ABSTRACT

The Pollination Ecology of *Hedysarum boreale* Nutt. (Fabaceae) and Evaluation of its Pollinating Bees for Restoration Seed Production

by

Katharine A. Swoboda, Master of Science

Utah State University, 2007

Academic Advisor: Dr. Edward W. Evans
Research Advisor: Dr. James H. Cane
Department: Biology

Federal land managers desire a consistent and cost-effective source of *Hedysarum boreale* Nutt. seed for rangeland restoration in the Great Basin and adjacent ecosystems. The breeding biology of *H. boreale* was assessed via hand pollination experiments at 2 sites in Cache County, Utah, USA in 2003. *H. boreale* was found to be self-compatible, but did not produce fruit and seeds in the absence of bee visitors. Xenogamy (out-crossing) treatments resulted in increased seed viability and decreased predispersal reproductive attrition. *H. boreale* was found to be homogamous during 2004 experiments designed to determine the timing and duration of stigma receptivity. *H. boreale* stigmas became receptive during the mature bud stage prior to flower opening (anthesis) and remained receptive for several days. *H. boreale* proved to be very rewarding in terms of floral resources; flowers contained abundant pollen grains and nectar of comparatively high sugar concentration.

The bee faunas of several natural populations of *H. boreale* in Utah and Wyoming, USA were systematically surveyed in 2004 and 2005. Each population was surveyed once per summer, sometimes in both years. Populations were surveyed at or just after peak bloom and during the early afternoon hours. An assortment of bee species in the families Apidae and Megachilidae were collected at *H. boreale* flowers. *Osmia* species proved to be an important component of *H. boreale* pollinator faunas. Three solitary, cavity-nesting candidate *Osmia* species were chosen and evaluated for their potential use as managed *H. boreale* pollinators: *O. bruneri* Cockerell, *O. lignaria* Say, and *O. sanrafaelae* Parker. Candidate pollinator species were chosen according to several criteria including range, phenology, floral preferences, life history, pollination efficacy, and body size.

The pollination efficacies of candidate *Osmia* species, other native bee species found to be abundant at *H. boreale*, and honeybees were compared via behavioral observations, foraging tempo, frequency of stigmatic contact, and pollen grains deposited per single flower visit. Females of *O. bruneri* and *O. sanrafaelae* were able to reproduce with *H. boreale* as their only pollen and nectar source. Nesting data from these species were combined with estimates of floral resource production by *H. boreale* to calculate stocking densities. In general, nesting by *O. lignaria* females was limited, suggesting that this species may not be the best option for managed pollination of *H. boreale* in most agricultural settings. *O. bruneri* and *O. sanrafaelae* proved to be effective *H. boreale* pollinators in terms of frequency of stigmatic contact and pollen grains deposited per single flower visit, and could be used for commercial production of *H. boreale* seed.

(127 pages)

ACKNOWLEDGMENTS

I would like to begin by thanking my research advisor, Dr. James H. Cane, for his helpful guidance and support during my thesis research and writing, and for making funding available for this research. I would also like to thank my academic advisor, Dr. Ted Evans, and my committee member, Dr. Eugene Schupp, for their helpful suggestions and support during my thesis research and writing. Rick and Claire Dunne of Wind River Seed Company in Manderson, Wyoming, graciously allowed the use of their 2-acre stand of *Hedysarum boreale* for the purposes of this research. Conversations with my committee members and with Rick Dunne inspired ideas for this research.

Many people at the USDA-ARS Bee Biology and Systematics Laboratory in Logan helped me tremendously. I offer my sincere thanks to Faye Rutishauser, Stephanie Miller, Melissa Weber, Joyce Knoblett, Kristal Watrous, Glen Trostle, Ellen Klinger, Theresa Pitts-Singer, Bill Kemp, Jordi Bosch, Olivia Messenger, Morgan Yost, James McDonald, and Carole Scoville, and the rest of the bee lab staff, technicians, and summer crews. Terry Griswold and Harold Ikerd assisted with species identifications. In addition, R.W. Brooks identified *Anthophora* specimens in 2005.

Several people at the USDA Forage and Range Laboratory in Logan offered assistance during my thesis research, including Tom Jones, Tom Monaco, Michael Peel, Kevin Connors, and Eamonn Leonard. I would also like to thank Nancy Shaw and Jim Ozenberger, both with the US Forest Service, for their help.

Many others contributed to my thesis research and writing. Dr. James Pitts kindly let me keep a desk in the insect collection. Likewise, Dr. Evans, Yukie Kajita, and Nicole Davidson kindly offered a much needed refuge in which to work, and excellent company

during the writing process. Joe Wilson kindly offered to take photographs of my bees. In addition, Red Fowler Jr. of Wind River Farm in Worland, Wyoming kindly looked after my bees during the summers of 2004 and 2005, and seemed to develop a keen interest in the blue orchard bee as a result.

I owe so much to my Utah family. Many thanks to Faye Rutishauser, Hans Millican, Heather Mickelson, Richard Swart, Amy Saunders, Ellen Klinger, Almut Vollmer, Bruce Christiansen, and Jayanti Mukharjee. I would like extend special thanks to Kishor Bhattarai for his endless kindness, patience, encouragement, and true companionship during the trials and tribulations that accompanied my thesis research and writing. I would not have been able to finish this thesis without Kishor. I would also like to thank my parents, Mary and Joseph Swoboda, and my lovely sister Amy, for the love, patience, and support they offered during my thesis research and writing. I cannot adequately express how much I appreciated their encouragement and enthusiasm. I would like to dedicate this thesis to Joe, Mary, and Amy.

This research was funded by the Great Basin Native Plant Selection and Increase Project funded through the USDI-BLM Great Basin Restoration Initiative and the USDA-FS Rocky Mountain Research Station.

Katharine Anne Swoboda

CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Ecological restoration aims to renew and maintain ecosystem health (SER 1995, 2005). If sustained ecosystem health is the ultimate goal of restoration, how do restoration ecologists know when they have succeeded? In practice, restoration success is primarily assessed via 3 major ecosystem attributes, namely vegetation structure, biotic diversity, and ecological function (Ruiz-Jaen and Aide 2005). Monitoring nutrient cycling and biological interactions (i.e. mycorrhizae, herbivory, and pollination) provide information about the health of restored ecosystems, while recovery of biological interactions is also critical for the long-term function of a restored ecosystem (Forup and Memmott 2005).

In response to the loss of ecological resilience and productivity within the Great Basin and adjacent ecosystems, the Bureau of Land Management (BLM) created the Great Basin Restoration Initiative (GBRI). As part of this initiative, the BLM and US Forest Service (USFS) intend to use seed of native plants for restoration whenever it is available (Forbis et al. 2006). *Hedysarum boreale* Nutt. is among several native forbs selected by the BLM and USFS for restoration purposes.

H. boreale was selected for several reasons. It is widely distributed throughout the Rocky Mountains and along the eastern edge of the Intermountain West. It is found in grasslands or on sagebrush slopes in the lowlands and in open fields or woodlands in the mountains (Northstrom and Welsh 1970). *H. boreale* exhibits considerable ecotypic variation for many characteristics including plant, flower, and leaflet size, and degree of pubescence (Northstrom and Welsh 1970). For this reason, Redente and Reeves (1981)

believe that *H. boreale* may be useful for re-vegetation of a wide variety of sites. Sufficient genetic variation was present among 11 *H. boreale* ecotypes studied by Johnson et al. (1989) to assure adaptation to a wide array of sites and facilitate improvement via breeding and selection. In fact, a seed-propagated cultivar of *H. boreale* named 'Timp' Utah sweetvetch was released for commercial seed production in 1994 (Stevens et al. 1994). Additionally, because it is a legume, *H. boreale* may help improve the nitrogen status of rangeland soils through nitrogen fixation and may do well in nitrogen-limited environments (Johnson et al. 1989). Nodulation by *H. boreale* plants in natural populations in Utah was observed by Athar (1996).

Hedysarum species have been cultivated as forage crops in several countries (Bassendowski et al. 1989). Meadows of perennial legumes are widely used as livestock forage in Mediterranean environments. The 2 most used species are sulla (*Hedysarum coronarium* L.) and sainfoin (*Onobrychis viciifolia* Scop.) (Martiniello and Ciola 1994); sainfoin is closely related to *Hedysarum* (Pohill 1981). Likewise, *H. boreale* can provide a considerable amount of early spring forage for wildlife and domestic livestock without the toxicity problems observed in other range legume species (Johnson et al. 1989). However, improper grazing has apparently eliminated *H. boreale* from much of its original range (Plummer et al. 1968).

The BLM and USFS would like to acquire tons of affordable *H. boreale* seed, but it is largely unavailable and expensive. Native bees and/or honeybees are needed to pollinate most of the forb species selected for restoration of the Great Basin and adjacent ecosystems. In order to produce seed at the scale desired by federal land managers, *H. boreale* will need to be farmed. Depending on the setting and acreage of the fields, it may

be necessary to provide a cavity-nesting or other manageable bee species to augment pollination by unmanaged native pollinators foraging in the area. Therefore, the overall goals of this research are to study the pollination ecology of *H. boreale* and evaluate its pollinating bees for use in seed production. The objectives of this research are threefold: 1) to determine the breeding biology of *H. boreale*; 2) to identify manageable bee pollinators for production of *H. boreale* seed in agricultural settings; and 3) to estimate, based on measures of the pollination efficacy of candidate bee pollinators and floral resource production by *H. boreale*, appropriate stocking densities for each candidate bee species (i.e. the optimal number of female bees needed to provide adequate pollination services for an acre of crop).

In Chapter 2, some basic questions concerning the breeding biology of *H. boreale* are addressed. Breeding system experiments focused on whether *H. boreale* is self-compatible and able to produce viable seeds in the absence of pollinators, and whether *H. boreale* fruit and seed yields increase in the presence of pollinators. Additional experiments addressed the timing and duration of stigma receptivity, and the duration of pollen viability following dehiscence. In addition, the floral resources (i.e. pollen and nectar) produced by *H. boreale* flowers were measured, scaled up, and used to predict the average amount of floral resources available to pollinators per acre of cultivated *H. boreale*.

The research described in Chapter 3 was designed to determine what types and species of bees are commonly found at *H. boreale* within its native range. Tepedino and Stackhouse (1987) extensively surveyed the bee fauna of a natural population of *H. boreale* in Wyoming. Systematic surveys of the bee faunas of several additional *H.*

boreale populations in Utah and Wyoming were conducted during this study. Based on survey results and on additional information found in the literature, candidate bee pollinators for production of *H. boreale* seed in agricultural settings were chosen according to several criteria. Criteria included range, floral preferences, life history, pollination efficacy, and body size (Bosch and Kemp 2002).

In Chapter 4, the foraging behavior and pollination efficacy of candidate pollinators are compared with those of abundant native bee species and honeybees at *H. boreale* flowers. In addition, nesting trials were run to evaluate *H. boreale* as a satisfactory source of pollen and nectar for adults and larvae of candidate pollinator species. Measures of nesting behavior were combined with estimates of the floral resources produced per acre of *H. boreale* from Chapter 2, and used to estimate appropriate stocking densities for each candidate pollinator species.

This research advances the identification and evaluation of bee pollinators of *H. boreale* for use in agricultural settings. Other aspects of pollinator biology are not directly addressed in this research (i.e. development and life cycle, parasites, predators, and pathogens) (Bosch and Kemp 2002). The next step for future research is to address the above aspects of pollinator biology, and to learn how to effectively manage candidate species in large numbers for commercial production of *H. boreale* seed.

LITERATURE CITED

Athar, M. 1996. Nodulation status of some legume species from Cache Valley and northern Utah. Phytologia 81:145-150.

Bassendowski, K.A., J.D. Smith, and R.E. Howarth. 1989. The potential value of *Hedysarum alpinum* var. *americanum* as a forage legume for the northern Canadian prairies. Canadian Journal of Plant Science 69:815-822.

Bosch, J., and W.P. Kemp. 2002. Developing and establishing bee species as crop pollinators: the example of *Osmia* spp. (Hymenoptera: Megachilidae) and fruit trees. Bulletin of Entomological Research 92:3-16.

Forbis, T.A., L. Provencher, L. Frid, and G. Medlyn. 2006. Great Basin land management planning using ecological modeling. Restoration Ecology 38:62-83.

Forup, M.L., and J. Memmott. 2005. The restoration of plant-pollinator interactions in hay meadows. Restoration Ecology 13:265-274.

Johnson, D.A., T.M.J. Ford, M.D. Rumbaugh, and B.Z. Richardson. 1989. Morphological and physiological variation among ecotypes of sweetvetch (*Hedysarum boreale* Nutt.). Journal of Range Management 42:496-501.

Martiniello, P., and A. Ciola. 1994. The effect of agronomic factors on seed and forage production in perennial legumes sainfoin (*Onobrychis viciifolia* Scop.) and French honeysuckle (*Hedysarum coronarium* L.). Grass and Forage Science 49:121-129.

Northstom, T.E., and S.L. Welsh. 1970. Revision of the *Hedysarum boreale* complex. Great Basin Naturalist 30:109-130.

Pohill, R.M. 1981. Hedysareae DC. Pages 367-370 *in* R.M. Pohill and P.H. Raven, editors, Advances in legume systematics. Royal Botanic Gardens, Kew, Richmond, Surrey.

Plummer, A.P., D.A. Christensen, and S.B. Monson. 1968. Restoring big-game range in Utah. Utah Division of Fish and Game, Publication 68-3.

Redente, E.F., and F.B. Reeves. 1981. Interactions between vesicular-arbuscular mycorrhiza and *Rhizobium* and their effect on sweetvetch growth. Soil Science 132:410-415.

Ruiz-Jaen, M.C., and T.M. Aide. 2005. Restoration success: how is it being measured? Restoration Ecology 13:569-577.

SER (Society for Ecological Restoration). 1995. Definition of ecological restoration. Society for Ecological Restoration, Madison, WI.

SER (Society for Ecological Restoration International Science & Policy Working Group). 2005. The SER international primer on ecological restoration (available from http//www.ser.org). Society for Ecological Restoration International, Tucson, AZ.

Stevens, R., E.D. McArthur, S.A. Young, G. Massay, R.S. Cuany, and D.A. Johnson. 1994. Notice of naming and release of 'Timp' Utah sweetvetch (*Hedysarum boreale*

Nutt.) for soil improvement and early spring forage for both wildlife and livestock. Upper Colorado Environmental Plant Center, Meeker, CO.

Tepedino, V.J., and M. Stackhouse. 1987. Bee visitors of sweetvetch, *Hedysarum boreale boreale* (Leguminosae), and their pollen-collecting activities. Great Basin Naturalist 47:314-318.

CHAPTER 2

THE BREEDING BIOLOGY OF HEDYSARUM BOREALE AND ESTIMATES OF ITS FLORAL RESOURCE PRODUCTION

INTRODUCTION

The development of a bee species as a new crop pollinator begins with the recognition of a pollination need in agriculture (Bosch and Kemp 2002). The Bureau of Land Management (BLM) and US Forest Service (USFS) would like to annually acquire large quantities of affordable *Hedysarum boreale* Nutt. seed for use in western rehabilitation efforts. *H. boreale* is a native perennial legume that is distributed throughout the Rocky Mountains and neighboring areas of the US Intermountain West. Seed of *H. boreale* is commonly collected from wildland stands. However, wildland collection is not a cost-effective way to obtain large quantities of seed (Johnson et al. 1989). Farming *H. boreale* for commercial seed production is therefore an essential step in meeting BLM and USFS needs. Pollination is an important aspect of seed production that is often overlooked during the crop development process. One objective of this research is to better understand the breeding biology of *H. boreale* to guide a more consistent, productive, and cost-effective production of its seed.

Pollination in angiosperms begins with pollen release (dehiscence) from the male part of a flower and ends with pollen deposition on a stigma (Fægri and van der Pijl 1979). Pollination can be followed by pollen germination and then by fertilization; however, authors commonly but incorrectly lump all of these processes together under the single title of pollination (Inouye et al. 1994). In this study, the term breeding biology

is used to refer not only to pollination, but also to additional processes such as pollen germination that may occur after pollination and influence its success. Stigma receptivity refers to the ability of the stigma to support germination of viable, compatible pollen (Shivanna 2003). The timing and duration of stigma receptivity can affect whether pollination translates into the production of viable seeds. Therefore, in this study, stigma receptivity and pollen germination are considered to be important components of breeding biology.

The first step in understanding the breeding biology of a species is to experiment with its breeding system and pollination needs. The term breeding system refers to the many aspects of sex expression in plants that affect the relative contributions to the next generation of individuals within a species (Wyatt 1983), which may include floral diversity, pollination biology and gene flow, multi-allelic self-incompatibility systems, self-fertilization and inbreeding, heteromorphy, dicliny, and agamospermy (Richards 1997). Traditionally, researchers of plant breeding systems have focused on mechanisms which promote or reduce out-crossing (Wyatt 1983, Dafni 1992). However, understanding the breeding system of a plant species is also important in assessing its dependence on pollinators, and may help to determine what type(s) of pollinators are required when seed production in a species is limited by pollination (Waser et al. 1996).

The breeding system and pollination needs of *H. boreale* are unknown; however, other legumes and closely related species may hold clues to its breeding biology. Legume breeding systems are very diverse. Some legumes are auto-pollinating and are able to produce fruits and seeds without flower manipulation (selfing species). Other legumes are obligate out-crossers and are unable to produce fruit or seeds without appropriate

pollinators. Many legumes exhibit intermediate breeding systems in which both selfing and out-crossing contribute to fruit and seed production (Free 1993). *Hedysarum coronarium* L. (sulla) is self-compatible (Louati-Namouchi et al. 2000). However, the relative positions of the stigma and anthers in *H. coronarium* flowers facilitate cross-pollination and discourage self-pollination in the absence of insect visitors (Free 1993). Sacchi (1950) found that *H. coronarium* plants caged to exclude insects set little or no seed. In fact, insects are necessary for both self- and cross-pollination in *H. coronarium* (Pinzauti and Magnani 1981). Baatout et al. (1991) analyzed reproduction and population structure in two subspecies of *Hedysarum spinosissimum* L. that exhibit marked variation in breeding systems. *H. spinosissimum* ssp. *euspinosissimum* reproduces primarily via selfing, while subspecies *capitatum* reproduces via out-crossing.

Breeding system experiments or artificial pollination procedures should be accompanied by a test of the timing and duration of the stigma's receptivity (Stone et al. 1995). Heslop-Harrison and Shivanna (1977) placed *Hedysarum* among a large group of taxa possessing a stigmatic surface with short to medium-height papillae that are wet when receptive; however, the timing and duration of stigma receptivity in *H. boreale* have yet to be documented. Stigma receptivity is an important stage in the flower life cycle that may greatly influence opportunities for self-pollination, chances for gametophytic selection, interference between male and female function, the relative importance of different pollinator types and species, pollination success at various stages in the flower life cycle, and rates of competition via improper pollen transfer (Galen et al. 1987, Dafni and Mottes Maues 1998). When the period of receptivity is very brief, as with some species, it may severely limit fruit and seed production (Egea et al. 1991,

Stpiczynska 2003). The oldest method used to determine the period of stigma receptivity is to manually pollinate flowers at various times of day or over a period of days, and then wait to see if seeds are produced. More recently, chemical tests have been developed to detect correlates of receptivity; some of these tests look for enzymatic reactions, and are based on the assumption that enzyme presence reflects receptivity (Kearns and Inouye 1993). Stigmatic esterase activity is commonly used as an indicator of stigma receptivity and to locate the receptive stigmatic surface (Bernhardt 1983).

The pollen-stigma relationship depends not only on the genetic interaction of both partners as dictated by incompatibility systems and on stigma receptivity, but also on pollen viability (Dafni et al. 2005). Pollen viability is 1 measure of male fertility; if a majority of pollen grains deposited on a receptive stigma are inviable, the probabilities of fruit and seed set are greatly diminished. Any failure of a pollen grain to germinate on an appropriate stigma and to later fertilize an ovule results in an unsuccessful pollination event. Evaluation of pollen viability and longevity is therefore an important first step in understanding the chances of a given pollen grain to germinate on the stigma (Dafni et al. 2005). In general, pollen germination is most successful just after anthesis and decreases with pollen age (Kearns and Inouye 1993).

In order to gain a better understanding of the breeding biology of *H. boreale*, the following questions are addressed: 1) is insect visitation required for effective pollination of *H. boreale* flowers; 2) do *H. boreale* fruit and seed yields increase in the presence of insect pollinators; 3) is *H. boreale* self-compatible, and if so, does *H. boreale* reproduce primarily via self- or cross-pollination; 4) when does the *H. boreale* stigma become

receptive and how long does it remain receptive; and 5) how long do *H. boreale* pollen grains remain viable following dehiscence?

In general, moving a plant species from patchy dispersion in natural populations to large dense stands in agricultural settings requires supplemental pollination (Free 1993). Therefore, once the breeding biology of a crop species has been determined, a next logical step is to assess which pollinators to supply if necessary, and how. Bees and legumes have been intimately associated with one another throughout their evolutionary histories. Bees are the principal pollinators of many legume species; similarly, legumes provide a major source of food for bees because they form 1 of the largest groups of angiosperms (Kalin Arroyo 1981). Pollen is a rich source of protein and, when mixed with nectar, is the food source fed to bee larvae; consequently, pollen is a very important resource for nesting female bees. Likewise, concentrations and quantities of nectar are important in the energy budget of pollinators. Nectar is the resource fueling bee foraging flights and is the carbohydrate in larval diets (Fægri and van der Pijl 1979). By estimating how much pollen and nectar bees need to sustain themselves and their progeny (see Chapter 4), and by quantifying average floral resources produced per acre or hectare of crop, it is possible to estimate an appropriate bee stocking density. The objective is to introduce an optimal number of female bees to provide sufficient pollination services (i.e. high fruit and seed yields) without exceeding carrying capacity and diminishing bee reproductive output (Richards 1996). Therefore, by estimating stocking density, both adequate pollination and sustainable bee reproduction can be obtained.

The objectives of this research are twofold: 1) to better understand the breeding biology of *H. boreale* to guide more consistent, productive, and cost-effective production

of its seed; and 2) to begin the process of estimating stocking densities of effective bee pollinators for commercial production of *H. boreale* seed via quantification of floral resources per acre of crop.

MATERIALS AND METHODS

Studies were conducted at 7 natural populations and 3 cultivated stands of *H. boreale* in Utah and Wyoming (Table 2-1). Plants of *H. boreale* at the Bee Biology and Systematics Laboratory were grown from seeds collected at Paradise and Wood Camp Hollow in northern Utah (Table 2-1; BBSL, PRD, and WCH). A 2-acre stand of certified 'Timp' Utah sweetvetch near Worland, Wyoming and a common garden experiment at Evans experimental farm near Logan, Utah were kindly made available for this research (Table 2-1; WRL and EF). The stand at EF had 400 plants from 6 *H. boreale* cultivars including 'Timp.'

Breeding Biology of *H. boreale*

BREEDING SYSTEM.—Fifteen plants at PRD were haphazardly chosen in early June 2003 to experimentally assess the breeding system of *H. boreale*. Five racemes at the bud stage were selected on each plant and marked with colored plastic tags to indicate treatment; four racemes were covered with small mesh bags (mesh size < 1 mm^2) to exclude insect visitors. Each plant received the following treatments: 1) positive control (open visitation), racemes were not bagged while in bloom, but were bagged at the end of the experiment to prevent loss of mature fruits; 2) autogamy, bagged flowers were marked with a black permanent marker on the standard petal at anthesis, but were

Table 2-1. Natural populations and cultivated stands of *H. boreale* arranged by state and county, with letter codes, and location and elevation data.

STATE: County	Site name	Letter code	Latitude, longitude	Elevation (m)
Natural populations				
UT: Cache	Cottonwood Canyon	CWC	41°53.41 N, 111°40.33 W	2670
UT: Cache	Mill Hollow	MH	41°44.97 N, 111°43.25 W	1640
UT: Cache	Paradise	PRD	41°34.54 N, 111°47.80 W	1609
UT: Cache	Wood Camp Hollow	WCH	41°48.42 N, 111°39.10 W	1743
UT: Weber	North Ogden Canyon	NOC	41°19.07 N, 111°56.04 W	1542
WY: Teton	Atherton Campground	ATC	43°38.45 N, 110°31.16 W	2207
WY: Teton	Horsetail Creek	HTC	43°38.26 N, 110°29.99 W	2124
Cultivated stands				
UT: Cache	Bee Biology and Systematics Lab	BBSL	41°45.27 N, 111°48.43 W	1408
UT: Cache	Evans Farm 'Timp'	EF	41°41.72 N, 111°49.91 W	1274
WY:Washakie	Worland 'Timp'	WRL	43°57.08 N, 107°58.48 W	1347

otherwise untouched throughout the experiment; 3) geitonogamy, bagged flowers were marked at anthesis and hand pollinated with pollen from another flower on the same plant; 4) xenogamy, bagged flowers were marked at anthesis and hand pollinated with pollen from a plant located at least 10 m away; and 5) distant-xenogamy, bagged flowers were marked at anthesis and hand pollinated with pollen from a plant located at least 100 m away. For manually pollinated flowers, a dry plant stem was first brushed across the stigma to disrupt any surface membrane and separate the stigmatic papillae before pollen was applied. Flowers were treated every 2 days beginning on 4 June. On each visit, flowers treated during the previous visit were re-treated to control for any effect of flower age on stigma receptivity. Treatment racemes were secured on 12 June and were individually collected in brown paper sacks once fruits were mature. The total numbers of fruits (loments), articles, and seeds produced on each treatment raceme were counted. Underdeveloped and fully developed articles were counted separately. Fully developed

articles were scored for the presence of a mature seed. This experiment, excluding the distant-xenogamy treatment, was repeated between 13 June and 2 July 2003 using 13 haphazardly selected plants at WCH.

Mature seeds from hand pollination experiments at PRD and WCH in 2003 were tested for germination during the spring of 2004. Seeds were cold, moist stratified at 4°C for 1 month prior to germination trials. Seeds from each treatment raceme were combined and soaked for 24 hours at room temperature in a 15 x 125 mm glass test tube filled with distilled water. The contents of each test tube were poured into a glass Petri dish and the article removed from each seed using forceps. Naked seeds were placed in a blender with a small amount of water (approximately 1:1 seeds/water); the blender was pulsed 3 to 4 times in order to scarify the seed coat. Scarification was confirmed visually. Seeds from each treatment raceme were then placed in a 100 x 15 mm polystyrene Petri dish with a circular sheet of blotter paper (83 mm diameter). The blotter paper was kept moist during the experiment using distilled water with Thiram fungicide. Seeds were incubated at 22°C and were monitored daily for germination.

TIMING AND DURATION OF STIGMA RECEPTIVITY.—Twenty-five plants at PRD were haphazardly chosen in early June 2004 to experimentally assess the general timing and duration of stigma receptivity in *H. boreale*. On 12 June, 6 racemes at the bud stage were chosen per plant, marked with colored plastic tags to indicate treatment, and bagged to exclude insect visitors. Treatments included the following: 1) positive control (open visitation), 1 raceme per plant was left open during bloom and was bagged at the end of the experiment to prevent loss of mature fruits; 2) bagged negative control, 1 raceme per plant remained bagged throughout the experiment; 3) morning visitation, 2

racemes per plant were exposed to pollinators once from 9-11 AM and were rebagged for the remainder of the experiment; and 4) afternoon visitation, 2 racemes per plant were exposed to pollinators once from 2-4 PM and were rebagged for the remainder of the experiment.

The effect of flower age on stigma receptivity was also assessed during this experiment using 4 flower age classes, including 0–24, 24–48, 48–72, and over 72 hours old. Starting on 13 June, morning visitation treatment racemes were examined just prior to 9 AM each day, and all newly opened flowers counted and marked for identification with a line of colored permanent marker ink applied along the base of the flower and pedicel into the stem. Similarly, afternoon visitation racemes were examined each day just prior to 2 PM and all newly-opened flowers counted and marked with ink. Four colors of ink were used in sequence to designate the day of application. Once a raceme had accumulated flowers in all 4 age classes, it was unbagged and exposed to pollinators during its designated morning or afternoon time period, and then re-bagged and left to mature fruits. Racemes were opened to visitation from 17 to 23 June. Racemes were collected on 8 July and placed individually in brown paper sacks. Fruits produced by flowers in each age class were counted for each treatment raceme.

The precise timing of stigma receptivity in *H. boreale* was evaluated using an enzymatic indicator of receptivity (α-naphthyl acetate with fast blue B salt; Mattsson et al. 1974, Kearns and Inouye 1993). Several *H. boreale* plants were grown from seed collected at WCH in August 2004. Seeds were germinated as described above, and grown in forestry propagation cells with 1:2 peat/sand mixture without fertilizer. Seedlings were moved to 1 gallon pots with soilless media (composed of equal parts peat, vermiculite,

sand, and Turface®, plus MagAmp®K, Osmocote®, and iron sulfate) in early spring. On 18 April 2005, greenhouse lights were set to provide daylight hours experienced on 1 June in Logan, Utah (Table 2-1; see BBSL coordinates). Plants began to bloom in early May. Separate trials were run to determine when the *H. boreale* stigma becomes receptive relative to 1) anthesis (trial A) and 2) anther dehiscence (trial B). Four flowers ranging from a mature bud to a fully open flower were tested for stigma receptivity in trial A; all 4 flowers had dehiscent anthers. Five flowers ranging from an immature bud with indehiscent anthers to a fully open flower with dehiscent anthers were tested for stigma receptivity in trial B; all flowers past the mature bud stage (n = 3) had dehiscent anthers. Another trial (C) was run to confirm results of the field experiment described above. In trial C, flower age classes were marked for 4 days prior to testing using colored permanent markers. One flower in each 24-hour age class was then chosen from replicate racemes on 3 plants (n = 12) and tested for receptivity. During each trial, 2.5 mg of α-naphthyl acetate (the substrate) was dissolved in 3 drops of acetone and mixed with 5 ml of phosphate buffer (0.1 M, pH 7.0) in a capped glass vial. Fast blue B (12.5 mg) was added to the solution, which was then vigorously shaken. A drop of solution was added to fresh *H. boreale* stigmas placed individually on precleaned glass slides. Stigmas were examined for a strong red coloration of receptive areas using a compound microscope at 20X. During each trial, a control solution including everything but the substrate was prepared and added to a fresh stigma matching the developmental stage of each experimental stigma.

POLLEN VIABILITY AND LONGEVITY.—Pollen viability and longevity were tested using an *in vitro* germination test described by Kearns and Inouye (1993). Six

‘Timp’ plants at EF were haphazardly chosen in 2005. Two secondary racemes at the bud stage were chosen on each plant, marked with colored plastic tags, and bagged to exclude insects. The effect of age on pollen viability was assessed using 4 flower age classes, including 0–24, 24–48, 48–72, and over 72 hours old; pollen age was scored as flower age in this experiment. Racemes were examined daily at 4 PM from 16 to 19 June. Each day, newly opened flowers were marked for identification with a line of colored permanent marker ink. Four colors of ink were used to designate the day of application. Racemes were marked and collected at 4 PM on 19 June and brought to the laboratory in floral water picks. One flower representing each age class was chosen, using the uppermost flower if several were available in any given age class. Therefore, pollen samples were obtained from the younger flowers within an age class, representing new, and approximately 24-, 48-, and 72-hour old pollen. Anthers were cut and dropped into 0.65-ml micro-centrifuge tubes with 250 µl of sterile water with a surfactant (0.02% Tween-80). Tubes were vortexed for 30 seconds to remove pollen from the anthers. Agar plates (100 x 50 mm) were prepared with boric acid and calcium nitrate after Kearns and Inouye (1993). Fifty µl of suspension was pipetted on to an agar plate and spread with a sterile media spreader. Plates were sealed with parafilm and incubated at 25°C for 48 hours, and then inverted and stored at 4°C. Plates were examined under a stereomicroscope at 125X. Germinated and un-germinated pollen grains were counted in 10 fields of view haphazardly chosen with closed eyes and controlled movement of the agar plate on the microscope stage. This experiment was repeated between 6 and 9 July 2005 using 6 haphazardly chosen plants at WCH.

Floral Resources

FLORAL RESOURCES PER FLOWER.—In order to estimate the number of pollen grains produced per flower, 10 plants at several sites were chosen in 2005 by blindly tossing a surveyor's flag over the shoulder; the flowering plant located closest to the flag was chosen. Two racemes on each plant were bagged at the bud stage; 1 new flower per raceme was subsequently collected and placed in a well of a tissue culture plate for transport to the laboratory. Standard and keel petals were carefully removed using forceps, and anthers cut into pre-cleaned 4 fl oz. capped glass vials with 25 ml of filtered ethanol. Samples were sonicated for up to 300 seconds, at which time anthers were examined microscopically to confirm complete pollen removal. Anthers were then removed using clean forceps, and 25 additional ml of filtered ethanol was added to each vial. Samples were sonicated for 142 seconds on average (n = 10) before 17 ml of the agitated solution were counted using a HIAC Royco Model 8000A Eight Channel Pollen Counter (HIAC/Royco Division of Pacific Scientific Co., Silver Spring, MD, 20904) with an attached HIAC Automatic Bottle Sampler Model ABS (Pacific Scientific Co., Silver Spring, MD 20904). Blank controls consisting of 50 ml of filtered ethanol were sonicated and counted after every 5 samples. Count values were multiplied by 2.94 (=50/17) to calculate the original quantity of pollen in each sample. Pollen samples (1 per plant) were collected from 17 haphazardly chosen plants at WRL on 11 May 2004. Pollen samples (2 per plant) were collected at BBSL, CWC, EF, and WCH in 2005. CWC samples were collected from mature buds instead of new flowers because the inaccessibility of this remote, high elevation site precluded bagging of racemes; mature buds were triangular, oriented perpendicular to the stem, and generally found just above an open flower.

Nectar was extracted from flowers using 1-µl disposable micro-pipettes (Drummond Scientific Co.). The sugar concentration of nectar samples was determined using 0-50% and 40-85% hand-held sugar refractometers modified for small volumes (Bellingham & Stanley Ltd., Tunbridge Wells, England); however, sugar concentrations could not be reliably obtained for flowers with ≤ 0.1 µl of nectar. In 2004, 20 samples were haphazardly chosen at BBSL from bagged racemes on several plants. In 2005, bagged racemes on 9 plants were allowed to accumulate nectar for several days. On 1 raceme per plant, nectar samples were extracted from 10 flowers starting with the uppermost open flower on each raceme and progressing downward.

FLORAL RESOURCES PER PLANT.—In order to estimate flower production by *H. boreale*, plants at several sites were chosen in 2005 using the surveyor's flag technique described previously. The total numbers of reproductive stems and racemes were counted on each plant. Five stems were then haphazardly chosen by reaching into the base of the plant with closed eyes. On each stem, the following flower stages were counted on all racemes beginning with the primary: buds, open flowers, finished flowers, bracts and/or flower abscission scars, and other. Finished flowers included those past their prime (i.e. the discolored standard petal was returning to the closed position) and fruits. Other flower stages included aborted buds and clusters of buds that failed to mature. Ten plants each at WCH, Atherton Campground in Teton County, Wyoming (Table 2-1; ATC), and Cottonwood Canyon in Cache County, Utah (Table 2-1; CWC) were sampled on 9, 13, and 29 July, respectively. Due to time constraints during the field season, flower production by 10 plants at PRD was estimated post bloom, while senesced biomass was collected from 6 plants at WRL on 18 August. Again, all reproductive stems

and racemes were counted on plants from PRD and WRL; bracts and/or flower scars were counted on a subset of racemes located on 5 haphazardly chosen stems.

FLORAL RESOURCES PER NATURAL POPULATION AND CULTIVATED STAND.—Population size was estimated during surveys of bee faunas at several natural populations of *H. boreale* (see Chapter 3). In order to estimate cultivated stand density, 2 acres of certified 'Timp' Utah sweetvetch at WRL were surveyed on 11 May 2004. Field dimensions were measured. Two rows of *H. boreale* were haphazardly chosen and counted; plants with flowers were tallied separately from those still in bud.

Data Analysis

In general, data are reported as mean ± standard error ($X \pm s_x$) unless otherwise indicated. ANOVA degrees of freedom are given in subscript brackets. A probability level of $P \leq 0.05$ was deemed significant in all analyses.

BREEDING SYSTEM.—The null hypothesis that frequency of fruit set was independent of pollination treatment at each site was tested with an R x C Test of Independence using the G-Test with William's correction (Sokal and Rohlf 1995). Data from racemes that set no fruit then were excluded from subsequent analyses. Proportional fruit set on racemes that set ≥ 1 fruit was calculated by dividing the number of fruits set by flowers treated. Proportional fruit set data from each site were individually analyzed using a completely randomized block design ANOVA with pollination treatment as a fixed factor and plant (block) as a random factor using the PROC GLM procedure of SAS Version 9.1 (SAS Institute Inc. 2004). Data from PRD and WCH were then combined and analyzed using a mixed model ANOVA with pollination treatment and site

as fixed factors and plant as a random factor using the PROC MIXED procedure of SAS Version 9.1; because distant-xenogamy pollinations were not preformed at WCH, these data were excluded from combined analyses. Treatment means were compared using REGWQ and Tukey-Kramer a posteriori tests in PROC GLM and PROC MIXED, respectively.

The proportion of fully developed articles was calculated by dividing the number of fully developed articles by the total number of all articles produced on a treatment raceme. Proportional seed development was calculated by dividing the number of fully developed articles with a mature seed by the total number of all fully developed articles produced on a treatment raceme. Proportional article and seed development data were analyzed in the same manner as proportional fruit set data (ANOVA only).

Proportional seed germination was calculated by dividing the number of mature seeds that germinated by the total number of mature seeds produced on a treatment raceme. Proportional seed germination data were analyzed in the same manner as proportional fruit set data (ANOVA only).

Proportional fruit set, article development, and seed development data from PRD and combined analyses were satisfactorily normalized using cube root transformations; the same data from WCH were normalized using $\log_{10}$ transformations. All seed germination data sets were normalized using arcsine square root transformations.

TIMING AND DURATION OF STIGMA RECEPTIVITY.—The null hypothesis that frequency of fruit set was independent of flower age was tested with an *R* x *C* Test of Independence using the *G*-Test with William's correction. Data then were excluded from subsequent analyses when 1) there were no flowers available in an age class, and 2) there

were no fruits set by flowers in an age class. Proportional fruit set was calculated by dividing the number of fruits set by the number of flowers available in an age class. Data were first analyzed in PROC MIXED using a mixed model ANOVA with visitation time (AM or PM) and flower age class as fixed factors and plant as a random factor. Data were then analyzed in PROC MIXED using a mixed model ANOVA with visitation date as an additional fixed factor; only data collected on 19, 20, and 22 June were used in this analysis as 76% of all visitation treatment racemes were opened to insect visitors on these dates. Treatment means were compared using Tukey-Kramer a posteriori tests.

POLLEN VIABILITY AND LONGEVITY.—Proportional pollen germination was calculated by first dividing the number of germinated pollen grains by the total number of pollen grains within a field of view, and then by averaging across 10 replicate fields of view. Proportional pollen germination data from each site were individually analyzed in PROC MIXED using a completely randomized block design ANOVA with pollen age class as a fixed factor and plant (block) as a random factor. Data from EF and WCH sites were then combined and analyzed in PROC MIXED using a mixed model ANOVA with site as an additional fixed factor. Treatment means were compared using Tukey-Kramer a posteriori tests. Data from EF, WCH, and combined analyses were satisfactorily normalized using square root transformations.

FLORAL RESOURCES.—Floral resource data sets (pollen grains/flower, racemes/plant, racemes/stem, and flowers/raceme) were individually analyzed in PROC GLM using completely randomized design ANOVA with site as a fixed factor and plant as a random factor. Treatment means were compared using REGWQ a posteriori tests.

Data sets for racemes/plant and racemes/stem were satisfactorily normalized using $\log_{10}$ transformations.

RESULTS

Breeding Biology of *H. boreale*

BREEDING SYSTEM.—Patterns of fruit set in response to pollination treatments were similar at both sites (Fig. 2-1). Autogamy yielded no fruit at either site (n = 705 flowers). Positive control, geitonogamy, and xenogamy treatments yielded some fruit on treated racemes at both sites, and distant-xenogamy yielded fruit at PRD. Frequency of fruit set depended on pollination treatment at both sites (PRD, n = 1417 flowers, G_{adj} = 454.25, df = 4, $P << 0.001$; WCH, n = 784, G_{adj} = 213.11, df = 3, $P << 0.001$).

Proportional fruit set on racemes that set ≥ 1 fruit varied with pollination treatment at the 2 sites (PRD, $F_{[3,40]} = 11.28$, $P < 0.0001$; WCH, $F_{[2,18]} = 13.58$, $P = 0.0003$) (Table 2-2). At PRD, positive controls yielded higher proportions of fruit than did geitonogamy, xenogamy, and distant-xenogamy treatments. At WCH, xenogamy yielded proportionally fewer fruits than both positive control and geitonogamy treatments. Combining data across sites, proportional fruit set again varied with pollination treatment ($F_{[2,39.7]} = 23.15$, $P < 0.0001$). All 3 pollination treatments were statistically different; positive controls yielded higher proportions of fruit than did geitonogamy ($P = 0.0025$) and xenogamy ($P < 0.0001$) treatments, while geitonogamy resulted in marginally more fruits than xenogamy ($P = 0.0390$).

On positive control racemes, mean numbers of articles per fruit were nearly identical at the 2 sites (2.56 ± 0.20 at PRD and 2.54 ± 0.16 at WCH). Geitonogamy,

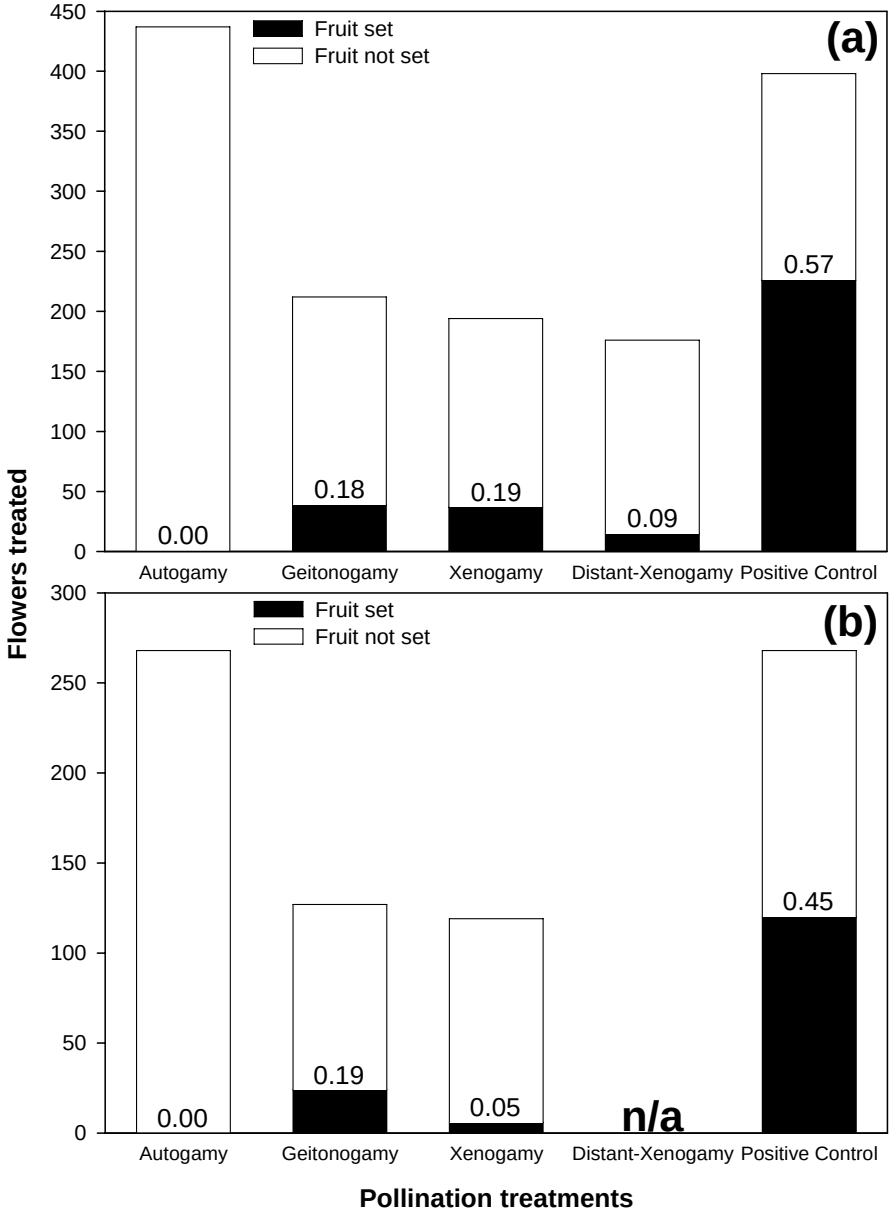

Fig. 2-1. Frequencies of fruit set for 5 breeding system treatments at Paradise and Wood Camp Hollow in 2003. The average number of flowers treated per raceme differed among treatments at both sites. (a) At PRD, an average of 29 flowers per raceme were treated on autogamy racemes, 14 on geitonogamy, 13 on xenogamy, 12 on distant-xenogamy, and 27 on positive controls. (b) At WCH, treated racemes averaged 24 flowers for autogamy, 12 for geitonogamy, 11 for xenogamy, and 24 for positive controls.

Table 2-2. Mean proportional fruit set (± standard error) on racemes that 1 or more fruits for 4 breeding system treatments at Paradise and Wood Camp Hollow in 2003. Sample sizes: PRD, positive control (n = 15 racemes), geitonogamy (9), xenogamy (12), distant-xenogamy (8); WCH, positive control (11), geitonogamy (5), xenogamy (5). Treatment means followed by different letters are statistically different ($P \leq 0.05$).

	PRD		WCH	
Pollination Treatment	$X \pm s_x$	Range	$X \pm s_x$	Range
Positive Control	$0.59^{a} \pm 0.05$	0.27 – 0.88	$0.48^{a} \pm 0.06$	0.25 – 0.85
Geitonogamy	$0.26^{b} \pm 0.05$	0.08 – 0.63	$0.40^{a} \pm 0.16$	0.15 – 1.0
Xenogamy	$0.25^{b} \pm 0.04$	0.07 – 0.41	$0.11^{b} \pm 0.05$	0.08 – 0.18
Distant-Xenogamy	$0.22^{b} \pm 0.08$	0.07 – 0.57	—	—

Table 2-3. Mean probability (± standard error) that an article is fully developed for 4 breeding system treatments at Paradise and Wood Camp Hollow in 2003. Sample sizes are identical to those in Table 2-2. Treatment means followed by different letters are statistically different ($P \leq 0.05$); treatment means were not significantly different at WCH.

	PRD		WCH	
Pollination Treatment	$X \pm s_x$	Range	$X \pm s_x$	Range
Positive Control	$0.81^{a} \pm 0.02$	0.67 – 0.92	0.89 ± 0.03	0.72 – 1.0
Geitonogamy	$0.72^{a} \pm 0.05$	0.54 – 1.0	0.97 ± 0.03	0.86 – 1.0
Xenogamy	$0.84^{a} \pm 0.06$	0.43 – 1.0	0.88 ± 0.12	0.40 – 1.0
Distant-Xenogamy	$0.96^{b} \pm 0.04$	0.71 – 1.0	—	—

xenogamy, and distant-xenogamy yielded means of 2.51 ± 0.41, 1.62 ± 0.16, and 1.91 ± 0.20 articles per fruit at PRD. At WCH, geitonogamy and xenogamy yielded somewhat fewer articles per fruit (1.74 ± 0.32 and 1.50 ± 0.32). Proportional article development xenogamy, and distant-xenogamy yielded means of 2.51 ± 0.41, 1.62 ± 0.16, and 1.91 ± 0.20 articles per fruit at PRD. At WCH, geitonogamy and xenogamy yielded somewhat varied with pollination treatment at PRD ($F_{[3,40]} = 7.09$, $P = 0.0006$), but was similar at WCH ($F_{[2,18]} = 1.16$, $P = 0.3356$) (Table 2-3). At PRD, distant-xenogamy resulted in more fully developed articles per fruit than positive control, geitonogamy, and xenogamy

Table 2-4. Mean probability (± standard error) that a fully developed article yields a mature seed for 4 breeding system treatments at Paradise and Wood Camp Hollow in 2003. Sample sizes: PRD, positive control (n = 13 racemes), geitonogamy (9), xenogamy (12), distant-xenogamy (8); WCH, positive control (9), geitonogamy (4), xenogamy (5). Treatment means were not significantly different at either site ($P \leq 0.05$).

	PRD		WCH	
Pollination Treatment	$X \pm s_x$	Range	$X \pm s_x$	Range
Positive Control	0.73 ± 0.05	0.43 – 1.0	0.66 ± 0.03	0.57 – 0.82
Geitonogamy	0.66 ± 0.10	0.08 – 1.0	0.71 ± 0.11	0.50 – 1.0
Xenogamy	0.81 ± 0.06	0.50 – 1.0	0.90 ± 0.10	0.50 – 1.0
Distant-Xenogamy	0.71 ± 0.11	0.33 – 1.0	—	—

treatments. When data were combined, site was the only significant fixed effect ($F_{[1,25.6]}$ = 11.48, $P < 0.0023$).

Fully developed articles did not always yield mature seeds. The probability of a fully developed article yielding a mature seed did not vary with pollination treatment at either site (PRD, $F_{[3,38]} = 0.56$, $P = 0.6477$; WCH, $F_{[2,15]} = 2.71$, $P = 0.0990$) (Table 2-4). When data were combined across sites, however, pollination treatment was a significant fixed effect ($F_{[2,35.6]} = 4.01$, $P = 0.0268$); fruits on xenogamy racemes were more likely to have articles with mature seeds than fruits on positive control racemes ($P = 0.0278$).

Patterns of seed germination were similar at the 2 sites, although significant treatment differences in proportional germination occurred for seeds from WCH only (PRD, $F_{[3,24]} = 1.35$, $P = 0.2810$; WCH, $F_{[2,5]} = 25.61$, $P = 0.0024$) (Fig. 2-2). At WCH, proportionately more seeds from xenogamy germinated than seeds from positive control and geitonogamy racemes. When data were combined, significant treatment differences were again observed ($F_{[2,30.8]} = 12.40$, $P < 0.0001$); a greater proportion of xenogamy seeds germinated than seeds from positive control ($P = 0.0007$) and geitonogamy ($P = 0.0002$) racemes.

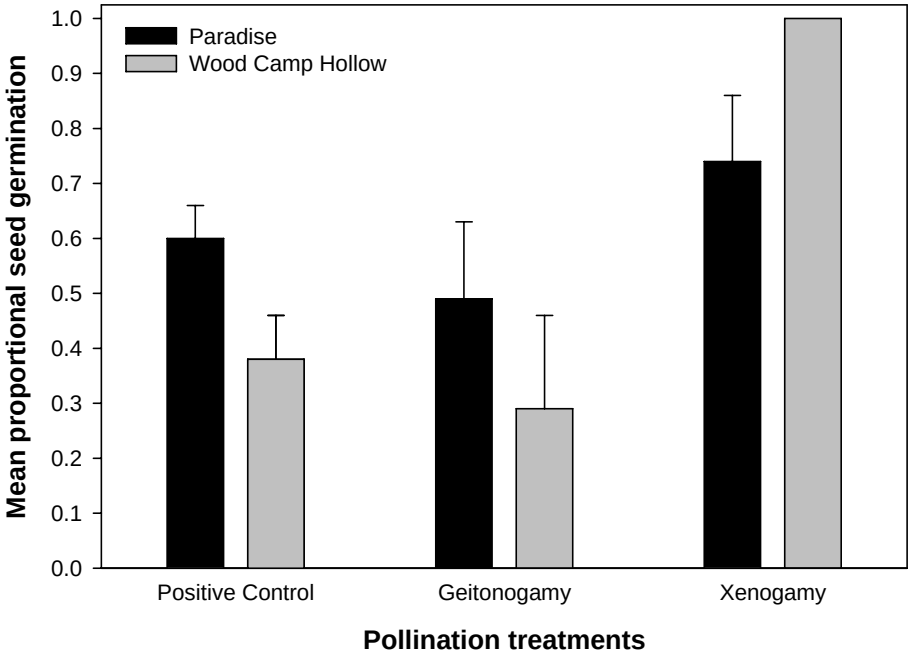

Fig. 2-2. Mean proportional seed germination (+ standard error) for 3 breeding system treatments at Paradise and Wood Camp Hollow in 2003. Distant-xenogamy data are not presented; mean proportional germination of distant-xenogamy seeds at PRD was 0.63 ± 0.16 (n = 8 racemes, 17 seeds). Sample sizes: PRD, positive controls (n = 13 racemes, 297 seeds), geitonogamy (8, 26), xenogamy (12, 36); WCH, positive controls (9, 150), geitonogamy (4, 26), xenogamy (4, 5). At WCH, proportionately more seeds from xenogamy germinated than seeds from positive control and geitonogamy racemes ($F_{[2,5]}$ = 25.61, P = 0.0024); treatment means were not significantly different at PRD.

TIMING AND DURATION OF STIGMA RECEPTIVITY.—*H. boreale* stigmas become receptive during the mature bud stage prior to anthesis and remain receptive for several days. Trials A and B were run to determine when the *H. boreale* stigma becomes receptive relative to anthesis and anther dehiscence, respectively. Anthesis refers to the period of time when a flower is fully open and functional; in this study, it was assumed that anthesis in *H. boreale* begins when the standard petal is fully open. In *H. boreale*, the anthers dehisce (release pollen) simultaneously in the bud and pollen is available at

anthesis (Tepedino and Stackhouse 1987). In trial A, all 4 experimental flowers (ranging from a mature bud to a fully open flower) exhibited a distinct red color at the tip of the style indicating receptivity. In trial B, the stigmas of 2 immature buds with indehiscent anthers exhibited no red coloration at the tip of the style; on the other hand, a mature bud with dehiscent anthers was very red compared to the 2 immature buds. Therefore, the *H. boreale* stigma appears to become receptive prior to anthesis, at approximately the same time that the anthers dehisce. None of the control flowers in trial A exhibited any red coloration; conversely, 4 of 5 controls in trial B exhibited some black discoloration near the base of the stigmatic papillae.

Trial C was run to determine how long the *H. boreale* stigma remains receptive. The style tips of all 12 flowers (3 flowers per 24-hour age class) were redder than controls. Thus, it appears that the stigma remains receptive for several days after anthesis. The duration of stigma receptivity was also assessed during the field trial at PRD in 2004. A quarter of racemes set ≥ 1 fruits in the oldest floral age class, indicating that the stigma is still receptive 72 hours after anthesis. However, frequency of fruit set depended on flower age (n = 1461 flowers, G_{adj} = 116.96, df = 3, $P << 0.001$) (Fig. 2-3). Percent fruit set for age classes that set ≥ 1 fruits varied with age class in both analyses (excluding date as a fixed effect, $F_{[3,46.8]} = 7.37$, $P = 0.0004$; with date, $F_{[3,57.1]} = 5.35$, $P = 0.0026$). Younger flowers (0 – 24 hours old) were more likely to yield fruit than older flowers (48 – 72 hours old, $P = 0.0006$; over 72 hours old, $P = 0.0106$). Time of day was not a significant effect in either analysis (excluding date, $F_{[1,32]} = 1.12$, $P = 0.2920$; with date, $F_{[1,80.2]} = 0.02$, $P = 0.8965$); however, there was a marginally significant interaction between time of day and date in the second analysis ($F_{[2,81]} = 3.39$, $P = 0.0385$).

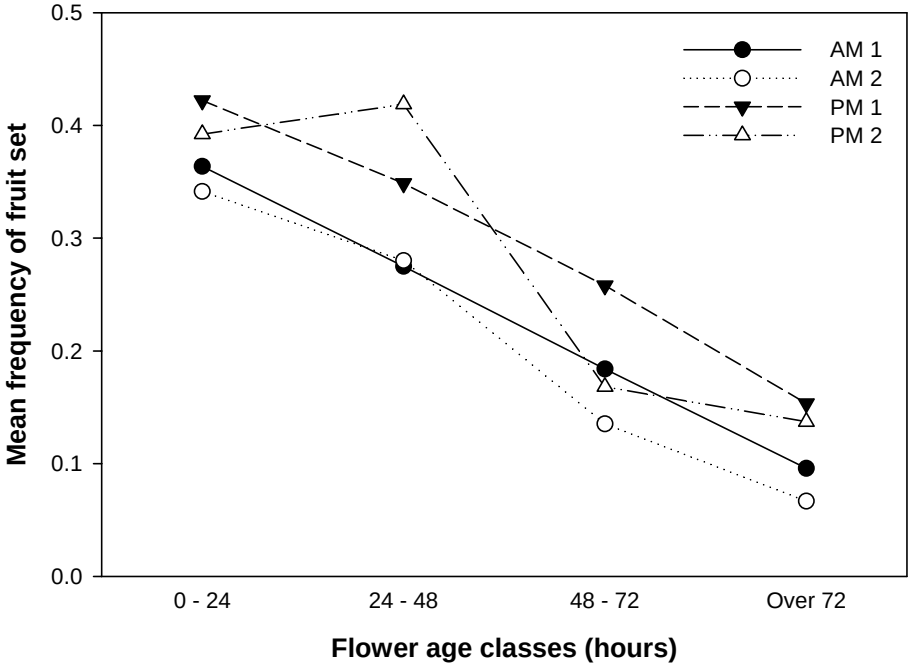

Fig. 2-3. Mean frequencies of fruit set for 4 flower age classes during morning (AM) and afternoon (PM) visitation periods at PRD in 2004. Frequencies were calculated by dividing fruits set by flowers available in an age class.

POLLEN VIABILITY AND LONGEVITY.—On average, only 23% of new pollen grains from plants at WCH germinated. Pollen viability decreased as pollen age increased at WCH; on average, only 18, 18, and 10% of approximately 24-, 48-, and 72-hour old pollen grains were viable. At WCH, there were significant differences between pollen age classes in percent germination ($F_{[3,15.3]} = 5.47$, $P = 0.0094$). Zero- to 48-hour old pollen germinated more often than did pollen ≥ 72 hours old. At EF, differences in germination between pollen age classes were only marginally significant ($F_{[3,15.2]} = 3.41$, $P = 0.0446$). There were significant differences between pollen age classes when data were combined across sites ($F_{[3,30.4]} = 6.34$, $P = 0.0018$). Pollen up to 72 hours old

germinated more often than did pollen older than 72 hours. There was a significant interaction between site and pollen age class, however ($F_{[3,30.4]} = 3.24$, $P = 0.0355$). Percent germination decreased steadily with pollen age at WCH, whereas germination at EF appeared to peak 2 days after anthesis and then decreased sharply.

Floral Resources

FLORAL RESOURCES PER FLOWER.—In general, *H. boreale* flowers produced large numbers of tiny pollen grains measuring < 30 microns in length. The number of pollen grains produced per flower varied among plants at a site and between plants at different sites (Table 2-5). Differences between sites were significant ($F_{[8,88]} = 2.81$, $P = 0.0079$). Plants at BBSL produced 1.5–1.8 fold more pollen grains per flower than did plants at EF and WCH. Several samples from EF and WCH had to be excluded from analyses due to deformed flower parts and/or excessive consumption of pollen by thrips.

In 2004, 20 haphazardly chosen flowers from bagged racemes on several plants at BBSL yielded 0.24 ± 0.02 µl of nectar per flower on average (range = 0.08 – 0.46). In 2005, nectar accumulated in bagged, unvisited flowers at BBSL, sometimes to a very large degree (over 2 µl) (Table 2-6). During the stigma receptivity experiment at PRD in 2004, approximately 3.6 ± 0.1 flowers per raceme opened during each 24-hour period ($n = 400$, range = 0 – 11). The first 4 flowers per raceme at BBSL in 2005 produced 0.29 ± 0.03 µl of nectar on average ($n = 36$, range = 0 – 0.61). Overall, the 9 plants sampled at BBSL in 2005 yielded nectar with 59.7 ± 2.1% sugar content on average (range = 45 – 66).

Table 2-5. Mean number of pollen grains (± standard error) produced by individual flowers collected at 2 natural populations and 3 cultivated stands of *H. boreale*. Two pollen samples per plant were taken from separate racemes at all sites except WRL where 1 sample was collected per plant. Treatment means followed by different letters are statistically different ($P \leq 0.05$).

		Number of pollen grains	
Site	*n*	$X \pm s_x$	Range
BBSL[1]	24	104,865[a] ± 5396	46,397 – 141,621
CWC[2]	26	87,945[a,b] ± 5440	36,697 – 139,938
EF[1] 'Timp'	14	59,140[c] ± 9331	15,850 – 126,568
WCH[2]	16	71,884[b,c] ± 6454	25,426 – 117,168
WRL[1] 'Timp'	17	87,061[a,b] ± 9521	30,424 – 180,741

[1]cultivated stand
[2]natural population

Table 2-6. Mean nectar in µl (± standard error) produced by consecutive flowers on bagged racemes from plants at the Bee Biology and Systematics Laboratory. Sample sizes: $n = 9$ racemes from separate plants. Flower 1 was the uppermost, and youngest, open flower on a raceme; flowers 2-10 progressed down the raceme in that order.

	µl of nectar			µl of nectar	
Flower	$X \pm s_x$	Range	Flower	$X \pm s_x$	Range
1	0.11 ± 0.04	0.0 – 0.35	6	0.52 ± 0.06	0.19 – 0.72
2	0.30 ± 0.06	0.0 – 0.59	7	0.56 ± 0.09	0.16 – 0.94
3	0.35 ± 0.06	0.08 – 0.58	8	0.72 ± 0.18	0.16 – 2.03
4	0.41 ± 0.07	0.07 – 0.61	9	0.88 ± 0.27	0.20 – 2.98
5	0.37 ± 0.06	0.09 – 0.68	10	0.77 ± 0.19	0.14 – 1.99

FLORAL RESOURCES PER PLANT.—Plants varied greatly in terms of overall flower production. Some plants produced only a few racemes while others produced hundreds of racemes (Table 2-7). Cultivated plants at WRL produced 4– to 6–fold more racemes than plants at ATC, CWC, PRD, and WCH ($F_{[4,41]} = 8.21$, $P < 0.0001$). The number of racemes produced per stem also varied between plants at different sites ($F_{[4,41]} = 2.87$, $P = 0.0349$) (Table 2-7). Plants at CWC generally had stems with only primary

Table 2-7. Mean values (± standard error) for 3 measures of floral resource production by *H. boreale* plants at 4 natural populations and 1 cultivated stand. Sample sizes: racemes/plant and racemes/stem, $n = 10$ plants (except WRL; $n = 6$); flowers/raceme, n = # racemes found on 5 stems/plant. Treatment means followed by different letters are statistically different ($P \leq 0.05$).

	Racemes per plant	Racemes per stem	Flowers per raceme
Site	$X \pm s_x$	$X \pm s_x$	$X \pm s_x$
ATC[1]	$32.40^{a} \pm 9.77$	$2.52^{a,b} \pm 0.30$	$18.00^{a,b} \pm 0.77$
CWC[1]	$33.80^{a} \pm 11.54$	$1.84^{a} \pm 0.19$	$14.92^{a} \pm 2.62$
PRD[1]	$32.30^{a} \pm 5.11$	$2.02^{a,b} \pm 0.15$	$26.97^{c} \pm 2.26$
WCH[1]	$46.80^{a} \pm 7.6$	$2.04^{a,b} \pm 0.14$	$22.32^{b,c} \pm 1.49$
WRL[2]	$196.5^{b} \pm 31.1$	$2.74^{b} \pm 0.22$	$25.16^{b,c} \pm 1.54$

[1]natural population
[2]cultivated stand

and secondary racemes, whereas plants at WRL tended to produce more racemes per stem.

The number of flowers produced per raceme ranged from 5 on a primary raceme at CWC to 52 on a secondary raceme at PRD. The number of flowers produced per raceme varied significantly between plants at different sites ($F_{[4,41]} = 6.83$, $P = 0.0003$) (Table 2-7). Plants at PRD and WRL produced racemes with more flowers than did plants at CWC. Plants at PRD also had more flowers per raceme than plants at ATC. In general, the number of flowers produced per raceme decreased between sequential racemes on a stem; for example, tertiary racemes often had fewer flowers than secondary racemes on the same stem.

FLORAL RESOURCES PER NATURAL POPULATION AND CULTIVATED STAND.—In 2005, natural populations ranged in size from 30 to 1100 flowering plants at CWC and ATC, respectively (Table 3-5). Most natural populations occupied approximately 1 acre or less; however, North Ogden Canyon in Weber County, Utah

(Table 2-1; NOC) and WCH occupied areas larger than 1 acre. The densities of flowering individuals per cultivated acre of *H. boreale* greatly exceeded that of natural populations. The 'Timp' Utah sweetvetch stand at WRL measured 26 by 315 m (8190 m^2 = 2.02 acres) with 35 rows evenly spaced at 0.75 m. Of 305 plants in the first sampled row, 266 were flowering and 39 were still in bud. In the second sampled row, 225 of 268 plants were flowering, while 43 were still in bud. The first sampled row was completely intact, whereas the second had stretches where plants were absent. At 286 plants per row on average, there were 10,010 plants on 2 acres or 5005 plants per acre. In the first and second sampled rows 87 and 84% of plants were flowering, respectively; on average, 85.5% of plants were in bloom. Therefore, there were 4279 flowering plants per acre at WRL on 11 May 2004.

On average, 'Timp' plants at WRL had 196.5 racemes with 25.2 flowers each (Table 2-7). Therefore, plants at WRL produced roughly 4952 (≈5000) flowers each over the course of the growing season. Based on estimates of available bloom made during surveys of bee faunas (see Chapter 3), only 16% of all flowers produced by a plant were available on an average day during mid bloom. With around 800 flowers per plant available daily (=4 flowers per raceme) and 5000 plants per acre, there are approximately new 4,000,000 flowers available per acre of crop on an average day during mid bloom. At 87,000 pollen grains per flower, roughly 3.5×10^{11} pollen grains are available to foraging female bees per acre of crop each day. If the first 4 flowers per raceme produce roughly 0.29 µl of nectar per day, then roughly 2.9×10^{5} µl of nectar are available to foraging females per acre of crop each day.

DISCUSSION

Hedysarum boreale is self-compatible, but requires insect visitation to produce fruits with viable seeds. Autogamy treatments yielded no fruit at either PRD or WCH, whereas geitonogamous pollinations yielded fruits with viable seeds at both sites. Therefore, fruit and seed production in *H. boreale* requires a floral visitor to move pollen, at the very least, within or between flowers on a plant (geitonogamy).

Boyd and Serafini (1992) studied predehiscence and postdehiscence reproductive attrition in the rare California shrub *Fremontodendron decumbens* Lloyd. They characterized bottlenecks that diminished sexual reproduction in *F. decumbens* from pollination to seedling establishment. In *H. boreale*, undeveloped articles, seedless articles, and articles with inviable seeds represent lost reproductive potential on positive control, geitonogamy, and xenogamy racemes (Fig. 2-4). In this study, *H. boreale* flowers to which outcross pollen was manually applied (xenogamy) were inexplicably less likely to set fruits than positive controls and geitonogamy (Fig. 2-1; Table 2-2). However, positive controls and geitonogamy yielded more undeveloped articles, seedless articles, and articles with inviable (ungerminated) seeds than xenogamy racemes (Fig. 2-4). Xenogamy, therefore, appears to offer a distinct advantage over geitonogamy in terms of reduced predehiscence reproductive attrition; fruits resulting from xenogamy are more likely to contain viable seeds than those resulting from geitonogamy. According to Northstrom and Welsh (1970), however, the evolutionary trend in *H. boreale* is toward fewer articles per fruit. Fruit initiation is often limited by pollen availability, whereas maternal resources seem to primarily limit fruit and seed maturation (Ehrlen 1992).

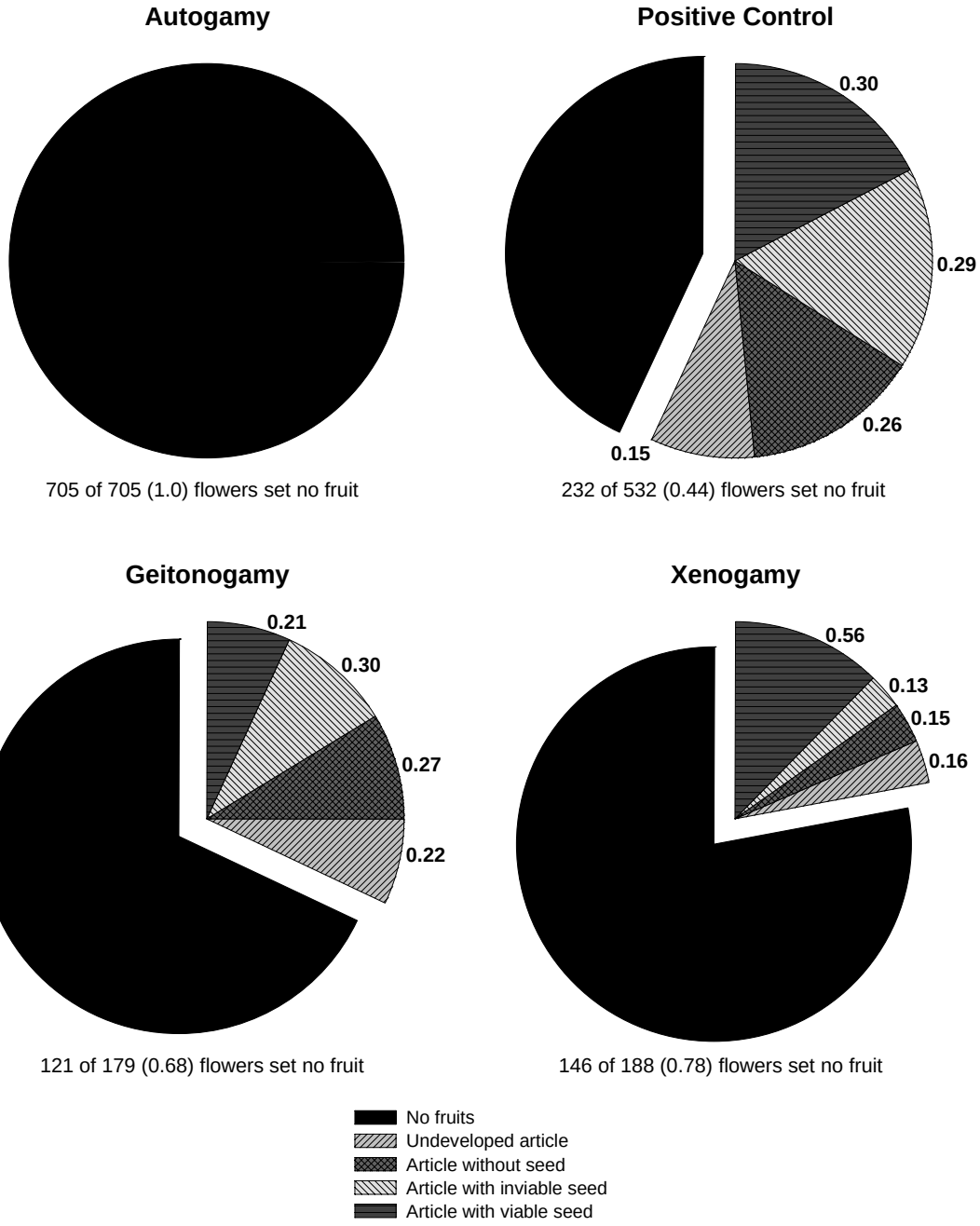

Fig. 2-4. Floral fates for 4 pollination treatments during breeding system experiments at PRD and WCH in 2003. Data from racemes that set ≥ 1 fruit were combined from PRD and WCH. Black wedges represent the proportion of treated flowers that set no fruit. Grey wedges represent the proportions of initiated ovules (summed across all fruits limited maternal resources, the ones most likely to mature are those that were set first, have the most seeds, or result from outcrossing (Stephenson 1981).

In this regard, undeveloped articles and seedless articles in *H. boreale* are likely the result of maternal selection after ovule fertilization (i.e. selective abortion of inferior seeds). In general, when pollinated flowers and immature fruits compete for limited maternal resources, the ones most likely to mature are those that were set first, have the most seeds, or result from outcrossing (Stephenson 1981).

Fabaceae is principally a bee-pollinated family (Kalin Arroyo 1981). Few insects other than bees were observed at *H. boreale* flowers during this study; therefore, all fruits and seeds produced on positive control (open visitation) racemes were assumed to result from bee pollination. In this study, it appears that bee pollination of *H. boreale* was a mixture of geitonogamy and xenogamy. The proportions of seedless articles and articles with inviable seeds were almost identical on positive control and geitonogamy racemes; viable seeds were produced more often on positive control racemes, however (Fig. 2-4). Because seeds resulting from xenogamy are often viable, the higher proportion of viable seeds on positive control racemes suggests that bee pollination of *H. boreale* is a mixture of geitonogamy and xenogamy. Seed germination results also support this idea; at both PRD and WCH, a greater proportion of xenogamy seeds germinated than seeds from positive controls, which in turn exceeded that of geitonogamy racemes (Fig. 2-2).

In this study, bees were superior pollinators of *H. boreale* compared to humans. Proportional fruit set on positive control racemes far exceeded proportional fruit set resulting from hand pollinations (Fig. 2-1; Table 2-2). However, bee pollination resulted in only 57 and 45% overall fruit set at PRD and WCH, respectively, during breeding system experiments at the 2 sites (Fig. 2-1). The development of a small number of fruits relative to flowers produced has been reported often in legumes (Zimmerman and Aide

1989). For example, open pollination treatments resulted in 45.7 and 57.3% fruit set at 2 populations of *H. coronarium* in Tunisia (Louati-Namouchi et al. 2000). In addition, Thomson (1938) observed that only 54% of sainfoin (*Onobrychis viciifolia* Scop) flowers produced fruits and seeds, while Bogoyavlenskii (1955) found than no more than 50% of sainfoin flowers on a raceme set seed (Free 1993). In hermaphroditic species, extra flowers help to increase attractiveness to pollinators, and contribute to increased paternal success through pollen export; extra flowers can also serve as an ovary pool against unforeseeable loss of flowers (Zimmerman and Aide 1989). Butterfly larvae (Lycaenidae), moth larvae (Gelechiidae), and adult grasshoppers were observed to consume *H. boreale* flowers at several sites.

Greater fruit and seed yields on positive control racemes suggest that there is some aspect of the pollination process in *H. boreale* that is difficult to replicate by hand pollination. The miniscule size of the stigma made it impossible to examine the intact living stigmatic surface for the presence of exudate. In general, the style of legume species is hollow with a central canal extending from just below the stigma to the ovary (Heslop-Harrison and Heslop-Harrison 1982). According to Shivanna and Sastri (1981), pollen grains do not germinate on the stigmas of hollow-styled taxa until exudate is released from within the stigmatic membrane. In *Trifolium pretense* and *Vicia faba*, the stigmatic membrane is relatively thin and breaks easily at maturity; however, in some of the self-infertile lines of *V. faba*, the stigmatic membrane is very thick and will not break until the flower is tripped (Lord and Kohorn 1986). Therefore, the gentle methods employed to rupture the stigmatic membrane in *H. boreale* flowers prior to hand

pollinations may have been ineffective. It may be necessary for the stigma to hit the keel petal suture or the underside of a bee for membrane rupture to occur.

Tripping devices promote outcrossing in many papilionaceous legumes. Tripping, in its simplest form, occurs when the staminal column is released from the keel petal as a pollinator alights on the wing petals and collects nectar from the nectary at the base of the ovary (Kalin Arroyo 1981). In legumes that require tripping, self-pollen does not germinate until a pollinator visits a flower. This mechanism assures potential for at least some outcrossing, especially if out-crossed pollen is competitively superior to self-pollen. Such a system occurs in *Hedysarum glomeratum* (de las Heras et al. 2001). A mechanical barrier prevents pollination within unvisited *H. glomeratum* flowers; only when pollinators visit the flowers are both self- and cross-pollination possible. *H. spinosissimum* ssp. *capitatum* (=*Hedysarum glomeratum*; White 2005) primarily reproduces via out-crossing (Baatout et al. 1991). *Hedysarum coronarium* L. and *Hedysarum humile* L. are both self-incompatible perennial herbs; in these species, tubes from self-sired pollen are arrested during development (Prados 1988, de las Heras et al. 2001). Shivanna and Sastri (1981) noted that wet stigmas are often associated with gametophytic self-incompatibility systems. In such systems, both compatible and incompatible pollen grains germinate on the stigma; however, incompatible pollen tubes grow more slowly through the style than compatible tubes or are totally arrested (Heslop-Harrison and Heslop-Harrison 1982). In this regard, it is possible that *H. boreale* self-pollen tubes grow more slowly than out-crossed pollen tubes, or are slowed down as they pass through the style.

Another possible explanation for the comparative failure of hand pollinations involves the papillae themselves. Pollen may not have been applied with sufficient force to push the pollen grains down amid the papillae and into contact with the stigmatic exudate upon its release. In this scenario, sequential flower visits may facilitate fruit and seed production in *H. boreale*. Pollen grains deposited on top of or just barely within the stigmatic papillae by a first flower visitor could be pushed further down into the papillae as a subsequent flower visitor deposits additional pollen onto the stigmatic surface. In *Onobrychis viciifolia* Scop (sainfoin), Bogoyavlenskii (1955) observed that the percentage of ovules fertilized increased from 10 to 50% with increase from 1 to 4 visits per flower. In addition, doubling the foraging population of honeybees increased seed yield by 60% in sainfoin (Bogoyavlenskii 1976). Sainfoin is closely related to *Hedysarum* (Welsh 1978). Therefore, if this scenario holds true for *H. boreale*, then enough bees should be stocked per acre of *H. boreale* to obtain multiple flower visits.

Results of stigma receptivity experiments show that the stigma becomes receptive in the mature bud stage prior to anthesis in *H. boreale* flowers and remains receptive for several days; pollen grains are also released during the mature bud stage. *H. boreale* flowers are therefore homogamous, as stigma receptivity and anther dehiscence occur synchronously in homogamous taxa (Fægri and van der Pijl 1979). Hence, there is no differential maturation of anthers and stigma to prevent selfing or promote out-crossing in *H. boreale*. Although the standard petal is the chief advertising organ of the typical legume flower (Fægri and van der Pijl 1979), several bee species in multiple genera (*Apis*, *Bombus*, and *Megachile*) were observed to push their way into flowers with half-open standards (see Chapter 4). The relative timing of stigma receptivity and anther

dehiscence appear to maximize the reproductive output of *H. boreale*; flowers are in fact 'ready' for visitation at anthesis.

Pollen viability is another factor important in fruit and seed production. In this study, patterns of pollen viability differed between the 2 sampled sites. At WCH, pollen viability decreased steadily with age, whereas viability at EF appeared to peak 2 to 3 days after anthesis. Unfortunately, these results were likely confounded by density issues. Density of pollen grains in/on the growth medium has been shown to affect germination (Kearns and Inouye 1993). In this study, 3 factors contributed to highly variable numbers of pollen grains per Petri dish: 1) 5- to 8-fold variation in the number of pollen grains produced per flower at WCH and EF, respectively (Table 2-5); 2) the tendency for older flowers to have fewer intact pollen grains due to thrips; and 3) incomplete dispersal of pollen grains on the growth medium despite addition of Tween-80 to the aqueous suspension. However, because pollen viability tends to decrease with pollen age (Kearns and Inouye 1993), it seems likely that results from EF were more confounded by density issues than those from WCH. Hand pollinations would likely fail to set fruit and viable seeds if older flowers were inadvertently selected as pollen donors.

Cultivation of *H. boreale* primarily enhanced bee forage by increasing plant size. In this study, cultivation increased the average number of racemes per plant nearly 4-fold (Table 2-7). In addition, *H. boreale* proved to be very rewarding (albeit variable) in terms of floral resources. Flowers contained abundant pollen that is actively harvested by nesting female bees. In addition, because *H. boreale* flowers open throughout the day, female bees have a more-or-less continuous supply of pollen. On average, *H. boreale* flowers offered concentrated nectar (60% sugar). Based on the diversity and abundance

of bees that visit *H. boreale* (see Chapter 3), it appears to be an attractive resource for nesting females wherever it is found. The presence of mass-flowering, highly rewarding crops was positively correlated with bumblebee densities in Europe (Westphal et al. 2003). Therefore, not only will a cultivated stand of *H. boreale* adequately support a population of managed pollinators, it might be a sufficiently rewarding resource to attract foraging native pollinators from outside the field.

In conclusion, *H. boreale* was found to be homogamous and self-compatible, but did not produce fruits in the absence of bee visitors. In this study, bee pollination of *H. boreale* flowers was likely a mixture of both selfing (via geitonogamy) and out-crossing (xenogamy). Xenogamy appeared to enhance long term reproductive success of *H. boreale* by increasing seed viability and by decreasing predispersal reproductive attrition. *H. boreale* proved to be very rewarding in terms of floral resources; flowers contained abundant pollen grains and nectar of comparatively high sugar concentration.

LITERATURE CITED

Baatout, H., D. Combes, and M. Marrakchi. 1991. Reproductive system and population structure in two *Hedysarum* subspecies. I. Genetic variation within and between populations. Genome 34:396-406.

Bernhardt, P. 1983. Dimorphic *Amyema melaleucae*: a shift towards obligate autogamy. Bulletin of the Torrey Botanical Club 110:195-202.

Bogoyavlenskii, S.G. 1955. Bees and sainfoin. Pchelovodstvo, Moskva 32:10-14.

_____. 1976. Effect of nectar productivity of plants on yield. Pages 118-124 *in* R.B. Kozin, editor, Pollination of entomophilous agricultural crops by bees. Amerind Publishing Co. Pvt. Ltd., New Delhi.

Bosch, J., and W.P. Kemp. 2002. Developing and establishing bee species as crop pollinators: the example of *Osmia* spp. (Hymenoptera: Megachilidae) and fruit trees. Bulletin of Entomological Research 92:3-16.

Boyd, R.S., and L.L. Serafini. 1992. Reproductive attrition in the rare chaparral shrub *Fremontodendron decumbens* Lloyd (Sterculiaceae). American Journal of Botany 79:1264-1272.

Dafni, A. 1992. Pollination ecology: a practical approach. Oxford University Press, New York. 272 pp.

Dafni, A., and M. Motte Maues. 1998. A rapid and simple procedure to determine stigma receptivity. Sexual Plant Reproduction 11:177-180.

Dafni, A., E. Pacini, and M. Nepi. 2005. Pollen and stigma biology. Pages 83-142 *in* A. Dafni, P. Kevan, and B. Husband, editors, Practical pollination biology. Enviroquest Ltd., Cambridge, Ontario.

de las Heras, M.A., P.J. Hidalgo, and J.L. Ubera. 2001. Stigmatic cuticle in *Hedysarum glomeratum*: structure and function. International Journal of Developmental Biology 45(S1):S41-S42.

Egea, J., L. Burgos, J.E. Garcia, and L. Egea. 1991. Stigma receptivity and style performance in several apricot cultivars. Journal of Horticultural Science 66:19-25.

Ehrlen, J. 1992. Proximate limits to seed production in a herbaceous perennial legume, *Lathyrus vernus*. Ecology 73:1820-1831.

Fægri, K., and L. van der Pijl. 1979. The principles of pollination ecology. 3rd edition. Pergamon Press, New York. 244 pp.

Free, J.B. 1993. Insect pollination of crops. 2nd edition. Academic Press, New York. 684 pp.

Galen, C., K.A. Zimmer, and M.E. Newport. 1987. Pollination and floral scent morphs of *Polemonium viscosum*: a mechanism for disruptive selection on flower size. Evolution 41:599-606.

Heslop-Harrison, J., and Y. Heslop-Harrison. 1982. Pollen-stigma interaction in the Leguminosae: constituents of the stylar fluid and stigma secretion of *Trifolium pratense* L. Annals of Botany 49:729-735.

Heslop-Harrison, Y., and K.R. Shivanna. 1977. The receptive surface of the angiosperm surface. Annals of Botany 41:1233-1258.

Inouye, D.W., D.E. Gill, M.R. Dudash, and C.B. Fenster. 1994. A model and lexicon for pollen fate. American Journal of Botany 81:1517-1530.

Johnson, D.A., T.M.J. Ford, M.D. Rumbaugh, and B.Z. Richardson. 1989. Morphological and physiological variation among ecotypes of sweetvetch (*Hedysarum boreale* Nutt.). Journal of Range Management 42:496-501.

Kalin Arroyo, M.T. 1981. Breeding systems and pollination biology in Leguminosae. Pages 723-769 *in* R.M. Pohill and P.H. Raven, editors, Advances in legume systematics. Royal Botanic Gardens, Kew, Richmond, Surrey.

Kearns, C.A., and D.W. Inouye. 1993. Techniques for pollination biologists. University Press of Colorado, Niwot. 583 pp.

Lord, E.M., and L.U. Kohorn. 1986. Gynoecial development, pollination, and the path of the pollen tube growth in the tepary bean, *Phaseolus acutifolius*. American Journal of Botany 73:70-78.

Louati-Namouchi, I., M. Louati, and A. Chriki. 2000. Mating system and multiple paternity in *Hedysarum coronarium* L. (Fabaceae). Agronomie 20:655-663.

Mattsson, O., R.B. Knox, J. Heslop-Harrison, and Y. Heslop-Harrison. 1974. Protein pellicle of stigmatic papillae as a probable recognition site in incompatibility reactions. Nature 247:298-300.

Northstrom, T.E., and S.L. Welsh. 1970. Revision of the *Hedysarum boreale* complex. Great Basin Naturalist 30:109-130.

Pinzauti, M., and G. Magnani. 1981. Ricerche comparative sull'-impollinazione della sulla (*Hedysarum coronarium* L.) in diverse localita toscane. Agricoltura Italia, Roma 110:117-127.

Prados, A. 1988. La morfologia floral de Hedysareae y su implicacion en la biologia de la reproduccion. Doctoral dissertation, Universidad de Cordoba, Spain.

Richards, A.J. 1997. Plant breeding systems. 2nd edition. Chapman and Hall, London. 529 pp.

Richards, K.W. 1996. Comparative efficacy of bee species for pollination of legume seed crops. Pages 81-103 *in* A. Matheson, S.L. Buchmann, C. O'Toole, P. Westrich, and I.H. Williams, editors, The conservation of bees. Academic Press, San Diego.

Sacchi, R. 1950. Investigation on the activity of bees as pollinators of sulla (sweetvetch). Annali Facoltà Agraria Università di Perugia 7:114-124.

SAS Institute Inc. 2004. SAS/STAT® 9.1 User's Guide. SAS Institute Inc., Cary, NC. 5136 pp.

Shivanna, K.R. 2003. Pollen biology and biotechnology. Science Publishers, Inc., Enfield, NH. 301 pp.

Shivanna, K.R., and D.C. Sastri. 1981. Stigma-surface esterase activity and stigma receptivity in some taxa characterized by wet stigmas. Annals of Botany 47:53-64.

Sokal, R.R., and F.J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research. 3rd edition. W.H. Freeman and Company, New York. 887 pp.

Stephenson, A.G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Annual Review of Ecology and Systematics 12:253-279.

Stone, J.L., J.D. Thomson, and S.J. Dent-Acosta. 1995. Assessment of pollen viability in hand pollination experiments: a review. American Journal of Botany 82:1186-1197.

Stpiczynska, M. 2003. Stigma receptivity during the life span of *Platanthera chlorantha* Custer (Rchb.) flowers. Acta Biologica Cracoviensia 45:37-41.

Tepedino, V.J., and M. Stackhouse. 1987. Bee visitors of sweetvetch, *Hedysarum boreale boreale* (Leguminosae), and their pollen-collecting activities. Great Basin Naturalist 47:314-318.

Thomson, J.R. 1938. Cross- and self-fertility in sainfoin. Annals of Applied Biology 25:695-704.

Waser, N.M., L. Chittka, M.V. Price, N.M. Williams, and J. Ollerton. 1996. Generalization in pollination systems, and why it matters. Ecology 77:1043-1060.

Welsh, S.L. 1978. Utah flora: Fabaceae (Leguminosae). Great Basin Naturalist 38:225-367.

Westphal, C., I. Steffan-Dewenter, and T. Tscharntke. 2003. Mass flowering crops enhance pollinator densities at landscape scales. Ecology Letters 6:961-965.

White, R. 2005. ILDIS (International Legume Database & Information Service) world database of legumes. [Online]. Available from: http://www.ildis.org/. Accessed November 2006.

Wyatt, R. 1983. Pollinator-plant interactions and the evolution of breeding systems. Pages 51-95 *in* L. Real, editor, Pollination biology. Academic Press Inc., Orlando, FL.

Zimmerman, J.K., and T.M. Aide. 1989. Patterns of fruit production in a neotropical orchid: pollinator vs. resource limitation. American Journal of Botany 76:67-73.

CHAPTER 3

THE TAXONOMIC COMPOSITION AND RELATIVE ABUNDANCE OF HEDYSARUM BOREALE POLLINATOR FAUNAS, AND SELECTION OF CANDIDATE BEE SPECIES FOR AGRICULTURAL SEED PRODUCTION

INTRODUCTION

The development of a bee species as a new crop pollinator begins with the recognition of a pollination need in agriculture (Bosch and Kemp 2002). The second step in the development of a new crop pollinator is to survey the bee fauna of a target crop species and choose candidate bee species for management (Bosch and Kemp 2002). Federal land managers would like to annually acquire large quantities of affordable *Hedysarum boreale* Nutt. seed for use in western rehabilitation efforts. *H. boreale* is a native perennial legume (Fabaceae) widely distributed throughout the Rocky Mountains and neighboring US Intermountain West. It is found in Canada from British Columbia to Alberta, and south through Idaho, Montana, and North Dakota, to Arizona and New Mexico in the United States (Northstrom and Welsh 1970). *H. boreale* typically flowers from early May to early August (Northstrom and Welsh 1970). In general, legume species are principally pollinated by bees, and are as such a major food source for bees. As a result, most legume species attract diverse bee species (Kalin Arroyo 1981). Not surprisingly, *H. boreale* has proven to be highly attractive to an array of bee species (Tepedino and Stackhouse 1987) and supports a wide bee fauna throughout its range.

This research aims to identify candidate bee pollinators for production of *H. boreale* seed in agricultural settings.

Several criteria must be considered when selecting candidate bee pollinators for a target crop. Candidate species should preferentially forage at flowers of the target crop (Torchio 1976). They should be able to effectively handle and pollinate flowers of the target crop (Bosch and Blas 1994). They should be native to areas where the crop is grown and active at the same time the crop is in bloom (Bosch and Kemp 2002). Ideally, candidate species should be active for a short period of time (i.e. a few weeks) and have a single generation per year (Parker and Torchio 1980). In addition, they should be able to tolerate nesting in high densities (Maeta 1990). In general, solitary, cavity-nesting bee species are sought for managed pollination of agricultural crops (Bosch and Kemp 2002). Many solitary species nest in pre-existing holes in wood and will readily accept artificial nesting domiciles (Parker and Torchio 1980); this habit has made it possible to manipulate and manage many solitary species in agricultural settings (Hurd 1979). Field and literature surveys are complementary ways to select candidate species. For example, once bee visitors to a target crop have been identified, available literature and/or museum records may provide additional information (Bosch and Kemp 2002).

The diversity and abundance of pollinators vary markedly in space and time (Herrera 1988). Therefore, in this study, field surveys were designed to provide a snapshot of the bees that commonly forage at *H. boreale* within its native range, instead of an exhaustive faunal list. Faunal snapshots were obtained by surveying *H. boreale* populations at or just after peak bloom, when a majority of pollinator species are likely to be present (Herrera 1988). In addition, faunal snapshots were obtained during the early

afternoon hours; Tepedino and Stackhouse (1987) extensively surveyed a Wyoming population of *H. boreale* and found that pollinators were most abundant during the afternoon hours. Widespread and comparatively abundant species were of particular interest, as were solitary, cavity-nesting species that meet the aforementioned criteria for selecting candidate pollinator species.

The development of a bee species as a new crop pollinator is only justified if the species is an effective pollinator of the target crop species (Bosch and Blas 1994). Currently, it is not known which bee species are effective pollinators of *H. boreale* because little work has been done on the pollination ecology of the plant. However, pollination efficacy is often related to pollinator body size (Richards 1996); for example, glossa length can influence whether or not and how fast a bee can manipulate a flower to probe for nectar and/or collect pollen. In fact, Kowalczyk (1973) postulated that smaller-bodied bees, especially solitary species, might not be effective pollinators of *H. boreale* ssp. *mackenzii* because of their small size relative to the size of the flower. Intertegular span and forewing length are 2 measures of body size not related to feeding or pollen collection (Wiens 1982) that have been used to estimate bee body size in other studies (Cripps and Rust 1989). Intertegular span is a good estimator of body musculature (Cane 1987), which is required to forcibly enter *H. boreale* flowers (Fægri and van der Pijl 1979). Therefore, in this study, bee body size was measured to determine whether there is a lower limit on body size that prevents access to pollen and nectar in *H. boreale* flowers.

Therefore, the objectives of this research are threefold: 1) to determine what types and species of bees are commonly found at *H. boreale* within its native range; 2) to

determine how bees that commonly forage at *H. boreale* flowers vary in body size; and 3) to select candidate bee species for *H. boreale* seed production in agricultural settings.

MATERIALS AND METHODS

The bee faunas of 7 natural populations of *H. boreale* in Utah and Wyoming, USA were systematically surveyed during the summers of 2004 and 2005 (Table 2-1; in Utah: Cottonwood Canyon (CWC), Mill Hollow (MH), North Ogden Canyon (NOC), Paradise (PRD), and Wood Camp Hollow (WCH); in Wyoming: Atherton Campground (ATC) and Horsetail Creek (HTC)). Each natural population was surveyed once per summer, sometimes in both years. In addition to natural populations of *H. boreale*, a 2-acre commercial seed field of 'Timp' Utah sweetvetch near Worland, Wyoming (Table 2-1; WRL) was surveyed in 2004. Likewise, a fallow field of sainfoin (*Onobrychis viciifolia* Scop.) near Geneva, Idaho (42º21.49 N, 111º5.36 W; 1906 m) was surveyed in 2005. All sites were surveyed between 12 and 2 PM. Thirty bees at *x* plants were collected at each site; if bees were scarce, then *x* bees at 300 plants were collected instead. Plants were haphazardly surveyed for the presence of bees, and all bees foraging on a plant collected. If a bee escaped collection, the formal count of plants surveyed was suspended until another bee was collected as a replacement. Replacement specimens were collected in order to preserve the correct ratio of bees foraging per plant, although minor changes in the species composition of the survey may have resulted. The sum of plants comprising each natural population was also estimated.

Bees were collected by insect net, killed, and pinned. Bumblebee workers (*Bombus* spp.) were always collected. However, in order to preserve queen bumblebees,

all possible effort was made to identify live individuals to species using pile coloration on the thorax and abdomen. If necessary, queens were netted, transferred to plastic vials in a cooler, and released once confidently identified to species. If there was any doubt as to the correct species identification, the individual was killed. The mandibles of female megachilids were opened when possible to aid identification. In addition, the corbiculae or scopa of all females were examined for the presence of bright yellow pollen (presumed to be from *H. boreale*) using a stereomicroscope.

Spatial and annual similarity in bee guild composition among surveyed sites was compared using Morisita's index (Krebs 1989). Pairwise comparisons were made for sites surveyed in 2004, in 2005, and in both years.

Flowering phenology was measured by haphazardly choosing 10 plants at each site surveyed in 2005 by blindly tossing a surveyor's flag over the shoulder. The flowering plant located closest to the flag was chosen. Ten racemes were haphazardly chosen on each plant and the following flower stages counted on each raceme: buds, open flowers, finished flowers, bracts and/or abscission scars, and other. Finished flowers included those past their prime (i.e. the discolored standard petal was returning to the closed position) and fruits. Other flower stages included aborted buds and clusters of aborted buds. Herbivory and other types of damage were noted.

Intertegular span (ITS) and forewing length were measured for females of solitary species collected during systematic surveys. Intertegular span was measured to the nearest 0.1 mm using a stereomicroscope with an ocular micrometer. Forewing length was measured to the nearest 0.01 mm using digital calipers. Dry mass (DM) in mg was calculated using the following equation from Bullock (1999): $ITS = 1.085 \cdot DM^{0.329}$.

These data were also collected for 5 females of each candidate species and for 5 honeybee workers.

RESULTS

Four natural populations were surveyed in 2004 (Table 2-1; in Utah: CWC and PRD; in Wyoming: ATH and HTC). Nineteen species of bees were collected representing 7 genera in the families Apidae and Megachilidae (Table 3-1.). Most of the specimens collected were from the genera *Bombus*, *Hoplitis*, *Megachile*, and *Osmia*, while nearly half of all specimens collected were *Osmia* (43%). All 59 specimens were female except for a *Hoplitis sambuci* male collected at PRD. A 2-acre commercial seed field of 'Timp' Utah sweetvetch at WRL was also surveyed in 2004. Seventeen bees in 7 species were collected, including 1 unidentified *Osmia* species (Table 3-1). *Bombus nevadensis* was the only species in common with other 2004 surveys. Four of the species collected at WRL were not collected during any of the systematic surveys in either year (*Anthophora porterae*, *Anthophora ursina*, *Apis mellifera*, and *Osmia integra*). Females of *A. ursina* were observed nesting among the furrowed rows of *H. boreale*.

In 2005, 6 natural populations were surveyed (Table 2-1; in Utah: CWC, MH, NOC, PRD, and WCH; in Wyoming: ATC). Twenty-two species in 6 genera were collected (Table 3-2.). All genera were from the families Apidae and Megachilidae, except 5 individuals of *Hylaeus* (Colletidae). *Bombus*, *Hoplitis*, *Megachile*, and *Osmia* were again well represented. However, *Osmia* accounted for only a quarter of specimens collected in 2005 as opposed to nearly half in 2004. Ten of the 72 specimens collected were male. On 14 July 2005, 5 bees from 4 *Bombus* species were collected at a fallow

Table 3-1. Results of 2004 systematic surveys of bee fauna by site and collection date (see Table 2-1; CWC = Cottonwood Canyon; PRD = Paradise; ATC = Atherton Campground; HTC = Horsetail Creek; and WRL = Worland). Fauna are listed by family and location collected. All specimens are female unless otherwise noted. *Bombus* specimens are queens unless notated by 'w' representing workers.

	Utah		Wyoming		
	CWC	PRD	ATC	HTC	WRL
Taxa	28 Jul	26 Jun	9 Jul	9 Jul	11 May
Apidae					
Anthophora porterae					1
Anthophora ursina					2
Apis mellifera					1
Bombus appositus			2w		
Bombus fervidus			1w		
Bombus huntii					9
Bombus nevadensis		1			1
Eucera frater		1			
Megachilidae					
Hoplitis hypocrita				1	
Hoplitis sambuci		8♀ (1♂)			
Megachile gemula		1			
Megachile frigida		1			
Megachile melanophaea			1	1	
Osmia albolateralis	1	1			
Osmia atrocyanea		4			
Osmia bucephala			2		
Osmia cyanella		6			
Osmia grindeliae			3	1	
Osmia integra					2
Osmia lignaria				1	
Osmia paradisica				1	
Osmia pusilla		1			
Osmia proxima				1	
Osmia simillima		1			
Osmia sp.					1
Totals	**1**	**26**	**9**	**6**	**17**

field of sainfoin near Geneva, Idaho. One *B. huntii* queen, 2 *B. bifarius* workers, and 1 worker each of *B. flavifrons* and *B. lapponicus* were collected. *B. huntii* was the only species in common with other 2005 surveys. Bee flies (Bombyliidae) were abundant at

Table 3-2. Results of 2005 systematic surveys of bee fauna by site and collection date (see Tables 2-1 and 3-1; MH = Mill Hollow; NOC = North Ogden Canyon; and WCH = Wood Camp Hollow). Fauna are listed by family and location collected. All specimens are female unless otherwise noted. All *Bombus* specimens are queens. 'X' refers to species collected in 2004, but not in 2005.

	Utah						Wyoming
	CWC	MH	MH	NOC	PRD	WCH	ATC
Taxa	29 Jul	2 Jul	3 Jul	5 Jun	30 Jun	9 Jul	12 Jul
Apidae							
Anthophora porterae	X						
Anthophora ursina	X						
Apis mellifera	X						
Bombus appositus		1	1				
Bombus fervidus					2		
Bombus griseocollis				4	1		
Bombus huntii		1					
Bombus rufocinctus				1			
Eucera frater					1	1	
Colletidae							
Hylaeus sp.		3			1	1	
Megachilidae							
Hoplitis hypocrita				2♀(2♂)	5♀(2♂)	1	
Hoplitis producta		1					
Hoplitis sambuci					3		
Megachile brevis					1		
Megachile gemula			1				
Megachile frigida			(1♂)		1	1	
Megachile melanophaea	1			2♀(5♂)	2	2	2
Osmia albolateralis	1					2	3
Osmia atrocyanea		2	1		1	1	
Osmia bucephala	X						
Osmia cyanella			1				
Osmia grindeliae							2
Osmia inermis	1						
Osmia integra	X						
Osmia lignaria	X						
Osmia longula						1	
Osmia paradisica	X						
Osmia pusilla	X						
Osmia proxima	X						
Osmia sanrafaelae				1			
Osmia simillima					1	1	
Osmia sp.	X						
Totals	**3**	**8**	**5**	**17**	**21**	**11**	**7**

this site, perhaps contributing to the obvious lack of other native bee species foraging on sainfoin.

Female bees commonly collected *H. boreale* pollen to provision their progeny; 86 of 92 females collected in the 2 years of surveys (excluding *Hylaeus*) had bright yellow pollen grains within their corbiculae or scopa. Pollen species were not determined. However, many females were observed to collect and/or groom and pack pollen while at *H. boreale* flowers.

Bee guild composition varied among sites surveyed within years and for those sites surveyed in both years. In 2004, several sites had no species in common ($C_\lambda = 0$) (Table 3-3). Even when sites shared species, C_λ ranged from 0.03 to only 0.26 in 2004. In 2005, several sites again had no species in common (Table 3-4). However, C_λ values resulting from 2005 site comparisons were much higher than those from 2004; C_λ ranged from 0.15 to > 1.0. Three sites were surveyed in both 2004 and 2005. Similarities of bee guild composition between sequential years at PRD, CWC, and ATC were 0.42, 0.56, and 0.67, respectively. MH was the only site surveyed twice in a single year; the C_λ value for this comparison was 0.48.

All sites were surveyed for bees at, or just after, peak bloom (Table 3-5). Finished flowers (those no longer available to pollinators) accounted for 55 percent of total potential bloom at MH and WCH, 57 percent at PRD, and 59 percent at NOC. Two sites were surveyed slightly later in bloom; finished flowers at ATC and CWC accounted for 71 percent of total potential bloom.

Females of solitary species ranged in size from 1.3 to 3.6 mm in intertegular span and from 4.92 to 11.17 mm in forewing length (Fig. 3-1). *Osmia* females ranged from 1.6

Table 3-3. Variability in bee guild composition among sites surveyed in 2004. Comparisons were made using Morisita's Index of Similarity.

	Utah		Wyoming		
Site	CWC	PRD	ATC	HTC	WRL
CWC	—	$C_\lambda = 0.06$	0	0	0
PRD	—	—	0	0	0.03
ATC	—	—	—	0.26	0
HTC	—	—	—	—	0
WRL	—	—	—	—	—

Table 3-4. Variability in bee guild composition among sites surveyed in 2005. Comparisons were made using Morisita's Index of Similarity. MH1 was surveyed on 2 July and MH2 on 3 July.

	Utah						Wyoming
Site	CWC	MH1	MH2	NOC	PRD	WCH	ATC
CWC	—	$C_\lambda = 0$	0	0.59	0.18	0.86	> 1.0[a]
MH1	—	—	0.48	0	0.22	0.63	0
MH2	—	—	—	0	0.15	0.38	0
NOC	—	—	—	—	0.70	0.69	0.49
PRD	—	—	—	—	—	0.86	0.15
WCH	—	—	—	—	—	—	0.95
ATC	—	—	—	—	—	—	—

[a] C_λ values typically range between 0 and 1; small sample sizes resulted in C_λ values >1.0

Table 3-5. Estimates of available bloom at sites surveyed in 2005. Sample sizes (*n*) reflect the total numbers of buds, flowers, finished flowers, and aborted buds counted at each site. Racemes with aborted flower heads were not included in bloom estimates.

				Percent (%)				#aborted
Site	Survey date	Plants in population	*n*	Buds	Flowers	Finished flowers	Aborted buds	flower heads
NOC	5 June	300	3206	24	17	59	0	0
PRD	29 June	500	2534	20	15	57	8	19
MH	2 July	600	1691	17	25	55	3	5
WCH	9 July	400	1907	27	14	55	4	17
ATC	13 July	1100	1674	10	17	71	2	11
CWC	29 July	30	1626	16	13	71	0	2

to 3.5 mm in intertegular span and 5.01 to 11.17 mm in forewing length (*O. pusilla* and *O. bucephala*, respectively). Females of solitary species ranged from 1.73 to 38.30 mg in dry mass.

DISCUSSION

A vast majority of the 8 genera and 31 species encountered during this research belong to the families Apidae and Megachilidae, while the family Colletidae was represented by a single species. Taxa from the families Andrenidae, Halictidae, and Melittidae were not encountered during this research. Tepedino and Stackhouse (1987) sampled extensively at Spread Creek Hill in Grand Teton National Park, approximately 20 km north of ATH and HTC sites in Bridger-Teton National Forest. They collected 4 genera not encountered during this research: Andrenidae, *Andrena* spp.; Apidae, *Nomada* sp. (♂ only); Halictidae, *Evylaeus* spp., *Halictus* sp. (♂ only). Therefore, it appears that the *H. boreale* bee fauna includes taxa from 5 of the 7 bee families.

Twenty of the 33 species (61%) collected by Tepedino and Stackhouse (1987) were also collected during the systematic surveys of bee fauna performed during this research. If the 3 species for which Tepedino and Stackhouse (1987) collected only male specimens are ignored, then 67% of their species were also collected during this research. The following species were collected by Tepedino and Stackhouse (1987), but not during this research: Apidae, *Bombus bifarius* Cresson, *Bombus flavifrons* Cresson, *Bombus lapponicus* F., *Bombus occidentalis* Greene, and *Psithyrus insularis* (Smith); Megachilidae, *Callanthidium formosum* (Cresson) (♂ only), *Megachile inermis* Provancher (♂ only), *Osmia bruneri* Cockerell, *Osmia cockerelli* Sandhouse (♂ only),

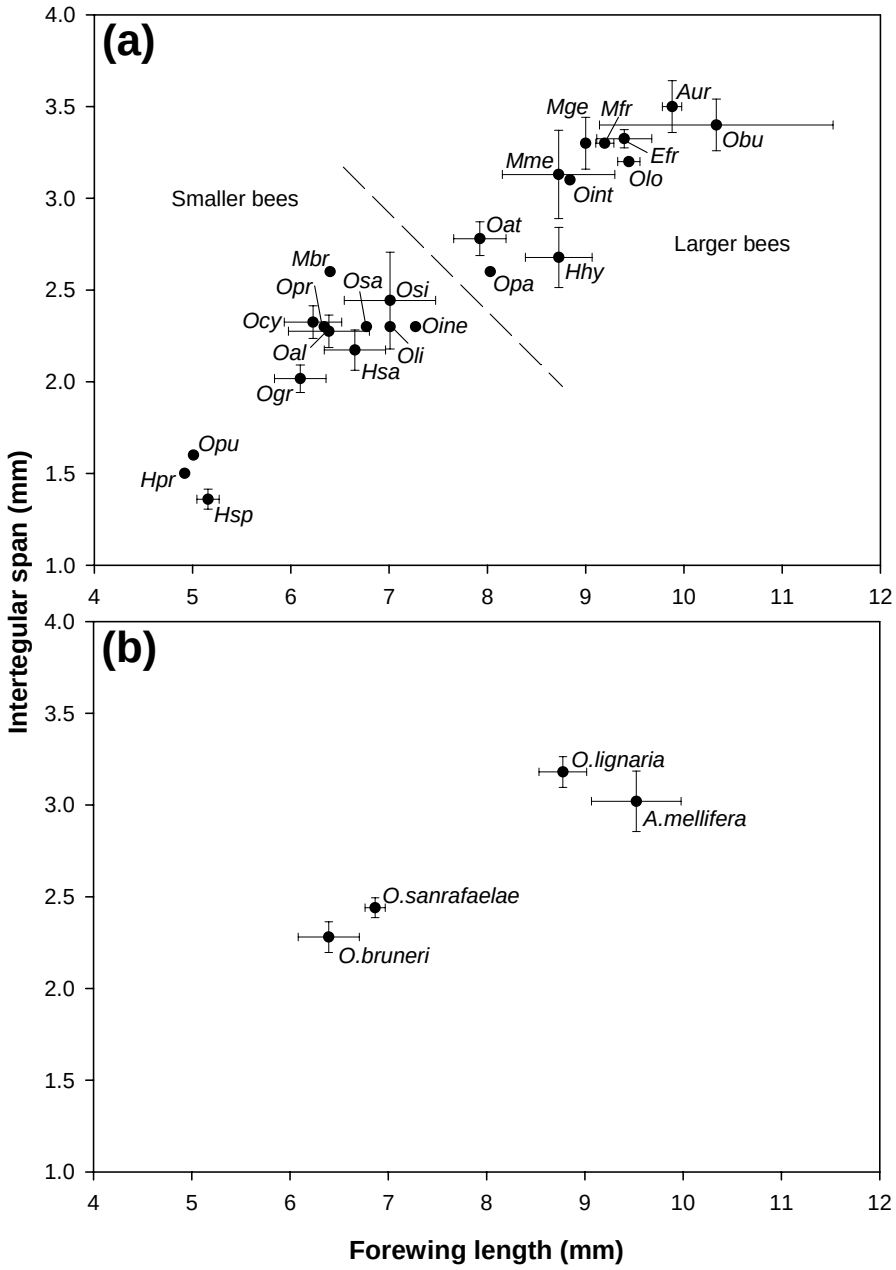

Fig. 3-1. Two measures of female body size (mean ± standard deviation) for (a) 24 solitary species collected during systematic surveys of bee fauna and for (b) candidate *Osmia* species and the commercially available pollinator *Apis mellifera*. Solitary species are coded by the first letter of their genus and first 2 or 3 letters of their species designation (e.g. *Hoplitis producta* is '*Hpr*').

Osmia nifoata Cockerell, *Osmia penstemonis* Cockerell, *Osmia tersula* Cockerell, and *Osmia tristella* Cockerell. In addition, 2 genera and 10 species were collected during systematic surveys that were not collected by Tepedino and Stackhouse (1987): Apidae, *Anthophora ursina* Cresson and *Anthophora porterae* Cockerell; Colletidae, *Hylaeus* sp.; Megachilidae, *Hoplitis sambuci* Titus, *Megachile brevis* Say, *Osmia cyanella* Cockerell, *Osmia integra* Cresson, *Osmia lignaria propinqua* Cresson, *Osmia proxima* Cresson, *Osmia sanrafaelae* Parker, and *Osmia simillima* Smith.

Furthermore, museum records from the USDA-ARS Bee Biology and Systematics Laboratory collection (Logan, UT) include the following taxa collected at *H. boreale* in addition to those encountered by Tepedino and Stackhouse (1987) and in this study: UT, Box Elder County, *Halictus rubicundus* (Christ) and *Megachile perihirta* Cockerell; UT, Grand County, *Anthidium dammersi* Cockerell, *Ashmeadiella cactorum* Cockerell, *Ashmeadiella erema* Michener, *Ashmeadiella foveata* Michener, and *Osmia cerasi* Cockerell; WY, Teton County, *Hoplitis albifrons* Kirby, *Hoplitis fulgida* Cresson, and *Osmia calla* Cockerell.

Kowalczyk (1973) observed 11 bee species at a population of *H. boreale* ssp. *mackenzii* in the Yukon Territory, Canada, and 5+ bee species at another nearby population. The most common species at each site were *Bombus bifarius* Cresson and *Bombus lucorum* L. In another study, males and females of *Megachile giliae* Cockerell were the most abundant visitors to a population of *H. boreale* near Fairbanks, Alaska (McGuire 1993); in fact, seed set by *H. boreale* was positively related to visitation rates of female *M. giliae*. Therefore, it appears that *Bombus* and *Megachile* species are important elements of the *H. boreale* pollinator fauna across its range, while smaller-

bodied species become more important at lower latitudes. The lack of other (often smaller) bee species in Alaska and Canada may simply illustrate the idea that larger body size is advantageous for ectotherms living in colder climates.

Richards and Edwards (1988) identified 6 bee species as pollinators of cultivated sainfoin in southern Alberta, Canada, including *Apis mellifera*, *Bombus fervidus*, *B. huntii*, *B. occidentalis*, *B. rufocinctus*, and *Megachile rotundata*. *Bombus* species were also prevalent at the Idaho population of sainfoin surveyed in 2005, and the pollinator faunas of *H. boreale* and sainfoin appear to share several species of bumblebees. However, it is not clear from the results of this study and from Richards and Edwards (1988) whether the pollinator faunas of *H. boreale* and sainfoin have other types of bees in common. The lack of additional megachilid species at sainfoin could simply be an artifact of the particular sites that were surveyed. Richards and Edwards (1988) surveyed a 7-year-old cultivated plot; the prevalence of *M. rotundata* at this site likely indicated the presence of a nearby managed population. In addition, the only systematic survey of a cultivated *H. boreale* stand completed during this research rendered a faunal list quite different from those of natural populations surveyed in the same year (Table 3-1; WRL). Therefore, it appears that natural populations and cultivated stands may support different portions of the larger pollinator fauna of a crop species. Perhaps sainfoin populations that have 'escaped' from cultivation support other species in common with *H. boreale*. Additional surveys, especially where *H. boreale* and sainfoin populations are sympatric, could help to elucidate whether or not this is true.

Morisita's index of similarity ranges from 0, indicating no similarity between samples, to around 1.0, indicating complete similarity. Seventy percent of pairwise

comparisons of sites had no species in common in 2004; in 2005, 29% of pairwise comparisons exhibited no similarity. Together, these results indicate that a diversity of bee species are attracted to *H. boreale*, and that several species are likely to be found foraging on *H. boreale* at the same time. In 2004, the faunal samples from ATC and HTC had the highest degree of similarity; *Megachile melanophaea* and *Osmia grindelidae* were the 2 species collected at both sites. In addition, *Bombus nevadensis* and *Osmia albolateralis* were also found at more than 1 site in 2004. In 2005, high degrees of similarity were found between sites when the following species were present: *Bombus* spp. (especially *B. appositus* and *B. griseocollis*), *Hoplitis hypocrita*, *Megachile frigida*, *M. melanophaea*, *Osmia albolateralis*, *Osmia atrocyanea*, and *Eucera frater*.

Morisita's index of similarity is nearly independent of sample size (except for samples of very small size) and has been recommended as the best overall measure of similarity for use in ecological studies (Wolda 1981, Krebs 1989). However, even though Morisita's index is the most robust measure of similarity for small sample sizes, the faunal samples from some sites in this study were too small. Therefore, some pairwise comparisons resulted in C_λ values greater than 1.0 (Table 3-4).

Differences in sample guild composition were not confounded by sampling sites at different stages of bloom (Table 3-6). On average, finished flowers accounted for 61% of potential bloom. There was considerable variation in bloom phenologies among plants within sites; for example, finished flowers ranged from 17 to 97% of potential bloom on plants at NOC, and from 26 to 73% at WCH. On average, plants had only 16% of potential bloom available at any one time, suggesting that *H. boreale* populations bloom for an extended period of time. Extended bloom in *H. boreale* might be an important

consideration when choosing a candidate pollinator species for managed seed production, or when choosing the timing of pollinator release. For example, in a particularly large field, it might be possible to maximize both seed set and bee reproduction by introducing 2 waves of managed, short-lived pollinators, the first just prior to the start of bloom and another to cover the mid part of bloom.

Osmia species are an important component of the *H. boreale* bee fauna (Tepedino and Stackhouse 1987; Tables 3-1 and 3-2). Three solitary, cavity-nesting *Osmia* were selected as candidate pollinator species in this study: *O. bruneri* Cockerell, *O. lignaria* Say, and *O. sanrafaelae* Parker. Several criteria were used to select these candidate pollinator species, including range, phenology, floral preferences, life history, pollination efficacy (addressed in Chapter 4), and body size (Bosch and Kemp 2002). The *Osmia* species collected at *H. boreale* during this study ranged 10-fold in size from *O. pusilla* at 3.26 mg to *O. bucephala* at 32.18 mg. They fell into 2 groups of comparatively small- and large-bodied species (Fig. 3-1), with several intermediate species (e.g. *O. atrocyanea* and *O. paradisica*), and a single tiny-bodied species (i.e. *O. pusilla*). Female *O. bruneri* and *O. sanrafaelae* were comparatively small-bodied, whereas female *O. lignaria* were comparatively large-bodied (the female collected during the 2004 systematic surveys was small for her species) (Fig. 3-1).

O. bruneri was not collected during any of the 2004 or 2005 systematic surveys of bee fauna conducted during this study. However, Tepedino and Stackhouse (1987) collected 5 females of *O. bruneri* foraging on *H. boreale* in Grand Teton National Park in Wyoming. All of the females were carrying pollen, and the average amount of *H. boreale* pollen carried in the scopa ranged from 29.3 to 89.5%. Based on these results, Tepedino

and Stackhouse (1987) suggested that *O. bruneri* could potentially be used as a managed pollinator of *H. boreale*.

O. bruneri visits a variety of flowers and is assumed to be polylectic. It is found throughout western North America, ranging from British Columbia to California and Montana to New Mexico (Hurd 1979). It has been collected as far east as Sioux County, Nebraska (BBSL museum records). Specimens of *O. bruneri* in the BBSL museum were collected between late April and mid-September; a majority of specimens were collected in June and July. Female *O. bruneri* were active throughout June in a study of the pollen foraging behaviors of *Osmia* females in Washoe County, Nevada (Cripps and Rust 1989). Female *O. bruneri* have been observed to nest in the old discarded nests of mud-daubing wasps and in other pre-existing cavities (Hicks 1926), and will utilize artificial domiciles (Frohlich 1983). Females use a green leaf-paste for cell partitions within their nests (Hicks 1926).

In 2004, a single *O. lignaria* female was collected as she foraged at *H. boreale* in Wyoming (Table 3-1). Females of *O. lignaria* have been observed to forage at redbud in Kansas (J.H. Cane, personal communication), and have used pollen from Western redbud (*Cercis occidentalis*) in their provision masses (Bosch and Kemp 2001). Redbud is a legume and its flowers resemble those of *H. boreale* in shape and color. Female *O. lignaria* have also been observed to use sweet pea pollen (Fabaceae: *Lathyrus* sp.) to construct provision masses (Bosch and Kemp 2001). *O. lignaria* is regionally polylectic (Rust 1990). Floral records for the western subspecies *O. lignaria propinqua* include 28 species in 19 families, and the legumes *Astragalus*, *Cercis*, *Lupinus*, and *Trifolium* (Rust 1974).

O. lignaria propinqua is found from March to July throughout western North America, ranging from British Columbia to southern California, and eastward to South Dakota and Texas. The eastern subspecies *O. lignaria lignaria* is found east of the Rocky Mountains (Rust 1974). Rau (1937) noted that *O. lignaria* females seem to prefer to nest in horizontal galleries, and will readily nest in close proximity to one another. Rau (1937) also noted that most *O. lignaria* daughters do not wander far from their natal nest to found new nests; thus, a "strong colony" of bees may build up within a few years. Much research has focused on developing *O. lignaria* as an orchard pollinator (detailed in Bosch and Kemp 2001). Females construct cell partitions from mud.

In 2005, a single *O. sanrafaelae* female was collected at *H. boreale* in Weber County, Utah (Table 3-2). In addition, several complete *O. sanrafaelae* nests were discovered in wooden nesting blocks placed near a 2-acre commercial seed field of 'Timp' Utah sweetvetch in 2004 (Table 2-1; WRL). This species was previously tested as a managed pollinator of other legumes including alfalfa (Parker 1985a, 1986a).

O. sanrafaelae was originally described by Parker (1985b) from the high desert areas of southern Utah. Specimens of *O. sanrafaelae* have since been collected in 10 counties in Utah, including Emery, Garfield, Grand, Kane, Piute, San Juan, Sevier, Uintah, Utah, and Wayne (BBSL museum records). One female was collected in June 2005 foraging at *Penstemon haydenii* (Scrophulariaceae) on the southeastern slope of Bear Mountain in Carbon County, Wyoming. Another female identified as *O. sanrafaelae* was collected 15 miles south of Burns, Harney County, in southeast Oregon in June 1955. It is possible that this female is actually *Osmia clarescens* Cockerell, as females of *O. sanrafaelae* and *O. clarescens* are not easily separable except by length of

the mouthparts (Parker 1985b). In any case, it appears that the distribution of *O. sanrafaelae* is wider than originally reported by Parker (1985b).

O. sanrafaelae is considered to be polylectic (Parker 1986b). Parker (1985b) collected *O. sanrafaelae* at *Astragalus* and *Cryptantha* flowers (Fabaceae and Boraginaceae, respectively). Many females were collected as they gathered *Oenothera* (Onagraceae) leaf material for cell partitions within their nests (Parker 1985b). In another study, *O. sanrafaelae* females exclusively provisioned their nests with pollen from legumes (alfalfa and/or sweet clovers); conversely, females did not collect pollen from morning glories, dill, composites, crucifers, and mints, all of which were in bloom at the same time as alfalfa and sweet clover (Parker 1989). Female *O. sanrafaelae* build cell partitions consisting of a 0.5 – 1.0 mm thick disc of masticated leaf, the inner surface of which is smooth and concave (Parker 1986b).

Parker (1986b) recovered *O. sanrafaelae* nests from cavities in dirt banks, and from wooden nesting blocks with 6 and 9 mm diameter holes. Nests in 9 mm holes contained more cells than those in 6 mm holes; females were able to pack more cells (up to 20 per nest) into wider holes by orienting provision masses across the burrow instead of along the burrow length (Parker 1986b). Nesting blocks placed on a variety of substrates (e.g. trees stumps, wooden fence posts, between rocks, set in cliff banks) were productive, suggesting that *O. sanrafaelae* females actively search for and use available nesting materials (Parker 1986b). In another study, females did not display aggressive behavior when nesting adjacent to one another, and were active for approximately 30 days (Parker 1989). Natural populations of *O. sanrafaelae* completed nesting by June and entered diapause by October (Parker 1985a).

O. sanrafaelae compared favorably with *Megachile rotundata* (F.) as an alfalfa pollinator. Seed production inside a field cage stocked with *O. sanrafaelae* was comparable to seed production by *M. rotundata* outside of the field cage (Parker 1985a). In another study, *O. sanrafaelae* individuals released near a 4-hectare field of alfalfa in Clark County, Nevada, remained in the area and visited the target crop (Parker 1986a). In both studies, females used alfalfa pollen and nectar as their only source of nutrients for themselves and their progeny. Based on such results, it appears that *O. sanrafaelae* could be used as a managed pollinator of legumes in some agricultural settings.

It is unlikely that the floral preferences of *O. sanrafaelae* will limit its use as a managed pollinator of *H. boreale*. In fact, *O. sanrafaelae* might eventually be used as a managed pollinator of other legumes on the GBRI Native Plant Selection and Increase Project list, such as *Astragalus* and *Lupinus*. Conversely, the use of *O. bruneri* and *O. lignaria* as managed pollinators of *H. boreale* may be limited by floral preference. For example, female *O. bruneri* have been observed to prefer pollen from *Phacelia hastata* Douglas & Lehmann (Hydrophyllaceae) over pollen from sympatric legume species (Cripps and Rust 1989). Similarly, female *O. lignaria* often prefer to collect pollen from plant species with multiple, exposed anthers on long filaments, such as *Phacelia hastata* and *Salix* spp. (Cripps and Rust 1989, Rust 1990). However, agricultural settings are highly variable, and it is likely that *O. bruneri* and *O. lignaria* could be used to pollinate *H. boreale* in settings where 'preferred' plant species like *Phacelia* are absent.

Other attributes of agricultural settings, such as location, latitude, bloom phenology, insecticide use, and proximity to natural habitat, may influence the appropriateness of using particular species as managed crop pollinators. For example, the

use of *O. sanrafaelae* is limited by its geographic distribution. At this point in time, it is unclear whether or not *O. sanrafaelae* naturally occurs outside of Utah and Wyoming; thus, it is probably safe to use *O. sanrafaelae* as a managed pollinator of *H. boreale* grown in Utah and Wyoming only. Conversely, both *O. bruneri* and *O. lignaria* are widely distributed throughout the western United States, and could be used to pollinate *H. boreale* in agricultural settings throughout the West. Depending on the agricultural setting, however, additional limitations may apply. For example, the use of *O. lignaria* may also be limited phenologically. *O. lignaria* usually flies from March to July, whereas *H. boreale* populations typically begin to bloom in late May. In agricultural settings where *O. lignaria* emerges early (e.g. lower latitudes), or where *H. boreale* blooms late, *O. lignaria* may not be a viable pollinator option for seed growers to use. Thus, the individual attributes of an agricultural setting should be considered when selecting 1 or more managed pollinator species.

In conclusion, an assortment of bees in the families Apidae and Megachilidae were collected at *H. boreale* flowers in 2004 and 2005. In both years, *Osmia* species were an important component of the *H. boreale* pollinator fauna. *Osmia* females collected at *H. boreale* formed 2 groups of comparatively small- and large-bodied species based on intertegular span and forewing length. Three solitary, cavity-nesting *Osmia* species were selected as candidate pollinator species in this study; *O. bruneri*, *O. lignaria*, and *O. sanrafaelae* were selected based on their range, phenology, floral preferences, life history, pollination efficacy, and body size. Their use as managed pollinators of *H. boreale* in some agricultural settings may be limited by location, latitude, and bloom phenology, and by what other types of crops are grown nearby.

LITERATURE CITED

Bosch, J., and M. Blas. 1994. Foraging behavior and pollinating efficacy of *Osmia cornuta* and *Apis mellifera* on almond (Hymenoptera, Megachilidae and Apidae). Applied Entomology and Zoology 29:1-9.

Bosch, J., and W.P. Kemp. 2001. How to manage the blue orchard bee as an orchard pollinator. Sustainable Agriculture Network, National Agricultural Library, Beltsville, MD. 88 pp.

_____. 2002. Developing and establishing bee species as crop pollinators: the example of *Osmia* spp. (Hymenoptera: Megachilidae) and fruit trees. Bulletin of Entomological Research 92:3-16.

Bullock, S.H. 1999. Relationships among body size, wing size and mass in bees from a tropical dry forest in Mexico. Journal of the Kansas Entomological Society 72:426-439.

Cane, J.H. 1987. Estimation of bee size using intertegular span (Apoidea). Journal of the Kansas Entomological Society 60:145-147.

Cripps, C., and R.W. Rust. 1989. Pollen foraging in a community of *Osmia* bees (Hymenoptera: Megachilidae). Environmental Entomology 18:582-589.

Fægri, K., and L. van der Pijl. 1979. The principles of pollination ecology. 3rd edition. Pergamon Press, New York. 244 pp.

Frohlich, D.R. 1983. On the nesting biology of *Osmia* (*Chenosmia*) *bruneri* (Hymenoptera: Megachilidae). Journal of the Kansas Entomological Society 56:123-130.

Herrera, C.M. 1988. Variation in mutualisms: the spatio-temporal mosaic of a pollinator assemblage. Biological Journal of the Linnean Society 35:95-125.

Hicks, C.H. 1926. Nesting habits and parasites of certain bees of Boulder County, Colorado. University of Colorado Studies 25:217-252.

Hurd, P.D., Jr. 1979. Superfamily Apoidea. Pages 1741-2209 *in* K.V. Krombein, P.D. Hurd, Jr., D.R. Smith, and B.D. Burks, editors, Catalog of Hymenoptera in America north of Mexico. Smithsonian Institution Press, Washington, D.C.

Kalin Arroyo, M.T. 1981. Breeding systems and pollination biology in Leguminosae. Pages 723-769 *in* R.M. Pohill and P.H. Raven, editors, Advances in legume systematics. Royal Botanic Gardens, Kew, Richmond, Surrey.

Kowalczyk, B.F. 1973. The pollination ecology of *Hedysarum alpinum* L. var. *americanum* (MCHX.) and *H. boreale* NVTT. var. *mackenzii* (Richards.) C.L. Hitchc. in the Kluane Lake Area of the Yukon Territory, Canada. Master of Science thesis, University of North Carolina, Chapel Hill.

Krebs, C.J. 1989. Ecological methodology. Harper Collins, New York. 654 pp.

Maeta, Y. 1990. Utilization of wild bees. Farming Japan 24:13-19.

McGuire, D.A. 1993. Interactions for pollination between two synchronously blooming *Hedysarum* species (Fabaceae) in Alaska. American Journal of Botany 80:147-152.

Northstrom, T.E., and S.L. Welsh. 1970. Revision of the *Hedysarum boreale* complex. Great Basin Naturalist 30:109-130.

Parker, F.D. 1985a. A candidate legume pollinator, *Osmia sanrafaelae* Parker (Hymenoptera: Megachilidae). Journal of Apicultural Research 24:132-36.

_____. 1985b. *Osmia sanrafaelae*, a new species from Utah's San Rafael Desert (Hymenoptera: Megachilidae). Journal of the Kansas Entomological Society 58:742-745.

_____. 1986a. Field studies with *Osmia sanrafaelae* Parker, a pollinator of alfalfa (Hymenoptera: Megachilidae). Journal of Economic Entomology 79:384-386.

_____. 1986b. Nesting, associates, and mortality of *Osmia sanrafaelae* Parker. Journal of the Kansas Entomological Society 59:367-377.

_____. 1989. Nest clustering as a means of managing *Osmia sanrafaelae* (Hymenoptera: Megachilidae). Journal of Economic Entomology 82:401-403.

Parker, F.D., and P.F. Torchio. 1980. Management of wild bees. Pages 144-160 *in* Beekeeping in the United States. USDA Agricultural Handbook 335.

Rau, P. 1937. The life-history of *Osmia lignaria* and *O. cordata*, with notes on *O. conjuncta*. Annals of the Entomological Society of America 30:324-343.

Richards, K.W. 1996. Comparative efficacy of bee species for pollination of legume seed crops. Pages 81-103 *in* A. Matheson, S.L. Buchmann, C. O'Toole, P. Westrich, and I.H. Williams, editors, The conservation of bees. Academic Press, San Diego.

Richards, K.W., and P.D. Edwards. 1988. Density, diversity, and efficiency of pollinators of sainfoin, *Onobrychis viciaefolia* Scop. Canadian Entomologist 120:1085-1100.

Rust, R.W. 1974. The systematics and biology of the genus *Osmia*, subgenera *Osmia*, *Chalcosmia*, and *Cephalosmia* (Hymenoptera: Megachilidae). Wasmann Journal of Biology 32:1-93.

_____. 1990. Spatial and temporal heterogeneity of pollen foraging in *Osmia lignaria propinqua* (Hymenoptera: Megachilidae). Environmental Entomology 19:332-338.

Tepedino, V.J., and M. Stackhouse. 1987. Bee visitors of sweetvetch, *Hedysarum boreale boreale* (Leguminosae), and their pollen-collecting activities. Great Basin Naturalist 47:314-318.

Torchio, P.F. 1976. Use of *Osmia lignaria* Say (Hymenoptera: Apoidea: Megachilidae) as a pollinator in an apple and prune orchard. Journal of the Kansas Entomological Society 49:475-482.

Wiens, J.A. 1982. On size ratios and sequences in ecological communities: are there no rules? Annales Zoologici Fennici 19:297-308.

Wolda, H. 1981. Similarity indices, sample size and diversity. Oecologia 50:296-302.

CHAPTER 4

THE COMPARATIVE FORAGING BEHAVIOR, POLLINATION EFFICACY, AND NESTING OF ABUNDANT NATIVE AND CANDIDATE BEE SPECIES, AND HONEYBEES, AT HEDYSARUM BOREALE

INTRODUCTION

The development of a bee species as a new crop pollinator begins with the recognition of a pollination need in agriculture (Bosch and Kemp 2002). The second step in the development a new crop pollinator is to survey the bee fauna of the target crop and choose candidate bee species (Bosch and Kemp 2002). The bee faunas of several natural populations of *H. boreale* were systematically surveyed in 2004 and 2005, and 3 candidate pollinator species chosen as a result: *Osmia bruneri* Cockerell, *Osmia lignaria* Say, and *Osmia sanrafaelae* Parker. After candidate pollinator species are selected, the next step in the development of a new crop pollinator is to study the foraging behavior and pollination efficacy of the candidate pollinator species (Bosch and Kemp 2002).

Definitions of and methods for assessing pollination efficacy abound (Inouye et al. 1994). Traditionally, pollinator abundance and flower visitation rates (herein referred to as foraging tempo) have been used to estimate the quantitative component of plant-pollinator interactions (Herrera 1989). However, strictly quantitative measures reveal little about the overall quality of flower visits by a particular pollinator species. Therefore, qualitative measures of pollination efficacy, such as frequency of pollen transfer and the number of pollen grains deposited on the stigma (Herrera 1987), should also be used to evaluate and compare pollinator species. According to Young (1988),

measures of pollination efficacy should be based on what is accomplished by a particular pollinator during a single flower visit, such as removing and depositing pollen grains, producing seeds, or influencing other aspects of plant reproduction (Inouye et al. 1994). In this study, both quantitative and qualitative measures of pollination efficacy, coupled with observation of foraging behaviors, were used to evaluate and compare candidate *Osmia* species with native bee species found to be abundant at *H. boreale*, and the commercially available pollinator *Apis mellifera*.

Frequency of stigmatic contact is influenced by the floral handling behaviors of pollinator species and the floral resources that they collect (Bosch and Blas 1994, Vicens and Bosch 2000). The *H. boreale* flower is an example of a typical flag blossom as described by Fægri and van der Pijl (1979). Nectar accumulates inside the filament sheath of flag blossoms and can only be accessed via 2 sinuses at the base of the sheath. The filament sheath is enclosed within the keel petal; thus, the only legitimate way for bees to probe flag blossoms for nectar is from the front. In addition, bees gain access to pollen by actively depressing the keel petal of flag blossoms. The pistil and filament sheath are rigid and do move with the keel petal. As a result, the anthers and stigma may contact the underside of a visiting bee provided that its body is sufficiently large (Fægri and van der Pijl 1979). Therefore, a pollinator's means of access to *H. boreale* nectar and pollen, and the consistency with which they collect nectar and pollen, can influence the likelihood of stigma contact. For example, some bees overcome the structural constraints of flag blossoms by robbing nectar (Inouye 1980). Some short-tongued bumblebees commonly rob nectar from legume flowers. Nectar robbers probe for nectar by biting or slicing a hole in the corolla near the nectaries, and do contact the stigma as a result. Such

behaviors diminish a species' pollination value; for example, nectar robbers may be very abundant but provide almost no pollination service.

Frequencies of stigmatic contact are sometimes related to the body size of a pollinator species relative to the size of the flower. For example, some small bee species collect pollen directly from individual anthers and never contact the stigma (Inouye 1980). In general, it is assumed that small bee species are not the best pollinators of large-flowered crops (Inouye 1980, Bosch and Kemp 2002). Several large-bodied bee species, such as *Megachile melanophaea*, are common and ubiquitous elements of the pollinator fauna on *H. boreale*. Bumblebees (*Bombus* spp.) were also present at many of the sites systematically surveyed in 2004 and 2005 (see Chapter 3); queens were easily identified on the wing based on pile coloration on the thorax and abdomen. Bumblebees can be superior pollinators of many crops (Free 1993). Together, these large-bodied species provide a benchmark by which to evaluate the pollination efficacies of the smaller-bodied candidate *Osmia* species and honeybees. In general, *Osmia* species that frequent *H. boreale* appear to fit into comparatively small and large-bodied groupings based on intertegular span and forewing length (Fig. 3-1). Females of *O. bruneri* and *O. sanrafaelae* fell within the small-bodied grouping, while *O. lignaria* females were comparatively large-bodied. Therefore, to gain a better understanding of how body size influences pollination efficacy, the following question is addressed: are comparatively small-bodied candidate *Osmia* species less effective pollinators of *H. boreale*, or can they compensate for their smaller size via particular flower handling behaviors?

H. boreale exhibits considerable ecotypic variation for several characters, including degree of pubescence, structure of the loment, shape and size of leaflets, and

size, and flower size (Northstrom and Welsh 1970). The average size of *H. boreale* flowers and their constituent parts may influence the pollination efficacy of some species, particularly those that are comparatively small-bodied relative to the size of the flower. Therefore, for highly variable species such as *H. boreale*, variation in flower size and morphology should be considered when evaluating the pollination efficacies of diverse species.

Strictly quantitative measures of pollination efficacy, such as pollinator abundance and foraging tempo, reveal little about the overall quality of flower visits by pollinator species. Likewise, simply measuring the frequency of stigmatic contact is not sufficient, as stigmatic contact does not guarantee pollen deposition. Therefore, in this study, the frequency of pollen deposition and the number of pollen grains deposited per single flower visit were used as additional measures of pollination efficacy. For some plant species, the relative pollination efficacy of a particular pollinator species is not independent of pollen and nectar removal and/or deposition by other flower visitors (Thomson and Thomson 1992). The style of *H. boreale* flowers is slender, curves upward toward the keel petal suture, and is topped by a minute stigma possessing many finger-like papillae. Because *H. boreale* flowers can be worked several times (Fægri and van der Pijl 1979), pollen grains deposited "on top" of the stigmatic papillae may be pushed down into the stigmatic papillae by subsequent flower visitors. Therefore, pollen grains deposited 'on top' of the stigmatic papillae may provide an indirect but important contribution to fruit and seed production in *H. boreale*.

Females of solitary bee species construct their own nests and provide food for their offspring without help from other bees. They provision each nest cell with enough

pollen and nectar to support complete larval growth, lay an egg, and close the cell using some kind of foreign material carried to the nest (e.g. leaves, leaf pulp, plant hairs, resin, pebbles, or mud) (Michener 2000). Several variables related to nesting can be used to estimate the pollination capacity of individual females, including the number of foraging trips needed to complete a provision mass, and the duration of foraging trips and other nesting behaviors (Bosch and Kemp 2002). Such information, coupled with estimates of average floral resources produced per acre of *H. boreale*, make it possible to estimate bee stocking density, or the optimal number of female bees needed per unit of crop to provide sufficient pollination services without exceeding carrying capacity and diminishing bee reproduction (Richards 1996). However, prior to estimating stocking densities for *O. bruneri*, *O. lignaria*, and *O. sanrafaelae*, some basic questions must be answered concerning their ability to nest using *H. boreale*. Can females nest and produce a full complement of progeny using *H. boreale* as their only source of pollen and nectar? Can nesting populations increase their numbers from year to year, or at the very least, reproduce enough to replace each nesting female? Having a positive return on female bees promotes sustainability, and helps to ensure that *H. boreale* plants receive adequate pollination from year to year.

Therefore, the first objective of this research is to quantitatively and qualitatively evaluate the pollination efficacy of candidate *Osmia* species in regard to other abundant native bee species found on *H. boreale*, and the commercially available pollinator *A. mellifera.* Quantitative measures of pollination efficacy used in this research include foraging tempo, a measure of how quickly bees move between *H. boreale* flowers. Qualitative measures of pollination efficacy include frequencies of stigmatic contact and

pollen deposition, and the number of pollen grains deposited within and on top of the stigmatic papillae of *H. boreale* flowers during single flower visits. The second objective of this research is to determine whether females of candidate *Osmia* species can nest using *H. boreale* as their only source of pollen and nectar, and to estimate how much pollen and nectar females need to sustain themselves and their progeny. This information, coupled with estimates of average floral resources produced per acre of *H. boreale* (see Chapter 2), will be used estimate an appropriate stocking density for each candidate *Osmia* species.

MATERIALS AND METHODS

Comparative Foraging Behavior and Pollination Efficacy of Abundant Native and Candidate Bee Species, and Honeybees, at *H. boreale*

STUDY SITES.—Data concerning the comparative foraging behavior and/or pollination efficacy of several bee species at *H. boreale* were collected at 4 sites in Cache County, Utah, USA in 2004 and 2005. Data for honeybees (*A. mellifera*) and queen bumblebees (*Bombus fervidus*, *Bombus huntii*, and *Bombus griseocollis*) were collected at a cultivated stand of *H. boreale* at Evans experimental farm near Logan, Utah in 2005 (Table 2-1; EF). Data for bumblebee queens (*Bombus appositus* and *Bombus nevadensis*) and 4 native megachilid species (*Megachile frigida*, *Megachile gemula*, *M. melanophaea*, and *Osmia simillima*) were collected at a natural population of *H. boreale* at Wood Camp Hollow in 2004 and/or 2005 (Table 2-1; WCH). Data for *O. bruneri* were collected within a net field cage at EF in 2005. Likewise, data for *O. lignaria* and *O. sanrafaelae* were collected within a net field cage at the Bee Biology and Systematics Laboratory in

Logan, Utah in 2005 (Table 2-1; BBSL). In addition, open nesting trials with *O. lignaria* females were conducted at a 2-acre stand of 'Timp' Utah sweetvetch near Worland, Wyoming in 2004 and 2005 (Table 2-1; WRL).

COMPARATIVE FORAGING BEHAVIOR.—The foraging behaviors of 11 bee species at *H. boreale* flowers were observed in 2004 and 2005, including *A. mellifera*, *B. appositus*, *B. fervidus*, *B. huntii*, *B. griseocollis*, *M. frigida*, *M. gemula*, *M. melanophaea*, *O. bruneri*, *O. lignaria*, and *O. sanrafaelae*. In both years, observations were made between approximately 10 AM and 3 PM during the peak activity period of each species (from May to July). Observations focused on honeybee workers, queen bumblebees, and females of solitary species. Females of those species observed in the open were haphazardly chosen for observation. Observations focused on behaviors that contribute to pollination, such as the frequency and means by which females foraged for nectar and/or pollen, and to positioning on the flower. Additional observations were made concerning fidelity in floral handling behaviors by foraging honeybees at EF in 2005.

FORAGING TEMPO.—The foraging tempos of 10 bee species were timed between May and July 2005. Foraging tempo was measured as the duration in seconds of 5 complete flower visits; timing began when a foraging female first visited a raceme and stopped when she landed on a sixth flower. Thus, this measure of foraging tempo includes both flower handling and interfloral flight times. Foraging tempos were timed between 10:30 AM and 2:30 PM. Foraging tempos for *O. lignaria* were collected on 28 and 29 May. Foraging tempos for *A. mellifera*, *B. fervidus*, *B. griseocollis*, and *B. huntii* were collected from 4 – 19 June. Foraging tempos for *O. sanrafaelae* were collected from 14 – 17 June. Foraging tempos for *O. bruneri* were collected on 20 and 21 June. Foraging

tempos for *B. appositus*, *M. frigida*, and *M. melanophaea* were collected from 6 – 9 July. Females of those species timed in the open were haphazardly chosen. Mode of interfloral travel was recorded for each timed duration of foraging tempo; in particular, it was noted whether females flew or walked between flowers, or combined both modes of travel.

FREQUENCY OF STIGMATIC CONTACT.—From May to July 2005, foraging females were observed for how often they touched the stigma with a part of their body bearing pollen (i.e. legitimate visitation). Numbers of legitimate and illegitimate flower visits, and reasons for illegitimate flower visits, were recorded for individual foraging females. Data were collected between 10:30 AM and 2:30 PM, at the same locations, and on the same dates as foraging tempos. Females of those species observed in the open were haphazardly chosen.

POLLEN GRAINS DEPOSITED PER SINGLE FLOWER VISIT.—Pollen deposition data were collected for 4 native bee species foraging on *H. boreale* at WCH between 2 and 9 July 2004, including *B. appositus*, *B. nevadensis*, *M. melanophaea*, and *O. simillima*. In 2005, pollen deposition data were collected for 7 bee species. Single flower visits by *A. mellifera*, *B. fervidus*, and *B. huntii* were obtained from 13 – 20 June. Single flower visits by *O. sanrafaelae* were obtained on 16 and 17 June. Single flower visits by *O. bruneri* were obtained on 20 and 21 June. Single flower visits by *M. melanophaea* were obtained between 8 and 15 July. In both years, data were collected between 10:30 AM and 5 PM.

In 2004, racemes at the bud stage were bagged on several haphazardly chosen plants at WCH. In 2005, 5 plants were haphazardly chosen at each of the 3 sites (BBSL, EF, and WCH) and several racemes bagged at the bud stage on each plant. In 2005 only,

1 flower was haphazardly chosen from 5 bagged racemes on each plant to serve as a control for pollen grains in the stigmatic papillae prior to bee visitation (n = 25 control flowers per site). In both years, experimental racemes were unbagged, cut near the base of the pedicel, placed in a florist's water pick secured to the end of a bamboo rod, and presented to foraging females (Kremen et al. 2002). Females of those species foraging in the open were haphazardly chosen. Flowers visited once by a female were immediately collected and placed in a well of a tissue culture plate for transport to the laboratory.

Petals of unvisited control and visited flowers were removed using forceps. The sexual column was excised at the base of the free portion of the stamens. The resulting length of style was gently removed and placed on a small slab of fuchsin gelatin on a glass slide for cleaning; the style was placed so that the stigma hung over the end of an unused portion of the gelatin slab. Forceps were used to roll the style across the gelatin to remove any errant pollen grains without letting any part of the stigma contact the gelatin. This procedure was performed under a stereomicroscope. Cleaned styles were then squashed individually in acetocarmine jelly on precleaned glass microscope slides (25 x 75 mm) heated on a stir plate. Squashed stigmas were viewed using an Inverted System Microscope (Model IX70 by Olympus) at 400X by focusing up and down throughout the papillae. For each squashed stigma, the number of pollen grains deposited within the papillae was counted at least twice to confirm counts.

Not all of the pollen grains left by a bee during a single flower visit were deposited within the stigmatic papillae. In fact, a portion of the total number of pollen grains left by a bee during a single flower visit was deposited on top of the stigmatic papillae. In order to measure the total number of pollen grains left by bee species during

single flower visits, other pollen grains appearing on the slide were viewed at lower magnification (100X) and counted separately from those deposited within the stigmatic papillae. In 2004, pollen grains were counted from margin to margin of the cover slip. In 2005, several slides were contaminated with pollen that remained on the style after it was cleaned. To solve this problem, dark distinctive pollen from an oriental poppy was applied with a fine tip paintbrush to stigmas of unvisited, new *H. boreale* flowers from BBSL. The basic trajectory of pollen off the top of the squashed stigmatic papillae was determined from the location of poppy pollen grains on the slide. Pollen trajectories from 5 flowers were sketched and pieced together, and used to distinguish those *H. boreale* pollen grains that were originally deposited on top of the stigmatic papillae from those that were due to contamination.

FLOWER MORPHOMETRICS.—Two racemes were bagged at the bud stage on each of the 5 plants chosen for pollen deposition experiments at BBSL, EF, and WCH in 2005. Approximately 5 flowers were haphazardly chosen on each raceme and measured using digital calipers in the laboratory. Four floral measures were repeated from Kowalczyk's (1973) study of *H. boreale* ssp. *mackenzii* populations in Alaska, including keel length, distance of stigma from nectary (along top plane), distance of stigma from nectary (along bottom plane), and length of the curved portion of style. The distance of the anther tips from the bottom plane of the sexual column was also measured in this study.

Nesting Trials with Candidate *Osmia* Species

OPEN NESTING TRIALS.—Female *O. lignaria* were released adjacent to 2 acres of 'Timp' Utah sweetvetch at WRL in 2004. A nesting shelter was set up on 11 May. Overwintered nesting straws containing approximately 40 *O. lignaria* females from Cache County, Utah, were placed in an emergence box at this time. Females were supplied with new nesting substrate composed of wooden blocks with drilled holes fitted with new paper straw inserts (9 mm diameter, 152 mm length). The shelter and nesting materials were retrieved on 11 July. Wooden nesting blocks were stored outside in a sheltered area at BBSL until early October, at which time plugged paper straw inserts were carefully removed from the blocks. On 14 October, the contents of nesting straws were viewed using X-rays (HP 4380N Faxitron, 25 KVP, 30 seconds, Industrex-M 8x10" film). The numbers of completed nest cells, dead eggs (indicated by intact complete provision masses), dead larvae/prepupae, and live adult progeny were counted in each straw. Because females were allowed to nest unobserved, pollen grains from intact provision masses were examined using fuchsin gelatin slides to determine whether females nesting using *H. boreale* pollen.

The open nesting trail described above was repeated in 2005. On 18 May, approximately 200 *O. lignaria* females were released at WRL. In addition to 4 wooden nesting blocks with paper straw inserts, these females were supplied with 8 bundles of 20 cardboard tubes plugged at 1 end with wall joint compound. The shelter and nesting materials were collected on 18 August. Nesting materials were again stored outside in a sheltered area at BBSL until early October, at which time paper straw inserts were carefully removed from the wooden nesting blocks. On 25 October, the contents of paper

nesting straws and cardboard tubes were viewed using X-ray exposure (25 KVP, 60 seconds) and counted as before.

FIELD CAGE TRIALS.—In 2005, net field cages measuring 6 x 6 x 2 meters were assembled at BBSL and EF prior to bloom. Wooden nesting blocks fitted with new paper straw inserts of appropriate diameter for the caged species (*O. bruneri*: 6 mm; *O. lignaria* and *O. sanrafaelae*: 9 mm) were mounted with wire to a T-post in the center of each cage. Overwintered nests collected from the wild were placed in an emergence box affixed to the top of each nesting block. Overwintered nests with approximately 15 females of *O. lignaria* from Cache County, Utah, were placed in an emergence box in the BBSL field cage on 14 May. Overwintered nests with approximately 20 females of *O. sanrafaelae* from Washakie County, Wyoming, were placed in the BBSL emergence box on 31 May. Overwintered nests with approximately 15 *O. bruneri* females from Cache County, Utah, were placed in an emergence box in the EF field cage on 28 May. Nesting blocks were removed from the field cages once females were done nesting. Blocks were stored outside in a sheltered area at BBSL until early October, at which time paper straw inserts were carefully removed from the blocks. The contents of paper nesting straws from each caged species were viewed using X-rays (25 KVP, 30 seconds) on 25 October and counted as before.

In order to preserve live adult progeny for use in subsequent research, cocoons were not dissected to determine the sex of adult progeny. Instead, published records of sex ratios for each species were used to estimate how many male and female progeny were produced during all of the open nesting and field cage trials described above.

Once caged females had been nesting for several days, nest entrance traffic was filmed using a camcorder mounted on a tripod. Nesting block traffic was taped during the following time periods: *O. lignaria* from 12 – 2 PM on 28 May, and 10 AM – 12 PM on 31 May; *O. sanrafaelae* from 11 AM – 3 PM on 14 June; and *O. bruneri* from 11 AM – 3 PM on 18 June. Tapes were transcribed for durations of foraging (collecting pollen and nectar, mud, or leaf pieces), provisioning (using pollen and nectar to make provision masses), and partitioning (building cell partitions from mud or masticated leaf) behaviors. Pollen and nectar foraging trips were measured as the time in seconds a female spent away from the nest when provisioning nest cells (as indicated by a female's exit, turn, and prompt re-entrance, abdomen first, into her nest to offload pollen). Provisioning was measured as the total time spent in the nest, from first entrance to final exit, when a female exited, turned, and promptly re-entered her nest to offload pollen. Mud and leaf foraging trips were measured as the time spent away from the nest when a female did not exit, turn, and promptly re-enter her nest; likewise, partitioning was measured as the time spent in the nest between mud or leaf foraging trips.

POLLEN GRAINS PER PROVISION MASS.—Intact complete provision masses were removed from *O. bruneri* and *O. sanrafaelae* nests on 25 October 2005. Provision masses were placed individually in particle counting vials and stored at −16°C. A 30-ml aliquot of filtered ethanol was added to each vial, which was sonicated for 10 – 20 minutes until the provision mass was completely dispersed. An additional 20-ml aliquot of filtered ethanol was then added to each vial. Vials were again sonicated, and a pipette used to extract a 1-ml aliquot of pollen in solution. This 1-ml aliquot was added to 49 ml

of filtered ethanol in another vial. Three such dilutions were performed for each provision mass. Pollen grains were counted using the method described in Chapter 2.

POLLEN GRAINS REMOVED PER SINGLE FLOWER VISIT.—Single flower visits were obtained to determine how many flowers a female must visit in order to complete a single provision mass. Additional racemes were bagged at the bud stage on the plants haphazardly chosen at BBSL and EF for pollen deposition experiments. These racemes were unbagged, cut at the base of the pedicel, placed in a florist's water pick secured to the end of a bamboo rod, and presented to pollen/nectar-foraging females of *O. bruneri* and *O. sanrafaelae*. Flowers visited by a female were immediately collected and placed in a well of a tissue culture plate; one unvisited control flower was collected from the same raceme as each experimental flower. Pollen grains in unvisited control flowers and visited flowers were counted using the method described in Chapter 2.

Data Analysis

In general, data are reported as mean ± standard error ($X \pm s_x$) unless otherwise indicated. ANOVA degrees of freedom are given in subscript brackets. A probability level of $P \leq 0.05$ was deemed significant in all analyses.

FORAGING TEMPO.—Foraging tempos were analyzed using completely randomized design ANOVA in the PROC GLM procedure of SAS Version 9.1 (SAS Institute Inc. 2004). Bee species and genus were used as fixed factors in 2 separate analyses. In additional, the foraging tempos of apid species (*A. mellifera*, *B. appositus*, *B. fervidus*, *B. griseocollis*, and *B. huntii*) were analyzed using a completely randomized design ANOVA with species and mode of interfloral travel as fixed factors using the

PROC MIXED procedure of SAS Version 9.1. Treatment means were compared using REGWQ and Tukey-Kramer a posteriori tests in PROC GLM and PROC MIXED, respectively. Foraging tempos were satisfactorily normalized using $\log_{10}$ transformations.

POLLEN GRAINS DEPOSITED PER SINGLE FLOWER VISIT.—Pollen deposition data collected in 2004 were analyzed using a completely randomized design ANOVA with bee species as a fixed factor in PROC GLM. Treatment means were compared using REGWQ a posteriori tests. Data were satisfactorily normalized by a $\log_{10}$ transformation of (the number of pollen grains within the stigmatic papillae + 1).

For pollen deposition data collected in 2005, the null hypotheses that the presence of pollen 1) within and 2) on top of the stigmatic papillae of unvisited control flowers is independent of collection site were tested by *R*X*C* Tests of Independence using the *G*-Test with William's correction (Sokal and Rohlf 1995). Likewise, the null hypotheses that the frequency of pollen deposition 1) within and 2) on top of the stigmatic papillae of flowers visited once by a bee is independent of bee species were tested by *R*X*C* Tests of Independence using the *G*-Test with William's correction. Data then were excluded from subsequent analyses when flowers had no pollen grains within or on top of the stigmatic papillae. Data for flowers with ≥ 1 pollen grain(s) within the stigmatic papillae were analyzed using a completely randomized design ANOVA with bee species as a fixed factor in PROC GLM. Likewise, data for flowers with ≥ 1 pollen grain(s) on top of the stigmatic papillae were analyzed using a completely randomized design ANOVA with bee species as a fixed factor in PROC GLM. In both analyses, treatment means were compared using REGWQ a posteriori tests. In addition, Pearson correlation analysis was used to test for positive associations between pollen grains deposited within and on top of

the stigmatic papillae during flower visits by individual bee species. T-tests were used to analyze the numbers of pollen grains deposited by *M. melanophaea* females within and on top of the stigmatic papillae of flowers in 2004 and 2005.

FLOWER MORPHOMETRICS.—Five measures of floral morphology were individually analyzed using completely randomized design ANOVA with site as a fixed factor and plant as a random factor in PROC MIXED. Treatment means were compared using Tukey-Kramer a posteriori tests. Distances from the stigma to the nectary, along both the top and bottom planes, were satisfactorily normalized using cube transformations. The lengths of both the curved portion of the style and of the free portion of the stamens were satisfactorily normalized using square transformations. In addition, Pearson correlation analysis was used to test for positive associations between the length of the curved portion of the style and the length of the free portion of the stamen in individual flowers.

RESULTS

Comparative Foraging Behavior and Pollination Efficacy of Abundant Native and Candidate Bee Species, and Honeybees, at *H. boreale*

COMPARATIVE FORAGING BEHAVIOR.—Megachilid species regularly collected both nectar and pollen from *H. boreale* flowers. For example, *O. lignaria* females simultaneously collected both nectar and pollen during most flower visits. While probing for nectar, *O. lignaria* females opened the keel petal with their fore- and midlegs (by pulling outward on the wing petals) and brushed their hind legs back and forth alongside the anthers to loosen and collect pollen. During most flower visits the anthers

and stigma of *H. boreale* flowers were located directly beneath the abdomen of *O. lignaria* females; females often patted their abdomen on top of the anthers as they collected pollen with their hind legs. Even females not intentionally collecting pollen appeared to contact the stigma during many flower visits. Females of *M. frigida*, *M. gemula*, and *M. melanophaea*, 3 native megachilid species similar in size to *O. lignaria*, were also observed to simultaneously collect nectar and pollen from *H. boreale* flowers much like *O. lignaria* females.

Female *O. bruneri* and *O. sanrafaelae* collected nectar and pollen in sequence. While probing for nectar, these smaller-bodied females opened the keel petal of *H. boreale* flowers with their fore- and midlegs, and loosened pollen with their hind legs. However, unlike *O. lignaria* females, *O. bruneri* and *O. sanrafaelae* females were not large enough to have their abdomen positioned directly above the anthers while they probed for nectar. Therefore, after they finished collecting nectar, females backed up on the keel petal and used their mid- and hind legs to scrape pollen from the anthers into their scopa while simultaneously patting their abdomen directly on top of the anthers. Female *O. bruneri* and *O. sanrafaelae* performed this back-up maneuver on most flower visits, and were observed to contact the stigma during many flower visits.

Bumblebee queens collected nectar but did not collect pollen directly from *H. boreale* flowers. Instead, bumblebee queens were observed to groom pollen from their bodies with their forelegs and pack it into their corbiculae, often while hanging from the keel petal of an *H. boreale* flower. The basic positioning of bumblebee queens on *H. boreale* flowers differed slightly among species. Queens of *B. fervidus* opened the keel petal with their forelegs and stabilized their large bodies by placing their midlegs on the

standard petal and their hind legs on the keel (or stem, adjacent flowers, etc.). Queens of *B. huntii* also opened the keel petal with their forelegs, but generally positioned their other pairs of legs on the keel petal itself. On rare occasions, *B. huntii* queens handled *H. boreale* flowers much like *B. fervidus* queens. Regardless of basic positioning, the anthers and stigma of *H. boreale* flowers tended to contact bumblebee queens on the underside of the thorax. Queens were often observed to groom pollen from this area of the body.

Honeybees exhibited 3 distinct behaviors when handling *H. boreale* flowers: legitimate nectar foraging, nectar thieving, and pollen gleaning. While probing for nectar, legitimate nectar foragers often grasped either side of the keel petal with their hind legs and were likely to contact the stigma when in this position. However, when legitimate nectar foragers were not aligned correctly on the keel petal, the style often slid up alongside the abdomen without making contact. Nectar thieves were never aligned correctly on *H. boreale* flowers, but were instead positioned aside the keel petal. Nectar thieves generally probed for nectar without opening the keel petal and rarely contacted the stigma as a result. Honeybees infrequently collected pollen directly from *H. boreale* flowers. Some legitimate nectar foragers brushed their hind legs back and forth alongside the anthers to loosen pollen, but this particular behavior was rare. More often, pollen gleaning foragers landed at the distal tip of the keel petal and pried it open by inserting their tongue into the suture. They then used their forelegs to scrape pollen from the anthers and transfer it to the underside of their thorax and abdomen. Pollen gleaners and legitimate nectar foragers alike were observed to groom pollen from their bodies and

pack it into their corbiculae. Similar pollen gleaning behavior was exhibited by the *Hylaeus* species collected during systematic surveys of bee fauna in 2005 (see Chapter 3).

Individual honeybees were observed to engage 1 or more foraging behaviors at sequential flowers. Of 38 honeybees, 32 exhibited a single type of foraging behavior during a sequence of flower visits; of these, 29 were legitimate nectar foragers, 2 were nectar thieves, and 1 was a pollen gleaner. Thirteen honeybees exhibited 2 behaviors during sequential flower visits; of these, 10 were both legitimate nectar foragers and nectar thieves, while 3 were both nectar thieves and pollen gleaners. Only 1 honeybee exhibited all 3 behaviors during a sequence of flower visits.

It appears that *H. boreale* nectar was accessible to most bee visitors including comparatively small-bodied species. Bees were not observed to rob nectar from *H. boreale* flowers, and flowers had no evidence of past nectar robbing (i.e. a hole cut near the base of the corolla). Therefore, nectar robbing behavior was not an important factor affecting the pollination of *H. boreale* flowers.

FORAGING TEMPO.—There were significant differences in the speed with which bee species foraged on *H. boreale* flowers ($F_{[9,187]} = 32.83$, $P < 0.0001$) (Table 4-1). On average, *B. fervidus* queens foraged faster than other species, while female *O. lignaria* and *O. sanrafaelae* were the slowest foragers. Overall, *Bombus* queens and female *Megachile* foraged significantly faster than did honeybees and *Osmia* females ($F_{[3,193]} = 60.48$, $P < 0.0001$).

A majority of bees began foraging near the bottom of *H. boreale* racemes and rotated around the raceme as they foraged upwards. Megachilid females invariably made short flights between flowers as they foraged up and between racemes. The interfloral

Table 4-1. Mean foraging tempos for 10 bee species at *H. boreale*. Foraging tempo was measured as the duration in seconds of 5 complete flower visits. Species are listed from fastest to slowest based on mean foraging tempo. Means followed by different letters are statistically different ($P \leq 0.05$).

		Foraging tempo (seconds)		
Species	*n*	$\bar{X}$	$s_{\bar{x}}$	Range
B. fervidus	20	12.5^{a}	1.4	6.5 – 26.7
B. huntii	20	17.4^{b}	1.1	10.8 – 26.8
M. melanophaea	21	18.2^{b}	1.6	9.8 – 36.39
M. frigida	20	19.4^{b}	2.1	12.2 – 48.0
B. griseocollis	17	19.9^{b}	1.8	9.1 – 36.4
B. appositus	16	$22.5^{b,c}$	2.5	12.0 – 50.6
O. bruneri	20	$28.1^{c,d}$	2.3	16.3 – 58.0
A. mellifera	20	$37.1^{d,e}$	2.4	18.6 – 54.8
O. sanrafaelae	22	$39.6^{e,f}$	2.8	18.5 – 71.8
O. lignaria	21	52.1^{f}	3.7	26.6 – 80.8

foraging patterns of honeybees and *Bombus* queens were more varied, however. Individuals either flew between flowers or climbed from flower to flower as they moved up a raceme; some individuals combined both modes of travel as they foraged. Mode of interfloral travel was not a significant factor influencing honeybee and bumblebee foraging tempos ($F_{[2,65]} = 0.62$, $P = 0.5414$). However, there was a significant interaction between travel mode and species ($F_{[7,65]} = 4.03$, $P < 0.0001$). Queens of *B. fervidus* and *B. huntii* foraged fastest when they flew between flowers, whereas *B. appositus* and *B. griseocollis* queens were fastest when they combined travel modes. Honeybees foraged fastest when climbing between flowers, followed by flying, but were comparatively slow foragers when they combined travel modes.

FREQUENCY OF STIGMATIC CONTACT.—Honeybees contacted the stigma less frequently than did other bee species (Fig. 4-1). Honeybees often landed just off center of the keel petal suture and caused the style to slip upward past their abdomen

without making contact. Likewise, some bumblebee queens were more likely to illegitimately visit flowers when walking between them or when using flowers like ladder rungs as they foraged up a raceme. When conditions were windy, honeybees appeared to confuse the standard and keel petals of *H. boreale* flowers. Other species were more adept at distinguishing between the standard and keel petals; for example, *M. melanophaea* females were observed to correctly visit flowers in unusual positions (such as inverted flowers). Nectar thieving was the most common reason for illegitimate flower visits among apid species, although *M. frigida* and *M. melanophaea* also thieved nectar

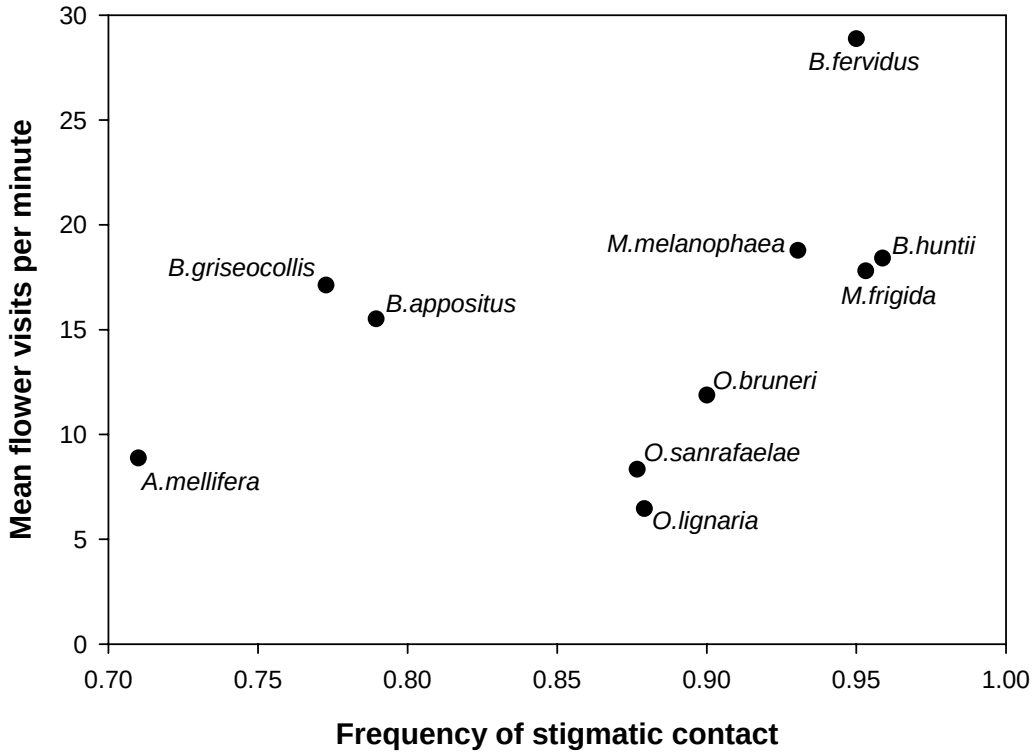

Fig. 4-1. Qualitative versus quantitative measures of pollination efficacy for 10 bee species at *H. boreale*. Mean flower visits per minute were derived from foraging tempo data (Table 4-1). Frequency of stigmatic contact sample sizes were as follows: *A. mellifera*, *n* = 100 flower visits; *B. appositus*, 19; *B. fervidus*, 100; *B. griseocollis*, 44; *B. huntii*, 97; *M. frigida*, 64; *M. melanophaea*, 115; *O. bruneri*, 100; *O. sanrafaelae*, 73. Faster, more consistent pollinators plot toward the upper right corner of the graph.

from *H. boreale* flowers. In contrast, failure to open the keel petal and expose the stigma was the most widespread reason for illegitimate flower visits among megachilids. When flowers had particularly long exerted styles, *O. bruneri* and *O. sanrafaelae* females commonly pushed the style and stigma away from their bodies while brushing the anthers with their hind legs. When females subsequently backed up to pat their scopal hairs on top of the anthers, the style was often angled away from their abdomen leaving the stigma untouched.

When quantitative (foraging tempo) and qualitative (frequency of stigmatic contact) measures of pollination efficacy are combined (Fig. 4-1), it is evident that some bee species are both faster and more consistent foragers at *H. boreale* flowers than others. Queens of *B. fervidus* were the fastest foragers and were nearly as consistent as *B. huntii* queens in frequency of stigmatic contact. On the other hand, honeybees were comparatively slow and inconsistent foragers.

POLLEN GRAINS DEPOSITED PER SINGLE FLOWER VISIT.—In 2004, mean numbers of pollen grains deposited within the stigmatic papillae by *B. appositus*, *B. nevadensis*, *M. melanophaea*, and *Osmia simillima* were not significantly different ($F_{[3,51]} = 0.75$, $P = 0.5266$) (Table 4-2). An unknown portion of pollen grains deposited on top of the stigmatic papillae were assumed to result from contamination. The clarity of fuchsin gelatin decreases over time; therefore, it was not possible to go back and determine which pollen grains resulted from contamination in 2004 using the same method employed in 2005 (oriental poppy pollen). These data were not analyzed as a result.

In 2005, the proportions of unvisited control flowers with pollen grains within and on top of the stigmatic papillae were independent of site (within the papillae,

Table 4-2. Mean pollen grains deposited within and on top of the stigmatic papillae during single flower visits by 4 native bee species in 2004. Control flowers were not collected in 2004. Mean pollen grains deposited within the stigmatic papillae were not statistically different ($P \leq 0.05$). Data for pollen grains deposited on top of the stigmatic papillae were not analyzed. n = number of flowers examined.

		Pollen deposited within the stigmatic papillae			Pollen deposited on top of the stigmatic papillae		
Species	n	X	s_x	Range	X	s_x	Range
B. appositus	14	5.1	1.5	0 – 15	26.0	14.2	2 – 206
B. nevadensis	11	8.0	1.8	0 – 17	49.6	8.7	19 – 120
M. melanophaea	23	7.3	1.6	0 – 26	31.1	5.3	2 – 106
O. simillima	7	3.3	0.8	0 – 6	67.0	38.9	9 – 297

Table 4-3. Mean number of pollen grains found within and on top of the stigmatic papillae of unvisited control flowers collected at 3 sites in 2005. n = number of flowers examined.

		Pollen grains within the stigmatic papillae			Pollen grains on top of the stigmatic papillae		
Site	n	X	s_x	Range	X	s_x	Range
BBSL	25	0.3	0.2	0 – 4	2.0	0.5	0 – 10
EF	25	0.4	0.2	0 – 4	7.8	2.5	0 – 48
WCH	25	0.9	0.6	0 – 14	4.4	0.9	0 – 15

$G_{adj[2]} = 2.19$, $P > 0.05$; on top of the papillae, $G_{adj[2]} = 3.85$, $P > 0.05$). In addition, the mean numbers of pollen grains within and on top of the stigmatic papillae were similar between sites (Table 4-3). Therefore, pollen grains deposited by bee species during single flower visits were analyzed without reference to site, and were compared to the combined control averages of 0.5 ± 1.8 and 4.8 ± 8.2 pollen grains within and on top of the stigmatic papillae, respectively.

The proportions of visited flowers and unvisited control flowers with pollen grains in the stigmatic papillae were dependent on treatment (control and bee species) in 2005 ($n = 299$, $G_{adj[6]} = 110.89$, $P << 0.0001$). For flowers with ≥ 1 pollen grain(s) within

the stigmatic papillae (n = 197), there were significant differences between treatment groups ($F_{[6,190]}$ = 8.27, P < 0.0001). Visits by all 6 bee species resulted in more pollen grains deposited within the stigmatic papillae than were found in the papillae of unvisited control flowers (Fig. 4-2). The proportions of visited flowers and unvisited control flowers with pollen grains on top of the stigmatic papillae were dependent on treatment in 2005 (n = 313, $G_{adj[6]}$ = 113.55, P << 0.0001). For flowers with ≥ 1 pollen grain(s) on top of the stigmatic papillae (n = 269), there were significant differences between treatment groups ($F_{[6,262]}$ = 15.31, P < 0.0001). Again, all 6 bee species deposited more pollen grains on top of the stigmatic papillae than were found on top of unvisited control stigmas (Fig. 4-2).

Pollen grains deposited within and on top of the stigmatic papillae in 2005 were significantly and positively correlated in flowers visited once by female *B. fervidus* (r = 0.50, P = 0.0085), *B. huntii* (r = 0.43, P = 0.0082), *M. melanophaea* (r = 0.45, P = 0.0054), *O. bruneri* (r = 0.72, P < 0.0001), and *O. sanrafaelae* (r = 0.51, P < 0.0096). However, pollen grains deposited within and on top of the stigmatic papillae were not significantly correlated in flowers visited once by honeybees (r = 0.1942, P = 0.0885).

Pollen grains deposited in the stigmatic papillae by female *M. melanophaea* in 2004 and 2005 did not differ ($t_{[58]}$ = 0.96, P = 0.3426). However, pollen grains deposited on top of the stigmatic papillae did differ according to year ($t_{[58]}$ = 3.48, P = 0.0010).

Female *O. bruneri* deposited 19.5 ± 2.7 pollen grains within the stigmatic papillae of *H. boreale* flowers on average (n = 33, range = 0 – 54) and 34.1 ± 24.6 pollen grains on top of the stigmatic papillae (n = 33, range = 0 – 112). On average, control flowers at

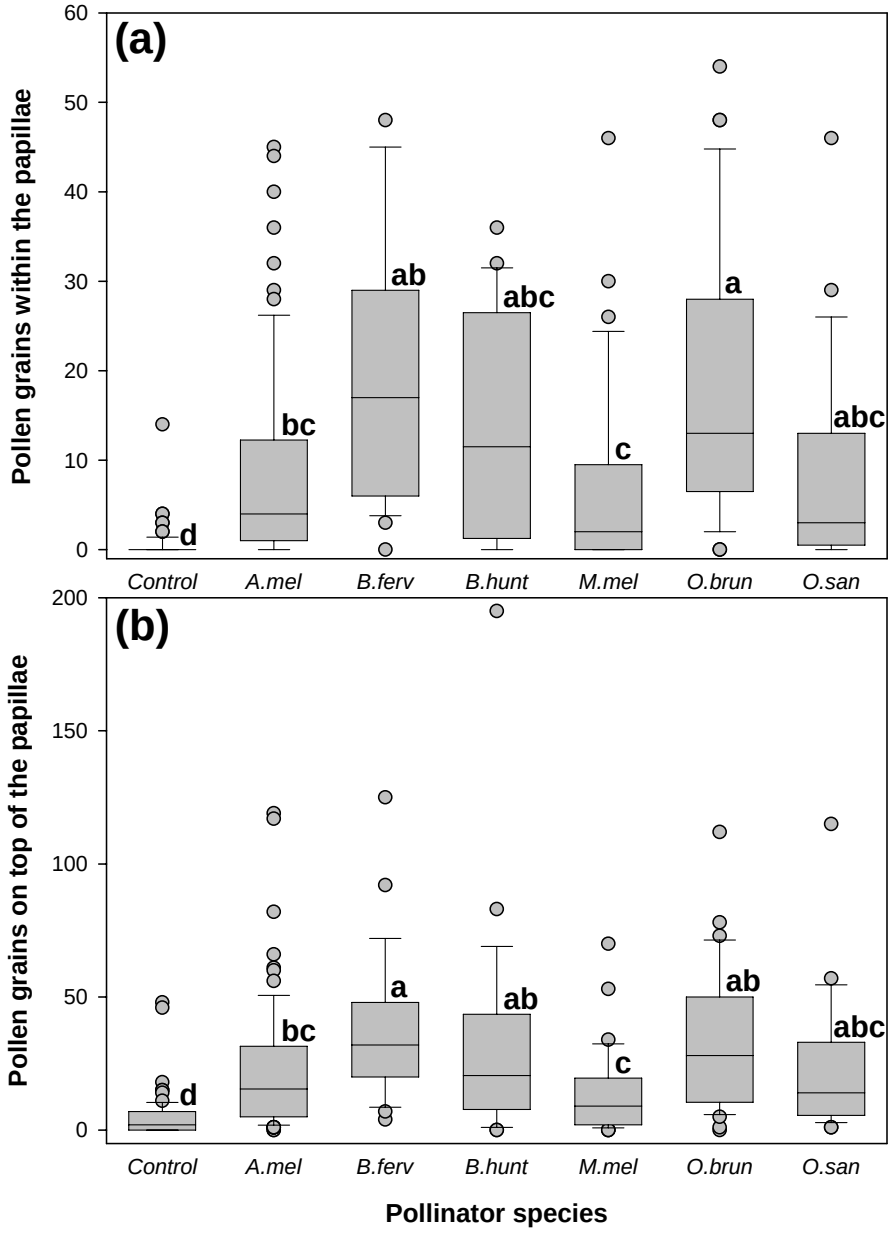

Fig. 4-2. Pollen grains deposited (a) within and (b) on top of the stigmatic papillae of *H. boreale* flowers during single visits by 6 bee species in 2005. Data were collected at 3 sites. Control: $n = 75$ flowers. Bee species: *A. mellifera*, $n = 78$ flower visits; *B. fervidus*, 27; *B. huntii*, 24; *M. melanophaea*, 37; *O. bruneri*, 33; *O. sanrafaelae*, 25. Plotted are the median and lower (25) and upper (75) quantiles (box), the 10th and 90th percentiles (whiskers), and outliers (points). Letters indicate significant treatment differences ($P \leq 0.05$).

EF had 0.4 ± 0.2 pollen grains within the stigmatic papillae (Table 4-3). Female *O. bruneri* deposited ≥ 1 pollen grain(s) within the stigmatic papillae during 31 out of 33 flowers visits. Therefore, it is likely that females contacted the stigma during at least 94% of flower visits. Likewise, female *O. sanrafaelae* deposited an average of 8.8 ± 2.3 pollen grains within the stigmatic papillae (n = 25, range = 0 – 46) and 23.8 ± 5.1 pollen grains on top of the stigmatic papillae (n = 25, range = 1 – 115) of *H. boreale* flowers. Control flowers at BBSL had 0.3 ± 0.2 pollen grains within the stigmatic papillae on average (Table 4-3). Females deposited ≥ 1 pollen grain(s) during 19 out of 25 or 76% of flowers visits.

The stigmatic threshold for fruit set and subsequent seed production in *H. boreale* is unknown. However, *H. boreale* seed is produced in loments with 2 to 8 articles (Stevens et al. 1996). Female *O. bruneri* deposited ≥ 8 pollen grains during 24 out of 33 flower visits (73%), whereas *O. sanrafaelae* females deposited 8 or more pollen grains during only 11 of 25 visits (44%). Therefore, *O. bruneri* females were more likely to deposit more pollen grains amid the stigmatic papillae than those needed to fertilize each ovule within an *H. boreale* ovary (assumed to be 8).

Just 2 single visits by *O. lignaria* females were obtained in 2005. During the first flower visit, a female deposited 38 and 76 pollen grains within and on top of stigmatic papillae, respectively. It appears that the female did not contact the stigma during the second flower visit; only 0 and 3 pollen grains were found within and on top of the stigmatic papillae, respectively.

FLOWER MORPHOMETRICS.—Flower size was not consistent between plants at different sites. Cultivated ‘Timp’ plants at EF produced significantly larger flowers

Table 4-4. Mean values (± standard deviation) for 5 measures of floral morphology for plants from 1 natural population and 2 cultivated stands of *H. boreale*. Sample sizes: BBSL, $n = 97$ flowers; WCH, 100; EF, 100. Means followed by different letters are statistically different ($P \leq 0.05$).

	Site		
Floral morphology measures (mm)	BBSL[1]	EF[1]	WCH[2]
Keel length	17.06[a] (± 1.54)	18.57[b] (± 1.70)	16.82[a] (± 1.22)
Distance of stigma from nectary (along top plane)	15.89[a] (± 1.95)	17.01[b] (± 1.59)	15.76[a] (± 1.32)
Distance of stigma from nectary (along bottom plane)	15.17[a] (± 1.83)	16.03[b] (± 1.49)	14.91[a] (± 1.24)
Length of curved portion of style	4.08[a] (± 1.08)	5.45[c] (± 0.97)	5.15[b] (± 0.91)
Length of free portion of stamen	3.74[a] (± 0.85)	4.44[b] (± 0.69)	4.34[b] (± 0.59)

[1]cultivated stand
[2]natural population

than did plants at BBSL and WCH in terms of keel length ($F_{[2,294]} = 40.08$, $P < 0.0001$), distance from stigma to nectary (along top plane) ($F_{[2,294]} = 20.09$, $P < 0.0001$), and distance from stigma to nectary (along bottom plane) ($F_{[2,294]} = 17.58$, $P < 0.0001$) (Table 4-4). The length of the curved portion of style tended to be comparatively short in flowers from BBSL plants, of medium length in flowers from WCH plants, and comparatively long in flowers plants at EF ($F_{[2,294]} = 10.56$, $P < 0.0001$). The length of the free portion of stamens was significantly shorter in flowers from BBSL plants than in flowers from the other sites ($F_{[2,294]} = 25.14$, $P < 0.0001$). The lengths of the curved portion of style and the free portion of the stamen were correlated at all sites (BBSL, $r = 0.59$; EF, $r = 0.63$; WCH, $r = 0.68$).

Nesting Trials with Candidate *Osmia* Species

OPEN NESTING TRIALS.—Approximately 40 *O. lignaria* females were released at WRL in 2004. X-rays of 32 resulting nests revealed 79 cells with a total of 72

live adult bees, 1 dead larva, and 6 unused provision masses. There were 2.45 cells per nest on average. Immature mortality was 9%. Assuming a sex ratio of 2♂:1♀ (Torchio and Tepedino 1980), 48 males and 24 females were produced across the 32 nests. There was a return of only 0.6 female progeny per nesting female. Pollen grains sampled from 3 intact provision masses were mounted on glass slides for identification; all 3 provision masses contained pollen resembling that of *H. boreale*. Another *Osmia* species nested in the blocks provided for *O. lignaria*, and was later identified as *O. sanrafaelae*. There were no intact provision masses in the *O. sanrafaelae* nests; therefore, it was not determined if females used *H. boreale* to provision their nests cells.

In 2005, few of the 200 *O. lignaria* females placed in the emergence box at WRL survived being held in cold storage for an extended period of time. None of the surviving females nested in the provided substrate. However, *O. sanrafaelae* females again nested in the substrate provided for *O. lignaria* (Table 4-5). Twenty-two complete nests were retrieved from WRL with around 172 male and 164 female progeny. Therefore, 22 nesting females produced 7.45 female progeny each. Pollen grains from 7 unused provision masses were examined, 4 of which contained pollen resembling that of *H. boreale* mixed with pollen from another source.

FIELD CAGE TRIALS.—Because *O. lignaria* females typically begin nesting in early spring (Bosch and Kemp 2001), overwintered nests containing *O. lignaria* adults were held at 4°C to delay emergence for as long as possible. Nests were moved to room temperature early on 14 May 2005 and to an emergence box in the BBSL field cage after dark on the same day. Males emerged immediately. Females began to emerge on 17 May. By 20 May, it appeared that several females were nesting in the provided substrate.

Table 4-5. Progeny produced by candidate *Osmia* species during open and caged nesting trials in 2005. During the open nesting trial, female *O. lignaria* were released adjacent to 2 acres of 'Timp' Utah sweetvetch at WRL. During caged nesting trials, females were enclosed in 6 x 6 x 2 m net field cages containing *H. boreale*. The numbers of nesting females were as follows: *O. lignaria*, *n* = 0 (open), *n* = 3 (caged); *O. sanrafaelae*, *n* = unknown (open), *n* = 7 (caged); and *O. bruneri*, *n* = 10 (caged).

			Progeny categories					Immature
Species	Nests	Total cells	Uneaten provisions	Dead larvae	Adults	♀♀	♂♂	mortality (%)
Open nesting trial								
O. lignaria	0	--	--	--	--	--	--	--
O. sanrafaelae	22	355	7	12	336	172	164	5.4
Field cage trials								
O. bruneri	16	99	16	2	81	27	54	18.2
O. lignaria	7	27	8	1	18	12	6	33.3
O. sanrafaelae	9	99	4	5	90	45	45	9.1

However, only 1 female had successfully completed a nest cell by 23 May. Only 2 more females had commenced nesting by 25 May, at which time 7 non-nesting females were ejected from the field cage. Females stopped nesting by 31 May. Therefore, females had between 6 and 8 days to nest in the field cage.

The 3 *O. lignaria* females who nested in the field cage produced 7 nests with around 3.9 cells each (Table 4-5). There was considerable immature mortality (33%), primarily at the egg stage. Twelve males and 6 females should have been produced across the 7 nests assuming a sex ratio of 2♂:1♀ (Torchio and Tepedino 1980). Therefore, the return of female progeny was 2 per nesting female.

The average foraging trip for pollen and nectar by *O. lignaria* females lasted 167 ± 14 seconds (*n* = 41, range = 36 – 440). During foraging tempo trials, females visited around 6.46 flowers per minute (Fig. 4-1). Therefore, *O. lignaria* females visited approximately 18 flowers per foraging trip. Females of *O. lignaria* that were tracked as

they foraged at 1 PM on 28 May 2005 visited 16 ± 4 *H. boreale* flowers per foraging trip (n = 13). Females spent similar amounts of time foraging for pollen and nectar and depositing these floral resources within the nest. Females spent 152 ± 17 seconds on average provisioning nest cells with pollen and nectar (n = 39, range = 66 – 507).

Female *O. lignaria* collected mud from shaded areas around the base of *H. boreale* plants and from beneath flaps of weed barrier. Females spent an average of 531 ± 81 seconds foraging for mud (n = 7, range = 366 – 975) and 345 ± 36 seconds adding to and manipulating cell partitions (n = 10, range = 221 – 604). The average duration of mud-foraging trips excludes an unusually long trip by female D8 lasting approximately 45 minutes. The female in nest H1 constructed an end plug during the taped time periods. Her foraging trips for mud lasted 43 ± 7 seconds on average (n = 32, range = 7 – 246). She spent an average of 92 ± 18 (n = 32) seconds adding to and manipulating the end plug (n = 32, range = 3 – 613).

In general, the durations of nesting behaviors were highly variable between individual *O. lignaria* females (Table 4-6). The female in D8 appeared to nest only sporadically and was no longer active on 31 May. Particular nesting behaviors were not synchronized between females; each female constructed nest cells at her own pace.

Overwintered nests containing *O. bruneri* and *O. sanrafaelae* were incubated at 29°C beginning on 25 and 27 May 2005, respectively. Males of both species began to emerge between 72 and 92 hours later. *O. bruneri* nests were moved to an emergence box in the EF field cage after dark on 28 May, while *O. sanrafaelae* nests were moved to the emergence box in the BBSL field cage after dark on 31 May. Female *O. bruneri* and *O. sanrafaelae* began to emerge on 3 and 4 June, respectively. Females of both species

Table 4-6. Mean durations of the foraging and cell provisioning/partitioning behaviors of 3 *O. lignaria* females nesting in a net field cage with *H. boreale* in 2005. Nest entrance traffic was videotaped from 12 – 2 PM on 28 May and from 10 AM – 12 PM on 31 May 2005. Data are reported as mean ± standard error (*n*). Means and standard errors are given in seconds; *n* = number of durations observed.

Female (nest)	Foraging for nectar/pollen	Provisioning nest cells	Foraging for mud	Building mud partitions
1 (D8)	—	—	377 ± 67 (5)	1187 ± 520 (4)
2 (F8)	152 ± 10 (34)	144 ± 18 (33)	—	—
3 (H1)	239 ± 62 (7)	197 ± 45 (6)	420 ± 26 (4)	314 ± 30 (5)

began to nest on 11 June following a week of unseasonably cold and wet weather. Bloom in both field cages lasted through 22 June. Thus, *O. bruneri* and *O. sanrafaelae* females had 12 days with comparatively good weather to nest.

Ten *O. bruneri* females nested in the EF field cage (Table 4-5). Sixteen nests, with an average of 6.2 cells each, were removed from the nesting block at the end of the summer. Frohlich (1983) described in detail the nesting biology of *O. bruneri*, but did not report the sex ratio of progeny in his study. According to sex ratio and investment theory (Fisher 1930), the sex ratio of a species should be 2♂:1♀ if producing a daughter requires twice as much effort as producing a son (Torchio and Tepedino 1980). Assuming a sex ratio of 1.9♂:1♀ (based on the ratio of average pollen grains in putative male and female provision masses), around 54 males and 27 females were likely produced across the 16 nests. However, many nests contained only male progeny upon examination, making an estimate of female progeny per mother bee unreliable. In addition, immature mortality was substantial (18%).

Seven *O. sanrafaelae* females nested in the field cage at BBSL (Table 4-5). Five completed nests (those with an entrance plug) and 4 incomplete nests were removed from

the nesting block at the end of the summer. Nests had 10 cells on average. Parker (1986) observed the sex ratio in completed nests to be 1.18♂:1♀, a ratio calculated by pooling data from nests with 6 and 9 mm diameters. Parker (1986) also observed the sex ratio in 9 mm nests to be 1♂:1.15♀. In order to correctly estimate the sex ratio of *O. sanrafaelae* progeny, nests with an even number of adult progeny were scored as 1♂:1♀. Conversely, nests with an odd number of adult progeny were scored depending on whether or not they were complete; the odd numbered bee was assumed to be male in completed nests and female in incomplete nests. Therefore, 45 of 90 adult bees should have been female, equaling 6.43 female progeny per mother bee.

Ten *O. bruneri* females completed 99 nest cells during the 12-day nesting period; therefore, each female completed 0.8 nest cells per day. Similarly, 7 *O. sanrafaelae* females completed 99 nest cells in 12 days, equaling 1.2 cells per female per day. Hourly observations of *O. sanrafaelae* nests on 15 June revealed that females varied in productivity from less than 1 to nearly 2 completed nest cells per day.

Female *O. bruneri* averaged 252 ± 26 seconds per pollen and nectar foraging trip (n = 52, range = 24 – 770), excluding an unusually long foraging trip by female G1 lasting around 18 minutes (Table 4-7). Females visited 11.9 ± 0.9 flowers per minute on average (Fig. 4-1). Therefore, *O. bruneri* females visited around 50 flowers per pollen and nectar foraging trip. Female *O. bruneri* spent 152 ± 16 seconds on average provisioning nest cells with pollen and nectar (n = 57, range = 24 – 651). Four unusually long provisioning stops by different females averaging 1240 ± 58 seconds were excluded from this estimate; it appears that females require around 20 minutes to complete the final touches on a provision mass and to lay an egg. Females spent roughly 60% of the time

Table 4-7. Mean durations of the foraging and cell provisioning/partitioning behaviors of 10 *O. bruneri* females nesting in a net field cage with *H. boreale* in 2005. Nest entrance traffic was videotaped from 11 AM to 3 PM on 18 June 2005. Data are reported as mean ± standard error (*n*). Means and standard errors are given in seconds; *n* = number of durations observed.

Female (nest)	Foraging for nectar/pollen	Provisioning nest cells	Foraging for leaf pieces	Building leaf partitions
1 (D8)	—	—	104 ± 26 (16)	102 ± 33 (16)
2 (E2)	268 ± 77 (10)	224[c] ± 117 (9)	77 ± 19 (7)	208 ± 93 (7)
3 (E8)	162 ± 16 (5)	124 ± 43 (6)	—	—
4 (F3)[a]	245 ± 55 (5)	94 ± 8 (6)	203 ± 96 (3)	146 ± 102 (4)
5 (F7)	196 ± 51 (6)	329[c] ± 152 (8)	242 ± 88 (5)	366 ± 122 (5)
6 (F8)	237 ± 47 (6)	170 ± 70 (7)	84 ± 14 (9)	172 ± 43 (10)
7 (G1)[b]	398 ± 251 (4)	114 ± 37 (4)	841 ± 468 (6)	66 ± 23 (7)
8 (G2)[b]	166 ± 117 (3)	504[c] ± 298 (4)	156 ± 46 (15)	106 ± 40 (15)
9 (H4)	501 ± 47 (8)	153 ± 27 (10)	—	—
10 (H7)	129 ± 37 (6)	349[c] ± 142 (7)	81 ± 18 (6)	214 ± 94 (6)

[a]The adult progeny in this nest were comparatively small, but otherwise healthy.
[b]*O. bruneri* nests commonly contain many long vestibular cells (Frohlich 1983). Adult progeny in nests G1 and G2 were spaced unusually close to one another; juvenile mortality in these nests was high compared to nests in which cell spacing was more typical.
[c]These estimates were skewed by abnormally long stops in the nest averaging 1240 ± 58 seconds.

they foraged for pollen and nectar depositing these floral resources in the nest. Therefore, each pollen and nectar foraging trip and subsequent deposit of resources lasted 404 seconds on average (6 min 44 sec).

The average foraging trip for leaf pieces by *O. bruneri* females lasted 125 ± 16 seconds (*n* = 61, range = 2 – 631). Females spent 160 ± 23 seconds building cell partitions on average (*n* = 63, range = 4 – 825). (Data from female G1 were excluded from both estimates.) Thus, females spent 22% less time foraging for leaf pieces than they spent adding those leaf pieces to cell partitions. In contrast, female *O. bruneri* spent

more time foraging for pollen and nectar than they spent depositing those resources in the nest.

It was not possible to distinguish between provisioning and partitioning behaviors of *O. sanrafaelae* females because they did not exit, turn, and promptly re-enter their nests to deposit pollen; due to the large cavity diameter (9 mm), females could simply somersault within the nest itself. Thus, the nesting behaviors of *O. sanrafaelae* females were measured as time spent away from the nest (foraging for pollen and nectar or leaf pieces) and time spent within the nest (provisioning or partitioning) (Table 4-8). Foraging trips away from the nest for pollen and nectar or leaf pieces lasted 198 ± 14 seconds on average (n = 133, range = 8 - 2779). Likewise, *O. sanrafaelae* females spent an average of 95 ± 4 seconds within the nest depositing pollen and nectar or leaf pieces (n = 258, range = 2 – 424). Therefore, female *O. sanrafaelae* spent 52% more time foraging for resources than they spent depositing those resources within the nest. Female *O. sanrafaelae* visited an average of 8.3 flowers per minute (Fig. 4-1), and spent 198 seconds on the average foraging trip. Therefore, *O. sanrafaelae* females likely visited around 27 flowers per pollen and nectar foraging trip.

POLLEN GRAINS PER PROVISION MASS.—There were comparable numbers of pollen grains in *O. bruneri* and *O. sanrafaelae* provision masses (Table 4-9). Provisions from the front of nests (mostly sons) contained roughly half the pollen grains as provisions from the back of nests (mostly daughters). Putative male (n = 2) and female (n = 3) provisions from *O. bruneri* nests had 4,523,000 and 8,563,000 pollen grains on average. Female *O. sanrafaelae* constructed putative male (n = 2) and female (n = 3)

Table 4-8. Mean durations of the foraging and cell provisioning/partitioning behaviors of 7 *O. sanrafaelae* females nesting in a net field cage with *H. boreale* in 2005. Nest entrance traffic was videotaped from 11 AM to 3 PM on 14 June 2005. Means are given in seconds for time spent away from the nest collecting pollen and nectar or leaf pieces, and for time spent within the nest provisioning or partitioning. *n* = number of durations observed.

Female	Away from nest (foraging)		Within nest (provisioning/partitioning)	
(nest)	$X \pm s_x\,(n)$	Range	$X \pm s_x\,(n)$	Range
1 (A1)	181 ± 14 (44)	8 – 396	83 ± 6 (43)	39 – 235
2 (A7)	213 ± 18 (38)	42 – 484	87 ± 9 (39)	24 – 366
3 (B3)	139 ± 20 (43)	12 – 668	122 ± 10 (42)	2 – 320
4 (B9)	138 ± 9 (51)	25 – 336	92 ± 10 (51)	12 – 424
5 (D3)	148 ± 9 (51)	21 – 270	80 ± 8 (51)	5 – 305
6 (F9)	318 ± 65 (21)	66 – 1284	113 ± 16 (22)	37 – 326
7 (G9)	779 ± 255 (10)	92 – 2779	126 ± 26 (10)	42 – 296

Table 4-9. Mean numbers of pollen grains in provision masses from *O. bruneri* and *O. sanrafaelae* nests. Provision masses collected from the front of nests were assumed to be male; those collected from the back were assumed to be female. Means for each provision mass are based on 3 replicate samples.

		Front of nest (♂)		Back of nest (♀)	
Species	Nest	X	s_x	X	s_x
O. bruneri	1[a]	2,470,000	149,000	5,422,000	177,000
O. bruneri	2[b]	5,448,000	54,000	9,079,000	857,000
O. bruneri	2[b]	5,651,000	141,000	8,205,000	202,000
O. bruneri	3	—	—	8,405,000	651,000
O. sanrafaelae	1	4,271,000	139,000	9,415,000	215,000
O. sanrafaelae	2	3,230,000	132,000	8,079,000	180,000
O. sanrafaelae	3	—	—	11,545,000	653,000

[a]This *O. bruneri* nest likely contained exclusively male progeny.
[b]Two male and 2 female provision masses were collected from an *O. bruneri* nest with considerable immature mortality.

provisions with averages of 3,750,000 and 9,680,000 pollen grains each. Female *O. sanrafaelae* were slightly larger than *O. bruneri* (Fig. 3-1). Therefore, it is appropriate that that *O. sanrafaelae* provisions were larger than those of *O. bruneri*.

Table 4-10. Mean number of pollen grains removed per single flower visit by pollen collecting *O. bruneri* and *O. sanrafaelae* females. n = 10 flower visits per species.

	Number of pollen grains		
	X	s_x	Range
O. bruneri			
Control flowers (EF)	50,965	9311	4768 – 85,429
Pollen removed per single visit	41,222	9451	0[a] – 77,956
% available pollen removed per single visit	65.1	11.5	0[a] – 97.7
O. sanrafaelae			
Control flowers (BBSL)	52,587	10,849	19,565 – 113,300
Pollen removed per single visit	28,007	6428	12,668 – 72,379
% available pollen removed per single visit	57.0	5.5	20.0 – 74.2

[a]The number of pollen grains removed by *O. bruneri* females exceeded the number of pollen grains in 2 corresponding control flowers.

POLLEN GRAINS REMOVED PER SINGLE FLOWER VISIT.—The number of pollen grains in unvisited control flowers at EF were extremely variable (Table 4-10). Therefore, if more pollen grains remained after a single visit than were found in the corresponding control, the removal value for that visit was set to zero. *O. bruneri* females removed about 65% of available pollen during single flower visits. *O. sanrafaelae* females removed a slightly lower percentage (57%) of available pollen per flower visit. It is likely that not all pollen removed from a flower ended up in a provision mass. On occasion, pollen was observed to fall from a flower as females brushed their hind legs alongside the anthers to loosen pollen.

DISCUSSION

Both *O. bruneri* and *O. sanrafaelae* females were effective pollinators of *H. boreale* based on frequency of stigmatic contact and pollen grains deposited within and on top of the stigmatic papillae of flowers. These comparatively small-bodied bees were able to compensate for their small size through specific flower handling behaviors, namely by backing up after probing for nectar and actively collecting pollen with their middle and hind legs. As a result, both species were able to effectively handle and pollinate *H. boreale* flowers, thereby meeting both their own needs and the pollination needs of the plant. Honeybees were also shown to deposit pollen within and on top of the stigmatic papillae of *H. boreale* flowers and could be used for managed seed production in some agricultural settings. Unfortunately, larger sample sizes are needed to determine if *O. lignaria* is also an effective pollinator of *H. boreale*.

Megachilid species consistently handled and moved between *H. boreale* flowers. Bumblebee queens consistently positioned themselves on *H. boreale* flowers; however, modes of interfloral travel employed by bumblebee queens were less consistent and influenced the legitimacy of some flower visits. By comparison, the floral handling behaviors and travel modes of honeybees were very inconsistent; such inconsistency results in part from their life history. Honeybees generally collect pollen or nectar during particular foraging trips, while pollen gathering generally involves a smaller percentage of foragers (Robinson 1977).

In terms of floral handling behaviors and frequency of stigmatic contact, nectar foraging honeybees were inferior pollinators of *H. boreale* compared to other species in this study. Heard (1994) compared the foraging behaviors and pollination efficacies of

honeybees and stingless bees (*Trigona carbonaria*) on macadamia. Stingless bees consistently foraged for pollen, and made intimate contact with the stigma during most flower visits. On the other hand, honeybees were largely nectar foragers and contacted the stigma less often as a result. Several studies have compared the pollination efficacies of honeybees and other bee species on particular crops (Bosch and Blas 1994, Vicens and Bosch 2000, Cane 2002). In all of these studies, nectar foraging honeybees were less effective pollinators than other bee species, often due to lower rates of stigmatic contact.

There appears to be a trade-off between the foraging tempo and pollination efficacy of flower visitors in some systems. In a study of native and introduced bees visiting lowbush blueberry in Nova Scotia, it was found that floral visitation rate alone was not a good indicator of pollination efficacy, as not all flower visits result in pollen deposition (Javorek et al. 2002). During foraging tempo trials, honeybees and female *Osmia* visited fewer flowers per minute than bumblebee queens and *Megachile* females (Fig. 4-1). Honeybees likely spent more time at individual flowers than other species because of their apparent ineptness in handling flowers. Because of their lack of speed and skill, honeybees actually had a longer time frame per flower visit in which to contact the stigma. As a result, in contrast to other comparative studies of pollen deposition (Wilson and Thomson 1991, Javorek et al. 2002), nectar gathering honeybees deposited pollen with similar efficacy as that of other species in this study.

Similarly, it is likely that flower visits by *O. bruneri* and *O. sanrafaelae* were longer than those of *Megachile* species because their small size required them to first probe for nectar and then back up to collect pollen during each flower visit. Both *M. frigida* and *M. melanophaea* were fast foragers and appeared to contact the stigma on

most flower visits (Fig. 4-1). However, during single visits to virgin flowers, female *M. melanophaea* deposited fewer pollen grains than *O. bruneri* and *O. sanrafaelae* (Fig. 4-2). Therefore, it appears that the extra step taken by the smaller-bodied *Osmia* to collect pollen might confer superior pollination efficacy at *H. boreale*.

Both *O. bruneri* and *O. sanrafaelae* females deposited pollen within and on top of stigmatic papillae of *H. boreale* flowers with similar efficacy as that of queen *B. fervidus* and *B. huntii*. However, *O. bruneri* females deposited more pollen grains on average than female *O. sanrafaleae*. This difference in the pollination efficacies of *O. bruneri* and *O. sanrafaelae* females might disappear if this research could be repeated. A week of unseasonably cold and wet weather just after *O. bruneri* was released contributed to a major outbreak of rust (identified as *Uromyces hedysari*) in the EF field cage. The health of *H. boreale* plants within the field cage at EF suffered as a result. Pollen grains from plants within the field cage (as viewed on slides of squashed stigmas from flowers visited by *O. bruneri*) were smaller that those from plants just outside the field cage and comparatively misshapen. Theoretically, more comparatively small and misshapen pollen grains should fit into a finite space (the stigmatic papillae) than healthy, full-sized pollen grains. Thus, the pollination efficacy of *O. bruneri* based on pollen deposition within the stigmatic papillae appears to be a slight overestimate. In contrast, pollen grain health likely did not influence the frequency with which *O. bruneri* females deposited pollen within the stigmatic papillae of *H. boreale* flowers.

The frequency with which *O. bruneri* females contacted the stigma (0.90) and the fraction of visits during which females deposited ≥ 1 pollen grain(s) within the stigmatic papillae (31/33 = 0.94) were in relative agreement. In contrast, the frequency of stigmatic

contact made by female *O. sanrafaelae* (0.88) was greater than the fraction of flowers in which females deposited ≥ 1 pollen grain(s) within the stigmatic papillae (19/25 = 0.76). Female *O. sanrafaelae* were skittish and not particularly amenable to the bamboo rod technique used to obtain single flower visits; these tendencies likely translated into more cautious flower visits during which *O. sanrafaelae* females deposited fewer pollen grains than they might have otherwise. Therefore, it seems likely that the pollination efficacy of *O. sanrafaelae* was underestimated during this study.

In this study, it was appropriate to compare single flower visits collected at 3 sites because the numbers of pollen grains found within and on top of the stigmatic papillae of unvisited control flowers were independent of collection site. However, subtle differences in the morphometrics of flowers collected at different sites may have influenced the appropriateness of pooling data across sites (Table 4-4). For example, when comparatively small-bodied *Osmia* species visited flowers with particularly long exerted styles, they commonly pushed the style and stigma away from their bodies while brushing the anthers with their hind legs; as a result, smaller-bodied *Osmia* females often failed to contact the stigma of flowers with long exerted styles. It appears that minute differences in floral morphology may significantly affect the pollination efficacy of certain pollinators. For *H. boreale* flowers, it is hypothesized that the distance from the nectary to the stigma (along the top plane) is more important for smaller-bodied pollinators, especially those species that sequentially collect nectar and then pollen. In terms of overall size, flowers from ‘Timp’ plants at EF were larger than flowers from BBSL and WDC. ‘Timp’ Utah sweetvetch is the only *H. boreale* cultivar currently available for commercial seed production. If ‘Timp’ plants regularly produce

comparatively large flowers, then bee body size may be a very important factor to consider when selecting bee species to pollinate 'Timp' plants.

Pollen grains deposited within the stigmatic papillae of *H. boreale* flowers likely contribute directly to fruit and seed production. However, the contribution of pollen grains deposited on top of the stigmatic papillae toward fruit and seed production is less clear. Unlike the explosive flag blossoms of some legumes, the *H. boreale* flower can be visited multiple times (Fægri and van der Pijl 1979). Therefore, pollen grains deposited on top of the stigmatic papillae have the potential to be pushed down into the papillae by the bodies of subsequent pollinators and/or by the deposition of more pollen grains on top of the stigmatic papillae. The relative patterns of pollen deposition within and on top of the stigmatic papillae varied among species in this study (Fig. 4-2). During single flower visits, *O. bruneri* and *B. fervidus* consistently deposited large numbers of pollen grains both within and on top of the stigmatic papillae. In contrast, pollen deposition by honeybees was more variable, as indicated by outliers on both pollen deposition graphs (Fig. 4-2) and the fact that pollen grains deposited by honeybees within and on top the stigmatic papillae of individual flowers were not significantly correlated.

Thomson and Thomson (1992) defined 3 classes of pollinators that differ in pollen removal and deposition: "good" bees remove and deposit relatively large amounts of pollen; "bad" bees remove and deposit less pollen than "good" bees; "ugly" bees remove a lot of pollen but deposit very little. According to this model, "bad" bees have very little effect on pollen dynamics, while "ugly" bees take large amounts of pollen out of circulation. It appears that *O. bruneri* and *O. sanrafaelae* females fall within the "good" pollinator category of Thomson and Thomson's model based on estimates of

pollen removal and deposition. The parameter for "good" bees in Thomson and Thomson's model was based on pollen removal and deposition by *Bombus occidentalis* queens; pollen removal by *B. occidentalis* queens was estimated to be around 70% (Thomson and Thomson 1989, Harder and Thomson 1989). Females of *O. bruneri* and *O. sanrafaelae* removed approximately 65 and 57% of available pollen from *H. boreale* flowers, respectively. In addition, both *O. bruneri* and *O. sanrafaelae* females deposited pollen within and on top of stigmatic papillae of *H. boreale* flowers with similar efficacy as that of bumblebee queens. However, a particular species' designation as "good, bad, or ugly" in Thomson and Thomson's model depends on the amount of pollen required by a plant species to maximize fruit and seed production. This information is not known for *H. boreale*. In addition, it appears that *H. boreale* produces a considerable number of ovules that do not develop into seeds (see Chapter 2); it is unknown what role pollen limitation may play in this phenomenon.

All 3 candidate species nested using *H. boreale* as their only source of pollen and nectar, albeit with varying degrees of success (Table 4-5). Nesting trials with female *O. lignaria* were particularly fraught with difficulty. In both years, potential nesting by *O. lignaria* was thwarted by weather. In 2004, bees had to endure over a week of winter weather just after release, and an unusually late snow in May. Conversely, in 2005, bees had to endure very hot temperatures just after release. In both years, weakened and/or stressed bees were less likely to successfully nest under such conditions. Initial results indicate that *O. lignaria* might be an excellent pollinator of *H. boreale*, provided that a setting can be found where *O. lignaria* is able to successfully emerge and nest. Females deposited an average of 19 pollen grains within the stigmatic papillae of flowers ($n = 2$),

and frequently contacted the stigma. Females of *O. bruneri* and *O. sanrafaelae* began to forage when temperatures reached approximately 70°F, while female *O. lignaria* were observed to forage at cooler temperatures. Thus, *O. lignaria* females may forage for more hours per day than other species, and could be used in colder agricultural settings. However, results of this study suggest that *O. lignaria* is not a good candidate species for seed production in some settings, especially those in which plants start blooming later in the season (unless bees could be obtained from an equally late-flying population).

The nesting successes of *O. bruneri* and *O. sanrafaelae* should both be considered minimum estimates. Due to a week of unseasonably cold weather followed by comparatively hot temperatures, bloom ended in both field cages prior to the end of nesting. In particular, *O. bruneri* females were nesting under sub-optimal conditions, and produced mostly male progeny as a result. Because female progeny are more costly to produce, *Osmia* species tend to produce more sons than daughters when stressed by poor weather, insufficient floral resources, and/or inadequate nesting substrates (Bosch and Kemp 2002). It is also possible that some *O. bruneri* females failed to mate. Philips and Klostermeyer (1978) noted that unmated *O. lignaria* females did not exhibit different nesting behaviors than mated females; they provisioned innermost nest cells as though they were to become female progeny.

In natural settings, megachilid females tend to construct and provision a single nest cell per day. However, in agricultural settings where abundant floral resources are found in close proximity to nests, megachilid females often construct and provision 2 nest cells per day (Philips and Klostermeyer 1978). Therefore, the estimates of 0.81 and 1.17

nest cells provisioned per day by *O. bruneri* and *O. sanrafaelae* females, respectively, indicate that floral resources may have been limited in both cages.

On an average day during mid bloom, there were roughly 4,000,000 new flowers available each day per acre of cultivated *H. boreale* (estimated on 11 May 2004 at WRL; see Chapter 2), each with 87,000 pollen grains on average (Table 2-5). Female *O. bruneri* and *O. sanrafaelae* removed approximately 65 and 57% of available pollen on average from *H. boreale* flowers, respectively. Therefore, female *O. bruneri* would likely remove 56,550 pollen grains from each flower at WRL, while female *O. sanrafaelae* would likely remove 49,590 pollen grains. Putative female provisions from *O. bruneri* and *O. sanrafaelae* nests have 8,563,000 and 9,680,000 pollen grains on average, respectively. Therefore, female *O. bruneri* and *O. sanrafaelae* would need to visit at least 151 and 195 flowers to provision a single female provision mass. Because megachilid females tend to provision 2 nest cells per day when floral resources are abundant and accessible, females of *O. bruneri* and *O. sanrafaelae* would need to visit at least 302 and 390 flowers per day, respectively. A seed grower would need to stock 13,245 and 10,256 female *O. bruneri* and *O. sanrafaelae* per acre, respectively, in order for each new *H. boreale* flower to be visited once by a pollen foraging female. This estimate of stocking density is likely an overestimate, as not all pollen grains removed from a flower end up in a provision mass. Some are lost during the collection process, while some are undoubtedly lost during transport to the nest.

In conclusion, both *O. bruneri* and *O. sanrafaelae* were able to effectively handle and pollinate *H. boreale* flowers. Females of both species were able to nest and produce progeny using *H. boreale* as their only source of pollen and nectar. Female *O.*

sanrafaelae were able to produce enough female progeny to replace themselves; female *O. bruneri* would likely have produced enough female progeny to replace themselves had they been nesting under less stressful conditions. Therefore, both species are excellent candidates for future development as managed pollinators of *H. boreale* seed crops. Additional research is needed to determine if *O. lignaria* is also an effective pollinator that could be used to pollinate *H. boreale* in commercial settings.

LITERATURE CITED

Bosch, J., and M. Blas. 1994. Foraging behavior and pollinating efficacy of *Osmia cornuta* and *Apis mellifera* on almond (Hymenoptera, Megachilidae and Apidae). Applied Entomology and Zoology 29:1-9.

Bosch, J., and W.P. Kemp. 2001. How to manage the blue orchard bee as an orchard pollinator. Sustainable Agriculture Network, National Agricultural Library, Beltsville, MD. 88 pp.

_____. 2002. Developing and establishing bee species as crop pollinators: the example of *Osmia* spp. (Hymenoptera: Megachilidae) and fruit trees. Bulletin of Entomological Research 92:3-16.

Cane, J.H. 2002. Pollinating bees (Hymenoptera: Apiformes) of U.S. alfalfa compared for rates of pod and seed set. Journal of Economic Entomology 95:22-27.

Fægri, K., and L. van der Pijl. 1979. The principles of pollination ecology. 3rd edition. Pergamon Press, New York. 244 pp.

Fisher, R.A. 1930. The genetical theory of natural selection. Clarendon Press, Oxford. 272 pp.

Free, J.B. 1993. Insect pollination of crops. 2nd edition. Academic Press, London. 684 pp.

Frohlich, D.R. 1983. On the nesting biology of *Osmia* (*Chenosmia*) *bruneri* (Hymenoptera: Megachilidae). Journal of the Kansas Entomological Society 56:123-130.

Harder, L.D., and J.D. Thomson. 1989. Evolutionary options for maximizing pollen dispersal of animal-pollinated plants. American Naturalist 133:323-344.

Heard, T.A. 1994. Behavior and pollinator efficacy of stingless bees and honey bees on macadamia flowers. Journal of Apicultural Research 33:191-198.

Herrera, C.M. 1987. Components of pollinator "quality": comparative analysis of a diverse insect assemblage. Oikos 50:79-90.

_____. 1989. Pollinator abundance, morphology, and flower visitation rate: analysis of the "quantity" component in a plant-pollinator system. Oecologia 80:241-248.

Inouye, D.W. 1980. The terminology of floral larceny. Ecology 61:1251-1253.

Inouye, D.W., D.E. Gill, M.R. Dudash, and C.B. Fenster. 1994. A model and lexicon for pollen fate. American Journal of Botany 81:1517-1530.

Javorek, S.K., K.E. Mackenzie, and S.P. Vander Kloet. 2002. Comparative pollination effectiveness among bees (Hymenoptera: Apoidea) on lowbush blueberry (Ericaceae: *Vaccinium angustifolium*). Annals of the Entomological Society of America 95:345-351.

Kowalczyk, B.F. 1973. The pollination ecology of *Hedysarum alpinum* L. var. *americanum* (MCHX.) and *H. boreale* NVTT. var. *mackenzii* (Richards.) C.L. Hitchc. in the Kluane Lake Area of the Yukon Territory, Canada. Master of Science thesis, University of North Carolina, Chapel Hill.

Kremen, C., N.M. Williams, and R.W. Thorp. 2002. Crop pollination from native bees at risk from agricultural intensification. Proceedings of the National Academy of Sciences of the United States of America 99:16812-16816.

Michener, C.D. 2000. The bees of the world. John Hopkins University Press, Baltimore, MD. 913 pp.

Northstrom, T.E., and S.L. Welsh. 1970. Revision of the *Hedysarum boreale* complex. Great Basin Naturalist 30:109-130.

Parker, F.D. 1986. Nesting, associates, and mortality of *Osmia sanrafaelae* Parker. Journal of the Kansas Entomological Society 59:367-377.

Philips, J.K., and E.C. Klostermeyer. 1978. Nesting behavior of *Osmia lignaria propinqua* Cresson (Hymenoptera: Megachilidae). Journal of the Kansas Entomological Society 51:91-108.

Richards, K.W. 1996. Comparative efficacy of bee species for pollination of legume seed crops. Pages 81-103 *in* A. Matheson, S.L. Buchmann, C. O'Toole, P. Westrich, and I.H. Williams, editors, The conservation of bees. Academic Press, San Diego.

Robinson, G. 1977. Honey bee foraging behavior. American Bee Journal 117:222-223,258.

SAS Institute Inc. 2004. SAS/STAT® 9.1 User's Guide. SAS Institute Inc., Cary, NC. 5136 pp.

Sokal, R.R., and F.J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research. 3rd edition. W.H. Freeman and Company, New York. 887 pp.

Stevens, R., K.R. Jorgensen, S.A. Young, and S.B. Monsen. 1996. Forb and shrub seed production guide for Utah. Utah State University Extension Electronic Publication AG 501.

Thomson, J.D., and B.A. Thomson. 1989. Dispersal of *Erythronium grandiflorum* pollen by bumblebees: implications for gene flow and reproductive success. Evolution 43:657-661.

_____. 1992. Pollen presentation and viability schedules in animal-pollinated plants: consequences for reproductive success. Pages 1-24 *in* R. Wyatt, editor, Ecology and evolution of plant reproduction. Chapman and Hall, New York.

Torchio, P.F., and V.J. Tepedino. 1980. Sex ratio, body size and seasonality in a solitary bee, *Osmia lignaria propinqua* Cresson (Hymenoptera: Megachilidae). Evolution 34:993-1003.

Vicens, N., and J. Bosch. 2000. Pollinating efficacy of *Osmia cornuta* and *Apis mellifera* (Hymenoptera: Megachilidae, Apidae) on 'red delicious' apple. Environmental Entomology 29:235-240.

Wilson, P., and J.D. Thomson. 1991. Heterogeneity among floral visitors leads to discordance between removal and deposition of pollen. Ecology 72:1503-1507.

Young, H.J. 1988. Differential importance of beetle species pollinating *Dieffenbachia longispatha* (Araceae). Ecology 69:832-844.

CHAPTER 5

SUMMARY

Results of this research will help to meet BLM and USFS desires for a more consistent and cost-effective source of *H. boreale* seed. *H. boreale* plants did not produce fruits in the absence of bee visitors. *H. boreale* was found to be homogamous and self-compatible. Bee pollination of *H. boreale* flowers was likely a mixture of selfing via geitonogamy and out-crossing (xenogamy). Out-crossing appeared to offer long-term advantages in reproductive success via increased seed viability and decreased predispersal reproductive attrition. *H. boreale* proved to be very rewarding in terms of floral resources; flowers contained abundant pollen grains and nectar of comparatively high sugar concentration.

Systematic surveys of bee faunas revealed that an assortment of bee species in the families Apidae and Megachilidae visit *H. boreale* flowers. Female bees often collected both nectar and pollen from *H. boreale* for provisioning their progeny. In general, *Osmia* species were an important component of *H. boreale* pollinator faunas in both survey years and at most sites. *Osmia* females formed 2 groups based on comparative body size. *Osmia bruneri* and *O. sanrafaelae* females fell in among comparatively small-bodied *Osmia* species, while *O. lignaria* fell among the larger-bodied species.

Three solitary, cavity-nesting candidate *Osmia* species were chosen and evaluated for their potential use as managed *H. boreale* pollinators. All 3 candidate *Osmia* species were able to reproduce with *H. boreale* as their only pollen and nectar source. In general, nesting success by *O. lignaria* females was limited, suggesting that this species may not be the best option for managed pollination of *H. boreale* flowers in most agricultural

settings. Both *O. bruneri* and *O. sanrafaelae* nesting results were encouraging, however; both species were able to provision enough female bees to replace themselves in subsequent years. In addition, both species proved to be effective pollinators of *H. boreale* flowers in terms of frequency of stigmatic contact and pollen grains deposited per single flower visit.