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Breeding Biology of the Threadstalk Milkvetch, Astragalus filipes (Fabaceae), with a Review of the Genus

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ABSTRACT.—*Astragalus* L. (Fabaceae) is an enormous and diverse plant genus with a cosmopolitan distribution, but relatively few breeding biologies are known for its member species. Threadstalk (or basalt) milkvetch, *Astragalus filipes* Torrey ex. A. Gray, is common and widespread throughout the U.S. Intermountain West, including the Great Basin. It is being studied and ultimately propagated for extensive rangeland restoration projects throughout the sagebrush steppe. Understanding the breeding biology of *A. filipes* will be necessary for reliable and consistent commercial seed production with this species. We examined reproductive output from four manual pollination treatments (autogamy, geitonogamy, xenogamy and distant xenogamy) in a common garden. As measures of fitness, we counted fruit and seed set, then germinated viable seeds, to assess reproductive output. This species is weakly self compatible; xenogamous pollen transfer results in nine times more seed per pollination. Pollen transfer between geographically distant seed accessions resulted in a decrease in seed germination, but no difference in fruit or seed set. Cross pollination by bees will be necessary for copious seed production by this species. In the wild, flowers of *A. filipes* are visited most commonly and ubiquitously by a diversity of *Osmia* bee species plus several bee species each of *Eucera*, *Anthidium*, *Bombus* and sometimes *Hoplitis*.

INTRODUCTION

Plants in the legume family (Fabaceae sensu *lato* Lindl.) are of global economic and ecological significance. The Fabaceae is second only to the grasses in economic importance and is only smaller than the Orchidaceae and Asteraceae in numbers of species. Papilionoid legumes are particularly valuable as ground cover, forage and food crops (Allen and Allen, 1981).

The genus *Astragalus* L. is the largest of the flowering plant genera (Frodin, 2004). Comprising some 3270 species, *Astragalus* is most diverse in the Sino-Himalayan region, Russia, the Andes mountains of South America and across western North America (Allen and Allen, 1981; Isely, 1998). Nearly 400 species of *Astragalus* occur in North America, with 156 species occurring in the Intermountain West alone (Barneby, 1964). Plants in this genus are economically significant as a source of gum tragacanth, as indicators of selenium and uranium and as toxic locoweeds in rangelands (Allen and Allen, 1981). The more widespread and common species can support diverse elements of the region's pollinating bee communities as well (*e.g.,* Green and Bohart, 1975; Clement *et al.*, 2006)

Few *Astragalus* breeding biologies are known despite the size, geographic extent and prevalence of the genus. A literature search revealed known breeding biologies for only 29 *Astragalus* species worldwide, accounting for <1% of all *Astragalus* species (Table 1). These

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few species have breeding biologies that span the range of self fertility, from self compatible to self incompatible; a few are obligately xenogamous. One annual species (*A. cymbicarpos* Brot.) is even cleistogamous (Gallardo *et al.*, 1993). This range of breeding biologies within the genus, coupled with a general paucity of knowledge about most species, makes predicting the breeding biology of a given *Astragalus* species dubious at best.

Our primary objective was to experimentally characterize the breeding biology of *Astragalus filipes* Torrey ex. A. Gray (threadstalk milkvetch or basalt milkvetch), which is widespread in western North American (Isely, 1998), and compare it with a compilation of other such studies with *Astragalus*. This species has been evaluated and now propagated for seed to use in future rangeland rehabilitation projects throughout its range (Shaw *et al.*, 2005). Wildland seed production is erratic and susceptible to beetle predation, making it more costly and unpredictable than cultivated seed production (Youtie and Miller, 1986; Cane, 2008a). The first tested germplasm for *A. filipes* (NBR-1) was recently released for commercial cultivation (Johnson *et al.*, 2008). Knowing a plant's breeding biology informs growers on whether or not they need pollinators for a seed crop, and the importance of outcrossing for consistent and copious seed production. We expect species that are good colonizers, as *A. filipes* is after fires, to be self-compatible (Kalin Arroyo, 1981; Bhattarai *et al.*, 2008). Conversely, plants like *A. filipes* with considerable genetic diversity within populations (B.S. Bushman, pers. comm.) often prove to be outcrossers; thus, we speculated that *A. filipes* might be at least moderately self compatible although likely to benefit from outcrossing. In addition, we report preliminary findings for the composition of the pollinator guild that visits *A. filipes* in the wild, to discover the kinds of pollinators that might be practical and suitable for this native seed crop.

METHODS

NATURAL HISTORY OF A. FILIPES

Astragalus filipes ranges from the southern Great Basin northward into the Columbia Plateau, with some disjunct populations in southern British Columbia, the San Bernardino mountains of southern California and northern Baja California (Barneby, 1964; Isely, 1998). In some parts of its range *A. filipes* is ''one of the truly common astragali … often occurring in colonies of great extent, sometimes in such quantity as to color the sagebrush hillsides with a wash of creamy, spicily fragrant blossoms'' (Barneby, 1964). Due to its lack of toxins for livestock (Williams and Barneby, 1977) and its extensive ecological and geographic range, *A. filipes* shows promise for restoration use (Shaw *et al.*, 2005; Bhattarai *et al.*, 2008). The fruits of *A. filipes* are presented on erect racemes easily accessible for mechanical seed harvest by combine. These factors make *A. filipes* a good candidate species for cultivated seed production.

PLANT ACQUISITION

Seeds were collected from wild *Astragalus filipes* populations in fall 2003 by Douglas Johnson and Kevin Connors (USDA-ARS-FRRL). Seed locations used for this experiment represent eight different Omernik Level IV Ecoregions (Omernik, 1987; Table 2). Seeds were germinated, and transplanted into forestry ''conetainers'' in Jan. 2004. A pair of small experimental arrays was established in a common garden at the USDA-ARS Bee Biology and Systematics Laboratory (BBSL), Logan, UT, USA. The silty clay-loam soil was amended with pea gravel for improved drainage. Seedlings were planted out in May 2004 into holes cut in weed barrier fabric to simplify weed control.

POLLINATION TREATMENTS

In May 2005, one array of *Astragalus filipes* plants was covered with a walk-in net field cage (6 × 6 × 2 m) to exclude pollinators and facilitate manual pollinations with minimal handling of the plants. In the caged array, three pollination treatments were assigned to separate tagged racemes on each plant, replicated for 24 plants (Fig. 1). Each flower (Fig. 1) on a raceme received the same pollination treatment. Racemes were chosen before bloom, using those with at least five buds. Number of flowers per raceme varied, and a range of four to 23 flowers were treated per raceme (mean = 12 flowers). The often numerous other racemes were not pollinated. The three manual pollination treatments were: (1) geitonogamy, in which pollen was transferred from flowers of an unmarked raceme to a recipient flower on the same plant; (2) xenogamy, in which flowers of the treatment raceme received pollen from another donor plant from within the same seed accession location as the recipient plant; (3) distant xenogamy, in which flowers of the treatment raceme received pollen from donor plants belonging to a different Ecoregion than that of the recipient flowers (Table 2, Fig. 2). This distantly outcrossed treatment allowed us to examine the possibility of outbreeding depression by transferring pollen between populations too distant for manual pollen transfer in the wild.

All pollen was transferred using the plush dorsal thoraces of dead honeybees as disposable fine brushes, which we first rubbed against the anthers of the donor flower(s) and then immediately pressed against the stigma of a recipient flower. Each honeybee was used to pollinate only a single raceme's open flowers of a given day, then discarded, as crosses were specific to a given plant (geitonogamy) or geographic source (outcrossing). Manual pollination required two persons, one to carefully depress a flower's keel to extend the style and expose the stigma, the other to brush the delicate stigma with the pollen-laden honey bee cadaver. Following pollen transfer, the bright orange pollen was visibly heaped on the tiny stigmas (10× magnification). We also retained one raceme per caged plant as a negative control treatment of autogamy, for which flowers were counted and marked but otherwise left unmanipulated. Each treated flower was marked on the banner petal with indelible ink. Flowers were counted and pollinated every other day at mid-late morning until the racemes produced no new flowers. Plants of the neighboring array were left uncaged as a positive control to evaluate pod and seed set resulting from incidental visits by bees available at BBSL, again mostly visiting mid-late morning. These plants served as our open visitation treatment.

SEED PRODUCTION

Once the fruits (pods) were mature, but just before dehiscence, the racemes were collected and returned to the laboratory. Total number of fruits per raceme was recorded, as well as each pod's content of plump seeds. Aborted ovules were tiny and disregarded. Plump seeds were allowed to dry and mature at 25 C for 2 mo before being placed in cold storage (4 C).

SEED GERMINATION

Seeds were stored dry in envelopes at 4 C for 6 mo. After storage, all plump seeds were poked with an insect pin to perforate the seed coat for better water permeation. Seeds from the same raceme were then placed together in a single well of a tissue culture plate submerged in distilled water imbued with a fungicide to prevent mold. These tissue culture plates were then placed in a dark cold room (4 C) and the seeds were monitored for germination. We recorded if the radicle was protruding from the seed coat and transplanted the germinating seed to a conetainer with a native soil mix.

Table 1.—Literature review of *Astragalus* breeding biologies. Pollinator genera are included, if known

Astragalus species	Breeding biology	Distribution	Pollinators	Reference
A. alpinus L.	obligate outcrosser	widespread, circumpolar subarctic & arctic	*Bombus* sp.	Kudo & Molau 1999
A. americanus (Hook.) M.E. Jones	obligate outcrosser	widespread, W.N.Am.	*Bombus* sp.	Kudo & Harder 2005
A. ampullarioides (S.L. Welsh) S.L. Welsh	self-compatible	Endangered (restricted), W.N.Am.	*Anthophora* sp., *Bombus* sp., *Dialictus* sp., *Osmia* sp.	Tepedino 2005
A. australis (L.) Lam. var. *olympicus* Isely	moderately self-compatible	Threatened (restricted), W.N.Am.	*Anthidium* sp., *Bombus* sp., *Megachile* sp., *Osmia* sp.	Kaye 1999
A. canadensis L.	obligate outcrosser	widespread, W.N.Am.	*Bombus* sp.	Platt *et al.* 1974
A. cibarius Sheldon	outcrossing beneficial	widespread, W.N.Am.	*Anthophora* sp., *Bombus* sp., *Eucera* sp.	Green & Bohart 1975
A. cicer L.	outcrossing beneficial	widespread crop, cosmopolitan	*Apis mellifera, Bombus* sp., *Megachile* sp.	Richards 1986
A. cremnophylax var. *cremnophylax,* North Rim population	self-compatible	Endangered (restricted), W.N.Am.	unknown	Allphin *et al.* 2005
A. cremnophylax var. *cremnophylax,* South Rim population	obligate outcrosser	Endangered (restricted), W.N.Am.	unknown	Allphin *et al.* 2005
A. cremnophylax var. *herronii*	self-compatible	Endangered (restricted), W.N.Am.	unknown	Allphin *et al.* 2005
A. cremnophylax var. *myriorraphis*	outcrossing beneficial	Endangered (restricted), W.N.Am.	unknown	Allphin *et al.* 2005
A. cymbicarpos Brot.	cleistogamous and chasmogamous are self-compatible	restricted, Iberian peninsula & North Africa	unknown	Gallardo *et al.* 1993, 1994
A. edulis Durieu ex Bunge	self-compatible	widespread, Europe to W. Asia	unknown	Gallardo *et al.* 1994
A. epiglottis L. subsp. *epiglottis*	self-compatible	widespread, Africa, Europe & Middle East	unknown	Gallardo *et al.* 1994
A. epiglottis L. subsp. *asperulus* (Dufour) Nyman	self-compatible	restricted, Spain	unknown	Gallardo *et al.* 1994
A. hamosus L.	self-compatible	widespread, Europe	unknown	Gallardo *et al.* 1994

TABLE 1.—Continued

Astragalus species	Breeding biology	Distribution	Pollinators	Reference
A. holmgreniorum Barneby	self-compatible	restricted, W.N.Am.	*Anthophora* sp., *Apis mellifera*	Tepedino 2005
A. humillimus A. Gray	self-compatible	Endangered (restricted), W.N.Am.	*Apis mellifera, Eucera* sp., *Osmia* sp.	Geer *et al.* unpublished
A. kentrophyta var. *tegetarius* (S. Wats.) Dorn	obligate outcrosser	widespread, W.N.Am.	*Anthidium* sp., *Osmia* sp.	Geer & Tepedino 1993; Geer *et al.* 1995
A. linifolius Osterh.	self-compatible, moderately autogamous	restricted, W.N.Am.	*Anthophora* sp., *Apis mellifera, Bombus* sp, *Osmia* sp.	Karron 1987, 1989
A. lonchocarpus Torrey	self-compatible	widespread, W.N.Am.	*Anthophora* sp., *Apis mellifera, Bombus* sp., *Osmia* sp.	Karron 1987, 1989
A. miser var. *oblongifolius* (Rydb.) Cron.	obligate outcrosser	widespread, W.N.Am.	*Bombus* sp., *Osmia* sp.	Geer & Tepedino 1993; Geer *et al.* 1995
A. monoensis Barneby	obligate outcrosser	restricted, W.N.Am.	*Anthidium* sp., *Apis mellifera, Bombus* sp., *Hoplitis* sp., *Osmia* sp.	Sugden 1985
A. montii Welsh	self-compatible	Endangered (restricted), W.N.Am.	*Anthidium* sp., *Osmia* sp.	Geer & Tepedino 1993; Geer *et al.* 1995
A. osterhouti M.E. Jones	moderately self-compatible	restricted, W.N.Am.	*Anthophora* sp., *Apis mellifera, Bombus* sp., *Osmia* sp.	Karron 1987, 1989
A. pectinatus (Hook.) G.Don	obligate outcrosser	widespread, W.N.Am.	*Anthophora* sp., *Bombus* sp., *Osmia* sp.	Karron 1989
A. striatus Nutt.	obligate outcrosser	widespread, W.N.Am.	*Bombus* sp.	Kudo & Harder 2005
A. tennesseensis Chapman	obligate outcrosser	restricted, Midwest U.S.	*Apis mellifera, Bombus* sp., *Xylocopa* sp.	Baskin *et al.* 1972
A. utahensis (Torr.) Torr. & A.Gray	outcrossing beneficial	widespread, W.N.Am.	*Anthophora* sp., *Bombus* sp., *Eucera* sp.	Green & Bohart 1975

TABLE 2.—Plant accession locations for manual pollination treatments. Distant xenogamy treatments were applied across different level IV Ecoregions (Omernik & Gallant, 1986; McGrath *et al.*, 2002; Bryce *et al.*, 2003; Thorson *et al.*, 2003). Superscript numbers indicate seed accessions which were crossed in distant xenogamy treatments and correspond to location numbers in Figure 2

Site name	County, State	Ecoregion IV	Ecoregion III	Latitude N	Longitude W	Elev (m)
Ellensburg[1]	Kittitas, WA	10g - Yakima Folds	Columbia Plateau	46°56′15.4″	120°15′22.9″	653
Clarno[1]	Wasco, OR	11a - John Day/Clarno Uplands	Blue Mountains	44°55′07.3″	120°31′09.2″	625
Otley Ranch[2]	Harney, OR	80g - High Lava Uplands	Northern Basin & Range	42°56′02.7″	118°36′48.6″	1502
Mountain City[2]	Elko, NV	80a - Dissected High Lava Plateau	Northern Basin & Range	41°48′10.7″	115°56′33.0″	1774
Warrior Mine[3]	Nye, NV	13v - Tonopah Sagebrush Foothills	Central Basin & Range	38°36′37.1″	117°50′08.9″	1888
Big Gulch[3]	Custer, ID	17e - Barren Mountains	Middle Rockies	44°20′43.3″	113°31′01.2″	2201
Black Mountain[4]	Owyhee, ID	80f - Owyhee Uplands & Canyons	Northern Basin & Range	43°08′07.3″	116°43′27.1″	1700
Champs Flat[4]	Lassen, CA	342Bd - Cottonwood – Skedaddle Mountains	Northern Basin & Range	40°42′07.7″	120°53′32.7″	1711

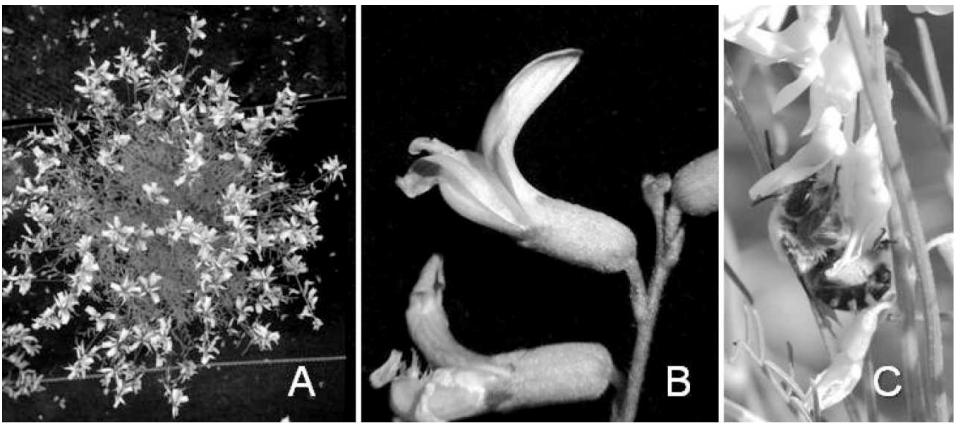

FIG. 1.—(A) Flowering 2 y old plant of *Astragalus filipes* in the experimental array, viewed from above. Pale structures are flowers atop their racemes (>50 racemes visible) (B) Open flower of *A. filipes* (keel is about 9 mm long) (C) Female *Eucera frater* (Cresson) bee foraging at flower of wild *A. filipes*

SEED COUNTS FROM WILD POPULATIONS

Seeds were collected in 2005 from four different wild *Astragalus filipes* populations: Pequop Summit, Elko County, NV (2); Big Gulch, Custer County, ID; and King Hill, Elmore County, ID. We counted the seed contents of 100 pods for each population to establish a baseline of wild seed set against which we compared our open visitation seed set at the Logan common garden.

BEE GUILDS FROM WILD POPULATIONS

The fauna of bees that visit *Astragalus filipes* (and other dominant forbs) are being collected from around the Great Basin and Snake River Plains as part of a larger study dealing with the effects of wildfire on bee communities. We have net collected bees visiting flowers of *A. filipes* at 24 populations growing in sagebrush basins and dry coniferous forests across five states (California, Idaho, Nevada, Oregon, Utah) during May and June. Identifications to species are ongoing for some genera, notably *Osmia*.

DATA ANALYSIS

We tested the null hypothesis that frequency of fruit set is independent of manual pollination treatment with an R×C Test of Independence using a G-Test with William's correction (Sokal and Rohlf, 1981). Rows were manual pollination treatments, and columns were counts of pods produced or failed pollinations (no pod). Individual flowers were used as independent replicates, as they arose from separate pollination events (often on different days as bloom proceed along a given raceme). We excluded open visitation data from this analysis, since that used different plants. A significant test for an overall effect of treatment on fruit set was followed by pairwise comparisons with G-Tests (Sokal and Rohlf, 1981). Only flowers setting fruits were used for subsequent seed set analyses.

We compared pollination treatments for the fraction of pods that contained one or more seeds (categorical analysis, CATMOD, SAS Institute, 2004). The linear model compared the proportion of seeded pods of each manual pollination treatments with our distant xenogamy treatment. We used distant xenogamy for comparison, as there was no significant difference in proportion of seeded pods between it and our xenogamy treatment. We made

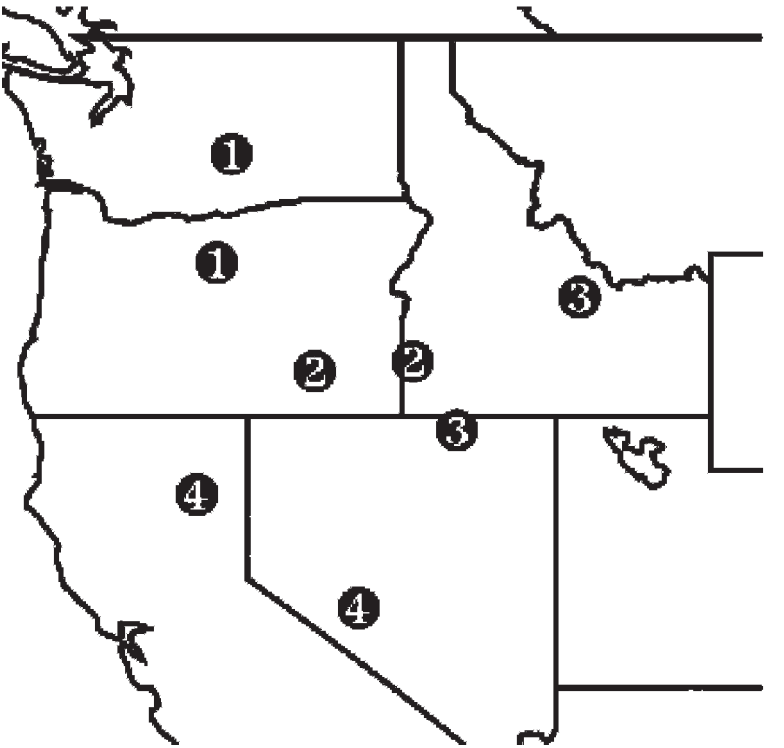

FIG. 2.—Seed source locations for common garden plants. Pairs of numbers indicate populations that were crossed in our distant outbreeding treatment. Populations crossed are from different Omernik level IV Ecoregions

three orthogonal pairwise contrasts: autogamy vs. geitonogamy, geitonogamy vs. outcross and freely-visited vs. outcross.

We used the Kruskal-Wallis test on ranked seed counts per pod, excluding empty pods, to compare seed counts per pod across pollination treatments (Proc NPAR1WAY; SAS Institute, 2004). Germination percentages (transformed to their arcsine values) for seeds from the different pollination treatments were analyzed with General Linear Model (GLM) ANOVA followed by REGWQ *a posteriori* tests. Seed counts per fruit were also compared using a GLM ANOVA for samples from the four wild populations and our open visitation treatment at the common garden. These data were satisfactorily transformed by adding one to each value, then applying a cube root transformation. Seed count means were compared using REGWQ *a posteriori* tests.

RESULTS

Overall, we manually pollinated 830 flowers and marked 795 more (372 flowers for autogamy and 423 freely-visited flowers) to track their reproductive fates. Collectively, the 835 pods that resulted contained 1065 seeds.

Cross pollination significantly enhanced fruit and seed set compared with the two self pollination treatments (autogamy and geitonogamy). The likelihood of fruit set differed

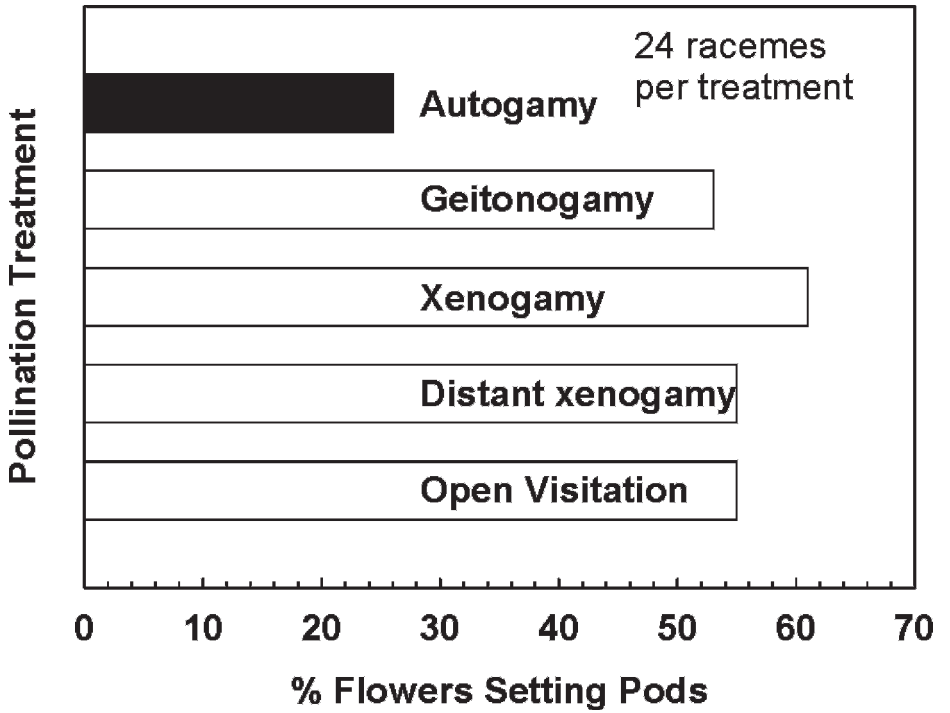

FIG. 3.—Proportion pod set per flower pollinated. Different fill denotes a significant difference ($P < 0.05$) between pollination treatments. Data includes seedless pods

among pollination treatments ($G_{[3,1191]} = 12.38$, $P < 0.001$, Fig. 3). Flowers of autogamous racemes were less likely to set fruit than any of the other pollination treatments ($G_{adj} = 107$, $P < 0.001$). The frequencies of seedless pods differed considerably between pollination treatments ($G_{[4,835]} = 255$, $P < 0.001$, Fig. 4). From the orthogonal contrasts, flowers of autogamous racemes produced more seedless pods than geitonogamous flowers ($G = 5.1$, $P = 0.024$), which in turn set more seedless pods than xenogamous racemes ($G = 11.2$, $P = 0.001$). Open visitation racemes set proportionately more seeded pods than xenogamous racemes ($G = 48.2$, $P < 0.001$). Pollination treatments also differed in the counts of seeds per pod, both for all pods ($H_{[3,204]} = 10.7$, $P = 0.014$, Fig. 5) as well as for the subset of seeded pods ($H_{[3,187]} = 9.9$, $P = 0.007$).

Percent germinable seed per raceme varied with pollination treatment ($F_{[4,59]} = 3.2$, $P = 0.02$, Table 3). Distant xenogamous seeds were significantly less likely to germinate than seeds from geitonogamy, xenogamous and open visitation treatments. Autogamous seeds were not evaluated as so few were produced. Seed germination spanned a 15 wk period, with a pulse of germination in the 10^{th} week for seeds from each treatment group.

Openly pollinated populations differed in seed set per fruit ($F_{[4,658]} = 12.9$, $P < 0.001$, Fig. 6). Openly visited plants in our common garden at BBSL set significantly fewer seeds per pod than did *Astragalus filipes* plants from four wild populations. Samples from the four wild populations had comparable seed counts per fruit.

The floral visitors foraging at flowers of *Astragalus filipes* are all bees (adult seed weevils are also present, but they are ovipositing in the young pods). Few of the visiting individuals and

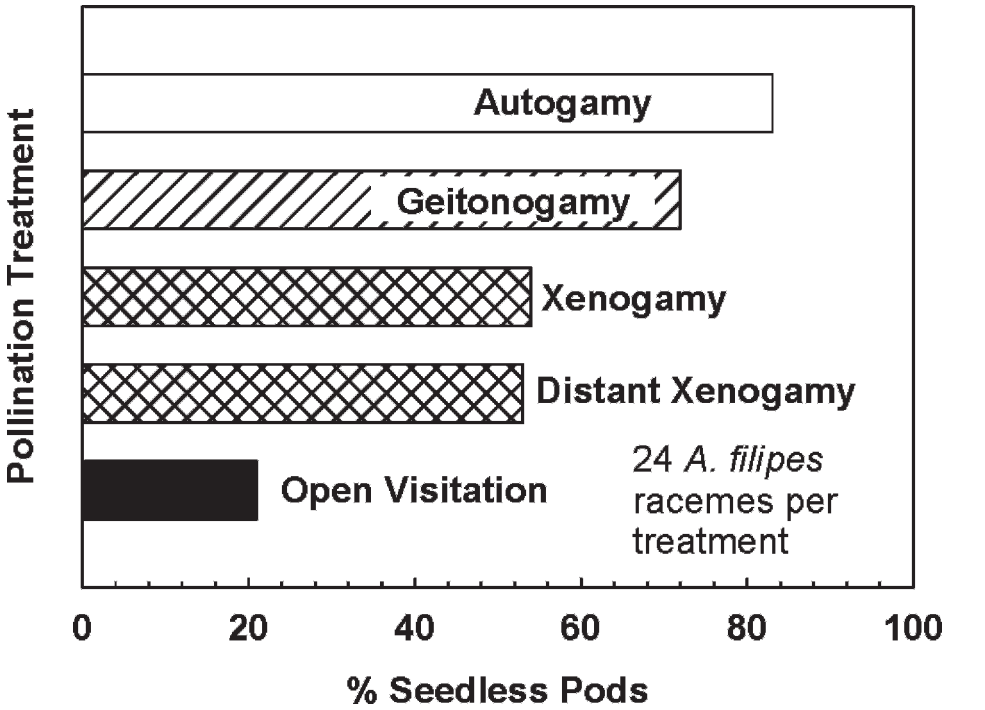

FIG. 4.—Proportion of the 835 pods resulting from pollination treatments that were seedless. Different fills denote significant differences ($P < 0.05$) between pollination treatments, such that in terms of seedless pods, autogamy > geitonogamy > either xenogamy > open visitation

species of bees foraging at flowers of *A. filipes* were social bees (bumblebees mainly) and none appears to be a specialist (=oligolege) for the genus *Astragalus*. Half of the 353 individual bees thus far sampled at *A. filipes* belong to 34 species of the bee genus *Osmia* (Megachilidae), with additional species in the genera *Anthidium, Bombus, Eucera* (Fig. 1) and *Hoplitis* also regularly found with the guild of bees sampled at *A. filipes* flowers.

DISCUSSION

Breeding biologies of papilionoid legumes are diverse, ranging from cleistogamous to obligately xenogamous (Kalin Arroyo, 1981). Though moderately self-compatible, *Astragalus filipes* and many other papilionoid legumes are bee pollinated (Kalin Arroyo, 1981). They benefit strongly from outcrossing facilitated by bee visitation. This combination of self-compatibility, but with reproduction enhanced considerably by outcrossing, is often classified as a ''mixed mating system'' (Neal and Anderson, 2005).

The autogamous (unmanipulated) treatment yielded significantly less fruit and seed set than any other treatment, indicating that most *Astragalus filipes* seed production does not result from mere autopollination. Cross pollination yielded nine times more seed than autogamy (93 vs. 11 seeds per 100 flowers) and three times more than geitonogamy (32 seeds per 100 flowers). Hence, although a colonizing individual can produce some progeny, even in the absence of pollinators, a substantial fraction of seed from well pollinated plants in a population likely result from cross pollination.

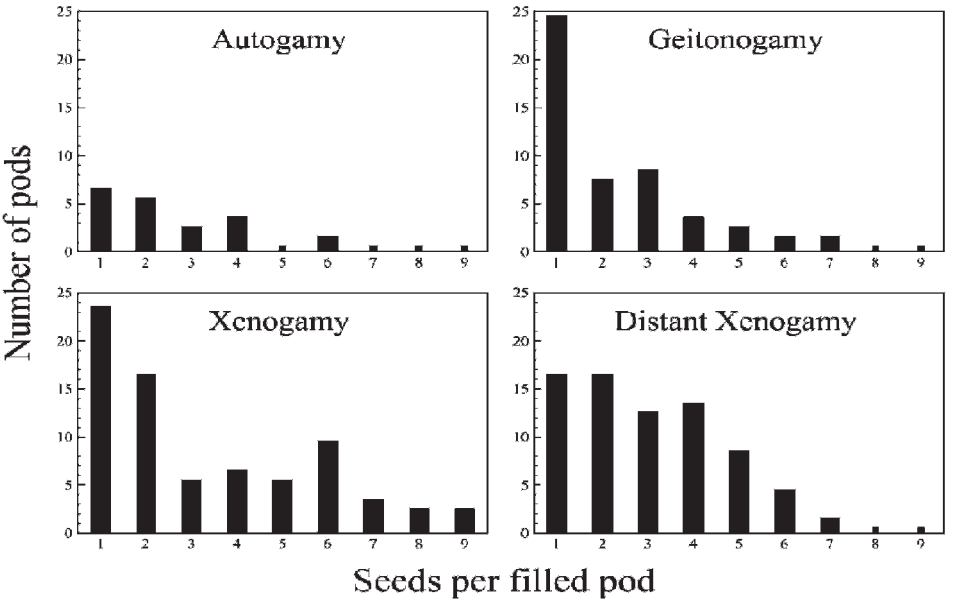

FIG. 5.—Ranked counts of seeds per pod, compared across manual pollination treatments

Openly visited flowers yielded the most seed per fruit, significantly more than the manual pollination treatments. These flowers likely received more frequent pollen deposition than did our manually pollinated flowers, each of which we pollinated only once. We observed bumble bee queens (*Bombus huntii* Greene, *B. fervidus* (Fabricius)) and workers (*B. huntii*) foraging at these uncaged plants frequently throughout the day. Similar to our results, Geer and Tepedino (1993) found that bees were superior pollinators to the experimenters for another *Astragalus* species. Despite the considerable fruit and seed set conferred by bumble bees in our common garden, our openly visited seed production was still less than that found in wild *A. filipes* populations (Fig. 6). Perhaps our pollinator guild, which was primarily just *Bombus,* was less effective than the species-rich guild of bees dominated by diverse *Osmia* species that we are finding associated with wild *A. filipes* throughout its range. Additionally, *A. filipes* plants may perform better in their respective native locations than in our common garden; a large comparison of accessions in a nearby common garden showed a wide range of performance (Bhattarai *et al.*, 2008). Furthermore, most wild plants that we have surveyed were larger and likely older than our young transplants.

TABLE 3.—Mean proportion (±standard error) for seed germination per raceme for four pollination treatments. Treatments were conducted at BBSL in 2005. n = number of plants. Letters following treatment means indicate statistical difference ($P \leq 0.05$)

Pollination treatment	N	$X \pm s_x$	Range
Open visitation	22	$0.73^{a} \pm 0.33$	0–1.0
Geitonogamy	11	$0.72^{a} \pm 0.38$	0–1.0
Xenogamy	16	$0.80^{a} \pm 0.24$	0.25–1.0
Distant xenogamy	15	$0.43^{b} \pm 0.29$	0–1.0

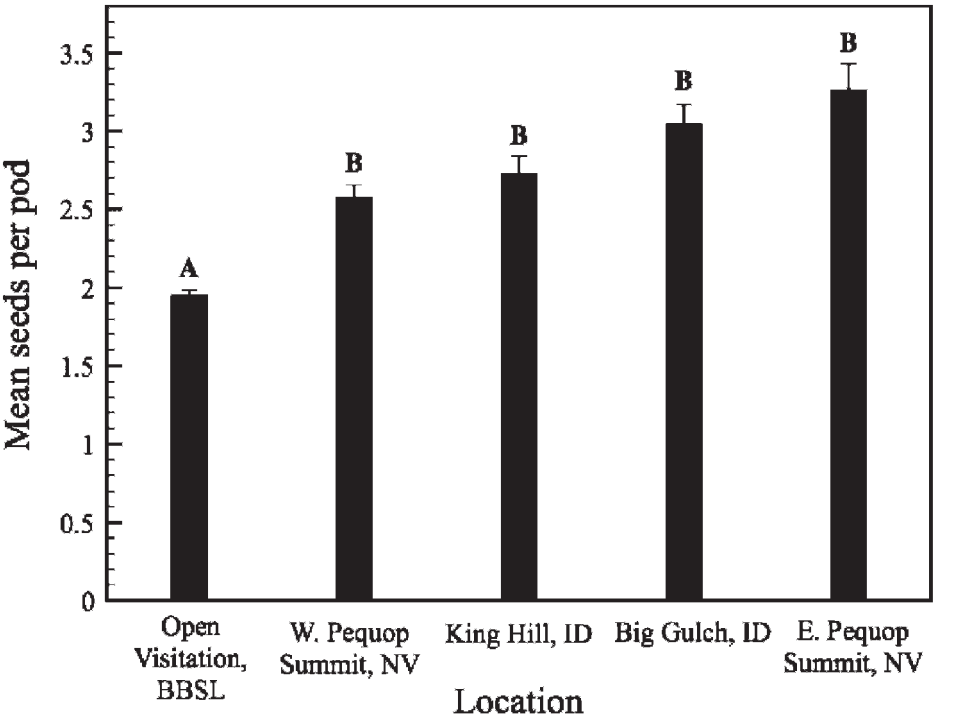

FIG. 6.—Mean count of seeds per seeded pods from wild populations and our open visitation plants. Data used are untransformed. Error bars show standard error. A different letter denotes a significant difference ($P < 0.05$) between populations

A smaller proportion of distant xenogamous seeds germinated compared with those from geitonogamy, within-population xenogamy or open visitation treatments. This negative effect of pollen transfer among geographically distant accessions on seed germination rates raises concern of possible outbreeding depression (Price and Waser, 1979), wherein locally adapted genotypes are disrupted or even swamped by mixing with other more distant populations. This concern is relevant to situations wherein farmed seed produced from distant germplasm accessions is seeding back into landscapes where conspecific populations persist (Monsen and Shaw, 2001; Hufford and Mazer, 2003; McKay *et al.*, 2005; Becker *et al.*, 2006). However, experimental trends with small sample sizes such as our's may not reasonably represent conditions at a larger scale. According to B.S. Bushman (pers. comm.) most detected genetic variability in *Astragalus filipes* is within populations, as is expected for a primarily outcrossing species. Outbreeding depression is less likely to be significant for species with genetically diverse populations.

A rich diversity of breeding biologies is represented among even the small sample (29) of *Astragalus* species that have been thus studied and reported. From our literature review (Table 1), half (15) of the studied species are self compatible. Of these self compatible species, five are widespread and ten have restricted distributions. Four more species are self compatible but benefit from xenogamous pollen transfer. One third (10) of the species are obligate outcrossers: seven are geographically widespread and three are restricted. Most threatened or endangered astragali are self compatible, though one endangered species in

Arizona, *A. cremnophylax* Barneby, varies from inbreeding to self incompatible, dependent on location and subspecies (Allphin *et al.*, 2005). Among the widespread species, five are self-compatible, seven are obligate outcrossers and three benefit from outcrossing. One unusual annual species from Spain and North Africa, *A. cymbicarpos* Brot., is even cleistogamous in some populations (Gallardo *et al.*, 1993). Predicting the breeding biology of a single *Astragalus* species, therefore, is speculative, owing to the diversity of breeding biologies, lack of correlates with life history or ecology and our general lack of knowledge about most species within this genus.

As in other papilionoid legumes, bees commonly visit *Astragalus* flowers for nectar and pollen (Kalin Arroyo, 1981). Among the ½ million bees pinned in the collections of the BBSL, 3400 specimens representing 192 bee species are labeled as having been taken at *Astragalus*. Among these floral hosts are 55 different species of *Astragalus*. No species of *Astragalus* is given for one third of the pinned specimens, probably due to the daunting identification challenge. Perhaps for this reason also, no bee in the BBSL collection was recorded from *A. filipes* until this study.

The remarkable diversity of *Osmia* bees thus far identified from specimens collected at *A. filipes* represents ¼ of the named species of *Osmia* in all of North America (Cane *et al.*, 2007). Abundant and diverse *Osmia* have been observed for other *Astragalus* species at other locations, with *Osmia* comprising more than 60% of the total flower visitor fauna sampled from three other *Astragalus* species over 3 y (Geer *et al.*, 1995). As in that study and others (Green and Bohart, 1975; Clement *et al.*, 2006), bees of the genera *Anthidium, Bombus, Eucera* (Fig. 1) and *Hoplitis* were also regular members of the guild of bees sampled at *A. filipes* flowers.

One of the cavity-nesting megachilid bees that forages at *Astragalus filipes*, *Osmia bruneri* Ckll., is widespread across the Intermountain West (Frohlich and Tepedino, 1986) where growers have recently begun to plant released germplasm of *A. filipes* (Johnson *et al.*, 2008). At the Logan lab, JHC is multiplying and evaluating a growing captive population of this species using nesting substrates, shelters and season management protocols developed and successfully implemented for a close relative, *O. aglaia* Sandhouse (Cane, 2008b). The population is multiplying 2–4-fold annually in straw-lined wooden (and polystyrene) nesting blocks, in agreement with an earlier report of their nesting success in captivity (Frohlich and Tepedino, 1986). Individual females have reproduced successfully when their foraging was confined to a cage over one of our *A. filipes* plots.

We found that *Astragalus filipes* has a mixed mating system, as it is self compatible but benefits greatly from outcrossing. Bees of several genera were regularly observed visiting *A. filipes* at numerous locations. Bees apparently transfer pollen better than manual pollination by researchers. Wild populations of *A. filipes* set significantly more seed per pod than did our open visitation treatment. Our research adds to the meager body of literature on breeding biologies of *Astragalus*, the largest genus of flowering plants, and will help guide successful production of this species for seed to be used in wildland restoration projects in the western USA.

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