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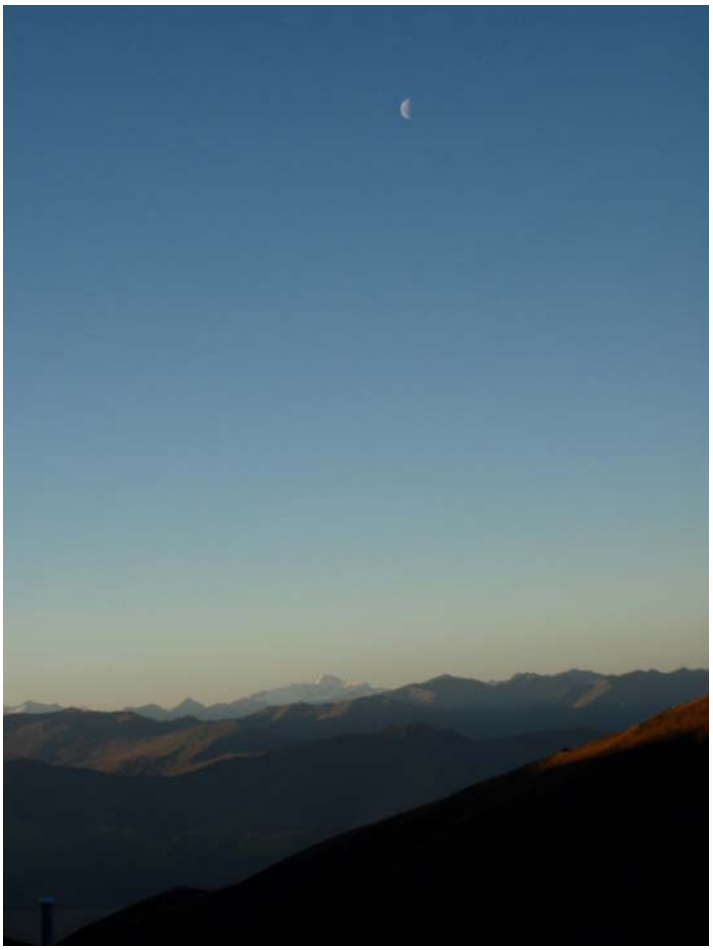
Pollination ecology of the New Zealand alpine flora



Referees: Prof. Dr. Claudia Erbar

Prof. Dr. Peter Leins

Your head is humming,
and it won't go, in case you don't know.
The piper's calling you to join him.

(Led Zeppelin 1971)

All data in the presented thesis was collected by myself. The plant phenology data was collected with Ms. Lisa R. Dobbie. The modelling in bee colour space was carried out in collaboration with Dr. Adrian Dyer, Monash University, Australia. The identification of floral scent compounds was done under supervisison of Dr. Andreas Jürgens, University of KwaZulu-Natal, South Africa. Photographs were taken by myself, Frank Wellenreuther and Dr. Alastair W. Robertson.

Table of contents

Index of figures

Index of tables

1 Summary

1.1 English summary

The interactions between flowers and the insects that pollinate them have fascinated scientists for more than 200 years. The last century saw the establishment of the fundamental concept of pollination syndromes which allows classification of flowers according to the agents that pollinate them demonstrating specialisation and co-evolution of plants and pollinators. This concept has recently been questioned and the contrary, ubiquitous generalisation and chance have been proposed to be the driving forces behind plant – pollinator interactions on an individual and community level. The present study was carried out to address the question of the level of pollinator dependence and generalisation in pollination systems in an alpine plant community in alpine New Zealand. Initial research in New Zealand alpine habitats had lead to the assumption of minor importance of insect pollination as the alpine flora in New Zealand in general is not very conspicuous and the available potential insect pollinators are mainly flies and short-tongued native bees. Therefore it had been proposed that the level of autogamy and generalisation in pollination interactions in a high-alpine habitat should be high.

However, it could be demonstrated that the majority of the 23 plant species in the alpine community depend on pollinator service to achieve reproductive success. A total of 87% of plant species under investigation are at least in part self-incompatible and therefore rely on pollinator service for outcross-pollen delivery. Moreover, it could be shown that the pollinators that transfer pollen do not choose plants at random. The pollination systems in the alpine community proved to consist of both rather specialised and rather generalised functional pollinator groups, moths and native bees belonging to the former and syrphid flies belonging to the latter. Furthermore, there was strong evidence that flower visitors do not automatically equal pollinators and that pollination efficiency differed between functional groups. When assessing the floral cues, e.g. flower colour and scent that attract a certain functional pollinator group, no clustering of the attractants in correlation with pollinator group could be demonstrated. However, the individual combination of colour and scent rendered each plant species distinct from most others. This novel feature of the alpine plant community may be interpreted as a way to facilitate associative pollinator learning. A foraging pollinator can easily memorisemdistinct flowers and subsequently proceed to direct visitation to repeat the experience of rewards. This way flower constancy and increased efficiency of pollen transfer is promoted allowing plants to benefit from adequate pollen delivery and xenogamous reproduction resulting in genetically diverse progeny that has a greater potential of survival in the challenging alpine environment.

1.2 Deutsche Zusammenfassung

Die Beziehung zwischen Blüten und Insekten, welche jene zur Bestäubung besuchen, fasziniert die Wissenschaft seit über 200 Jahren. Im letzten Jahrhundert wurde das fundamentale Konzept der Bestäubungssyndrome eingeführt, welches die Einteilung von Blüten nach ihrem jeweiligen Bestäubungsmechanismus erlaubt. Bestäubungssyndrome gelten allgemein als ein Beispiel von Spezialisierung und Coevolution in einer mutualistischen Beziehung. In jüngster Zeit wurde das Konzept der Bestäubungssyndrome jedoch in Frage gestellt. Stattdessen wurden „allgemeine Generalisierung“ und Zufall als Ursachen von Pflanze-Bestäuber-Interaktionen angenommen.

Die vorliegende Arbeit beschäftigt sich mit dem Grad der grundsätzlichen Abhängigkeit von Insektenbestäubung und dem Ausmaß an Generalisierung in Bestäubungssystemen in einer alpinen Pflanzengemeinschaft in Neuseeland. In frühen Untersuchungen der neuseeländischen Alpenflora wurde der Insektenbestäubung nur eine untergeordnete Rolle beigemessen, da angenommen wurde, dass die Blüten alpiner Pflanzen in Neuseeland grundsätzlich unscheinbar seien und die vorhandenen potentiellen Bestäuber zudem in erster Linie zu den Fliegen oder einheimischen Bienen zählen, welche wiederum als ineffektive Bestäuber gelten. Aus diesem Grund wurde vemutet, dass sowohl der Anteil an autogamer Reproduktion der Pflanzen als auch das Ausmaß der Generalisierung des Bestäubungssystems hoch sind.

In der vorliegenden Arbeit konnte gezeigt werden, dass der Reproduktionserfolg der Mehrheit der 23 Pflanzenarten der alpinen Pflanzengesellschaft sehr wohl vom Bestäuberservice der Insekten abhängig ist. Eine Mehrheit von 87% der Pflanzenarten ist zumindest zum Teil selbstinkompatibel und benötigt Insekten für den Pollentransport. Darüber hinaus ergab eine Untersuchung der Interaktionssysteme, dass die Bestäuber die Blüten nicht zufällig aufsuchen. Die Bestäubungssysteme in der alpinen Pflanzengesellschaft beinhalten sowohl Spezialisten als auch Generalisten, wobei erstere unter den Motten und einheimischen Bienen und letztere besonders unter den Schwebfliegen zu finden sind. Außerdem wurde deutlich, dass Blütenbesucher nicht automatisch auch Bestäuber sind und dass die Effizienz der Pollenübertragung von der funktionellen Gruppe des Bestäubers abhängt.

Die Untersuchung der Blütenmerkmale Farbe und Duft, die grundsätzlich mit einer funktionellen Bestäubergruppe korrelieren können, ließ keine Einteilung in Gruppen ähnlicher Blüten in Abhängigkeit von der vorherrschenden Bestäubergruppe zu. Es wurde jedoch deutlich, dass die Blüten der jeweiligen Pflanzenarten durch die individuelle Kombination von Blütenfarbe und -duft klar voneinander abgegrenzt werden können. Dieser

ungewöhnliche Umstand wird im Zusammenhang mit der Förderung von assoziativem Lernen der jeweiligen Bestäuber interpretiert**:** Einem bestäubenden Insekt wird das Lernen von bestimmten Blütentypen erleichtert, wenn sich die Blüten in der Gesellschaft stark unterscheiden. Auf diese Art wird die Blütenstetigkeit unter den Bestäubern gefördert und somit gleichzeitig die Effizienz der Bestäubung gesteigert. Dies erlaubt den Pflanzen der alpinen Pflanzengesellschaft in Neuseeland von ausreichender Pollenübertragung und xenogamer Reproduktion zu profitieren. Diese wiederum führt zu einer erhöhten genetischen Vielfalt unter den Nachkommen und erleichtert somit das Überleben der Art unter den harschen klimatischen Bedingungen des alpinen Habitats.

2 Introduction

2.1 The principles of pollination ecology – then and now

> *"There is a grandeur in this view of life, with its several powers, having been originally breathed by the Creator into a few forms or one, and that, whilst this planet has gone cycling on according to the fixed law of gravity, from so simple a beginning endless forms most beautiful and most wonderful have been, and are being evolved."*
>
> (Darwin 1859)

The relationship between angiosperms and pollinating insects is one that goes back into the middle Cretaceous during the Mesozoic, when the first flowering plants occurred and with them their early primitive beetle pollinators (Diels 1916, Grant 1950, but see also Gottsberger 1974). Apparently insect pollination among other innovations was a great success (Mulcahy 1979) and the rise of the angiosperms during the early Palaeocene and their continuous radiation until today took place simultaneously to an excessive expansion and radiation among pollinating insects (Burger 1981, Crane *et al*. 1995).

Relationships between plants and their pollinators were well established by the time scientists began to notice and comment on them. The earliest record of the recognition that insects may play a crucial role in the reproductive success of plants was published by the German naturalist Joseph Gottlieb Kölreuter in 1761. Influenced by the studies of Camerarius who had demonstrated the existence of sexual reproduction in plants in 1691, Kölreuter proceeded to define three alternative ways how plants might achieve pollination, i.e. the transfer of pollen to the stigma. He distinguished autogamy, i.e. self-pollination („*Ohne fremde oder äußerliche Beyhülfe, ganz allein*“), anemophily, i.e. wind pollination („*Durch den Wind...*“) and anthophily, i.e. pollination by insect visitors foraging for nectar („*Durch Insekten beim Nektarsaugen an den Blüten...*“). By 1793 Christian Konrad Sprengel had published his classic work on the structure and fertilisation of flowers. He described many features of plants in the context of insect pollination such as the function of nectaries, flower colours and odours and many more. Sprengel’s conclusions were based on extensive observation of a large number of plant species and his explanations and definitions are remarkably accurate. He also introduced an early system of categorisation of flower classes which would ultimately evolve into the concept of pollination syndromes. However, the scientific investigation of pollination proceeded to the next level when Charles Darwin entered the stage and convincingly introduced the concept of natural selection and adaptation as a result (1859). Darwin effectively placed the study of pollination in its modern evolutionary framework (Waser

2006). While Darwin's studies were essentially concentrated on plants and description of pollinators plays a minor role, the Italian botanist Federico Delpino made an effort to describe pollinators (1868-1875) and developed two general schemes for the classification of flowers, one of them grouping flowers according to the agent of pollination that they show morphological adaptations to. Vogel (1954) went on to propose six floral styles that can be seen as the direct precursor of the seminal work by Faegri and van der Pijl (1966) who attempted to "formulate the general principle of pollination ecology, applicable anywhere." They finally introduced the term "pollination syndrome" which has become a standard concept and a household name in pollination ecology.

Pollination syndromes are defined as a suite of floral traits, such as flower size, shape and colour, and rewards such as pollen or nectar, that are an adaptation to a taxonomic order or functional group of animals, and in return by those animals to a particular group of flowers that may or may not have a close phylogenetic relationship to each other. This concept has been very popular over the past 50 years and it has been widely applied in the study of pollination ecology. An overwhelming number of flower features has been investigated in the context of pollination syndromes interpreting all kinds of traits with increasing detail as evidence of co-evolution. A basic principle underlying this conclusion is the assumption of a mutualistic relationship between flowers and their pollinators that becomes more and more effective as plant and pollinator become increasingly adapted to each other (Stebbins 1970, Gilbert and Raven 1975, Crepet 1983, 1984).

A mutualism is defined as an interaction that is beneficial to both participants. The majority of angiosperms rely in whole or part on animals for pollination (Nabhan and Buchmann 1997, Renner 1998) so there is a clear benefit from the plants' point of view. Pollinators in return receive food, shelter and nesting material among other benefits (Leins and Erbar 2008) in a true mutualistic situation. There is also considerable evidence of deceit from both participants of the interaction (Leins and Erbar 2008). However, a mutualistic relationship does by no means imply cooperation (Waser and Price 1983, Howe 1984, Westerkamp 1997). Animals do not deliberately pollinate flowers. They perceive them first and foremost as sources of food or other needed resources, and while foraging for rewards offered they inadvertently contact the reproductive organs of flowers. This causes the transfer of pollen to the pollinators body which is turn is being transferred onto the stigmatic surface of the same or the next flower resulting in self- or cross-pollination, respectively. It is a generally accepted principle that Darwin (1862) was the first to demonstrate that self-pollination is not optimal for genetic recombination and may decrease the genetic diversity and fitness of a population. Therefore a

great many plants have adaptations which maximise their potential for cross-pollination (Leins and Erbar 2008).

The first step on the way to cross-pollination is the attraction of pollinators unless abiotic agents like wind or water serve the purpose of pollen transfer. Flowers advertise on the pollinator market in order to encourage visits using honest and sometimes dishonest signals to achieve visitation. Within the scope of the mutualistic relationship, plants and animals have rather different objectives. A plant can maximise its reproductive fitness by maximum pollen dispersal between individuals of the same species. At the same time it may need to avoid stigma contamination with heterospecific pollen deposition and will benefit from minimising the resources invested in pollinator rewards. So an ideal pollinator would visit many flowers of the same species in rapid succession, effectively transfer pollen and exhibit floral constancy while not using or wasting any pollen and ideally no other reward as well (Glover 2007). On the other hand, attractive plants for pollinators are defined by large and easily accessible rewards so that the pollinator can conserve energy. Moreover, a flower visitor should switch between flowers of many species if they are spatially close and offer equally attractive rewards. Clearly, the interests of plants and pollinators diverge. Therefore highly specialised interactions on the species-species level are very rare, since they are also very prone to disruption with potentially catastrophic consequences, e.g. when a plant looses its specialist pollinator (Terry *et al.* 2005). In an evolutionary context plant – pollinator mutualism might have most likely followed a middle road between extreme specialisation, i.e. the exclusive interaction of one plant species with a single pollinator species, and extreme generalisation where interaction between plants and pollinators occur entirely on a random basis. The concept of pollination syndromes resides in this middle ground predicting the co-evolution and adaptation between plants and pollinator guilds, i.e. groups of similar flowers with groups of insects with similar morphology, behaviour and demands. Although this concept is intrinsically appealing due to the order it proposes, the idea that plants and animals continue to co-evolve heading for increasingly close matching has evoked criticism in more recent times.

Although the existence and applicability of pollination syndromes had been questioned early on (Robertson 1928, van Steenis 1980) a heated debate was initiated when Waser *et al.* (1996) among others (Herrera 1996, Ollerton 1996) published their view that generalisation dominates in pollination systems as a rule. In an empirical approach they evaluated a large number of data sets of pollination systems and concluded that in the vast majority of cases, plants were visited by a large number of insect species, often from multiple genera or orders.

Thereupon, Fenster *et al*. (2004) retorted by reassessing the same data of Waser *et al*. with respect to functional pollinator groups rather than taxonomic groups. In conclusion they found specialisation between plants and a functional pollinator guild in about 75% of the cases. Vogel (2006) himself, one of the founders of the concept of pollination syndromes, pointedly reminded that syndromes should not be interpreted as fixed and rigid entities but the culmination of comparative investigation. In the recent literature there are many cases of evidence for the existence of pollination syndromes (Hargreaves 2004, Machado and Lopes 2004, Wilson *et al.* 2004) as well as against it (Herrera 1993, Hingston and McQuillan 2000, Zhang *et al.* 2005, Ollerton 1998, Ollerton *et al.* 2007). However, the most striking question that emerges, and that has not been satisfactorily addressed, is the question of the effectiveness of the observed flower visitors as pollinator (Johnson and Steiner 2000). Most comparative community pollination surveys measure visitation frequency. Thus they equal flower visitors with pollinators and assume equal pollination potential for all visitors. However, these assumptions have not been tested as the amount of pollen delivered by pollinators is usually not quantified in community pollination studies due to the immense amount of work required. The question whether a flower is euphilic (specialised) or allophilic (generalistic) and in turn visited and pollinated by eutropous (specialised) or allotropous (generalistic) pollinators should be investigated in a modular approach where generalisation on the flower visitation level does not automatically equal generalisation on the pollination effectiveness level. A plant may have many visitors, among them several pollinators of whom one or more may be the most effective in terms of conspecific pollen delivery (Schemske and Horvitz 1984). On the other hand, the establishment of such a scenario where one pollinator is mainly responsible for effective pollen delivery while other opportunistic co-visitors (Vogel 2006) fulfil minor pollination duties, if any, may take some time to evolve in an evolutionary context. The level of specialisation may therefore depend on the succession stage of a community.

Most evidence for specialism on the community level derives from studies in saturated communities, e.g. in tropical or subtropical ecosystems with intricate flowers (Vogel 1954, 1981, 1990, van der Pijl and Dodson 1966, Grant and Grant 1968, Kampny 1995, Johnson *et al.* 1998, Goldblatt *et al.* 2000, Perret *et al.* 2001, Weigend and Gottschling 2006). Conversely, a relatively young ecosystem with many open-access flowers should exhibit a high degree of generalisation. The pollinator service in such a system should be unreliable with heterospecific pollen deposition a commonly observed feature since generalist pollinators can be expected to show reduced floral constancy.

My aim in the presented thesis was to investigate a plant community that is predicted to display high levels of generalisation in order to test whether the generalised pollination system can be confirmed when the pollination function and effectiveness of the observed flower visitors is considered. The native flora of New Zealand in general and particularly the alpine assemblages grant interesting examples of supposedly generalised pollination systems with mostly open-access flowers (Newstrom and Robertson 2005). There is evidence that most of the plant families encountered in the recent alpine flora first arrived in New Zealand during the late Tertiary and the New Zealand flora is known as one of comparatively little appeal in terms of spectacular flowers and intricate pollination systems (Mark and Adams 1993). This might explain why virtually no investigation on pollination has been carried out in the New Zealand alpine areas to date.

2.2 Pollination in the New Zealand flora – what do we know?

"We have one very stupid white gentian"
(Butler 1860, cited in Cockayne 1967)

The Flora of New Zealand, an isolated oceanic archipelago in the Southern Hemisphere, is characterised through the predominance of small white or pale, dish or bowl-shaped flowers (Newstrom and Robertson 2005). Pollination in New Zealand has previously been characterised as having low rates of self-incompatibility and a lack of specialised pollination, as well as little pollinator dependence (Godley 1979, Lloyd 1985). These features have been interpreted as supportive of "Baker's Rule" (Baker 1955, 1967), which suggests that long-distance colonisation selects for autogamous breeding systems where plants can self-pollinate to provide reproductive assurance. The prevailing view of New Zealand pollination systems is one of extreme generalisation with extensive pollinator sharing, and unpredictable, imprecise pollinator service that may or may not be effective (Newstrom and Robertson 2005). Several aspects of the breeding systems of the flora have been interpreted in this context (Wallace 1880, Thomson 1927, Heine 1937, Godley 1979, Lloyd 1985, Webb and Kelly 1993). It was suggested that outcrossing was more commonly promoted by sexual dimorphism rather than by self-incompatibility (Baker 1967), that herkogamy (i.e., spatial separation of anthers and stigma) and dichogamy (i.e., temporal separation of male and female function) in outbreeding hermaphrodites were means of separating male and female function to increase the efficiency of otherwise imprecise and unspecialised pollination processes (Lloyd and Webb 1986, Webb

and Lloyd 1986), and, finally, that there was a strong trend towards autogamy (i.e., self-pollination), particularly in alpine herbs (Raven 1973, Wardle 1978). However, New Zealand alpine pollination in terms of the pollination syndrome concept has not been examined.

Potential insect pollinators in New Zealand include all orders usually involved in pollination, but important families are missing and some are extremely low in diversity or abundance compared with other areas of similar size (Godley 1979, Lloyd 1985). The most significant disparity is the complete lack of large social bees, both long- and short-tongued. Indigenous bees are not large (with the biggest about the size of worker honeybees), usually solitary (a few have some social structure) and all short-tongued (Donovan 2007). Bees are the most important pollinators worldwide (Leins and Erbar 2008) because they depend on pollen and nectar for their brood, unlike most other insect groups. Still the flowers in New Zealand alpine habitats evolved largely without their particular influence. Butterflies are also scarce and largely replaced by day-flying moths, another unusual feature of the New Zealand alpine flora. The only published community study on flower visitation so far has been conducted by Primack (1983) in montane and subalpine habitats in the South Island and supports the predominance of rather unspecialised entomophilous pollination. However, given the fact that Primack's survey remains the only comprehensive published record on pollination in alpine New Zealand, it appears remarkably vague in describing the applied method of sampling. Furthermore, the insects included in the data set were not necessarily pollinators after all ("No attempt was made to determine whether an insect was actually carrying pollen on its body", Primack 1983, p. 317). Thus the interactions Primack demonstrates may or may not be pollination events and it is entirely possible that the high level of generalisation evident here exists only on the visitor level. In an earlier publication on montane "pollinator" assemblages Primack (1978) had already concluded that "flowers are visited by whatever pollinators are immediately available".

Pollination systems in alpine New Zealand present an excellent opportunity to test whether a generalised visitation pattern is evidencing a generalised pollination pattern, in other words whether there is no difference in the number of visitors and the number of pollinators of given plant species. The postulated generalisation of the New Zealand system would predict a high degree of heterospecific pollen transfer among plants in an alpine community. Furthermore the prediction that alpine plants in New Zealand are predominantly autogamous remains to be verified. The present thesis was designed to address three fundamental questions in a logic order which will be introduced in the following subchapters.

2.3 Do plants in alpine New Zealand depend on pollinator service at all?

> *"...self-fertilising plants achieve an increase in immediate fitness at the expense of a decreased flexibility."*
>
> (Lloyd 1965)

Every perfect, i.e. hermaphroditic, flower is endowed with the potential to self-pollinate and fertilise its own ovules if no mechanisms to avoid self-pollination and/or self-fertilisation have been developed. Sexual reproduction without the need for another individual or pollen vector may be advantageous under certain circumstances (Glover 2007). Especially pioneer species that regularly colonize new habitats where single propagules may travel considerable distances will benefit from such a scenario of independent reproduction (Baker 1955), a principle also known as Baker's Rule. This principle may be applied to actual island ecosystems surrounded by a large body of water as well as islands in a figurative sense where ecosystems are separated from each other by geographical and climatic barriers.

The alpine flora of New Zealand is an example of both described situations as it occurs on mountain ranges that are in part distinctly separated from each other by river valleys on an island in the South Pacific. There is evidence that most of the plant families encountered in the recent alpine flora first arrived in New Zealand during the late Tertiary undergoing considerable radiation with the recent uplift of the mountain habitats over the last 5-2 million years ago (Winkworth *et al.* 2005). This suggests a young system were self-pollination would certainly be advantageous in terms of reproductive assurance, especially in an alpine ecosystem with severe and highly unpredictable weather patterns on an archipelago which is known for its relatively depauperate pollinator fauna (Newstrom and Robertson 2005). On the other hand xenogamy, i.e. cross-fertilisation, has been demonstrated as the most common sexual system in alpine plants world-wide (Körner 2003). In continental mountain floras, plants commonly increase the display are of their flowers in order to attract pollinators, in some cases sacrificing considerable carbon gain through self-shadowing (Fabbro and Körner 2004, but see also Totland *et al.* 2000). Self-fertilising plants are prone to inbreeding depression, i.e. the reduction of viability and /or fertility of inbred offspring in comparison with those from outcrossed matings (Barrett and Harder 2006). This has been identified as the primary selective pressure resulting in strategies to avoid self-fertilisation wherever possible (Barrett 2002). Inbred plants within a population exhibit a similar genotype and are therefore limited in their ecological amplitude. Alpine plants however should strive for genetic

variability in order to maintain flexibility to meet the challenge of a highly unpredictable environment.

In case of the New Zealand alpine flora there is very little data available regarding the sexual systems of alpine plants. Alpine *Parahebe* (Garnock-Jones 1976) have been shown to be adapted to autogamy while *Ourisia macrocarpa* is self-compatible but predominantly outcrossing (Schlessman 1986). Outbreeding has also been demonstrated for alpine *Ranunculus* (Fisher 1965). On a community level no studies assessing the sexual systems of plants have been carried out.

Several methods have been proposed to predict and subsequently assess aspects of the sexual system of flowering plants. Cruden (1977) introduced the pollen/ovule ratio (P/O ratio) on the assumption that flowers that habitually self-pollinate do not risk substantial pollen loss to vectors and will therefore produce lower amounts of total pollen grains. This results in low P/O ratios whereas predominantly outcrossing species will produce considerably more pollen in relation to ovule number. Thus, P/O ratios can be seen as a measure of the pollination efficiency (Cruden 1977, 2000) and may represent the best indicator of the breeding system (Plitmann and Levin 1990). P/O ratios have been calculated for a large number of species (Erbar and Langlotz 2005) with mixed results regarding the conformity of P/O ratio and breeding system. P/O ratios may be affected by many factors, among them pollination mechanisms, pollen vectors and life form (Small 1986, Cruden 2000, Jürgens *et al.* 2002, Erbar and Langlotz 2005). However, they provide a useful and well-established tool to gain first insights into the reproductive system of a plant.

Erbar and Enghofer (2001) pointed out that besides the P/O ratio the pollen load on the stigma at the end of female anthesis in relation to the number of ovules (P-S/O ratio) provides insight into the pollinator service and the potential for pollen tube competition. Pollen tube competition as a result of ovule enclosure in a carpel and insect pollination delivering pollen to stigmas has been linked to the overwhelming success of the angiosperms (Mulcahy 1979). Pollen tube competition provides the basis for prezygotic selection which acts as an example of the survival of the fittest (Erbar 2003). Therefore the P-S/O ratio can be employed to measure two different aspects of the pollination system: the effectiveness of the pollinator service in terms of pollen limitation, i.e. the reduction in potential seed production when some ovules remain unfertilised due to insufficient pollen delivery as well as the plants' ability to autonomously self-pollinate when pollinators are absent, i.e. experimentally excluded. Although P/O ratio and P-S/O ratio provide valuable insights into the reproductive systems of plant, they can only be indicators of the breeding system. Fruit and seed set represent the

ultimate measure of reproductive success (Glover 2007) and therefore have to be investigated in depth in pollinator exclusion experiments and through experimental hand-pollination. If breeding system experiments reveal a fraction of alpine plants in New Zealand to be pollinator dependent, then the question about the nature of these indispensable pollinators arises.

2.4 Are alpine pollination networks in New Zealand entirely generalised?

> *"My main object, therefore, in drawing up this paper, is to urge on both entomologists and botanists to a closer examination of their collected material from this standpoint of the inter-relation of the two groups."*
>
> (Thomson 1927)

From an ecological point of view, the interactions between plants and their pollinators can be visualised as a network where both sets of interaction partners, the plants and the pollinators, are depicted as nodes. If a pollinator visits the flowers of a plant, this will be indicated by a link connecting them. In a mutualistic relationship both partners benefit from such a link that ultimately sustains their survival as a species. Therefore mutualistic networks have been called the architecture of biodiversity (Bascompte and Jordano 2007).

The number of interactions depicted by links in a network can vary greatly (Waser *et al.* 1996). In principle, species with a low number of interactions are called ecological specialists and species displaying a multitude of interactions are called ecological generalist (Ferry-Graham *et al.* 2002). An interesting discovery regarding the structure of mutualistic networks is the fact that most interactions are asymmetric, e.g. a specialised plant is mostly visited by a few generalist pollinators while specialist pollinators visit mainly a few generalist plants (Petanidou and Ellis 1996, Bascompte *et al.* 2003, Dupont *et al.* 2003, Vázquez and Simberloff 2002, Vázquez and Aizen 2004). This finding appears quite unexpected as it contradicts the intuitive assumptions that generalists will choose other generalists while specialists visit specialists (Vázquez and Aizen 2004 and references therein). This phenomenon has been illustrated with the “nested” structure mutualistic networks appear to have (Bascompte *et al.* 2003, Dupont *et al.* 2003, Ollerton *et al.* 2003). Nested structures reveal themselves if the interaction matrix between plants and animals is arranged according to the number of interactions (Fig 1.1)

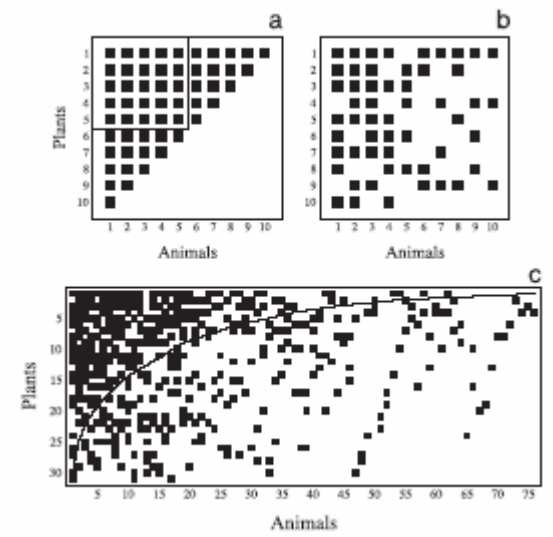


Fig. 2.1 Plant animal mutualistic interaction matrices (Bascompte *et al.* 2003, modified). Numbers on axis label plant and animal species which a arranged according to number of interaction in decreasing order. Black squares depict an observed interaction. (a) perfectly nested, (b) random, (c) realistic mutualistic network distribution.

A random structure would be the opposite of a nested structure, also called a checkerboard pattern. In a network where all interactions were entirely the product of chance, the interaction matrix would look like a checkerboard. Nestedness of the interaction patterns has been proposed to be related to species abundance (Dupont *et al.* 2003, Vázquez and Aizen 2004, Vázquez 2005). These authors have argued that abundant visitor species visit many plant individuals and, because they choose plant individuals randomly, many plant species. As a result, rare plant species are visited by few individuals and thus by few visitor species that are most likely ecologically generalised. Nestedness is explained by random choices skewed by species abundance. However, Stang *et al.* (2006) have shown that morphological constraints restrict choices and visitors therefore do not visit flowers at random.

The pollination system in alpine New Zealand is thought to be extremely generalised to random (Primack 1978, 1983). However, due to reasons discussed above the evidence for such a conclusion is not convincing. Therefore the plant-pollinator network patterns in a community of the New Zealand alpine flora will be re-assessed with modern methods. If a nested structure can be demonstrated, a certain level of specialisation in the community can expected. In case of a significantly nested interaction pattern, visitors will not choose plants at random but will visit certain species in a directed manner. Some visitor species are expected to be allophilic while others may be euphilic. If a nested pattern can be demonstrated, flower visitor must be able to discriminate between flowers of different species. The cues they might employ while foraging is the third question I am going to address.

2.5 Which floral traits maintain interaction patterns between flowers and insect visitors?

> *"Flowers rank amongst the most beautiful productions of nature¸ but they have been rendered conspicuous on contrast with green leaves, and in consequence at the same time beautiful, so that they may be easily observed by insects"*
>
> (Darwin 1859)

If non-random interaction patterns between plants and their pollinators occur, pollinators must by definition be able to make informed choices before visiting a flower. The main attractants that characterise and advertise a flower are floral shape, colour and scent. Pollination systems have been described as biological markets in which animals choose between 'products' (flower species) on the basis of quality (e.g. nectar and pollen quality and quantity), and in which plants might compete for 'customers' (pollinators) (Chittka and Schürkens 2001). Plants will benefit from reliable pollinator service that is more likely to be provided if pollinators express floral constancy. The sensory system of a pollinator will determine which signals are effective to promote memorisation of a certain flower attribute. There is evidence that relatively subtle changes in floral characters, sometimes produced by a single mutation, can substantially affect pollinator behaviour (Waser and Price 1985, Comba *et al.* 2000).

Plants possess a multitude of chemical pathways to colour all parts of their display units (Leins and Erbar 2008). Furthermore is has been demonstrated that colour patterns on petals mimic parts of the androeceum within a flower acting as signals, e.g. for visitors foraging for pollen (Osche 1979, 1983, Lunau 2007 and references therein). The flowers of angiosperm plants in general present us with an overwhelming diversity of colour signal designs, however, the flora of New Zealand, especially in alpine areas is essentially white or pale with few exceptions (Mark and Adams 1993, Körner 2003). On the other hand, on a community level white, UV-absorbing flowers are the most common in practically all temperate European and Mediterranean habitats (Chittka *et al.* 2004). It has been proposed that flowers in the New Zealand alpine zone lack colour because it may be a disadvantage in attracting available pollinators as they are not adapted to bright colours and that floral scent may be a more effective lure (Mark and Adams 1993). However, the extent to which alpine flowers in New Zealand do differ in colour in relation to pollinator colour perception and ability to distinguish them from the ambient background and flowers of different species from each other has never been quantified. Although flowers in New Zealand alpine areas may look rather inconspicuous to the human observer, they may still be highly visible and attractive to insect

pollinator, especially if floral display includes UV-signals that some or even the majority of insects can perceive (Briscoe and Chittka 2001).

While pollinator vision in relation to flower colour has received considerable attention from the beginnings of flower visitor observations, the role of floral scent as an attractant or repellent in context with pollination syndromes or generalised and specialised interactions has rarely been considered in an integrated approach (Raguso 2008). However, the emission of floral volatiles is one of the important key features of angiosperm flowers for attracting pollinators (Dobson 2006). The composition of floral odours is often very complex with sometimes more than 50 or more chemical compounds in a single flower (Knudsen *et al.* 2006). The floral odour patterns of single species, or species with the same pollinator type, are often interpreted in relation to the olfactory preferences of the pollinating agents. Pattern analysis of floral bouquets revealed similarities in the floral odour composition of plants with similar types of pollinators (e.g. Ollerton and Raguso 2006), independent of the phylogenetic relatedness of the plant species. Similar odour compositions have for example been found in plants adapted to bats (Knudsen and Tollsten 1995, Bestmann *et al.* 1997), flies (Kite and Hetterscheid 1997, Jürgens *et al.* 2006), beetles (Thien *et al.* 1975,Yasukawa *et al.* 1992, Jürgens *et al.* 2000), and moths (Knudsen and Tollsten 1993, Raguso and Pichersky 1995, Miyake *et al.* 1998).

Despite the growing number of floral scent analyses that support the idea of floral scent being an important component of pollination syndromes, there is no information on the odour diversity on the community level, either in New Zealand or world-wide. The three question related to the analysis of odour composition on the community level are: (a) How high is the overlap in the chemical composition of the floral volatiles within plant communities, (b) do we find similar odour patterns in plants visited and pollinated by the same functional flower visitor groups, (c) or alternatively, show plants with similar pollination syndromes distinct odour patterns to attract pollinators with specific signals that can then be learned by them?

The community analysis of the constitution of the main attractants flower colour and scent will provide exciting insight into the underlying mechanisms that allow insects in the alpine community discrimination and choice of certain flowers over others.

In summary, this thesis aims to investigate an alpine plant community with respect to its flower visitor assemblage, pollinator dependence of reproductive success, the level of generalisation in existing pollination systems and the attractants that may allow pollinators the perception and potential discrimination of flowers of different plant species in the community.

3 Materials and methods

3.1 Study area

All experiments were conducted in the valley below Lake Alta at Rastus Burn Recreation Reserve within the Remarkables Ecological District, Otago Conservancy, New Zealand (Fig. 3.1). Lake Alta (45º03.761' S, 168º 48.823' E at 1800m a.s.l.) is located at the northern end of the Remarkables mountain range.

The Remarkables Ecological District in general is characterised by extremely steep, rugged, strongly glaciated North-South trending schist mountains up to an altitude of 2343 m a.s.l. in the rain shadow of the Main Divide. It mainly consists of strong steepland soils, bare rock, scree and deeper loess derived soils on easier slopes. The main vegetation forms in the alpine zone above the tussock grassland are fellfield, herbfield, cushion and snowbank communities (Mark and Bliss 1970). Experiments were carried out in the Rastus Burn Basin in altitudes between 1650 m and 1800 m a.s.l (Fig 3.1).

The experimental area can be divided into three sections: the lower plateau at about 1650 m a.s.l., the middle plateau at about 1750 m a.s.l. and the lake shore at around 1800 m a.s.l (Fig. 3.2). The lower part is dominated by a bog community where *Donatia*, *Phyllachne* and *Dracophyllum* are prominent genera (Mark and Adams 1993), the middle part can be described as an alpine herbfield with key genera including *Celmisia*, *Ranunculus*, *Anisotome*, *Gingidum* and *Ourisia* (Mark and Adams 1993), and the lake shore which is a combination of rocky alpine fellfield and herbfield on the southern slope dominated by genera such as *Celmisia*, *Ranunculus*, *Dolichoglottis*, *Gentianella*, *Aciphylla*, *Chionohebe* and *Hectorella* (Mark and Adams 1993). The whole area is generally snow-free between the summer months of December to late March.

The experimental area is part of a ski field that has operated during the winter months (June to October) since 1984. It is also popular with walkers during the summer months. There are concerns about the impact of continued development and damage by snow grooming and related activities (Fahey and Wardle 1998). However, despite some apparent damage especially to the wetland communities (e.g. Ski groomer track marks), the study area represents a largely intact alpine community and was therefore considered adequate as an experimental site.

Fig. 3.1 The experimental site (a) in July during the ski season and (b) in February during the field season.

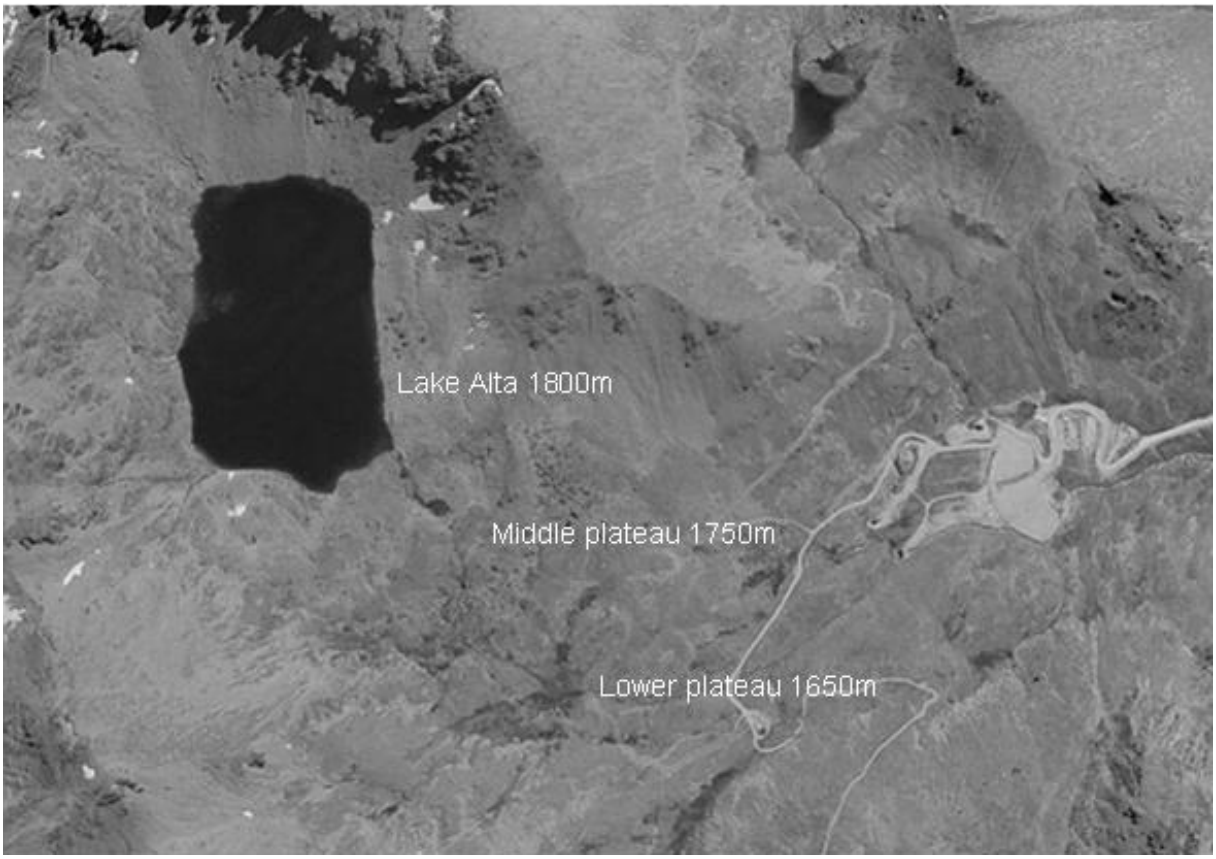


Fig. 3.2 Map of the experimental area in the Remarkables ski field area.

3.2 Study plants

A total of 23 plant species was included in the community analysis. Among those were members of the families of Asteraceae (6), Campanulaceae (1), Ericaceae (2), Gentianaceae (1), Lobeliaceae (1), Onagraceae (1), Orobanchaceae (1), Portulacaceae (1), Plantaginaceae (4), Ranunculaceae (2), Stylidiaceae (1), Thymelaeaceae (1) and Violaceae (1). Plant species were chosen with respect to abundance and accessibility. In Table 3.1 they are introduced with taxonomic classification and abbreviated names that will be used for simplification. A detailed description of each plant species can be found in Appendix 7.1. An attempt was made to subject all species to all experiments presented in this thesis. However, due to the very different morphology and considerable fluctuation in flowering patterns between the seasons the data set remains incomplete for some species. An overview of all experimental data obtained for the 23 species of the alpine community is presented in Chapter 3: Results. Voucher specimens for all plant species were collected and will be stored in the Otago Herbarium Collection, Botany Department, University of Otago, New Zealand.

Table 3.1 Species of the alpine plant community at Remarkables Ski Field, listed with taxonomic affiliations, abbreviations used for simplification in graphs and throughout text, blossom class (Faegri and van der Pijl 1979) and mode of access (Endress 1994). (Identification of plants after Mark and Adams 1993. Taxonomical nomenclature: http://nzflora.landcareresearch.co.nz/)

Species	Family	Abbreviation	Blossom	Access
Anaphalioides bellidioides (G.Forst.) Glenny (1997)	Asteraceae	*A. bellidioides* (AB)	dish/ bowl	open
Brachyglottis bellidioides (Hook.f.) B.Nord. (1978)	Asteraceae	*B. bellidioides* (BB)	dish/ bowl	open
Brachyscome sinclairii Hook.f. (1864)	Asteraceae	*B. sinclarrii* (BS)	dish/ bowl	open
Celmisia sessiliflora Hook.f. (1864)	Asteraceae	*C. sessilifolia* (CS)	dish/ bowl	open
Chionohebe densifolia (F.Muell.) B.G.Briggs and Ehrend. (1976)	Plantaginaceae	*C. densifolia* (CD)	gullet	directed
Chionohebe thomsonii (Buchanan) B.G.Briggs and Ehrend. (1976)	Plantaginaceae	*C. thomsonii* (CT)	gullet	directed
Craspedia lanata (Hook.f.) Allan (1961)	Asteraceae	*C. lanata* (CL)	dish/ bowl	open
Dolichoglottis lyallii (Hook.f.) B.Nord. (1978)	Asteraceae	*D. lyallii* (DL)	dish	open
Dracophyllum muscoides Hook.f.	Ericaceae	*D. muscoides* (DM)	bowl	open
Epilobium porphyrium G. Simpson (1945)	Onagraceae	*E. porphyrium* (EP)	dish/bowl	open
Euphrasia zelandica Wettst. (1896)	Orobanchaceae	*E. zelandica* (EZ)	gullet	directed
Gaultheria nubicola D.J.Middleton (1990)	Ericaceae	*G. nubicola* (GN)	bowl	open
Gentianella corymbifera (Kirk) Holub (1968)	Gentianaceae	*G. corymbifera* (GC)	dish/ bowl	open
Lobelia glaberrima Heenan (2008)	Lobeliaceae	*L. glaberrima* (LG)	gullet	directed
Montia sessiliflora (G. Simpson) Heenan (2007)	Portulacaceae	*M. sessiliflora* (MS)	dish/ bowl	open
Ourisia caespitosa Hook.f. (1853)	Plantaginaceae	*O. caespitosa* (OC)	gullet	directed
Ourisia glandulosa Hook.f. (1864)	Plantaginaceae	*O. glandulosa* (OG)	gullet	directed
Phyllachne colensoi (Hook.f.) Berggr.	Stylidiaceae	*P. colensoi* (PC)	bowl	open
Pimelea oreophila C.J.Burrows	Thymelaeaceae	*P. oreophila* (POr)	dish/bowl	open
Psychrophila obtusa (Cheeseman) W.A.Weber (1982)	Ranunculaceae	*P. obtusa* (POb)	dish/ bowl	open
Ranunculus gracilipes Hook.f. (1864)	Ranunculaceae	*R. gracilipes* (RG)	dish/ bowl	open
Viola cunninghamii Hook.f. (1852)	Violaceae	*V. cunninghamii* (VC)	tube	directed
Wahlenbergia albomarginata Hook. (1852)	Campanulaceae	*W. albomarginata* (WA)	bell	directed

3.3 Laboratory equipment

All preparations were carried out under a dissecting microscope (SZ 40 Olympus) with Dumont Inox Swiss jewellers' forceps (E120 5). All analytical work was carried out under a compound microscope (CH-2 Olympus) with a 10-fold lens (E A10 0.25 160/-) or a 40-fold lens (E A40 0.65 160/0.17). All pictures were taken under a compound microscope (BH-2 Olympus) with a mounted camera (Nikon E995).

3.4 Assessing the reproductive system

3.4.1 Pollen/ovule ratios

The pollen/ovule ratio after Cruden (1977) as an indicator of a plant's reproductive system is calculated from the total number of pollen grains per flower divided by the number ovules produced by the same flower. Over the course of the field season from November 2007 to March 2008 25 well-developed buds that were not yet releasing pollen were collected per species and stored in 70% EtOH. Ten mature buds were used for pollen and ovule counting resulting in a total of ten replicates per species. For the gynodioecious *Phyllachne colensoi* ovules of female and hermaphrodite flowers were quantified. For the Asteraceae five inflorescences were randomly chosen and pollen grains of five florets from each inflorescence were counted resulting in a total of five replicate plants each with five florets counted per plant. The number of female florets was also calculated and factored into the final P/O ratio.
Preparation for ovule counting was carried out under the dissecting microscope in a drop of 70% EtOH. All ovules were considered fertile (Cruden 1977). Preparation for pollen counting varied according to species. The anthers of most species were dissected under the dissecting microscope in 0.7 M mannitol solution in volumes between 50 and 300 µl. For the Asteraceae a solution of 0.7 M mannitol and 70% EtOH (1:1) was utilised to dissolve pollenkitt. The number of pollen grains was counted using a haemocytometer under the compound microscope counting all nine squares. The average count from these squares was extrapolated by taking in account the proportion of suspension counted to obtain an estimate of the number of pollen grains per flower and then this count was divided by the number of ovules to calculate the pollen/ovule ratio. *Epilobium porphyrium* (Onagraceae) and *Montia sessiliflora* (Portulacaceae) pollen numbers were counted directly under the dissecting microscope without the haemocytometer as pollen numbers were very low. Then, assuming that all pollen grains are viable, pollen numbers per flower were divided by the number of ovules of the

same flower to calculate the pollen/ovule ratio. For the dioecious species *Pimelea oreophila* the P/O ratio was calculated assuming a sex ratio of female and male flowers of 1:1. For the gynodioecious species *Phyllachne colensoi* the pollen number of hermaphrodite flowers were used to calculate P/O of hermaphrodite flowers as well as of female flowers assuming a ratio of 1:1. P/O ratios were averaged per species and the 23 species of the community were then classified after Cruden (1977) (Table 3.2).

Table 3.2 Classification of reproductive system classes of plants depending on the pollen/ ovule ratio after Cruden (1977).

Reproductive system	P/O ratio ± SE
Cleistogamy	4.7 ± 0.7
Obligate autogamy	27.7 ± 3.1
Facultative autogamy	168.5 ± 22.1
Facultative xenogamy	796.6 ± 87.7
Xenogamy	5859.2 ± 936.5

3.4.2 Pollen on stigma/ovule ratios under natural and experimental conditions

The numbers of conspecific pollen grains that are being deposited on a receptive stigma reflect the effectiveness of the pollinator service, i.e. the transfer of self and/ or outcross pollen, in a community as well as the plant's ability to autonomously self-pollinate, i.e. to transfer pollen from the anthers to the stigma within the same flower without any interference of a vector. Comparing pollen transfer to stigmas under open conditions and under pollinator exclosure serves as a measure of the dependency of a flower on pollinator service. Furthermore, in comparison with ovule numbers needing fertilisation pollen deposition under natural conditions gives evidence of pollen limitation or pollen competition. Assuming transfer of viable and compatible pollen grains, the potential for pollen competition arises if the Pollen on stigma/ovule ratio (P-S/O ratio) is greater than one, and every ovule receives more than one pollen grain for fertilisation (Erbar and Enghofer 2001). This situation is beneficial for the maternal plant as only the fittest male gametophytes will be able to fertilise the available ovules. However, if the P-S/O ratio is below one, the plant is pollen-limited in the number of seeds that can be fertilised.

In order to quantify the number and composition of deposited pollen grains under natural conditions and under pollinator exclosure the following experiment was carried: 24 unopened

ripe buds of a species were labelled with coloured wire and placed under mesh bags to exclude insect visitation. Subsequently 12 mesh bags were removed when flowers entered the female stage and the stigmas became receptive. Receptivity data was obtained from Dobbie (unpublished M.Sc. thesis) The 12 receptive virgin stigmas were exposed to insect visitation for 48 hours while the other 12 receptive stigmas remained under bags. Thereafter the 12 enclosed stigmas and the 12 stigmas subject to potential pollinator visitation were collected. In order to count pollen grains deposited on the stigma the 24 flowers were dissected and their stigmas excised with forceps avoiding additional contamination with pollen from their own anthers. Each stigma was then stained with a solution of methylene green-phloxine B (1% in 50% EtOH respectively) in a ratio of 1:3 (Dafni *et al.* 2005, p. 142) for five minutes. Thereafter each stigma was transferred to a clean microscope slide into a drop of glycerine, covered with a cover slip and gently squashed. To seal the slide the edges of the cover slip with the specimen were sealed with clear nail polish. When the nail polish had set rendering a semi-permanent slide the stigma was examined under the compound microscope determining the type and number of pollen grains deposited. The pollen loads of visited and enclosed stigmas in relation to mean ovule number were statistically compared in order to assess the pollinator service under natural conditions (including transfer of self and outcross pollen) as well as the plants ability to autonomously self-pollinate under bags. Thus the potential for pollen competition and the occurrence of pollen limitation with and without insect visitation was explored. It was possible to complete this data set for 20 out of 23 species under investigation.

3.4.3 Fruit and seed set under natural and experimental conditions

The fruit and seed set of 22 species was scored over the field seasons 2006/07 and 2007/08. Seed developmental status was assessed by morphological characters (Webb and Simpson 2001). The production of morphologically fully developed seeds served as a measure of reproductive output and success. To characterise the breeding system 15 sets of four buds respectively were marked with coloured wire. Four treatments were applied to flowers as they entered female anthesis: 15 flowers respectively received (a) mesh bags in order to exclude pollinators and check for autonomous self-pollination or apomixis, (b) mesh bags in order to exclude pollinators and application of self-pollen to receptive stigmas and check for self-compatibility in case of pollinator-mediated self-pollination, (c) supplemental application of outcross pollen to receptive stigmas in order to check for pollen limitation or (d) no treatment at all to check for natural seed set, i.e. natural open flowers as controls (Fig 3.3).

Donor plants for supplemental hand-cross pollination were chosen from a plant patch distinctly separate and at least 10 m away from the experimental site to ensure donors were genetically distinct from recipients. Pollen of three donor anthers from three different plants was applied using forceps and/ or a fine paintbrush (Fig. 3.4). Hand-self pollination was carried out transferring pollen from the anthers to the stigma within a flower using forceps and/or a fine paintbrush. Equipment was repeatedly cleaned between flowers with 70% EtOH and stream water to avoid contamination with pollen from previous flowers. After all treatments were completed for all 60 flowers of a species and plants were subsequently monitored several times a week to observe imminent seed dispersal. Fruits were collected immediately before seed dispersal while the fruit was ripe but still closed.

Fig. 3.3 Pollinator exclusion experiments on *Viola cunninghamii* (Violaceae). Two flowers are under bags to assess autonomous self-pollination and seed set after hand-self pollination. The supplemental hand-cross pollination is marked in blue and the natural flower is marked in red.

Fig. 3.4 Hand cross-pollination on *Viola cunninghamii* (Violaceae) using forceps. The black serrated rim above the flower is a magnifying jewellers' eye-piece worn attached to one eye to improve handling of very small plants.

After collection the fruits were stored in paper bags and later the fruits and seeds per flower were counted under the dissecting microscope or with the naked eye, size permitting. Only well developed seeds were counted (Webb and Simpson 2001) while visibly shrivelled, small or deformed seeds were discarded. From these data three indices of breeding system (Bawa 1974, Ruiz and Arroyo 1978, Larson and Barrett 2000) were calculated. The self-compatibility index (SCI) is the hand-pollinated self/hand-cross ratio for seed production, the autonomous selfing index (ASI) is the pollinator-excluded/natural ratio and the pollination limitation index (PLI) is the natural/ hand-cross ratio (truncated at 0, Larson and Barrett 2000). Indices as a measurement of breeding system were chosen because they allow comparison with other national and international studies, directly measure the effect size and emphasise biological rather than statistical effects related to sample size (Newstrom and Robertson 2005). In general, failure to set fruit regardless of the treatment was common in all species.

3.5 Analysing the plant-pollinator network

3.5.1 Plant phenology

Plant phenology was characterised using a transect approach in the field season 2007/08. To assess the resources available to insect visitors throughout the season the number of open flowers or inflorescences along a 1500m transect that cut through the entire experimental area was counted within continuous quadrats of 1 m^2 (Fig 3.5). The spatial position of each quadrat with flowering plants was recorded with a GPS (Garmin GPS60 with Map Source software). The numbers of flowers and buds along the transect were recorded on a weekly basis for a total of 15 weeks starting on 05.12.07 and finishing on 14.03.08.

Fig. 3.5 Counting grid to assess phenology along a transect.

3.5.2 Insect phenology

Flower visitor phenology is difficult to measure independent of the plants that are visited by them since this is the time when insectd are most likely to be observed. However, basing the phenology on the list of insects seen on plants gives at least useful information on the presence of insects as flower visitors. However, the relative appeal of a visited plant species factors into the abundance of insect visitors and cannot provide a neutral assessment of the

numbers of insects present. Therefore the assessment of insect phenology from the visitor observations carried out (see *3.5.3*) is limited. The results are qualitative rather than quantitative and will be displayed in presence-absence format only.

3.5.3 Visitor observations

Flower visitor observations were carried out at all 23 plant species over the course of the field seasons 2006/07 and 2007/08. The data sets in both seasons were obtained by two observers simultaneously. The information on visitor composition and visit frequency represent an essential first step to assess the pollination life history of a plant. Observations were carried out at four plant species per week per observer allowing monitoring of a total of up to eight plant species simultaneously. However, most plant species' peak flowering periods lasted considerably longer than the experimental observation period of five consecutive days. To allow for comparisons, all observations were conducted during the peak flowering time of each species.

Observations were carried out in blocks of 30 min of continuous undisturbed monitoring on at least eight occasions (Minimum total of observation time 240 min). After 30 min of observation 15 min were spent catching the main flower visitors that had been observed in the 30 min period as voucher specimens. At the beginning of each observation period temperature, wind speed, cloud cover and the number of flowers observed were recorded. In order to randomise observation time for all species, the following observation schedule was employed for all species under observation in a given week (Table 3.3).

Table 3.3 Weekly observation schedule for plant species.

	Day 1	Day 2	Day 3	Day 4	Day 5
10:00-10:45	Species A	Species B	Species C	Species D	Spare day
10:45-11:30	Species B	Species C	Species D	Species A	
11:30-12:15	Species C	Species D	Species A	Species B	
12:15-13:00	Species D	Species A	Species B	Species C	
Lunch					
14:00-14:45	Species A	Species B	Species C	Species D	
14:45-15:30	Species B	Species C	Species D	Species A	
15:30-16:15	Species C	Species D	Species A	Species B	
16:15-17:00	Species D	Species A	Species B	Species C	

By following this schedule each plant species was provided with the same opportunity to be observed during insect visitation because each species was observed at least once in each time slot. That way a skew towards favourable or less favourable observation slots was avoided and possible changes in flower visitor activity over the course of a day were likely to be recorded. Day 5 as a spare day provided opportunity to complete the schedule in case there were data gaps due to unfavourable weather as well as extra time for observation in a randomly assigned order if the basic schedule was already complete. Observations were not carried out in medium to heavy rain or if the ambient temperature was below 10°C since initial observations in the first field season demonstrated no insect activity under such conditions. Night time observations at dusk were carried out occasionally over the course of both seasons when the weather was calm and temperatures after dark were mild.

Flower visitors were recorded by eleven coarse categories which are easily recognisable under field conditions. The categories based on taxonomy and size consisted of big flies, medium flies, small flies, black hover flies, striped hover flies, soldier flies, beetles, butterflies and moths and native bees as well as the non-native bumble bees and honey bees. Categorisation was essential since a large proportion of species of New Zealand Diptera and other native insects remain undescribed and are very hard to identify under field conditions. Visitors were systematically collected after each observation period at the observation site as well as at random times all over the field site whenever insect activity was high. It was attempted to catch as many flower visitors as possible to obtain voucher specimens of a large proportion of the visitor spectrum of the different flowering plant species, but flower visitors were never disturbed during observation times. Collection date and time, plant species collected on and visitor behaviour were recorded and stored with each insect caught. Insects were caught in killing jars loaded with Ethylacetate ($CH_3CO_2{\cdot}C_2H_5$) and subsequently stored at -18°C until pinning. The insect voucher collection will be stored at the Botany Department, University of Otago, New Zealand.

3.5.4 Pollen library

A pollen library of pollen mounted in fuchsin gel (Kearns and Inouye 1993) on microscopic slides was created for all the flowering plant species in the experimental area. It served as a reference collection to identify pollen on insect bodies as well as pollen being deposited on virgin stigmas. Pollen was collected fresh from opening anthers as species came into bloom. It was transferred onto a 2mm x 2mm fuchsin cube on a microscope slide and then the fuchsin block was melted carefully on a slide warmer not allowing it to boil. A cover slip was placed

on the molten fuchsin with pollen grains imbedded to seal it. After 24 hours the fuchsin had hardened and the stain had sufficiently coloured the pollen grains to allow storage as a permanent reference. Further sealing with nail polish was not necessary. The pollen grains of each species were measured and described employing the categories of Moar (1993). The reason pollen grains could not directly be compared to Moar (1993) was that he used SEM techniques and acetolysed pollen grains which considerably alter the appearance of pollen grains but facilitate identification of pollen from peat cores which must be treated to remove soil and other organic matter. Therefore the easier and more practical fuchsin method was chosen for the reference collection. A description of the complete pollen reference collection can be found in Appendix 7.1.

3.5.5 Pollen loads on insect bodies

Pollen loads on insect bodies reflect the insects' feeding history and indicate whether insects could potentially pollinate the flower they were collected on while visiting this flower and touching anthers and/ or stigma. Pollen loads were collected from frozen specimens under the dissecting microscope with very small fuchsin cubes. Fuchsin gel is naturally sticky so pollen can be easily dabbed off the insects' body parts. Prior to pollen collection insects were softened in a relaxation chamber for a minimum of 2 hours (Walker and Crosby 1988). The insect was manipulated with forceps and a fuchsin cube mounted to a very fine insect needle in order to try to collect all pollen grains the insect might be carrying. An attempt was made to remove every single pollen grain; however pollen located around the mouthparts sometimes proved impossible to remove. A minimum number of ten pollen grains per plant species was the minimum to be recorded.

3.5.6 Pollen loads on receptive stigmas

The amount and composition of the pollen load that is deposited on a receptive stigma ultimately determines a plan's reproductive output in a given season. Deposition of outcross pollen in numbers that exceed the number of ovules to be fertilised would be most beneficial for the maternal plant. In self-compatible plants sufficient pollen deposition of self pollen on the stigma may achieve the same effect independent of a pollinator. However, dependence on outcross pollen that has to be delivered by pollinators poses the risk of stigma clogging with unwanted heterospecific pollen of other plant species. Therefore the degree of contamination

of receptive stigmas indicates the reliability of the pollinator service and allows conclusions about the floral fidelity of the visiting insects.

Stigma contamination was measured using stigmas from the experimental set-up introduced in subchapter *3.4.2*. As described above the number of con- and heterospecific pollen grains deposited on stigmas under natural open conditions was determined. The degree of stigma contamination with heterospecific pollen was calculated allowing conclusions about pollinator movements between plant species and floral constancy of alpine pollinators.

3.5.7 Analysis of pollination network parameters

In order to describe the pollination network structure two commonly analysed parameters were calculated: nestedness and the connectance of the link structure (fill). Nestedness in general serves as a measure of order in an ecological system, referring to the order in which the number of species is related to area or other factors. The more a system is "nested" the more it is organised.

To test whether observed patterns were correlated with the nestedness of interactions, the species in the plant–flower visitor matrix were arranged according to the number of interactions with their potentially mutualistic partners in descending order. A commonly used estimate of nestedness was used: system temperature T (Atmar and Patterson, 1993) by using the Nestedness Calculator software, which was developed by Atmar and Patterson in 1995 (AICS Research, University Park, NM). System temperature T is a measure of the number of deviations of unexpected presences and absences in the observed matrix above and below a calculated boundary threshold of a perfectly nested matrix. For each of these unexpected presences or absences, a normalized measure of global distance to the boundary is calculated, and these values are averaged. System temperature T has values ranging from 0° to 100° with T = 0° representing a perfectly nested matrix (no disorder). The colder the temperature of the actual matrix, the more organised and non-random is the system. In a perfectly nested matrix with less than 50% fill the observed interactions will form a concave meniscus in the upper-left corner of the matrix. A matrix is considered significantly nested if the observed T value is smaller than a benchmark value (5%) of 1,000 randomly gathered T values using matrices of similar size and fill (Stang *et al.* 2007). From the temperature T a nestedness index N can be calculated (N = (100 – T)/ 100). Hence nestedness N assumes values between 0 and 1 with 1 representing a perfectly nested and 0 representing an entirely random interaction pattern. In order to compare networks of different sizes, relative nestedness N* can be calculated ($N^* = (N - N_R)/ N_R$) (Nielsen and Bascompte 2007).

The measurement used most commonly to characterize community-wide specialisation is the 'connectance' index (*C*) (Bluethgen *et al.* 2006). Connectance is defined as the quotient between the number of actual interactions between insects and plants divided by the number of potentially possible interactions. Connectance ranges between values of 0 and 1 with high values indicating a high level of generalisation in the community. These calculations as well as all graphical analysis of the networks were done with the software PAJEK 1.23.

3.6 The floral attractants

3.6.1 Flower colour analysis

Flower colour is a primary attractant to allure pollinators. In order to gain objective and comparable measurements of flower colour colour-reflectance spectra were obtained for a total of 19 species of the community.

Perceived colour is the proportion or wavelength of light that is reflected by an object surface such as a petal. Two different types of reflection can be distinguished: total reflection where the law of reflection applies (Angle of incidence = Angle of reflection) and diffuse reflection, where the light is scattered due to the uneven surface of the object. Therefore, when measuring colour reflectance spectra the relative amount of light (%) being reflected is compared to a white standard with a reflectance of 100% across the entire spectrum.

Colour reflectance spectra were measured using an Ocean Optics photo-spectrometer (USB 4000 Plug and Play) with a Xenon light source that allowed measurements in the UV range of the spectrum (λ = 250 nm – 800 nm). Petals of flowers were mounted on black tape (Fig. 3.6) and then inserted into the experimental set-up (Fig. 3.7).

Fig. 3.6 Mounting technique for colour measuremnts. Petals of *Ourisia glandulosa* and *Ranunculus gracilipes* mounted on black tape before measuring colour reflectance spectra.

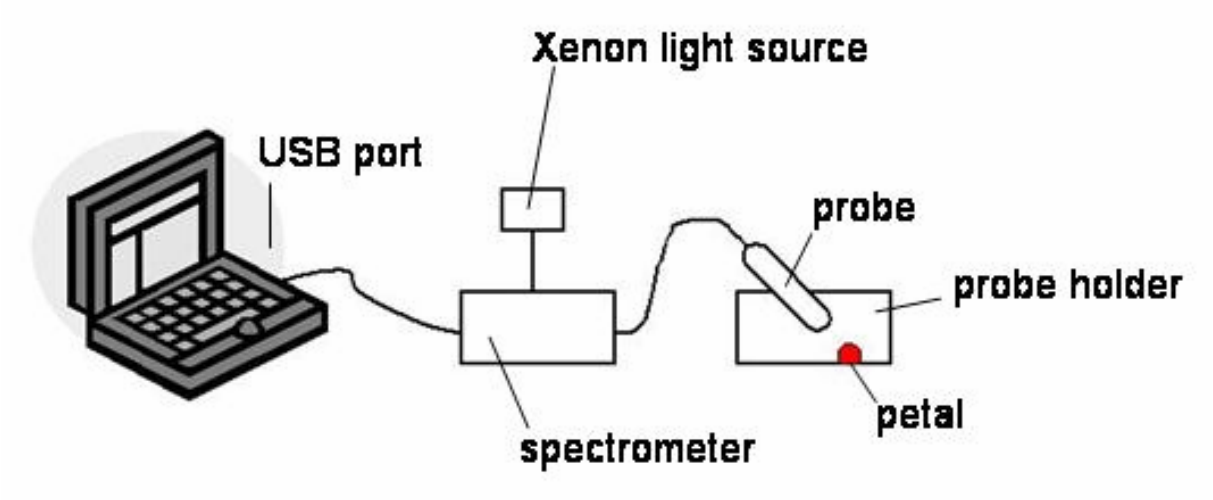


Fig. 3.7 Experimental set-up of colour reflectance measurements (objects not to scale).

Technical specifications:
Ocean Optics USB4000 fibre optic spectrometer optimised for 250-800nm wavelengths
PX2 Pulsed Xenon light source
R400 UV-Vis reflection probe
RPH1 probe holder

The probe contains a fibre-optic light guide that emits light from the Xenon light source in seven 400µm-thin cables that are assembled in a circle around the central detector that measures the light that is being reflected from the object. The probe holder maintains a constant distance to the object at a constant angle of 45°. The detector transports the light into the photometer where it is being split by a prism. Then the light is being analysed by an array of diodes. Results computed by the USB 4000 spectrometer were analysed with the software Ocean Optics Spectra Suite. Petals of five different flowers per species were measured freshly after collection. The light and dark standards were renewed after completion of measurements for one species. Finally the five replicate measurements were averaged for graphic display. The petals of all species were measured at the base and the tip of the petal. In case of colour irregularities such as nectar guides or coloured vein patterns distinct measurements where attempted. However, given the extremely small size of some of the flowers exact measurement proved difficult. Therefore a certain degree of arbitrariness is to be expected.
The averaged reflectance spectra of the 19 species were subsequently modelled in a hexagon colour space with 'typical' photoreceptors (350, 440 and 540nm) for trichromatic bees (Kevan *et al.* 2001) using a Stavenga vitamin A1 visual template (range 300-650nm) as described by Dyer (1999). Colour loci of stimuli were calculated in a hexagon space (Chittka 1992)

considering D65 illumination (Judd 1964) which was corrected for photon flux, and the visual system being adapted to leaf green (Dyer and Chittka 2004) as a background for the petal colour stimuli. The colour distance between each species in hexagon units was calculated. It represents the measure of a bees' potential ability to distinguish between the flowers making choices based on colour stimuli.

3.6.2 Floral scent analysis

Floral scent sampling - Floral scent was collected using dynamic headspace extraction methods and analysed by coupled gas chromatography and mass spectrometry (GC-MS). In total, 47 samples from 19 species were analysed.

The samples were taken by enclosing inflorescences in polyacetate bags. Air from these bags was then pumped through small cartridges (micro vials) filled with 1 mg of Tenax® and 1 mg of carbotrap® activated charcoal at a flow rate of 50 ml/min (Fig 3.8). Controls were taken from an empty polyacetate bag sampled for the same duration. The samples were collected in the field during day time. Samples were taken according to growth form from single inflorescence or where this was not possible, from whole flowering cushions. Scent was accumulated in the bags for 15 min before the sample was taken for another 15 min (Fig 3.9).

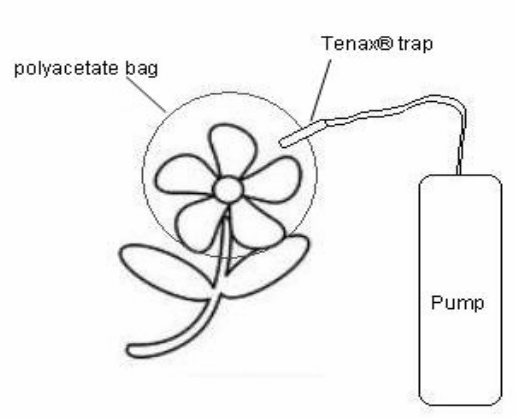


Fig. 3.8 Model of floral scent sampling technique.

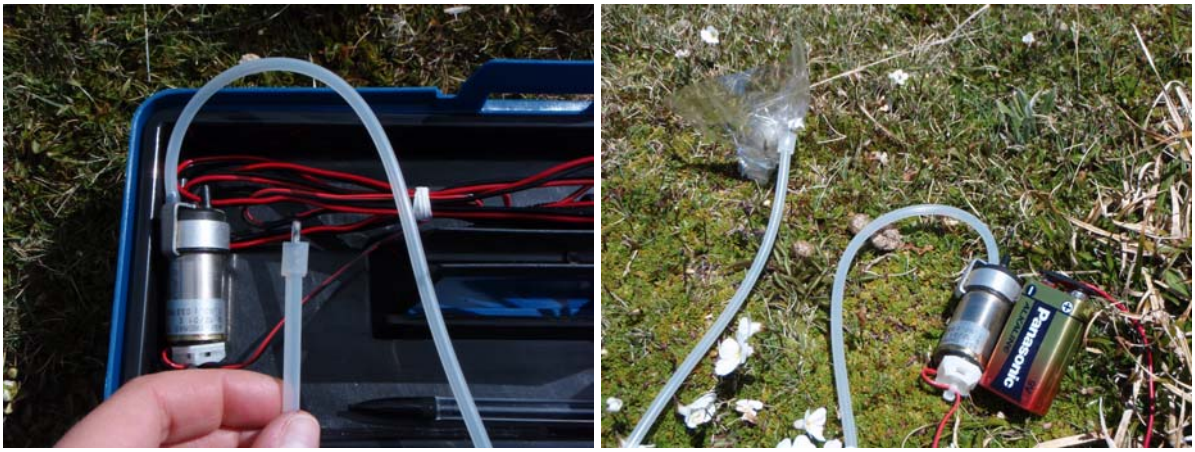


Fig. 3.9 Collection of floral odours in the field. (a) Pump and odour trap (b) Set-up during sampling on *Ourisia glandulosa* in polyacetate bag.

Gas chromatography-mass spectrometry (GC-MS) analysis of floral scent - GC-MS analysis of these samples was carried out using a Varian CP-3800 GC (Varian, Palo Alto, California) with a 30 m x 0.25 mm internal diameter (film thickness 0.25 µm) Alltech EC-WAX column coupled to a Varian 1200 quadrupole mass spectrometer in electron-impact ionisation mode. Cartridges were placed in a Varian 1079 injector equipped with a "Chromatoprobe" thermal desorbtion device. This device allows the thermal desorption of small amounts of solids or liquids contained in quartz microvials (Amirav and Dagan 1997). The Chromatoprobe micro vial was loaded into the probe, which was then inserted into the modified GC injector. The flow of helium carrier gas was 1 ml min^{-1}. The injector was held at 40 °C for 2 min with a 20:1 split and then increased to 200 °C at 200 °C min^{-1} in splitless mode for thermal desorption. After a 3 min hold at 40 °C, the temperature of the GC oven was ramped up to 240 °C at 10 °C min^{-1} and held there for 12 min. Compounds were identified using the Varian Workstation software with the NIST05 mass spectral library and verified, where possible, using retention times of authentic standards and published Kovats indices. Compounds present at similar abundance in the controls were considered to be contaminants and excluded from analysis.

Statistical Analysis - The Primer 6 program (Clarke and Warwick 2001, Clarke and Gorley 2006) was used to assess the variability in scent of the investigated species. Semi-quantitative data of compounds (percentages = relative amounts with respect to total peak areas) were used because the total amount of emitted volatiles varied greatly among different individuals (see also Dötterl *et al.* 2005). Multidimensional scaling (MDS) was used, based on Bray–Curtis similarities, to detect similarities among samples. To obtain the scent matrix, mean

relative amounts of compounds were calculated for the different species, and these values were used to calculate the Bray–Curtis similarities finally used for the analysis. To evaluate how well or poorly the particular configuration produces the observed distance matrix, the stress value is given. The smaller the stress value, the better the fit of the reproduced ordination to the observed distance matrix (Clarke 1993).

3.7 Data Analysis

All data was entered into Microsoft© Excel Office XP Professional. Unless specifically stated all graphs were calculated with Microsoft© Excel Office XP Professional. Unless specifically stated all statistical analysis was carried out with SAS/STAT® Software.

4 Results

4.1 Summary of all experimental work

In Table 4.1 a complete overview over all the experimental data obtained for the 23 species under investigation has been compiled. It was attempted to complete all experiments listed in Table 4.1 for all species in the community. Due to very different morphology and considerable fluctuation in flowering patterns between the seasons the data set remains incomplete for some species.

Table 4.1 Summary of all experimental work in the alpine plant community. Data collected comprises of pollen/ovule ratio (P/O), pollen on stigma/ovule ratio (P-S/O), fruit and seed set under natural and experimental conditions (FS), phenology (PH), flower visitor observations (VO), pollen loads on insect bodies (PI), pollen loads on receptive stigmas (PS), flower colour (C) and floral scent (S). (Hyphen= no data available). [1]Gynodioecious species with female flowers and hermaphrodite flowers [2]Dioecious species

Species	P/O	PS/O	FS	PH	VO	PI	PS	C	S
A. bellidioides	x	x	x	(x)	x	x	x	x	x
B. bellidioides	x	x	x	x	x	x	x	x	x
B. sinclarrii	x	x	x	x	x	x	x	x	x
C. sessilifolia	x	x	x	x	x	x	x	x	-
C. densifolia	x	x	x	x	x	x	x	-	x
C. thomsonii	x	-	x	(x)	x	x	-	-	-
C. lanata	x	-	x	x	x	x	-	x	x
D. lyallii	x	x	x	x	x	x	x	x	x
D. muscoides	x	x	x	x	x	x	x	x	x
E. porphyrium	x	x	x	x	x	x	x	x	x
E. zelandica	x	x	x	x	x	x	x	x	x
G. nubicola	x	x	x	x	x	x	x	x	x
G. corymbifera	x	x	x	x	x	x	x	x	x
L. glaberrima	x	-	x	x	x	x	-	x	x
M. sessiliflora	x	x	x	x	x	x	x	x	x
O. caespitosa	x	x	x	x	x	x	x	x	x
O. glandulosa	x	x	x	x	x	x	x	-	x
P. colensoi [1]	x	x	x	x	x	x	x	x	x
P. oreophila [2]	x	x	-	x	x	x	x	x	x
P. obtusa	x	x	x	x	x	x	x	-	-
R. gracilipes	x	x	x	x	x	x	x	x	x
V. cunninghamii	x	x	x	x	x	x	x	x	-
W. albomarginata	x	x	x	x	x	x	x	x	x

4.2 Reproductive system

The reproductive system of a plant encompasses all aspects of sexuality during a plants life cycle and can vary greatly between species, individuals and even between seasons on a temporal scale. Here, several important aspects regarding the dependence on pollinators have been investigated.

4.2.1 Pollen/ovule ratios

A wide spectrum of P/O ratios and corresponding reproductive systems was observed in the alpine plant community under investigation. Apart from cleistogamy, all reproductive system classes after Cruden (1977) were present (Fig. 4.1).

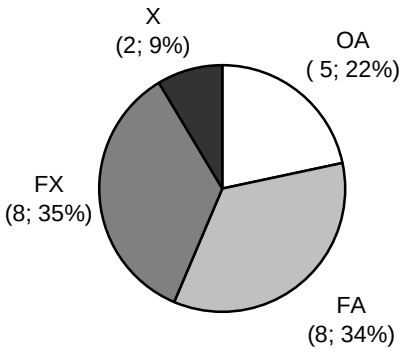


Fig. 4.1 Distribution of P/O ratios in the alpine plant community. Obligate autogamy (OA), facultative autogamy (FA), facultative xenogamy (FX) and xenogamy (X). In brackets number of species and proportion (%).

The P/O ratios for all species under investigation are displayed in Table 4.2. The lowest P/O ratio with a value of 24 pollen grains per ovule was found in *E. porphyrium*. Overall five species could be classified as obligately autogamous. These species represent less than a fourth of the entire community.

The highest P/O ratio of 5467 pollen grains per ovule was observed in *C. sessiliflora* which classifies this species one of the two obligately outcrossing species of the community. The majority of the species in this alpine ecosystem seems to be adapted to a variable or intermediate reproductive strategy with approximately equal proportions of facultative autogamous (34%) and facultative xenogamous (35%) species. These reproductive systems allow for reproductive assurance as well as outbreeding whenever possible.

The dioecious species *P. oreophila* shows a rather high ratio of 1267 pollen grains per ovule. Compared to this the P/O ratio of 164 for the gynodioecious *P. colensoi* seems extremely low.

Table 4.2 Pollen/ovule ratios and reproductive system (RS) for all 23 species of the alpine community (values rounded). Reproductive system classes after Cruden (1977): obligate autogamy (OA), facultative autogamy (FA), facultative xenogamy (FX), and xenogamy (X) were demonstrated. (Species ranked with increasing P/O ratio.)

[1]Gynodioecious species were the ratio of male to hermaphrodite flowers and the functional gender of plants is not known
[2] Dioecious species were the ratio of male to female plants is 1:1. Due to similar floral display the ratio of male and female flowers is assumed to be 1:1.

Species	Pollen grain number ± SE	Ovule number ± SE	P/O ratio ± SE	RS
D. muscoides	16000 ± 1265	176 ± 14	94 ± 13	OA
E. porphyrium	1471 ± 105	76 ± 7	24 ± 4	OA
G. nubicola	9933 ± 1576	143 ± 9	71 ± 12	OA
O. caespitosa	22711 ± 2858	224 ± 8	102 ± 14	OA
W. albomarginata	27467 ± 3212	379 ± 49	75 ± 7	OA
C. densifolia	25467 ± 3798	65 ± 4	380 ± 46	FA
C. thomsonii	6400 ± 348	27 ± 1	237 ± 10	FA
E. zelandica	2733 ± 427	24 ± 2	121 ± 25	FA
L. glaberrima	11667 ± 464	71 ± 2	167 ± 9	FA
M. sessiliflora	1364 ± 54	3 ± 0,2	431 ± 26	FA
O. glandulosa	66000 ± 9450	282 ± 28	231 ± 17	FA
P. colensoi [1]	3067 ± 402	20 ± 3	164 ± 4	FA
V. cunninghamii	10333 ± 2550	25 ± 3	447 ± 110	FA
A. bellidioides	1052 ± 120	1 ± 0	1052 ± 120	FX
B. sinclarrii	3185 ± 332	1 ± 0	3185 ± 332	FX
C. lanata	2519 ± 103	1 ± 0	2519 ± 103	FX
D. lyallii	3333 ± 754	1 ± 0	3333 ± 754	FX
G. corymbifera	38667 ± 3180	65 ± 3	592 ± 39	FX
P. obtusa	86489 ± 10190	25 ± 2	3476 ± 474	FX
P. oreophila [2]	1267 ± 114	1 ± 0	1267 ± 114	FX
R. gracilipes	40600± 5646	22 ± 2	1879 ± 337	FX
B. bellidioides	3837 ± 792	1 ± 0	3837 ± 792	X
C. sessilifolia	5467 ± 547	1 ± 0	5467 ± 547	X

Within taxonomic plant families similar P/O ratios could be demonstrated. All members of the Asteraceae show high numbers of pollen grains in relation to ovules where values range from 1052 for *A. bellidioides* to 5467 for *C. sessiliflora*. Members of the Ranunculaceae also score high P/O ratios with 3476 for *P. obtusa* and 1879 for *R. gracilipes*. Therefore all the Asteraceae and Ranunculaceae investigated could be classified as facultative or obligate xenogamous. All members of the former Scrophulariaceae (now Plantaginaceae and Orobanchaceae) show a uniform trend to facultative autogamy with P/O ratios between 102 for *O. caespitosa* and 380 for *C. densifolia*. Finally, both Ericaceae, *D. muscoides* and *G. nubicola* exhibit very low P/O ratios of 94 and 71 pollen grains per ovule respectively.

Evidently the taxonomic relationships between plants of the same families are reflected in their similar reproductive systems.

4.2.2 Pollen on stigma/ovule ratios under natural and experimental conditions

The number of conspecific pollen grains that are being deposited on a receptive stigma allows conclusions about the effectiveness of the pollinator service as well as the potential for pollen tube competition during fertilisation (Erbar and Enghofer 2001). If an insufficient number of pollen grains is being deposited, the plant is pollen-limited and thus unable to achieve full seed set.

Pollen on stigma/ovule ratios (P-S/O ratios) on natural open and experimentally enclosed stigmas were calculated for 20 species of the community (Fig 4.2). There is considerable variation throughout the community. The lowest natural P-S/O ratio of 0.3 could be demonstrated in *W. albomarginata* while the highest number of deposited pollen grains per ovule of 87.2 was found in *C. sessiliflora*. Overall all the members of the Asteraceae score high P-S/O values with a mean of 41.7 for all five species (Fig 4.2 a).

The majority of plant species in the community seems to receive adequate pollen delivery under natural conditions to receptive stigmas to fertilise all ovules. The common ratio appears to be one to four pollen grains per ovule which in theory, assuming that self-pollination and self-incompatibility do not play a role, is sufficient to achieve full seed set. Moreover, in most species the opportunity for pollen tube competition and pre-zygotic selection arises. However, four species receive less pollen grains than required under natural conditions. *C. densifolia*, *O. caespitosa, O. glandulosa*, *P. colensoi*, and *W. albomarginata* all have a P-S/O ratio of below one. At the end of the experimental period the number of pollen grains on the stigma is less than the number of ovules to be fertilised. These species appear to be pollen limited, i.e. the reproductive success of these species is limited by inadequate pollen delivery to receptive stigmas. Therefore no selection pressure may lie on the male gametophytes germinating as they do not have to compete for ovules to fertilise. However, stigmatic longevity data (Dobbie, unpublished M.Sc. thesis) suggest that at least *O. caespitosa* and *O. glandulosa* maintain receptive stigmas for more than 96 hours which is twice the experimental period. Thus, in these species autonomous or pollinator-mediated pollen transfer may happen at a later stage. On the other hand Dobbie's data suggests that stigmas of *W. albomarginata* remain receptive for 24 hours only while the receptivity period of *P. colensoi* and *C.*

densifolia stigmas has not been investigated. Therefore, from the stigma load data set pollen limitation can only be deduced for *W. albomarginata*.

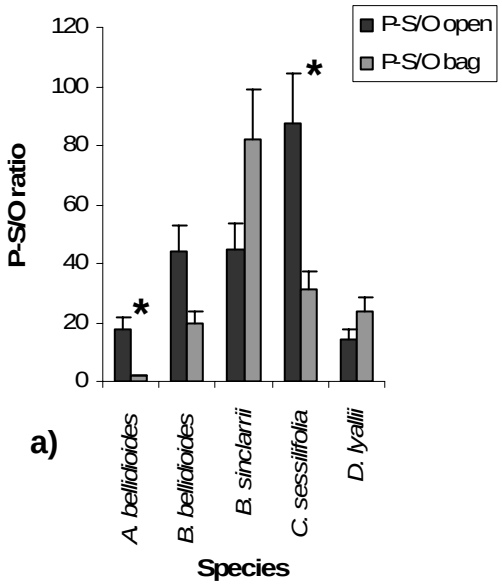
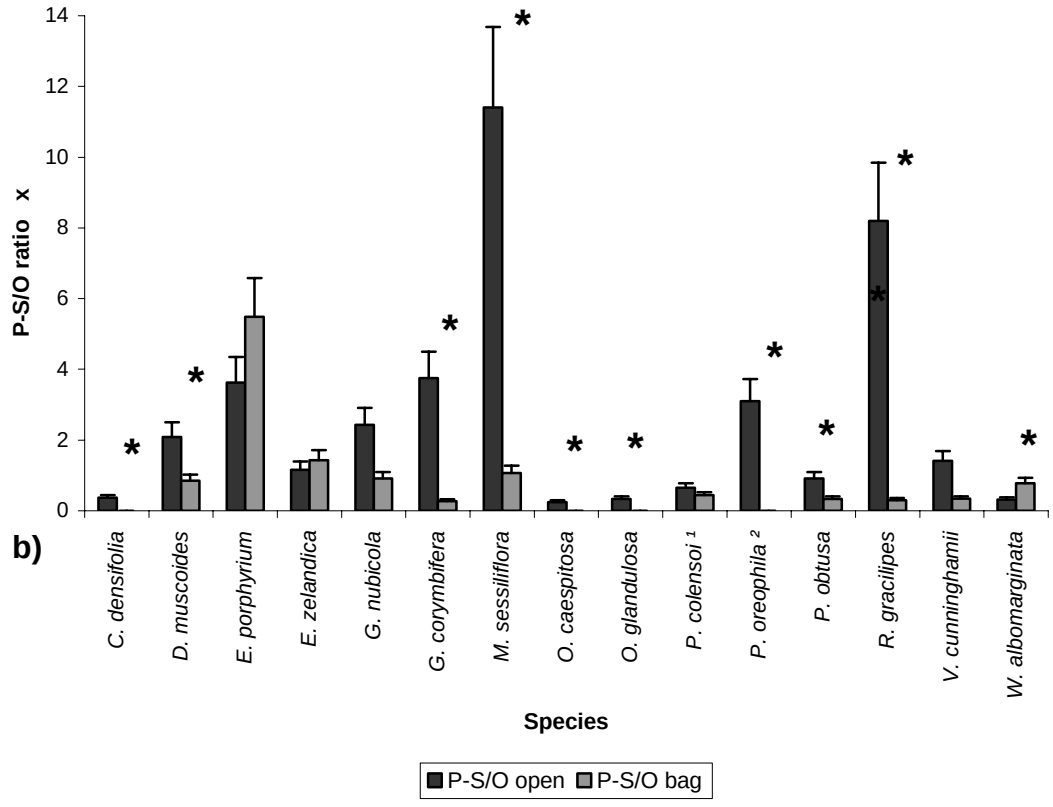


Fig. 4.2 Pollen on stigma/ovule ratios found on open and bagged stigmas for 20 species of the alpine plant community. Mann Whitney U-Test, $p<0.05$ marked with an asterisk. (a) High P-S/O ratios of the Asteraceae. (b) Low P-S/O ratios of other species.

[1] Gynodioecious species with female flowers and hermaphrodite flowers
[2] Dioecious species



The ability of a self-compatible plant to autonomously self-pollinate, i.e. to transfer pollen to its own receptive stigma without a vector indicates the independency of pollinator visitation.

To explore the potential for autonomous self-pollination the P-S/O ratios of open and enclosed stigmas were compared with a Mann-Whitney U-Test for non-parametric data after testing for normality with the Kolmogorov-Smirnov procedure (Fig. 4.2). Where P-S/O ratios decreased significantly with insect exclusion a dependency on pollinators for seed set is likely (Fig. 4.3).

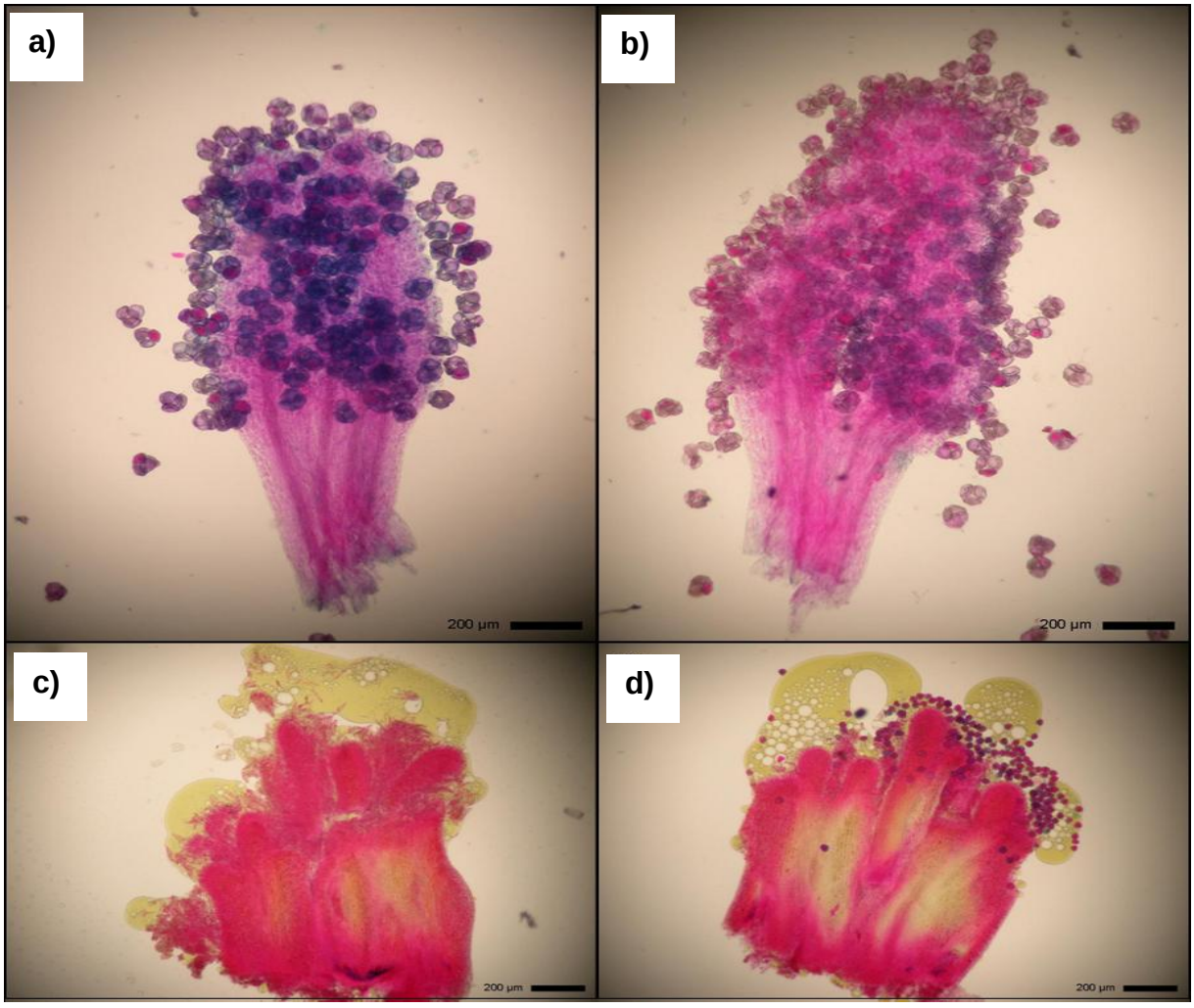


Fig. 4.3 Pollinator dependency and autonomous self-pollination. No significant difference in *E. porphyrium*: typical pollen loads on bagged (a) and open (b) stigmas. Pollen loads depend on insect visitation in *D. muscoides*: typical pollen loads on bagged (c) and open (d) stigmas.

A slight majority of the plant species under investigation (12, 60%) show a significantly increased P-S/O ratio under natural conditions and are therefore classified as pollinator-dependent. Some pollen may still be transferred without a vector but the number of pollen grains in generally well below 50% of the pollen load under natural conditions. The P-S/O ratio indicates still adequate pollen deposition in *A. bellidioides* and *C. sessiliflora* but drops below the minimum value of 1 in the ten remaining species. *C. densifolia, O. caespitosa* and *O. glandulosa* do not manage to transfer any pollen at all without insect visitation. In these species the indicated natural pollen limitation becomes extreme under insect exclusion. The same is obviously valid for females of the dioecious *P. orephila* where autogamy is not an option due to spatial separation of male and female function. In this experimental set-up it is not possible to distinguish whether outcrossing or pollinator-mediated selfing takes place in homoecious species under natural conditions, but a dependence on pollinator visitation for pollen transfer can be confirmed.

Overall, these twelve species can be clearly distinguished from the remaining eight species (40%) that show no significant difference between the two treatments. In those cases, flowers are independent of insect pollinators and manage sufficient or even more effective pollen transfer without a vector. *W. albomarginata* stands out in increasing its P-S/O ratio under insect exclusion by 145% from 03. to 0.8 as pollen loss to visitors is prevented . However, pollen deposition is still not sufficient.

There is no apparent taxonomic trend in pollinator-dependency and ability to autonomously self-pollinate within the members of the different plant families. Apart from the two members of the Ranunculaceae all other plant families include autonomously selfing as well as pollinator-dependent species.

The apparent pollinator-dependence of more than half of the plant species in the community indicates the importance of flower-visiting insects as pollen vectors in this ecosystem. However, in combination with the investigations on self-compatibility (see *4.2.3*) true pollinator independence can finally only be confirmed for the fully self-compatible species *E. porphyrium, G. nubicola* and *P. colensoi*. The other five species may achieve adequate pollen transfer but due to incompatibility barriers these pollen grains will not fertilise ovules.

4.2.3 Fruit and seed set under natural and experimental conditions

The ultimate goal of any flowering plant is to set fruit and produce fruits with a maximum number of seeds. While the reproductive output does not exclusively depend on pollination but also on constraints such as resource limitation in the habitat or herbivory, successful pollen transfer is a crucial step in all but apomictic plant species. In order to achieve adequate pollination plants may employ different reproductive strategies. The data set presented here examines the breeding systems in three indices.

The results for the fruit set of 22 plant species are presented in Table 4.3. Species were classified as self-compatible where the hand-self to hand-cross ratio of seed set SCI > 0.80, partially compatible where 0.20 < SCI < 0.80, and self-incompatible where SCI < 0.20 (Bawa 1974, Ruiz and Arroyo 1978), autonomously selfing where the bagged to natural seed set ratio ASI > 0.5. The degree of pollen limitation was classified as high where the pollen limitation index (1 – natural/hand-cross seed set) PLI > 0.75, medium where 0.25 < PLI < 0.75, and low where PLI < 0.25.

Table 4.3 Experimental tests of breeding systems in the alpine community. The self-compatibility index (SCI) is the hand-self/hand-cross ratio, the autonomous selfing index (ASI) is bagged/natural ratio, and the pollen limitation index (PLI) (1- natural/hand-cross). Reproductive output was scored at the level of fruit and seed set and is displayed as seeds per flower (fruit set x seed set).

Species	SCI	ASI	PLI
A. bellidioides	0	0.01	0
B. bellidioides	0	0.02	0
B. sinclarrii	0	0	0
C. sessilifolia	0.55	0.02	0
C. densifolia	1.35	0	0
C. thomsonii	0.04	0.12	0
C. lanata	0.14	0.31	0.09
D. lyallii	0.23	0.48	0
D. muscoides	0.26	0.06	0
E. porphyrium	1.54	0.93	0
E. zelandica	0.09	0.11	0
G. nubicola	0.35	0.72	0.35
G. corymbifera	0.15	0.15	0
L. glaberrima	0	0	0
M. sessiliflora	0.03	0.03	0
O. caespitosa	0.03	0	0
O. glandulosa	0	0	0
P. colensoi	1.38	1.28	0
P. obtusa	0.37	0	0
R. gracilipes	0.01	0.01	0
V. cunninghamii	0.08	0.01	0
W. albomarginata	0	0	0

Furthermore the relative fruit set in correlation with the experimental treatment is shown in Fig. 4.4 and 4.5.

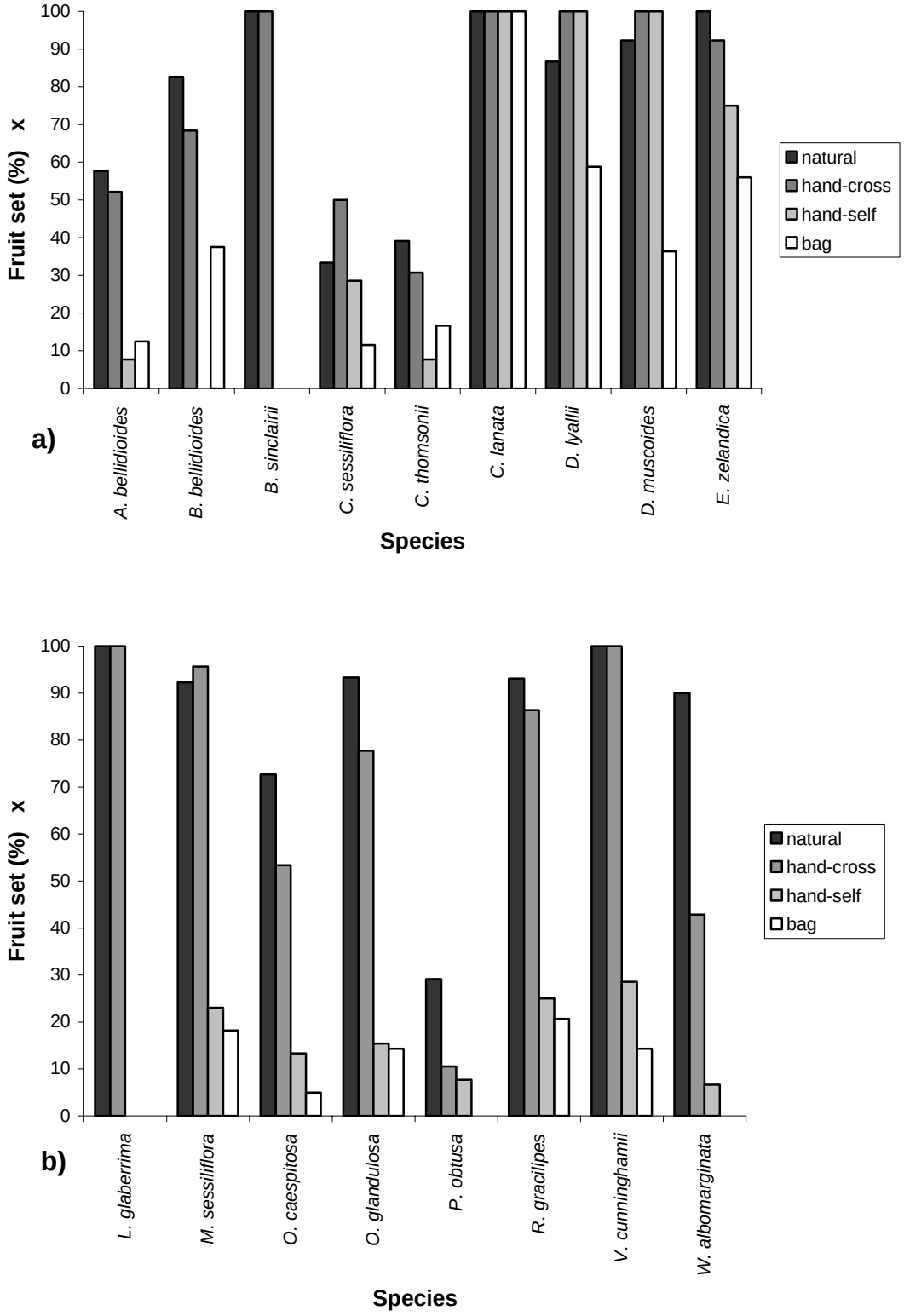


Fig. 4.4 Subset of plant species in the alpine community that could be classified as pollinator-dependent due to SCI and ASI indices (Table 4.3).

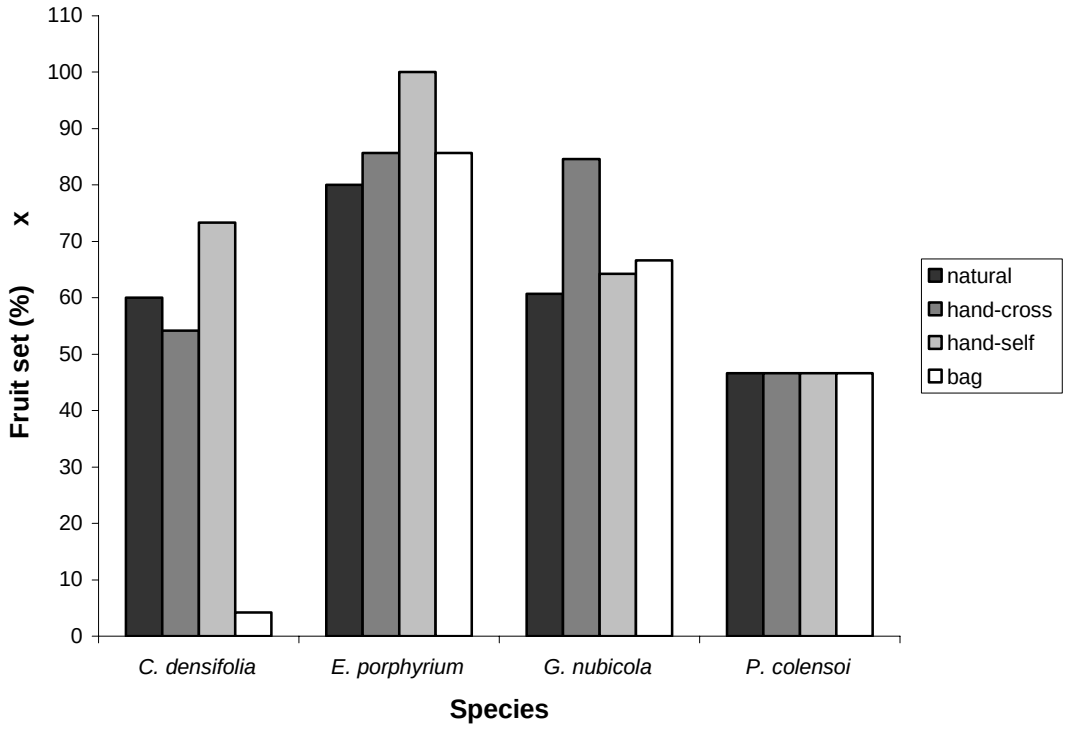


Fig. 4.5 Subset of plant species in the alpine community that could be classified as pollinator-independent due to SCI and ASI indices (Table 4.3) or show show high pollinator-mediated selfing ability in case of *C.densifolia*.

Flowers of the 18 plant species presented in Fig. 4.4 a-b are pollinator-dependent as the calculated SCI and ASI indices show. Full self-incompatibility (SCI < 0.20) could be demonstrated for the species *A. bellidioides, B. bellidioides, B. sinclairii, C. thomsonii, C. lanata, E. zelandica, G. corymbifera L. glaberrima, M. sessiliflora, O. caespitosa, O. glandulosa, R. gracilipes, V. cunninghamii* and *W. albomarginata* which represents 64% of the alpine community. Partial self-incompatibility (0.20 < SCI < 0.80) could be demonstrated for the species *C. sessilifolia, D. lyallii, D. muscoides, G. nubicola* and *P. obtusa* which represents a further 23% of the alpine community. The remaining species *C. densifolia, E. porphyrium* and *P. colensoi* are fully self-compatible (SCI > 0.80). *C. densifolia* stands out as being dependent on pollinator-mediated self-pollination. Furthermore autonomous self-pollination (ASI > 0.50) could be confirmed for the species *E. porphyrium, G. nubicola* and *P. colensoi* which equal 13% of the alpine plant community. All other species were not capable of autonomous self-pollination according to the ASI index. Data from the stigma pollen load (see *4.2.2.*) corresponds with this result. Very low pollen limitation (0.25 < PLI <

0.75) was detected for *C. lanata* and moderate for *G. nubicola* which equals 9% of all species under investigation.

4.2.4 Summary of the reproductive system results

The reproductive system for the 23 species of the alpine community (Table 4.4) could be deduced employing three indicators, i.e. pollen/ovule ratio and pollen on stigma/ovule ratio under natural conditions and without insect visitation. In correlation with the data on the actual reproductive systems of the plant species in the community a correct prediction could be made in approximately half of the cases (48%, 11 species). For three species (*B.bellidoides, B. sinclairii* and *D. lyallii*) the autonomous selfing potential was interpreted as autonomous autogamy while the breeding system revealed self-incompatibilty and thus obligate xenogamy for the former two and facultative xenogamy for the latter. For another four species (*D. muscoides, G. corymbifera, L. glaberrima* and *M. sessiliflora*) the P/O ratio was too low to match the demonstrated breeding system of obligate xenogamy. For the remaining five species contrasting results were recorded and the predictions about the reproductive system did not match the data for fruit and seed set. Overall the great majority of plant species (87%, 20 species) in the alpine community exhibit a xenogamous pollination system that requires pollination by insect visitors. Only three species are autonomously autogamous species that do not depend on pollinators to set fruit (13%).

Table 4.4 Conclusive summary of all experimental data investigating the reproductive system of 23 species of the community. Pollen/ovule ratio (P/O): obligate autogamy (OA), facultative autogamy (FA), facultative xenogamy (FX) and xenogamy (X) (Cruden 1977), pollen on stigma/ovule ratio (P-S/O): pollen sufficient (PS), pollen-limited (PL), Autonomous selfing potential (AS): pollinator required (PR), autonomous autogamy (AA), Fruit and seed set under natural and experimental conditions (FS): autonomous autogamy (AA), pollinator-mediated autogamy (PMA) facultative xenogamy (FX) and xenogamy (X), pollen-limitation (PL). (Species ranked with increasing pollinator-dependency with respect to breeding system). (Hyphen = no data available)

[1]Gynodioecious species were the ratio of male to hermaphrodite flowers and the functional gender of plants is not known

[2] Dioecious species were the ratio of male to female plants is 1:1. Due to similar floral display the ratio of male and female flowers is assumed to be 1:1.

Species	P/O	P-S/O	AS	FS
E. porphyrium	OA	PS	AA	AA
P. colensoi [1]	FA	PL	AA	AA
G. nubicola	OA	PS	AA	AA/ PL
C. densifolia	FA	PL	PR	PMA
C. sessilifolia	X	PS	PR	FX
D. lyallii	FX	PS	AA	FX
D. muscoides	OA	PS	PR	FX
P. obtusa	FX	PS	PR	FX
A. bellidioides	FX	PS	PR	X
B. bellidioides	X	PS	AA	X
B. sinclarrii	FX	PS	AA	X
C. thomsonii	FA	-	-	X
E. zelandica	FA	PS	AA	X
G. corymbifera	FA	PS	PR	X
L. glaberrima	FA	-	-	X
M. sessiliflora	FA	PS	PR	X
O. caespitosa	OA	PL	PR	X
O. glandulosa	FA	PL	PR	X
P. oreophila [2]	FX	PS	PR	X
R. gracilipes	FX	PS	PR	X
V. cunninghamii	FA	PS	AA	X
W. albomarginata	OA	PL	PR	X
C. lanata	FX	-	-	X/PL

4.3 Plant-pollinator network

The following subchapters describe the different aspects of the observed plant-pollinator network. The sequence of flowering plants in the community and insect phenology are briefly described and then the observed plant-flower visitor interactions are analysed. The relationships between plants and their insect visitors on a community level are best visualised and considered using a network approach where plants and insects are nodes that are connected by links. Three different methods for network quantification result in three different network patterns that describe the alpine community: (a) visitor observation network, (b) presence-absence data for insects collected on flowering plants and (c) pollen loads on insect bodies.

4.3.1 Plant phenology

The times and patterns of flowering in the alpine community over the season 2007/08 are displayed in Fig. 4.6. Individual flowering graphs may be found in App. 7.2.

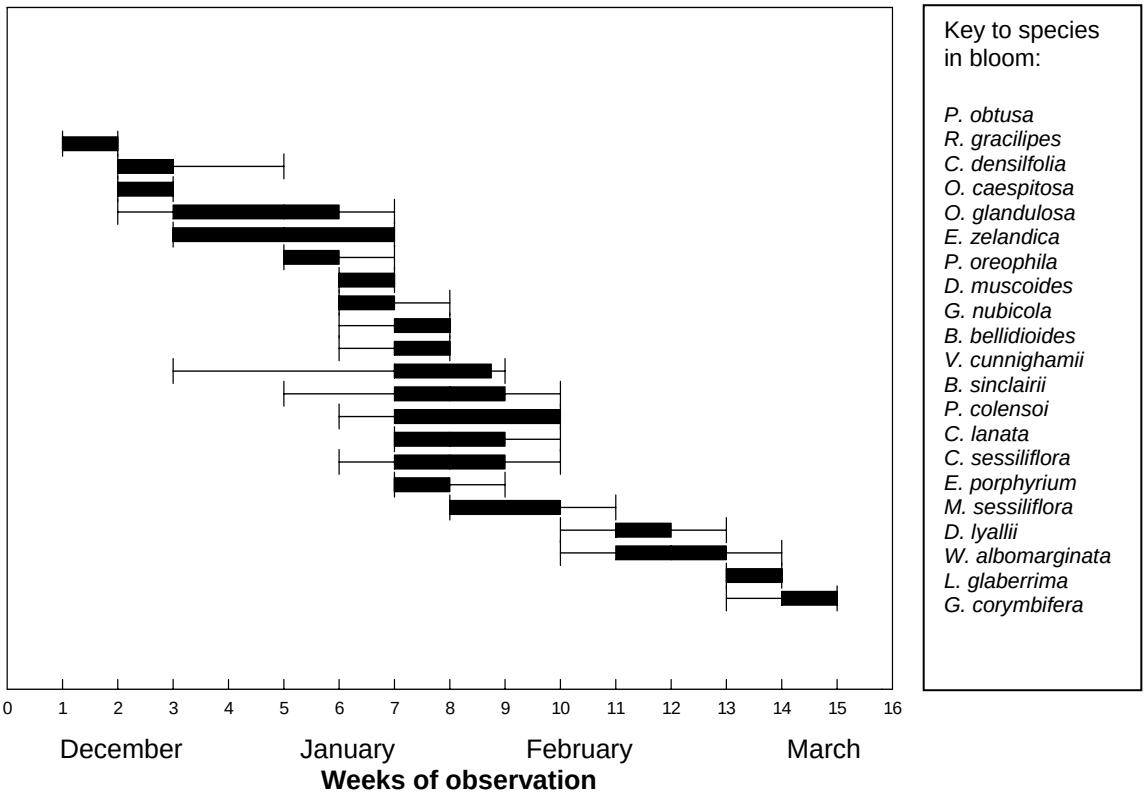


Fig. 4.6 Flowering sequences of 21 plant species in the alpine community. For each species, the line illustrates its total flowering period and the bar depicts peak flowering during which more than half of the plants on the transect displayed open flowers.

Two of the species under investigation, *C. thomsonii* and *A. bellidioides* where not recorded during transect observations as they grew in locations outside the transect. Their flowering peaks are in week 3 and in week 9, respectively (estimate deduced from flower visitor observation data and pollinator exclusion experiments).

The majority of flowers reach peak flowering between mid-January and early February. Therefore potential competition for pollination and cross-contamination of stigmas of one plant species with heterospecific pollen grains of another should be most frequently observed around this time. Typical spring flowers are *P. obtusa* and *R. gracilipes* with the former flowering immediately after snow-melt in early December. Towards autumn, *L. glaberrima* and *G. corymbifera* complete the flowering season for the alpine community in late March. Consistent with patterns observed around the globe the members of the Ranunculaceae set the stage of the annual flowering cycle while the curtain falls with the gentians (Körner 2003).

4.3.2 Insect phenology

The data for insect phenology and activity patterns are derived from the visitor observation experiments (Table 4.5). They can therefore supply qualitative information only and serve as a very broad record of insect life histories in the Remarkables Mountains. A bias because of the relation to flower visitation is to be expected. The information may serve as a benchmark and evidence for general trends among flower visitors. The minimum temperature where activity was recorded was relatively low. Flower visitors became generally active at temperatures above 15°C and abundant at temperatures above 20°C.

Table 4.5 Insect phenology data extracted from flower visitor observations.

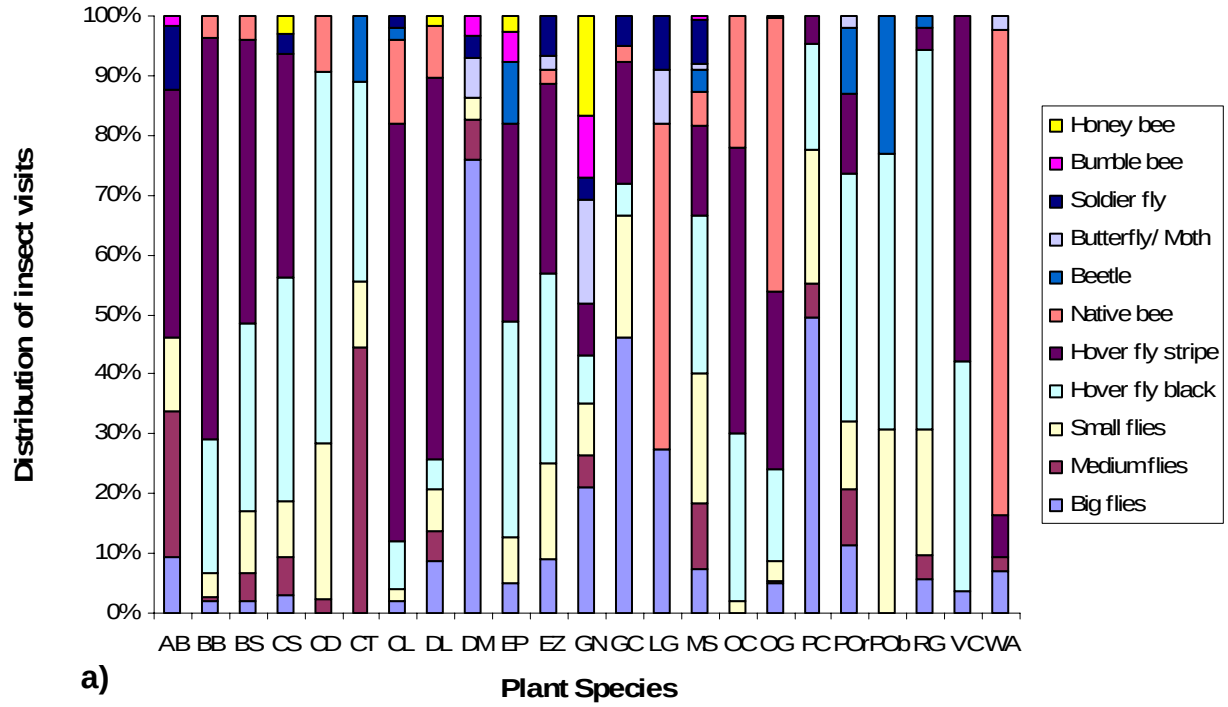
Visitor class	First record	Last record	Tmin (°C)	Activity period
Big flies	16. Jan	08. Mar	11.0	am/ pm
Medium flies	18. Dec	07. Feb	14.9	am/ pm
Small flies	28. Nov	21. Feb	11.0	am/ pm
Hover flies black	05. Dec	20. Feb	14.4	am/ pm
Hover flies stripe	07.Dec	19. Feb	6.9	am/ pm
Soldier flies	16. Jan	07. Mar	12.2	am/ pm
Beetles	28. Nov	05. Feb	11.4	am/ pm
Butterfly/ moth	18. Jan	08. Mar	9.0	pm/ dusk
Native bees	03. Jan	08. Mar	13.2	am/ pm
Honey bees	11. Jan	22. Feb	16.4	pm
Bumble bees	16. Jan	05. Feb	23.5	pm

Especially the non-native honey bee and bumble bee visitors were recorded only in one out of three field season and the period of their activity in the study area coincided with the peak flowering of *Gaultheria nubicola* where they could be seen nectar foraging in high numbers on warm afternoons with temperatures well over 20°C.

4.3.3 Visitor observations

All 23 species of the alpine community were subject to flower visitor observations that yielded a quantitative characterisation of the visitor spectrum and the individual visit frequencies of the eleven flower visitor categories. In Fig. 4.7a the relative visitation is displayed for each plant species. Most plants are visited by several visitor classes in varying frequencies.

Ten plant species receive more than 50% of their visits from one visitor class. Species with predominantly big fly visitation (Families Tachinidae, Muscidae) are *D. muscoides* and *P. colensoi*. Flowers of *B. bellidioides, C. densifolia, C. lanata, D. lyallii* and *V. cunninghamii* receive most of their visits from striped hover flies of the Genus *Allograpta* (Syrphidae). Black hover flies of the Genus *Platycheirus* are the major flower visitors of *Ranunculus gracilipes* while native bees of the Genera *Hylaeus* (Colletidae) and *Leioproctus* (Colletidae) are the main visitors of *L. glaberrima, O. glandulosa* and *W. albomarginata.*



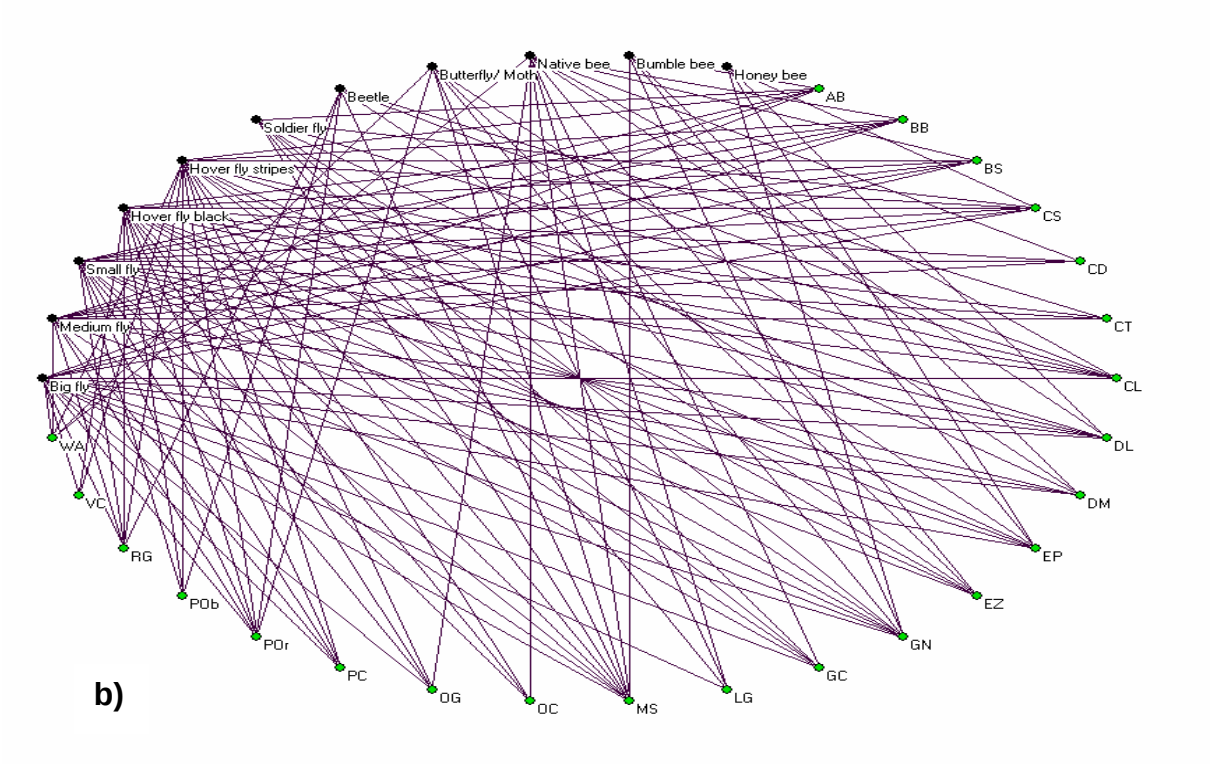
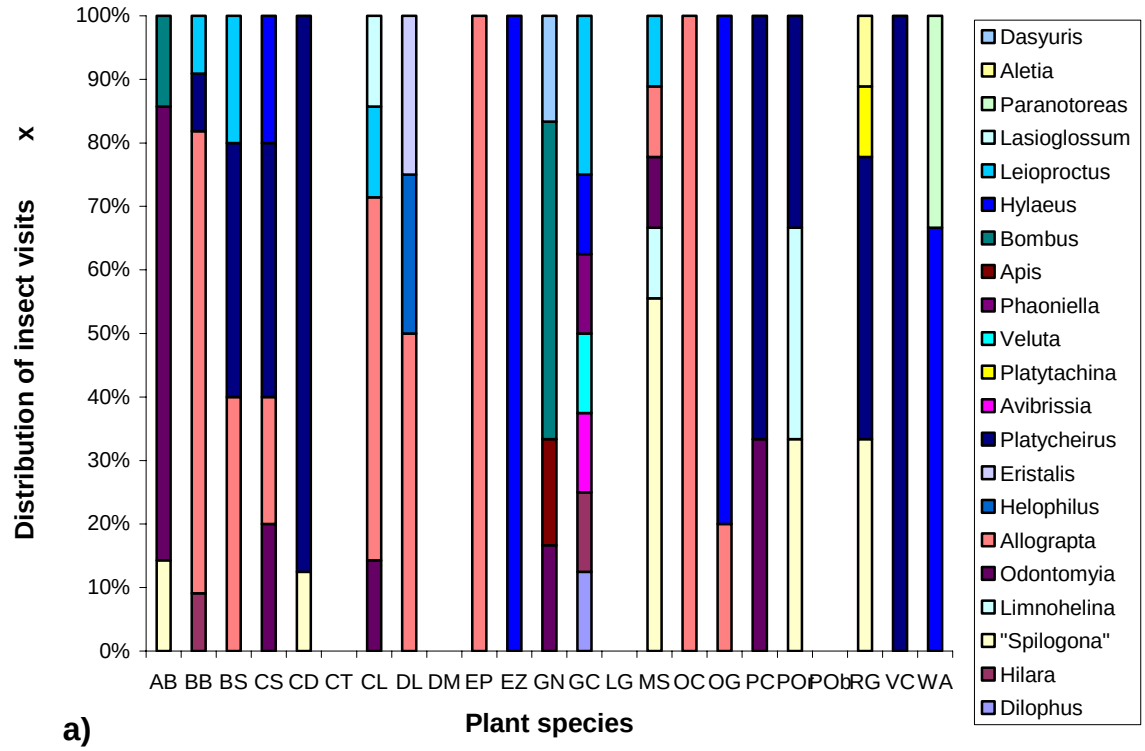


Fig. 4.7 Insect visitation in 23 species of the community. (a) Quantitative frequency of insect visitation by different visitor classes. (b) Qualitative interaction network of insect visitor classes and plant species.

Fig. 4.7b depicts the flower visitor – plant network. Plants are connected to their insect visitors through qualitative links were all interactions are of equal weight and the frequency of interactions is not considered. Flies maintain the majority of the connections, however most species are connected by a number of links (for detailed analysis of the network parameters see *4.3.6*). It is essential to bear in mind that this network depicts potential pollinator visits. From the observation data no conclusion can be drawn to the biological relevance of the visitors as pollinators of the plants.

4.3.4 Pollen loads on insect bodies

A total of 244 insect specimens that touched anthers and/or stigma during their flower visits were collected during two field seasons. Out of this total a subset of 132 specimens carried pollen on their bodies. Those potential pollinators could be identified to genus level and resulted in 21 different classes of flowers visitors. In total, of the order Diptera March Flies (Bibionidae: *Dilophus*), Dance Flies (Empididae: *Hilara*), Muscid flies (Muscidae: *"Spilogina", Limnohelina*), Soldier Flies (Stratiomyidae: *Odontomyia*), Hover Flies (Syrphidae: *Allograpta, Helophilus, Platycheirus*) and Tachinid Flies (Tachinidae: *Avibrissima, Platytachina, Veluta, Phaoniella*) were present. Furthermore, of the order Hymenoptera there were Plasterer bees (Colletidae: *Hylaeus, Leioproctus*), Sweat bees (Halictidae: *Lasioglossum*) and honey and bumble bees (Apidae: *Apis* and *Bombus*). Moths of the order Lepidoptera were also present (Geometridae: *Paranotoreas, Dasyuris*, Noctuidae: *Aletia*). Full descriptions of the taxonomy can be found in App.7.4. Details about the host plant were always recorded. The data derived from the insect voucher collection could be analysed in two different ways. Firstly, a flower visitor – plant network was described linking insect specimens in a matrix with the plants they were caught on (Fig. 4.8 a-b). This was done to compare methodologies commonly used in network studies where insect visitors are being collected and no actual visit frequency data is being recorded.



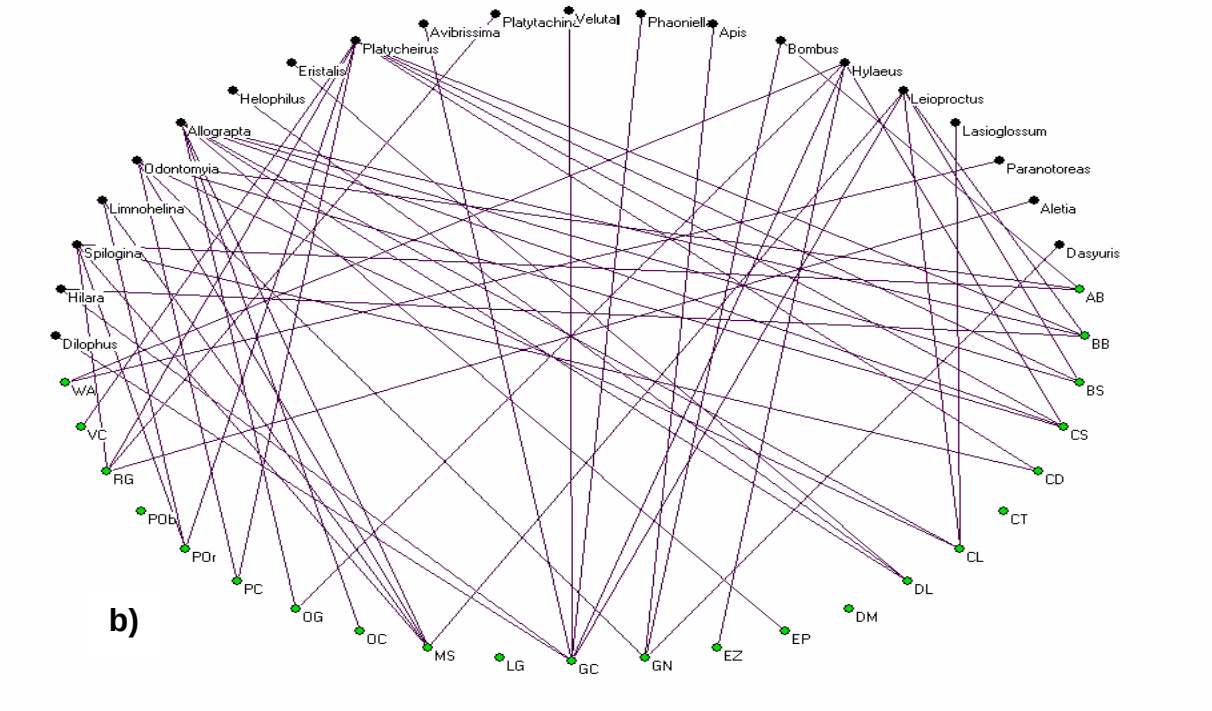


Fig. 4.8 Insect voucher specimens collected on 23 plant species of the community. (a) Quantitative frequencies of insects caught on plant species. (b) Qualitative interaction network based on the voucher specimens collection.

Although voucher specimens were collected on all 23 plant species of the community, not all of the visitors carried pollen and were therefore not included in the potential pollinator network. On the basis of this exclusion not all plant species in the community received potential pollinator visits and the frequency of visits declined overall (Fig. 4.8a). The number of visits and the distribution of visitor classes declines considerably which is to be expected as the number of interactions recorded here is lower than the number of visits recorded during victor observations. Overall, the mere collection of flower visitors paints a much simpler picture of the interaction network (Fig. 4.8b)

Secondly, in addition to identification of the potential pollinators the pollen loads they were carrying were collected and analysed. The presence of pollen grains of a plant species was considered indirect evidence of a visit at some point in time. Given the fact that most insects are pollen feeders and groom frequently pollen on insect bodies was considered to be viable unless apparent damage was obvious during analysis. For pollen identification a category of unidentified pollen (UP) had to be added that contained mainly Asteraceae pollen which looks very similar across species and genera. If the pollen load information is added to the interaction matrix constructed from the voucher specimens, the picture changes dramatically (Fig 4.9). Although the data set was corrected for forbidden links where plant and insect phenologies did not overlap, the number of interactions more than triples (for detailed analysis of the network parameters see *4.3.6*). All plant species are being visited in this scenario although *Viola cunninghamii* receives visits from *Platycheirus* hover flies alone. *Allograpta* hoverflies maintain interactions with 23 out of 24 possible host plants and can be classified as the most generalistic flower visitor. Moths of the genera *Aletia* and *Dasyuris* seem most limited in the range of flowers that they visit being only recorded for two plant species each (Fig 4.9a). Overall the network derived from data on host plants and pollen loads of potentials pollinators appears a lot more satisfying as it captures a higher proportion of the pollination-relevant interactions that are occurring in the community (Fig 4.9b). The information extracted from this matrix is also superior to the visitor observations in its qualitative evidence for pollination. During the visitor observations it was not possible to determine whether visitors were carrying pollen loads of conspecific pollen while the voucher specimens could readily be analysed with respect to pollinator service (see *4.3.5*). However, the information on visit frequencies is also very valuable and indispensable as a pollinator may not be

very efficient in pollen transfer per visit but still effective through an increased number of visits.

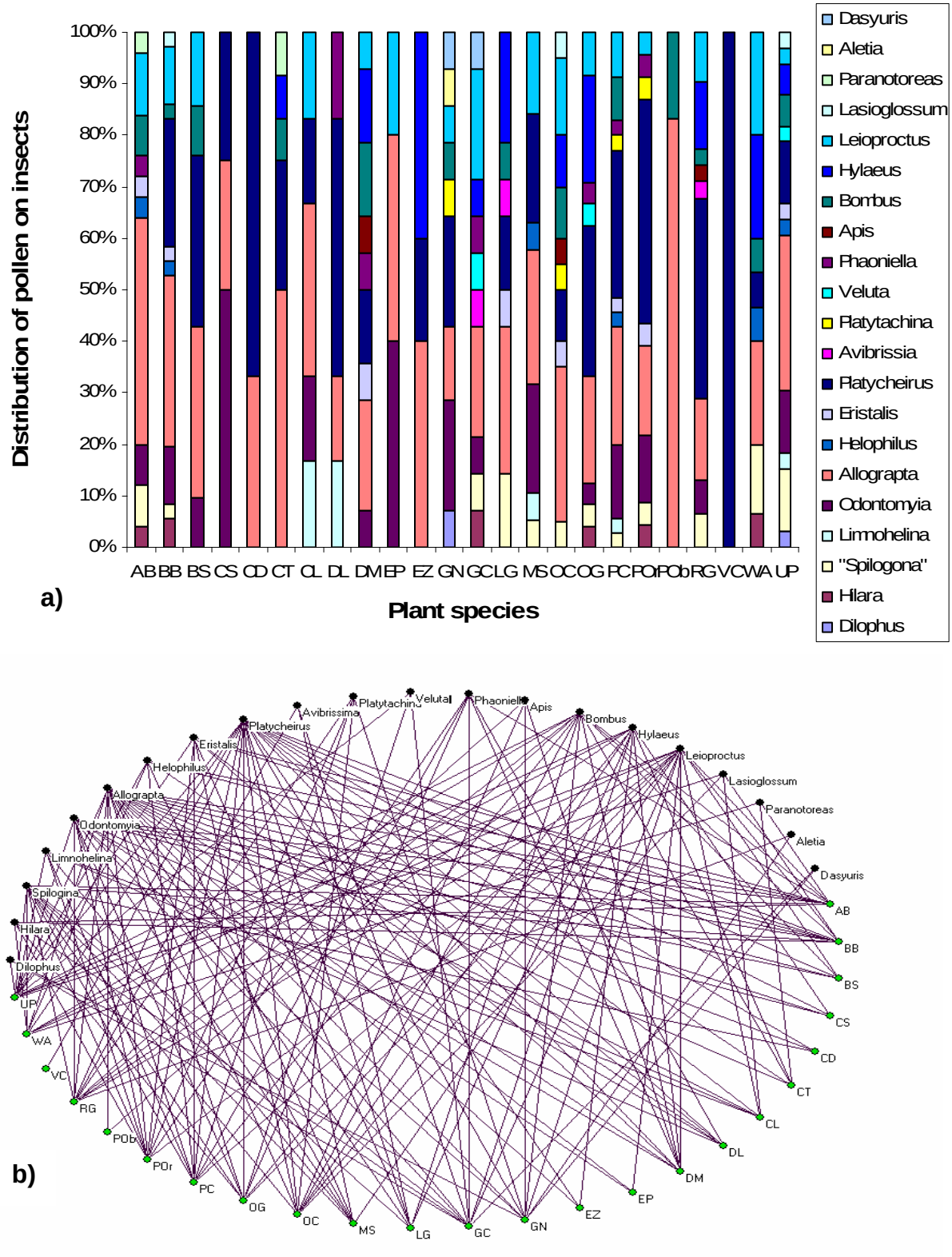


Fig. 4.9 Insect voucher specimens and their respective pollen loads colleted on 23 species of the community. (a) Quantitative frequencies of pollen load composition on insect bodies, (b) combined network of host plant and pollen load information.

4.3.5 Pollen loads on receptive stigmas

Visitation frequencies and insect pollen loads as presented above an indicators of potential pollination events within a community. However, actual pollen delivery can only be measured by analysing pollen loads on receptive stigmas. The degree of contamination with heterospecific pollen, i.e. stigma clogging, could be recorded for 20 species of the alpine community. A look at the pollen loads found on insect bodies from the potential pollinators point of view suggests a considerable degree of generalisation in plants being visited and therefore low floral fidelity in the flower visitors in this system (Fig. 4.10).

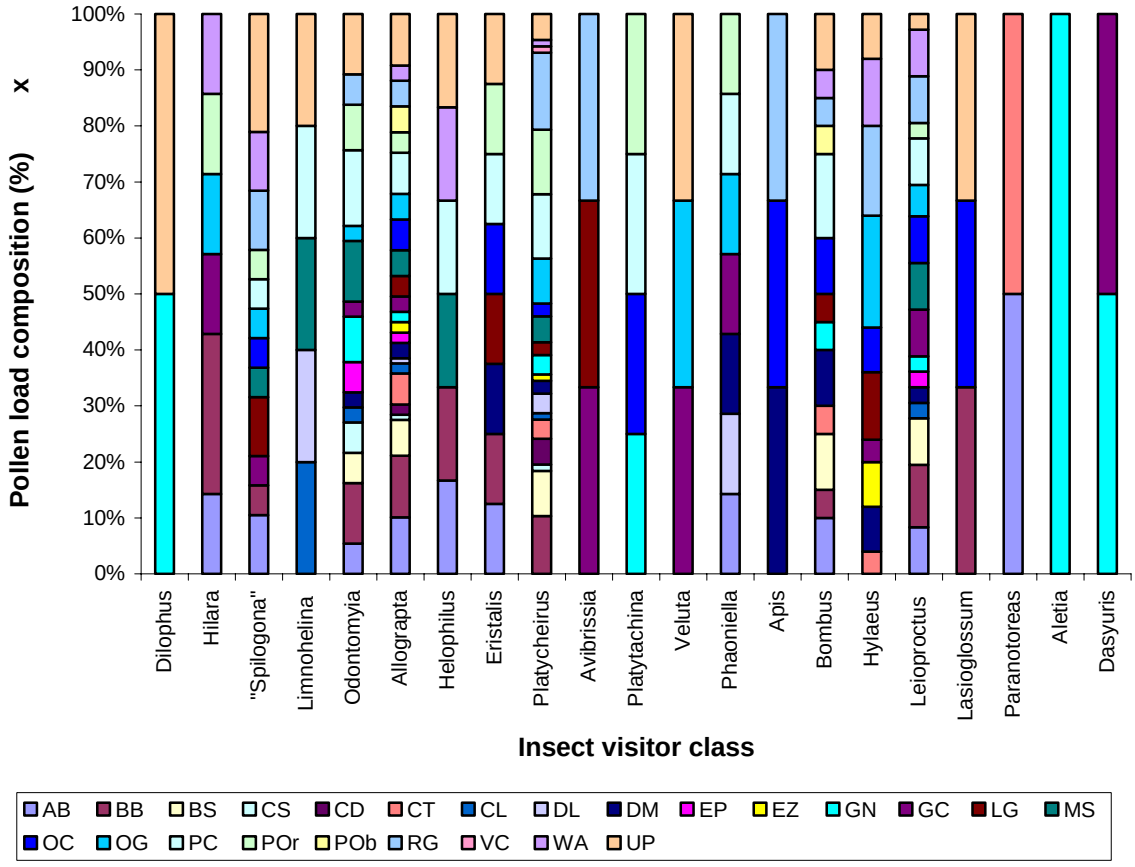


Fig. 4.10 Relative pollen amounts carried by the different visitor classes in the alpine community.

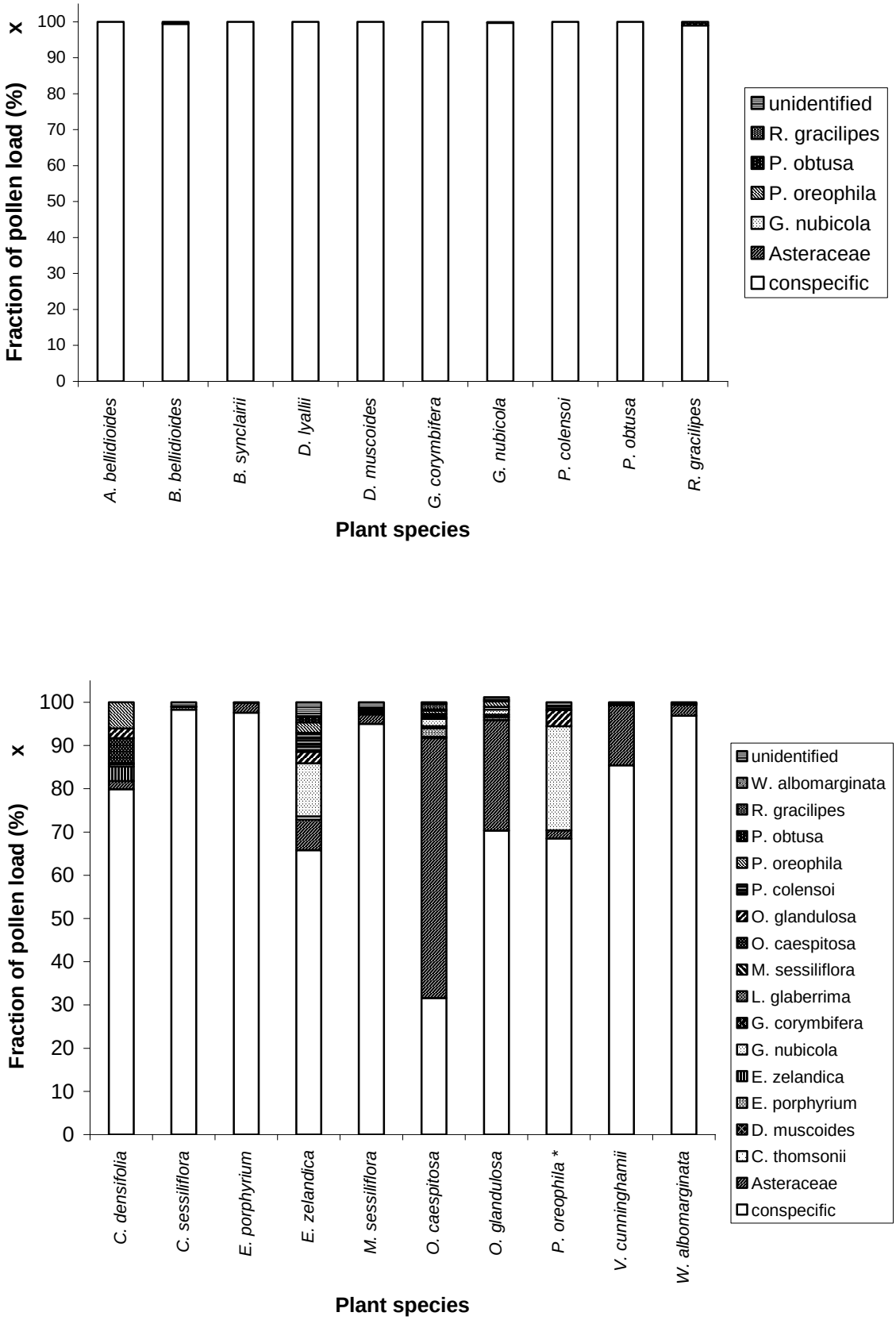


Fig. 4.11 Composition of the pollen loads on receptive stigmas (*P.oreophila=dioecious species).

Apart from the lepidopterans (Paranotoreas, *Aletia, Dasyuris*) and the Bibionid fly (*Dilophus*) all insects carry diverse pollen loads suggesting that floral fidelity is low and insects switch readily between flowers of different plants. However, the delivery of pollen to receptive stigmas may still be accurate, e.g. if the pollen is being attached to different body parts of the insect during the visit of a flower of one particular species. Pollinator service is inadequate if the stigma is being contaminated with heterospecific pollen because this decreases the available space for conspecific pollen on the stigmatic surface having a detrimental effect on the plants' reproductive output and fitness. Given the fact that flower visitors carry such diverse pollen loads the potential for stigma contamination is high. Fig 4.11 illustrates the degree of stigma contamination with heterospecific pollen within the alpine community. Conspecific pollen transfer could always be detected and the fraction of heterospecific pollen was often marginal with values of less than 1%. However, *Ourisia caespitosa* received up to 70% of heterospecific pollen, clearly restricting germination of conspecific pollen grains. From these findings a network could be calculated depicting interspecific pollen transfer (Fig 4.12). The links connecting species in this 1-mode network equal at least one pollinator that transfers pollen between species. Species that have several arrows pointing at them receive heterospecific pollen from many sources and stigma contamination is potentially high. Species that have no arrows pointing at them do not experience stigma clogging with heterospecific pollen. All species displayed conspecific pollen on their stigmas which is not indicated by an arrow here.

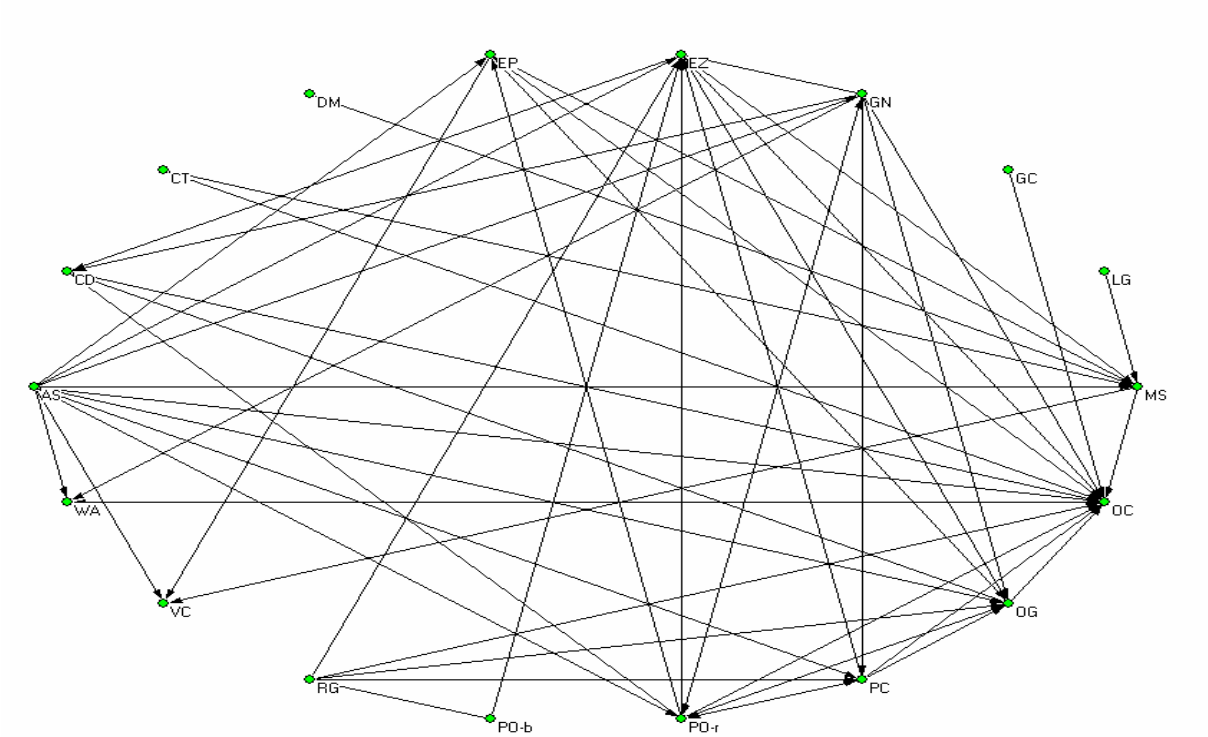


Fig. 4.12 Stigma contamination on receptive stigmas. In this network, plan species are displayed as green dots. Insect visitors are represented by black links. A link between two plants species indicates at least one pollinator that visits flowers of both species. Arrows indicate the direction of pollen transfer. AS equals unidentified Asteraceae pollen. Note: conspecific pollen transfer not depicted by arrow but always documented.

4.3.6 Comparing the emerging networks

In order to compare the three networks drawn from different data sources describing the flower visitor – plant network in the alpine community four commonly recognised parameters were calculated (Table 4.6)

Table 4.6 Summary of network parameters. Size equals plant species x animal taxa or group, PI equals potential interactions, DI equals documented interactions, Fill equals connectance index, DC equals degree of centralisation, N equals absolute nestedness, N* equals relative nestedness, p-value equals the significance level obtained testing for nestedness with Nestedness Temperature Calculator

Network	Size (PxA)	PI	DI	Fill (%)	DC	N	N*	p-value
Observations	23 x 11	253	131	53.0	0.39	0.71	0.61	< 0.05
Voucher	23 x 21	483	56	11.6	0.15	0.84	0.19	0.39
Pollen	24 x 21	504	175	38.0	0.34	0.78	0.78	<0.0001

Connectance is the proportion of the interactions realised in the network out of the total possible, a measure of the generalisation level of the community. Pollination networks have, in general, a relatively low connectance. Nestedness occurs when generalists species interact both with other generalists and also with specialists ones, whereas specialist species only interact with generalists. This leaves a dense core of generalist species interacting between them and with a periphery of specialist species attached to the core, the so-called core-periphery structure. To compare networks of different sizes relative nestedness N* was calculated (Nielsen and Bascompte 2007). The degree of centralisation illustrates the differences in species connectivities. Networks with a high DC have some highly generalist species (species with a high connectivity) and a high number of specialist species (those with a very low connectivity; Wasserman and Faust 1994, de Nooy *et al.* 2004). The generalists may be envisioned as highly connected hubs between the specialist species in the periphery. Both nestedness and centralisation reflect a high level of asymmetric interactions where specialists interact mostly with generalists.

The observations network and the pollen-load network appear fairly similar in structure while the voucher-specimens network differs considerably since the number of documented interactions is so low. A look at the network graphs presented above already illustrated this. The levels of connectance (Fill) for the link-rich networks are relatively high with a fill of 53% being one of the highest published (Nielsen and Bascompte 2007). This indicates a high level of generalisation in the interaction patterns. The voucher specimen network fails to deliver the same conclusion with a connectance of 11.6%.

In terms of the degree of centralisation again the link-rich networks have similar values which represent an intermediate degree of centralisation compared to literature values (Petanidou *et al.* 2008). This indicates an asymmetric distribution of links in the community. The voucher-specimens network data suggests a much lower degree of centralisation because a fewer number of interactions has been recorded and therefore this network is built with very little sampling effort. If conclusions about a community are solely based on this sampling method, they may be misleading.

Nestedness as a measure of asymmetry of interactions in the community is significant for both high-interaction networks while the voucher-specimens network does not display a nested structure. Networks are directly comparable via the relative nestedness N* and again the observation and the pollen-load network score similar

values with the pollen-load network being the most nested of the three. The voucher-specimens network has a low relative nestedness. Overall, it does not display the interaction patterns in the community as accurately as the other two networks.

All the interaction networks drawn contain qualitative interaction information only. The pollen-loads-on-insects-bodies network is the most important as these interactions can be regarded as confirmed potential pollination events since the visitors are definitely carrying pollen. A visit from an insect carrying pollen may be beneficial for the plant if conspecific pollen is deposited and detrimental if stigma contamination with heterospecific pollen occurs. The analysis of the flower visitor – plant networks suggests a moderate to high degree of generalisation. Therefore the potential for unwanted pollen delivery may be high.

If the 2-mode network depicting interactions between visitors and plants is transformed to a 1-mode network where plants are represented by nodes and insects by the links connecting them, a direct comparison between the contamination potential and the actual contamination is possible (Fig. 4.13). The connectance of the potential-contamination-through-cross-visitation network (Fig. 4.13a) is very high while the connectance of the actual-contamination-observed network (Fig. 4.13b) is considerably lower. Obviously only a fraction of the visits by insects to flowers in the community are depicting actual pollination events. However, they may display the potential for interactions outside the plant – pollinator mutualism, such as illegitimate robbing of rewards or herbivory. To describe plant – pollinator relationships in detail it appears to be essential to measure actual pollination events rather than interaction frequencies on the visitation level only. While the majority of flower visitors in the community may display low floral fidelity and a rather generalistic foraging behaviour, the plants do not necessarily suffer from inaccurate pollinator delivery as predicted by interaction pattern alone.

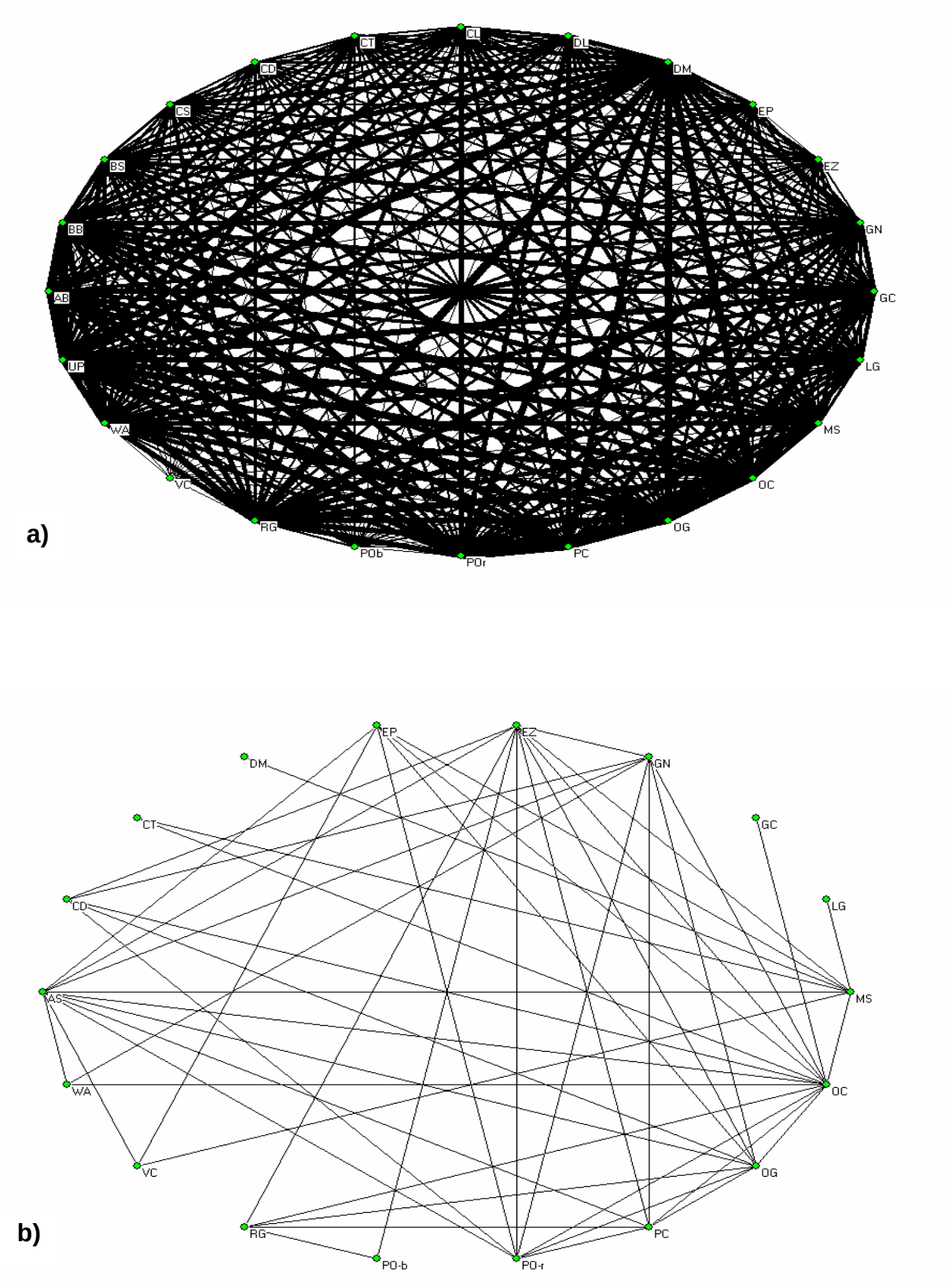


Fig. 4.13 Stigma contamination with heterospecific pollen deposition by pollinators. (a) Contamination potential derived from the number of interactions in the community (b) Actual contamination demonstrated in the community (plants green nodes, visitors black links, each link represents at least one insects transferring pollen. Both networks transformed to undirected 1 mode network format).

4.4 Floral attractants

The main signals that flowers employ to attract their insect visitors are colour and scent. An analysis of both has been carried out to characterise the way plant advertise their rewards to insects in the New Zealand alpine flora.

4.4.1 Flower colour

Flower colour reflectance spectra from the petals of 19 species were obtained (Fig. 4.14). The single reflectance spectra for all species can be found in App. 7.3. Most species in the community have white, pale pink or blue or strongly yellow corollas. The species *B. bellidoides, D. lyallii* and *R. gracilipes* exhibit a strong UV mark around 360 nm. The spectra differ in intensity but a similar trend can be detected for most of them. The reflectance curves have steep gradients at about 390nm and 500nm, which fits with previous findings that Northern hemisphere flower colours evolved to suit the visual capabilities of trichromatic pollinators (Chittka and Menzel 1992, Chittka 1996).

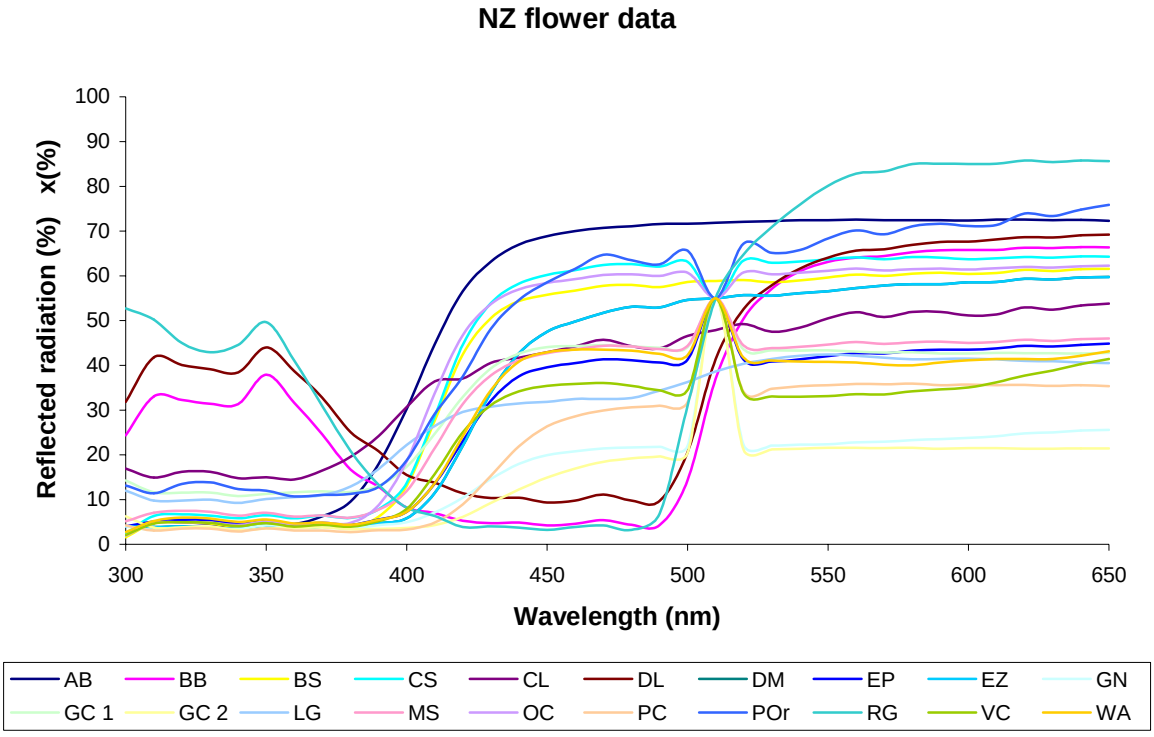


Fig. 4.14 Colour reflectance spectra for petals of 19 species of the community.

In order to asses the colour difference between flowers in the community and to test whether a pollinator could distinguish between flowers making colour based choices a model was calculated plotting the colours in a bee colour space hexagon (Fig. 4.15). The absolute distance for all flower colours can be found in App. 7.3. The bee colour space is model is the only model to date that allows modelling colours according to animal perception incorporating actual receptor measurements. A considerable proportion of the flower visitors in the community are native and introduced bees so the presented results apply to them first and foremost. Moreover, there is evidence that the visual system of most insects is similar to a certain extent so the findings may be of relevance to other visitor groups as well.

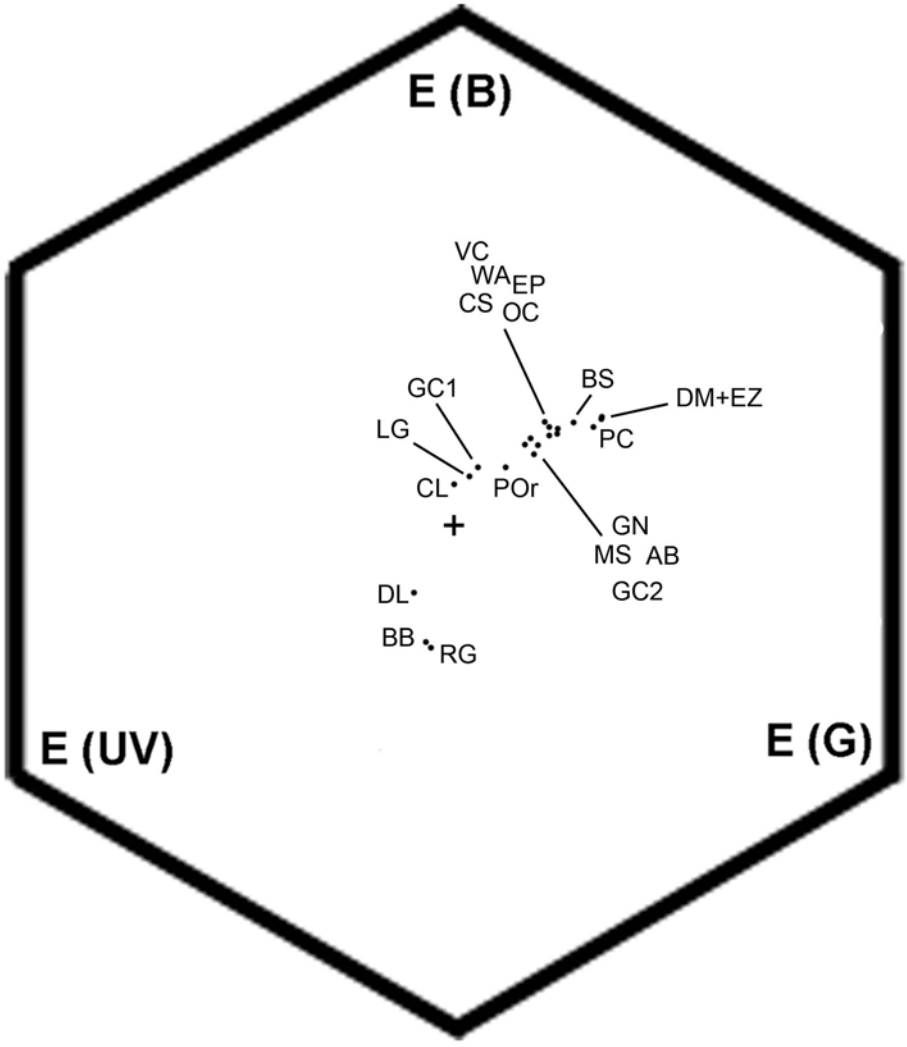


Fig. 4.15 Hexagon model of bee colour space. Trichromatic visual system with three receptor types (G=green 540 nm, B=blue 440 nm, UV= UV 350 nm). (GC1= petal tip, GC2= petal base).

The flower colours cluster into several groups that are below the discrimination threshold of an average bee. The minimum colour distance that has been demontrated

to be discriminated by bees with approximately 70% accuracy. is ≈ 0.05 hexagon units (Dyer and Chittka 2004). The following groups emerge: (1) *A. bellidoides, G. corymbifera* (petal base),*G. nubicola* and *M. sessiliflora* form one cluster, (2) *D. muscoides, E. zelandica* and *P. colensoi* another, (3) *C. sessiliflora, E. porphyrium, O. caespitosa, V. cunninghamii* and *W. albomarginate* a third and (4) *C. lanata, G. corymbifera* (petal tip), *L.glaberrima* and *P.oreophila* a fourth. All theses flowers appear white or near white to humans. (5) *B. bellidoides* and *R. gracilipes* cluster in human yellow while (6) *D. lyallii* is also yellow and represents the only flower that is distinguishable from all other flowers (for portrait pictures of all flowers see App. 7.1). All flowers were distinguishable from a green leaf background. An average bee would not be able to discriminate between flowers within these six clusters and would require additional cues like morphology or scent to make a choice. However, learning has been shown to improve bees' colour choices down to a threshold of 0.01 hexagon units (A. Dyer pers. communication) so association with positive or negative rewards experienced may potentially increase discrimination ability based on colour choice.

4.4.2 Floral scent

In the flowers of the 19 investigated species 98 volatile compounds were detected of which 95 were identified at least to compound class. A summary of the floral scent chemistry showing the relative amounts of volatiles, and their distribution among the five main chemical compound classes is given in Table 4.7 and Table 4.8 and Figures 4.16 and 4.17. The compounds are ordered in classes, which to some degree reflect their biosynthetic origin (see Knudsen *et al.* 1993). The present analyses identified a wide variety of volatile compounds across all species examined, including monoterpenoids, sesquiterpenoids, fatty acid derivatives, benzenoids, sulphur-containing compounds and nitrogen-bearing compounds. More than half of the occurring compounds were isoprene derivatives, including 34 sesquiterpenoids, and 24 monoterpenoids. Benzenoid compounds made 14 of the compounds (including benzenoid alcohols, aldehydes and esters) and 24 of the compounds were fatty-acid derivatives (Table 4.7 and 4.8). The remaining compounds comprise one nitrogen-bearing compound (Indole) and one sulphur-containing compound (Benzothiazole). Three compounds could not be identified. Most of the compounds were detected only in small relative amounts, and only 18 compounds (eight aliphatics, five benzenoids,

and five isoprenoids) reached a relative amount of at least 20% in any of the species. The number of scent compounds varied markedly between species, ranging from six in *W. albomarginata* and *G. corymbifera* to 28 compounds in *O. caespitosa*.

The most widespread compound was Benzaldehyde occurring in all investigated species. Other common compounds were Limonene (11 species), 3-Hexene-1-ol acetate (11 species), Linalool (1,6-Octadiene-3-ol, 3,7-dimethyl) (11 species), Benzyl alcohol (11 species), p-Anisaldehyde (9 species), *beta*-Caryophyllene (9 species), and Butyl acetate (8 species). Different species emitted quite different volatiles or volatile patterns, and 51 of the 98 detected compounds were found only in one of the studied species. In general, scent of most species was either dominated by high relative amounts of benzenoids (e.g. Benzaldehyde, Benzyl alcohol, Methyl benzoate, p-Anisaldehyde), monoterpenoids (e.g. Linalool, Hotrienol see Fig. 4.17a) and b) or aliphatics (e.g. Butyl acetate, *cis*-3-Hexen-1-ol, 3-Hexen-1-ol acetate). With the exception of seven species (*B. sinclairii*, *E. porphyrium*, *E. zelandica*, *G. nubicola*, *O.caespitosa*, *O. glandulosa*, *P. oreophila*) all species had a high relative content (>30%) of aliphatics (Fig. 4.18) that was associated with a high content of benzenoids (Fig. 4.18). Four of the seven species with a low content of aliphatics had high relative contents of sesquiterpenes (>20%) (*B. sinclairii, E. zelandica,O. caespitosa*, *O. glandulosa*) and two of them (*P. oreophila, G. nubicola*) had high relative amounts of monoterpenes (>20%).

The Bray Curtis-MDS analysis based on the relative abundance of the compounds showed no clear groupings and almost all species formed one cloud (Fig. 4.19). *P. colensoi* was the only species separated from the other species, due the dominance of a single compound in its floral odour 4-Hexen-1-ol acetate that made 90.1% of the relative amount in the species.

Neither taxonomic relatedness nor breeding system nor flower visitor association seems to explain the pattern of floral scent distribution in the community. There was no correlation between the odour composition or the number of compounds and the breeding system of the investigated species (Fig. 4.20). For example in species with high autonomous selfing capability (*E. porphyrium, G. nubicola, P. colensoi*) the number of compounds emitted did not differ from plants that depend on pollination by insects (Fig. 4.20). In the same way the data gave no evidence for a link between flower visitor and pollinator assemblages and floral scent patterns. This indicates, although single species have species specific odour patterns, that species do not form

clusters within the community that are characterized by distinct odour types and are associated with different pollinator types. This may imply that odour does not play a major role in attracting flower visitors. However, interestingly a trend for plants that grow in cushions (and other very small plants with minuscule flowers) could be detected. They form a group in the odour space (Figure 4.20). These plants are characterised by high relative amounts of Limonene, p-Anisaldehyde and Benzyl alcohol compounds that could be involved in attracting pollinators. It can be speculated that in cushion plants, where visual features are less prominent than in plants with erect inflorescences, odours might play an important role to attract flower visitors.

Table 4.7 Compounds isolated by GC-MS from headspace samples of 10 species (26 samples) of a New Zealand alpine community. Compounds are listed in order of increasing retention time within each compound class. RRT = Relative Retention Time. Relative amounts (%). tr = trace amount (< 0.1 % of total sample). AB = *Anaphalioides bellidioides*, BB = *Brachyglottis bellidioides,* BS = *Brachyscome sinclairii*, CD = *Chionohebe densiflora*, CL = *Craspedia lanata*, DL = *Dolichoglottis lyallii*, DM = *Dracophyllum muscoides*, EP = *Epilobium porphyrium*, EZ = *Euphrasia zelandica*, GC = *Gentianella corymbifera*.

Compound	**RRT**	**Criteria** *	AB	BB	BS	CD	CL	DL	DM	EP	EZ	GC
Number of samples analysed			1	3	3	3	1	3	3	3	3	3
Aliphatics												
Ketones												
2-Nonanone	1137	b	-	-	-	-	36.7	-	-	-	-	-
2-Butanone	1243	a	-	4.1	-	-	-	-	-	-	-	-
2-Undecanone	1452	a	-	-	-	-	10.7	-	-	-	-	-
Alcohols												
1-Butanol	799	a	-	29.6	-	tr	-	-	-	-	-	-
trans-3-Hexen-1-ol	1098	b	-	1.7	-	-	-	-	-	-	-	-
cis-3-Hexen-1-ol	1124	b	3.0	-	-	36.8	-	-	-	-	-	-
1-Octen-3-ol	1209	c	0.3	-	-	-	-	-	-	-	-	-
1-Hexadecanol	2318	a	-	-	-	-	1.8	-	-	1.3	11.0	24.3
Aldehydes												
2-Hexenal, (E)-	895	a	0.7	-	-	-	-	-	-	-	-	-
Esters												
Butyl acetate	706	b	16.5	14.8	tr	-	15.4	42.1	20.5	-	-	-
Hexyl acetate	985	b	4.4	-	-	0.9	-	-	-	-	-	-
4-Hexen-1-ol, acetate	1042	a	3.5	-	-	-	-	-	-	-	-	-
3-Hexen-1-ol, acetate	1045	b	32.7	11.5	-	52.2	-	-	10.6	-	-	5.4
2-Hexen-1-ol, acetate, (E)-	1065	a	0.4	-	-	-	-	-	-	-	-	-
Unidentified hydrocarbon ester	1721	a	-	-	-	-	1.5	-	-	-	-	-
Hexadecanoic acid, 1-methylethyl ester	2180	a	-	-	-	-	2.0	-	-	-	-	-
Acids												
Acetic acid	1223	b	-	tr	-	-	6.9	-	-	-	-	28
Hydrocarbon acid	1823	a	-	-	-	-	0.4	-	-	-	-	-
Benzenoids												
Methoxybenzene	1075	a	-	-	-	-	-	18.5	-	68.6	-	-
Benzaldehyde	1318	c	6.7	26.7	3.0	6.9	2.3	11.5	7.0	8.7	7.2	25.8
Methyl benzoate	1431	c	28.2	-	-	tr	-	-	-	-	-	5.0
Benzyl alcohol	1739	c	-	11.6	-	-	2.8	10.0	0.9	7.3	0.6	11.4
Phenylethyl alcohol	1761	a	-	-	-	-	-	-	1.9	-	0.2	-
Unidentified benzenoid compound	1851	a	-	-	-	-	1.5	10.6	-	2.9	1.6	-

Compound	**RRT**	**Criteria** *	AB	BB	BS	CD	CL	DL	DM	EP	EZ	GC
p-Anisaldehyde	1900	c	-	-	-	-	0.7	-	43.8	0.6	0.4	-
Isoprenoids												
Monoterpenes												
Limonene	884	a	3.1	tr	-	-	-	5.5	2.6	2.3	17.9	-
beta-Phellandrene	915	a	-	tr	tr	-	-	-	-	-	-	-
Eucalyptol	901	a	-	tr	6.2	-	-	-	-	-	-	-
Linalool	1330	c	0.2	-	4.7	tr	16.6	-	3.6	0.8	tr	-
Lilac aldehyde A	1369	b	-	-	-	-	-	-	2.4	-	-	-
Lilac aldehyde B	1382	b	-	-	-	-	-	-	2.9	-	-	-
Lilac aldehyde D	1412	b	-	-	-	-	-	-	1.4	-	-	-
beta-Terpineol	1460	b	-	-	0.6	-	-	-	-	-	-	-
Lilac alcohol B	1582	b	-	-	-	-	-	-	1.9	-	-	-
2,6-Dimethyl-3,7-Octadiene-2,6-diol	1790	b	-	-	-	-	0.4	-	-	-	-	-
Sesquiterpenes												
Unidentified Sesquiterpene	1273	a	-	-	tr	-	-	-	-	-	-	-
Unidentified Sesquiterpene	1331	a	-	-	2.1	-	-	-	-	-	-	-
Unidentified Sesquiterpene	1376	a	0.1	-	-	-	-	-	-	-	7.5	-
Unidentified Sesquiterpene	1388	a	-	-	11.8	-	-	-	-	-	-	-
Unidentified Sesquiterpene	1401	a	-	-	-	-	-	-	-	-	6.7	-
Unidentified Sesquiterpene	1403	a	-	-	tr	-	-	-	-	-	-	-
beta-Caryophyllene	1422	c	0.2	-	42.7	2.3	-	-	-	7.5	1.9	-
Unidentified Sesquiterpene	1429	a	-	-	tr	-	-	-	-	-	-	-
Allo-Aromadendrene	1471	a	-	-	-	-	-	-	-	-	32.7	-
Unidentified Sesquiterpene	1487	a	-	-	-	-	-	-	-	-	2.7	-
alpha-Humulene	1492	b	-	-	17.8	0.9	-	-	-	-	6.1	-
Unidentified Sesquiterpene	1501	a	-	-	-	-	-	-	-	-	0.1	-
Unidentified Sesquiterpene	1519	a	-	-	3.7	-	-	-	-	-	-	-
Germacrene D	1541	b	-	-	tr	-	-	-	-	-	-	-
Unidentified Sesquiterpene	1561	a	-	-	1.5	-	-	-	-	-	-	-
Unidentified Sesquiterpene	1569	a	-	-	1.4	-	-	-	-	-	-	-
Unidentified Sesquiterpene	1573	a	-	-	-	-	-	-	-	-	0.4	-
Unidentified Sesquiterpene	1578	a	-	-	0.3	-	-	-	-	-	-	-
Unidentified Sesquiterpene	1616	a	-	-	4.2	-	-	-	-	-	-	-
Sulphur-containing compounds												
Benzothiazole	1820	b	-	-	-	-	0.3	1.9	-	-	-	-
Nitrogen-containing compounds												

Compound	**RRT**	**Criteria** *	AB	BB	BS	CD	CL	DL	DM	EP	EZ	GC
Indole	2302	c	-	-	-	-	-	-	0.3	-	3.0	-
Total number of compounds			14	11	19	9	15	7	13	9	17	6

* Compound identification criteria= comparison of MS with published data (e.g. NIST library), b = comparison of MS and retention time with published data, c = comparison of MS and retention time with authentic standard.

Table 4.8 Compounds isolated by GC-MS from headspace samples of nine species (21 samples) of a New Zealand alpine community. Compounds are listed in order of increasing retention time within each compound class. RRT = Relative Retention Time. amounts (%). tr = trace amount (< 0.1 % of total sample). GN = *Gaultheria nubicola,* LG = *Lobelia glaberrima*, MS = *Montia sissiflora*, OC = *Ourisia caespitosa*, OG = *Ourisia glandulosa*, PC = *Phyllachne colensoi*, POr = *Pimelea oreophila*, RG = *Ranunculus gracilipes*, WA = *Wahlenbergia albomarginata.*

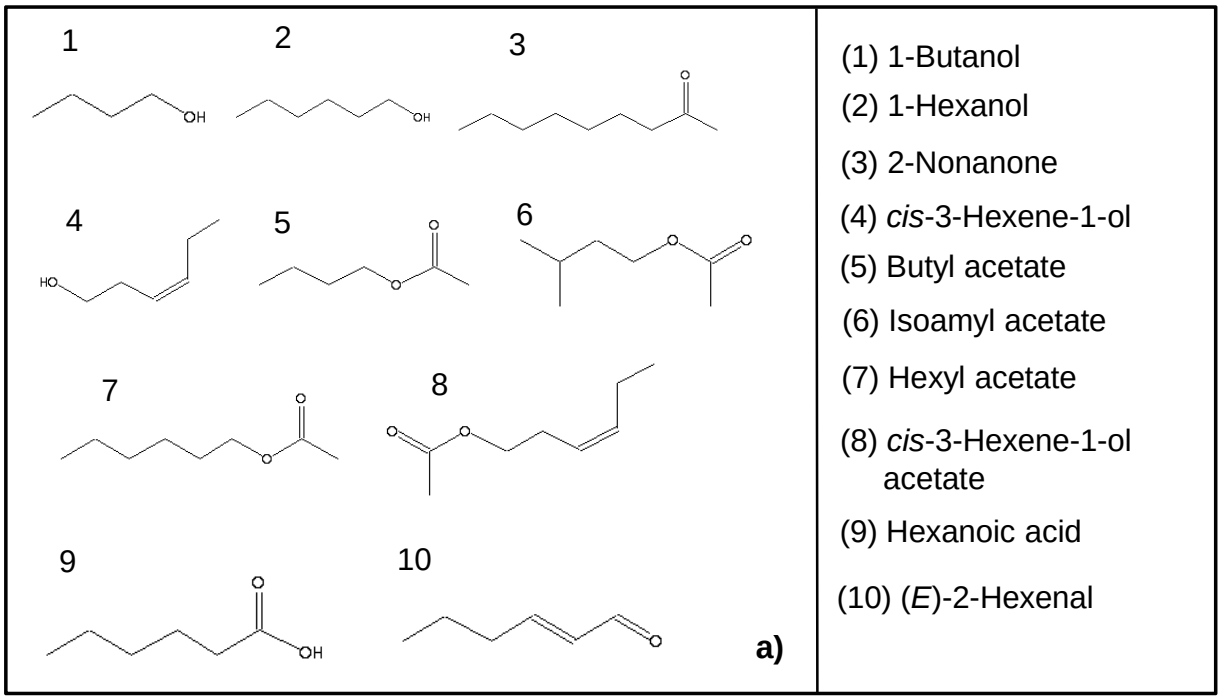
Compound	**RRT**	**Criteria ***	GN	LG	MS	OC	OG	PC	POr	RG	WA
Number of samples analysed			2	2	3	3	3	1	3	2	2
Aliphatics											
Ketones											
2-Butanone	1243	a	-	-	-	0.4	-	-	-	-	-
Alcohols											
1-Butanol	799	a	-	-	-	-	-	-	-	-	21.2
Hydrocarbon alcohol	873	a	-	-	-	-	-	1.7	-	-	-
1-Hexanol	1095	c	-	-	-	-	2.0	-	-	-	-
trans-3-Hexen-1-ol	1098	b	0.1	-	tr	-	-	-	-	-	-
cis-3-Hexen-1-ol	1124	b	-	-	-	1.1	-	0.1	-	6.0	-
Esters											
Butyl acetate	706	b	-	25.3	73.5	-	-	-	-	-	-
Isoamyl acetate	755	b	-	-	-	-	-	3.1	-	-	-
Hexyl acetate	985	b	-	-	-	-	-	-	-	tr	-
4-Hexen-1-ol, acetate	1042	a	-	-	-	-	-	90.1	-	-	-
3-Hexen-1-ol, acetate	1045	b	1.3	-	-	0.7	1.5	-	7.5	28.9	12.3
Unidentified hydrocarbon ester	1721	a	-	-	-	-	-	0.7	-	-	-
Unidentified hydrocarbon ester	1726	a	-	-	-	-	-	0.7	-	-	-
Acids											
Acetic acid	1223	b	-	4.6	3.5	-	-	-	-	14.5	30.1
Butanoic acid, 2-methyl-	149-	a	0.1	-	-	-	-	-	-	-	-
Hexanoic acid	1702	b	-	0.3	-	-	-	-	-	-	-
Hydrocarbon acid	1823	a	-	0.1	-	-	-	-	-	-	-
Benzenoids											
para-Cymene	979	b	-	-	-	0.6	0.6	-	-	-	-
1-Methoxy-4-methyl-benzene	1202	b	-	-	-	-	-	-	20.1	-	-
Benzaldehyde	1318	c	4.0	5.7	6.5	3.5	9.4	0.6	0.9	21.9	26.1
Methyl benzoate	1431	c	-	-	-	0.2	tr	-	tr	tr	-
Unidentified benzenoid compound	1642	a	-	-	-	-	-	-	0.9	-	-
Phenylethyl acetate	1662	b	-	1.4	-	-	-	-	-	-	-
Benzyl alcohol	1739	c	0.3	1.3	4.2	-	-	-	0.3	-	-
Phenylethyl alcohol	1761	b	0.2	5.6	-	-	-	-	-	-	-

Compound	**RRT**	**Criteria ***	GN	LG	MS	OC	OG	PC	POr	RG	WA
Unidentified benzenoid compound	1851	a	-	0.9	5.8	-	-	0.2	-	-	-
p-Anisaldehyde	1900	c	0.7	0.1	4.3	-	-	0.1	-	-	5.0
para-Cresol	1949	b	-	-	-	-	-	-	4.0	-	-
Benzyl benzoate	2594	c	-	0.4	-	-	-	-	-	-	-
Isoprenoids											
Monoterpenes											
Limonene	884	b	2.5	6.1	2.2	-	tr	-	-	tr	-
beta-Phellandrene	915	a	-	-	-	-	-	-	-	-	-
Eucalyptol	901	b	-	-	-	-	tr	-	-	-	-
cis-Ocimene	927	c	-	-	-	-	-	-	9.3	-	-
Unidentified Monoterpene	944	a	-	-	-	0.6	-	-	-	-	-
trans-Ocimene	957	b	-	-	-	-	1.1	-	13.2	tr	-
trans-Linalool oxide (furanoid)	1206	c	3.7	-	-	-	-	-	tr	-	-
trans,trans-2,6-Dimethyl-1,3,5,7-octatetraene	1220	b	1.5	-	-	-	-	-	1	-	-
cis-Linalool oxide (furanoid)	1259	c	0.5	-	-	-	-	-	-	-	-
Linalool	1330	c	40.6	-	-	-	18.5	-	16.9	6.4	-
Hotrienol	1413	b	29.8	-	-	-	-	-	-	-	-
Epoxylinalool	1575	a	1.2	-	-	-	-	-	-	-	-
cis-Linalool oxide (pyranoid)	1592	b	-	-	-	-	-	-	tr	-	-
Unidentified monoterpene	1610	a	0.1	-	-	-	-	-	-	-	-
2,5-Hexanediol, 2,5-dimethyl-	1613	a	-	38.0	-	-	-	-	-	-	-
2,6-Dimethyl-3,7-Octadiene-2,6-diol [d]	1790	b	9.7	-	-	-	-	-	3.8	-	-
Sesquiterpenes											
alpha-Cubebene	1226	a	-	-	-	1.7	-	-	-	-	-
Unidentified Sesquiterpene	1230	a	-	-	-	-	-	0.3	-	-	-
Nerolidol	1241	b	-	-	-	tr	-	-	-	-	-
Unidentified Sesquiterpene	1273	a	-	-	-	2.3	-	-	-	-	-
beta-Bourbonene	1309	a	-	-	-	1.4	-	-	-	-	-
Unidentified Sesquiterpene	1331	a	-	-	-	1.8	-	0.7	-	-	-
Unidentified Sesquiterpene	1401	a	-	-	-	-	tr	1.9	-	-	-
Unidentified Sesquiterpene	1403	a	-	-	-	-	5.9	-	-	-	-
beta-Caryophyllene	1422	c	-	-	-	25.5	55.9	-	16.3	9.6	-
Unidentified Sesquiterpene	1429	a	-	-	-	tr	-	-	-	-	-
Unidentified Sesquiterpene	1461	a	-	-	-	0.6	-	-	-	-	-
(Z)-*beta*-Farnesene	1484	a	-	-	-	1.5	-	-	-	-	-
Unidentified Sesquiterpene	1487	a	0.3	-	-	-	-	-	-	-	-

Compound	**RRT**	**Criteria ***	GN	LG	MS	OC	OG	PC	POr	RG	WA
alpha-Humulene	1492	b	-	-	-	-	2.3	-	1.2	-	-
Unidentified Sesquiterpene	1501	a	0.6	-	-	3.1	-	-	-	-	5.2
Unidentified Sesquiterpene	1519	a	-	-	-	3.7	-	-	-	-	-
Germacrene D	1541	b	1.9	-	-	11.5	-	-	tr	-	-
Unidentified Sesquiterpene	1561	a	-	-	-	5.6	-	-	-	-	-
Unidentified Sesquiterpene	1569	a	tr	-	-	-	-	-	-	-	-
Unidentified Sesquiterpene	1574	a	-	-	-	13.8	-	-	-	-	-
alpha-Farnesene	1576	b	-	-	-	-	2.9	-	2.5	-	-
Unidentified Sesquiterpene	1597	a	1.0	-	-	2.8	-	-	-	-	-
Unidentified Sesquiterpene	1603	a	-	-	-	2.1	-	-	-	-	-
Unidentified Sesquiterpene	1611	a	-	-	-	5.1	-	-	-	-	-
Unidentified Sesquiterpene	1629	a	-	-	-	0.6	-	-	-	-	-
Unidentified Sesquiterpene	1640	a	-	-	-	0.8	-	-	-	-	-
Unidentified Sesquiterpene	1686	a	-	-	-	0.6	-	-	-	-	-
trans-Nerolidol	1867	a	-	-	-	-	-	-	2.0	-	-
Sulphur-containing compounds											
Benzothiazole	1820	b	-	0.2	-	-	-	-	-	-	-
Unknowns[+]											
m/z: 201*, 73, 57, 111, 95	1126		-	10.2	-	-	-	-	-	-	-
m/z: 189*, 149, 91, 81, 41, 164, 119	1400		-	-	-	8.4	-	-	-	-	-
m/z: 96, 42, 68, 54	1410		-	-	-	-	-	-	-	12.7	-
Total number of compounds			21	15	8	28	14	12	18	11	6

[*] Compound identification criteria: a = comparison of MS with published data (e.g. NIST library), b = comparison of MS and retention time with published data, c = comparison of MS and retention time with authentic standard.

[+] Mass fragments for unknowns are listed with the molecular ion first (if known) marked with *, followed by the base peak and other fragments in decreasing order of abundance.



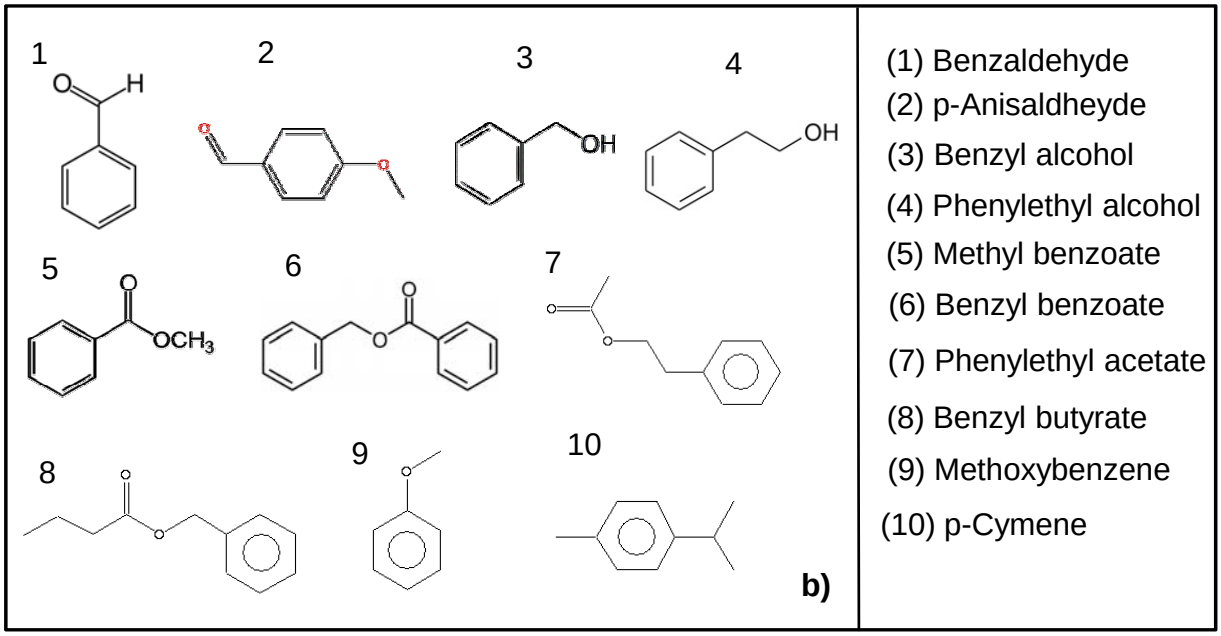
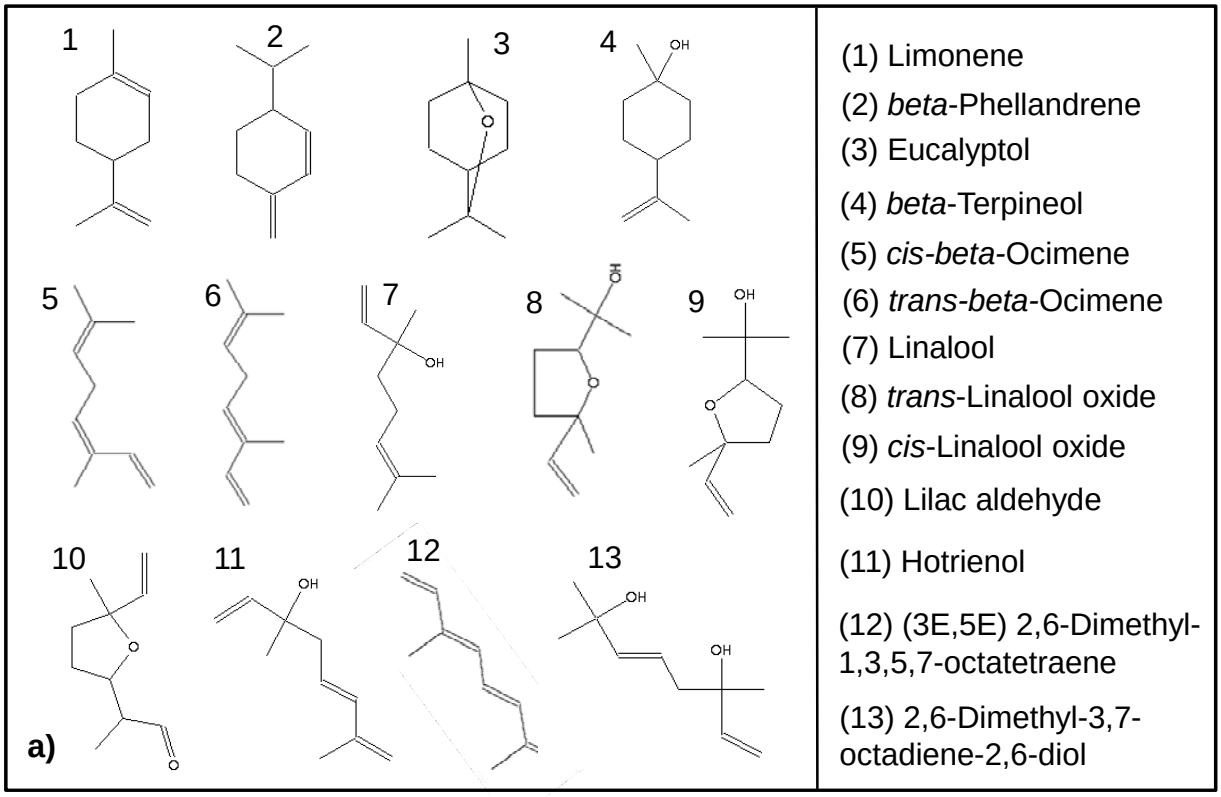


Fig. 4.16 Chemical structures of some aliphatic compounds (a) and benzenoids (b) found in the floral odour of an alpine New Zealand community.



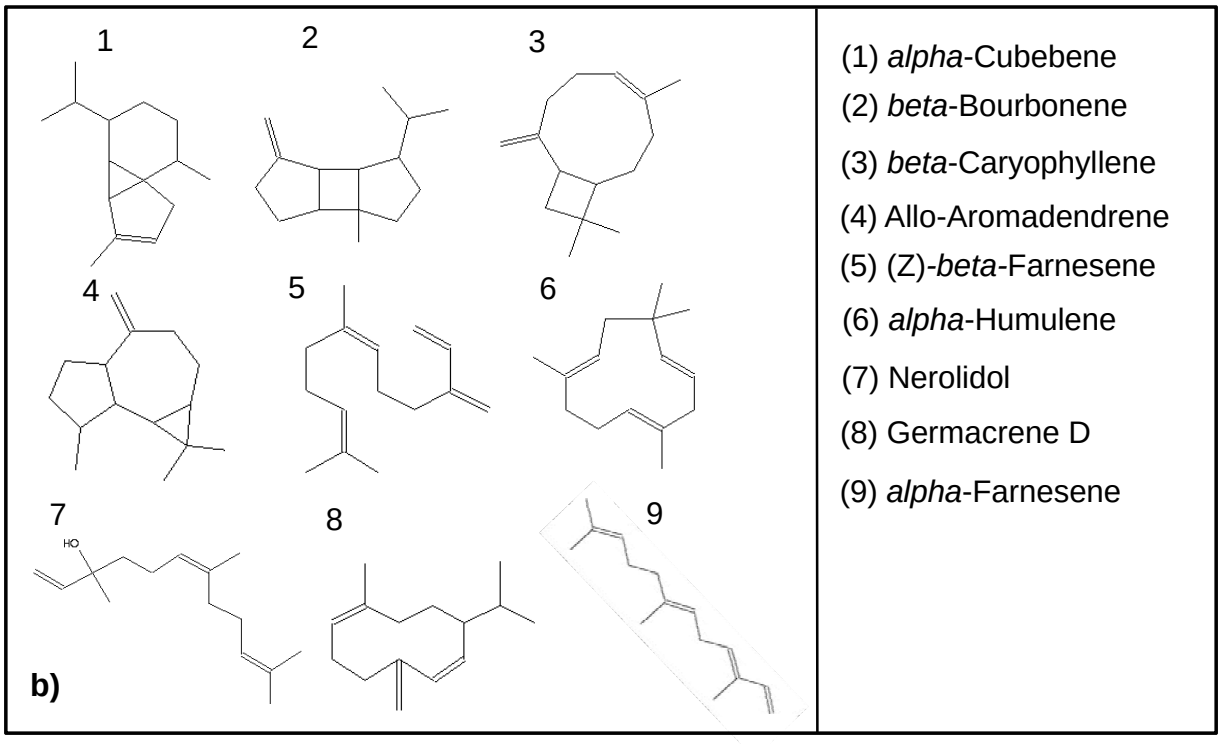


Fig. 4.17 Chemical structures of some monoterpenoids (a) and sesquiterpenoids (b) found in the floral odour of an alpine New Zealand community.

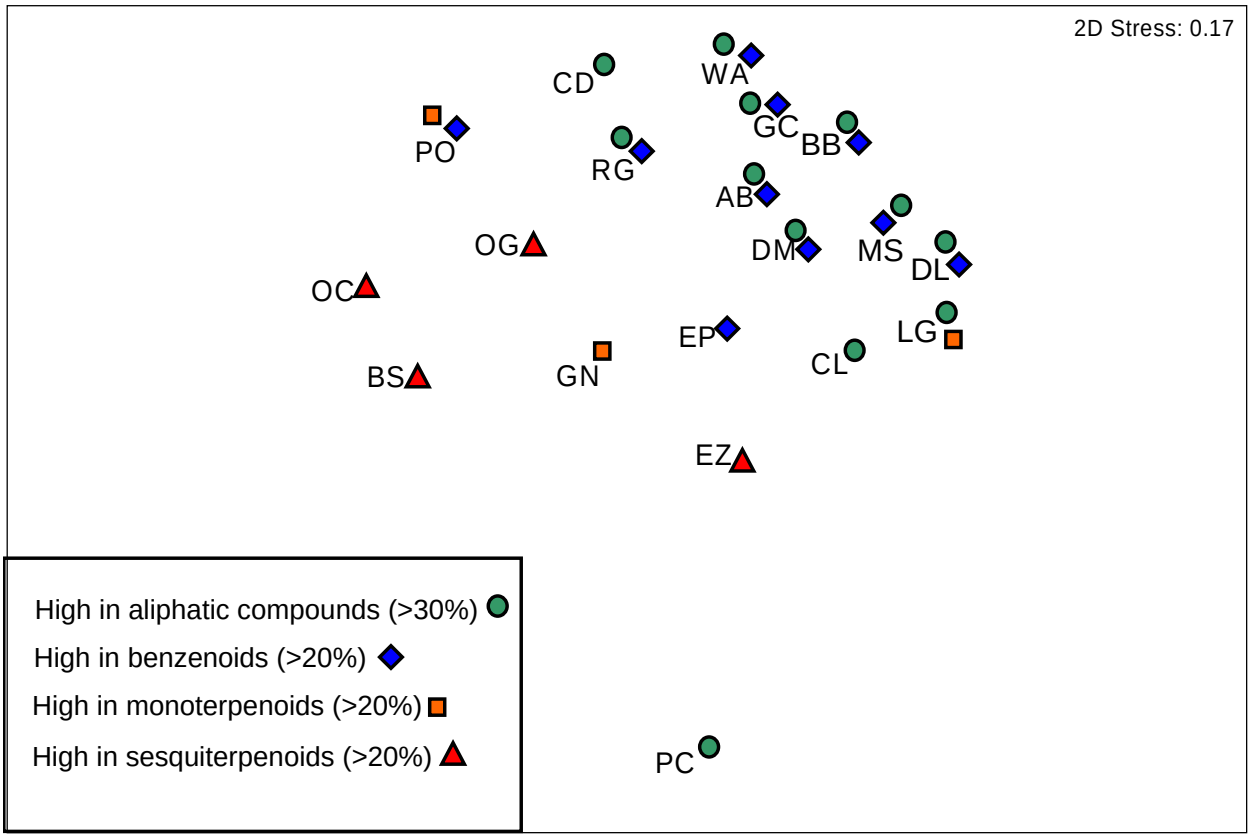


Fig. 4.18 Non-linear multidimensional scaling (NMDS) of the floral scent profiles of 47 samples (19 species) of an new Zealand alpine community based on the based on Bray–Curtis similarities – relative amounts. Relative amounts of compound classes (aliphatic compounds, benzenoids, monoterpenoids and sesquiterpenoids) for each species. Abbreviations: AB = *Anaphalioides bellidioides*, BB = *Brachyglottis bellidioides,* BS = *Brachyscome sinclairii*, CD = *Chionohebe densiflora*, CL = *Craspedia lanata*, DL = *Dolichoglottis lyallii*, DM = *Dracophyllum muscoides*, EP = *Epilobium porphyrium*, EZ = *Euphrasia zelandica*, GC = *Gentianella corymbifera*, GN = *Gaultheria nubicola,* LG = *Lobelia glaberrima*, MS = *Montia sissiflora*, OC = *Ourisia caespitosa*, OG = *Ourisia glandulosa*, PC = *Phyllachne colensoi*, POr = *Pimelea oreophila*, RG = *Ranunculus gracilipes*, WA = *Wahlenbergia albomarginata*.

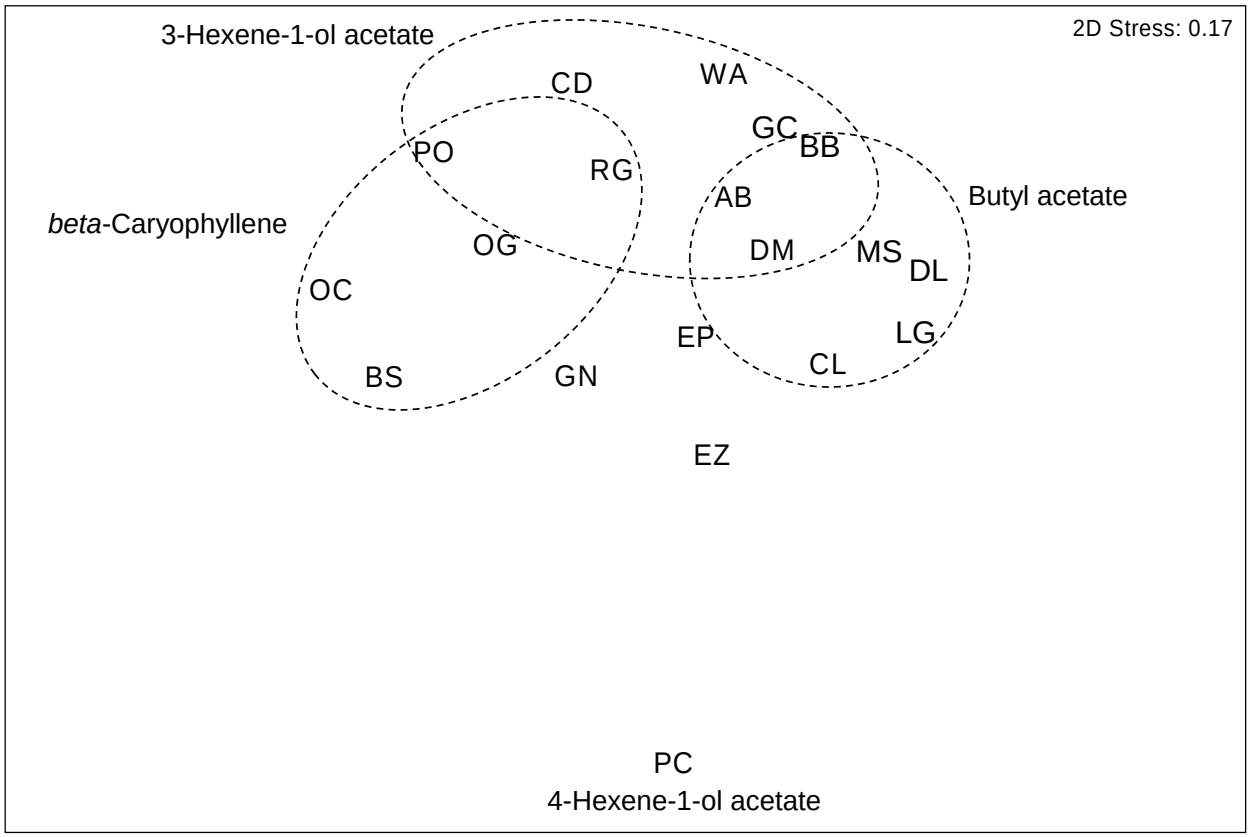


Fig. 4.19 Non-linear multidimensional scaling (NMDS) of the floral scent profiles of 47 samples (19 species) of an New Zealand alpine community based on the based on Bray–Curtis similarities – distribution. The average relative abundance was used for the analysis in cases, where more than one sample per species was collected. Species in the circle on the left side showed relative high amounts of *beta*-Caryophyllene. Species in the circle on the right side had relative high amounts of Butyl acetate. Species in the circle in the middle were characterized by relative high amounts of 3-Hexene-1-ol acetate. Stress value = 0.17. Abbreviations: *AB = Anaphalioides bellidioides, BB = Brachyglottis bellidioides, BS = Brachyscome sinclairii, CD = Chionohebe densiflora, CL = Craspedia lanata, DL = Dolichoglottis lyallii, DM = Dracophyllum muscoides, EP = Epilobium porphyrium, EZ = Euphrasia zelandica, GC = Gentianella corymbifera, GN = Gaultheria nubicola, LG = Lobelia glaberrima, MS = Montia sissiflora, OC = Ourisia caespitosa, OG = Ourisia glandulosa, PC = Phyllachne colensoi, POr = Pimelea oreophila, RG = Ranunculus gracilipes, WA = Wahlenbergia albomarginata.*

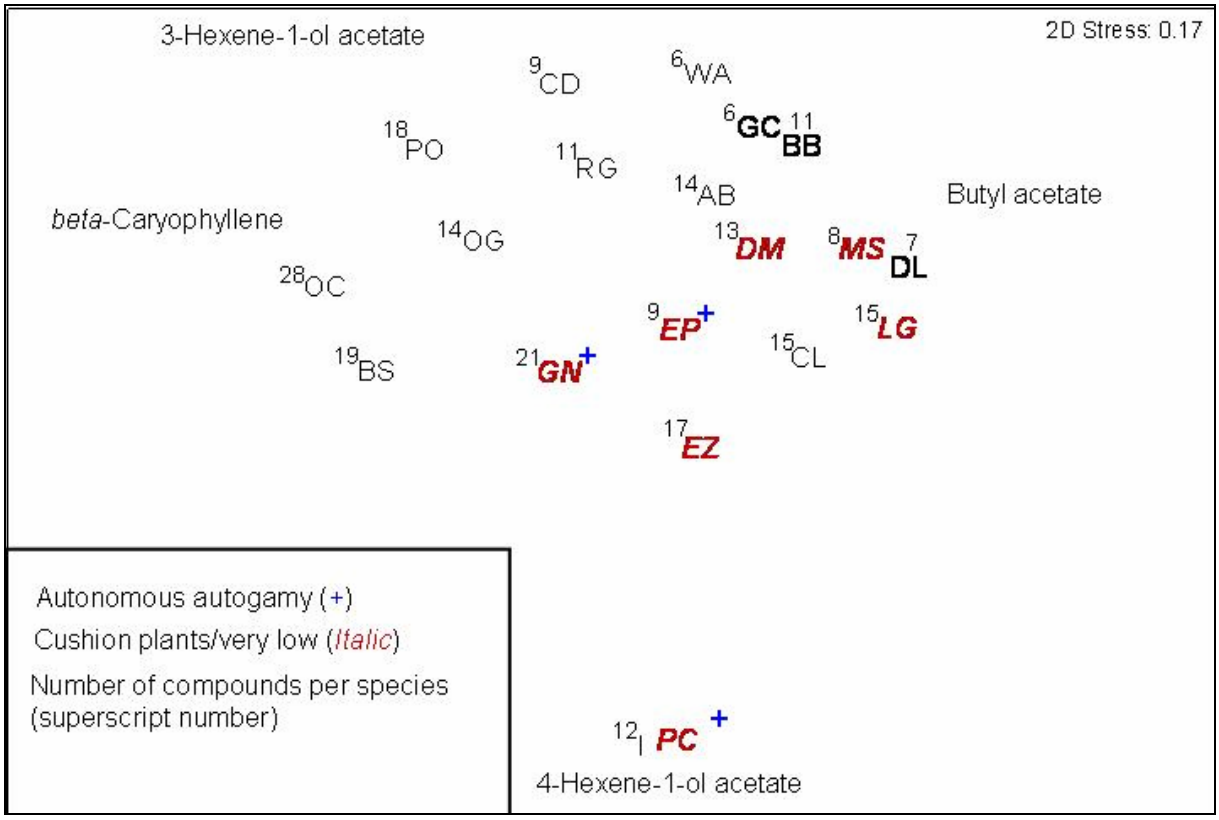


Fig. 4.20 Non-linear multidimensional scaling (NMDS) of the floral scent profiles, the occurrence of autonomous autogamy, low growing plants, and number of scent compounds per species of 19 species (47 samples) of an New Zealand alpine community based on the based on Bray–Curtis similarities. Abbreviations: *AB = Anaphalioides bellidioides, BB = Brachyglottis bellidioides, BS = Brachyscome sinclairii, CD = Chionohebe densiflora, CL = Craspedia lanata, DL = Dolichoglottis lyallii, DM = Dracophyllum muscoides, EP = Epilobium porphyrium, EZ = Euphrasia zelandica, GC = Gentianella corymbifera, GN = Gaultheria nubicola, LG = Lobelia glaberrima, MS = Montia sissiflora, OC = Ourisia caespitosa, OG = Ourisia glandulosa, PC = Phyllachne colensoi, POr = Pimelea oreophila, RG = Ranunculus gracilipes, WA = Wahlenbergia albomarginata.*

5 Discussion

The present thesis was designed to answer a set of questions in logical order. The results presented in Chapter 4 will now be discussed with respect to (a) the evidence they provide in relation to the original question and (b) the adequacy of the different methods employed to answer the questions posed.

5.1 Do plants in alpine New Zealand depend on pollinator service at all?

On a taxonomic level xenogamy could be confirmed for all members of the Asteraceae in the New Zealand alpine plant community. Asteraceae are, among others families, e.g., Rhamnaceae, Iridaceae or Solanaceae, renowned for high rates of self-incompatibility (Kress and Beach 1994, Bianchi *et al.* 2000). Further xenogamists in the community apart from the dioecious and gynodioecious members of the Thymelaeaceae and Stylidiaceae, respectively, include both members of the Ranunculaceae as well as the studied members of the families of Gentianaceae, Lobeliaceae, Orobanchaceae, Portulacaceae and Violaceae. Discrepancies on the taxonomic level arise within the Ericaceae and the Plantaginaceae where some members are xenogamous and others autonomously autogamous. Further autogamists belong to the families of the Onagraceae and the Stylidiaceae which are represented by the hermaphrodite flowers of the gynodioecious *Phyllachne colensoi*. Overall this suggests a constancy of the breeding system on the taxonomic level, i.e. the members of similar taxonomic affiliation will often exhibit a similar breeding system. For the genera *Epilobium*, *Ourisia* and *Ranunculus* previously published trends in breeding systems, i.e. autonomous autogamy in *Epilobium* and xenogamy in *Ourisa* and *Ranunculus*, could be confirmed for alpine New Zealand species (Brockie 1959, Fisher 1965, Raven and Raven 1976, Schlessman 1986).

Overall, dependence on pollinator service could be demonstrated for 20 plant species (87%) of the alpine community of which 14 species displayed full self-incompatibility (64%) and five species partial self-incompatibility (23%). Only three species (13%) could be classified as fully self-compatible and autonomously autogamous and thus, pollinator-independent. In contrast to these findings, high rates of autogamy have been predicted for New Zealand alpine herbs (Raven 1973, Wardle 1978) in general and several studies on small-stature New Zealand herbs of forests and alpine situations did in fact reveal high rates of autonomous selfing (e.g., *Cardamine*, Pritchard 1957, *Epilobium*, Brockie 1959 and Raven and Raven 1976, *Parahebe*, Garnock-Jones 1976). Moreover, New Zealand in general is frequently cited as having high levels of self-compatibility, as expected under Baker's Rule (Raven 1973, Godley 1979, Webb and Kelly 1993, Barrett 1996, Anderson *et al.* 2001, Bernadello *et al.*

2001, Schueller 2004). However, until the review of Newstrom and Robertson (2005) this remained a theoretical prediction rather than a verified fact. Newstrom and Robertson (2005) compiled an extensive data set on plant sexual reproduction in New Zealand and concluded that a majority of 79% of the New Zealand's plant species (including woody and herbaceous growth forms) are to some degree self-compatible and 21% of the entire New Zealand flora included in the evaluation is capable of autonomous autogamy. Thus, the rate of self-compatibility and autonomous self-pollination in the alpine community is very low compared to the other New Zealand plants. In comparison with alpine systems on a global scale, e.g. an alpine meadow in Chile (Arroyo and Squeo 1990) and a subalpine meadow in Canada (Pojar 1974) self-compatibility and autonomous selfing rates in alpine New Zealand appear very low.

The fact that in the alpine community under investigation both the percentage of self-compatible and autonomously self-pollinating species are substantially lower than the values for the entire New Zealand flora and alpine floras elsewhere suggests a more distinctive focus on outbreeding as well as a more pronounced pollinator dependency than expected. Globally several recent studies suggest that outcrossing is the predominant breeding system in alpine plants (Gugerli 1998, Bingham and Ranker 2000, Körner 2003). This meets the expectation for plants coping with a highly stochastic environment where genetic variability and the inferred ecologic flexibility are vital. It has been shown that regular self-fertilisation leads to inbreeding depression within a population and that strategies to avoid self-fertilisation are being evolved wherever possible (Barrett 2002). Thus, bottleneck effects will be avoided. The plant species of the New Zealand alpine community seem to follow a global trend in favouring a xenogamous breeding system that allows them the genetic flexibility that would be compromised by regular autogamy (Lloyd 1965).

Apart from pollen delivery the reproductive success of plant species in a New Zealand alpine community may not solely depend on pollination. In a challenging environment with harsh and unpredictable conditions, adequate pollination may not necessarily result in full fruit and seed set, or any reproductive output at all. For most species included in the investigation, failure to set fruit was common regardless of the experimental treatment. Resource limitation and herbivory among others have been demonstrated to influence seed set more distinctively than pollination (Herrera 1993). In conclusion, the plant species in the New Zealand alpine community can be characterised as an assemblage of predominantly outcrossing, fully or partially self-incompatible species that are pollinator-dependent in terms of reproductive success. The majority of flowers received adequate pollen delivery under natural conditions in

the seasons recorded. This is surprising, as it has been shown that pollen limitation in general is very common in the New Zealand flora (Newstrom and Robertson 2005). However, pollen limitation in terms of fruit set limitation could only be demonstrated for *Gaultheria nubicola*, and pollen-limitation in terms of seed set for *Craspedia lanata*. These results from fruit and seed set experiments differ from the P-S/O ratios that had been calculated as an indicator for pollen limitation in natural flowers as introduced by Erbar and Enghofer (2001). While P-S/O ratios indicated pollen limitation for five species, none of them have been shown to suffer inadequate pollen delivery in terms of fruit and seed set under natural conditions. Most likely the limited experimental period of 48 hours for stigma exposure to pollinators for measuring P-S/O ratios (that was based on preliminary stigmatic receptivity data, Dobbie, unpubl. M.Sc. thesis) accounts for this discrepancy. Flowers in alpine habitats have general increased longevity (Kalin-Arroyo 1981, Primack 1985, Blionis *et al.* 2001) suggesting long periods of stigmatic receptivity to maximise pollen delivery. Furthermore, stigmatic receptivity and flower longevity strongly depend on pollen delivery and may be extended if no pollen is delivered (e.g., Arditti 1976, Gori 1983, Primack 1985, Proctor and Harder 1995), so that under unfavourable natural conditions flowers may last much longer than 48 hours in order to receive sufficient pollen. The P-S/O ratios demonstrated in the alpine community cannot be integrated into a larger global data set as almost no other data on pollen delivery to the stigma in relation to ovules has been published. Available ratios for one Asteraceae and one Ranunculaceae (Erbar 2003) as well as several *Epilobium* species (Snow 1986, Müller 2000) suggest corresponding values. In conclusion, the pollinator service in New Zealand alpine habitats may not be significantly less effective than in continental Northern hemisphere habitats. However, the overall moderate rates of fruit set suggest that a plant may fail to set fruit due to other constraints apart from insufficient pollination, such as e.g. resource limitation.

P/O ratio and P-S/O ratio as indicators of the breeding system

Apart from the quantification of the actual breeding system of plants in the alpine community, two indicators of the plants' reproductive system, the pollen/ovule ratio (P/O ratio) (Cruden 1977) and Pollen on stigma/ovule ratio (P-S/O ratio) (Erbar and Enghofer 2001), have been calculated and will be discussed here with respect to accuracy of the prediction. The P/O ratio has often been employed to gain insight into plant breeding systems (Erbar and Langlotz 2005 and references therein) with mixed results regarding the accordance of predicted and observed breeding system. Consistently with this overall trend, accordance of P/O ratios with the

experimental evaluation of the breeding system of plants in the alpine community varied. Xenogamy was indicated for ten species, all of which indeed employ xenogamy as their breeding system during the studied seasons. Correspondingly, autogamy was indicated for four species, and was confirmed by breeding system experiments. The remaining eight species results are ambiguous, that is the P/O ratio was too low to support the breeding system that was demonstrated. Reasons for this can be multifold as P/O ratios depend on many different factors, among them breeding and sexual system, pollen vector, dispersal unit and ecological constraints (Cruden 2000, Jürgens *et al.* 2002, Erbar and Langlotz 2005). Species with very low P/O ratios generally showed some features that may optimise pollen dispersal, e.g. tetrad pollen dispersal units (PDUs) in *D. muscoides, E. porphyrium* and *G. nubicola*, viscin threads in *E. porphyrium* and morphological adaptations in zygomorphic flowers of *E. zelandica, O. caespitosa* and *O. glandulosa* that allow precise pollen deposition and therefore may minimise the risk of pollen waste. These mechanisms have been interpreted as adaptations to pollen protection so that optimised pollen dispersal may be reflected in decreased P/O ratios (Cruden 2000).

In a comparison on the taxonomic level (data compiled by Erbar and Langlotz 2005), almost all P/O ratios of New Zealand alpine species correspond with the ratios demonstrated for congeners on a global scale. One exception is the comparably high P/O ratio of *Viola cunninghamii* (447) which differs strikingly from the usually low values given in literature for other *Viola* species (Cruden 1973, 1977). However, those *Viola* species were usually cleistogamous. Many *Viola* species are reportedly insect-pollinated (Beattie 1976) and their P/O values may be much higher and similar to *V. cunninghamii* studied here.

Overall, it seems that the family-typical P/O ratio levels that have evolved under different conditions before the genera arrived in New Zealand have more or less been conserved. The relatively short time frame since the formation of the alpine habitats in New Zealand (Wardle 1978, Winkworth *et al.* 2005) suggests limited opportunity for major changes.

In addition to the P-S/ O ratio under natural condition (test for pollen limitation, see above), the stigmatic pollen load under pollinator exclusion was employed to assess the potential for autonomous self-pollination in the alpine community. In most cases these results corresponded with the breeding system. However, in case of three members of the Asteraceae as well as *E. zelandica*, *V. cunninghamii* and *W. albomarginata* the autonomous P-S/O ratio suggested pollinator independence while the breeding system experiments revealed xenogamy. All species display high levels of self-incompatibility in the breeding system experiments. In case of the Asteraceae with their secondary pollen presentation in form of a

pump mechanism (Leins and Erbar 2008), most likely the number of pollen grains on the stigma was overestimated because self-pollen grains were still adhering to the stigmatic lobes. This does not neccessarily result in pollen tube germination if the plant has no or low self-compatibility. In general, secondary pollen presentation on the style or stigma indicates that some form of self-incompatibilty may be present (Leins and Erbar 2008). In case of *E. zelandica* and *V. cunninghamii*, similar mechanisms of self-incompatibility may prevent self-fertilisation even if no secondary pollen presentation occurs.

Secondary pollen presentation may also account for the overall conflicting results in *W. albomarginata*. This Campanulaceae employs a proterandous stylar brush mechanism that is very typical for members of this family (Erbar and Leins 1989). The stylar brush structure allows flowers of *W. albomarginata* to exhibit almost complete dichogamy and herkogamy (Lloyd and Yates 1982) under natural conditions. However, the autonomous P-S/O ratio suggests that pollinator-independent transfer of pollen is possible when pollinators are fully absent. In fact the absence of pollen removal by insects explains the observed significant increase in pollen load on the stigma compared to open flowers. However, this does not result in fruit set. Although self-compatibility in controlled hand-self pollination experiments has been demonstrated (Lloyd and Yates 1982) in other *Wahlenbergia* populations, the studied flowers in the Remarkables population depended on pollen dispersal by insect visitors. This may reflect a decline in pollen viability if pollinators cannot access the stylar brush where pollen is being presented. As flowers enter the female stage of anthesis and some self-pollen is transferred onto the stigma, its quality may have suffered from continuous exposure. In combination with low levels of self-compatibility self-pollination does not result in self-fertilisation in *W. albomarginata*.

In conclusion, both P/O and P-S/O ratio as indicators may provide interesting insights into the breeding system of a plant species; however final conclusions should always be based on a synthesis of indicators and actual breeding system experiments. Thus it becomes possible to capture a multitude of information about the reproductive system of a plant.

5.2 Are alpine pollination networks in New Zealand entirely generalised?

In order to assess the mutualistic plant-pollinator network in the alpine community in the Remarkables Mountains three different levels of investigation were carried out. Interactions were quantified (a) as visitation frequencies in plant observations, (b) presence-absence data from the insect voucher specimens caught on flowers of all the plant species under

investigation and (c) by analysing the pollen load that the collected specimens were carrying. Both the visitation network and the pollen-load network found in the alpine plant community in New Zealand were significantly nested when species were ordered after linkage level and therefore not randomly distributed as previously proposed (Primack 1978, 1983). Nestedness in general implies that specialised plants attracted a smaller subset of animals visiting more generalised plant species, and that specialised animals fed on a subset of the food plant species of more generalised animals. Therefore, specialised plants were most likely to receive visits from generalised animals, and specialised animals were most likely to utilise generalised food plants (Dupont *et al.* 2003).

Comparison of the three different levels of investigation reveals that the observation and the pollen load network yield similar network characteristics while the voucher network shows a significant decrease in nestedness, i.e. the interaction distribution is equal compared to the asymmetric patterns of the other two networks. Given the fact that all three networks describe the same system it becomes obvious that the presence-absence data of the voucher network is insufficient in displaying all features of the network. The sampling effort with this method would have to be considerably increased in order to capture most of the interactions within a community. Therefore studies that quantify presence-absence data only should be approached with caution (e.g., Primack 1978, 1983). The considerable increase in information by sampling pollen loads of flower visitors has been previously demonstrated (Kanstrup and Olesen 1999, Phillip *et al.* 2006) The advantage of the pollen load identification over the voucher collection alone is that the pollen loads on a voucher may reflect multiple previous flower visits of the captured insect specimens, whereas the capture of a specimen from a flower confirms only the actual visit. Because of the obviously limited value of the voucher collection in terms of network analyses, my discussion will focus on the pollen-load network with respect to overall network structure, and then proceed to examine the information on visit frequencies obtained from insect observations to finally correlate these findings with the actual pollen delivery to receptive stigmas.

The pollen-load network provides the most accurate information on connectance and link distribution in the alpine community as the specimens included are all carrying pollen and they are all identified to genus level. Insects differed in the diversity of the composition of their pollen loads, e.g. syrphid flies carried pollen of all plant species in the community while some moths carried only pollen from a single species. Most international studies describe plant-flower visitor interactions at the species level (e.g., McMullen 1993, Olesen and Jordano 2002, Olesen *et al.* 2002, Dupont *et al.* 2003). This was not possible for the New

Zealand alpine insect fauna as a considerable number of taxa has not yet been described. Therefore the results obtained from this investigation may be coarser in the definition of visitor categories; however, this is not necessarily a disadvantage. Evidence for specialised interactions in the studied network based on such coarse categories is certainly strong, whereas the strength of evidence for generalisation based on the same coarse data must be relatively weak. Compared to the three different New Zealand montane and alpine networks analysed by Primack (1983) (network parameters subsequently analysed in Dupont *et al.* 2003) that are presumably based on presence-absence sampling (observation method not stated, no examination for pollen loads confirmed), the number of realised interactions in the Remarkables-pollen-load network is rather intermediate with 175 interactions compared to 120, 346 and 376 records of presence on a flower at Primack's study sites. However, the level of connectance, which acts as a measure of generalisation in a community (Bluethgen *et al.* 2006), in the Remarkables network is very high (C=38%) compared to the other New Zealand networks (C=11%, 6% and 6%, respectively). Primack's interpretation of his findings might therefore have to be re-evaluated under consideration of modern statistical network analysis methods.

Globally, most plant – flower visitor networks on oceanic island are characterised by a small size (Olesen and Jordano 2002). As a consequence the connectance is high suggesting a substantial generalisation level. Further, linkage level for insular plants is shown to be lower than on the mainland (Olesen and Jordano 2002, Phillip et al. 2006). High altitude networks on the other hand usually show a low connectance and significantly nested patterns (Arroyo *et al.* 1982, Inouye and Pyke 1988, Elberling and Olesen 1999, Dupont *et al.* 2003). The network on the Remarkables Mountains therefore appears rather generalised on the interaction level. However, none of the current network analysis methods factors visit frequencies to assess interaction patterns on a qualitative and quantitative level.

The flower visitation frequencies in the alpine community on the Remarkables Mountains reveal that about half of the plant species in the assemblage receive more than 50% of their insect visits from one visitor class only. The flower visitor fauna in the Remarkables community is fly-dominated corresponding to the global pattern for high altitude or latitude systems (Primack 1983, Lloyd 1985, Inouye and Pyke 1988, Elberling and Olesen 1999, Larson *et al.* 2001). However, the proportion of fly genera differs considerably from Northern Hemisphere system where muscid flies are most prominent (Elberling and Olesen 1999) while syrphid flies dominate in New Zealand contradicting global patterns (Pont 1993). Furthermore several genera of native bees and some moths are involved in flower visitation as well.

Approximately half of all the collected flower visiting insects were carrying pollen on their bodies suggesting differences in the acquisition of pollen loads when visiting different types of flowers, supposedly due to morphological contraints (Dobbie unpubl. M.Sc. thesis) or different life stages of the insects collected. In illustration of considerable differences in visitation frequencies the flowers of *W. albomarginata* are visited by native bees approximately 80% of the time while *G. nubicola* is visited by nine different visitor classes in approximately equal proportions. These two species represent the two extremes of a continuum throughout the alpine community. Although there is no common statistical procedure that incorporates visitation frequencies into network analysis (but see Bluethgen *et al.* 2006) the recorded differences in visitation frequencies suggest a different relative importance of flower visitors that is lost when analysing data in a binary format only. Therefore visit frequencies provide important information about the biological impact of a link in a network structure, even if this cannot be factored mathematically with the presently available methods (A.M.González, pers. communication).

Overall it can be confirmed that the flower visitor – plant network in the New Zealand alpine community is not a random assemblage. Network parameter analysis of qualitative data as well as quantitative visitation frequency observations suggest a mixed assembly of more or less specific interactions while none of them is exclusive. Syrphid flies are the most opportunistic flower visitors and cushion plants like *G. nubicola* have the broadest visitor spectrum. Moths and beetles on the other hand seem to be limited in the types of flowers they visit while *V. cunnighamii* has the narrowest visitor spectrum of all plant species in the community most likely due to its floral spur that restricts access to the nectar for short-tongued insects. Surprisingly, pollen load data shows that only about half of the collected flower visiting insects were carrying pollen on their bodies (despite being caught during flower visits). Morphological constraints (Dobbie, unpubl. M.Sc. thesis) and/ or different age and flower visitation history of the insects collected may be responsible for this.

Pollination efficiency in the alpine community was assessed by the delivery of pollen to receptive stigmas. Most species in the community do not suffer from pollen limitation so pollination services appear to be adequate for full fruit and seed set. However, the composition of the pollen load on the stigma allows conclusion about the level of flower constancy among pollinators. On a community level, this type of analysis has to my knowledge been carried out only once in a very small-scale network (Philipp *et al.* 2006) and has never been assessed in other alpine systems. Phillip *et al*. (2006) conducted their survey in the Galapagos Islands and reported low levels of stigma contamination.

In 14 species of the alpine community the contamination with heterospecific pollen was below 10% of the total pollen load. Among them are all three of the autogamous species. In *Psychrophila obtusa* absent contamination is not surprising as this species flowers before any other in the alpine community and phenological overlap is low. All other species co-flower with several others. In *Chionohebe densifolia, Euphrasia zelandica, Montia sessiliflora, Ourisia caespitosa, Ourisia glandulosa, Pimelea oreophila* and *Viola cunninghamii* the contamination with heterospecific pollen is considerably higher, peaking at up to 70% in *O. caespitosa*. All these flowers are predominantly visited by syrphid flies that are the most generalistic of all flower visitors in the alpine community. Evidently syrphid flies exhibit little floral constancy being opportunistic feeders. Therefore the degree of illegitimate pollination caused by syrphid flies is high in the present system. On the other hand, the low ratio of heterospecific to conspecific pollen delivery in most other plant species suggests a reliable pollinator service despite the very low presence of major taxonomic groups prominent in continental Northern hemisphere systems, e.g. bumble bees (Körner 2003).

The demonstrated correlation between generalistic pollinators and high degrees of stigma contamination provides an example of the predicted differences in pollinator performance according to functional visitor group (Schemske and Horwitz 1984, Vogel 2006). On the other hand, there is no reason why plants should not benefit from low levels of conspecific pollen transfer when the alternative would be no pollen transfer at all. In conclusion, there is no evidence that plant species in the New Zealand alpine community suffer from overall high levels of stigma contamination due to the lack of floral constancy of pollinators.

Overall, the findings from the New Zealand alpine plant community indicate that there is indeed considerable variation in the degree of generalisation in plant – pollinator interactions. Although the community exhibits several features that have been linked with generalisation, such as a high dominance of fly pollinators (Elberling and Olesen 1999), the small size of the network (Phillip *et al.* 2006) and easily accessible flowers (Stang *et al.* 2006), generalisation appears to be species-dependent and furthermore related to the level of investigation. There is certainly a considerable degree of generalisation on the flower visitor – plant level. However, this becomes less relevant at the level of actual pollen delivery to receptive stigmas where a sufficient degree of conspecific pollen delivery could be demonstrated in most cases while the degree of contamination with heterospecific pollen varied greatly among species. Clearly, not all flower visitors are pollinators, and not all pollinators are "good" pollinators in terms of conspecific pollen transfer and flower constancy - *multi sunt vocati, pauci vero electi* (Vogel 2006). In the worst case scenario from the plants' point of view insect visitors may be nothing

but herbivores. Therefore a modular approach in accessing plant – pollinator interactions is proposed where the different levels of the relationship are investigated individually wherever feasible. Thus, it will be less likely to generalise where generalisation may not be appropriate.

5.3 Which floral traits maintain interaction patterns between flowers and insect visitors?

In the New Zealand alpine plant community on the Remarkables Mountains the majority of plant species has been shown to be pollinator-dependent and furthermore not entirely generalised in the subset of insects groups that visit and potentially pollinate them. Now the morphological, visual and olphactory cues that might be responsible for visitation patterns remain to be evaluated.

The classic pollination syndrome concept traditionally focuses on morphological traits among other things (Vogel 1954, Faegri and van der Pijl 1966). The blossom class and flower access classification (Faegri and van der Pijl 1979, Endress 1994) in the alpine community revealed mainly open-access flowers in dish or bowl shapes with several directed-access gullet flowers as well. However, corolla tubes width in gullet flowers were not effectively excluding visitation from one or more of the insect visitor groups (Dobbie unpubl. M.Sc. thesis). The only flower with a truly intricate design was *Viola cunninghamii* where the nectar is hidden in a nectar spur and flower visitors may be excluded by morphological constraints. The predominance of shallow open-access flowers in a community has been linked to an increased level of generalisation on the flower visitation level (Stang *et al*. 2006). Flower visitors are predominantly short-tongued and small to medium in size (Newstrom and Robertson 2005) therefore being able to effectively access and potentially pollinate the flowers they visit. The pollination syndrome concept would predict generalised pollinators in the majority of plant species in the alpine community due to the lack of morphological restriction imposed on flower visitors. On the other hand, sufficiently different morphology between radial-symmetric and zygomorphic flowers, e.g. between the capitulum of the Asteraceae and the gullet flowers of the Plantaginaceae, indicates that insects might be able to discriminate based on morphology (Campbell and Bischoff, unpubl. data). In conclusion, flower morphology on the community level does not place major restrictions on flower visitor access but flower visitors may be able to discriminate between several flower types based on morphology alone.

Flower colour is one of the most striking features of floral display and the interaction between insect visitors and flowers mediated by colour has received considerable attention from an early stage (Frisch 1950, Menzel 1967) The analysis of the visual flower cues present in the alpine community based on a model in bee colour space (Kevan *et al.* 2001) revealed a clustering of petal colours into six distinct groups. An average bee would be able to discriminate between these six groups in a choice based on colour alone. Furthermore all colours proved to be distinguishable from a green background. Therefore the long-standing claim that insect in New Zealand alpine communities may not be able to choose the flowers they visit by colour cues (Mark and Adams 1993) can be rejected. Bees cannot only detect all flowers in the alpine community by colour cues; they can also potentially discriminate between at least six clusters. There is good evidence that the findings from the bee colour space model may be applicable to other insect visitor groups as well. It has been suggested that most of the recent insect taxa have basic bauplan of UV-blue-green-trichromacy (Briscoe and Chittka 2001). Foraging muscid and syrphid flies have been shown to perceive colour (Lunau 1988, Pickens 1990) and some syrphids display a pronounced innate preference for the colour yellow (Lunau and Maier 1995). The spectral range of some lepidopterans has been demonstrated to be among one of the widest reported for any animal, some ranging from 300nm to 700 nm (UV to red) (Silberglied 1984, Lunau and Maier 1995). Therefore it may be assumed that the other visitor classes apart from hymenopterans in the alpine community can utilise colour cues to distinguish between several plant species of the alpine community as well.

A look at other flower colour surveys on a global scale reveals a pattern that is expressed in New Zealand alpine flowers as well. The reflectance curves have steep gradients at about 390nm and 500nm, representing wavelengths where hymenopterans are most sensitive to changes in colour (Chittka and Menzel 1992). This corresponds with the previous findings that Northern hemisphere flower colours evolved to suit the visual capabilities of trichromatic pollinators (Chittka and Menzel 1992). Based on the phylogenetic evidence obtained by mapping the evolution of wavelengths positioning of insect spectral receptors onto the phylogenetic tree of the arthropods (Chittka 1996) the finding from the New Zealand alpine plant community suggests that the New Zealand flower visitors to which the flowers evolved possessed a trichromatic visual system as well. On the other hand, this finding may demonstrate the relatively young age of the plant community suggesting that flower colour may have evolved before the recent arrival and subsequent radiation of New Zealand alpine plants (Winkworth *et al.* 2005). In conclusion, flower colour signals are sufficient cues for

flower visitor to allow for discrimination from a green background as well distinguish several groups of similar coloured flowers in choices based on flower colour alone.

Floral odours represent the third major pollinator attractant a flower may possess (Leins and Erbar 2008). They have been shown to play an important role for the chemical communication between plants and their pollinators (Pellmyr and Thien 1986, Dobson 1994, Knudsen *et al.* 2006). From a plant's point of view floral volatiles serve to attract potential pollinators that are searching for suitable food resources such as nectar or pollen. From a flower visitor's point of view floral scent is used during foraging to identify and discriminate among rewarding and less rewarding flowers. Like flower colour that serves as an advertisement for flower visitors floral odours may also affect pollinator choice and have subsequent effects on plant reproduction. It is believed that the floral scent composition of plants reflects adaptations towards the olfactory requirements of efficient pollinators (Raguso 2001).

This is the first study to my knowledge (confirmed by R. Raguso, pers. communication) that investigates the floral odour composition on the community level. Most of the 98 compounds identified in this study are well known and widespread floral scent compounds (Knudsen *et al.* 2006). Linalool, Limonene, Benzyl alcohol and *beta*-Caryophyllene, some of the most widespread compounds in the samples of the investigated species, all occur in more than 50% of the families of seed plants (Knudsen *et al.* 2006). Linalool for example is often found in the floral fragrances of moth-pollinated plant taxa (e.g. Miyake *et al.* 1998) though it is not restricted to moth adapted flowers (Raguso and Pichersky 1999) and occurs widely in many diurnal flowers pollinated by bees (Borg-Karlson *et al.* 1996) and beetles (Thien *et al.* 1975).

Interestingly, different plant species in the alpine community emitted quite different volatiles. Overall, 51 of the 98 detected compounds were found only in one of the studied species. The occurrence of very widespread chemicals on the one hand and a high degree of species specific components on the other hand is also reflected in the results of the Bray Curtis-MDS analysis that revealed a poor separation into clustered groups.

Striking is the predominance of aliphatics, particularly typical green leave volatiles (e.g. *trans*-3-Hexen-1-ol, 3-Hexen-1-ol, acetate, Hexanoic acid) in many of the species and the relatively low content of monoterpenes and/or sesquiterpenes in these species. Contrary to this there is a tendency in some species towards high relative amounts of monoterpenes (*Gaultheria nubicola, Lobelia glaberrima*, *Pimelea oreophila*) or sesquiterpenes (*Ourisia glandulosa*, *Ourisia caespitosa*, *Brachyscome sinclairii*, *Euphrasia zelandica*) in others. The

only trend that could be detected was the predominance of high relative amounts of Limonene, p-Anisaldehyde and Benzyl alcohol in cushion plants with minuscule flowers while plant species with an erect growth form emit primarily green leaf volatiles. This finding may suggest the superior importance of scent as a pollinator cue when floral visual display is rather inconspicous. In conclusion, the floral scent profiles of the plant species in the New Zealand alpine community are all very different from each other and do not reflect similarities on a taxonomic or syndrome level. However, some small plant species with a prostrate growth form emit strong floral scents while most erect plant species are characterised by neutral green leaf odours only suggesting that the importance of floral scent as an attractant for pollinators may vary.

All characteristics of the alpine plant community that have been investigated are displayed in a comprehensive table (Table 5.1) that allows comparison on the community level.

Species	Family	Breeding system	Blossom class	Access	Colour	Scent	Main visitor group
P. oreophila (POr)	Thymelaeaceae	X	dish/bowl	open	4	M/B	all fly groups
G. nubicola (GN)	Ericaceae	AA/PL	bowl	open	1	M	all groups equal
M. sessiliflora (MS)	Portulacaceae	X	dish/ bowl	open	1	A/B	all groups equal
P. obtusa (POb)	Ranunculaceae	FX	dish/ bowl	open	-	-	Hover/ Muscid flies/ Beetles
C. thomsonii (CT)	Plantaginaceae	X	gullet	directed	-	-	Muscid flies
W. albomarginata (WA)	Campanulaceae	X	bell	directed	3	A/B	Native bees
L. glaberrima (LG)	Lobeliaceae	X	gullet	directed	4	A/M	Native bees
C. sessilifolia (CS)	Asteraceae	FX	dish/ bowl	open	3	-	Syrphid flies
D. lyallii (DL)	Asteraceae	FX	dish	open	6	A/B	Syrphid flies
A. bellidioides (AB)	Asteraceae	X	dish/ bowl	open	1	A/B	Syrphid flies
B. bellidioides (BB)	Asteraceae	X	dish/ bowl	open	5	A/B	Syrphid flies
B. sinclarrii (BS)	Asteraceae	X	dish/ bowl	open	2	S	Syrphid flies
C. lanata (CL)	Asteraceae	X	dish/ bowl	open	4	A	Syrphid flies
E. porphyrium (EP)	Onagraceae	AA	dish/bowl	open	3	B	Syrphid flies
E. zelandica (EZ)	Orobanchaceae	X	gullet	directed	2	S	Syrphid flies
C. densifolia (CD)	Plantaginaceae	PMA	gullet	directed	-	A	Syrphid flies
O. caespitosa (OC)	Plantaginaceae	X	gullet	directed	3	S	Syrphid flies
R. gracilipes (RG)	Ranunculaceae	X	dish/ bowl	open	5	A/B	Syrphid flies
V. cunninghamii (VC)	Violaceae	X	tube	directed	3	-	Syrphid flies
O. glandulosa (OG)	Plantaginaceae	X	gullet	directed	-	-	Syrphid flies/ native bees
D. muscoides (DM)	Ericaceae	FX	bowl	open	2	A/B	Tachinid flies
G. corymbifera (GC)	Gentianaceae	X	dish/ bowl	open	1/4	A/B	Tachinid flies
P. colensoi (PC)	Stylidiaceae	AA	bowl	open	2	A	Tachinid flies

Tab. 5.1 Comprehensive summary of all aspects of the alpine plant community in the Remarkables Mountains. All Sorted and colour coded according to main visitor group (Visitation in more than 50% of recorded events) Colour codes: brown=all groups/ blue=several fly visitors/ pink=muscid flies/ lavender=native bees/ yellow= syrphid flies/ orange= syrphid flies+native bees/ green= tachinid flies. Abbreviations: Breeding system X= xenogamy, FX= facultative xenogamy, PMA= pollinator-mediated autogamy, AA= autonomous autogamy. Scent: A= aliphatic compunds, B= benzenoid compounds, M= monoterpenoid compounds, S= sesquiterpenoid compounds (Scent classification according to the predominant compound). Flower colour groups according to clusters demonstrated in hexagon bee colour space.

The comprehensive overview presented in Table 5.1 suggests that the flowers of plant species in the New Zealand alpine plant community may be predominantly visited by one group of visitors, however this visitation patterns is not reflected in common trends regarding floral attractants in correlation with main visitor group. On the contrary, species that are related on a taxonomic level and are visited by the same main insect visitors, e.g. members of the Asteraceae or the Plantaginaceae visited by syrphid flies, differ remarkably in the combination of characteristics that defines their flowers. This suggests that in the New Zealand alpine community, floral constancy and efficient pollination are promoted through associative learning rather than through co-adaptation of plants and pollinators.

Associative learning ability, e.g. the ability of an insect to combine the experience of a reward when visiting a certain flower with a feature of this flower, has been demonstrated for the majority of all insects tested (Weiss 2001). Bees excel at learning associative tasks, (e.g. Chittka and Thomson 2001, Chittka and Raine 2006) but learning ability has also been demonstrated for flies, hover flies and lepidopterans (Kugler 1950, Fukushi 1989, Hartlieb 1996, Fan *et al.* 1997) mostly with respect to visual cues. Furthermore, pollinators have been shown to use fragance cues for distance orientation, approach, landing, feeding, and associative learning (Williams 1983, Metcalf 1987, Dobson 1994). The learning capacities of individual pollinators suggest that in the New Zealand alpine community, flower constancy is achieved when individual foraging pollinators learn to associate a certain flower type that is distinct from other flower types in the community through the combination of morphology, flower colour and scent with a certain reward. In subsequent visits the pollinator may strive to repeat the experience and will discriminate between available flowers based on a learned stimulus. Overall a plant would benefit from standing out from other plants species so that pollinators may notice it easily and recognise it in subsequent visits. In such a scenario plants may be able to exploit innate preferences of insects that may be fortified by experience, such as syrphid fly preferences for the colour yellow (Lunau 1988). Furthermore, similar odours in low growing plants might be explained by the fact that different plant species exploit the learned behaviour of insects for finding flowers of low-growing plants that is probably based mainly on olfaction and not on vision.

In conclusion, the majority of plant species in the New Zealand alpine community receives visits from rather generalised flower visitors such as syrphid flies and native bees. However, this may not be a major disadvantage for these plants. Flower types characterised by their floral morphology, colour and scent may be distinct and allow pollinators to discriminate between flower types and actively choose to visit a certain flower type that has been

previously encountered and associated with a certain reward. Furthermore, plants may actually benefit from a set of pollinators that does not employ the foraging strategies of social bees, e.g. extensive pollen foraging to sustain the colony. It is often assumed that social bees are superior pollinators because of their efficient handling of flowers (Kevan and Baker 1983). However, other pollinator groups may not be as constant, but they also do not exploit floral rewards as heavily as social bees. Evidently, the magnitude of pollination that is achieved in the New Zealand alpine community with the services of the present native pollinator fauna is sufficient and sufficiently constant for alpine plants to reproduce successfully.

5.4 General conclusions and future research

The considerable upheaval that the world of pollination biology experienced with the publication of three major challenges to the concept of specialisation in the relationships between plants and their pollinators a decade ago (Herrera 1996, Ollerton 1996, Waser *et al.* 1996) still reverberates and has by no means to date been settled. However, there is no gain in painting the matter of the nature of plant-pollinator interaction strictly in black and white. Pollination syndromes should not be portrayed as rigid entities that do not allow variation, and correspondingly generalisation should not be perceived as the renunciation of the evolution of adaptive traits. Generalised pollination may be adaptive, as demonstrated in the family of the Asteraceae (Vogel 2006). Most importantly, a modular approach when determining specialisation or generalisation should by all means be adopted. Evidently the different levels of an interaction, i.e. the visitation level and the pollination level, demand equal attention and should not be equated unless investigated separately and in all thoroughness. Future research on the community level would benefit enormously from the differentiation of flower visitors and pollinators and furthermore, effective and ineffective pollinators (Leins and Erbar 2008). Furthermore, new methodologies are being developed, e.g. floral scent analysis, and may contribute considerably to our understanding of plant – pollinator relationships (Raguso 2008). After all, generalisation and specialisation, if approached on the different levels of biological meaning and depending on plant or insect perspective, do not necessarily exclude each other. Most likely, a synthesis of concepts will solve the evolutionary conundrum that is being presented to us. However, understanding nature may always remain the supreme challenge in the times to come.

6 References

Anderson GJ, Bernardello G, Stuessy TF, Crawford DJ 2001. Breeding system and pollination of selected plants endemic to Juan Fernandez Islands. American Journal of Botany 88: 220–233.

Amirav A and Dagan S 1997. A direct sample introduction device for mass spectrometry studies and GC-MS analysis. European Journal of Mass Spectrometry 3: 105–111.

Arditti J 1976. Post-pollination phenomenon in orchid flowers. Orchid Review 84: 261-268.

Armstrong JE and Irvine AK 1989. Floral biology of *Myristica insipida* (Myristicaceae), a distinctive beetle pollination syndrome. American Journal of Botany 76: 86–94.

Arroyo MTK, Primack R, Armesto J 1982. Community studies in pollination ecology in the high temperate Andes of central Chile. I. Pollination mechanisms and altitudinal variation. American Journal of Botany 69: 82–97.

Arroyo MTK and Squeo F 1990. Relationship between plant breeding systems and pollination. In: Kawano S (ed.). Biological approaches and evolutionary trends in plants, pp. 205–227. Academic Press, New York.

Atmar W and Patterson BD 1993. The measure of order and disorder in the distribution of species in fragmented habitat. Oecologia 96: 373-382.

Baker HG 1955. Self-compatibility and establishment after "long-distance" dispersal. Evolution 9: 347–349.

Baker HG 1967. Support for Baker's law – as a rule. Evolution 21: 853–856.

Barrett SCH 1996. The reproductive biology and genetics of island plants. Philosophical Transactions of the Royal Society of London Series B 351: 725–733.

Barrett SCH, Harder LD, Worley AC 1996. The comparative biology of pollination and mating in flowering plants. Philosophical Transactions of the Royal Society of London Series B 351: 1271–1280.

Barrett SCH 2002. The evolution of plant sexual diversity. Nature Reviews Genetics 3: 237–284.

Barrett SCH and Harder LD 2006. David G. Lloyd and the evolution of floral biology: from natural history to strategic analysis. In: Harder LD and Barrett SCH (eds.). The ecology and evolution of flowers, pp. 1-21. Oxford University Press, New York.

Barth FG 1985. Insects and flowers: the biology of a partnership. Princeton University Press, Princeton.

Bascompte J, Jordano P, Melián CJ, Olesen JM 2003. The nested assembly of plant-animal mutualistic networks. Proceedings of the National Academy of Sciences USA 100: 9383-9387.

Bascompte J and Jordano P 2007. Plant-animal mutualistic networks: the architecture of biodiversity. Annual Review of Ecology, Evolution and Systematics 38: 567-593.

Bawa KS 1974. Breeding systems of tree species of a lowland tropical community. Evolution 28: 85-92.

Beattie AJ 1976. Plant dispersion, pollination and gene flow in *Viola*. Oecologia 25: 291-300.

Bernardello G, Anderson GJ, Stuessy TF, Crawford DJ 2001. A survey of floral traits, breeding systems, floral visitors, and pollination systems of the angiosperms of the Juan Fernandez Islands (Chile). Botanical Review 67: 255–308.

Bestmann HJ, Winkler L, von Helversen O 1997. Headspace analysis of volatile flower scent constituents of bat-pollinated plants. Phytochemistry 46: 1169-1172.

Bianchi MB, Gibbs PE, Prado DE, Vesprini JL 2000. Studies on the breeding systems of understorey species of a Chaco woodland in NE Argentina. Flora 195: 339–348.

Bingham RA and Ranker TA 2000. Genetic diversity in alpine and foothill populations of *Campanula rotundifolia* (Campanulaceae). International Journal of Plant Science 161(3): 403-411.

Blionis GJ, Halley JM, Vokou D 2001. Flowering phenology of *Campanula* on Mt Olympos, Greece. Ecography 24: 696–706.

Blüthgen N, Menzel F, Blüthgen N 2006. Measuring specialisation in species interaction networks. Bio Med Central Ecology 6: 9.

Borg-Karlson A-K, Unelius CR, Valterova I, Nilsson LA 1996. Floral fragrance chemistry in the early-flowering shrub *Daphne mezereum* (Thymelaeaceae). Phytochemistry 41: 1477-1483.

Briscoe A and Chittka L 2001. The evolution of colour vision in insects. Annual Review of Entomology 46: 471-510.

Brockie WB 1959. Breeding systems in New Zealand species of *Epilobium* L. Transactions of the Royal Society of New Zealand 87: 189–194.

Burger WC 1981. Why are there so many kinds of flowering plants? Bioscience 31: 572-581.

Chittka L 1992. The colour hexagon: a chromaticity diagram based on photoreceptor excitations as a generalized representation of colour opponency. Journal of Comparative Physiology A 170: 533-543.

Chittka L and Menzel R 1992. The evolutionary adaptation of flower colours and the insect pollinators' colour vision systems. Journal of Comparative Physiology A, 171: 171-181.

Chittka L 1996. Does bee colour vision predate the evolution of flower colour? Naturwissenschaften 83: 136-138.

Chittka L and Schürkens S 2001. Successful invasion of a floral market. Nature 411: 653.

Chittka L and Thomson JD (eds.) 2001. Cognitive ecology of pollination - animal behavior and floral evolution. Cambridge University Press, Cambridge.

Chittka L, Ings TC, Raine NE 2004. Chance and adaptation in the evolution of island bumblebee behaviour. Population Ecology 46: 243-251.

Chittka L and Raine NE 2006. Recognition of flowers by pollinators. Current Opinion in Plant Biology 9: 428-435.

Clarke KR 1993. Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18: 117-143.

Clarke KR and Warwick RM 2001. Change in marine communities: an approach to statistical analysis and interpretation, 2nd edition. Primer-E, Plymouth.

Clarke KR and Gorley RN 2006. Primer v6: User Manual/Tutorial. Primer-E, Plymouth: 1-91.

Cockayne L 1967. New Zealand plants and their story. 4th ed. Government Printer, Wellington, NZ.

Comba L, Corbert SA, Hunt H, Outram S, Parker JS, Glover BJ 2000. The role of genes influencing the corolla in pollination of *Antirrhinum major*. Plant, Cell and Environment 23: 639 - 647.

Crane PR, Friis EM, Pedersen KR 1995. The origin and early diversification of angiosperms. Nature 374: 27-33.

Crepet WL 1983. The role of insect pollination in the evolution of angiosperms. In: Real L (ed.). Pollination biology, pp. 31-48. Academic Press, New York.

Crepet WL 1984. Advanced (constant) insect pollination mechanisms: pattern of evolution and implication visa-vis angiosperm diversity. Annals of the Missouri Botanical Garden 71: 607–630.

Cruden RW 1973: Reproductive biology of weedy and cultivated *Mirabilis* (Nyctaginaceae). American Journal of Botany 2: 802–809.

Cruden RW 1977 Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. Evolution 31: 32–46.

Cruden RW 2000. Pollen grains: why so many? Plant Systematics and Evolution 222: 143–165.

Dafni A, Kevan P, Husband B 2005. Practical Pollination Biology. Enviroquest Ltd., Cambridge, Ontario, Canada.

Darwin C 1859. The origin of species. Mentor Edition (1958). Penguin, New York.

Darwin C 1862. The various contrivances by which British and foreign orchids are fertilized by insects. Murray, London.

de Nooy W, Mrvar A, Batagelj V 2005. Exploratory social network analysis with Pajek. Cambridge University Press, New York.

Diels L 1916. Käferblumen bei den Ranales und ihre Bedeutung für die Phylogenese der Angiospermen. Berichte der Deutschen Botanischen Gesellschaft 34: 758-77.

Dobbie L. Morphological matching between flowering plants and their visitors in a New Zealand alpine plant community. Unpublished M.Sc. thesis. University of Otago, Dunedin, N.Z.

Dobson HEM 1994. Floral volatiles in insect biology. In: Bernays EA (ed.). Insect-plant interactions, pp. 47-81. CRC Press, Boca Raton, FL.

Dobson HEM, Raguso RA, Knudsen JT, Ayasse M 2005. Scent as an attractant. In: Dafni A, Kevan P, Husband BC (eds.). Practical pollination biology, pp. 197-230. Enviroquest Ltd., Cambridge, ON, Canada.

Dobson HEM 2006. Relationship between floral fragrance composition and type of pollinator. In: Dudareva N and Pichersky E (eds.). Biology of floral scent. CRC Press, Boca Raton: 147-198.

Donovan BJ 2007. Apoidea (Insecta: Hymenoptera). Fauna of New Zealand 57.

Dötterl S, Wolfe LM, Jürgens A 2005. Qualitative and quantitative analyses of flower scent in *Silene latifolia*. Phytochemistry 66: 203–213.

Dupont YL, Hansen DM, Olesen JM 2003. Structure of a plant-flower-visitor network in the high-altitude sub-alpine desert of Tenerife, Canary Islands. Ecography 26: 301-310.

Dyer AG 1999. Broad spectral sensitivities in the honeybee's photoreceptors limit colour constancy. Journal of comparative physiology A 185: 445-453.

Dyer AG and Chittka L 2004. Biological significance of discriminating between similar colours in spectrally variable illumination: bumblebees as a study case. Journal of comparative physiology A 190: 105-114.

Elberling H and Olesen JM 1999. The structure of a high latitude plant-flower visitor system: the dominance of flies. Ecography 22: 314-323.

Endress PK 1994. Diversity and evolutionary biology of tropical flowers. Cambridge University Press, Cambridge.

Erbar C and Leins P 1989. On the early floral development and the mechanisms of secondary pollen presentation in *Campanula, Jasione and Lobelia*. Botanische Jahrbücher für Systematik 111: 29-55.

Erbar C and Enghofer J 2001. Untersuchungen zum Reproduktionssystem der Wegwarte (*Cichorium intybus*, Asteraceae): Pollenportionierung, Narbenbelegung und Pollenschlauchkonkurrenz. Botanische Jahrbücher für Systematik 123: 179-208.

Erbar C 2003. Pollen tube transmitting tissue: place of competition of male gametophytes. International Journal of Plant Sciences 164: 265-277.

Erbar C and Langlotz M 2005. Pollen to ovule ratios: standard or variation - a compilation. Botanische Jahrbücher für Systematik 126: 71-132.

Fabbro T and Körner C 2004. Altitudinal differences in flower traits and reproductive allocation. Flora 199: 70-81.

Faegri K and van der Pijl L 1966. The principles of pollination ecology. Pergamon Press, Oxford.

Fahey B and Wardle K 1998. Likely impacts of snow grooming and related activities in the West Otago ski fields. Science for Conservation 85. Department of Conservation, Wellington, NZ

Fan R-J, Anderson P, Hansson BS 1997. Behavioural analysis of olfactory conditioning in the moth *Spodoptera littoralis* (Biosd.) (Lepidoptera: Noctuidae). Journal of Experimental Biology 200: 2969-2976.

Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD 2004. Pollination syndromes and floral specialisation. Annual Review of Ecology, Evolution and Systematics 35: 375-403.

Ferry-Graham LA, Bolnick DI, Wainwright PC 2002. Using functional morphology to examine the ecology and evolution of specialisation. Integrative and Comparative Biology 42: 265-277.

Fisher FJF 1965. The alpine *Ranunculi* of New Zealand. DSIR, Wellington, NZ.

Frisch K von 1950. Bees - their vision chemical senses and language. Cornell University Press, Ithaca, NY.

Fukushi T 1989. Learning and discrimination of coloured papers in the walking blowfly *Lucilia cuprina*. Journal of Comparative Physiology A 166: 57-64.

Garnock-Jones PJ 1976. Breeding systems and pollination in New Zealand *Parahebe* (Scrophulariaceae). New Zealand Journal of Botany 14: 291–298.

Gilbert LE and Raven PH 1975. Coevolution of animals and plants. Symposium V. First International Congress of Systematic and Evolutionary Biology, Boulder, Colourado, August 1973. University of Texas Press, Austin and London.

Glover BJ 2007. Understanding flowers and flowering: an integrated approach. Oxford University Press, Oxford.

Godley E 1979. Flower biology in New Zealand. New Zealand Journal of Botany 17: 441-466.

Goldblatt P and Manning JC 1999. The long-proboscid fly pollination system in *Gladiolus* (Iridaceae). Annals of the Missouri Botanical Garden 86: 758–774.

Goldblatt P, Bernhardt P, Manning JC 2000. Adaptive radiation of pollination mechanisms in the African genus *Ixia* (Iridaceae: Crocoideae). Annals of the Missouri Botanical Garden 89: 564-577.

Gori DF 1983. Post-pollination phenomena and adaptive floral changes. In: Jones CE and Little RJ (eds.). Handbook of experimental pollination biology, pp. 31-49. Van Nostrand Reinhold, New York.

Gottsberger G 1974. The structure and function of the primitive angiosperm flower: a discussion. Acta Botanica Neerl. 23(4): 461-471.

Grant V 1950. The pollination of *Calycanthus occidentalis*. American Journal of Botany 37: 294-297.

Grant V and Grant KA 1968. Flower pollination in the Phlox family. Columbia University Press, New York and London.

Gugerli F 1998. Effect of elevation on sexual reproduction in alpine populations of *Saxifraga oppositifolia* (Saxifragaceae). Oecologia 114: 60–66.

Hargreaves A, Johnson SD, Nol E 2004. Do floral syndromes predict specialisation in plant pollination systems? An experimental test in an "ornithophilous". African Protea. Oecologia 140, 295-301.

Hartlieb E 1996. Olfactory conditioning in the moth *Heliothis virescens*. Naturwissenschaften 83: 87-88.

Heine EM 1937. Observations on the pollination of New Zealand flowering plants. Transactions of the Royal Society of New Zealand 67: 133-148.

Herrera CM 1993. Selection on floral morphology and environmental determinants of fecundity in a hawk moth-pollinated violet. Ecological Monographs 63(3): 251-275.

Herrera CM 1996. Floral traits and plant adaptation to insect pollinators: a devil's advocate approach. In: Lloyd DG and Barrett SCH (eds.). Floral biology, pp. 65-87. Chapman and Hall, New York.

Hingston AB and McQuillan PB 2000. Are pollination syndromes useful predictors of floral visitors in Tasmania? Austral Ecology 25: 600–609.

Howe HF 1984. Constraints on the evolution of mutualism. American Naturalist 123: 764-777.

Inouye DW and Pyke GH 1988. Pollination biology in the Snowy Mountains of Australia: comparisons with montane Colourado. Australian Journal of Ecology 13: 191-210.

Johnson SD, Linder HP, Steiner KE 1998. Phylogeny and radiation of pollination systems in *Disa* (Orchidaceae). American Journal of Botany 85: 402–411.

Johnson SD and Steiner KE 2000. Generalisation versus specialisation in plant pollination systems. Trends in Ecology and Evolution 15(4): 140-143.

Johnson SD, Brown M 2004. Transfer of pollinaria on birds' feet: a new pollination system in orchids. Plant Systematics and Evolution 244: 181-188.

Jordano P 1987. Patterns of mutualistic interactions in pollination and seed dispersal: connectance, dependence, and coevolution. American Naturalist 129: 657-677.

Judd DB, MacAdam DL, Wyszecki G 1964. Spectral distribution of typical daylight as a function of correlated colour temperature. Journal of the Optical Society of America 54: 1031-1040

Jürgens A, Webber AC, Gottsberger G 2000. Floral scent compounds of Amazonian Annonaceae species pollinated by small beetles and thrips. Phytochemistry 55: 551-558.

Jürgens A, Witt T, Gottsberger G 2002. Pollen grain numbers, ovule numbers and pollen-ovule ratios in Caryophylloideae: Correlation with breeding system, pollination, life form, style number, and sexual system. Sexual Plant Reproduction 14(5): 279-289.

Jürgens A, Dötterl S, Meve U 2006. The chemical nature of fetid floral odours in stapeliads (Apocynaceae-Asclepiadoideae-Ceropegieae). New Phytologist 172, 452-468.

Kalin-Arroyo MT, Armesto JJ, Villagran C 1981. Plant Phenological Patterns in the High Andean Cordillera of Central Chile. Journal of Ecology 69(1): 205-223.

Kampny CM 1995. Pollination and flower diversity in Scrophulariaceae. Botanical Review 61: 350–366.

Kanstrup J and Olesen JM 1999. Plant-flower visitor interactions in a neotropical rain forest canopy: community structure and generalisation level. In: Totland Ö et al. (eds.). Scandinavian pollination biology, pp. 32-42. Norwegian Academy of Science and Letters, Oslo.

Kearns CA and Inouye DW 1993. Techniques for pollination biologists. University Press of Colourado, Colourado.

Kevan PG and Baker HG 1983. Insects as flower visitors and pollinators. Annual Reviews in Entomology 28: 407-453.

Kevan PG, Chittka L, Dyer AG 2001. Limits to the salience of ultraviolet: lessons from colour vision in bees and birds. Journal of Experimental Biology 204: 2571-2580.

Kite GC and Hetterscheid WLA 1997. Inflorescence odours of *Amorphophallus* and *Pseudodracontium* (Araceae). Phytochemistry 46: 71-75.

Knudsen JT, Tollsten L, Bergström G, 1993. Floral scents - a checklist of volatile compounds isolated by head-space techniques. Phytochemistry 33: 253-280.

Knudsen JT and Tollsten L 1993. Trends in floral scent chemistry in pollination syndromes: floral scent composition in moth-pollinated taxa. Botanical Journal of the Linnean Society 113: 263-284.

Knudsen JT and Tollsten L 1995. Floral scent in bat-pollinated plants - a case of convergent evolution. Botanical Journal of the Linnean Society 119: 45-57.

Knudsen JT, Eriksson R, Gershenzon J, Ståhl B 2006. Diversity and distribution of floral scent. The Botanical Review 72: 1-120.

Kölreuter JG 1761. Vorläufige Nachricht von einigen das Geschlecht der Pflanzen betreffenden Versuchen und Beobachtungen. Gleditsche Buchhandlung, Leipzig.

Körner C 2003. Alpine plant life. Functional plant ecology of high mountain ecosystems. Springer, Berlin.

Kress WJ and Beach JH 1994. Flowering plant reproductive systems. In: McDade LA, Bawa KS, Hespenheide HA, Hartshorn GS (eds.). La Selva: ecology and natural history of a neotropical rainforest, pp. 161-182. University of Chicago Press, Chicago.

Kugler H 1950. Der Blütenbesuch der Schlammfliege (*Eristalomyia tenax*). Zeitschrift für vergleichende Physiologie 32: 328-347.

Larson BMH and Barrett SCH 2000. A comparative analysis of pollen limitation in flowering plants. Biological Journal of the Linnean Society 69: 503-520.

Leins P and Erbar C 2008. Blüte und Frucht. Morphologie, Entwicklungsgeschichte, Phylogenie, Funktion und Ökologie. 2. Aufl., Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.

Lloyd DG 1965. Evolution of self-compatibility and racial differentiation in Leavenworthia (Cruciferae). Contributions from the Gray Herbarium of Harvard University 195.

Lloyd DG 1979. Some reproductive factors affecting the selection of self-fertilisation in plants. American Naturalist 113: 67–79.

Lloyd DG and Yates JMA 1982. Intra-sexual selection and the segregation of pollen and stigmas in hermaphrodite plants, exemplified by *Wahlenbergia albomarginata* (Campanulaceae). Evolution 36: 903–916.

Lloyd DG 1985. Progress in understanding the natural history of New Zealand plants. New Zealand Journal of Botany 23: 707–722.

Lloyd DG and Webb CJ 1986. The avoidance of interference between the presentation of pollen and stigmas in angiosperms. I. Dichogamy. New Zealand Journal of Botany 24: 135–162.

Lloyd DG and Schoen DJ 1992. Self- and cross-fertilisation in plants. I. Functional dimensions. International Journal of Plant Sciences 153: 358-369.

Lunau K 1988. Innate and learned behaviour of flower-visiting hoverflies: flower-dummy experiments with *Eristalis pertinax* (Scolopi) (Diptera: Syrphidae). Zoologische Jahrbücher (Physiologie) 92: 487-499.

Lunau K and Maier EJ 1995. Innate colour preferences of flower visitors. Journal of Comparative Physiology A 177: 1-19.

Lunau K 2007. Stamens and mimic stamens as components of floral colour patterns. Botanische Jahrbücher für Systematik 127: 13–41.

Machado IC and Lopes AV 2004. Floral traits and pollination systems in the Caatinga, a Brazilian tropical dry forest. Annals of Botany 94(3): 365-376.

Mark AF and Bliss LC 1970. The high-alpine vegetation of Central Otago, New Zealand. New Zealand Journal of Botany 8: 381-451.

Mark AF and Adams NM 1993. New Zealand alpine plants. 3rd edition. AH & AW Reed Ltd., Wellington, Sydney, London.

McMullen CK 1993. Flower-visiting insects of the Galapagos Islands. Pan-Pacific Entomologist 69: 95–106.

Menzel R 1967. Untersuchungen zum Erlernen von Spektralfarben durch die Honigbiene (*Apis mellifera*). Zeitschrift für vergleichende Physiologie 56: 22-62.

Metcalf RL 1987. Plant volatiles as insect attractants. Critical Reviews in Plant Sciences 5: 251-301.

Miyake T, Yamaoka R, Yahara T 1998. Floral scents of hawkmoth-pollinated flowers in Japan. Journal of Plant Research 111: 199-205.

Moar NT 1993. Pollen grains of New Zealand dicotyledonous plants. Manaaki Whenua Press, Lincoln

Mulcahy DL 1979. The rise of the Angiosperms: a genecological factor. Science 206: 20–23.

Müller M 2000. Vergleichende Blütenbiologie an verschiedenen Epilobium-Arten. Diploma thesis. University of Heidelberg.

Nabhan GP and Buchmann SL 1997. Services provided by pollinators. In: Daily GC (ed.). Nature's services: societal dependence on natural ecosystems, pp. 133-150. Island Press, Washington DC.

Nagamitsu T and Inoue T 1997. Cockroach pollination and breeding system of *Uvaria elmeri* (Annonaceae) in a lowland mixed-dipterocarp forest in Sarawak. American Journal of Botany 84: 208–213.

Newstrom L and Robertson A 2005: Progress in understanding pollination systems in New Zealand. New Zealand Journal of Botany 43: 1-59.

Nielsen A and Bascompte J 2007. Ecological networks, nestedness and sampling effort. Journal of Ecology 95(5): 1134–1141.

Ollerton J 1996. Reconciling ecological processes with phylogenetic patterns: the apparent paradox of plant-pollinator systems. Journal of Ecology 84: 767-769.

Ollerton J 1998. Sunbird surprise for syndromes. Nature 394: 726-727.

Ollerton J, Johnson, SD, Cranmer L, Kellie S 2003. The pollination ecology of an assemblage of grassland asclepiads in South Africa. Annals of Botany 92: 807-834.

Ollerton J and Raguso R 2006. The sweet stench of decay. New Phytologist 172: 382-385.

Ollerton J, Alarcon R, Waser N, Price MV, Watts S, Cranmer L, Hingston A, Peter C, Rotenberry J 2007. A global test for the pollination syndrome hypothesis. Submitted.

Olesen, JM, Eskildsen LI and Venkatasamy S 2002. Invasion of oceanic island-pollination networks: importance of invader complexes and endemic super generalists. Diversity and distributions 8: 181–192.

Olesen JM and Jordano P 2002. Geographic patterns in plant/pollinator mutualistic networks. Ecology 83: 2416-2424.

Olesen JM and Valido A 2003. Lizards as pollinators and seed dispersers: an island phenomenon. Trends in Ecology and Evolution 18: 177–181.

Osche G 1979. Zur Evolution optischer Signale bei Blütenpflanzen für die Evolution. Biologie in unserer Zeit 9: 161-170.

Osche G 1983. Optische Signale in der Coevolution von Pflanze und Tier. Berichte der Deutschen Botanischen Gesellschaft 96: 1-27.

Pauw A 1998. Pollen transfer on bird's tongues. Nature 394: 731–732.

Pellmyr O and Thien LB 1986. Insect reproduction and floral fragrances: keys to the evolution of the angiosperms? Taxon 35(1): 76-85.

Perret M, Chautems A, Spichiger R, Peixoto M, Savolainen V 2001. Nectarsugar composition in relation to pollination syndromes in Sinningieae (Gesneriaceae). Annals of Botany 87: 267–273.

Petanidou T and Ellis WN 1996. Interdependence of native bee faunas and floras in changing Mediterranean communities. In: Matheson A et al. (eds.): The conservation of bees, pp. 201-226. Academic Press, New York.

Petanidou T, Kallimanis AS, Tzanopoulos J, Sgardelis SP, Pantis JD 2008. Long-term observation of a pollination network: fluctuation in species and interactions, relative invariance of network structure and implications for estimates of specialisation. Ecology Letters 11(6): 564-75.

Philipp M, Böcher J, Siegismund HR, Nielsen LR 2006. Structure of a plant-pollinator network on a pahoehoe lava desert of the Galápagos Islands. Ecography 29: 531-540.

Pickens LG 1990. Colourimetric versus behavioural studies of face fly (Diptera: Muscidae) vision. Environmental Entomology 19: 1242-1252.

Pijl L van der and Dodson CH 1966. Orchid flowers. Their pollination and evolution. The Fairchild Tropical Garden and University of Miami Press, Coral Gables, FL.

Plitmann U and Levin DA 1990. Breeding system in the Polemoniaceae. Plant Systematics and Evolution 170: 205–214.

Pojar J 1974. Reproductive dynamics of four plant communities of southwestern British Columbia. Canadian Journal of Botany 52: 1819–1834.

Pont AC 1993. Observations on anthophilous Muscidae and other Diptera (Insecta) in Abisko National Park. Sweden. Journal of Natural History 27: 631-643.

Primack RB 1978. Variability in New Zealand montane and alpine pollinator assemblages. New Zealand Journal of Ecology 1: 66–73.

Primack RB 1983. Insect pollination in the New Zealand mountain flora. New Zealand Journal of Botany 21: 317–333.

Primack RB 1985. Longevity of individual flowers. Annual Reviews of Ecology and Systematics 16: 15-35.

Pritchard GG 1957. Experimental taxonomic studies of species of *Cardamine* Linn. in New Zealand. Transactions of the Royal Society of New Zealand 85: 75–89.

Proctor HC and Harder LD 1995. Effect of pollination success on floral longevity in the orchid *Calypso bulbosa* (Orchidaceae). American Journal of Botany 82: 1131-1136.

Proctor M, Yeo P, Lack A 1996. The natural history of pollination. Harper Collins, London.

Raguso RA and Pichersky E 1999. A day in the life of a linalool molecule: chemical communication in a plant-pollinator system. Part 1: Linalool biosynthesis in flowering plants. Plant Species Biology 14: 95-120.

Raguso R 2001. Floral scent, olfaction, and scent-driven foraging behavior. In: Chittka L and Thomson JD (eds.). Cognitive ecology of pollination. Cambridge University Press, Cambridge.

Raguso RA 2008. Start making scents: the challenge of integrating chemistry into pollination ecology. Entomologia Experimentalis et Applicata 128(1): 196-207.

Raven PH 1973. Evolution of sub-alpine and alpine plant groups in New Zealand. New Zealand Journal of Botany 11: 177–200.

Raven PH and Raven TE 1976. The genus *Epilobium* (Onagraceae) in Australasia: a systematic and evolutionary study. DSIR Bulletin 216: 1–321.

Renner SS 1998. Effects of habitat fragmentation on plant–pollinator interactions in the tropics. In: Newbery DM, Prins HHT, Brown ND (eds.). Dynamics of tropical communities, pp. 339-360. Blackwell Science, Oxford.

Robertson C 1928. Flowers and insects: lists of visitors to four hundred and fifty-three flowers. Carlinville, IL.

Robertson AW and Lloyd DG 1991. Herkogamy, dichogamy and self-pollination in six species of *Myosotis* (Boraginaceae). Evolutionary Trends in Plants 5: 53-63.

Ruiz T and Arroyo MTK 1978. Plant reproductive ecology of a secondary deciduous tropical forest in Venezuela. Biotropica 10: 221-230.

Schlessman MA 1986. Floral protogyny, self-compatibility and the pollination of *Ourisia macrocarpa* (Scrophulariaceae). New Zealand Journal of Botany 24: 651-656.

Schemske DW and Horvitz CC 1984. Variation among floral visitors in pollination ability: a precondition for mutualism specialisation. Science 255: 519–521.

Schueller SK 2004. Self-pollination in island and mainland populations of the introduced hummingbird-pollinated plant, *Nicotiana glauca* (Solanaceae). American Journal of Botany 91: 672–681.

Silberglied RE 1984. Visual communication and sexual selection among butterflies. In: Vane-Wright RI and Ackery PR (eds.). The biology of butterflies. Academic Press, London.

Small E 1986. Pollen-ovule patterns in tribe Trifoliae (Leguminosae). Plant Systematics and Evolution 160: 195-205.

Snow AA 1986. Pollination dynamics in *Epilobium canum* (Onagraceae): consequences for gametophytic selection. American Journal of Botany 73: 139-151.

Sprengel CK 1793. Das entdeckte Geheimnis der Natur im Bau und in der Befruchtung der Blumen. Vieweg, Berlin. (Translated into English by Haase P 1996: In: Lloyd DG and Barrett SCH (eds.). Floral biology. Chapman and Hall, New York).

Stang M, Klinkhamer PGL, Van Der Meijden E 2006. Size constraints and flower abundance determine the number of interactions in a plant–flower visitor web. Oikos 112: 111-121.

Stang M, Klinkhamer PGL, Van Der Meijden E 2007. Asymmetric specialisation and extinction risk in plant-flower visitor webs: a matter of morphology or abundance? Oecologia 151: 442-453.

Stebbins GL 1970. Adaptive radiation of reproductive characteristics in angiosperms. I: pollination mechanisms. Annual Review of Ecology and Systematics 1: 307-326.

Steenis CG van 1980: The pollination syndrome. Flora Malesia Bull. 33: 3437–3439.

Terry LI, Walter GH, Donaldson JS, Snow E, Forster PI, Machin PJ 2005. Pollination of Australian Macrozamia cycads (Zamiaceae): effectiveness and behavior of specialist vectors in a dependent mutualism. American Journal of Botany 92: 931-940.

Thien LB, Heimermann WH, Holman RT 1975. Floral odours and quantitative taxonomy of *Magnolia* and *Liriodendron*. Taxon 24: 557-568.

Thomson GM 1927. The pollination of New Zealand flowers by birds and insects. Transactions and Proceedings of the New Zealand Institute 57: 106–125. [issued separately 1926.]

Totland O, Eide W, Grytnes JA 2000. Is there a typical alpine flower? Det Norske Videnskamp-Akademi. I. Matematisk Naturvidenskapelige Klasse, Skrifter, Ny Serie 39: 139-148.

Vázquez DP and Simberloff D 2002. Ecological specialisation and susceptibility to disturbance: conjectures and refutations. American Society of Naturalists 159: 606–623.

Vázquez DP and Aizen MA 2004. Asymmetric specialisation: a pervasive feature of plant-pollinator interactions. Ecology 85: 1251-1257.

Vázquez DP 2005. Degree distribution in plant-animal mutualistic networks: forbidden links or random interactions? Oikos 108: 421-426.

Vogel S 1954. Blütenbiologische Typen als Elemente der Sippengliederung dargestellt anhand der Flora Südafrikas. Botanische Studien Heft 1. Fischer, Jena.

Vogel S 1981: Bestäubungskonzepte der Monokotylen und ihr Ausdruck im System. Berichte der Deutschen Botanischen Gesellschaft 94: 663–675.

Vogel S 1990: Radiación adaptativa del síndrome floral en las familias neotropicales. Bol. Acad. Nac. Ci. Córdoba, Argentina 59: 5–30.

Vogel S 2006. Floral syndromes: empiricism versus typology. Botanische Jahrbücher 127(1): 5-11.

Walker AK and Crosby TK 1988. The preparation and curation of insects (revised edition). Department of Scientific and Industrial Research, Wellington, N.Z.

Wallace AR 1880. Island life. Macmillan, London.

Wardle P 1978. Origin of the New Zealand mountain flora, with special reference to trans-Tasman relationships. New Zealand Journal of Botany 16: 535–550.

Waser NM and Price MV 1983. Optimal and actual outcrossing in plants, and the nature of plant-pollinator interactions. In: Jones CE and Little RJ (eds.). Handbook of experimental pollination biology. Van Nostrand Reinhold, New York.

Waser NM, Chittka L, Price MV, Williams NM, Ollerton J 1996. Generalisation in pollination systems, and why it matters. Ecology 77: 1043–1060.

Waser NM 2006. Specialisation and generalisation in plant-pollinator interactions: a historical perspective. In: Waser NM and Ollerton J (eds.). Plant pollinator interactions. University of Chicago Press, London.

Wasserman S and Faust K 1994. Social network analysis. (Structural analysis in the social sciences 8). Cambridge University Press, Cambridge.

Webb CJ and Lloyd DG 1986. The avoidance of interference between the presentation of pollen and stigmas in angiosperms II. Herkogamy. New Zealand Journal of Botany 24: 163–178.

Webb CJ and Kelly D 1993. The reproductive biology of the New Zealand flora. Trends in Ecology and Evolution 8: 442-447.

Webb CJ and Simpson MJA 2001. Seeds of New Zealand: gymnosperms and dicotyledons. Manuka Press, Christchurch, NZ.

Weigend M. and Gottschling M. 2006: Evolution of funnel-revolver flowers and ornithophily in Nasa (Loasaceae). Plant Biology 8: 120–142.

Weiss MR 2001. Vision and learning in some neglected pollinators: beetles, flies, moths, and butterflies. In: Chittka L and Thomson JD (eds.). Cognitive ecology of pollination. Cambridge University Press, Cambridge.

Westerkamp C 1997. Keel blossoms: bee flowers with adaptations against bees. Flora 192: 125-132.

Williams NH 1983. Floral fragrances as cues in animal behavior. In: Jones CE and Little RJ (eds.). Handbook of experimental pollination biology, pp. 51-69. Van Nostrand Reinhold, New York.

Wilson P, Castellanos MC, Hogue JN, Thomson JD, Armbruster WS 2004. A multivariate search for pollination syndromes among *Penstemons*. Oikos 104: 345–361.

Winkworth RC, Bryant D, Lockhart PJ, Havell D, Moulton V 2005. Biogeographic interpretation of splits graphs: least squares optimization of branch lengths. Systematic Biology 54: 56–65.

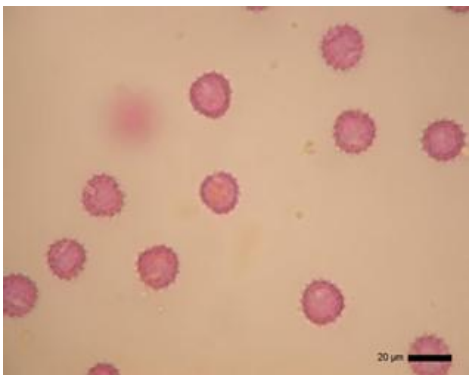
Yasukawa S, Kato H, Yamaoka R, Tanaka H, Arai H, Kawano S 1992. Reproductive and pollination biology of Magnolia and its allied genera (Magnoliaceae). I. Floral volatiles of several Magnolia and Michelia species and their roles in attracting insects. Plant Species Biology 7: 121-140.

Zhang L, Barrett SCH, Gao JY, Chen J, Cole WW, Liu Y, Bai Z-L, Li Q-L 2005. Predicting mating patterns from pollination syndromes: the case of "sapromyiophily" in *Tacca chantrieri* (Taccaceae). American Journal of Botany 92: 517-524.

7 Appendix

7.1 Details of plant species under investigation

Asteraceae

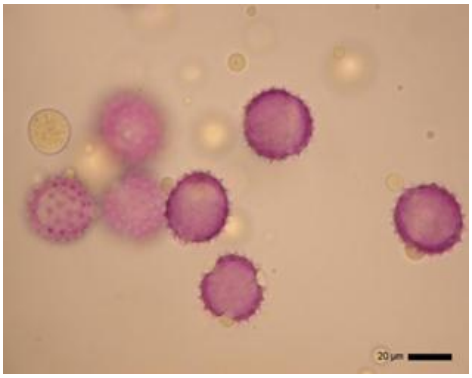


Anaphalioides bellidioides *(G. Forst.) Glenny 1997*

Mean number of pollen grains: 1052 ± 120
Mean number of ovules: 1
P/O ratio: 1052 ± 120
Mean number of seeds natural: 9.15
Mean umber of seeds hand-cross: 10.18
Mean number of seeds hand-self: 0.08
Mean number of seeds bag: 0.5
Main visitor class: syrphid flies

Pollen description

Monad, isopolar, shape spheroidal, circular in polar view; P axis 17.5-25 µm, E plane 17.5-20 µm; tricolporate, ectoapertures c. ⅔ length of grain, narrowing abruptly towards poles, endoapertures lalongate, c. 10 µm long , tapering; exine c. 1 µm thick, cavate, echinate, tectate, baculate, tectum perforate, spines 3-4 µm long, base about as broad as long, sides slightly concave, tips acute, 4-5 across mesocolpia

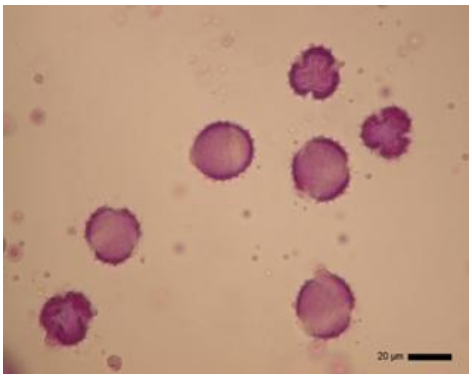


Brachyglottis bellidioides *(Hook. f.) B. Nord*

Mean number of pollen grains: 3837 ± 792
Mean number of ovules:1
P/O ratio: 3837 ± 792
Mean number of seeds natural: 21.22
Mean umber of seeds hand-cross: 12.53
Mean number of seeds hand-self: 0.5
Mean number of seeds bag: 3.25
Main visitor class: syrphid flies

Pollen description

Monad, isopolar, shape spheroidal; P axis 25-27.5 µm, E plane 25-30 µm; tricolporate, ectoapertures overall length often > ⅔ grain, ending abruptly towards poles, endoapertures lalongate, tapering, c. 18 µm long; exine 2 µm thick, cavate, echinate, tectate, baculate, baculum fine; tectum finely perforate, spines c. 4 µm long, about as broad, sides slightly concave, usually sharply pointed, sometimes awl-shaped, 5-6 across mesocolpia

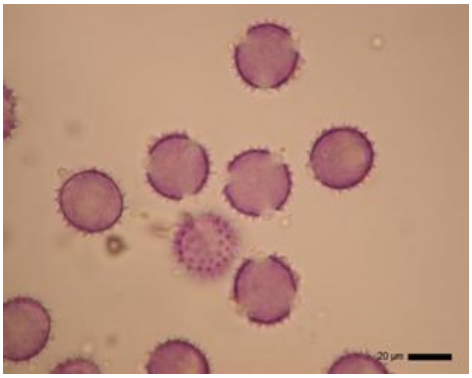


Brachyscome synclairii *Hook. f.*

Mean number of pollen grains: 3185 ± 332
Mean number of ovules: 1
P/O ratio: 3185 ± 332
Mean number of seeds natural: 52.89
Mean umber of seeds hand-cross: 51.00
Mean number of seeds hand-self:0
Mean number of seeds bag:0
Main visitor class: syrphid flies

Pollen description

Monad, isopolar, shape spheroidal; P axis 20-22.5 µm, E plane 20-25 µm; tricolporate, c. ⅔ length of grain, endoapertures lolongate, c. 7-8 µm long; Exine 2 µm thick, cavate, echinate, tectate, baculate, tectum smooth sparingly perforate, spines short, 2 µm long, sides concave, tapering to acute point, 5-6 across mesocolpia

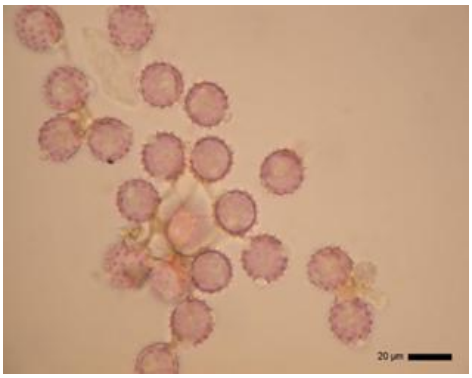


Celmisia sessiliflora *Hook. f.*

Mean number of pollen grains: 5467 ± 547
Mean number of ovules: 1
P/O ratio: 5467 ± 547
Mean number of seeds natural: 9.92
Mean umber of seeds hand-cross: 7.05
Mean number of seeds hand-self: 5.35
Mean number of seeds bag: 0.77
Main visitor class: syrphid flies

Pollen description

Monad, isopolar, shape spherical, circular in polar view; P axis 25-27.5 µm, E plane 25-27.5 µm; tricolporate, ectoapertures often > ⅔ length of grain, endoapertures lolongate, c. 10 µm long; exine 2 µm thick, cavate, echinate, tectate, baculate, tectum perforate, spines 2-4 µm long

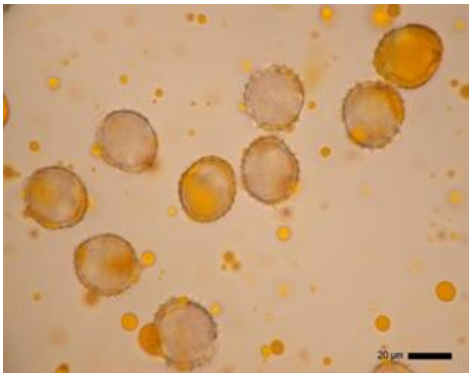


Craspedia lanata. *G. Forst*

Mean number of pollen grains: 2519 ± 103
Mean number of ovules: 1
P/O ratio: 2519 ± 103
Mean number of seeds natural: 103.42
Mean umber of seeds hand-cross: 151.00
Mean number of seeds hand-self: 23.25
Mean number of seeds bag: 20.33
Main visitor class: syrphid flies

Pollen description

Monad, isopolar, shape spheroidal, circular in polar view; P axis 15-20 µm, E plane 17.5-20 µm; tricolporate, short ectoapertures, ⅓ length of grain, ends blunt or rounded, endoapertures lalongate , c. 10 µm long , rounded at ends; exine 2 µm thick, cavate, echinate, tectate, baculate, tectum perforate, spines 3 µm long, as broad as long, sides slightly concave, tips acute, 4-5 across mesocolpia



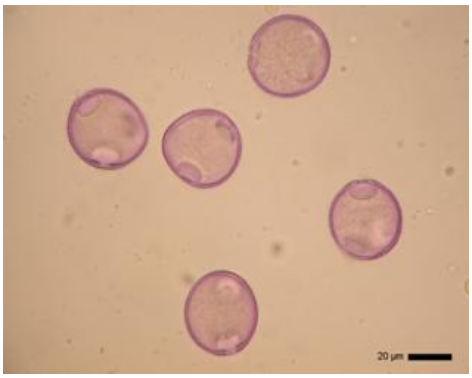
Dolichoglottis lyallii *(Hook. f.) B.Nord.*

Mean number of pollen grains: 3333 ± 754
Mean number of ovules: 1
P/O ratio: 3333 ± 754
Mean number of seeds natural: 35.40
Mean umber of seeds hand-cross: 32.25
Mean number of seeds hand-self: 11.29
Mean number of seeds bag: 22.12
Main visitor class: Syrphid flies

Pollen description

Monad, isopolar, shape spheroidal, circular in polar view; P axis 25 µm, E plane 25-30 µm; tricolporate, endoapertures lolongate, c. 14 µm long , blunt; exine 2 µm thick, cavate, echinate, tectate, baculate, baculum usually fine, tectum perforate, spines 4-5 µm long, 6-7 across mesocolpia

Campanulaceae



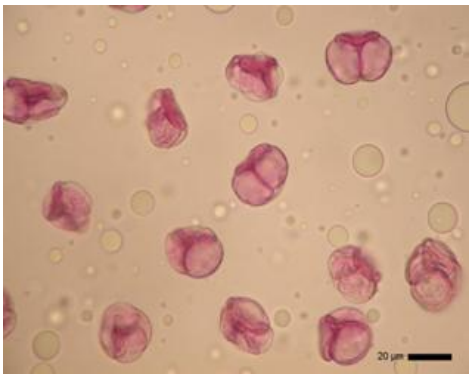
Wahlenbergia albomarginata *Hook.*

Mean number of pollen grains: 27467 ± 3212
Mean number of ovules: 379 ± 49
P/O ratio: 75 ± 7
Mean number of seeds natural: 184.35
Mean umber of seeds hand-cross: 69.86
Mean number of seeds hand-self: 3.13
Mean number of seeds bag: 0
Main visitor class: native bees

Pollen description

Monad, isopolar or subisopolar, shape spheroidal, subtriangular or almost circular in polar view; P axis 32.5-37.5 µm, E plane 35-40 µm; triporate, pores circular, 5-6 µm diameter; exine 2 µm thick, spinulose, semitectate, baculate, tectum slightly reticulate

Ericaceae

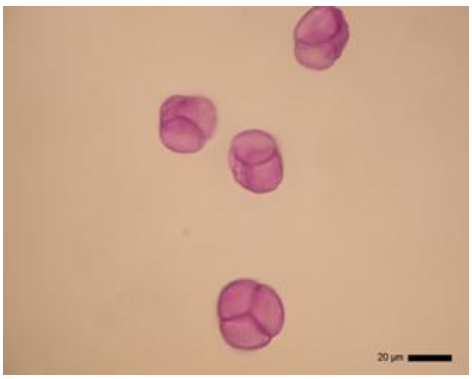


Dracophyllum muscoides *Hook. f.*

Mean number of pollen grains: 16000 ± 1265
Mean number of ovules: 176 ± 14
P/O ratio: 94 ± 13
Mean number of seeds natural: 13.85
Mean umber of seeds hand-cross: 14.33
Mean number of seeds hand-self: 3.67
Mean number of seeds bag: 2.36
Main visitor class: Tachinid flies

Pollen description

Tetrahedral tetrad, lobed; tetrads 22.5-30 µm, single grains 17.5-20 µm; tricolporate, ectoapertures short, 14 µm long, 2 µm wide, tapering to acute point, confluent with apertures of adjoining grains, endoapertures lalongate, 9 µm long; exine 1.5-2 µm thick, tectate, baculate, tectum smooth



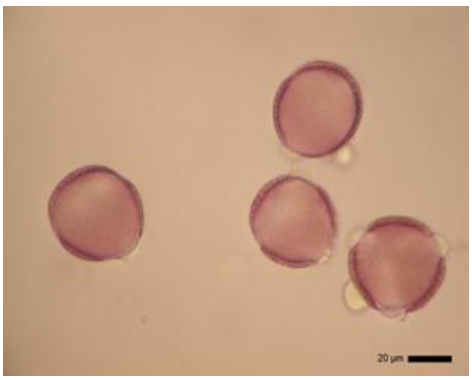
***Gaultheria nubicola** D. J. Middleton*

Mean number of pollen grains: 9933 ± 1576
Mean number of ovules: 143 ± 9
P/O ratio: 71 ± 12
Mean number of seeds natural: 49.79
Mean umber of seeds hand-cross: 59.71
Mean number of seeds hand-self: 51.36
Mean number of seeds bag: 43.67
Main visitor class: all visitor classes

Pollen description

Tetrahedral tetrad, subtriangular, convex in Äquatorialebene; tetrads 27.5-32 µm, single grains 20-22.5 µm; tricolporate, ectoapertures short, 14 µm long, 2 µm wide, tapering to acute point, confluent with apertures of adjoining grains, endoapertures lalongate, 9 µm long; exine 2 µm thick at ectoapertures , otherwise c. 1 µm, tectate, baculate

Gentianaceae



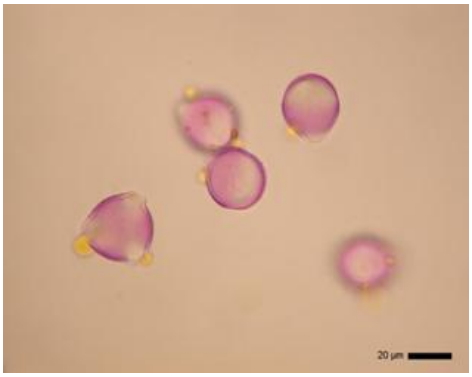
***Gentianella corymbifera** (Kirk.) Holub*

Mean number of pollen grains: 38667 ± 3180
Mean number of ovules: 65 ± 3
P/O ratio: 592 ± 39
Mean number of seeds natural: 31.13
Mean umber of seeds hand-cross: 27.01
Mean number of seeds hand-self: 8.00
Mean number of seeds bag: 4.30
Main visitor class: Tachinid flies

Pollen description

Monad, isopolar, rounded at poles, subtriangular in polar view, on average slightly convex, size varies, P axis 37.5-42.5 µm, E plane 40-45 µm; tricolporate, angulaperturate, ectoapertures long, ⅔ length of grain, tapering to acute point, endoapertures more or less circular; exine very thick, up to 5 µm in mesocolpia, 2 µm at ectoapertures, semitectate, baculate, parallel striped-reticulate, tectum c. 1 µm thick

Lobeliaceae



Lobelia glaberrima *Heenan*

Mean number of pollen grains: 11667 ± 464
Mean number of ovules: 71 ± 2
P/O ratio: 167 ± 9
Mean number of seeds natural: 73.13
Mean umber of seeds hand-cross: 70.25
Mean number of seeds hand-self: 0
Mean number of seeds bag: 0
Main visitor class: Native bees

Pollen description

Monad, isopolar, flattened at poles, circular in polar view; P axis 25-27.5 µm, E plane 27.5-32.5 µm; tricolporate, angulaperturate, ectoapertures very long, 2 µm wide, endoapertures lalongate, c. 17 µm long; exine 2 µm thick, slightly tapered at apertures, semitectate, baculate, reticulate

Onagraceae

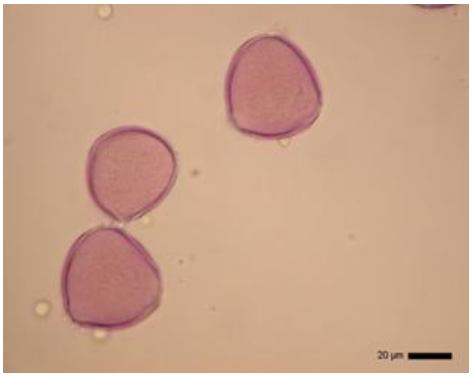
Epilobium porphyrium *G. Simpson.*

Mean number of pollen grains: 1471 ± 105
Mean number of ovules: 76 ± 7
P/O ratio: 24 ± 4
Mean number of seeds natural: 43.5
Mean umber of seeds hand-cross: 39.05
Mean number of seeds hand-self: 51.64
Mean number of seeds bag: 40.37
Main visitor class: Syrphid flies

Pollen description

Loose tetrads, viscin threads on proximate end, single grains subisopolar, flat at poles, elliptic in polar view, subtriangular in polar view; tetrads 85-90 µm, single grains 42.5-55 µm; angulaperturate, tricolporate, erect pores, pores round or oval, vestibulate, and tapering to acute point; exine width variable, c. 2 µm, tectate, baculate, surface slightly striate, bacula very short,

Orobanchaceae



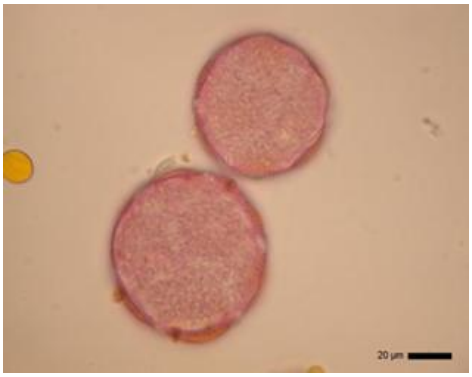
Euphrasia zelandica *Wettst.*

Mean number of pollen grains: 2733 ± 427
Mean number of ovules: 24 ± 2
P/O ratio: 121 ± 25
Mean number of seeds natural: 14.96
Mean umber of seeds hand-cross: 11.62
Mean number of seeds hand-self: 2.79
Mean number of seeds bag: 2.72
Main visitor class: Syrphid flies

Pollen description

Monad, isopolar, shape oblate or oblate spheroidal, rounded or flattened at poles, subtriangular in polar view, slightly convex; P axis 40-47.5 µm, E plane 45-47.5 µm; tricolpate, sometimes tetracolpate, angulaperturate, ectoapertures c. ⅔ length of grain, 8 µm wide at equator, exine very thin, 1µm thick, semitectate, baculate, reticulate or rugulate

Portulacaceae



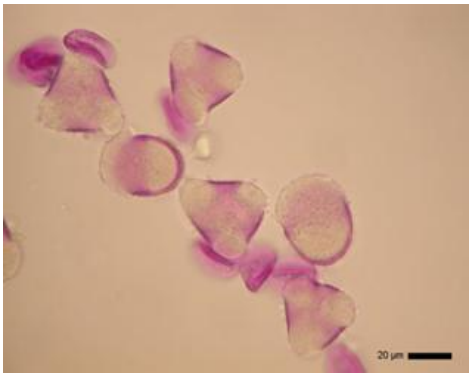
Montia sessiliflora *(G. Simpson) Heenan*

Mean number of pollen grains: 1364 ± 54
Mean number of ovules: 3 ± 0,2
P/O ratio: 431 ± 26
Mean number of seeds natural: 2.42
Mean umber of seeds hand-cross: 2.52
Mean number of seeds hand-self: 0.54
Mean number of seeds bag: 0.43
Main visitor class: all visitor classes

Pollen description

Monad, apolar, shape spherical; size varies, 72.5-80 µm; pericolpate, ectoapertures 12-30, mostly 20, narrow, up to 14 µm long, length variable, einige syncolpat; exine up to 5 µm thick, thinner at apertures, baculate, tectate, tectum slightly perforate, occasional spinulae, spines vary in length

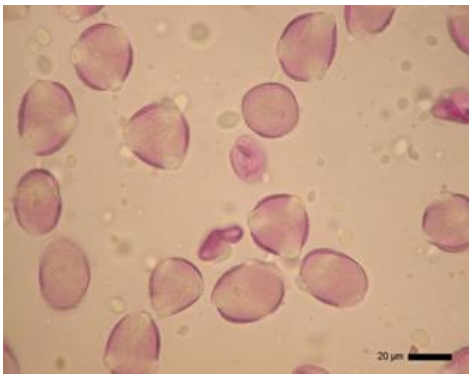
Plantaginaceae



Chionohebe densifolia *(F. Muell.) B. G. Briggs & Ehrend.*

Mean number of pollen grains: 25467 ± 3798
Mean number of ovules: 65 ± 4
P/O ratio: 380 ± 46
Mean number of seeds natural: 10.00
Mean umber of seeds hand-cross: 8.42
Mean number of seeds hand-self: 13.40
Mean number of seeds bag: 0.5
Main visitor class: Syrphid flies
Pollen description

Monad, isopolar, elongate rounded or flattened at poles; P axis 25-30 µm, E plane 30-37.5 µm; tricolpate, sometimes dicolpate, exine very thin, simplibaculate, bacula fine, occasional striped texture, semitectate, reticulate, reticulum fine and smooth



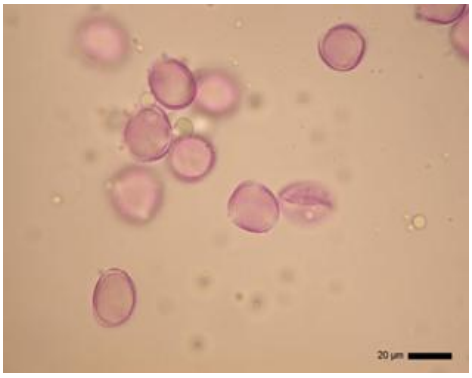
Chionohebe thomsonii *(Buchanan) B. G. Briggs & Ehrend*

Mean number of pollen grains: 6400 ± 348
Mean number of ovules: 27 ± 1
P/O ratio: 380 ± 46
Mean number of seeds natural: 2.00
Mean umber of seeds hand-cross: 1.31
Mean number of seeds hand-self: 1.23
Mean number of seeds bag: 0.54
Main visitor class: Muscid flies
Pollen description

Monad, isopolar, elongate, , rounded or flattened at poles; P axis 20-25 µm, E plane 22.5-30 µm; tricolpate, exine very thin, semitectate, baculate, reticulate

Ourisia caespitosa *Hook. f*

Mean number of pollen grains: 22711 ± 2858
Mean number of ovules: 224 ± 8
P/O ratio: 102 ± 14
Mean number of seeds natural: 23.18
Mean umber of seeds hand-cross: 22.67
Mean number of seeds hand-self: 2.73
Mean number of seeds bag: 0.52
Main visitor class: Syrphid flies

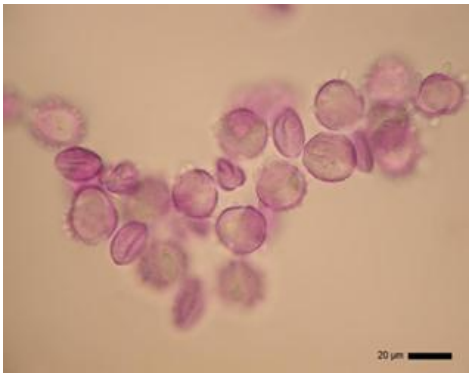


Pollen description

Monad, isopolar, shape spheroidal, subtriangular or circular in polar view; P axis 17.5-20 µm, E plane 22.5-25 µm; tricolpate, ectoapertures slightly sunken, very long, c. 2 µm wide at equator, tapering to rounded end; exine 1.5 µm thick, thinner at poles and along aperture edges, semitectate, baculate, reticulate, reticulum fine

Ourisia glandulosa *Hook. f.*

Mean number of pollen grains: 66000 ± 9450
Mean number of ovules: 282 ± 28
P/O ratio: 231 ± 17
Mean number of seeds natural: 72.48
Mean umber of seeds hand-cross: 84.96
Mean number of seeds hand-self: 0.36
Mean number of seeds bag: 0.29
Main visitor class: Syrphid flies and native bees



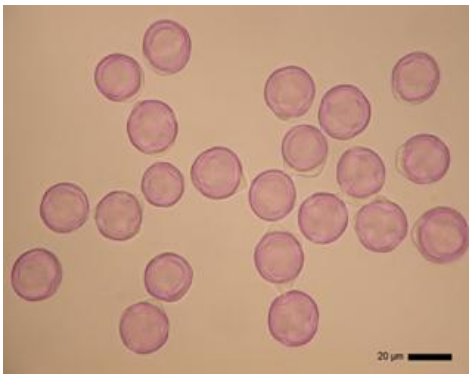
Pollen description

Monad, isopolar, shape spheroidal, subtriangular or circular in polar view; P axis 22.5-25 µm, E plane 22.5-25 µm; tricolpate, ectoapertures slightly sunken, very long, 2 µm wide at equator, rounded ends; exine 1.5 µm thick, thinner at poles and along aperture edges, semitectate, baculate, reticulate, reticulum heterobrochate

Ranunculaceae

Psychrophila obtusa *(Cheeseman) W. A. Weber*

Mean number of pollen grains: 86489 ± 10190
Mean number of ovules: 25 ± 2
P/O ratio: 3476 ± 474
Mean number of seeds natural: 3.92
Mean umber of seeds hand-cross: 0.74
Mean number of seeds hand-self: 0.54
Mean number of seeds bag: 0
Main visitor class: Syrphid and Muscid flies

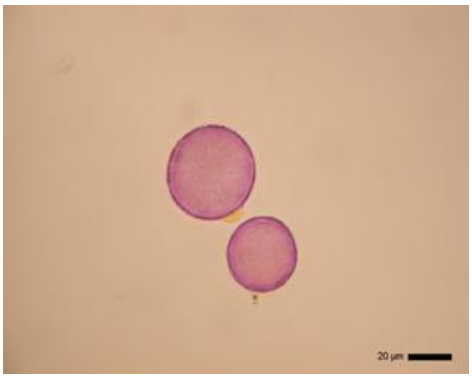


Pollen description

Monad, isopolar, shape spheroidal; P axis 17.5-25 µm, E plane 20-25 µm; tricolpate, ectoapertures ⅔ length of grain, narrow; exine 1µm thick, baculate, tectate, perforate, spines short and dense

Ranunculus gracilipes *Hook. f.*

Mean number of pollen grains: 40600± 5646
Mean number of ovules: 22 ± 2
P/O ratio: 1879 ± 337
Mean number of seeds natural: 14.48
Mean umber of seeds hand-cross: 9.95
Mean number of seeds hand-self: 0.58
Mean number of seeds bag: 0.45
Main visitor class: Syrphid flies



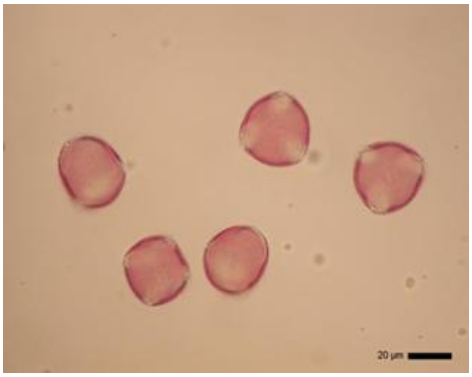
Pollen description

Monad, isopolar, shape flattened; circular in polar view; P axis 25-30 µm, E plane 27.5-32.5 µm; pericolpate, ectoapertures vary in size, sometimes syncolpate; exine thin, 1-2 µm thick, baculate, tectate, tectum slightly perforate,

Stylidiaceae

Phyllachne colensoi *(Hook. f.) Berggr.*

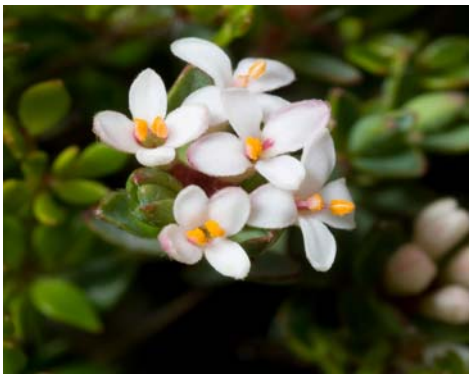
Mean number of pollen grains: 3067 ± 402
Mean number of ovules: 20 ± 3
P/O ratio: 164 ± 4
Mean number of seeds natural: 3.33
Mean umber of seeds hand-cross: 2.27
Mean number of seeds hand-self: 3.13
Mean number of seeds bag: 4.27
Main visitor class: Tachinid flies



Pollen description

Monad, isopolar, shape flattened at poles, subtriangular in polar view, convex; P axis 25-30 µm, E plane 30-32 µm; tricolpate, rarely tetracolpate, angulaperturate, ectoapertures broad, slightly tapered rounded ends, ⅔ length of grain; exine very thin, 1 µm thick, tectate, baculate, tectum spinulose, spines not very dense, very short, 0.5 µm long

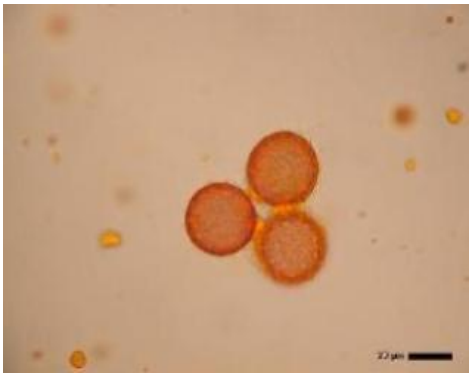
Thymelaeaceae



Pimelea oreophila *C. J. Burrows*

Mean number of pollen grains: 1267 ± 114
Mean number of ovules: 1
P/O ratio: 1267 ± 114
dioecious

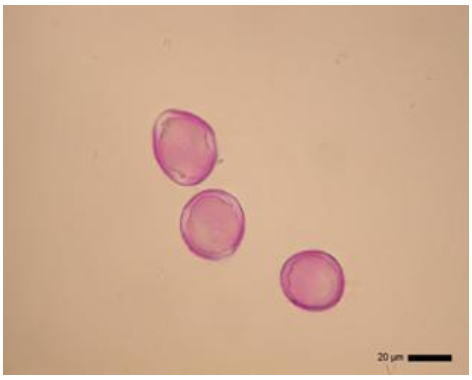
Main visitor class: all fly visitors



Pollen description

Monad, apolar, shape spherical, 30-35 µm; periporate, c. 30 pores, 2-3 µm diameter; exine very thick, 3-4 µm, semitectate, baculate, tectum spinulose, spines very short, 0.5 µm long

Violaceae



Viola cunninghamii *Hook. f.*

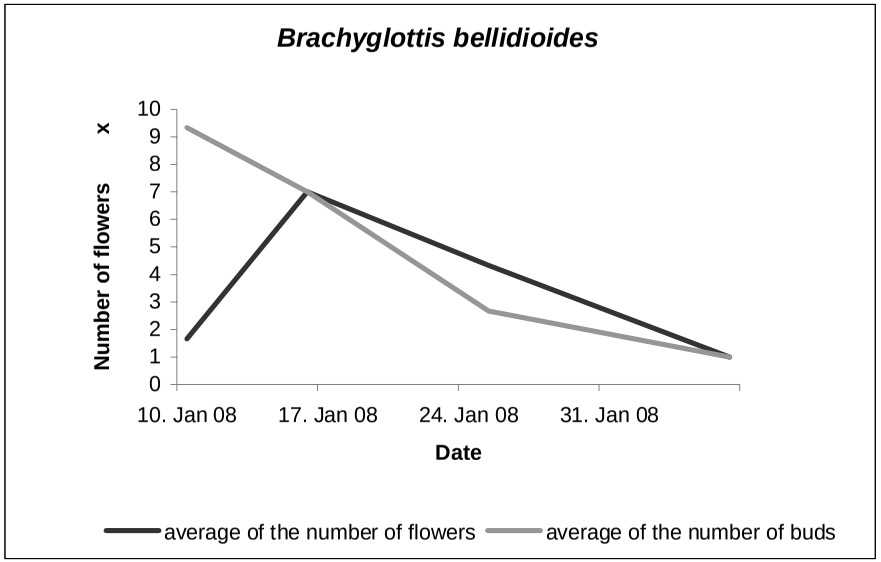
Mean number of pollen grains: 10333 ± 2550
Mean number of ovules: 25 ± 3
P/O ratio: 447 ± 110
Mean number of seeds natural: 17.4
Mean umber of seeds hand-cross: 14.1
Mean number of seeds hand-self: 5.57
Mean number of seeds bag: 2.0
Main visitor class: Syrphid flies

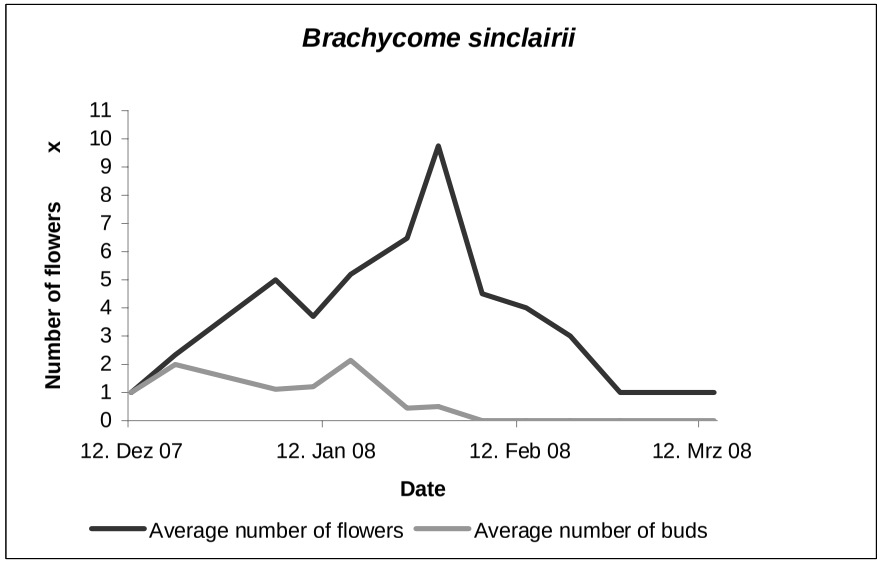
Pollen description

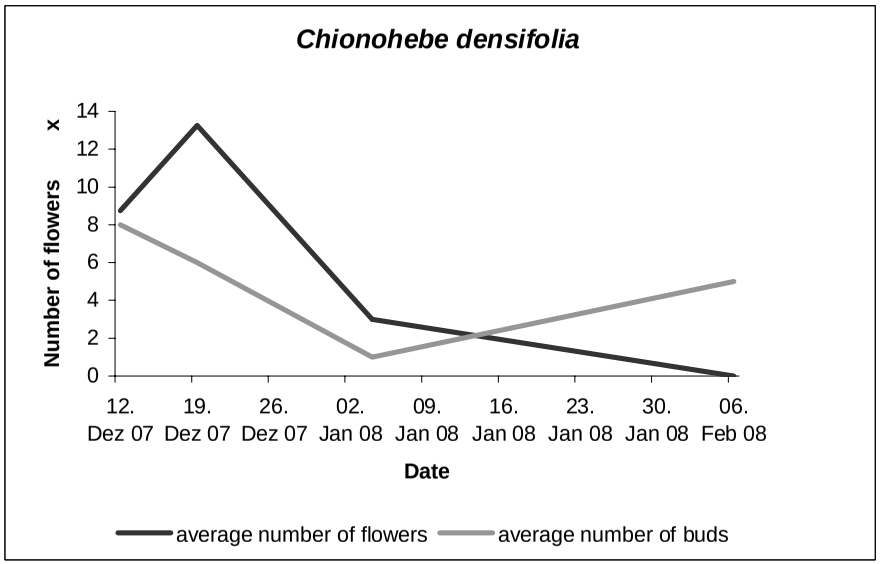
Monad, isopolar, shape spheroidal, circular in polar view; P axis 22.5-32.5 µm, E plane 27.5-32.5 µm; tricolporate, broad ectoapertures. 4 µm wide, very long, tapering to acute point, edges irregular; exine very thin, < 1 µm thick, tectate, baculate, scabrate

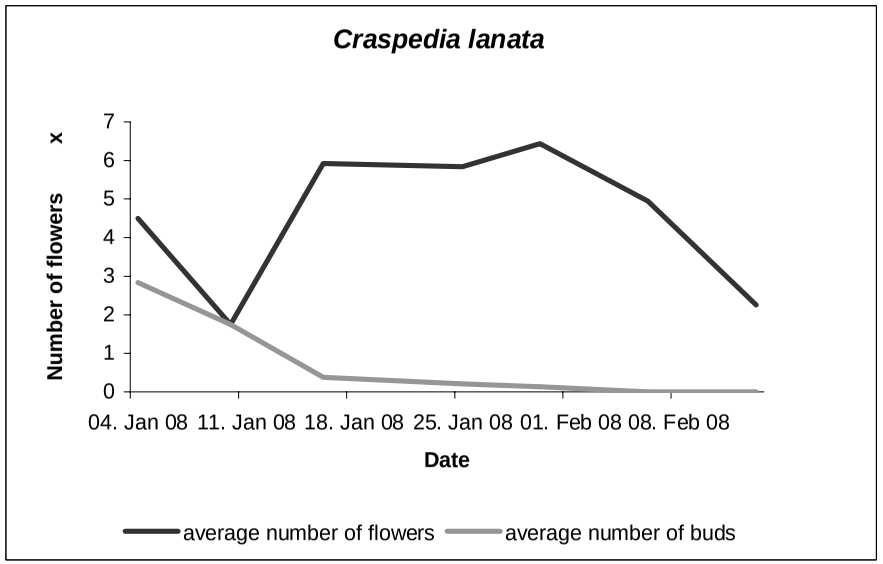
7.2 Phenology of plant species under investigation

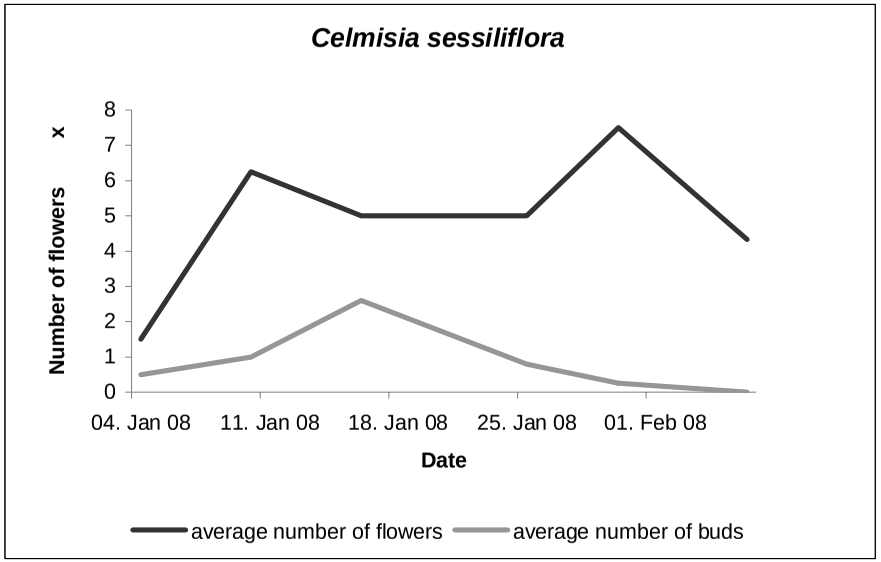
Single species flowering phenology graphs are presented for 21 species of the alpine community.

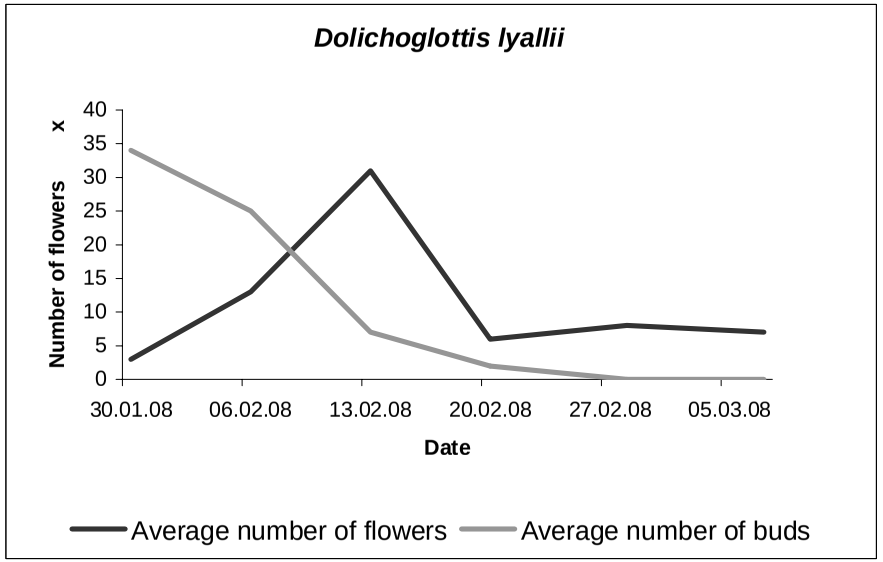

Brachyglottis bellidioides
Number of flowers x
10
9
8
7
6
5
4
3
2
1
0
10. Jan 08
17. Jan 08
24. Jan 08
31. Jan 08
Date
average of the number of flowers
average of the number of buds

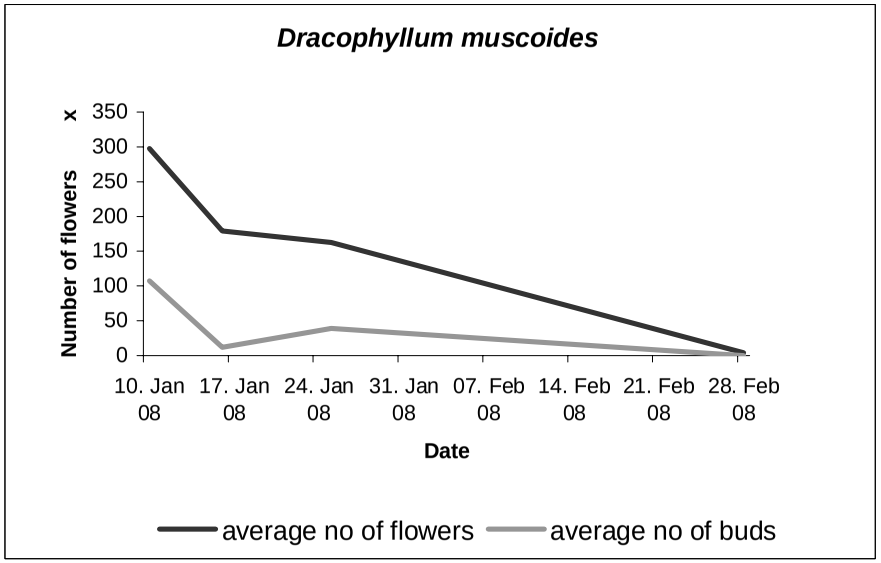

Brachycome sinclairii
Number of flowers x
11
10
9
8
7
6
5
4
3
2
1
0
12. Dez 07
12. Jan 08
12. Feb 08
12. Mrz 08
Date
Average number of flowers
Average number of buds

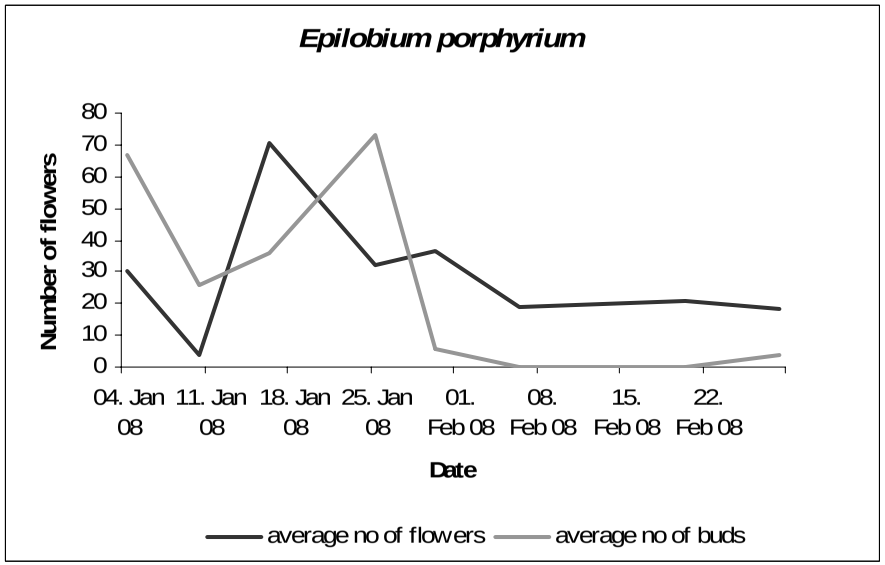

Chionohebe densifolia
Number of flowers x
14
12
10
8
6
4
2
0
12. Dez 07
19. Dez 07
26. Dez 07
02. Jan 08
09. Jan 08
16. Jan 08
23. Jan 08
30. Jan 08
06. Feb 08
Date
average number of flowers
average number of buds

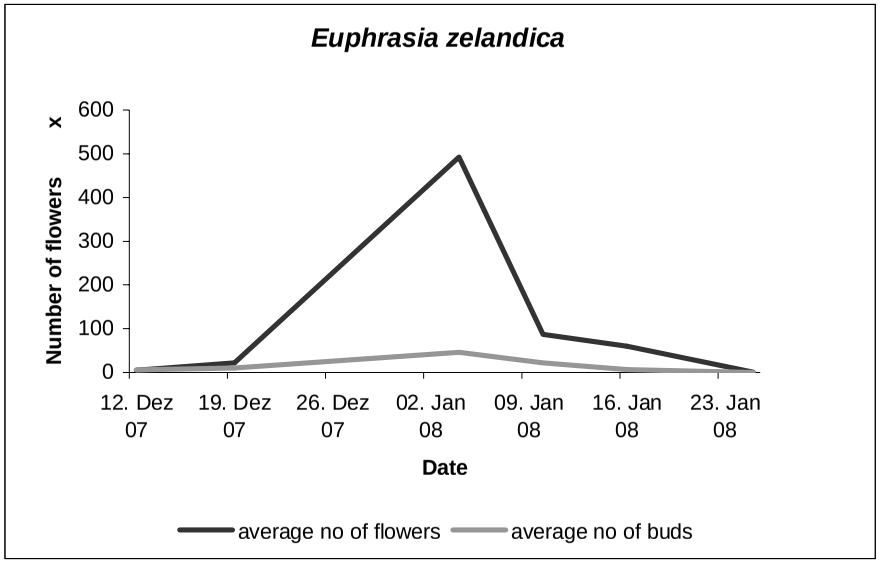

Craspedia lanata
Number of flowers x
7
6
5
4
3
2
1
0
04. Jan 08
11. Jan 08
18. Jan 08
25. Jan 08
01. Feb 08
08. Feb 08
Date
average number of flowers
average number of buds

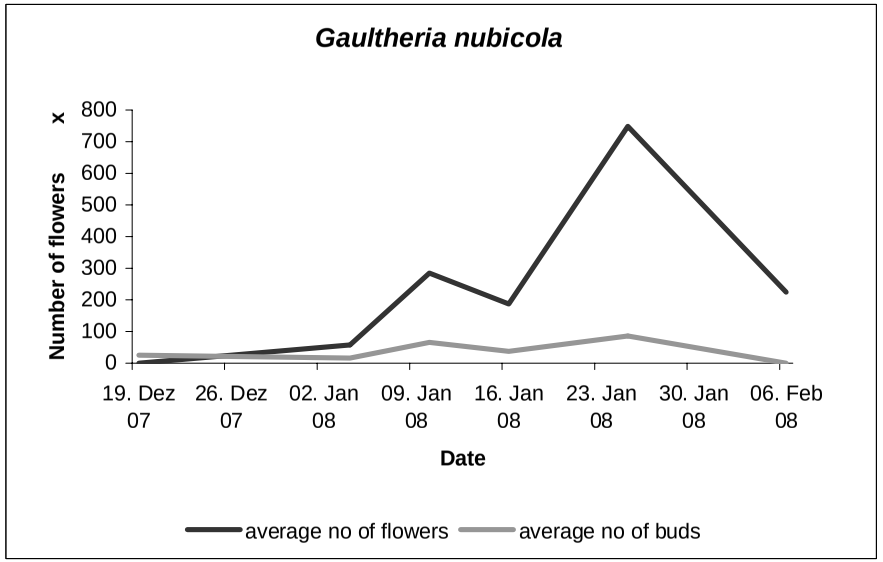

Celmisia sessiliflora
Number of flowers x
0
1
2
3
4
5
6
7
8
04. Jan 08
11. Jan 08
18. Jan 08
25. Jan 08
01. Feb 08
Date
average number of flowers
average number of buds

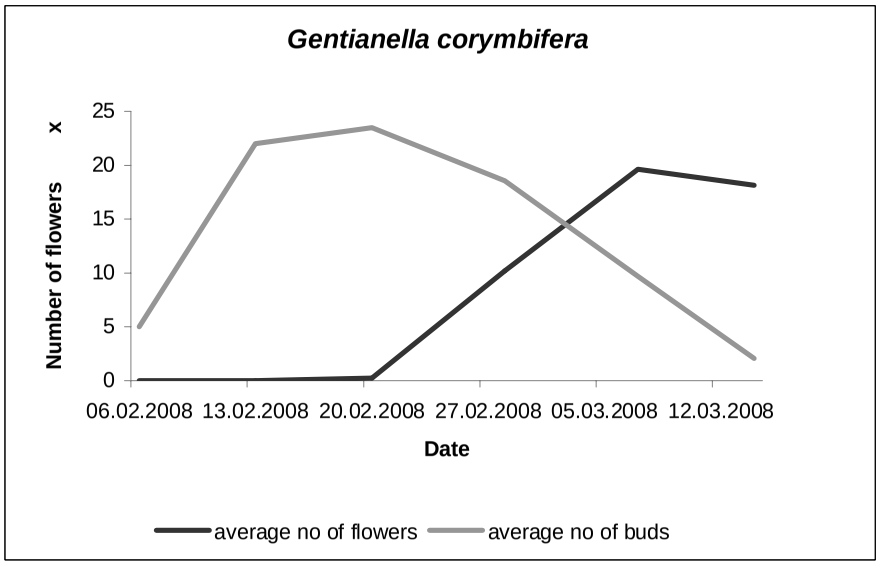

Dolichoglottis lyallii
Number of flowers x
0
5
10
15
20
25
30
35
40
30.01.08
06.02.08
13.02.08
20.02.08
27.02.08
05.03.08
Date
Average number of flowers
Average number of buds

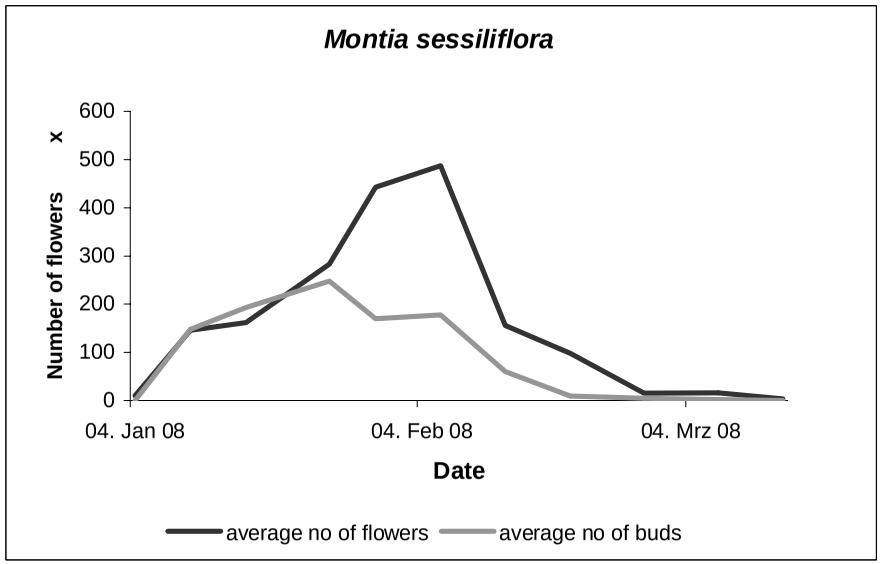

Dracophyllum muscoides
Number of flowers x
0
50
100
150
200
250
300
350
10. Jan 08
17. Jan 08
24. Jan 08
31. Jan 08
07. Feb 08
14. Feb 08
21. Feb 08
28. Feb 08
Date
average no of flowers
average no of buds

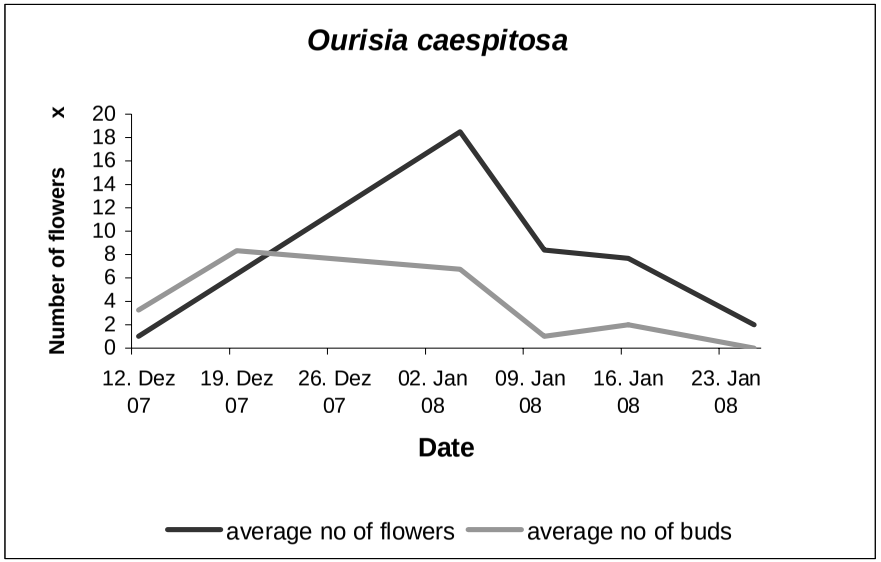

Epilobium porphyrium
Number of flowers
0
10
20
30
40
50
60
70
80
04. Jan 08
11. Jan 08
18. Jan 08
25. Jan 08
01. Feb 08
08. Feb 08
15. Feb 08
22. Feb 08
Date
average no of flowers
average no of buds

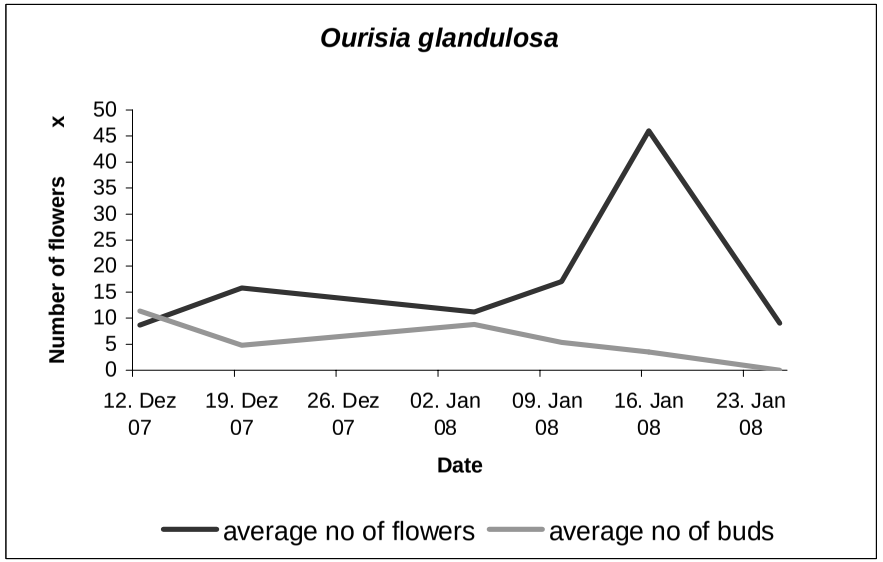

Euphrasia zelandica
Number of flowers x
600
500
400
300
200
100
0
12. Dez 07
19. Dez 07
26. Dez 07
02. Jan 08
09. Jan 08
16. Jan 08
23. Jan 08
Date
average no of flowers
average no of buds

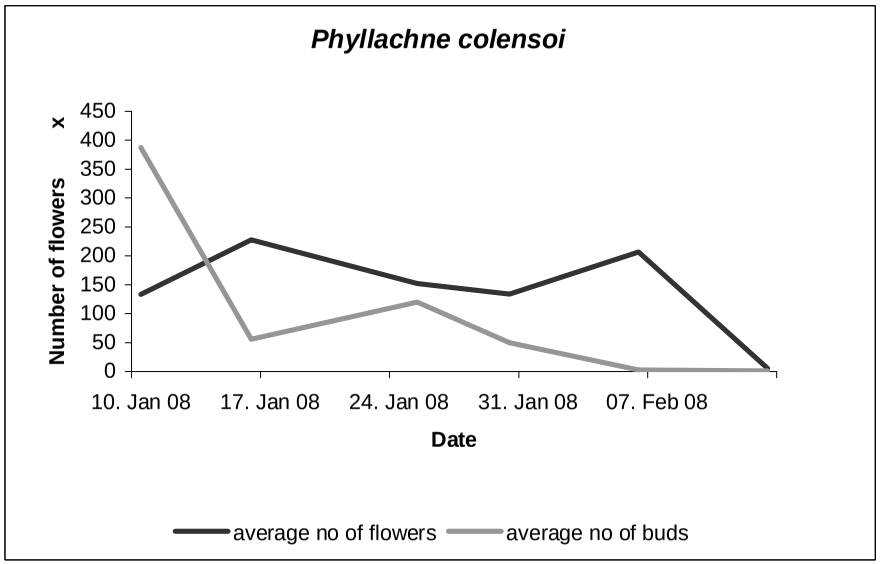

Gaultheria nubicola
Number of flowers x
800
700
600
500
400
300
200
100
0
19. Dez 07
26. Dez 07
02. Jan 08
09. Jan 08
16. Jan 08
23. Jan 08
30. Jan 08
06. Feb 08
Date
average no of flowers
average no of buds

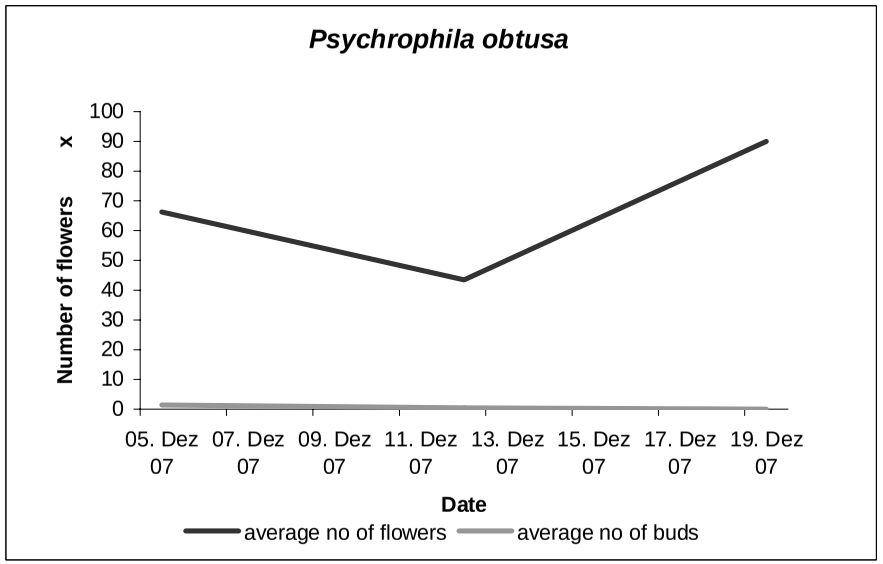

Gentianella corymbifera
Number of flowers x
25
20
15
10
5
0
06.02.2008
13.02.2008
20.02.2008
27.02.2008
05.03.2008
12.03.2008
Date
average no of flowers
average no of buds

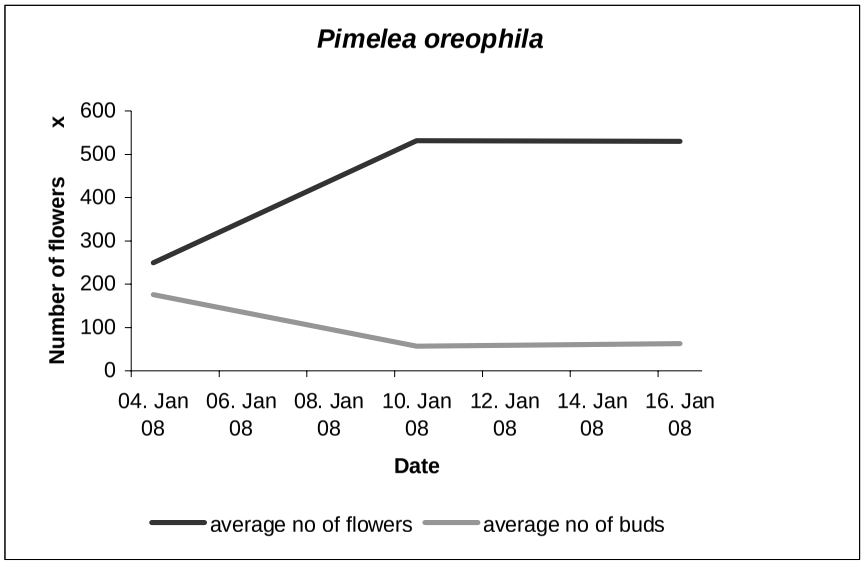

Montia sessiliflora
Number of flowers x
600
500
400
300
200
100
0
04. Jan 08
04. Feb 08
04. Mrz 08
Date
average no of flowers
average no of buds

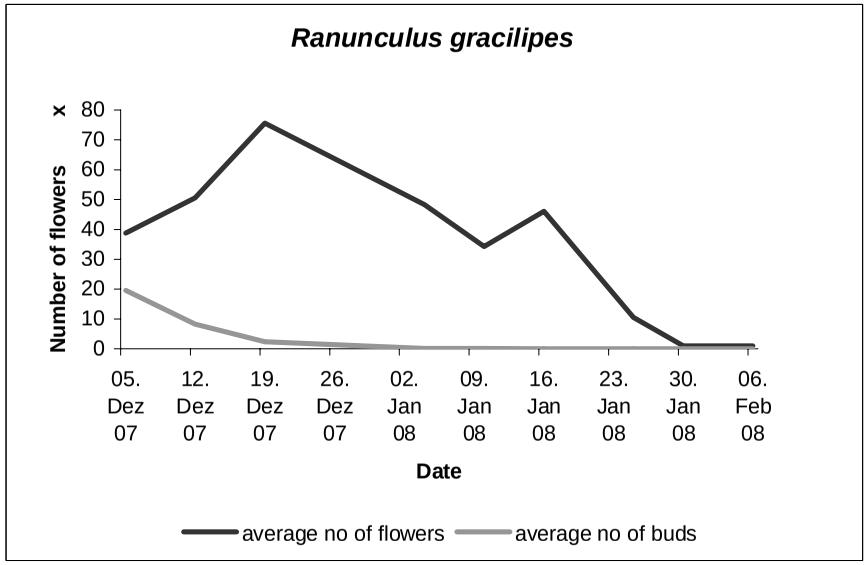

Ourisia caespitosa
Number of flowers x
20
18
16
14
12
10
8
6
4
2
0
12. Dez 07
19. Dez 07
26. Dez 07
02. Jan 08
09. Jan 08
16. Jan 08
23. Jan 08
Date
average no of flowers
average no of buds

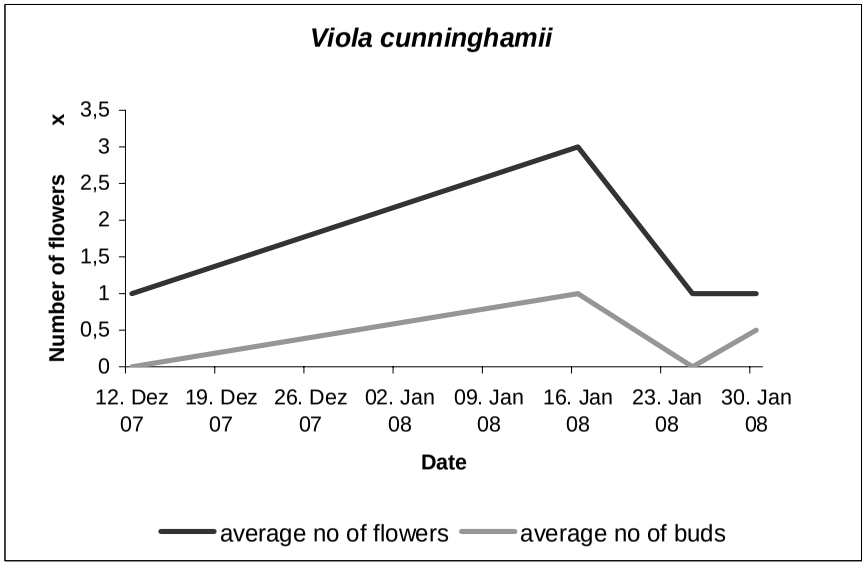

Ourisia glandulosa
Number of flowers x
50
45
40
35
30
25
20
15
10
5
0
12. Dez 07
19. Dez 07
26. Dez 07
02. Jan 08
09. Jan 08
16. Jan 08
23. Jan 08
Date
average no of flowers
average no of buds

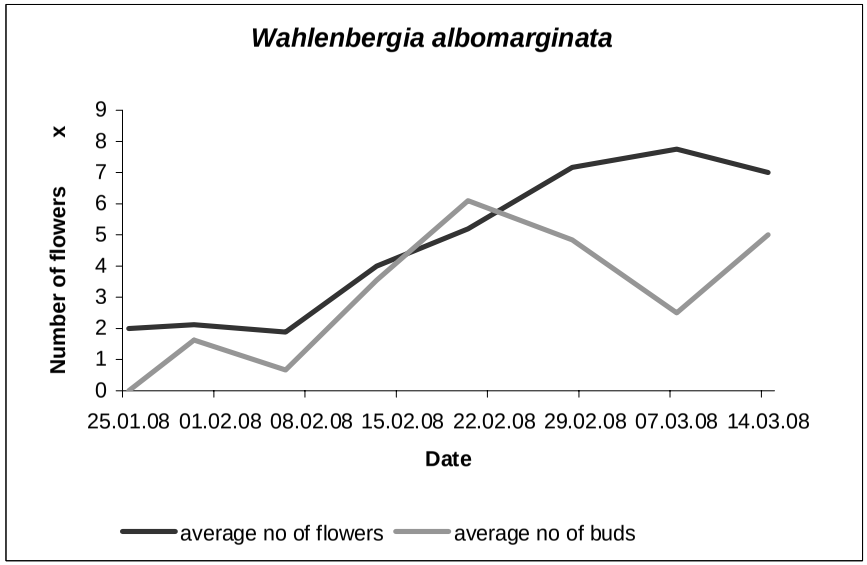

Phyllachne colensoi
Number of flowers x
450
400
350
300
250
200
150
100
50
0
10. Jan 08
17. Jan 08
24. Jan 08
31. Jan 08
07. Feb 08
Date
average no of flowers
average no of buds


Psychrophila obtusa
Number of flowers x
100
90
80
70
60
50
40
30
20
10
0
05. Dez 07
07. Dez 07
09. Dez 07
11. Dez 07
13. Dez 07
15. Dez 07
17. Dez 07
19. Dez 07
Date
average no of flowers
average no of buds


Pimelea oreophila
Number of flowers x
600
500
400
300
200
100
0
04. Jan 08
06. Jan 08
08. Jan 08
10. Jan 08
12. Jan 08
14. Jan 08
16. Jan 08
Date
average no of flowers
average no of buds

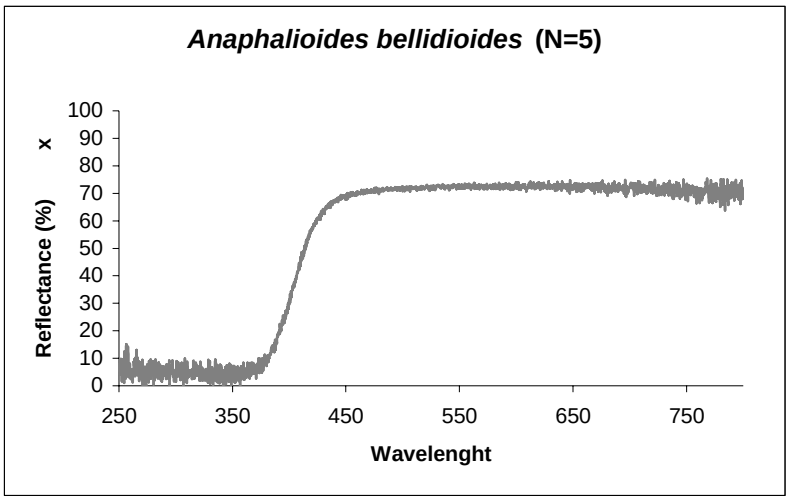

Ranunculus gracilipes
Number of flowers x
80
70
60
50
40
30
20
10
0
05. Dez 07
12. Dez 07
19. Dez 07
26. Dez 07
02. Jan 08
09. Jan 08
16. Jan 08
23. Jan 08
30. Jan 08
06. Feb 08
Date
average no of flowers
average no of buds

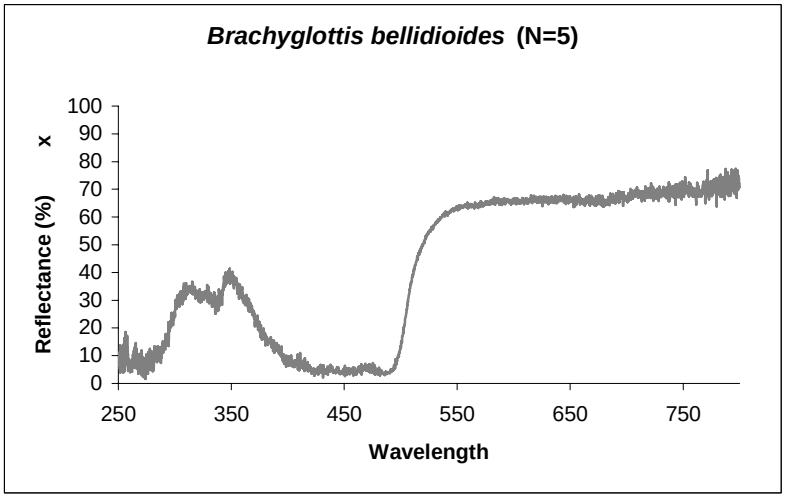

Viola cunninghamii
Number of flowers x
3,5
3
2,5
2
1,5
1
0,5
0
12. Dez 07
19. Dez 07
26. Dez 07
02. Jan 08
09. Jan 08
16. Jan 08
23. Jan 08
30. Jan 08
Date
average no of flowers
average no of buds

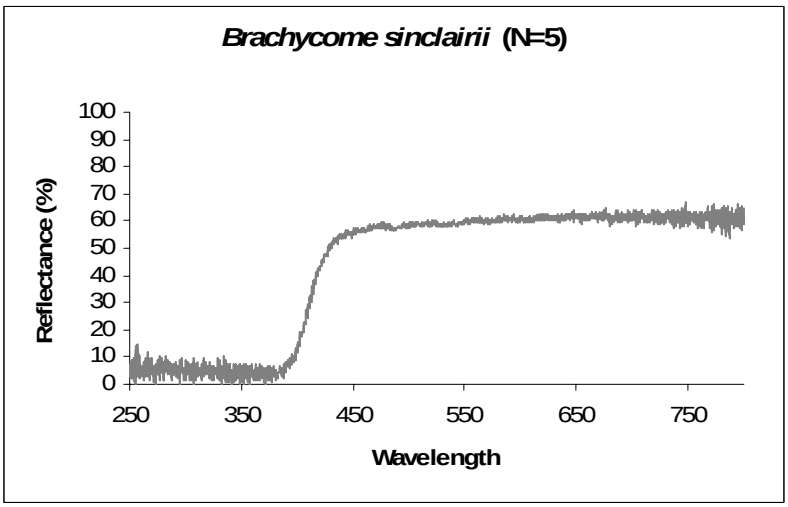

Wahlenbergia albomarginata
Number of flowers x
9
8
7
6
5
4
3
2
1
0
25.01.08
01.02.08
08.02.08
15.02.08
22.02.08
29.02.08
07.03.08
14.03.08
Date
average no of flowers
average no of buds

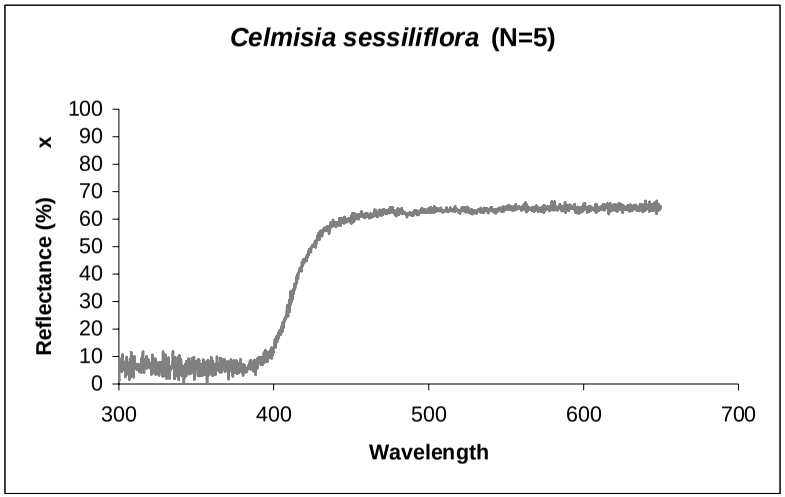
7.3 Spectral reflectance curves of plant species under investigation

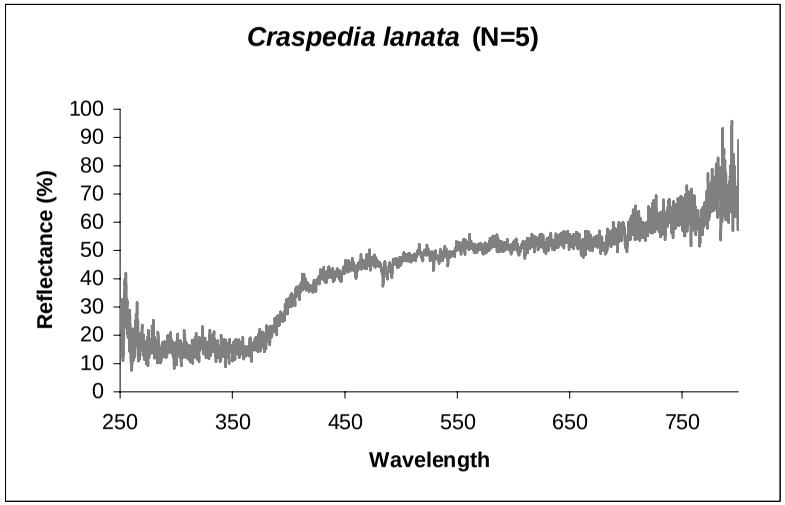
Single species reflectance graphs are presented for 18 species of the alpine community.

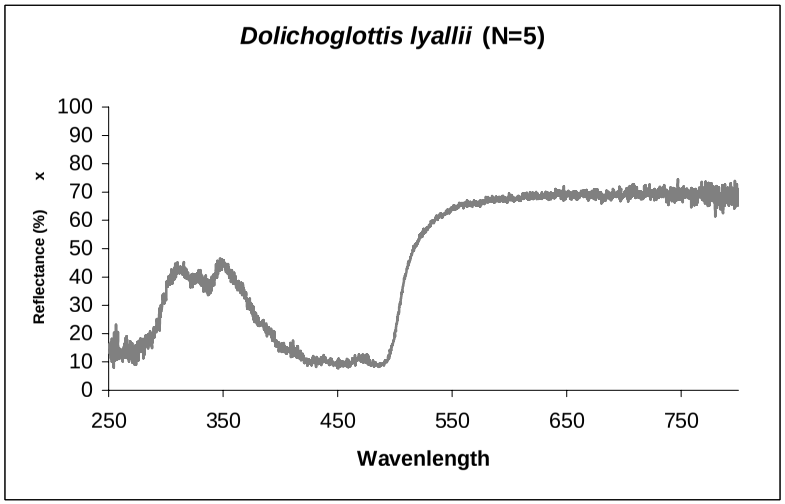


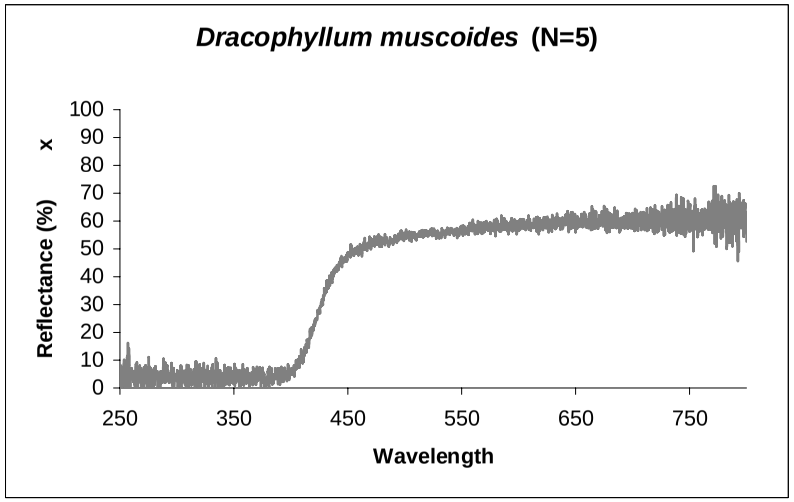


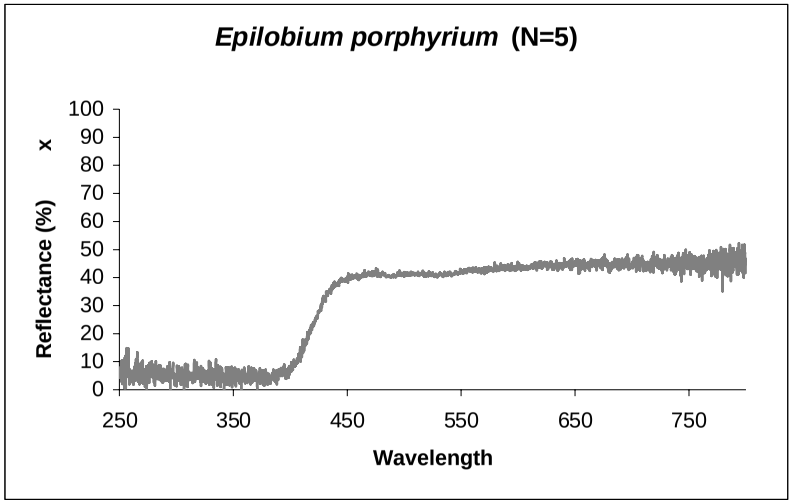


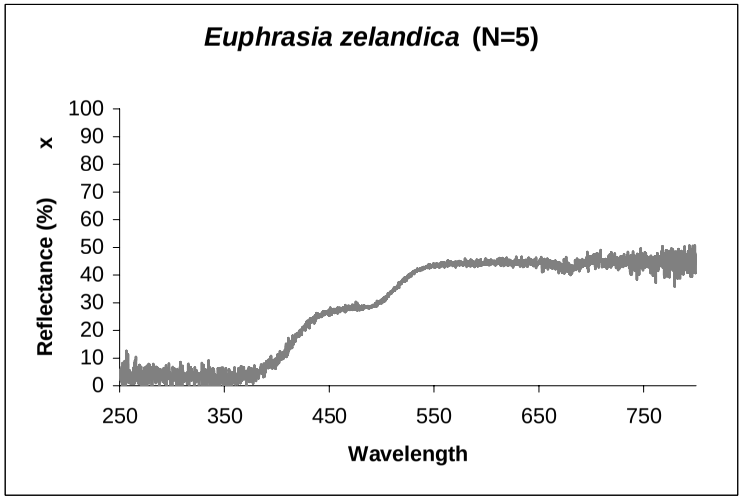

Celmisia sessiliflora (N=5)
Reflectance (%)
100
90
80
70
60
50
40
30
20
10
0
300
400
500
600
700
Wavelength

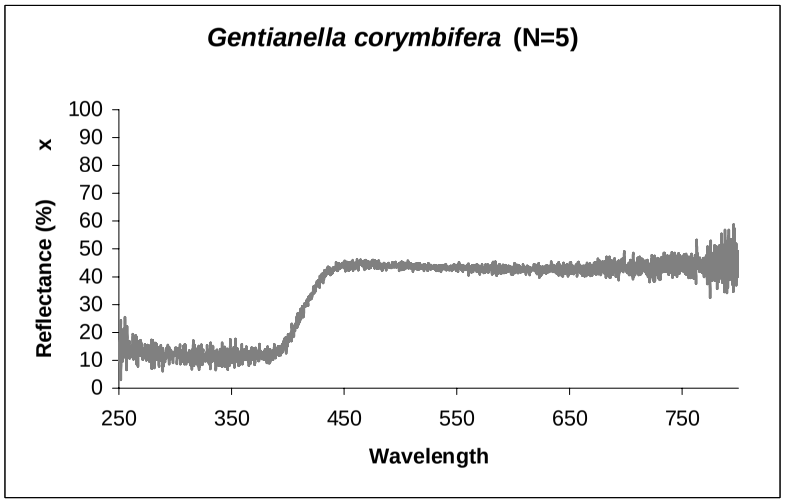

Craspedia lanata (N=5)
Reflectance (%)
100
90
80
70
60
50
40
30
20
10
0
250
350
450
550
650
750
Wavelength

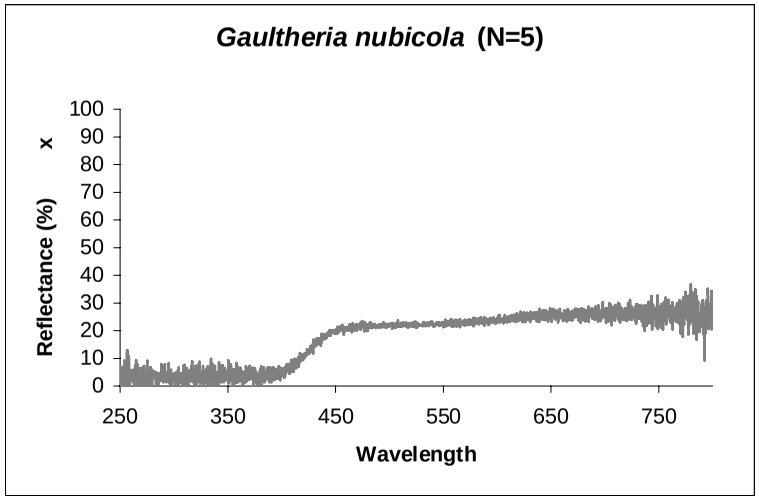

Dolichoglottis lyallii (N=5)
Reflectance (%)
100
90
80
70
60
50
40
30
20
10
0
250
350
450
550
650
750
Wavenlength

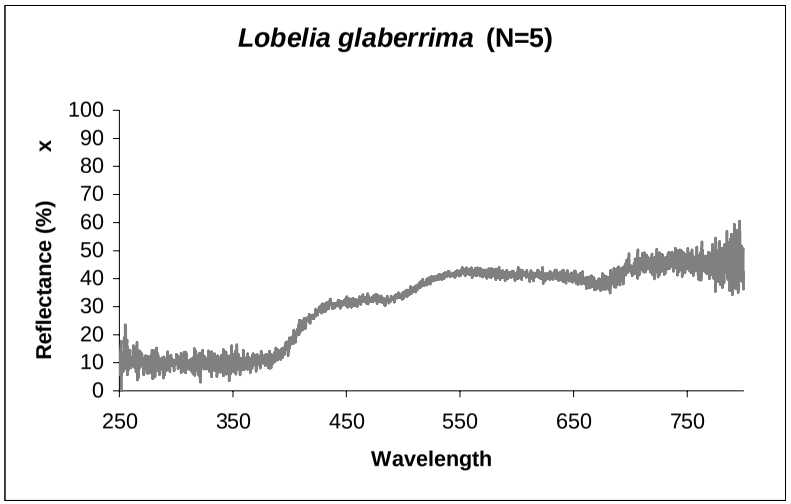

Dracophyllum muscoides (N=5)
Reflectance (%)
100
90
80
70
60
50
40
30
20
10
0
250
350
450
550
650
750
Wavelength

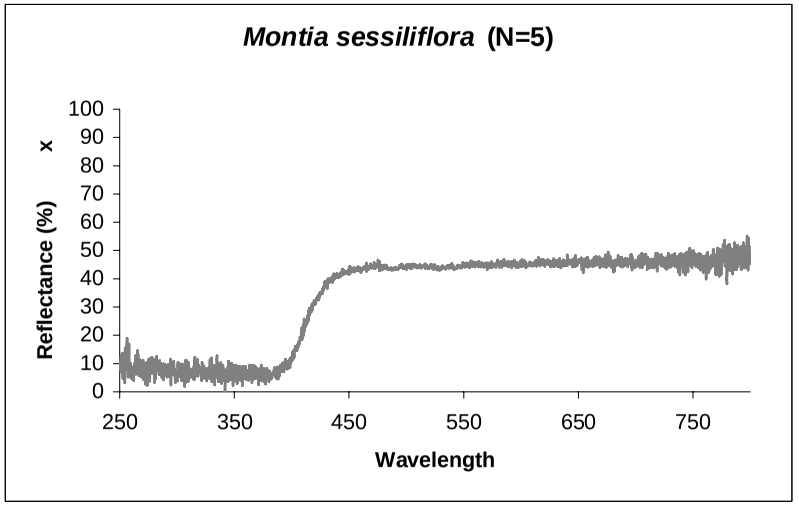

Epilobium porphyrium (N=5)
Reflectance (%) x
100
90
80
70
60
50
40
30
20
10
0
250
350
450
550
650
750
Wavelength

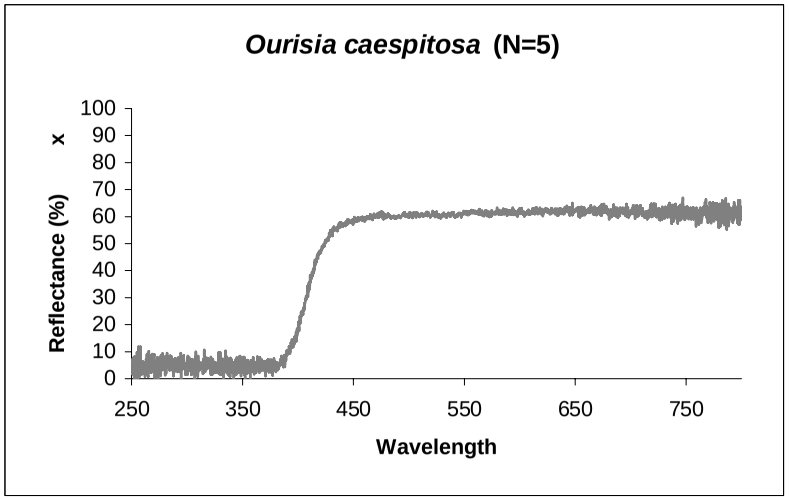

Euphrasia zelandica (N=5)
Reflectance (%) x
100
90
80
70
60
50
40
30
20
10
0
250
350
450
550
650
750
Wavelength

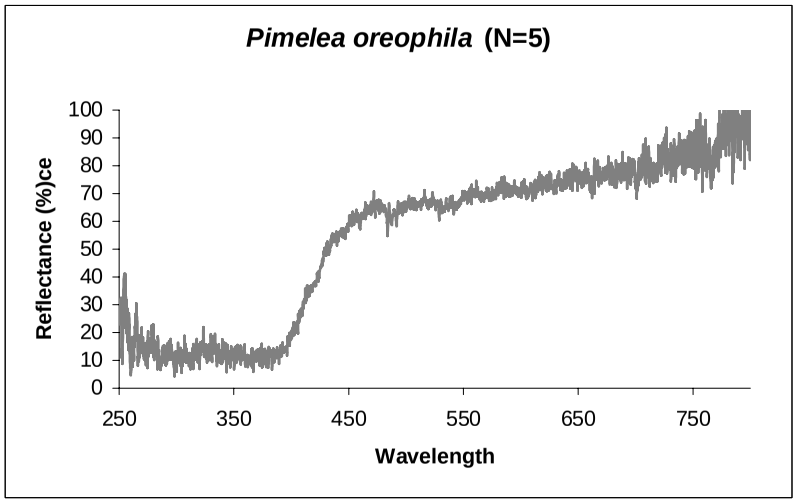

Gentianella corymbifera (N=5)
Reflectance (%) x
100
90
80
70
60
50
40
30
20
10
0
250
350
450
550
650
750
Wavelength

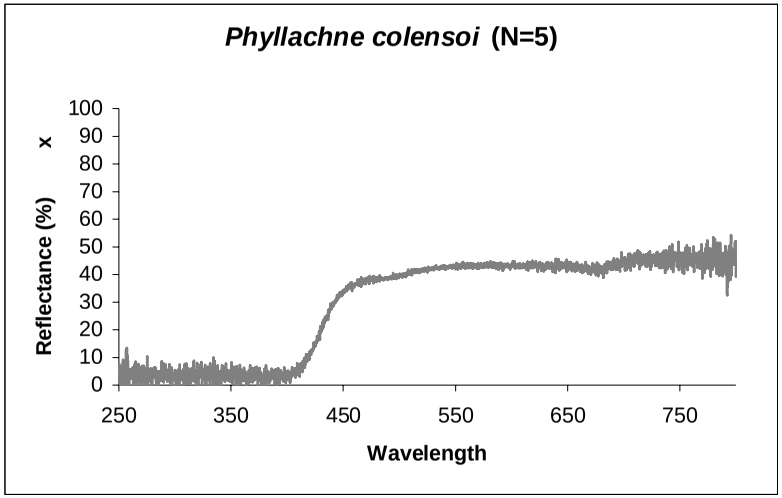

Gaultheria nubicola (N=5)
Reflectance (%) x
100
90
80
70
60
50
40
30
20
10
0
250
350
450
550
650
750
Wavelength

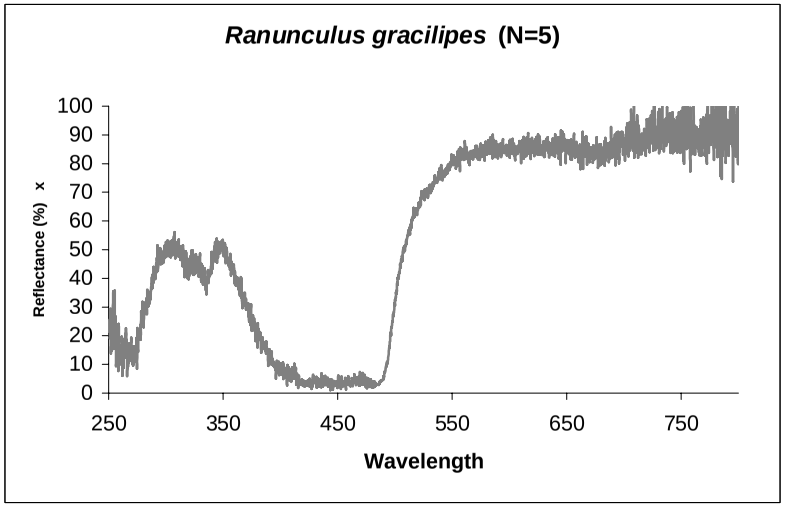

Lobelia glaberrima (N=5)
Reflectance (%) x
100
90
80
70
60
50
40
30
20
10
0
250
350
450
550
650
750
Wavelength

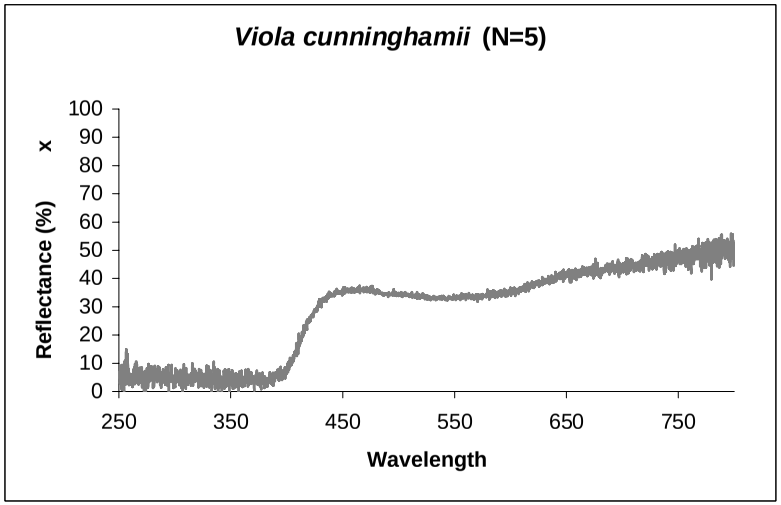

Montia sessiliflora (N=5)
Reflectance (%) x
100
90
80
70
60
50
40
30
20
10
0
250
350
450
550
650
750
Wavelength

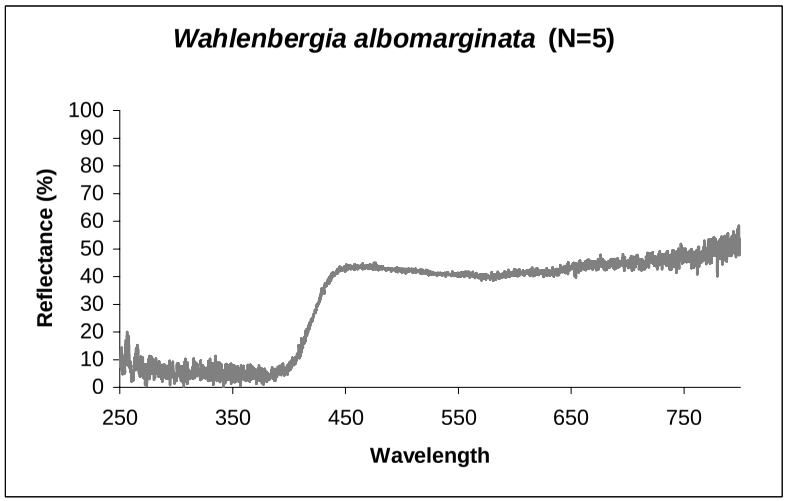

Ourisia caespitosa (N=5)
Reflectance (%) x
100
90
80
70
60
50
40
30
20
10
0
250
350
450
550
650
750
Wavelength


Pimelea oreophila (N=5)
Reflectance (%)ce
100
90
80
70
60
50
40
30
20
10
0
250
350
450
550
650
750
Wavelength


Phyllachne colensoi (N=5)
Reflectance (%) x
100
90
80
70
60
50
40
30
20
10
0
250
350
450
550
650
750
Wavelength


Ranunculus gracilipes (N=5)
Reflectance (%) x
100
90
80
70
60
50
40
30
20
10
0
250
350
450
550
650
750
Wavelength


Viola cunninghamii (N=5)
Reflectance (%) x
100
90
80
70
60
50
40
30
20
10
0
250
350
450
550
650
750
Wavelength


Wahlenbergia albomarginata (N=5)
Reflectance (%)
100
90
80
70
60
50
40
30
20
10
0
250
350
450
550
650
750
Wavelength

Furthermore, colour distance measurements in bee colour space are presented with the actual distance measurements in matrix format where the distances of all species to each other may be looked up.

Table 7. 1 X- and Y- coordinates for petal colours of 18 species of the alpine community

	x	y
AB	**0,1716**	**0,1545**
BB	**-0,0547**	**-0,2445**
BS	**0,2394**	**0,1957**
CS	**0,1928**	**0,1671**
CL	**0,0062**	**0,0744**
DL	**-0,0738**	**-0,1457**
DM	**0,2956**	**0,2091**
EP	**0,2075**	**0,1846**
EZ	**0,2955**	**0,2091**
GN	**0,1572**	**0,1644**
GC 1	**0,0497**	**0,1111**
GC 2	**0,1641**	**0,1324**
LG	**0,0380**	**0,0890**
MS	**0,1467**	**0,1565**
OC	**0,2064**	**0,1798**
PC	**0,2782**	**0,1900**
POr	**0,1033**	**0,1058**
RG	**-0,0471**	**-0,2559**
VC	**0,1854**	**0,1970**
WA	**0,1975**	**0,1899**
LEAF	0,0000	0,0000

Table 7. 2 Colour distance chart in bee colour space for species AB to GN

	AB	BB	BS	CS	CL	DL	DM	EP	EZ	GN
AB	0,0000	0,4587	0,0793	0,0247	0,1838	0,3877	0,1355	0,0469	0,1355	0,0175
BB	0,4587	0,0000	0,5294	0,4803	0,3246	0,1006	0,5731	0,5029	0,5731	0,4606
BS	0,0793	0,5294	0,0000	0,0547	0,2629	0,4633	0,0578	0,0337	0,0578	0,0879
CS	0,0247	0,4803	0,0547	0,0000	0,2084	0,4110	0,1110	0,0229	0,1110	0,0357
CL	0,1838	0,3246	0,2629	0,2084	0,0000	0,2341	0,3192	0,2296	0,3192	0,1759
DL	0,3877	0,1006	0,4633	0,4110	0,2341	0,0000	0,5121	0,4339	0,5121	0,3867
DM	0,1355	0,5731	0,0578	0,1110	0,3192	0,5121	0,0000	0,0914	0,0000	0,1454
EP	0,0469	0,5029	0,0337	0,0229	0,2296	0,4339	0,0914	0,0000	0,0913	0,0542
EZ	0,1355	0,5731	0,0578	0,1110	0,3192	0,5121	0,0000	0,0913	0,0000	0,1453
GN	0,0175	0,4606	0,0879	0,0357	0,1759	0,3867	0,1454	0,0542	0,1453	0,0000
GC 1	0,1294	0,3706	0,2077	0,1537	0,0569	0,2849	0,2647	0,1741	0,2646	0,1200
GC 2	0,0234	0,4358	0,0984	0,0450	0,1682	0,3659	0,1522	0,0680	0,1522	0,0328
LG	0,1488	0,3462	0,2279	0,1734	0,0350	0,2600	0,2842	0,1946	0,2842	0,1411
MS	0,0249	0,4488	0,1006	0,0473	0,1628	0,3741	0,1578	0,0670	0,1578	0,0131
OC	0,0430	0,4982	0,0366	0,0186	0,2263	0,4295	0,0938	0,0050	0,0938	0,0515
PC	0,1123	0,5473	0,0392	0,0884	0,2955	0,4864	0,0258	0,0708	0,0258	0,1236
POr	0,0839	0,3843	0,1631	0,1085	0,1021	0,3076	0,2183	0,1307	0,2182	0,0797
RG	0,4650	0,0137	0,5348	0,4863	0,3345	0,1134	0,5776	0,5088	0,5776	0,4674
VC	0,0447	0,5026	0,0539	0,0309	0,2172	0,4297	0,1108	0,0254	0,1108	0,0431
WA	0,0439	0,5024	0,0422	0,0234	0,2235	0,4316	0,0999	0,0114	0,0999	0,0477
LEAF	0,1716	-0,0547	0,2394	0,1928	0,0062	-0,0738	0,2956	0,2075	0,2955	0,1572
LEAF	0,2309	0,2506	0,3092	0,2551	0,0746	0,1633	0,3620	0,2778	0,3620	0,2275

Table 7. 3 Colour distance chart in bee colour space for species GC to WA

	GC 1	GC 2	LG	MS	OC	PC	POr	RG	VC	WA
AB	0,1294	0,0234	0,1488	0,0249	0,0430	0,1123	0,0839	0,4650	0,0447	0,0439
BB	0,3706	0,4358	0,3462	0,4488	0,4982	0,5473	0,3843	0,0137	0,5026	0,5024
BS	0,2077	0,0984	0,2279	0,1006	0,0366	0,0392	0,1631	0,5348	0,0539	0,0422
CS	0,1537	0,0450	0,1734	0,0473	0,0186	0,0884	0,1085	0,4863	0,0309	0,0234
CL	0,0569	0,1682	0,0350	0,1628	0,2263	0,2955	0,1021	0,3345	0,2172	0,2235
DL	0,2849	0,3659	0,2600	0,3741	0,4295	0,4864	0,3076	0,1134	0,4297	0,4316
DM	0,2647	0,1522	0,2842	0,1578	0,0938	0,0258	0,2183	0,5776	0,1108	0,0999
EP	0,1741	0,0680	0,1946	0,0670	0,0050	0,0708	0,1307	0,5088	0,0254	0,0114
EZ	0,2646	0,1522	0,2842	0,1578	0,0938	0,0258	0,2182	0,5776	0,1108	0,0999
GN	0,1200	0,0328	0,1411	0,0131	0,0515	0,1236	0,0797	0,4674	0,0431	0,0477
GC 1	0,0000	0,1163	0,0250	0,1071	0,1711	0,2417	0,0538	0,3795	0,1607	0,1675
GC 2	0,1163	0,0000	0,1333	0,0297	0,0635	0,1278	0,0663	0,4420	0,0681	0,0666
LG	0,0250	0,1333	0,0000	0,1280	0,1913	0,2605	0,0674	0,3553	0,1828	0,1888
MS	0,1071	0,0297	0,1280	0,0000	0,0640	0,1356	0,0668	0,4557	0,0560	0,0608
OC	0,1711	0,0635	0,1913	0,0640	0,0000	0,0725	0,1269	0,5040	0,0272	0,0135
PC	0,2417	0,1278	0,2605	0,1356	0,0725	0,0000	0,1941	0,5519	0,0930	0,0807
POr	0,0538	0,0663	0,0674	0,0668	0,1269	0,1941	0,0000	0,3917	0,1228	0,1263
RG	0,3795	0,4420	0,3553	0,4557	0,5040	0,5519	0,3917	0,0000	0,5091	0,5085
VC	0,1607	0,0681	0,1828	0,0560	0,0272	0,0930	0,1228	0,5091	0,0000	0,0140
WA	0,1675	0,0666	0,1888	0,0608	0,0135	0,0807	0,1263	0,5085	0,0140	0,0000
LEAF	0,0497	0,1641	0,0380	0,1467	0,2064	0,2782	0,1033	-0,0471	0,1854	0,1975
LEAF	0,1217	0,2108	0,0968	0,2145	0,2737	0,3369	0,1478	0,2602	0,2706	0,2740

7.4 Insect visitor groups

Allograpta spec. (Diptera: Syrphidae) on *Brachycome sinclairii* (Asteraceae)

Platycheirus spec. on *Phyllachne colensoi* (Stylidiaceae)

Tachinid fly (Tachinidae) on *Dracophyllum muscoides* (Ericaceae)

Soldier fly (Stratiomyidae) on *Brachycome sinclairii* (Asteraceae)

Leioproctus spec. (Hymenoptera: Colletidae) on *Dolichoglottis lyallii* (Asteraceae)

Hylaeus matamoko (Hymenoptera: Colletidae) on *Ourisia glandulosa* (Plantaginaceae)

Apis mellifera (Hymenoptera: Apidae) on *Gaultheria nubicola* (Ericaceae)

Day-flying moth on *Gaultheria nubicola* (Ericaceae)

Table 7. 4 Taxonomic information on all insect voucher specimens included in the investigation

ID #	Identification	Order:Family	Host plant
203	*Dilophus harrisoni* (Hardy, 1951)	Dip: Bibionidae	GC
163	*Hilara* sp. 'smooth femur', f	Dip: Empididae	GC
205	*Hilara* sp. 'spiny femur', f	Dip: Empididae	BB
165	*"Spilogona"* sp.A, f	Dip: Muscidae	MS
241	*"Spilogona"* sp.A, f	Dip: Muscidae	RG
242	*"Spilogona"* sp.A, f	Dip: Muscidae	RG
243	*"Spilogona"* sp.A, f	Dip: Muscidae	
118	*"Spilogona"* sp.A, m	Dip: Muscidae	RG
206	*"Spilogona"* sp.B	Dip: Muscidae	AB
95	*"Spilogona"* sp.C	Dip: Muscidae	POr
90	*"Spilogona"* sp.D 'red tibiae'	Dip: Muscidae	CD
89	*"Spilogona"* sp.E 'foretarsus dilated'	Dip: Muscidae	MS
196	*Limnohelina* sp., f	Dip: Muscidae	MS
96	*Limnohelina* sp., m	Dip: Muscidae	POr
86	*Odontomyia* sp., f	Dip: Stratiomyidae	GN
194	*Odontomyia* sp., f	Dip: Stratiomyidae	PC
197	*Odontomyia* sp., f	Dip: Stratiomyidae	CS
198	*Odontomyia* sp., f	Dip: Stratiomyidae	AB
200	*Odontomyia* sp., f	Dip: Stratiomyidae	MS
201	*Odontomyia* sp., f	Dip: Stratiomyidae	CL
199	*Odontomyia* sp., m	Dip: Stratiomyidae	AB
69	*Allograpta ?pseudoropalus* (Miller, 1921), f	Dip: Syrphidae	BS
223	*Allograpta ?pseudoropalus* (Miller, 1921), f	Dip: Syrphidae	CL
231	*Allograpta ?pseudoropalus* (Miller, 1921), f	Dip: Syrphidae	BB
236	*Allograpta ?pseudoropalus* (Miller, 1921), f	Dip: Syrphidae	CL
237	*Allograpta ?pseudoropalus* (Miller, 1921), f	Dip: Syrphidae	BB
62	*Allograpta ?pseudoropalus* (Miller, 1921), f	Dip: Syrphidae	VC
224	*Allograpta ?pseudoropalus* (Miller, 1921), f	Dip: Syrphidae	CL
144	*Allograpta ?pseudoropalus* (Miller, 1921), m	Dip: Syrphidae	BB
71	*Allograpta ?pseudoropalus* (Miller, 1921), m	Dip: Syrphidae	OC
75	*Allograpta ?pseudoropalus* (Miller, 1921), m	Dip: Syrphidae	BB
222	*Allograpta ?pseudoropalus* (Miller, 1921), m	Dip: Syrphidae	EP
232	*Allograpta ?pseudoropalus* (Miller, 1921), m	Dip: Syrphidae	BB
233	*Allograpta ?pseudoropalus* (Miller, 1921), m	Dip: Syrphidae	OC
221	*Allograpta ?ropalus* (Walker), f	Dip: Syrphidae	DL
72	*Allograpta* sp. ("*ortas*" sensu Miller), f	Dip: Syrphidae	OC
218	*Allograpta* sp. nr *flavofaciens*	Dip: Syrphidae	BB
195	*Helophilus* sp. nr *hectori* Miller, 1924	Dip: Syrphidae	DL

ID #	Identification	Order:Family	Host plant
193	*Allograpta ?pseudoropalus* (Miller, 1921), m	Dip: Syrphidae	DL
228	*Allograpta ?pseudoropalus* (Miller, 1921), m	Dip: Syrphidae	BB
230	*Allograpta ?pseudoropalus* (Miller, 1921), m	Dip: Syrphidae	DL
226	*Allograpta ?pseudoropalus* (Miller, 1921), m	Dip: Syrphidae	BB
227	*Allograpta ?pseudoropalus* (Miller, 1921), m	Dip: Syrphidae	CL
229	*Allograpta ?pseudoropalus* (Miller, 1921), m	Dip: Syrphidae	CS
234	*Allograpta ?pseudoropalus* (Miller, 1921), m	Dip: Syrphidae	BS
238	*Allograpta ?pseudoropalus* (Miller, 1921), m	Dip: Syrphidae	MS
61	*Platycheirus ?howesii* Miller 1921	Dip: Syrphidae	PC
110	*Platycheirus ?howesii* Miller 1921	Dip: Syrphidae	RG
208	*Platycheirus ?howesii* Miller 1921	Dip: Syrphidae	BS
209	*Platycheirus ?howesii* Miller 1921	Dip: Syrphidae	VC
210	*Platycheirus ?howesii* Miller 1921	Dip: Syrphidae	OG
211	*Platycheirus ?howesii* Miller 1921	Dip: Syrphidae	OG
212	*Platycheirus ?howesii* Miller 1921	Dip: Syrphidae	OG
213	*Platycheirus ?howesii* Miller 1921	Dip: Syrphidae	BB
214	*Platycheirus ?howesii* Miller 1921	Dip: Syrphidae	PC
215	*Platycheirus ?howesii* Miller 1921	Dip: Syrphidae	OG
216	*Platycheirus ?howesii* Miller 1921	Dip: Syrphidae	OG
217	*Platycheirus ?howesii* Miller 1921	Dip: Syrphidae	CS
219	*Platycheirus ?howesii* Miller 1921	Dip: Syrphidae	RG
204	*Platycheirus* sp.	Dip: Syrphidae	RG
220	*Platycheirus* sp. 'yellow & black tibiae'	Dip: Syrphidae	CS
116	*Platycheirus* sp. 'yellow knees'	Dip: Syrphidae	CD
82	*Platycheirus* sp. 'yellow knees'	Dip: Syrphidae	BS
179	*Platycheirus* sp. 'yellow knees'	Dip: Syrphidae	CD
180	*Platycheirus* sp. 'yellow knees'	Dip: Syrphidae	CD
181	*Platycheirus* sp. 'yellow knees'	Dip: Syrphidae	CD
182	*Platycheirus* sp. 'yellow knees'	Dip: Syrphidae	CD
207	*Platycheirus* sp. 'yellow knees'	Dip: Syrphidae	POr
117	*Platycheirus* sp. 'yellow knees'	Dip: Syrphidae	CD
120	*Platycheirus* sp. 'yellow knees'	Dip: Syrphidae	RG
141	*Platycheirus* sp. 'yellow knees'	Dip: Syrphidae	CD
168	*Avibrissia sp.'black palpi'*	Dip: Tachinidae	GC
153	*Platytachina sp., cf. difficilis Malloch, 1938*	Dip: Tachinidae	RG
172	*Veluta albicincta Malloch, 1938*	Dip: Tachinidae	GC
167	*Phaoniella sp., 'yellow palpi'*	Dip:Tachinidae	GC
121	*Apis mellifera*	Hym: Apidae	GN
102	*Bombus terrestris*	Hym: Apidae	AB
103	*Bombus terrestris*	Hym: Apidae	GN

ID #	Identification	Order:Family	Host plant
158	*Bombus terrestris*	Hym: Apidae	GN
177	*Bombus terrestris*	Hym: Apidae	GN
112	*Hylaeus matamoko*	Hym: Colletidae	EZ
130	*Hylaeus matamoko*	Hym: Colletidae	OG
132	*Hylaeus matamoko*	Hym: Colletidae	OG
133	*Hylaeus matamoko*	Hym: Colletidae	WA
137	*Hylaeus matamoko*	Hym: Colletidae	C. haastii
139	*Hylaeus matamoko*	Hym: Colletidae	OG
184	*Hylaeus matamoko*	Hym: Colletidae	CS
244	*Hylaeus matamoko*	Hym: Colletidae	OG
111	*Leioproctus fulvescens*	Hym: Colletidae	BB
171	*Leioproctus pango*	Hym: Colletidae	GC
108	*Leioproctus pekanui*	Hym: Colletidae	BS
162	*Leioproctus pekanui*	Hym: Colletidae	GC
174	*Leioproctus pekanui*	Hym: Colletidae	MS
183	*Leioproctus pekanui*	Hym: Colletidae	CL
145	*Lasioglossum maunga*	Hym: Halictidae	CL
124	*Hylaeus matamoko*	Hym:Colletidae	WA
161	*Hylaeus matamoko*	Hym:Colletidae	GC
178	*Paranotoreas ferox Butler, 1877*	Lep: Geometridae	WA
157	*Aletia pandaPhilpott, 1920*	Lep: Noctuidae	GN
97	*Dasyuris austrina Philpott, 1928;*	Lep:Geometridae	RG

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