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RESEARCH

What is pollinating the critically threatened calcicolous plants in the Waitaki Valley?

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Abstract: Understanding the key pollinators of rare plants is important for a holistic assessment of ecosystem health, as the impact on a given species can travel through the network, affecting other species. Many specialist calcicolous (limestone-specific) plants are rare. Conservation management plans have been developed to assist in the survival of these rare plant species, but these rarely include information on pollinators. To increase our understanding of limestone ecosystem functioning, we investigated the pollinators of three critically threatened plant species, *Lepidium sisymbrioides*, *Carmichaelia hollowayi*, and *Gentianella calcis* subsp. *calcis* in two Otekaike limestone reserves, Wai O Toura Scenic Reserve and Waipata Scientific Reserve. We used community sampling and 10-minute observations to investigate which insect species are the most likely pollinators of these three plants species. Observed flower visitors were caught, identified, and swabbed for pollen. The ability to deposit conspecific pollen on stigmas was tested for selected insect species via single-pollinator visit assays to female *L. sisymbrioides* flowers. The community sampling experiments show that the most likely pollinators of *L. sisymbrioides* are the endemic bee, *Lasioglossum sordidum*, and Tachinid flies, including native *Pales* sp., and endemic *Mallochomacquartia vexata*. The most likely pollinator for *G. calcis* subsp. *calcis* is the endemic hover fly, *Melanostoma fasciatum*. This species was the most frequent visitor, with over half the hover flies captured carrying *G. calcis* subsp. *calcis* pollen. The native bee *Leioproctus pango* is the most likely pollinator for *C. hollowayi*, as it was the most observed visitor and nearly all captured bees were carrying *C. hollowayi* pollen. This research contributes to the literature on New Zealand's naturally uncommon ecosystems and provides an in-depth look into plant-pollinator interactions of these under-researched plant species.

Keywords: arthropods, endemic, limestone, New Zealand, observational, pollen, pollinators, uncommon ecosystems

Introduction

New Zealand is home to over 70 naturally uncommon ecosystems (Williams et al. 2007), and limestone outcrops (cliffs, tors and scarps) are one of these. New Zealand limestone habitats contain a high diversity of specialist plant species (Williams et al. 2007), supporting over 152 calcicolous (limestone-specific) plants, which represent 5.6% of the 2718 taxa of vascular plants in New Zealand (Rogers et al. 2018). These calcicolous plants likely have always existed in small populations across a network of naturally uncommon ecosystems. However, due to quarrying, deforestation, pastoral grazing and weed invasion, the vast majority of limestone plants are now confined to remnant populations, and almost all have restricted and decreasing distributions (Heenan & Rogers 2019). For example, the distributional range of some calcicolous plants can be 10 square metres or less (e.g. *Australopyrum calcis* subsp. *calcis*, *Brachyscome montana*, and *Carmichaelia hollowayi*; Heenan & Rogers 2019). Hence, many plants suffer from inbreeding depression due to very small population sizes (Heenan & Rogers 2019). A change in farming practices, with conversion from extensive sheep grazing to intensive dairy farming, also puts these plant populations at risk. Out of the 152 calcicolous plant species, 73 are ranked Data Deficient or Threatened (of which 44 are classified as Nationally Critical, the highest threat rank in Aotearoa New Zealand), and 71 are ranked At Risk (de Lange et al. 2010; Rogers et al. 2018). Management usually focuses on individual plants and sometimes their immediate habitat (Corlett 2016), but mostly excludes any consideration of their pollinators.

An insect can be considered a pollinator if, in the process of visiting flowers, it transfers pollen from an anther to the receptive stigmatic surface of the same or another flower of the same species (Proctor et al. 1996). Pollinators are critical to the reproduction of the majority of flowering plants, and they often allow cross-fertilisation and gene flow within and across plant populations (Proctor et al. 1996; Ollerton et al. 2011). Understanding the degree of reliance on pollinators is important when managing small plant populations (Lord 2022).

This study examined the potential pollinators of the

Nationally Critical species, *Lepidium sisymbrioides*, *Carmichaelia hollowayi*, and *Gentianella calcis* subsp. *calcis* (hereafter *Gentianella calcis*) on limestone habitat in the Waitaki Valley, North Otago. While *L. sisymbrioides* can also be found on schist soils, the other two species are restricted to Otekaike Limestone outcrops and bluffs. Both *C. hollowayi* and *G. calcis* are capable of self-pollination but likely rely on entomophily to maintain gene flow within and between populations (de Lange et al. 2004; de Lange 2022; Lord 2022). *Lepidium sisymbrioides* is entirely reliant on entomophily and is one of only two dioecious *Lepidium* species in the world (Bateman 1955; Soza et al. 2014). Currently, there are no studies documenting invertebrate species involved in the pollination of *L. sisymbrioides* and *C. hollowayi* in the Waitaki populations. Some information has been provided on insects observed visiting *G. calcis*, but the study focused on whether the plants could self-pollinate rather than thoroughly documenting pollinators (Lord 2022). Thus, we aimed to investigate which species are likely to be pollinators for *L. sisymbrioides*, *C. hollowayi*, and *G. calcis* using community observations and sampling of insects to examine whether the focal plant pollen was on their bodies. Single-pollinator visitation assays were used to determine whether conspecific pollen is being deposited onto the stigma of female *L. sisymbrioides* plants. Pollen on insect bodies is evidence that a species might be an effective pollinator (Delaplane et al. 2013).

Methods

Site and plant selection for invertebrate and plant community sampling

This study was conducted over the 2021–2022 and 2022–2023 Spring, Summer, and Autumn seasons, during the flowering period of focal plants, at two study sites: Wai O Toura Scenic Reserve (formerly Gards Road Scenic Reserve) and Waipata

Scientific Reserve (also known as Earthquakes Scientific Reserve), in the lower Waitaki River Valley (Fig. 1). Wai O Toura contains *L. sisymbrioides* and *C. hollowayi* (Fig. 2). Waipata contains mainly *G. calcis* and a few individuals of *L. sisymbrioides* (Fig. 2). These reserves are managed by the Department of Conservation.

Study species

Lepidium sisymbrioides Hook.f. (Brassicaceae) is an endemic, dioecious cress identifiable by its rosettes of pinnate leaves and low-growing profile with proportionally long flower stalks bearing many small white flowers (Fig. 3a). Plant sex can be distinguished by the seed pods grown by female plants after flowering and by the visible anthers on the flowers of male plants (Heenan et al. 2007). Both sexes flower from November to December (Heenan et al. 2007). *Lepidium sisymbrioides* can be found in North Otago on the south side of the upper Waitaki River, and in Central Otago near Falls Dam in the upper Manuherika Gorge and in the Kawarau Gorge near Cromwell (based on circumscription of Heenan et al. 2007). Plants are often found on cliff faces. At Wai O Toura Scenic Reserve, the population of *L. sisymbrioides* is 73 individual plants, some found on the cliff edge and some in the grass which overlays limestone pavement areas back from the cliff edge. Of these 73 plants, 22 (13 female, 9 male) were observed in this study, selected on the basis of accessibility, whether they had enough flower stalks, and whether they had ID tags. There are also 12 plants at Waipata Scientific Reserve, but these were not included in this study as only two female plants flowered.

Gentianella calcis Glenny et Molloy subsp. *calcis* (Gentianaceae) is an endemic, calcicolous gentian with narrow, deep green rosette leaves, and flower stalks bearing relatively large, solitary, bisexual white flowers with a conspicuous yellow eye (Fig. 3b). It flowers through March and April (de Lange et al. 2004). *Gentianella calcis* is found at two sites in the Waitaki River Valley (Heenan et al. 2007), one on private
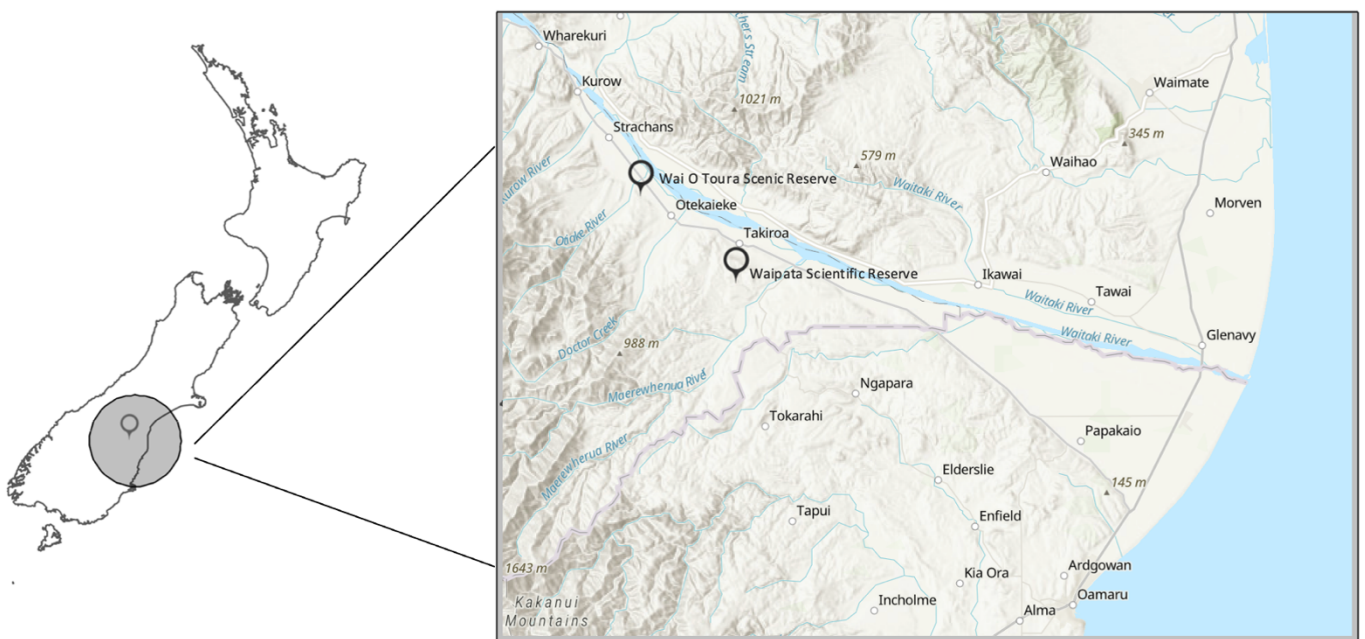

Figure 1. Study sites on limestone in Waitaki Valley, North Otago, New Zealand: Wai O Toura Scenic Reserve (−44.807904, 170.534274) and Waipata Scientific Reserve (−44.853920, 170.610830).

Figure 2. (a) locations of plants across Waipata Scientific Reserve, the squares represent *Gentianella calcis* subsp. *calcis* (*n* = 70). (b) locations of plants across Wai O Toura Scenic Reserve, triangles represent *Lepidium sisymbrioides* (*n* = 20) and circles *Carmichaelia hollowayi* (*n* = 6). Note that for *G. calcis* and *L. sisymbrioides*, symbols do not represent each individual plant location, but rather locations of clusters of plants.

Figure 3. Flowers of (a) *Lepidium sisymbrioides*, (b) *Gentianella calcis* subsp. *calcis*, and (c) *Carmichaelia hollowayi*. Photo credit: Sharn Milliken.

property and the other at Waipata Scientific Reserve, which contains roughly 100 wild and 80 translocated plants of this species. Ten wild plants and 60 translocated plants were observed in this study.

Carmichaelia hollowayi G. Simpson (Fabaceae) is an endemic, calcicolous broom that can be distinguished from other native brooms by its low-growing profile with yellow-tipped, erect branches that appear leafless (Fig. 3c). *Carmichaelia hollowayi* produces small, white-lilac bisexual flowers, normally over a period of two to four weeks from towards the end of December to early January (Heenan 1995; de Lange 2022). *Carmichaelia hollowayi* is known only on limestone outcrops on the south bank of the Waitaki River and is restricted to three known sites: Wai O Toura Scenic Reserve and two other sites on private property and/or in dangerous locations. Wai O Toura Scenic Reserve has a *C. hollowayi* population of eight plants, six of which were observed. The other two were on a cliff edge and not accessible.

Pollinator community sampling

Survey and sampling of pollinators were conducted on days with no rain and low wind. Observations for *L. sisymbrioides* occurred over a 40 day period (November and December 2021), within which weather conditions and logistics allowed for seven days of sampling. *Gentianella calcis* observations occurred over a 45 day period (March and April 2022), with six suitable days for sampling, and *C. hollowayi* observations over a 20 day period (December 2022 and January 2023), with seven suitable days for sampling. Plants were monitored on-site using 10-minute continuous observation periods, which included observation and capture. One period of sampling was conducted each day for each study plant, meaning each plant would be individually observed for 10 minutes. Observations were done between mid-morning and mid-afternoon.

During each pollinator observation period for *L. sisymbrioides*, the types of insects visiting (e.g. flies, bees, grasshoppers) and the number of flower stalks visited were recorded, along with the sex of the plants and DOC tag ID (for those plants that DOC had tagged prior). The total number of flower stalks produced per plant was recorded at the end of the flowering season in 2021. *Gentianella calcis* plants were identified by site and plot (subpopulation) number, the plots consisting of both natural and translocated plants. Plant size was not measured. For each observation period, the types of insects visiting individual flowers were recorded. For *C. hollowayi*, each plant was given a colour ID which was recorded along with the types of insects visiting during each observation period. The number of flowers per plant was recorded midway through the flowering period when plants were in peak bloom.

Night observations were undertaken for both *L. sisymbrioides* and *G. calcis* on one occasion, a warm night with little wind. *Carmichaelia hollowayi* was not observed at night because the plants are located on a cliff edge, which was deemed a hazard.

Pollen load sampling

During each observation period for each species any insect that contacted a flower was caught, if possible (some were missed, but no species proved noticeably harder to catch than others), and placed in a vial labelled with the date, time, and site. Insects were stored at −20°C and later identified to the lowest taxonomic level possible. Species identification was

through consultation with specialist entomologists or through research grade identifications on iNaturalist. Glycerine jelly (one part gelatine: two parts glycerine; Appendix S1) was used to swab pollen from the collected insects (Souza et al. 2021). Depending on the size of the insect, a square of jelly between 2 and 4 mm wide was cut out and stuck with an insect pin (size 000, 1 or 3). Insects were lightly swabbed around the legs, head, and thorax to extract pollen. Solitary bees are tiny, so swabbing for pollen unavoidably included the scopa. The glycerine jelly was then placed onto a microscope slide, melted on a hotplate, and a coverslip was added and sealed with clear nail polish. An Olympus BX51 compound microscope at 400x magnification was used to image the pollen grains. They were then identified to the lowest taxonomic level possible with the aid of a pollen library (Appendix S3).

To create the pollen reference library, flowers were taken from not only each of the study species but from all other plants flowering between November–December 2021 and March–April 2022 at the study sites. The flowers were transported back to the Zoology Department and frozen. Pollen was removed from anthers, and microscope slides were prepared as for the pollen swabbed from insects. The pollen library included samples of every vascular plant species in flower at either site, with images of their pollen, an image of the plant, date collected, and scientific and common names (Appendix S3).

Single-visit pollinator experiment

Nine female plants of *Lepidium sisymbrioides* at Wai O Toura Scenic Reserve with two or more flower stalks were used to investigate the effectiveness of single pollinator visits. Seven of these plants were located in grassy areas, and two on the cliff (Fig. 2b). Plants with only one flower stalk were not selected because covering the entire stalk with a pollen bag would remove that individual from the gene pool of this critically threatened species, reducing the number of plants available for reproduction in that season.

The single-pollinator experiment took place in November 2022. At the start of the experiment one whole flower stalk bearing multiple flowers was bagged per plant. We used fine organza bags (hereafter called pollen bags) tied with a drawstring closure around the stalk below the flowers (Fig. 4). DOC tags were used to identify each plant. Bags were put in place while flower buds were still closed to ensure no pollinators visited before the experiment started (Fig. 4). The bags were placed on the stalks before flowers had opened and were only removed for observations. Once the flowers had fully opened, the pollen bag was removed on each observation day and the stalk was observed until a single pollinator had visited a single flower. If a pollinator did not visit within 30 minutes, the observation would stop, and the bag would be replaced. Visitors were identified at the site to the nearest taxonomic order or family. Once a pollinator had visited, the visited flower was removed and placed into a labelled vial, and the flower stalk was then re-bagged until the next observation day. Cotton wool was placed into the vial to prevent flower movement and loss of pollen during transport. In the laboratory, stigmas were lightly swabbed with glycerine jelly cubes to remove pollen. The glycerine jelly was then processed in the same manner as the reference pollen. All pollen grains were identified to the lowest taxonomic level possible and counted. Observations were limited to days with ideal conditions (e.g. warm, low wind, zero precipitation) for insect flight. Adverse weather limited the number of days during which observations

Figure 4. Bagged female *Lepidium sisymbrioides* plants. One stalk per plant was bagged and only plants with two or more stalks were chosen for bagging. Bags are secured using a drawstring. Photo credit: Tom Waterhouse.

could be carried out to a total of four. Unvisited flowers were not checked for pollen, as *L. sisymbrioides* is unlikely to be wind-pollinated.

Data analysis

For each focal plant species, the most common insect visitors were compared to the insects that carried pollen of the focal plant. We used linear mixed effect (lmer) models to investigate the influence of plant size (number of flowers or flowering stalks) on the number of plant visitors. Models were conducted in R (version 4.2.1, R Core Team 2022), using the packages lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017). Individual plant identity was included as a random variable due to repeated observations of the same plants. Linear mixed effect models were also used to analyse the influence of flower sex on the number of visitors to *L. sisymbrioides* flowers. Plant size was included as a covariate, sex as a fixed factor, and plant identity as a random variable. Because the number of insects visiting was count data, the data were log-transformed to improve normality, and the residuals of the models checked for normality.

Results

Insect community visiting flowers

A total of 129 insects were observed on *L. sisymbrioides* flowers over seven days of sampling, 115 insects on *G. calcis* over six days of sampling, and 93 insects on *C. hollowayi* over seven days of sampling (Appendix S2). For both *L. sisymbrioides* and *G. calcis,* Diptera was the most commonly observed taxonomic order, while for *C. hollowayi,* Hymenoptera was the most common taxonomic order (Appendix S2). A total of 78 insects were caught on *L. sisymbrioides*, 63 on *G. calcis*, and 32 on *C. hollowayi* (Appendix S2).

The most abundant native visitors to *L. sisymbrioides* were *Lasioglossum sordidum* solitary bees and *Mallochomacquartia vexata* flies. An abundant exotic visitor was *Copidosoma* sp., a chalcid wasp. Common native visitors to *G. calcis* were small hover flies (*Melanostoma fasciatum*) and Pterophoridae. The striped dung fly, *Oxysarcodexia varia*, was the most abundant exotic visitor. The most common native visitor to *C. hollowayi* flowers was the solitary bee *Leioproctus pango.* There was only one exotic visitor, a species of ladybird beetle, *Coccinella undecimpunctata*, that is not considered to be a pollinator (see discussion).

The pollen reference library consisted of 37 species flowering at either of the sites over the course of the study. Nine species were native, eight were introduced Asteraceae species, six were introduced Fabaceae, and the remainder were a variety of other introduced species. The pollen morphologies of native species were sufficiently distinctive for identification to species level. However, to avoid overstating species representation in pollen loads, other pollen types were grouped into introduced Asteraceae, introduced Fabaceae, or "other/known" (Appendix S2). For insects caught on each of the three study species, pollen of that species was among the most frequently found pollen types, but pollen loads tended to be diverse. Visitors to *L. sisymbrioides* flowers carried the most diverse pollen loads, and visitors to *C. hollowayi* carried the least diverse loads. Visitors to *G. calcis* carried more pollen from introduced Asteraceae than conspecific pollen.

Plant size (number of flowering stalks) significantly influenced the number of arthropods visiting *L. sisymbrioides* flowers (Est = 0.033; SD = 0.014; $t = 2.27$; $p = 0.042$). When an unusually large plant with 29 flower stalks was removed from the analysis, and the effect became marginally non-significant ($t = 2.09$; $p = 0.05$). Flowers per plant significantly influenced the number of insects visiting *C. hollowayi* flowers (Est = 0.011; SD = 0.003; $t = 3.352$; $p = 0.002$). There was no significant effect of sex on arthropod count on male

vs. female *L. sisymbrioides* plants (Est = 0.30; SD = 0.23; *t* = 1.32; *p* = 0.20).

Potential pollinators

Lepidium sisymbrioides

The five most common arthropods observed visiting *L. sisymbrioides* were chalcid wasps (*Copidosoma* sp.), a spider mite (Tetranychidea), an endemic bee (*Lasioglossum sordidum*), and two species of Tachinid fly (*Pales* sp. and *Mallochomacquartia vexata*) (Fig. 6). These top five arthropods comprised 60% of all identified visitors. *Lepidium sisymbrioides* pollen was found on 71% of *L. sordidum* captured. Tachinid flies, *M. vexata*, were captured six times, with 50% carrying *L. sisymbrioides* pollen. Chalcid wasps were observed 20 times, but only 20% of them carried *L. sisymbrioides* pollen. Braconid wasps (Microgastrinae) were observed 4 times, but only one carried *L. sisymbrioides* pollen. Long-legged flies (Dolichopodidae), bush-cricket (*Conocephalus* sp.) and cluster flies (*Pediculata* sp.) carried *L. sisymbrioides* pollen, but all were only captured once. Willowherb yellow moth (*Anachloris subochraria*), a field cricket (*Bobilla* sp.), robber fly (*Neoitamus melanopogon*), and short-horned grasshopper (*Phaulacridium marginale*) were all captured once but carried no *L. sisymbrioides* pollen (Fig. 6a). Two insects were observed visiting *L. sisymbrioides* plants at night: one was a robber fly (*N. melanopogon*), and the second was a field cricket (*Bobilla* sp.); neither had any pollen on them.

Gentianella calcis

The top five insects captured visiting *G. calcis* were the native hover fly (*Melanostoma fasciatum*; Fig. 5), striped dung flies (*Oxysarcodexia varia*), a long-legged fly (Dolichopodidae), a plume moth (Pterophoridae), and cluster flies (*Pediculata* sp.). Hover flies were the most frequently caught and observed insect with 46 total observations and 29 caught, accounting for 46% of all identified visiting species. Out of the 29 captured hover flies, 48% carried *G. calcis* pollen. Striped dung flies were observed four times, with three captured individuals carrying pollen. Insects such as *Apis mellifera* (honey bees), *Calophora quadrimaculata* blowflies, and Tachinid flies were only observed once, and each carried *G. calcis* pollen (Fig. 6b). Night observations did not detect insects visiting *G. calcis*.

Carmichaelia hollowayi

The endemic bee *Leioproctus pango* (Fig. 5), the endemic large hover fly (*Melangyna novaezelandiae*), the endemic orange-spotted ladybird beetle (*Coccinella leonina*), a native field katydid (*Conocephalus sp.*), a species of long-legged fly (Dolichopodidae), and the exotic eleven-spotted ladybird (*C. undecimpunctata*) visited *C. hollowayi*. *Leioproctus pango* was the most commonly caught and observed insect, with 29 bees caught and 76 observed. *Leioproctus pango* made up 85% of all identified visiting species. Of the six insects observed visiting *C. hollowayi*, only three carried *C. hollowayi* pollen: *L. pango*, the field katydid, and *M. novaezelandiae* (Fig. 6c). Out of the 29 captured *L. pango*, 92% carried *C. hollowayi* pollen. The field katydid and *M. novaezelandiae*, each only observed once, also carried *C. hollowayi* pollen (Fig. 6c).

Single pollinator assays

The single-visit pollinator assays demonstrated that *L. sisymbrioides* pollen is being deposited onto female *L. sisymbrioides* flowers via an insect pollinator (Appendix Figure S5). Out of the 13 insects (10 flies, two wasps and one *Lasioglossum sordidum* bee) observed visiting flowers after

Figure 5. *Melanostoma fasciatum* visiting *G. calcis* (left), and *Leioproctus pango* visiting *C. hollowayi* (right). Photos: Sharn Milliken.

bags were removed, nine deposited conspecific pollen on the stigma. An average of 8.92 grains of pollen were deposited per visit (SD = 21.77), with a range of 1–80. The highest deposit of 80 grains was by a fly (*Pales* sp., Tachinidae). Eight out of the ten flies observed deposited pollen, and the one native bee also was successful in depositing pollen. In contrast, the two wasps did not deposit any pollen grains on stigmas (Fig. 7).

Discussion

This study investigated the potential insect pollinators of three limestone-inhabiting, rare plant species: *Lepidium sisymbrioides*, *Carmichaelia hollowayi*, and *Gentianella calcis* subsp. *calcis*. Flower visitors can be defined as pollinators only if specific requirements are met: (1) pollen transfer to,
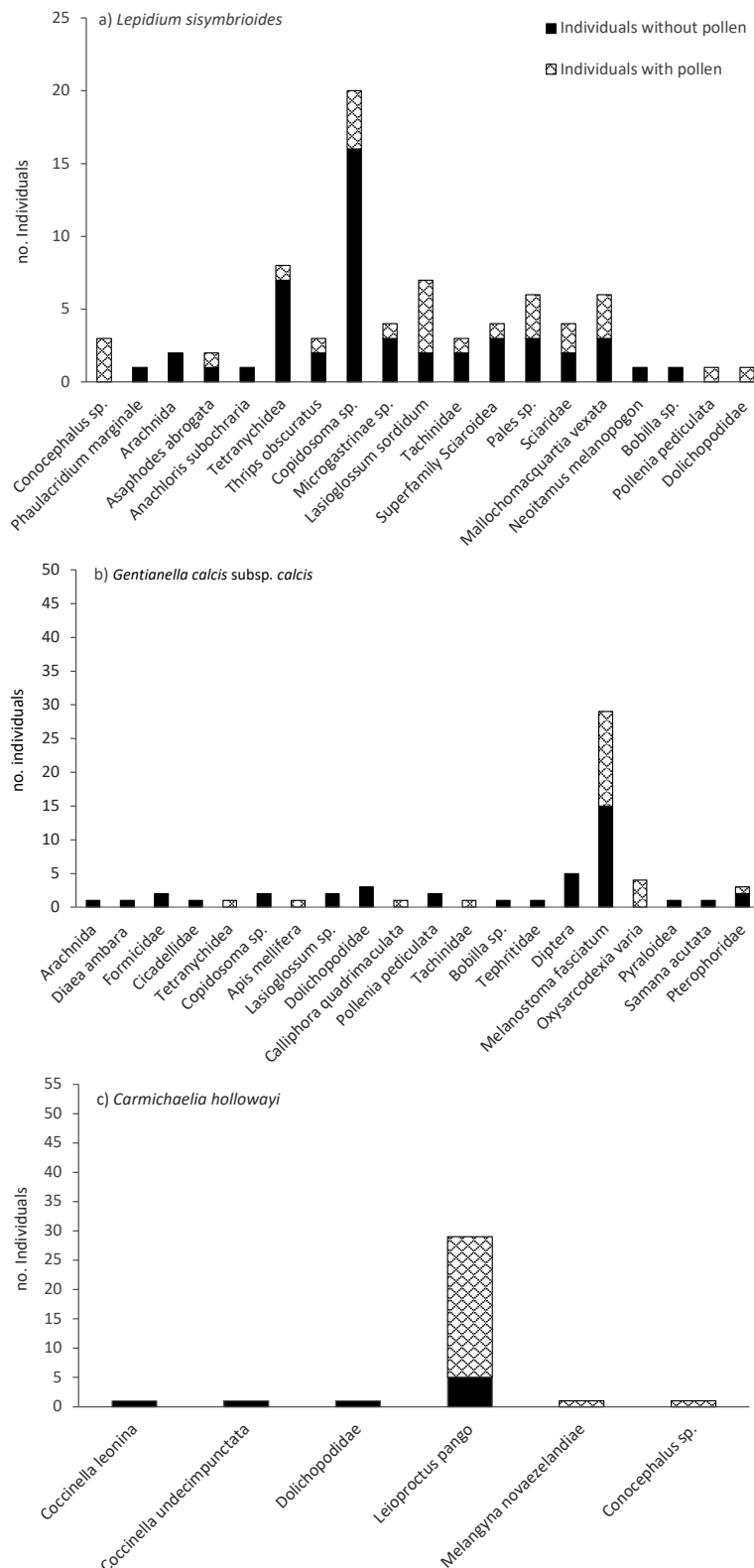

Figure 6. Abundances of captured arthropods (filled) and those carrying pollen (patterned). (a) *Lepidium sisymbrioides*, *n* = 81 total arthropods captured over seven days from 4 November–14 December 2021. (b) *Gentianella calcis* subsp. *calcis*, *n* = 62 insects captured over six days from 17 March–29 April 2022. (c) *Carmichaelia hollowayi*, *n* = 33 insects captured over seven sampling days from 14 December 2022–3 January 2023.
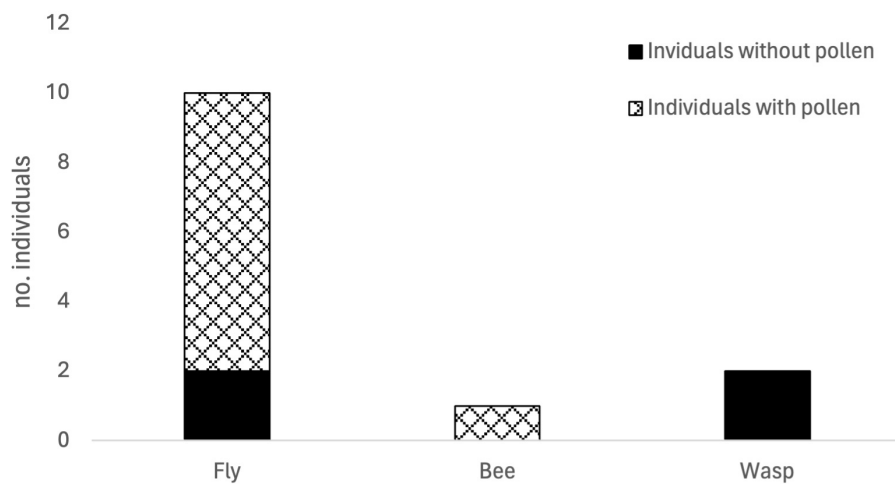
Figure 7. Abundance of captured insects and those that deposited pollen onto *Lepidium sisymbrioides* flowers (patterned) from single pollinator assays, *n* = 13 total insects captured on four days of sampling between 1–15 November 2022.

(2) pollen transport by, (3) and pollen transfer from insect to stigma of receiver flower (Wallace et al. 2002). Based on species abundance and their ability to carry pollen, the most likely pollinators for *L. sisymbrioides* are *Lasioglossum sordidum* bees and Tachinid flies, including native *Pale*s sp*.,* and endemic *Mallochomacquartia vexata.* The most likely pollinator for *G. calcis* is the hover fly *Melanostoma fasciatum. Leioproctus pango* bees are the most likely pollinators for *C. hollowayi*. As expected, *L. sisymbrioides* and *C. hollowayi* plants with more flowers attracted more insects. This study confirmed insect deposition of conspecific pollen onto female *L. sisymbrioides* flowers. In the single-pollinator visitation assays, Tachinid flies were the most effective, most frequently depositing pollen and depositing the highest pollen count out of the 13 insects caught.

Pollinators of *Lepidium sisymbrioides*

This study provides the first evidence of effective pollinators for New Zealand members of the *Lepidium* genus. *Lasioglossum sordidum* were observed visiting *Lepidium* stigmas and *Lepidium* pollen was attached to their hairs. This finding corroborates a report of *L. sordidum* foraging behaviour and their effectiveness as pollinators (Wallace et al. 2002). *Lasioglossum sordidum* bees are therefore likely to be the most important insect pollinator of *L. sisymbrioides.* However, Tachinid flies also likely play a large role, with 70% of flies visiting *L. sisymbrioides* carrying pollen, and *Pales* sp. depositing the most pollen onto female *Lepidium* flowers in the single-visit pollinator assays. The effectiveness of flies as pollinators is thought to be generally low due to small pollen loads compared with bees (Proctor et al. 1996; Newstron-Lloyd 2013), though they still carry a higher pollen load than other insects such as beetles or wasps (Newstron-Lloyd 2013). Flies can be especially important as pollinators when bees are less abundant (Ssymank et al. 2008; Lord et al. 2013). While native bees have not been surveyed at the limestone reserves, they were frequently observed at the start of our 2021 field season, but were not common during our 2022 field season. Concerns have long been raised about declines in native bee species (Donovan 1980) and there is clear evidence of recent declines in both wild and domesticated pollinators around the globe (Potts et al. 2010; Dicks et al. 2021). Declines in native bees would leave a considerable gap in pollination services that flies may be able to fill (Ssymank et al. 2008). Although

Copidosoma is not a likely primary pollinator, these wasps could still contribute as secondary pollinators in the absence of bees, because pollen can attach to the small number of hairs on their bodies (Jousselin et al. 2003; Shuttleworth & Johnson 2009; Brock et al. 2021). Each *Lepidium* flower has just two ovules, so low pollen loads can still provide adequate pollination.

Pollinators of *Gentianella calcis* subsp. *calcis*

While *G. calcis* was visited by a large variety of insects, the only common floral visitor was *Melanostoma fasciatum*, a hover fly. This species is found throughout New Zealand, where it occurs in grasslands and other habitats with low-growing vegetation (Hickman et al. 1995). Hover flies are important pollinators in many ecosystems (Doyle et al. 2020). Hover flies have a small proboscis and can consume nectar from exposed blooms only, like those of *G. calcis* (Hickman et al. 1995). Field experiments show *M. fasciatum* prefer Papaveraceae and Ranunculaceae families (Holloway 1976); these flowers have similar morphologies to the actinomorphic free-petalled flower of *G. calcis*. Of other New Zealand *Gentianella* species, alpine *G. corymbifera* is most commonly visited by solitary bees and Tachinid flies (Primack 1983; Bischoff 2008), subantarctic *G. concinna* is visited by noctuid moths (Buxton et al. 2019), and *G. lineata* and *G.gibbsii* are thought to be mainly self-pollinated (Glenny 2004).

Pollinators of *Carmichaelia hollowayi*

The most common floral visitor to *C. hollowayi* was the solitary bee *Leioproctus pango*. Out of 33 insects caught, 28 were *L. pango,* and c. 93% of those were carrying a large abundance of *C. hollowayi* pollen. *Leioproctus pango* were observed crawling into the papilionaceous flowers of *C. hollowayi*; the small size and flattened body of solitary bees makes them the perfect size for fitting inside the flower. The fertilisation of plants with papilionaceous flowers is thought to have evolved under selective pressure from bee pollinators (Aronne et al. 2012). Primack (1983) recorded that solitary bees were the main visitors to flowers of *C. angusta*; however, other New Zealand species in this genus can be fly pollinated (*C. grandiflora,* Primack 1983) or bird pollinated (Heenan & de Lange 1999).

Pollinator preferences

The signals, rewards, and morphology of a flower affect the

suite of pollinators that will be attracted to that plant species (Kehrberger & Holzshuh 2019). *Lepidium sisymbrioides* and *G. calcis* produce multi-flowered stalks, whereas *C. hollowayi* has individual pedicellate flowers (de Lange 2022). As stated above, *C. hollowayi*'s small papilionaceous-shaped flower suggests it has evolved to attract specialist insect pollinators, mainly bees (Aronne et al. 2012). Insect visitors to *C. hollowayi* must cope with the typical Fabaceae flower structure; anthers are concealed in the keel which is pushed down as an insect enters, so the pollen and stigma rub the underside of the insect's bodies. In contrast, *G. calcis* and *L. sisymbrioides* pollinators can contact pollen on their heads, tarsi and the ventral portion of their bodies. The increased contact with pollen may explain why *L. pango* collected on *C. hollowayi* had more pollen attached to their bodies compared to *L. sordidum* bees that were collected on *L. sisymbrioides*. Further research could include imaging bees before removing the pollen. These images would indicate which body segments pollen is most abundant on and allow comparisons between bees caught on *C. hollowayi* and bees caught on the open flowers of *L. sisymbrioides*.

Factors influencing bee pollination

Lepidium sisymbrioides plants were already flowering when observations commenced in November 2021 and native bees were very common on the very first sampling day. New female and male *L. sordidum* emerge from their nesting tunnels when the soil starts to warm around October, are most abundant in January, then typically rapidly decline during February (Donovan 1980; Bennet et al. 2018). Therefore, *L. sordidum* observed on *L. sisymbrioides* should have increased in frequency as the study went on, though it did not (Appendix S6). It is possible *L. sordidum* was attracted to other plants that were flowering in this area.

Observations of *C. hollowayi* started in late December 2022, which is when *Leioproctus* bees are at their peak population abundance. *Leioproctus* bees are active for a much shorter period than *L. sordidum*, starting in mid-October, peaking in December and ending abruptly before February (Donovan & Maynard 2010; Bennet et al. 2018). Of the 76 *L. pango* bees observed visiting *C. hollowayi*, 72 were on just two plants, located next to each other on a sheltered cliff edge. *Leioproctus pango* were observed visiting these two *C. hollowayi* plants then flying back to their nests only a metre away. Fewer bees were observed on the *C. hollowayi* plants that were furthest away (100–200 m) from the observed nests. Most New Zealand native bees forage over short distances and stay in a radius of approximately 100 m from their nests (Kasper 2021). Hence, the distant *C. hollowayi* and *L. sisymbrioides* plants were possibly outside the foraging range of *L. pango* nesting on the cliff edge. It is surprising that there were not any *L. sordidum* visiting *C. hollowayi*, considering they flower while *L. sordidum* is at peak population density. The *L. sordidum* population may have already been declining in late December, considering they were seen in abundance in early November, or they may have come out of their nesting tunnels earlier than mid-October, as they are sensitive to changes in soil temperature (Donovan 1980). *Lasioglossum sordidum* has a larger foraging range than most of New Zealand's native bees, being approximately 200–250 m (Rader et al. 2011; Bennet et al. 2018). The distance that bees forage can be related to several factors, including proximity to nesting sites and flower preference (Stewart 2002). The nesting site of *L. sordidum* at Wai O Toura was never located, so it is hard to determine whether the lack of visits by *L. sordidum* to *C.*

hollowayi flowers was due to flower preference, nest locality, or population density. Future studies should locate *L. sordidum* nesting sites and study their activity across the season, as well as test their plant preferences to determine the extent to which invasive plants are competing for pollinator services. In similar communities where native flowers are out-numbered by attractive, open access introduced flowers, native pollinators can transfer significant amounts of introduced pollen to the stigmas of native plants, reducing their reproductive output (Miller et al. 2018).

Factors influencing insect visitation

Plant size tended to influence insect visitation rates for both *L. sisymbrioides* and *C. hollowayi*, with an increased number of visitors observed on plants with more flowers, which is consistent with other literature (Connor & Rush 1996; Grindeland et al. 2005; Caraballo-Ortiz et al. 2011). For example, increases in both flower number and plant size were linked to increased visitation in wild radish, *Raphanus raphanistrum* (Connor & Rush 1996). Likewise, in matabuey (*Goetzea elegans*), a positive relationship between the number of flowers and the rate of pollinator visits was observed (Caraballo-Ortiz et al. 2011).

Surprisingly, we did not find a difference in insect visitation between male and female *L. sisymbrioides* plants, in contrast to studies that found that plant sex in dioecious species affects visitation rates (Delph & Lively 1992; Olsen 1996; Koski et al. 2018). Our finding may reflect the complementary rewards sought by bees and flies, which were both important flower visitors. Dipteran floral visitors primarily seek nectar, collecting pollen accidentally, whereas bees are attracted to both, and other taxa predominantly seek pollen (Cane 2016; Zhu et al. 2017; Villa et al. 2019). These behavioural differences can impact pollination if, for example, insects remove large amounts of pollen from the male plants in dioecious plants, but never visit a female plant (Koski et al. 2018). In dioecious species, male flowers often allocate more energy to produce either more nectar or more or larger flowers than female plants (Kay et al. 1984; Waelti et al. 2009). These differences in the degree of attractiveness and amount of floral reward have been shown to affect the pattern of visitation by pollinators, with pollinators typically preferring the more rewarding sex, which is usually males (Delph & Lively 1992; Olsen 1996; Koski et al. 2018). Hence, it is surprising that *L. sisymbrioides* sex did not influence the visitation rates of insects, though there was a trend of higher visitation in male plants, so perhaps the relatively small sample size across seven observation days may have limited our ability to detect a significant difference between the sexes. However, the difference in attractiveness is likely minimal in *L. sisymbrioides*, as flowers are small and petals are commonly absent in both sexes. Where present in male flowers, petals are 1.5 mm long, while female flowers lack petals entirely. Nectar rewards are likely to be the same, as both sexes are described as having nectariferous floral glands, and stamens in female flowers abort, so female flowers would not offer a pollen reward (Allan 1961).

Limitations

Several limitations in the community sampling experiment need to be considered. There were some inconsistencies in plant observations: some plants were overlooked, or observations were cut short due to bad weather. The experiment aimed to observe each study plant over its entire flowering period. However, *Lepidium sisymbrioides* had started flowering at

the end of October before observations began, meaning some insects might have been missed. Night observations were attempted at both sites, with only two insects observed despite weather conditions on these nights being favourable. Future studies should attempt more night observations to check for night pollinators given that moths are suspected to visit other open access white flowered species in New Zealand (Buxton et al. 2018). Finally, although over 200 insects were observed, the capture rate was much lower. While best efforts were made to capture all visitors of the plants in this study, not all were caught. This may have introduced a capture bias, though we do not feel that any particular taxa were more likely to evade capture than others.

The single-visit pollinator experiments were limited by weather and plant number. The number of female *L. sisymbrioides* plants suitable for observation (those with two or more stalks) was lower than originally expected, and less data was gathered. Also, the female *L. sisymbrioides* finished flowering earlier than the male plants, which means there was a shorter observation period available for these experiments.

Conclusions

Pollination does not appear to be a limiting factor for *G. calcis* or *L. sisymbrioides,* given the diversity and number of insects visiting flowers and carrying pollen. Further, both species produce large amounts of viable seeds (Tom Waterhouse, Department of Conservation, pers. comm.). In contrast, our data shows that pollination of *C. hollowayi* is limited and only performed by one species of native bee. This likely explains low seed production and poor viability of seeds (Tom Waterhouse, Department of Conservation, pers. comm.). *Carmichaelia hollowayi* is already at a disadvantage due to its small population size, but its flower morphology probably limits effective pollination to native bees. Any declines in their specialist pollinators would further put this species at risk. Conservation efforts should thus focus on testing pollination limitation by trialling hand-pollination between plants, recreating bee habitat and ex-situ propagation of plants for translocation back into the wild. This would increase the potential for cross-pollination, as wild plants may at present be too far apart to allow genetic mixing.

The introduced flowering species at the study sites could be beneficial for maintaining populations of pollinators, but also detrimental to the pollination of the less common native species due to competition for visits and stigma clogging. Introduced Asteraceae are often visited by New Zealand native bees and flies, which can then deposit large amounts of such pollen on the stigmas of native species (Miller et al. 2018). The abundance of pollen from introduced Asteraceae on insects visiting *G. calcis* is the most concerning as stigma clogging could reduce seed production.

Limestone outcrops are uncommon ecosystems that play a significant role in New Zealand's biodiversity. The findings of this study contribute to increasing the understanding of plant-pollinator interactions and help build a better management plan for future conservation.

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Additional information and declarations

Author contributions: SM, CL, JL, and SJ conceptualised the research and method development; SM undertook field and lab work; SJ, CL and JL provided supervision; SM and SJ undertook the analysis; all authors were involved in writing, reviewing, and editing.

Data and code availability: All data is available at https://doi.org/10.17605/OSF.IO/7V8AP

Ethics: This research was conducted in collaboration with the New Zealand Department of Conservation.

Conflicts of interest: The authors declare no conflicts of interest.

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Supplementary material

Additional supporting information may be found in the supplementary material file for this article:

Appendix S1. Supplementary methods.

Appendix S2. Supplemental data.

Appendix S3. Supplemental library.

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