2002, Vamosi and Otto 2002). Our results fully support the notion of hermaphrodites' maleness biased sexual allocation. Hermaphrodites receive more visits, whereas the visits to females were of better quality, although fewer in number. Gynodioecy has resulted from mutations in



a random manner (Schnable and Wise 1998, Budar *et al.* 2003), but this study contributes to the body of evidence on how gynodioecy is maintained within the populations via ecological interactions. Based on the results it can be argued that the differential trade-offs between the sexes give rise to the within-species diversity in the form of plant sexes differentiating due to directional selection. In our videorecorded study material, it became apparent that the floral size (i.e. petal length and opening) and length of the style were key features in determining the pollen receipt and export in a flower. Although the nectaries are similarly placed in the sexes, the smaller female flowers consistently had a shorter style and a slightly more closed corolla with shorter petals, which directed pollinator insects to land on the stigma, or reach over it when reaching for nectar. In other words, while hermaphrodite sex expression is consistent with the goal of attracting visitors, female flower size is an adaptation to maximize pollen receipt by various insects. The trade-off is understandable in the context, that a female plant would need only a single high quality pollinator visitation, whereas a hermaphrodite benefits of the consecutive visits as long as there remains pollen in the stamen.

In our study, different flower visitor groups were evaluated in their pollination probability. It was found that the small female flowers were probable to be pollinated by various visitor groups, although by far the highest pollination probability was associated with bumblebees. Hermaphrodite flowers on the other hand were only pollinated by bumblebees, and even then, much less likely than the females. Therefore, within-species diversity is also related to the identity of visitors. For example, these results give reason to argue that in different environmental conditions, or when pollinator populations of certain species dwindle, within-species diversity is an important trait of the plant population. Hughes *et al.* (2008) cite within-species diversity as integral to the preservation of species, for it allows the adaptation of the population to new conditions. According to the results of the study I, for a *G. sylvaticum* population to possess diversity in its floral morphologies and sexes would mean that some plants in the population are still effectively pollinated, should the pollinator community change.

Any traits of plants that affect pollination are likely to have pronounced effects to the plant evolution and the degree of observed within-species diversity. In the case of *G. sylvaticum*, the selection pressure caused by pollinator behaviour in the different sizes of flowers has contributed to the degree of diversity; its birth and maintenance in *G. sylvaticum* populations. These results can be summarized by concluding that within-species diversity is emphasized in relation to the plants' interactions with pollinator insects. Diversity enables the plant to interact among various insects, which should eventually contribute to the stability of the population. Furthermore, different plant morphologies are promoted and moulded in respect to the type of interaction with pollinator insects that best serve the purpose of maximizing the fitness of the morphs.

## 4.2 Floral antagonists, plant genotype and plant reproduction (II)

Many studies attest to the negative effects of florivory on plant reproduction. There are both direct (e.g. Stephenson 1981, Marshall and Ellstrand 1988, Zhang *et al.* 2011) and indirect (Mothershead and Marquis 2000, Leavitt and Robertson 2006) effects on the reproduction of plants. Furthermore, evidence states that in many cases pre-dispersal seed predators also affect plant reproduction (Brody 1992, Kon *et al.* 2005, Xu *et al.* 2015). In the study II, our first goal was to define whether the florivory and pre-dispersal seed predation by *Zacladus geranii* had an impact on *G. sylvaticum* reproduction, before assessing its potential interaction with within-species diversity of *G. sylvaticum*. Rather than addressing the effects of *Z. geranii* according to certain reproductive parameter, it was noted that effects of *Z. geranii* may differ according to the stages of seed development. In other words, the effects may include for example wilting of flowers and fruits, reduced seed set as well as predation on pre-dispersal seeds.

No evidence was found of florivory affecting the seed set of a developed *G. sylvaticum* fruit, although it was hypothesized that the direct and indirect effect of *Z. geranii* could damage the ovules and impair pollination services leading to less seeds produced. We hypothesized that such mechanisms would underlie our study system as well, since many other studies support the idea of florivory affecting seed production (Mothershead and Marquis 2000, Leavitt and Robertson 2006, McCall and Irwin 2006). However, despite the lack of effects on seed production, we noted that the flowers with *Z. geranii* damage had much higher probability of being aborted than did the healthy, or manually punctured flowers. This denotes that the plant rather actively aborts flowers with *Z. geranii* florivory, or damage itself is connected to flower wilting. Damage to flowers has been found to cause floral abortions in some cases (Goto *et al.* 2010). Overall, only 60.1 % of all flowers in this study developed seeds. It has been proposed by other studies that plants produce more flowers than set seeds, and that this surplus can serve as a pre-dispersal seed predator sink and back-up, should other flowers fail (Holtsford 1985, Ghazoul and Satake 2009). If *Z. geranii* damaged flowers (roughly 4–5 % of count flowers) wilt and resources from them can be allocated to surplus flowers, the plant should be able to compensate for the damage. *Z. geranii* damaged flowers, more at risk of being aborted, could leave more resources for the rest of the flowers and their developing seeds as proposed by McNaughton (1983). This could explain why we did not find effects on seed production from florivory.

Though florivory did not affect the seed production, we found that pre-dispersal seed predation negatively affected the seed set, and when present in a fruit, the decrease in seed production was more than the loss of the predated seed alone. Since increased floral abortions by the plant were offset by the plant, we propose that in the quantities it is found in nature, *Z. geranii* mainly influences plants by pre-dispersal seed production rather by contributing to abortions and directly destroying seeds. Arguably effects of florivory and pre-dispersal seed

production are difficult to compare, since evaluation of the respective compensatory mechanisms is difficult. Upon these results it can be argued that *Z. geranii* has some potential to impose selective pressure and affect *G. sylvaticum* evolution.

A study by Tsuchimatsu *et al.* (2014) indicated that *Z. geranii* direct their feeding behaviour towards certain Geranium genotypes and certain flower morphologies. Since a connection can be made between similar traits preferred by florivores and pollinator insects (Galen 1999, Adler and Bronstein 2004, Theis 2006, McCall and Irwin 2006, Cardel and Koptur 2010), we would also expect that the same genotypic morphs are preferred for both groups. Therefore, either the increment in pollination (and florivory) would be offset in comparison with the low pollinator visitation rates or low florivore burden, respectively. In the data from study II, some of the genotypes differed from each other in the count of *Z. geranii* damaged flowers. This is likely to be a conservative estimate since the high mobility of *Z. geranii* causes the dispersion to be even among the *G. sylvaticum* population when viewed over a longer timescale. Furthermore, according to our measurements, there is reason to argue that the dispersion of *Z. geranii* is mostly density dependent among plants, since the number of damaged flowers in different plants remains surprisingly constant.

In terms of genotypic variance, it is interesting to note that plants differ both in terms of their father and mother clones in both the amount of florivory and pre-dispersal seed predation (II). These results denote a genotypic difference in *Z. geranii* preference which goes further than what was predicted by other variables like plant sex, colour, abortion rate or flowering period. Although Tsuchimatsu *et al.* (2014) did not have other proxies for plant genotypic variation than colour, our results agree that plant morphologies indeed affect florivore preference, and genotypic background has similar effect than in the case of pollinator preference (Genung *et al.* 2010).

### 4.3 Within-species diversity and pollinator competition (III)

According to the three studies in this thesis, within-species diversity of a plant has consequences to the fitness of different morphologies of the plant *G. sylvaticum* (I), and the plant within-species differences also affect *Z. geranii* florivory and pre-dispersal seed predation (II). Therefore, the missing component is the significance of plant within-species diversity to pollinators. Pollinators depend on diversity in terms of floral continuation which ensures that food is consistently available over the active season of the pollinator insects (Ebeling *et al.* 2008). Furthermore, diversity in floral resources is an aspect contributing to overall pollinator health. We approach the significance of plant within-species diversity from the viewpoint of pollinator competition. Pollinators visit the same flowers, pre-empting them and horizontally transporting pathogens. Niche partitioning exists among pollinator species to allow their coexistence. We determined to investigate whether within-species diversity of the plant *G.*

*sylvaticum* could enable niche partitioning between native pollinators and introduced honeybee, which is the most abundant introduced pollinator in Finland.

Literature notes that honeybee presence negatively affects native pollinators (Goulson 2003, Cappellari *et al.* 2022). For example, bumblebee worker size has been noted to decrease in response to honeybee presence (Goulson and Sparrow 2009), and there are other cases denoting the negative effects via various pathways (Mallinger *et al.* 2017, Nanetti *et al.* 2021). In the study III, the competition between honeybees and the native bumblebees was pronounced, as the presence of honeybees at the study site lowered the visitation frequency of bumblebees. *G. sylvaticum* was the most abundant flowering plant. It is therefore likely that bumblebees in presence of the competition shifted their foraging focus to other plants. Some pollinator species could do this better, as is shown by the fact that the long tongued *Bombus pascuorum* visitation rates were most pronounced to decrease, as they could avoid competition with the short-tongued (Balfour *et al.* 2013) honeybees. Other pollinator species, namely the rest of the groups in focus, hoverflies and solitary Hymenoptera were not affected by honeybee competition in the parameters measured, although our data only addresses the quantity of visits, not the actual effects to pollinator health (III).

The study assembly consisted of various genotypes of *G. sylvaticum*. As a result, it was noted that the bumblebee foraging rate shifts in response to honeybee presence were not uniform across all of the genotypes. The honeybee is generally cited as a generalist in its flower species visitations (Crane 1999, Steffan-Dewenter and Tscharntke 1999). This same trend was observed for individual *G. sylvaticum* genotypes as well. As honeybees did not discriminate between their specific genotypes, but bumblebees did, it is likely that bumblebee foraging patterns were determined by a specific, undefined goal. Therefore, within-species diversity gives bumblebees the aspect of variety from where to choose from according to their needs, and therefore within-species diversity should be counted as a beneficial component of a flower population to the native pollinators. However, since honeybees were noted to compete differently with different species of bumblebees, further studies will have to focus on effects of individual bumblebee species.

#### **4.4 Florivores, pollinators and the plant within-species diversity: a synthesis**

Various interactions take place between plants and pollinators (Bierzychudek 1981, Ghazoul 2006, Abrahamczyk *et al.* 2017, Sauquet and Magallón 2018), between plant (Temeles *et al.* 2016, Thomson and Page 2020), between pollinator species (Strange *et al.* 2011, Thomson and Page 2020, Taggar *et al.* 2021) as well as between plants and florivores/pre-dispersal seed predators (McCall and Irwin 2006, Althoff *et al.* 2013, Xu *et al.* 2015, Boaventura *et al.* 2022). Based on the three

studies included in this thesis, viewing a system through within-species diversity furthers depth into understanding the trophic level interactions. The results in the original studies agree with the literature that such interactions exist between trophic levels. Furthermore, during this thesis, within-species diversity of plants was found to have consequences to all investigated parties, the floral antagonists (II), pollinators (III) and the plants themselves (I).

Pollinator insects, when presented competition, may niche partition between plant genotypes (III). Although partitioning between different genotypes was noted, inter-species differences should be greater than intra-species differences due to the degree of specialization and divergence. Importance of within-species diversity was noted, as it can allow resource partitioning and therefore also coexistence between native and introduced species (III). The necessity of this is attested by the differential efficiency of native and introduced species to pollinate, and the resulting loss of pollination services, should the native pollinators be replaced with introduced ones (I).

Niche partitioning between pollinators also has to do with pollinator preference, which correlates with florivore preferences as well (Theis 2006). An interesting question is whether the plant genotype influences pollinator (III) and florivore (II) behaviour similarly in terms of their preferences. Although in the original studies included in this thesis, both pollinator and florivore preferences were measured, but the plants and genotypes used in studies II and III are not the same. Still, based on the results it can be stated that both florivores/pre-dispersal seed predators and pollinators of *G. sylvaticum* interact with the plant genotype, and further studies should find it interesting to examine the extent of overlap between these groups.

The interplay between preferences and function of a visitor direct plant evolution and can contribute to within-species diversity (Temeles *et al.* 2016). In terms of a florivore affecting pollination and pollinators of a flower, the interactions are complex. In fact, since florivores (II) and pollinators (III) have differential preferences to their food sources, and different visitors are not equally efficient pollinators (I), florivores may cause unforeseeable consequences to plants. These effects should also carry to plant genotypes as well, and therefore florivore behaviour could also induce selection pressures at the population level. Viewing florivory by species specific interactions between florivores and pollinators is however beyond the scope of this thesis.

The concept of species is mundanely used when studying the interactions between plants and their floral visitors. This thesis highlights the importance of within-species diversity of plants in their interactions between floral mutualists and antagonists. Along the taxonomic gradient of diversity, similar patterns emerge at species and within-species level. I conclude that sufficient understanding on species interactions requires consideration over different levels of diversity, and accounting for them in a flexible and holistic manner in nature conservation and science.

## 5 FUTURE DIRECTIONS

“If you had unlimited resources to spend, where would you go from here?” is a question that is posed to many doctoral candidates. Therefore, let us spend a moment to consider the questions that have risen as a result of the answers gained during this thesis.

Especially the plant sex as an aspect of plant within species diversity has received wide attention and has been studied from various viewpoints. We've established its importance in accordance with intrinsic plant goals (I), florivores (II) and pollinators (III) while also accounting for other aspects of plant within-species diversity. As we now know that diversity is maintained and emphasized in a gynodioecious population via the plant sexes' interaction with pollinator insects, it would be very interesting to expand this approach to other traits of diverse populations of plants. Gynodioecy has been explained via resource allocation and enemy avoidance hypotheses, but this work has also introduced a link to insect behaviour (I). Therefore, it would be worthwhile to consider the interactions between the pollinator insects and plant sex, and genotypes could be viewed with more depth. For example, questions like do other traits than small flower size in relation to pollinator interactions of the sexes affect insect behaviour towards their primary sexual functions, drive pollen receipt in females or do males have other traits that direct pollinator behaviour towards more efficient pollen export?

We've also established that pollinators (III) as well as florivores and pre-dispersal seed predators (II) prefer certain plant genotypes. However, these answers give rise to more questions than what we have been able to address. It would be especially interesting to study the different colour patterns and colours and their effects on florivore and pollinator behaviour, and the interaction of the two more deeply. There are also various aspects of florivore-pollinator interactions that are left unexplored in terms of plant genotype.

One of the motivations for this thesis is to help further the goals of protection of pollinators. We've highlighted the interaction of within-species diversity with pollinator competition, especially between native pollinators and honeybees. From where we stand, we can conclude that two matters call for

further attention. Firstly, in midst of the current pollinator crisis and widely spread farming of honeybees, the effects of honeybees on native pollinators must be evaluated more widely and administered as necessary. Secondly, a door has been opened leading to further understanding on how within-species diversity may affect the competition between pollinator insects and especially in the case of introduced species. Plant within-species diversity and its effects on pollinators must be evaluated in more detail to better conservation efforts.

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## YHTEENVETO (RÉSUMÉ IN FINNISH)

### **Metsäkurjenpolven pölytys, kukkiensyönti ja lajinsisäinen monimuotoisuus**

Pölytys on tapahtuma, jossa kukan naarastoimisen kukan emin luotille siirtyy siitepölyä koirassukusoluja tuottavilta heteiltä. Pölytys voidaan karkeasti jakaa eläinpölytykseen ja abioottiseen, eli veden tai tuulen avulla tapahtuvaan pölytykseen. Eläinpölytyksessä siitepölyn kuljetuksesta vastaa aktiivisesti liikkuva eläin, joka voi olla hyönteinen, lintu tai nisäkäs. Maailmanlaajuisesti 80 % kasveista tarvitsee eläinpölytystä, ja Euroopassa viljelykasvilajeistamme 85 % on riippuvaisia siitä.

Pölyttäjähyönteiset valikoivat ravintokasvinsa eri tekijöihin nojaten. Eri kasvilajien tarjoamien resurssien laatu ja määrä vaihtelevat, ja täten ne eroavat toisistaan pölyttäjien näkökulmasta. Kukkia valikoidaan myös niiden värin, muodon ja tuoksun perusteella, ja varsinkin symmetriset, kookkaat ja voimakkaan väriset kukat vetoavat pölyttäjiin. Pölyttäjien välille kuitenkin syntyy kilpailua, jos ne suosivat samoja kukkia. Pölyttäjien välinen kilpailu näkyy tehokkuuden laskuna kukkaresurssien keräämisessä, ja lisäksi loiset ja taudinaiheuttajat voivat siirtyä pölyttäjistä toiseen kukkien välityksellä. Ekologinen eriytyminen pölyttäjien välillä kuitenkin mahdollistaa rinnakkaiselon ja vähentää kilpailua. Ekologinen eriytyminen vuorokauden- tai vuodenaikaisessa esiintymisessä tai kukkakasvilajien valinnassa tarkoittaa käytännössä sitä, että eri pölyttäjälajien toiminta keskittyy tiettyihin ajankohtiin tai kasvilajeihin, vähentäen päällekkäisiä kukkavierailuja ja kilpailua. Vierasperäiset pölyttäjälajit eivät kuitenkaan omaa resurssienjakoa samalla tavoin kuin kotoperäiset, pitkään keskenään vuorovaikuttaneet lajit. Vierasperäiset pölyttäjät voivatkin uhata kotoperäisiä pölyttäjiä, sillä ne ovat tehokkaita kilpailijoita ja levittävät monenlaisia taudinaiheuttajia, joihin kotoperäiset lajit eivät ole välttämättä tottuneet. Suomessa kiistatta runsain vierasperäinen pölyttäjä on tarhamehiläinen, joka pystyy selviytymään täällä ainoastaan ihmisen avustamana. Tarhamehiläispesiä on Suomessa n. 70000 ja jokaisessa pesässä voi olla jopa 65000 mehiläistä. Tarhamehiläisen on osoitettu kilpailevan luonnonpölyttäjien kanssa haitallisin seurauksin, ja sen tiedetään myös levittäneen tauteja luonnon pölyttäjiin.

Kasvien kukintoja syövät florivorit vaikuttavat myös vahvasti kasveihin ja pölyttäjiin. Kun vauriot ovat kukkien toissijaisissa lisääntymisrakenteissa, kohdistuvat vaikutukset kasviin epäsuorasti pölyttäjien kautta. Vaurioituneet kukinnot eivät houkuttele pölyttäjiä yhtä tehokkaasti muuttuneen ulkomuotonsa vuoksi ja jäävät näin ollen pölyttymättä tai saavat vähemmän vierailuja. Kukkiensyönnin suorat vaikutukset kohdistuvat kasvin lisääntymiseen silloin, kun kasvin primääriset lisääntymisrakenteet, emit ja heteet ja näiden sisältämät solumolut, kärsivät vahinkoja. Monet kukkiensyöjät syövät kukkien lisäksi kehittyviä siemeniä, ja vaikuttavat tuntuvasti selviytyvien siementen määrään. Vaikka kukkiensyöjät kuluttavat kokonaisuudessaan vähemmän solukkoa, on niillä suuri

vaikutus kasveihin juurikin lisääntymisrakenteiden tärkeyden vuoksi. Kuten kasvinsyöjät, kukkiensyöjätkin ovat merkittävästi ohjanneet kasvien evoluutiota.

Kasvien kukilla tapahtuvat antagonistiset ja mutualistiset vuorovaikutukset keskittyvät pitkälti pölyttäjien ja kukansyöjien mieltymyksiin. Siinä missä pölyttäjät ovat riippuvaisia kasvien monimuotoisuudesta, myös kukkia syövät hyönteiset valikoivat isäntäkasvinsa samoin periaattein. Monet pölyttäjähyönteiset vierailevat metsäkurjenpolvella, jonka pääasialliset pölyttäjät ovat kimalaiset. Pölyttäjille kukkakasvilajien monimuotoisuus mahdollistaa monipuolisuuden ravinnonlähteissä, ja kukkajatkumon, eli kukkien tarjonnan läpi koko kasvukauden. Kukkia syövät hyönteiset arvottavat kukkia mm. niiden ravintoarvon ja ulkomuodon osalta. Monimuotoisuus ei ole kuitenkaan pelkkää lajiston monimuotoisuutta, vaan siihen liittyy myös geneettinen, lajinsisäinen komponentti. Tietyn kasvilajin sisällä monet piirteet, kuten meden ravintoarvo, kukkien väri ja määrä, sekä kukinta-aika voivat vaihdella. Geneettinen monimuotoisuus voisi toimia kuten lajistollinenkin monimuotoisuus, ja sillä voi olla merkitystä kasveille, pölyttäjille, kasvinsyöjille ja näiden välisille vuorovaikutuksille.

Tässä väitöskirjassa ja siihen sisältyvissä kolmessa artikkelissa tarkastellaan kasvilajin geneettistä monimuotoisuutta suhteessa pölyttäjiin sekä kukkia syöviin hyönteisiin. Tutkimusasetelmat on toteutettu Konnevedellä sijaitsevalla metsäkurjenpolvikentällä. Koealalla kasvaa 531 kasvia, vanhempaisklooneja sekä näiden jälkeläisiä, joiden tausta tunnetaan. Metsäkurjenpolvi (*Geranium sylvaticum*) on geneettisesti monimuotoinen, naaraskaksikotinen, Suomessa laajalti yleisenä tavattava kasvi. Naaraskaksikotisena eli gynodieekkisenä populaatiot koostuvat naaraskasveista sekä hermafrodiiteista, jotka ovat sekä koiras- että naarastoimisia. Metsäkurjenpolven kukilla elää kurjenpolvikärsäkäs (*Zacladus geranii*), joka syö isäntäkasvinsa kukkien osia, näkyvimmin kukkien terälehtiä. Lisäksi kärsäkäs munii kehittyvään siemenaiheeseen, josta siemenen sijaan kuoriutuu kurjenpolvikärsäkäs.

Ensimmäisessä tutkimuksessa tarkastellaan metsäkurjenpolven naaraskaksikotisuutta uudesta näkökulmasta. Työn hypoteesi oli, että naaraskukkien pieni koko on sopeuma pölytetyksi tulemiseksi. Koeasetelmassa videokuvattiin metsäkurjenpolvilla vierailevia hyönteisiä, ja myöhemmin videomateriaalista arvioitiin vierailijoiden käyttäytymistä ja niiden todennäköisyyttä kantaa ja välittää siitepölyä eri sukupuolisiin kukkiin. Tutkimuksessa havaittiin, että pienemmissä naaraspuolisissa kukissa pienikokoisemmatkin pölyttäjät koskettivat todennäköisemmin emiä, sillä koiraspuolisten kukkien suuren koon vuoksi hyönteiset usein laskeutuivat terälehdelle, ryömivät sitä myöten juomaan mettä kuitenkin koskettamatta lisääntymisrakenteita. Suuren kokonsa vuoksi metsäkurjenpolven hermafrodiittikukat kuitenkin saavat naaraita enemmän vierailuja ja varmistavat näin siitepölyn tehokkaan levikin.

Toisessa tutkimuksessa paneuduttiin metsäkurjenpolven ja kurjenpolvikärsäkkään väliseen suhteeseen. Aiemmissä tutkimuksissa on havaittu kurjenpolvikärsäkkään valikoivan isäntäkasvinsa mm. värin perusteella. Tässä kokeessa tarkoituksena olikin määrittellä kurjenpolvikärsäkkään vaikutus metsäkurjenpolven lisääntymismenestykselle ja sen genotyyppikohtaiset mieltymykset. Koe tapahtui

kolmivaiheisesti. Ensinnä valikoitujen kohdekasvien kukintaa seurattiin vähintään joka toinen päivä. Kasveista laskettiin kukinnan parametrit kuten kukkien määrä, sukupuoli ja avonaisten emien määrä sekä kurjenpolvikärsäkkään voittamien kukkien määrä. Joka toinen päivä kohdekasveista etsittiin kärsäkkään voittamia kukkia, terveitä kukkia sekä rei'itettiin itse kukkia niin, että vauriot näyttivät kärsäkkään aiheuttamilta. Kasvukauden lopulla kasvit kerättiin ja niiden kokonaissiementuotanto sekä kärsäkkään voittamat siemenet laskettiin. Tutkimuksessa havaittiin, että kurjenpolvikärsäkäs ei vaikuttanut kukkien siementuotantoon, mutta lisäsi kukan todennäköisyyttä kuihtua. Kasvi todennäköisesti abortoi kukkia, joissa se havaitsee kärsäkkään läsnäolon tai pölytystodennäköisyys on heikompi. Kärsäkkään mieltymyksistä kertoo, että tietyissä kasvigenotyypeissä havaittiin runsaammin kärsäkkäiden florivoriaa sekä siementensyönteitä. Tutkimuksen mukaan kurjenpolvikärsäkäs voi luoda tietynasteisen valintapaineen metsäkurjenpolven genotyypeille.

Kolmannessa tutkimuksessa tarkasteltiin metsäkurjenpolven geneettistä monimuotoisuutta ja sen merkitystä pölyttäjähyönteisten, erityisesti vierasperäisen tarhamehiläisen ja kimalaisten väliselle kilpailulle. Kokeessa mehiläispesä sijoitettiin kentän välittömään läheisyyteen ja kahden päivän jälkeen se vietiin pois. Genotyypiltään erilaisia kurjenpolviyksilöitä seurattiin tarkkailujaksoissa ja kaikki kasvissa vierailleet pölyttäjät tunnistettiin ja niiden vierailemat kukat laskettiin läpi kahden kasvukauden. Tutkimuksessa havaittiin, että tarhamehiläisen läsnäolo vaikutti merkittävästi kimalaisten vierailuihin. Kimalaisten vierailut kukissa vähenivät, ja kimalaiset suosivat eri genotyyppejä kuin ilman mehiläisiä. Tämä viittaa siihen, että geneettisellä monimuotoisuudella on merkitystä vierasperäisten ja kotoperäisten lajien kilpailun lievittämisessä. Tarhamehiläiset vierailivat kurjenpolvigenotyypeissä huomattavasti vähemmän valikoiden kuin kimalaiset. Tutkimuksen nojalla voidaan sanoa, että tarhamehiläinen vaikuttaa luonnonpölyttäjästä tuntevimmin juuri kimalaisiin, ja tämä tulisi huomioida niin luonnonsuojelussa kuin mehiläistarhauksen säännöstelyssä.

Yhteenvedona voidaan sanoa, että kasvin lajinsisäinen monimuotoisuus vaikuttaa kasvien, pölyttäjien ja kasvinsyöjien vuorovaikutuksiin useissa yhteyksissä, vastaten laadullisesti lajiston monimuotoisuuden tasolla havaittavia vuorovaikutuksia. Näin ollen kokonaiskuvaksi tutkimuksista muodostuu tarve huomioida populaatioiden lajinsisäinen monimuotoisuus luonnonsuojelussa sekä pölytysökologiassa aiempaa paremmin.

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## ORIGINAL PAPERS

### I

# ***GERANIUM SYLVATICUM* INCREASES POLLINATION PROBABILITY BY SEXUALLY DIMORPHIC FLOWERS**

by

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# *Geranium sylvaticum* increases pollination probability by sexually dimorphic flowers

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## Abstract

Sexual dimorphism is expressed as different morphologies between the sexes of a species. Dimorphism is pronounced in gynodioecious populations which consist of female and hermaphrodite individuals. The small size of female flowers in gynodioecious species is often explained by resource re-allocation to seed production instead of large flowers. However, pollinator attraction is critical to female fitness, and factors other than resource savings are needed to explain the small size of female flowers. We hypothesized that the floral size dimorphism in the perennial gynodioecious *Geranium sylvaticum* (L.) is adaptive in terms of pollination. To test this “pollination hypothesis,” we video recorded the small female and large hermaphrodite *G. sylvaticum* flowers. We parameterized floral visitor behavior when visiting a flower and calculated pollination probabilities by a floral visitor as the probability of touching anther and stigma with the same body part. Pollination probability differed in terms of flower sex and pollinator species. Bumblebees had the highest pollination probability. The small female flowers were more likely to receive pollen via several pollinator groups than the large hermaphrodite flowers. The pollen display of hermaphrodites matched poorly with the stigma display of hermaphrodites, but well with that of females. Although the small size of female flowers is commonly explained by resource re-allocation, we show that sexual dimorphism in flower size may increase the main reproductive functions of the females and hermaphrodites. Dimorphism increases pollination probability in females and fathering probability of the hermaphrodites likely driving *G. sylvaticum* populations towards dioecy.

## KEYWORDS

disruptive selection, flower size, *Geranium sylvaticum*, gynodioecy, pollination, sexual dimorphism

## TAXONOMY CLASSIFICATION

Behavioural ecology, Botany, Entomology, Evolutionary ecology, Population ecology

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## 1 | INTRODUCTION

Sexual dimorphism in plants refers to the morphological differences between sexes. Sexual differences in vegetative traits are usually absent. Although some intrinsic differences are present in the primary sex organs, sexual dimorphism refers to, and is most pronounced in, differences in flower or inflorescence morphology (Ashman, 2005). Sexual dimorphism is most notable in dioecious and gynodioecious species. In dioecious species, the two sexes are expressed in different plant individuals. Gynodioecious populations consist of female individuals that bear flowers with only the female function, and hermaphrodite individuals with both the female and male function (Ågren & Willson, 1991; Eckhart & Chapin, 1997; Miller & Venable, 2003). Approximately 6% of angiosperms are dioecious (Renner & Ricklefs, 1995) and gynodioecy is present in 2.2% of angiosperm families, while 0.5% of dicot species are gynodioecious (Godin & Demyanova, 2013). Gynodioecious species are proposed to arise as cytoplasmic determinants followed by mutations that cause the loss of the male function in hermaphrodite flowers (Budar et al., 2003; Schnable & Wise, 1998).

Despite the understanding of the mechanisms of how gynodioecy may arise, it is challenging to explain for many reasons. The loss of male function entails that females lose half of the reproductive fitness of the hermaphrodites associated with pollen. Due to this in-born disadvantage of the females, females must compensate for the lost half of their reproductive fitness in comparison to hermaphrodites (Lewis, 1941; Lloyd, 1976). In the absence of alleviating factors, the increased contribution to the gene pool of the offspring should at least account for the fitness derived from pollen. The female compensation in fertility is usually less than the required compensation which in some cases can be expected as high as 200%, although the compensation depends on the sex ratio of the population and the mechanism of male sterility (Charlesworth & Charlesworth, 1978; Lewis, 1941). Increased seed viability and increased offspring fitness resulting from avoidance of inbreeding depression in females have been suggested to reduce female disadvantage further (Dufay & Billard, 2012; Puterbaugh et al., 1997). Females may gain benefits by avoiding inbreeding depression (Baker, 1959; Charlesworth & Charlesworth, 1978; Sakai et al., 1997), but the resulting benefit is difficult to evaluate. As some gynodioecious species show little inbreeding depression (Mutikainen & Delph, 1998), female advantage by cross-pollination may not be universal.

The consequent loss of fitness along the male function is not the only problem posed by females in gynodioecious species. Fitness in females is critically dependent on pollinators visiting female flowers after visiting the pollen-bearing hermaphrodite flowers. As a general rule, in sexually dimorphic species, the female flowers are significantly smaller than those of the larger, showier hermaphrodite flowers (Ågren & Willson, 1991; Barret & Hough, 2013; Miller & Venable, 2003). Female flowers may also provide less nectar to the pollinators (Delph & Lively, 1992; Klinkhamer et al., 1991; Varga, Nuortila, & Kytöviita, 2013) and intrinsically lack pollen. Because

pollinators strongly discriminate between flowers and prefer large and showy (Bond & Maze, 1999; Martin, 2004), symmetric flowers (Moller, 1995) with ample rewards (Delph & Lively, 1992; Varga & Kytöviita, 2010), hermaphrodite flowers are predicted to be selected for these traits in promotion of their male function (Vaughton & Ramsey, 1998). In line with the showiness and rewards, insects visit hermaphrodite flowers more frequently than those of the females in most gynodioecious species (Asikainen & Mutikainen, 2005a; Cuevas et al., 2008; Van Etten & Chang, 2014; Varga & Kytöviita, 2010).

Furthermore, many pollinators exhibit flower constancy, i.e., behavior where the pollinator learns fidelity toward a specific rewarding plant species or morph (Waser, 1986). Flower constancy is proposed to be based on the handling skills required to access rewards (Ishii & Kadoya, 2016), visual appearance (Gegeer & Laverty, 2005; Ishii & Masuda, 2014), and olfactory cues (Laska et al., 1999; Wright & Schiestl, 2009) of the flower that the pollinator learns to favor. Flower constancy is considered an important aspect of the evolutionary ecology of plant-pollinator interactions as it improves the pollination services received by the plant. For instance, it reduces the probability of clogging the stigma with the pollen of other species (Morales & Traveset, 2008; Muchhala & Thomson, 2012). On the other hand, it reduces the amount of wasted pollen in terms of transport to intraspecific recipient flowers (Schmid et al., 2016). The flower constancy and consequent potential passing over the females by the pollen carriers are aggravated by the fact that there are usually fewer females in a gynodioecious population (Asikainen & Mutikainen, 2003; Chang, 2006). This often leads to minority disadvantage (Levin, 1972) and females receive less visits by pollinators which mainly forage the most common morphs (Levin, 1972; Van Etten & Chang, 2014). Females cannot equal hermaphrodites in frequency (Charlesworth & Charlesworth, 1978) to escape minority disadvantage (Levin, 1972), but females could attract pollinators more efficiently (Glaetli & Barrett, 2008) and counteract the minority disadvantage by increased floral attraction. Furthermore, female flowers may compensate for smaller flower size by remaining in the receptive phase longer (Ashman & Stanton, 1991). Despite these potential counteractive measures, females have been frequently shown to receive fewer pollinator visits than hermaphrodites or males (Asikainen & Mutikainen, 2005a; Bond & Maze, 1999; Cuevas et al., 2008; Van Etten & Chang, 2014; Varga & Kytöviita, 2010) although not universally in all studies (e.g., Cervantes et al., 2018).

In hermaphrodite flowers, the male function may pose different evolutionary selection pressures on floral morphology than the female function (Barret, 2002). Hermaphrodites are subject to the cost of increased inbreeding depression resulting from self-pollination (Charlesworth & Charlesworth, 1987; Varga, Vega-Frutis, & Kytöviita, 2013). Arising from the different evolutionarily stable strategies in the sexes, pollinator-limited males are also proposed to allocate on floral display and reward (Thomson & Brunet, 1990). In gynodioecious populations, hermaphrodites gain most of their fitness through the male function due to the presence of females (Charlesworth, 1981; Lloyd, 1976; Vamosi & Otto, 2002). This should

select for larger floral displays and pollen production in hermaphrodites (Vaughton & Ramsey, 1998) because the male function is promoted by pollen export and thus ultimately attractiveness to pollinators.

Most studies explain sexual flower size dimorphism in gynodioecious species by different aspects of resource allocation and trade-offs (e.g., Ashman, 1992, 1994; Delph et al., 1996; Miller & Venable, 2003). Seed production demands a substantial portion of plant resources (Ashman, 1992). For example, Ashman (1992) found that *Sidalcea oregana* plants allowed to make seeds allocated 20% less biomass to floral structures, and in turn, plants that were not, produced 40% more floral biomass the next year than the plants that were allowed to produce seeds the first year. The higher allocation in seed set in females vs. hermaphrodites has been suggested to be possible via enhanced resource allocation to female function (Ashman, 1994; Chang, 2006). The decreased size of the corolla as well as the loss of stamens in females may leave more resources for seed production (Ashman, 1994; Eckhart, 1992). We argue that the benefit gained from re-allocating floral biomass to seed mass is inadequate given that the small flower size handicaps pollination (Bond & Maze, 1999; Martin, 2004). It would be more economic for the plant to re-allocate resources to seeds from less critical sources such as older parts of foliage or roots rather than the critical floral display. The difference in flower size between sexes is a general phenomenon, and we propose that factors other than resource savings are needed to explain the apparent mismatch between costs and benefits of the smaller flower size in females in gynodioecious plant populations.

In this work, we explore an alternative, but not necessarily exclusive hypothesis to explain sexual dimorphism. We focus on *Geranium sylvaticum*, a gynodioecious perennial plant with sexually dimorphic populations consisting of female and hermaphrodite individuals. The female flowers are smaller than the hermaphrodite ones (Asikainen & Mutikainen, 2005a; Varga & Kytöviita, 2010), provide less nectar (Varga, Nuortila, & Kytöviita, 2013), and naturally no pollen as a reward for pollinators. The female flowers are visited less frequently by insect visitors (Asikainen & Mutikainen, 2005a; Varga & Kytöviita, 2010). We hypothesize that the small size of female flowers in *G. sylvaticum* is adaptive because it increases pollination probability in females and thus the fitness gained by female function in females and male function in hermaphrodites. We test this “pollination hypothesis” by comparing the probability of pollen transport from anther to receptive stigma (I) between hermaphrodite flowers and (II) between hermaphrodite and female flowers. Support for the hypothesis that sexual dimorphism is adaptive will be evidenced if (I) is smaller than (II). Furthermore, we compare the probability of pollen transport from an anther to a stigma by the most common floral visitors of *G. sylvaticum*. We hypothesize that the small size of female flowers in *G. sylvaticum* is an adaptation to pollination by bumblebees and expect that bumblebees rather than the other common floral visitors are responsible for pollen transport between flowers. Each insect visitor has characteristics that determine its

specific pollination efficiency (Motten, 1986). These are how frequently and how faithfully the insect visits a given host, how much pollen it carries during visits, and how the visitor morphology and foraging behavior match with the flower morphology. In the present work, we investigate the latter point related to visitor behavior and how it matches the morphology of the two sexes of *G. sylvaticum*.

## 2 | MATERIALS AND METHODS

### 2.1 | Study organism

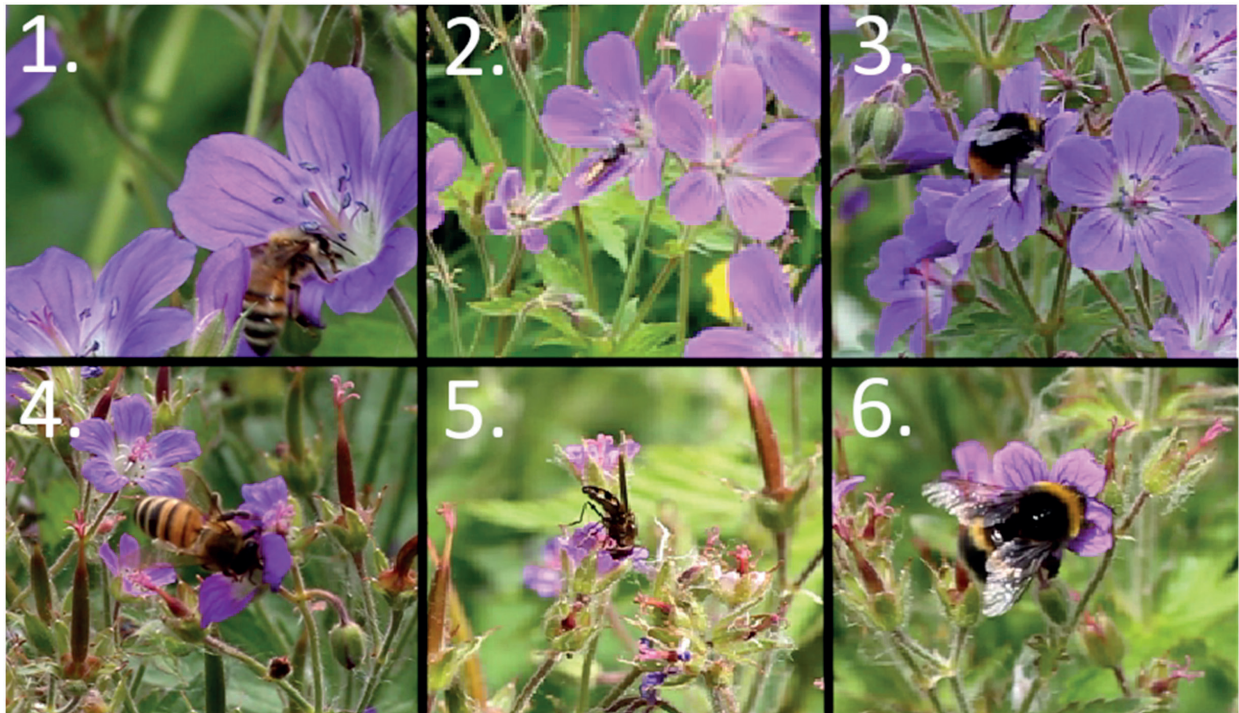
*Geranium sylvaticum* (L.) is a self-compatible perennial with Eurasian distribution (Stroh, 2014). *Geranium sylvaticum* is common in meadows but thrives also in shade (Korhonen et al., 2004), in particular when nutrient availability is high (Hokkanen, 2003). The plant is gynodioecious, and the proportion of female plants varies between 0% and 23% between populations (Asikainen & Mutikainen, 2005a; M.-M. Kytöviita, personal observations). Both female and hermaphrodite flowers offer nectar as a reward for pollinators (Varga, Nuortila, & Kytöviita, 2013). The fruit matures in 3 weeks after fertilization and is a schizocarp with five locules and the maximum number of seeds per fruit is five.

### 2.2 | Field measurements

We estimated pollen transport probabilities by quantifying floral visitors and their behavior in detail in video recorded *G. sylvaticum* plants. The plants were growing in an experimental site of the University of Jyväskylä established in an old field year 2008 at Konnevesi Finland (62°35'17.4"N 26°14'03.2"E). Altogether seven female plants and 13 hermaphrodite plants were video recorded when in full bloom between June 14 and 18, 2021. The plants were of the same age and size and were composed of 34 floral shoots on average. Alternating between random female and hermaphrodite plants, a portion of the inflorescences were recorded on average in 40-min intervals. Multiple cameras ensured that the temporal variability in insect activity did not affect behavior in the sexes differently.

The plants were video recorded during the most active period of insects (9:00 a.m. to 6:00 p.m.). The image was focused so that 10–15 fully open flowers in a plant could be followed simultaneously with a sufficient accuracy to distinguish the pollinator behavior (Figure 1). The hardware used for recording included Canon EOS 550D digital camera with 55–250mm objective set to 250mm, as well as portable computer-run cameras with the use of the application OBS studio ver. 26.1.1. (64 bit). Altogether 30h of video data were gathered on the 20 plant individuals, of which a total of 13h were gathered on the hermaphrodite plants, and 17h on the female plants. Female plants were recorded more to compensate for the expected lesser visitation rates in females versus hermaphrodites.





**FIGURE 1** Examples of the video material illustrating the behavior of different insect pollinators and the different sexes of the plant *Geranium sylvaticum*. 1. *Apis mellifera* visiting a non-receptive hermaphrodite flower touching the anthers with the head. 2. A Syrphidae resting in a receptive hermaphrodite flower. The fly slips under the anthers and makes little contact with reproductive structures. 3. *Bombus pratorum* visiting a receptive hermaphrodite flower. The bee has climbed over the reproductive structures so that the thorax contacts both the anthers and the stigma. 4. *Apis mellifera* visiting a female flower. Due to the small size of the flower, the bee reaches the nectaries over the receptive stigma and touches the stigma surfaces with ventral side of the thorax. 5. A Syrphidae visiting a female flower making contact with the stigma while reaching the nectaries across the stigma. 6. *Bombus soroensis* on a female flower. Due to the small size of the flower, the bee has planted itself over the flower for easiest access to nectaries. It touches the stigma with its ventral side of thorax.

### 2.3 | Visitation parametrization

The flower-visiting insects were assigned to seven groups (hereafter visitor groups) which consisted of bumblebees in the genus *Bombus* (hereafter *Bombus*), honeybees *Apis mellifera* (L.) (hereafter *Apis*), hoverflies of the family Syrphidae (hereafter Syrphidae), and solitary Hymenoptera, Diptera, Hemiptera, and Coleoptera. Syrphidae were intentionally separated from Diptera in general due to their distinct behavior and abundance, and *Apis mellifera* from other eusocial bees because *Apis mellifera* is farmed in Finland and does not occur naturally.

The behavior of an insect was parameterized so that a contact with the reproductive surface of an anther or stigma in the flower was noted along with the body part of the insect that had made the contact. The body parts were classified as follows: head ventral, head dorsal, foot, leg, thorax ventral, thorax dorsal, abdomen ventral, and abdomen dorsal. We only report visitations where it was possible to distinguish the movements of an insect within a flower and whether it had contacted the floral reproductive organs. In addition to the movements of the insect, the stigma phase (receptive/non-receptive) and the time of visit were recorded. Visitation frequencies by insect group per hour

were extrapolated by following the flowers visible on the screen for the length of the video. These data are not based on the sexual organ contact data as all visitations were usable to estimate the data on frequency.

When the fruits were ripe in August, the schizocarps were collected and dried (60°C, 12 h). Based on the seed scars in the schizocarps, the average seed production per flower, total seed production per plant, and the ratio of undeveloped-to-developed schizocarps were counted.

### 2.4 | Data analysis

In the probability estimations and statistical analyses, we only used the contacts with the ventral side of the insect's body (i.e., ventral side of head, thorax, and abdomen). This is because *G. sylvaticum* flowers are sternotribic (Kozuharova, 2002), and dorsal contacts by the insects were ineligible. Dorsal contacts would not transmit pollen; although an insect could touch anthers with dorsal side, it could not land upside down on the stigma. Correspondingly, there were a few dorsal anther contacts (mainly with the head), but no dorsal stigma contacts in the video material.

The probability of pollination was calculated and defined as the probability of an insect contacting an anther with a certain part of the body and contacting the stigma of a flower with the same body part.

The probabilities of pollen transport between the compared groups were calculated with the basic formula of the probability of two independent events:

$$P(A \cap B) = P(A) \times P(B)$$

where  $P(A)$  is the probability of anther contacts (anther contacts/visits) in the visitor group, and  $P(B)$  is the probability of contacting stigma in the receptive phase (stigma contacts/visits) in the same visitor group. This method of calculating pollination probability focuses on insect behavior when visiting a flower but does not estimate the holistic pollinator efficiency (Motten, 1986).

The probabilities of pollination were calculated separately in the two plant sexes (females and hermaphrodites) and the different pollinator groups, so that ventral contacts to anthers and stigma were taken into account, respectively, for all body parts (head, thorax, and abdomen) and summed to get the final pollination likelihood.

The data were statistically analyzed as follows. The data on anther or stigma contacts were analyzed with generalized linear regression model (GLM, with the logit link function and binomial family distribution). In the GLM, the frequency of ventral stigma or anther contact (yes/no) was set as the response variable and the plant sex (not in the model for anther contact) and visitor group as predictor variables. The visitor group analysis was repeated by setting each visitor group as the reference level to compare the visitor groups with each other. In the analyses, visits by Diptera, Coleoptera, Hemiptera, and solitary Hymenoptera were not tested as there were not enough visits to draw reliable conclusions. For visitations per flower per hour frequency data, negative binomial generalized linear model with log link function was used, with visitation rate per flower per hour set as the response variable and sex and the number of floral shoots were used as predictor terms. Analyses for effects on mean or overall seed production were conducted with GLM using the Gaussian family distribution with logit link function for the response variable, which was either the mean seed production per flower in a plant or total seed production. Models with the ratio of undeveloped to developed schizocarps as the response variable were conducted with quasibinomial distribution family and logit link function. As predictor terms, visitation rates by insect groups per flower in an hour, sex, and number of floral shoots were used, depending on the optimal model determined by AIC values and/or the distribution of residuals.

Within each insect group, the differences in frequencies in stigma contacts between plant sexes were analyzed with two-sample Z-test for probabilities using a subset of data at a time containing only one visitor group. To test the statistical significance of the differences in the pollination probabilities between the plant sexes, we resampled the data by randomly selecting pairs of anther contact (0/1) and stigma contact (0/1) iterated 5000 times. If there was a contact (1) on both anther and stigma, it was taken as a

probable pollination event. These resampled data conformed to the aforementioned grouping so that all combinations of sex, body part, and visitor class were present. The random-pair data were used in two-sample Z-test to analyze the differences in the anther–stigma random frequencies of successful pollinations between the female and hermaphrodite groups in the respective body parts (head ventral, thorax ventral, and abdomen ventral) and in the respective visitor groups (Syrphidae, *Bombus* and *Apis*).

The data were analyzed using the statistical programming software R, ver. 4.0.2. (64 bit).

### 3 | RESULTS

Altogether, we recorded 536 insect visits in the study plants. Of the visits, 406 were observed in flowers with receptive stigma. In *G. sylvaticum* flowers, the stigma lobes are closed when non-receptive and open in the receptive female phase (Varga, Nuortila, & Kytöviita, 2013). The recorded insects belonged to a range of taxa: bumblebees (*Bombus pratorum* (L.), *B. soroensis* (Fabricius), *B. lucorum coll.* (L.), *B. sporadicus* (Nylander), *B. pascuorum* (Scopoli), *B. hypnorum* (L.), *B. terrestris* (L.), *B. bohemicus* (Seidl), and *B. lapidarius* (L.)), honey bee (*Apis mellifera* (L.)), syrphid flies (e.g., *Sphaerophoria scripta* (L.), *Syrphus ribesii* (L.), *Microdon* sp., *Cheilosia* sp., and *Helophilus pendulus* (L.)), and solitary Hymenoptera (*Lasioglossum* sp. and *Corynis obscura* (F.)) accompanied with various species of Diptera; various Brachycera and some small Nematocera. At the site, occasionally Coleoptera such as *Zacludus geranii* (Paykull), *Corizus hyoscyami* (L.), *Coreus marginatus* (L.), *Pentatoma rufipes* (L.), and *Dolycoris baccarum* (L.) were observed as well as some butterflies (*Vanessa atalanta* (L.), *Anthocharis cardamines* (L.), *Aglais io* (L.), and *Araschnia levana* (L.)) and some moths (Geometridae). In terms of the plant sexes (hermaphrodite/female), we recorded 68/163 visits by *Bombus*, 63/88 by Syrphidae, 9/1 by Solitary Hymenoptera, 45/32 by *Apis*, and 14/15 by Diptera, respectively. It should be noted that these data are simply the recorded visitations that were distinguished in the videos, and do not represent differences in frequency of visitations between sexes, but the number of visits we video recorded (size of data) and on which the contact probability calculations are based on.

#### 3.1 | Visitation frequency

Pooling all of the insect groups, the female plants received 12.5 visits per flower per hour and hermaphrodites received 20.14 visits per hour. However, these overall visitation rates were not statistically significantly different between the sexes ( $df = 45$ ,  $AIC = 354.58$ , Estimate = 0.48,  $z = 1.26$ ,  $p = .208$ ). Visitation frequencies by the three focal insect groups in females/hermaphrodites were 5.44/9.33 in *Bombus* ( $df = 45$ ,  $AIC = 981.11$ , Estimate = 0.53,  $z = 4.88$ ,  $p < .001$ ), 1.48/2.61 in *Apis* ( $df = 45$ ,  $AIC = 351.13$ , Estimate = 0.56,  $z = 2.69$ ,  $p = .007$ ), and 3.8/4.08 in Syrphidae ( $df = 45$ ,  $AIC = 218.43$ , Estimate = 0.07,  $z = 0.126$ ,  $p = .899$ ). Visitation rates by *Bombus* and



*Apis*, but not by Syrphidae, were statistically significantly smaller in females than in hermaphrodites.

### 3.2 | Anther contacts

Overall, the anthers in hermaphrodite flowers had .34 probability to be contacted during a visit. The probability to contact anthers with the ventral side of the body of an insect during a floral visit was .78 in *Bombus*, .07 in *Apis*, and .06 in Syrphidae.

The probability to contact anthers with ventral side by members of Syrphidae did not differ from that of *Apis* ( $df = 69$ ,  $AIC = 52.1$ , Estimate = 0.48,  $z = 0.38$ ,  $p = .70$ ) but was lower than that of *Bombus* ( $df = 69$ ,  $AIC = 52.1$ , Estimate = 4.36,  $z = 3.74$ ,  $p < .01$ ). The probability to contact anthers with ventral side by *Apis* was inferior to that by *Bombus* ( $df = 69$ ,  $AIC = 52.1$ , Estimate 3.88,  $z = 4.20$ ,  $p < .01$ ). Ventral anther contact hierarchy was thus established as *Bombus* > Syrphidae, *Apis*.

The visitor probabilities to contact anthers are visualized in Figure 2.

### 3.3 | Stigma contacts

The stigma contacts were influenced by plant sex. Females had an overall higher probability of receiving a contact to the receptive stigma by a floral visitor ( $p = .72$ ) than hermaphrodites ( $p = .16$ )

( $df = 367$ ,  $AIC = 331.75$ , Estimate = 2.50,  $z = 6.15$ ,  $p < .01$ ). The probability to contact a female/hermaphrodite receptive stigma with the ventral side in the main visitor groups was .87/.63 in *Bombus* ( $df = 180$ ,  $AIC = 154.25$ , Estimate = 1.37,  $z = 2.60$ ,  $p = .01$ ), .34/<.001 in *Apis* ( $df = 58$ ,  $AIC = 49.213$ , Estimate = 0.34,  $z = 3.76$ ,  $p < .001$ ), and .70/<.001 in Syrphidae ( $df = 108$ ,  $AIC = 120.98$ , Estimate = 0.7,  $z = 7.18$ ,  $p < .001$ ).

Between visitor group comparisons revealed that in both sexes the stigma contact probability in the group Syrphidae was greater than in *Apis* ( $df = 367$ ,  $AIC = 331.75$ , Estimate = -1.49,  $z = -3.59$ ,  $p < .01$ ) but less than in *Bombus* ( $df = 367$ ,  $AIC = 331.75$ , Estimate = 2.94,  $z = -6.95$ ,  $p < .01$ ). Also, *Apis* had smaller probability to contact the stigma than *Bombus* ( $df = 367$ ,  $AIC = 331.75$ , Estimate = 2.94,  $z = 6.95$ ,  $p < .01$ ). Stigma contact hierarchy was thus established as *Bombus* > Syrphidae > *Apis*.

Visitor probabilities to make stigma contacts in female and hermaphrodite flowers are illustrated in Figure 3.

### 3.4 | Pollination probability

The probability of transporting pollen from the anthers of a hermaphrodite plant to the stigma of hermaphrodite plant by a specific pollinator group during a single visit was .47 in *Bombus* and <.001 in the case of *Apis* and Syrphidae. The probability to make contact with the anthers of a hermaphrodite plant and then make a contact with the stigma in a female plant was .68 in terms of *Bombus*, .04 of Syrphidae,

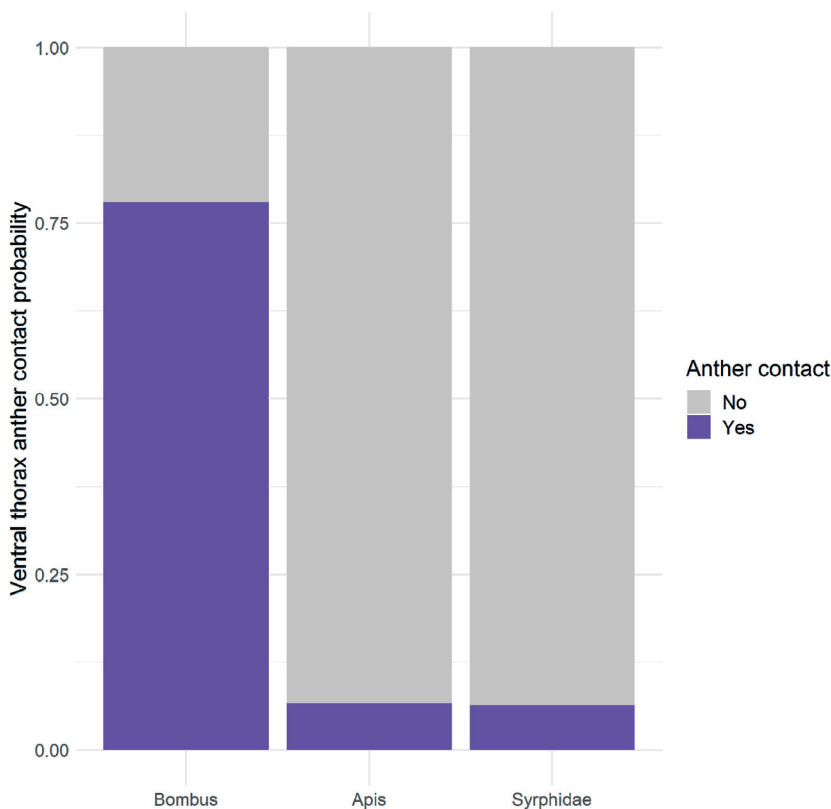


FIGURE 2 Average anther contact probabilities by different visitor groups (*Bombus*, *Apis*, and Syrphidae) in the hermaphrodite *Geranium sylvaticum* flowers ( $N = 199$ ). Anther contacts are binary (yes, no), the contacts represent ventral thorax contacts only.

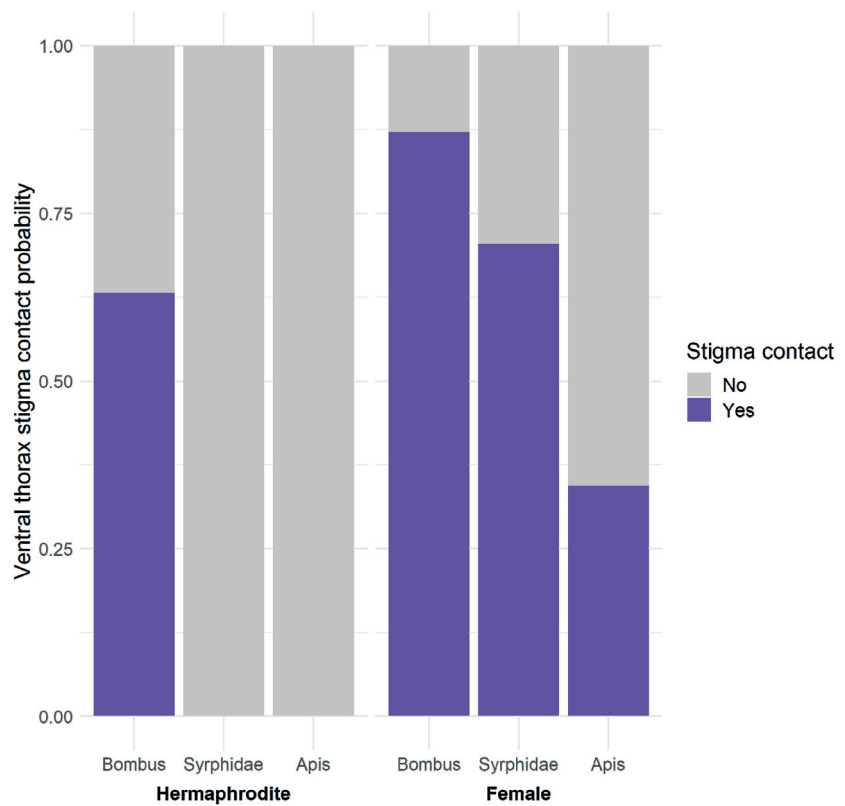
and .017 of *Apis*. The difference between the plant sexes was statistically significant in *Bombus* head ventral, thorax ventral, and abdomen ventral pollination probabilities (Table 1). Similarly, the pollination probability with *Apis* head ventral, thorax ventral, and abdomen ventral differed statistically significantly between the sexes (Table 1). In Syrphidae, pollination probabilities differed significantly between the sexes in head ventral and thorax ventral contacts (no data were recorded on abdomen ventral contacts; Table 1). In general, the hermaphrodite plants had inferior probability to be pollinated by any pollinator group compared to that of females. The statistical significances and test values between the different visitor groups and body parts in female/hermaphrodite plants are shown in Table 1.

Without sex discrimination, the likelihood to contact anthers and then any stigma was .64 in *Bombus*, .04 in Syrphidae, and .01 in *Apis*.

The possibility for autogamous pollination occurred only in the visitor group *Bombus*. In 16.2% of visits, *Bombus* touched both the anthers and the stigma with the same body part, but taking the insect behavior within the flower into account it was estimated that only 5.4% of *Bombus* visits in the hermaphrodites could have potentially led to autogamous pollination.

According to the calculated probability values, the pollination probabilities in both plant sexes rank as *Bombus* > Syrphidae > *Apis* in the three main visitor groups.

**FIGURE 3** Average stigma contact probabilities by the different visitor groups (*Bombus*, *Apis*, and Syrphidae) in the hermaphrodite and female *Geranium sylvaticum* flowers. Stigma contacts are binary (yes, no), the contacts represent ventral thorax contacts only.



**TABLE 1** Statistics, probabilities, and *p*-values from a two-sample Z-test for equality of proportions test depicting the comparisons between the probability to pollinate female (F) versus hermaphrodite (H) *Geranium sylvaticum* flowers in different visitor groups (rows) and their respective ventral side body parts (columns).

VISITOR CLASS	Head	Thorax	Abdomen
<i>Bombus</i> , F vs. H plants	$\chi^2 = 807.48$ , $df = 1$ $p_{(F)} = .15$ , $p_{(H)} < .001$ $p < .01$	$\chi^2 = 252.96$ , $df = 1$ $p_{(F)} = .64$ , $p_{(H)} = .49$ $p < .01$	$\chi^2 = 9.1$ , $df = 1$ $p_{(F)} < .001$ , $p_{(H)} = .002$ $p < .01$
<i>Apis</i> , F vs. H plants	$\chi^2 = 2$ , $df = 1$ $p_{(F)} < .001$ , $p_{(H)} < .001$ $p = .13$	$\chi^2 = 58.36$ , $df = 1$ $p_{(F)} = .012$ , $p_{(H)} < .001$ $p < .01$	$\chi^2 = 13.08$ , $df = 1$ $p_{(F)} = .003$ , $p_{(H)} < .001$ $p < .01$
Syrphid, F vs. H plants	$\chi^2 = 5.15$ , $df = 1$ $p_{(F)} = .001$ , $p_{(H)} < .001$ $p = .02$	$\chi^2 = 168.84$ , $df = 1$ $p_{(F)} = .34$ , $p_{(H)} < .001$ $p < .01$	NA

Note: Syrphidae abdomen ventral contact probabilities were not comparable as no contacts occurred within this group.

### 3.5 | Seed production

Hermaphrodites produced on average  $1605 \pm 996$  seeds per plant and females  $2654 \pm 402$ . The difference between the sexes was statistically significant ( $df = 12$ ,  $AIC = 240.28$ , Estimate =  $-758.44$ ,  $t = -2.16$ ,  $p = .05$ ). Females produced statistically significantly more seeds also per flower ( $3.51 \pm 0.47$  seeds per flower) than the hermaphrodites ( $1.81 \pm 0.40$  seeds per flower) ( $df = 12$ ,  $AIC = 23.12$ , Estimate =  $-1.66$ ,  $t = -0.10$ ,  $p < .0001$ ). Sex affected the ratio of wilted flowers to schizocarps (distinguished from flowers by missing seeds or swollen ovaries and elongated stigma), between females (6.8% of all flowers did not develop into schizocarps) and hermaphrodites (26% of all flowers did not develop) ( $df = 12$ , Estimate =  $1.45$ ,  $t = 2.76$ ,  $p = .02$ ).

Bumblebee visitation rate per flower per hour was positively related to the mean number of seeds produced per flower in a plant ( $df = 10$ ,  $AIC = 18.9$ , Estimate =  $0.02$ ,  $t = 2.29$ ,  $p = .05$ ). Visitation rate by Syrphidae was also positively related to the mean production of seeds per flower ( $df = 10$ ,  $AIC = 19.03$ , Estimate =  $0.03$ ,  $t = 0.01$ ,  $p = .05$ ), but *Apis* visitation rate did not have any statistically significant relationship ( $df = 10$ ,  $AIC = 24.123$ , Estimate =  $0.01$ ,  $t = 0.70$ ,  $p = .50$ ). The bumblebee visitation rate reduced the ratio of undeveloped flowers to developed schizocarps marginally significantly ( $df = 10$ , Estimate =  $-0.04$ ,  $t = -2.34$ ,  $p = .04$ ). Also, Syrphidae visitation ratio had a statistically significant negative effect on the ratio of undeveloped flowers to developed schizocarps ( $df = 10$ , Estimate =  $-0.05$ ,  $t = -3.0$ ,  $p = .01$ ), but *Apis* had no effect on the undeveloped-to-developed schizocarps ratio ( $df = 10$ , Estimate =  $-0.03$ ,  $t = -1.4$ ,  $p = .18$ ).

## 4 | DISCUSSION

Although sexual dimorphism has arisen in several distinct genera (Miller & Venable, 2003; Thomson & Brunet, 1990), the underlying evolutionary effectors are not clear (Charlesworth, 1981; Delph et al., 1996; Thomson & Brunet, 1990). To explain dimorphism between the sexes, arguments for non-adaptive (reviewed by Delph et al., 1996), anti-selfing (Baker, 1959; Kawagoe & Suzuki, 2003; Wilmer, 2011), and resource allocation hypotheses (Ashman, 1994; Chang, 2006; Delph et al., 1996; Eckhart, 1992) have been forwarded. The morphology of a flower is a compromise between different selection pressures (Galen, 1999). Larger flowers often receive higher visitation rates and have been proposed to evolve due to directional selection promoting increased floral attraction (Martin, 2004; Stanton & Preston, 1988). Visitation rates have been frequently shown to be positively linked with flower size (Bond & Maze, 1999; Martin, 2004; Van Etten & Chang, 2014). However, the reverse has not been documented previously: how small, visually unattractive flowers could make up for the loss of visitation rates.

The size of the sex organs in the flower plays a crucial role in the pollination probability. Due to developmental constraints,

corolla size in a flower increases in size in symmetry with the other parts of a flower (Moyrold & Glover, 2017; Paterno et al., 2020). In *G. sylvaticum* flowers, the smaller petal size is associated with smaller style length and larger petals with longer styles (Asikainen & Mutikainen, 2005a). When the stigma is in the receptive phase, the style is typically longer in hermaphrodite flowers than in females (Asikainen & Mutikainen, 2005a). The long style length in hermaphrodite flowers has positive and negative effects on reproduction. The hermaphrodite stigma in the receptive phase protrudes over the anthers. This has positive effects as a means of prevention of autogamy in *Geranium* species (Konarska & Mazierowska, 2020; Philipp, 1985) in addition to the partial protandry in this species (Asikainen & Mutikainen, 2005a; Varga, Nuortila, & Kytöviita, 2013). However, the long style length has negative effects on the female function in hermaphrodites as it reduces the probability of pollen transfer on stigmas by pollinators as is demonstrated in this study. The style length has been shown to have a relatively narrow optima for pollen deposit and pollinator contact probability in *Brassica napus* flowers (Cresswell, 2000).

Larger flowers are advantageous in male function in the way of pollen transport from hermaphrodite flowers (Ashman, 1992). In agreement with our study, Ashman (1992) found that, although longer petals contributed to a better pollen export, the petal length was a poor predictor of pollen deposition. Concluding from the contacts to *G. sylvaticum* reproductive organs in our study, pollen display in hermaphrodites matched stigma display in hermaphrodite flowers poorly. In contrast, female flower morphology was a better match to the hermaphrodite pollen display. Accordingly, different aspects of morphology promote different sexual functions. Hermaphrodite morphology is adapted to pollen export (Ashman, 1992; Asikainen & Mutikainen, 2005a; Bond & Maze, 1999). In females, flower morphology that maximizes pollen receipt on stigma according to hermaphrodite pollen display should be selected because it is the sole function of the female flowers.

The small size of female flowers is often explained by the resource re-allocation hypothesis stating that the energy and nutrient investment difference between hermaphrodite and female flowers may be allocated to seed production (Ashman, 1994; Chang, 2006; Eckhart, 1992). In this work, we challenge the non-adaptive and resource re-allocation hypotheses in explaining the floral dimorphism in gynodioecious plants. We specifically tested the "pollination hypothesis" that flower size variation in *G. sylvaticum* is adaptive because it enhances probability of a visitor contacting stigma, and thus promotes pollination probability in females. We stress that we did not measure pollen deposition, but probability of pollen deposition. We base this estimate on the assumption that only when an insect makes a ventral contact with the receptive stigma lobes, pollen is deposited. In the case when the receptive stigma lobes are not contacted, pollen cannot be transmitted. In support of the pollination hypothesis, the stigmas in the small female flowers were more likely to be contacted by visitors than the stigmas in hermaphrodite plants. This indicates that the balance between visitor attraction and consequent pollen

transmission on one hand and pollen deposition on stigmas on the other hand may act as drivers in *G. sylvaticum* sexual dimorphism.

The stepping-stone hypothesis for the evolution of dioecy requires that the hermaphrodites in a gynodioecious population are biased toward maleness and ultimately lose their female role (Lloyd, 1976; Spigler & Ashman, 2012). One of the explanations for this is the aggravated competition for females through the male function (Lloyd, 1976). Consequently, hermaphrodites in gynodioecious populations are expected to be biased toward maleness (Goldman & Wilson, 1986; Spigler & Ashman, 2012). Assuming a similar number of visits, although smaller than the hermaphrodite flowers, the female flowers are more likely to be pollinated during a single visit than the hermaphrodite ones according to our data. As *G. sylvaticum* female flowers have been shown to receive fewer visits than hermaphrodites previously (Asikainen & Mutikainen, 2005a; Varga & Kytöviita, 2010) and in this work, this estimate is conservative. This is supported also in other studies, as—although hermaphrodite *G. sylvaticum* flowers do in general receive more visits than females (Asikainen & Mutikainen, 2005a; Varga & Kytöviita, 2010)—the fruit set is frequently lower in hermaphrodites than in females (Asikainen & Mutikainen, 2005b; Varga & Kytöviita, 2010, present study). Consequently, our work supports the stepping-stone hypothesis as hermaphrodite flowers had much lower pollination probability than female flowers during a single visit and lower seed set suggesting lower fitness gains through female function in hermaphrodites.

Flowers are subject to both directional and disruptive selection (Galen, 1999; Galen et al., 1987). Directional selection occurs when, for example, a trait of a plant positively affects pollinator visitation rates creating a selective pressure for increase in the expression of such trait (e.g., see Galen, 1989). In the case of *G. sylvaticum*, sexual dimorphism is the result of disruptive selection fueled by the different flower size optima for female and male fitness. The disruptive selection agents are the pollinating insects that behave differently in *G. sylvaticum* flowers of different sexes. The fact that we observed disruptive selection between the sexes of *G. sylvaticum* also supports the gynodioecy–dioecy pathway hypothesis (Dufay et al., 2014; Spigler & Ashman, 2012). Generally, floral characteristics such as showiness are promoted as the plants benefit from increased number of visitations (Martin, 2004; Van Etten & Chang, 2014), but disruptive selection by insect behavior may help explain the evolution of sexually dimorphic flowers. If the differential selection pressures on the sexes persist, *G. sylvaticum* may evolve toward dioecy.

Although we note that disruptive selection drives floral sexual dissimilarity in size, floral constancy may limit the evolutionarily stable degree of dimorphism. Due to the floral constancy behavior of the insects (Waser, 1986), the flowers need to be perceived as similar enough to be constantly visited. The scarcity of females can also cause minority disadvantage (Levin, 1972), which further reduces the visitation rates in *G. sylvaticum* females (Van Etten & Chang, 2014). We only distinguished the flowers by their size which was sex-specific and the presence/absence of anthers. In addition to corolla size, several other factors such as odor, color, and their relations to bee memory and handling skills (Chittka et al., 1999;

Ishii & Masuda, 2014; Waser, 1986) could be responsible for floral constancy. Bees possess notable olfactory discrimination abilities (Laska et al., 1999). Pollinators relying on cues such as odor or the color spectra could explain why, e.g., the bumblebees do not always discriminate between the sexes of *G. sylvaticum* (Asikainen & Mutikainen, 2005a) despite the dimorphism in size and the fact that females produce less nectar (Varga, Nuortila, & Kytöviita, 2013). In this experiment, bumblebee visitation rates were notably higher in hermaphrodites suggesting that at least occasionally bumblebees may favor the more rewarding sex. The morphological size dimorphism in *G. sylvaticum* may be furthered if the floral constancy of pollinators is more tightly linked to factors other than the size of flowers.

The pollen and stigma displays are linked via the morphology and behavior of the pollinator insects. Insect morphology imposes selective pressure on flowers to match the reproductive displays of the sexes. In a previous study on *Cucurbita maxima*, bumblebees carried considerably more pollen on their bodies than honeybees (Kamo et al., 2022). Due to their pollen transport capacity and behavior within the flower, bumblebees also effectively deposited more pollen than honeybees or other floral visitors (Kamo et al., 2022). Honeybee, *Apis mellifera*, is a non-native farmed insect in Finland. Our results suggest that although pollinating to some degree, it is inferior to native pollinators. Honeybee visitation rates did not influence the mean seed production per flower in our study plants, but *Bombus* and Syrphidae visitation rates did. Syrphid flies have been shown to be the most common floral visitors in *G. sylvaticum* (Bauman et al., 2021; Varga & Kytöviita, 2010). However, in our study Syrphid flies were likely to pollinate female flowers and even then only to a relatively small degree. *Bombus* had the highest likelihood of all of the visitor groups to contact *G. sylvaticum* anthers and stigma. This work supports our previous work that members of the genus *Bombus* are the primary pollinators of *G. sylvaticum* (Varga & Kytöviita, 2010). Bumblebees were noted to be behaviorally and morphologically effective pollinators also in a closely related hermaphroditic *Geranium* species (Kandori, 2002). In contrast to hermaphrodites, the small size of female stigma facilitated stigma contacts to a small degree also by honeybees and Syrphid flies. Because hermaphrodites were only pollinated by *Bombus*, but females by several insect groups, sexual size dimorphism could assure reproduction in both sexes in the face of fluctuating pollinator populations.

Although effective pollinators, bumblebees were the only visitor group that had the potential to effectuate self-pollination in the hermaphroditic flowers (in 5.4% of all visits by *Bombus* the insect touched an anther and then stigma in the same flower in a manner that could cause pollination). Avoidance of inbreeding is one of the mechanisms that has been proposed to drive gynodioecy (Baker, 1959; Charlesworth & Charlesworth, 1987). The effects of inbreeding vary between species and populations (Keller & Waller, 2002). Some inbreeding depression in *G. sylvaticum* has been observed in terms of lower germination rate in self-pollinated vs. crossed offspring (Varga, Vega-Frutis, & Kytöviita, 2013). Given that on average bumblebees visit 2–3 flowers within a plant (Asikainen & Mutikainen, 2005a), and

that there is a small degree of anther-to-stigma contacts in the same flower (this study), the self-pollination rate effectuated by *Bombus* may have some consequences. It should be noted, however, that the effects of inbreeding would be diminished in gynodioecious populations where higher proportion of offspring would be the result of crossbreeding (Baker, 1959). Altogether, it is likely that both the pollination hypothesis and the anti-selfing hypothesis (Baker, 1959; Kawagoe & Suzuki, 2003) explain the dimorphism in gynodioecious populations.

## 5 | CONCLUSIONS

Our results demonstrate disruptive selection in flower size and are in line with the stepping-stone hypothesis in explaining gynodioecy in *G. sylvaticum*. Supporting our “pollination hypothesis” dimorphism in *G. sylvaticum* seems to be adaptive in terms of optimizing female and male fitness in females and hermaphrodites, respectively. The two sex morphs are linked and selected by pollinator behavior.

Various insect species visited the flowers, but it is apparent that the two sex morphs are most efficiently pollinated by bumblebees whose visitation rates were also linked with seed production. The female function of hermaphrodites was dependent on bumblebees, whereas the female function in females was supported by several insect groups. This may promote sexual dimorphism depending on local insect fauna and its fluctuations and should be studied further. Farmed honeybees provided inferior pollination services compared to native pollinators and did not link with seed production or the ratio of wilted flowers to developed schizocarps. Altogether these results highlight the importance of pollinator diversity and of bumblebees in particular in plant sexual reproduction.

### AUTHOR CONTRIBUTIONS

**Jaakko O. S. Soininen:** Conceptualization (equal); data curation (lead); formal analysis (lead); funding acquisition (lead); investigation (equal); methodology (equal); project administration (supporting); resources (supporting); software (lead); validation (equal); visualization (lead); writing – original draft (equal); writing – review and editing (equal). **Minna-Maarit Kytöviita:** Conceptualization (equal); data curation (supporting); formal analysis (supporting); funding acquisition (supporting); investigation (equal); methodology (equal); project administration (equal); resources (lead); software (supporting); supervision (lead); validation (equal); visualization (supporting); writing – original draft (equal); writing – review and editing (equal).


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### DATA AVAILABILITY STATEMENT

Data are deposited in the JYX repository (Library of the University of Jyväskylä). <https://doi.org/10.17011/jyx/dataset/84567>.

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## II

**PLANT SEX AND PARENTAL GENOTYPE AFFECT  
FLORIVORY AND SEED PREDATION BY *ZACLADUS*  
*GERANII* IN *GERANIUM SYLVATICUM***

by

Soininen J.O.S. & Kytöviita M.-M. 2023

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### III

## MANAGED HONEYBEES AFFECT THE FORAGING BEHAVIOUR OF BUMBLEBEES IN *GERANIUM* *SYLVATICUM*

by

Soininen J.O.S., Paukkunen J. & Kytöviita M.-M. 2023

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