Australian Mountain Pollinators in a Changing World

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Candidate's Declaration

This thesis contains no material that has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge, it contains no material previously published or written by another person, except where due reference is made in the text.

Joshua Michael Coates

Date: 21/12/25

Preface

This thesis comprises several thematically linked peer-reviewed publications, compiled here under the ‘Thesis by Compilation’ format. Each of these papers has been published in, or submitted to, scientific journals at the time of submission of this thesis. Therefore, there is unavoidable repetition between some chapters, which serve as separate stand-alone publications and may duplicate some content from the introduction and methods, for example. Chapters 2 and 5 are already published. I have responded to minor revisions on Chapter 3 and I expect it to be published in the coming weeks. Chapter 4 has been submitted and is in peer-review. The layout and structure of each paper may vary according to the requirements of each journal, though some changes have been made to formatting to ensure consistency throughout the thesis. Figures and tables are numbered within each chapter, rather than continuously throughout the thesis. References are included at the end of each chapter. Data from each chapter are archived and available online via Dryad, according to each journal’s data accessibility requirements.

Where chapters are comprised of co-authored publications, I use ‘we’; elsewhere I use ‘I’. In all instances, I was the primary author and major contributor to each chapter presented here. I led the development of experimental design, conducted all fieldwork and data collection, and led data analysis and writing of manuscripts. Throughout these efforts, I was assisted by several collaborators, each of which are separately credited through coauthorship or Acknowledgements on each paper.

The individual papers included in this Thesis by Compilation are:

Chapter 2: Coates J.M., Evans M.J., Scheele B.C., Encinas-Viso F., Florez Fernandez J., Lumbers J., Cunningham S.A. 2024. Influence of climate, weather and floral associations on pollinator community composition across an elevational gradient. Oikos 12: e10688.

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Author Contribution:

JMC: Conceptualization (lead); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); project administration (lead); resources (lead); validation (lead); visualisation (lead); writing – original draft (lead); writing – review and editing (lead).

MJE: Data curation (supporting); formal analysis (supporting); investigation (supporting); visualisation (supporting); writing – original draft (supporting); writing – review and editing (supporting).

BCS: Conceptualization (supporting); formal analysis (supporting); investigation (supporting); writing – original draft (supporting); writing – review and editing (supporting).

FEV: Conceptualization (supporting); formal analysis (supporting); investigation (supporting); writing – original draft (supporting); writing – review and editing (supporting).

JFF: investigation (supporting); writing – original draft (supporting); writing – review and editing (supporting).

JL: investigation (supporting); writing – original draft (supporting); writing – review and editing (supporting).

SAC: Conceptualization (equal); investigation (supporting); methodology (supporting); writing – original draft (supporting); writing – review and editing (supporting).

Chapter 3: Coates J.M., Takeshima, N., Encinas-Viso F., Cunningham S.A. in review. Against the odds: Nesting Specialization and Foraging Ecology Provide Insights into Climate Change Responses in a Mountain Bee. Insect Conservation and Diversity.

Word Length: 9,149

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NT: Conceptualization (supporting); writing – original draft (supporting).

FEV: Conceptualization (supporting); formal analysis (supporting); investigation (supporting); writing – original draft (supporting).

SAC: Conceptualization (equal); investigation (supporting); methodology (supporting); writing – original draft (supporting).

Chapter 4: Coates J.M., Evans M.J., Scheele B.C., Cunningham S.A. in review. Hotspots on Cold Mountains: Hot Flowers as Pollinator Refuges in Mountain Ecosystems. Functional Ecology

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MJE: formal analysis (supporting); visualisation (supporting); writing – original draft (supporting); writing – review and editing (supporting).

BCS: Conceptualization (supporting); formal analysis (supporting); investigation (supporting); writing – original draft (supporting).

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Chapter 5: Coates, J. M., Keaney, B., Scheele, B.C., Cunningham, S.A. 2023. Endangered Bogong moths (*Agrotis infusa*) forage from local flowers after annual mass migration to alpine sites. Global Ecology and Conservation 44: e02482.

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BK: Formal analysis (supporting); investigation (supporting); project visualisation (supporting); writing – original draft (supporting); writing – review and editing (supporting).

SAC: Conceptualization (equal); investigation (supporting); methodology (supporting); writing – original draft (supporting); writing – review and editing (supporting).

Communications

All research included here was intended to be published in peer-reviewed journals. In addition to these publications, I was fortunate to be able to communicate my research through radio, conference presentations and posters and community presentations. I was directly involved in the following science communications:

Date	Outlet	Communication
November 2025	*Australian National Botanical Gardens seminar series*, Canberra	I delivered a community presentation on my research in mountain pollination ecology
September 2025	*Radford Primary School,* Canberra	I delivered content on insect conservation to students
September 2025	*Floriade Festival,* Canberra	Through my role at Invertebrates Australia, I organised and delivered an entomology workshop across several weekends at Floriade Festival
August 2024	*National Science Week Story Time Takeover*, Canberra	I delivered a "scientist takeover" story time session at Gungahlin library, where I read insect stories to young kids and delivered hands-on insect content
March 2025	*Enlighten Festival*, Canberra	I delivered insect outreach content at Enlighten Festival's entomology exhibit
March 2025	*ABC Radio National*	I appeared on ABC Radio National with Renee Krosch to talk about insect diversity and conservation in Australia
February 2025	*Bogong Moth Summit,* Canberra	Bogong moths (*Agrotis infusa*) Forage from Local Flowers Over Summer Alpine Activity Period, Conference poster presentation
January 2025	*National Youth Science Forum*, Canberra	I delivered entomology and pollination ecology content to National Youth Science Forum Students at ANU
August 2024	*International Congress of Entomology ICE2024*, Kyoto	Newly Discovered Cold-Tolerant Bee That Favours Life in the Subalpine Woodland of Australia's Highest Mountains, Conference presentation
July 2023	*SydBee,* Sydney	Ecology of a Newly Discovered, Uniquely Adapted Alpine Native Bee, Conference presentation
July 2023	*Ecological Society of Australia: ESA2023*, Darwin	Plant-Pollinator Networks in Alpine Australia: Implications Under Climate Change, Conference presentation
December 2022	*ABC Radio*, Canberra	I appeared on ABC Radio with Ross Solly to talk about Bogong moth conservation and my research on Bogong moth pollination ecology

December 2022	*Australian Institute of Alpine Studies conference*, Jindabyne	Bogong moths (*Agrotis infusa*) Forage from Local Flowers Over Summer Alpine Activity Period, Conference presentation
December 2021	*Australian Institute of Alpine Studies conference*, Jindabyne	Plant-Pollinator Networks in Alpine Australia: Implications Under Climate Change, Conference presentation
September 2021	*Ginninderra Rotary Club*, Canberra	I delivered a community presentation on local Canberran pollinator species
June 2021	*Australian National Bee Association*, Sydney	*Exoneura* nest in the stems of cultivated *Rubus* plants and act as effective crop pollinators, Conference presentation
March 2021	*Ainslie Primary School,* Canberra	I delivered content on native bees and bee-friendly gardens to students

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This research was conducted on the traditional lands of the Ngarigo, Ngunnawal and Wiradjuri Nations. I acknowledge the lasting connection with the Australian Alps held by Traditional Owners, developed over many thousands of years. I'm grateful for my experiences on this land and recognise that this research was conducted on unceded land.

Throughout my Ph.D. candidature, I have been generously supported by countless individuals. Their expertise and support reduced many hurdles in this project, and I wouldn't have had nearly as much fun as I did without them all. My memory is a fallible thing at the best of times, but here follows some of the standout contributions.

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Saul: despite being one of the busiest people in the school, you have always made time for me. Until starting as an Honours student in your lab all those years ago, doing a Ph.D. was never really on my radar. You helped to channel my passion for insects and the natural world into research curiosity, and for this I will always be grateful. Thank you for believing in my potential as an ecologist and inspiring me to realise my capacity. Your characteristic optimism and good humour have made a world of difference these last few years, helping to decouple some of the grind from this journey.

Ben: thank you for your consistent warmth and approachability. You were available whenever I needed guidance, and your speedy, constructive feedback made a huge difference in the writing of my thesis and each of its papers.

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John Evans: while you were not officially on my supervisory panel, my project has benefitted from your advice immensely. At the onset of my candidature, wrangling with statistical analysis and R was always the most intimidating part of the Ph.D. process. While it was an uphill battle at times, I now feel like I’ve come out the other side with a solid quantitative head on my shoulders, thanks in no small part to you. I’ve referred to you many times as “John the Stats Wizard”, and I’m grateful for the stats/magic that you’ve lent to my research.

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Thanks to the many specialists that helped me to identify and understand the diversity of insects in my project, including Dr. Hermes Escalona, Dr. Michael Braby, Dr. Michael Batley, Dr. Jaime Florez-Fernandez, Dr. Vanessa Kellermann, Dr. Carmen da Silva and Dr. Ken Green.

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Last, but certainly not least, to my partner Sam: your support throughout this thesis has made a world of difference. Thank you for to the countless times you've listened patiently while I practiced my conference presentations (I'm sure you could've presented my talk yourself in Kyoto last year), for putting up with me while I groaned about broken R code, for your patience while I worked endless long days and weekends, and for somehow maintaining interest in and remembering the minutiae of my project. I could write a whole thesis full of the ways in which you've helped me throughout this journey, but I've just written this thesis and one is plenty!

Abstract

Insect pollinators inhabiting cold environments face considerable challenges to survival and reproduction. As ectotherms, insects rely on external temperatures to regulate their body temperature. In mountain ecosystems, where temperatures are frequently low, unpredictable, and variable, maintaining an optimal body temperature for flight is energetically demanding and can restrict periods of activity. Mountain environments are also characterised by long periods of snow cover and short but intense flowering seasons, which further limits activity. These conditions result in pollinator communities that differ markedly from those in warmer climates. Mountain environments are also particularly vulnerable to climate change, with warming temperatures, reduced snow cover, and earlier onset of snowmelt already recorded. Understanding how pollinators respond to these pressures is critical, as climate change is altering both the activity and distribution of species in mountain environments.

In my thesis, I investigate how pollinators in mountain environments respond to climate and weather across multiple ecological scales. I consider processes at broad spatial scales, such as changes in community composition along climate gradients, down to the microhabitat experienced by an insect inside an individual flower. I first focus on the whole pollinator community, before focussing in on the ecology of particular insect species, including a newly discovered cold-adapted bee and the culturally significant Bogong moth, to explore how specialised life histories interact with environmental constraints. By linking patterns from communities to species and from climate to microhabitat, I aim to provide an integrated understanding of the impact of abiotic conditions on pollinators in mountain environments under climate change.

Chapter 2

While ectothermic insects are influenced by temperature, activity is also reliant on suitable biotic conditions, such as the presence of host plants. This chapter disentangles the influence of abiotic and biotic factors on pollinator assemblages across an elevational gradient (930–2000 m a.s.l.). Diptera dominated interactions at higher elevations, whereas Hymenoptera and Coleoptera were more frequent at lower elevations. This pattern was driven by abiotic conditions, with

cold-tolerant Diptera more frequent at high elevations, as well as host-plant floral associations (fly-attracting flowers at high elevations, bee-attracting flowers at lower elevations). While previous work has focussed only on abiotic conditions, this research suggests that shifts in pollinator assemblages with elevation are shaped by the combined effects of environmental filtering and floral community composition.

Chapter 3

While Chapter 2 showed reductions in Hymenoptera activity with increasing elevation, I regularly observed one bee at high-elevation sites, which were otherwise dominated by Diptera. This previously unstudied bee in the genus *Exoneura* appeared to be displaying unique traits that allow it to persist in cold, high-elevation environments. In this chapter, I explore how this bee thrives in high-elevation (~1500–1900 m a.s.l.) subalpine woodlands. Unlike other *Exoneura* species that nest in pithy stems, this bee excavates nests in dead branches of snow gum (*Eucalyptus pauciflora* subsp. *niphophila*). *Exoneura* nest abundance peaked at the tree line where it is coldest, potentially allowing this bee to benefit from a release from competition from other bees. Their foraging trips were also concentrated within just 30 m of nest sites, so that their presence in the landscape closely mapped snow gum stands. Biophysical modelling showed that activity windows were strongly constrained by temperature but are projected to expand with climate warming. This bees' unique nesting biology and close association with snow gums reveal adaptations that enable persistence in cold environments and highlight potential shifts under climate change.

Chapter 4

Insects are highly mobile organisms, enabling them to select suitable microhabitats to buffer against unfavourable weather. Their small size also allows them to exploit refuges inaccessible to larger organisms. Among the microhabitats available to mountain pollinators, flowers are of particular interest. In this chapter, I quantify the thermal environments within flowers and their influence on insect warming to investigate the role of floral warming on mountain insect activity. Flowers provided thermal refuges that were consistently warmer than ambient air temperature, with species-level variation in flower temperature linked to flower shape. Crucially, I also show that warm flower microhabitats influenced insects' behaviour, with Diptera interacting more frequently with warm flower when ambient conditions were cool. This research suggests that floral microhabitats may serve as refuges for thermoregulation, underscoring the importance of fine-scale microclimates in decoupling insect body temperature from cold environments.

Chapter 5

The Bogong moth (*Agrotis infusa*) is a keystone species that undergoes long-distance migration to the Australian Alps, where individuals escape the summer heat by sheltering in high-elevation caves. Recently listed as Endangered, this species has undergone sharp population declines, yet its feeding behaviour during the aestivation life stage was previously unclear, with some studies suggesting that they enter dormancy and do not feed during aestivation. In this chapter, I trapped Bogong moths during their aestivation period and used pollen analysis to demonstrate that they regularly feed on flowers at high-elevation sites. Almost all individuals carried pollen, often in large quantities, representing a diverse assemblage of plants. Importantly, moths were carrying pollen collected from endemic subalpine and alpine plants. This indicates generalist foraging habits and local feeding while in the mountains, challenging previous suggestions of non-feeding during aestivation. These findings suggest Bogong moths may play a previously overlooked role in mountain pollination networks, and their continued decline may have consequences for pollination services in these ecosystems.

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Chapter 1: Introduction

1.1 Pollination in the Cold

With the majority of angiosperms relying on insect pollen vectors, insect pollination supports plant reproduction and ecosystem services in a broad range of environments (Ollerton *et al.*, 2011). By transferring pollen among plants, pollinators influence seed set, outcrossing rates and plant population structure (Knight *et al.*, 2005). Pollinator movement also governs gene flow among geographically isolated plant populations, limiting inbreeding (Jordano, 2010). Variation in pollinator activity and pollen transfer can cascade to shifts in plant abundance that influence community structure (Bascompte and Jordano, 2006). Because of their role in shaping ecosystem structure, environmental constraints on insects' ecology can have ecosystem-level consequences (Burkle *et al.*, 2013). As a result, conditions that limit pollinator activity can alter plant demography and influence community turnover across environmental gradients.

As ectotherms, insects rely on environmental temperatures to ensure appropriate body temperature for activity. Their metabolism is governed by steep thresholds at lower temperature bounds, enabling activity such as basking, flight and foraging (Lahondère, 2023). In cold environments, where insects are likely to spend more time near these low temperature thresholds, abiotic constraints on pollinator performance are amplified (Willmer, 1983). As a result, pollinator activity in cold climates can be limited to narrow windows, both within a day and throughout the year. Because pollen transfer depends on when insects can fly, thermal limits trickle down to plant reproductive outcomes, linking abiotic conditions directly to ecosystem processes (Stone and Willmer, 1989).

The profitability of foraging depends on whether energy gained from flowers offsets the costs of foraging and thermoregulation. Net energy gain can be expressed as the energetic benefits obtained from food (especially nectar) minus the energy expended during thermoregulation, flight and foraging activities (Pyke, 1978; Lahondère, 2023). Low ambient temperatures increase the energy required to initiate foraging activities, with wind also increasing convective heat loss, so that inclement conditions shift the cost-benefit balance against foraging. This can lead to reduced growth periods and metabolic rates that can be life-limiting for species (Lefebvre *et al.*, 2018). Insects compensate for this behaviourally by selecting warm microhabitats, shortening foraging distances or delaying activity until foraging is advantageous (Kenna *et al.*, 2021). The result is that insects' activity in marginal environments is clustered spatially and temporally into brief opportunities for activity that permit profitable foraging (Denlinger and Lee Jr, 2010).

1.2 Mountain Ecosystems as a Natural Laboratory for Thermal Ecology

High-elevation environments are conducive to the study of thermoregulatory phenomena that underpin pollinator activity due to the presence of shifts in conditions across small geographic areas (Sundqvist *et al.*, 2013). Conditions such as temperature, precipitation, snow fall and atmospheric pressure can change a lot even with small increases in altitude (Körner, 2007), with temperature typically decreasing by 0.6ºC per 100m of elevation gain (Guo *et al.*, 2013). Topographic variation in mountain environments also provides a variety of microhabitats that enable organisms to buffer the effects of cold conditions (Opedal *et al.*, 2015), by creating create a mosaic of temperatures across a small area (Rae *et al.*, 2006). At the spatial scale experienced by insects themselves, surfaces on alpine plants can buffer against air temperature significantly, matching conditions typical of averages experienced at lower elevations (Scherrer and Kãrner, 2009).

Pollinator species assemblages in mountain environments are often distinct from their lowland counterparts due to unique cold-tolerant adaptations necessary to persistent in the cold (Junker and Larue-Kontić, 2018; Lefebvre *et al.*, 2018). For instance, Diptera typically dominate high-elevation pollinator communities, whereas in lower-elevation environments Hymenoptera are more common (Arroyo *et al.*, 1982; Arroyo *et al.*, 1985; Inouye and Pyke, 1988; Hodkinson, 2005; Hoiss *et al.*, 2012; Lefebvre *et al.*, 2018). Similar shifts towards Diptera dominant-pollination systems with increasing latitude have also been observed in the Arctic (Kevan, 1973; Kearns, 1992). Diptera tend to dominate high-elevation and high-latitude assemblages because they exhibit greater cold tolerance and cold-adapted life-history traits (Lefebvre *et al.*, 2018). A notable exception to this trend are

Bombus, a genus of bees that exhibit specialised cold-tolerant traits and are widespread in high-elevation and latitude environments (Minachilis *et al.*, 2020), though they are absent from mainland Australia, and present only as an exotic invader in Tasmania. Because pollinators vary in their effectiveness as pollen vectors (Bischoff *et al.*, 2013), these elevation-associated shifts in dominant pollinators have important impacts on plant reproductive success (McCabe and Cobb, 2021).

1.3 Pollination in the Australian Alps

Much of the research on mountain pollinators comes from the Northern Hemisphere (Santamaría *et al.*, 2014; CaraDonna *et al.*, 2017; Junker and Larue-Kontić, 2018; Lefebvre *et al.*, 2018; Zhao *et al.*, 2019; Ahmad *et al.*, 2023; Matsubara *et al.*, 2023), while Australia's mountain systems remain comparatively understudied. Australia's high country is also distinct from mountain environments elsewhere, with lower maximum elevations and accordingly warmer temperatures and shorter periods of snowfall (Verrall *et al.*, 2021), so conclusions from other regions are not necessarily directly applicable in Australia. Australia's unique biographic history has also produced high levels of endemism and rich biodiversity (Crisp and Cook, 2013). Alpine environments are particularly rare in Australia, covering less than 1% of the continent's land mass, yet contain a high level of endemism (Costin et al., 2000). In their seminal work, Inouye and Pyke (1988) characterised pollination networks in Australia's high country at Charlotte Pass, using visitation surveys conducted across one flowering season from elevations ranging from 1860m to 2040 m asl. Analogous with mountain pollination networks overseas (McCabe *et al.*, 2019), they found a dominance of flies visiting flowers at their sites, with fewer bees. More recent work in this ecosystem has also shown that flies were more abundant than bees in mountain communities (Stock and Pickering, 2002; Milla and Encinas-Viso, 2020), that pollinator taxa differed in the pollen-transfer capacity (Encinas-Viso *et al.*, 2024), and that microclimatic heterogeneity drives fine-scale turnover in community composition and plant-pollinator interactions over short distances (Encinas-Viso *et al.*, 2022). Goodwin *et al.* (2021) showed that abiotic factors such as temperature, humidity and windspeed impacted upon pollinator visitation levels at Charlotte Pass, allowing greater prevalence of flies over bees.

Collectively, this literature points to generalised, fly-dominated systems, but key gaps remain that underpin the structuring of cold-adapted pollinator communities in this environment. It remains unclear how biotic and abiotic drivers structure pollinator activity and networks across scales, particularly how these effects shift from microhabitat to landscape scales.

1.4 Climate Change in the Australian Alps

High-altitude environments are predicted to see some of the earliest and most significant impacts of climate change (Pickering and Buckley, 2003). Gallagher *et al.* (2009) demonstrate that mean annual temperatures in Alpine Australia have already increased by 0.74ºC between 1950 and 2007. This will alter mountain ecosystems and the structures and networks that underpin them, with some predicting loss of these ecosystems by 2100 (Williams *et al.*, 2007). Australia's alps have already begun to see the effects of climate change, with reduced annual snowfall (Slatyer *et al.*, 2017), a shortening of the snow season, increased temperature averages (Gallagher *et al.*, 2009; Sanchez-Bayo and Green, 2013) and occurrence of extreme weather events (Guo *et al.*, 2013) already recorded. Nash *et al.* (2013) and Farkas *et al.* (2025) demonstrate that Australian ground-dwelling arthropod taxa in the alps show varied responses to warming, pointing to future changes in community composition. Clarifying the thermal and ecological mechanisms linking climate change to pollinator activity is essential for forecasting pollinator biodiversity trajectories in Australia's highest ecosystems.

1.5 Thesis Scope and Aims

The aim of this thesis is to understand the thermal ecology ectothermic insect pollinators in the Australian Alps. By examining pollinators at multiple ecological scales, from whole communities down to individual species, from climates down to microhabitats, I aim to provide an integrated understanding into how Australian mountain pollinators are responding to changing conditions.

In Chapter 2, I examine the separate impacts of climate, weather and floral community composition on pollinator diversity and plant-pollinator networks along an elevation gradient. By using elevation as a correlate for climate, this approach disentangles the relative contributions of long-term abiotic conditions, short-term weather variation, and host-plant availability in shaping pollinator diversity and interaction networks.

In Chapter 3, I focus on a previously unstudied bee with unique, cold-tolerant life history traits. I show that this *Exoneura* bee is able to persist in cold environments, which are otherwise dominated by fly pollinators, due to their novel nesting biology within snow gum (*Eucalyptus pauciflora* subsp. *niphophila*) branches and their ability to satisfy ecological needs with limited activity windows and accessing resources close to the nest. I also demonstrate that climate change will significantly alter their activity levels, which will result in changes in competitive dynamics and trophic interactions in the community.

In Chapter 4, I zoom in even further, to the scale experienced by mountain insects while visiting individual flowers. I show that most mountain flowers are significantly warmer than ambient air temperature, with flower shape driving floral warming. I also show for the first time that these warm microclimates influence frequency of pollinator interactions, by providing a thermal reward that enables flower visitors to buffer against ambient weather conditions.

In Chapter 5, I investigate the pollination ecology of an Endangered, culturally significant, keystone species, the Bogong moth. This nocturnal insect has a unique relationship with the cold mountain climate, undertaking an annual migration to the area each year to escape the summer heat of their natal habitats. In this chapter, I show that this species performs a previously unrecognised role as a pollinator to endemic alpine plants and that they are still seeking nectar during their aestivation period, challenging previous ideas about their dormancy and energetics during aestivation. These results point to further ecological importance in these environments during their summer migration to the mountains.

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Chapter 2: Influence of Climate, Weather and Floral Associations on Pollinator Community Composition Across an Elevational Gradient

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2.1 Foreword

This chapter partitions the effects of daily weather conditions, site-level climate, and floral community structure on pollinator assemblages across elevations in the Australian Alps. Prior work has largely attributed elevational shifts in pollinator communities to abiotic conditions, particularly temperature and precipitation, which act as environmental filters on ectothermic pollinators and drive predictable changes in species turnover with elevation. As elevation increases, low temperatures increase thermoregulatory costs and decrease foraging suitability, narrowing windows in which foraging yields a positive energy balance. Pollinator community change with elevation reflects where and when positive energy budgets can be achieved given abiotic conditions and the distribution of floral rewards.

This chapter's key contribution is to clarify the relative importance of broad climatic context versus weather and floral associations. By introducing the 'floral association hypothesis', this chapter shows for the first time that floral community also structures pollination communities along elevation gradients. This research also provides a data-rich baseline for understudied mountain pollination systems in Australia.

In many respects, this chapter comprises the keystone paper of the thesis. It shaped the overall conception of my studies, and subsequent chapters develop questions that emerged from its findings. Overwhelmingly, it represents the most significant field sampling effort in my thesis, resulting in a data-rich exploration of the broad drivers structuring plant-pollinator networks in the Australian Alps.

2.2 Abstract

Insect pollinators, which are ectothermic, are especially sensitive to abiotic conditions, which often drive predictable patterns of pollinator species turnover along environmental gradients. However, pollinator activity is also reliant on suitable biotic conditions, such as the presence of host plants. High-elevation environments provide a useful setting to examine the relative contribution of abiotic and biotic factors in shaping species interactions as they are often characterised by strong environmental gradients over short geographic distances. Here, we examined pollination interaction networks across an elevational gradient from 930-2000m a.s.l. in southern Australia, to determine the underlying patterns of pollinator activity and their interactions with flowers. Interaction frequency of Diptera increased at high elevations, while interaction frequency of Hymenoptera and Coleoptera decreased. We provide evidence that this elevational pattern of activity is partly driven by floral associations, with interactions dominated by Hymenoptera-attracting plant families at lower elevations (Proteaceae, Fabaceae) and a Diptera-attracting family at high elevation (Asteraceae). Pollinator activity was also influenced by weather conditions, with reduced activity for all three orders at lower temperatures, and Diptera active across the broadest range of temperature, humidity and wind conditions. We suggest that changes across elevation gradients in pollinator community composition are driven by both direct responses to abiotic conditions such as temperature, as well as the elevational distribution patterns of associated flowering plants. Despite these distinct shifts in composition of the pollinator assemblage with elevation, pollination network structure was stable across the elevational gradient, with moderate levels of specialisation and low levels of connectance and nestedness present across the gradient. By considering both abiotic conditions and biotic processes, our results provide insight for predicting the impacts of upslope vegetation shifts on pollinator communities in the face of climate change.

2.3 Key Words

Pollinator Distribution, Elevational Gradients, Ecological Networks, Alpine Ecosystems

2.4 Introduction

Understanding how biotic communities are shaped along environmental gradients by abiotic conditions, such as weather and climate, and biotic processes, such as competition, is central to ecology (Pellissier *et al.*, 2010; Wisz *et al.*, 2013). Research has primarily focussed on the role of abiotic processes in structuring species distributions, with biotic processes receiving relatively less attention (Gutiérrez *et al.*, 2005; Araújo and Luoto, 2007). However, biotic processes can affect how species respond to abiotic conditions along environmental gradients (Davis *et al.*, 1998; Hargreaves *et al.*, 2015), and vice versa, so that both need to be considered in tandem (Wisz *et al.*, 2013). Subsequently, to understand factors controlling species distributions, research is needed that examines biotic processes alongside abiotic conditions (Gilman *et al.*, 2010).

High-elevation environments, such as alpine and subalpine ecosystems, offer a unique opportunity to study the mechanisms that underpin ecosystem structure, due to the substantial changes in abiotic and biotic processes across relatively small geographic ranges (Sundqvist *et al.*, 2013). Because abiotic conditions such as temperature, precipitation, and solar radiation change rapidly across short distance with elevation gain, elevation is often used as a proxy for climate (Pepin *et al.*, 2022). For instance, average temperatures decrease by 1^{o}C with every 100m of elevation gain (Rolland, 2003). Subalpine and alpine ecosystems are subject to harsh abiotic conditions, such as low temperatures and high precipitation levels, which drive unique patterns in biotic interactions (Adedoja *et al.*, 2018). Species assemblages in these ecosystems are distinct from their lowland counterparts, and often vary predictably along elevational gradients (Junker and Larue-Kontić, 2018; Lefebvre *et al.*, 2018). Alpine ecosystems are also predicted to see some of the earliest and most significant impacts of climate change (Pickering and Buckley, 2003), highlighting the need to understand the mechanisms structuring contemporary mountain ecosystems.

The distribution of pollinators is limited by their responses to environmental conditions, causing variation in community assemblage across elevational gradients (McCabe *et al.*, 2019). This variation often drives predictable turnover among pollinator taxa across elevation gradients. In this study, we focus on the observation that Diptera species dominate high-elevation pollinator communities, compared to in lower-elevation environments where Hymenoptera species typically dominate

(Arroyo *et al.*, 1982; Inouye and Pyke, 1988; Hodkinson, 2005a; Hoiss *et al.*, 2012; Lefebvre *et al.*, 2018). Similar shifts towards Diptera-dominant pollination systems with increasing latitude have also been observed (Kevan, 1973; Kearns, 1992). These studies indicate that pollinators respond directly to abiotic conditions, such as temperature, that covary with elevation and latitude, suggesting that because Diptera species display greater cold tolerances and life history traits that allow them to persist in colder climates, they dominate high-elevation and high-latitude environments (Lefebvre *et al.*, 2018). Elevational shifts in dominant pollinators could have important effects on plant reproductive success (McCabe and Cobb, 2021), because pollinators vary in their effectiveness as pollen vectors (Bischoff *et al.*, 2013).

While it is recognised that abiotic conditions are important to explaining elevational patterns in pollinator community composition (Arroyo *et al.*, 1982; Inouye and Pyke, 1988; Hodkinson, 2005a; Hoiss *et al.*, 2012; Lefebvre *et al.*, 2018), the interrelated effects of weather and climate remain unresolved (but see Arroyo *et al.* (1982)). Weather refers to the short-term variability in abiotic conditions and is often characterised by rapid fluctuations, whereas climate describes long-term average weather conditions (Drake, 1994; Baker *et al.*, 2017). Weather may influence flight and foraging activity levels (Goodwin *et al.*, 2021), emergence, migration and mating timing, as well as weather-induced mortality (Drake, 1994). Conversely, climate provides the broader context and long-term patterns that shape the composition, physiology, distribution, and ecological dynamics of pollinator communities, and includes impacts that span multiple generations (Drake, 1994).

While most research into changes in pollinator communities along elevational gradients has focussed on abiotic mechanisms (Hoiss *et al.*, 2012; Hoiss *et al.*, 2015; Minachilis *et al.*, 2020; McCabe and Cobb, 2021), biotic processes are also likely to be important, but have received comparatively little study (Wisz *et al.*, 2013; Pires *et al.*, 2020; Ahmad *et al.*, 2023). Many pollinators have been shown to favour flowers of a set of related species (e.g., within a genus or family) (Harder and Johnson, 2009). It is expected, therefore, that the identity and availability of flowers will influence pollinator distribution along elevational gradients (Pires *et al.*, 2020; Ahmad *et al.*, 2023). Just as pollinators follow typical elevational patterns of distribution, plants also respond to abiotic conditions and vary predictably across elevational gradients (Pellissier *et al.*, 2010; Basnett *et al.*, 2019). Plants vary along elevation gradients in terms of their floral traits (Zhao and Wang, 2015; Basnett *et al.*, 2019; Ahmad *et al.*, 2023), vegetative traits (Junker and Larue-Kontić, 2018), floral cover (Hoiss *et al.*, 2012), and community composition (Sundqvist *et al.*, 2013; Silva Mota *et al.*, 2018). Pires *et al.* (2020) showed that butterfly communities were strongly associated with plant community composition across an elevational gradient.

Understanding pollination network structure across environmental gradients allows us to characterise how interactions between plants and pollinators impact ecosystem functioning and resilience (Lara-Romero *et al.*, 2019). Network metrics quantify the structural components of a network (Dormann, 2020), facilitating comparison between sites with different species composition. Other studies have demonstrated that pollination network metrics change along elevation gradients (Hoiss *et al.*, 2012), often in consistent directions. For instance, high-elevation pollination networks are often characterised by high levels of generalisation (Inouye and Pyke, 1988; Lara-Romero *et al.*, 2019) and increased nestedness (Dupont *et al.*, 2003; Miller-Struttmann and Galen, 2014; Inouye, 2020), compared to lowland systems, which typically display decreased levels of nestedness (Bascompte *et al.*, 2003) and specialisation (Brosi, 2016). These structural network metrics can provide insights into how pollination networks will change with climate change (Adedoja *et al.*, 2018).

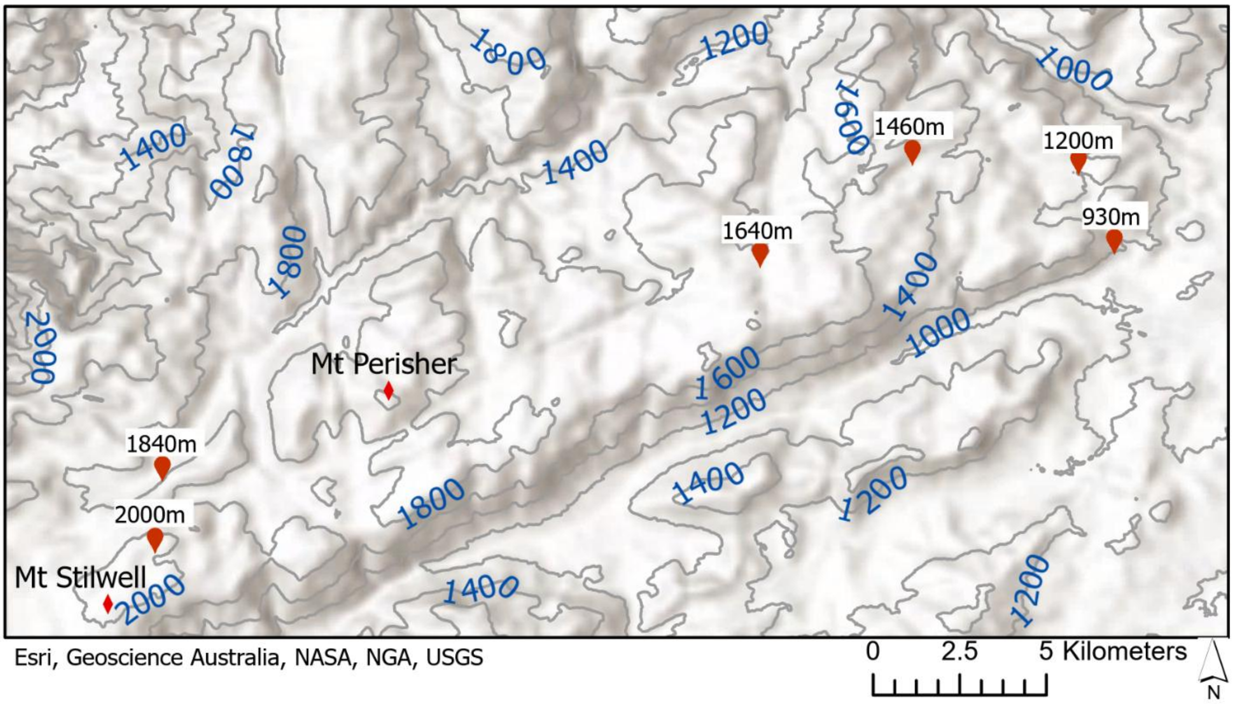
In this study, we examine the mechanisms underlying pollinator communities by sampling pollination networks across an elevational gradient from ~930m to 2000m a.s.l. in Kosciuszko National Park, New South Wales, Australia. A general pattern that has been observed in pollinators activity across elevation gradients on multiple continents is the dominance of Diptera species at high elevations, as distinct from lowland ecosystems interaction networks dominated by Hymenoptera species (Inouye, 2020; McCabe and Cobb, 2021). This study examines the effects of climate, weather, and the floral community on pollinators across an elevational gradient. We examined the impact of climate, which is correlated with elevation, by sampling sites along an elevation gradient. We also repeatedly sampled sites across the active season (October-March) to explore the impact of day-to-day variability in weather. We sought to determine how pollinator activity is driven by the separate influences of abiotic conditions (weather and climate), biotic interactions (plant-pollinator associations), or both. While other studies have examined these factors separately, our approach integrates the investigation of both abiotic factors and biotic interactions to elucidate the complex dynamics driving pollinators in subalpine and alpine ecosystems.

2.5 Methods

2.5.1 Study Area

We conducted flower visitor surveys in Kosciuszko National Park, New South Wales, Australia from October 2021 to March 2022. This period captured the onset of flowering at montane and subalpine sites following snowmelt, as well as the subsequent peak alpine flowering period (Costin *et al.*, 2002). Being a whole of season study, our survey accommodated the peak of flowering for almost all the plant species present across each of the elevation bands (see below).

To capture an elevational gradient, we selected six study sites, with two plots per site, starting from ~930m to above the tree line at ~2000m, with sites approximately every 200m of elevation gain (930m, 1200m, 1460m, 1640m, 1840m, 2000m), with approximately 25km of horizontal extent. This design emphasised repeated sampling throughout the season within each study site, rather than replication of sites at each elevation at the expense of repeated sampling at each site. All study sites were located along the northeast side of the mountain range, within the Perisher Valley area (Figure 1).



Esri, Geoscience Australia, NASA, NGA, USGS

Figure 1. Location of six study sites in Kosciuszko National Park, Australia. Contour map labels shown in blue (meters above sea level). Mount Perisher is located at -36.4094, 148.3863.

2.5.2 Sampling Protocol

We conducted sampling sessions during a wide range of weather conditions. While flower visitor surveys are often only conducted during fine weather (Brittain *et al.*, 2013), Goodwin *et al.* (2021) showed that in Australian alpine ecosystems, pollinators remain active in other weather conditions, with different taxa responding distinctly to conditions. To capture this range of responses, our study primarily sampled visitors in warmer weather (>10°C), but we also regularly conducted samplings in cooler conditions (5 to 10°C) to include species tolerant of lower temperatures (Supporting Information Table 1). Temperature during sampling sessions ranged from 5ºC to 28ºC, windspeed ranged from 0 to 23kmh, and humidity ranged from 27 to 93%. Due to a strong La Nina event, temperatures during the sample period were below long-term averages, while rainfall was above average (Bureau of Meterology, 2023).

At each of the six elevation bands, we established two 50x50m plots nearby (50-100m) to one another. We chose plots in order to maximise the diversity and abundance of flowering plants at each site. We observed pollinators at each study site while plants were flowering. We sampled several times per week at each plot across the sample period and ensured that each plot was sampled during different times of day. Each of our sampling periods consisted of 30 minutes of observation, whereupon we walked through each plot and recorded which pollinator species were visiting which plant species and the frequency of each interaction. We only recorded a visit if an insect appeared to contact the reproductive parts of a flower. We counted all insect taxa that visited flowers (Diptera, Hymenoptera, Lepidoptera, Orthoptera, Coleoptera) and took efforts to ensure that sampling was conducted so that observers' shadows did not land on flower-visiting insects. We identified insect species on the wing (i.e., without collection), collecting voucher specimens for later identification when necessary. We identified plants in the field using field guides and advice from locally-expert botanists. At the beginning and end of each sampling period, we recorded air temperature, windspeed, and humidity using a Kestrel 3000 Weather Meter (Kestrel Australia, Victoria, Australia), averaging these values for each sampling period. We sampled between 9am and 5pm from October 2021 to February 2022. Because the flowering season was shorter at the higher sites, our sampling effort was unevenly spread across sites (Supporting Information Table 1), with a higher sampling effort at lower sites.

Whereas we had expertise in identification of Hymenoptera and Diptera, we sought identification assistance from Hermes Escalona for Coleoptera and Michael Braby for Lepidoptera at The Australian National Insect Collection, CSIRO, and Michael Batley for cryptic Hymenoptera at The Australian Museum. Using reference material at the Australian National Insect Collection, we identified insects to the highest taxonomic resolution, in most case species, with morphospecies identifiers assigned when necessary. We deposited all 1422 insect specimens into the Australian National Insect Collection.

2.5.3 Statistical Analysis

2.5.3.1 Network Metrics

We conducted all statistical analysis in R version 4.2.2 (R Core Team, 2023). We used the *networklevel()* and *grouplevel()* functions in the 'bipartite' package (Dormann *et al.*, 2009) to compute the commonly-used pollination network metrics of connectance, network nestedness (Almeida-Neto and Ulrich, 2011), pollinator diversity (calculated as Shannon diversity of pollinators), and network specialisation (H_2') at species-level (Bascompte *et al.*, 2003; Jordano *et al.*, 2003; Blüthgen *et al.*, 2008). We tested the significance of network metrics by comparison with null models created using the *vaznull* function (Dormann *et al.*, 2009). These null models randomized plant-pollinator interactions while maintaining network connectance, number of interactions, and species richness. We generated 500 null models and calculated *z*-scores to determine significance (Encinas-Viso *et al.*, 2022).

2.5.3.2 Impact of Abiotic Conditions on Pollinator Activity

We used generalised linear mixed models (GLMM) using the *glmmTMB* function within the 'glmmTMB' package (Brooks *et al.*, 2017) to determine the impact of elevation and weather on pollinator activity within each observation period. Whereas bipartite network analysis was conducted at insect species-level, this analysis was conducted at order-level, following similar studies across environmental gradients (Adedoja *et al.*, 2018; Lefebvre *et al.*, 2018; McCabe and Cobb, 2021). We conducted modelling separately for each of the most frequent insect orders observed (Diptera, non-Formicidae Hymenoptera, Coleoptera), with other taxa (Lepidoptera, Orthoptera, Formicidae, Hemiptera) excluded from analysis due to a low sample size. We included temperature, windspeed, humidity, flower species richness, and elevation as predictor variables, with pollinator activity levels within each observation period as the response variable. Data were pooled for the two adjacent plots at each site, so that site was treated as the replicate. While weather varied with each sampling session, elevation (a proxy for climate) was linked to study site. By including elevation and weather variables separately, we were able to determine if there is a residual effect of elevation after allowing for the separate effects of weather variables. We scaled all predictor variables to allow for direct comparison. We also included the quadratic term for elevation in each model, to test for curvilinear responses that might suggest an elevational envelope where pollinators are best adapted (Lefebvre *et al.*, 2018). To check for correlations between predictor variables, we computed Pearson's correlation coefficients using the *cor()* function in the 'stats' package, following Dormann *et al.* (2013). Because all variables showed coefficients < 0.7, we kept all variables in the analysis. We assumed negative binomial error distributions for count response variables because overdispersion was present using Poisson distributions. We included sample date as a random effect, to control for potential temporal non-independence (Zuur *et al.*, 2009). We inspected histograms of residuals to check for normality, variable inflation factor for collinearity, and overall model performance using the *check_model()* function in the 'performance' package. We accounted for zero inflation, where present, by fitting zero-inflation models, specifying the same model formula for the zero-inflation component as the conditional component of the model in the *glmmTMB* specification.

2.5.3.3 Pollinator Floral Associations

To determine the influence of floral associations on pollinator community dynamics across elevation, we modelled interaction frequency separately for the four plant families whose flowers were most frequent in our visit observations, i.e., Asteraceae, Proteaceae, Ericaceae and Fabaceae. While there were key genera and species within these families common between some sites (Supporting Information Table 3 (Costin *et al.*, 2000)), we conducted this analysis at family level because there were too few genera or species that occurred widely across our transect. While species-level specialisation is relatively rare and many pollinators are generalists, able to collect food from many plant species (Olesen and Jordano, 2002), specialisation commonly occurs at higher levels of phylogeny (such as family) (Grant and Grant, 1965; Tremblay, 1992; Ollerton and Liede, 2008; Zhang *et al.*, 2010; Chartier *et al.*, 2014). Family-level groupings can also capture shared functional traits, such as flower shape, which are significant in understanding pollinator behaviour and preferences (Harder and Johnson, 2009; Zhang *et al.*, 2010; Xiang *et al.*, 2023). This analysis allowed us to determine whether certain plant families were visited more by one of the three orders of pollinators, and whether these patterns changed with elevation. We constructed generalised mixed models using the *glmmTMB()* function in the 'glmmTMB' package, with the linear and quadratic terms for elevation as predictor variables (Lefebvre *et al.*, 2018). We performed model specification and validation using the same methods as above GLMMs.

We quantified the frequency of flower visits by the three insect orders to these four plant families at each elevation, to examine whether the pattern was influenced by elevation. In the absence of floral community surveys conducted independently from flower visitor observations, this approach does not provide a measure of floral abundance at each site, and instead quantifies the use of each plant family by pollinators at each elevation band (Dormann, 2020). This approach places the focus only on those plants that are visited by pollinators, and does not provide a measure of flower abundance that is independent of pollinator use.

Additionally, we analysed phenology of key plant and pollinator taxa across our study system, using Generalised Additive Models (see Supporting Information).

2.5.3.4 Activity Niche Hypervolumes

To quantify differences in activity niche between each pollinator order, we constructed multidimensional hypervolumes. This method allowed us to compare the combined influence of environmental variables, enabling more nuanced comparisons than are possible with two-dimensional methods (Blonder *et al.*, 2014). While regression modelling shows insects' responses to each predictor variable individually, activity niche hypervolumes reveal how the combination of weather variables influenced insect activity. We constructed hypervolumes using the 'hypervolume' package in R (Blonder *et al.*, 2018), using the support vector machine method, with default parameters. We used temperature, humidity, and windspeed as variables for each hypervolume. We centre scaled each of these variables using the *scale* function in the 'base' package. To avoid differences in hypervolume size resulting from disparity in sample size between pollinator orders, we used the *resample_data* function within the 'fabricatr' package (Blair *et al.*, 2023) to resample each order to $n = 1200$ (Blonder *et al.*, 2014). We used the function *hypervolume_volume* to quantify and compare the volume of each hypervolume. The outer boundaries of the hypervolumes illustrate differences in each order's activity niche breadth. To calculate niche dissimilarity between each pollinator order, we used the *kernel.beta* function within the 'BAT' package (Mammola and Cardoso, 2020). This function computes three dissimilarity measures: 'β_{total}', a measure of the shared space between hypervolumes, with 1 indicating no shared space and 0 indicating complete overlap. '$\beta_{\text{replacement}}$' measures the replacement of hypervolume space between two hypervolumes, while 'β_{richness}' measures the difference in the amount of space enclosed by each hypervolume (Carvalho and Cardoso, 2020; Mammola and Cardoso, 2020).

2.6 Results

We sampled a total of 167 insect species from 5,131 individual pollinator visits to flowers across 121.5 hours of observation. For all sites combined, Diptera were the most frequent flower visitors (45.7%), followed by Coleoptera (27.16%), Hymenoptera (22.2%), and Lepidoptera (4.4%). This pattern varied with elevation, with Diptera dominance increasing with elevation. The introduced species *Apis mellifera* made up 37% of Hymenoptera visits and Apoidea more broadly made up 86% of Hymenoptera visits. The frequency of *Apis mellifera* across the elevational gradient was consistent with the pattern for Hymenoptera more broadly. Because our study was located within a large National Park where beekeeping is prohibited, *A. mellifera* present at our study site most likely originated from wild nests. For a full list of plant and pollinator species occurrences at each site, see Supporting Information Table 2 and 3.

At the site level, there was variation in pollinator species richness with elevation. At all elevations, Diptera was the most species-rich order. Hymenoptera richness peaked at the lowest elevation and then declined with elevation, so that Diptera composed a greater proportion of the pollinator species with increasing elevation. Coleoptera maintained low levels of species richness across all elevations (Table 1).

Table 1. Species richness for all pollinators and the three major pollinator orders observed during flower visitor surveys at each elevation.

Elevation (m a.s.l.)	Pollinator richness	Hymenoptera richness	Diptera richness	Coleoptera richness
1000	72	22	36	9
1200	64	21	26	14
1400	55	18	22	5
1600	59	11	34	9
1800	61	6	34	7
2000	55	3	36	7

2.6.1 Network Structure

Pollination network metrics showed no discernible pattern across the elevational gradient. Moderate levels of network specialisation (H2’) were present across all sites. Low levels of both network connectance and nestedness were present across all sites, though nestedness was statistically insignificant at most sites (Table 2). See

Supporting Information Figures 1 to 6 for bipartite pollinator network diagrams for each site.

Table 2. Pollination network metrics and pollinator diversity for the three major pollinator orders at each elevation. Pollinator diversity refers to Shannon diversity of pollinators at each site. z-values and p-values presented below each metric in parentheses.

Elevation	Pollinator diversity	Network specialisation (H_2')	Connectance	Network nestedness
1000m	0.965 * (-10.218, <0.05)	0.527 * (36.61, <0.05)	0.102 * (-15.922, <0.05)	7.916 * (6.577, <0.05)
1200m	1.12 * (-5.858, <0.05)	0.492 * (19.7, <0.05)	0.079 * (-16.77, <0.05)	3.050 (-1.057, 0. 291)
1400m	1.045 * (-2.25, 0.02)	0.461 * (17.41, <0.05)	0.115 * (-20.687, <0.05)	9.416 * (3.234, <0.05)
1600m	1.11 * (-4.016, <0.05)	0.454 * (11.324, <0.05)	0.113 * (-14.742, <0.05)	9.154 (1.372, 0.17)
1800m	0.967 * (-7.868, <0.05)	0.502 * (13.612, <0.05)	0.109 * (-17.574, <0.05)	7.548 * (2.018, <0.05)
2000m	1.076* (-4.082, <0.05)	0.510 * (11.667, <0.05)	0.110 * (-21.2, <0.05)	8.031 (0.266, 0.789)

2.6.2 Effect of Weather and Elevation on Pollinator Activity

We removed time of day as a predictor from each model to achieve the most parsimonious model, as its inclusion was not influential (ΔAICc >2) and caused model convergence issues. Insect activity varied in response to weather and elevation with each order responding differently. Coleoptera and Hymenoptera showed similar responses to elevation, with a strong decrease in activity with elevation gain. Conversely, Diptera activity showed a considerable increase with elevation, with strong statistical significance. No significant effect was found for the quadratic term for elevation (Figure 2, Supporting Information Table 4). Temperature had the most significant effect on insect activity, with a positive relationship present in all orders. Diptera displayed higher tolerance to cooler temperatures, commencing activity at lower temperatures than Hymenoptera and Coleoptera, while activity levels for Hymenoptera and Coleoptera increased more sharply with temperature (Figure 2, Supporting Information Table 4). Insect activity had a positive relationship with humidity, with some variation present among orders (Figure 2). However, this relationship was statistically significant only for Coleoptera activity levels (Supporting Information Table 4). Activity levels for all orders displayed a negative relationship with windspeed, though with limited statistical significance (Figure 2, Supporting Information Table 4). Flower species richness was not a significant predictor of activity (Supporting Information Table 4).

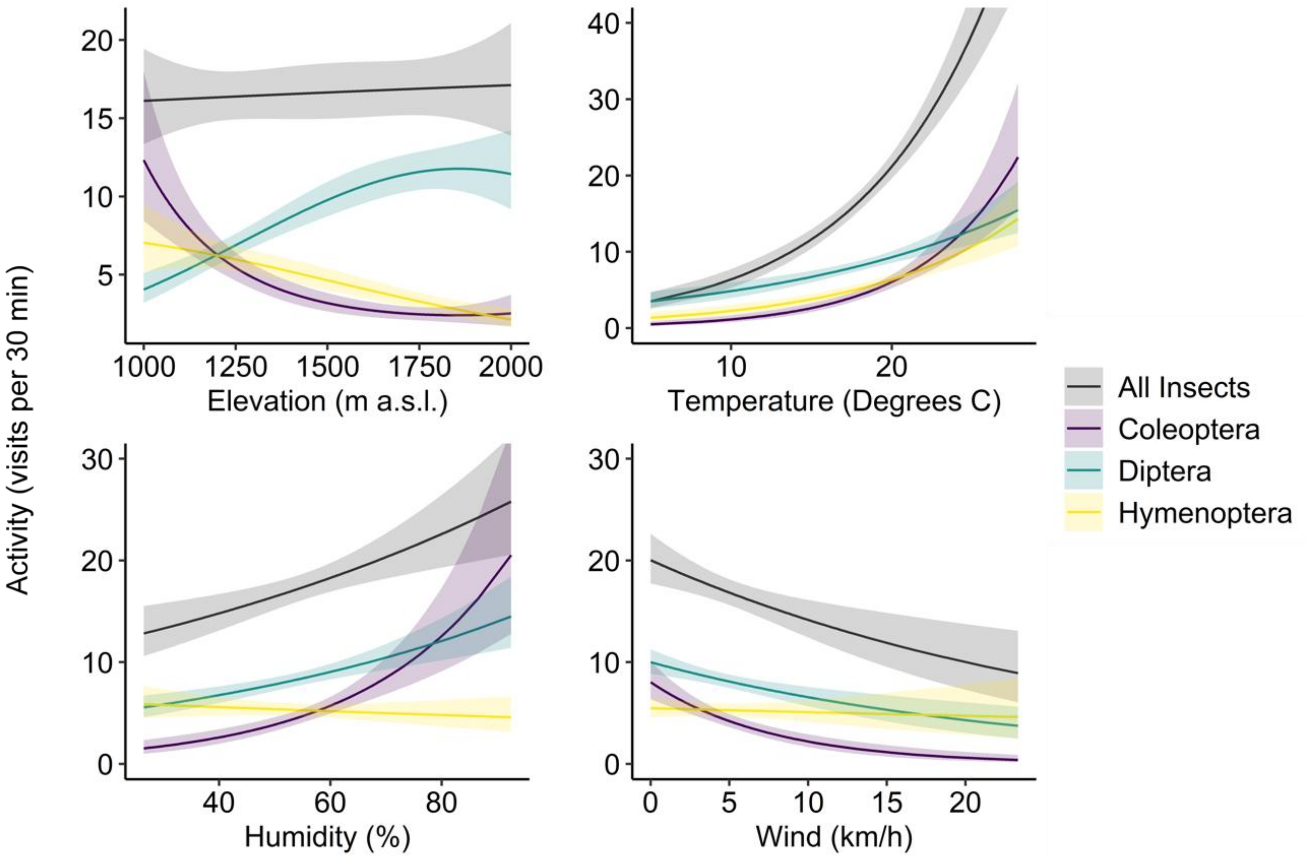


Figure 2. Predicted response plots for elevation and weather predictors for each of the main pollinator orders.

2.6.3 Pollinator Floral Associations

We observed visits to 69 plant species from 27 families. Three quarters of all visits were recorded from just four plant families: Asteraceae (25.8%), Proteaceae (21.9%), Ericaceae (14.7%) and Fabaceae (12.2%). The frequency of visits to these families changed with elevation. Visits to Proteaceae and Ericaceae were more frequent at lower elevations and decreased with elevation while the frequency of visits to Asteraceae increased (Figure 3). Species richness within these plant families followed similar trends (Table 3).

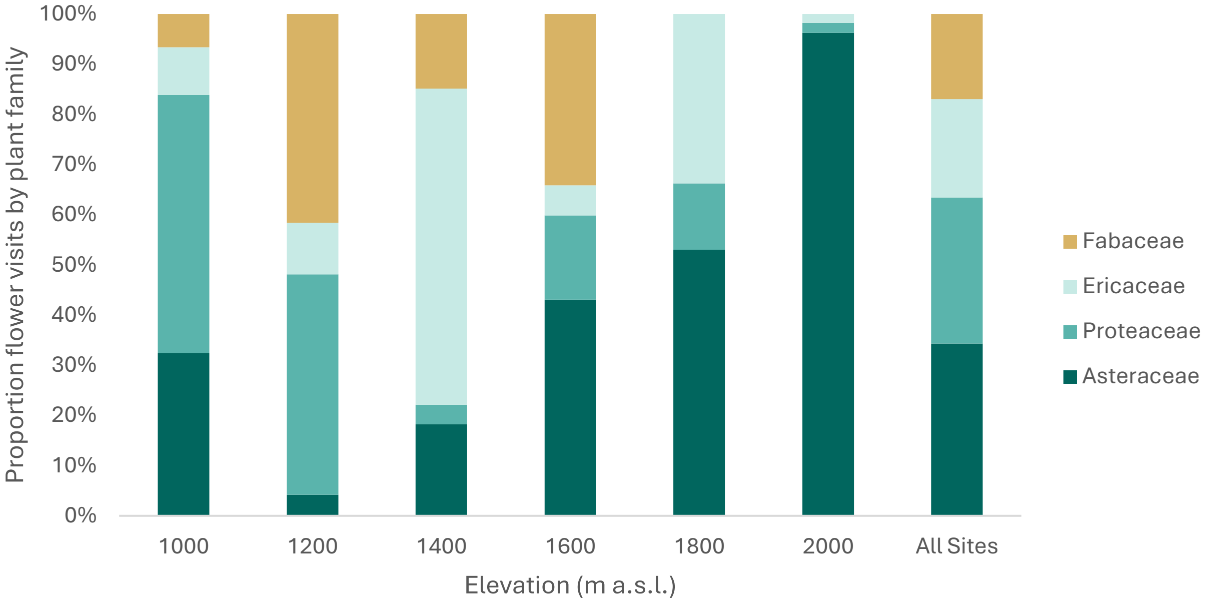


Figure 3. The four plant families most frequently observed in the flower visitor survey.

Table 3. Species richness for the four major plant families observed during flower visitor surveys at each elevation.

Elevation (m a.s.l.)	Asteraceae richness	Ericaceae richness	Fabaceae richness	Proteaceae richness
1000	2	2	4	2
1200	5	3	6	3
1400	5	3	2	3
1600	5	2	4	2
1800	8	3	0	1
2000	8	3	0	1

Some plant species also displayed strong associations with pollinator orders, which may be driving much of the family-level signal. For instance, almost half of Hymenoptera and Coleoptera visits were made to *Grevillea lanigera* and *Hakea lissosperma* (Proteaceae), respectively; species that are typical of woodland sites (Duncan, 2010) and which were not observed at higher-elevation sites. Conversely, Diptera were most abundant on Asteraceae species *Olearia phlogopappa, Craspedia lamicola*, and *Celmisia costiniana*, which are highly abundant at high elevations (Inouye and Pyke, 1988; Costin *et al.*, 2000; Duncan, 2010; Encinas-Viso *et al.*, 2022). For the full plant and pollinator species checklist, see Supporting Information Table 2 and 3.

We observed differences in the frequency of visits by elevation when the four main plant families were modelled separately for the three main orders of pollinators. Hymenoptera were very frequent on Proteaceae at the lowest elevations, and Fabaceae at the mid elevations. At the highest elevations their visit frequencies were low across all four families. Diptera were most frequently seen on Asteraceae at the lowest and highest elevations, peaking at the highest sites. At mid elevations, visits to Ericaceae were most frequent. Proteaceae and Fabaceae were relatively rarely visited at any elevation. The pattern for Coleoptera was similar to Diptera, although they occasionally visited Proteaceae, which Diptera did not (Figure 4).

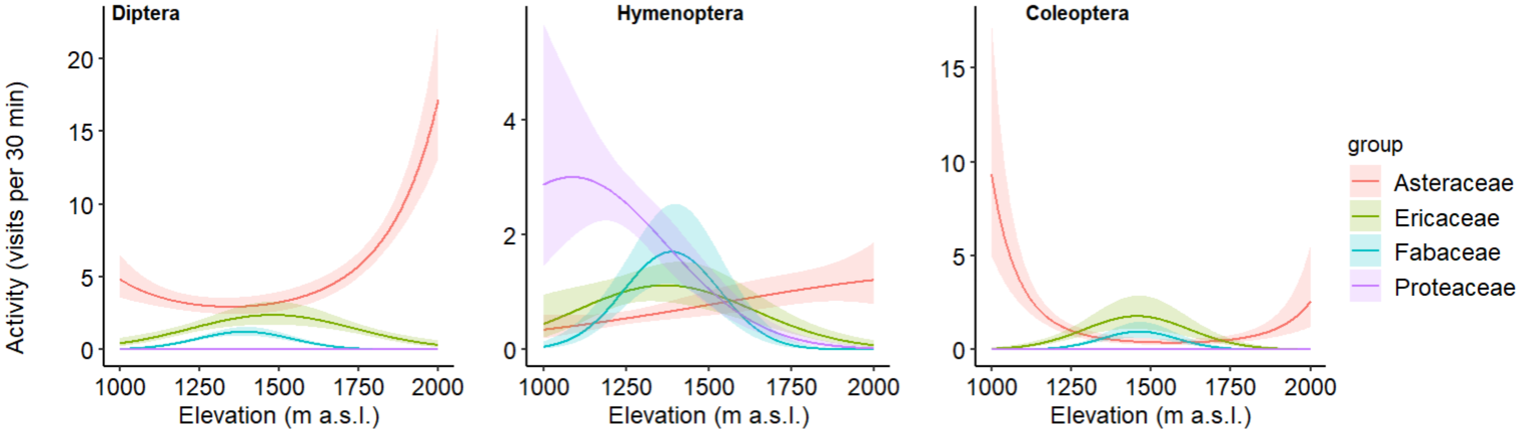


Figure 4. Response plots showing pollinator visits to four main plant families across the elevation gradient.

2.6.4 Plant and Pollinator Phenology

The timing of flowering varied with elevation, with lower sites commencing and finishing earlier than higher sites. Phenological patterns were also present in key plant and pollinator taxa, with variation in activity across the season. While Hymenoptera and Coleoptera flower interactions were more frequent earlier in the season at lower sites, Diptera flower visits were more frequent later in the season and at higher sites. Similarly, Proteaceae and Fabaceae interactions were more frequent earlier in the season and at lower sites, while Asteraceae interactions were more frequent later in the season and at higher sites (Supplementary Information Figures 8 to 11).

2.6.5 Activity Niche Hypervolumes

Among the pollinator orders, Diptera exhibited a broader activity niche breadth than Coleoptera and Hymenoptera. Pairwise niche plots revealed that Diptera were more tolerant of windy and humid conditions, as well as cold and windy conditions (Figure 5). The centroids of Hymenoptera and Coleoptera were indicative of an activity niche characterised by warmer conditions compared to Diptera. Diptera's hypervolume (50.39) was also more than twice that of Hymenoptera (20.59) and Coleoptera (22.67) (Supporting Information Figure 7), indicating a larger activity niche breadth. Conversely, Hymenoptera shows the narrowest breadth, and its niche centroid indicating lower tolerance for adverse conditions. Coleoptera occupies an intermediate position between Diptera and Hymenoptera in terms of its activity niche.

Metrics of niche dissimilarity also show that Diptera differed from Coleoptera and Hymenoptera, which were comparatively similar (Supporting Information Figure 7). For β_{total} (a measure of shared space between two hypervolumes), activity niche was relatively similar between the three orders. However, β_{richness} (net difference in the space enclosed in each hypervolume) was higher, and $\beta_{\text{replacement}}$ (the replacement of niche space between two hypervolumes) was lower in pairwise comparisons including Diptera.

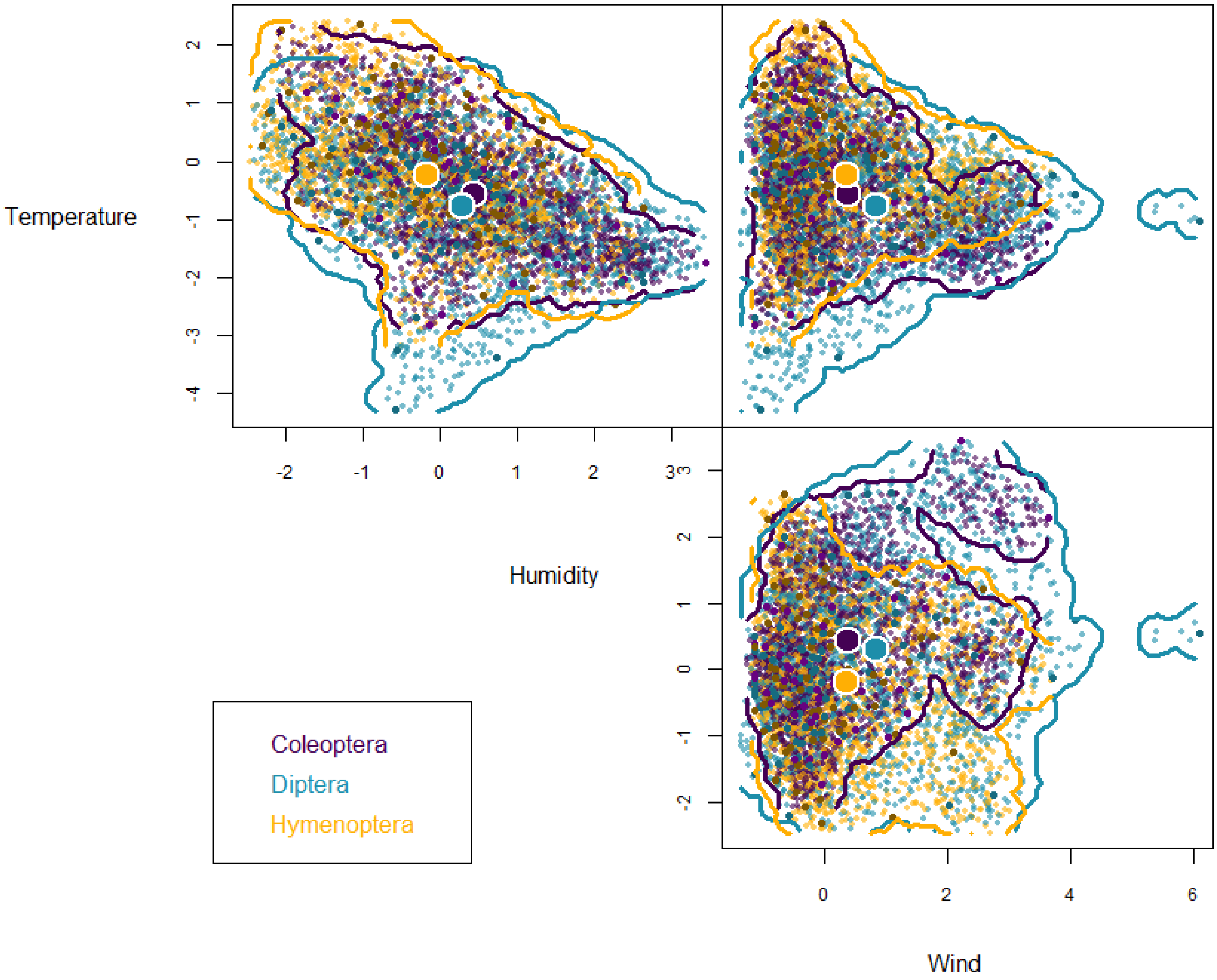


Figure 5. **Pairwise niche plots characterising the range of weather conditions (activity niche) under which taxa from each order were observed interacting with flowers. Solid datapoints indicate input data, while opaque datapoints show points randomly generated during modelling. Centroid is indicated by large circle. Outer bounds indicated by coloured line.**

2.7 Discussion

Here we demonstrate that while abiotic and biotic processes both drive changes in pollinator network composition along an elevational gradient, network structure remained relatively stable. Our results demonstrate that weather, climate, and the floral community all influence pollinator interaction frequencies across an elevation gradient. Pollinator composition changed with elevation, consistent with similar systems globally (Lara-Romero *et al.*, 2019; Chesshire *et al.*, 2021), with an increased activity of Diptera species with elevation, while the activity of Coleoptera and Hymenoptera decreased. Overall, pollinator species richness declined with elevation, consistent with other studies (Medan *et al.*, 2002; Adedoja *et al.*, 2018). However, network structure did not follow trends seen elsewhere (Inouye and Pyke, 1988; Hoiss *et al.*, 2012; Lara-Romero *et al.*, 2019; Inouye, 2020), with little change with increased elevation and unusual network properties compared to other alpine systems. We show that the association between elevation and pollinator interaction frequency is largely shaped by climate, which regulates both the geographical distribution and the population sizes of pollinators and flowering plants. Notably, each pollinator order also displayed associations with flower families that were consistent with plant family interaction frequency and richness across the elevational gradient, suggesting additional mechanisms through which climate influences pollinator distributions. However, within species' distributions, pollinator activity is strongly influenced by weather, with Diptera displaying a broader activity niche breadth, indicative of greater tolerance of colder, windier, and more humid conditions.

2.7.1 Effect of Weather and Elevation on Insect Activity

Although individual Diptera, like most ectothermic insects, are more active in warm weather (Mellanby, 1939), there were nevertheless more Diptera species active overall at higher elevations, where the climate is coldest. This may be explained by some Diptera species' increased cold tolerance and subsequent broader activity niche breadth, which allows them to remain active under colder conditions. While our analysis aggregated pollinator data at the order-level, there may also be patterns at family, genus and species level underlying the observed order-level pattern. Due to the higher species richness of Diptera at high-elevation sites, there were more species within this order compared to other pollinator orders, allowing for greater diversity of functional traits. This functional trait diversity may explain why Diptera's activity niche was more than twice that of Coleoptera and Hymenoptera, with different Diptera species displaying functional traits that allow them to survive across a broader range of conditions. It may be that some high-elevation Diptera species are more generalised in their feeding habits, such as some adult Calliphoridae (Parry *et al.*, 2021) and Tabanidae (Mullens, 2019) species that feed on non-floral resources such as animal waste and blood. Diptera larvae also often develop in carrion or aquatic environments, where they are not reliant on floral resources (Davis *et al.*, 2023). Species such as these may therefore have increased capacity to wait out unfavourable weather or operate in an environment where floral resources are only available for a limited part of the season. Conversely, bees require pollen and nectar to complete their life cycle, with adults provisioning young with these food resources (Michener, 2000).

Other studies have shown similar elevational patterns (McCabe and Cobb, 2021) and suggest that other pollinators cannot persist in cold climates, resulting in a vacant pollinator niche, which is subsequently occupied by Diptera species (Adedoja *et al.*, 2018; Lefebvre *et al.*, 2018). This “release from competition hypothesis” suggests that Diptera activity increases relative to other weather-sensitive insects because Diptera species that are active at high elevations have less competition for floral resources. Similar patterns of release from competition with elevation gain have also been observed in bees (Hoiss *et al.*, 2012), ants (Machac *et al.*, 2011), and hummingbirds (Graham *et al.*, 2009). This hypothesis implies that there is strong overlap in resource use, with pollinator taxa from different orders competing over the same flowers. Our data, however challenge this idea because they show that the three orders of insect pollinators have very different frequencies of interaction when comparing across the more frequent plant families.

2.7.2 Pollinator Floral Associations

While much research has focused on how elevation shapes the distribution of pollinators directly (McCabe *et al.*, 2019), our results reveal that this abiotic regulation occurs at least in part through a biotic pathway, via the effects of elevation on plant species distribution. We show that there are patterns of floral associations, whereby different flower visiting insects exploit some plant families more than others, so that changes in the plant community with elevation are therefore expected to drive changes in pollinator community composition, such as the increase in Diptera at higher elevations. Alongside the direct effect of weather and elevation, we observed taxaspecific floral associations, with pollinator orders showing different patterns of association when comparing across the four most frequently observed plant families. Asteraceae, which increase with elevation and are recognised as typical of alpine vegetation in the area (Inouye and Pyke, 1988; Costin *et al.*, 2000; Duncan, 2010; Encinas-Viso *et al.*, 2022), were most commonly visited by Diptera at all sites, and were rarely visited by Hymenoptera at any sites. Foremost among these were the Asteraceae species *Craspedia lamicola* and *Celmisia costiniata,* which are particularly abundant at high elevation (Costin *et al.*, 2000; Duncan, 2010). Conversely, at lower elevations, Hymenoptera were the most common visitors to Proteaceae and Fabaceae, whereas these plant families were scarcely visited by Diptera. For example the Proteaceae species as *Grevillea lanigera* and *Hakea lissosperma* were frequently visited at lower sites by Hymenoptera and Coleoptera species, but do not occur at our high-elevation sites (Duncan, 2010).

Phenological observations revealed temporal dynamics underlying the floral associations. For instance, both Asteraceae and Diptera showed increased interactions later in the season and at higher elevations, while Hymenoptera, Proteaceae and Fabaceae played a bigger role earlier in the season and at lower elevations. This limited overlap in floral resource use indicates that pollinators are not competing strongly for the same flowers in this system, showing an effect of floral association that contributes to the elevational distribution of pollinators. If floral resource composition changes across an elevational gradient, as seen in our study system, floral association may play a bigger role in determining pollinator distribution than previously recognised.

The frequency of Asteraceae species in communities is well recognised in high-elevation and latitude systems elsewhere (Silva Mota *et al.*, 2018; Pires *et al.*, 2020; Baskin and Baskin, 2023), and may also contribute to the frequency Diptera species in these locations. Some other studies have also indicated the importance of flowering plants in determining insect activity across elevational gradients (Pellissier *et al.*, 2010; Basnett *et al.*, 2019), demonstrating the importance of floral traits (Pellissier *et al.*, 2010; Basnett *et al.*, 2019; Ahmad *et al.*, 2023) and floral cover (Hoiss *et al.*, 2012). Pires *et al.* (2020) also demonstrated that floral community was more important than abiotic conditions in determining Lepidoptera richness and abundance across an elevational gradient. We therefore conclude that the elevational patterns in the activity of pollinators in our data is likely driven by both differences in the abundance of their associated plants, alongside the direct influence of climate on pollinators as suggested by other studies (Adedoja *et al.*, 2018; Lefebvre *et al.*, 2018). The pattern could be also be influenced by other factors that vary with elevation that we did not survey, such as canopy cover (McCabe *et al.*, 2019), precipitation (Devoto *et al.*, 2005), nesting resource availability (Bates *et al.*, 2011), or the occurrence of extreme weather events (Hoiss *et al.*, 2015).

The floral associations that we document could reflect coevolutionary relationships between flowers and their associated pollinators. Mutualistic plant-pollinator interactions drive ecological divergence (Ehrlich and Raven, 1964; Thompson and Cunningham, 2002), including through trait matching (Lunau, 2004) and geographical distribution (Thompson and Cunningham, 2002). For instance, a coevolutionary association between Diptera and Asteraceae may explain why Diptera species are common visitors to Asteraceae at all elevations in our study, even the lower elevations where Coleoptera and Hymenoptera species are more abundant. In our study system, the dominance of Diptera-attracting Asteraceae at high elevations may have evolved due to the availability of Diptera at high elevations. High-elevation plants may have evolved features that allow them to persist in environments where their preferred, cold-tolerant pollinators, such as Diptera species, are distributed (Pellissier *et al.*, 2010). Alternatively, Diptera species might have evolved an association with Asteraceae because these flowers are abundant at high elevations, with plant distribution responding primarily to abiotic conditions that vary with elevation (Körner, 2007; Junker and Larue-Kontić, 2018) and the association between Diptera and Asteraceae at lower elevations might have followed from the association formed first at high elevation.. There is evidence to support both plant-mediated (Körner, 2007) and pollinator-mediated (Basnett *et al.*, 2021) community assemblage in alpine pollination networks. While we cannot determine the direction of coevolution in our study, we can observe that floral use patterns were consistent across elevations, with patterns present that demonstrate consistent plant-insect associations.

2.7.3 Network Structure

While the shift in pollinator community composition shown here follows trends seen elsewhere, with increasing dominance of Diptera at high elevations (Kearns, 1992; Inouye, 2020), pollination network structure did not show that same pattern presented in other elevation gradient studies. In other studies, a decrease in specialisation (Lara-

Romero *et al.*, 2019; Classen *et al.*, 2020), nestedness (Classen *et al.*, 2020) and connectance (Lara-Romero *et al.*, 2019), and pollinator diversity (Arroyo *et al.*, 1982; Arroyo *et al.*, 1985; Hodkinson, 2005b) have been detected with increasing elevation. In contrast, we saw no decrease in pollinator diversity with elevation, and otherwise moderate levels of network specialisation, low levels of connectance and very low nestedness with no consistent changes across the gradient in any of these measures. Network specialisation was higher than expected for alpine communities, with values similar to montane networks in the Andes (Watts *et al.*, 2016). Nestedness values were also unusually low for plant-pollinator networks (Toju *et al.*, 2015), though they were not statistically significant compared to null models. Encinas-Viso *et al.* (2022) similarly found low levels of connectance and moderate levels of network specialisation working in sites nearby to our highest sites. Network nestedness and connectance have been shown to relate to the stability and resilience of mutualistic networks (e.g., plant-pollinator communities) (Encinas-Viso *et al.*, 2022), as well as network robustness (i.e., based on the number of secondary extinctions) (Dunne *et al.*, 2002; Estrada, 2007). Low levels of pollination network nestedness and connectance may indicate low resilience to disturbance and extinction events (Piazzon *et al.*, 2011; Bascompte and Scheffer, 2023). The difference between our results and other similar studies may be explained by the relatively limited elevational extent of the Australian alps compared to other systems. For instance, while we sampled a transect of ~1000m elevational extent up to ~2000m a.s.l., similar studies conducted in the Andes sampled an elevational extent of ~1400m up to ~3600m a.s.l. (Arroyo *et al.*, 1982; Arroyo *et al.*, 1985) and ~1400m up to ~3300m a.s.l. (Medan *et al.*, 2002), while studies in the Himalayas sampled an extent of ~1400m up to ~4200m a.s.l. (Basnett *et al.*, 2019) and ~1200m up to ~3900m a.s.l. Global variation in the altitude of the tree line may also be a factor, with Australia's tree line relatively low at ~2000m a.s.l. (Costin *et al.*, 2000), compared to 4200m a.s.l. in the Himalayas (Shi *et al.*, 2020) and ~4800m a.s.l. in the Andes (He *et al.*, 2016). Our study system also lacks cold-adapted *Bombus* bees that dominate many mountain pollination networks in the Northern Hemisphere (Minachilis *et al.*, 2020). However, some other studies have also found unexpected network properties across elevational gradients (Ramos-Jiliberto *et al.*, 2010; Minachilis *et al.*, 2020; Chesshire *et al.*, 2021), indicating network metrics do not always predictably vary with elevation as is often suggested.

2.8 Conclusion

By studying the effects of weather, elevation and floral community, our results provide insights into how pollinator communities are structured along elevational gradients. Other studies illustrate that climate change will impact pollination networks in alpine environments by driving asynchronous range shifts, with many pollinator species shifting upslope as temperatures warm (Parmesan *et al.*, 1999; Forister *et al.*, 2010). Our data suggest that such shifts could change community composition at high-elevation sites. For instance, Hymenoptera may play a greater role in mountain pollination networks, where previously they were restricted by cold temperatures. Similarly, McCabe and Cobb (2021) suggest that Hymenoptera populations may expand their range under current warming predictions, shifting upslope by as much as 200-400 metres in mountain environments (McCabe and Cobb, 2021). However, by showing that floral associations also influence pollinator activity, we suggest that these shifts will also hinge on the availability and presence of suitable plants as well as suitable temperatures. Our findings also indicate that upslope shifts in Hymenoptera species may not necessarily lead to increased competition for Diptera species if they visit different flowers.

Our study represents a comprehensive analysis of the interplay between abiotic and biotic factors in shaping pollinator communities across an elevational gradient in mountain ecosystem in Australia. Our findings underscore the importance of considering both floral associations and abiotic factors in understanding pollinator distribution and network dynamics, where previously research on the elevational distribution of pollinators primarily considered abiotic factors. We present the 'floral association hypothesis' as a key explanatory factor, highlighting the role of plant community composition in driving pollinator preferences and distributions. Our study contributes to the broader understanding of mountain ecosystems, illustrating the complex relationships between pollinators, plants, and environmental gradients. Future research should continue to explore these interactions, particularly in light of climate change and its potential impacts on these sensitive ecosystems.

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2.11 Supplementary Material

Table S1. Heatmap showing spread of temperatures sampled at each elevation. Greener cells indicate higher sampling frequency.

Temperature (Degrees Celsius)	1000m a.s.l.	1200m a.s.l.	1400m a.s.l.	1600m a.s.l.	1800m a.s.l.	2000m a.s.l.
5	0	0	0	1	0	0
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	0	0	0	0	0	0
9	0	1	1	1	0	0
10	0	2	1	0	0	0
11	2	3	0	1	0	0
12	0	1	0	1	0	1
13	0	2	2	2	2	2
14	2	0	2	2	2	1
15	2	0	2	4	3	3
16	2	3	1	1	4	4
17	4	1	4	3	3	5
18	5	6	4	4	4	5
19	4	3	3	0	2	7
20	4	9	6	2	3	7
21	7	6	3	3	7	1
22	6	1	2	0	3	2
23	4	2	2	5	2	1
24	5	8	2	2	1	0
25	2	0	2	0	0	0
26	2	2	0	0	0	0
27	0	0	1	0	0	0
28	1	1	0	0	0	0
29	0	0	0	0	0	0
30	0	0	0	0	0	0
	0	0	0	0	0	0
Total sampling periods per site	52	51	38	32	36	39

Table S 2. Insect taxa observed during plant-pollinator surveys at each elevation.

Insect Taxa	1000m	1200m	1400m	1600m	1800m	2000m	Total
ORDER COLEOPTERA	**674**	**105**	**199**	**112**	**78**	**59**	**1227**
indet. Coleoptera	1			1		1	3
Family Belidae		**1**					**1**
Rhinotia sp.		1					1
Family Buprestidae		**2**	**1**	**3**		**19**	**25**
Buprestidae sp. 6		2	1	3			6
Castiarina montigena						19	19
Family Cantharidae		**1**	**188**	**75**	**21**	**29**	**314**
Chauliognathus lugubris		1	188	75	21	29	314
Family Carabidae	**14**	**20**					**34**
Carabidae sp. 1	14	20					34
Family Cerambycidae		**1**					**1**
Ochyra sp. 1		1					1
Family Chrysomelidae	**1**	**5**	**5**		**1**		**12**
Chrysomelidae sp. 2		1	5				6
Chrysomelina sp. 1	1	2					3
Chrysomelidae sp. 1		2			1		3
Family Cleridae	**3**		**4**	**12**	**7**		**26**
Cleridae sp. 1			4	7	7		18
Cleridae sp. 2	3			5			8
Family Coccinellidae		**12**				**1**	**13**
Coccinellini sp. 1		1					1
Coccinellini sp. 2		10					10
Coccinellini sp. 3						1	1
Microweiseinae sp. 1		1					1
Family Curculionidae						**1**	**1**
Curculionidae sp. 2						1	1

Family Elateridae					**1**		**1**
Crepidomenus sp. 1					1		1
Family Lycidae	**9**			**1**			**10**
Porrostoma rhipidium	9			1			10
Family Mordellidae	**48**		**1**	**8**	**16**	**2**	**75**
Mordellidae sp. 1	48		1	8	16	2	75
Family Phalacridae	**1**	**1**					**2**
Phalacridae sp. 1	**1**	1					2
Family Scarabaeidae	**2**			**12**	**32**	**6**	**52**
Diphucephela sp. 1				7			7
Diphucephela sp. 2				5	1		6
Phyllotocus rufipennis	2				31	6	39
Family Tenebrionidae	**595**	**62**					**657**
Alleculinae sp. 1	1						1
Atoichus bicolor	594	62					656
ORDER DIPTERA	**304**	**246**	**425**	**247**	**404**	**441**	**2067**
indet. Diptera	21	4	19	19	65	32	160
Subsection Acalyptratae				**1**		**1**	**2**
indet. Acalyptratae sp. 2				1			1
indet. Acalyptratae sp. 6						1	1
Family Bibionidae			**9**	**8**			**17**
Dilophus cf. *dubius*				1			1
Dilophus cf. *longirostris*			8	7			15
Plecia dimidiata			1				1
Family Bombyliidae	**23**				**3**		**26**
Aleucosia sp. 1					3		3
Aleucosia sp. 2	3						3
cf. *Staurostichus* sp. 1	5						5
cf. *Staurostichus* sp. 2	15						15

Family Calliphoridae	**51**	**10**	**25**	**30**	**103**	**16**	**235**
Calliphora (Paracalliphora) auger	2						2
Calliphora (Paracalliphora) sp. 1	8	2	3	8	22	4	47
Chrysomya sp. 1					1		1
cf. *Calliphora (incertae sedis)* sp. 1	2	2	12	15	51	1	83
cf. *Calliphora (incertae sedis)* sp. 2		1	3		1		5
cf. *Calliphora (incertae sedis)* sp. 3		3		2	10	2	17
cf. *Calliphora (incertae sedis)* sp. 4	39	2	7	5	18	9	80
Family Chloropidae	**1**	**1**	**10**				**12**
cf. *Tricimba* sp. 2			10				10
Chloropidae sp.1	1	1					2
Family Conopidae	**1**						**1**
cf. *Camrasiconops* sp. 1	1						1
Family Empididae	**3**	**37**		**1**	**11**	**33**	**85**
Empididae sp. 1		1				1	2
Empididae sp. 3	1					7	8
Empididae sp. 4	2	36				1	39
Empididae sp. 5				1		1	2
Empididae sp. 6					11	23	34
Family Lauxaniidae	**4**	**18**	**1**	**25**	**36**	**211**	**295**
cf. *Rhagadolyra* sp. 1	1	9					10
cf. *Sapromyza* sp. 1	2						2
cf. *Sapromyza* sp. 2		7					7
cf. *Sapromyza* sp. 3						1	1

Incurviseta sp. 1					10	167	177
Incurviseta sp. 2				6		2	8
Lauxaniidae sp. 2			1			3	4
Lauxaniidae sp. 3					6	1	7
Lauxaniidae sp. 5					1		1
Poecilohetaerus sp. 1	1	2		19	19	37	78
Family Muscidae	**8**	**7**	**10**	**26**	**13**	**27**	**91**
cf. *Limnophora* sp. 1	1			2			3
Coenosia sp. 1					1	1	2
Coenosia sp. 2	2			3	2		7
Helina sp. 1	2	5		1		1	9
Helina sp.2	1	1		1	3		6
Musca vetustissima	1	1		12	1	23	38
Muscidae sp. 1			10	7	5	2	24
Pyrelia sp. 1	1				1		2
Family Pelecorhynchidae			**4**			**1**	**5**
Pelecorhynchus sp. 2			4			1	5
Family Platystomatidae		**1**	**10**	**2**	**2**	**14**	**29**
Platystomatinae gen.1 sp.1		1	10	2	2	14	29
Family Polleniidae					**1**		**1**
Pollenia sp. 1					1		1
Family Rhagionidae	**5**	**1**		**4**	**3**		**13**
Atherimorpha sp. 1	5	1		4	3		13
Family Rhinidae		**1**		**1**			**2**
Stomorhina discolor		1		1			2
Family Sciaridae				**4**			**4**
Sciaridae sp. 4				3			3
Sciaridae sp.2				1			1
Family Stratiomyidae	**2**		**7**			**3**	**12**

Chiromyza sp. 1						2	2
Odontomyia sp. 1	2		7			1	10
Family Syrphidae	**169**	**161**	**307**	**107**	**76**	**45**	**865**
Eristalis tenax					1	4	5
Melangyna viridiceps	161	148	307	100	72	41	829
Simosyrphus grandicornis	5			1	1		7
Sphaerophoria macrogaster				4	2		6
Syrphidae sp. 1	1						1
Syrphidae sp. 2		1					1
Triglyphus sp. 1	2	12		2			16
Family Tabanidae	**2**		**8**		**17**	**2**	**29**
Scaptia sp. 1	2		8		12	2	24
Scaptia sp. 2					5		5
Family Tachinidae	**12**	**2**	**13**	**9**	**25**	**5**	**66**
aff. *Australotachina* sp. 1					1		1
Austronilea sp. 1						2	2
cf. *Cuphocera* sp. 1	1					1	2
cf. *Pilimyia* sp. 1				2			2
Cuphocera sp. 1		1					1
Cylindromyia sp. 1	1						1
Cylindromyia sp. 2	3						3
Exechopalpus sp. 1			1	1			2
Exoristini gen. 1 sp. 1	5	1	1	3			10
Exoristinae gen. 1 sp. 1	1				1	5	7
Exoristinae gen. 2 sp. 1						1	1
Exoristinae gen. 3 sp. 1	1						1
Leskiini gen. 1 sp. 1				1			1
Leskiini gen. 2 sp. 1			1				1
Rutilia (Rutilia) sp. 2	1		5				6

Senostoma sp. 1			5	2	3	1	11
Senostoma sp. 2					3		3
Senostoma sp. 3					18	1	19
Siphonini gen. 1 sp. 1	1						1
Family Tephritidae		**3**	**1**	**9**	**48**	**45**	**106**
Tephritidae sp. 2		1	1	4			6
Tephritidae sp. 3		1			48	43	92
Tephritidae sp. 2		1		5		2	8
FAMILY FORMICIDAE	**9**	**3**	**12**	**1**			**25**
Formicidae	**9**	**3**	**12**	**1**			**25**
indet. Formicidae	9	3	12	1			25
ORDER HEMIPTERA			**1**				**1**
indet. Hemiptera			1				1
ORDER HYMENOPTERA	**394**	**591**	**263**	**129**	**142**	**89**	**1608**
indet. Hymenoptera	12	26	3	2	2	12	57
Family Apidae	**318**	**512**	**83**	**77**	**99**	**46**	**1135**
Apis mellifera	169	282	28	69	15	45	608
Exoneura sp. 1	130	223	55	8	80		496
Exoneura sp. 2	19	7			4	1	31
Family Chrysididae			**94**	**10**	**11**	**12**	**127**
Chrysididae sp. 1			90	7	3	5	105
Chrysididae sp. 2			4	3	8	7	22
Family Colletidae	**14**	**44**	**18**	**2**	**3**		**81**
Euhesma sp. 1		4					4
Euhesma sp. 2					3		3
Hylaeus (Prosopisteron) sp. 2		2					2
Hylaeus (Prosopisteron) sp. 3		1					1
Hylaeus (Prosopisteron) sp. 1		8					8
Hylaeus (Pseudhylaeus) sp. 1		3					3

Hylaeus daviesiae		1					1
Hylaeus probligenatus		3					3
Hylaeus semipersonatus		1					1
Leioproctus maculatus			6				6
Leioproctus plumosis			1				1
Leioproctus plumosus	1		5	1			7
Leioproctus sp. 1		9	6	1			16
Leioproctus sp. 2	6	2					8
Leioproctus sp. 3	6						6
Trichocolletes aeratus		2					2
Trichocolletes fuscus	1	8					9
Family Halictidae	**44**	**7**	**27**	**20**	**16**	**4**	**118**
Homalictus punctatus		3					3
Homalictus sphecodoides		3					3
Lasioglossum (Austrevylaeus) sp. 1	4						4
Lasioglossum (Austrevylaeus) sp. 2	6						6
Lasioglossum (Chilalictus) brazieri	5						5
Lasioglossum (Chilalictus) imitans	2					1	3
Lasioglossum (Chilalictus) lanarium			1	3	4		8
Lasioglossum (Chilalictus) mundulum			2	1			3
Lasioglossum (Chilalictus) opacicalle	4				2	2	8
Lasioglossum (Chilalictus) sp. 1	7						7

Lasioglossum (Parapschecodes) subrussatum	10	1	5		3		19
Lasioglossum (Parasphecodes) alticum	6		6	16	7	1	36
Lasioglossum (Parasphecodes) atronitens			1				1
Lasioglossum (Parasphecodes) olgae			2				2
Lasioglossum (Parasphecodes) sp. 1			10				10
Family Ichneumonidae	**2**						**2**
Ichneumonidae sp. 1	2						2
Suborder Symphyta	**3**	**2**	**38**	**18**	**11**	**15**	**87**
Symphyta sp. 1	2	1	34	18	10	15	80
Symphyta sp. 2	1	1	4		1		7
Family Vespidae	**1**						**1**
Vespula germanica	1						1
ORDER LEPIDOPTERA	**16**	**5**	**103**	**12**	**11**	**55**	**202**
indet. Lepidoptera	3	1	10	2	2	9	27
Family Erebidae	**1**				**1**		**2**
Nyctemera amicus	1				1		2
Family Geometridae					**1**		**1**
Chrysolarentia sp. 1					1		1
Family Lycaenidae			**1**				**1**
Zizina otis			1				1
Family Nymphalidae	**11**		**28**	**3**	**4**	**45**	**91**
Heteronympha cordace	11			1			12
Oreixenica orichora			1		3	45	49
Vanessa itea			15		1		16

Vanessa kershawi			12	2			14
Family Papilionidae			**27**	**2**			**29**
Graphium macleayanum			27	2			29
Family Pieridae	**1**	**4**	**37**	**5**	**3**	**1**	**51**
Pieris rapae	1	4	37	5	3	1	51
ORDER ODONATA			**1**				**1**
Indet. Odonata			1				1
Total	**1397**	**950**	**1004**	**501**	**635**	**644**	**5131**

Table S 3. Plant taxa observed during plant-pollinator surveys at each elevation.

Plant taxa	1000m	1200m	1400m	1600m	1800m	2000m	Total
Asphodelaceae		**1**					**1**
Dianella longifolia		1					1
Asteraceae	**384**	**35**	**118**	**164**	**176**	**440**	**1317**
Brachyscome scapigera						61	61
Brachyscome spathulata		1	94	2		4	101
Cassinia aculeata		11					11
Celmisia costiniana					64	164	228
Craspedia aurantia					28	53	81
Craspedia lamicola					70	77	147
Craspedia maxgrayii			1				1
Hypochaeris radicata	22	15	5	15	4		61
Leptorhynchos squamatus s.l.					1		1
Leucochrysum albicans					4		4
Microseris lanceolata						3	3
Olearia phlogopappa	362		9	125	2		498
Ozothamnus secundiflorus		5		19	3	3	30
Senecio pinnatifolius var. *pleiocephalus*						75	75
Taraxacum officinale		3	9	3			15
Brassicaceae				**2**	**3**		**5**
Barbarea grayi				1			1
Cardamine sp.				1	3		4
Caryophyllaceae	**15**						**15**
Stellaria pungens	15						15
Ericaceae	**113**	**86**	**408**	**23**	**112**	**8**	**750**
Acrothamnus hookeri	68	56	53		1	5	183
Epacris breviflora	45		24				69

Epacris glacialis						1	1
Epacris impressa		1					1
Epacris paludosa			331	11			342
Epacris petrophila				12	109		121
Leucopogon fletcheri		29					29
Richea continentis					2	2	4
Fabaceae	**78**	**348**	**96**	**130**			**652**
Acacia melanoxylon		2					2
Acacia obliquinervia		8	7				15
Acacia penninervis	1	114					115
Acacia siculiformis	5	4					9
Bossiaea foliosa			89	35			124
Daviesia mimosoides		185					185
Dillwynia prostrata	1						1
Hovea montana				93			93
Mirbelia oxylobioides	71	35		1			107
Pultanaea subspicata				1			1
Gentianaceae						**1**	**1**
Gentianella mulleriana						1	1
Geraniaceae	**3**				**1**		**4**
Geranium potentilloides var. *abditum*					1		1
Geranium retrorsum	3						3
Hypoxidaceae		**4**					**4**
Hypoxis hygrometrica		4					4
Lamiaceae	1				**198**	**29**	**228**
Ajuga australis	1						
Prostanthera cuneata					198	29	227
Myrtaceae	**168**		**18**		**2**	**33**	**221**

Baeckea gunniana			18		2	33	53
Leptospermum polygalifolium	168						168
Oxalidaceae	**2**						**2**
Oxalis perennans	2						2
Plantaginaceae	**2**			**35**			**37**
Veronica derwentiana				35			35
Veronica gracilis	2						2
Poaceae		**2**					**2**
Poaceae sp.		2					2
Polygonaceae						**1**	**1**
Rumex acetosella						1	1
Portulacaceae				**5**			**5**
Neopaxia australasica				5			5
Proteaceae	**608**	**367**	**25**	**64**	**44**	**9**	**1117**
Callistemon sieberi		2	6				8
Grevillea australis				58	44	9	111
Grevillea lanigera	133	320	3				456
Hakea lissosperma	474	45	16	6			541
Ranunculaceae		**4**	**181**	**8**	**61**	**34**	**288**
Ranunculus graniticola		2	181	8	60		251
Ranunculus gunnianus					1	34	35
Ranunculus scapiger		2					2
Rosaceae	**11**						**11**
Rubus parvifolius	11						11
Rubiaceae	**11**		**1**				**12**
Asperula pusilla	11		1				8
Rutaceae					**28**	**47**	**75**
Nematolepis ovatifolia					28	47	75

Santalaceae		**101**					**101**
Exocarpos strictus		101					101
Scrophulariaceae					**6**	**39**	**45**
Euphrasia collina					6	39	45
Thymelaeaceae			**156**	**67**	**4**	**3**	**230**
Pimelea alpina					4	3	4
Pimelea axiflora				67			67
Pimelea linifolia			156				156
Violaceae	**1**	**2**		**2**			**5**
Melicytus dentatus				2			2
Viola betonicifolia	1	2					3
Winteraceae			**1**	**1**			**2**
Tasmannia xerophila			1	1			2
Grand Total	**1397**	**950**	**1004**	**501**	**635**	**644**	**5131**

Table S 4. Significance values from generalised linear mixed models of interaction frequency by insect order against each predictor variable. For each model, p value is shown with significance indicated at α = 0.05, with z value shown in parentheses. Asterisks indicate statistically significant results (p <0.05).

Predictor	All visitors	Hymenoptera	Diptera	Coleoptera
Elevation	0.411 (0.822)	<0.001 * (-3.31)	<0.001 * (4.11)	<0.001 * (-4.02)
Elevation^2	0.577 (-0.558	0.569 (-0.569)	0.15 (-1.44)	0.19 (1.32)
Temperature	<0.001 * (7.219)	<0.001 * (4.316)	0.001 * (3.18)	<0.001 * (4.92)
Windspeed	0.011 * (-2.551)	0.72 (-0.349)	0.091 (-1.69)	0.007 * (-2.67)
Humidity	0.017 * (2.394)	0.336 (-0.961)	0.09 (2.59)	0.002* (3.11)
Flower richness	0.508 (0.662)	0.824 (-0.223)	0.72 (0.35)	0.37 (-0.89)

Table S 5. Significance values from generalised linear mixed models of interaction frequency by plant family against each predictor variable. For each model, p value is shown with significance indicated at α = 0.05, with z value shown in parentheses. Asterisks indicate statistically significant results (p <0.05).

Predictor	Asteraceae	Ericaceae	Fabaceae	Proteaceae
Elevation	0.227 (1.21)	0.2 (-1.282)	<0.001 * (-5.65)	<0.001 * (-6.96)
Elevation^2	<0.001 * (3.825)	<0.001 * (-3.962)	<0.001 * (-5.95)	0.963 (-0.172)

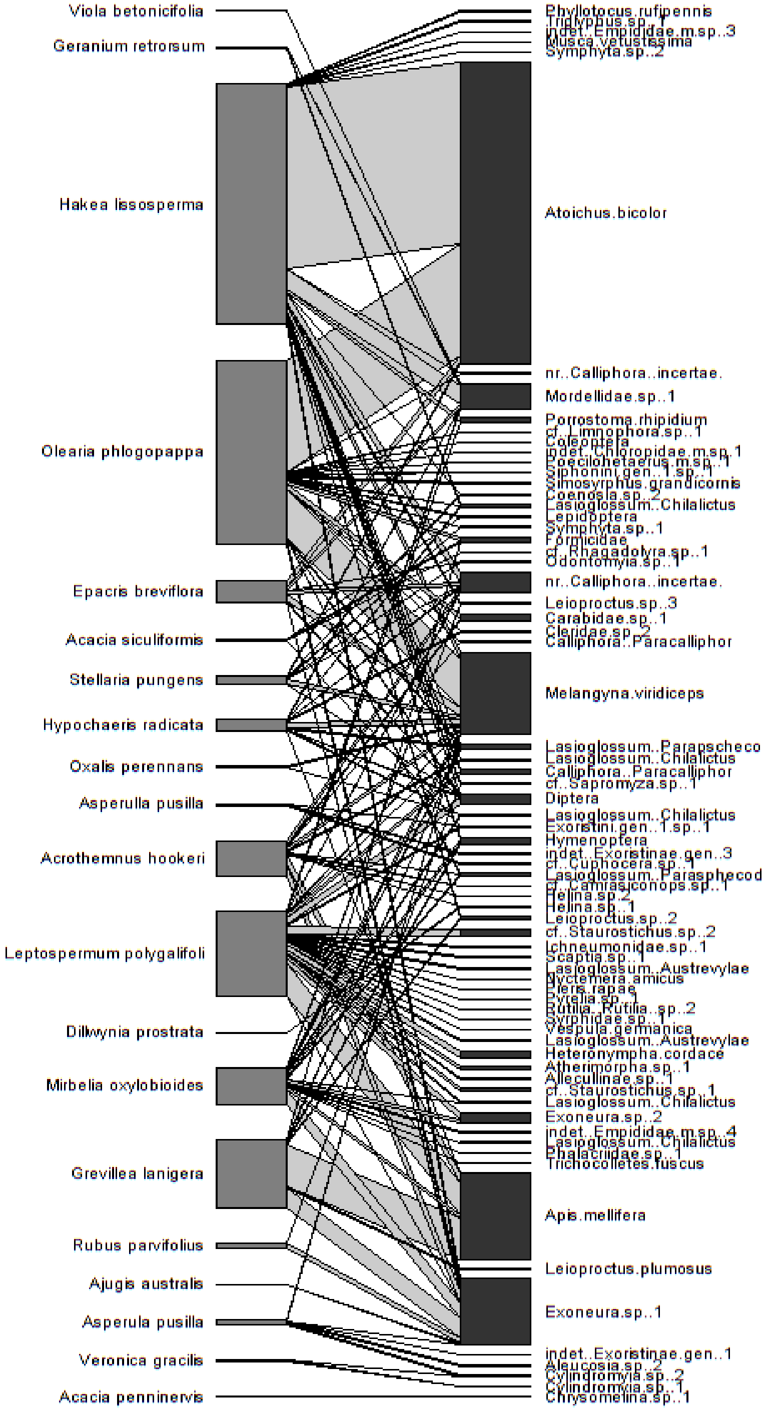


Figure S 1. Bipartite pollinator network plot for 1000m a.s.l. site, with plants on the left and pollinators on the right.

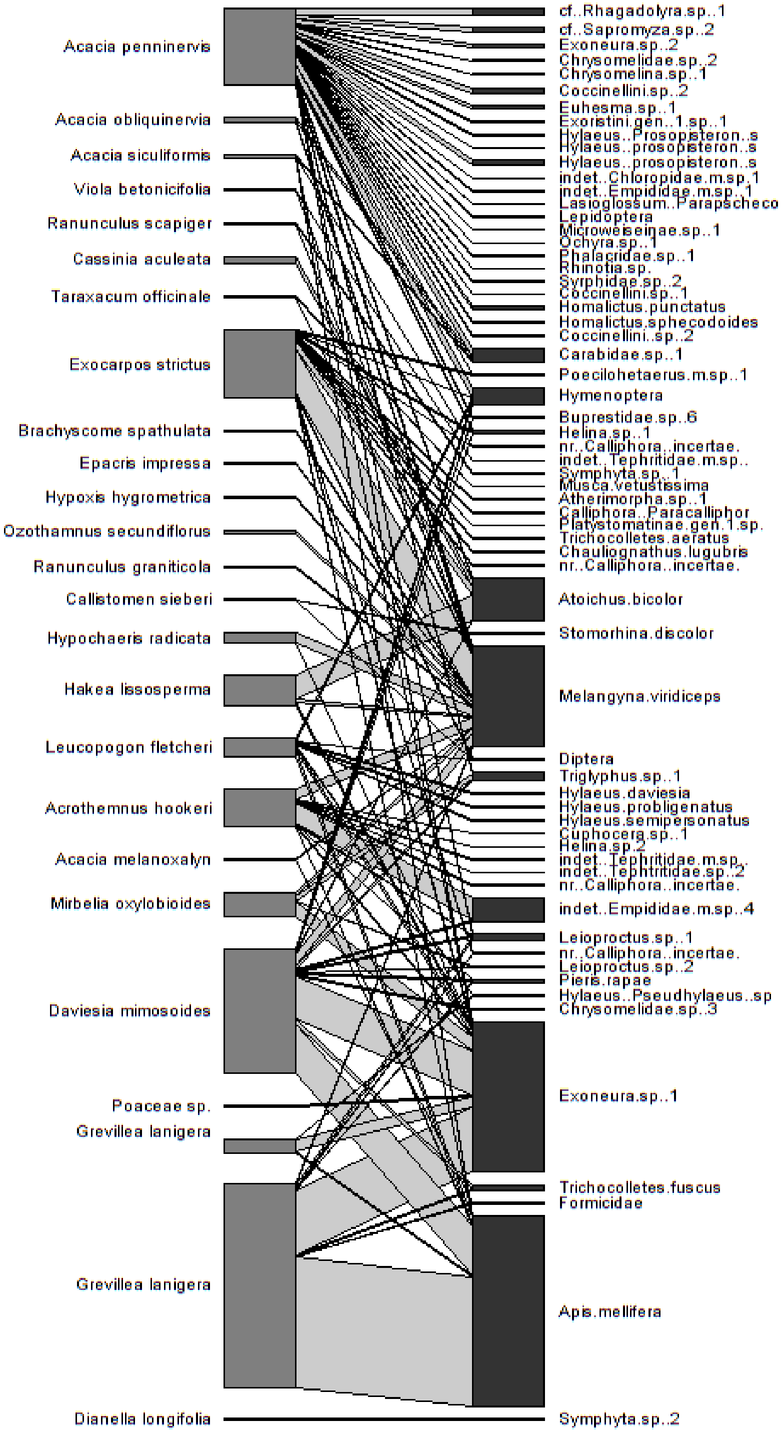


Figure S 2. Bipartite pollinator network plot for 1200m a.s.l. site, with plants on the left and pollinators on the right.

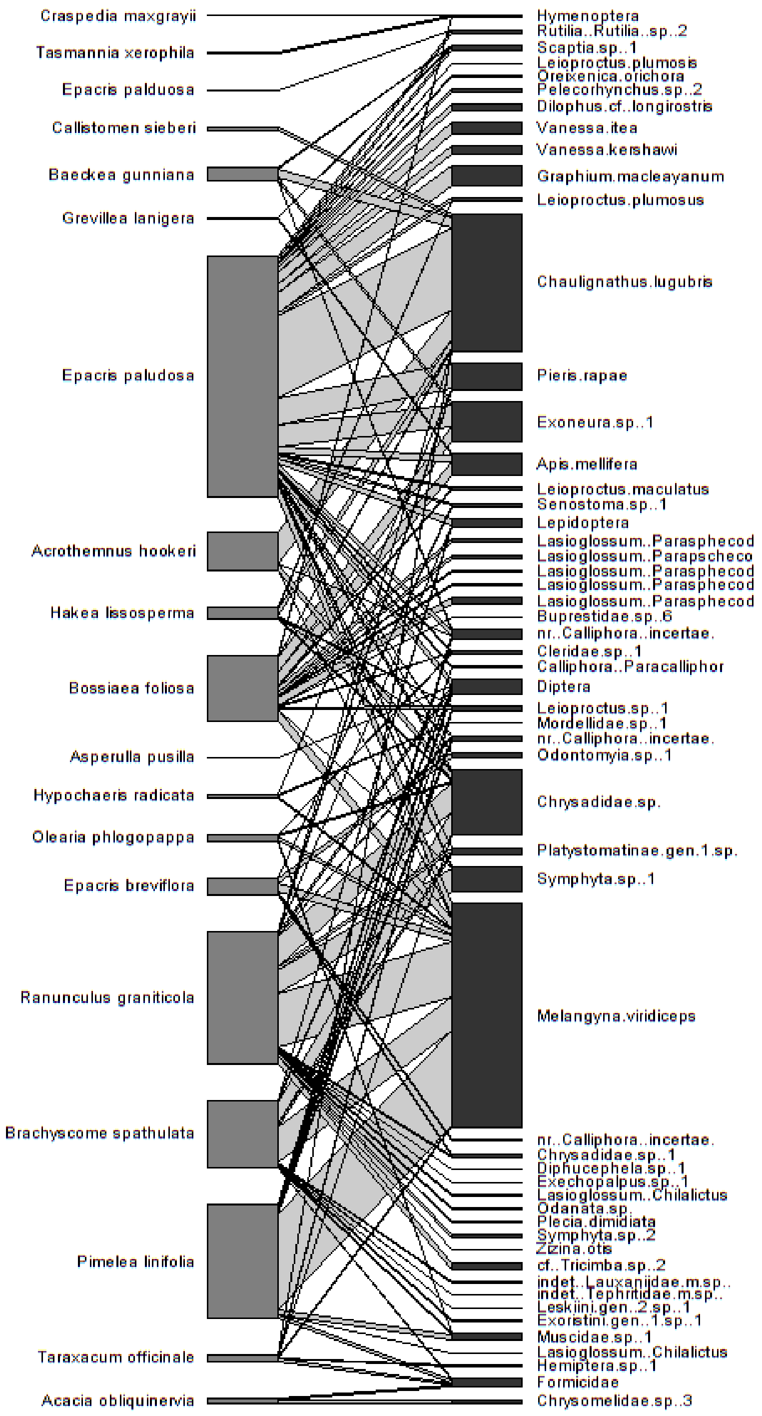


Figure S 3. Bipartite pollinator network plot for 1400m a.s.l. site, with plants on the left and pollinators on the right.

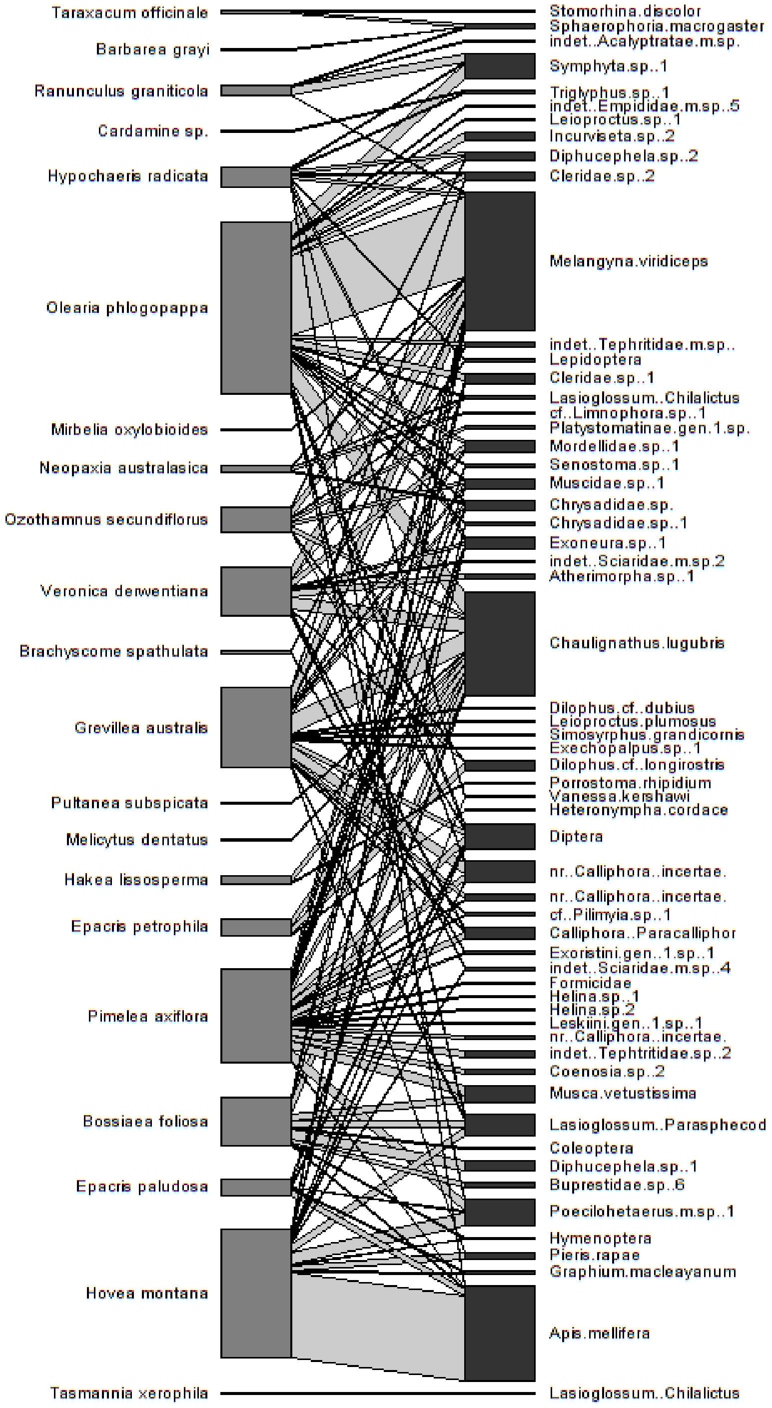


Figure S 4. Bipartite pollinator network plot for 1600m a.s.l. site, with plants on the left and pollinators on the right.

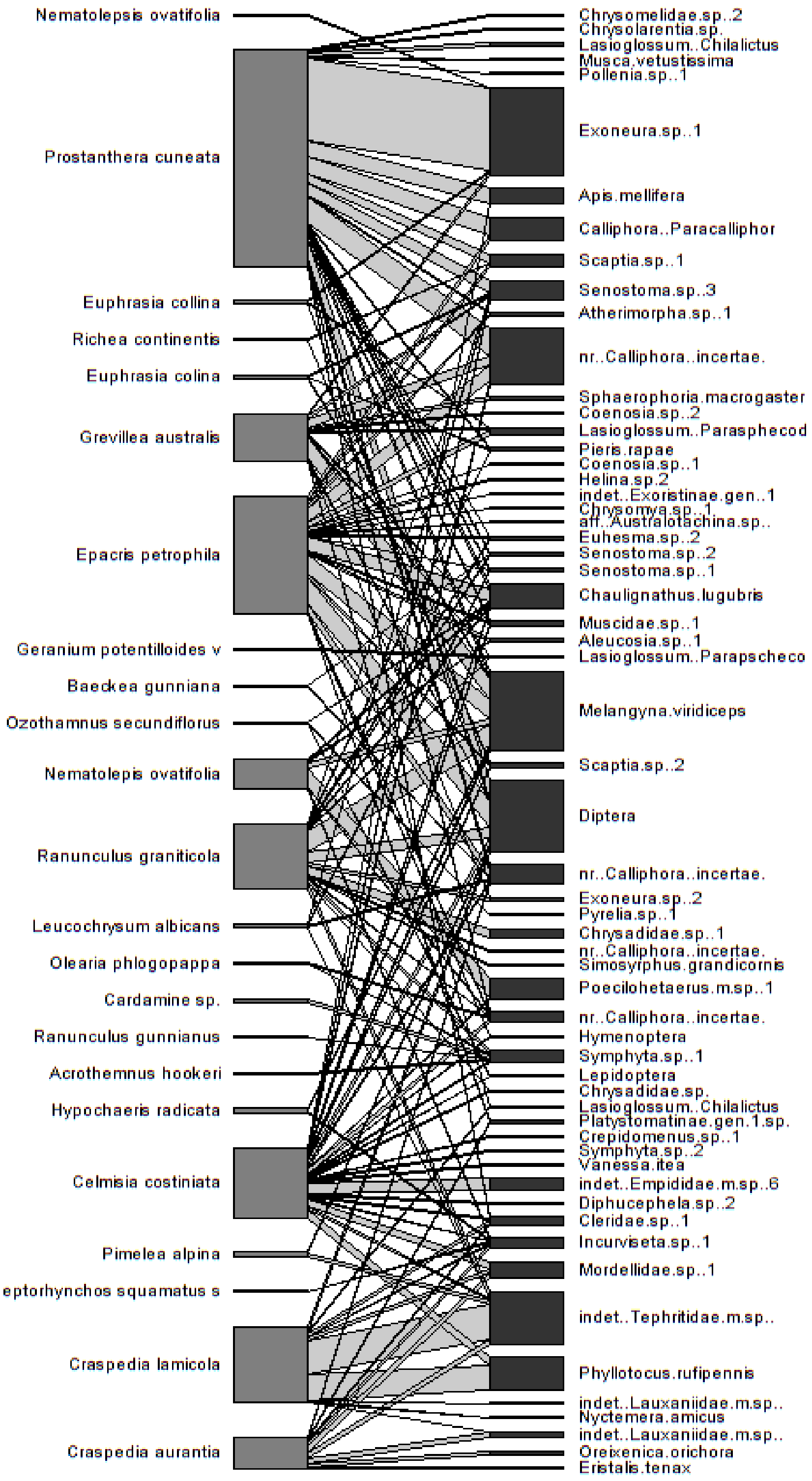


Figure S 5. Bipartite pollinator network plot for 1800m a.s.l. site, with plants on the left and pollinators on the right.

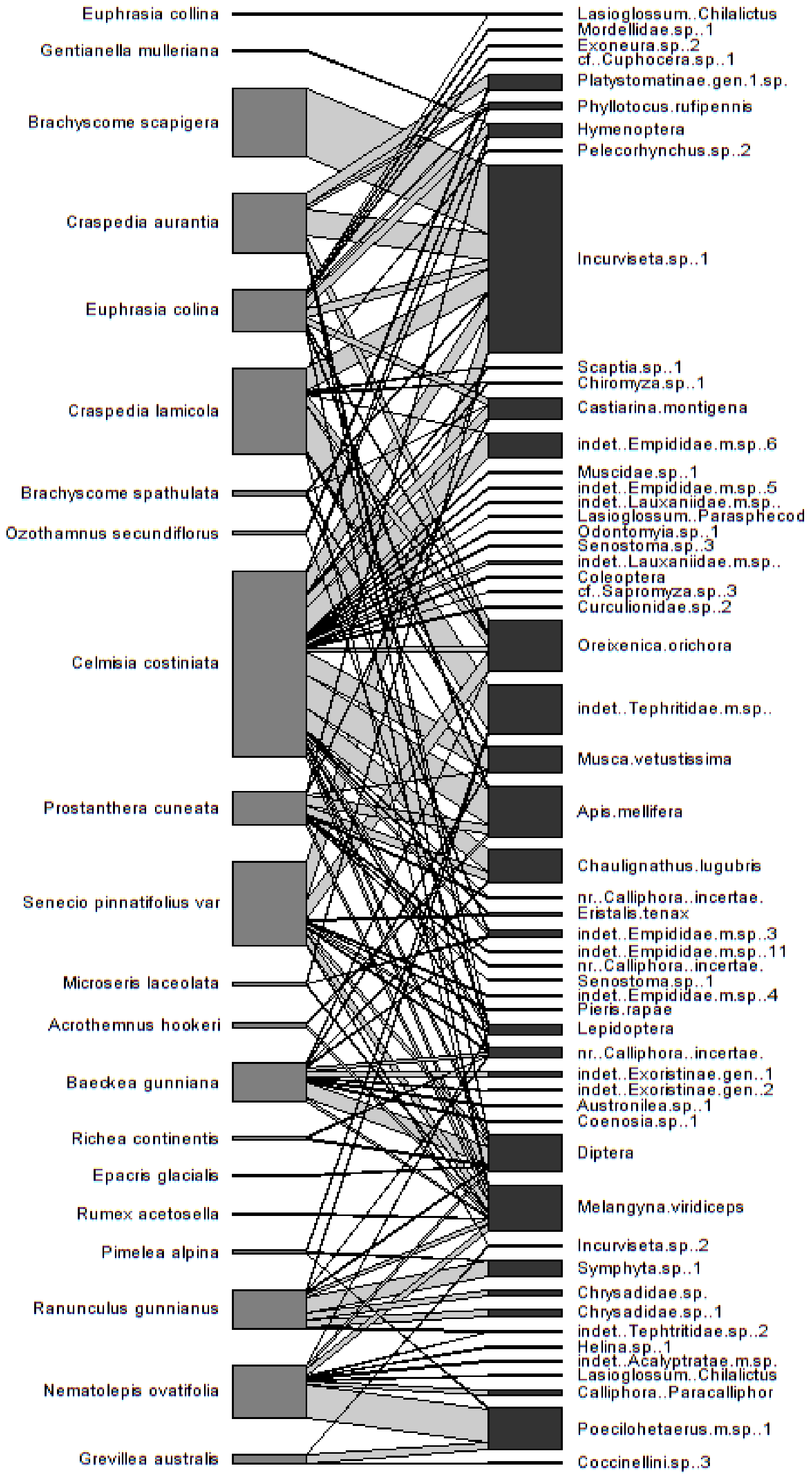


Figure S 6. Bipartite pollinator network plot for 2000m a.s.l. site, with plants on the left and pollinators on the right.

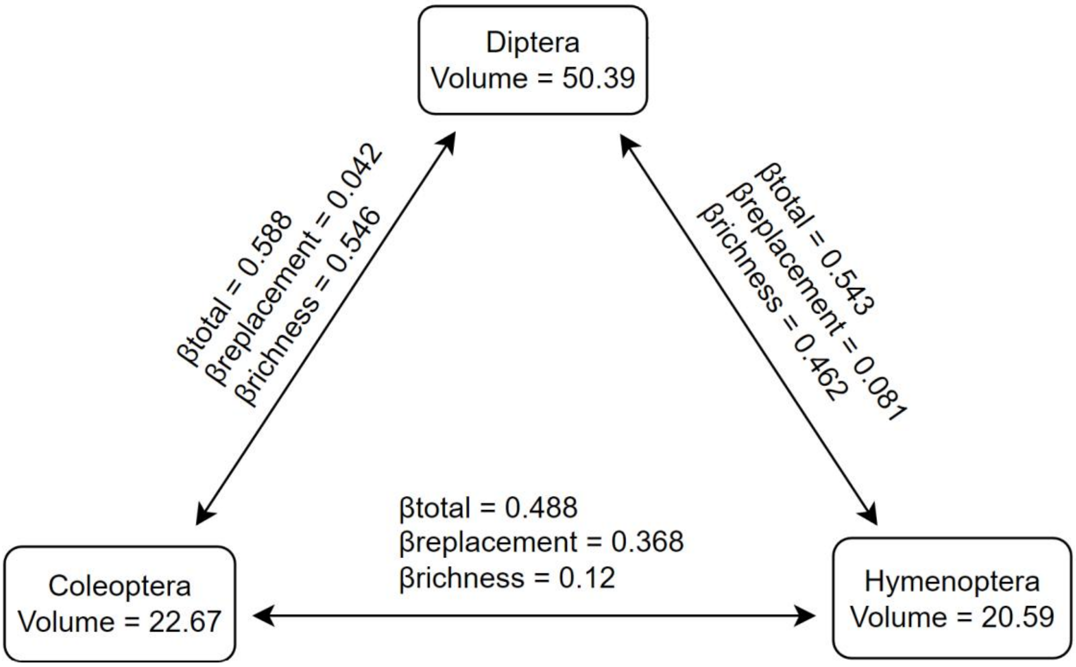


Figure S 7. Niche hypervolume volume and dissimilarity metrics for each pollinator order and their pairwise comparisons.

2.11.1 Phenology Analysis

To investigate insect and floral phenology throughout the sampling period, we constructed generalised additive models (GAMs) using the *gam* function in the mgcv package (Wood and Wood, 2015). We used insect or plant interactions as the response variable, with Sample Date as the predictor and a Poisson distribution. We used the *gam.check* function to validate models and iteratively adjusted k values to achieve an optimal amount of basis functions (Supplementary Information Table 5). While there were insufficient data to construct valid GAMs for each plant and insect taxa after subsetting data at the site level, we constructed plots to visualise patterns phenological changes across the elevation gradient.

For all sites combined, Sample Date had a significant effect on insect and floral taxa (Supplementary Information Table 5), showing an effect of phenology on insect and plant community dynamics. The direction and strength of this relationship varied between taxa. For instance, later in the season there was an increase in both Diptera and Asteraceae interactions, while other taxa showed more stable phenological patterns, with only moderate declines present as the season progressed (Supplementary Information Figure 8 and 9, Table 5). Site level plots showed that plant and insect phenology were highly variable, with a shift in phenological patterns across the elevation gradient. For instance, upper sites were dominated by Diptera and Asteraceae later in the sampling season, while earlier in the season lower sites showed variable activity from other taxa (Supplementary Information Figure 10 and 11).

Table S 6. Model summary and gam.check results for GAMs with the smooth term of Sample Date as the predictor variable and activity as response variable for all sites combined.

Model	Basis functions (k)	k-Index	edf	Adj R^2	Chi^2	p-value
Asteraceae	30	0.9	27.38	0.282	853.8	< 0.05
Ericaceae	20	1.03	18.96	0.085	483.2	< 0.05
Fabaceae	30	0.95	22.18	0.111	283.7	< 0.05
Proteaceae	30	0.99	20.89	0.168	952.6	< 0.05
Hymenoptera	30	1	26.7	0.106	543.7	< 0.05
Diptera	30	1.07	27.95	0.254	655.8	< 0.05
Coleoptera	30	1.01	26.14	0.039	731.2	< 0.05

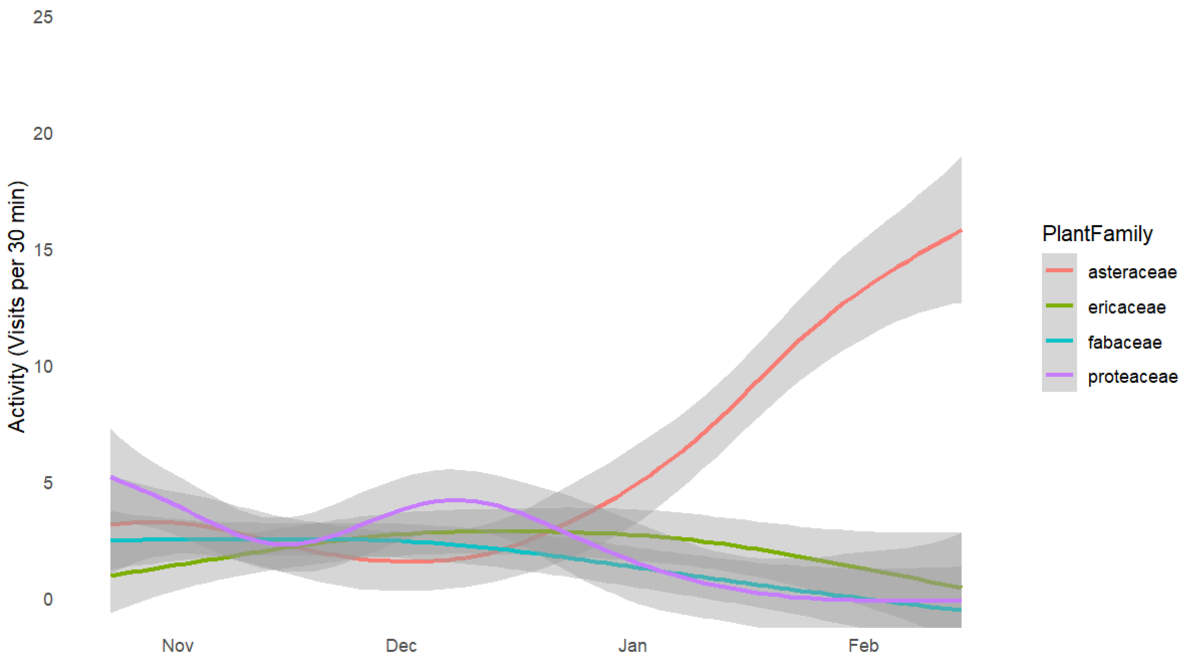


Figure S 8. Response plots showing effect of phenology on four main plant families across the sampling season for all sites compiled.

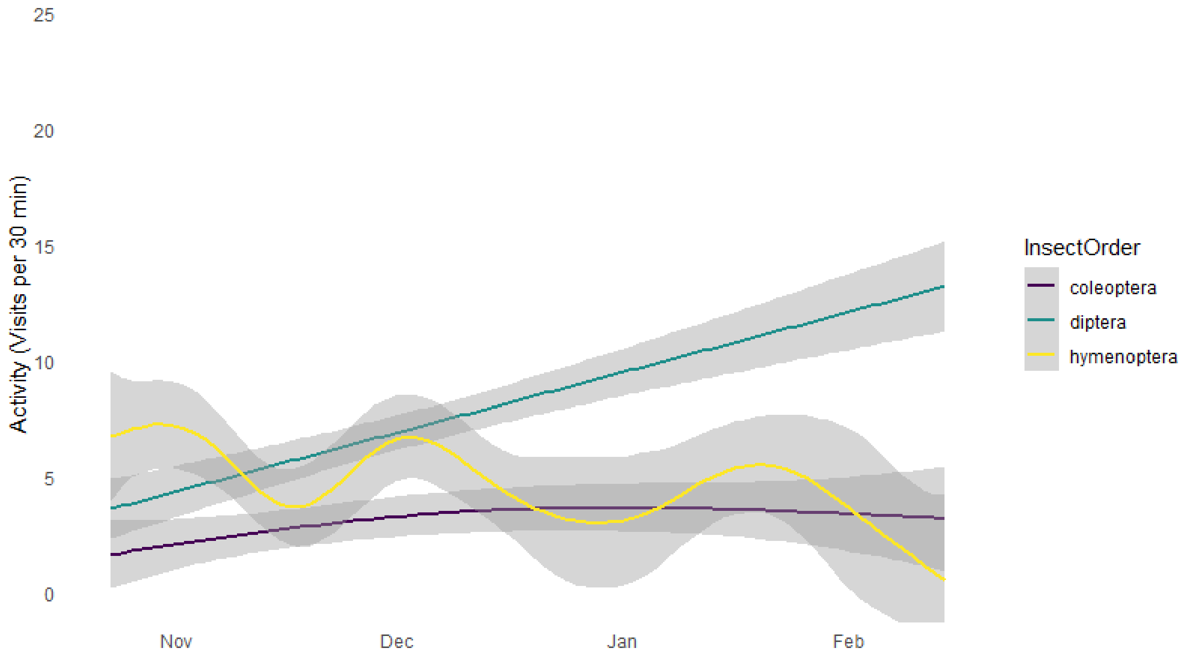


Figure S 9. Response plots showing effect of phenology on three main insect orders across the sampling season for all sites compiled.

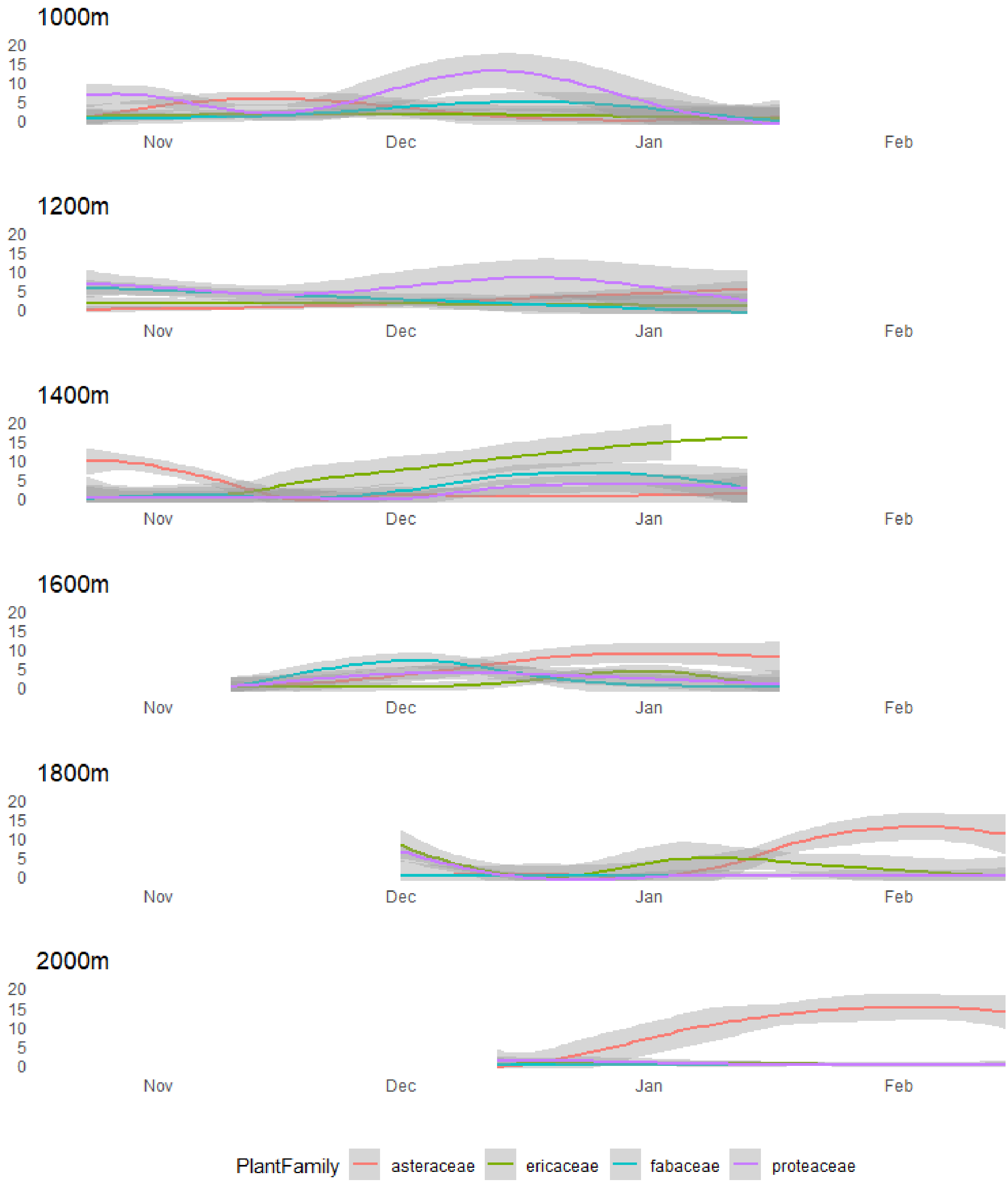


Figure S 10. Plots showing effect of phenology of interactions considering four main plant families across the sampling season at individual elevation.

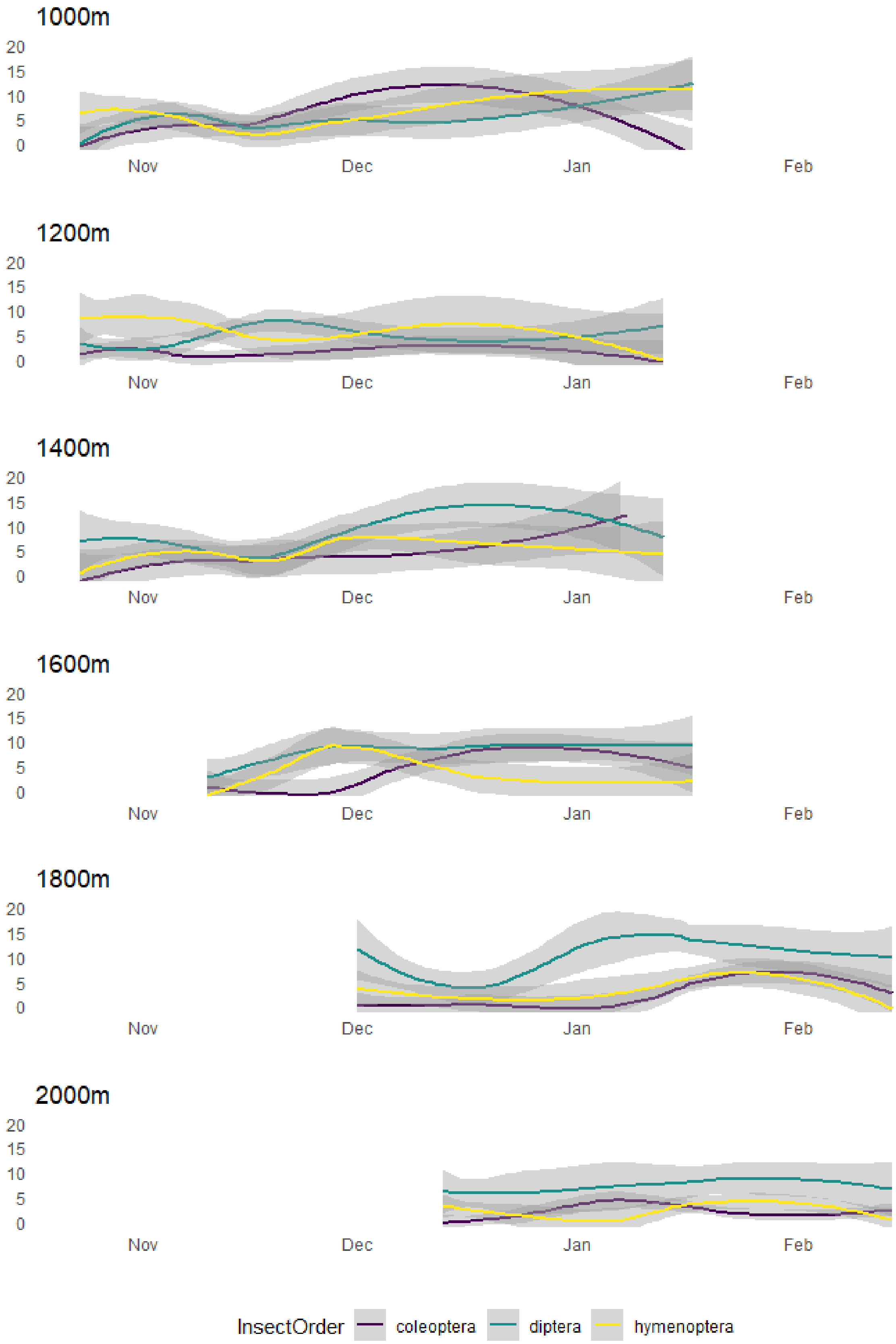


Figure S 11. Plots showing effect of phenology of interactions considering three main insect orders across the sampling season at individual elevation.

2.11.2 References

Wood, S., Wood, M.S., 2015. Package ‘mgcv’. R package version 1, 729.

Chapter 3: Against the Odds: Nesting Specialisation and Foraging Ecology Provide Insights into Climate Change Responses in a Mountain Bee

Citation: Coates J.M., Takeshima, N., Encinas-Viso F., Cunningham S.A. in review. Against the odds: Nesting Specialization and Foraging Ecology Provide Insights into Climate Change Responses in a Mountain Bee. Insect Conservation and Diversity.

3.1 Foreword

This chapter investigates the biology of a newly discovered bee that manages to survive in Australia’s coldest ecosystems, in an environment that is otherwise inhospitable to most other bees. While Chapter 2 documented distinct elevational shifts in pollinator community composition, with a shift away from bees to flies at high elevations, Chapter 3 examines an exception to this pattern. Building on the community-level focus of the previous chapter, this study focuses on species-level life history traits in a specialised cold-climate bee. By linking behaviour, microhabitat choice and thermal physiology to flight thresholds and foraging profitability, it connects species-level traits to the energetic constraints that structure mountain pollinator communities and their responses to warming.

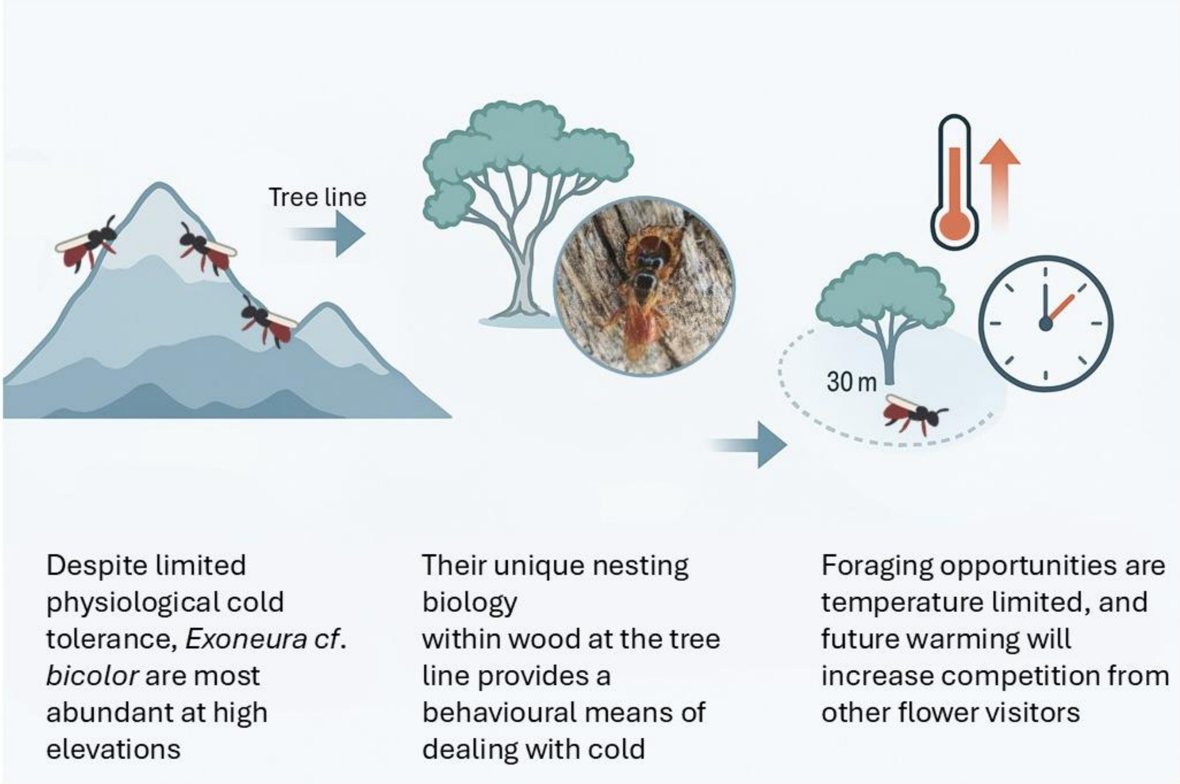
This chapter's key contribution is to reveal how a previously unstudied (and still undescribed) bee lives in a generally inhospitable environment, with climate change modelling providing insight into future activity. This research combines extensive field sampling, custom-made camera traps, spatial analysis and climate change modelling to examine the life history traits that underpin this bees' persistence in cold climates.

My first experience with pollination ecology during my Honours research was on a species of *Exoneura*, albeit in a very different environment, examining their role as agricultural pollinators. For this reason, I especially took satisfaction in this chapter, where I was able to continue working on a genus close to my heart, but this time on a previously unstudied species.

3.2 Abstract

1. Climate change is rapidly altering temperature regimes in mountain environments, reshaping the distribution and activity of pollinators. Studies of insect pollinator assemblages in mountains show a general pattern of decreasing bee (Apoidea) activity with increasing elevation.
2. Here we report a unique *Exoneura* bee, that counters this trend and thrives in cold, high-elevation (~1500-1900m a.s.l.) woodlands at the tree line. Whereas other members of the genus at lower elevation nest in narrow pithy or hollow plant stems, this bee has a unique nesting biology, burrowing into the dead branches of the only tree in Australia's subalpine zone (snow gum, *Eucalyptus pauciflora* subsp. *niphophila*).
3. Using a combination of observational surveys, spatial mapping, temperature tolerance tests, and biophysical modelling we investigated various aspects of the bee's ecology. We found that this species' nest abundance peaked at the tree line (approx. 1900m m a.s.l). Foraging behaviour was highly dependent on the presence of snow gums for nesting, with activity concentrated within 30m of snow gum stands.
4. Biophysical modelling showed that the daily and annual foraging activity window was significantly limited by temperature, and that it will substantially increase under climate change projections.
5. This study reveals several unique traits of a new bee species that shows a close association with snow gums, providing insights into its flourishing in the changing conditions of subalpine Australia's high-elevation environments.

3.3 Graphical Abstract



Despite limited physiological cold tolerance, *Exoneura cf. bicolor* are most abundant at high elevations

Their unique nesting biology within wood at the tree line provides a behavioural means of dealing with cold

Foraging opportunities are temperature limited, and future warming will increase competition from other flower visitors

3.4 Keywords

climate change*,* *Exoneura*, foraging behaviour, pollinator activity, snow gum

3.5 Introduction

In mountain ecosystems, climate change is driving some of the earliest and most pronounced shifts in abiotic conditions (Pickering and Buckley, 2003), with impacts recorded on mountain pollinator communities (Buckley and Kingsolver, 2012; Inouye, 2020). Abiotic factors impact pollinators' activity levels, thermoregulation, phenology and mortality (Hoiss *et al.*, 2015). As a result, pollinator communities in high-elevation environments are often compositionally distinctive (Coates *et al.*, 2024), such as the reduced diversity of bees (Apoidea) in high-elevation pollinator communities, where they are replaced by flies (Diptera) (McCabe and Cobb, 2021). Because pollinators differ in their capacity to transport and deposit pollen effectively, variation in pollinator community composition can strongly influence plant reproductive success and community structure (Herrera, 1987). Given that insect pollinators are ectotherms, it is essential to understand the thermal sensitivity underpinning their foraging activity in order to predict how climate change may alter pollination dynamics in these vulnerable environments.

Insect pollinators persist in cold environments due to a combination of behavioural and physiological traits that allow them to maintain foraging activity. For instance, *Bombus* bees, important alpine pollinators in the northern hemisphere, display both behavioural (e.g., vibrating flight muscles to generate warmth) and physiological traits (e.g., insulation via dense setae, large body size for increased thermal mass) that allow them to forage in the cold (Heinrich, 2004; Minachilis *et al.*, 2020). Other bee species rely on their nesting site to provide passive thermal insulation from ambient temperatures (Jones and Oldroyd, 2006; Antoine and Forrest, 2021). These traits allow pollinators to maintain thermally suitable conditions required for flight and foraging activity.

By coupling climate data with information on species' traits and thermal activity thresholds, such as through using biophysical modelling approaches, we can predict how climate warming will alter the daily or seasonal duration available for essential behaviours like foraging (Briscoe *et al.*, 2023). For instance, Buckley and Kingsolver (2012) use biophysical modelling to show how available flight time for *Colias* butterflies declined for species at a warmer, lower-elevation site, but increased for species in colder, high-elevation sites. Biophysical modelling has also been used to assess how vertebrate ectotherms' activity time will shift under climate change (Kearney *et al.*, 2008; Muñoz *et al.*, 2022; Gao *et al.*, 2024), with Gao *et al.* (2024) demonstrating greater increases in activity time at higher-elevation sites.

Australia's unique biogeographic history means that its bee fauna is distinct, accounting for approximately 10% of global bee species richness (Michener, 2007). Australia is home to endemic taxa such as the family Stenotritidae, an unusually high proportion of species in the family Colletidae, Euryglossinae (Michener, 2007) and the genus *Exoneura* (Schwarz *et al.*, 2006). On the other hand, several taxa with widespread global distributions, such as Adrenidae, Mellitidae, *Apis* and *Bombus*, are not found natively in Australia (Batley and Hogendoorn, 2009). Whereas the cold-tolerant *Bombus* species are often abundant at high elevations elsewhere in the world (Minachilis *et al.*, 2020), Coates *et al.* (2024) showed that *Exoneura* were the only abundant bee in subalpine woodlands.

Exoneura is an endemic Australian stem-nesting bee genus (Cronin and Schwarz, 1999a), representing 67 of the 197 Australian Apidae species (Michener, 1965; Batley and Hogendoorn, 2009). Due to their facultatively social behaviour, the genus has been the focus of several studies on the evolution of sociality in insects (Michener, 1965; Schwarz, 1986; Schwarz and O'keefe, 1991; Bernauer *et al.,* 2021). *Exoneura* are typically stem-nesters, excavating burrows into exposed pithy plant stems, such as in native *Dicksonia* tree ferns and in *Rubus* stems (Schwarz, 1986; Coates *et al.*, 2022). Researchers have suggested that *Exoneura* are incapable of burrowing into hard materials due to their small mandibles and weak musculature (Rayment, 1951; Silberbauer and Schwarz, 1995), although *E. robusta* Cockerell have been observed nesting in rotting *Eucalyptus* wood (Cronin and Schwarz, 1999b). Because they exhibit little morphological variation, *Exoneura* are difficult to identify to species, and while it has long been recognized that the genus requires taxonomic revision (Schwarz *et al.*, 1986) the taxonomy remains poorly resolved. Genetic sequencing by Takeshima *et al.* (in prep) indicates that the *Exoneura* present in subalpine woodlands cluster closely with *E. bicolor* Smith, but may represent an undescribed species. Accordingly, we refer to *Exoneura* present in our study area as *E.* cf. *bicolor*, reflecting their unresolved taxonomy. In other studies, *E. bicolor* primarily nests within pithy plant stems (Cane and Michener, 1983; Schwarz, 1986; Schwarz and O'keefe, 1991; Silberbauer and Schwarz, 1995), with some anecdotes also noting occasional nesting in decaying *Eucalyptus* branches (Silberbauer, 1992; Silberbauer and Schwarz, 1995), and opportunistic use of holes bored in wood by beetles (Rayment, 1951; Michener, 1965).

In this study, we examine the unique traits of *E.* cf. *bicolor* in the subalpine woodlands of Australia’s highest mountains. We investigate the behavioural and physiological traits that enable this subalpine bee to thrive in cold, high-elevation habitats, and use these traits to make predictions about their response to climate change. In our subalpine study area, we have observed *E.* cf. *bicolor* burrowing within large woody branches and trunks of dead snow gum (*Eucalyptus pauciflora* subsp. *niphophila* (Maiden & Blakely) L.A.S.Johnson & Blaxell), progressively excavating nests over a period of days. This nesting habit is unusual among other stem-nesting *Exoneura*, which are often considered incapable of burrowing into wood (Rayment, 1951; Silberbauer and Schwarz, 1995). We examine what role the bees' unique nesting substrate plays in shaping their abundance at high-elevation and foraging activity. We also quantify the bees' thermal activity window and thermal tolerance limits to parameterize biophysical models, which we use to quantify the bees' annual activity window and forecast how that will be affected by climate warming.

3.6 Methods

3.6.1 Study Sites

We conducted fieldwork in Kosciuszko National Park, New South Wales, Australia from November 2022 to February 2023. Previous work by Coates *et al.* (2024) indicates that this period captures *E.* cf. *bicolor*'s activity period in the subalpine zone, which also coincides with the onset of peak flowering (Costin *et al.*, 2000). Most of our sampling was conducted at Charlotte Pass (~1850m a.s.l., 36° 25' 55.5672" S, 148° 19' 44.076" E), where we surveyed nest activity, foraging distances and collected bees for thermal assays. An additional site, downslope at Perisher (~1720 m a.s.l., 36° 24′ 16.1″ S, 148° 24′ 50.0″ E), was also sampled for nest activity and the nest survey extended down to Sawpit Creek at 1400m a.s.l.

3.6.2 Nest Abundance with Elevation

To determine the elevational extent of subalpine *E.* cf. *bicolor* populations, we surveyed dead *Eucalyptus* branches for nest entrances, sampling along an elevational gradient. Sites were located from approximately 1400 to 2000m a.s.l. along Kosciuszko Road. Several other *Eucalyptus* species are found in montane forests at the bottom of this gradient, including *E. dalrympleana* Maiden*, E. camphora* Baker and *E. stellulata* Sieber, while from ~1600m a.s.l., only snow gums are present (Duncan, 2010). We determined 1400m a.s.l. as the lower bound for the survey after no nests were found below 1530m. To select sample sites, we examined this gradient in seven bands each covering 100m of elevation and calculated the horizontal extent within each elevation band along the main road in the park. We used a random number generator to select three sites to sample within each elevation band for a total of 21 sites (Supplementary Information Table 1).

At each site, we walked both north and south on either side of the road until we reached a *Eucalyptus* stand, where we surveyed the first 20 suitable branches for the occurrence of nest entrances. Initial observations of nest sites indicated that only dead branches that were free of bark and of at least 10cm in diameter were colonized, and so only these were included as suitable branches in the survey. We also did not survey branches that were difficult to access, above 2m in height. Nest entrances were defined as those where either an *E.* cf. *bicolor* individual could be seen within the nest, or where the characteristic rim created by the species during nest excavation was visible at the nest entrance (Supplementary Information Figure 1).

3.6.3 Foraging and Canopy Cover

To determine how *E.* cf. *bicolor* foraging behaviour relates to the presence of snow gum nesting sites, we conducted pollinator visitation surveys along transects with increasing distance from snow gum stands. We established seven transects near Charlotte Pass, ensuring a spread of aspects and slope gradients (upslope, downslope and cross-slope). We ensured that snow gum stands nearby to plots had dead snow gum branches with active *E.* cf. *bicolor* nests. In each transect we conducted sampling at three plots along increments (near, middle, far) leading away from snow gum stands. Plots were circular, with a 30m radius. We conducted consecutive 20-minute surveys, one at each of the three plots, where we recorded all visits of *E.* cf. *bicolor* to flowers, recording a visit when the reproductive parts of a flower were contacted.

We analysed the spatial relationship between trees and foraging locations using Geographic Information System (GIS) mapping. We obtained high resolution LiDAR (Light Detection and Ranging) point cloud data for the study area collected via a ALS50 (SN092) sensor (Geoscience Australia, 2023). These data was pre-processed to ICSM Classification Level 3 (Intergovernmental Comittee on Surveying and Mapping, 2008), whereby ground return (bare earth) and non-ground return (e.g., vegetation, buildings) data points are distinguished, which enabled us to determine canopy cover within the study area.

Using the LAS toolbox in ArcGIS Pro 3.1.3 (Esri Inc., 2023), we initially processed the LiDAR data into LAS format using the *Convert LAS* tool. Using the *Make LAS Dataset Layer* tool, we filtered out LAS data points according to the LAS

1.3 standard (The American Society for Photogrammetry & Remote Sensing, 2010), to create a dataset containing only LiDAR Point Classes 4 (Medium vegetation) and 5 (High Vegetation). We visually inspected this dataset by overlaying satellite imagery, to ensure that only snow gum canopy was captured within this output raster. We then rasterized, standardized and inverted this data, to create a raster containing values of 1 where snow gum canopy is present, and values of 0 where absent. Finally, this raster was converted to a vector layer containing only snow gum canopy cover.

We then quantified the proportion of canopy cover within a radius distance of each plot by creating circular buffers around each plot using the *Buffer* tool. We created ten different sized buffers, (10m to 100m radius) to determine the distance from snow gum canopy that best predicted *E.* cf. *bicolor* abundance. Finally, we used the *Tabulate Intersection* tool to quantify the overlap (in M^2) of snow gum canopy cover within each circular buffer.

3.6.4 Activity at the Nest

To monitor the effect of temperature on activity at the nest entrance, we placed camera traps with iButton temperature loggers at *E.* cf. *bicolor* nest entrances at Perisher and Charlotte Pass. We built five camera traps following Droissart *et al.* (2021), which consisted of a Raspberry Pi Zero W microcomputer, Raspberry Pi infrared camera module with 2.6mm 5MP lens, 30,000mAh battery pack, 128gb SD card, and a waterproof case. Each camera trap was paired with two iButton temperature loggers placed within a Stevenson screen, which monitored ambient air temperature every 5 minutes at a height of 1.2m. Video files and iButton temperature logger timestamps were synchronised, so that we could pair ambient temperature with activity. We mounted cameras on poles placed ~15-20cm from nest entrances, positioned so that their shadow did not fall across the nest entrance. Cameras were set to record continuously from 6am until 8pm, capturing the full window of potential activity from this diurnal species. Cameras captured consecutive one-hour long videos until the battery discharged, usually after 3-5 days of recording. We then collected cameras and recharged them overnight, redeploying them the next day.

We processed video files after each camera was collected from the field following Droissart *et al.* (2021). Similar ex situ motion detection methods have previously been shown to be effective in detecting pollinator movement (Azarcoya-Cabiedes *et al.*, 2014; Weinstein, 2015). We processed videos with DVR-Scan software (Castellano, 2025) to filter out motion sequences. We initially determined the ideal threshold for motion detection by incrementally adjusting the motion detection threshold using video files that contained *E.* cf. *bicolor* activity. This allowed us to reduce the possibility of false negatives, while limiting review time created by false positive detections, such as those triggered by movement from wind, rain or snow (Weinstein, 2015).

We manually reviewed motion-sequenced video files to record the occurrence of *E.* cf. *bicolor* activity. When possible, we determined foraging trip time when only one bee was recorded arriving and departing, without interruption from another bee accessing the nest entrance in the intervening period.

3.6.5 Critical Thermal Limits

To determine the critical thermal limits of *E.* cf. *bicolor*, we collected individuals by hand netting from flowers. We then transported them to the lab within 3 hours to conduct thermal assays. Bees were placed into individual 55x20mm plastic dram vials with air holes in the lid and fluon coating the interior walls to prevent them from climbing away from the heat source. We placed vials within holes milled into a high-density foam block to insulate against ambient temperature, with the bottom of each vial contacting a Peltier plate. We used a thermoelectric Peltier plate (Model Number CP-121HT, TE Technology, INC.) attached to a controller (Model Number TC-720, TE Technology, INC.). To record the temperature experienced by bees within each vial, we recorded temperature during thermal assays from K-type thermocouple loggers (DT-847U, Instrument Choice) placed within empty vials alongside the vials containing bees.

For each experimental run, we placed up to 14 bees in an array of vials. We held bees at 22ºC for 10 minutes, before ramping down to -10ºC at a rate of 0.25ºC/min. We monitored bees continuously for signs that they had reached their lower thermal limits (CT_{min}). CT_{min} was defined as the point at which an individual is no longer able to right itself after being flipped on its back (Kingsolver and Umbanhowar, 2018). We then allowed bees to cool further, until they ceased all movement and entered into a chill coma (MacMillan and Sinclair, 2011). We removed bees from the Peltier plate following chill coma and allowed them to equilibrate at room temperature. We then immediately began upper thermal limit (CT_{max}) trials, by returning bees to the Peltier plate and holding them at 22ºC for 10 minutes, before ramping to 60ºC at a rate of 0.25ºC/min. We observed bees continuously for signs of CT_{max}. CT_{max} was defined as the onset of involuntary muscular spasms, which is commonly used as an upper temperature threshold for ectotherms (Lutterschmidt and Hutchison, 1997; Oyen and Dillon, 2018). Finally, heat death temperature was also recorded.

3.6.6 Analysis

We conducted all analysis within R Studio Version 4.2.2 (R Core Team, 2022). To determine the influence of elevation on *E.* cf. *bicolor* nest abundance, we constructed a generalised linear model using the *glm.nb* function in the 'MASS' package (Venables and Ripley, 2002) and used a negative binomial model due to the presence of over-dispersed count data. We checked statistical significance using the *summary* function and performed model validation using the *check_model* function within the 'performance' package (Lüdecke *et al.*, 2021).

To analyze the relationship between *E.* cf. *bicolor* abundance and canopy cover within each circular buffer at each plot, we developed zero-inflated negative binomial models incorporating canopy cover as a conditional variable to account for zero inflation. The analysis was performed using the *zeroinfl* function from the 'pscl' package (Jackman, 2024), specifying a negative binomial distribution for the count data and a logit link function for the zero-inflation model component. To evaluate each model's fit and compare it against a simpler model without zero inflation, we employed the Vuong's closeness test, using the *vuong.test* function in the 'mpath' package (Wang, 2024). We assessed model diagnostics to ensure the robustness and appropriateness of each model's fit. Pearson residuals were extracted using the *resid* function in the 'stats' package (R Core Team, 2022), specifically tailored for zero-inflated models. We plotted the predicted values against the residuals for visual inspection of patterns that might indicate deviations from model assumptions.

To determine which sized buffer best predicts *E.* cf. *bicolor* abundance, we constructed a series of zero-inflated negative binomial models corresponding to different distances ranging from 10 meters to 100 meters. Model selection was conducted using the *aictab* function from the 'AICcmodavg' package (Mazerolle, 2023).

To analyse the effect of temperature on *E.* cf. *bicolor* activity, we constructed a generalized linear mixed model (GLMM) using the *glmmTMB* function in the ‘glmmTMB’ package (Brooks *et al.*, 2017). We used a binomial distribution with nest activity as the response variable, with fixed effects for temperature and its quadratic term, as well an interaction term between site and temperature to assess potential differences between Perisher and Charlotte Pass. To address time autocorrelation within each observation day, we aggregated the data into 30-minute intervals and used an autoregressive AR(1) function within model specification (Harrison, 2021; Kristensen, 2024). We set time as a factor with explicit levels, grouped by date as a grouping variable using the ‘lubridate’ package (Grolemund and Wickham, 2011). We included a random effect for Date and Nest ID to account for variation among nests. We performed model diagnostics using the *check_model* function in the ‘performance’ package (Lüdecke *et al.*, 2021).

To determine the influence of temperature on foraging trip length, we constructed a GLMM with foraging time as the response variable and average, minimum and maximum temperature during foraging trip as the predictor variable, and Nest ID as a random effect. To test for a difference in the temperature at which bees either depart from or return to the nest, we conducted a two-sample t test.

We created mechanistic niche models at each of our two study sites that predict *E.* cf. *bicolor* activity using the ‘NicheMapR’ package (Kearney and Porter, 2020), parameterizing models using field-collected data (Supplementary Information Table 2). These models integrate a species’ biological attributes (physiology, behaviour and morphology) with abiotic conditions (topography, locality, climate) to model biophysical traits such as activity time (Kearney and Porter, 2020). We used the *micro_silo* function to produce hourly estimates of microclimatic conditions during our study period at Charlotte Pass (148.323187, -36.443593; 1850m a.s.l.), where the species was most abundant according to our sampling and where our camera traps were set up. This function downloads climate data collected from the Australian Government’s SILO database, which uses Bureau of Meteorology observational data spatially interpolated to create a gridded raster for our study site during the observation period (Jeffrey *et al.*, 2001).

We then parameterized the *ectotherm* function using physiological traits sampled from individuals collected in the field. Because air temperature is typically coupled with body temperature for small insects (Stevenson, 1985), we treated air temperature sampled from our iButtons as insect body temperature. To avoid the influence of outliers, we used the 1st percentile of activity temperature (15.75°C) recorded from camera traps for minimum foraging temperature (TF_{min}). Because this species is limited primarily by cold conditions, temperatures recorded in the field were not indicative of maximum foraging temperature (TF_{max}) or preferred body temperature (T_{pref}). For instance, the maximum temperature we recorded was 28°C, while Jaboor *et al.* (2022) showed that the maximum foraging temperature for *E. robusta* was 38.82°C. We used this value of 38.82°C for TF_{max}, but it did not influence model outcomes as the study site never approached this temperature. We saw no evidence of basking at nest entrances prior to departing for foraging, so minimum basking temperature (TB_{min}) was not considered.

We performed Pearson correlation analyses using the *cor.test* function in the 'stats' package (R Core Team, 2022) to validate modelled microclimate and nest activity versus data observed in the field for our sample dates. At Charlotte Pass, modelled microclimate ($r = 0.93$, $p < 0.01$, Supplementary Information Figure 2) and activity ($r = 0.74$, $p < 0.01$) were strongly positively correlated with observations, indicating that the model predicted microclimate and nest activity well. However, at Perisher, while modelled microclimate accurately predicted temperature ($r = 0.88$, $p < 00.1$) activity was only moderately correlated with observed data ($r = 0.66$, $p < 00.1$), indicating a weaker relationship between model predictions and observed nest activity. Because model validation indicated a strong relationship between modelled and observed data at Charlotte Pass but not Perisher, subsequent climate change predictions were only performed at Charlotte Pass, where our field data also indicated the bee is most abundant.

To predict shifts under climate change scenarios, we compared annual foraging activity under present climate conditions to two climate change scenarios in 2100 (Mi *et al.*, 2022; Muñoz *et al.*, 2022; Gao *et al.*, 2024). We averaged activity over ten years of SILO climate data (2015-2024) to represent the present climate. Representative Pathway Concentration (RCP) 4.5 represents a moderate climate change scenario where greenhouse gas emissions peak around 2040 and then decline, leading to predicted global average temperature increase of 2-3°C. RCP 8.5 represents a severe scenario where emissions continue unabated and predicted global average temperature may increase by 4.3-6.1°C (Muñoz *et al.*, 2022). To model these scenarios, we set the warm parameter within *micro_silo* to 2.5°C for RCP4.5, and 5.2°C for RCP8.5 (Gao *et al.*, 2024), thereby projecting future climates for this site that use the same 10-year base line.

3.7 Results

3.7.1 Nest Abundance with Elevation

Across 21 sites, we sampled a total of 840 *Eucalyptus* branches which contained 372 *E.* cf. *bicolor* nests. Nest abundance per branch range from 0 to 2.1 (mean=0.56±0.51). Nests were only found in snow gum (*E. pauciflora* subsp. *niphophila)* branches, with no nests found in other *Eucalyptus* species at lower sites. No nests were found below 1530m a.s.l. There was a statistically significant effect of elevation on nest abundance (p<0.05), with an increase in nest abundance observed with increasing elevation (Figure 1).

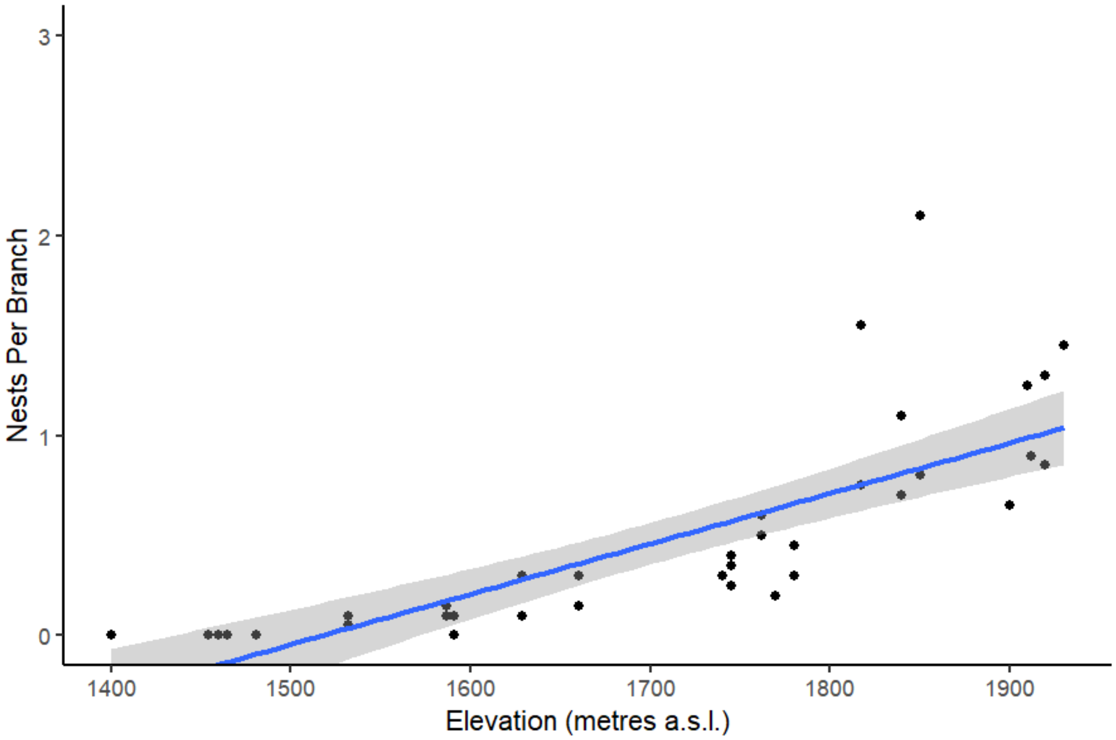


Figure 1. *Exoneura* cf. *bicolor* nest abundance per branch across elevation, from a sample of 20 branches at each site. Grey band shows 95% Confidence Interval.

3.7.2 Foraging and Canopy Cover

Having considered buffer sizes ranging from 10 to 100m, model comparison revealed that the model with a 30m buffer best explained the variation in *E.* cf. *bicolor* abundance with proximity to nearby canopy cover (AICc=252.05, ΔAICc>2, Supplementary Information Table 3). The functional relationship was positive, with *E.* cf. *bicolor* activity increasing with snow gum canopy cover within 30m, with very low activity when canopy cover is below 3% (Figure 2).

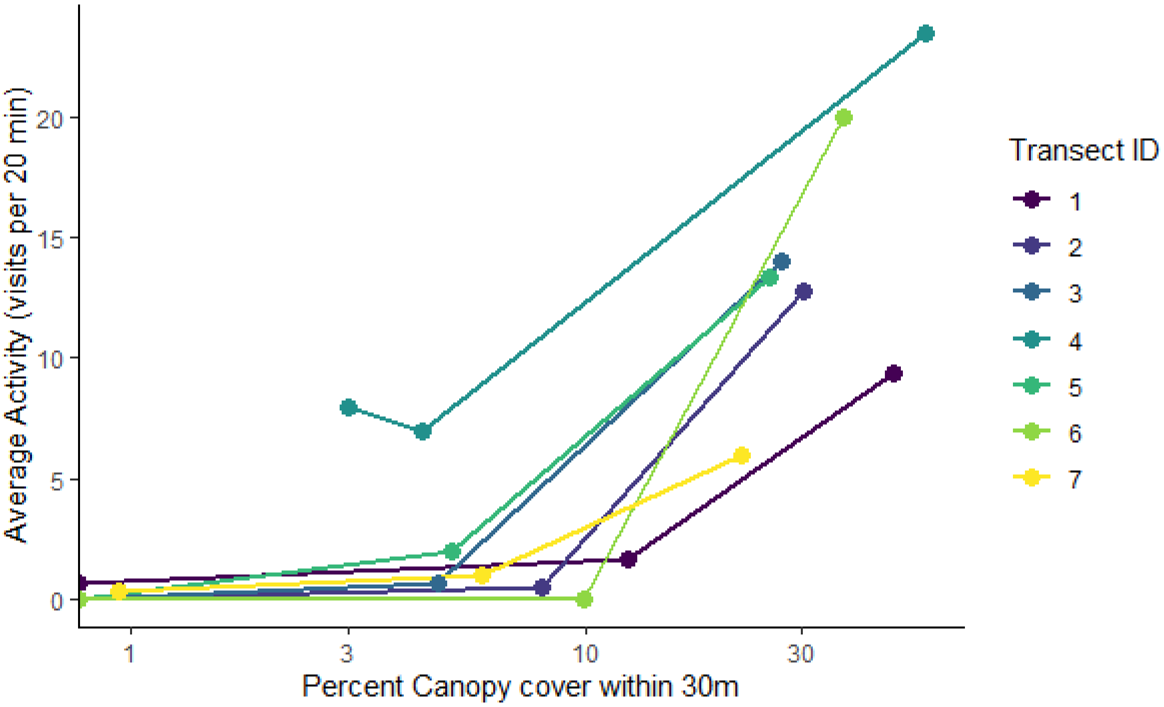


Figure 2. The effect of snow gum (*Eucalyptus pauciflora* subsp. *niphophila*) canopy cover on *Exoneura* cf. *bicolor* visitation rate within a 30m buffer. Grey band shows 95% Confidence Interval. Each line displays data from one of the seven transects, with three points showing far, mid and near plots.

3.7.3 Activity at the Nest

We captured 1287 hours of footage across 50 days, in which 825 separate instances of *E.* cf. *bicolor* activity were observed from 16 different nest entrances. In several instances, we observed multiple adults inhabiting the same nest. The range of temperatures captured was -4.5 to 28°C, with *E.* cf. *bicolor* active above 14.5°C. Activity increased with temperature and became most likely at ~17°C (Figure 3). We did not detect an upper temperature threshold, with activity continuing up to the maximum recorded temperature of 28°C. There was a significant effect of both temperature and the quadratic term for temperature on *E.* cf. *bicolor* activity ($p<0.05$). Site and the interaction between site and temperature were not significant, indicating no difference between the temperature response for populations at the two study sites. We found no difference between nest departure temperatures and arrival temperatures (paired t, $t(289)=0.31766$, $p>0.05$)

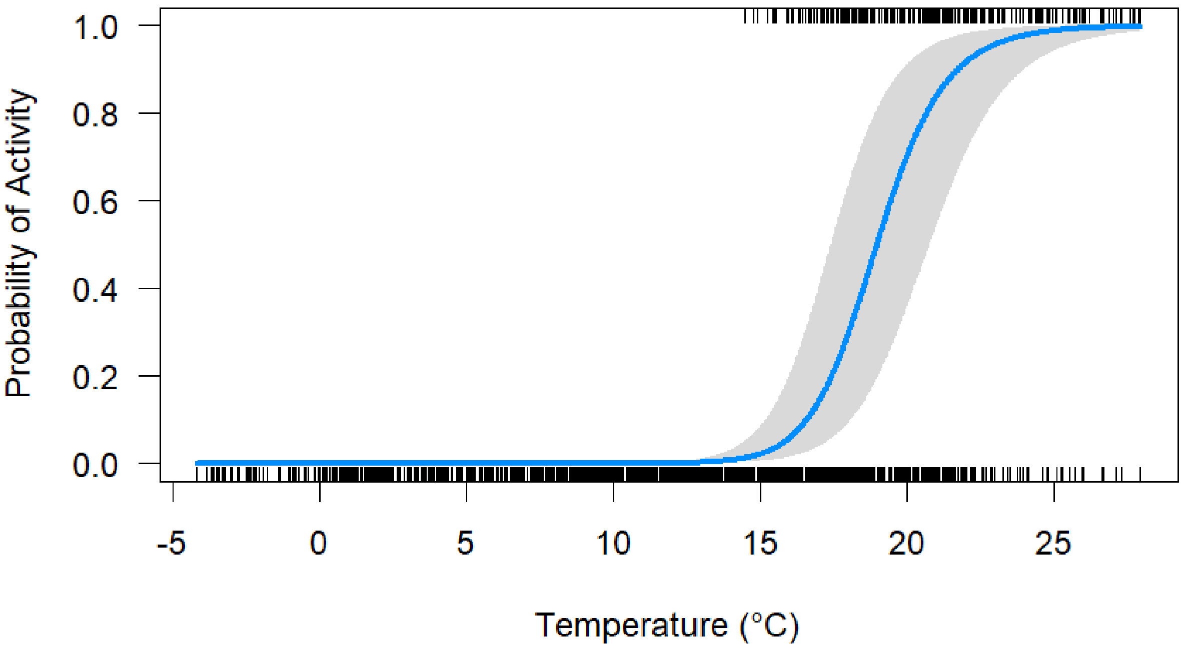


Figure 3. Binomial plot showing the effect of temperature on probability of *Exoneura* cf. *bicolor* activity. Grey band shows Standard Error. Lines along top and bottom of plots show individual observations for the presence and absence of activity, respectively.

We were able to determine trip duration on 81 separate foraging trips. Trips ranged from 5 to 90 minutes, with a mean trip duration of 27.3±16.9 minutes. We found no significant effect of minimum, maximum or average temperature during the foraging trip on foraging trip duration ($p>0.05$).

3.7.4 Thermal Limits and Biophysical Modelling of Activity

We collected 51 female *E.* cf. *bicolor* individuals for thermal assays: 34 from Charlotte Pass and 16 from Perisher. CT_{min} occurred at 3.5±2.1°C (range 0 to 7.2°C).

Chill coma occurred at -0.16±1.8°C (range -3.3 to 4.6°C). CT_{max} occurred at 44.6±2.9°C (range 37.7 to 51.6°C). Heat death occurred at 47.1±2.7°C (range 37.7 to 52.8°C). T tests (Supplementary Information Table S4) found no statistical significance of sampling site on chill coma, CTmax, or heat death (all $p>0.05$) while CT_{min} varied between sites ($p < 0.05$). Thresholds from Charlotte Pass bees were then used to parameterize our biophysical models for this site. No evidence of heterothermic behaviour, such as warming the body through vibrating flight muscles, was observed during assays.

Under present climate parameters, predicted foraging activity at Charlotte Pass spanned from November to March, peaking in January and February, with 89.1 days and 552.7 hours of annual activity available. Under RCP4.5, foraging activity increased to 132.6 days and 964.6 hours, representing a 48.8% increase in active days and a 74.5% increase in active hours. Under RCP8.5, activity rose further to 172.7 days and 1492.1 hours, representing a 93.9% increase in active days and a 170.0% increase in active hours.

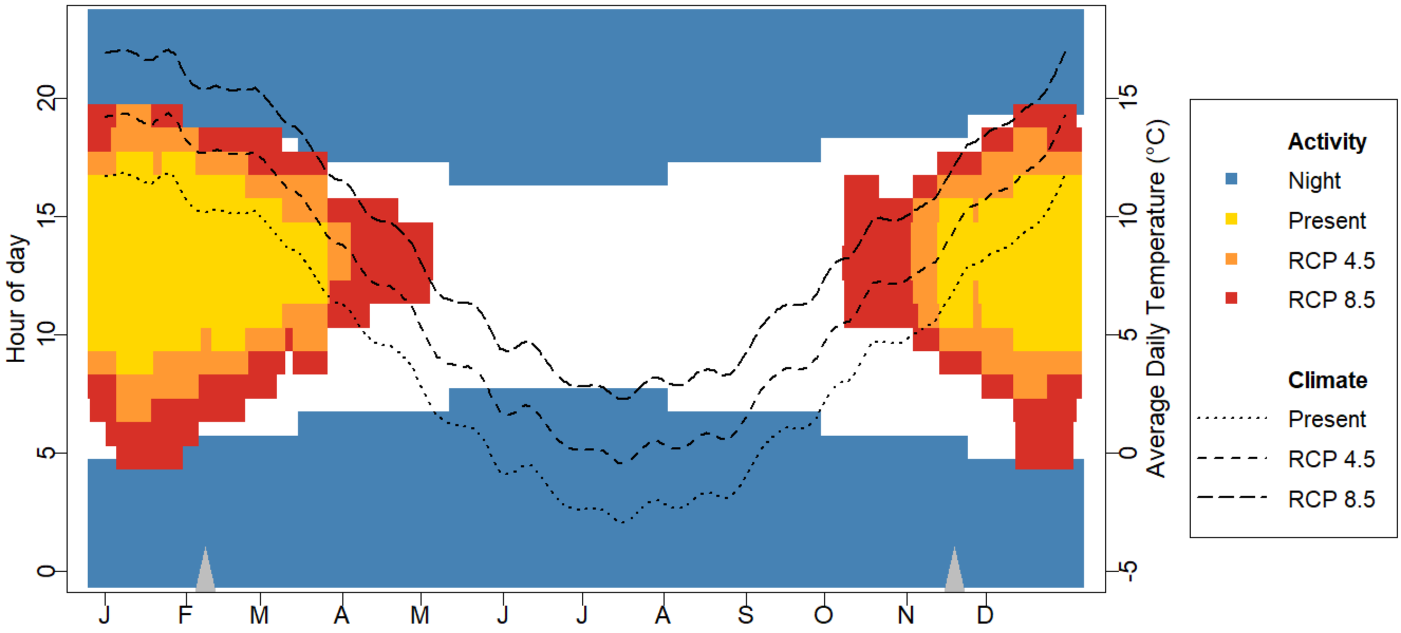


Figure 4. Predicted annual activity period for *Exoneura* cf. *bicolor* at Charlotte Pass, shown for present climate, RCP 4.5 and RCP 8.5 climate scenarios. Lines indicate average daily temperature, with axis on right. Grey triangles on bottom axis indicate the field data collection period (November to February).

3.8 Discussion

Here we investigate the ecology of a bee species that manages to survive in Australia's coldest environments, representing a unique cold-tolerant Australian bee. We show that the traits that allow *E.* cf. *bicolor* bee to thrive in cold climates are unique from the strategies that allow other well-studied cold-tolerant bees, such as *Bombus* (Oyen and Dillon, 2018), to persist in cold mountain environments. By documenting features of *E.* cf. *bicolor*'s nesting, foraging behaviour and thermal ecology, we show that despite physiological constraints that limit the availability of foraging activity, their nesting strategy may offset some of the metabolic costs and temperature thresholds that restrict pollinator activity in mountains ecosystems. However, our findings also highlight vulnerabilities under climate change.

3.8.1 Wood Nesting Strategy

We have documented that *E.* cf. *bicolor* have shifted to wood nesting from the typical stem nesting habit displayed by other *Exoneura* (Michener, 2007), and suggest that this behaviour has enabled this bee to persist in cold, high-elevation locations where they benefit from reduced competition for floral resources from other bees. The elevational distribution of *E.* cf. *bicolor* nests in our study, with no nests found below 1530m a.s.l., closely mirrors the elevational distribution of snow gums, which are the only trees above 1600m a.s.l. (Duncan, 2010). Wood as a nesting substrate provides important thermal insulation benefits, buffering nest temperatures from cold ambient conditions (Jones and Oldroyd, 2006). Nesting in wood is likely to be thermally advantageous in this environment relative to soil-nesting or stem nesting. Beyond thermoregulation, wood cavities are longer-lived than stems, enabling multi-season persistence and natal nest reuse by daughters (Silberbauer & Schwarz 1995). Takeshima *et al.* (in prep) showed that *E.* cf. *bicolor* were present on flowers down to 900m a.s.l. near our study area, even though we found no nests in wood below 1530m a.s.l. in our survey. It is unclear whether the *E.* cf. *bicolor* collected at lower sites nearby (Takeshima *et al.,* in prep) nest in wood or stems. Importantly it is also yet to be determined if the populations of this newly discovered bee are interbreeding or discontinuous.

Evidence for the relatively low competition for floral resources at higher elevations comes from surveys of flower visitors in these sites (Inouye and Pyke, 1988; Coates *et al.*, 2024). Coates *et al.* (2024) showed that at Charlotte Pass, *Exoneura* comprised 71% of visits from bees to flowers, representing a higher proportion than for *Exoneura* at lower elevation sites along an elevational gradient down to 900m a.s.l, where *Exoneura* comprised 38% of bee visits. This study also showed that bees tended to visit different plant families than flies, which were most abundant at high-elevation sites, so that resource partitioning may allow for reduced floral competition between flies and *E.* cf. *bicolor* at these sites (Coates *et al.*, 2024).

3.8.2 Foraging Opportunities

Given that *E.* cf. *bicolor* restricts foraging activity to temperatures above 15.75°C, there are limited opportunities for thermally suitable foraging conditions in this environment. As central place foragers with a fixed nesting site, *E.* cf. *bicolor* are unable to move to track suitable microclimatic conditions (Kenna *et al.*, 2021). During our observations from November to February, inclement weather was common, with temperatures failing to surpass 15.75°C for a total of 14 days, including a period of seven consecutive days where no activity was recorded. This contrasts with flies, the most abundant pollinator in this system, which Coates *et al.* (2024) showed were able to forage at lower temperatures. Other cold-adapted bees, such as *Bombus*, can forage at temperatures as low as 5-10°C (Corbet *et al.*, 1993), while *E.* cf. *bicolor's* thermal physiology restricts foraging opportunities in this environment.

While bees of this body size are typically capable of flying up to 1 km (Greenleaf *et al.*, 2007) *E.* cf. *bicolor* were primarily foraging within 30m of nests in areas around snow gum stands. Optimal foraging theory proposes that animals will maximise their net energy gain by balancing the costs of foraging with the energetic value of the resources obtained (Stephens and Krebs, 1986). The fact that *E.* cf. *bicolor* achieve a net energy gain foraging in such a small area, and in only limited suitable foraging times, suggests that nectar and pollen is relatively abundant. These short flights may also reduce the risk of being caught too far from the nest if the temperature suddenly drops. In cold environments, low temperatures raise the metabolic costs of flight and reduce the efficiency of foraging for pollinators (Harrison and Fewell, 2002). The strategy of using short-distance flights to take advantage of the short periods of thermally suitable weather may seem to be tenuous, but the high abundance of *E.* cf. *bicolor* in this landscape (Coates *et al.*, 2024) indicates that it can support a thriving population. Short foraging bouts may also enable this relatively small bee to unload pollen resources more often than larger bees. This short-distance foraging pattern also means that *E.* cf. *bicolor*'s geographic presence in the landscape is closely tied to the presence of snow gum stands, which form a mosaic at the tree line and in nearby frost hollows. Their role as pollinators is also expected to diminish rapidly with distance from snow gums.

3.8.3 Future Foraging Activity Under Climate Change

Our biophysical modelling suggests a substantial expansion in the activity window of *E.* cf. *bicolor* under future warming scenarios. While this offers insight into how thermal constraints will relax, the scale of predicted change introduces uncertainty, particularly when considering how interacting ecological processes may influence outcomes. Biophysical models offer a framework for predicting a species' fundamental niche, but they do not account for all biotic influences that constitute its realized niche (Kearney and Porter, 2009). As such, our projections reflect physiological potential rather than direct predictions of behavioural outcomes. Other biotic factors that influence *E.* cf. *bicolor*'s survival, such as changes in competition dynamics, associations with flowers, and impacts on immature life stages will also determine their response to climate change but are not captured by our model's predictions. For instance, in *E. robusta*, warmer conditions have been linked to increased brood production and more frequent social nesting (Cronin and Schwarz, 1999a), while studies of butterflies suggest different vulnerabilities across larval and egg life stages compared to adults (Buckley and Kingsolver, 2012; Kiekebusch *et al.*, 2024).

Given the current low bee diversity at high elevations in this area (Coates *et al.*, 2024; Encinas-Viso *et al.*, 2024), warming may introduce increased competition for floral resources from bees moving up from lower elevations, or increased competition from less-abundant bees already present in the area, such as *Lasioglossum* species (Encinas-Viso *et al.*, 2022; Coates *et al.*, 2024). Similar range shifts have been observed in other alpine pollinators, including *Bombus* (Pyke *et al.*, 2016) and butterflies (Konvicka *et al.*, 2003; Forister *et al.*, 2010), with species moving upslope to track their preferred temperature envelope. This shift in competition dynamics may be especially pronounced at our highest sites, where *E.* cf. *bicolor* populations were most abundant but will be unable to move upslope due to the absence of wood for nesting above the current tree line. Snow gums are unlikely to keep pace with upslope shifts in climate under rapid climate change as they are a slow-growing tree and are currently experiencing dieback that appears to be linked to climate change (Charerntantanakul *et al.*, 2025). In contrast, ground-nesting bee species, which make up the majority of non-*Exoneura* bee diversity present nearby and downslope (Coates *et al.*, 2024) are expected to be able to move more freely without losing access to required nesting sites.

Biophysical modelling indicated that *E.* cf. *bicolor*'s seasonal window will expand under climate change, which may have consequences for their association with flowers. While our models shows that *E.* cf. *bicolor* will be active earlier and later in the season under warming, it is unknown how flowering times of key plants will respond to the same climate signal. Earlier flowering under climate change has been observed in some Australian alpine species (Gallagher *et al.*, 2009), as well as in alpine systems globally (Studer *et al.*, 2005), but responses are variable across taxa and years (Kudo, 2014) and so may not match predicted shifts in early-season *E.* cf. *bicolor* foraging. Late-season foraging similarly depends on timing and duration of flowering for different species. The predicted increase in late-season activity for *E.* cf. *bicolor* could translate into decreased foraging success, with comparable mismatches showing declining overlap between pollinators and late-flowering species in the Rocky Mountains (Iler *et al.*, 2013) and Arctic systems (Høye *et al.*, 2013; Schmidt *et al.*, 2016). While such dynamics are plausible in our system, a better understanding of floral phenological responses is required to assess this risk.

3.8.4 Cold-Climate Strategies

This study indicates that the traits that make *E.* cf. *bicolor* successful in cold, high-elevation environments are distinct from those of the better known and well-studied bees such as *Bombus*. *Bombus*, a cold-tolerant bee genus distributed in every continent except Australia and Antarctica (Williams and Osborne, 2009), display unique behavioural and physiological traits (Heinrich, 2004) that allow them to fly at temperatures as low as 5-10°C (Corbet *et al.*, 1993). In our study, *E.* cf. *bicolor* were unable to fly below 14.5°C, with probability of activity increasing at approximately 17°C, indicating that their physiological limits afford only a relatively narrow window for activity in this environment. Despite these indications that *E.* cf. *bicolor*'s temperature thresholds present significant limits to activity in this environment, the abundance of nests across our elevational gradient was highest at the tree line where it is coldest, with Coates *et al.* (2024) also demonstrating that they are abundant on flowers at these sites. *E.* cf. *bicolor*'s behavior of nesting in well-developed branches of snow gum appears to be the key trait that allows them to persist in this environment. During the spring-summer foraging period, nesting in snow gums provides shelter overnight and during cold, inactive days, but may be even more advantageous when populations overwinter, when ambient temperatures regularly drop below -10°C at Charlotte Pass (Bureau of Meterology, 2025). In alpine environments overseas, *Bombus* queens overwinter in a state of diapause (Whitehorn *et al.*, 2025). While the overwintering behaviour of *E.* cf. *bicolor* at our sites area is unknown, at lower elevations elsewhere in Australia, stem-nesting *E. bicolor* adults overwinter alongside developing brood (Silberbauer and Schwarz, 1995). While further research is needed, it is possible that, like lower-elevation *E. bicolor*, *E.* cf. *bicolor* females also overwinter within their nests, where the thermally buffered environment of snow gum wood may provide critical protection during sub-zero winters.

3.9 Conclusions

E. cf. *bicolor* is a previously undocumented case of a cold-adapted bee that makes up for its limited physiological cold tolerance with displaying behavioural traits that enable persistence in Australia's coldest environments. The success of *E.* cf. *bicolor* at high elevations appears to hinge on its unique wood nesting behaviour, which likely provides it with a thermally buffered environment. In contrast to other physiologically cold-tolerant pollinators, *E.* cf. *bicolor* remains inactive during unfavourable weather, waiting for brief warm periods to forage. By sheltering in these nests and foraging for abundant nearby resources during suitable weather, *E.* cf. *bicolor* benefits from abundant floral resources and reduced competition from other bees. However, its capacity to maintain this low-effort, high-reward strategy may be compromised under climate change. While we predict future warming will extend the bees' foraging window, it may also introduce changes in competition dynamics, potential for phenological mismatches, and the inability to expand their range above the tree line. As warming permits more competitors to move upslope, *E.* cf. *bicolor* may face increased competition without the ability to shift its range. In this context, its reliance on nesting within snow gums may shift from an advantage to a liability. These findings highlight the vulnerability of insect pollinators to climate change, with broader implications for the stability of alpine plant-pollinator networks. Future research on *E.* cf. *bicolor*'s nesting biology (Tierney *et al.*, in prep), ecology during other life stages, particularly while overwintering, and on their thermal ecology at other elevations (Takeshima *et al.*, in prep), will help to reveal how they mediate survival under extreme winter conditions.

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3.12 Supplementary Material

Table S 1. Sites selected for nest surveys. Each elevational band contained 3 study sites, randomly selected along Kosciuszko Road using a random number generator.

Elevation band	Location	Horizontal extent along Kosciuszko Road	RNG x3	GPS Coordinates
13-1400m	Top of montane zone	1.6	2 (0.32km) 3 (0.48km) 8 (1.28km)	• -36.35075638688645, 148.5455851558787 • -36.34946079743964, 148.54482654028584 • -36.34412365423711, 148.53998246534525
14-1500m	Below Wilsons Valley	2.1km	2 (0.42km) 5 (1.05km) 8 (1.68km)	• -36.34663631502185, 148.53298207469143 • -36.34716811181311, 148.52616299521603 • -36.35035720279291, 148.52141776131208
15-1600m	Wilsons Valley Chain Bay to Rennix walking track, then again around Sponars	4.6km	8 (3.68km) 10 (4.6km) 4 (1.84km)	• -36.36168630315609, 148.5027662600055 • -36.36531277788674, 148.47790100380917 • -36.361414326725914, 148.48440153771634
16-1700m	Rainbow lake walking track to Smiggins	5.3km	1 (0.53km) 4 (2.12km) 2 (1.06km)	• -36.37271683165397, 148.47007370651505 • -36.37581347066811, 148.4665890594266 • -36.393705546183355, 148.43164236616525
17-1800m	Just after Smiggins to just after Perisher (a), then inbetween Perisher and Charlotte Pass (b)	a)3.6km b)4.5km 8.1km total	6 (4.86km) 7 (5.67km) 9 (7.29km)	• -36.42146198860946, 148.3809877232048 • -36.425770477559624, 148.36985143591278 • -36.42691531807462, 148.3502415949011
18-1900m	Perisher gap (a), otherwise from Charlotte Pass village turnoff along Summit Track (b)	a)400m b)2.2km 2.6km total	7 (1.82km) 4 (1.04km) 2 (0.52km)	• -36.43068286903584, 148.3332227508486 • -36.432134351956684, 148.3278372891131 • -36.436124515051674, 148.32144658139572
19-2000m	Stillwell walking track	1km	10 (1km) 4 (0.4km) 8 (0.8km)	• -36.43905337549333, 148.3242741320121 • -36.44221564935072, 148.32516653130506 • -36.44372886660407, 148.32620362307082

Figure S 1. Examples of *Exoneura* cf. *bicolor* nest entrances, with the characteristic rim that individuals create at nest entrances visible. From left to right: newly excavated nest showing frass at nest entrance, newer nest with rim not yet dried, typical older nest with rim dried to a grey colour, *E.* cf. *bicolor* individual at entrance of nest.

Table S 2. Relevant parameters used in ectotherm model.

Parameter	**Description**	**Unit**	**Value**	**Source**
CT_{max}	Critical thermal maxima	°C	47.1	This study
CT_{min}	Critical thermal minima	°C	0.16	This study
TF_{min}	Minimum foraging temperature	°C	15.75	This study
TF_{max}	Maximum foraging temperature	°C	38.82	(Jaboor *et al.*, 2022)
W_w	Wet body mass	g	0.015	This study
Percent wet	Fraction of body surface acting as a free-water surface		0.01	Estimated
Percent eyes	Fraction of eyes surface acting as a free-water surface		0	Estimated
Burrow	Can the animal move underground?		False	
Shape	Body shape		Cylinder	

Table S 3. Candidate models included in selection process for *Exoneura* cf. *bicolor* foraging distance analysis.

Model	AICc	Delta_AICc	AICcWt
model_30m	252.0482	0.0000	0.5709
model_40m	254.3852	2.3370	0.1775
model_100m	256.5423	4.4941	0.0604
model_90m	257.1865	5.1383	0.0437
model_50m	257.1879	5.1397	0.0437
model_80m	257.9316	5.8834	0.0301
model_20m	258.0073	5.9591	0.0290
model_70m	258.2332	6.1850	0.0259
model_60m	259.0244	6.9761	0.0174
model_10m	264.1112	12.0630	0.0014

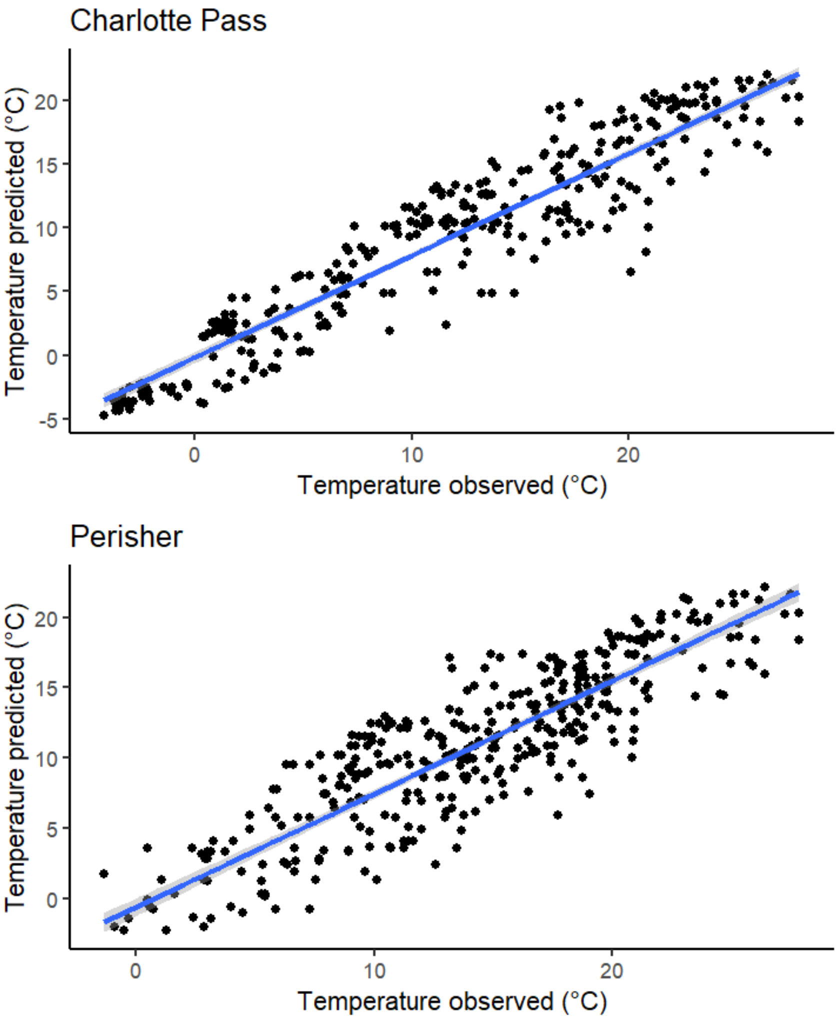
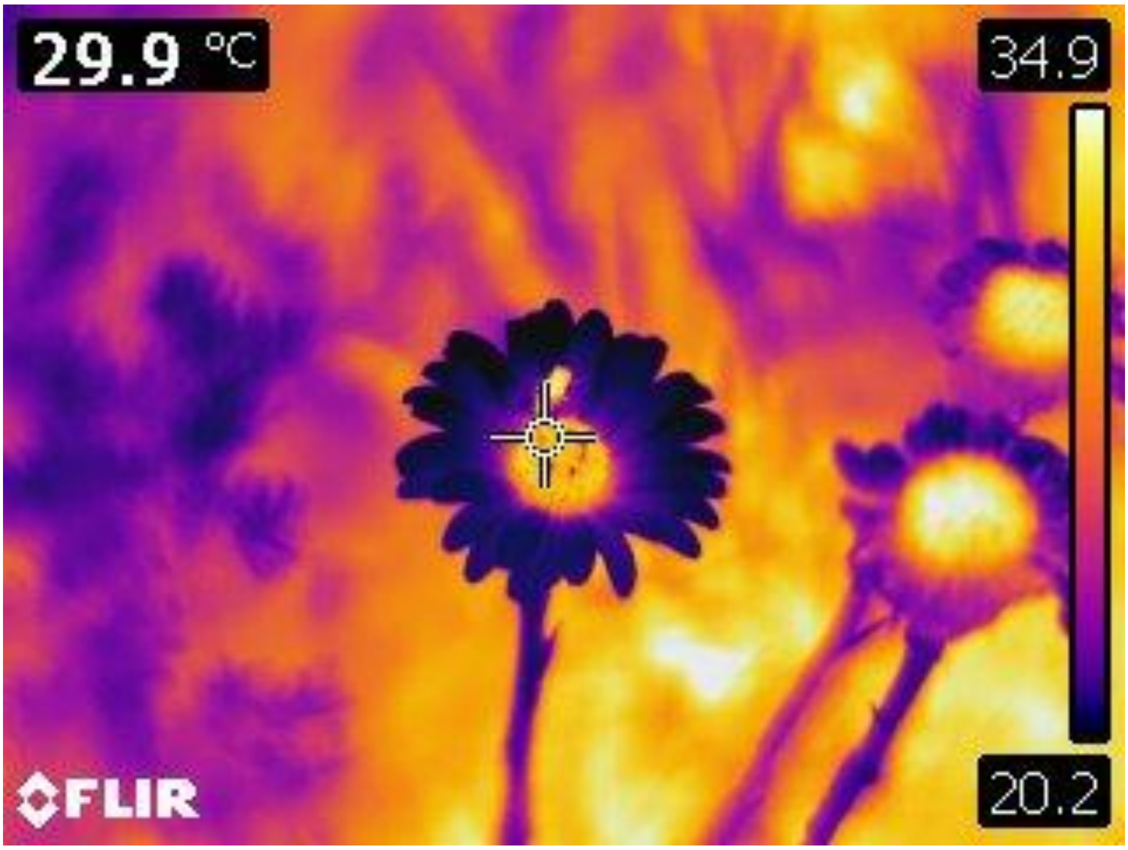


Figure S 2. Correlation between observed temperatures and predicted temperatures from the ‘micro_silo’ package.

Table S 4. T test results from comparison of mean thermal limits (°C) for bees collected at Charlotte Pass versus Perisher study sites. p-values are two-sided. Welch's t-test was used (unequal variances).

Threshold	**t**	**df**	**p**
CT_{min}	-4.17	31.54	2.20e-04
Chill coma	-1.72	47.98	0.092
CT_{max}	-0.60	36.62	0.553
Heat death	1.32	38.40	0.195

Chapter 4: Hotspots on Cold Mountains: Hot Flowers as Pollinator Refuges in Mountain Ecosystems



Citation: Coates J.M., Evans M.J., Scheele B.C., Cunningham S.A. in review. Hotspots on Cold Mountains: Hot Flowers as Pollinator Refuges in Mountain Ecosystems. Functional Ecology

4.1 *Foreword*

This chapter focuses on the microhabitat conditions experienced by pollinators while foraging on flowers. I investigate whether mountain flowers provide functionally meaningful thermal refuges for ectothermic pollinators. Using community-wide sampling of floral microclimates with infrared thermography paired with pollinator visitation data from Chapter 2, I test whether fine-scale thermal heterogeneity within flowers influences pollinator activity. I seek to understand whether flowers offer a thermal benefit that reduces thermoregulatory costs and increases suitable foraging opportunities.

This chapter's key contribution is as the first community-level research showing that insect visitation frequency is positively associated with flower temperatures when ambient air temperatures are cool, thereby connecting ambient temperature, floral microclimate, and insect activity across a mountain plant-pollinator assemblage. By connecting flower-scale thermal heterogeneity to pollinator activity levels, this chapter offers a mechanistic understanding of the conditions that govern pollinator behaviour.

4.2 Abstract

Mountain environments constrain the activity of ectothermic pollinators. Yet, fine-scale thermal refuges might buffer these effects. Pollinators spend much of their time on flowers, which begs the question: do flowers offer thermal benefits that influence pollinator activity? To test whether subalpine and alpine flowers function as warm microhabitats for insect pollinators, we quantified flower temperatures across a diverse plant community in the Australian alps and examined the relationship with the frequency of flower-insect interactions. We found that most plant species exhibited floral temperatures above ambient air temperatures, with the difference sometimes exceeding 10 °C. Environmental conditions, especially UV Index, were associated with enhanced warming, while increased relative humidity was associated with lower flower temperatures. Among the floral traits we examined, shape was the strongest predictor of warming, with globular, cup- and star-shaped flowers warming most strongly, whereas tubular and bell-shaped flowers only warmed slightly above air temperature. Pollinator body temperature was also typically warmer than floral tissues. Notably, we found that when ambient conditions were cool, warm flowers received significantly more insect visits, especially from Diptera. This effect weakened as ambient temperature increased. Our results indicate that some flowers may provide insects with a thermal reward when ambient conditions were otherwise activity limiting. Our results offer the first community-level insights suggesting that naturally warmer flowers attract more visitors during cold conditions, thereby connecting ambient temperature, floral microhabitat, and insect activity across a mountain plant-pollinator assemblage.

4.3 Introduction

Mountain environments are commonly characterised by cold climates and dynamic weather conditions, imposing considerable thermal constraints for ectothermic insects (Goodwin *et al.* 2021). These conditions limit foraging activity, increase energetic costs of thermoregulation, and can reduce survival and reproduction. This often drives pollinator community assembly at high-elevation sites, where only cold-tolerant species can persist. For instance, mountain plant-pollinator networks are typically dominated by Diptera species, which remain active under cooler conditions than most bees and display a broader foraging niche than other taxa (McCabe and Cobb, 2021; Coates *et al.*, 2024). *Bombus* bees are an exception, using endothermy and physical traits such as dense hairs to remain active in cold conditions (Kenna *et al.*, 2021). Some insects buffer against ambient conditions through behavioural use of fine-scale habitat features, with their small body size and mobility allowing them to exploit microhabitats and thereby enable activity during unfavourable conditions (Pincebourde and Casas, 2019).

Because pollinators spend a large proportion of their time visiting flowers, the thermal conditions within flowers are of particular relevance to pollinators in mountain ecosystems. Previous studies highlight how cold-climate flowers can form warm microhabitats, sometimes several degrees above ambient conditions. Some alpine flowers can actively warm their surface through processes such as heliotropism (Kevan, 1975; Stanton and Galen, 1993), chemical exogenesis and diurnal opening and closing (van Doorn and Van Meeteren, 2003). A survey of a floral community in the Swiss Alps showed that flowers averaged ~6 °C warmer than ambient air temperature, emphasising the potential for floral warming to buffer plants and pollinators against cold stress (Körner, 2014). Across six sub-Antarctic megaherbs, inflorescences were reported up to ~9–11 °C warmer than ambient air. However, these species have large, dark, densely packed flower heads, so the magnitude of warming may not be representative of most flowers (Little *et al.*, 2016). Accordingly, Shrestha *et al.* (2018) found that across 30 Australian species from diverse ecosystems, fewer than half exhibited floral temperatures above ambient, and some were cooler than air, indicating that warm floral microhabitats are not universal. While these studies demonstrate examples of floral warming, they do not directly link flower temperatures to pollinator behaviour.

Warm floral microhabitats may be important for pollinator activity and thermoregulation, especially in cold climates. By basking within warm flowers, insects in cold environments can reduce the energetic demands of flight and thermoregulation and increase activity levels. For instance, insects basking within *Dryas integrifolia* flowers in the Arctic reach up to 15 °C above ambient air temperature (Kevan, 1975). Lab experiments have also shown that *Tetragonula carbonaria* prefer flowers with warmer nectar, with more pronounced effects during cool temperatures (Norgate *et al.*, 2010) and that *Bombus terrestris* show a preference for warmer flowers (Dyer *et al.*, 2006) and can detect 2°C temperature differences within flowers (Harrap *et al.*, 2017). By painting flowers in the field with reflective paint to reduce solar heat gain, Apland *et al.* (2025) showed that Diptera favoured warmer flowers in cool ambient conditions but that this effect switched as ambient conditions warmed. Similarly, by manipulating the orientation of sunflower heads to reduce solar warming, Creux *et al.* (2021) showed that sun-facing flowers received more visits and produced more seeds. Warmer flowers may also enhance nutritional rewards via increased nectar production (Petanidou and Smets, 1996). Such thermal rewards could reduce the costs of foraging in challenging conditions, extending activity windows via a thermal reward. However, most evidence of insect floral warming derives from lab assays or single-species studies, and community-level, in situ tests of how natural floral warming alters plant-pollinator interactions across taxa are lacking.

Floral traits strongly influence the capacity of flowers to generate and retain heat (Van der Kooi *et al.*, 2019). Previous studies suggest that flower shape may be particularly important, with upward-facing, bowl- or cup-shaped corollas acting as “solar ovens,” that reflect and focus radiation onto central reproductive structures and create boundary layers that reduce heat loss (Kevan, 1975; Herrera, 1995). Other floral traits such as size, orientation and colour can also influence floral warming (Van der Kooi *et al.*, 2019). Larger flowers and dense inflorescences can provide greater thermal mass, allowing them to retain heat for longer, while orientation enables flowers to maximise solar gain throughout the day (Van der Kooi *et al.*, 2019). Recent work that compared floral warming in eight species in a subalpine meadow further shows that open flower morphologies warmed more than closed flowers, especially when flowers were yellow, whereas closed flowers were thermally more stable (Apland *et al.*, 2025). Together, these findings indicate that multiple traits interact to shape floral thermal properties.

Floral warming can also directly benefit plant reproductive performance by increasing the rate of reproductive tissue development. Small increases in floral temperature accelerate pollen germination and tube growth and shorten fertilisation time, lowering the risk of subsequent embryo abortion (Stanton & Galen, 1989; Stephenson, 1981; van der Kooi *et al.*, 2019). For instance, experimental warming of early-spring *Helleborus foetidus* increased the number of pollen tubes reaching the ovary (Herrera & Medrano, 2016). By keeping floral reproductive tissues within suitable temperature ranges, warming can also help buffer against cold snaps and resulting damage (Stephenson, 1981; van der Kooi *et al.*, 2019). These benefits are most evident in cold-limited systems, where even small thermal increments can translate into higher seed set (van der Kooi *et al.*, 2019).

Taken together, this literature establishes that flowers can be warmer than ambient conditions and that warming can influence plant function and insect activity. However, most evidence comes single-species studies, often under lab conditions or with experimental manipulation of flowers with only a limited set of field conditions examined. Additionally, lab-based studies that measure thermal ecology of insects often fail to capture the fine-scale conditions organisms experience in the field (Terblanche *et al.*, 2007). Flower temperature data are also rarely surveyed alongside pollinator interactions, limiting inference about consequences for insects. To advance research on this topic requires in situ, season-long, community-level data that couple natural variation in floral temperatures with observed pollinator interactions to the same flowers.

In this study, using infrared (IR) thermography and concurrent flower interaction surveys within an Australian mountain ecosystem, we: (1) Quantified floral temperatures across species at the community level; (2) Determined the influence of environmental conditions on floral warming; (3) Tested whether flower traits predict floral warming; and (4) Quantified the frequency of insect interactions as a function of floral temperature to assess whether warmer flowers receive more visits during cool conditions. Our study presents a novel approach to linking flower thermal characteristics with community-level plant-pollinator interactions in situ. This integrative field design, combining IR thermography and floral interaction surveys, allows us to quantify how flower microhabitats function as a meaningful thermal refuge for insects in a mountain plant-pollinator community.

4.3.1 Methods

4.3.2 Study Area

We conducted fieldwork in Kosciuszko National Park, New South Wales, Australia across two flowering seasons from October 2021 to March 2022 and November 2022 to February 2023. This period captured the onset and peak of flowering following snowmelt. Being a whole-of-season study, our survey accommodated the peak of flowering for almost all the plant species present (Costin *et al.*, 2002). All study sites were located within subalpine and alpine ecosystems along the northeast side of the mountain range, from Perisher Valley at ~1600m a.s.l. to Mt. Stilwell at ~2050m a.s.l.

4.3.3 Sampling Procedure

4.3.3.1 Infrared Thermography

We collected high-resolution IR thermographs taken with an IR camera (Model FLIR T420, FLIR Systems, Inc., Wilsonville, OR, USA) from October 2021 to March 2022 and November 2022 to February 2023. This camera captures long-wave infrared radiation from approximately 7.5 to 13µm using a 320 × 240 pixel (76,800-pixel) uncooled microbolometer detector, translating radiance into temperature with a thermal sensitivity better than 0.045 °C at 30°C and an accuracy ±2%. We used an emissivity value of 0.98, consistent with previous studies on flowers (Harrap *et al.* 2021) and other plant tissues (Scherrer and Körner 2010). We took images from the sun-facing side of each flower under low wind, and paired them with a digital photograph to enable species identification.

We aimed to sample all flowering species in our subalpine and alpine study area between the hours of 9am and 5pm, photographing multiple individuals per species where possible to capture variation in environmental conditions. We collected data across all weather conditions except during rain, snow, or strong wind. For species with compound inflorescences, such as the numerous Asteraceae present at our study area, we considered the whole inflorescence as one functional flowering unit, and hereafter refer to these as “flowers” for consistency. We also took images of flowers when there were pollinators present, allowing quantification of pollinator body temperatures.

We measured ambient conditions using a handheld weather meter (Skywatch BL-500; JDC Electronic SA, Switzerland) placed 1m above the ground, set to continuously record air temperature, windspeed, relative humidity and ultraviolet (UV) Index, which we matched with each IR image. We used UV Index as a proxy for solar irradiance because UV radiation co-varies with the visible and IR components of sunlight. UV Index is modified by cloud cover, atmospheric aerosols, surface reflectance, and elevation, providing a convenient summary metric of incident shortwave conditions (Caldwell *et al.*, 2007).

4.3.4 Floral Traits

We compiled species-level floral trait information from Australian botanical resources (Centre for Biological Information Technology, 2025; Lucidcentral, 2025; Royal Botanic Gardens and Domain Trust, 2025), extracting family, flower size, colour and shape. From these traits, we calculated midpoints for reported size ranges, and standardised colour and shape into harmonised categories (Table S1). In the few instances where species-level identification could not be reached from our digital images due to morphological similarity, we used genus-level traits and verified them against our digital images and species with known distributions in Kosciuszko National Park (Table S1). In these instances, *Epacris* sp. comprises *E. glacialis*, *E. petrophila* and *E. paludosa*; *Craspedia* sp. comprises *C. aurantia* and *C. lamicola*; *Geranium sp.* comprises *G. retrorsum* and *G. potentilloides* var. *abditum*; and *Brachyscome* sp. comprises *B. spathulata*, *B. aculeata* and *B. nivalis*.

4.3.5 Infrared Image Processing

We processed all IR images using ThermImageJ (Giacometti and Tattersall, 2024), an ImageJ plugin designed for extracting calibrated temperature data from thermal imagery, following algorithms developed by FLIR (Minkina and Dudzik, 2009). ThermImageJ calculates temperatures from IR images by using the radiometric values stored in each pixel, along with relevant weather parameters (Giacometti and Tattersall, 2024). We specified object distance, ambient temperature and relative humidity conditions as recorded from our temperature logger during imaging. We excluded images with poor focus or extreme reflectance artefacts prior to analysis. We manually defined regions of interest (ROI) polygons around petals, floral centres, and insects. This approach captured the fine-scale thermal heterogeneity within flowers, where centres and petals often differed in temperature. Insects, by contrast, displayed relatively uniform thermal profiles and were represented by a single ROI (Figure 1). We ensured that ROIs were larger than the camera's minimum spot size to ensure reliable measurements (Playà-Montmany and Tattersall, 2021). We extracted the temperature values from each ROI for subsequent statistical analyses in R.

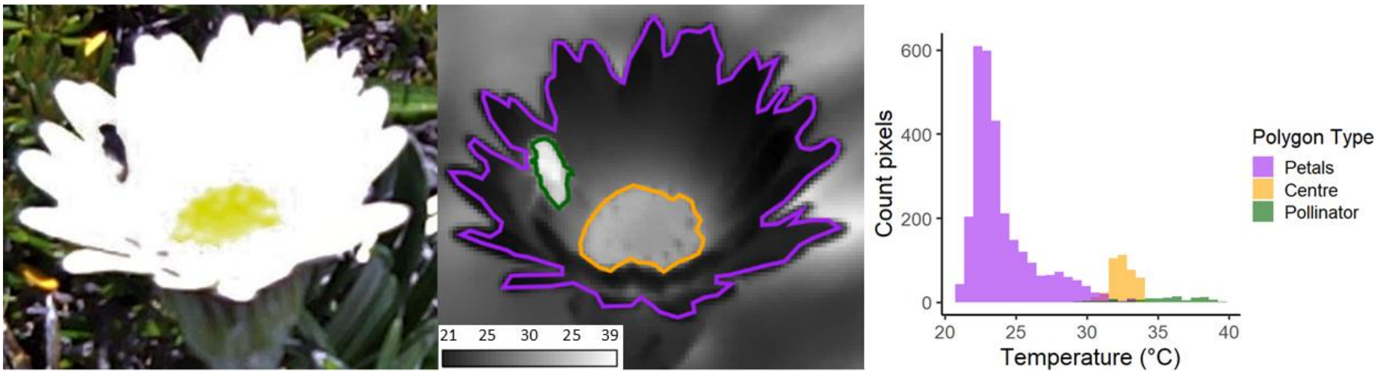


Figure 1. From left to right: Digital photograph of flower, infrared thermograph of flower with Region of Interest (ROI) polygons overlaid, and histogram of pixel temperature values from within each ROI.

4.3.6 Pollinator Interactions

We used plant-pollinator interaction data reported in Coates *et al.* (2024), restricting analyses to the sites between 1600–2000 m a.s.l., which were the same as those surveyed with IR thermography. We conducted surveys from October 2021 to March 2022, with two 50 × 50 m plots in each of the three sites. We included these sites in IR thermography sampling, often on the same day. We sampled pollinator interactions several times per week at each plot across the sample period and ensured that each plot was sampled during different times of day. In each plot, we conducted 30-minute surveys, during which we recorded interactions when insect visitors contacted floral reproductive parts. At the beginning and end of each sampling period, we recorded air temperature, windspeed, and relative humidity using a Kestrel 3000 Weather Meter (Kestrel Australia, Victoria, Australia), averaging these values for each sampling period. Sampling spanned a wide range of conditions (5–28 °C; 0–23 km/h wind; 27–93% RH) to capture interactions under both marginal and favourable weather. Across 107 surveys, we observed 1,780 plant-pollinator interactions. Full methodological details for these surveys are provided in Coates *et al.* (2024).

4.3.7 Statistical Analysis

4.3.7.1 Species-level Floral Warming

We conducted all statistical analyses in R version 4.2.2 (R Core Team, 2025). To characterise the floral microhabitat at the species level, we analysed variation in floral warming for each flower species. The response variable was the difference between the 95th percentile temperature and ambient temperature (ΔT_{max}). We used the 95th percentile for ΔT_{max}, rather than the maximum values, to reduce the influence of outliers. We excluded species with fewer than six replicates (6 species out of the 43 sampled) to avoid estimates associated with sparsely represented species (Bolker *et al.*, 2009). We fitted linear mixed-effects models with flower species, UV Index, wind speed, and relative humidity as fixed effects to control for environmental variation, and image ID included as a random effect. We tested species-level effects using ANOVA with Satterthwaite-adjusted degrees of freedom from the lmerTest package (Kuznetsova *et al.* 2017) and verified model assumptions using the performance package (Lüdecke *et al.* 2021).

4.3.7.2 Environmental Conditions

To assess the influence of environmental conditions on floral and pollinator ΔT_{max}, we fitted linear mixed-effects models using the lme4 package in R (Bates *et al.* 2015). Because most insect species were sparsely represented, we treated all flower-visiting insects (Diptera, Hymenoptera, Coleoptera) as a single group when modelling their body temperature. Separate models were constructed for pollinators and flower ΔT_{max}, to assess difference in temperature between these ROIs. We included fixed effects for UV Index, wind speed, relative humidity, and flower species. We included image ID as a random effect to account for non-independence in instances when we measured multiple flowers within the same image. We tested significance of fixed effects by evaluating model coefficients and verified model assumptions using the performance package (Lüdecke *et al.* 2021).

4.3.7.3 Floral Traits

To identify which floral traits most strongly influenced flower and pollinator maximum warming capacity, we fitted separate linear mixed-effects models for $\Delta Tmax$ for flowers and pollinators. We retained all species in trait-based analyses, including those with fewer replicates excluded for the species-level analysis, so that their attributes contributed to the broader trait categories. Because a global model including all traits was rank-deficient due to collinearity among predictors, we instead fitted single-trait models to evaluate each floral trait independently alongside environmental covariates. We fitted separate models for flowers and pollinators, with fixed effects for floral colour, shape, size, and family, and species identity with UV Index, wind speed, and relative humidity as fixed effects to control for environmental variation and photo ID as a random effect. We compared trait models using Akaike's Information Criterion (AIC) and tested fixed effects with ANOVA using Satterthwaite-adjusted degrees of freedom (lmerTest; Kuznetsova *et al.* 2017). Model assumptions were checked with the performance package (Lüdecke *et al.* 2021).

4.3.7.4 Pollinator Interactions

To test whether floral temperatures affected pollinator interaction frequency, we modelled pollinator interactions for 24 flower species that had also been surveyed using IR thermography. We classified species into three equal-frequency terciles (low, moderate, high ΔT_{max}). We modelled the interaction of ambient temperature (°C) and ΔT_{max} for each tercile using generalized linear mixed models. We included wind speed, relative humidity, and elevation as fixed effects, with random intercepts for date and plant species. We restricted analysis to surveys conducted ≥10 °C because observations below 10 °C were sparse (Figure S1), risking unstable estimates.

We then modelled pollinator interactions within the three main taxonomic orders (Diptera, Coleoptera, Hymenoptera), testing for the interaction of ambient temperature, ΔT_{max} and order in a zero-truncated negative binomial generalised linear mixed model. We used the same covariates (wind speed, humidity, elevation) and random intercepts (date, plant species) as the community level analysis above. We checked model assumptions with the performance (Lüdecke *et al.* 2021) and DHARMa packages (Hartig, 2016).

4.4 Results

We collected a total of 375 infrared images, from which we extracted temperature data for 898 individual flowers from 43 species representing 18 families. We also imaged 125 insects on the flowers of 22 plant species. Environmental conditions during flower imaging spanned a UV Index of 0 to 8, ambient temperatures of 11.2 to 23.1°C, relative humidity of 26% to 90.2%, and windspeed of 0 to 8.3 km/h. Across 53.5 hours of pollinator interaction sampling, we recorded 1397 pollinator interactions to 24 flower species for which we had temperature data.

4.4.1 Species-level Floral Warming

Flower species differed in their floral warming capacity, with species identity contributing to floral warming (ΔTmax, $p < 0.001$, Table S2). Flower ΔTmax spanned a wide range across species, reaching values >10 °C above ambient in the warmest species and approaching ambient conditions in the coolest. Confidence intervals often overlapped for mid-ranked species but were clearly separated at the warm and cool extremes (Figure 2).

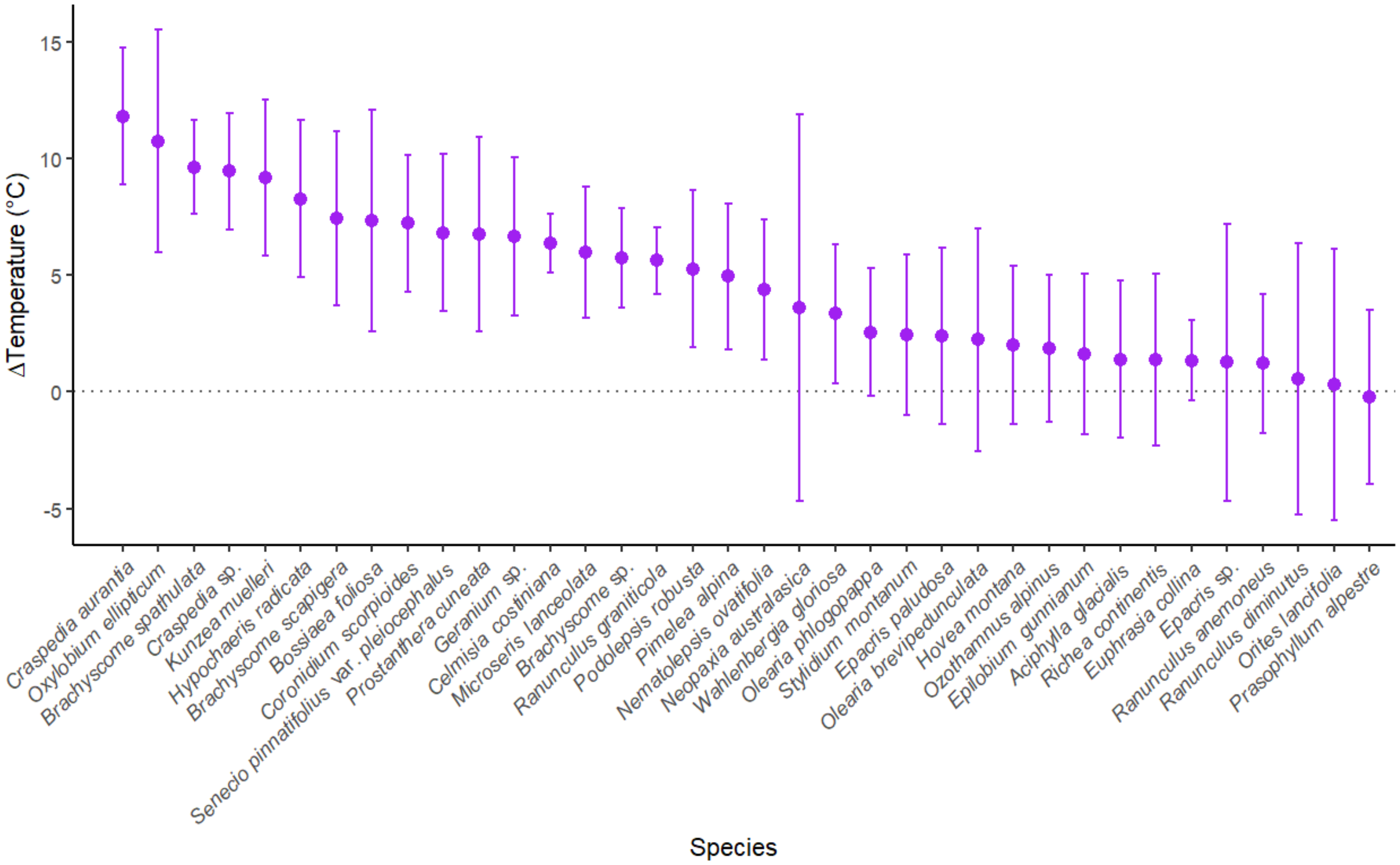


Figure 2. Species-level difference between maximum flower temperature and ambient temperature (ΔTmax), with species ordered in descending order. Plot shows difference between maximum flower temperature and ambient temperature (ΔTmax). Figure shows model-adjusted

4.4.2 Environmental Conditions

Environmental conditions, particularly UV Index, strongly influenced both pollinator and floral ΔT_{max}, with pollinators showing greater warming with increased UV Index. UV Index was a consistent positive predictor of warming for pollinators and flowers ($p < 0.05$). Pollinators, which were usually darker in colour, were generally 3-4°C warmer than flowers at all levels of UV Index. ΔT_{max} increased with UV Index for both ROIs, with the steepest slope for pollinators, indicating they heated more rapidly in strong sunlight. Flowers also warmed with increasing UV, but at lower rates (Figure 3). Relative humidity had no effect on pollinator temperatures ($p = > 0.05$) but reduced ΔT_{max} in flowers ($p < 0.01$). Windspeed did not significantly affect ΔT_{max} for pollinators or flowers ($p > 0.05$, Table S2).

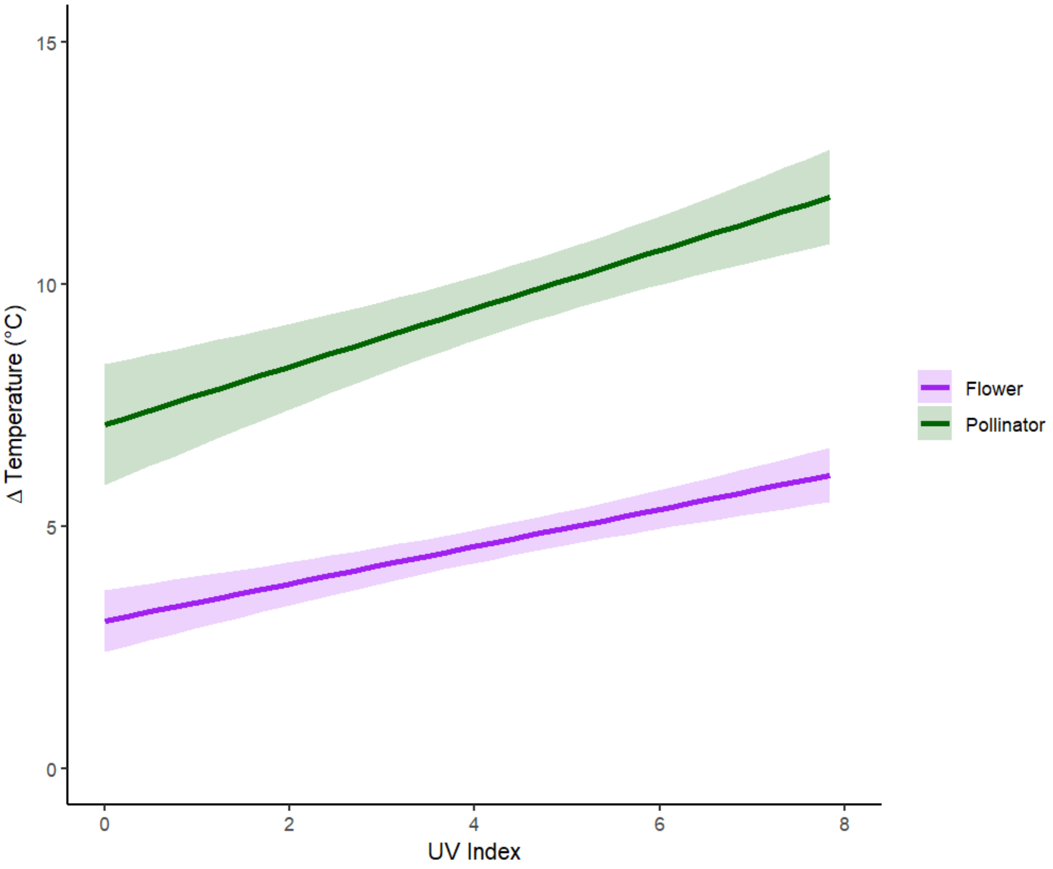


Figure 3. Relationship between UV Index and the difference between maximum flower temperature and ambient temperature (ΔTmax) for pollinators, flower centres, and petals. Lines show predicted values from linear mixed-effects models, with shaded ribbons representing ± 1 standard error.

4.4.3 Floral Traits

Among the floral traits we assessed, flower shape was the strongest predictor of ΔT_{max} ($p < 0.001$, Table S3), with model selection indicating strong effects of shape on ΔT_{max}, whereas colour, family, and size class were not supported as predictors ($\Delta AICc > 50$, Table S4). Shape was also better supported than species identity in model selection, indicating that species-level differences in floral warming are likely partly mediated by variation in flower shape. Globular heads were the warmest morphology, averaging more than 10 °C above ambient air temperature. Radiate heads, star-like, cup-shaped and pea flowers also exhibited elevated heating, typically 5–7 °C above ambient. By contrast, bell-shaped flowers were cooler (~2–3 °C above ambient), while tubular, umbel/clustered and "other" morphologies were the coolest, averaging close to ambient temperatures (Figure 4).

For on-flower pollinators, body temperature was contingent on floral morphology, with some shapes enhancing insect warming more than others. Flower shape significantly predicted polinator ΔT_{max} ($p < 0.001$, Table S3). Although flower family also improved model fit (Table S5), collinearity between family and shape (VIF > 15) indicated that family effects were largely confounded with shape. We therefore emphasise floral shape as the more mechanistic predictor, consistent with floral shape being the best predictor of flower ΔT_{max}. Contrasts of flower versus pollinator ΔT_{max} revealed shape-dependent differences. Pollinators were significantly warmer than flowers in radiate, star-like, tubular and umbel/clustered morphologies (1.7–4.8 °C; $p < 0.01$), and especially in cup-shaped flowers (~6 °C warmer; $p < 0.0001$). By contrast, globular heads were warmer than their pollinators (~3 °C; $p < 0.0001$), and no significant difference was detected in bell-shaped flowers (Figure 4). Pea and "other" morphologies could not be estimated due to limited pollinator data.

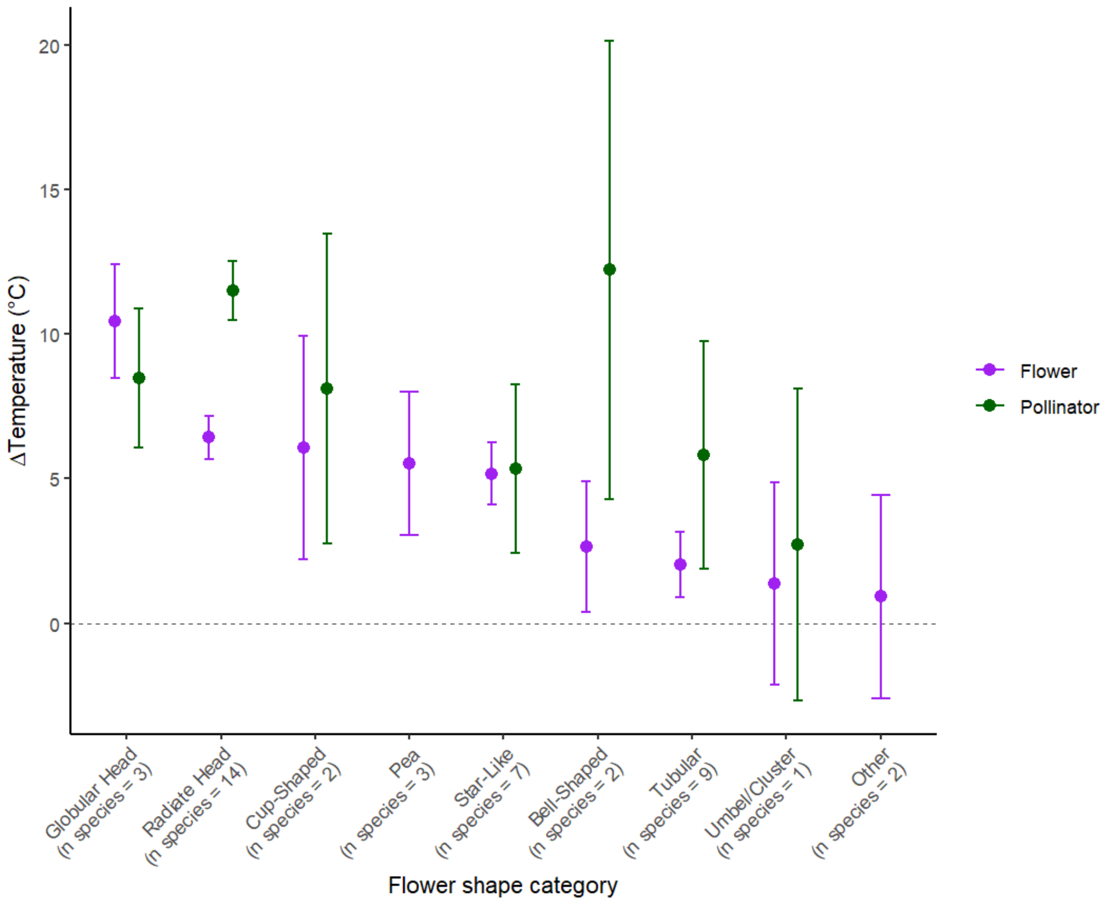


Figure 4. Effect of flower shape on the difference between maximum flower/pollinator temperature and ambient temperature (ΔTmax), ordered in descending order by flower ΔTmax. Figure shows model-adjusted means with 95% confidence interval error bars. The number of species within each shape category is indicated below labels on the x axis.

4.4.4 Pollinator Interactions

High ΔT_{max} flowers had the highest predicted interactions in cooler conditions while interactions on Low and Moderate ΔT_{max} flowers were highest in warmer conditions (Figure 5). There was a significant interaction between flower ΔT_{max} and ambient temperature in determining insect-flower interaction frequency ($p < 0.05$). For High ΔT_{max} flowers, insect interaction frequency was greater when ambient air temperature was lower ($p < 0.05$). Conversely, interaction frequency increased with ambient air temperature for Low and Moderate ΔT_{max} flowers, though this effect was not significant ($p > 0.05$). Elevation, wind speed, and relative humidity were not significant predictors (all $p > 0.05$, Table S6).

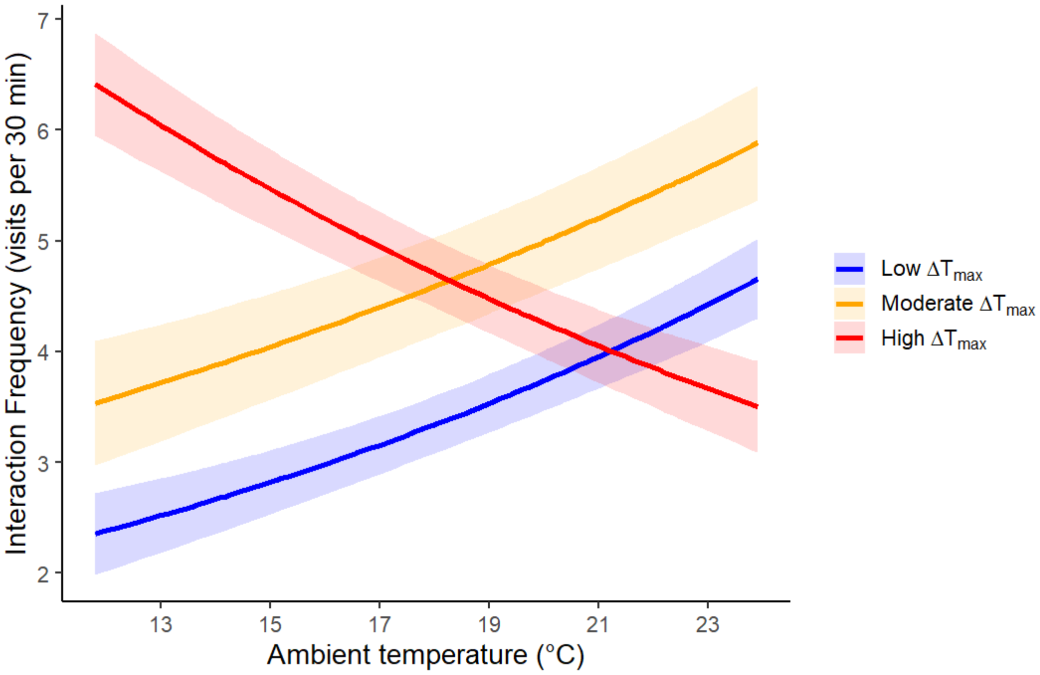


Figure 5. Relationship between ambient temperature and pollinator interaction frequency by the difference between maximum flower temperature and ambient temperature (ΔTmax). Lines show predictions from a generalized linear mixed-effects model, with shaded ribbons representing ± 1 standard error.

Across pollinator orders, the interaction between ΔT_{max}, ambient temperature and insect interaction frequency differed. For Diptera, the interaction of ambient temperature and floral temperature for High ΔT_{max} flowers was significant ($p > 0.001$), whereas it was not for Hymenoptera or Coleoptera (both $p > 0.05$, Table S7). This indicates that Diptera interactions with high ΔT_{max} flowers are more frequent under colder ambient conditions. Consequently, the community level pattern, where High ΔT_{max} flowers have more frequent interactions with insects at low ambient temperatures, appears to be primarily driven by Diptera (Figure 6).

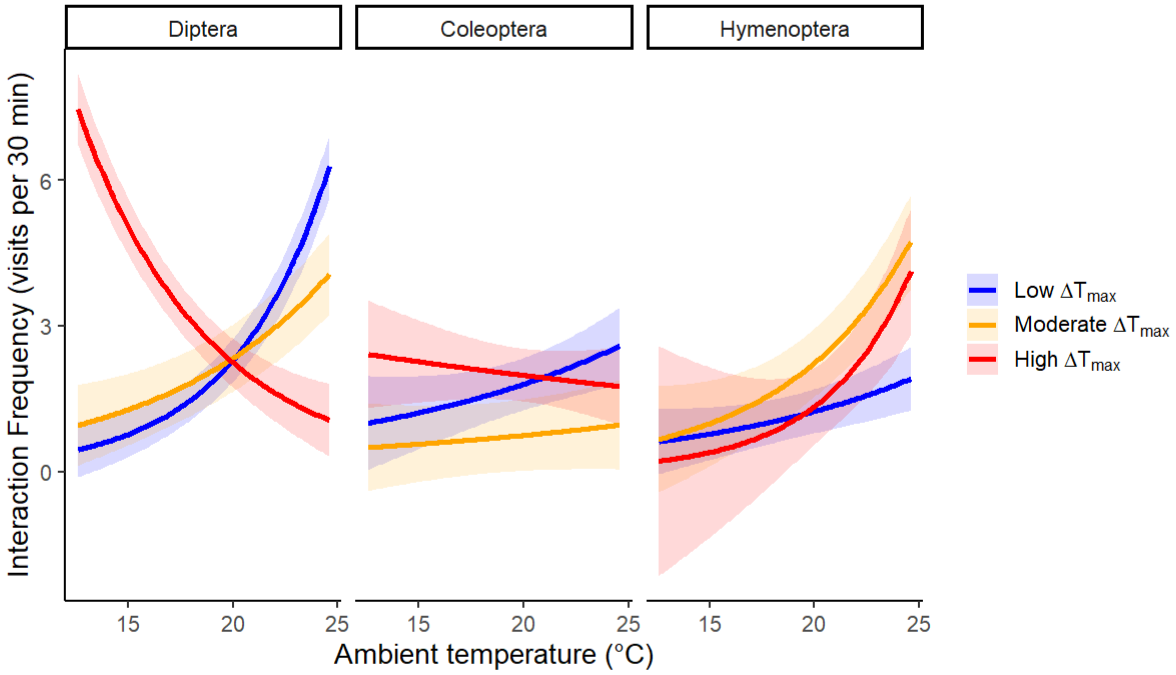


Figure 6. Relationship between ambient temperature and pollinator interaction frequency by the difference between maximum flower temperature and ambient temperature (ΔTmax) within the three major pollinator orders. Lines show predictions from generalized linear mixed-effects models, with shaded ribbons representing ± 1 standard error.

4.5 Discussion

Cold environments are thermally challenging for ectothermic insect pollinators, with ambient air temperatures commonly limiting activity levels (Oyen and Dillon, 2018; Lahondère, 2023; Coates *et al.*, 2024). Using community-level surveys of flower microhabitats and pollinator interactions, we show that subalpine and alpine flowers offer thermal conditions that allow pollinators to buffer against ambient conditions. Critically, we provide the first community-level, in-situ evidence that naturally warmer flowers are associated with more frequent pollinator interactions under lower ambient air temperatures. Warmer flowers received more visits under lower ambient conditions, especially from Dipteran pollinators, and this effect weakened as ambient temperature rose. Almost all plant species provided flower microhabitats that were warmer than ambient conditions. Pollinators visiting flowers were also significantly warmer than ambient temperature and typically warmer than the flowers themselves, potentially because many small pollinators have low thermal inertia and can behaviourally thermoregulate during foraging to capitalise on warm microhabitats (Heinrich, 2013). Floral shape was the strongest trait predictor of warming, with both floral tissues and pollinator body temperature warmer on globular, cup- and star-shaped flowers, whereas tubular and bell-shaped flowers only warmed slightly above air temperature. These results show that floral microhabitats function as thermal refuges that influence pollinator activity when temperatures are otherwise limiting.

Among the environmental variables we measured, UV Index was the strongest predictor of ΔT_{max} for flowers and pollinators, with relative humidity also influencing flower ΔT_{max}. UV Index likely mirrored longwave solar radiation and cloud levels (Caldwell *et al.*, 2007), explaining why it influenced ΔT_{max}. Relative humidity is also correlated with cloud cover (Saito and Baba, 1988), so that in our data, relative humidity's effect on ΔT_{max} may be associated with reduced solar radiation through increased cloud cover. Under sunny, low-humidity conditions, flower petals concentrate solar energy to warm floral tissues and insects visiting them. These dynamics match "solar oven" flower observations in Arctic and alpine environments (Kevan, 1975; Herrera, 1995; Dietrich & Körner, 2014; Morales *et al.*, 2025). Together, the positive UV Index and negative relative humidity effects indicate that radiative solar gains primarily determine ΔT_{max} in our data. Other studies also show that alpine plant tissues warm with solar radiation but decline with relative humidity (Morales *et al.*, 2025), and that flower temperature increases with solar radiation (Anderson *et al.*, 2013; Dietrich and Körner, 2014), with other morphological features such as petal angle and reflectance influencing the capture of solar radiation (Koski *et al.*, 2024).

Across the flower species we examined, floral warming capacity varied markedly, yet the majority of flowers showed positive ΔT_{max}, including up to 10 °C above ambient conditions. These findings mirror observations of sub-Antarctic megaherbs where inflorescences can be 9–11 °C warmer than ambient conditions (Little *et al.*, 2016), and flowers from the Swiss Alps that averaged ~6°C warmer than ambient conditions (Dietrich and Körner, 2014). By contrast, a survey of 30 Australian plants from lowland ecosystems found more inconsistent patterns in floral temperature, with less than half of species producing floral microhabitats that were warmer than ambient conditions, and some species showing evidence of cooling below ambient conditions (Shrestha *et al.*, 2018). Additional to the possible benefit of enhanced pollen outcrossing from increased pollinator interactions, floral warming has important functional advantages for reproductive success, such as enhanced pollen tube number (Herrera and Medrano, 2017) and embryo formation (Stephenson, 1981).

Flower shape was the strongest predictor of floral warming. Globular, radiate-, pea, cup- and star-shaped flowers all showed $\Delta T_{max} >$ °5C. These flower morphologies present upward-facing surfaces, which are better able to capture solar energy and create boundary layers that reduce heat loss (Herrera, 1995). In contrast, tubular, bell-shaped and umbel flowers were relatively cooler in our data (though still warmer than ambient), possibly because their narrow corolla opening limits solar penetration and heat retention. For instance, Rejšková *et al.* (2010) found that bell-shaped flowers were cooler than other open flowers in their study and Van der Kooi *et al.* (2019) described how tubular flowers are limited in warming capacity due to the limited heat absorption and retention of these shapes. In a recent study that examined the impacts of flower shape and colour on floral warming in eight subalpine species, open flowers warmed more than closed flowers (Apland *et al.*, 2025). These patterns imply that flower morphology not only determines pollinator attraction, such as through visual appeal and filtering access (Dafni and Kevan, 1997), but also the temperature of floral microhabitats.

Pollinators visiting flowers were consistently warmer than ambient conditions and typically warmer than floral tissues. This is likely due to a combination of insects' physical properties and their ability to select warmer microhabitats and adjust their posture to maximise warming. Due to their small scale, dark colouration, high cuticular absorbance and low heat capacity, insects are well suited to absorb solar radiation. By exploiting centimetre-scale thermal mosaics, such as those within flowers, insects are also able to thermoregulate behaviourally so that their body temperature exceeds ambient conditions and even that of surrounding floral tissues. (Lahondère, 2023). This allows pollinators to reduce the constraints from cool ambient termperatures, with warm floral microhabitats reducing the time and energy insects must spend elsewhere to thermoregulate and achieve flight temperatures (Cooley, 1995). For instance, insects basking inside Arctic *D. integrifolia* flowers were up to 15 °C warmer than ambient (Kevan, 1975). These flowers are able to provide pollinators with a thermal benefit, additional to the typical nutritional award offered by nectar and pollen (Cooley, 1995). This dynamic also benefits plants, by attracting more pollinators to improve reproductive success.

Floral warming translated into measurable changes in pollinator interaction frequency at the community level. Warmer flowers received more visits under cold ambient conditions, with this response reducing with increasing ambient temperatures. In an environment that is subject to low temperatures, warm floral microhabitats allow insects increased opportunity for foraging during inclement weather conditions, allowing them to increase activity and reduce energetic costs associated with thermoregulation. Studies on experimentally warmed flowers and artificial flowers, show that pollinators can detect temperature differences, which influences their choice of flowers (Dyer *et al.*, 2006; Harrap *et al.*, 2017; Creux *et al.*, 2021; Apland and Koski, 2025). Building on this evidence, our study offers the first community-level demonstration that naturally warmer flowers attract more visitors during cold conditions, thereby connecting ambient temperature, floral microhabitat, and insect activity across a mountain plant-pollinator assemblage.

Patterns in pollinator activity levels associated with floral warming were primarily driven by Diptera, while Coleoptera and Hymenoptera did not show similar patterns of response to high ΔT_{max} flowers. Because Diptera are the most active order at low temperatures in this system (Coates *et al.*, 2024), it is unsurprising that they are driving the community-level pattern. These order-specific responses likely reflect differences in thermal physiology and behaviour, with Diptera able to achieve flight at lower ambient conditions compared to Hymenoptera (Heinrich, 1974). Several Diptera species are able to forage at lower ambient temperatures, allowing them to exploit relatively warm flowers to provide an advantage by enabling an earlier foraging window under conditions that exclude species from other orders. Apland and Koski (2025) experimentally warmed flowers and showed that Diptera preferred warmer flowers under cool ambient temperatures but switched to cooler flowers when ambient temperatures were high, whereas bees altered interaction frequency with air temperature but showed little direct response to flower temperature. This study also found that flies foraged longer and basked more in warmer flowers under cool air temperatures and that a shift towards Diptera at higher elevations may help to drive pollinator-mediated selection for warm floral microhabitats in mountain environments (Apland and Koski, 2025).

Disentangling the effect of thermal reward from other floral cues can be challenging because flowers also vary in colour, shape, accessibility, or nutritional rewards, all of which are likely to influence pollinators' behaviour. Other studies have addressed this issue by experimentally altering floral temperature while keeping other floral traits constant, either in artificial (Dyer *et al.*, 2006; Norgate *et al.*, 2010; Harrap *et al.*, 2017) or real flowers (Creux *et al.*, 2021; Apland and Koski, 2025). Our approach instead compares floral warming across a community of species that naturally differ across other floral cues, allowing us to test for a temperature effect on pollinator interaction frequency over and above other sources of variation. Our results are consistent with findings from manipulative studies, despite using different methods in a different study system, indicating generality in this pattern. Therefore, our community-scale field analysis provides complementary evidence to experimental manipulations, demonstrating temperature effects under naturally varying trait assemblages and environments.

4.6 Conclusion

Mountain pollinators operate under considerable thermal constraints, yet the flowers of some plant species provide warm microhabitats that may buffer cold stress and provide thermal refuges. Our community-level field measurements show that most flower species maintain floral temperatures above ambient air temperature, but the magnitude of this warming varies with flower morphology. Crucially, warmer flowers received more visits under lower ambient temperatures, with Diptera driving this response, and the effect weakened as temperatures rose. Our results suggest that floral microhabitats function as a thermal refuge that may expand the activity window for flower visiting Diptera when flight is otherwise temperature-limited.

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4.8 Supplementary Material

Table S 1. Species-level floral trait dataset compiled from Australian botanical resources (Centre for Biological Information Technology, 2025; Lucidcentral, 2025; Royal Botanic Gardens and Domain Trust, 2025). For each species, we extracted family, flower size range, colour and shape, and shape information. Where size was reported as a range, we calculated the midpoint. Colour and shape were standardised into harmonised categories for analysis. 'Interaction data' indicates the 24 flower species included in the pollinator interaction analysis.

Species	Family	Flower size (mm)	Flower colour description	Flower Colour	Flower Shape	Interaction data
Achillea millefolium	Asteraceae	7.5	each flower-head has about 5 petals (white, pink, reddish-pink or mauve)	white	radiate head	
Aciphylla glacialis	Apiaceae	2.75	white or cream to greenish	white	umbel/cluster	
Bossiaea foliosa	Fabaceae	6.5	bright yellow petals	yellow	pea	X
Brachyscome obovata	Asteraceae	9.5	white, bluish, mauve or tinged purple petals	purple	radiate head	
Brachyscome scapigera	Asteraceae	8.35	white to mauve petals with yellow centres	white	radiate head	X
Brachyscome sp.	Asteraceae	15.5	white 'petals' with yellow centres	white	radiate head	X
Brachyscome spathulata	Asteraceae	15	pale blue to mauve (rarely white) with yellow centres	purple	radiate head	X
Caladenia sp.	Orchidaceae	25	pale to deep pink flowers	pink	spider-like	
Celmisia costiniana	Asteraceae	45	white 'petals' tipped mauve on underside with yellow centres	white	radiate head	X
Chionogentias muelleriana	Gentianaceae	12.5	white with grey violet or purplish veins	white	cup-shaped	
Coronidium scorpioides	Asteraceae	11	pale yellow to lemon-yellow intermediate 'petals' with yellow centres	yellow	radiate head	
Craspedia aurantia	Asteraceae	25	yellow to orange brown globular heads	yellow	globular head	X

Craspedia leucantha	Asteraceae	25	yellow or pale yellow or orange globular heads	yellow	globular head	X
Craspedia sp.	Asteraceae	17.5	yellow globular heads	yellow	globular head	X
Epacris paludosa	Ericaceae	5	white tubular flowers	white	tubular	X
Epacris sp.	Ericaceae	5	white often pink-tipped	white	tubular	X
Epilobium gunnianum	Onagraceae	11.5	purplish pink to purplish red, mauve, pale pink or white	pink	tubular	
Euphrasia collina	Orobanchaceae	14.5	white to purple, blue or pink, sometimes with yellow blotch	purple	tubular	X
Geranium sp.	Geraniaceae	7.5	pink, sometimes white petals with veins	pink	star-like	X
Hovea montana	Fabaceae	8.75	pink, mauve, purple to violet or white with yellow spot	purple	pea	X
Hypochaeris radicata	Asteraceae	9	yellow flower heads with many ray florets	yellow	radiate head	X
Kunzea muelleri	Myrtaceae	2.25	cream to yellow round petals	yellow	star-like	
Microseris lanceolata	Asteraceae	40	yellow heads with yellow 'petals'	yellow	radiate head	X
Nematolepsis ovatifolia	Rutaceae	10	white flowers (pink outside in bud)	white	star-like	X
Neopaxia australasica	Montiaceae	14	white or pale pink	white	star-like	X
Olearia brevipedunculata	Asteraceae	18.5	white 'petals' with yellow centres	white	radiate head	
Olearia phlogopappa	Asteraceae	20	white 'petals' with yellow centres	white	radiate head	X
Orites lancifolia	Proteaceae	5	white to pale yellow	white	tubular	
Oxylobium ellipticum	Fabaceae	10	yellow to orange with red markings and red keel	yellow	pea	
Ozothamnus alpinus	Asteraceae	5.75	dull-yellow florets with outer bracts dull yellow to pale brown, often tinged red to magenta, inner bracts white	yellow	bell-shaped	
Pimelea alpina	Thymelaeaceae	6.5	pinkish red to pink or white	pink	tubular	X

Pimelea ligustrina	Thymelaeaceae	11.25	creamy white to white flowers (rarely pinkish) with greenish to reddish-brown or purplish bracts	white	tubular	
Pimelea linifolia	Thymelaeaceae	11.5	white, occasionally pink or yellow	white	tubular	
Podolepsis robusta	Asteraceae	32.5	yellow to orange petals and yellow centres	yellow	radiate head	
Prasophyllum alpestre	Orchidaceae	10	white, purplish and green	white	other	
Prostanthera cuneata	Lamiaceae	12	white petals with tiny dark purple spots in the throat	white	cup-shaped	X
Ranunculus anemoneus	Ranunculaceae	22.5	creamy white petals	white	star-like	
Ranunculus diminutus	Ranunculaceae	4.75	yellow glossy petals	yellow	star-like	X
Ranunculus graniticola	Ranunculaceae	13	yellow petals often with dark centres	yellow	star-like	X
Richea continentis	Ericaceae	6	greenish or creamy-white flowers	white	tubular	X
Senecio pinnatifolius var. *pleiocephalus*	Asteraceae	12.5	yellow petals with yellow centres	yellow	radiate head	X
Stylidium montanum	Stylidiaceae	9.5	dark pink to magenta flowers	pink	tubular	
Taraxacum officinale	Asteraceae	50.5	bright yellow ray flowers	yellow	radiate head	
Wahlenbergia gloriosa	Campanulaceae	18	purple to purplish violet or deep blue flowers	blue	bell-shaped	

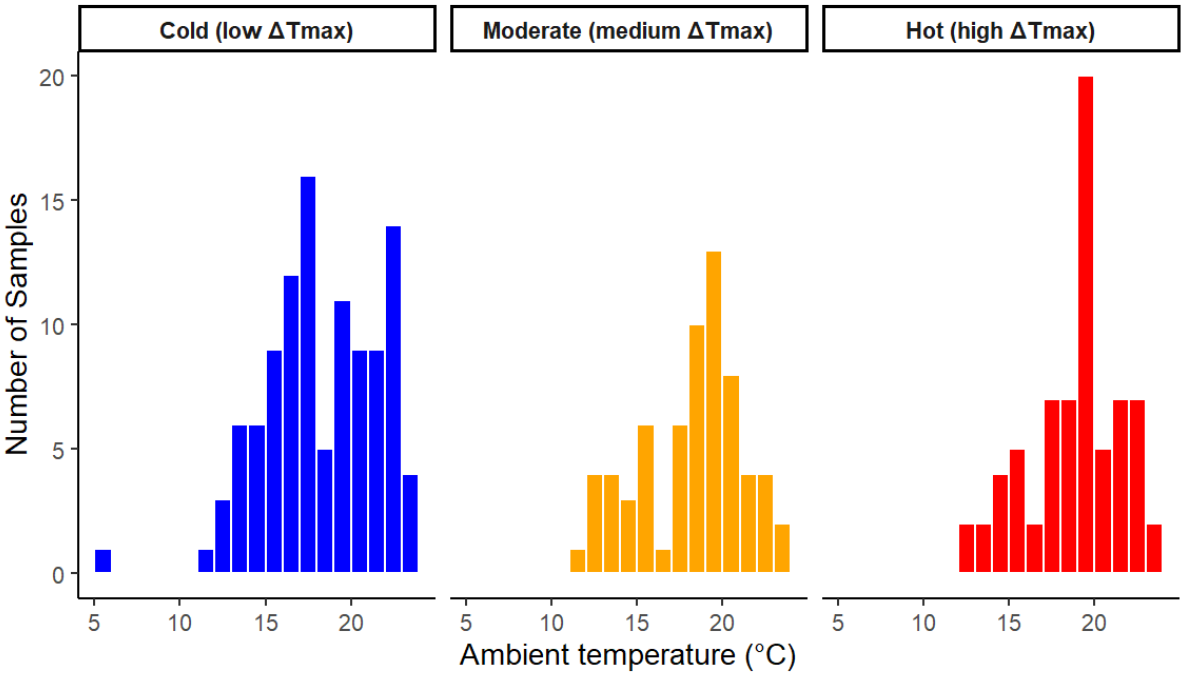

Figure S 1. Spread of samples across ambient temperatures for each flower ΔTmax tercile.

Table S 2. Significance values from linear mixed-effects models of ΔTmax for pollinators and flowers. For each model, p value is shown with asterisks indicating significance at α = 0.05, and z/F statistic is shown. Because flower species has many levels, the table reports an omnibus F-test instead of listing coefficients for each species.

Predictor	**Pollinators**		**Flowers**	
	z/F	p	z	p
UV Index	2.60	0.012 *	3.05	0.003 *
Wind speed	0.19	0.847	1.35	0.179
Relative humidity	0.80	0.429	-3.12	0.002 *
Flower species (overall)	F=3.39	<0.001 *	F=3.74	<0.001 *

Table S 3. Significance values from linear mixed-effects models of ΔTmax for pollinators and flowers. For each model, p value is shown with asterisks indicating significance at α = 0.05, and z/F statistic is shown. Because flower shape has many levels, the table reports an F-test instead of listing coefficients for each shape.

Predictor	Pollinators		Flowers	
	z/F	p	z	p
UV Index	3.87	<0.001 *	3.10	0.002 *
Wind speed	0.27	0.789	1.62	0.106
Relative humidity	1.03	0.308	-3.56	<0.001 *
Flower shape (overall)	F=6.05	<0.001 *	F=10.10	<0.001 *

Table S 4. Model selection comparing flower warming trait predictors. Models are ranked by AICc; lower values indicate better support. ΔAICc is relative to the top-ranked model. Columns report the number of estimated parameters (K), AICc, ΔAICc, AICc weights, cumulative weights, and log-likelihood (LL).

Model	K	AICc	ΔAICc	AICc weight	Cum. weight	LL
Flower Shape	15	3409.45	0.00	0.98	0.98	-1689.43
Flower Species	47	3416.91	7.46	0.02	1.00	-1658.54
Flower Family	23	3438.74	29.28	0.00	1.00	-1695.68
Flower Colour	10	3458.60	49.14	0.00	1.00	-1719.16
Flower Size	11	3464.89	55.44	0.00	1.00	-1721.28
Environmental variables only	6	3471.11	61.66	0.00	1.00	-1729.50

Table S 5. Model selection comparing pollinator warming trait predictors. Models are ranked by AICc; lower values indicate better support. ΔAICc is relative to the top-ranked model. Columns report the number of estimated parameters (K), AICc, ΔAICc, AICc weights, cumulative weights, and log-likelihood (LL).

Model	K	AICc	ΔAICc	AICc weight	Cum. weight	LL
Flower Family	14	458.64	0.00	0.92	0.92	-212.14
Flower Shape	12	463.64	5.00	0.08	1.00	-217.53
Flower Species	24	476.91	18.27	0.00	1.00	-203.74
Flower Size	10	478.00	19.36	0.00	1.00	-227.43
Environmental variables only	6	478.17	19.53	0.00	1.00	-232.52
Flower Colour	9	480.13	21.49	0.00	1.00	-229.80

Table S 6. Significance values from a negative-binomial GLMM of pollinator activity. For each model, p value is shown with asterisks indicating significance at α = 0.05, and z/F statistic is shown. Because elevation has several levels, the table reports an F-test instead of listing coefficients.

Predictor	z/F	p
Ambient Temperature	1.32	0.185
ΔT_{max} medium	0.76	0.445
ΔT_{max} high	0.94	0.348
Elevation	F=1.63	0.442
Wind	-1.11	0.267
Humidity	-0.19	0.849
Temperature × ΔT_{max} medium	-0.27	0.788
Temperature × ΔT_{max} high	-2.00	0.046 *

Table S 7. Significance values from truncated negative-binomial GLMM of pollinator activity, with Diptera as the reference insect order and ΔTmax low as the reference ΔT group. For each model, p value is

shown with asterisks indicating significance at α = 0.05, and z/F statistic is shown. Because elevation has several levels, the table reports an F-test instead of listing coefficients.

Predictor	z/F	p
Ambient Temperature	2.93	0.003 *
ΔT_{max} medium	0.21	0.836
ΔT_{max} high	0.90	0.367
Elevation	F=1.91	0.385
Wind	-1.23	0.218
Humidity	0.90	0.367
Ambient Temperature × ΔT_{max} medium	-1.07	0.285
Ambient Temperature × ΔT_{max} high	-3.78	<0.001 *
Ambient Temperature × Insect order: Coleoptera	-1.16	0.247
Ambient Temperature × Insect order: Hymenoptera	-1.37	0.172
ΔT_{max} medium × Insect order: Coleoptera	-1.92	0.054
ΔT_{max} high × Insect order: Coleoptera	-0.44	0.657
ΔT_{max} medium × Insect order: Hymenoptera	0.80	0.423
ΔT high × Insect order: Hymenoptera	-0.67	0.501
Ambient Temperature × ΔT_{max} medium × Insect order: Coleoptera	0.46	0.646
Ambient Temperature × ΔT_{max} high × Insect order: Coleoptera	1.45	0.146
Ambient Temperature × ΔT_{max} medium × Insect order: Hymenoptera	1.04	0.300
Ambient Temperature × ΔT_{max} high × Insect order: Hymenoptera	1.78	0.074

Chapter 5: Endangered Bogong moths (*Agrotis infusa*) Forage from Local Flowers After Annual Mass Migration to Alpine Sites

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5.1 Foreword

The Bogong moth is an iconic long-distance migratory insect with deeply rooted cultural significance to Indigenous peoples. The species, now listed as Endangered by the IUCN, has long been recognised for its key role as a prey resource in mountain ecosystems, but previous research suggested that they do not play a role as flower visitors while at their alpine aestivation sites. This chapter tests that assumption by analysing the pollen carried by Bogongs caught nearby to their high-elevation aestivation sites. Unlike other insects in this thesis, Bogongs migrate to the mountains precisely because of the cold climate, escaping the summer heat of their lowland breeding grounds to enter into aestivation in alpine caves.

This chapter's key contribution is to establish the previously unrecognised role of Bogong moths as flower visitors to endemic mountain plants. Using microscopy to identify and quantify pollen swabbed from their proboscis, I show that Bogongs visit a broad variety of plants, displaying generalist foraging habits. Crucially, I also demonstrate that they carry pollen collected from endemic mountain plants, evidencing feeding during their residence in the mountains. These findings revise our understanding of Bogong's ecological importance in mountain ecosystems, indicating a previously unappreciated role as mountain pollinators in these systems.

5.2 Abstract

Migration plays an important role in the life cycle of many insect species, allowing them to escape unfavourable seasonal conditions. The Bogong moth (*Agrotis infusa*) is a keystone species that undertakes a long-distance annual migration, with billions of individuals aestivating in summer in the Australian Alps. This species has undergone drastic population declines in the past five years, with steady long-term declines also recorded since the 1980s, and has recently been recognised as Endangered by the IUCN. Despite the role of Bogong moths as a keystone species in the Australian sub-alpine and alpine zones, their feeding habits during their time in the mountains remain understudied, although earlier research suggested that they do not feed during their summer aestivation. To examine whether Bogong moths visit flowers during the summer, we collected pollen from moths caught at a high-elevation site in the Australian Alps over the 2021/22 summer period and then identified and counted pollen using light microscopy. We show for the first time that Bogong moths visit a broad range of plant species during their summer activity period and characterise the plant assemblage visited. Almost all the 129 moths sampled carried pollen in large quantities, with a mean of 521 grains per moth. Individual moths varied considerably in pollen load and species composition, suggesting generalist flower visitation. The pollen taxa present on moths indicates local flower feeding at high-elevation sites, rather than pollen carried from the lowland origins of migrants. The presence of pollen throughout summer indicates continual flower feeding activity. These results provide important new insights into the ecology of Bogong moths and suggest they could play a previously unrecognised role in pollination in sub-alpine and alpine ecosystems.

5.3 Key words

Insect migration, Noctuidae, aestivation, flower visitation, pollination

5.4 Highlights

- Bogong moths (*Agrotis infusa*) forage from local flowers while present in Australian mountain ecosystems during annual mass migration.
- Bogong moths carried pollen from a large variety of plant taxa, suggesting a generalist feeding habit.

- Pollen load was maintained across the duration of the summer aestivation period, suggesting continued feeding.
- Moths may act as important pollinators in their mountain ecosystem, broadening theory functional role in these landscapes.

5.5 Introduction

Species that undertake large mass migrations have attracted scientific attention, with many studies focusing on the process of migration itself (Kimura, 2021). As migrating species can occur in great numbers, there is also the likelihood that they can be important to ecological processes as they interact with other species in the places they move through or occupy. This study focuses on the possible ecological impacts of an insect involved in one of the most remarkable mass migrations known. Migration has been documented in several lepidopteran species (Kimura, 2021). These migrations can occur across multiple life cycles, most famously in the Monarch butterfly (*Danus plexippus*; Brower *et al.*, 2006), or within a single adult phase, such as many moths in the family Noctuidae (e.g., Bogong moth (*Agrotis infusa*) (Common, 1954; Hendricks, 1998; Kimura, 2021). Noctuids are recorded flying up to 1600km (Hendrix and Showers, 1992), often from lowland breeding grounds to higher-altitude locations, before travelling back to lowland breeding grounds to complete their life cycle (Common, 1954; Hendricks, 1998; Kimura, 2021).

Noctuid moths have been noted as one of the most common nocturnal flower visitors (MacGregor *et al.*, 2015; Hahn and Brühl, 2016), and migratory moths have been observed to visit flowers for nectar while migrating (Hendrix and Showers, 1992; Gregg, 1993; Del Socorro and Gregg, 2001) and at migration sites (Kevan, 1997). At aestivation sites moths (and butterflies) are thought to enter a period of reduced metabolic demands (Common, 1954). While in-transit foraging is likely driven by the energetic needs of migrating moths, such visits could also contribute to pollination services. However, difficulties associated with sampling migratory insect populations mean that behaviour while migrating is often poorly understood.

The Bogong moth (*Agrotis infusa*) is a nocturnal noctuid moth widely known due to its cultural and historic significance to Indigenous peoples and mass migration in eastern Australia (Flood, 1980). Bogong moths complete the reproductive stages of their life cycle in lowland breeding grounds in seasonally dry inland plains in eastern Australia. Larvae then pass through six instars, feeding through winter months and maturing in the early spring. From their breeding grounds, newly emerged adult moths migrate eastward to the Australian Alps by the billions annually. They aestivate over the summer in high-elevation caves and rock crevices, sheltering from the summer heat, before emerging after summer to return to their breeding grounds (Green *et al.*, 2021). The adults have been observed in aestivation throughout mountain peaks above ~1700m a.s.l. in southeastern Australia (Green *et al.*, 2021). Common (1954) describes up to 17,000 moths per 1m^2 lining the walls of aestivation caves. Bogong moths provide a significant input of nutrients to the Australian Alps during their annual migration, and are important as prey species to birds, reptiles, fish and mammals in the Alps (Green, 2010b). Bogong moths are also documented as an important food source during traditional gatherings of Indigenous Australians, with a long history of Indigenous peoples gathering nearby to mountain aestivation sites in summer to eat Bogong moths and engage in cultural ceremony (Flood, 1980).

The Bogong moth has experienced significant population declines in recent decades (Green *et al.,* 2021). Historical records collected from summer aestivation sites show that numbers have declined steadily since the 1980s and then sharply from 2017. Initial declines have been attributed to changes in farming practices at the moths' breeding grounds, whereas the sharp decline in numbers since 2017 is likely due to the severe drought experienced during this period (Green *et al.*, 2021). Bogong moth aestivation will likely be impacted by climate change (Lownds *et al.*, 2023) and arrival and departure times have changed significantly since the 1950s (Caley and Welvaert, 2018). Given these concerns, the species has been listed as endangered under the IUCN Red List (International Union for the Conservation of Nature, 2022).

Little is known about flower visitation by Bogong moths during their time in the Australian Alps. Bogong moths initially arrive at lower elevations in the mountains as early as mid-September, some months before they enter aestivation (Green, 2010a) and may be exploiting floral resources for sugar-rich nectar as they slowly make their way to high-elevation aestivation sites. Incidental observations suggest that they visit flowers across their migratory route including *Prostanthera, Leucopogon, Correa* (Cockburn, 1981), *Epacris*, *Grevillea* and *Eucalyptus* (Common, 1981) and they have been observed foraging from *Epacris paludosa* in the Australian Alps (Inouye and Pyke, 1988). In his seminal work on the species, Common (1954) recorded few observations of Bogong moths actively feeding from the abundant *Eucalyptus niphophila* and conducted experiments where flowers were offered to captive adults, and concluded that the species does not feed while in aestivation.

The activity period for Bogong moths in the mountains corresponds with the flowering period for almost all of the plant species in the alpine and subalpine plant communities (Costin *et al.,* 2000). There are, therefore, plentiful floral resources for potential visitation. Annual light trapping in Kosciuszko National Park to assess population size suggests that Bogong moths may exploit flowers, as the moths appear to leave a nectar residue in the traps (M. Schroder, pers. comm., 20 January 2021). One recent study suggests that Bogong moths carry pollen on their body from various shrub species (Keaney, 2022), but this initial survey was limited by small sample size. A recent study showed that moths undergo regular night flights from their aestivation sites (Wallace *et al.*, 2022), the purposes of which are unclear. This nocturnal activity may be motivated by energy needs or for other reasons, such as to visit water sources.

In this study, we examine whether Bogong moths undertake nocturnal flower visitation during their summer aestivation in the Australian Alps. We used analysis of pollen loads from field-collected insects to identify the plant taxa that Bogong moths interact with. This technique has been effectively used in many previous studies on insect flower visitors (Alarcón, 2010; Banza *et al.*, 2015; Encinas-Viso *et al.*, 2022; Popic *et al.,* 2012; Zhao *et al.,* 2019) including noctuid moths (Chang *et al.,* 2018; Devoto *et al.,* 2010). Identification of plant taxa from pollen morphology has a long history and well-developed methods (Kearns and Inouye, 1993). The most widely used alternative technique is to observe flowers, hoping to witness visits, but this method is biased towards the plant species chosen for observation and is extremely difficult to apply for night active insects (Banza *et al.,* 2015; Macgregor *et al.*, 2015). We analysed pollen loads on moth specimens collected during their October 2021 to February 2022 activity period in Kosciuszko National Park, New South Wales, to examine evidence for flower use. We aimed to learn more regarding feeding habits during the migratory phase of the species' life cycle and determine whether their activity may be relevant to pollination outcomes. We also examined whether patterns of activity change over the sampling period, as most of the population finished migrating and entered into summer aestivation.

5.6 Methods

5.6.1 Study Area

Moths were collected from Charlotte Pass (1800m a.s.l., 36° 25' 55.5672" S, 148° 19' 44.076" E), Australia, nearby to a potential aestivation site (Warrant *et al.*, 2016), between October 2021 and February 2022. This period coincides with the moths' annual aestivation period in the Alps and with the flowering peak in the sub-alpine and alpine zones (Inouye and Pyke, 1988). Home to the continent's highest mountains, the area is characterised by prolonged periods of snow coverage in the colder months, and high levels of climatic variability year-round. During the summer (December-February) the mean maximum temperature is 16.8°C, the mean minimum temperature is 4.7°C and mean rainfall is 137mm (Bureau of Meteorology, 2022). Weather conditions during the sampling period were cooler and wetter than average, due to a strong La Niña phase of the El Niño Southern Oscillation.

5.6.2 Field Sampling

Moths were collected under NSW Government Biodiversity Conservation Act 2016 scientific collection permit #SL102555 and in a manner compliant with NSW animal ethics regulation. Nocturnal light-trapping sessions were conducted on nights with fine local weather, as Bogong moth flight activity is moderated by weather conditions (Wallace *et al.*, 2022). On each night of sampling, a light trap (12W UV black light and 180 x 160cm night collecting tent) was erected soon after sunset, as Common (1954) found that Bogong moths are most active at this time. Each sampling session continued until 10 individuals were captured, or until it was clear that conditions were not favourable for trapping. Sampling sessions typically lasted from 1-3 hours, but unfavourable weather conditions meant that the target of 10 specimens per night was sometimes unachievable. Moths were collected into individual, sterile sample jars straight from the light trap sheet, then euthanised and stored in a freezer. The light trap was thoroughly cleaned between each sampling effort to avoid pollen contamination between sampling nights.

5.6.3 Pollen Identification

To liberate pollen, Bogong specimens were mounted under a dissecting microscope. A small quantity of fuchsin jelly (approximately 1-2mm^3) was held on a pin which was then used to collect pollen grains from each moth. Pollen was collected from the head, eyes and proboscis because they are most likely to contact anthers when flower feeding (Banza *et al.*, 2015; Devoto *et al.*, 2011). The jelly was then placed on a microscope slide, which was heated to 50ºC to melt the jelly before a coverslip was placed on top of the sample to seal it. Slides then cooled and the perimeter of each coverslip was sealed with acrylic paint to prevent desiccation. To aid in pollen identification, reference pollen collections were collected from all flowering plants in the study area, and mounted to microscopes slides in a similar way.

Light microscopy was used to identify and count pollen to the lowest possible taxonomic resolution. This method was chosen because it allowed us to determine the proportion and quantity of pollen grains present in each sample. However, achieving species-level taxonomic resolution is often difficult due to morphological similarities among some taxa (Liu *et al.*, 2017; Suchan *et al.*, 2019). Pollen grains were examined under 25x and 40x magnification, following similar protocol to LeCroy *et al.* (2012). All pollen grains were counted on each slide, rather than using a transect-based approach, to ensure that species with low abundance were detected (Arstingstall *et al.*, 2021). Pollen identification was conducted at the Department of Archaeology and Natural History in the Australian National University, using physical reference material contained in The Australasian Pollen and Spore Atlas collection. To guide identification, we also used pollen collected from reference flowers, online resources (APSA Members, 2007) published literature (Thornhill, 2011; Macphail and Hope, 2018) and our own observations of flowering phenology at the study site during the sampling period.

5.6.4 Analysis

The geographical distribution and flower phenology of each of the plant taxa detected was determined using literature and online databases (Atlas of Living Australia, 2022; Costin *et al.*, 2000; The NSW Plant Information Network System, 2022). To determine whether pollen was collected from the local high-elevation area, plant taxa were grouped into bands according to their elevational extent, with alpine defined as above 2000m, subalpine between 1500-2000m, montane between 1000-1500m and lowland below 1000m (Costin *et al.*, 2000; Körner, 1999; Körner, 2012). For 11 pollen types, identity could not be determined and so they were unable to be assigned an elevational distribution. The pattern of pollen load across the sampling period was visualised using Tilia 3.0.1 (Grimm, 2016). Plant taxa with <10 pollen grains (23 taxa, 77 grains total) were excluded from this analysis.

5.7 Results

Across 19 nights in the October-February sampling period, 129 moths were collected. Almost all moths (95%) carried pollen. A total of 67330 pollen grains was counted, with pollen load varying greatly among individuals (mean= 521, stdev= 1447.8, max = 10905). No significant difference (t(124) = -0.251, p = 0.8) was found in the pollen load between male (mean= 494, stdev=1182, n= 61) and female moths (mean=561, stdev=1710, n=65).

A total of 56 plant taxa were identified with 9 species-, 31 genus-, 5 family-level, and 11 morphospecies-level classifications. All plant taxa identified had distributions within at least montane elevations, with high representation in subalpine and alpine endemic taxa present (Table 1). All plant taxa identified were in flower in Kosciuszko National Park during the sampling period. Several taxa identified were endemic only to subalpine and alpine zones (e.g., *Aciphylla, Richea, Grevillea australis*).

Table 1. Summary of pollen data collected from 122 Bogong moths (*Agrotis infusa*) carrying pollen. Data pooled for whole sampling period. Plant taxa listed from most to least abundant within each distribution. Each moth is included only once within distribution categories according to the highest elevation pollen it carried, so that 'moths, N' sums to 122.

		Pollen grains		Moths	
Plant taxa	Distribution	N	Percent of total	N	Percent of total
Aciphylla, Richea, Grevillea australis	Alpine to subalpine	12308	18.3%	12	9.9%
Nematolepis ovatifolia, Celmisia	Alpine to montane	1425	2.1%	9	7.4%
Hakea lissosperma	Subalpine to montane	6	>0.1%	2	1.6%
Baeckea, Leucopogon, Epacris other*, Stackhousia, Kunzea, Epacris paludosa, Orites, Pimelea,* Poaceae, *Brachyscome,* Lamiaceae, *Exocarpus, Plantago, Senecio, Rumex, Oxylobium, Podocarpus, Cassinia, Wahlenbergia, Micrantheum, Leptorhynchos, Tasmannia*	Alpine to lowland	26205	38.9%	81	66.9%
Eucalyptus other*, Tetratheca, Eucalytpus pauciflora*-type, *Eucalyptus dalrympleana*-type, *Lomatia, Grevillea lanigera, Malva*	Subalpine to lowland	13723	20.4%	10	8.3%
Leptospermum, Eucalyptus rubida-type, *Monotoca, Acacia, Mirbelia oxyloboides, Banksia, Callitris, Dodonea, Hibbertia, Epacris impressa,* Brassicaceae sp. cf. *Rorippa*	Montane to lowland	8553	12.7%	6	4.9%
11 unidentified morphotypes	Distribution indeterminate	5110	7.6%	2	1.6%

Pollen load species composition varied greatly both across the season and within a given sampling night. Moths carried pollen from between 1 to 6 taxa (mean = 2.5, stdev= 1.5), while the average number of plant taxa collected from all moths on a sampling night was 3 (stdev = 2). The most common families present (Figure 1) were Myrtaceae (50.2%), Ericaceae (18.9%) and Apiaceae (11.5%; though all Apiaceae pollen present was from one moth specimen, which carried 7689 *Aciphylla* grains). Only four pollen taxa identified were wind pollinated

(Poaceae, *Callitris, Rumex, Podocarpus*), which represented 0.06% of pollen grains counted.

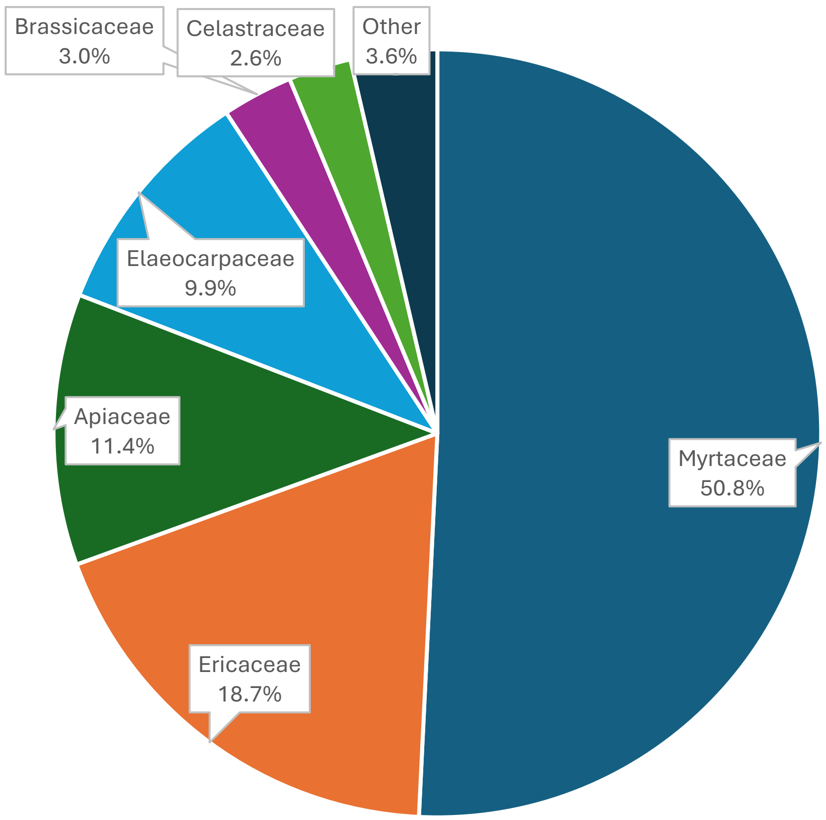

Figure 1. Proportion of plant families present in Bogong moth (*Agrotis infusa*) pollen load. 10 most common families shown, with 17 uncommon families grouped as 'Other' (Table S1, supplementary material).

No temporal pattern was discernible in total pollen load during the sampling period. However, the distribution of plant taxa appeared to shift during the sampling period. In weeks 1 to 5 only one moth carried pollen from a plant taxon in either of the highest distributional bands, whereas from weeks 6 to 12 this pollen was frequent (Figure 2).

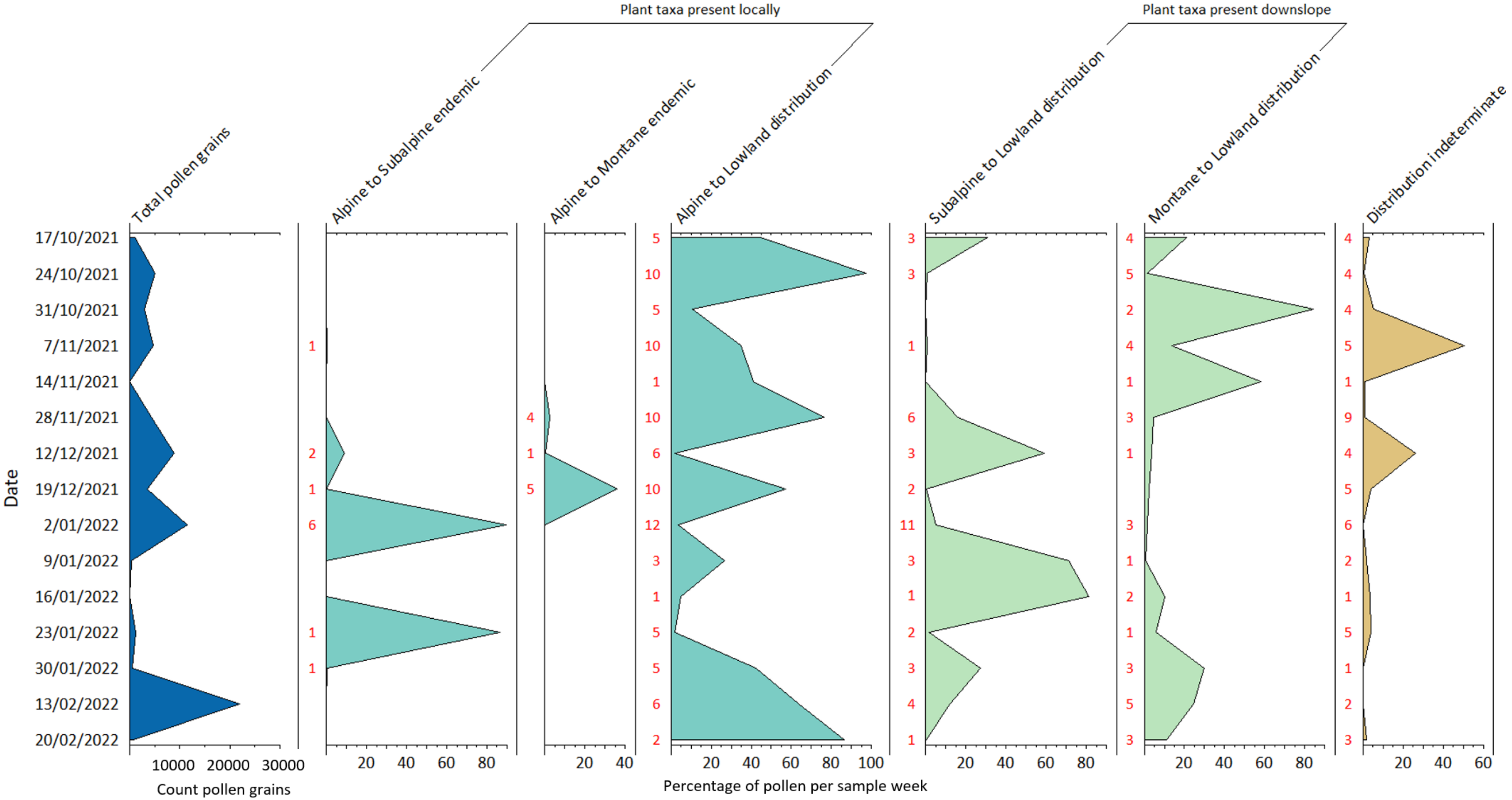

Figure 2. Altitudinal distribution of plant taxa found in Bogong moth (*Agrotis infusa*) pollen loads throughout the sampling period. Pollen count is binned by sampling week. Red numbers indicate the number of moths collected per sampling week for each distribution. Left panel shows total pollen grain count, other panels show proportion of pollen carried in each sampling week. Figure created using Tilia 3.0.1.

5.8 Discussion

Bogong moths collected at our high-elevation study site carried large quantities of pollen collected from plant taxa typical of the local, high-mountain plant communities across the summer months. Almost one-fifth of pollen collected was from plants endemic to sub-alpine and alpine zones. The majority of pollen grains (79.7%) were from plant taxa with distributions that include the sub-alpine and alpine zone (Table 1, Figure 2) where these moths were collected. Therefore, although some of these plant taxa have distributions that extend to lower elevations, the pattern is most consistent with the hypothesis that Bogong moths are locally foraging on flowers. Furthermore, plant taxa within this broader distribution were also characteristic of subalpine and alpine ecosystems (Table 1; Costin *et al.,* 2000), suggesting the possibility that they were also collected locally. The dominance of insect-pollinated plants in the moths' pollen loads suggests they are actively foraging from flowers rather than being incidentally exposed to pollen. Wind-pollinated species made up just 0.06% of pollen present on moths' bodies. If pollen adhered to moths' bodies only incidentally while in contact with other surfaces, wind-pollinated taxa such as the abundant local grass species would be expected to be more heavily represented (Timerman and Barrett, 2021).

While the presence of pollen from plant species restricted to high elevations confirms that much of the pollen carried on moths' bodies was collected locally, it is difficult to know how long pollen may adhere to a moth's body. This introduces uncertainty surrounding when and where pollen was collected, with the possibility that some of the pollen was collected from lowland ecosystems while enroute to alpine aestivation sites, rather than after arriving at aestivation sites. Research suggests great variability in pollen retention among flower-visiting insect species. Some studies indicate capacity for long-distance pollen retention among migratory insects (Ahmed *et al.*, 2009), including lepidoptera generally (Suchan *et al.*, 2019), Noctuidae (Hendrix *et al.*, 1987) and in another *Agrotis* species (Hendrix and Showers, 1992). However, Del Socorro and Gregg (2001) showed that sunflower pollen retention declined significantly on the bodies of a noctuid moth, *Helicoverpa* *armigera,* after just two days. Gregg (1993) also showed that pollen present on the bodies of *H. arimgera* and *H. punctigera* was primarily indicative of plants nearby to the sample sites, while pollen from inland sites at the start of the moths' migration route was uncommon. While some pollen grains are expected to be lost with time, leading to higher numbers of pollen from recently visited flowers, more research is needed on pollen retention in insects (Suchan *et al.*, 2019).

In his seminal work on the Bogong moth, Common (1954) made two important observations that have informed our expectations regarding flower use by Bogong moths. Common made fewer than a dozen observations of moths feeding from flowers, all on *E. niphophila*. Following this observation, experiments were conducted using a series of Petri dishes containing sucrose, honey, water and fermenting molasses. From these experiments, which elicited little feeding response, the author concluded that Bogong moths do not actively engage in foraging while in aestivation. On the other hand, our data indicate that almost all moths caught at light traps are carrying pollen, and the pollen identity indicates local foraging rather than pollen brought up from the lowlands. Common (1954) also experimentally blocked the exit of a small aestivation cave for a period of 3-4 months so that moths could not exit at night and then observed substantial moth mortality, especially in the latter summer. This result could reflect a lack of nectar feeding opportunities or limited access to water. While our study suggests that flower feeding during summer may be important to the moths, further research is needed to determine the role of nectar feeding by Bogong moths in the Australian Alps, including teasing apart the importance of water versus carbohydrate acquisition from nectar and separating flower visitation habits pre- vs. post-aestivation.

We do not know whether the moths we collected at light traps were on their way to or from a period of aestivation, or if they were taking flights from their aestivation caves, as observed by Wallace *et al.* (2022). The apparent shift in pollen composition (Figure 2) might be explained by a greater number of recently arrived moths in the early weeks, and more local activity in later weeks. If aestivation were associated with a complete cessation of flower feeding, one might expect a reduced frequency of moths or reduced pollen loads in the period

December-February, but this was not apparent in our data. This may provide evidence of flower feeding during aestivation and might provide an explanation for the activity observed at aestivation caves by Wallace *et al.* (2022), which alternatively could also be explained by foraging for water. This finding is also consistent with the foraging habits of *Euxoa auxilliaris*, another migratory noctuid moth which feeds during their annual alpine activity period in the Rocky Mountains (Kevan, 1997). However, we cannot determine whether moths caught in our study were feeding while still enroute to aestivation sites, or after leaving caves during night flights. While Warrant *et al.* (2016) recorded an aestivation site nearby to our subalpine sampling location at Charlotte Pass, the majority of aestivation sites in NSW are recorded in the alpine zone, at elevations upwards of 2000m a.s.l. Sampling Bogong moth pollen loads at these higher elevation sites would help to clarify feeding habits during aestivation.

The discovery that Bogong moths are flower feeding while in the Alps, presents the possibility that the conservation of flowering plants in the Alps could be important to sustaining Bogong moth populations. Declines in pollinator abundance are often associated with a loss of host plant populations, and so conserving these plants can be an important step in supporting populations (Biesmeijer *et al.*, 2006; Schmidt *et al.*, 2020). For instance, conserving nectar-producing plants nearby to Monarch butterfly hibernation sites has been identified as an important step in supporting migratory populations (Brower *et al.*, 2006).

While we show that Bogong moths carry large quantities of pollen while in the Australian Alps, further research is needed to determine whether moths are effectively transferring conspecific pollen when visiting flowers, and whether this is important to alpine plants. Given that in many cases hundreds or thousands of pollen grains were found on an individual moth's body (Figure 2), and that moths historically occur in these high-altitude environments in their billions throughout the flowering season (Green *et al.*, 2021), it is quite possible Bogong moths are important to reproduction and gene exchange for some plant species. While observing nocturnal pollination is difficult (Banza *et al.*, 2015), others have shown that visits from noctuid moths lead to conspecific pollen transfer (Buxton *et al.*, 2022) and effective pollination (Esposito *et al.*, 2017;

Xiong *et al.*, 2020). We recommend further study into the contribution of Bogong moths to plant pollination in the Australian Alps, such as with pollinator exclusion experiments (Benning, 2015; Van Zandt *et al.*, 2020) or lab-based pollen transfer experiments (Buxton *et al.*, 2022).

The large variation in plant species composition in the pollen sample suggests that Bogong moths are generalist flower visitors. The high frequency of Myrtaceae in the sample may well reflect that species of Myrtaceae (including *Eucalyptus*) are among the most widespread and abundant plants in the region (Costin *et al.,* 2000), and typically have nectar-rich flowers that are open to a wide range of visitors (Nicolson, 1994). Previous authors have also demonstrated generalist flower-visiting habits among other Noctuidae species (Ribas-Marquès *et al.*, 2022). Generalists visit a larger variety of flowers but may be less effective pollinators per-visit than specialists, that selectively visit flowers of fewer plant species (Morales and Traveset, 2008; Maldonado *et al.*, 2013). However, pollinator abundance is also an important consideration in determining pollination success and may be more important than degree of specialisation, as frequent visitors can deposit large amounts of pollen when considering the totality of their visits (Sahli and Conner, 2006).

Light microscopy enabled us to determine sufficient taxonomic resolution necessary to confirm that Bogong moths are feeding on local plants in the sub-alpine and alpine zones. However, due to similarities in pollen morphology (particularly in some groups, such as Myrtaceae), species-level identification was not possible in most cases. While light microscopy is commonly used in pollination ecology (Ribas-Marquès *et al.*, 2022), genetic approaches, such as DNA-metabarcoding, can help with pollen identifications at a finer taxonomic resolution (Macgregor *et al.*, 2019; Suchan *et al.*, 2019; Encinas-Viso *et al.*, 2022). The use of these techniques may have allowed us to further clarify species present in our data. Traditional light microscopy techniques and DNA metabarcoding are increasingly used in tandem (Liu *et al.*, 2017; Suchan *et al.*, 2019) and this approach should be adopted in future work on the Bogong moth.

5.9 Conclusion

We show that the mass-migrating and post-arrival moths Bogong moths are feeding from sub-alpine and alpine plants while in the Australian Alps, introducing several areas for further research. Our findings provide new insights into the migratory and aestivating phase of the Bogong moth's life cycle and may lend insight into the conservation of this declining species. Our work introduces the possibility that Bogong moths are important for plant reproductive success in Australia's mountain ecosystems. We recommend further study on Bogong moth flower feeding to examine plant pollination outcomes, the dormancy and foraging habits of aestivating moths, and their reliance on floral resources while at aestivation sites.

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5.12 Supplementary Material

Table S 1. Pollen data for each plant taxa found in Bogong moth (*Agrotis infusa*) pollen loads. Distributions defined as: lowlands (L) = <1000m a.s.l., montane (M) = 1000-1500m a.s.l., subalpine (S) = 1500-2000m a.s.l., alpine (A) = >2000m a.s.l.

Plant taxon	**Plant Family**	**Sum Grains**	**Proportion of grains**	**Number of moths carrying pollen type**	**Distribution**
Baeckea	Myrtaceae	14543	21.81	16	L, M, S, A
Aciphylla	Apiaceae	7689	11.53	1	S, A
Tetratheca	Elaeocarpaceae	6637	9.95	2	L, M, S
Leptospermum	Myrtaceae	5701	8.55	12	L, M
Richea	Ericaceae	4606	6.91	8	S, A
Leucopogon	Ericaceae	4089	6.13	17	L, M, S, A
Eucalyptus pauciflora-type	Myrtaceae	3324	4.99	33	L, M, S
Eucalyptus rubida-type	Myrtaceae	2681	4.02	8	L, M
Epacris other	Ericaceae	2331	3.5	24	L, M, S, A
Eucalyptus other	Myrtaceae	2203	3.3	25	L, M, S
Brassicaceae sp. cf. *Rorippa*	Brassicaceae	2007	3.01	5	N/A
Stackhousia	Celastraceae	1783	2.67	4	L, M, S, A
Myrtaceae sp. indet.	Myrtaceae	1771	2.66	7	N/A
Kunzea	Myrtaceae	1614	2.42	5	L, M, S, A
Epacris paludosa	Ericaceae	1476	2.21	3	L, M, S, A

Nematolepis ovatifolia	Rutaceae	1422	2.13	10	M, S, A
Eucalyptus dalrympleana-type	Myrtaceae	1413	2.12	24	L, M
Eucalyptus swollen-type	Myrtaceae	934	1.4	9	N/A
Rhamnaceae sp. cf. *Spyridium*	Rhamnaceae	346	0.52	1	N/A
Orites	Proteaceae	190	0.28	2	L, M, S, A
Lomatia	Proteaceae	121	0.18	6	L, M, S
Pimelea	Thymelaeaceae	99	0.15	10	L, M, S, A
Monotoca	Ericaceae	77	0.12	4	L, M
Acacia	Fabaceae	36	0.05	3	L, M
Grevillea lanigera	Proteaceae	24	0.04	1	L, M, S
Poaceae	Poaceae	23	0.03	18	L, M, S, A
Unknown A	N/A	19	0.03	1	N/A
Cf. *Plantago*	N/A	19	0.03	2	N/A
Brachyscome	Asteraceae	17	0.03	5	L, M, S, A
Mirbelia oxyloboides	Fabaceae	16	0.02	2	L, M
Banksia	Proteaceae	16	0.02	6	L, M
Callitris	Cupressaceae	13	0.02	3	L, M
Grevillea australis	Proteaceae	13	0.02	7	S, A
Lamiaceae sp.	Lamiaceae	8	0.01	3	L, M, S, A

Dodonaea	Sapindaceae	6	0.01	2	L, M
Hakea lissosperma	Proteaceae	6	0.01	2	M, S
Asteraceae (Tubuliflorae) cf. *Carthamus*	Sapindaceae	6	0.01	2	N/A
Exocarpus	Santalaceae	5	0.01	1	L, M, S, A
Plantago	Plantaginaceae	5	0.01	1	L, M, S, A
Senecio	Asteraceae	5	0.01	5	L, M, S, A
Indet. Zonocolpate sp.	N/A	5	0.01	1	N/A
Hibbertia	Dilleniaceae	4	0.01	4	L, M
Rumex	Polygonaceae	4	0.01	3	L, M, S, A
Oxylobium ellipticum	Fabaceae	4	0.01	1	L, M, S, A
Epacris impressa	Ericaceae	3	<0.01	3	L, M
Podocarpus	Podocarpaceae	3	<0.01	3	L, M, S, A
Celmisia	Asteraceae	3	<0.01	1	M, S, A
Cassinia	Asteraceae	2	<0.01	1	L, M, S, A
Malva	Malvaceae	1	<0.01	1	L, M, S
Wahlenbergia stricta	Campanulaceae	1	<0.01	1	L, M, S, A
Micrantheum	Asteraceae	1	<0.01	1	L, M, S, A
Leptorhynchos	Asteraceae	1	<0.01	1	L, M, S, A

Tasmannia	Winteraceae	1	<0.01	1	L, M, S, A
Urticaceae	Urticaceae	1	<0.01	1	N/A
Restionaceae	Restionaceae	1	<0.01	1	N/A
Asteraceae (Liguliflorae) cf. *Taraxacum*	Asteraceae	1	<0.01	1	N/A

Chapter 6: Conclusion

Together, this thesis demonstrates that mountain pollinators are influenced by the interactions of biotic and abiotic conditions operating across different spatial and temporal scales. Chapter 2 showed that climate, weather and floral community composition influence changes in pollination networks and communities in mountain environments. Chapter 3 focussed on a unique bee that counters the broader ecological trends displayed by other bees in this habitat, displaying life history traits that allow it to persist in the cold. Chapter 4 quantified the diversity of floral microhabitats experienced by pollinators in the Australian Alps, showing that pollinator choice is influenced by the thermal rewards. Chapter 5 focussed on the pollination ecology of a nocturnal, Endangered species, showing that the Bogong moth plays a previously underappreciated ecological role as a flower visitor in the Australian Alps.

Mountain ecosystems provide steep environmental gradients and constricted flowering and activity windows, offering valuable natural experiments for cross-scale research. In the Australian Alps, elevation gradients compress shifts in temperature, snow cover, and species distribution over short geographic distances, while weather variability imposes strong hourly and daily fluctuations. In my thesis, I have leveraged these natural components of Australia's Alps to connect patterns in pollinator community composition and life history traits to abiotic and biotic mechanisms. This research documents a hierarchy of drivers and mechanisms, where climate limits community composition, weather influences foraging activity, and plant community composition determines when and how interactions occur, including through floral microhabitats. Framing this system in this way clarifies how abiotic and biotic filters interact to produce observed patterns of insect distribution and flower visitation, providing a template for predicting responses to environmental change.

This scale-explicit view clarifies potential climate change responses in pollinator communities in the Australian Alps. In Chapter 2, I show that pollinator community distribution is governed by elevation-linked climate envelopes and local plant assemblages. This suggests that with continued warming, highly mobile pollinator species may shift upslope faster than their host plants are able to relocate, driving spatial mismatches. In Chapter 3 I show that *Exoneura* bees are highly susceptible to fluctuations in weather conditions, and that predicted warming will expand their foraging opportunities at high-elevation sites. This may lead to changes in competition dynamics and plant-pollinator phenological mismatches. In Chapter 4 I show that mountain flowers offer functionally important thermal microhabitats, which afford some resilience by buffering against broader unsuitable climatic conditions. In a warmer future, these thermal benefits that aid foraging under cold conditions may lose value, or even become liabilities, during warm periods. In Chapter 5 I show that Bogong moths are important flower visitors in mountain ecosystems, and that if their population declines with continued climate change (among other drivers), that there may be consequences for plant reproductive success in the Australian Alps. Together, these results predict shifts in pollinator foraging and floral visitation that may cascade to changes in plant reproductive outcomes and ecosystem structure.

Going forward, management actions should prioritise protecting plant communities that sustain long flowering windows and conserving key flower resources, particularly at high elevations where activity windows are most constrained. Future research should link microclimate and behaviour to phenology, visitation, and plant reproductive outcomes (including community-scale nocturnal sampling) to identify vulnerable interactions and inform targeted conservation. Importantly, while this thesis primarily examined pollinator activity and interaction structure, translating these changes into consequences for plant reproduction remains a key next step, requiring explicit measurements of pollen limitation, pollen transfer, and seed set. Predicting plant-pollinator community responses will benefit from extending a thermal ecology lens beyond flower-visiting adults, as examined in my thesis, to include temperature sensitivity across other life stages (e.g., development, overwintering, and emergence), which may impose constraints that are not apparent from adult foraging alone. Finally, community-level nocturnal sampling is needed to resolve the contribution of night-active taxa to pollination networks and to determine how nocturnal shifts in visitation may mediate resilience or vulnerability under ongoing climate change.