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Breeding biologies, pollinators, and seed beetles of two prairie-clovers, *Dalea ornata* and *Dalea searlsiae* (Fabaceae: Amorpheae), from the Intermountain West, USA

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BREEDING BIOLOGIES, POLLINATORS, AND SEED BEETLES OF TWO PRAIRIE-CLOVERS, *DALEA ORNATA* AND *DALEA SEARLSIAE* (FABACEAE: AMORPHEAE), FROM THE INTERMOUNTAIN WEST, USA

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ABSTRACT.—Two prairie-clovers, *Dalea ornata* (Douglas ex Hook.) Eaton & J. Wright and *Dalea searlsiae* (A. Gray) Barneby, are perennial forbs found sporadically in the U.S. Intermountain West. Their seed is desirable for use in rangeland restoration. We experimentally characterized the breeding biologies of *D. ornata* and *D. searlsiae* in a common garden, surveyed their pollinator guilds, and sampled their seed predators. The 2 *Dalea* species, being primarily xenogamous, have comparable pollination requirements. For flowers manually pollinated with outcross pollen, an average of 42% of *D. ornata* flowers and 39% of *D. searlsiae* flowers yielded plump large seeds filled with endosperm. Both species proved to be self-compatible, but far fewer seeds resulted from either manual pollination with self-pollen (11% seed set for *D. ornata* and 7% for *D. searlsiae*) or unassisted autogamy (5% and 6% seed set, respectively). Limited surveys of the prairie-clovers' pollinator guilds in ruderal or cheatgrass-infested habitats revealed sparse visitation solely by wild bees, primarily of the genera *Anthidium, Colletes, Bombus, Eucera,* and *Melissodes.* Beetles (*Acanthoscelides oregonensis* Johnson and *Apion amaurum* Kissinger) infested seed sampled from 18 of 25 *D. ornata* populations across a 3-state region. Productive farming of the seed of these prairie-clovers for rangeland restoration in the western United States will require supplementation of bees for pollination and exclusion of seed beetles.

RESUMEN.—Dos especies de daleas, *Dalea ornata* (Douglas ex Hook.) Eaton & J. Wright y *Dalea searlsiae* (A. Gray) Barneby, son plantas herbáceas perennes que se encuentran esporádicamente en la región intermontañosa del oeste de los Estados Unidos. Sus semillas son útiles para la restauración de pastizales. Caracterizamos experimentalmente la biología de la reproducción de *D. ornata* y *D. searlsiae* en un experimento de jardín común; examinamos sus gremios de polinizadores y tomamos muestras de los depredadores de sus semillas. Las 2 especies de *Dalea,* siendo primordialmente xenógamas, tienen requisitos comparables para la polinización. En las flores que fueron polinizadas manualmente con polen ajeno, un 42% en promedio de las flores de *D. ornata* y un 39% de las flores de *D. searlsiae* produjeron semillas grandes y voluminosas llenas de endospermo. Ambas especies resultaron ser auto-compatibles, pero resultaron muchas menos semillas tanto de la polinización manual con polen propio (11% de semillas en *D. ornata* y 7% en *D. searlsiae*) como de autogamia no asistida (5% y 6% de semillas, respectivamente). Muestreos limitados de los gremios de polinizadores en hábitats dominados por plantas ruderales o por *Bromus tectorum* (cheatgrass) revelaron visitas escasas conformadas exclusivamente por abejas silvestres, principalmente de los géneros *Anthidium, Colletes, Bombus, Eucera* y *Melissodes.* Dos especies de escarabajos (*Acanthoscelides oregonensis* Johnson y *Apion amaurum* Kissinger) infestaron las muestras de semillas en 18 de las 25 poblaciones de *D. ornata* a lo largo de una región que abarca tres estados. Habrá que proporcionar abejas para la polinización y excluir o controlar los escarabajos depredadores de semillas para tener una cosecha productiva de las semillas de estas daleas con el fin de usarlas en esfuerzos por restaurar pastizales en el oeste de los Estados Unidos.

The 161 described species of the monophyletic genus *Dalea* Lucanus (prairie-clovers) are distributed throughout much of the Americas, from southwestern Canada through Central America and the Caribbean, southward to Argentina. Their diversity is centered in Mexico (115 species) and North America (23 endemic species; Barneby 1977). Prairie-clovers are mostly perennial forbs or small shrubs bearing numerous flowering racemes or spikes that are densely crowded with small hermaphroditic flowers during May–July. Two species of prairie-clovers are widely distributed but infrequent in the sagebrush-steppe and pinyon-juniper woodlands of the western United States: *Dalea ornata* (Douglas ex Hook.) Eaton & J. Wright and *Dalea searlsiae* (A. Gray) Barneby. The northerly *D. ornata* occurs in the Columbia Plateau, Blue Mountains, Snake River Plain, and Northern Basin and Range ecoregions of the Intermountain region. The southerly range of *D. searlsiae* extends through the Central Basin

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and Range, Colorado Plateau, and Arizona/New Mexico Plateau ecoregions (Barneby 1977). Because of their potential to rehabilitate native plant communities of the Intermountain West as part of a native forb component for seed mixes (Cane 2008), these 2 *Dalea* species are being cultivated by private growers and by federal and state plant nurseries (Bhattarai et al. 2010, 2011). Similarly, a close relative, *Dalea purpurea* Vent., is extensively farmed to produce large quantities of seed for prairie restoration, conservation set-asides, and roadside beautification in the Midwest.

Farming seed crops often requires pollinator supplementation (Free 1993) and seed predator control. To evaluate pollination needs, seed growers must first understand the reproductive biology of a plant species. However, only one published study experimentally documents the reproductive biology of any *Dalea* species (Cane 2006) or other species of the tribe Amorpheae. The objectives of our study were to characterize the reproductive biologies of *D. ornata* and *D. searlsiae,* determine the identities and contribution of pollinators to seed set, and identify seed predators from wildland seed collections.

METHODS

Plant Source and Propagation

Plants were grown from seed collected from the wild. Seed for *D. searlsiae* came from Patterson Pass, Lincoln County, Nevada. Plants of *D. ornata* were grown by Clearwater Native Nursery from seed collected in the Deschutes Basin, Oregon. These 15–20 plants were transplanted with 1-m spacing into neighboring blocks of a common garden at the Pollinating Insect Research Unit (PIRU) in Logan, Utah, and grew for 1–3 years with periodic drip irrigation. The clay loam soil at the field site was augmented with compost to improve drainage.

Pollination

Five to 7 large individuals of each species, matched for size and vigor, were chosen and tagged. Individual pollinator exclusion bags were not used because the densely crowded floral spikes of *Dalea* transfer pollen by contact with the bag's mesh (Cane 2006). To exclude pollinators, each array of plants was enclosed in a walk-in field cage (7 × 7 × 2 m) made of Lumite screening (Synthetic Industries, Chicopee, GA). Three concurrently maturing floral spikes with flower buds were tagged on each plant. For *D. searlsiae,* each tagged plant's earliest spike was allowed to reach full bloom prior to caging in order to evaluate seed set resulting from unfettered bee visitation to flowers (an opportunity noticed too late to implement for *D. ornata* 2 years earlier). After caging, other spikes on the same plants were tagged for our other pollination treatments.

From late June to mid-July in 2006 (*D. ornata*) and 2008 (*D. searlsiae*), 3 manual pollination treatments were applied daily to open flowers of tagged spikes of the 5–7 caged plants: autogamy (unassisted autopollination), geitonogamy (transfer of self-pollen), and xenogamy (outcrossing). Each plant's 3 tagged spikes were assigned a different treatment. Geitonogamous pollination involved rubbing recipient virgin stigmas with anthers clipped from flowers on an untagged spike from the same plant. Donor flowers for xenogamy were taken from other untagged flowering spikes. For *D. ornata,* all flowers in a spike were treated. For the less-crowded floral spikes typical of *D. searlsiae,* a central 5-cm length of each spike was marked with thread to delimit flowers used in pollination treatments. The numbers of flowers produced were later recovered by counting the persistent floral calyces (Cane 2006). Optical visors were used to observe each tiny stigma and confirm its applied load of bright orange pollen.

Seed Production

Plants remained caged after flowering to guard against seed predators and herbivores. Once seeds were mature, but before they were shed, spikes were individually bagged, clipped, and returned to the laboratory. Although each flower has 2 ovules, only 1 seed per flower ever matures (Barneby 1977). After drying for >7 days, seeds and spent flowers were stripped from each spike. Each tagged spike's flowers were dissected for their complement of large, plump seeds. Unlike large, plump seeds, noticeably smaller or shrunken seeds rarely contained endosperm in *D. purpurea* (Cane 2006); this observation was confirmed with X-ray imagery for a subset of 50 seeds each of *D. ornata* and *D. searlsiae* (HP 4380N Faxitron, 25 KV, 30 s exposure, medium grain industrial film).

The 2 species and 3 pollination treatments were compared for their yields of large, plump seeds using general linear models (Littell et al.

1996). Data were analyzed by ANOVA (Proc GLM), with *Dalea* species and pollination treatment as fixed effects and plants as unreplicated random blocks. The residual sums of squares were weighted by the counts of flowers pollinated per spike. The seed production of each floral spike's treated flowers was divided by its flower production; this percentage was arcsine-transformed. The distribution of residuals was judged to be adequately normal. Treatments were compared by 2 orthogonal contrasts: (1) autogamy versus geitonogamy, and (2) autogamy plus geitonogamy versus xenogamy. For data available only for *D. searlsiae,* we conducted additional ANOVA tests to compare seed set between (1) freely visited and manually outcrossed spikes and (2) 2008 pollination results and results of 2007 preliminary experiments. Means are reported with their associated standard errors; degrees of freedom for test statistics are subscripted.

Pollinator Faunas

Bees were net-collected as they visited flowers of 2 populations of *D. searlsiae* in northwestern Utah (UTAH: *Box Elder Co.*—Pigeon Mountain and Rabbit Springs) and 3 populations of *D. ornata* (IDAHO: *Elmore Co.*—north of King Hill; OREGON: *Malheur Co.*—Succor Creek; WASHINGTON: *Benton Co.*—Benton City). All plants examined were counted and their visiting bees collected during a walking census. Additional collections were made at cultivated plots of both species growing in Cache County, Utah.

Seed Predators

The seed collections of *D. ornata* that we checked for seed predators originated from the following counties: IDAHO: *Canyon, Elmore* (2), and *Owyhee* (2) *Cos.;* OREGON: *Crook, Jefferson, Malheur* (2), *Sherman* (2), *Umatilla,* and *Wheeler* (2) *Cos.;* WASHINGTON: *Asotin, Benton, Franklin, Walla Walla,* and *Wallowa Cos.* (locality details in Bhattarai et al. 2010). Site elevations ranged from 110 m to 1300 m. Seeds were manually stripped from spikes in the field during July, bagged separately by site, and returned to Logan. Each sample was scrutinized for emergence holes in seeds; all live or dead beetles were recovered within 2 months of collection. Species of *Acanthoscelides* were identified by Clarence Johnson and Jesus Napoles; species of *Apion* were identified by JHC using the keys and descriptions provided by Kissinger (1968). Vouchers are deposited in the insect collection of Utah State University.

Results and Discussion

Plants of both *Dalea* species produced copious bloom in the common garden. Seventeen *D. ornata* plants had 117 spikes (SE = 13). Each spike possessed 151 flowers (SE = 12, n = 19). Across 2 years, spikes of *D. searlsiae* plants averaged 131 flowers (SE = 7, n = 62). Equivalent production of flowering spikes per young plant (110) and flowers per spike (182) was reported for *D. purpurea* in the same garden; those yielded an estimated 22,000 seeds annually per well-pollinated plant (Cane 2006).

Overall, percent seed set differed significantly among spikes ($F_{3,30} = 22.9$, $P < 0.0001$). Both *D. ornata* and *D. searlsiae* responded similarly to pollination treatments ($F_{1,30} = 0.8$, $P > 0.37$). Production of large, plump seeds varied significantly among pollination treatment groups ($F_{2,30} = 33.9$, $P < 0.0001$). Geitonogamy was equivalent to autogamy in average percent seed set for both species ($F_1 = 0.04$, $P > 0.8$; Fig. 1). Manual outcrossing (xenogamy) between plants quadrupled seed production compared to manual selfing of the maternal plant (geitonogamy) for both species, and manual outcrossing also yielded significantly more seed than the combination of autogamy and geitonogamy ($F_1 = 68$, $P < 0.0001$; Fig. 1). For *D. searlsiae,* the open-pollinated early spikes set as many seeds as did spikes on the same plants that were later caged and manually outcrossed ($F_{1,8} = 0.9$, $P > 0.37$; Fig. 1). Treatment differences were consistent across 2 years for *D. searlsiae* ($F_{2,58} = 51.6$, $P < 0.0001$), with no added year effect or year × treatment interaction ($P > 0.6$). The poor seed set resulting from geitonogamy can be interpreted as either limited self-compatibility or early acting inbreeding depression (Seavey and Bawa 1986).

Outcrossing yielded comparable gains in seed yield for another prairie-clover, *D. purpurea* (Cane 2006). In that study, flowers of outcrossed spikes yielded more than twice the seed of manually self-pollinated spikes, which in turn were 10-fold more productive than spikes in the autogamy treatment. For *D. ornata* and *D. searlsiae,* outcrossed spikes yielded 4 times the seed of selfed ones and 10-fold more

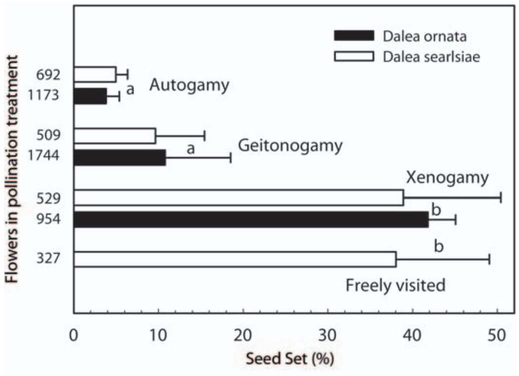

Fig. 1. Pollination treatments compared for the proportion of large seeds set per flowering spike for *Dalea ornata* and *Dalea searlsiae.* Treatment bars followed by different letters are statistically different from each other ($P \leq 0.05$); the 2 species responded similarly.

than autogamous spikes (Fig. 1). We conclude that seed production by all 3 *Dalea* species is limited by the supply of outcross pollen.

For flowering plant species that are primarily self-incompatible, much of their genetic variation is represented within populations. This was reported for *D. purpurea* using allozyme and RAPD markers (Gustafson et al. 2002). For AFLP markers isolated from geographic surveys of *Dalea* from the Intermountain region, much of each species' diversity (molecular variance) is apportioned also within populations, both for *D. ornata* (69%; Bhattarai et al. 2010) and *D. searlsiae* (76%; Bhattarai et al. 2011), consistent with a hypothesis for substantial gene flow (pollen flow, seed dispersal, or both). The seed of *D. ornata* growing in our common garden originated in the Deschutes River Valley of interior Oregon, home of a regional genetic grouping distinct from populations elsewhere in the species' geographic range (Bhattarai et al. 2010). Because the breeding biology of *D. ornata* from this genetically distinct population was similar to that of *D. purpurea* and *D. searlsiae* (primarily xenogamous), we expect that our results will be broadly representative for *D. ornata* growing in other regions too.

A few species of the Amorpheae have been surveyed for their native faunas of floral visitors. These species hosted diverse guilds of native bees, which are undoubtedly their primary pollinators (reviewed in Cane 2006). From 847 flowering plants of wild *D. ornata* and *D. searlsiae* surveyed at 6 sites, we collected 114 bees representing 22 species: ANDRENIDAE: *Andrena* sp.; APIDAE: *Bombus* (*bifarius, centralis, fervidus, griseocollis, huntii, morrisoni, nevadensis*), *Epeolus* sp., *Eucera* (*actuosa, edwardsii*), *Melissodes* sp., *Triepeolus* sp.; COLLETIDAE: *Colletes* (*Daleae* group sp. novo); HALICTIDAE: *Dialictus* sp., *Halictus* (*farinosus, ligatus*), *Lasioglossum* sp.; MEGACHILIDAE: *Anthidium atripes, Ashmeadiella* sp., *Hoplitis hypocrita, Megachile* sp. The prevalent bees were from the genera *Anthidium, Bombus, Colletes, Eucera,* and *Melissodes.* At 5 cultivated plots of *D. ornata* near Logan, Utah, and Ontario, Oregon, the sparse bee faunas consisted mostly of *Bombus, Dialictus,* and *Halictus tripartitus,* plus managed honey bees, *Megachile rotundata* and *Osmia bruneri*, when provided. These bees were also taken at flowering *D. purpurea* planted nearby (Cane 2006). In the extensive bee collections at PIRU, bees labeled from *Dalea* are generally sparse and not diverse, except for numerous individuals of the oligolectic bee *Colletes petalostemonis.* With so few sites sampled (and only the most northerly ones of *D. searlsiae*), we expect that, with more collection effort, more bee species will be found visiting these 2 *Dalea* species in the Intermountain West.

The beetles we found infesting wild-collected seed of *D. ornata* were *Acanthoscelides oregonensis* Johnson (Chrysomelidae: Bruchinae) and *Apion* (*Pseudapion*) *amaurum* Kissinger (Brentidae: Apioninae). Multiple adults—76 of *Acanthoscelides oregonensis,* 80 of *Apion amaurum*—were found infesting seed lots from 13 and 14 of the 23 sampled locations, respectively. Seed from 10 locations had mixed infestations; no beetles were found in 6 of the seed lots. The brentid *A. amaurum* is in Kissinger's *varicorne* group and represents a new seed host record and much greater geographic coverage within the Intermountain West, which was previously represented by only 3 specimens (Kissinger 1968). Seven other *Apion* species have been reported from *Dalea,* but none from either *D. ornata* or *D. searlsiae* (Kissinger 1968, 1988). C.D. Johnson first described *Acanthoscelides oregonensis* from a few specimens reared from *D. ornata* seed; he considered the species rare in collections (Johnson personal communication). As with *Apion amaurum,* each tiny larva develops within a single seed, and, as is the case for most other *Acanthoscelides,* larvae pupate in the seed, from which the adults later emerge (Johnson 1981).

The dependence of *Dalea* seed production on outcrossing has implications for rangeland restoration. If *Dalea* spp. are seeded sparsely as a minor component of a seed mix, resulting plants will likely be spaced too widely to be sequentially visited during the foraging trip of a given bee. Consequently, plants would be rarely outcrossed by pollinators, resulting in poor seed sets. Similar density-dependent outcomes have been shown for several other self-incompatible insect-pollinated plants (e.g., Knight 2003, Wolf and Harrison 2001). The problem of density-dependent seed set combined with limited *Dalea* seed for planting could be practically addressed by planting seed more thickly in scattered clumps. Bees should be more likely to visit multiple neighboring plants sequentially during their foraging rounds, ensuring more cross-pollination and seed set among the clumped plants. Such a seeding pattern can be accommodated by modern rangeland seed drills with little or no extra effort. This restoration seeding strategy would be applicable to any self-incompatible wildflower whose seed is expensive and/or scarce in commercial markets.

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