PLANT SPECIES BIOLOGY

Plant Species Biology (2012) 27, 138–146 doi: 10.1111/j.1442-1984.2011.00339.x

Breeding biology and incremental benefits of outcrossing for the restoration wildflower, *Hedysarum boreale* Nutt. (Fabaceae)

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Abstract

Northern sweetvetch (*Hedysarum boreale* Nutt.) is an herbaceous perennial legume of the Rocky Mountains, USA, whose seed is desired for rehabilitating degraded plant communities. Through experimental pollinations, the necessity of pollinators was shown by the failure of autogamy, despite stigmas first becoming receptive in the bud in close proximity to the dehiscing anthers. Nonetheless, the species proved to be self-fertile, initiating as many fruits through selfing as outcrossing. Incremental benefits of outcrossing only later manifested in superior fruit development, seed maturation and seed germination. Farming of *H. boreale* can yield abundant viable seed if adequately visited by pollinating bees.

Keywords: breeding biology, Fabaceae, fruit set, *Hedysarum*, outcrossing, seed production.

Received 29 November 2010; accepted 9 May 2011

Introduction

Restoring degraded or locally extirpated native plant communities can be aided by reseeding with formerly prevalent native plant species. Although planted as seed mixes, component species are frequently grown in pure managed stands to simplify harvest timing, cleaning, certification, pricing and marketing of seed. Although the seeds of grasses and most shrubs used for restoration in the western USA arise from auto pollination or wind pollination, many North American wildflowers are showy and depend on guilds of wild pollinators for fruit and seed set. Farming of most native wildflowers for restoration seed will only succeed if the wildflower's breeding biology and pollination ecology are understood and used to guide management of effective pollinators on-farm.

For several decades, northern sweetvetch (*Hedysarum boreale* Nutt.) has been studied as a restoration wildflower (Redente 1982; Johnson *et al*. 1989). Its seed has been harvested from the wild or grown on farms, yielding limited quantities of expensive seed for restoration. This herbaceous perennial legume occurs throughout the Rocky Mountains from northern New Mexico to Alaska, where it is found in grasslands or on sagebrush slopes at lower elevations and higher up in open meadows or woodlands (Northstrom & Welsh 1970). Its showy, pink, papilionaceous flowers reportedly attracted only bumblebees and solitary leafcutting bees (*Megachile* spp.) in studies in Canada and Alaska (Kowalczyk 1973; McGuire 1993). In contrast, a rich diversity of non-social bees avidly worked its flowers in western Wyoming, USA (Tepedino & Stackhouse 1987). Studies of the European species *Hedysarum coronarium* revealed considerable self-fertility, but a reliance on bees for pollination (Sonet & Jacob-Remacle 1987).

One of the three large genera of the legume tribe Hedysareae, *Hedysarum* (180 species) spans the Palearctic, from Japan westward across northern Asia to Scandinavia, and southwards around the Mediterranean Basin (Lock 2005). Several species, including *H. boreale*, are native to western North America. Species of *Hedysarum* are found from alpine/arctic habitats to boreal and montane forests to shrub-steppe grasslands. Many of the species are valued as fodder plants for livestock; the foliage, roots and seeds are highly palatable to wildlife as well.

The purpose of the present study was to establish the breeding biology of *H. boreale*, including ancillary information about floral maturation, anther dehiscence and

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stigma receptivity. The consequences of outcrossing were tracked for subsequent fruit and seed development, as well as seed viability, comparing manually pollinated flowers with those openly visited by bees. In addition, rates of reproductive attrition resulting from self-pollination, cross pollination and open pollination were compared through fruit set and development, seed maturation and seed viability.

Materials and methods

Study sites

The following studies were conducted at two lower canyon wild populations of *H. boreale* in Cache County, Utah, USA: Paradise (41°34′N, 111°47′W, 1609 m a.s.l.) and Wood Camp Hollow (41°48′N, 111°39′W, 1743 m a.s.l.). The natural population at Paradise consisted of approximately 500 plants growing on a steep, northeast-facing slope. The roughly 400 plants in the Wood Camp Hollow population were found on a gradual, west-facing slope amid sagebrush (*Artemesia* sp.).

Breeding system

The breeding system of *H. boreale* was experimentally assessed in 2003 using 15 plants at Paradise and 11 plants at Wood Camp Hollow. Five racemes on each plant were chosen at the bud stage and tagged by treatment using small, brightly colored plastic clips. Flowers on one raceme of each plant received one of the following treatments: (i) autogamy: flowers unmanipulated; (ii) geitonogamy: flowers manually pollinated with pollen from another flower on the same plant; (iii) xenogamy: flowers manually pollinated with pollen from a plant located approximately 10 m distant; and (iv) positive control: flowers freely visited by pollinators. Small, fine-mesh bags (mesh size <1 mm^2) were used to exclude pollinators from racemes in treatments 1–3; racemes remained bagged at all times except when manual pollinations were carried out. Autogamy racemes were left bagged throughout the experiment; positive control racemes were bagged only at the end of the experiment to prevent later loss of mature fruits. To determine if the degree of outcrossing influences fruit and seed production in *H. boreale*, plants at Paradise received an additional treatment: distant xenogamy: flowers on one raceme were manually pollinated with pollen from a plant located >100 m away.

For manually pollinated flowers, a dry grass stem was first brushed across the stigma to try to gently rupture any surface membrane (pellicle: Shivanna & Sastri 1981) and to separate the stigmatic papillae before pollen was applied. Flowers were manually pollinated every other day from 4 to 12 June 2003 at Paradise and from 13 June to 2 July 2003 at Wood Camp Hollow. All open flowers were pollinated on each visit, including those pollinated on previous visits, to accommodate any effect of flower age on stigma receptivity. Racemes were individually collected once fruits were mature. *Hedysarum boreale* fruits ('loments') disarticulate into flat, circular, one-seeded articles (Northstrom & Welsh 1970), which may or may not be fully developed and contain a fully developed seed at maturity (Fig. 1). Therefore, the total numbers of fruits, articles and seeds produced on each treated raceme were counted. Underdeveloped and fully developed articles were counted separately. Fully developed articles were scored for the presence of a mature seed.

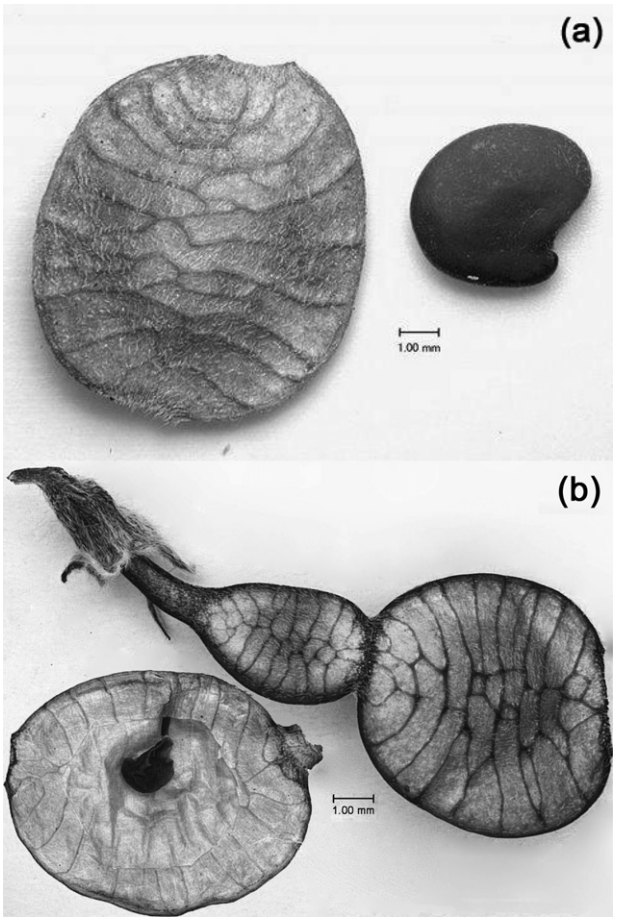

Fig. 1 (a) Image of a mature *Hedysarum boreale* article and seed. (b) Images of an *H. boreale* fruit with one mature and one underdeveloped article, and a fully developed article without a mature seed (inset; the top of the article was cut away to expose the immature seed).

Statistical comparison was complicated by the fact that most flowers in the autogamy and manual pollination treatments failed to produce fruit. Therefore, pollination treatments were first compared within sites for the overall proportion of flowers that set fruit using a *G*-test with

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William's correction (Sokal & Rohlf 1995). For those racemes that set at least one fruit, pollination treatments were then compared for proportional fruit set (number of fruits set/total number of flowers treated on a raceme), proportional article development (number of fully developed articles/total number of articles on a raceme) and proportional seed development (number of fully developed articles with a mature seed/total number of fully developed articles on a raceme). An ANOVA was carried out using the MIXED procedure of SAS v. 9.2 (SAS Institute 2008) with pollination treatment and site as fixed factors and plant as a random factor. Significance was set at $\alpha = 5\%$; treatment means were compared using Tukey–Kramer a posteriori tests. Data were checked for normality and homoscedasticity and were transformed when necessary.

Seed germination

Mature seeds from the breeding system experiments were tested for viability by germination in spring 2004. Seeds were cold, moist stratified at 4°C for 1 month prior to the germination trials. Seeds from each treated raceme were removed from their articles, combined and soaked in distilled water for 24 h at room temperature. Because sweet-vetch seeds require mechanical scarification (Redente 1982), the naked seeds were placed in a blender with a small amount of water (approximately 1:1 seeds/water) and pulsed 3–4 times to scratch their hard seed coats. Scarification was confirmed visually. Seeds from each treatment raceme were then placed in a Petri dish on blotter paper moistened with distilled water and Thiram fungicide. Seeds were incubated at 22°C and monitored daily for germination.

An ANOVA using the MIXED procedure of SAS was used to analyze proportional seed germination (number of mature seeds that germinated/total number of mature seeds on a raceme) with pollination treatment and site as fixed factors and plant as a random factor. Data were analyzed in the same manner as the proportional fruit set, proportional article development and proportional seed development data from the breeding system experiments in 2003.

Effect of time of day and flower age on fruit set

Twenty-five plants at Paradise were chosen in early June 2004 to experimentally assess the effect of time of day and flower age on fruit set in *H. boreale*. Racemes were chosen and tagged as before. Two treatments were used to assess the effect of time of day on fruit set: (i) morning visitation: two racemes per plant were exposed to pollinators once from 9.00–11.00 AM; and (ii) afternoon visitation: two racemes per plant were exposed to pollinators once from 2.00–4.00 PM. On morning and afternoon visitation racemes, four age classes were used to assess the effect of flower age on fruit set: (i) 0–24 h old; (ii) 24–48 h old; (iii) 48–72 h old; and (iv) over 72 h old. Small fine-mesh bags were used to exclude pollinators from treatment racemes. In addition, one raceme per plant served as a negative control and remained bagged throughout the experiment. Another raceme on each plant served as a positive control; it remained open to pollinators during the experiment and was bagged at the end of the experiment to capture mature fruits.

Starting on 13 June 2004, morning and afternoon visitation racemes were examined just prior to 9.00 AM or 2.00 PM each day, respectively. All newly opened flowers were counted and marked for identification using a sequence of colored permanent marker ink. Once a raceme had accumulated flowers in all four age classes, it was unbagged and exposed to pollinators once during its designated morning or afternoon time period. It was then rebagged and left to mature fruits. Different racemes were opened daily to visitation from 17 to 23 June, and were individually collected on 8 July. Fruits produced by flowers in each age class were counted on each raceme.

Statistical comparison was again complicated by the fact that many flowers failed to produce fruit. Therefore, flower age classes were first compared within morning and afternoon visitation treatments for the overall proportion of flowers that set fruit using a *G*-test with William's correction (Sokal & Rohlf 1995). Frequency of fruit set data (number of fruits set/total number of flowers available in an age class) were then excluded from subsequent analyses when either: (i) there were no fruits set by any flowers in an age class; or (ii) on the rare occasion when there were no flowers available in an age class. An ANOVA was carried out using the MIXED procedure of SAS with time of day, flower age class and replicate raceme as fixed factors and plant as a random factor. Significance was determined at $\alpha = 5\%$; treatment means were compared using Tukey–Kramer a posteriori tests. Data were checked for normality and homoscedasticity before analysis.

Timing and duration of stigma receptivity

The timing and duration of stigma receptivity in *H. boreale* were evaluated using an enzymatic stain indicator of receptivity (α-naphthyl acetate with fast blue B salt; Mattsson *et al*. 1974; Kearns & Inouye 1993). Seeds collected at Wood Camp Hollow in 2004 were germinated as above, grown in forestry propagation cells and transplanted to gallon pots with soil-less media in early spring 2005. On 18 April, greenhouse lights were set to provide the long daylight hours experienced at the beginning of June in Logan, Utah. Plants began to bloom in early May.

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Separate trials were run to determine when the *H. boreale* stigma becomes receptive relative to anther dehiscence and anthesis. The anthers in an *H. boreale* flower dehisce (release pollen) simultaneously in the bud. Thus, pollen is available when flowers first open (Tepedino & Stackhouse 1987). To determine when the stigma becomes receptive relative to anther dehiscence, the following flowers taken from the same plant were tested for receptivity: an immature bud with indehiscent anthers; a mature bud with indehiscent anthers; a mature bud with dehiscent anthers; an opening flower with dehiscent anthers; and a fully open flower with dehiscent anthers. Another trial was run to determine when the stigma becomes receptive relative to the start of anthesis; anthesis is defined as the period during which a flower is fully open and functional. In the present study, anthesis was taken to begin when the standard petal was fully upright. To determine when the stigma becomes receptive relative to anthesis, four flowers from the same plant, ranging from a mature bud with dehiscent anthers to a fully open flower, were tested for receptivity.

A third trial was run to determine how long the *H. boreale* stigma remains receptive. Racemes on three plants were chosen and tagged; flower age classes (0–24, 24–48, 48–72 and over 72 h) were marked as before for 4 days prior to testing. The uppermost flower in each age class was then tested for receptivity.

During each trial, a control solution including everything but the substrate (α-naphthyl acetate) was prepared and added to a fresh stigma matching the developmental stage of each treated stigma. Control and treated stigmas were examined microscopically (20×) for a strong red staining of receptive areas.

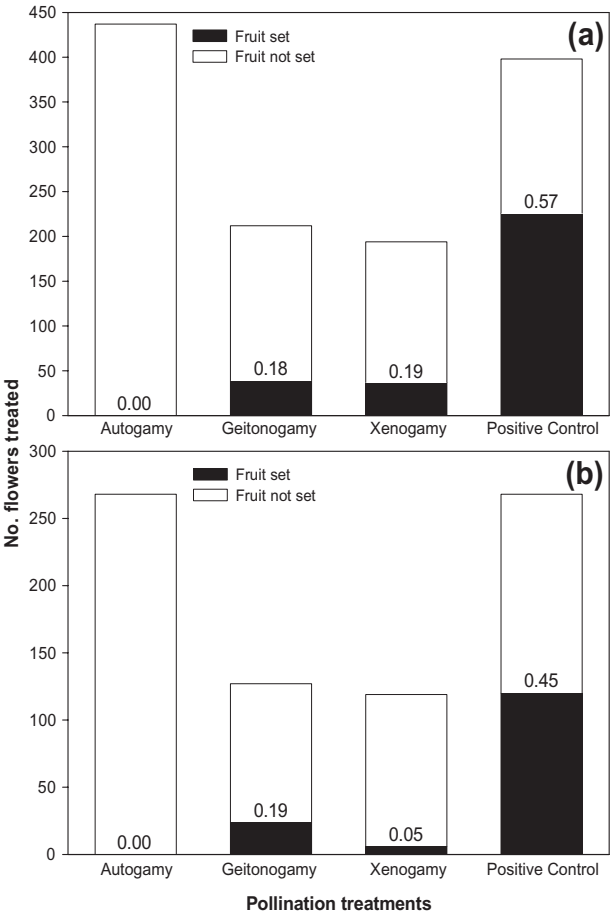

Fig. 2 Frequencies of fruit set for four breeding system treatments at (a) Paradise and (b) Wood Camp Hollow in 2003. Average number of flowers treated per raceme: Paradise: autogamy ($n = 29$ flowers), geitonogamy (14), xenogamy (13), positive control (27); Wood Camp Hollow: autogamy (24), geitonogamy (12), xenogamy (11), positive control (24).

Results

Breeding system

Patterns of fruit set were similar at the two sites (Fig. 2). Autogamy yielded no fruit at either site, whereas the geitonogamy, xenogamy and positive control (freely visited) treatments yielded some fruit on treated racemes at both sites. Overall, at both sites, the frequency of fruit set depended on the pollination treatment (Paradise: $G_3 = 429$, $P < 0.0001$; Wood Camp Hollow: $G_3 = 213$, $P < 0.0001$).

For proportional fruit set on racemes that set at least one fruit, the interaction between pollination treatments and sites was not significant ($F_{2,51} = 2.98$, $P = 0.06$). Therefore, proportional fruit set data were combined across sites. Proportional fruit set varied with pollination treatment ($F_{2,51} = 23.50$, $P < 0.0001$) (Table 1). Positive controls yielded greater proportions of fruit than did geitonogamy and xenogamy, whereas geitonogamy resulted in more fruits than xenogamy.

On positive control racemes, the mean numbers of articles per fruit were nearly identical at the two sites (Table 2). Overall, manual pollination treatments yielded fewer articles per fruit on average, with the exception of geitonogamy at Paradise. At both sites, however, some fruits resulting from each pollination treatment had articles that were not fully developed; such articles never contained seeds that were full-sized and mature. The interaction between pollination treatments and sites was not significant for proportional article development ($F_{2,51} = 2.01$, $P = 0.14$). Therefore, data were combined across sites. The probability that an article is fully developed did not vary with pollination treatment ($F_{2,51} = 1.47$, $P = 0.24$) (Table 1).

Similarly, even fully developed articles did not always contain a mature, full-sized seed. The interaction between pollination treatments and sites was not significant for

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Pollination treatment	Fruits†	Fully developed articles‡	Fully developed articles with mature seeds§
Geitonogamy	$0.31^{a} \pm 0.24$ (14)	0.81 ± 0.18 (14)	$0.67^{ab} \pm 0.26$ (13)
Xenogamy	$0.21^{b} \pm 0.13$ (17)	0.85 ± 0.22 (17)	$0.83^{a} \pm 0.21$ (17)
Positive control	$0.54^{c} \pm 0.19$ (26)	0.84 ± 0.10 (26)	$0.70^{b} \pm 0.16$ (22)

† Number of fruits set/total number of flowers treated on a raceme. ‡ Number of fully developed articles/total number of articles on a raceme. § Number of fully developed articles with a mature seed/total number of fully developed articles on a raceme. Treatment means followed by different letters are statistically different ($P \leq 0.05$).

Table 1 Comparisons of proportional fruit set, article development and seed development data (mean ± standard deviation) (n = racemes) for three pollination treatments combined across the Paradise and Wood Camp Hollow sites in 2003

	Articles/fruit		Fully developed articles†	
Pollination treatment	PRD	WCH	PRD	WCH
Geitonogamy	2.51 ± 1.22	1.74 ± 0.71	$0.72^{a} \pm 0.15$	0.97 ± 0.06
Xenogamy	1.62 ± 0.57	1.50 ± 0.71	$0.84^{ab} \pm 0.21$	0.88 ± 0.27
Distant xenogamy	1.91 ± 0.57	–	$0.96^{b} \pm 0.10$	–
Positive control	2.56 ± 0.79	2.54 ± 0.53	$0.81^{a} \pm 0.08$	0.89 ± 0.10

† Number of fully developed articles/total number of articles on a raceme. Sample sizes: PRD: geitonogamy ($n = 9$ racemes), xenogamy (12), distant xenogamy (8), positive control (15); WCH: geitonogamy (5), xenogamy (5), positive control (11). Treatment means followed by different letters are statistically different ($P \leq 0.05$).

Table 2 Mean number of articles per fruit (± standard deviation) and mean probability that an article is fully developed (± standard deviation) for four pollination treatments at Paradise (PRD) and three pollination treatments at Wood Camp Hollow (WCH) in 2003

proportional seed development ($F_{2,46} = 0.90$, $P = 0.41$). Therefore, data were combined across sites. The probability of a fully developed article yielding a mature seed varied with pollination treatment ($F_{2,46} = 3.51$, $P = 0.0380$) (Table 1). Fruits on xenogamy racemes were more likely to have articles with mature seeds than fruits on positive control racemes.

Seed germination

Patterns of seed germination were similar at the two sites (Fig. 3). The interaction between pollination treatments and sites was not significant for proportional seed germination ($F_{2,44} = 3.02$, $P = 0.059$). Therefore, data were combined across sites. Significant treatment differences in proportional seed germination were observed ($F_{2,44} = 13.38$, $P < 0.0001$). A greater proportion of xenogamy seeds (mean ± $s = 0.81 \pm 0.34$) germinated than seeds from geitonogamy (0.42 ± 0.37) and positive control (0.51 ± 0.25) treatments.

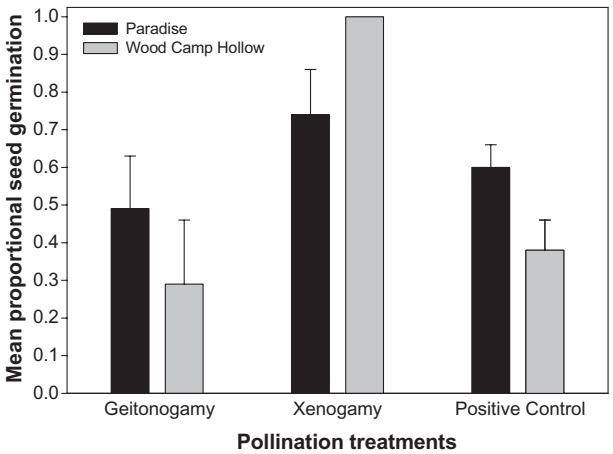

Fig. 3 Mean proportional seed germination (+ standard error) for three breeding system treatments at Paradise and Wood Camp Hollow in 2003. Proportional seed germination was calculated by dividing the number of mature seeds that germinated by the total number of mature seeds produced on a treatment raceme. Sample sizes: Paradise: geitonogamy ($n = 8$ racemes, 26 seeds), xenogamy (12, 36), positive control (13, 297); Wood Camp Hollow: geitonogamy (4, 26), xenogamy (4, 5), positive control (9, 150).

Degree of outcrossing (distant xenogamy vs xenogamy)

Distant xenogamy resulted in more fully developed articles per fruit than either the geitonogamy or positive control treatments ($F_{3,40} = 6.22$, $P = 0.0014$) (Table 2). Only 9% of flowers that received the distant xenogamy treatment at Paradise set fruit, compared with 19% of flowers that received the xenogamy treatment. Xenogamy exceeded distant xenogamy for proportional fruit set ($X = 25$ *vs* 22%), the proportion of fully developed articles with mature seeds (81 *vs* 71%) and the proportion of

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Table 3 Mean frequencies of fruit set ± standard deviation (n = racemes) for four flower age classes during morning (AM) and afternoon (PM) visitation periods at Paradise in 2004

	Flower age (h)			
Treatment	0–24	24–48	48–72	Over 72
AM	0.35 ± 0.40 (45)	0.28 ± 0.33 (49)	0.16 ± 0.25 (46)	0.08 ± 0.18 (43)
PM	0.41 ± 0.39 (48)	0.38 ± 0.35 (49)	0.21 ± 0.28 (49)	0.15 ± 0.26 (44)

Frequencies were calculated by dividing the number of fruits set by the total number of flowers available in an age class.

germinable seeds (74 *vs* 63%), although none of these comparisons were significant.

Effect of time of day and flower age on fruit set

Nearly 40% of new flowers (0–24 h old) set fruit, whereas only 10% of 72-h-old flowers set fruit. Overall, the frequency of fruit set depended on flower age for both morning ($G_3 = 59.49$, $P < 0.0001$) and afternoon ($G_3 = 56.99$, $P < 0.0001$) visitation (Table 3). For frequency of fruit set data, the interaction between flower age classes and replicate morning and afternoon racemes was not significant for either treatment (morning: $F_{3,70} = 0.23$, $P = 0.88$; afternoon: $F_{3,92} = 0.57$, $P = 0.64$). Therefore, data were combined across replicate morning and afternoon racemes. The interaction between time of day and flower age classes was not significant ($F_{3,170} = 0.75$, $P = 0.52$), nor did time of day have a significant effect on the frequency of fruit set ($F_{1,170} = 2.16$, $P = 0.14$). However, the frequency of fruit set did vary with flower age class ($F_{3,170} = 10.94$, $P < 0.0001$). Younger flowers (0–24 h old; 24–48 h old) were more likely to yield fruit than older flowers (48–72 h old; over 72 h old).

Timing and duration of stigma receptivity

Stigmas of *H. boreale* become receptive during the mature bud stage prior to anthesis and remain receptive for several days. In the trial run to determine when the stigma becomes receptive relative to anther dehiscence, two buds with indehiscent anthers (one immature and one mature) exhibited no red staining at the tip of the style; this lack of red coloration indicates that the buds were not yet receptive. In contrast, the stigma of a mature bud with dehiscent anthers stained very red. Therefore, it appears that the *H. boreale* stigma becomes receptive at about the same time as the anthers dehisce. In the trial run to determine when the stigma becomes receptive relative to anthesis, the stigmas of all four flowers (ranging from a mature bud with dehiscent anthers to a fully open flower) stained red. Therefore, it appears that the stigma in *H. boreale* flowers becomes receptive before anthesis begins. In both trials, none of the stigmas of the control flowers exhibited any red coloration.

Regarding the duration of *H. boreale* stigma receptivity, the stigmas of the three replicate flowers in each age class all stained redder than the controls. Therefore, it appears that the stigma in *H. boreale* flowers remains receptive for at least 72 h after anthesis.

Discussion

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

Few potential pollinators other than bees were observed on *H. boreale* flowers during this study. Therefore, all fruits and seeds produced on positive control (open visitation) racemes were likely the result of pollination by bees. In the present study, bees were better pollinators of *H. boreale* than we were; proportional fruit set on positive control racemes far exceeded proportional fruit set resulting from our manual pollinations (Fig. 2; Table 1). However, bee pollination resulted in only 57 and 45% overall fruit set at Paradise and Wood Camp Hollow, respectively, during breeding system experiments at the two sites (Fig. 2). That no more than half the flowers set fruits, even when well pollinated, has been reported often in legumes (Zimmerman & Aide 1989), including other species of *Hedysarum*. For example, open pollination treatments resulted in 57 and 46% fruit set in two populations of *H. coronarium* in Tunisia (Louati-Namouchi *et al*. 2000). Extra flowers in hermaphroditic species can increase the attractiveness of displays to pollinators, contribute to increased paternal success through pollen export and serve as an ovary pool to hedge against unforeseeable loss of flowers (Zimmerman & Aide 1989) or bouts of inclement weather. At both sites in our study, larvae of an herbivorous butterfly (*Lycaeides melissa*) and moth (Gelechiidae), as well as adult grasshoppers, were seen consuming and/or damaging a few *H. boreale* flowers.

Flowers of *H. boreale* to which outcross pollen was manually applied (xenogamy) were inexplicably less

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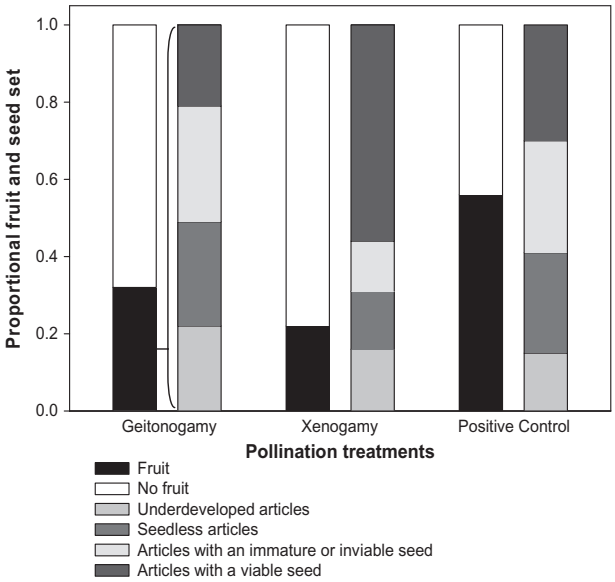

Fig. 4 Floral fates for four pollination treatments during breeding system experiments at Paradise and Wood Camp Hollow in 2003. Data from racemes that set one or more fruits were combined from the two sites. White bars represent the proportion of treated flowers that set no fruit. Grey bars represent the proportions of initiated ovules (summed across all fruits resulting from a particular treatment) that developed into four 'floral fate' categories. The underdeveloped article, seedless article and article with immature or inviable seed categories represent lost reproductive potential (i.e. reproductive attrition); inviable seeds were those that failed to germinate. In contrast, the class of articles with a viable seed represents the proportion of initiated ovules that reached their full reproductive potential.

likely to set fruits than those that received geitonogamy and positive control treatments (Fig. 2; Table 1). However, geitonogamy and positive control treatments yielded more underdeveloped articles, seedless articles and articles with inviable (immature or ungerminated) seeds than xenogamy racemes (Fig. 4). Boyd and Brum (1983) studied predispersal reproductive attrition in the creosote bush *Larrea tridentata* by characterizing bottlenecks that diminished its sexual reproduction. Wind damage, insect and animal predation, flower bud browning, loss of 'normal' flowers, empty fruits, suboptimal pollination and seed death were responsible for a 92% loss of reproductive potential in one population of creosote bush during a single blooming season. In *H. boreale*, underdeveloped articles, seedless articles and articles with inviable seeds represent reproductive potential lost from geitonogamy, xenogamy and positive control racemes (Fig. 4). Although xenogamy produced fewer fruits than geitonogamy in the present study, xenogamy offers a distinct advantage over geitonogamy in terms of less subsequent reproductive attrition. Fruits resulting from xenogamy were twice as likely to contain viable seeds as those resulting from geitonogamy (Fig. 4).

In the present study, it appears that bee pollination of *H. boreale* involves a mixture of geitonogamy and xenogamy. The proportions of seedless articles, as well as those articles with inviable seeds, were almost identical on positive control and geitonogamy racemes. However, viable seeds were produced more often at positive control racemes (Fig. 4). Because seeds resulting from xenogamy were often viable (Fig. 3), the higher proportion of viable seeds on positive control racemes suggests that bees delivered both self and outcross pollen to *H. boreale* flowers. Ehrlen (1992) concluded that fruit initiation of another legume was often limited by pollen availability, whereas maternal resources primarily limited fruit and seed maturation. 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, that had the most seeds or those that resulted from outcrossing (Stephenson 1981). Regarding *H. boreale*, underdeveloped and seedless articles, and articles with immature or inviable seeds, seem likely to be the result of maternal selection after ovule fertilization (i.e. selective abortion of inferior seeds).

The inferiority of our manual pollinations did not result from a mismatch between the timing of pollen application and stigma receptivity, however. The results of the stigma receptivity experiments show that the stigma in *H. boreale* flowers becomes receptive in the mature bud stage, just prior to anthesis. Because their anthers also shed pollen during the mature bud stage (Tepedino & Stackhouse 1987), flowers of *H. boreale* are homogamous. That is, anther dehiscence and stigma receptivity co-occur and there is no differential maturation of anthers and stigma to limit selfing or promote outcrossing (Fægri & van der Pijl 1979). The relative timing of anther dehiscence and stigma receptivity appears to maximize the reproductive output of *H. boreale*. Flowers are in fact 'ready' for visitation even before the standard petal is fully erect. Although the standard petal is the chief advertising organ of the typical legume flower (Fægri & van der Pijl 1979), several bees (honeybees, bumblebees and *Megachile* spp.) were observed to push their way into flowers in which the standard petal was not yet fully erect.

It is unclear why bees were better *H. boreale* pollinators in the present study. 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 or that is overlooked by manual pollination. In general, the style of legume flowers is hollow, with a central canal extending from just below the stigma down to the ovary (Heslop-Harrison & Heslop-Harrison 1982). Pollen grains do not germinate on the stigmas of hollow-styled taxa until exudate is released from within the stig-

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matic membrane (Shivanna & Sastri 1981). The stigmatic membrane in some legume species is relatively thin and breaks easily at maturity, whereas the stigmatic membrane in other legumes is very thick and will not break until the flower is tripped (Lord & Kohorn 1986). The miniscule size of the stigma of *H. boreale* made it impossible to examine the intact living stigmatic surface in the field for the presence of exudate. Therefore, our gentle methods used to rupture the stigmatic membrane in *H. boreale* flowers prior to manual pollinations may have been ineffective. In order for membrane rupture to occur, it may be necessary for the stigma to forcefully hit the keel petal suture or the bristly underside of a visiting bee as the flower is tripped.

Tripping mechanisms 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 lands on the wing petals, forces them apart, and proceeds to collect nectar from the nectary at the base of the ovary (Kalin Arroyo 1981). For legumes that require tripping, self-pollen does not germinate until a pollinator visits the flower. Such a system is reported for *Hedysarum glomeratum* (=*H. spinosissimum* ssp. *capitatum*: International Legume Database and Information Service 2005). A mechanical barrier prevents pollination within unvisited *H. glomeratum* flowers; only when pollinators visit the flowers are both self-pollination and cross-pollination possible (de las Heras *et al*. 2001). Based on the fact that autogamy failed to yield any fruit in the present study, it is likely that a similar mechanism exists in *H. boreale*. Such a mechanism assures the potential for at least some outcrossing, particularly if outcross pollen is competitively superior to self-pollen.

Another possible explanation for the comparative failure of hand pollinations involves the stigmatic papillae themselves. Pollen may not have been applied with sufficient force to push individual grains down amid the papillae and into contact with the stigmatic exudate. In this scenario, sequential flower visits may facilitate fruit and seed production in *H. boreale*. Pollen grains deposited on top of, or just within, the stigmatic papillae could be pushed further down into the papillae as subsequent pollinators deposit more pollen. McGuire (1993) reported that seed set per flower in *H. boreale* increased with increasing visitation rates by female *Megachile giliae*, suggesting that seed set in some individuals was limited by pollinator visits. In sainfoin (*Onobrychis viciifolia* Scop.), a close relative of *Hedysarum* (Welsh 1978), the percentage of fertilized ovules increased from 10 to 50% with an increase from one to four pollinator visits per flower (Bogoyavlenskii 1955). Similarly, doubling the foraging population of honeybees increased seed yield by 60% in sainfoin (Bogoyavlenskii 1976). Therefore, to ensure that adequate pollen is pushed down amid the stigmatic papillae, it appears that enough bees should be stocked for cultivated *H. boreale* to obtain multiple flower visits.

In conclusion, *H. boreale* was found to be homogamous and self-compatible, but produced no fruit without bee visitation. Therefore, growers who plan to farm *H. boreale* for seed should provide managed bee pollinators if populations of local wild bee pollinators are not abundant. In the present study, younger flowers were more likely to set fruit (Table 3). Thus, seed growers should stock enough bees so that each new or day-old flower is visited at least once. Ideally, to maximize fruit and seed yields, enough bees should be provided to obtain multiple flower visits. Finally, because xenogamy appears to enhance the long-term reproductive success of *H. boreale* by increasing seed viability and by decreasing reproductive attrition, seed growers should provide a bee pollinator whose foraging behavior promotes outcrossing. For example, in fruit orchards, blue orchard bees (*Osmia lignaria*) tend to visit fewer flowers per tree, instead moving frequently from tree to tree and row to row. Such foraging behaviors promote outcrossing (Bosch & Kemp 2001). In another study, both honeybees and alfalfa leafcutting bees (*Megachile rotundata*) promoted outcrossing by moving laterally between rows more often than along rows of bean cultivars (*Vicia faba*) (Currie *et al*. 1990). Supplying a pollinator with comparable foraging behavior would be ideal for maximizing fruit and seed yields in *H. boreale*.

Acknowledgments

This research was funded by the Great Basin Native Plant Selection and Increase Project funded through the U.S. Department of the Interior, Bureau of Land Management (USDI-BLM) Great Basin Restoration Initiative and the U.S. Department of Agriculture, Forest Service (USDA-FS) Rocky Mountain Research Station. Vince Tepedino and Edward Evans provided detailed, insightful comments on drafts of this manuscript.

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