

Pollen Limitation and Reproductive Success in Five South Puget Sound Prairie Plants

by

Savannah Richard

A Thesis
Submitted in partial fulfillment
Of the requirements for the degree
Master of Environmental Studies
The Evergreen State College
December 2021

©2021 by Savannah Richard. All rights reserved.

This Thesis for the Master of Environmental Studies Degree

by

Savannah Richard

has been approved for

The Evergreen State College

by

Sarah Hamman

Sarah Hamman Ph.D.

Member of Faculty

December 14, 2021

Date

ABSTRACT

Pollen Limitation and Reproductive Success in Five South Puget Sound Prairie Plants Savannah Richard

Disruption of pollen distribution can negatively impact pollen quality and quantity, leading to reduced seed set. Pollen limitation is measured by comparing pollen supplementation treatments to naturally pollinated treatments. If pollen supplemented treatments increase in reproductive success then pollen limitation is occurring. Pollen limitation is caused by land degradation and environmental disruptions that change pollinator networks and pollen deposition. Better understanding the patterns of pollen limitation and reproductive success can aide in restoration efforts for sensitive plant species. This study included three native south Puget Sound prairie plant species (*Eriophyllum lanatum*, *Lupinus lepidus*, and *Plectritis congesta*) and two non-native invasive plant species (*Hypochaeris radicata* and *Leucanthemum vulgare*). These plants were sampled across six restoration sites varying in land use and restoration treatment history. No evidence of pollen limitation was found but reproductive success was shown to vary among restoration sites. *L. lepidus* had the highest seed quantity at a high-quality restoration site. This result aligned with previous predictions of native plant species having higher reproductive success at high-quality restoration sites. This differs from *P. congesta* which had the highest seed quality at a low-quality restoration site. These findings highlight the complex interactions of plant life history, habitat fragmentation, and restoration practices on plant reproductive success.

Table of Contents

List of Figures	v
List of Tables	vi
Acknowledgements	vii
Introduction	1
Research Questions and Hypothesis	4
Literature Review	6
Background	6
Pollen Deposition and Fertilization	7
Evolution of Floral Morphology in Relationship to Pollination	9
Theoretical Framework	13
Pollen Supplementation Studies	19
Habitat Fragmentation	21
Invasive Plant Species	24
Restoration Applications	26
Methods	28
Study Site	28
Study Species	31
Experimental Design	32
Data Collection	32
Statistical Analysis	33
Results	36
Treatment Effect	36
Seed Quality – Individual Seed Weight	36
Seed Quality – Seed Viability	38
Quantity – Proportion of Fertilized Seeds	39
Discussion	42
Treatment Effect	42
<i>Plectritis congesta</i>	43
<i>Lupinus lepidus</i>	45
<i>Eriophyllum lanatum</i>	46
<i>Hypochaeris radicata</i>	46
<i>Leucanthemum vulgare</i>	47
Conclusion	48

List of Figures

Fig 1: Willamette Valley Puget Trough Georgia Basin.....	2
Fig 2: Pollen Deposition Diagram	6
Fig. 3: Sequential and Simultaneous Flowering Diagram	12
Fig. 4: Haig and Wetoby Equilibrium Model	14
Fig. 5: Stochastic Resource Allocation	16
Fig. 6: Stochastic Resource Allocation in Low and High Quality Environments	17
Fig. 7: IPU and Resource Allocation	21
Fig. 8: Pollinator Trait Responses to Habitat Fragmentation	22
Fig. 9: Ecological Restoration Practices	26
Fig. 10: Plant Species.....	31
Fig. 11: <i>P. congesta</i> Seed Weight.....	37
Fig. 12: <i>L. vulgare</i> Total Individual Seed Weight	37
Fig. 13: <i>H. radicata</i> Fertilized Seed Weight.....	38
Fig. 14: <i>H. radicata</i> Proportion of Viable Seeds	39
Fig. 15: <i>L. lepidus</i> Proportion of Fertilized Seeds	40
Fig. 16: <i>H. radicata</i> Proportion of Fertilized Seeds.....	40

List of Tables

Table 1: CNLM Restoration Treatment Application	28
Table 2: Plant Life History	30

Acknowledgements

This project was made possible by a grant from The Evergreen State College Master's in Environmental Studies thesis fund. This grant assisted with purchasing laboratory supplies. I would also like to thank the Science Support Center, Jenna Nelson, John Kirkpatrick, and Dylan Fischer for facilitating lab access during the COVID-19 pandemic. I would especially like to thank Susan Waters for providing me with the opportunity to intern on multiple projects and introducing me to the world of pollination ecology. Susan Waters also graciously shared her data, seed samples and pollination ecology knowledge with me. She also assisted me with coding in R and statistical analysis. I would also like to thank Sanders Freed and the Center of Natural Lands Management for providing management plans for the sites used in this study.

My success during the thesis process was highly influenced by the support and guidance of my thesis advisor Sarah Hamman. Sarah greatly aided in the thesis writing process and statistical analysis. Her feedback and expertise were essential for my successful completion of this project. I would also like to thank members from my cohort: Tim Atkinson and Sarah Larson, for their advice and support. Lastly, I would like to thank my life partner Jesse Hunnicutt who has been a constant source of emotional support and humor.

Introduction

Pollination services are essential to plant reproduction and underpin healthy functioning ecosystems. Although important, pollination ecology receives little research especially in the context of ecological restoration science. Animal mediated pollination is responsible for 88% of flowering plant species sexual reproduction globally (Cariveau et al. 2020). This means most of the plants used in restoration are impacted by changes in pollinator networks and pollen limitation.

Plant reproduction hinges on pollinator networks, pollen deposition and pollen quality. If there is a disruption in the pollination process this has impacts on not only plant reproduction but plant populations and ecological functions (Harder and Barrett 1996, Ashman et al. 2004, Aizen and Harder 2007). This is of particular concern for the restoration of rare plants or endangered plants that are experiencing limited pollen quantity or quality. Limits in pollen deposition can change plant community compositions and drive evolutionary changes such as an increase in self-pollination (Knight et al. 2005).

A major contributing factor to pollen limitation is land degradation and land use conversion which results in fragmented landscapes. Such ecological disruptions can alter pollinator networks and restrict access to plant populations (Knight et al. 2005). Invasive plant species colonization can also play a role in pollen limitation. Invasive species often produce excessive amounts of pollen which can effectively clog plant stigmas and limit access to conspecific pollen (Arceo-Gomez et al. 2016).



Fig 1: Willamette Valley Puget Trough Georgia Basin extending from British Columbia to Southern Oregon (Hamman et al. 2011).

South Puget Sound Prairies

The Pacific Northwest is the unlikely home to rare prairie ecosystems. Known as the Willamette Valley-Puget Trough-Georgia Basin Ecoregion, prairie habitat extended from British Columbia to the Willamette Valley, Oregon, now only fragmented remnants remain (Fig.1) (Hamman et al. 2011). In Washington remnant prairies exist in the South Puget Sound region clustered in Joint Base Lewis-McChord (JBLM) and near Olympia. These prairies were formed by the retreating of the Vashon glacier over 14,000 years ago. This geologic event caused glacial outwash soils rich in gravel, which drain quickly and are ideal for grasslands (Kruckeberg 1991).

Climatologically, South Puget Sound prairies differ significantly from midwestern prairies. The growing season for perennial forbs and grasses begins in the winter and extends into spring. While many perennial native plants experience a summer dormancy, due to drought conditions (Sinclair et al. 2006). Midwestern prairie vegetation primarily consists of perennial grasses with less forb diversity. The Pacific Northwest experiences increased levels of precipitation from October to May, but the Puget Sound trough exists in a rain shadow, shielded from high levels of precipitation by the Olympic

Mountain range (Bowcutt and Hamman 2016). The average rainfall for Olympia, Washington for 2020 was 127 cm, while the mean temperature was 16°C (NOAA). The temperate climate of the Pacific Northwest also means that temperatures rarely drop below freezing in lowland regions. This contrasts with Midwestern U.S. prairies that experience freezing temperatures throughout the fall and winter months.

Pacific Northwest prairies contain mosaics of plant communities ranging from Garry oak woodlands, savannas, grasslands, and wetlands. The primary vegetation composition consists of perennial grass and forbs, annual forbs and sparsely dispersed shrub and trees (Sinclair et al. 2006). Of the recorded 278 plant species on South Puget Sound prairies in the early 2000's, 59% were native and the remaining 41% were nonnative (Dunwiddie et al. 2006). Of the 23,000 acres of Puget Sound lowland prairies only 3,000 acres have predominantly native plant species (Storm 2006).

The disappearance of prairies in this region began with the arrival of European colonists. Starting in the 1800's prairie land was used for agriculture and grazing livestock. As the Euro American population expanded so did the development of residences, towns, and roads. This development heavily fragmented prairies, impacting animal populations and altering pollinator networks. Introduction of invasive plant species and tree encroachment further degraded prairie sites (Kruckeberg 1991).

Prior to European settler colonization, Indigenous peoples-maintained prairie lands through frequent, low intensity, controlled burns. These burns promoted growth of plant species used for food, medicine, and fibers. Burning prairies also eliminated tree establishment and created an open landscape that attracted elk and deer (Storm 2002). The disappearance of fire

from the landscape has resulted in reduced native plant and animal diversity, proliferation of invasive species and encroachment of trees and shrubs.

Since the cessation of Indigenous land management practices such as controlled burns, 95-99% of western Washington prairies have been lost (Hamman et al. 2011). A small portion of prairies remain although scattered and heavily fragmented. Currently prairie sites are managed by Washington State Department of Fish and Wildlife, Washington State Department of Natural Resources, Joint Base Lewis McChord (JBLM) and non-profit land trusts such as the Center for Natural Lands Management and Capital Land Trust. Restoration efforts include prescribed burns, herbicide treatments, Douglas fir removal, mowing and mechanical removal of invasive species and native planting and seeding.

Research Questions and Hypothesis

This thesis project aims to address three over-arching questions. (1) Is pollen limitation occurring for any plant species? (2) Does reproductive success vary by restoration site? (3) How do native and non-native plant species differ in reproductive success among sites? The first question will address if pollen limitation is occurring by comparing hand pollinated treatments to open pollination. If seed quantity or quality is significantly higher in hand pollinated treatments, then we can presume that pollen limitation is occurring. If there is no difference between treatments, then pollen limitation is not occurring. The second question investigates the impact of restoration sites on seed quantity and quality. The third question is a qualitative comparison of non-native invasive species and native species reproductive success among restoration sites.

Six prairie restoration sites were chosen in the south Puget Sound region with varying land use histories and restoration treatments. Some sites have decades of invasive species management and native plant reintroduction while others have low-quality prairie with a limited

history of restoration. If a restoration site is particularly associated with pollen limitation for multiple plant species, then the restoration history can be examined to improve future practices.

For this study I hypothesized that plant species experiencing pollen limitation will be associated with prairie sites with limited restoration history. I also surmise that native plants will have greater reproductive success at comparatively more restored sites while invasive species will be reproductively successful no matter the restoration history of the site. The null hypothesis for this study is that seed quantity and quality are not affected by restoration site or pollination treatment.

Literature Review

Background

Flowering plants rely on abiotic and biotic vectors as a means of sexual reproduction, due to their immobile nature. This process is known as pollination and involves a multitude of interactions that influence reproduction and floral evolution (Harder and Barrett 1996, Knight et al. 2006). For successful animal-facilitated pollination to take place, a pollinator must deposit pollen from one member of a species to another (*Fig.2*). Intraspecific plant species pollen, also known as conspecific pollen, must land on the receptive area of the plant, the stigma. Chemical reactions then occur triggering the growth of a pollen tube to the ovum. Once the pollen tube reaches the ovum fertilization occurs and seeds are produced (Cheung 1996).

Pollen limitation is a potential outcome that may occur when there is insufficient pollen quantity being produced or distributed. The quality of pollen can also negatively influence plant reproduction, resulting in aborted ovules or infertile seeds (Harder and Barrett 1996, Ashman et al. 2004). Pollen limitation can result from a multitude of external factors such as habitat

A A theoretical small population of plants showing typical pollen export, import, and ovule fertilisation

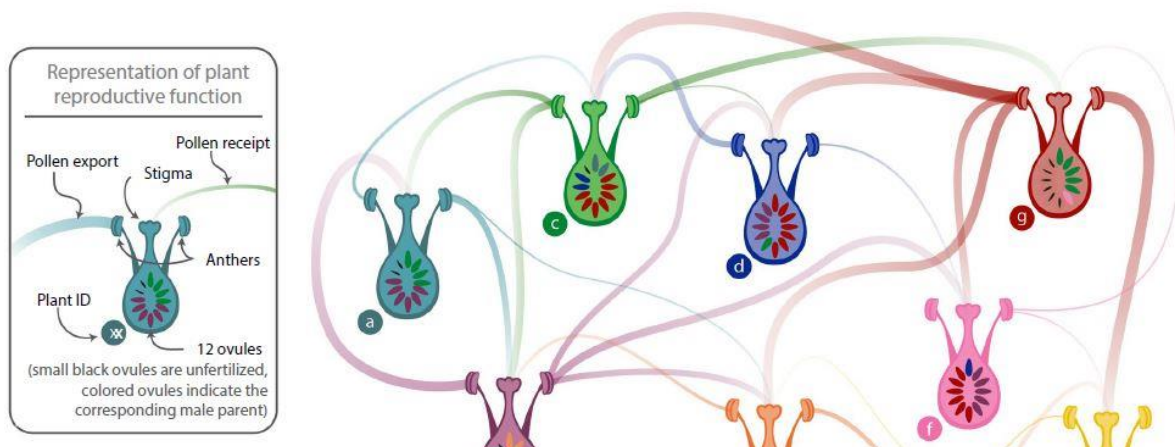


Fig 2: Pollen Deposition Diagram. Pollen deposition diagram displaying the complex process of insect mediated pollination among hermaphroditic plants of the same species. Anthers are shown exporting pollen while stigmas are shown receiving pollen. Ovules are colored to match the corresponding fertilizing individual (Anderson and Minnaar 2020).

fragmentation, decreases in pollinator populations or environmental disruptions (Alvarez 2002, Knight et al. 2005, Aguilar et al. 2006, Newman et al. 2013). The evolutionary life history and reproductive strategy of plants can also play an integral role in pollen limitation.

Plant populations experiencing pollen limitation can be negatively impacted through reduced reproductive success and population declines. Chronic pollen limitation can lead to plants selecting for self-pollination as a preferred reproductive strategy. This switch to self-pollination greatly reduces genetic complexity in plant populations and could potentially lead to plant communities being less evolutionarily adaptable. Although, plants that have evolved to be hermaphroditic or partially self-compatible have a much lower rate of pollen limitation. It is thought that these reproductive strategies are used to avoid pollen limitation (Knight et al. 2005).

Pollen limitation studies are often used to assess the availability of pollen for a plant species in a specific environment. To test for pollen limitation, researchers implement pollen supplementation experiments. This type of natural experiment involves supplementing a portion of flowers with conspecific pollen and allowing a control group to be openly pollinated. Reproductive success is then quantified by seed set or other metrics to account for the supplemental pollen impact. If the hand pollinated treatment has a higher rate of reproductive success when compared to the control group, then pollen limitation is determined to be occurring (Ashman et al. 2004).

Pollen Deposition and Fertilization

Pollination can be a major driving force in floral evolution. Pollen donors compete to fertilize ovules by locating females, excluding other pollen donors, and being accepted by female

receptive floral anatomy (Burd 1994, Snow 1994). Locating female flowers involves strategies such as having a similar phenology, sharing pollinators, or increasing patch density. Research has shown that reproductive success is not limited by the male flower population but by the ability of males to locate females (Burd 1994). This is especially true for dioecious plants that have male and female flowers on separate plants. There is less possibility of selfing but more energy spent locating intraspecific plants of the opposite sex.

Hermaphroditic flowers differentiate reproductive structures on temporal or physical scales to avoid self pollination (Harder and Barrett 1996). When self pollination occurs, it can cause pollen discounting, which reduces the amount of pollen grains for cross-pollination. To limit the effects of pollen discounting, strategies such as having separate timing for male and female reproductive parts within an individual inflorescence are used. Herkogamy is another common strategy which involves a physical separation of anthers and stigmas. Heterostyly is a form of herkogamy where the stigma grows at a different length than the anthers. This can reduce pollen discounting costs across other floral species morpho-types rather than intraspecific individual flowers (Harder and Barrett 1996).

These evolutionary competitive dynamics also play out on the microscopic scale during post-pollination on the stigma. Plants can exert little sexual selection control over the pollen being deposited on the stigma but post pollination process such as the rate of pollen tube growth or fertilization rates are a few ways in which selection can take place (Burd 1994, Snow 1994). After male pollen granules germinate on the stigma a pollen tube grows towards the ovary. Pollen tube growth rates vary depending on genetic makeup. Biochemical reactions can occur on the surface of the stigma inhibiting pollen tube growth. This can occur for self-incompatible plants when self-pollen is deposited on the stigma (Cheung 1996). Post pollination selection is

referred to as “female choice” due to the selective pressures exerted on the pollen from the stigma (Snow 1994).

Evolution of Floral Morphology in Relationship to Pollination

Plant life history and physiology, impact the ways in which pollen is produced, received, and distributed (Harder and Barrett 1996, Fenster et al. 2004, Knight et al. 2005). Plants associated with abiotic pollen distribution such as wind, tend to produce larger quantities of pollen and have smaller, less colorful floral displays. This contrasts with animal pollinated plants that often produce larger and more colorful floral display to attract pollinators (Harder and Barrett 1996, Fenster et al. 2004). Elaborate floral displays and nectar rewards require allocation of more resources. This resource allocation causes less pollen to be produced than wind pollinated flowers but there is higher efficiency at conspecific pollination (Knight et al. 2005).

Flowering plants associated with specialist pollinators tend to be more impacted by pollen limitation than generalist pollinator associated plants (Knight et al. 2005, Fernández et al. 2012, Hagen et al. 2012, Newman et al. 2013). Plants associated with specific insect functional groups co-evolve morphological structures that aide in pollination (Harder and Barrett 1996, Fenster et al. 2004). This leads to a reduction in diversity of overall pollinator visitations but improves conspecific pollination rates and limits heterospecific pollen transfer. The limited diversity of pollinator visits means specialist associated plants are more susceptible to pollen limitation due to changes in pollinator networks or patch density (Knight et al. 2005, Fernández et al. 2012, Newman et al. 2013). Pollinator preference is often expressed through morphological patterns such as radially symmetrical flowers attracting generalist pollinators and bilaterally symmetrical flowers attracting specialist pollinators (Harder and Barrett 1996). These morphological

expressions correspond with low pollen limitation for radially symmetrical flowers and a higher risk of pollen limitation among bilaterally symmetrical flowers (Knight et al. 2005).

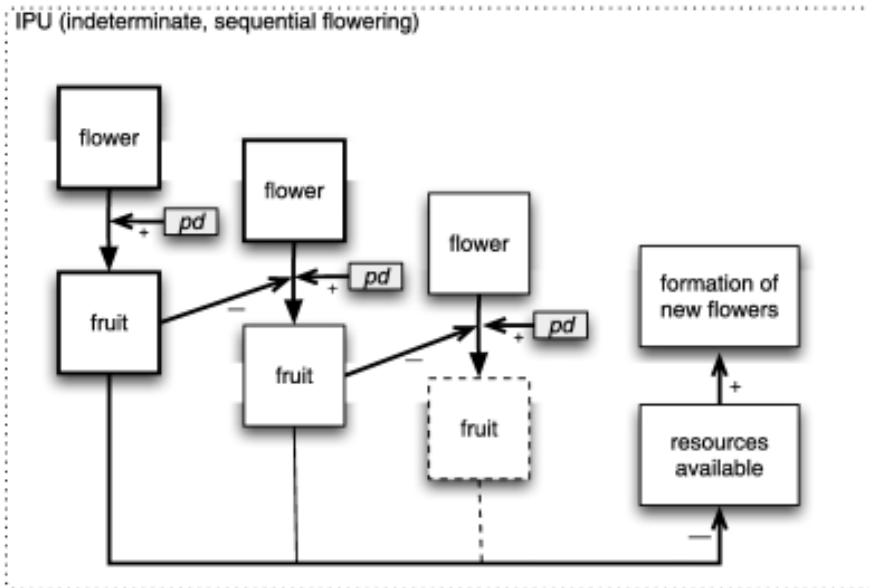
It is commonly observed that hermaphroditic flowers are more prevalent than mono-sexual (dioecious) flower types (Knight et al. 2005). A potential advantage of hermaphroditic flowers is the ability for outcrossing and self-pollination (Lloyd 1992). By retaining both modes of reproduction hermaphroditic flowers can ensure reproduction when stochastic environmental variations occur. Hermaphroditic flowers are also known to over produce floral structures compared to seed capsule production (Ehrlen 1991, Burd 1998). This could be due to pollen limitation where not all inflorescences are receiving adequate pollen deposition. Alternatively, Cohen and Durkas (1990) conclude that hermaphroditic floral production increases in relation to increased pollination variance. Hermaphroditic flower structures also require less resource investment than complete female flowers (Wesselingh 2007). This means the overproduction of flowers in hermaphroditic flowers is explained by the low cost of flower production and an increased variance in pollination (Ehrlen 1991, Burd 1998).

Pollen deposition can vary among multiple inflorescences on one plant depending on arrangement and bloom time. Sequential flowering or when flowers bloom on the same plant at varying times, allows the opportunity for a response to low pollen deposition. If pollen deposition is low a sequential blooming plant can reallocate resources to produce more subsequent flowers (Wesselingh 2007). Whereas a plant that has a synchronous bloom of flowers all at the same time does not have the opportunity to reallocate resources to produce more flowers later (*Fig.3*). Instead, synchronous blooming plants invest in more flowers than sequential blooming plants and have higher rates of fruit abortion (Casper and Neissenbaum 1993).

The spatial arrangement of inflorescence on a plant can also cause varying amounts of pollen deposition among flowers. Accounting for how flowers are arranged in relation to one another and how many flowers per inflorescence are all important factors when considering variations in pollen deposition. (Wesselingh 2007). Basal flowers on plants with sequential blooms, tend to bloom earlier and produce high quantity and quality seeds, while distal flowers bloom later and produce less seed set (Diggle 1995, Ashman and Hitchens 2000). The spatial arrangement of flowers on a stalk can influence pollinator interaction and ultimately seed set.

Phenology of flowers on a plant is also an important factor in pollen deposition. Plants with extended floral bloom times tend to not experience as much variability in seed set as plants with shorter bloom cycles (Galen and Stanton 1991). This is due to variation in environmental conditions and pollinator networks and visitations. Galen and Stanton (1991) found that late season blooming alpine *Ranunculus* experienced reduced seed weight compared to earlier season flowers. This is a general pattern described for sequentially blooming flowers. Early season basal flowers tend to serve as resource sinks and produce higher quantity and quality seeds. Later season flowers suffer from limited resources and tend to produce seeds of less quantity and quality (Casper and Neissenbaum 1993).

(a)



(b)

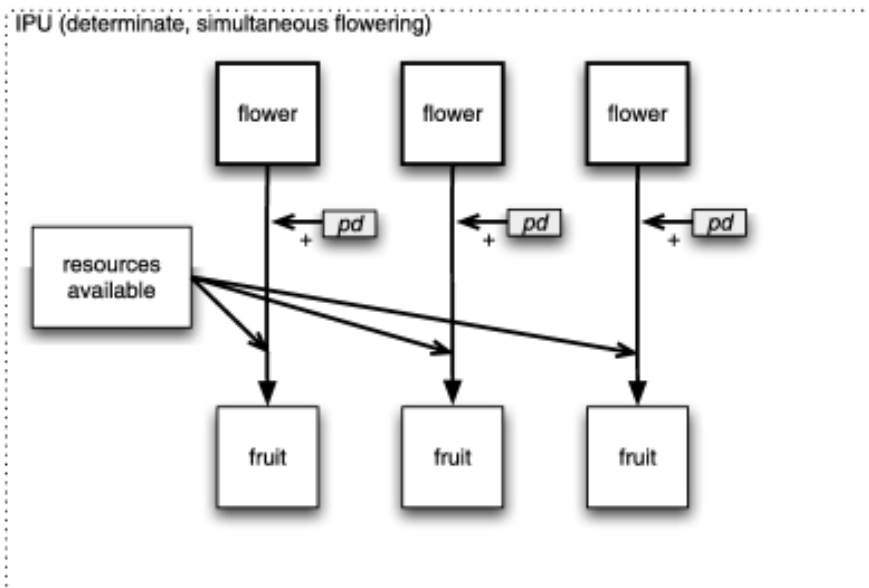


Fig. 3: Sequential and Simultaneous Flowering Diagram

(a) Shows a sequential flowering plant where (pd) represents pollen deposition. The thickness of lines shows the strength of pollen deposition. Diagram (b) represents simultaneous flowering where a set number of resources are predetermined no matter the pollen deposition (pd) (Wesselingh 2007).

Theoretical Framework

Pollen limitation research is often carried out using pollen supplementation studies based on multiple theories foundational to evolutionary biology. The optimality theory is based on Darwin's theory of natural selection and is used in evolutionary biology modeling to determine fitness. Natural selection presumes that organisms are in constant competition to evolve adaptive traits or behaviors to maximize fitness. This maximization of fitness translates to the assumption that in an ideal environment, organisms will reproduce and function at an optimum rate (Parker and Smith 1990, Knight et al. 2005). If this is not the case, external pressures such as competition or environmental changes may be to blame. For pollen limitation studies, the optimality theory assumes that a plant existing in an optimal abiotic and biotic environment will not be any more reproductively successful when more pollen is introduced. If the plant has higher reproductive success when pollen is introduced this would indicate that an ecological disturbance is taking place which is limiting pollen (Knight et al. 2005).

Rosenheim et al. (2010) outlines Liebig's law of the minimum which states that plant growth is limited by a single nutrient or external factor. When Liebig's law is applied in ecological experiments, typically nutrients are added and then plant fitness is quantified to determine which is the limiting growth factor. This law also applies to pollen limitation experiments. If more pollen is added through hand pollination and this positively impacts plant reproduction, then we can assume pollen availability is a key limiting factor. Depending on the plant and environment there may be other simultaneous limiting factors negatively impacting plant reproduction.

In Haig and Westoby's (1988) seminal paper, they created a graphical model describing plant resource allocation and reproductive success. The authors reasoned that ovule fertilization

would increase when increased resources were allocated to pollinator attraction; this is known as the fitness gain curve. The ability of ovules to mature and form seeds would decrease because of the resources diverted to pollinator attraction. On the graphical model this is known as the resource cost curve (Fig. 4). Haig and Westoby (1988) refer to the intersection of the fitness gain curve and the resource costs curve as equilibrium. They argued that plants would evolve traits to maximize attractiveness to pollinators while maintaining enough resources for adequate seed production. If a plant population were to reach equilibrium, then supplementation of pollen would have no effect on seed set.

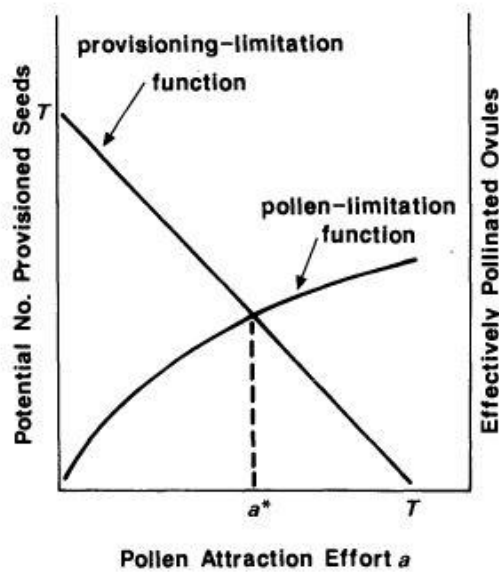


Fig. 4: Haig and Westoby Equilibrium Model. The pollen-limitation function increases as resources are spent in pollen attraction effort. The provisioning-limitation function decreases as resources are allocated to pollen attraction. This reduces effectively pollinated ovules and seed set. The intersection of the provisioning-limitation function and the pollen-limitation function (a^*) represents the equilibrium (Haig and Westoby 1988).

Burd (2008) revisited the Haig and Westoby equilibrium model and revised it by adding stochastic variation. Stochastic variation includes variability in pollen supply, resource allocation and environmental factors from year to year. The first model (*Fig. 5*) represents a plant species response to stochastic resource allocation. The line labeled 'R' represents mean resource constraint, while the dotted lines on either side represent high and low variations of resource allocation. 'F' represents number of ovules fertilized as resources are allocated to pollinator attractiveness. There are also two dotted lines on either side of F to represent the upper and lower stochastic variations. This model shows an equilibrium (a^*) at the same point as Haig and Westoby's model, where lines F and R intersect. The dashed line ' S_{t+R} ' represents average seed set, which would be impacted by resource variability.

The second model created by Burd (2008) (*Fig. 6*) represents a plant species existing in two habitats, where one is richer in resources than the other. ' R_1 ' represents a level of resource allocation while R_0 represents a low level of resource allocation. ' S_0 ' represents the range of seed fertilization in a low-quality environment while S_1 represents the range of seed fertilization in a high-quality environment. The low-quality environment with limited resource allocation has a lower mean seed set (a_0) than the high-quality environment with increased resource allocation (a_1). This model differs from the Haig and Westoby equilibrium model, showing how stochastic variations in environmental resources cause variations in seed set. Burd's second model can be applied in pollen limitation studies to better understand how environmental conditions can impact resource allocation in plants. In poor quality habitats with habitat fragmentation or low species richness, the plant may be operating at a lower than optimal equilibrium.

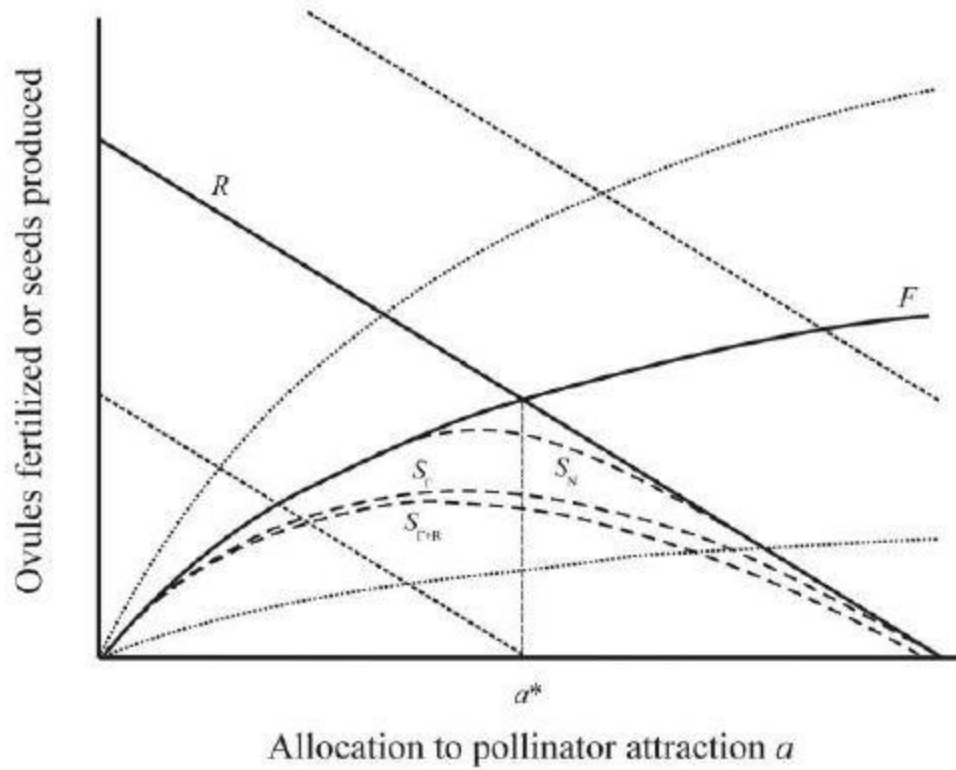


Fig.5: Stochastic Resource Allocation

The Burd (2008) model shows a plant species that has stochastic resource allocation, where F represents mean ovule production and the dotted lines on either side represent low and high variations in response to resource allocation that is driven by resource constraint (R).

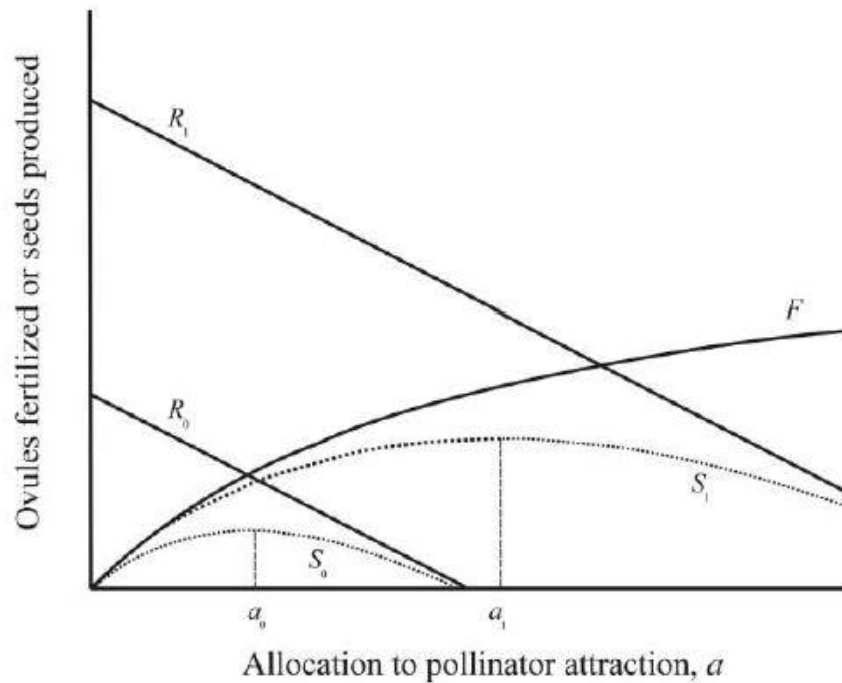


Fig.6: Stochastic Resource Allocation in Low and High Quality Environments

Burd's (2008) second revised model represents a single plant species' ovule production response (F) to its allocation to pollinator attraction, driven by a stochastic environment, where S_0 represents a low quality environment and S_1 represents a high quality environment.

Recent theoretical framework analysis and meta-analysis has suggested that pollen limitation may not only be a consequence of ecological disturbance but also a response to stochastic variation in pollen delivery, plant life history and ovule fertilization (Ashman 2004, Knight 2006, Burd 2008). Pollen limitation may vary among plant species even if co-flowering in the same vicinity. This could be due to differing life histories and reproductive strategies such as rates of self-compatibility or specialist pollinator associations. Plants with higher rates of self-compatibility tend to be less impacted by pollen limitation (Knight 2006).

The prevalence of pollen limitation detected in studies suggests that plants may be adapted to stochastic environments that experience varied amounts of pollen distribution (Burd 1994). Knight et al. (2006) suggests that the phenomenon of frequent pollen limitation may be

due to a hedge-betting strategy of over-producing flowers in case an influx of pollen distribution occurs. Such an adaptation would allow a plant to take advantage of pollen fluctuations to create a higher seed set. This hedge-betting strategy theory conflicts with Haig and Westoby's model which is predicated on the concept that resource allocation has costs for plants, hence plants would tend to not over-produce flowers. Knight et al.'s (2006) meta-analysis reveals that a high percentage of pollen limitation studies have found a high proportion of flowers to set seed, indicating an overproduction of flowers. If plants are existing at equilibrium then we would expect that flower to seed set ratios would be equal.

Cohen and Dukas' (1990) findings point to both resource allocation and hedge-betting strategies. Cohen and Dukas (1990) conclude that over production of hermaphroditic flowers in relationship to seed set, is due to increased variance in pollination and resource allocation. Hermaphroditic floral structures require less resources for production than a complete female flower (Wesselingh 2007). Over production of flowers is also an adaptation to stochastic pollination, allowing plants to take advantage of influxes of conspecific pollen (Ehrlen 1991, Burd 1993).

An opposing theory to pollen limitation is genetic load theory. This theory suggests that self-incompatible plants dependent on outcrossing pollination have higher genetic loads than self-compatible plants. This theory presumes that low levels of sexual reproduction may be due to genetic load rather than a lack of availability of pollen (Charlesworth et al. 1990). If genetic load is the underlying cause of reduced plant reproduction, then pollen supplementation through hand pollination would produce negligible impacts compared to naturally pollinated plants (Burd 1994). To entirely resolve the issue of genetic load being an underlying cause, selfing rates of plants would need to be determined.

Pollen Supplementation Studies

In recent years there has been considerable reexamination of pollen limitation studies methodologies. This has been due in part to reviews of pollen supplementation experiments revealing that the majority of research resulted in pollen limitation (Burd 1994, Ashman et al. 2004, Knight et al. 2005). This can be interpreted as most plants studied are experiencing pollen limitation or there are faults in the experimental design. Seed set is often counted as the only measurement of pollen limitation. This leads to the appearance of pollen limitation without taking into consideration pollen deposition, phenology, and plant life history. Due to the ongoing debate surrounding quantifying pollen limitation, many studies have cited the need for more measurements that quantify pollen limitation such as pollen quantity and quality (Burd 1994, Ashman et al. 2004, Knight et al. 2006, Aizen and Harder 2007).

A common proxy measurement for pollen quantity is seed set, due to the direct effects of pollen deposition and seed production. Pollen quality can impact post-pollination process of ovule fertilization and viable seed development. Low quality pollen can result in unsuccessful pollen tube growth, unfertilized or aborted seeds (Ashman et al. 2004, Aizen and Harder 2007). Poor pollen quality can occur for self-incompatible plants when self-pollen is deposited on the stigma and for self-compatible plants experiencing an inbreeding depression that receive self-pollen (Aizen and Harder 2007). Pollen quality can be accounted for by measuring pollen tube growth, seed viability rates, seed weight, counting aborted seeds or determining pollen deposition ratios on the stigma (Ashman et al. 2004). Seed weight has been found to correspond to seedling survivability and plant establishment (Galen and Stanton 1991, Aizen and Harder 2007). Accounting for pollen quality in pollen limitation experiments can provide a more accurate picture of pollen distribution and plant reproduction.

A meta-analysis of pollen limitation studies done by Knight et al. (2006), found several ways in which to improve pollen limitation studies moving forward. Their research found that previous hand pollination experiments did not measure amount of pollen being applied and applied pollen as a singular treatment. Over pollinating flowers could reduce the production of more flowers in the future due to resource allocation amongst flowers. A single pollen supplementation treatment does not capture flowers produced later in the season. Unequal resource allocation could also take place if fractions of plants are supplemented with pollen (Zimmerman and Pyke 1988). More research still needs to be done on best practices when conducting pollen supplementation experiments but treating all flowers multiple times throughout the phenology period can account for variables such as resource allocation.

Determining an appropriate morphological unit of measurement for plants sampled in pollen limitation studies is an important factor to consider when designing pollen supplementation experiments. Wesselingh (2007) suggests using an integrated physiological unit (IPU) which is used to group morphological arrays into functional units or subunits. An IPU can be an individual flower, inflorescence, or an entire plant (*Fig. 7*). Plant life history is an important factor when considering an appropriate IPU. For instance, perennial plant species are often able to re-allocate unused resources to be used in preceding seasons, while annual plants cannot (Diggle 1995, Casper and Neissenbaum 1993, Wesselingh 2007). In some cases it has been shown that inflorescences comprised of many florets act as a single morphological unit, for instance the ray and disc florets that compose an Asteraceae inflorescence. If singular florets are removed there is not a significant difference in pollen or seed production (Herrera 1991).

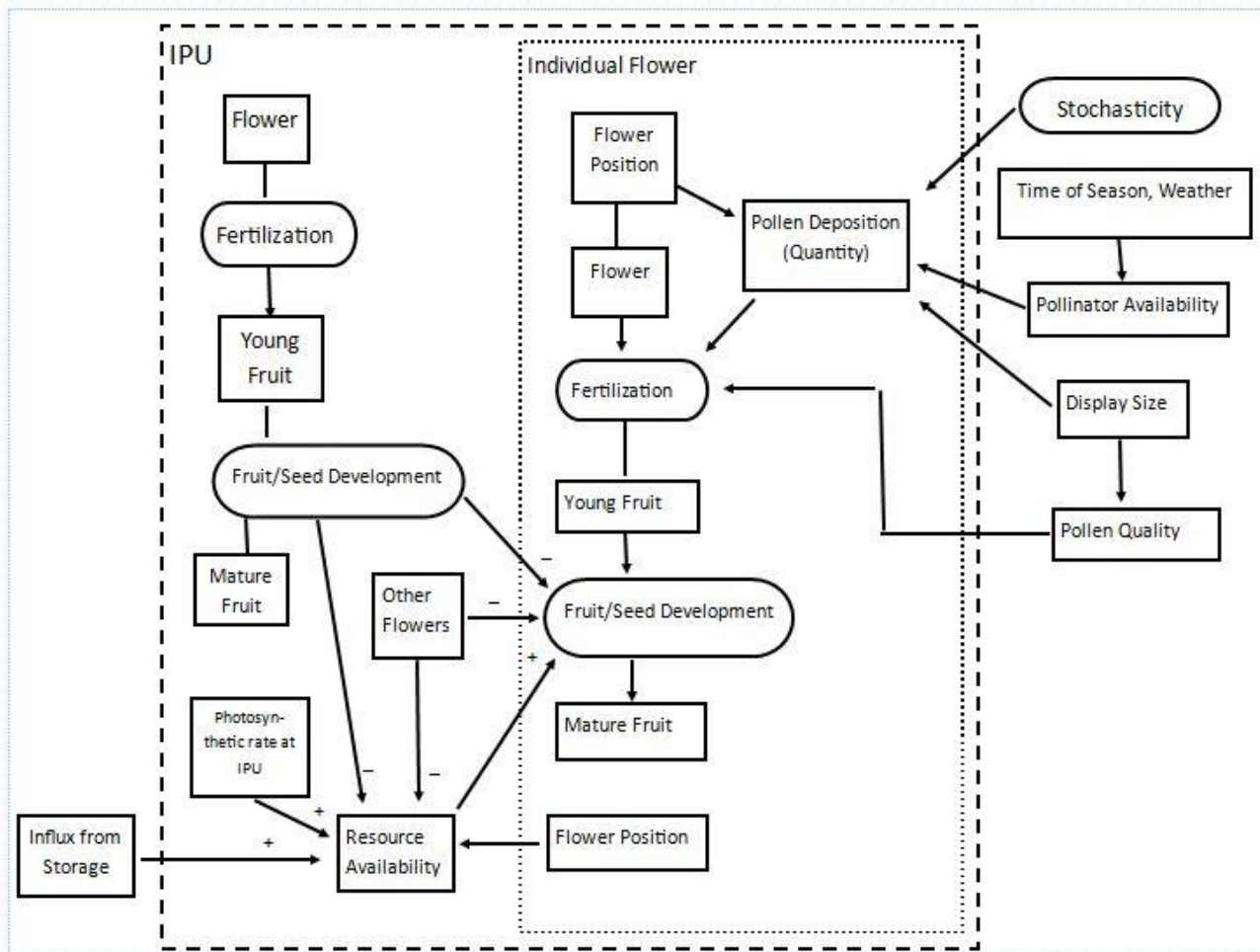


Fig.7: IPU and Resource Allocation

This diagram shows integrated physiological unit (IPU) of an entire plant (dashed line). Dotted lines indicate individual flower. Arrows denote allocation of resources (Wesselingh 2007).

Habitat Fragmentation

Anthropogenic environmental disturbances through changes in land use have caused global biodiversity declines. Habitat loss and fragmentation are driving forces in changing pollinator networks and plant communities (Hagen et al. 2012, Newman et al. 2013, Cariveau et al. 2020). This occurs through large expanses of habitat with intricate plant – animal interactions being transformed into several small, isolated patches. This spatial separation disrupts pollinator networks, alters plant community composition, and contributes to pollen limitation and

ultimately reduces plant reproduction. (Knight et al. 2005, Aguilar et al. 2006, Hagen et al. 2012, Kaiser-Bunbury 2017, Bennett et al. 2018). These cascading effects of alterations in pollinator – plant relationships are important to understand when attempting to restore ecosystem functions and conserve sensitive plant species.

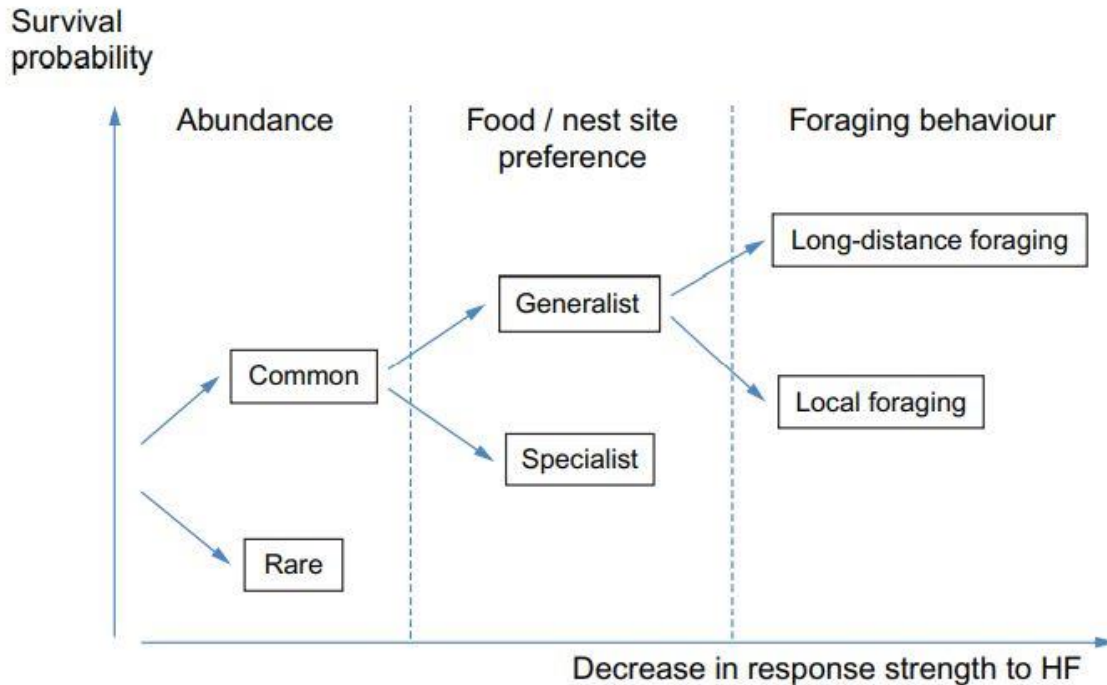


Fig.8: Pollinator Trait Responses to Habitat Fragmentation
 A framework displaying differing pollinator traits and responses to habitat fragmentation (HF) (Hagen et al. 2012).

Research has shown that varying plant life histories and reproductive strategies react differently to fragmented habitats (Aguilar et al. 2006, Hagen et al. 2012, Bennett et al. 2018). Plants with generalist associated pollination syndromes have shown a greater capability to cope with fragmented habitats on a localized scale (*Fig.8*) (Newman et al., 2013). Alternatively, Aguilar et al. (2006) found that generalist and specialist pollinator associated plants both responded negatively to fragmented habitats with reduced seed set. In addition, self-incompatible plant reproduction were negatively impacted by fragmentation, while self-compatible plants were not. This outcome illustrates the adaptive advantages of self-compatibility and the

vulnerability to ecological changes self-incompatible plants.

For rare plant species habitat fragmentation can be especially dangerous, driving population declines. Ashman et al. (2004) suggests the Allee effect plays a role in pollen limitation and reduced reproduction. The Allee effect is a biological concept that refers to the positive association between population size and fitness. In pollination ecology low density floral patches attract fewer pollinators and thus suffer from pollen limitation. The Allee effect can exacerbate this situation by negatively impacting seed quantity or quality. In self-compatible plant communities, selfing rates increase as patch density declines (Fernández et al. 2012). This can lead to an inbreeding depression due to lack of genetic out crossing.

Overall plant population size can also cause Allee effects regardless of floral patch density. If plant species are self-incompatible then the reduction in available compatible mates will produce pollen limitation symptoms and lower seed set (Ashman et al. 2004). A reduced species population could also suffer from increases in heterospecific pollen transfer, which can block receptive areas on the stigma for conspecific pollen (Arceo-Gomez et al. 2016). These scenarios would happen regardless of pollinator visitations or reduced patch density due to lack of outcrossing.

Declines in plant populations can also lead to pollen limitation and potential Allee effects (Agren 1996). Small population size can lead to reduction in self-compatible alleles leading to inbreeding (Hagen et al. 2012). This can lead to the Allee effect and threaten small populations with reproductive incompatibility and inability to self-pollinate (Knight 2004). The Allee effect is known to impact small populations more severely and can lead to risk of extinction. Better understanding minimum population sizes for plant species can aide in reintroduction efforts of threatened or endangered plant species.

Changes in floral patch densities is another form of habitat fragmentation that can lead to pollen limitation. Reductions in floral patch densities has been associated with increases in pollen limitation (Knight 2004). Newman et al. (2013) found that fragmentation on a localized spatial scale drastically impacted plant reproduction. Bare percent ground cover of over 40% was found to be associated with total reproductive failure of plants sampled. These findings demonstrate that fragmentation on a local level can drastically impact pollen distribution and subsequently depress reproduction.

Invasive Plant Species

Introduced invasive plant species are one of the largest ecological threats to native plant species causing habitat fragmentation and disruptions of pollinator networks resulting in pollen limitation and reduced reproductive capacity (Flory and Claye 2009, Morales and Traveset 2009, Orrock and Witter 2010). Invasive plant species often have adaptive characteristics that allow them to alter their environment and transform conditions for co-flowering native plant communities. This can include changing plant community composition, floral patch densities and altering pollinator networks (Lopezaraiza et al. 2007, Orrock and Witter 2010). Some invasive plants can produce large quantities of pollen which can effectively clog native plants stigmas and block conspecific pollen reception (Kanchan and Chandra 1980, Arceo-Gomez and Ashman 2016). These environmental disruptions can ultimately negatively impact native plant pollen distribution and plant reproduction (Flory and Claye 2009, Morales and Traveset 2009, Orrock and Witter 2010).

Invasive plant species often share pollinators with native plant species, causing either facilitation or disruptions in visitations or pollen quality (Orrock and Witter 2010). In some

cases, invasive plant species can act as a pollinator magnet, drawing more pollinator visitations to floral patches (Lopezaraiza et al. 2007, Ramula and Pihlaja 2012, Waters et al. 2014). This pollinator magnet effect can impact native plants differently depending on pollinator preferences. A pollination network analysis by Lopezaraiza et al. (2007) found that invasive plant species *I. glandulifera* attracted an increase of pollinator richness and abundance to the co-flowering patch. Although *I. glandulifera* received the most pollinator visitations and native flowers received increases in heterospecific pollen transfer. This study reveals the pollinator magnet effects of invasive species on native plant communities and the disproportionate rewards reaped by the invading species.

Introduced invasive species have been shown to cause both facilitative and competitive effects on native plant communities (Morales and Traveset 2009, Waters et al. 2014). Research by Waters et al. (2009) showed that two native plants have differing responses to the introduction of an invasive plant species. One native (*M. laciniata*) had reduced seed set in floral patches with the invasive species (*H. radicata*). While the other native (*E. lanatum*) experienced a higher seed set in the presence of the invasive non-native. This research highlights the complex nature of invasive plant species colonization impacts on native plant communities.

Additionally, Morales and Traveset's (2009) research shows that morphological similarity to the invading plant species is related to facilitative impacts on native plants. Their research revealed native plant seed set was reduced in the presence of invasive species. However, native plants that had morphological similarities to the invasive species did not have reduced seed set or pollinator visitation.

Restoration Applications

Common ecological restoration practices used to improve prairie habitat include prescribed burns, invasive species removal, mowing, seeding and out-planting native species. Restoration treatments applied on the landscape scale can increase habitat quality and address habitat connectivity. Treatments focused on the local scale can more directly impact biological communities and pollination functions (Fig.9). These treatments can be tailored to include plant-

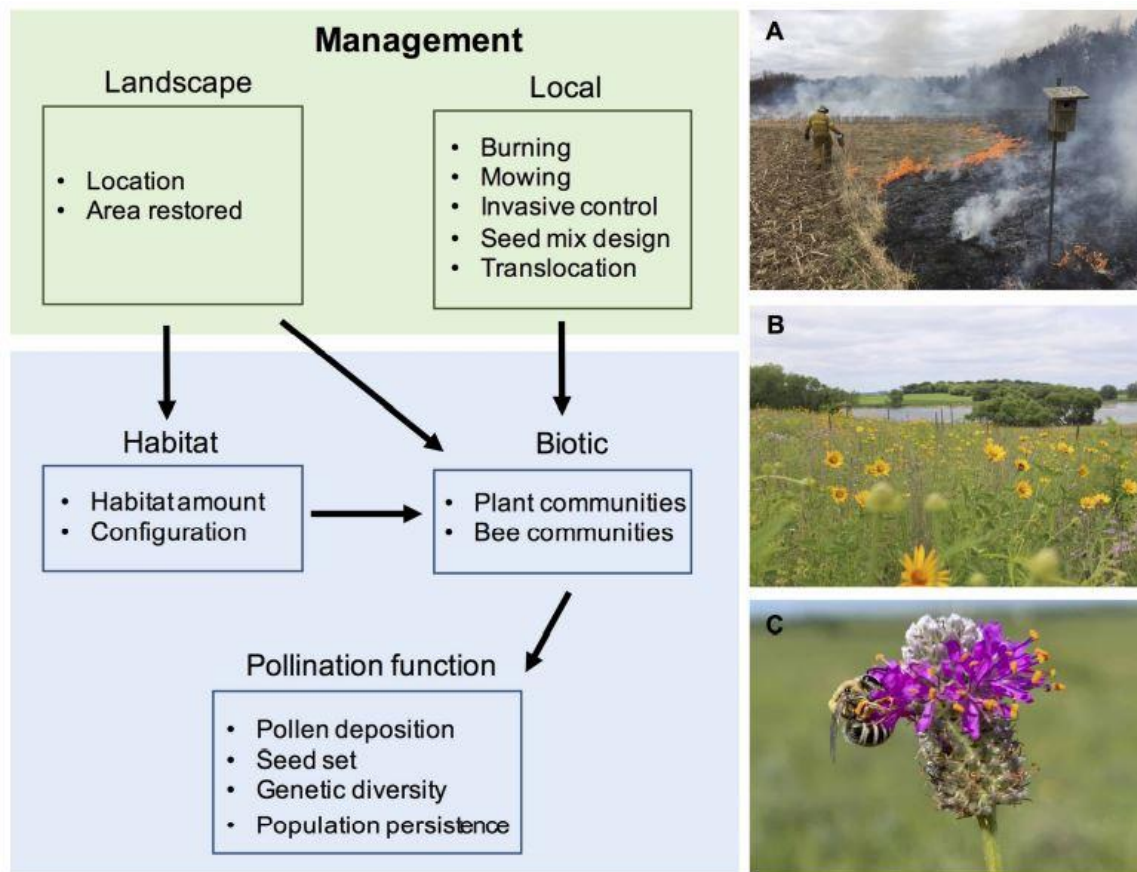


Fig.9: Ecological Restoration Practices

Restoration treatments on the landscape level have impacts on habitat. Restoration treatments on the local level have direct impacts on biotic communities and pollination function (Cariveau 2020).

pollinator relationships such as increasing floral patch densities, altering disturbance regimes, and improving pollinator diversity. Restoration treatments such as invasive species removal have been shown to improve pollinator network diversity, increase pollinator visitation rates and

increase seed set (Flory and Claye 2009, Morales and Traveset 2009, Orrock and Witter 2010, Kaiser-Bunbury et al. 2017).

Restoring pollinator diversity has been associated with improving native plant seed set and establishment. Research done by Albrecht et al. (2012) found a positive association between pollinator diversity and native plant seed set, although this effect began to wane with the highest level of pollinator diversity causing declines in seed set. This research shows that pollinator diversity alone will not uniformly improve native plant seed set. It is likely that plant association with specific pollinators and pollinator functional group abundance are important components to include with diversity (Sabatino et al. 2021). Using increased biodiversity as a restoration metric may not be appropriate for every plant community. Better understanding site specific pollinator network – plant relationships and measuring reproductive success could prove to be a more useful tool in restoring pollination functions.

A promising approach in restoring ecological pollination function, is to improve habitat connectivity (Kaiser-Bunbury 2017, Betts et al. 2019). Focusing on connecting restoration site locations can bridge pollinator communities and support plants with self-incompatible life histories in reproduction (Aguilar et al. 2006). This approach along with site specific pollen limitation, reproductive success, and plant-pollinator relationships, are all important components in restoring pollination functions to degraded ecosystems.

Methods

Study Site

Sampling sites were established from spring to summer of 2020, on six Puget Trough prairie restoration sites. These sites vary in restoration history and land management techniques applied (Table 1). Glacial Heritage Preserve is owned by Thurston County and was managed by the Nature Conservancy and the Center for Natural Lands Management since 1994. Glacial Heritage Preserve is renowned for having some of the highest quality prairie ecosystems in south Puget Sound. In recent years Glacial Heritage was the site of Taylor’s checkerspot butterfly and *Castilleja levisecta* reintroduction (personal correspondance, Sanders Freed).

Table 1: CNLM Restoration Treatment Application for Each Site (Unpublished data, Susan Waters).

Sites	Site acreage	Prescribed Burns	Total burned acreage	Herbicide	Seeding	Total seeding acreage	Total plugs planted
Glacial Heritage (GH)	1020	2001-2020	956.29	2007-2020	2001-2020	384.205	371,151
Wolf Haven (WH)	36	2009-2020	22.21	2009-2019	2009-2020	22.218	93,554
Tenalquot (TQ)	126	2007-2020	64.6	2008-2019	2008-2020	66.094	199,539
Johnson Prairie (JP)	194	2009-2019	316.39	2009-2019	2014-2019	35.58	22,025
Cavness (CV)	655	2009-2019	34.16	2014-2019	2011-2019	48.97	45,448
Deschutes (DS)	140	2015-2019	23	2015-2019	2016-2020	39.32	0

This site was originally dominated by Scotch broom, requiring years of intensive mowing and invasive removal. Prescribed burns have taken place regularly since 2001 on 956 acres of the 1020 total acres (unpublished data, Susan Waters). The Scotch broom population has been successfully reduced through regular herbicide treatments and mechanical and hand removal. Other nonnative forbs targeted for herbicide treatments include *Hypochoeris radicata*, *Leucanthemum vulgare* and *Jacobaea vulgaris*.

Wolf Haven Preserve is in Tenino, Washington and is owned by Wolf Haven International. The 36 acres prairie is managed by CNLM and contains rare plant species such as *Castilleja levisecta*. This restoration site is small in comparison to other sites included in this study but boasts high quality prairie. Regular prescribed burns have occurred at this site since 2009 with 22 acres burned and seeded (Unpublished data, Susan Waters).

Tenalquot Prairie Preserve is a 126 acres site established in 2006 and located near the town of Rainier and JBLM. This property is predominantly grassland bordered by an oak and Douglas fir forest. Prescribed burning has been conducted since 2007 and herbicide treatments and native plant seeding began in 2008 (Unpublished data, Susan Waters). Established populations of *Castilleja levisecta* currently exist at Tenalquot. This was achieved through plug plantings and seedings beginning in 2007. The success of native plant community establishment at this site has made it eligible for introduction of the federally listed Taylor's Checkerspot butterfly (personal correspondence, Sanders Freed).

Johnson Prairie is 194 acres of high-quality prairie located in a designated military training area on Joint-Base Lewis McChord. This restoration site has a history of military related ecological disturbances, which has benefited native prairie communities that are adapted to disturbance regimes. CNLM began prescribed burn and herbicide treatments in 2009 and started native seeding in 2014, however large section of this site are considered very high quality, with diverse and abundant native species (unpublished data, Susan Waters).

Cavness Ranch is managed by CNLM through a conservation easement established in 2005. Cavness Ranch is 613 acres near Tenino, Washington. This site has a long history of varied land use such as logging, agriculture, and cattle ranching. Currently, land within the conservation easement is being used for Christmas tree farming and cattle grazing. This property

also has a diversity of habitats including wetlands, riparian forest, Oregon white oak - Douglas fir forest, mixed forest, and grassland (personal correspondence, Sanders Freed).

Restoration at Cavness Ranch began in 2009 with prescribed burning prescribed burning. Native seeding and plug planting followed starting in 2011, then herbicide treatments started in 2014 (unpublished data, Susan Waters). This site is heavily fragmented due to varying ecosystems and historical land use activities throughout the property.

The 140 acres Deschutes River Preserve was acquired by CNLM in 2014. This property was previously used as an equestrian center and cattle ranch. Of the sites sampled in this study Deschutes River Preserve has had the least amount of restoration treatments. The majority of this property (100 acres) is degraded prairie grasslands with little native plant cover. The remaining portion of this property is dominated by Oregon white oak and Douglas fir forests. A high priority restoration goal for this site is to improve habitat for the federally listed Mazama pocket gophers, which has been observed at this site (personal correspondence, Sanders Freed).

Table 2: Plant life history (Andersson 2008, Adderly et al. 2015).

Plant Species	Life History	Floral Morphology	Endemic Status	Pollination Syndrome	Phenology	Self-Compatibility
<i>Plectritis congesta</i>	Annual	Many small pink flowers forming a terminal cluster	Native	Ground bee preference	April-June	Self-compatible (70% outcrossing)
<i>Lupinus lepidus</i>	Perennial	Racemes of blue pea flowers	Native	Bombus sp.	May- August	Self-incompatible
<i>Eriophyllum lanatum</i>	Perennial	Yellow inflorescence composed of many ray and disc florets	Native	Generalist	May-August	Self-compatible
<i>Hypochaeris radicata</i>	Perennial	Yellow inflorescence composed of many ray and disc florets	Non-native	Generalist	May-October	Self-incompatible
<i>Leucanthemum vulgare</i>	Perennial	Inflorescence composed of white ray florets and yellow disc florets	Non-native	Generalist	May-October	Self-incompatible

Restoration treatments such as prescribed burns and herbicide treatments began in 2015 followed by native seeding in 2016 (unpublished data, Susan Waters). Deschutes River Preserve has the shortest restoration history of all sites sampled in this study and has the highest proportion of degraded low-quality prairie.

Study Species

Five common prairie plant species were chosen to sample: *Eriophyllum lanatum*, *Plectritis congesta*, *Lupinus lepidus*, *Leucanthemum vulgare* and *Hypochaeris radicata*. (Fig.10). The plants chosen in this study have varying floral morphologies, life histories and pollination syndromes (Table 2). Of these five plant species three are native species: *Eriophyllum lanatum*, *Plectritis congesta* and *Lupinus lepidus*. Two plants are nonnative: *Leucanthemum vulgare* and *Hypochaeris radicata*. Among the plants chosen, four are perennial (*Eriophyllum lanatum*, *Lupinus lepidus*, *Hypochaeris radicata* and *Leucanthemum vulgare*) and one is an

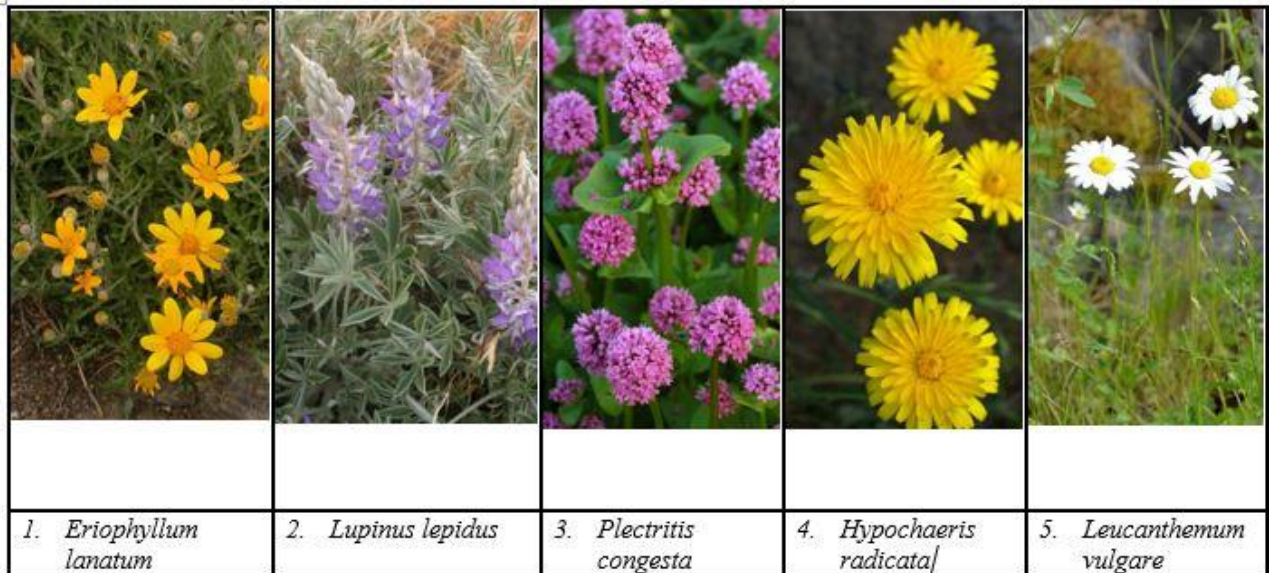


Fig. 1049: Plant Species chosen for this study.

Photo credits: 1,2,3: Legler, Ben (2004), 4: Houck, Douglas (2012), 5: Skotland, Bruce (2004) Burke Herbarium Image Collection.

annual (*Plectritis congesta*). These plant species cannot be compared amongst each other due to varying life histories, pollinator syndromes and floral morphologies.

Experimental Design

At each site selected plant species were located, although not all plant species occurred at each site. Plants were randomly selected at each site then inflorescence were randomly selected on each plant for sampling. Inflorescence were then subjected to one of two treatments: open pollination or hand pollination. These paired treatments were conducted ten times for each species at each site. Selected hand pollinated plants had their stigmas dusted with a paintbrush containing conspecific pollen from that site. Sampled flowers were hand pollinated once then were snooded with fine mesh nylon bags, as to not allow for pollinator access. Flowers selected for open pollination were not hand pollinated nor snooded. This treatment group was allowed access to pollinators.

Data Collection

Physical characteristics of each sampled plant were recorded such as plant height, flowering stalk, and number of flowers. These characteristics did not apply evenly to each plant species due to varying floral morphologies. Seeds were cleaned and counted by hand or using a dissecting microscope. Due to the quantity of seed produced by *L. vulgare*, a count estimation technique was used. Fertile seeds were visibly distinguished from infertile or immature seeds. Fertile and infertile seeds were both counted, providing a total seed count. Fertile seeds were then collected and weighed using an analytical scale, to determine individual fertile seed weight.

Two plant species were chosen for Tetrazolium staining process to determine seed viability *P. congesta* and *H. radicata*. These plants were chosen to examine native and non-native and annual and perennial differences. Seeds were stained using *Tetrazolium: An important test for physiological seed quality evaluation* (Franca-Neto et al. 2019). A stain was created using 1% 2,3,5-triphenyltetrazolium chloride in a solution of deionized water. pH was adjusted to a 6.8-7.4 range. All fertilized seed samples were soaked in the staining solution for 48 hours at room temperature. Seeds were then dissected to determine embryo staining. Red staining indicated viability while no staining or a white embryo indicates non-viability.

To determine pollen limitation seed quantity and quality were compared to open pollination and hand pollination treatments. Seed quantity was measured calculating proportion of fertilized seeds out of total seed set. Seed quality was determined by individual seed weight for each sample. Proportion of viable seeds out of total seed set was used as an additional seed quality measurement for *P. congesta* and *H. radicata*.

Statistical Analysis

Individual fertilized seed weight was calculated by dividing total fertilized seed set weight by total seed set. For *L. vulgare*, fertilized, immature or aborted seeds were all included in seed set count. Therefore, seed weight is a measurement of total individual seed weight for *L. vulgare*.

Fertilized seed set was divided by total seed set to get a percentage of fertilized seed. For *L. vulgare*, fertilized seed was indistinguishable from non-fertile seeds, so total seed count was measured. Number of viable seeds was divided by total seeds to get the percentage of viable seeds. Where needed the percentage values were arcsine square root transformed to better fit a normal distribution.

Distribution normality for all other response variables were tested by graphing them out in a histogram then running a Shapiro-Wilkes test. For non-normally distributed data, Akaike Information Criterion (AIC) tables were used to compare negative binomial regression, gamma, and Poisson distributions to select the best fit. Gamma distributions were used for nonparametric continuous data and negative binomial regression, or Poisson were used for count or proportional data.

Two factor mixed effect models were selected for each dependent variable. Covariates such as height and number of flowering stalks were included in model selection as to account for plant robustness. Pair number was included in the models as a random effect. Several nested models were compared using the (AIC). Eight models were used in the selection process: (1) site and treatment interactions, pair number as a random effect and number of flower stalks and height (2) site and treatment interactions and flower stalks (3) site and treatment interactions and height (4) site and treatment interactions (5) site and treatment (6) site (7) treatment (8) a null model.

For all normally distributed parametric data a general linear model (GLM) was used. A one-way analysis of variance (ANOVA) test was run on models selected with three or less independent factors that had a normal distribution. An alpha of 0.05 was set for all tests. If ANOVA tests had significant variables, then a Tukey post hoc test was run to compare each variable against each other for significance.

For non-normally distributed continuous variables a gamma distributed general linear mixed model (GLMM) was fit an estimated marginal means post hoc test was run to evaluate pairwise comparisons. A pairwise p-value matrix was created to compare restoration sites amongst each other. Non-normally distributed data that did not fit general linear models or

general linear mix model distributions, were tested using the Kruskal Wallace test and then a Wilcoxon rank sum test was used to make pairwise comparisons.

Results

Treatment Effect

Pollen supplementation treatments and open pollination treatments were not significant factors in any mixed models for any of the plant species in this study. This results means that neither open or hand pollination treatment had any significant impact on seed quality or quantity among restoration sites.

Seed Quality – Individual Seed Weight

Individual seed weight was found to significantly vary among sites for *L. vulgare*, *P. congesta* and *H. radicata*. *L. vulgare* total individual seed weight varied significantly by restoration site (Appendix A Table 1). Seed weight was significantly higher at Johnson ($p=0.046$) and Wolf Haven ($p=0.014$) than at Tenalquot (*Fig.11*). *P. congesta* fertilized seed weight had the highest mean at Cavness when compared to all other restoration sites (*Fig.12*) (Appendix A Table 2). Site was also significant for *H. radicata* individual fertilized seed weight (Appendix A Table 1). A pairwise comparison for *H. radicata* showed that Glacial Heritage fertilized seed weight was significantly lower than Deschutes ($p=0.048$) and Tenalquot ($p<0.001$) (*Fig.13*). Seed weight did not significantly vary among restoration sites for *E. lanatum* (Appendix A Table 3) or *L. lepidus*.

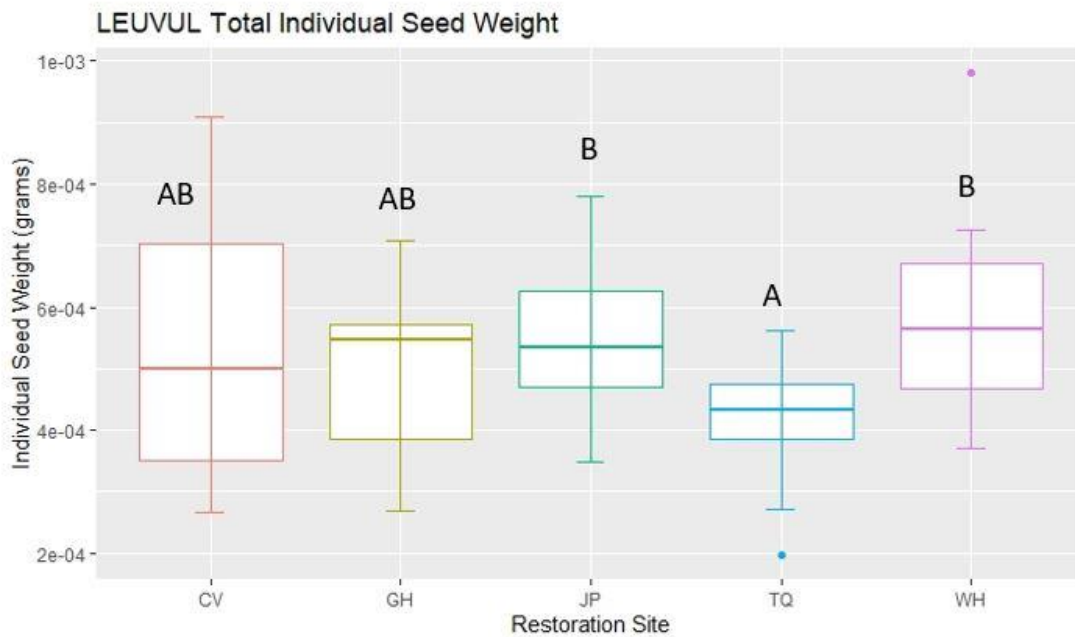


Fig. 11: *L. vulgare* Total Individual Seed Weight
 Significant differences in means are depicted by letters. Error bars denote one standard deviation from the mean.

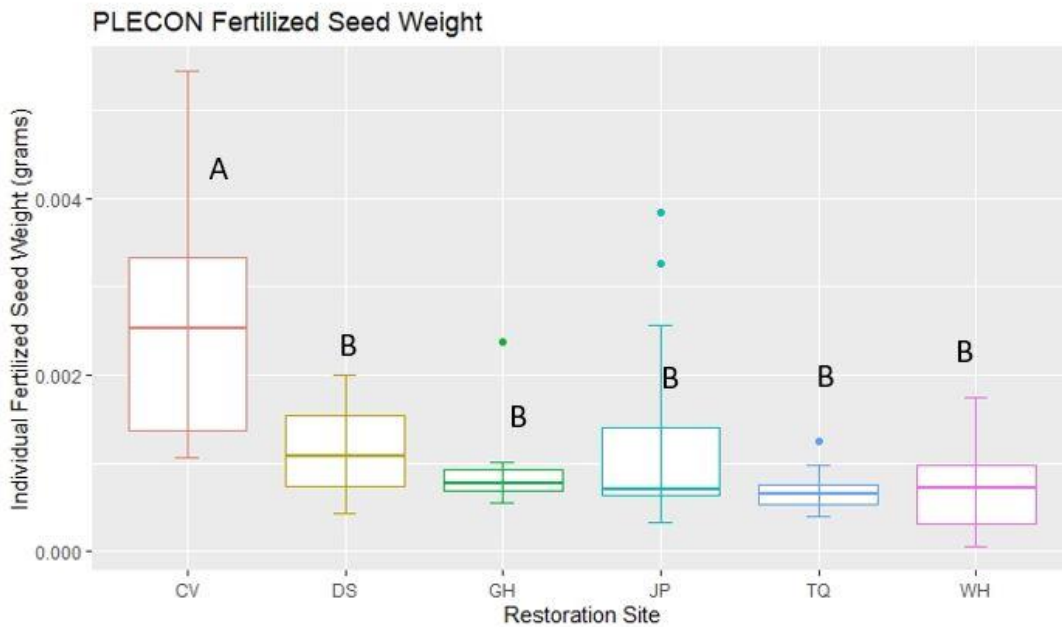


Fig. 12: *P. congesta* Fertilized Seed Weight
P. congesta individual fertilized seed weight by restoration site. Error bars depict one standard deviation from the mean. Significant differences in means are depicted using differing letters.

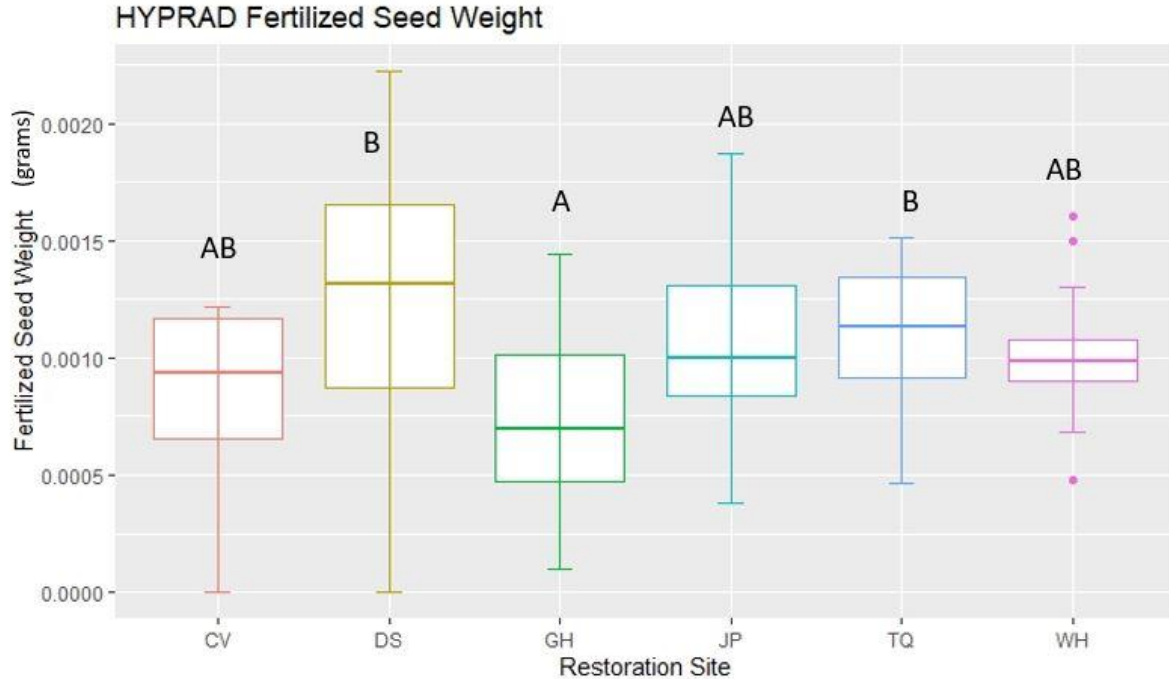


Fig. 13: *H. radicata* Fertilized Seed Weight

Individual fertilized seed weight for *H. radicata*. Significant differences in means denoted by differing letters. Error bars represent one standard error from the mean.

Seed Quality – Seed Viability

Proportion of fertilized seed viability varied among sites significantly for *H. radicata*

(Appendix A Table 4), but no significant site variations were found for *P. congesta* (Appendix A Table 5). Sites Cavness, Wolf Haven and Deschutes had significantly lower mean seed viability than Glacial Heritage, Tenalquot and Johnson Prairie (Fig.14).

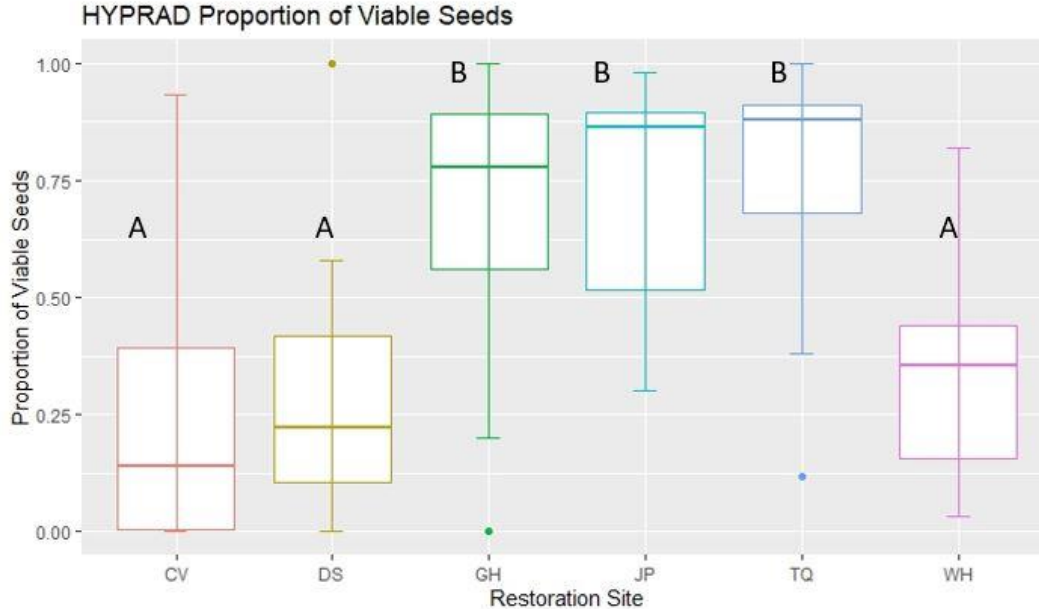


Fig.14: *H. radicata* Proportion of Viable Seeds
 Proportion of viable seeds for *H. radicata*. Significantly different means are represented by differing letters. Error bars represent one standard deviation from the mean.

Quantity – Proportion of Fertilized Seeds

The proportion of fertilized seed counts for *L. Lepidus* varied significantly by restoration site (Appendix A Table 6). A pairwise comparison of each site showed that Glacial Heritage had the highest mean proportion of fertilized seeds compared to Cavness ($p < 0.001$) Tenalquot ($p < 0.001$) and Wolf Haven ($p < 0.001$) (Fig.15). *H. radicata* also had restoration site significance for the proportion of fertilized seed counts (Appendix A Table 7). A pairwise comparison of each site revealed that Deschutes has significantly lower proportion of fertilized seeds when compared to Johnson Prairie ($p = 0.037$), Tenalquot ($p = 0.007$), and Cavness ($p = 0.02$). Tenalquot has a significantly higher proportion of fertilized seeds than Deschutes ($p = 0.007$), and Wolf Haven ($p = 0.02$) (Fig.16). Proportion of fertilized seeds did not significantly vary among sites for

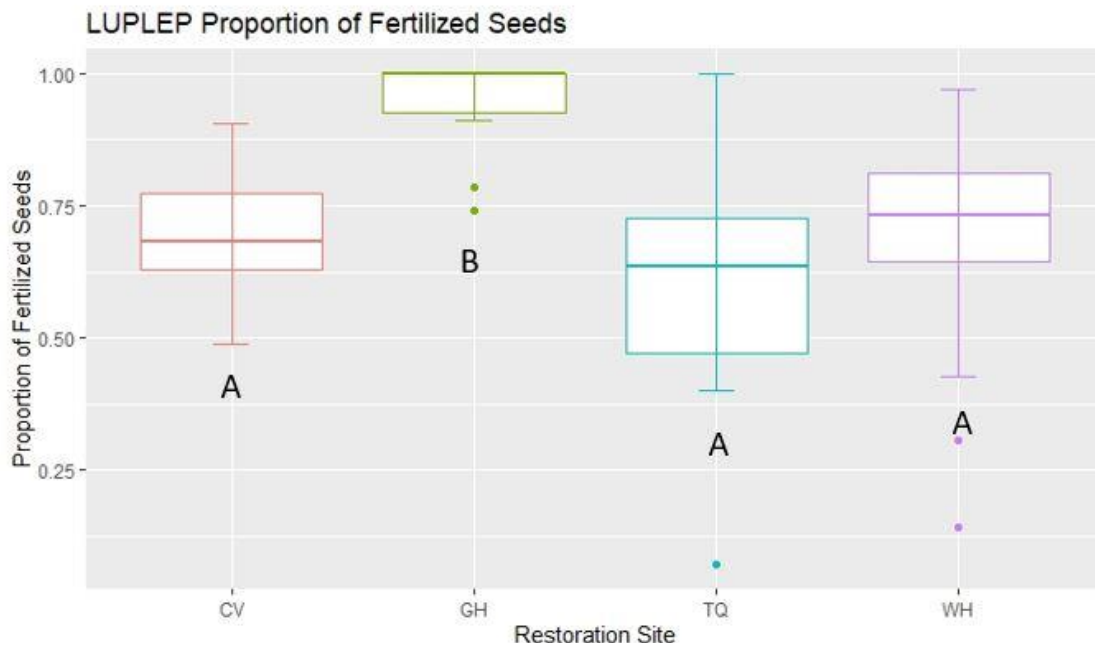


Fig.15: *L. lepidus* Proportion of Fertilized Seeds

L. lepidus proportion of fertilized seeds by site. Means were calculated using the Wilcoxon sum rank test (Appendix A Table 6). Significant differences in means are represented by differing letters. Error bars represent one standard error from the mean.

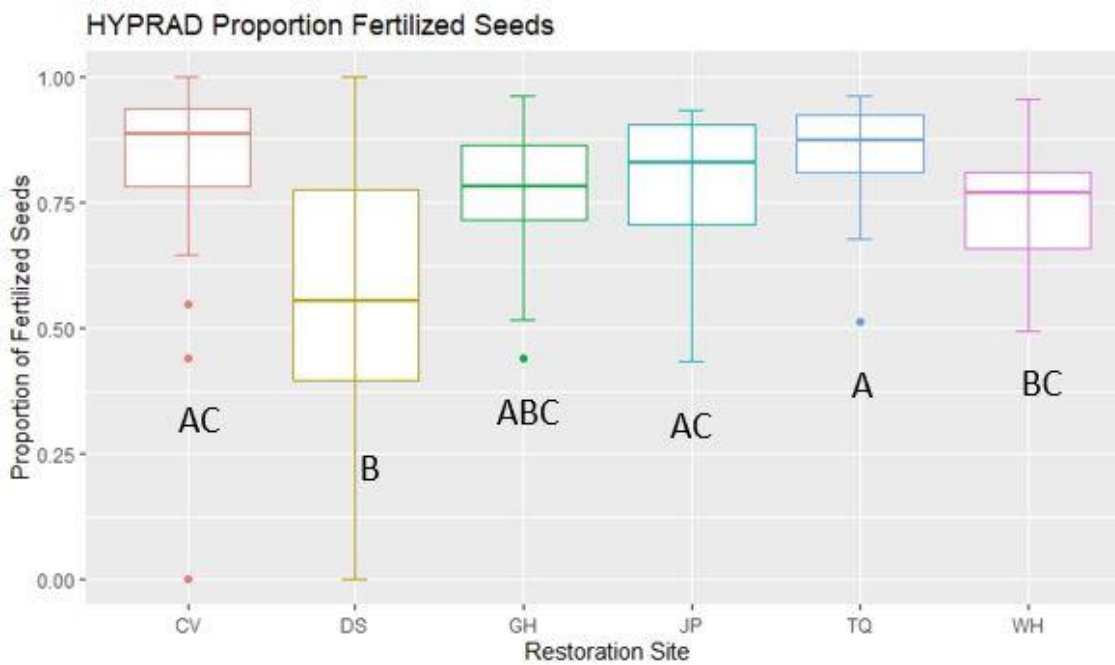


Fig.16: *H. radicata* Proportion of Fertilized Seeds

H. radicata proportion of fertilized seeds by restoration site. Differing letters signify significant differences in means. Error bars represent one standard deviation from the mean.

L. vulgare (Appendix A Table 8), *P. congesta* (Appendix A Table 9), or *E. lanatum* (Appendix A Table 10).

Discussion

All plants sampled displayed varying responses in reproductive success across all restoration sites. Pollen limitation was not found to be occurring for any plant species. Variation in reproductive success is most likely due to the different life histories represented by the five plants chosen. These results highlight the ecological differences among restoration sites resulting from fragmentation and land use history.

Treatment Effect

In this study, there was no significant difference between pollen supplementation treatments and open pollination treatments. For some mixed models, treatment was included but for all measurements of seed quality and quantity treatment was not a significant factor. This could be an indicator of the lack of pollen limitation among plant species and restoration sites. If this is the case then this would indicate that the plants sampled have adequate pollen distribution and in tact pollinator networks.

The overall lack of treatment significance could also indicate a field sampling error when conducting pollen supplementation treatments. Hand pollination treatments were conducted on subsets of an entire plant including a flower head or inflorescence. Number of flowering stalks per plant sampled were accounted for and in some cases multiple flowering stalks from the same plant were treated.

Selecting an appropriate integrated physiological unit (IPU) of measurement is an important component of creating an accurate pollen limitation experiment. An IPU is described by Wesselingh (2007) as a unit of measurement used to group morphological arrays or functional subunits. In pollination studies this often means sampling subunits such as individual flowers or treating an entire plant as one unit. This is done to account for variability of resource allocation

depending across plant life histories and reproductive strategies. Perennial plant species can store unused resources in corms, rhizomes, and bulbs, for future use (Vico et al. 2016). Annual plants are unable to store unused resources, which allows them to put extra energy into seed production. These resource allocation trade offs vary depending on plant life history.

The complex nature of assigning an appropriate IPU for pollen limitation studies has been researched by many pollination ecologists. In analysis of pollen limitation methodologies researchers suggest categorizing the entire plant as an IPU and conducting multiple hand pollination treatments throughout the plant's life cycle (Zimmerman and Pyke 1988, Ashman 2004). Multiple hand pollination treatments could account for varying flowering times and variations in seasonality. While measuring the entire plant as an IPU may be appropriate for some plants this would depend on plant life history and taxonomy.

In future pollen limitation experiments selecting an appropriate IPU for each plant species should be carefully considered. This decision should be based on plant life history, plant morphology and phenology. The addition of multiple hand pollination treatments throughout the flowering season to plants such as Lupine that bloom sequentially could improve the accuracy of pollen limitation detection.

Plectritis congesta

P. congesta was found to have higher seed quality at the low-quality prairie restoration site Cavness. This outcome did not fit the original hypothesis predicting native plants would have higher seed quality and quantity at high quality prairie sites. A study done by Ramula and Pihlaja (2012) found that meadow plant species experienced declines in species richness after non-native invasions. Although, invaded native meadow species also showed an increase in

reproductive output. This could be due to increased competition for resources that a non-native invasion creates or the invasive species altering pollinator networks. Invasive species can change pollinator networks and act as a magnet species by attracting pollinators to floral patches which can temporarily positively impact native plant reproduction.

Research conducted by Trowbridge et al. (2016) found that *P. congesta* had increased in reproductive success at sites where it was seeded after one burn treatment. This finding was temporary, lasting only eight growing seasons before drastic declines in establishment were seen. This contrasts with native perennials used in this study (*E. lanatum*, *A. millefolium* and *R. occidentalis*) that had long-term persistence and increased abundance associated with a single burn and seeding treatment. Differing plant life histories may explain these differing responses to restoration treatments.

This aligns with Dunwiddie et al.'s (2014) research regarding the loss of native annuals throughout Northwest prairie systems. Annuals such as *P. congesta* could require repeated burn treatments to stimulate reproductive outputs. At Cavness, where the increase in *P. congesta* seed quality was observed, regular burning and seeding is part of the restoration plan (personal communication, Sanders Freed). This could explain the increase in seed quality seen at this low-quality site. Long term research is required to observe establishment rates and overall reproductive success of this species at Cavness.

Research done by Waters et al. (2014) on two native Coast Salish prairie plants (*E. lanatum* and *M. laciniata*) and one non-native (*H. radicata*), compared pollinator visitation rates and plant seed set at native and invasive dominated neighborhoods. Native *E. lanatum* had more pollinator visits and higher seeds set at the plot with invasive plants while *M. laciniata* had more pollinator visits and higher seed set at native dominated floral communities. This research

reveals the facilitative and competitive interactions invasive plants have on native plant communities.

Lupinus lepidus

In contrast with *P. congesta*, *L. lepidus* had significantly higher seed set at the high-quality prairie site Glacial Heritage. This aligns with the original hypothesis of higher seed quality occurring for native plants and higher-quality prairie sites. Glacial Heritage has undergone over twenty years of invasive species removal and seasonal burning greatly reducing non-native plant species richness and abundance. Reduction of invasive plant populations has been shown to increase native plant diversity, species richness and biomass (Flory and Claye 2009).

One of the most impactful environmental disturbances facing native plant populations is the invasion of non-native plant species. Mechanisms by which invasive plants colonize new environments include high seed set production, heterospecific pollen transfer and changes in pollination networks (Flory and Claye 2009). Invasive plant species not only alter environmental conditions, but also increase competition for resources among native plants communities. This is achieved through changes in floral patch density, abundance, and distribution, resulting in reductions in reproductive success (Flory and Claye 2009, Orrock and Witter 2010). Meadow dwelling forbs adapted to high levels of light exposure have been found to be particularly negatively impacted by non-native plant invasions when compared to other habitat types (Alvarez 2002).

Eriophyllum lanatum

E. lanatum was the only plant species sampled that had no significant difference of seed quantity or quality among restoration sites. This is most likely due to *E. lanatum* self-compatibility or asexual reproduction (Appendix B Figure 1). This adaptation allows plants to be less impacted by environmental changes such as habitat fragmentation and pollinator networks (Aguilar et al. 2006). Evolving asexual reproduction while retaining the ability to sexually reproduce is an adaptive strategy when experiencing environmental disruptions that would impact pollination (Knight et al. 2005). This could explain why *E. lanatum* showed no significant variations among restoration sites for reproduction success.

Hypochoeris radicata

The results for *H. radicata* reveal complex relationships between seed quality, quantity, and restoration sites. Cavness Ranch had significantly higher fertilized seed quantity when compared to Deschutes, Glacial Heritage and Johnson Prairie. For restoration sites such as Cavness, seed quality and quantity do not align. Seed quality measured by viability varied among sites with Cavness, Deschutes and Wolf Haven significantly lower seed viability. Glacial Heritage, Johnson Prairie and Tenalquot had significantly higher seed viability. Fertilized seed weight also presented nuanced results with Glacial Heritage, having significantly lower seed weight when compared to Tenalquot and Deschutes.

These findings show that measurements of seed quality and quantity do not vary uniformly among restoration sites. The restoration site Cavness had high seed quantity with low seed viability. This result shows that *H. radicata* produced high seed quantity at Cavness but

those seeds had comparatively low viability. This result could reveal environmental stressors such as increased co-flowering competition that influences resources allocation.

Measurements of seed quality, such as seed weight and seed viability, are typically expected to reveal similar results (Burd 1994, Ashman et al. 2005, Knight et al. 2006). This was not the case for *H. radicata* at Glacial Heritage where seed viability was relatively high and seed weight was significantly low when compared to other restoration sites. These conflicting results illustrate the complexities of environmental differences at heavily fragmented sites.

Leucanthemum vulgare

The results of *L. vulgare* revealed that seed weight was significantly lower at Tenalquot when compared to Wolf Haven and Johnson Prairie. Seed weight is correlated to seedling survivorship and plant establishment (Galen and Stanton 1991, Aizen and Harder 2007). This could indicate that the *L. vulgare* population at Tenalquot is being impacted by restoration treatments causing low seed weight. *L. vulgare* is specifically listed as an invasive species of restoration focus in the CNLM Tenalquot management plan (personal correspondence, Sanders Freed).

Conclusion

This study produced surprising results that drastically differed from earlier hypotheses and predictions. Pollen limitation was not detected for any plant species sampled at any restoration site. The absence of treatment significance may be due to field sampling methods. Alterations to pollen limitation field sampling methodologies have been outlined in this study and could be applied to future pollen limitation studies.

All species sampled had varying responses in reproductive success among restoration sites. *L. lepidus* seed quality followed the hypothesis that native plant species would display higher reproductive success at the more restored sites. In contrast, *P. congesta* did not respond as expected, producing the highest seed quality at a low-quality prairie site. Invasive plant species reproductive success among sites showed more nuanced site specific results. Including other variables such as soil chemistry, soil moisture and plant species richness in future studies on plant reproductive success will elucidate these complex results (Gornish 2016).

The findings in this study can be utilized by restoration managers to better understand variations in reproductive success among plant populations for restoration sites. In the future measurements of pollen limitation and reproductive success can be added to site specific monitoring efforts. This can be especially important for assessing plant establishment for rare plants of conservation concern. Better understanding complicated plant-pollinator interactions can aid in restoring ecological functions to degraded landscapes and add to the greater body of knowledge within pollination ecology.

Bibliography

- Adderley, Lorraine & Vamosi, Jana. (2015). Species and Phylogenetic Heterogeneity in Visitation Affects Reproductive Success in an Island System. *International Journal of Plant Sciences*. 176. 186-196. [10.1086/679617](https://doi.org/10.1086/679617).
- Aguilar, R., Ashworth, L., Galetto, L., & Aizen, M. A. (2006). Plant reproductive susceptibility to habitat fragmentation: Review and synthesis through a meta-analysis. *Ecology Letters*, 9(8), 968–980. <https://doi.org/10.1111/j.1461-0248.2006.00927.x>
- Aizen, M. A., & Harder, L. D. (2007). EXPANDING THE LIMITS OF THE POLLEN-LIMITATION CONCEPT: EFFECTS OF POLLEN QUANTITY AND QUALITY. *Ecology*, 88(2), 271–281. <https://doi.org/10.1890/06-1017>
- Albrecht, M., Schmid, B., Hautier, Y., & Müller, C. B. (2012). Diverse pollinator communities enhance plant reproductive success. *Proceedings of the Royal Society B: Biological Sciences*, 279(1748), 4845–4852. <https://doi.org/10.1098/rspb.2012.1621>
- Alvarez, M. E., & Cushman, J. H. (2002). COMMUNITY-LEVEL CONSEQUENCES OF A PLANT INVASION: EFFECTS ON THREE HABITATS IN COASTAL CALIFORNIA. *Ecological Applications*, 12(5), 1434–1444. [https://doi.org/10.1890/1051-0761\(2002\)012\[1434:CLCOAP\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2002)012[1434:CLCOAP]2.0.CO;2)
- Anderson, B., & Minnaar, C. (2020). Illuminating the incredible journey of pollen. *American Journal of Botany*, 107(10), 1323–1326. <https://doi.org/10.1002/ajb2.1539>
- Andersson, S. (2008). Pollinator and nonpollinator selection on ray morphology in *Leucanthemum vulgare* (oxeye daisy, *Asteraceae*). *American Journal of Botany*, 95(9), 1072–1078. <http://www.jstor.org/stable/41922355>

- Arceo-Gómez, G., Abdala-Roberts, L., Jankowiak, A., Kohler, C., Meindl, G. A., Navarro-Fernández, C. M., Parra-Tabla, V., Ashman, T.-L., & Alonso, C. (2016). Patterns of among- and within-species variation in heterospecific pollen receipt: The importance of ecological generalization. *American Journal of Botany*, *103*(3), 396–407. <https://doi.org/10.3732/ajb.1500155>
- Arceo-Gómez, G., Schroeder, A., Albor, C., Ashman, T.-L., Knight, T. M., Bennett, J. M., Suarez, B., & Parra-Tabla, V. (2019). Global geographic patterns of heterospecific pollen receipt help uncover potential ecological and evolutionary impacts across plant communities worldwide. *Scientific Reports*, *9*(1), 1–9. <https://doi.org/10.1038/s41598-019-44626-0>
- Ashman, T., & Hitchens, M. S. (2000). Dissecting the causes of variation in intra-inflorescence allocation in a sexually polymorphic species, *Fragaria virginiana* (Rosaceae). *American Journal of Botany*, *87*(2), 197–204. <https://doi.org/10.2307/2656906>
- Ashman, T.-L., Knight, T. M., Steets, J. A., Amarasekare, P., Burd, M., Campbell, D. R., Dudash, M. R., Johnston, M. O., Mazer, S. J., Mitchell, R. J., Morgan, M. T., & Wilson, W. G. (2004). POLLEN LIMITATION OF PLANT REPRODUCTION: ECOLOGICAL AND EVOLUTIONARY CAUSES AND CONSEQUENCES. *Ecology*, *85*(9), 2408–2421. <https://doi.org/10.1890/03-8024>
- Bennett, Joanne. M., Steets, Janette. A., Burns, Jean. H., Durka, W., Vamosi, Jana. C., Arceo-Gómez, G., Burd, M., Burkle, Laura. A., Ellis, Allan. G., Freitas, L., Li, J., Rodger, James. G., Wolowski, M., Xia, J., Ashman, T.-L., & Knight, Tiffany. M. (2018). *Data from: GloPL, a global data base on pollen limitation of plant reproduction* (Version 1, p. 2274543 bytes) [Data set]. Dryad. <https://doi.org/10.5061/DRYAD.DT437>
- Betts, M. G., Hadley, A. S., & Kormann, U. (2019). The landscape ecology of pollination. *Landscape Ecology*, *34*(5), 961–966. <https://doi.org/10.1007/s10980-019-00845-4>

- Burd, M. (1994). Bateman's Principle and Plant Reproduction: The Role of Pollen Limitation in Fruit and Seed Set. *Botanical Review*, 60(1), 83–139.
- Burd, M. (2008). The Haig-Westoby Model Revisited. *The American Naturalist*, 171(3), 400–404.
<https://doi.org/10.1086/527499>
- Bowcutt, F., & Hamman, S. (2016). Vascular Plants of the South Sound Prairies. The Evergreen State College.
- Cariveau, D. P., Bruninga-Socular, B., & Pardee, G. L. (2020). A review of the challenges and opportunities for restoring animal-mediated pollination of native plants. *Emerging Topics in Life Sciences*, 4(1), 99–109. <https://doi.org/10.1042/ETLS20190073>
- Casper, B. B., & Niesenbaum, R. A. (1993). Pollen versus resource limitation of seed production: a reconsideration. *Current Science*, 210-214.
- Charlesworth, D., Morgan, M. T., & Charlesworth, B. (1990). INBREEDING DEPRESSION, GENETIC LOAD, AND THE EVOLUTION OF OUTCROSSING RATES IN A MULTILOCUS SYSTEM WITH NO LINKAGE. *Evolution*, 44(6), 1469–1489.
<https://doi.org/10.1111/j.1558-5646.1990.tb03839.x>
- Cheung, A. Y. (1996). The pollen tube growth pathway: its molecular and biochemical contributions and responses to pollination. *Sexual Plant Reproduction*, 9(6), 330-336.
- Cohen, D., & Dukas, R. (1990). The Optimal Number of Female Flowers and the Fruits-to-Flowers Ratio in Plants Under Pollination and Resources Limitation. *The American Naturalist*, 135(2), 218–241. <https://doi.org/10.1086/285040>
- Cruzan, M. B., & Barrett, S. C. (1996). Postpollination mechanisms influencing mating patterns and fecundity: an example from *Eichhornia paniculata*. *The American Naturalist*, 147(4), 576-598.

- Diggle, P. K. (1995). Architectural effects and the interpretation of patterns of fruit and seed development. *Annual Review of Ecology and Systematics*, 26(1), 531-552.
- Dunwiddie, P. W., Alverson, E. R., Martin, R. A., & Gilbert, R. (2014). Annual Species in Native Prairies of South Puget Sound, Washington. *Northwest Science*, 88(2), 94–105.
<https://doi.org/10.3955/046.088.0205>
- Ehrlén, J. (1991). Why do plants produce surplus flowers? A reserve-ovary model. *The American Naturalist*, 138(4), 918-933
- Fenster, C. B., Armbruster, W. S., Wilson, P., Dudash, M. R., & Thomson, J. D. (2004). Pollination Syndromes and Floral Specialization. *Annual Review of Ecology, Evolution, and Systematics*, 35(1), 375–403. <https://doi.org/10.1146/annurev.ecolsys.34.011802.132347>
- Fernández, J. D., Bosch, J., Nieto-Ariza, B., & Gómez, J. M. (2012). Pollen limitation in a narrow endemic plant: Geographical variation and driving factors. *Oecologia*, 170(2), 421–431.
<https://doi.org/10.1007/s00442-012-2312-1>
- Ferrero-Serrano, Á., Hild, A. L., & Meador, B. A. (2011). Can invasive species enhance competitive ability and restoration potential in native grass populations?. *Restoration Ecology*, 19(4), 545-551.
- Flory, S. L., & Clay, K. (2009). Invasive plant removal method determines native plant community responses. *Journal of Applied Ecology*, 46(2), 434–442. <https://doi.org/10.1111/j.1365-2664.2009.01610.x>
- França-Neto, J. de B., & Krzyzanowski, F. C. (2019). Tetrazolium: An important test for physiological seed quality evaluation. *Journal of Seed Science*, 41(3), 359–366.
<https://doi.org/10.1590/2317-1545v41n3223104>

- Galen, C., & Stanton, M. L. (1991). Consequences of Emergence Phenology for Reproductive Success in *Ranunculus adoneus* (*Ranunculaceae*). *American Journal of Botany*, 78(7), 978–988. <https://doi.org/10.2307/2445177>
- Gornish, E. S., & Ambrozio dos Santos, P. (2016). Invasive species cover, soil type, and grazing interact to predict long-term grassland restoration success. *Restoration Ecology*, 24(2), 222–229.
- Haig, D., & Westoby, M. (1988). On Limits to Seed Production. *The American Naturalist*, 131(5), 757–759. <https://doi.org/10.1086/284817>
- Hagen, M., Kissling, W. D., Rasmussen, C., De Aguiar, M. A. M., Brown, L. E., Carstensen, D. W., Alves-Dos-Santos, I., Dupont, Y. L., Edwards, F. K., Genini, J., Guimarães, P. R., Jenkins, G. B., Jordano, P., Kaiser-Bunbury, C. N., Ledger, M. E., Maia, K. P., Marquitti, F. M. D., McLaughlin, Ó., Morellato, L. P. C., ... Olesen, J. M. (2012). Biodiversity, Species Interactions and Ecological Networks in a Fragmented World. In *Advances in Ecological Research* (Vol. 46, pp. 89–210). Elsevier. <https://doi.org/10.1016/B978-0-12-396992-7.00002-2>
- Hamman, S. T., Dunwiddie, P. W., Nuckols, J. L., & McKinley, M. (2011). Fire as a Restoration Tool in Pacific Northwest Prairies and Oak Woodlands: Challenges, Successes, and Future Directions. *Northwest Science*, 85(2), 317–328. <https://doi.org/10.3955/046.085.0218>
- Harder, L. D., & Barrett, S. C. H. (1996). Pollen Dispersal and Mating Patterns in Animal-Pollinated Plants. In D. G. Lloyd & S. C. H. Barrett (Eds.), *Floral Biology: Studies on Floral Evolution in Animal-Pollinated Plants* (pp. 140–190). Springer US. https://doi.org/10.1007/978-1-4613-1165-2_6
- Herrera, J. (1991). ALLOCATION OF REPRODUCTIVE RESOURCES WITHIN AND AMONG INFLORESCENCES OF *LAVANDULA STOECHAS* (*LAMIACEAE*). *American Journal of Botany*, 78(6), 789–794. <https://doi.org/10.1002/j.1537-2197.1991.tb14480.x>

- Kaiser-Bunbury, C. N., Mougal, J., Whittington, A. E., Valentin, T., Gabriel, R., Olesen, J. M., & Blüthgen, N. (2017). Ecosystem restoration strengthens pollination network resilience and function. *Nature*, 542(7640), 223–227. <https://doi.org/10.1038/nature21071>
- Kanchan, S., & Chandra, J. (1980). POLLEN ALLELOPATHY—A NEW PHENOMENON. *New Phytologist*, 84(4), 739–746. <https://doi.org/10.1111/j.1469-8137.1980.tb04786.x>
- Knight, T. (2004). Floral density, pollen limitation, and reproductive success in *Trillium grandiflorum*. *Oecologia*, 137, 557–563. <https://doi.org/10.1007/s00442-003-1371-8>
- Knight, T. M., Steets, J. A., Vamosi, J. C., Mazer, S. J., Burd, M., Campbell, D. R., Dudash, M. R., Johnston, M. O., Mitchell, R. J., & Ashman, T.-L. (2005). Pollen Limitation of Plant Reproduction: Pattern and Process. *Annual Review of Ecology, Evolution, and Systematics*, 36(1), 467–497. <https://doi.org/10.1146/annurev.ecolsys.36.102403.115320>
- Knight, T., Steets, J. A., & Ashman, T.-L. (2006). A QUANTITATIVE SYNTHESIS OF POLLEN SUPPLEMENTATION EXPERIMENTS HIGHLIGHTS THE CONTRIBUTION OF RESOURCE REALLOCATION TO ESTIMATES OF POLLEN LIMITATION. *American Journal of Botany*, 93, 271–277. <https://doi.org/10.3732/ajb.93.2.271>
- Krukeberg, A. 1991. The Natural History of Puget Sound Country. University of Washington Press, Seattle, Washington
- Lloyd, D. G. (1992). Self- and Cross-Fertilization in Plants. II. The Selection of Self- Fertilization. *International Journal of Plant Sciences*, 153(3, Part 1), 370–380. <https://doi.org/10.1086/297041>
- Lopezaraiza-Mikel, M. E., Hayes, R. B., Whalley, M. R., & Memmott, J. (2007). The impact of an alien plant on a native plant–pollinator network: An experimental approach. *Ecology Letters*, 10(7), 539–550. <https://doi.org/10.1111/j.1461-0248.2007.01055.x>

- Morales, C. L., & Traveset, A. (2009). A meta-analysis of impacts of alien vs. native plants on pollinator visitation and reproductive success of co-flowering native plants. *Ecology Letters*, *12*(7), 716–728. <https://doi.org/10.1111/j.1461-0248.2009.01319.x>
- National Oceanic and Atmospheric Administration (NOAA). National Weather Service. <https://www.weather.gov/> accessed 11/2/2021.
- Newman, B. J., Ladd, P., Brundrett, M., & Dixon, K. W. (2013). Effects of habitat fragmentation on plant reproductive success and population viability at the landscape and habitat scale. *Biological Conservation*, *159*, 16–23. <https://doi.org/10.1016/j.biocon.2012.10.009>
- Noland, S., Carver, L. (2011). *Prairie Landowner Guide for Western Washington*. The Nature Conservancy, Seattle, WA.
- Orrock, J. L., & Witter, M. S. (2010). Multiple drivers of apparent competition reduce re-establishment of a native plant in invaded habitats. *Oikos*, *119*(1), 101–108. <https://doi.org/10.1111/j.1600-0706.2009.17831.x>
- Parker, G. A., & Smith, J. M. (1990). Optimality theory in evolutionary biology. *Nature*, *348*(6296), 27–33. <https://doi.org/10.1038/348027a0>
- Ramula, S., & Pihlaja, K. (2012). Plant communities and the reproductive success of native plants after the invasion of an ornamental herb. *Biological Invasions*, *14*(10), 2079–2090. <https://doi.org/10.1007/s10530-012-0215-z>
- Rosenheim, J. A., Alon, U., & Shinar, G. (2010). Evolutionary Balancing of Fitness-Limiting Factors. *The American Naturalist*, *175*(6), 662–674. <https://doi.org/10.1086/652468>
- Sabatino, M., Rovere, A., & Meli, P. (2021). Restoring pollination is not only about pollinators: Combining ecological and practical information to identify priority plant species for restoration

of the Pampa grasslands of Argentina. *Journal for Nature Conservation*, 61, 126002.

<https://doi.org/10.1016/j.jnc.2021.126002>

Snow, A. A. (1994). Postpollination selection and male fitness in plants. *The American Naturalist*, 144, S69-S83.

Storm, L. (2002). *Patterns and Processes of Indigenous Burning How to Read Landscape Signatures of Past Human Practices*. International Society of Ethnobiology, University of Georgia Press pp. 496-508 ref. 49.

Trowbridge, C. C., Stanley, A., Kaye, T. N., Dunwiddie, P. W., & Williams, J. L. (2017). Long-term effects of prairie restoration on plant community structure and native population dynamics. *Restoration Ecology*, 25(4), 559–568. <https://doi.org/10.1111/rec.12468>

Vico, G., Manzoni, S., Nkurunziza, L., Murphy, K., & Weih, M. (2016). Trade-offs between seed output and life span—a quantitative comparison of traits between annual and perennial congeneric species. *New Phytologist*, 209(1), 104-114.

Waters, S. M., Fisher, S. E., & Hille Ris Lambers, J. (2014). Neighborhood-contingent indirect interactions between native and exotic plants: Multiple shared pollinators mediate reproductive success during invasions. *Oikos*, 123(4), 433–440. <https://doi.org/10.1111/j.1600-0706.2013.00643.x>

Wesselingh, R. A. (2007). *Pollen limitation meets resource allocation: Towards a comprehensive methodology*. *New Phytologist*, 174(!), 26-37.

<https://nph.onlinelibrary.wiley.com/doi/full/10.1111/j.1469-8137.2007.01997.x>

Zimmerman, M., & Pyke, G. H. (1988). Reproduction in *Polemonium*: Assessing the Factors Limiting Seed Set. *The American Naturalist*, 131(5), 723–738.

Appendices

Appendix A

Table 1
ANOVA Table
Individual Seed Weight by Site

Plant Species	Model	DF	SS	MS	F	P
<i>Lupinus lepidus</i>	ANOVA	3	0.003	<0.001	1.546	0.215
<i>Hypochaeris radicata</i>	ANOVA	5	<0.001	<0.001	3.636	0.004
<i>Leucanthemum vulgare</i>	ANOVA	4	<0.001	<0.001	3.132	0.018

Table 2
Plectritis congesta
Individual Seed Weight by Site
P-values from General Linear Mixed Model using Gamma Loglink Model

Site	CV	DS	GH	JP	TQ	WH
CV		0.004	0.001	0.016	<0.001	<0.001
DS			0.934	1.00	0.092	0.090
GH				0.928	0.708	0.07
JP					0.095	0.080
TQ						1.00
WH						

Table 3*Eriophyllum lanatum*

Fertilized Seed Weight

P-values from Pairwise Comparison of Means

Site	CV	GH	JP	TQ	WH
CV		1.00	1.00	0.977	0.890
GH			1.00	0.988	0.881
JP				0.988	0.890
TQ					0.538
WH					

Table 4*Hypochoeris radicata*

Proportion of Stain Viable

P-values from Wilcoxon Rank Sum Test Pairwise Comparison Table

Sites	CV	DS	GH	JP	TQ
DS	0.588				
GH	0.002	0.002			
JP	<0.001	<0.001	0.834		
TQ	<0.001	<0.001	0.525	0.531	
WH	0.199	0.332	0.002	<0.001	<0.001

Table 5
Plectritis congesta
 ANOVA Table
 Proportion Stain Viable

	DF	SS	MS	F	P
Site	5	0.440	0.087	1.327	0.262

Table 6
Lupinus lepidus
 Proportion of Fertilized Seed
 P-values from Wilcoxon Rank Sum Test Pairwise Comparison Table

Site	CV	GH	TQ
GH	<0.001	-	-
TQ	0.23	<0.001	-
WH	0.23	<0.001	0.08

Table 7*Hypochaeris radicata*

Proportion of Stain Viable

P-values form Wilcoxon Rank Sum Test Pairwise Comparison Table

Sites	CV	DS	GH	JP	TQ
DS	0.020				
GH	0.140	0.073			
JP	0.272	0.037	0.629		
TQ	0.881	0.007	0.093	0.184	
WH	0.073	0.131	0.475	0.256	0.020

Table 8*Leucanthemum vulgare*

Total Number of Seeds

ANOVA Table

	DF	SS	MS	F Value	P
Site	3	42130	14043	1.578	0.202
Treatment	1	6157	6157	0.692	0.408
Height	1	95903	95903	10.775	<0.001

Table 9*Plectritis congesta*

Proportion Fertilized Seed

P-values from Estimated Marginal Means Pairwise Comparison Table

Site	CV	DS	GH	JP	TQ	WH
CV	-	0.833	0.402	0.249	0.141	0.175
DS			0.999	0.999	0.993	0.996
GH				1.00	.999	1.00
JP					0.999	1.00
TQ						1.00
WH						

Table 10*Eriophyllum lanatum*

Proportion Fertilized Seeds

P-values from the Wilcoxon Sum Tank Test Pairwise Comparison Table

Site	CV	GH	JP	TQ
GH	0.87			
JP	0.90	0.90		
TQ	0.87	0.90	0.90	
WH	0.87	0.72	0.72	0.72

Appendix B

Self-compatibility data for south Puget Sound prairie plant species. Mean seed set for open and closed pollination treatments (unpublished data, Susan Waters).

