

# **Pollen limitation and network asymmetry in an endangered oak-savannah ecosystem**

**by**

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## Abstract

Understanding how changes in biodiversity affect plants and plant-pollinator communities is important for their conservation. I measured the effect of conspecific density and co-flowering diversity on pollen limitation in six plant species at six sites over two years to investigate the effect of the co-flowering community on plant reproduction. Plant patches with high conspecific density generally had higher pollen limitation, although the effect was weak and overall pollen limitation was rare. Co-flowering diversity had little effect on pollen limitation. I also collected flower visitors off plants to produce a plant-pollinator interaction network. Networks were highly asymmetric; in general, interacting species did not rely equally on one another for either floral resources or pollination. Larger networks contained more specialized pollinators that were strongly dependent on the plants they visited, producing very negative network asymmetry. Furthermore, introduced plants but not an introduced pollinator, integrated into the networks similarly to native species.

**Keywords:** pollen limitation; plant-pollinator interactions; facilitation; introduced species; asymmetric specialization; floral density

# Dedication

To my teachers,  
you are all way cool scientists.

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# Chapter 1: Introduction

Worldwide, the value of ecosystem services is conservatively estimated to be at least equal to global GNP (Costanza *et al.* 1997). One important ecosystem service is pollination. By weight, 35% of all the food produced in the world relies on biotic pollination (Klein *et al.* 2007), a service that is estimated to be worth €153 billion (approximately \$200 billion CAD) (Potts *et al.* 2010). Animal pollination is also vital for the reproduction of 87% of flowering plants (Ollerton *et al.* 2011). It is evident that the conservation of plant-pollinator communities is crucial for both managed and wild plants.

Biodiversity is important for many ecosystem services, including pollination, and the effect of biodiversity has been examined at multiple scales. At large scales, biodiversity is widely recognized as a driver of ecosystem functions such as rates of nutrient cycling (Hooper *et al.* 2005). In more diverse ecosystems productivity is generally less variable through time and more resistant to perturbations (Loreau 2000). Individual population sizes fluctuate more when species richness is high, but community stability, measured as the change in biomass, is increased (Worm and Duffy 2003). Therefore the maintenance of biodiversity is important for community persistence. However, at small scales, predicting the consequences of diversity is less clear because species identity may affect the outcome of specific interactions. For example, local diversity can decrease the likelihood of species invasions by reducing niches available for invaders, but conversely nitrogen fixing plants can increase nutrient availability, facilitating invasions (Hooper *et al.* 2005). Keystone species can dominate ecosystem

functions, leading some to question the whether biodiversity is important at small scales (Thompson and Starzomski 2007). Nevertheless, understanding the factors needed for maintenance of biodiversity and ecosystem function remains a major initiative in ecology.

Despite global efforts to preserve biodiversity, it is still threatened by anthropogenic disturbance. Across terrestrial biomes, human-induced land use change, climate change and biotic introductions are expected to be the main causes of biodiversity loss in the next century (Sala *et al.* 2000). Threats to biodiversity are severe when considered individually, however in combination they are likely even more detrimental and are predicted to have non-linear effects on biodiversity through multiple mechanisms (Sala *et al.* 2000). For example, evidence suggests that habitat fragmentation interacts with habitat loss to affect species richness more than does either disturbance alone (Swift and Hannon 2010).

Species do not exist in a vacuum however, and the presence or abundance of one species affects the presence and abundance of those with which it interacts. In short, interactions between species allow for biodiversity feedbacks, causing further changes in biodiversity. Most research in this area has come from examination of antagonistic relationships between species. In herbivore-consumer systems, the effect of changes in diversity depends on the degree of competition (Thébault *et al.* 2007). When competition is high, the loss of a species results in more secondary extinctions, but the effect on overall biomass is dampened, probably due to competitive release and subsequent compensation from other species. Research from host-parasite systems has similarly shown that parasitism can increase biodiversity by reducing the abundance of dominant species, allowing others to thrive (Hudson *et al.* 2006). Clearly, interactions

between species are important not only for shaping communities, but also for increasing their resistance to disturbance.

Unlike antagonistic interactions, the role of mutualistic interactions between species has traditionally seen less investigation and has been largely isolated to examining pairwise comparisons (Stanton 2003). However, more recently the examination of multispecies mutualisms, including plant-ant and plant-frugivore interactions, and in particular plant-pollinator mutualisms, has been the subject of intense research (Vázquez *et al.* 2009). The interactions that occur between plants and pollinators can affect fitness however these interactions are complex making predictions about their effects very difficult, therefore there has been a push to examine plant-pollinator interactions in a community context (Strauss and Irwin 2004) which may be especially useful for understanding how mutualisms respond to anthropogenic disturbance.

Plant-pollinator mutualisms are predicted to be negatively affected by disturbances which affect species abundance or phenology (Tylianakis *et al.* 2008). Reviews of the effects of disturbance on pollinators have shown that they are sensitive to habitat loss and fragmentation (Brown and Paxton 2009; Winfree *et al.* 2009). Pollinators in small populations can also suffer from reduced genetic diversity (Cameron *et al.* 2011). Some of the main threats to plant biodiversity are land use change (Niedrist *et al.* 2009) and introduction of invasive species (Sharma *et al.* 2005). The mutualistic relationship between plants and pollinators also means that disturbances which affect one trophic level can also impact the other. Bee diversity is strongly positively correlated with plant diversity (Potts *et al.* 2003) and concurrent declines in plants and pollinators have been reported in Europe (Biesmeijer *et al.* 2006). Although it is not known whether the decline has been precipitated by the loss of plants or pollinators, it is likely that the

two are linked (Aguilar *et al.* 2006). In some cases, pollinator decline has led to reduced plant reproduction through the reduction of pollen receipt, called pollen limitation (Knight *et al.* 2005). However the local plant community can also affect pollen delivery if surrounding individuals compete with a plant for pollinator visits or act as beacons and attract more pollinators to the plant, thus increasing pollen delivery (Knight *et al.* 2005). In addition, small plant populations may also suffer from reduced mate availability, limiting reproduction (Elam *et al.* 2007). Therefore disturbances which change plant diversity can affect pollination through multiple mechanisms.

From the pollinator's perspective, loss of plants reduces floral resources and has been strongly implicated in pollinator declines (Potts *et al.* 2010). The link between plant and pollinator persistence has led to concern that coextinctions could severely affect plant-pollinator communities (Dunn *et al.* 2009) and it is now commonly recognized that plant and pollinator communities must be considered in unison for conservation to be effective (Kearns *et al.* 1998). This has led to increased research based on network theory to examine the entire suite of plant-pollinator interactions in a community. Early research on plant-pollinator networks described only which species interacted, that is, they simply identified pollinators and associated them with plant species. These studies showed that plant-pollinator networks have consistent structure. For example, the number of interactions that occurs between species is surprisingly invariant despite significant spatial and temporal changes in the identity of plants and pollinators present (reviewed by Vázquez *et al.* 2009). Recent research has measured the frequency of interactions between plants and pollinators (ie. the number of times a pollinator species visits a plant species) to produce weighted, or quantitative networks. Studies examining quantitative networks show that there are only a few species which interact with many others and most species interact with only a few. This can lead to a property called



interaction strength asymmetry (or simply asymmetry), where species differ in how much they rely on each other for either pollen delivery or for floral resources. Asymmetry maintains biodiversity (Bascompte *et al.* 2006; Bastolla *et al.* 2009) and simulations suggest that it contributes to the resistance of plant-pollinator communities to random extinctions (Kaiser-Bunbury *et al.* 2010) because most species are only weakly relied upon. Thus the network approach has contributed to our understanding of how plant-pollinator communities will be affected by anthropogenic changes. Yet, though network asymmetry is important for network resistance, there have been very few studies which have empirically measured how changes in diversity affect network asymmetry, probably due to the difficulty in collecting data to produce high resolution plant-pollinator networks.

In this thesis, I examine how diversity affects the pollination process and the network structure of a plant-pollinator community in an endangered oak-savannah ecosystem. I begin in Chapter 2 by describing variation in pollen limitation in six focal forb species over multiple sites and two years. I also asked whether local plant diversity or the diversity of visiting pollinators affects pollen limitation in focal species. In Chapter 3, I describe the structure of plant-pollinator networks and ask whether interaction asymmetry changes with diversity. Furthermore, I investigate whether introduced species interact similarly to native species in networks. My research has implications for oak-savannah ecosystem conservation, because I identify important plants and pollinators which support many other species that could be the focus of conservation efforts. My research also adds to our understanding of the effects of biodiversity on ecosystem functions such as pollen delivery and how it may be changed by disturbance which alters species composition. Research on plant-pollinator mutualisms is critical in order to make informed decisions about the conservation of terrestrial ecosystems.

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## Chapter 2: The effect of the co-flowering community on pollen limitation

### Introduction

Worldwide, more than 60% of plant species' reproduction is believed to be limited by the receipt of pollen (Burd 1994; Knight *et al.* 2005). Pollen limitation (PL) is an enigma; it has been commonly documented even though it is expected to have potentially large negative consequences such as reduced individual fitness and slow population growth (Ashman *et al.* 2004; Law *et al.* 2010). Understanding why PL occurs has therefore become a major initiative in ecology.

Pollen limitation is a complex problem and many, often opposing, mechanisms have been proposed to explain it. Mechanistic explanations for PL focus on aspects of populations or communities that affect pollinator visit rates. Simply put, inadequate pollen deposition is likely if pollinator visit rate is low. Plant patches with high conspecific density are more efficient for foraging pollinators and as a result may have higher per capita visit rate by pollinators, potentially reducing PL (Grindeland *et al.* 2005; de Jong *et al.* 2005; Dauber *et al.* 2010). However in dense patches pollinators transfer most pollen to nearby plants, which may be related (Watkins and Levin 1990) thus at very high densities, plants may suffer from inbreeding depression. Early-acting inbreeding depression (ie. at the seed stage) would increase observed PL (Aizen and Harder 2007). In patches with very high conspecific density plants can also suffer from intraspecific competition for pollinators, again increasing observed PL (Zorn-Arnold and Howe 2007).

Thus there may be an optimal conspecific density at which plant patches are the most attractive to pollinators without suffering the effects of inbreeding depression or competition for pollinators.

Local heterospecific plant diversity can also influence pollinator visit patterns. In some cases, the presence of co-flowering plants increases visit rate to focal species (e.g. Hegland *et al.* 2009). However increased visit rate due to facilitation by co-flowering plants may not translate into increased seed production if pollinators transfer more heterospecific pollen between plants (Knight *et al.* 2005). Heterospecific pollen can prevent germination of conspecific pollen (Waser and Fugate 1986; Galen and Gregory 1989). One study showed that a higher proportion of heterospecific pollen grains were deposited in the presence of other plant species (Caruso 1999), however in this case the deposition of heterospecific pollen did not affect seed set. Some studies that directly measured the effect of co-flowering heterospecific plants on PL focussed on interactions between pairs of species, rather than the plant community (eg. Caruso 1999; Moeller 2004). One of the few studies that directly examined the effect of the co-flowering community on PL, including conspecific and heterospecific plants, was that of Jakobsson *et al.* (2009) who found that PL decreased with the number of conspecifics and increased with the number of heterospecific individuals in *Armeria maritima*, but not in *Ranunculus acris*.

Within a community, different plant species are likely to vary in their attractiveness to pollinators and their overall rate of visitation by pollinators, such that more attractive plants should have lower PL than less attractive ones (Knight *et al.* 2005). Across communities, there may be variation in the pollinator community that leads to site-specific differences in PL. For instance, if pollinator abundance is high, visit rate may be especially high (Liu and Koptur 2003) which mitigates PL by increasing

pollen transfer (Witjes *et al.* 2011). Because pollinator species can differ in their effectiveness at pollen transfer (Adler and Irwin 2006), it is reasonable to predict that a diverse assemblage of pollinators may mitigate PL. In one case, it has been demonstrated that low pollinator diversity results in increased PL, in part because the most common visitors were inefficient at pollen transfer (Gómez *et al.* 2010). However a recent review shows that PL is rarely linked to the pollinator community suggesting that more detailed observation of pollinator effectiveness is required (Davila *et al.* submitted).

Despite these expectations of site-specific influences on PL, and the growing interest in PL since recent reviews (Ashman *et al.* 2004; Knight *et al.* 2005), few studies examine PL through multiple sites or years. Therefore I measured PL in six plant species across six sites and two years, and assessed how it varied given the local (patch-scale) floral neighbourhood and the (site-scale) pollinator community. I expected that PL would be reduced when conspecific density and co-flowering plant diversity were high due to facilitation of pollination, and when pollinator diversity and abundance were high due to greater total pollen delivery. My experiments were conducted on perennial plant species, most of which have low autofertility, at the northern range limit of a highly fragmented ecosystem. Because perenniality, obligate outcrossing, and habitat fragmentation are all predicted to increase PL (Aizen *et al.* 2002; Knight *et al.* 2005; Alonso *et al.* 2010), I expected PL to be widespread in my study, improving my ability to determine how it is affected by my explanatory variables.

## **Methods**

### **Study system**

Research occurred in an oak-savannah and associated ecosystem of the Cowichan Valley in south-eastern Vancouver Island, British Columbia, Canada. This

ecosystem is highly endangered and supports many at-risk vascular plants. Due to human development and fire suppression this ecosystem is extremely fragmented with less than 5% of the historical habitat remaining. Invasive shrubs and grasses are common (Fuchs 2001). Taken together, these threats may make PL an important factor influencing reproduction in plants of this ecosystem. Study sites were located along a plant diversity gradient with the highest diversity at coastal sites. Coastal sites were the Cowichan Garry Oak Reserve (GO) and the Mount Tzouhalem Ecological Reserve (MZ); inland sites were the Cowichan Lake Forestry Station (FS) and Mesachie Mountain (MM). Mid sites, Stoltz Meadows (SM) and Cowichan River Provincial Park (CR), were located approximately midway between coastal and inland sites. Most sites were surrounded by closed canopy coniferous forest, except the coastal sites, which were also partially surrounded by suburban development. In this spring-blooming ecosystem, forbs are generally in bloom from mid-April to mid-July.

## **Focal species**

I examined PL in six native wildflowers. Because the active pollinator species and their abundance vary within a season (Parachnowitsch and Elle 2005), I chose focal species with varying phenology which allowed me to examine PL over most of the flowering season. Focal species were: *Dodecatheon hendersonii* and *Erythronium oregonum* (early), *Camassia quamash* and *Delphinium menziesii* (mid), and *Allium acuminatum* and *Brodiaea hyacinthina* (late). All species are polycarpic perennials and produce a single inflorescence of perfect flowers.



## Autofertility

If plants are able to autonomously self-pollinate then lack of outcross pollen may not result in PL. I measured autofertility by comparing seed production between pollinator excluded and hand-outcrossed plants. I performed ten replicates for each species except *C. quamash*, which was measured previously (Elle and Neame, unpublished data). Although the degree of autofertility for a species can vary among populations, this is usually associated with among-population morphological variation (Kennedy and Elle 2008). Thus, I assumed that autofertility would be a species trait for my focal species, and so I estimated it at a single site. To reduce the potential for differences in resource availability and plant vigour to influence seed set results, I located each pair of plants within 1 m of each other, and chose plants with the same number of flower buds at the start of the experiment. I surrounded all plants with nets to exclude pollinators. Pollinator enclosure nets were supported by frames to prevent the netting from brushing against flowers and potentially transferring pollen. Seed production by hand-outcrossed plants is interpreted as the maximum production possible given the presence of nets. Seed production by the pollinator excluded plants is interpreted as due to autonomous autogamy.

I performed one-way t-tests by species to determine whether seed production differed between plants which received outcross pollen and those which reproduced autonomously. In this analysis I assumed that seeds produced through outcrossing and autonomous autogamy were equally viable and ignore the potential effects of inbreeding depression, which may be substantial for some species.

## Pollen limitation

I performed PL experiments over two years on all species except *D. menziesii*, which I studied in year 2 only. At each of six sites I attempted to use a sample size of 30 paired stems for each focal species that was present, however in some instances this was not possible: *Brodiaea hyacinthina* and *Allium acuminatum* were uncommon at the Cowichan Lake Forestry Station thus I only manipulated 12 pairs of *B. hyacinthina* at this site in year 1 and 20 pairs of *A. acuminatum* in year 2. I standardized each pair for flower number as above and supplemented all flowers on one stem (S) with the other acting as a naturally pollinated control (C). As in the autofertility experiments, S and C stems within pairs were located within 1 m in an attempt to control for spatial variation in the resource and pollination environment. Not all species were present at every site. Distribution of most species within sites was patchy so in year 1 I set up experiments in patches of approximately 10-20 individuals each, rather than randomly locating individuals across the sites, to ensure I could find two individuals with the same flower number. In year 2 when I additionally measured floral density and diversity (see below) I made an effort to segregate pairs spatially, attempting an inter-pair distance of at least 5 m.

I used a mixture of pollen from at least five source plants per species and site for supplementation. For species with large stigmas (*C. quamash* and *E. oregonum*) I applied pollen using a paint brush dipped in a vial of collected dehiscing anthers. I used a mechanical sonicator to collect pollen from *D. hendersonii* and pollinated flowers by dipping the stigma into the vial of collected pollen. Pollen was applied with a pin dipped in pollen for other species (*A. acuminatum*, *B. hyacinthina* and *D. menziesii*). Both *A. acuminatum* and *D. menziesii* have flowers with inaccessible stigmas making handling effects a concern so I sham pollinated control plants by opening the surrounding petals

with forceps to simulate hand pollination. Each flower was supplemented once except for *E. oregonum* which produces only one flower and was pollinated twice to ensure pollination. After supplementing each flower I marked the pedicel with correction fluid; pedicels of flowers on control plants were also marked as a control. I repeated pollinations every one to three days until all flowers on an inflorescence were pollinated. I attempted to collect fruit when unripe to avoid loss of seeds. However, in some cases seed predation occurred or fruit had begun to dehisce prior to collection. If the number of seeds produced by apparently predated or dehiscing fruit was more than two standard deviations from the mean produced for that species, site and treatment combination, the plant was not used in analyses calculating PL.

To assess whether seed production differed between treatments, years, and among sites, I performed an analysis of variance (ANOVA) with total seed production per plant as the response variable (as recommended by Knight *et al.* 2005). All analyses were performed in SAS (SAS 9.2, SAS Institute Inc. 2008). I ln-transformed seed number to reduce heteroscedasticity using  $\ln(\text{seeds}+1)$  to avoid undefined values that would occur if a plant produced zero seeds. To interpret results, I subsequently performed post-hoc analysis using an unpaired two-sample t-test comparing seed production between S and C stems for each species  $\times$  site  $\times$  year combination. Pollen limitation occurs if S plants produce significantly more seeds than C plants. All values are presented as mean  $\pm$  SE. At CR most *D. menziesii* died (possibly due to disease) and thus I excluded all individuals from this site from the analysis.

## **Pollinator assemblage and pollen limitation**

Changes in the relative number of pollinating species of a plant (pollinator assemblage) have been shown to influence degree of PL observed (Gómez *et al.* 2010).

To quantify the effect size of PL, I used the log response ratio modified from Knight *et al.* (2005):  $\ln R = \ln\left(\frac{S+1}{C+1}\right)$ , where S and C were total number of seeds produced for pollen supplemented and control plants in a pair, respectively. I added one seed to all seed totals as some plants in both S and C treatments produced zero seeds, leading to undefined ratios. A positive response ratio indicates PL.

To describe the pollinator assemblage, I collected putative pollinators on each focal species using insect nets. Collection of insect visitors was only done on warm, sunny days between 10 AM and 4 PM. Two separate collectors netted insects for 10 min per species per date, only collecting those that contacted the reproductive organs of focal species' flowers. Effectiveness of insect visitors as pollinators has not been determined, but I use visit rate (visitors collected per 10 minute period) as a surrogate as this has been shown to approximate effectiveness well (Sahli and Conner 2007). I attempted to complete a similar number of collection periods for focal species at all sites where they occurred, however weather conditions meant this was not always possible. I standardized for effort by dividing the total number of pollinators by the number of collection periods (range = 2-10) for each species × site × year combination. All collected insects were identified to the lowest taxonomic level possible for analysis.

I calculated insect visitor diversity for each species × site × year combination as Simpson's index of diversity,  $D = 1 - \frac{\sum_{i=1}^S n_i(n_i-1)}{N(N-1)}$ , where  $n_i$  = the total number of individuals of the  $i^{\text{th}}$  species and N = the total number of individuals of all species: a value of 0 indicates low diversity whereas a 1 indicates high diversity. This index is less influenced by samples with few individuals than other indices (Payne *et al.* 2005). However this measure of diversity gives no indication of visit rate. Therefore I weighted diversity by the number of pollinators collected to give the highest weight to plant

species that have the most diverse pollinator assemblage and the highest visit rate. I used linear regression to test the relationship between weighted diversity (ln transformed to improve normality) and PL.

## **Co-flowering community**

To examine the effect of floral co-flowering density and diversity on PL, I counted the number of conspecific and heterospecific flowering stems in 1 m<sup>2</sup> circular quadrats centred between the two plants comprising a C/S pair in year 2. In each quadrat I counted the number of inflorescences of all species with open flowers. Because I measured floral density once per focal species per site, I counted the number of stems which would likely have open flowers at the same time as the focal plants. I did not count wind-pollinated species (grasses, some forbs), forbs with very small flowers which were unlikely to be attractive to pollinators (such as *Myosotis discolor*) or forbs that were not in bloom at the same time as the focal species. In each quadrat I measured conspecific density and heterospecific diversity during the peak flowering period for each focal species at a site, which I defined as the time when most experimental individuals had open flowers. Diversity and PL were expressed as above.

To show how co-flowering plant density and diversity affect PL of focal species, I used the Akaike's Information Criterion corrected for small sample sizes (AICc) to choose the best regression model. For each species I performed the analysis both within and across sites to examine the spatial variation in effects of the co-flowering community on PL. Candidate models were all combinations of conspecific density, richness and diversity in 1 m<sup>2</sup> plots using each focal pair from the year 2 data as a replicate. To improve normality I performed a reciprocal transform on conspecific density and a log transform on richness. Diversity was arcsine square-root transformed

as is recommended for proportions (Quinn and Keough 2007). I tested for an optimum effect of density on PL by including a squared heterospecific density term in the set of candidate models. However, models including this term had virtually identical AICc values to models without it, thus I included only a linear conspecific density term in the final models. In each case, no model received overwhelming support as the best model (no AICc model weight  $\geq 0.9$ ) so I performed model averaging across all candidate models (Burnham and Anderson 2002).

## Results

### Autofertility

Excluding pollinators significantly reduced seed production in all focal species except *A. acuminatum* (Fig. 2-1). The mean difference in seed production between autonomously self-pollinated and outcrossed *A. acuminatum* was weakly non-significant ( $P = 0.07$ ) and autonomously self-pollinated plants produced many seeds compared to controls. All nine *A. acuminatum* individuals that survived the autofertility experiment were able to produce seeds, thus I feel confident that this species has some autofertility.

Both *D. hendersonii* and *E. oregonum* each produced seeds when pollinators were excluded. For these early-flowering species however, enclosure frames were unstable and may have moved in wind, brushing against plant reproductive organs artificially causing self-pollination to occur (enclosure design was improved for later-flowering species to prevent this). In *D. hendersonii* the netting probably transferred pollen in just a few individuals (four) which were the only experimental plants to produce seeds (Fig. 2-1). This seems likely as the congener, *D. meadia* does not autonomously self-pollinate (Macior 1964). However, since many (8/10) experimental *E. oregonum* plants produced seeds I cannot rule out that this species is capable of some limited

amount of autonomous self-fertilization. *Camassia quamash* produced very few seeds autonomously (Elle and Neame, unpublished data). Both *D. menziesii* and *B. hyacinthina* were unable to produce seeds autonomously.

## Pollen limitation

My analysis indicated that there was considerable variation in seed production left unexplained by my model (ANOVA  $R^2 = 0.36$ ). In general seed production increased with pollen supplementation ( $S = 28.80 \pm 1.09$  seeds,  $C = 26.66 \pm 1.18$ ), but the main effect of treatment was not significant (Table 2-1). However, the significant four-way interaction that I observed indicates that the response of my focal species to pollen supplementation treatments depends on site and year. This suggests species-specific spatial and temporal variation in PL. Species differed in the number of seeds they produced (Table 2-1, Fig. 2-2). *Dodecatheon hendersonii* and *D. menziesii* had the highest seed production when pooling across years and sites,  $71.36 \pm 3.25$  and  $67.12 \pm 4.13$ , respectively. *Allium acuminatum* produced the fewest seeds, only  $9.45 \pm 0.32$  per plant. Differences in species-specific seed production likely explain the significant site effect (Table 2-1) because not all species were present at all sites; for example, I observed the highest overall seed production at MM simply because *D. menziesii* was the only species in which I measured PL at that site. In some species, seed production never responded to supplemental pollination (*D. menziesii*, *A. acuminatum*), whereas other species responded at some sites but not others. For example, *B. hyacinthina* was pollen limited or showed a strong trend towards PL at FS in both years (Fig. 2-2), however PL did not occur in this species at other sites. Responses to supplemental pollination also varied across years with some species being pollen limited at a site in one year but not the next. In fact, aside from the case of *B.*

*hyacinthina* mentioned above, no species was pollen limited in both years at a single site.

Seed production varied between years (Fig. 2-2). When I removed species/site combinations where species were not manipulated in both years (*D. menziesii*, *B. hyacinthina* at FS and *A. acuminatum* at FS) seed production was 16% higher in the second year (year 1 =  $21.24 \pm 0.83$ , year 2 =  $24.74 \pm 1.15$ ). However, species responded differently (species  $\times$  year interaction, Table 2-1). For example, *D. hendersonii*, *B. hyacinthina* and *A. acuminatum* produced 67, 28 and 66% more seeds, respectively, in the second year while *E. oregonum* produced 100% more in the first year (Fig. 2-2). Overall seed production in *C. quamash* remained virtually the same between years.

There was also variation in seed production among sites (Table 2-1). Within species, there was a tendency for higher seed production at coastal sites (GO and MZ) compared to inland sites (FS or MM) for species that were present at those sites (Fig. 2-2). Despite this variation in seed production, the site  $\times$  treatment effect was not significant in my ANOVA (there were no sites that were, overall, more pollen limited than other sites), and instead, PL occurred in one species at four sites, two species at a fifth, and no species at my sixth site. However, at the latter site (MM) I only measured seed production in a single species and year.

## **Pollinator assemblage**

Despite expecting that PL would decline with increased number and diversity of floral visitors, there was no relationship between PL and weighted pollinator diversity ( $P > 0.05$ ) when pooling across all species, sites and years (Fig. 2-3). This occurred despite variation among species in pollinator assemblage. *Camassia quamash* had by



far the highest visit rate among focal species (Table 2-2). The lowest visit rates were observed in *E. oregonum* and *D. menziesii*, but these species were not, in general, more pollen limited than *C. quamash*.

## **Co-flowering community**

When pooling across sites within species, increased conspecific density increased PL in *D. hendersonii*, *C. quamash* and *B. hyacinthina*, suggesting competition for pollinators. No combination of measured variables explained PL in *E. oregonum*, *D. menziesii* or *A. acuminatum* when considering data pooled across all included sites. Of the 23 species × site combinations, 17 included a significant effect of conspecific density; PL increased with density in 12 of 17 cases. However, there was variation in both the magnitude and direction of effects and no species showed consistent responses to density across all sites where it occurred. For example, PL increased with conspecific density of *C. quamash* at four sites, but PL decreased as conspecific density increased at another (Table 2-3, Fig. 2-4). In addition, density effects appeared more important at some sites than others; all species present at FS showed significant effects of density on PL, but only 4/6 species at GO did. The only site at which I did not observe an effect of density was MM, where I only manipulated one species. The effects of co-flowering plant richness and diversity on PL were very minor. Richness did not significantly affect PL in any case (Table 2-3). I only observed significant effects of diversity in two species; PL increased with diversity in *C. quamash* at SM and decreased with diversity in *A. acuminatum* at MZ. In both cases, PL also increased with density for that species at that site.

## Discussion

### Patterns of seed production and pollen limitation

I showed that wildflowers of this oak-savannah ecosystem are sometimes pollen limited; however PL was less prevalent than has been reported elsewhere. Across all combinations of species, sites, and years supplemental pollen only significantly increased seed production in 16% of cases (6 of 38 combinations). By treating multiple populations (sites) and years as independent data points Knight *et al.* (2005) report pollen limitation in 63% of records. Burd (1994) showed that 62% of species were pollen limited. Even when excluding *A. acuminatum* (which is able to autonomously self-pollinate), PL was rare in my study. The lack of frequent PL is unexpected, as perennials are predicted to be more prone to PL in any given year due to a longer life-span during which fitness can be gained (Knight *et al.* 2005).

My results emphasize the importance of considering multiple years and sites when examining population-level effects of PL. Pollen limitation in this oak-savannah ecosystem is site- and year-specific and it would be unwise to extrapolate the results of one site or year to others. For example, *C. quamash* was pollen limited in three sites in the first year of my study but was not pollen limited at any site the following year (Fig. 2-2). The few other studies which have examined PL across multiple sites and years show similar results. Huang and Guo (2002) found that PL was also dependent on the interaction between site and year in *Liriodendron chinense* and Knight (2003) shows a trend for a similar effect in *Trillium grandiflorum*. It has been suggested that plants are forced to balance the resources they allocate to pollinator attraction (showy flowers, nectar) and seed production. Haig and Westoby (1988) predicted that ideally plants would achieve an optimal trade-off of resources to result in maximum seed production,

but that perturbations to resources and pollen receipt (resulting in PL) could lower realized seed production. My results suggest that in this ecosystem, PL is rarely a significant perturbation to seed production at the population level. Instead, seed production may be largely resource limited, with variation in resource availability contributing to the large inter-site and inter-annual differences in seed production that I observed. Resource addition experiments would clarify this issue.

## **Importance of pollinators and the co-flowering community**

The significant inter-annual and spatial variation in seed production that I observed may reflect variation in resources which could affect seed production, coupled with variation in pollination effectiveness among sites and years. Variation in degree of PL has been shown to be caused by differences in the diversity of the pollinator assemblage (Gómez *et al.* 2010), although this finding is less common than might be expected (Davila *et al.* submitted). However, I did not observe any relationship between pollinator diversity or abundance and PL, despite variation among sites and species in these variables. These results indicate that variation in the pollination environment is inadequate to explain the observed pattern of PL in my study.

Other research has shown that high conspecific density increases seed production of focal plants (Knight 2003; Dauber *et al.* 2010). This may occur if increased attractiveness of dense patches to pollinators leads to higher per-capita visit rate and increased pollen transfer (facilitation). However, my results suggest that while co-flowering density does affect PL, the effects were often minor or not in the predicted direction. In most cases, PL increased with conspecific density, suggesting competition for pollinators rather than facilitation. However, the effect size was small in the case of *D. menziesii*, *B. hyacinthina* and *A. acuminatum* (Fig. 2-4) and although the effect of

conspecific density was greater in the remaining species, it was variable, with no species responding the same to conspecific density across all sites. Differences among sites may have to do with site factors that were not the focus of my research. For instance, in *C. quamash* PL increased with density (competition) at four sites. The single negative effect of conspecific density (facilitation) was at a large site with dense patches of *C. quamash* that were widely separated from each other. I hypothesize that with this configuration, dense patches are more attractive to pollinators by reducing travel between foraging locations compared to the smaller sites where patches are much closer together. Future work should include the configuration of plant patches as well as conspecific density.

Compared to conspecific density, diversity of the co-flowering community was less important, significant in only two models. Species richness never had a significant effect. I observed reduced PL with more diverse co-flowering patches surrounding *C. quamash* at one site which may indicate facilitation from co-flowering species. Facilitation of pollination can occur when co-flowering species share pollinators (Moeller 2004). It then follows that plant species visited by the richest pollinator assemblage would be involved in facilitative interactions, as they are more likely to share pollinators with co-flowering species. Indeed, *C. quamash* had by far the highest pollinator richness (Table 2-2) and was the only species to show reduced PL in diverse patches and showed a trend for increasing PL with visitor diversity. The only other species to respond to diversity was *A. acuminatum* which showed weakly increasing PL with plant diversity at one site, perhaps indicating some competition for pollinators; however this species is autofertile which should reduce PL. Given that these diversity effects were relatively minor and only occurred in two cases however, these results should be interpreted with some caution.

In most cases diversity had no effect on PL in my study which contrasts with Hegland *et al.* (2009) who found that interactions between species (either facilitation or competition for pollen) were common. While my pollinator collection methods do not allow me to address whether diverse patches within sites have higher per capita visit rate, my results suggest that if they do, it rarely translates into reduced PL.

## **Methodological considerations**

One of the drawbacks of studies which examine pollen limitation using within-plant controls is that plants may re-allocate resources to flowers which receive more pollen, leading to greater measured PL than actually occurs (Knight *et al.* 2005). In my study I avoided this issue by measuring PL at the whole plant level. Despite attempting to control for resource differences by locating paired stems close together (within 1 m) and controlling for flower number within pairs, there was considerable variation in seed production between individuals within pairs, and many plants in both treatments produced no seeds at all. While this variation may reflect the stochasticity of pollination, it may also suggest that resources vary over shorter distances than I assumed. If resources vary at the microscale, measuring PL at the within-plant level may be desirable, if it can be demonstrated that the effect of resource re-allocation on seed production is low.

## **Conclusions**

In this chapter I demonstrated that PL can be rare even in plants with perennial life histories that occur in a fragmented ecosystem, both of which are normally associated with high PL. That the co-flowering and pollinator communities had very little, if any, impact on PL was unexpected, however this may be due to the general lack

of PL in my focal ecosystem. My results highlight the complexity of pollination and demonstrate that at local scales, the causes of PL remain highly context dependent. Perhaps more importantly however, my research, along with a similar comprehensive multispecies study (Hegland and Totland 2008), suggests that PL may not be as common as has previously been reported. It is unclear whether studies of PL which find non-significant results are underreported (Burd 1994). Regardless, I believe that in order to properly assess the impact of PL on plant communities, research must include information on the natural spatial and temporal variation in seed production which, as I have shown, may outweigh variation in PL.

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## Tables

**Table 2-1. Analysis of variance (ANOVA) testing whether total seed production per plant differs with treatment, control (C) vs. pollen supplementation (S), for six wildflower species at six sites and two years. Significant effects are indicated in bold.**

| Source of variation               | <i>df</i> | MS     | <i>F</i> | <i>P</i>         |
|-----------------------------------|-----------|--------|----------|------------------|
| Species                           | 5         | 141.47 | 91.81    | <b>&lt;.0001</b> |
| Treatment                         | 1         | 4.16   | 2.70     | 0.1006           |
| Site                              | 5         | 20.57  | 13.35    | <b>&lt;.0001</b> |
| Year                              | 1         | 0.01   | 0.00     | 0.9513           |
| Site × Year                       | 4         | 3.91   | 2.54     | <b>0.0383</b>    |
| Site × Treatment                  | 5         | 0.89   | 0.58     | 0.7167           |
| Treatment × Year                  | 1         | 0.18   | 0.12     | 0.7325           |
| Species × Treatment               | 5         | 1.55   | 1.01     | 0.4120           |
| Species × Site                    | 11        | 17.04  | 11.06    | <b>&lt;.0001</b> |
| Species × Year                    | 4         | 47.97  | 31.13    | <b>&lt;.0001</b> |
| Species × Site × Treatment        | 11        | 2.94   | 1.91     | <b>0.0343</b>    |
| Species × Treatment × Year        | 4         | 2.34   | 1.52     | 0.1943           |
| Site × Treatment × Year           | 4         | 2.57   | 1.67     | 0.1546           |
| Species × Site × Treatment × Year | 14        | 8.97   | 5.82     | <b>&lt;.0001</b> |
| Error                             | 2027      | 1.54   |          |                  |

**Table 2-2. Summary of pollinator assemblages of wildflower species for which pollen limitation was assessed. Pollinator abundance is the total number of pollinators collected on that species standardized for number of collection periods. Richness is the total number of pollinating species collected on that plant species across all sites and years. Diversity is quantified as the average Simpson’s index of diversity pooled across sites and years for a species.**

| Plant species                  | Visiting insect community |          |           |
|--------------------------------|---------------------------|----------|-----------|
|                                | Abundance                 | Richness | Diversity |
| <i>Dodecatheon hendersonii</i> | 1.33                      | 6        | 0.58      |
| <i>Erythronium oregonum</i>    | 0.24                      | 5        | 0.10      |
| <i>Camassia quamash</i>        | 9.72                      | 48       | 0.78      |
| <i>Delphinium menziesii</i>    | 0.48                      | 14       | 0.14      |
| <i>Brodiaea hyacinthina</i>    | 2.13                      | 28       | 0.73      |
| <i>Allium acuminatum</i>       | 1.93                      | 15       | 0.78      |

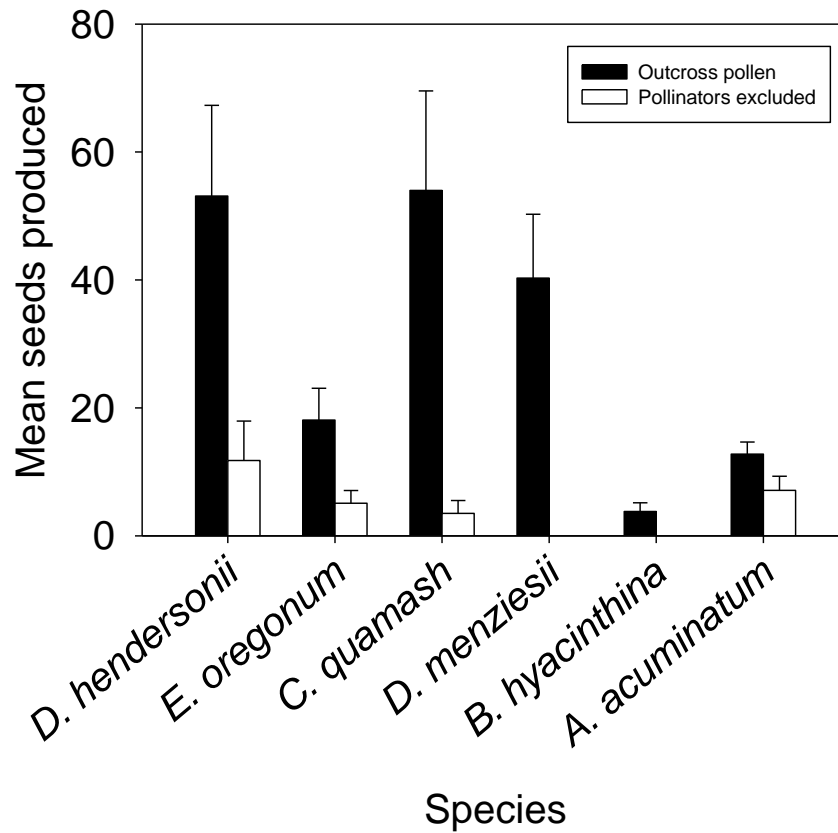
**Table 2-3. Averaged models using AICc explaining the relationship between pollen limitation (PL) and conspecific density and diversity in 1 m<sup>2</sup> quadrats around focal stems. Richness was never significant in any model and is not shown. Analysis was performed by pooling species across sites and for each species × site combination. I present model averaged parameters ± SE for each combination as well as highest R<sup>2</sup> among all models. Only significant parameters shown (parameters with confidence intervals which do not include zero).**

| Species               | Site   | Model parameter |               |              | Highest R <sup>2</sup> |
|-----------------------|--------|-----------------|---------------|--------------|------------------------|
|                       |        | Intercept       | Density       | Diversity    |                        |
| <i>D. hendersonii</i> | Pooled | -3.17 ± 1.59    | 5.27 ± 0.91   | —            | 0.07                   |
|                       | GO     | —               | 8.00 ± 0.93   | —            | 0.24                   |
|                       | MZ     | —               | —             | —            | —                      |
| <i>E. oregonum</i>    | Pooled | —               | —             | —            | —                      |
|                       | GO     | 8.92 ± 1.26     | -12.08 ± 1.51 | —            | 0.29                   |
|                       | MZ     | —               | 5.84 ± 0.74   | —            | 0.20                   |
|                       | SM     | —               | —             | —            | —                      |
|                       | CR     | —               | 1.69 ± 0.81   | —            | 0.02                   |
| <i>C. quamash</i>     | Pooled | -3.53 ± 1.65    | 5.15 ± 1.33   | —            | 0.03                   |
|                       | GO     | —               | 6.24 ± 0.82   | —            | 0.13                   |
|                       | MZ     | -12.23 ± 2.98   | 21.87 ± 1.86  | —            | 0.29                   |
|                       | SM     | -14.15 ± 1.59   | 15.69 ± 1.24  | -3.08 ± 1.23 | 0.38                   |
|                       | CR     | —               | 3.18 ± 0.83   | —            | 0.04                   |
|                       | FS     | —               | -9.33 ± 0.94  | —            | 0.09                   |
| <i>D. menziesii</i>   | Pooled | —               | —             | —            | —                      |
|                       | GO     | —               | 4.17 ± 0.74   | —            | 0.05                   |
|                       | MZ     | —               | -2.51 ± 0.74  | —            | 0.06                   |
|                       | SM     | —               | 2.06 ± 0.58   | —            | 0.06                   |
|                       | MM     | —               | —             | —            | —                      |
| <i>B. hyacinthina</i> | Pooled | —               | 1.00 ± 0.42   | —            | 0.05                   |
|                       | GO     | —               | —             | —            | —                      |
|                       | MZ     | —               | 0.32 ± 0.11   | —            | 0.06                   |
|                       | SM     | —               | -3.99 ± 0.63  | —            | 0.08                   |
|                       | FS     | —               | 3.60 ± 0.76   | —            | 0.02                   |

| Species              | Site   | Model parameter |              |             | Highest R <sup>2</sup> |
|----------------------|--------|-----------------|--------------|-------------|------------------------|
|                      |        | Intercept       | Density      | Diversity   |                        |
| <i>A. acuminatum</i> | Pooled | —               | 0.45 ± 0.21  | —           | 0.03                   |
|                      | GO     | —               | —            | —           | —                      |
|                      | MZ     | —               | 2.95 ± 0.44  | 0.96 ± 0.38 | 0.28                   |
|                      | FS     | —               | -1.16 ± 0.21 | —           | 0.06                   |

## Figures

Figure 2-1. The mean number of seeds produced when pollinators were excluded with netting and plants were hand-outcrossed. All species produced significantly more seeds when receiving outcross pollen than when reproducing through autonomous autogamy ( $P < 0.05$  for all tests), except *A. acuminatum* ( $P = 0.07$ ). Both *D. menziesii* and *B. hyacinthina* were unable to produce any seeds autonomously. Error bars  $\pm 1$  SE. Data for *C. quamash* from Elle and Neame (unpublished data).





**Figure 2-2. Mean total seed production  $\pm$  SE for focal species by treatment (supplemented vs. control), sites, and years. Graphs are paired by early, mid, and late season bloomers (top to bottom). Sites indicated with two-letter abbreviations; coastal sites are GO and MZ, mid sites CR and SM, and inland sites MM and FS (see text for site names). Pollen additions were not performed on *D. menziesii* in year 1. I did not perform pollen additions on species which were uncommon as was the case in year 1 with *A. acuminatum* (at FS) and *B. hyacinthina* (at MZ). \* = significant differences between treatments for species  $\times$  site  $\times$  year combinations.**

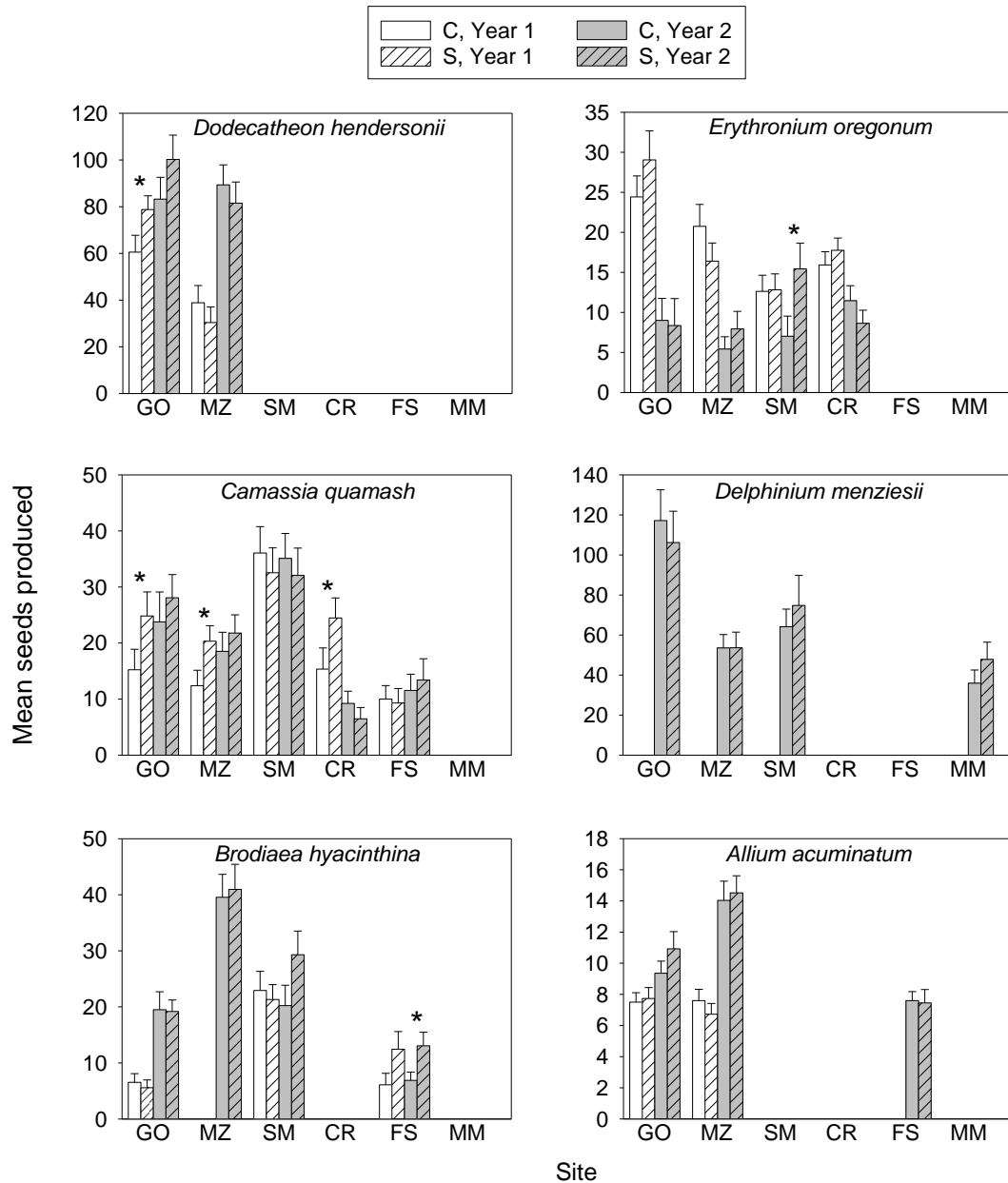
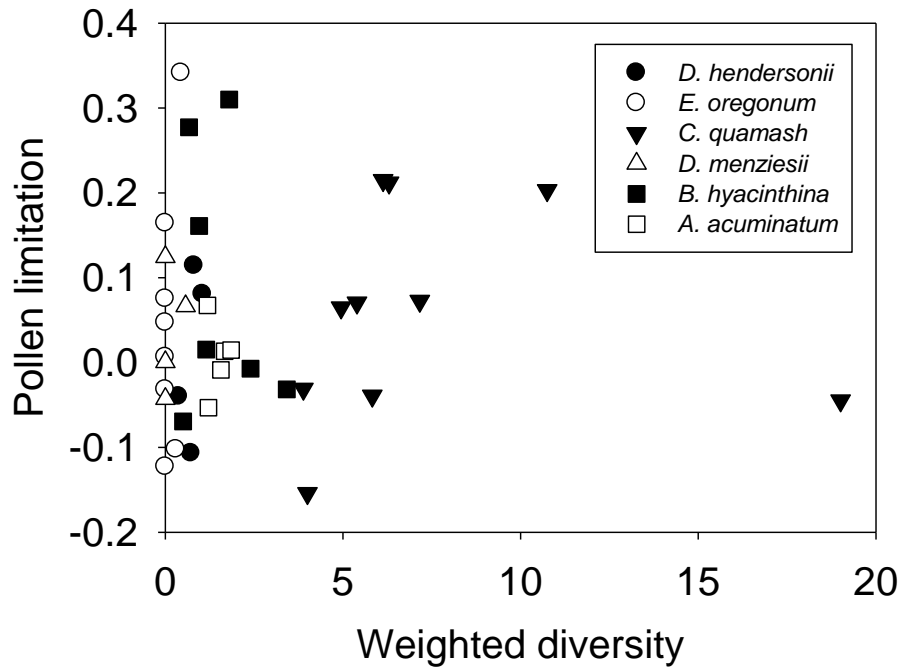
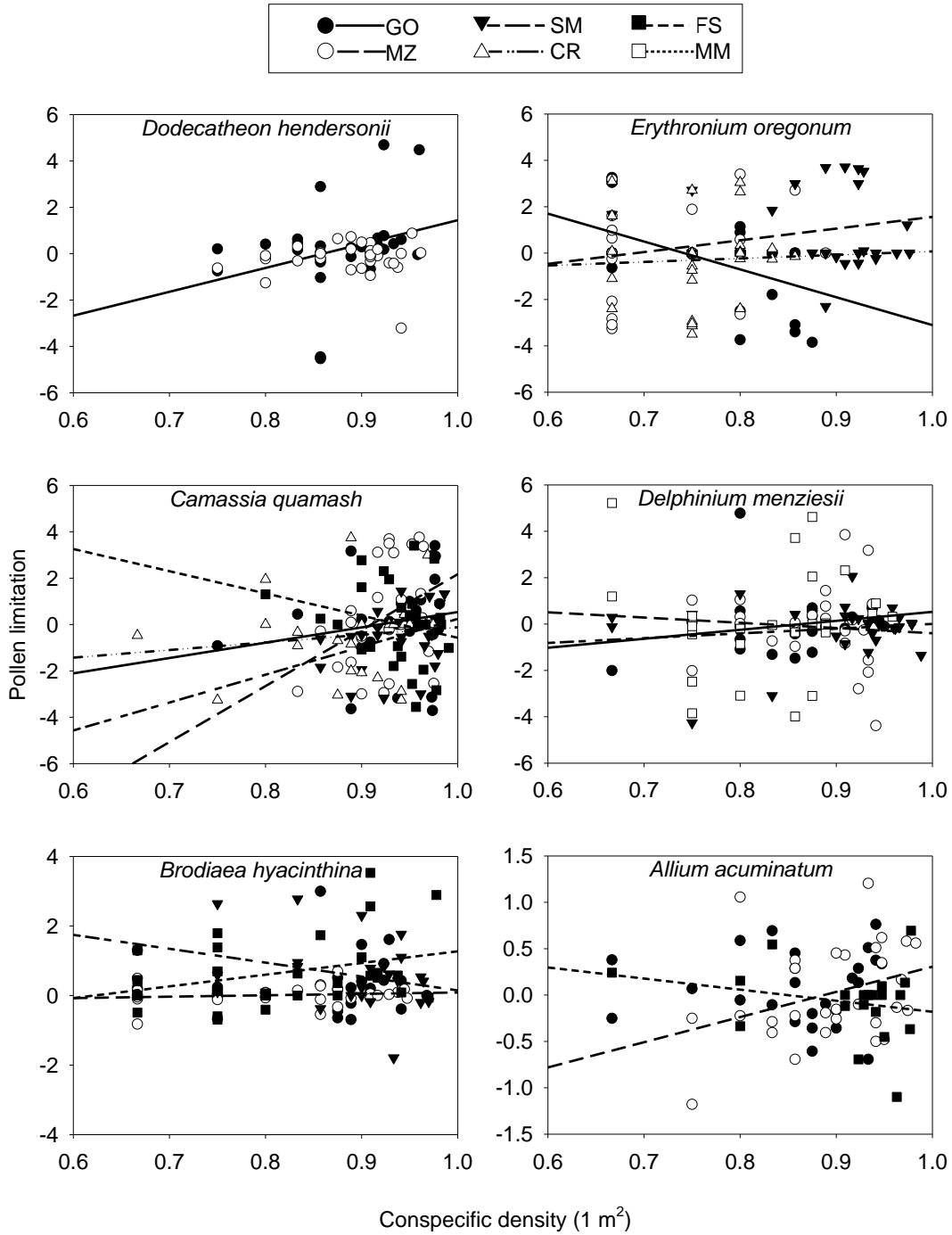


Figure 2-3. The relationship between weighted diversity of the pollinator assemblage and pollen limitation for six wildflower species at six sites and two years. Weighted diversity is the product of the Simpson's index of diversity and the standardized visit rate of putative pollinators for each species  $\times$  site  $\times$  year combination. Pollen limitation is the log response ratio. Across all species, sites and years there was no overall relationship between PL and weighted diversity.



**Figure 2-4. Relationship between PL and conspecific density in 1 m<sup>2</sup> quadrats around focal stems for six wildflower species. Conspecific density = 1-1/(density+1); values close to 1 indicate high density. Pollen limitation is the log response ratio; positive values indicate pollen limitation. When sites within species were analysed separately, there was a significant relationship between PL and conspecific density in at least one site in all species.**



# Chapter 3: Network size, interaction strength asymmetry, and the integration of introduced species into a plant-pollinator network

## Introduction

Pollinator populations are declining worldwide, and some species are threatened with extinction (Potts *et al.* 2010; Cameron *et al.* 2011). Concurrent declines in plant populations have also been observed although it is unknown whether the loss of pollinators has led to the loss of plants or vice versa (Biesmeijer *et al.* 2006). Given that 87.5% of angiosperms require animal pollinators for reproduction (Ollerton *et al.* 2011), plant and pollinator communities should be jointly considered in conservation decisions. Network approaches, which focus on interactions between species rather than simple estimates of biodiversity *per se*, may be the best approach to take when seeking to conserve mutualisms like pollination (Elle *et al.* in press).

Most research on plant-pollinator networks has focussed on describing their structure and has shown that it is surprisingly consistent: specialists are common, which combined with nestedness (Bascompte *et al.* 2003) results in strong interaction strength asymmetry (hereafter asymmetry) in species interdependency. Biologically, in plant-pollinator networks, asymmetry means that species differ in how much they rely on one another for the products of the interaction, such as pollen delivery to plants or food rewards (nectar and pollen) to pollinators (Vázquez *et al.* 2007; Bascompte *et al.* 2006). Simple descriptions of network structure are useful for comparing networks, but we know

surprisingly little of how ecological processes and anthropogenic changes affect that structure. Both plants and pollinators are threatened by anthropogenic changes such as habitat destruction and alteration, which can reduce species richness (Dirzo and Raven 2003; Winfree *et al.* 2009; Neame *et al.* in press). Habitat modification can also lead to the introduction of non-native plants (Richardson *et al.* 2000) and also some widespread introduced pollinators (Goulson 2003). Introduced plants and pollinators can interact with many species in the community (Aizen *et al.* 2008; Bartomeus *et al.* 2008) however until recently, the impact of changes in species richness and presence/absence of introduced species on plant-pollinator network structure has seen little empirical investigation.

If many species are lost due to anthropogenic disturbance, networks collapse can occur, where most species lose their interactions partners. How network collapse occurs depends greatly on which species are lost. Asymmetric interactions mean that the removal of specialists can be tolerated as they are weakly relied upon by their interaction partners, because those partners tend to be generalized and have many additional interaction partners (Kaiser-Bunbury *et al.* 2010). Generalists are important for network resistance and simulations show that networks are expected to collapse faster when generalists are removed because they interact with many species (Memmott *et al.* 2004). Hence, the presence of generalists can compensate for perturbation more readily than the presence of specialists (Memmott *et al.* 2007; Tylianakis *et al.* 2010). Generalists are also important in network assembly and disassembly; as new species are observed in a network they preferentially interact with generalists that are already present (Olesen *et al.* 2008). Although specialist pollinators are thought to be more vulnerable than generalists to disturbances such as habitat fragmentation, one study examined real networks and showed that because specialist plants interact with the

most generalized pollinators, both specialist and generalist plants show equal responses to fragmentation (Ashworth *et al.* 2004), thus the presence of generalists in asymmetric interactions with specialists contributes to the resistance of networks to disturbance. This finding suggests that small networks will be as resistant to disturbance as large ones, however the few studies that have examined this question directly have used modelling of qualitative interaction networks and have ignored interaction strength (Memmott *et al.* 2004; but see Kaiser-Bunbury *et al.* 2010). The only study that I am aware of that has empirically measured how interaction strength changes across a diversity gradient was that of Albrecht *et al.* (2010). Their study showed that networks became more symmetric as they contained more species meaning that in general species relied less on each other, which may make them more vulnerable to random extinctions.

Anthropogenic change can lead to the introduction of new species (Mack *et al.* 2000), but the impact of species introductions on plant-pollinator networks is equivocal. Introduced plants may be considered beneficial if they increase the floral resources available (Lopezaraiza-Mikel *et al.* 2007) or act as pollinator “beacons” and increase pollen delivery rate to surrounding species (Bartomeus *et al.* 2008). Introduced insects such as the European honeybee, *Apis mellifera*, can be very effective pollinators (Sahli and Conner 2007) and may be considered beneficial for the reproduction of some plants (Cayuela *et al.* 2011). However, introduced species are often extremely generalized (Lopezaraiza-Mikel *et al.* 2007; Carvalheiro *et al.* 2010). As a consequence of high generality, introduced species can alter plant-pollinator networks by being involved in many very highly asymmetric interactions which reduces overall interaction strength between native species and makes the introduced species disproportionately important (Aizen *et al.* 2008). Plants in particular are often very strongly relied upon by pollinators

using them as food sources (Vázquez *et al.* 2007), thus introduced plants may become more integrated into networks than introduced pollinators.

In this chapter, I describe plant-pollinator networks from six sites sampled over two years, and examine how the networks vary across a natural diversity gradient. I expected that larger, more species-rich networks would be more asymmetric because they would have proportionately more specialist species. I also examined how introduced species invade networks. I expected that introduced species would interact with many species and be strongly relied upon by those species, as indicated by positive asymmetry values. I compared introduced focal species to the most generalized native species and other native species of similar generalization to the focals. I expected that the highly generalized native species would interact indiscriminately with other native species, supporting species of both positive and negative asymmetry, and that introduced species would interact in a similar manner, indicating high integration into the network. I conclude by summarizing the practical implications of my findings for plant and pollinator conservation.

## **Methods**

### **Quantification of plant-pollinator interactions**

I constructed plant-pollinator interaction networks, concurrently with pollen limitation experiments in Chapter 2, at six sites over two years for a total of 12 networks. To estimate the frequency of interactions between plants and insects I netted insects directly off flowers during good weather (warm, minimal wind). I collected flower visitors (hereafter pollinators) by plant species between 1000 and 1600 hours. Each site was sampled every 2-3 days, with two 10 min netting rounds per plant species for each site/date combination. I attempted to allocate netting equally over different times of day

and between different collectors. This method allocates netting effort more evenly between plant species than transect netting and is more likely to detect rare species and their interactions (Gibson *et al.* 2011), so is more appropriate for heterogeneous habitats like an oak-savannah. In order to quantify plant-pollinator interactions, I captured only insects which made physical contact with the reproductive organs of the flower. However, the resulting interaction web likely includes some true pollinators as well as other visitors which may not have transferred pollen. I attempted to allocate equal effort among sites and especially for species that were present at multiple sites, but weather, phenological differences, and differences in plant population density resulted in somewhat unequal sample sizes. Some plant species with very small (putatively unattractive) flowers, such as *Myosotis discolor* and *Galium aparine* were not included in the study. Collected insects were identified to the lowest taxonomic level possible. The few hummingbirds and butterflies that visited flowers during our study were identified in the field without being captured.

## **Connectance, asymmetry and specialization**

I created plant-pollinator interaction matrices for all site and year combinations. A species may be able to interact with more species than are available at a single site or year, so I also combined data across all sites and years into a master matrix to assess the true generality and asymmetry if all species were present. This combined matrix had 220 pollinator and 42 plant species. Matrices can be visualised as columns for each pollinator species and rows for each plant species. Each value in the matrix represents an interaction between a pollinator species and a plant species. Due to unequal netting effort, I standardized all matrices by netting effort on each plant species.



To calculate connectance and asymmetry I used the Bipartite package (Dormann *et al.* 2008) in R (R Development Core Team, 2011). Connectance is the number of realized interactions out of the total number of possible interactions and varies between 0 (no species interact) and 1 (every insect interacts with every plant). It is a measure of how much species share interaction partners; values close to 1 indicate redundancy of interaction partners which is regarded as contributing to network resistance (Memmott *et al.* 2004).

Asymmetry was calculated from interaction strengths (Vázquez *et al.* 2007). Interaction strength ( $S$ ) describes the effect that one species has on another. A species will have the strongest interactions with those species with which it interacts most frequently, ie. the species that accounts for the largest proportion of all its interactions. Interaction strength describes the effect that species  $i$  in one trophic level has on species  $j$  in the other trophic level and is expressed as the proportion of all interactions of species  $j$  that are with species  $i$ . Thus the effect of species  $i$  on species  $j$ ,  $S_{ij}$ , is calculated as:

$$S_{ij} = \frac{f_{ij}}{\sum_{m=1}^I f_{mj}}$$

Where  $f_{ij}$  is the frequency of interactions between species  $i$  and  $j$ ,  $I$  is the total number of species in that trophic level and  $m$  is the total number of species that interact with species  $j$ . If interactions with species  $i$  make up a large portion of the total interactions with species  $j$ , then species  $i$  strongly interacts with species  $j$  and is strongly relied upon by species  $j$  as an interaction partner. The difference in interaction strengths between species  $i$  and species  $j$  quantifies interaction strength asymmetry: the relative imbalance in how much interaction partners rely on each other (Fig. 3-1). The difference in interaction strengths between species  $i$  and  $j$ ,  $d_{ij}$ , is calculated as:

$$d_{ij} = S_{ij} - S_{ji}$$

Values of  $d_{ij}$  range between 1 and -1. Values close to 1 indicate that species  $j$  relies on species  $i$  for most of its interactions, but that species  $i$  relies very little on species  $j$ . For example, if species  $i$  were a plant and species  $j$  a pollinator, a  $d_{ij}$  value close to 1 would indicate that most of the pollinator's visits are to that plant, but visits to the plant by that pollinator make up very few of the total visits to the plant. This is interpreted as the pollinator relying on the plant for floral resources, but the plant relying very little on that pollinator species for pollen delivery compared to other pollinator species. Asymmetry, the overall imbalance in interaction strengths for a species, can then be calculated as the average  $d_{ij}$  value of all the species with which a focal species (species  $j$ ) interacts. I performed analyses on both a master web which pooled data from across all sites and on site x year combinations, as indicated below. Network level asymmetry was calculated as the average unweighted asymmetry of all species in the network.

I used ANOVA to ask how asymmetry varied by year, site, trophic level (plant or pollinator) and specialization. Specialization was a categorical variable based on species degree (the number of interaction partners for a species). Species were divided into 'specialized', 'generalized' and 'very generalized' categories. All species in the specialized category had three or less interaction partners, an operational definition of specialized which is similar to that of Ashworth *et al.* (2004). There is little consensus on how many interaction partners a species must have to classify as generalized and the terms "generalist" and "super generalist" have been loosely attributed to species with very high degree (Richardson *et al.* 2000). Nevertheless I attempted to reflect this classification by placing species in similar categories which were scaled differently between plants and pollinators to reflect differences in the variation in degree between

trophic levels. Generalized plants had 4-20 interaction partners and very generalized plants had > 20. Generalized pollinators had 4-10 interaction partners and very generalized pollinators had > 10. These somewhat arbitrary categories aim to differentiate those species which are extreme generalists and interact with a considerable portion of the community from those that are less extreme. Results of the ANOVA are presented as mean asymmetry  $\pm$  SE.

I predicted that smaller networks may not support specialized species leading to asymmetry values closer zero. To test this, I first used linear regression to test whether asymmetry varied by network size (performed in SAS 9.2; SAS Institute Inc. 2008). Others have defined network size as the total number of plants plus pollinators (Dormann *et al.* 2009), however since my goal was to compare asymmetry to the richness of possible interactions, I defined network size as the product of the number of plants and pollinators. Thus the richest sites have the potential to have the most interactions. Second, I investigated whether the relationship between network size and asymmetry was caused by the presence of specialists. To test for a nonlinear relationship I used Akaike's Information Criterion (AIC) to find the best of two polynomial models (1<sup>st</sup> or 2<sup>nd</sup> order) to describe the shape of each regression, using R (R Development Core Team, 2011). I then performed an analysis of covariance (ANCOVA) with network size as the covariate, specialization as a categorical variable, and number of species within a specialization category as the response variable. I interpreted a significant specialization  $\times$  network size interaction as evidence that species with different specialization levels are differently maintained in networks of different sizes.

## Integration of introduced species

Defining integration is difficult, however Memmott and Waser (2002) used species degree (number of interaction partners) of introduced and native species as a measure of integration, where species with more interaction partners are considered more integrated. Aizen *et al.* (2008) expanded the concept of integration by measuring interaction strengths; if introduced species have strong interactions, then they are well integrated. Here I use both the degree of introduced species and their asymmetry as measures of how they integrate into the network.

I compared the asymmetry values for introduced focal species to native species in the same trophic level. First, I compared introduced species to the most generalized (highest degree) native species under the assumption that the most generalized native, by definition, is highly integrated. I also compared each introduced species to a native species with the most similar degree to the introduced. When there were several native species with identical degree, I compared asymmetry of focal introduced species to all of them. Species which have more interaction partners are also more likely to interact with species of low (negative) asymmetry. I used logistic regression to test whether introduced species were more likely to interact with the highest (most positive), over the lowest (most negative) asymmetry species in the opposite trophic level, similar to the approach used by others (Lopezaraiza-Mikel *et al.* 2007). I expected that introduced species would interact with more low asymmetry species (negative or zero asymmetry) than do native species of similar degree. Significant results of logistic regressions are presented with odds ratios which describe, for every unit increase in asymmetry, how many times more likely an interaction partner is to interact with the focal species. Since asymmetry is confined between -1 and 1, I present odds ratios for a 0.1 unit increase in asymmetry. A large odds ratio indicates that the focal species is more likely to interact

with more positively asymmetric species in the opposite trophic level, and can be considered less integrated as it is relied upon by species which, in general, rely weakly on their interaction partners. Thus the difference in odds ratio between introduced and native species indicates differences in how those species integrate into the network.

## Results

### Connectance, asymmetry and specialization

The results of the ANOVA included significant three-way interactions indicating that asymmetry differed between trophic levels depending on the site and year combination, and depending on inter-annual variation in asymmetry among the different specialization categories. In short, there is interannual and spatial variation in the amount that plants and pollinators rely on their interaction partners (Table 3-1).

Plants had more positive asymmetry than pollinators, however the difference in asymmetry between trophic levels was greater for specialized species (plants =  $-0.09 \pm 0.25$ , pollinators =  $-0.81 \pm 0.01$ ) than for generalized (plants =  $0.10 \pm 0.03$ , pollinators =  $-0.62 \pm 0.02$ ) and very generalized species (plants =  $0.31 \pm 0.02$ , pollinators =  $-0.24 \pm 0.02$ ). Pollinator asymmetry decreased more in year 2 (year 1 =  $-0.56 \pm 0.02$ , year 2 =  $-0.58 \pm 0.02$ ) than plant asymmetry did (year 1 =  $0.22 \pm 0.03$ , year 2 =  $0.21 \pm 0.03$ ). The reduction in asymmetry in year two was also smaller for very generalized species (year 1 =  $-0.03 \pm 0.03$ , year 2 =  $-0.04 \pm 0.03$ ) than for generalized species (year 1 =  $-0.40 \pm 0.03$ , year 2 =  $-0.42 \pm 0.04$ ) or specialized species (year 1 =  $-0.79 \pm 0.02$ , year 2 =  $-0.81 \pm 0.02$ ).

Mean square values of the ANOVA indicate that most of the variation in asymmetry was accounted for by differences between trophic levels and specialization

(Table 3-1). Plants had more positive asymmetry than pollinators, and asymmetry values varied with specialization as expected (very generalized > generalized > specialized).

The plant, *Plectritis congesta* interacted with the most other species (had the largest degree) of any species in the study, being visited by 60 pollinators across all years and sites. Among pollinators, *Bombus bifarius* had the largest degree, visiting a total of 26 plant species (see appendix A, B). There were notable differences in generality between plants and pollinators: 80 pollinators (36 %) were only observed visiting one plant species whereas just a single plant, *Saxifraga occidentalis* (2 %), was visited by a single species of pollinator (Bruchidae sp.1).

Asymmetry was significantly more negative in larger networks ( $R^2 = 0.44$ ,  $P = 0.02$ ; Fig. 3-2). The linear model best described how the number of species in each specialization category changed with network size (AIC = -84.70), followed by the quadratic model (AIC = -82.93). Since the difference in AIC between the two best models was < 2, I cannot rule out the existence of a nonlinear relationship between asymmetry and network size (Burnham and Anderson 2002). However, since I had no reason to expect a non-linear relationship, I chose the simpler linear model as the best. The significant interaction between specialization and network size indicated that the number of species in each specialization category responded differently to network size. The number of specialized species increased with network size faster than the number of generalized and very generalized species (Fig. 3-3). Small networks are dominated by generalists whereas large networks have a higher proportion of specialists.

Connectance, the number of unique interactions in a network out of the total possible interactions (network size), varied between 0.9 and 0.13 (Table 3-2) this means

that very few of the possible interactions between species actually occur. Combining all data across all sites and years yielded a network with connectance of 0.10 which means that of all the possible interactions between plants and pollinators, 10% were actually observed to occur. These values indicate high fidelity and low sharing of interaction partners.

The average asymmetry of all species in the master network was -0.41 which means that overall, species relied strongly on their interaction partners. Both trophic levels contained species with positive and negative asymmetry (Fig. 3-4). In some cases, asymmetry was very negative and in both trophic levels there were some species that were extremely dependent on their interaction partners, with asymmetry values approaching -1. Only a single plant species had asymmetry below -0.5, *Saxifraga occidentalis*, whereas a third of pollinator species (n = 76) had (low) asymmetry below -0.9. The plant with the most positive asymmetry was *Symphoricarpus albus* (0.43); the pollinators with the most positive asymmetry were *Bombus flavifrons* and *Bruchidae* sp.1 (both 0.11). There were also more plants (n = 37, 88 %) than pollinators (n = 10, 5 %) with positive asymmetry values, which indicates that most plants are relied upon for their floral rewards more than they rely on particular pollinator species for pollen delivery.

## **Integration of introduced species**

I compared introduced species to native species of similar degree to see whether introduced species integrated into the network in the same way as native species.

There was only one relatively abundant introduced pollinator at our study sites, the European honeybee, *Apis mellifera* (single specimens of *Anthidium manicatum*, *Megachile rotundata* and *Osmia caerulescens*, and five specimens of *Lasioglossum*

*zonulum* were also caught). *Apis mellifera* had an asymmetry value of 0 which means that on average it was involved in symmetric interactions with plants. However, since most pollinators had negative asymmetry it was in general more strongly relied on by plants than were other pollinators (Fig. 3-4a). However, the most generalized pollinator, the bumblebee *Bombus bifarius*, had a positive asymmetry value (0.09). The asymmetry values of native species with identical degree to *A. mellifera* varied: the bumblebee *Bombus mixtus* had slightly higher asymmetry (0.01) whereas the sweat bee *Halictus rubicundus* had lower asymmetry (-0.04). *Apis mellifera* largely visited plants with the most positive asymmetry whereas all native pollinators tested visited plants with both positive and negative asymmetry (Table 3-3, Fig.3-5a-d).

Introduced plants varied in asymmetry. There were five introduced plants at my study sites: Scotch broom (*Cytisus scoparius*, Fabaceae), dovefoot geranium (*Geranium molle*, Geraniaceae), hairy cat's ear (*Hypochaeris radicata*, Asteraceae), self-heal (*Prunella vulgaris*, Lamiaceae) and hairy vetch (*Vicia hirsuta*, Fabaceae). All introduced plants had positive asymmetry (Fig. 3-4b). *H. radicata* had the most positive asymmetry of any introduced plant (0.22) followed by *C. scoparius* (0.19), *P. vulgaris* (0.15), *G. molle* (0.13) and *V. hirsuta* (0.07). *Hypochaeris radicata* was the only introduced plant that had more positive asymmetry than the most generalized plant, *P. congesta*, which had an asymmetry value of 0.18. *Hypochaeris radicata* was also the only plant that had more positive asymmetry when compared to native species of similar degree (Table 3-3). Other introduced plants all had much less positive asymmetry when compared to native plants with similar degree.

All plants tested, native or introduced, interacted mostly with pollinators that had positive and neutral asymmetry and few that had negative asymmetry meaning they were more likely to interact with pollinators which, in general, rely weakly on plants



(Table 3-3, Fig. 3-5e-i). Plants varied in which pollinators they interacted with however. The odds ratios for the introduced plants *H. radicata* and *C. scoparius* were lower than native species of similar degree indicating they were visited by more negative asymmetry pollinators than the native species. In contrast, the other introduced plants, *Geranium molle*, *Prunella vulgaris* and *Vicia hirsuta*, all had larger odds ratios when compared to a native species with similar degree, indicating they were more likely to be visited by positive and neutral (rather than negative) asymmetry pollinators than the native plants (Table 3-3).

## Discussion

### Asymmetry and network size

My prediction that smaller networks would be less asymmetric (asymmetry values closer to zero) was supported. This indicates that in smaller networks pairs of interacting species are more likely to rely equally on each other in symmetric interactions. This result contrasts with another study that simulated bipartite networks and showed that interactions became more symmetric (asymmetry closer to zero) as network size increased (Dormann *et al.* 2009) and another empirical study which showed that large networks have more mutually weak (symmetric) interactions with asymmetry near zero (Albrecht *et al.* 2010). However both of these studies removed singleton interactions which would cause networks to become less asymmetric. Since my goal was to examine the effect of the richness of possible interactions (network size) on asymmetry, I included singleton interactions in my analysis which may explain why the networks I observed showed the opposite pattern: large networks had proportionately more specialists, which lead to more asymmetric interactions. Specifically, the presence of uncommon pollinators likely caused network asymmetry to

become more negative. From a conservation perspective, this result suggest that large oak-savannah plant-pollinator networks will be more resistant to random species losses than small networks.

## **Integration of introduced species**

The introduced European honeybee, *Apis mellifera*, integrated into the network differently than native pollinators. Although *A. mellifera*, had lower asymmetry than the most generalized native species, the bumblebee, *Bombus bifarius*, it still had relatively high positive asymmetry for a pollinator indicating it was more strongly relied upon than most other pollinators in my measured networks. I expected that high asymmetry would be due to a generalized species interacting with specialists, but the significant logistic regression and large odds ratio for *A. mellifera* suggests that it had high asymmetry despite interacting mostly with high positive asymmetry (generalized) plants. This result was surprising, but could occur if *A. mellifera* was a very frequent visitor to many positive asymmetry plants which would make it strongly relied upon by those plants, thus these plants would rely weakly on most pollinators, but strongly on *A. mellifera* specifically. Among native pollinators, the bumblebee *Bombus bifarius* and the sweat bee, *Halictus rubicundus* both visited the same number of plant species as *A. mellifera*; however *B. bifarius* visited more plants with middle to low asymmetry whereas *H. rubicundus* showed a trend towards visiting more positive asymmetry plants. Thus *A. mellifera* integrates differently than one native species (*B. bifarius*) but similarly to another (*H. rubicundus*).

Despite having high degree, the introduced pollinator, *A. mellifera*, was the only pollinator tested that was more likely to visit high (positive) asymmetry plants than plants with low asymmetry (negative or near zero). In contrast to the introduced pollinator,

introduced plants interacted similarly to native species: all plants, native or introduced were much more likely to be visited by pollinators with asymmetry that is near zero or positive than negative. The most generalized introduced plant, *Hypochaeris radicata* (Asteraceae), had even more positive asymmetry than the native, high-degree generalist *P. congesta* (Valerianaceae), indicating that it is more strongly relied on as a source of floral resources than *P. congesta*. Furthermore the odds ratio for *P. congesta* was 1.51 compared to 1.42 for *H. radicata*, indicating that *P. congesta* is more likely to interact with high (near zero or positive) asymmetry pollinators than *H. radicata*, which suggests the introduced species is more integrated into the network. When compared to *Ranunculus occidentalis* (Ranunculaceae), a native plant with similar degree, *H. radicata* had slightly higher asymmetry and a lower odds ratio for the logistic regression suggesting it is more strongly relied upon by negative asymmetry pollinators. Therefore *H. radicata* was a particularly important plant for pollinators that in general rely strongly on the plants they interact with.

Other introduced plant species varied in how they integrated into networks. *Geranium molle* (Geraniaceae), *Prunella vulgaris* (Lamaceae) and *Vicia hirsuta* (Fabaceae) all had lower asymmetry (closer to zero) and larger odds ratios when compared to native species with similar degree, indicating that when compared to native species, these introduced species are visited by pollinators that in general rely the least on the plants they interact with. Taken together this indicates that they are not well integrated into the network. Compared to native plants with similar degree, *Cytisus scoparius* (Fabaceae) had lower asymmetry (more negative) than all three of the native species, and a lower odds ratio than two of them. Thus compared to native species with similar degree, *C. scoparius* is generally less strongly relied upon but does still interact with some low asymmetry pollinators.

Comparisons among introduced plants show that *H. radicata* was well integrated into the network: it was visited by the most pollinators, had the most positive asymmetry and the lowest odds ratio, indicating it is relied upon by many negative asymmetry pollinators (likely specialists). In general, as introduced plants decreased in degree, their asymmetry also decreased (moved towards zero) and they supported fewer low asymmetry species. My results support those of another study that found that introduced plants attracted mostly generalist pollinators (Memmott and Waser 2002). I showed that most introduced plants were visited by pollinators which had positive or near zero asymmetry, which are likely mostly generalists.

Differences in how introduced species integrated into networks at my study sites may be due to differences in floral morphology. Floral morphology is probably the main way that plants restrict pollinators (Stang *et al.* 2006). Actinomorphic flowers, like those of *H. radicata*, are not restrictive and may be accessed by a variety of pollinators, which may explain why it is so well integrated with the network. The same is true of *G. molle*, though it was not visited by as many species as *H. radicata*, perhaps because it has less conspicuous flowers, or because *H. radicata* blooms late in the season when there is less available forage for pollinators. In contrast, flowers of *V. hirsuta*, *P. vulgaris*, and *C. scoparius* are all bilaterally symmetrical, which has been suggested to restrict pollinators (Neal *et al.* 1998), and *V. hirsuta* may additionally restrict access by all but long-tongued pollinators. These restrictions may reduce the number of pollinator species which visit these plants, in turn preventing them from becoming highly integrated into the network.

## **Implications for conservation**

Asymmetry can be used to make conservation decisions (Elle *et al.* in press). Asymmetric interactions such as those I observed are thought to contribute to overall

network resistance because in asymmetric networks few species rely mutually strongly on each other (Ashworth *et al.* 2004; Bastolla *et al.* 2009). Asymmetry can largely be explained by abundance (Vázquez *et al.* 2007) as abundant species are more likely to interact with uncommon ones in asymmetric interactions. Thus asymmetry is likely the result of many uncommon specialized species which rely on relatively few, highly generalized abundant species. One result of these properties is that networks are resistant to the loss of random species; however a consequence of negative asymmetry is that networks are more vulnerable to the loss of species with high positive asymmetry. High positive asymmetry species are often abundant generalists, which may be buffered from fluctuations in abundance or even presence of their interaction partners (Memmott *et al.* 2007) and are resistant to changes in network structure. However, networks may be more vulnerable to some anthropogenic disturbances which affect common pollinator species preferentially, as has been the case with the range decline of some formerly widespread bumblebee species in North America (Cameron *et al.* 2011).

Species with high positive asymmetry often support many uncommon specialists. Although specialists are not important for network structure, if the goal of conservation is preservation of biodiversity, then uncommon species must be considered in network analysis. In addition the network approach can be used to identify and conserve generalists, the relatively few positively asymmetric species which contribute to the persistence of many uncommon species. Because plants had higher asymmetry than pollinators in my study (as in Vázquez *et al.* 2007), the conservation of high asymmetry plants in particular would be an efficient way to maintain biodiversity. For example, four pollinator species were caught exclusively on the native plant, *P. congesta*. Introduced plants may also support uncommon pollinators; indeed, six pollinator species were only observed visiting *H. radicata*. The pollinators that were observed visiting exclusively *P.*

*congesta* or *H. radicata* were singletons so it is possible that they visit other plants as well. However my results do show that some high asymmetry plants can provide forage that allows for the persistence of uncommon pollinator species. The higher proportion of specialists in large networks also suggests that the effect of generalists has increasing benefits for biodiversity as networks become larger.

Another study found that among introduced pollinators, *A. mellifera* visited many plant species and was a frequent visitor to the most generalized endemic plants while other introduced pollinators visited fewer plants (Olesen *et al.* 2002). My results agree with this work, showing that *A. mellifera*, may actually have a superficial effect on network structure even if it visits many plant species because few of those plants rely strongly on it for pollen delivery. My results suggest that the effect of *A. mellifera* on networks in this oak-savannah ecosystem may be minor. *Apis mellifera* can be considered a super generalist (Richardson *et al.* 2000), therefore introduced pollinators that are even less generalized, and likely interact with fewer species in weaker interactions, would have an even smaller impact on plant-pollinator networks. Therefore invasion by pollinators is unlikely to be a significant threat to network stability. It has also been suggested that *A. mellifera* could be used in conservation to increase pollination of common native plants (Cayuela *et al.* 2011), but could they be useful for pollinating uncommon plants as well? My results suggest that in the oak-savannah ecosystems of British Columbia, Canada, honeybees mostly visit common plants and are unlikely to visit uncommon plants and so *A. mellifera* would not be useful for the pollination of rare species.

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## Tables

**Table 3-1. Analysis of variance (ANOVA) testing whether asymmetry varies by trophic level (plant vs. animal), specialization (specialized, generalized, and very generalized) and year for the two years and six sites for which networks were assembled.**

| Source of variation                  | <i>df</i> | MS    | <i>F</i> | <i>P</i> |
|--------------------------------------|-----------|-------|----------|----------|
| Level                                | 1         | 19.47 | 232.24   | <.0001   |
| Specialization                       | 2         | 8.04  | 95.92    | <.0001   |
| Year                                 | 1         | 0.54  | 6.45     | 0.01     |
| Site                                 | 5         | 0.07  | 0.88     | 0.50     |
| Level × Specialization               | 2         | 0.55  | 6.60     | <0.01    |
| Level × Year                         | 1         | 0.45  | 5.40     | 0.02     |
| Level × Site                         | 5         | 0.04  | 0.51     | 0.80     |
| Specialization × Year                | 2         | 0.29  | 3.41     | 0.03     |
| Specialization × Site                | 10        | 0.08  | 0.94     | 0.50     |
| Year × Site                          | 5         | 0.14  | 1.72     | 0.13     |
| Level × Specialization × Year        | 2         | 0.34  | 4.01     | 0.02     |
| Level × Specialization × Site        | 6         | 0.11  | 1.33     | 0.24     |
| Level × Year × Site                  | 5         | 0.20  | 2.44     | 0.03     |
| Specialization × Year × Site         | 10        | 0.10  | 1.21     | 0.28     |
| Level × Specialization × Year × Site | 6         | 0.03  | 0.34     | 0.92     |
| Error                                | 932       | 0.08  |          |          |

**Table 3-2. Number of pollinator and plant species, network size, connectance and asymmetry for six oak-savannah sites over two years, and for a master network that combines data across sites and years. Site abbreviations are: the Cowichan Garry Oak Reserve (GO), the Mount Tzouhalem Ecological Reserve (MZ), Stoltz Meadows (SM), Cowichan River Provincial Park (CR), Cowichan Lake Forestry Station (FS) and Mesachie Mountain (MM). Sites are arranged from those near the coast (generally higher species richness) to those further inland (generally lower species richness).**

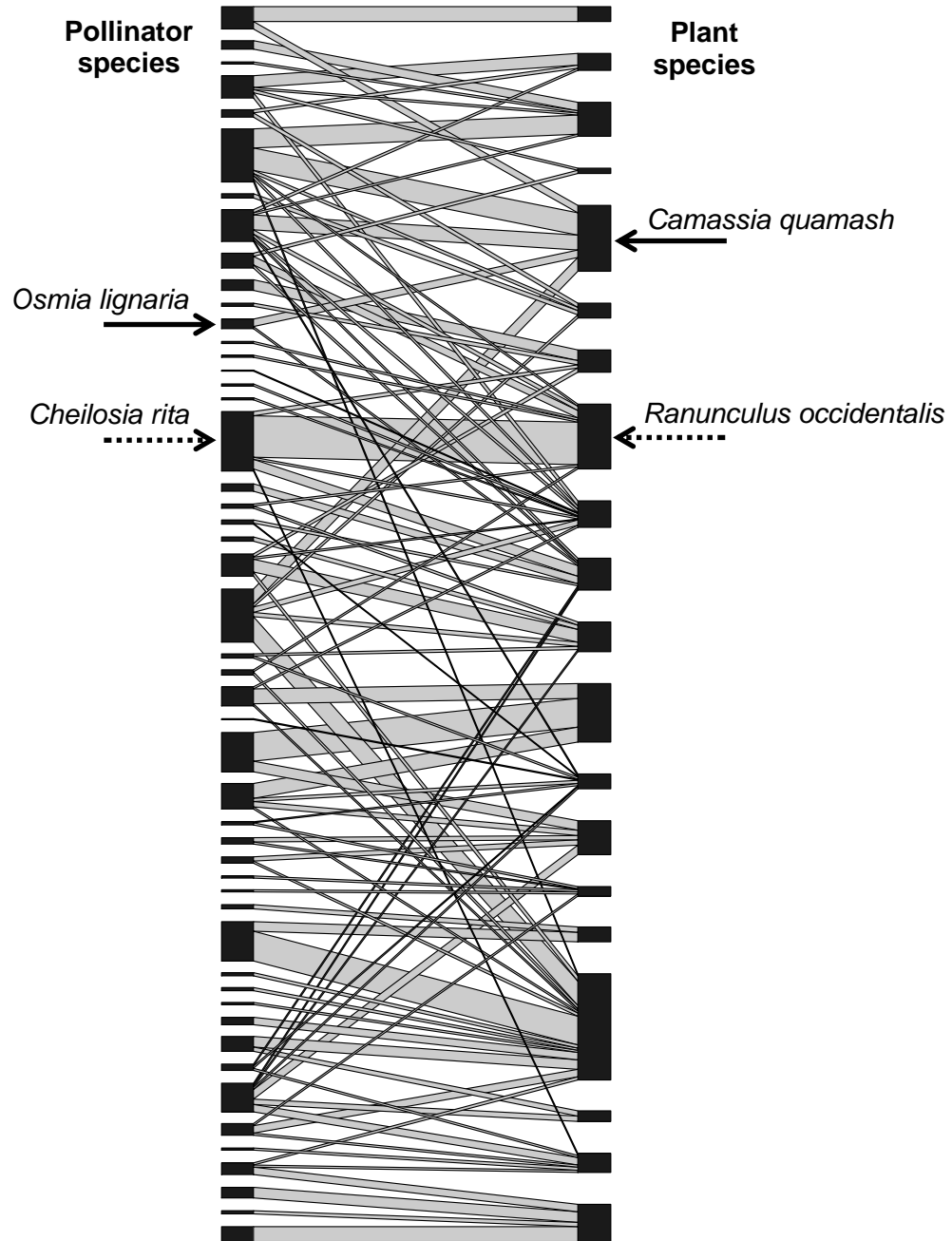
| Site   | Year | Pollinators | Plants | Network size | Connectance | Asymmetry |
|--------|------|-------------|--------|--------------|-------------|-----------|
| GO     | 1    | 77          | 24     | 1848         | 0.09        | -0.39     |
| GO     | 2    | 83          | 25     | 2075         | 0.09        | -0.40     |
| MZ     | 1    | 73          | 21     | 1533         | 0.11        | -0.37     |
| MZ     | 2    | 66          | 23     | 1518         | 0.09        | -0.39     |
| SM     | 1    | 77          | 23     | 1771         | 0.11        | -0.39     |
| SM     | 2    | 71          | 22     | 1562         | 0.09        | -0.43     |
| CR     | 1    | 46          | 17     | 782          | 0.13        | -0.34     |
| CR     | 2    | 50          | 20     | 1000         | 0.11        | -0.30     |
| FS     | 1    | 50          | 18     | 900          | 0.11        | -0.39     |
| FS     | 2    | 67          | 21     | 1407         | 0.09        | -0.39     |
| MM     | 1    | 45          | 15     | 675          | 0.11        | -0.35     |
| MM     | 2    | 44          | 18     | 792          | 0.10        | -0.34     |
| master | 1,2  | 220         | 42     | 9240         | 0.10        | -0.41     |

**Table 3-3. Results of logistic regression testing whether introduced species, and comparable native species, interacted with more positive asymmetry species in the opposite trophic level. Each introduced species was compared to native species in its trophic level with the nearest degree (comparisons indicated with the same letter beside species name). Degree (D), asymmetry (A), coefficients of logistic regression (B), standard error (SE), and Wald test of significance based on a chi-square distribution are shown. Since asymmetry values are constrained between -1 and 1, the odds ratios for a more biologically meaningful change of 0.1 are also indicated.**

|                                  | Introduced? |    |       |       |      |    |       |         |            |  |
|----------------------------------|-------------|----|-------|-------|------|----|-------|---------|------------|--|
| Pollinator species               | N           | D  | A     | B     | SE   | df | Wald  | P       | Odds ratio |  |
| <i>Bombus bifarius</i>           | N           | 26 | 0.09  | 2.52  | 1.91 | 1  | 1.75  | 0.1860  | 1.29       |  |
| <i>Apis mellifera</i>            | Y           | 16 | 0.00  | 10.70 | 4.32 | 1  | 6.14  | 0.0132  | 6.80       |  |
| <i>Bombus mixtus</i>             | N           | 16 | 0.01  | 1.87  | 2.04 | 1  | 0.84  | 0.3596  | 1.21       |  |
| <i>Halictus rubicundus</i>       | N           | 16 | -0.04 | 6.13  | 3.34 | 1  | 3.37  | 0.0663  | 1.85       |  |
| Plant species                    |             |    |       |       |      |    |       |         |            |  |
| <i>Plectritis congesta</i>       | N           | 60 | 0.18  | 4.11  | 0.68 | 1  | 36.29 | <0.0001 | 1.51       |  |
| <i>Hypochoeris radicata</i>      | Y           | 42 | 0.22  | 3.52  | 0.72 | 1  | 24.07 | <0.0001 | 1.42       |  |
| <i>Ranunculus occidentalis</i>   | N           | 38 | 0.21  | 4.12  | 0.84 | 1  | 23.89 | <0.0001 | 1.51       |  |
| <i>Geranium molle</i>            | Y           | 17 | 0.13  | 7.04  | 1.86 | 1  | 14.29 | 0.0002  | 2.02       |  |
| <i>Sanicula crassicaulis</i>     | N           | 17 | 0.18  | 4.55  | 1.33 | 1  | 11.76 | 0.0006  | 1.58       |  |
| <i>Prunella vulgaris</i>         | Y           | 16 | 0.15  | 4.72  | 1.41 | 1  | 11.31 | 0.0008  | 1.60       |  |
| <i>Mahonia aquifolium</i>        | N           | 16 | 0.27  | 2.26  | 0.90 | 1  | 6.33  | 0.0118  | 1.25       |  |
| <i>Cytisus scoparius</i>         | Y           | 14 | 0.19  | 3.74  | 1.26 | 1  | 8.78  | 0.0030  | 1.45       |  |
| <i>Arctostaphylos columbiana</i> | N           | 15 | 0.21  | 4.64  | 1.43 | 1  | 10.54 | 0.0012  | 1.59       |  |
| <i>Camassia leichtlinii</i>      | N           | 15 | 0.24  | 5.58  | 1.65 | 1  | 11.48 | 0.0007  | 1.75       |  |
| <i>Lomatium nudicaule</i>        | N           | 15 | 0.38  | 2.25  | 0.92 | 1  | 5.89  | 0.0152  | 1.25       |  |
| <i>Vicia hirsuta</i>             | Y           | 9  | 0.07  | 10.80 | 3.43 | 1  | 9.93  | 0.0016  | 2.95       |  |
| <i>Minuartia tenella</i>         | N           | 9  | 0.12  | 9.03  | 2.98 | 1  | 9.17  | 0.0025  | 2.47       |  |
| <i>Rosa gymnocarpa</i>           | N           | 9  | 0.13  | 8.89  | 2.95 | 1  | 9.10  | 0.0026  | 2.43       |  |

## Figures

Figure 3-1. An example interaction network from one site. Black bars represent each species, and the width of the bars is proportional to the number of interactions between species. Grey bars connect interacting species and the width of bars is proportional to interaction frequency. When one species is more relied upon than the other, the interaction is asymmetric. Solid black arrows show two species involved in an asymmetric interaction: the bee, *Osmia lignaria*, visits *Camassia quamash* almost exclusively, but *C. quamash* is in turn visited by many pollinators and so relies weakly on *O. lignaria*. In contrast, dashed arrows indicate a more symmetric interaction: the hoverfly, *Cheilosia rita*, mainly visits *Ranunculus occidentalis* and in turn *R. occidentalis* is visited mostly by *C. rita*.



**Figure 3-2. Network-level asymmetry (mean asymmetry of all plants and pollinators at a site) measured for six oak-savannah sites in two years. Site/year combinations differ in network size, the product of the number of plants and animals observed. Asymmetry became more negative as networks became larger indicating that in general there is a greater difference in interaction strengths between species.**

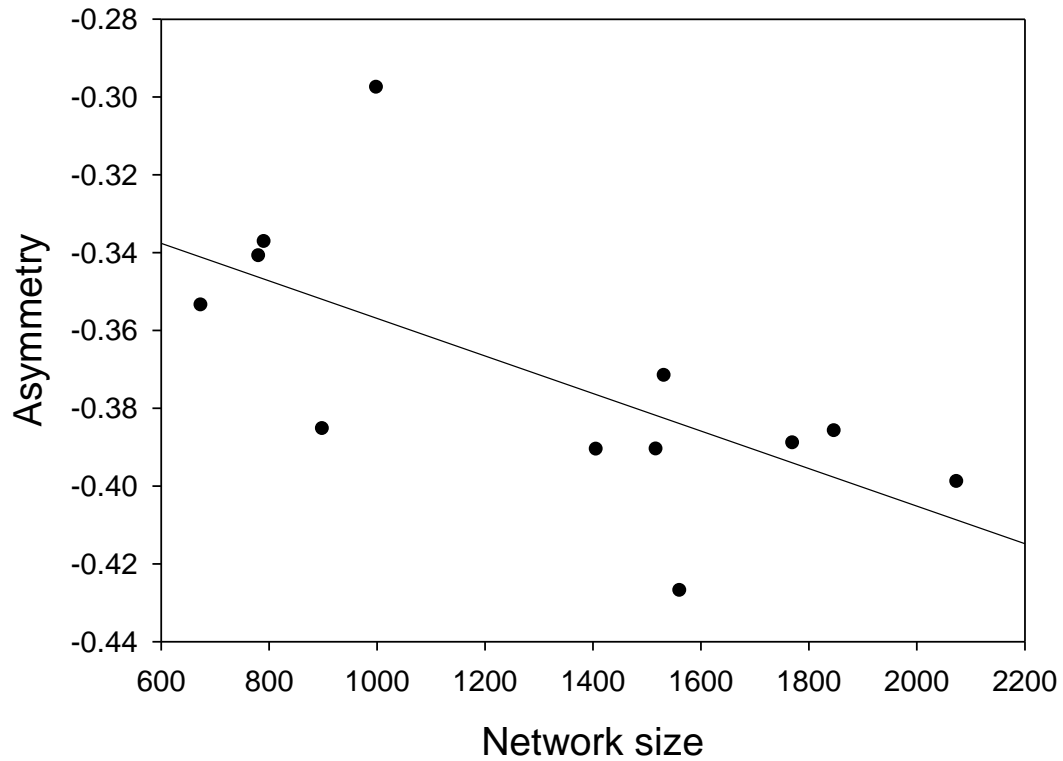
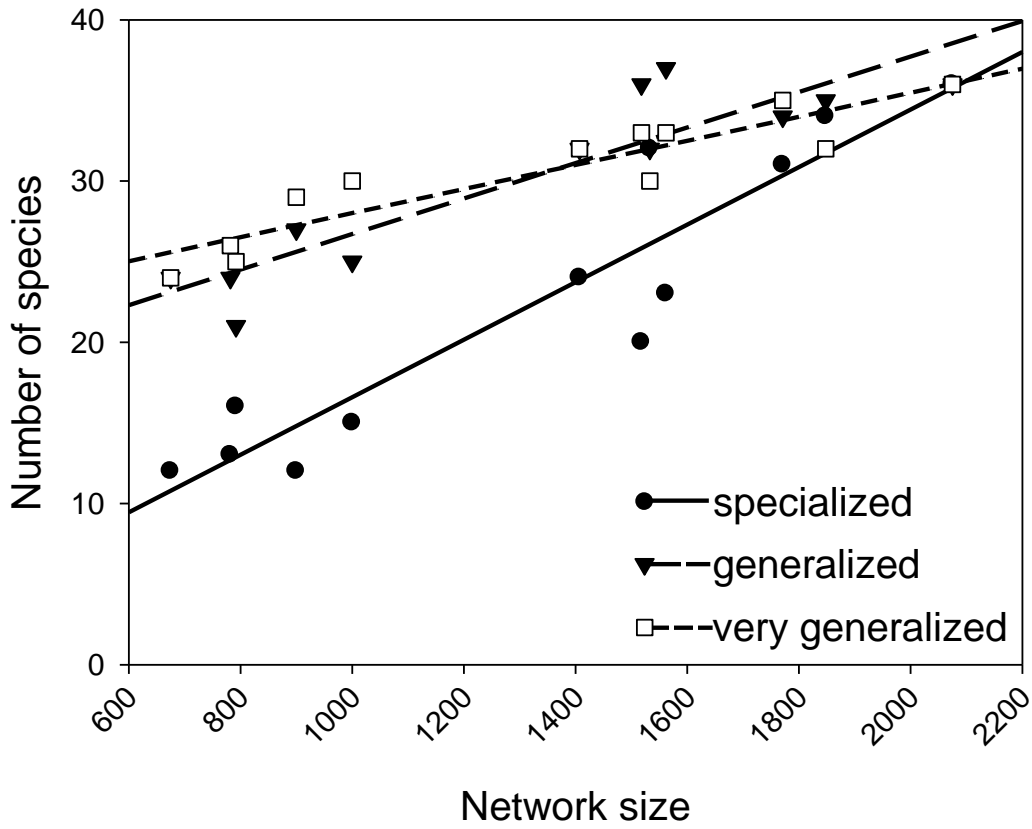




Figure 3-3. ANCOVA showed a significant interaction between network size and pollinator specialization. AIC revealed that a linear relationship best fit the data. As networks become larger, they contain proportionately more specialized species.



**Figure 3-4. Asymmetry of introduced species (black) with native species for comparison (white). Species are ranked by asymmetry with the high (most positive) asymmetry species on the right. The only abundant introduced pollinator was *Apis mellifera* (a); there were five introduced plant species (b): *Hypochaeris radicata*, *Geranium molle*, *Prunella vulgaris*, *Cytisus scoparius* and *Vicia hirsuta*.**

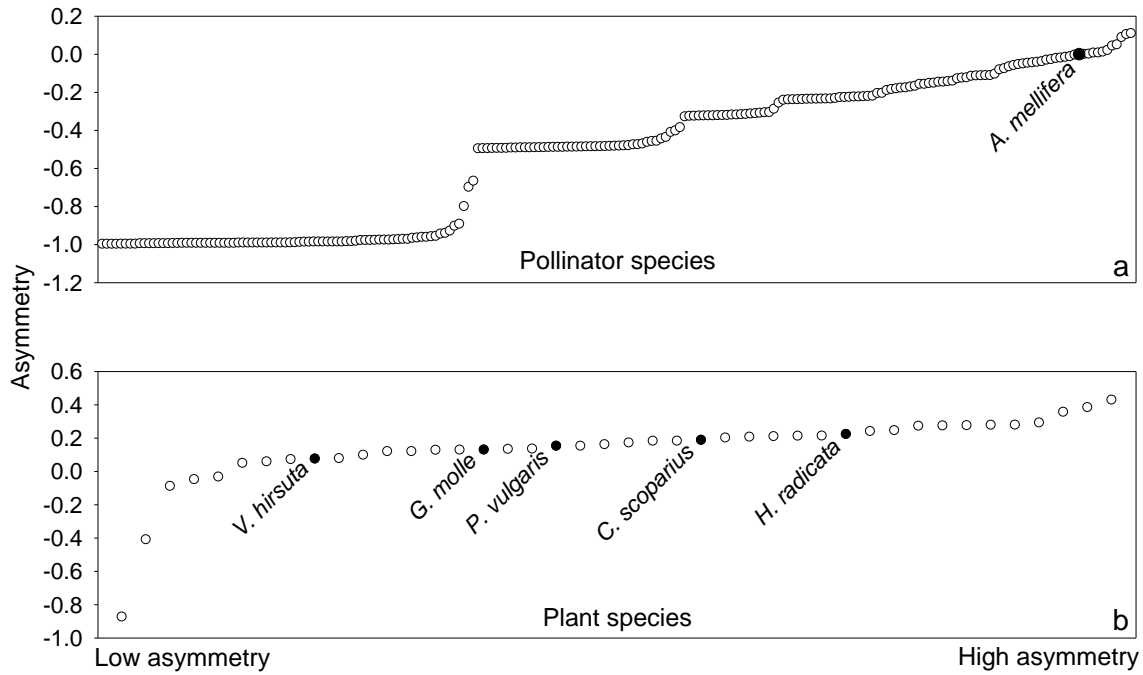
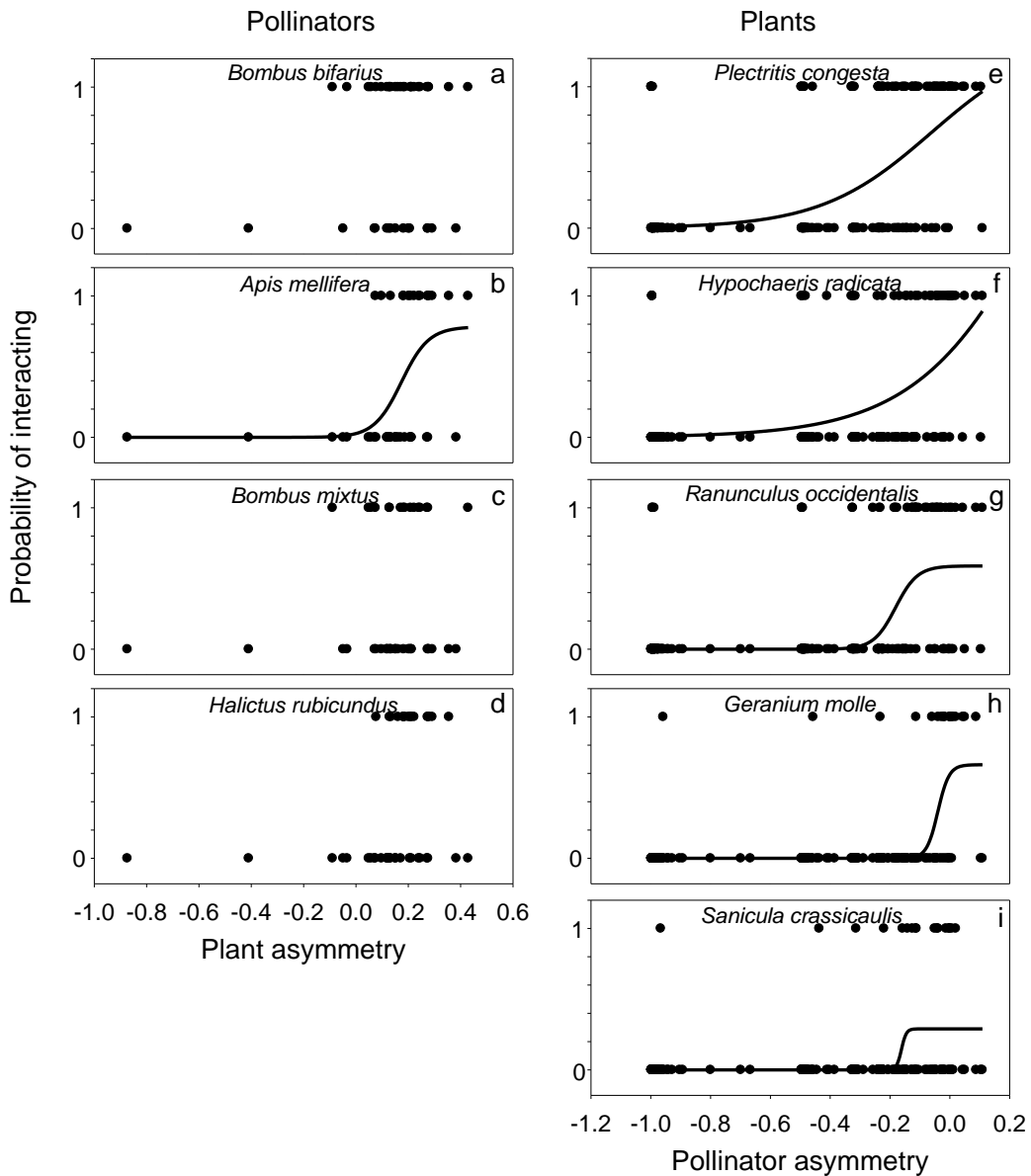


Figure 3-5. Patterns of interaction of introduced species; the most generalized native species and native generalist species with identical degree are provided for illustration. 1 = interacts, 0 = does not interact. The native generalist pollinator, *Bombus bifarius* (a), visits both high and low asymmetry plants. The introduced pollinator, *Apis mellifera* (b), mostly visits high (most positive) asymmetry plants. By comparison *Bombus mixtus* (c) visits both high and low asymmetry plants, however, similar to *A. mellifera*, *Halictus rubicundus* (d) shows a trend towards visiting mostly high asymmetry plants. All plants tested were visited mostly by high asymmetry pollinators. The most generalized native plant, *Plectritis congesta*, is visited by several pollinators with low asymmetry (e). The native plant *Hypochaeris radicata* (f) was visited by many negative asymmetry pollinators, similar to *Ranunculus occidentalis* (g), a native species with similar degree. In contrast *Geranium molle* (h) was visited by fewer negative asymmetry pollinators than the native plant, *Sanicula crassicaulis* (i).



## Chapter 4: Conclusion

### Pollen limitation

Pollen limitation (PL) is purported to be a common phenomenon (Burd 1994; Knight *et al.* 2005). However I found that PL was rare and no population was more prone to pollen limitation than others. To explain PL, most studies have examined either the effects of the plant community (Dauber *et al.* 2010; Hegland *et al.* 2009; Knight 2003) or pollinator community (Gómez *et al.* 2010; Witjes *et al.* 2011) on pollination success; however few studies have measured both as I do in this thesis. My result is therefore surprising because pollen limitation was only weakly dependent on the local plant community and not at all related to pollinator diversity. Other studies that have measured PL in multiple populations or years showed that although common, it was highly variable and that even if significant pollen limitation occurred, most showed that it varied across seasons and sites (Burd 1994). Inter-site and inter-annual variation points to multiple unmeasured factors which affect PL and suggests that for a given population there may be no single universal cause. More likely, several factors act simultaneously to affect pollen delivery and it may be difficult to predict which will be the main cause of PL in a particular site or year.

Many of the world's biodiversity hotspots support endemic plants which are threatened the most by pollen limitation (Alonso *et al.* 2010). Although the plant and pollinator community had little effect on PL in my study, these factors are thought to be important in global diversity hotspots and an important step in conservation of plants in

hotspots will be to identify whether this is true. A useful study would directly measure pollen transfer and visit rate along a plant diversity gradient to uncover if, and when, pollen limitation occurs due to transfer of heterospecific pollen or simply reduced pollinator visit rate due to increased competition with other plants. This research would also need to be combined with a study which examined the effect of inbreeding to determine at what population density inbreeding with conspecifics begins to decrease reproduction.

Could the prevalence of PL —said to occur in over 60% of plant populations— be overstated in the literature? An early analysis of pollen limitation (Burd 1994) which showed that PL was common, acknowledged that a “file drawer effect” may occur, where studies which did not detect pollen limitation were not published. The author also argued that most studies were performed on large and dense populations in which pollen limitation is presumably less likely to occur, which would counteract any publication bias. Another, more recent review which came to a similar conclusion attempted to avoid publication bias by using unpublished, as well as published, data (Knight *et al.* 2005) however the authors did not specify how many unpublished studies were included and it is unknown how many studies in which pollen limitation is not observed go unreported. Therefore, it remains possible that a systematic publication bias in pollen limitation studies exists which would overstate the prevalence of pollen limitation.

Even if pollen limitation is the rule rather than the exception, pollen limitation does not necessarily have demographic consequences. While some studies have shown that population growth rate is sensitive to the effect of PL on fecundity (Law *et al.* 2010) and others have shown the opposite (Ramula *et al.* 2007), in reality most studies, including my own, omit demographic data. However since pollen limitation was rare in

my study, it is unlikely that it limits population growth in this case. The crucial link between PL and population decline remains elusive not just for my research, but for the field in general.

## Interaction networks

The plant-pollinator networks that I observed are similar to those of all other studies that I am aware of. There are few generalists and many specialists, leading to interaction strength asymmetry. Furthermore, I showed that at the network level, asymmetry varied with the number of possible interactions and that this is likely driven by the increasing number of specialists in large webs. The amount which network properties are the result of simple sampling effects is debated in the literature. Unpredictable weather, common in spring-flowering communities, meant I was unable to sample pollinators evenly across species and sites, possibly leading to bias. Though simple properties such as connectance are sensitive to sampling effort, they asymptote as sampling effort increases. In contrast, network level asymmetry is insensitive to sampling intensity (Dormann *et al.* 2009), thus my asymmetry results are unlikely to be the result of sampling artefacts.

With some exceptions, few studies have been able to show empirically how interaction networks assemble and disassemble and how interaction asymmetry changes as assembly or disassembly occurs. I showed that small networks contain mainly generalists, and as they increase in size, proportionately more specialists are added. Asymmetry becomes more negative in large networks because interactions between generalists and specialists are strongly asymmetric. A study of a high latitude plant-pollinator network which was sampled every day throughout its season showed that as species were added to the network, they preferentially interacted with the most

generalized species (Olesen *et al.* 2008). These interactions would likely be very asymmetric. However another study showed that pollinator generality (degree weighted by interaction strength) increased in large networks implying that pollinators increased their diet breadth as new plant species became available (Albrecht *et al.* 2010). Therefore it is likely that as networks become larger they become more asymmetric for two reasons. First, because they contain increasingly more specialists but also because generalists become even more generalized as more species are present for them to interact with.

Published plant-pollinator networks are asymmetric which contributes to their resistance to species loss and networks in the oak-savannah are also highly asymmetric suggesting they are likewise resistant. However, we still do not know how much of the pollination function species are able to compensate for when other species in the same trophic level are absent. In most studies simulating network extinctions it is assumed that all species are equally effective pollinators or sources of floral rewards and that as long as a species has at least one interaction partner remaining, it will persist (Memmott *et al.* 2004; Kaiser-Bunbury *et al.* 2010). If it is assumed that all species are equally effective partners (ie. equal pollination efficiency or source of floral rewards), then a species could tolerate the loss of a frequent interaction partner as long as less frequent interaction partners remain, causing networks to remain intact until many species are removed. While simulations based on these assumptions are a useful starting point for investigations into network disassembly, ultimately they are unrealistic. In reality, pollinators likely differ in effectiveness, but interaction frequency is more important than per visit effectiveness meaning that the most frequent pollinators of a plant are the most important even if they transfer less pollen per visit (Vázquez *et al.* 2005). Therefore it is

possible that species which lose their most frequent interaction partners could suffer population declines, even if other interaction partners remain.

Biodiversity is important for ecosystem functions, but its role in plant-pollinator communities is difficult to ascertain. In their examination of plant-pollinator communities, most studies have predicted that initial biodiversity losses will be compounded by cascading extinctions and changes in the floral community which affect pollen limitation. My results are encouraging since networks in the oak-savannah ecosystem of Vancouver Island, BC, despite extreme fragmentation, are very asymmetric, contributing to their resistance to random species losses. Furthermore, plants in this ecosystem are rarely pollen limited and pollination is only weakly affected by the local plant community. Nevertheless, the oak-savannah ecosystem is still threatened by anthropogenic disturbance (Fuchs 2001) and continued monitoring is required if the effects of disturbance are to be detected early enough for conservation plans to be effective. The monitoring of species with high, positive asymmetry, for example bumblebees and the generalist plants *Symphoricarpus albus* and *Holodiscus discolor*, should be of concern since conserving these species would contribute to the conservation of the many others that rely strongly on them. As plants are in general strongly relied upon by many insects, the conservation of plants in particular would be an efficient way to preserve diversity by maintaining forage for many insects. Furthermore managers should exercise caution when removing high asymmetry introduced plants as this may remove an important source of forage for insects. Instead, removal of introduced species should be performed in stages as has been suggested elsewhere (Carvalho *et al.* 2008). Although recommendations such as these would not be apparent if conservation were considered only at the species level, conservation of particular species may still be effective if they are put into a community context allowing conservationists to identify



those species that contribute to network, and community, resistance and stability.

Therefore community level monitoring with a focus on conservation of particular species is likely an efficient means of supporting the existence of important plant-pollinator mutualisms not only in the oak-savannah, but other ecosystems as well.

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## **Appendices**

## Appendix A.

### Summary data for netted pollinators

Summary data for all netted pollinator species and morphospecies in study. Degree and asymmetry are from the master network which combines data from all sites and years.

Specialization category: v=very generalized, g=generalized and s=specialized.

| Family              | Pollinator species / morphospecies | Degree | Asymmetry | Specialization category |
|---------------------|------------------------------------|--------|-----------|-------------------------|
| Andrenidae          | <i>Andrena amphibola</i>           | 2      | -0.44     | s                       |
|                     | <i>Andrena angustitarsata</i>      | 7      | -0.05     | g                       |
|                     | <i>Andrena astragali</i>           | 4      | -0.16     | g                       |
|                     | <i>Andrena auricoma</i>            | 6      | -0.11     | g                       |
|                     | <i>Andrena caerulea</i>            | 4      | -0.18     | g                       |
|                     | <i>Andrena chlorogaster</i>        | 1      | -0.96     | s                       |
|                     | <i>Andrena cressonii</i>           | 4      | -0.24     | g                       |
|                     | <i>Andrena hemileuca</i>           | 1      | -0.98     | s                       |
|                     | <i>Andrena knuthiana</i>           | 1      | -0.99     | s                       |
|                     | <i>Andrena lupinorum</i>           | 4      | -0.24     | g                       |
|                     | <i>Andrena melanothroa</i>         | 4      | -0.23     | g                       |
|                     | <i>Andrena microchlora</i>         | 2      | -0.48     | s                       |
|                     | <i>Andrena milwaukeensis</i>       | 1      | -0.97     | s                       |
|                     | <i>Andrena nigrocaerulea</i>       | 11     | 0.00      | v                       |
|                     | <i>Andrena nivalis</i>             | 1      | -0.99     | s                       |
|                     | <i>Andrena pallidifovea</i>        | 1      | -0.99     | s                       |
|                     | <i>Andrena prunorum</i>            | 2      | -0.50     | s                       |
|                     | <i>Andrena rufosignata</i>         | 1      | -0.99     | s                       |
|                     | <i>Andrena saccata</i>             | 8      | -0.12     | g                       |
|                     | <i>Andrena salicifloris</i>        | 4      | -0.24     | g                       |
|                     | <i>Andrena scurra</i>              | 3      | -0.31     | s                       |
|                     | <i>Andrena sigmundi</i>            | 2      | -0.49     | s                       |
|                     | <i>Andrena sola</i>                | 2      | -0.48     | s                       |
| <i>Andrena</i> sp.2 | 1                                  | -0.80  | s         |                         |
| <i>Andrena</i> sp.5 | 1                                  | -0.99  | s         |                         |

| Family             | Pollinator species / morphospecies | Degree | Asymmetry | Specialization category |
|--------------------|------------------------------------|--------|-----------|-------------------------|
| Apidae             | <i>Andrena</i> sp.6                | 1      | -0.99     | s                       |
|                    | <i>Andrena subtilis</i>            | 1      | -0.99     | s                       |
|                    | <i>Andrena thaspiae</i>            | 1      | -0.98     | s                       |
|                    | <i>Andrena transnigra</i>          | 4      | -0.24     | g                       |
|                    | <i>Andrena trevoris</i>            | 1      | -0.96     | s                       |
|                    | <i>Andrena vicina</i>              | 1      | -0.99     | s                       |
|                    | <i>Andrena vicinoides</i>          | 1      | -0.99     | s                       |
|                    | <i>Andrena vierecki</i>            | 1      | -0.99     | s                       |
|                    | <i>Andrena w.scripta</i>           | 2      | -0.49     | s                       |
|                    | <i>Panurginus atriceps</i>         | 6      | 0.00      | g                       |
|                    | <i>Perdita nevadensis</i>          | 1      | -0.99     | s                       |
|                    | <i>Anthophora pacifica</i>         | 1      | -0.98     | s                       |
|                    | <i>Anthophora porterae</i>         | 1      | -0.67     | s                       |
|                    | <i>Apis mellifera</i>              | 16     | 0.00      | v                       |
|                    | <i>Bombus bifarius</i>             | 26     | 0.09      | v                       |
|                    | <i>Bombus flavifrons</i>           | 19     | 0.10      | v                       |
|                    | <i>Bombus melanopygus</i>          | 21     | 0.05      | v                       |
|                    | <i>Bombus mixtus</i>               | 16     | 0.01      | v                       |
|                    | <i>Bombus sitkensis</i>            | 2      | -0.50     | s                       |
|                    | <i>Bombus vosnesenskii</i>         | 3      | -0.31     | s                       |
|                    | <i>Ceratina acantha</i>            | 20     | 0.01      | v                       |
|                    | <i>Ceratina nanula</i>             | 6      | -0.15     | g                       |
|                    | <i>Epeolus compactus</i>           | 1      | -0.97     | s                       |
|                    | <i>Epeolus minimus</i>             | 1      | -0.97     | s                       |
|                    | <i>Eucera frater</i>               | 4      | -0.24     | g                       |
|                    | <i>Habropoda cineraria</i>         | 3      | -0.32     | s                       |
|                    | <i>Habropoda miserabilis</i>       | 1      | -1.00     | s                       |
|                    | <i>Nomada</i> sp.1                 | 4      | -0.23     | g                       |
|                    | <i>Nomada</i> sp.10                | 3      | -0.32     | s                       |
|                    | <i>Nomada</i> sp.11                | 4      | -0.24     | g                       |
|                    | <i>Nomada</i> sp.2                 | 1      | -0.99     | s                       |
|                    | <i>Nomada</i> sp.3                 | 1      | -1.00     | s                       |
| <i>Nomada</i> sp.4 | 7                                  | -0.13  | g         |                         |
| <i>Nomada</i> sp.7 | 3                                  | -0.32  | s         |                         |

| Family        | Pollinator species / morphospecies | Degree | Asymmetry | Specialization category |
|---------------|------------------------------------|--------|-----------|-------------------------|
| Bibionidae    | <i>Bibio</i> sp.1                  | 1      | -0.99     | s                       |
|               | <i>Bibio</i> sp.2                  | 1      | -0.93     | s                       |
|               | <i>Dilophus</i> sp.1               | 3      | -0.26     | s                       |
| Bombyliidae   | <i>Anthrax</i> sp.2                | 1      | -0.98     | s                       |
|               | <i>Bombylius major</i>             | 14     | 0.04      | v                       |
|               | <i>Bombylius xanthothrix</i>       | 4      | -0.21     | g                       |
|               | <i>Conophorus</i> sp.2             | 2      | -0.46     | s                       |
|               | <i>Systoechus oreas</i>            | 2      | -0.48     | s                       |
| Bruchidae     | <i>Bruchidae</i> sp.1              | 20     | 0.11      | v                       |
| Buprestidae   | <i>Buprestidae</i> sp.2            | 5      | -0.17     | g                       |
| Calliphoridae | <i>Lucilia illustris</i>           | 2      | -0.49     | s                       |
| Cerambycidae  | <i>Cerambycidae</i> sp.6           | 4      | -0.21     | g                       |
|               | <i>Leptura chrysocoma</i>          | 1      | -0.94     | s                       |
| Chrysididae   | <i>Chrysididae</i> sp.1            | 4      | -0.22     | g                       |
| Cimbicidae    | <i>Cimbicidae</i> sp.1             | 1      | -0.98     | s                       |
| Cleridae      | <i>Cleridae</i> sp.1               | 5      | -0.17     | g                       |
| Colletidae    | <i>Colletes fulgidus</i>           | 3      | -0.32     | s                       |
|               | <i>Colletes kincaidii</i>          | 3      | -0.31     | s                       |
|               | <i>Hylaeus basalis</i>             | 4      | -0.24     | g                       |
|               | <i>Hylaeus modestus</i>            | 5      | -0.18     | g                       |
|               | <i>Hylaeus nevadensis</i>          | 1      | -0.99     | s                       |
| Conopidae     | <i>Myopa</i> sp.1                  | 1      | -1.00     | s                       |
|               | <i>Myopa</i> sp.2                  | 2      | -0.49     | s                       |
|               | <i>Physocephala</i> sp.1           | 2      | -0.49     | s                       |
|               | <i>Thecophora</i> sp.2             | 2      | -0.49     | s                       |
| Curculionidae | <i>Rhynchites bicolor</i>          | 1      | -0.89     | s                       |
| Elateridae    | <i>Elateridae</i> sp.3             | 2      | -0.49     | s                       |
|               | <i>Elateridae</i> sp.4             | 1      | -0.99     | s                       |
|               | <i>Elateridae</i> sp.5             | 1      | -0.99     | s                       |
| Empididae     | <i>Empididae</i> sp.1              | 5      | -0.12     | g                       |
| Halictidae    | <i>Halictus confusus</i>           | 11     | -0.07     | v                       |
|               | <i>Halictus ligatus</i>            | 1      | -1.00     | s                       |
|               | <i>Halictus rubicundus</i>         | 16     | -0.04     | v                       |
|               | <i>Lasioglossum athabascense</i>   | 2      | -0.40     | s                       |

| Family        | Pollinator species / morphospecies | Degree | Asymmetry | Specialization category |
|---------------|------------------------------------|--------|-----------|-------------------------|
|               | <i>Lasioglossum colatum</i>        | 1      | -0.99     | s                       |
|               | <i>Lasioglossum cressonii</i>      | 15     | -0.01     | v                       |
|               | <i>Lasioglossum egregium</i>       | 1      | -0.97     | s                       |
|               | <i>Lasioglossum incompletum</i>    | 1      | -0.99     | s                       |
|               | <i>Lasioglossum knereri</i>        | 22     | 0.00      | v                       |
|               | <i>Lasioglossum laevisimum</i>     | 4      | -0.22     | g                       |
|               | <i>Lasioglossum mellipes</i>       | 1      | -0.99     | s                       |
|               | <i>Lasioglossum nevadense</i>      | 15     | -0.04     | v                       |
|               | <i>Lasioglossum olympiae</i>       | 21     | 0.02      | v                       |
|               | <i>Lasioglossum ovaliceps</i>      | 2      | -0.46     | s                       |
|               | <i>Lasioglossum pacatum</i>        | 2      | -0.48     | s                       |
|               | <i>Lasioglossum pacificum</i>      | 3      | -0.32     | s                       |
|               | <i>Lasioglossum sisymbrii</i>      | 1      | -1.00     | s                       |
|               | <i>Lasioglossum</i> sp.1           | 3      | -0.33     | s                       |
|               | <i>Lasioglossum</i> sp.2           | 2      | -0.49     | s                       |
|               | <i>Lasioglossum</i> sp.3           | 13     | -0.04     | v                       |
|               | <i>Lasioglossum</i> sp.4           | 5      | -0.19     | g                       |
|               | <i>Lasioglossum</i> sp.5           | 7      | -0.11     | g                       |
|               | <i>Lasioglossum</i> sp.6           | 22     | 0.01      | v                       |
|               | <i>Lasioglossum titusi</i>         | 2      | -0.41     | s                       |
|               | <i>Lasioglossum zonulum</i>        | 4      | -0.24     | g                       |
|               | <i>Sphecodes</i> sp.1              | 2      | -0.49     | s                       |
|               | <i>Sphecodes</i> sp.2              | 2      | -0.48     | s                       |
|               | <i>Sphecodes</i> sp.3              | 4      | -0.22     | g                       |
|               | <i>Sphecodes</i> sp.4              | 1      | -0.99     | s                       |
| Hesperiidae   | <i>Erynnis propertius</i>          | 5      | -0.19     | g                       |
| Ichneumonidae | <i>Ichneumonidae</i> sp.1          | 1      | -0.99     | s                       |
|               | <i>Ichneumonidae</i> sp.4          | 1      | -1.00     | s                       |
| Incurvariidae | <i>Adela septentrionella</i>       | 11     | -0.02     | v                       |
| Lonchaeidae   | <i>Lonchaeidae</i> sp.1            | 1      | -0.90     | s                       |
| Lycaenidae    | <i>Icaricia</i> sp.1               | 4      | -0.23     | g                       |
|               | <i>Lycaenidae</i> sp.1             | 4      | -0.23     | g                       |
| Megachilidae  | <i>Anthidium manicatum</i>         | 1      | -1.00     | s                       |
|               | <i>Ashmeadiella cactorum</i>       | 1      | -0.98     | s                       |



| Family       | Pollinator species / morphospecies | Degree | Asymmetry | Specialization category |
|--------------|------------------------------------|--------|-----------|-------------------------|
|              | <i>Coelioxys sodalis</i>           | 1      | -0.99     | s                       |
|              | <i>Dianthidium subparvum</i>       | 1      | -1.00     | s                       |
|              | <i>Heriades carinatus</i>          | 1      | -0.99     | s                       |
|              | <i>Megachile frigida</i>           | 1      | -0.99     | s                       |
|              | <i>Megachile gemula</i>            | 3      | -0.32     | s                       |
|              | <i>Megachile melanophaea</i>       | 1      | -0.99     | s                       |
|              | <i>Megachile nivalis</i>           | 2      | -0.49     | s                       |
|              | <i>Megachile perihirta</i>         | 3      | -0.32     | s                       |
|              | <i>Megachile relativa</i>          | 1      | -1.00     | s                       |
|              | <i>Megachile rotundata</i>         | 1      | -0.99     | s                       |
|              | <i>Osmia albolateralis</i>         | 12     | -0.03     | v                       |
|              | <i>Osmia bakeri</i>                | 2      | -0.46     | s                       |
|              | <i>Osmia bella</i>                 | 1      | -0.98     | s                       |
|              | <i>Osmia bucephala</i>             | 1      | -1.00     | s                       |
|              | <i>Osmia caerulescens</i>          | 1      | -0.96     | s                       |
|              | <i>Osmia coloradensis</i>          | 4      | -0.22     | g                       |
|              | <i>Osmia cyanella</i>              | 2      | -0.49     | s                       |
|              | <i>Osmia densa</i>                 | 3      | -0.32     | s                       |
|              | <i>Osmia dolerosa</i>              | 15     | -0.02     | v                       |
|              | <i>Osmia juxta</i>                 | 3      | -0.32     | s                       |
|              | <i>Osmia kincaidii</i>             | 4      | -0.22     | g                       |
|              | <i>Osmia lignaria</i>              | 11     | -0.05     | v                       |
|              | <i>Osmia malina</i>                | 4      | -0.24     | g                       |
|              | <i>Osmia nigrifrons</i>            | 1      | -0.99     | s                       |
|              | <i>Osmia odontogaster</i>          | 4      | -0.23     | g                       |
|              | <i>Osmia pusilla</i>               | 10     | -0.05     | g                       |
|              | <i>Osmia texana</i>                | 3      | -0.33     | s                       |
|              | <i>Osmia trevoris</i>              | 5      | -0.11     | g                       |
|              | <i>Osmia tristella</i>             | 13     | -0.02     | v                       |
| Mordellidae  | <i>Mordellidae</i> sp.1            | 3      | -0.29     | s                       |
| Muscidae     | <i>Muscidae</i> (metallic) sp.1    | 1      | -0.98     | s                       |
| Papilionidae | <i>Parnassius clodius</i>          | 1      | -0.99     | s                       |
| Phalacridae  | <i>Phalacridae</i> sp.1            | 3      | -0.33     | s                       |
| Pompilidae   | <i>Pompilidae</i> sp.1             | 1      | -0.99     | s                       |

| Family                   | Pollinator species / morphospecies | Degree | Asymmetry | Specialization category |
|--------------------------|------------------------------------|--------|-----------|-------------------------|
| Sphecidae                | <i>Ammophila</i> sp.1              | 2      | -0.49     | s                       |
|                          | <i>Sphecidae</i> sp.1              | 2      | -0.49     | s                       |
| Sphingidae               | <i>Hemaris diffinis</i>            | 2      | -0.49     | s                       |
| Symphyta                 | <i>Symphyta</i> sp.1               | 1      | -0.98     | s                       |
| Syrphidae                | <i>Blera</i> sp.1                  | 1      | -0.98     | s                       |
|                          | <i>Cheilosia rita</i>              | 10     | -0.03     | g                       |
|                          | <i>Chrysotoxum fasciatus</i>       | 3      | -0.32     | s                       |
|                          | <i>Criorhina</i> sp.1              | 1      | -0.99     | s                       |
|                          | <i>Dasysyrphus</i> sp.1            | 8      | -0.10     | g                       |
|                          | <i>Epistrophe emarginata</i>       | 1      | -1.00     | s                       |
|                          | <i>Epistrophe nitidicollis</i>     | 2      | -0.49     | s                       |
|                          | <i>Eriozona erraticus</i>          | 2      | -0.49     | s                       |
|                          | <i>Eristalis anthophorinus</i>     | 2      | -0.49     | s                       |
|                          | <i>Eristalis arbustorum</i>        | 2      | -0.49     | s                       |
|                          | <i>Eristalis dimidiatus</i>        | 1      | -0.70     | s                       |
|                          | <i>Eristalis obscurus</i>          | 1      | -0.99     | s                       |
|                          | <i>Eristalis transversa</i>        | 1      | -0.97     | s                       |
|                          | <i>Eumerus narcissi</i>            | 2      | -0.47     | s                       |
|                          | <i>Eupeodes curtus</i>             | 3      | -0.31     | s                       |
|                          | <i>Eupeodes lapponicus</i>         | 2      | -0.50     | s                       |
|                          | <i>Eupeodes latifasciatus</i>      | 8      | -0.11     | g                       |
|                          | <i>Eupeodes volucris</i>           | 6      | -0.14     | g                       |
|                          | <i>Hadromyia crawfordi</i>         | 2      | -0.49     | s                       |
|                          | <i>Heringia</i> sp.1               | 1      | -0.99     | s                       |
|                          | <i>Heringia</i> sp.2               | 2      | -0.49     | s                       |
|                          | <i>Lejops</i> sp.1                 | 2      | -0.49     | s                       |
|                          | <i>Lejota</i> sp.1                 | 1      | -0.96     | s                       |
|                          | <i>Melanostoma mellinum</i>        | 5      | -0.14     | g                       |
|                          | <i>Paragus</i> sp.2                | 8      | -0.08     | g                       |
|                          | <i>Parasyrphus</i> sp.1            | 5      | -0.18     | g                       |
|                          | <i>Pipiza</i> sp.1                 | 1      | -0.99     | s                       |
| <i>Platycheirus</i> sp.1 | 2                                  | -0.49  | s         |                         |
| <i>Scaeva pyrastris</i>  | 7                                  | -0.13  | g         |                         |
| <i>Sericomyia lata</i>   | 1                                  | -0.99  | s         |                         |

| Family      | Pollinator species / morphospecies | Degree | Asymmetry | Specialization category |
|-------------|------------------------------------|--------|-----------|-------------------------|
|             | <i>Sphaerophoria philanthus</i>    | 12     | -0.06     | v                       |
|             | <i>Sphaerophoria sulphuripes</i>   | 2      | -0.48     | s                       |
|             | <i>Sphecomyia</i> sp.1             | 2      | -0.44     | s                       |
|             | <i>Syrphus opinator</i>            | 4      | -0.24     | g                       |
|             | <i>Temnostoma aequale</i>          | 1      | -0.99     | s                       |
|             | <i>Toxomerus occidentalis</i>      | 9      | -0.08     | g                       |
|             | <i>Volucella bombylans</i>         | 5      | -0.15     | g                       |
|             | <i>Xylota</i> sp.1                 | 2      | -0.48     | s                       |
| Tachinidae  | <i>Epalpus signifer</i>            | 2      | -0.39     | s                       |
|             | <i>Gymnosoma fulginosa</i>         | 4      | -0.15     | g                       |
|             | <i>Peleteria</i> sp.2              | 1      | -0.99     | s                       |
|             | <i>Tachinidae</i> (Large)          | 3      | -0.31     | s                       |
|             | <i>Tachinidae</i> (Medium)         | 6      | -0.16     | g                       |
|             | <i>Tachinidae</i> (Small)          | 3      | -0.32     | s                       |
|             | <i>Tachinidae</i> sp.1             | 2      | -0.47     | s                       |
|             | <i>Trichopoda</i> sp.1             | 1      | -0.99     | s                       |
|             | <i>Trichopoda</i> sp.2             | 2      | -0.49     | s                       |
| Trochilidae | <i>Selasphorus rufus</i>           | 1      | -0.94     | s                       |
| Vespidae    | <i>Ancistrocerus</i> sp.1          | 1      | -1.00     | s                       |
|             | <i>Dolichovespula maculata</i>     | 2      | -0.49     | s                       |
|             | <i>Eumeninae</i> sp.1              | 1      | -0.99     | s                       |
|             | <i>Polistes gallica</i>            | 1      | -0.99     | s                       |
|             | <i>Polistes</i> sp.1               | 6      | -0.15     | g                       |
|             | <i>Polistes</i> sp.2               | 1      | -0.99     | s                       |
|             | <i>Vespula</i> sp.2                | 1      | -0.97     | s                       |

## Appendix B.

### Summary data for plants

Summary data for all plant species in study. Degree, asymmetry and specialization category are per Appendix A.

| Family          | Plant species                    | Degree | Asymmetry | Specialization category |
|-----------------|----------------------------------|--------|-----------|-------------------------|
| Apiaceae        | <i>Lomatium nudicaule</i>        | 15     | 0.38      | g                       |
|                 | <i>Lomatium utricultum</i>       | 49     | 0.20      | v                       |
|                 | <i>Sanicula crassicaulis</i>     | 17     | 0.18      | g                       |
| Asteraceae      | <i>Achillea millefolium</i>      | 35     | 0.28      | v                       |
|                 | <i>Balsamorhiza deltoidea</i>    | 5      | -0.09     | g                       |
|                 | <i>Eriophyllum lanatum</i>       | 49     | 0.27      | v                       |
|                 | <i>Hypochaeris radicata</i>      | 42     | 0.22      | v                       |
| Berberidaceae   | <i>Mahonia aquifolium</i>        | 16     | 0.27      | g                       |
| Brassicaceae    | <i>Cardamine nuttallii</i>       | 3      | 0.13      | s                       |
| Caprifoliaceae  | <i>Symphoricarpus albus</i>      | 24     | 0.43      | v                       |
| Caryophyllaceae | <i>Cerastium arvense</i>         | 32     | 0.13      | v                       |
|                 | <i>Minuartia tenella</i>         | 9      | 0.12      | g                       |
| Ericaceae       | <i>Arctostaphylos columbiana</i> | 15     | 0.21      | g                       |
|                 | <i>Arctostaphylos uva-ursi</i>   | 18     | 0.17      | g                       |
| Fabaceae        | <i>Cytisus scoparius</i>         | 14     | 0.19      | g                       |
|                 | <i>Vicia hirsuta</i>             | 9      | 0.07      | g                       |
| Geraniaceae     | <i>Geranium molle</i>            | 17     | 0.13      | g                       |
| Lamiaceae       | <i>Prunella vulgaris</i>         | 16     | 0.15      | g                       |
| Liliaceae       | <i>Allium acuminatum</i>         | 18     | 0.16      | g                       |
|                 | <i>Allium cernuum</i>            | 22     | 0.28      | v                       |
|                 | <i>Brodiaea coronaria</i>        | 23     | 0.21      | v                       |
|                 | <i>Brodiaea hyacinthina</i>      | 28     | 0.29      | v                       |
|                 | <i>Camassia leichtlinii</i>      | 15     | 0.24      | g                       |
|                 | <i>Camassia quamash</i>          | 48     | 0.21      | v                       |
|                 | <i>Erythronium oregonum</i>      | 4      | 0.15      | g                       |

| Family           | Plant species                   | Degree | Asymmetry | Specialization category |
|------------------|---------------------------------|--------|-----------|-------------------------|
|                  | <i>Triteleia howellii</i>       | 2      | -0.41     | s                       |
|                  | <i>Zygadenus venenosus</i>      | 16     | 0.28      | g                       |
| Portulacaceae    | <i>Montia parvifolia</i>        | 20     | 0.05      | g                       |
| Primulaceae      | <i>Dodecatheon hendersonii</i>  | 8      | 0.06      | g                       |
| Ranunculaceae    | <i>Delphinium menziesii</i>     | 12     | 0.13      | g                       |
|                  | <i>Ranunculus occidentalis</i>  | 38     | 0.21      | v                       |
| Rosaceae         | <i>Fragaria virginiana</i>      | 21     | 0.12      | v                       |
|                  | <i>Holodiscus discolor</i>      | 50     | 0.36      | v                       |
|                  | <i>Rosa gymnocarpa</i>          | 9      | 0.13      | g                       |
|                  | <i>Rosa nutkana</i>             | 26     | 0.25      | v                       |
| Saxifragaceae    | <i>Lithophragma parviflorum</i> | 11     | -0.03     | g                       |
|                  | <i>Saxifraga integrifolia</i>   | 3      | 0.07      | s                       |
|                  | <i>Saxifraga occidentalis</i>   | 1      | -0.87     | s                       |
| Scrophulariaceae | <i>Collinsia parviflora</i>     | 29     | 0.10      | v                       |
|                  | <i>Mimulus alsinoides</i>       | 7      | -0.05     | g                       |
|                  | <i>Mimulus guttatus</i>         | 22     | 0.08      | v                       |
| Valerianaceae    | <i>Plectritis congesta</i>      | 60     | 0.18      | v                       |