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Ecological Dynamics and Evolutionary Implications

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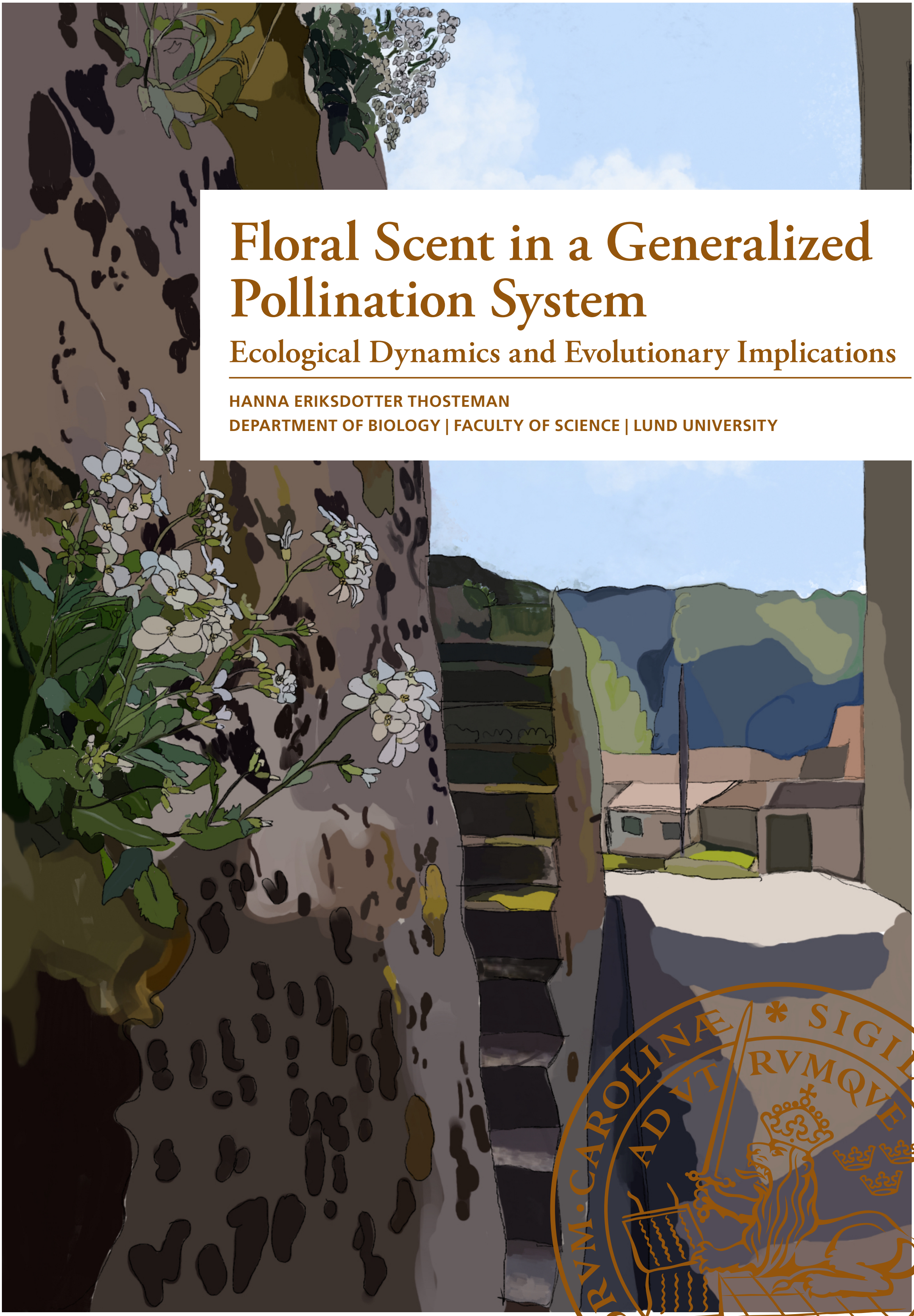

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Ecological Dynamics and Evolutionary Implications

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DOCTORAL DISSERTATION

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Abstract

The extraordinary diversity of flowering plants has long captivated biologists and evolutionary ecologists. Plant-insect relationships are recognized as a major driver of this diversity, with pollinators playing a crucial role in angiosperm speciation and trait diversification. Numerous observational and experimental studies have shown that pollinators influence the evolution of floral traits—such as size, shape, color, and scent—through their preferences and selective pressures. While much of our understanding of plant phenotypic diversification stems from specialized systems, most plants participate in more generalized interactions. This bias has created a significant gap in our knowledge of how phenotypes diversify in relation to pollinator communities in more generalized species. In this thesis, I investigate the drivers of intraspecific variation in floral scent and morphology within *Arabis alpina*, a pollination-generalist species. Through a series of experiments, I examine the interplay between floral traits, pollinator communities, and environmental factors to understand how mainly floral scent, but also plant-pollinator trait-matching, evolves across the species range. In Chapter I, I found that while floral scent shares biosynthetic links with other phytochemical traits, it may evolve independently under pollinator-mediated selection. Chapter II demonstrated that floral scent emission is consistent across various conditions, despite significant differences in scent composition and pollinator community between neighboring populations. Chapter III revealed that almost all insect visitors contribute effectively to pollination, and have variable levels of morphological trait-matching, suggesting they could influence floral trait evolution. In Chapter IV, I showed that pollinators consistently preferred local over foreign flowers, indicating a possible mechanism for the observed geographic variation in scent composition. Collectively, these findings suggest that floral scent variation in *A. alpina* is shaped by pollinator-mediated selection, potentially as a locally adapted trait. This work contributes to the growing understanding of how floral scent as well as other floral traits evolve in pollination-generalist species, emphasizing its significance in generalized plant-insect interactions.

Key words: *Arabis alpina,* floral scent, floral scent diversity, generalist phenotypes, intraspecific variation, phenotypic evolution, phenotypic integration, plant-pollinator trait-matching, pollinator community, spatial and temporal variation

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II. **Thosteman, HE,** Eisen, K, Montgomery, C.M, Pace, L, Friberg, M. Spatial and temporal floral scent variation in the generalist-pollinated *Arabis alpina.* (2024). Submitted manuscript.

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IV. **Thosteman, HE,** Susheel, A, Montgomery C.M, Pace, L, Eisen, K, Santana, P, Friberg, M. Local pollination generalization predicts floral scent diversity in a perennial herb. (2024). Manuscript.

Author contributions

I. MF and **HET** designed the experiment. **HET**, HP, SB, MCM, CDM, MF, JH, LP and JB collected data, and **HET** performed the analyses with support from KE, HP and JB. **HET** led the writing of the manuscript with substantial contributions from JB, MCM, HP, KE, MF and input and edits from all co-authors.

II. **HET**, MF and KE designed the experiment. **HET**, KE, MF, LP, and CMM collected the data. **HET** analyzed the data and led the writing of the manuscript with substantial contributions from all authors.

III. **HET**, MF and PS designed the experiment. **HET**, CMM, SSJB and AS collected the data. **HET**, AS and PS analyzed the data, and **HET** led the writing of the manuscript with substantial contributions from MF and PS, and input edits from all authors.

IV. **HET**, MF, KE and PS designed the experiment. **HET**, AS, CMM, KE and MF collected the data. **HET** performed the data analysis with conceptual contributions from PS, MF and AS. **HET** led the writing of the manuscript with substantial contributions from MF and PS, and input edits from all authors.

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Abstract

The extraordinary diversity of flowering plants has long captivated biologists and evolutionary ecologists. Plant-insect relationships are recognized as a major driver of this diversity, with pollinators playing a crucial role in angiosperm speciation and trait diversification. Numerous observational and experimental studies have shown that pollinators influence the evolution of floral traits—such as size, shape, color, and scent—through their preferences and selective pressures. While much of our understanding of plant phenotypic diversification stems from specialized systems, most plants participate in more generalized interactions. This bias has created a significant gap in our knowledge of how phenotypes diversify in relation to pollinator communities in more generalized species. In this thesis, I investigate the drivers of intraspecific variation in floral scent and morphology within *Arabis alpina*, a pollination-generalist species. Through a series of experiments, I examine the interplay between floral traits, pollinator communities, and environmental factors to understand how mainly floral scent, but also plant-pollinator trait-matching, evolves across the species range. In **Chapter I**, I found that while floral scent shares biosynthetic links with other phytochemical traits, it may evolve independently under pollinator-mediated selection. **Chapter II** demonstrated that floral scent emission is consistent across various conditions, despite significant differences in scent composition and pollinator community between neighboring populations. **Chapter III** revealed that almost all insect visitors contribute effectively to pollination, and have variable levels of morphological trait-matching, suggesting they could influence floral trait evolution. In **Chapter IV**, I showed that pollinators consistently preferred local over foreign flowers, indicating a possible mechanism for the observed geographic variation in scent composition. Collectively, these findings suggest that floral scent variation in *A. alpina* is shaped by pollinator-mediated selection, potentially as a locally adapted trait. This work contributes to the growing understanding of how floral scent as well as other floral traits evolve in pollination-generalist species, emphasizing its significance in generalized plant-insect interactions.

Svensk populärvetenskaplig sammanfattning

Vår planet är en fantastisk plats. Den är inte bara hem åt människor, utan även åt mikroorganismer, djur och växter, som tillsammans skapar de ekosystem som vi är en del av. En av de mest fascinerande aspekterna av livet på jorden är den enorma diversitet vi omges av, i form av olika arter eller utseenden inom arter. Extra häpnadsväckande är mångfalden av blommande växter, där evolutionen har lett till otaliga kombinationer av färg, form och storlek på det reproduktiva organ som blomman utgör. Forskning har visat att växters interaktioner med insekter är en av de starkaste drivkrafterna bakom variationen i blomkaraktärer, och särskilt viktiga är pollinerare.

De insekter som besöker blommor i jakt på födoämnen såsom pollen och nektar, bidrar till växtens förökning genom pollinering. Dessa pollinerare är ofta till stor del beroende av de resurser som växterna erbjuder, och självinkompatibla växter, dvs växter som inte kan pollineras med individens eget pollen, är ofta helt beroende av att locka till sig effektiva pollinerare för lyckad reproduktion. Under dessa förhållanden bör blomkaraktärer som maximerar blommans attraktiva egenskaper för de bäst lämpade pollinerarna vara under naturlig selektion. Under det senaste dryga seklet har mycket forskning riktats mot hur pollinerare påverkar evolutionen av blomstorlek, form, färg och antal, medan mindre uppmärksamhet har riktats mot blomdoft – en del av blommans karaktär som kan ha stor betydelse för att locka till sig pollinerare. Särskilt lite vet vi om hur blomdoft fungerar som signal till pollinerare hos växter som interagerar med många olika typer av obesläktade pollinerare, så kallade generalister. Faktum är att vi vet mycket lite om hur blomfenotyper (blommans utseende och funktion) generellt evolverar hos generalister, inte bara blomdoft utan även andra aspekter av blomman såsom storlek och form, och hur dessa varierar i relation till pollinerarsamhällen.

I min forskning undersöker jag vilka drivkrafter som ligger bakom variationen i blomdoft, hur den generalistiska fenotypen har och kan diversifieras, och om detta kan kopplas till variation mellan pollinerarsamhällen. Ett sätt att studera dessa aspekter är att fokusera på en vanligt förekommande art där blomfenotyper varierar mellan platser där den förekommer (populationer). En sådan art är fjälltrav (*Arabis alpina*) som tillhör familjen korsblommiga växter (Brassicaceae) och därmed är släkt med många av våra vanligaste grödor, såsom raps, kål och senap. Fjälltrav förekommer i bergsmiljöer på många platser på norra halvklotet, och tidigare forskning visar att många växtkaraktärer varierar mellan dessa platser. Till exempel är fjälltravspopulationer i Sverige, Spanien, Frankrike och Schweiz självkompatibla, vilket innebär att de kan befrukta sig själva. I Italien och Grekland däremot är populationerna nästan helt självinkompatibla, vilket innebär att de behöver pollen från andra individer för att föröka sig. Dessa populationer är alltså helt beroende av pollinerare för att sätta frön. Det är därför intressant att blomfenotypens egenskaper, som är viktiga för att locka pollinerare, varierar kraftigt mellan populationerna. Blomstorlek, till exempel, skiljer sig åt mellan fjälltravs-populationerna. Dessutom skiljer sig fjälltravens blomdoft både i mängd och sammansättning mellan självkompatibla och självinkompatibla populationer, och även mellan olika självinkompatibla populationer. Självinkompatibla populationer har större blommor och doftar mer och annorlunda än självkompatibla, men det finns också stor variation mellan de självinkompatibla populationerna.

Blomdoft är en komplex del av blommans fenotyp, och kan bestå av några få till flera hundra doftmolekyler av olika kemiskt ursprung och som kan vara mer eller mindre kemiskt besläktade med varandra. Doft kan variera på flera olika sätt, till exempel skiljer sig blommor åt både i vad de doftar (kvalitativt) och hur mycket de doftar (kvantitativt). Blomdoft skiljer sig som sagt inte bara åt mellan arter, utan kan även variera mellan populationer inom en art. Dessutom finns det arter som ändrar, ökar eller minskar sin doft beroende på tid på dygnet, för att öka chansen att rätt pollinerare lockas till blommorna. Den hittills samlade forskningen indikerar att variation i blomdoft är länkat till pollinerare, både till vilka arter av pollinerare som besöker växterna, och i förhållande till pollinerarnas dygnsrytm. När jag vill studera varför blomdoft skiljer sig åt mellan fjälltravs-populationer, och om detta är kopplat till dess pollinerare, finns det därför flera aspekter jag måste ta hänsyn till, vilket jag beskriver i mer detalj lite längre ned.

I min avhandling har jag valt att även uppmärksamma en omstridd del inom pollinationsbiologin, nämligen huruvida pollinerare och de blommor som de besöker är morfologiskt anpassade till varandra. Det finns forskning som visar att växter och insekter som uppvisar sådan anpassning ofta får fördelar genom mer effektiv pollination för växten samt ökad tillskansning av resurser för insekten. Sådan anpassning sker ofta genom karaktärsmatchning, till exempel en lång nektarsporre hos växten och en lika lång sugsnabel hos insekterna. En sådan matchning leder ofta till att de reproduktiva delarna av blomman, pistillen och ståndarna, kommer i kontakt med rätt delar på insektens kropp, och pollen flyttas mer effektivt mellan växtindivider. Mycket av det vi vet om karaktärs-matchning kommer från mer specialiserade interaktioner, och vi saknar ofta en förståelse för hur detta fungerar hos generalistväxter. I teorin bör de insekter som är mest effektiva att pollinera generalistväxterna också vara bäst matchade med blommans morfologi. Genom att studera vilka insekter som är mest effektiva och om dessa matchar blommans morfologi kan vi förstå mer om hur olika insekter påverkar växters både morfologiska och kemiska fenotyp.

För att studera hur blomdoft, blommans utseende och pollinerare samvarierar har jag valt att genomföra ett flertal experiment, både i en växthusmiljö och i de naturliga miljöer där fjälltraven återfinns. I **kapitel I** undersökte jag om det fanns några andra möjliga förklaringar till varför blomdoft varierar mellan fjälltravspopulationer, som till exempel kemiska släktskap eller andra hinder. Sådana kemiska avvägningar kan begränsa blomdoftens möjligheter att evolvera fritt i förhållande till pollinerardriven selektion, oberoende av andra kemiska aspekter i växten, till exempel kemiska försvarsmekanismer. I min forskning fann jag inga sådana avvägningar, och variationen i blomdoft som vi ser mellan olika fjälltravs-populationer är därför potentiellt ett resultat av pollinerardriven selektion.

I **kapitel II** ville jag ta reda på under vilken tid på dygnet självinkompatibla, italienska populationer blir pollinerade och om detta samvarierar med ökad, minskad eller förändrad blomdoft. Jag fann övervägande bevis för att fjälltraven pollineras dagtid, men att detta inte åtföljs av en ökad doftproduktion. Blomdoften utsöndras istället i lika mängd under dygnets alla timmar, oberoende av ljus eller mörker, temperatur och pollinerarnas dygnsrytm. Detta kan bero på att blomdoft inte är energimässigt kostsamt för fjälltrav, något som är svårt att fastslå utan mer detaljerade studier. Jag fann också att de två italienska populationerna jag inkluderade i studien skiljde sig åt i blomdoft samt i vilka pollinerare som besökte blommorna trots att de befann sig bara 4 km ifrån varandra. Detta indikerar att det kan finnas ett samband mellan doft och pollinerare även hos fjälltravspopulationer.

Baserat på min kunskap om vilken tid på dygnet fjälltraven pollineras, undersökte jag i **kapitel III** vilka insekter som mest effektivt pollinerade fjälltravens blommor, om dessa skiljde sig åt mellan populationer, samt om det fanns något samband mellan effektivitet och morfologisk matchning mellan fjälltravsblommorna och de insekter som besökte dem. Därför utökade jag antalet studerade italienska populationer till sju för att kunna se mönster över större landskap. Bin med långa tungor visade sig vara de mest effektiva pollinerarna i alla populationer, men eftersom de inte förkom i lika stor utsträckning överallt, fanns det visst mått av variation i hur viktiga olika pollinerare var i de olika populationerna. Vad som framför allt var intressant var att i den population där en pollinerare var som mest effektiv var ofta samma population där den hade som lägst matchning med blomman. Generellt fanns ett mönster av att ju kortare sugsnabel i förhållande till blommans djup, desto bättre och effektivare pollinering, oavsett insektsart. På grund av de beteendeskillnader jag såg mellan olika insektsgrupper, och variationen i deras effektivitet mellan populationer, är det troligt att fjälltravens blomfenotyp formas av summan av alla interagerande insekter. Jag fann inga tydliga tecken på att växten hade utvecklat lokala specialiseringar beroende på pollinerare, utan i stället verkade fjälltraven anpassa sig till många olika insektsgrupper, i linje med teorin om adaptiv generalisering – det vill säga att en växt kan understödja många olika pollinerare och samtidigt dra nytta av deras varierande pollinationsförmåga. Pollinerarnas mängd och deras karaktärsmatchning med blommorna varierade mellan olika populationer. Detta indikerar att blommornas egenskaper kan utsättas för varierande selektion beroende på vilka pollinerare som är vanligast i ett visst område. Fjälltraven kan alltså evolvera enligt ett mönster som kallas "adaptiv vandring", där insekter med kortare sugsnabel utgör ett starkare selektionstryck i de populationer där de är vanliga.

Efter att ha etablerat att fjälltraven besöks av många olika insekter som nästan alla bidrar till dess reproduktion, återvände jag i **kapitel IV** till blomdoften. Här ville jag undersöka mer i detalj hur blomdoftsammansättningen varierade i förhållande till det lokala pollinerarsamhället och om pollinerare föredrog doften från sin hemma-population. Genom att jämföra mångfalden av besökande insekter till fjälltraven med blomdoftens mer specifika sammansättning och kemiska diversitet, kunde jag visa att populationer med hög mångfald av pollinerare också utsöndrade blomdoft av högre kemisk diversitet. Detta kan bero på att olika arter av pollinerare föredrar olika kemiska ämnen, och ju fler kemiska ämnen en blomma utsöndrar, desto fler olika typer av pollinerare lockar den till sig. Dessutom fann jag att pollinerare generellt föredrog att besöka blommor från sin hemmapopulation i förhållande till blommor från en population med främmande doftsammansättning. Eftersom pollinerare kan föredra olika kemiska ämnen, men också snabbt kan lära sig att associera ämnen med resurser, kan det vara så att de föredrar blommor med bekant doft eftersom de vet att dessa kan tillgodose deras behov. Därför är det troligt att lokal anpassning till enskilda pollinerare eller grupper av pollinerare har format blomdoftsvariationen vi ser hos den italienska fjälltraven.

Sammanfattningsvis visar min forskning att fjälltraven har en fenotyp som samvarierar med pollinerarsamhällen över dess utbredningsområde. Genom att studera hur generalistväxter samverkar med pollinerare och hur detta kan leda till anpassningar i blommans fenotyp, kan vi dra slutsatser om hur evolutionen verkar även hos dessa arter. I min avhandling har jag visat att även om fjälltrav är en generalist och besöks av många olika typer av insekter, finns det starka kopplingar mellan pollinerare, blomdoft och delvis morfologi. Slutligen bidrar dessa resultat till vår förståelse för hur diversitet skapas och bibehålls genom evolution.

Definitions and abbreviations

Insect visitor	Insect species that does not contribute to pollination.
Pollinator	Insect species that to various degrees contribute to pollination.
Population	Geographically, genetically and/or phenotypically distinct population with limited gene-flow to surrounding populations.
SC	Self-Compatible
Scent profile **Scent bouquet** **Scent blend**	The floral scent composition of a species, population or individual.
SI	Self-Incompatible
SV	Single Visit, referring to Single Visit pollen deposition.
VOC	Volatile Organic Compound

Prologue

I lie on my stomach for hours, the sun's warmth spreading across my back. With a small stick, I trace patterns in the grass, gently prodding at the shadowy, mysterious world beneath the blades. My nose hovers close, where tiny critters scurry about, intent on some secret journey.

In my hand, the paper is damp, carefully folded over and over until it tears as I try to open it. A sweet scent lingers from the glass jar where it rested, warm and familiar. Inside, thick, yellow peas lie swollen and slippery, larger now than when I first tucked them away. A small, green sprout pushes through the pea, eager to break free, to stretch towards the light. A life cradled in my palm.

My fingers are smudged with dirt, black streaks marking my cheeks, my nose, my forehead. The seeds are planted in pots of every shape and size, their identities forgotten in my eager haste. I never seem to do this the way it’s meant to be done. The windowsill is crowded, a jumble of life learning to share. I too must learn to coexist with them. I check them often - an hour passes, then another, and another. The wait is always too long; my eagerness, too great.

The beech tree towers above me, it gives me vertigo as I stand at its base. It smells green, sweet, and fresh, mingled with the earthy scent of soil, leaves, and rain. The branches rise in layers, I could climb it. My hand slides across the smooth, grey bark - no, green? I can’t quite tell. The sunlight filters through the leaves, casting the world in shades of green and grey. I want to climb, but my hands slip, and they too become green, or grey, or perhaps both.

It trickles down my neck, wet and cold. It mixes with the salt on my skin, my hair clinging to my cheeks. The world is silent under my hood, only the slight rustling of my movements and the gentle sound of rain drops. A small universe sticks to my fingers as I hold the green, fragile stem in my hand. Broken seeds, a small, brown leaf. Across a few blades of grass, a small beetle scurries to find the highest point on my fingers. The flower is damp, its scent hidden beneath the translucent veil of water. I press it against my nose and lips, wanting to sense it.

I crave you, green world of chaos and serenity. You rest in my heart, and I always come back to you. The safety, the certainty. The challenge. We can work together, and we struggle. I may forget, but you always remind me.

Introduction

Life on Earth exhibits remarkable diversity, with flowering plants standing out as a particularly striking example. The vast array of flower colors, scents, sizes, and shapes has long captivated researchers across disciplines, from studies of their medicinal and cultural significance to investigations in taxonomy, physiology, chemistry, and evolutionary ecology (Darwin, 1862; Harder and Johnson, 2009; Stebbins, 1970). A leading hypothesis for the observed variation in floral phenotypes centers on the intricate interactions between plants and their pollinators (Caruso et al., 2019; Castellanos et al., 2004; Fenster et al., 2004; Schiestl and Johnson, 2013). A majority of plants rely on animals for reproduction or seed dispersal (Ollerton et al., 2011), and many animals depend on plants as food sources, mating sites or oviposition substrates (Price, 1997). In the majority of plant species, reproductive output is tightly linked to efficient pollination where compatible pollen is transported by an animal vector and successfully deposited on a stigma (Ollerton et al., 2011). In such systems, plant traits that optimize attraction and fit of pollinators should be favored by selection (Caruso et al., 2019; Fenster et al., 2004).

Research into pollination biology provides many examples of floral structures that have evolved in response to natural selection imposed by pollinators. When floral traits vary among species or populations, this variation is often linked to differences in pollinator communities and the selection pressures from diverse plant-pollinator interactions (Fenster et al., 2004; Schiestl and Johnson, 2013; Thompson, 2013, 1999; Thomson et al., 2000). Floral scent is an important pollinator attractant in many systems (Raguso, 2008a), and has been shown to vary among species (e.g., Byers et al., 2013; García et al., 2023; Powers et al., 2020) and within species (Friberg et al., 2019; Gross et al., 2016; Petrén et al., 2021; Xu et al., 2020). Intraspecific, genetically determined, floral scent variation suggests locally divergent selection across the geographical range of a plant species, potentially driven by differences in pollinator preferences (Raguso, 2008b). However, the inherent complexity and multidimensional nature of floral scent profiles, coupled with their role in mediating diverse species interactions, presents a significant challenge in identifying both the targets and agents of selection driving floral scent diversification (Opedal et al., 2022).

Most of our understanding of floral scent as a pollinator attractant, and how floral scent phenotypes vary among populations, comes from studies on specialized pollination systems (Raguso, 2008b). However, it has become clear that floral scent plays a crucial role also in more generalized pollination systems, as evidence of floral scent profiles linked to generalist pollinator communities is growing (e.g., Gross et al., 2016; Johnson and Hobbhahn, 2010). Changes in floral scent profiles of more generalized species can alter insect visitation networks (Larue et al., 2016), and there is increasing evidence of generalist floral scent profiles having the ability to rapidly evolve in response to pollinator-mediated selection (e.g., Gervasi and Schiestl, 2017; Ramos and Schiestl, 2019). Still, we are only beginning to understand the role of floral scent, and what drives intraspecific scent variation in more generalized plant species. Further, floral scent should be studied in conjunction with other traits, such as morphological characteristics or other aspects of plant chemistry, to gain a more holistic perspective. This integrated approach may offer deeper insights into how multiple factors interact to shape floral trait variation.

The perennial herb *Arabis alpina* offers a valuable opportunity to study the ecological and evolutionary drivers behind among-population variation in floral scent. Unpublished data indicate that this species is visited by insects from various orders, suggesting it is involved in a generalized pollination system (Petrén, 2020), a relatively understudied group in the context of floral scent signaling. Further, previous research has documented a genetic basis of floral scent variation in *A. alpina* (Luizzi et al., 2021), partly linked to differences in mating systems, but substantial variation also occurs within mating system (Petrén et al., 2021). Here, self-compatible populations emit less scent compared to self-incompatible populations (Petrén et al., 2021). However, there is ample floral scent variation both in emission rate and in the compounds emitted also among populations within mating systems, and phylogenetic analyses indicate that the observed scent variation is larger than what can be explained solely by phylogeography or genetic drift (Petrén, 2020). Further, floral scent variation occurs at both large and small geographic scales, suggesting that the strength and/or direction of selection varies across environments. Indeed, divergent selection on specific floral scent compounds has been observed in outcrossing populations, underscoring the potential role of pollinators as agents of selection (Petrén, 2020). Collectively, these findings suggest that intraspecific variation in floral scent in *A. alpina* represents an adaptive trait shaped by interactions with local pollinator assemblages.

Yet, several aspects of the *A. alpina* scent phenotype remain underexplored, limiting our ability to conclusively identify pollinators as selective agents. For instance, very few studies of any plant species have attempted to detect potential biosynthetic constraints or phytochemical correlations that may influence floral scent diversification. Although considered a pollination generalist, the identity of insect visitors is available only from two *A. alpina* populations (Petrén 2020), and there is no evidence for how efficiently different insect visitors contribute to pollination, or if pollinator effectiveness varies among populations. *Arabis alpina* further offers an excellent opportunity to study how other aspects of the generalist floral phenotype, such as flower morphology, diversifies in relation to variation in pollinator communities. Understanding also how the morphological generalist phenotype evolves in a diverse pollinator community is essential for understanding the evolution of plant chemical traits, as these may be linked or can highlight the importance of certain pollinators as particularly important for the phenotypic diversification across landscapes. Additionally, comprehensive studies are needed to examine how spatial and temporal patterns of floral scent emission correlate with pollinator activity and community composition across diverse populations.

In this thesis, I aim to explore the ecology and evolution of floral scent in *Arabis alpina*, with a focus on its interactions with pollinator assemblages and their activity pattern. I begin by examining biosynthetic correlations and constraints as potential drivers of intraspecific scent variation (**I**), and then I investigate the role of pollinator communities in shaping floral trait variation (**II**, **III**, **IV**). My research highlights how floral scent interacts with pollinator assemblages and how this dynamic may influence the evolutionary trajectories of plant populations. By considering both the temporal and spatial dimensions of scent variation, this work seeks to deepen our understanding of the role of floral scent in plant-insect interactions and its broader ecological and evolutionary significance across diverse environments.

Aims of the thesis

The central focus of my thesis is to examine the factors driving intraspecific floral scent variation in the pollination-generalist herb *Arabis alpina*.

(**I**) In a series of greenhouse experiments, I begin by exploring biosynthetic constraints on floral scent variation, addressing key questions such as:

1. Are attraction and defense compounds part of an integrated scent phenotype, or can floral scent evolve independently from other plant chemistry in response to pollinator-mediated selection?

2. Does phenotypic integration between attraction and defense compounds vary among populations and mating systems?

(**II**) To further investigate the temporal and spatial scales of floral scent variation in *A. alpina* and its relationship with insect activity, I conduct both greenhouse and field experiments, focusing on the following:

3. During which periods of the day do insects visit and pollinate flowers?

4. Does insect community composition differ between populations at small spatial scales?

5. Is there evidence of synchronization of the emission and/or composition of floral scent and insect visitor activity?

6. Does floral scent composition vary among floral tissues, and are specific tissues responsible for the observed floral scent compound variation among populations?

(**III**) Building on these findings, I examine the insect community in detail to assess the relative importance of various insect visitors to *A. alpina* and their potential role as selective agents on floral scent and morphology:

7. Which insect groups are the primary pollinators of *A. alpina,* and do these groups vary among populations?

8. Is there evidence of trait matching between flower and insect morphology, and if so, does this correlate with pollinator efficiency?

(**IV**) Finally, I delve into the relationship between floral scent composition and pollinator assemblages in *A. alpina*, while also considering the influence of environmental factors on floral scent, by addressing:

9. Do populations with high floral scent structural diversity also interact with an insect community of high diversity?

10. Is the structural diversity of floral scent related to variation in the co-flowering community?

11. To identify patterns of local adaptation, I ask if pollinators prefer to visit flowers from their local environment emitting familiar scents over flowers with foreign scent composition.

Pollinators and floral phenotypes

A commonly held theory imposes that evolutionary diversification of floral traits, including size, display, shape, color, and scent, is driven by pollinator-mediated, diversifying natural selection (Darwin, 1862; Fenster et al., 2004). While it is important to acknowledge that non-selective forces like genetic drift and gene flow also contribute to the observed variation between and within species, the gathered evidence points to pollinators being a key driving force behind this diversification (Delle-Vedove et al., 2017).

In this section, I discuss the role of pollinators in the context of more generalized pollination systems, where floral phenotypes are simultaneously subjected to multiple vectors of selection. I address the issue of assessing the relative importance of visiting insects and how that relates to plant-pollinator fit traits (**III**) before exploring how phenotypes in more generalized plant species have diversified in relation to diverse communities of pollinators (**II**, **IV**).

Pollinators – who are they?

Animals interacting with flowers and to at least some extent contribute to the successful reproduction of the plant are considered pollinators. The requirement for successful pollination includes adequate removal of pollen by a vector that more or less efficiently transfers the pollen to a compatible flower where pollination is achieved by pollen deposition on a receptive stigma (Ollerton et al., 2011).

Depending on the interacting partners, pollination efficiency for each visiting species may fall along a continuous spectrum from suboptimal to optimal (Stebbins, 1970). Efficiency may be influenced by factors such as pollinator fit (meaning the morphological fit between flower and pollinator) and pollen turnover (meaning the proportion of removed pollen successfully transported from donor to receiver) (e.g., Castellanos et al., 2003). Although pollinators include for example birds (e.g., Castellanos et al., 2004), bats (e.g., Fleming et al., 2009), lizards (e.g., González-Castro and Siverio, 2024) and even shrews (Wester, 2011), I will use the term pollinator to refer to insects alone in the following sections, as they are the group of animals responsible for pollination in *A. alpina*. Knowing what pollinators or pollinator groups contribute to successful fertilization may help us identify potential agents of selection acting on floral phenotypes.

Efficiency

Most of our understanding of pollinator efficiency comes from more specialized systems (e.g., Fenster et al., 2004) and it is widely known that not all insects contribute equally to pollination in any given plant species (Stebbins, 1970). In specialized systems where a plant species interacts with only one or a limited number of related insect taxa, determining the identity of the main pollinator may be straightforward. In more generalized systems, however, identifying the per insect level of contribution to reproductive success can be both complex and cumbersome, because efficiency must be assessed for each type of pollinator – of which there can be many (King et al., 2013; Ollerton et al., 2024). Merely using visitation rate as a proxy for pollinator efficiency is also not a reliable method in most systems (King et al., 2013). Instead, a combination of a quantitative (e.g., visitation rate) and qualitative (e.g., single visit pollen deposition) estimation is necessary to fully separate insect visitors into pollinators and insects that do not contribute to successful fertilization (Gómez et al., 2022; King et al., 2013; Ollerton et al., 2024).

In chapter **III**, I quantify the pollinator communities interacting with different *A. alpina* populations, and by assessing both visitation rates and single visit pollen deposition and pollen germination, I explore the relative contributions by several functional groups to successful pollination. In my thesis, I follow previous literature when defining pollinator effectiveness (Gómez et al., 2022). The term 'effectiveness' refers to the combined effect of pollen quality and quantity, together describing the relative importance, or effectiveness, of the pollinators investigated.

Plant-pollinator fit

Efficiency is often linked to plant-pollinator fit, a term describing how well the morphology of a flower matches the morphology of visiting insects, such that optimal pollen removal and deposition is achieved (Patchett et al., 2017). Floral fit traits may include flower tube length, nectar-stigma distance and corolla opening restriction (Caruso et al., 2019). Sometimes plant-pollinator fit traits co-evolve via reciprocal selection (Thompson, 2013), such as the morphological match between the long-proboscid fly *Moegistorhynchus longirostris* (Diptera: Nemestrinidae) and the long-spurred iris *Lapeirousia anceps*, where the degree of trait matching is positively correlated with plant reproduction (Pauw et al., 2009). An extreme example such as the relationship between *M. longirostris* and *L. anceps* will effectively filter out unwanted, less efficient visitors (*cf.* Castellanos et al., 2004).

However, plant-pollinator fit traits are not limited to highly specialized interactions and do not necessarily result in pollinators with a less precise match being excluded. For instance, the beardtongue *Penstemon strictus* (Plantaginaceae) has floral traits corresponding with a 'bee-pollination syndrome' with wider corolla opening, shorter corolla tube length, gradual pollen presentation and low pollen dosage (Castellanos et al., 2003). Despite this apparent bee-syndrome, hummingbirds are removing and depositing pollen at similar rates to bees in this system (Castellanos et al., 2003).

As successful pollination typically requires close contact between the two partners, proboscis length and corolla depth are frequently used metrics in studies of plant-pollinator fit and trait matching (Anderson et al., 2010; Opedal, 2021; Thompson, 2013). Meta-analyses show that trait matching between flowers and their pollinators is rarely perfect, and that flowers often exhibit exaggerated morphological features compared to their interacting partners (Anderson et al., 2010). Interestingly, a precise match in traits does not always result in the most favorable outcome for either the plant or the pollinator (Toju and Sota, 2005). Trait matching can also fluctuate across populations, if selection pressures vary among ecological communities (Anderson et al., 2010).

Still, pollinators often gain advantages from visiting flowers with corolla depths that align with their morphology, such as reduced handling time and lower competition (Zhao et al., 2022). For instance, in studies of generalized Asteraceae, bees with varying tongue lengths were found to prefer flowers whose corolla depths closely matched their tongue length, likely due to a reduced handling time (Klumpers et al., 2019). Thus, when specific pollinator species or functional groups play a key role in a community, we might expect some degree of trait matching between flowers and those pollinators due to their relative importance.

In chapter **III**, I examine the level of trait-matching between flowers and insect functional groups in *A. alpina*, and to what extent a closer trait-matching relates to a higher reproductive success. Investigating how trait-matching vary across functional groups and communities in more generalized systems will deepen our understanding of how plant-insect interactions drive the evolution of plant traits.

Phenotypic evolution in generalized systems

In a specialized context it is possible to describe what is referred to as pollination syndromes, i.e. flower adaptations that cater to single pollinating species or functional groups (Fenster et al., 2004). Such pollination syndromes are examples of convergent evolution, where closely or distantly related plant species interacting with the same type of pollinators have repeatedly evolved similar suits of traits. Common examples include hummingbird-pollinated flowers which are often tubular, red and nectar rich (Fenster et al., 2006; Grant, 1966), and moth-pollinated flowers which are often white, scented and receptive at dusk (Fægri and Van Der Pijl, 1979). Although pollination syndromes can be useful when describing examples of co-evolution and diversification of plant traits, they do not accurately capture the full extent of floral trait and plant-pollinator interaction diversity (Ollerton et al., 2009) and shed little light on how generalized plant phenotypes are maintained (Ollerton, 1996).

Plant populations involved in generalized pollination systems often interact with diverse pollinator communities (Ollerton, 1996; Ollerton et al., 2009). As a result, pollinator-mediated selection on generalized phenotypes can be viewed as the cumulative effect of all selective pressures—whether conflicting or additive—imposed by the local pollinator community (Armbruster, 2016). The evolutionary response to these combined pressures may lead to several phenotypic outcomes, three of which are discussed below.

First, if the strength of selection on plant reproductive success varies significantly across pollinators in different populations, local specialization may evolve. This scenario assumes a dominant functional group of pollinators within each population. Under such conditions, populations may become locally specialized while remaining regionally or globally generalized (Ollerton et al., 2007).

Second, plants may evolve phenotypic traits that accommodate multiple groups of pollinators through adaptive generalization (Ohashi et al., 2021). In this process, selection imposed by different pollinators can shape distinct traits, as seen in the corolla tube depth and restriction of *Aloe krausii*, which is pollinated by both bees and sunbirds (Hargreaves et al., 2019). Bees outperform sunbirds in flowers with shorter, wider tubes, but when the tube is narrow, both groups are effective pollinators, regardless of tube length (Hargreaves et al., 2019). A similar strategy is found in compound flowers of Apiaceae and Asteraceae, where floret arrangement maximizes pollen transfer from pollinators of varying morphologies, despite poor trait-matching between individual taxa and florets (Ohashi et al., 2021). In this scenario, pollinators may exhibit similar effectiveness even if their trait-matching varies considerably.

Third, adaptive wandering can drive intraspecific variation among generalized populations (Thomson and Wilson, 2008). When pollinator communities differ among populations, so too may the relative strength of selection (Thompson, 2013). As a result, plant populations may evolve distinct phenotypes that cater to their local pollinators without excluding visits from other pollinators (Thomson and Wilson, 2008). In species displaying phenotypic variation across geographic scales, adaptive wandering driven by divergent pollinator communities may explain the observed patterns.

Experimental evidence for how pollinator-mediated selection has contributed to floral trait diversification is largely lacking from more generalized species. However, a recent study on how strength and direction of selection varies among populations of the generalist *Viscaria vulgaris* (Caryophyllaceae) suggests that between-population divergence in pollinator assemblages correlates with divergence in strength and direction of selection on floral phenotypes (Torres-Vanegas et al., 2024). These results support the Grant-Stebbins model stating that patterns of phenotypic selection on floral traits shift in response to differences in the composition and characteristics of local pollinator communities (e.g., Herrera et al., 2006; Opedal, 2021), strengthening the hypothesis that also more generalized species may be locally adapted to the pollinator community (Ohashi et al., 2021).

While traditional models of selection in generalist plants focus on visitor-mediated trade-offs, or the balance of conflicting selection pressures, other hypotheses such as adaptive generalization and adaptive wandering offer new perspectives on how phenotypic variation is shaped. These theories highlight the potential for generalized species to adapt dynamically to diverse pollinator groups and local environments, enabling them to navigate the complexities of multiple selective forces without conforming to strict syndromes. This flexibility may play a crucial role in the evolution of generalized plant species. In chapters **III** and **IV**, I explore among-population variation in pollinator communities and floral phenotypes and discuss my findings from the perspective of how traits diversify in more generalist species.

Phenotypic integration, conflicting selection pressures and trade-offs

The evolution of a focal trait can be limited or enhanced by the degree to which it is correlated with other traits (Hansen, 2003). Experiments aimed at detecting selection on single traits may therefore over- or underestimate the intensity of selection acting on the specific trait, as the observed selection may be enhanced or obscured by selection acting upon correlated traits (Lande and Arnold, 1983). Many plant traits are part of such integrated phenotypes, which means that any constraints due to trait correlations may affect the potential for single traits to respond and diversify in response to natural selection. For instance, correlated traits can be subjected to conflicting selection pressures generating evolutionary constraints (e.g., male and female reproductive traits) (Armbruster and Schwaegerle, 1996; Willi, 2013). Many composite traits, such as plant defense and attraction compounds, may have multiple functions and could thus be subjected to multiple agents of selection and trade-offs (Irwin et al., 2004; Kessler and Halitschke, 2009). Hence, to understand local trait diversification it is important to understand how the evolution of a focal trait is influenced by the evolution of other traits and multiple agents of selection that may impose unaligned selection pressures (Armbruster and Schwaegerle, 1996; Ehrlich and Raven, 1964). In chapter **I**, I explore the potential for trait correlations to constrain floral scent and leaf chemistry evolution.

Phenotypic integration among phytochemicals

Plants produce a range of phytochemicals crucial for attracting mutualists and deterring herbivores (Kessler and Halitschke, 2009; Knudsen et al., 2006). These compounds, synthesized via a limited number of biosynthetic pathways of which a handful are well-documented, often exhibit phenotypic integration due to their shared origins (Dudareva et al., 2013; Junker et al., 2018; Sun et al., 2016). Volatile

Organic Compounds (VOCs) involved in phytochemical attraction and defense are emitted from floral and leaf tissues, and are classified based on biosynthetic pathways and molecular structures (Knudsen et al., 2006; Sun et al., 2016). For instance, the Shikimate pathway produces aromatic compounds such as benzenoids and phenylpropanoids, while the MEP-pathway produces terpenoids, and fatty acid derivatives are produced by the LOX-pathway (Dudareva et al., 2013). Although compounds can have different biosynthetic origin, there is still considerable crosstalk between pathways (via, for example, shared substrates; Dudareva et al., 2013) and VOCs can vary in their degree of structural relatedness. Compounds originating from the same biosynthetic pathway are structurally more similar and often more integrated compared with those from a different pathway (Dudareva et al., 2013; Junker et al., 2018; Pellmyr and Thien, 1986; Schiestl, 2010).

Phenotypic variation in composite traits that are potentially phenotypically integrated presents a challenge for identifying targets of selection. In addition to the shared biosynthesis of floral scent attraction compounds and vegetative volatile defense compounds, there are potential links between volatile and tissue bound defense compounds through shared biosynthetic pathways. Glucosinolates, the non-volatile defense compounds present in Brassicales, vary among and within species (Buckley et al., 2019; Hopkins et al., 2009), and may contribute to variation in defense volatiles through their role as precursors to volatile isothiocyanates (Hopkins et al., 2009; Schiestl, 2014). Thus, there are multiple layers of potential correlation between plant chemical attraction and defense compounds that may complicate studies aimed at identifying agents and targets of selection on chemical phenotypes. However, traditionally, studies that identify intraspecific variation and local adaptation in plant chemical ecology, are focused on either the attracting (e.g., Dormont et al., 2019; Friberg et al., 2019; Gross et al., 2016; Petrén et al., 2021) or the defensive aspects of chemical traits (Benedek et al., 2019; Buckley et al., 2019; Wurst et al., 2008), and few studies have asked to what extent defensive and attractive chemical emissions co-vary (but see Junker et al., 2018).

Studying the diversification and correlation of traits in parallel may help researchers avoid misinterpreting the agents and targets of selection responsible for generating variation (Armbruster and Schwaegerle, 1996; Sletvold, 2019). In **chapter I**, I investigate whether plant attraction- and defense chemistry in *A. alpina* are free to evolve separately and explore the potential for floral scent to respond independently to pollinator-mediated selection. If floral scent and leaf defense compounds are part of an integrated phenotype, the observed intraspecific variation in floral scent composition may reflect patterns of indirect selection, where defense compounds are the primary targets. Conversely, if floral scent is uncorrelated with defense compounds, it may respond independently to pollinator-mediated selection, a process potentially responsible for the intraspecific scent variation in this species.

Mating systems and phenotypic integration

The transition from outcrossing to selfing is one of the most common and well-known evolutionary shifts in plants (Barrett, 2010; Bush, 2005), and variation in outcrossing rates among conspecific populations is widespread (Barrett, 2010). Pollinator-dependent, outcrossing plants typically invest more in floral attraction traits, whereas more selfing species generally evolve traits such as reduced petal size and altered anther orientations that collectively are referred to as the selfing syndrome (Lande and Schemske, 1985; Sicard and Lenhard, 2011; Toräng et al., 2017).

Floral scent emission has been shown to decrease when species evolve self-fertilization, although the magnitude of change varies among systems (Doubleday et al., 2013; Majetic et al., 2019; Petrén et al., 2021; Raguso et al., 2007; Sas et al., 2016). The reduction of traits associated with pollinator attraction in selfing populations and species may be related to metabolic or ecological costs. When pollinator dependency is reduced, there is less need for costly traits such as large, brightly colored and highly scented flowers (Doubleday et al., 2013). In fact, in the absence of pollinators these traits may even be non-adaptive, as risk of detection by antagonists increases (Doubleday et al., 2013). Thus, the loss of self-incompatibility may change the selective forces acting on plant signaling.

In the context of phenotypic integration among attraction and defense compounds, the diversification of mating systems may have several different outcomes for the phytochemical phenotype. Although research on this subject is lacking, there are a few hypotheses on how a transition from outcrossing to selfing may affect plant chemistry. First, a prior meta-analysis examining phenotypic integration of floral morphological traits between selfing and outcrossing plant species found that selfing species exhibited higher levels of integration in both entire flowers and in sexual organs compared to outcrossing species (Fornoni et al., 2015). If this pattern also extends to phytochemistry, we may expect higher phenotypic integration among floral scent compounds in selfing and self-compatible species and/or populations, as the reduced pollinator-mediated selection is likely to preserve developmental connections between these compounds. Furthermore, self-incompatible populations could show a low level of integration within floral volatiles and between floral and foliar volatiles, which would enable an adaptive response to selection pressures exerted by pollinators. This pattern would align with the Berg hypothesis, which suggests that floral structures are usually evolutionarily decoupled from vegetative tissues, allowing for differential responses to selective pressures from antagonists and mutualists (Berg, 1959).

Arabis alpina populations express a wide range of outcrossing rates, from autonomously selfing to exclusively outcrossing (Laenen et al., 2018, see Methods section), which provides an excellent opportunity to test these hypothesis in a single species. In chapter **I**, I investigate the levels of phenotypic integration within and between floral scent, foliar volatiles and glucosinolates to expand our knowledge on how these traits are affected by mating system.

Conflicting selection pressures and trade-offs

There are multiple reasons for why plant attraction and defense traits may be correlated across populations. Firstly, the plant population is potentially subjected to selection from both antagonists (herbivores, florivores, seed predators, nectar robbers) and mutualists (pollinators, seed dispersers). Multiple simultaneous vectors of selection could indicate that the locally optimal trait value may vary both temporally and spatially, depending on the local insect community and the relative importance of selection from antagonist and mutualist insects (Schiestl, 2015). Second, a phytochemical signal may have multiple functions (Irwin et al., 2004). For example, monoterpene phytochemicals function as floral pollinator attractants in many species (Andersson and Dobson, 2003; Dötterl and Vereecken, 2010; Parachnowitsch et al., 2013; Riffell et al., 2009) but as insect repellents in others (Junker and Blüthgen, 2010; Schiestl, 2010).

Moreover, many compounds, such as terpenoids, that are traditionally described as defense compounds are structurally similar to pollinator attracting floral scent compounds (Pellmyr and Thien, 1986) and share biosynthetic pathways (Dudareva et al., 2013). Hence, it is plausible that conflicting selection pressures exerted by mutualists and antagonists may result in trade-offs and constraints on attraction and/or defense compounds that co-vary. This scenario was highlighted in an experiment by Ramos and Schiestl (2020), that revealed potential trade-offs between floral scent and defense compounds in *Brassica rapa* (Brassicaceae). They observed significant differences in floral scent evolution between test groups where herbivores were absent or present, suggesting that conflicting selection pressures are imposed by mutualist and antagonist interactions – a theory also supported by field experiments on other species (Kessler and Halitschke, 2009; Strauss and Irwin, 2004; Theis and Adler, 2012).

To maximize fitness, plants should emit scent to attract pollinators and simultaneously avoid detection by antagonists (Theis et al., 2007). A phytochemical signal intended for pollinator attraction can also act as a detection signal for enemies. For example, in an experiment on *Cucurbita pepo* var. *texana,* researchers enhanced the natural floral fragrance known to attract the main pollinator species, and observed increased attraction of florivore beetles and subsequent decrease in seed set (Theis and Adler, 2012). The reduction in seed set was likely caused by pollinators rejecting flowers infested with florivore beetles (Theis and Adler, 2012).

Enhanced floral scent can also directly deter pollinators. In a dosage experiment researchers investigated the effects of floral scent on pollinator and antagonist attraction. It was observed that plants with low to natural amounts of a single compound attracted pollinators and were more likely to be damaged by antagonists. When the concentration of the compound was artificially increased, antagonists were deterred, but so also the pollinators (Galen et al., 2011). This result further indicates that a given phytochemical signal intended for either pollinator attraction or enemy defense can come at a cost of attraction or deterrence of the ‘wrong’ receiver. Additionally, not all compounds in a floral scent bouquet may be biologically active (i.e. generating a response in foraging pollinators) (Schiestl et al., 2011). In fact, there may be a fitness cost associated with emitting non-active compounds if they are correlated with reduced attractiveness to pollinators or increased risk of enemy detection (Schiestl et al., 2011).

Another, less studied, aspect of costs associated with floral scent emission is the metabolic component. The biosynthetic pathways involved in VOC production require energy and may thus be subject to allocation trade-offs (Agrawal, 2011). However, studying the metabolic costs of floral scent can be difficult, as it requires stable environments where the many confounding factors such as genetic relatedness, herbivore protection, temperature regime et cetera, are controlled for.

One way of measuring metabolic costs via allocation trade-offs is by manipulating water and nutrient availability. Here, a metabolic cost of floral scent production could be observed through lowered emission or altered composition in response to limited resource access. Yet, the few studies that have attempted these experiments report conflicting results. For instance, in *Lithophragma bolanderi* (Saxifragaceae), the floral scent emitted by single flowers is unaffected by changes in nutrient availability (Friberg et al., 2017). In *A. alpina* (Brassicaceae), increasing nutrient availability has a slightly positive correlation with floral scent emission, but this pattern is not associated with any observable fitness trade-off (Luizzi et al., 2021). In both these cases, plants responded to the increased nutrient levels by producing a higher number of flowers, thus emitting more floral scent at the level of the inflorescence (Friberg et al., 2017; Luizzi et al., 2021), making it difficult to disentangle putative costs of floral scent in relation to the cost of producing additional floral tissue. Other experiments show that floral scent can be highly affected by nutrient and water stress (Knudsen and Gershenzon, 2020 and references therein). Thus, there is a considerable gap in our understanding of how metabolic trade-offs affect floral scent emissions.

Collectively, the floral scent phenotype is likely the result of multiple agents of selection acting simultaneously, which relate to variation in the reproductive success and survival of the plant in its current environment (Sletvold, 2019). In chapter **I**, I study how floral scent VOCs are related to defense volatiles to discover any phenotypic trade-offs between floral and leaf volatiles in *A. alpina*. In chapter **II,** I focus on the pollinator attraction aspect of the *A. alpina* floral scent phenotype to understand if there are any signs of metabolic costs related to abiotic factors, or temporal variations potentially associated with ecological costs.

Pollinators and floral scent

Floral scent is a multidimensional trait, meaning that it consists of multiple components and aspects rather than being a single, uniform characteristic. The complex blend of various VOCs that each have its own chemical properties, emission patterns, and ecological functions make scent a part of the floral phenotype that can vary on multiple levels. Pollinators can use floral scent as a foraging cue (Raguso, 2008b) and depending on species they can respond to single compounds or compound blends (Andersson and Dobson, 2003; Raguso and Light, 1998). Thus, pollinators may influence floral scent variation at multiple levels. In this section, I discuss spatial and temporal variation in floral scent (**II, IV**) in relation to pollinator-mediated selection before ultimately addressing how pollinators may affect floral scent composition through innate and learned olfactory preferences (**IV**).

Spatial variation

On spatial scales, floral scent emission rate (quantitative variation) and composition (qualitative variation) may vary among conspecific populations (Delle-Vedove et al., 2017), among individuals within a population (e.g., Eisen et al., 2022a; Gfrerer et al., 2023; Powers et al., 2022) and between tissues of individual flowers (García et al., 2021). Within species, pollinator-mediated selection may diverge scent compositions among populations. For instance, considerable intraspecific variation in floral scent composition was found among populations within the *Ophrys sphegodes* and *O. arachnitiformis* species complexes (Orchidaceae) (Mant et al., 2005). Coupled with pollinator behavioral experiments and molecular analysis, researchers were able to determine that the most likely explanation for the intraspecific variation in floral scent was pollinator-mediated selection (Mant et al., 2005). Similar patterns have been observed also in more generalized systems. In *Gymnadenia odoratissima* (Orchidaceae), researchers found divergent selection on floral scent compounds across populations growing on different altitudes with different pollinator communities (Gross et al., 2016). The accumulated evidence points in the direction of pollinators having substantial effect on floral scent phenotypes in many specialized and generalized systems, to the extent that intraspecific scent variation may be a locally adapted phenotype.

As previously mentioned, floral scent emissions may vary also within individual flowers, as different floral tissues emit different VOCs. Traditionally, petals have been considered as the main source of scent emissions in the flower (García et al., 2021), but also other floral tissues such as reproductive organs may contribute significantly to floral scent variation (García et al., 2021). One hypothesis explaining among-tissue variation is that scent functions as both long-distance and short-distance attraction (Raguso and Willis, 2002), where fine-scale tissue specific scent emissions may aid pollinators in landing, to discriminate among flowers and even to locate rewards (García et al., 2021 and references therein). Another hypothesis relates to differential functions of floral tissues, where petals are responsible for pollinator attraction and therefore emit VOCs that appeal to pollinators, while compounds associated with defense are emitted from reproductive organs as protection against florivores (García et al., 2021 and references therein).

In chapters **II** and **IV**, I explore pollinator communities and floral scent variation in *A. alpina* to understand at what spatial scales we can observe floral scent variation and if this relates to variation in pollinator communities. Further, in chapter **II**, I dissect the floral scent composition in floral tissues and examine the spatial separation of floral scent emissions to understand the physiology underlying the observed intraspecific variation.

Temporal variation

Quantitative and qualitative variation may occur on temporal scales, for example diurnally or throughout plant ontogeny. Plants should emit more scent while their main pollinators are active to maximize pollinator attraction (Raguso, 2008b). This theory is based on the assumption that emission of floral scent carries a metabolic and/or ecologic cost, but the evidence for this is inconclusive (see 'Conflicting selection pressures and trade-offs'). What the accumulated evidence shows is that floral scent can decrease and increase temporally, with strong correlation to pollinator activity, in many species (e.g., Chapurlat et al., 2018; Friberg et al., 2014; Hoballah et al., 2005; Raguso et al., 2003).

Ontogenetic changes in floral scent emission and/or composition can signal flower maturity, as has been observed in *Cirsium arvense* (Asteraceae) where young and mature flowers emitted substantially more scent compared with buds or older flowers (Theis and Adler, 2012). In other species, scent varies across different hours of the day, with the highest emission matching peak pollinator activity. In fact, temporal variation can be found at even smaller scales, such as up- or downregulation of individual compounds or compound groups. In *Petunia axillaris*, emissions of benzenoid compounds (benzaldehyde, benzyl alcohol and methyl benzoate) were highly correlated with the activity patterns of its main pollinator *Manduca sexta* (Sphingidae) (Hoballah et al., 2005). However, not all species have scent phenotypes that correlate to pollinator activity (e.g., Waelti et al., 2008). Hence, ecological and evolutionary research on floral scent suggests that variations in scent emission across space and time tend to be species-specific and/or influenced by ecological context.

In chapter **II**, I explore temporal variation in floral scent emission and composition in *A. alpina* in relation to pollinator activity. Finding at what scales floral scent varies in a more generalized system will help us understand the role of floral scent in plant-insect interactions and at what levels we may expect floral scent to operate as a mediator.

Pollinator preferences and floral scent diversity

Many pollinators actively use olfactory cues alone or in combination with other stimuli when foraging (Raguso, 2008b; Raguso and Willis, 2002). Although floral scent bouquets can consist of many hundreds of VOCs, not all compounds elicit responses in all pollinating species (Dötterl and Gershenzon, 2023a). Variation in pollinator preferences among populations creates a mosaic of strength and direction of pollinator-mediated selection on floral scent across geographic landscapes, as described in the previous section on spatial variation.

Floral scent preferences may be innate or acquired through olfactory learning. For instance, sphingid moths frequently visit flowers that emit sesquiterpenes (Knudsen and Tollsten, 1993), while bees show strong innate preferences for specific scent compounds such as limonene, benzyl alcohol, linalool, and indole (Dötterl and Gershenzon, 2023a; Rachersberger et al., 2019). Additionally, both bees and bumblebees can rapidly associate different scent compounds, originating from various biosynthetic pathways, with food rewards (Dötterl and Gershenzon, 2023a; Wright and Schiestl, 2009). Thus, for a generalist plant where many different pollinating species contribute to fitness, it may be advantageous to emit a wide range of floral scent compounds to attract as many of these species as possible (Dötterl and Gershenzon, 2023a). This theory aligns closely with the interaction diversity hypothesis, which proposes that plants interacting with insects from various mutualistic and/or antagonistic taxa will produce and emit compounds from multiple, increasingly diverse biosynthetic pathways (Whitehead et al., 2021).

Measuring floral scent diversity is not straightforward. Traditional diversity indices such as Simpson or Shannon are not suitable for phytochemical diversity, as they do not account for structural diversity. Consider two conspecific plant populations that each emit five compounds in equal proportions. Using traditional measures, both populations would have similar diversity index scores. However, in one population the five compounds originate from two different biosynthetic pathways, while in the other population all compounds are from the same pathway. In that case, one can argue that the former population is structurally more diverse compared to the latter population. As insects are able to discriminate between individual VOCs and even between VOCs originating from the same pathway (e.g., Andersson and Dobson, 2003; Carvalho et al., 2014; Raguso and Light, 1998), structural diversity can have great impact on the number of insects that are attracted to the floral scent. Recently, a comprehensive method for incorporating chemical complexity and shared substructures was released in the form of a novel statistical approach (available as a package in the statistical software R) that allows scientists to readily integrate compound diversity in their research (Junker, 2018; Petrén et al., 2023, see chapter **IV** Methods). Floral scent diversity may be indirectly affected by the surrounding plant community. In natural populations, individual plant species are embedded within broader networks of co-flowering plants that either compete for pollinators or facilitate pollination (Bronstein et al., 2006). As co-flowering community diversity increases, it can lead to greater pollinator diversity (Bronstein et al., 2006), which, in line with the interaction diversity hypothesis, may drive increased phytochemical diversity in individual generalist species (Whitehead et al., 2021).

The ability to measure floral scent diversity significantly advances the field of pollination ecology, especially considering this part of the floral scent phenotype has been comparatively understudied in relation to other aspects (Petrén et al., 2023a). In fact, no studies to date have tested the relationship between phytochemistry diversity and pollinator community diversity in the context of floral scent, but mainly focused on plant-herbivore interactions (e.g., Richards et al., 2015; Salazar et al., 2016).

As previously mentioned, understanding the chemical preferences of individual pollinators is an important facet when studying how floral scent phenotypes diverge across populations. Through for instance Y-tube behavioral assays and electroantennogram responses it has been possible to identify active key compounds and host discrimination among pollinators (e.g., Friberg et al., 2014; Raguso and Light, 1998). These trials are informative when identifying the responses of single compounds or compound blends on individual pollinators but will not tell us how the wide pollinator community responds to, for instance, conspecific host plants of diverging scent compositions. If the floral scent phenotype is locally adapted, through adaptive generalization or adaptive wandering, we expect the pollinator community to prefer the local floral scent, which can be tested in translocation experiments (Armbruster et al., 1992).

In chapter **IV**, I address the relationships between floral scent diversity, pollinator community diversity and co-flowering diversity in natural *A. alpina* populations. Additionally, by conducting a translocation experiment, I explore the scent preferences of individual pollinator species. Together, these experiments aim to in detail investigate how individual pollinator preferences may drive floral scent diversity within a community, as well as diversification across the larger geographic distribution.

Methodology

All experiments presented in this thesis were collaborative in nature with many people involved in the different stages of data collection, analyses and writing. However, for the purpose of this section of my thesis I will continue to present Methodology, Results and Discussion in first-person singular.

Study species

The small-statured, white-flowered perennial herb *Arabis alpina* L. (Brassicaceae), or alpine rock-cress (Figure 1), originated in Asia Minor, and spread to Central and Northern European mountains through a single migration event, while populations occurring in East and North African populations spread via two separate events from Asia Minor (Koch et al., 2006). *Arabis alpina* thrives in high altitude, shaded, moist and rocky habitats with calcareous bedrock (Mossberg and Stenberg, 2010).

Figure 1. An *Arabis alpina* (alpine rock-cress) individual growing on a moss-covered, vertical rock. This picture was taken by H. Thosteman in the It16 population (Capistrello) in April 2023. The dried scapes and fruits of the previous year are visible hanging to the right, next to the current season's flowering parts.

In the last decade, this diploid plant has become a model species for evolutionary ecology and genomic research, because of its relatively short generation time and ease of cultivation, as well as the possibility to perform observational and experimental studies in natural populations (Wötzel et al., 2022).

Arabis alpina displays a substantial amount of intraspecific variation in several traits. Firstly, European populations differ in outcrossing rates. Scandinavian populations, with individuals that are self-incompatible, represent one extreme on the continuous scale (Laenen et al., 2018; Toräng et al., 2017). Populations in Spain, France, and Switzerland are outcrossing but possess the ability to self – with the help of insect vectors (Buehler et al., 2012; Laenen et al., 2018; Toräng et al., 2017). In Italy and Greece outcrossing rates are high, self-incompatibility dominates, and populations are almost exclusively reliant on pollinators for reproduction (Laenen et al., 2018). Secondly, floral size and anther orientation vary among populations: self-compatible individuals tend to have smaller flowers and anthers angled towards the stigma, whereas self-incompatible populations have larger flowers and anthers oriented outwards (Petrén et al., 2021; Toräng et al., 2017). Thirdly, floral scent emission and glucosinolate composition vary among populations (Buckley et al., 2019; Petrén et al., 2021). Here, self-incompatible populations emit greater amounts of scent which is often dominated by the compound benzaldehyde (Petrén et al., 2021). In contrast, self-compatible populations, emit little or no scent, with a profile that is often dominated by a mix of compounds (Petrén et al., 2021). Self-incompatible populations located at close geographic distance may also have differing floral scent profiles (Petrén et al., 2021). For glucosinolates, a recent study revealed prominent compositional variation along an elevational gradient among Swiss populations, potentially related to herbivore defense (Buckley et al., 2019).

In this thesis, a total of 29 populations were used in all experiments combined (Table 1). These populations span the entire mating system range, from autonomously selfing Swedish individuals, to completely outcrossing plants from Greece and Italy.

Table 1. List of *Arabis alpina* populations used in this thesis. Population indicates short form used in the chapters. Location name indicates local name of location. Region refers to country where the population was located. Coordinates are provided as accurate as possible in degrees, minutes, seconds. Altitude indicates meters above sea level (m asl). Mating system is either self-compatible (SC), SC-mixed (self-compatible with modest outcrossing rate), or self-incompatible (SI). Used in chapter refers to in which chapters the population is studied.

POPULATION	LOCATION NAME	REGION	LAT, LONG	ALTITUDE (M ASL)	MATING SYSTEM	USED IN CHAPTER
Aal 04	Küttigen	Switzerland	47°25'48.4"N; 8°01'27.8"E	816	SC-mixed	I
Aal 12	Montagne-de-Courtelary	Switzerland	47°10'34.3"N; 7°07'30.0"E	1050	SC-mixed	I
Aal 29	Piz Albana	Switzerland	46°29'07.6"N; 9°46'22.0"E	2850	SC-mixed	I
Aal 34c	Obersee	Switzerland	47°05'04.9"N; 9°01'00.4"E	988	SC-mixed	I
Aal 36	Hinterrugg	Switzerland	47°09'15.7"N; 9°18'24.3"E	2288	SC-mixed	I
Aal DMB	Col des Martinets	Switzerland	46°12'37.2"N; 7°04'14.7"E	2618	SC-mixed	I
Aal FC	Fild de Cassons	Switzerland	46°52'49.3"N; 9°15'52.0"E	2659	SC-mixed	I
Aal PB	Pilatus/Blumfenpad	Switzerland	46°58'34.8"N; 8°15'11.3"E	1879	SC-mixed	I
Aal SFH	Schrattenflu	Switzerland	46°50'28.0"N; 7°58'46.2"E	1735	SC-mixed	I
E3	Grandiella	Spain	43°14′N; 5°56′W	1171	SC-mixed	I
Fr1	Lautaret	France	45°03′N; 6°24′E	2196	SC-mixed	I
Fr2	Les Tronchets	France	44°57′N; 6°36′E	1983	SC-mixed	I
Fr3	Sain-Dalmas de Tende	France	44°03′N; 7°30′E	2227	SC-mixed	I
Fr4	Fontanalba	France	44°06′N; 7°31′E	1787	SC-mixed	I
G4	Raftanei	Greece	39°30'54"N; 21°01'59"E	1176	SI	I
IT1	Pietracamela	Italy	42°31'23.8"N; 13°33'15.5"E	1005	SI	III, IV
IT2	Prati di Tivo	Italy	42°30'24.8"N; 13°34'03.7"E	1635	SI	I
IT4	Tornimparte	Italy	42°15'7.93"N; 3°19'8.54"E	900	SI	I, II, III, IV
IT5	Trasacco	Italy	41°57'35.2"N; 13°33'00.9"E	656	SI	I, III, IV
IT6	Scanno	Italy	41°50′N; 13°56′E	1542	SI	I
IT8	Gorfigliano	Italy	44°08′N; 10°15′E	850	SI	I
IT9	Porreta	Italy	44°05′N; 10°19′E	1002	SI	I
IT10	La Chiesola	Italy	42°14'18.20"N; 13°21'58.09"E	1710	SI	I, II, III, IV
IT13	Gole di Celano	Italy	42°05'09.8"N;13°34'03.9"E	980	SI	I
IT15	Rocca di Mezzo	Italy	42°12'43"N; 13°31'26"E	1285	SI	I, III, IV
IT16	Capistrello	Italy	41°56'31"N; 13°21'43"E	1547	SI	I, III, IV
IT17	Cerri	Italy	42°15'14"N; 13°29'24"E	1410	SI	I
IT18	Monte Sant'Angelo	Italy	41°42'29"N; 15°57'11"E	813	SI	I, III, IV
S1	Abisko	Sweden	68°24′N; 18°19′E	769	SC	I

Plant cultivation (I, II, IV)

The seeds used in **chapters I, II** and **IV** were field collected, and to maximize the genetic variation among plants, I grew seeds from a minimum of 5 (range 5-15) seed families per population for the different experiments. I sowed seeds into wells containing a mix of 2/3 moist potting soil ("YrkesPlantjord" Weibulls Horto AB, Sweden) and 1/3 of 6-8mm clay pearls (LECA, Saint-Gobain Byggprodukter AB, Sweden) with a thin top layer of wet topsoil ("Plugg och Såjord", Weibulls Horto AB, Sweden). In each well, I evenly spaced five seeds from the same seed family and subsequently put them in a dark stratification chamber maintaining 4-6°C for 1 week. Then, I moved the seeds to a germination chamber maintaining summer conditions of 18°C 165 µmol m^{-2} s^{-1} days and 16°C nights. As seedlings started to emerge, I transplanted individual plants into 7×7×7cm pots containing the same soil mixture as described above and kept them in summer conditions for vegetational growth.

To induce flowering, I moved the plants to a vernalization chamber maintaining 7-8°C, 25–30 µmol m^{-2} s^{-1} days and 4-6°C nights. After 10-14 weeks I again moved to a chamber maintaining summer conditions. The first flowers opened after 10-15 days. Individuals from the It4 population did not flower as expected during the first season (i.e., too few flowers per plant and too few flowering plants for adequate sample size). Therefore, I repotted these individuals into marginally larger pots (10 × 10 × 7 cm) and allowed them a second flowering season after I had moved them into vernalization a second time. After 10-15 weeks, or 30 weeks for **chapter II**, I moved the It4 individuals to summer conditions for another flowering season.

Occasionally, during non-experimental periods, the plants were treated with pesticides to kill aphids. Pest (aphid) control was performed by a professional by spraying 14 mg/m^2 of the pesticide Teppeki ® (NordiskAlkali, Malmö, Sweden) over the entire plant common garden.

Sampling of phytochemicals (I, II, IV)

Floral volatiles (I, II, IV)

All floral volatile collections followed the dynamic headspace method outlined in Raguso & Pellmyr (1998) and adapted for *A. alpina* by Petrén et al. (2021). This method allows for simultaneous quantitative and qualitative analysis of the floral scent bouquet and eventually results in emission rates (ng/flower/hour) of individual compounds as well as total scent emission.

To collect scent volatiles, I enclosed intact inflorescences with known number of open flowers in a thin plastic bag. I inserted a Teflon tube scent trap connected to a tube leading to an air pump via a flowmeter into the plastic bag (Figure 2). Air was then pulled through the trap at a rate of 200ml/min for 3 consecutive hours. To not create a vacuum, I cut a small hole in one top corner of the bag. For each collection bout I added an ambient control sample by collecting air from an empty plastic bag.

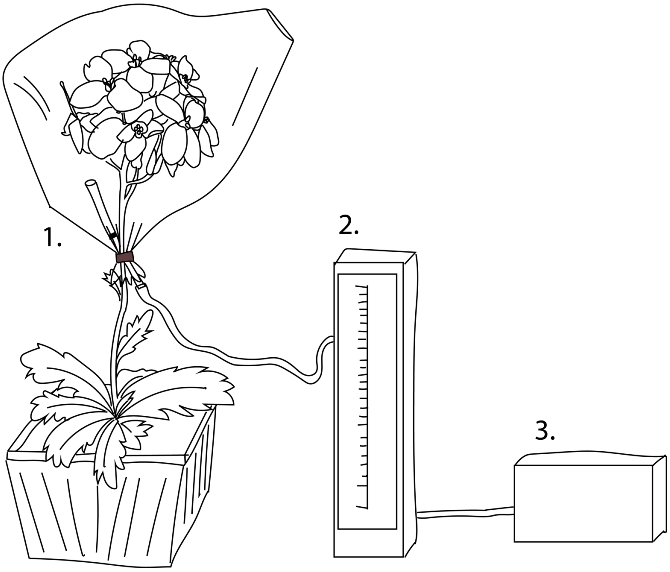

Figure 2. Illustration of floral scent collection set up. 1) Teflon tube containing an absorbent filter inserted into plastic bag. 2) Flow meter pulling air at a rate of 200ml/min. 3) Air pump responsible for air flow through the system. Illustration by H.E. Thosteman.

Immediately after scent collection, I transported the traps to the lab where I eluted them in 300µl of hexane into glass vials. The vials were stored in a -20°C freezer until further analyses. To prepare for analysis, I concentrated each sample to 50µl by gently evaporating hexane under a moderate flow of nitrogen gas. Then, I added 5µl of a 0.03% solution of toluene in hexane to each sample as internal standard.

I used gas chromatography/mass spectrometry (GC/MS) for compound annotation and quantification (abundance relative to internal standard). The gas chromatograph heats the sample, separating the molecules in the solution. The different molecules in the mixture then travel along a column towards a detector that identifies at what time compounds hit the detector. Lighter substances travel faster along the heated column and register at the detector earlier than heavier substances. The mass spectrometer, which is coupled to the GC, then bombards the incoming substances with electrons, breaking the molecules into ions. The ions are then sorted into their mass-to-charge ratio and identified according to their mass spectra. The result is a chromatogram displaying a time sequence where peaks indicate the location and identity of the compounds present in the sample. The area under each peak represents the relative abundance of the compound.

I identified compounds manually in a software called Xcalibur Qual Browser v.1.4 (Thermo Electron Corp., 19982003, Waltham, MA, USA) (**I**) or Chemstation (v. D 00.01.27, Agilent Technologies, Santa Clara, CA, USA) (**II, IV**) by comparing the chromatogram results to NIST library suggestions (NIST MS Search 2.0, 2008), Kovats retention indices in the literature, occasionally to synthetic standards and to previous findings in *A. alpina* (Petrén et al., 2021). I then converted the amount of each compound into ng/flower/hour, adjusted sample content according to the internal standard, and filtered compound content and emission rate by comparing samples with corresponding ambient control samples (*cf.* Eisen et al., 2022).

Solid Phase Micro Extraction (SPME) (II)

In **chapter II,** I investigated tissue specific scent emission of flowers using Solid Phase Micro Extraction (SPME). This method is a powerful tool for capturing presence or absence and relative abundance of compounds in a sample at a snapshot in time (Barták et al., 2003). While dynamic headspace sampling can accurately capture scent emission rates, it requires a more complex set up, solvents and standards. SPME is simpler and more versatile, allowing for precise scent collection from even very small objects. Here, a superabsorbent fiber or filament is inserted into a container with the sample, and compounds filling the airspace around the sample will reach the SPME through passive diffusion (Barták et al., 2003).

I collected floral scent emitted from petals, reproductive organs, sepals as well as whole flowers using SPME. I put the plant tissues in clean 2.5 ml glass containers and the sealed the opening with a thin plastic bag and left them to equilibrate for 1 hour. Then, I inserted the SPME fiber through the bag into the container without touching the sample. The collection period lasted for 30 minutes, after which I carefully retracted the fiber from the container and brought it to the GC/MS for analysis. On two occasions, I stored samples in 6-8°C for 24-48 hours before analysis. The GC/MS protocol, and identification and quantification of chromatogram peaks were identical as for samples collected using dynamic headspace.

Foliar volatiles (I)

I collected foliar volatiles using the dynamic headspace method, following an identical protocol to the method used for floral volatiles. Here, I enclosed intact leaves still attached to the plant in the plastic bag from which the air was pulled through the same system as for floral scent. I always captured foliar volatiles after floral scent collection.

The annotation and quantification of individual compounds followed the same protocol as for floral scent. I standardized samples by leaf tissue dry weight, resulting in a final unit of volatile emission of ng/gram/hour.

Glucosinolates (I)

To extract glucosinolates, I collected fresh leaf tissue and immediately flash froze it before they were freeze-dried in preparation for analysis. I analyzed the samples using High-Performance Liquid-Chromatography (HPLC) following the protocol outlined in Grosser and Van Dam, (2017). HPLC is an effective method for detecting concentrations of compounds in a solution. As with GCMS, the solution is transported through a column where molecules in the sample move faster or slower depending on how easily they bind to the column, thus separating the different compounds in the solution. A detector then identifies the compounds using mass spectrometry and UV-reflection. Annotation and quantification were performed automatically by the MassHunter qualitative software, and I then converted the final amounts of glucosinolates into µlmol g^{-1} dry tissue weight.

Integration of phytochemicals (I)

The aim of this chapter was to explore correlations between, or levels of phenotypic integration among, floral scent, foliar defense volatiles, and glucosinolates. In this experiment, I sampled 28 populations of *A. alpina* plants cultivated in greenhouse conditions and used dynamic headspace to collect floral scent from 340 individuals, foliar volatiles from 115 individuals and HPLC to collect glucosinolates from 159 individuals. I calculated an integration index (degree of correlation between compounds corrected for sample size and total number of compounds emitted by the population or species) between compound groups and for the three groups separately. To understand if any of the three compound groups showed a higher or lower degree of among-compound integration, I compared integration indices between the groups using a Kruskal-Wallis test. Then, I compared level of integration among floral volatiles in self-incompatible populations with self-compatible populations using a Wilcoxon signed-rank test, to investigate if self-incompatible populations were less integrated. This test was also coupled with a comparison of strength of correlation between compounds, where I tested the correlation coefficients *r* in self-compatible and self-incompatible populations using a Mann-Whitney U test. For self-incompatible and self-compatible populations, I also compared level of inter-group (e.g., floral volatile × foliar volatile) integration using bootstrapped data where I calculated the probability of the observed difference between integration indices in a two-tailed t-test. Lastly, it was important to understand if the level of inter-group integration was greater or smaller than intra-group integration to explore how free the three groups of phytochemicals were to evolve independently of one another. Because of the data structure (see **chapter I** for details), I compared correlation coefficients *r* instead of integration indices using a Kruskal Wallis test followed by Dunn's test to identify differences between groups.

To visualize the correlation networks, I used a software called Cytoscape, where nodes corresponded to individual compounds and edges reflected the strength of correlation between two or more compounds. The networks made it possible to identify both the strength and direction (positive or negative) of the correlations.

Synchronization of scent emission and insect activity (II)

This chapter covered several greenhouse and field experiments aimed at exploring the temporal and spatial scales at which scent varies in relation to insect activity. I sampled floral scent across 24 hours (dawn, day, dusk and night) from two closely related Italian populations (cultivated under greenhouse conditions) using dynamic headspace sampling and controlling for light and temperature. From the same populations, I also sampled tissue specific scent emissions using SPME. I then visited the two populations in the field, where I performed day-time insect surveys to quantify insect communities, and pollinator exclusion experiments to compare seed set between plants accessible to only diurnal or only nocturnal pollinators. I compared floral scent emission rates between the four different times of day using a linear mixed model with scent emission as response variable, time of day (dawn, day dusk or night) as fixed factor, and plant individual nested within start group as random variables. Start group indicated what time of day a cycle was initiated for a specific group of plants, to correct for any exhaustion in scent emission after several rounds of collection. I tested differences in compound compositions between the time periods and between floral tissues in a PERMANOVA and visualized in Non-Metric Multidimensional Scaling (NMDS) plots. I identified differences in seed set from the pollinator exclusion experiment using a Kruskal-Wallis test followed by Dunn's test, and to understand how insect communities differed between the two populations I again used a PERMANOVA followed by an NMDS plot.

Pollinator effectiveness and trait matching (III)

Arabis alpina is a generalist herb and visited by insect species belonging to several orders. However, because insect species differ in the amount of compatible pollen they transfer and deposit (i.e. effectiveness) and how often they visit, insects may not be of equal importance for the pollination success of the plant. Additionally, effectiveness can be linked to the degree of trait-matching between the insect and the flower. Thus, this chapter was aimed at investigating pollinator effectiveness and its correlation with degree of trait-matching between insects and flowers.

I collected SV data from three populations, quantified the pollinator communities in an additional four populations and measured insect and flower morphology in six populations of Italian *A. alpina*.

Following Gómez et al., (2022), I estimated visitor effectiveness using two components: the quantity component (QTC) and the quality component (QLC). The QTC represents the total number of pollen grains deposited by a species or functional group on a single visit multiplied by its visitation rate. The QLC represents the probability that one of the deposited pollen grains fertilizes an ovule. I obtained QTC and QLC values for five functional groups in the three populations, and extrapolated the QTC and QLC data from the functional groups present in the SV dataset to insects observed in the insect surveys that belonged to the same functional groups. I calculated the relative pollinator effectiveness by combining the QTC and QLC data in a regression analysis using the effectiveness_plot() function in the effect.lndscp package in R (Jordano, 2019).

Because of unbalanced datasets, trait matching between insect tongue lengths and flower corolla depth was measured using resampled data. Here, I created a match ratio describing the relationship between the two morphological structures, where I could distinguish between tongues being longer or shorter than the corolla or having perfect match.

To assess whether functional groups with higher pollination effectiveness were better matched to flower morphology, I calculated mean relative effectiveness for each group by multiplying QTC and QLC values, then log-transformed the result. Then, I calculated the mean match ratio for each group and tested the correlation between effectiveness and match ratio using the cor.test function in R. Due to data limitations, only three functional groups were included, as both metrics had to come from the same population to ensure accuracy.

Finally, to understand the biological relevance of the effectiveness for each SV-scored insect, I tested how many ovules a single functional group would be able to fertilize during the receptive stage of a single flower, if they were the sole pollinator. I did this by estimating the opening time for a single flower to 32 hours and calculated the percentage of ovules fertilized within this time span. I estimated the number of available ovules to 21, based on previous results in closely related Italian populations (Petrén et al., 2023b).

Floral scent and insect community diversity (IV)

In my final chapter, I returned to the relationship between floral scent and insect communities in a combined greenhouse and field study involving seven Italian populations. Here, my aim was to link intraspecific variation in floral scent to insect community variation, and to further investigate if a high floral scent compound diversity was related to a high insect community diversity according to the interaction diversity hypothesis. Additionally, I was interested in exploring the effect of co-flowering community on *A. alpina* floral scent diversity. To discover any indications of insects preferring local over foreign floral scent compositions, or *vice versa*, I also performed translocation experiments between populations with diverging scent profiles.

I performed floral scent collections in greenhouse conditions using dynamic headspace. I compared floral scent compound compositions among populations using a PERMANOVA and subsequent NMDS plot. I calculated floral scent diversity using the chemodiv R package (Petrén et al., 2023a), which uses traditional diversity indices but also accounts for compound dissimilarities caused by differential molecular structures.

I estimated insect community diversity from insect community surveys conducted in the natural populations. Here, I calculated Shannon diversity indices and compared community composition between populations using a PERMANOVA and subsequent NMDS plot.

I surveyed co-flowering communities using timed transects across the entire *A. alpina* flowering season. I determined a plant species to be co-flowering if it occurred in the *A. alpina* population and had at least one open flower at the same time as *A. alpina*. I compared co-flowering community compositions using a PERMANOVA and subsequent NMDS plot and calculated co-flowering community diversity using the Shannon diversity index.

To understand how floral scent diversity was related to insect community diversity or co-flowering community diversity, I performed two separate Pearson correlations where I tested co-flowering community diversity (Shannon) or insect community diversity (Shannon) against floral scent diversity.

I was also interested in exploring the effect of geographic distance on insect communities and floral scent divergence between populations. Therefore, I created two dissimilarity matrices for insect community and floral scent that were incorporated into two Mantel tests where the two matrices were tested against geographic distance.

The translocation experiments included transporting live flower scapes between populations with divergent scent profiles and observing insect visitors to local and foreign flowers. I compared the results in several generalized mixed models using Template Model Builder with a negative binomial distribution (glmmTMB function in glmmTMB package, Brooks et al., 2017). Here, I compared models with presence/absence data as response variable and various combinations of flower origin (local or foreign), effort (date surveyed) and insect functional group or taxa as random or fixed factors.

Results and discussion

Integration of phytochemicals (I)

This chapter was aimed at discovering patterns of phenotypic integration among attraction and defense plant chemistry potentially constraining evolution. I found that, in general, floral scent volatiles, foliar volatiles and glucosinolates were largely uncorrelated, and experienced higher intra-group correlation compared to inter-group correlation (Figure 3).

The low level of inter-group correlation was reflected in both integration indices and correlation coefficients and indicated that all three compound groups are to a great extent free to evolve independently in response to selection. For research focusing on floral scent as target of pollinator mediated selection, these results are encouraging as few previous studies have examined the potential for floral scent evolution being constrained by correlational selection on biosynthetically related compounds (but see Ramos and Schiestl, 2020).

The general lack of phenotypic integration between floral and foliar volatiles sharing biosynthetic pathways was an intriguing result. One potential explanation for this pattern is the Berg hypothesis which suggests an evolutionary decoupling of floral traits from vegetative parts of the plant, leading to a more conserved flower morphology ensuring pollinator fit (Armbruster et al., 1999). It is tempting to use similar reasoning for the lower levels of phenotypic integration among floral and foliar volatiles detected in this study if this phenomenon extends to phytochemistry.

Although the overall phenotypic integration between compounds with different functional properties was low, there were a few instances of single floral volatiles being positively correlated with foliar volatiles, and in some cases also with glucosinolates. Further studies are needed to understand and disentangle the functional relationship between these compounds, because not all of them shared biosynthetic background. The few cases where foliar volatiles were positively correlated with glucosinolates may be explained by a biosynthetic linkage, since the volatile isothiocyanates are derived from glucosinolates (Fahey et al., 2001), and could signal the defense status of a plant.

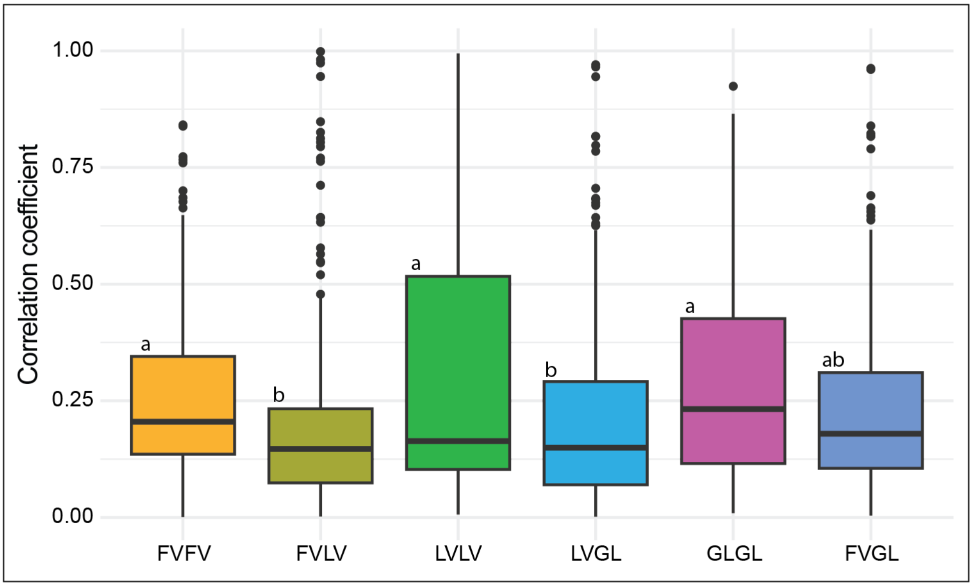

Figure 3. Comparison of correlation coefficient (r) values between types of compound interactions in *Arabis alpina* plants. FV, floral volatile; GL, glucosinolate; LV, leaf (foliar) volatile. The correlation coefficient (interaction) between two compound groups is denoted for example FVFV (floral volatile interacting with floral volatile) or FVLV (floral volatile interacting with leaf (foliar) volatile). Letters above boxes indicate significant differences between groups. All correlation coefficients are based on Pearson's correlations (r) with threshold = 0. Boxplot showing the distribution of the data. The central box represents the interquartile range (IQR), with the bottom and top edges indicating the first quartile (Q1) and third quartile (Q3), respectively. The line inside the box represents the median. The whiskers extend to the smallest and largest values within 1.5 times the IQR from Q1 and Q3. Data points outside this range are considered outliers and are plotted individually.

In this chapter, I sampled populations with different outcrossing rates, ranging from self-compatible/selfing to self-incompatible/outcrossing to understand how mating system may affect the phenotypic integration of plant chemistry. Contrary to my predictions, I found no evidence of reduced integration within attraction compounds or between attraction and defense compounds in self-incompatible populations. First, I found similar levels of intra-group integration among floral compounds in self-compatible and self-incompatible populations. Yet, floral compounds emitted from self-incompatible individuals had significantly stronger correlations suggesting that they experience more interdependence than those emitted by self-compatible plants. Self-incompatible scent profiles being more strongly intercorrelated may relate to variation in pollinator dependence among populations with different mating systems, because self-incompatible populations may experience stronger selection on certain floral compounds that potentially affect pollinator attraction, leading to a more frequent co-occurrence (Knudsen et al., 2004; Knudsen and Tollsten, 1993). Second, floral and foliar volatiles showed similar levels of integration in self-compatible and self-incompatible individuals. *Arabis alpina* displays a wide range of outcrossing rates across its geographic range: from autonomously selfing Scandinavian populations to completely self-incompatible populations in the Mediterranean. In the present study, self-incompatible populations were compared with outcrossing populations that have evolved self-compatibility. Therefore, differences in integration due to decoupling of floral scent and foliar defense compounds may not be as pronounced in this comparison, as the self-compatible populations used in this study may still experience selection on floral scent compounds as a distinct group rather than together with foliar defense compounds (Ramos and Schiestl, 2020).

In this chapter, I found no evidence of integration between floral scent—key for pollinator attraction—and biosynthetically related foliar defense compounds, indicating that floral scent can evolve independently in response to e.g. pollinator-mediated selection. Additionally, there were no strong negative correlations between compounds, suggesting that trade-offs do not constrain phytochemical expression. This study supports the rarely tested hypothesis that phytochemical diversification within species is driven by independent selection forces.

Synchronization of scent emission and insect activity (II)

In this chapter, I focused on the diel rhythms of scent emission and pollinator activity in *A. alpina*. I quantified the insect communities and scent compositions of both populations as well as scent emissions from specific floral tissues in order to explore the scales at which we can detect floral scent variation, and how it relates to insect assemblages.

From the pollinator exclusion experiments I could determine that both populations were predominantly pollinated during daytime (Figure 4a). In both populations, plants available to pollinators during nighttime also produced seeds, a pattern that was more pronounced in It10 than in It4. It is possible that crepuscular or nocturnal pollinators were responsible for pollinating these flowers, as other members of the Brassicaceae family, such as *Hesperis matronalis*, are known to be visited by pollinators at all hours of the day (Majetic et al., 2009; Mitchell and Ankeny, 2001). However, in my experiment, when arriving at the field site, I noticed on several occasions how day-flying insects visited the plants early in the mornings, potentially providing sufficient pollination to result in seed production also in the night-open treatment. To fully exclude crepuscular and nocturnal pollinators in *A. alpina*, future experiments need to include pollinator observations also during these hours.

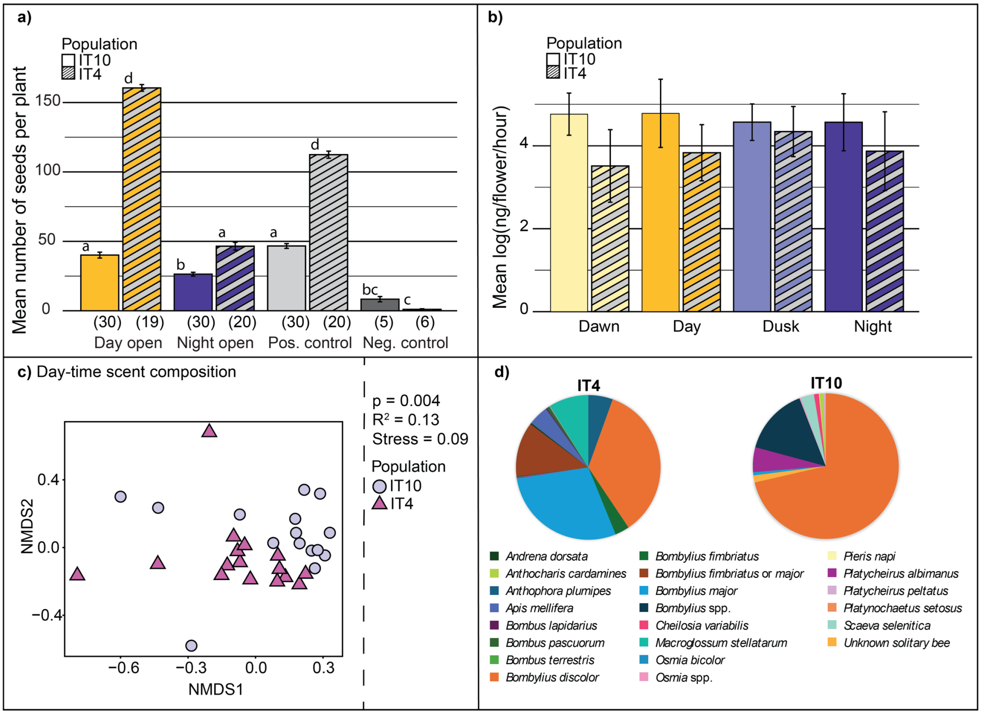

Figure 4. Seed set, diel scent emissions, scent composition and pollinator assemblages in the two populations of *Arabis alpina*. a) Mean number of seeds produced per plant in the four treatments and two populations. Pos. = Positive, Neg. = Negative. Filled boxes represent It10 and striped boxes represent It4. Error bars indicate +/- SE. Letters denote significant differences. Numbers in brackets below bars indicate sample size. **b)** Mean log(nanograms/flower/hour) floral scent emission in It10 (filled bars) and It4 (striped bars) across the four different times periods. Error bars indicate +/- SE. **c)** Compound composition visualized in an NMDS plot of floral scents emitted from both populations during daytime. **d)** Mean insect communities (mean visits/flower/hour) in the two populations in 2022.

Although being predominantly daytime pollinated, floral scent emissions did not follow the expected pattern of up- and down-regulation in relation to pollinator activity (Figure 4b). Under controlled conditions, *A. alpina* individuals emit similar amounts of scent of similar compositions across all sampling periods, irrespective of changes in light or temperature conditions. There are three possible explanations for the observed pattern: first, floral scent emission may instead be regulated through plant ontogeny, where scent emissions peak when the individual flower is most receptive (*cf.* Theis et al., 2007). Ontogenetic regulation of both scent emission and composition may be ecologically advantageous if florivory and herbivory are present on immature buds and developing fruits, as reduced scent emissions or increased emissions of defense compounds may deter enemies (Theis et al., 2007). Although I did not observe any significant damages caused by florivores or herbivores in any of our study populations, there are indications that herbivores do infest *A. alpina* plants at other sites (Buckley et al., 2019). Ontogenetic regulation of floral scent may also explain the level of intra-population variation I consistently find across populations. If, by chance, plants with flowers of different ages are sampled and compared, they will appear to diverge in composition in relation to their population neighbors. However, ontogenetic changes in floral scent are unlikely in *A. alpina* considering the stability of population-level variation. Second, floral scent emission may not carry an ecologic or metabolic cost in this system, as previous results on scent emission and compositional changes in relation to water and nutrient availability have indicated (Luizzi et al., 2021). As previously stated, low levels of florivory and herbivory were observed in the two focal populations, as well as in other Italian populations. Thus, there may be no need to adjust scent emissions to avoid attracting enemies. Related to this theory, is the third potential explanation for the stable scent emission. There may be costs associated with the regulation and/or genetic expression of floral scent itself (Kabera et al., 2014; Lang et al., 2009). As floral scent biosynthesis can be related to production of other important parts of the floral phenotype, such as phytohormone regulation, metabolic processes and protection against abiotic and biotic stressors (Kabera et al., 2014), downregulation of floral scent may have negative side-effects.

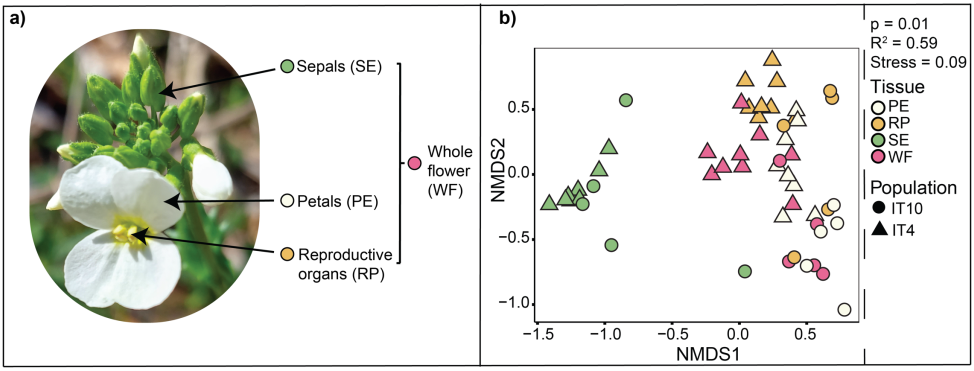

Figure 5. Tissue specific scent emissions from three floral structures: petals (PE), sepals (SE) and reproductive organs (RP), as well as whole flower (WF) sampled from *Arabis alpina*. a) Schematic showing the sampled tissues (note that sepals were sampled from the same open flower for which I sampled petals and reproductive organs). **b)** Non-metric Multidimensional Scaling plot visualizing compound composition of all tissues sampled from the two populations (It4: circles, It10: triangles).

The two populations in this study were located only 4.2 km apart, yet they emitted distinct floral scent compositions (Figure 4c). Detecting divergence in floral scent composition at such small geographic scale is intriguing, as it suggests a potential for divergent forms and/or strengths of selection across neighboring environments (*cf.* Parachnowitsch et al., 2012). When studying the spatial separation of floral scent emission in floral tissues (Figure 5a), I found that reproductive organs and petals were responsible for the observed divergence in floral scent composition between the two populations, emitting various aromatic compounds, while sepals were clearly separated from the other tissues (Figure 5b).

Since petals are part of the pollinator attraction module, and reproductive organs are typically classified as a pollinator fit- or reward-trait (Caruso et al., 2019), it is not surprising that these two tissue types emit aromatic compounds associated with pollinator attraction (García et al., 2021). Pollinator attraction mediated via floral scent likely has a long-distance and a short-distance signaling component (García et al., 2021) and it is therefore plausible that emissions from these tissues attract pollinators at these two scales simultaneously.

Overall, *A. alpina* scent emissions mainly consist of aromatic compounds, and a few of these compounds such as benzaldehyde and phenylacetaldehyde have been shown to be under divergent selection in previous research on this species (Petrén, 2020). Phenylacetaldehyde in particular has been shown to be positively correlated with nectar volume in *A. alpina*, potentially functioning as an honest signal (Eisen et al., 2023). Thus, it was interesting to discover that one of the populations included in this study, It10, emitted more than twice as much of this compound in the daytime scent bouquets compared with It4. This compound is known to attract bumblebees in other Brassicaceae species (e.g., Gervasi and Schiestl, 2017), and many lepidopteran, dipteran and hymenopteran species to a variety of plants (Dötterl and Gershenzon, 2023b; Huber et al., 2005). This result indicates that pollinator-mediated selection may play a part in shaping the evolution of floral scent differentiation in this species.

Indeed, insect communities differed between the two populations (Figure 4d), with the It4 community being mixed compared with the It10 community which was dominated by *Bombylius discolor* visits. Bombyliid flies were the dominant visitors in both populations, but It4 had higher occurrence of other species as well, such as *Macroglossum stellatarum* (hummingbird hawkmoth, Sphingidae), *Apis mellifera* (honeybee, Apidae) and several solitary bee species as well as bumblebees. In It10, syrphid flies were generally more common, and *Anthocharis cardamines* (orange-tip butterfly, Pieridae) was only sighted here. It is tempting to relate the difference in floral scent composition to the variation in insect communities. However, to determine if these two patterns are related, behavioral assays are needed to investigate insect responses to floral scent compounds and bouquets (see chapter **IV** for a deeper discussion).

Pollinator effectiveness and trait matching (III)

Across the seven populations included in the effectiveness trials, I found that long-tongued bees were overall the most effective pollinator where they occurred, mainly driven by the high probability of fertilizing an ovule after a single visit (Figure 6a). In fact, this was the pollinator most important for reproduction in all populations except It5, where macrolepidopterans were of higher importance. The variation in pollinator effectiveness across populations was primarily driven by differences in their relative abundance (visitation rate, Figure 6a), which fluctuated substantially between populations. Conversely, variation in effectiveness between functional groups was largely influenced by their individual efficiency (probability of fertilizing ovules). For instance, long-tongued flies were abundant in almost all populations but were comparatively poor pollinators per visit, while short-tongued bumblebees were relatively rare but efficient on single visits.

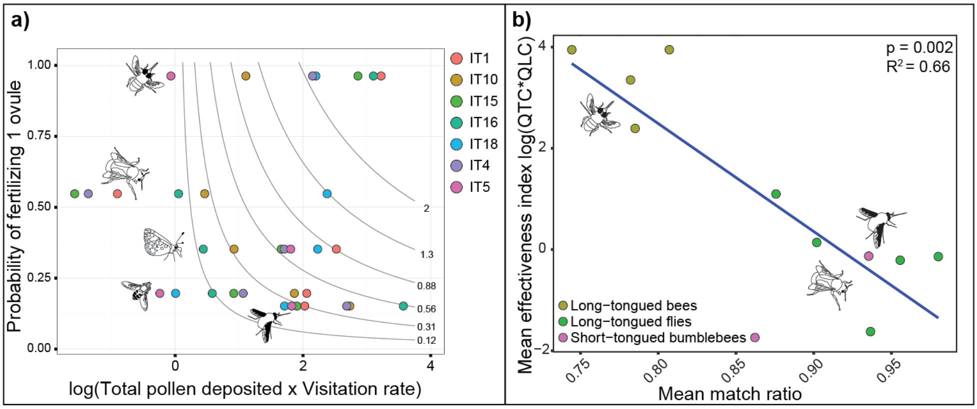

Figure 6. The relative pollinator effectiveness and its relation to plant-pollinator trait-match in Italian *Arabis alpina* populations. **a)** The effectiveness landscape of five functional groups (from the top: long-tongued bees, short-tongued bumblebees, macrolepidopterans, hoverflies and long-tongued flies). Data points represent mean values for specific functional groups in specific populations. Clines indicate mathematical relationship between the x and y axes across different regions of the graphs, and the corresponding isocline values are listed on the right of the clines. The x-axis represents the quantity component (QTC) and is calculated as mean total pollen grains deposited on a single visit, multiplied with the functional group specific visitation rate. The QTC is log-transformed. The y-axis represents the quality component (QLC) and is calculated as the mean number of germinated tubes on a single visit by a specific functional group, divided by 21 which is the mean number of ovules present in an *A. alpina* style. **b)** Correlation between match ratio and effectiveness for three functional groups in six *A. alpina* populations. Mean effectiveness index was calculated as log(quality * quantity), and Mean match ratio was calculated as the mean match for all insects belonging to a specific functional group in each population. The correlation was calculated using the cor.test function in R.

Contrary to my prediction, the pollinators of highest relative effectiveness were not also highly matched with flower morphology. Instead, I found a negative correlation between pollinator effectiveness and match ratio (Figure 6b), indicating that pollinators with high effectiveness generally had shorter tongues relative to the flower corolla depth. Most insects visiting *A. alpina* forage for nectar, pollen, or both (Ollerton et al., 2024). The nectaries are located at the base of the lower sepals, near the pedicel, requiring nectar foragers to insert their tongue through the corolla. Pollinators with shorter tongues make close contact with the anthers near the corolla opening, enhancing pollen removal and deposition. Pollen foragers of various tongue lengths often crawl across the corolla, contacting both anthers and the stigma. Some long-tongued bees and bumblebees also sonicate while foraging, releasing large amounts of pollen. These behaviors, rather than tongue length alone, may better predict pollination success across different functional groups.

Although pollinators varied in effectiveness among functional groups and populations, I found that all functional groups except hoverflies would successfully pollinate at least half of all available ovules within the 32-hour receptive window of the flower, if they were the sole visitors. When many visiting insects have similar impact on the reproductive outcome of the plant, there are multiple vectors of selection acting simultaneously on the floral phenotype (Torres-Vanegas et al., 2024). In the case of *A. alpina*, pollinator abundance varies among populations, driving much of their relative importance. Abundance-driven relative importance was shown also in the generalist herb *Knautia arvensis* (Caprifoliaceae), where the relative abundance of pollinating insects varied substantially among populations and years (Ollerton et al., 2024), generating a potential mosaic of changing selection pressures along both spatial and temporal axes. It is therefore likely that the vectors of selection acting on the *A. alpina* phenotype vary not only among populations, but also within populations, among years. Thus, the opportunity for strong, directional selection on certain floral traits, such as corolla depth, may be limited and variable.

I found no evidence of clear pollinator filtering in the populations, as most functional groups visited and performed an adequate pollination service where they occurred. *Arabis alpina* flowers thus seem to cater to a wide range of pollinators of varying sizes and behaviors, in accordance with the adaptive generalization theory (Ohashi et al., 2021). However, given the variation in pollinator abundance and trait matching across populations, floral phenotypes may experience different selection pressures. This suggests that floral traits could evolve through adaptive wandering, with pollinators having shorter tongues relative to corolla depth exerting stronger selection in the populations where they are more common (Ohashi et al., 2021; Thomson and Wilson, 2008).

In this chapter, I was able to establish *A. alpina* as a functional and ecological pollination generalist. Although relative pollinator effectiveness varied among populations, I found no evidence of local morphological specialization to specific pollinator groups. Instead, regardless of functional group, pollinators with a shorter tongue relative to flower corolla depth typically performed a better pollination service. However, as pollinator relative abundance was the main driver of variation in effectiveness among populations, abundance may also vary within populations among years. Thus, there is likely little potential for strong directional selection on tongue-corolla trait-match in this system. Still, these factors do not preclude variation in strength and direction of selection on other aspects of the floral phenotype, such as corolla size which has been shown to function as an honest signal in this system (Eisen et al., 2023), or floral scent.

Floral scent and insect community diversity (IV)

In this chapter, I investigated the relationship between floral scent diversity and local pollinator assemblage and preferences in Italian populations of *A. alpina*. Specifically, I tested the interaction diversity hypothesis which suggests that plants that interact with a diverse insect community, and/or located in a community with high plant diversity, should produce and emit a higher diversity of phytochemicals (Whitehead et al., 2021).

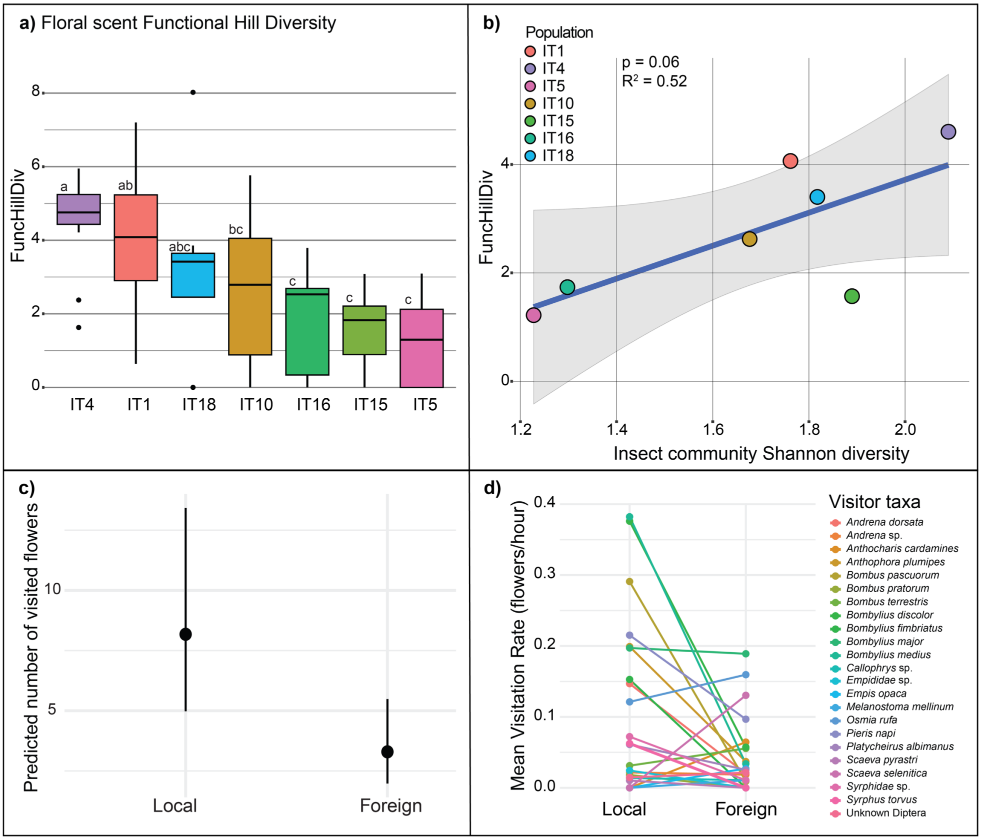

Figure 7. Floral scent diversity (FuncHillDiv) and its relationship with insect community diversity (a-b), and insect preferences for local and foreign flowers (c-d) in Italian *Arabis alpina* populations. **a)** Functional Hill Diversity of floral scent in seven *A. alpina* populations. Boxplot displaying the FuncHillDiv show median values and upper and lower quartiles, with whiskers extending up to 1.5 times the interquartile range. Letters indicate groups of statistical differences. **b)** Visualization of the positive linear relationship between FuncHillDiv and insect community diversity. Shaded area reflects 95% confidence interval. **c)** Predicted number of flowers visited by all insects to local and foreign flowers. The visualization is based on the best fitted model where flower origin was included as fixed factor. Whiskers indicate 95% confidence intervals. **d)** Visitation rate to local and foreign flowers by specific taxon. Horizontal lines connect identical taxon visiting both local and foreign flowers.

This theory has primarily been tested in the context of plant defense, but the same reasoning could be extended also to plant-pollinator interactions. Further, I subjected pollinator assemblages to local and foreign flowers in a translocation experiment to determine if there were any signs of adaptation of floral scent profiles to the local pollinator community, or *vice versa*.

The seven populations included in the floral scent diversity survey had surprisingly variable floral scent diversity indices (Figure 7a), despite almost all compounds being aromatic. This indicates that even within compound classes there can be differences in compound structural properties influencing the diversity score (Petrén et al., 2023a). Aromatic compounds include e.g., ketones, esters, aldehydes and alcohols (Dudareva et al., 2013; Knudsen et al., 2006), and pollinators are indeed able to discriminate also between compounds from the same fundamental biosynthetic pathway, such as the shikimate pathway producing aromatic compounds (e.g., Andersson and Dobson, 2003; Carvalho et al., 2014; Raguso and Light, 1998). The observed variation in floral scent diversity was positively correlated with insect community diversity (Figure 7b), suggesting that the interaction diversity hypothesis is also relevant in the context of plant-pollinator interactions. The floral scent diversity was largely independent of the co-flowering community. This pattern did therefore not agree with the interaction hypothesis at a community level, which has been shown for communities of different *Piper* species (Richards et al., 2015). But, to formally test this hypothesis, future research should include variation of floral scents emitted from co-flowering species that share pollinators with *A. alpina*.

Floral scent divergence (dissimilarity) between populations was highly correlated with geographic distance (Figure 3a in chapter **IV**). However, as all populations but It18 were located within a 36 km radius, the positive relationship implied that scent divergence increased rapidly even at short geographic distances. In contrast, no such relationship was discovered between geographic distance and insect community divergence, suggesting that populations located in proximity could experience disparate insect communities, or *vice versa*. Together, these results indicate that there is a relationship between local floral scent diversity and local insect community diversity, where populations interacting with a community of higher diversity, also produce and emit floral scents of higher structural diversity.

The second key finding of this study was the pattern of higher insect visitation rates to local compared to foreign flowers in the translocation experiment (Figure 7c-d). In more specialized systems, pollinators are highly efficient in detecting the scent of their local host plants (e.g., Carvalho et al., 2014; Friberg et al., 2014; Suinyuy et al., 2015), but similar studies in more generalized systems are scarce. However, when manipulating floral scent blends from two co-occurring, more pollination-generalized plant species visited by separate pollinators in their natural habitat, Larue et al. (2016) showed that the insect networks became disrupted and overlapping. The results from Larue et al. (2016) indicate that floral scent can be important for attraction and interaction specificity, suggesting that pollinators can discriminate between scents of hosts and 'non-hosts' also in more generalized systems.

Although the general pattern showed that insects preferred the local over foreign flowers, insects responded differently both between and within taxonomic family and/or genus (Figure 7d). For instance, among bombyliid flies both *Bombylius medius* and *B. discolor* strongly preferred local flowers over foreign, while *B. major* showed no preference. The opposite pattern occurred among syrphid flies, where most species preferred the local flowers or had no preference, while *Scaeva selenitica* almost exclusively visited foreign flowers. For bombyliid flies, it was interesting to observe strong preferences for local flowers, as these pollinators are typically considered to be visually oriented (Kastinger and Weber, 2001). As floral morphology was a confounding factor in this experiment, it is possible that the bombyliid flies were susceptible to some morphological cue related to the flower, such as flower size, color or iridescence. However, when comparing flower sizes between the populations included in each translocation pair, I found that size varied extensively among individuals within a population, and sizes overlapped to great extent between populations, suggesting that flower size may not be the main discriminating factor for foraging bombyliid flies and other insects. To fully disentangle what cue these insects use to discriminate between local and foreign *A. alpina* flowers, future studies should aim to perform bioassays where chemical and visual cues are separated. Syrphid flies are primarily visual foragers (Klecka et al., 2018; Raguso, 2020), but are known to respond also to multimodal cues including floral scent (Nordström et al., 2017). Thus, it is plausible that syrphids have learned to associate local scents with flower morphology, hence the relatively universal preference for local flowers in the translocation trials.

Insect groups that to higher degree rely on olfactory cues when foraging include lepidopterans (e.g., Wang et al., 2023), bees and bumblebees (e.g., Gomez Ramirez et al., 2023). In this experiment, *Pieris napi* (Lepidoptera: Pieridae) followed the general pattern and visited local flowers twice as often as foreign flowers. In contrast, *A. cardamines* (Pieridae) never visited local flowers but were observed visiting the foreign ones. The observed pattern of disparate preferences for *P. napi* and *A. cardamines* could be attributed to innate preferences for certain floral compounds, or olfactory learning, where the compounds detected by the butterflies are associated with known food sources. However, it is difficult to speculate around the preference of butterflies, as visits from them were rare compared to e.g., bombyliid flies, and the sample size may thus be too small for any definitive pattern of preference. All bees and bumblebees except *Osmia rufa* (syn. *O. bicornis*, Megachilidae) preferred local over foreign scents, in accordance with previous research showing strong influence of both innate (Dötterl and Gershenzon, 2023b; Rachersberger et al., 2019) and learned preferences for floral scents similar to those emitted by *A. alpina* plants (Dötterl and Gershenzon, 2023b; Wright and Schiestl, 2009), meaning that visiting bees and bumblebees may have learnt to associate the local floral scent blend with food reward.

Although species responded differently to the introduction of foreign flowers, the overall pattern of higher visitation to the locally sourced inflorescences was clear. Together with the positive correlation between floral scent diversity and insect community diversity, the results from this study point in the direction of local co-variation between floral scent and pollinators. Naturally, there are non-mutually exclusive scenarios under which this pattern could have occurred. First, the floral phenotype could be a result of local adaptation to the local insect community, where plant-pollinator interactions have shaped the scent diversity to maximize the number of attracted pollinator species. Local adaptations of floral scent have been documented in several, more specialized pollination systems (e.g., Friberg et al., 2014; Suinyuy et al., 2015). However, in this instance, the local adaptation is likely the result of selective pressures exerted by an entire community of insect species. Second, olfactory learning may play a part for many insect species, where the local pollinator community has learned to recognize the *A. alpina* scent and associate it with a reward. In this scenario, floral scent could diverge across populations through non-adaptive processes, with pollinators in each local population learning to recognize and visit the local floral scent phenotype. Nevertheless, two key pieces of evidence suggest that local selection is driving floral scent variation in *A. alpina*. First, the variation in floral scent among populations exceeds what would be expected under a neutral model (Eisen et al., in prep), indicating that mechanisms other than genetic drift are contributing to this diversity. Additionally, *A. alpina* interacts with a community of insect visitors that also pollinate various other plant species. For many of these insects, *A. alpina* might not be a significant resource, making it less likely for them to learn specific *A. alpina* scent profiles. To thoroughly investigate the role of local adaptation in this system, future research should focus on the significance of *A. alpina* within each local plant-pollinator network and evaluate the evolutionary fitness (e.g., seed set) of both local and non-local *A. alpina* plants.

Conclusions

This thesis investigates the drivers of phenotypic evolution, with a particular focus on intraspecific variation in floral scent within the pollination-generalist *Arabis alpina*. Through a series of experiments, I examine various aspects of the floral phenotype and pollinator community, aiming to piece together a fuller picture of how floral scent diversifies across the species' range.

In Chapter **I**, I discovered that while floral scent compounds are biosynthetically linked to other phytochemical traits, they appear largely free from evolutionary constraints, allowing floral scent to evolve independently under pollinator-mediated selection. Chapter **II** revealed that floral scent emission incurs little to no cost—plants emit similar amounts of scent regardless of time of day, temperature, or pollinator activity. Despite this consistency, scent compositions and insect communities differed significantly between two closely situated populations.

In Chapter **III**, I found that most insect visitors to *A. alpina* contributed to successful pollination, suggesting they could play a significant role in shaping floral trait evolution. Finally, in Chapter **IV**, I examined how pollinators influence floral scent diversity and found that they consistently preferred the local scent profile over a foreign one, offering a potential explanation for the observed correlation between floral scent diversity and pollinator community variation across different regions.

Collectively, my research suggests that floral traits, and specifically floral scent variation in *A. alpina* populations may have evolved through pollinator-mediated selection, potentially as locally adapted traits. However, further investigation is needed to fully understand the role of pollinators in the evolution of floral scent and their influence on floral morphology. First, since pollinator importance can vary across years (Ollerton et al., 2024). and this study provides only a snapshot, continued surveys across multiple seasons are essential to capture temporal variation in pollinator communities as well as variation in strength and direction of selection on the floral phenotype. Second, phylogenetic studies among closely located populations are required to assess the influence of phylogeography and genetic drift on scent variation.

Nevertheless, this thesis serves as a comprehensive investigation of how evolutionary trade-offs, abiotic and biotic factors shape phenotypic evolution in a generalist species. Specifically, it adds to the growing evidence highlighting the importance of floral scent also in generalized plant-insect interactions.

Acknowledgements

My friend CGPT tells me there are about 100 people (plus an unknown number of cats, dogs, and composers) in this part. That's an awful lot of people, I didn't even know I knew that many people. But apparently, I do. A typical Swede interacts with anything between 10,000 and 40,000 people in her lifetime. I don't know where I fall on that scale, probably 'lagom'. But among that lagom number of people, there are about 100 (plus cats, dogs, and composers) who have really mattered for this thesis to happen. It takes a village to raise a child, and it takes a department to raise a PhD.

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