

BREEDING BIOLOGY AND BEE GUILD OF DOUGLAS' DUSTYMAIDEN, *CHAENACTIS DOUGLASII* (ASTERACEAE, HELENIEAE)

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ABSTRACT.—Douglas' dustymaiden, *Chaenactis douglasii* (Hook.) Hook. & Arn., is a widespread, inconspicuous, short-lived perennial wildflower that blooms in early summer and is found in basin sagesteppe to upper montane areas throughout the U.S. Intermountain West. The species is proving practical to grow for seed and is expected to be used for western rangeland rehabilitation. Through manual pollination experiments, *C. douglasii* was found to be only weakly self-fertile; 15% of flowers from geitonogamy and autogamy treatments yielded filled achenes. In contrast, 57% of out-crossed flowers produced achenes filled with endosperm, with every capitulum yielding some fertile seeds. Freely visited flowers from a wild population produced 91% fertile achenes, indicating that seed production was not pollinator limited. Floral visitors to *C. douglasii* were sparse, consisting entirely of bees, most of them floral generalists. Museum specimens of bees taken at *C. douglasii* and 3 closely related congeners comprise 175 species in 39 genera and all 6 North American bee families. A population of the manageable mesolectic cavity-nesting bee, *Osmia californica*, when released at one sagesteppe site, provisioned its cells primarily with pale, spiny pollen resembling that of the *C. douglasii* growing at the site. This bee and the honey bee appear most promising for pollinating dustymaiden that is being farmed for seed.

RESUMEN.—La doncella de Douglas, *Chaenactis douglasii* (Hook.) Hook. & Arn., es una flor silvestre ampliamente distribuida, no conspicua, de corta vida y perenne que florece a principios de verano desde las estepas de salvia hasta las áreas montañosas a lo largo del oeste de los Estados Unidos. Ha resultado práctico cultivarla por sus semillas y se espera que se las utilice para la rehabilitación de las tierras de pastoreo del oeste. Mediante experimentos manuales de polinización, se comprobó que esta especie es apenas auto-fértil; 15% de las flores provenientes de los tratamientos de geitonogamia y autogamia produjeron achenios cubiertos. En contraste, el 57% de las flores desarrolladas por fecundación cruzada produjeron achenios cubiertos con endospermo, con cada capítulo produciendo algunas semillas fértiles. Las flores de una población silvestre que fueron visitadas libremente produjeron un 91% de achenios fértiles, lo que indica que la producción de semillas en ese lugar no estuvo limitada por la polinización. Los visitantes florales de *C. douglasii* fueron escasos, y consistieron totalmente de abejas, la mayoría de las cuales fueron generalistas. Especímenes de abejas de museo colectadas en *C. douglasii* y tres congénéricas estrechamente relacionadas comprenden 175 especies en 39 géneros y las seis familias de abejas de América del Norte. Una población de abejas manejables que anidan en cavidades, *Osmia californica*, cuando fue liberada en un sitio de estepa de salvia, provisionó a sus celdas principalmente con polen pálido y espinoso que se asemejaba al de *C. douglasii* que crecía en el sitio. Esta abeja y la abeja de miel parecen ser las más propicias para polinizar las plantas doncellas que se cultivan para semillas.

The 18 species of *Chaenactis* compose the tribe Chaenactideae, a monophyletic clade of uncertain phylogenetic placement within the Helenieae (Baldwin et al. 2002). The flowers of all *Chaenactis* species are entirely discoid (lack petals) and bisexual. The genus is restricted to western North America, where species like *C. douglasii* are widespread, although often inconspicuous. Within western North America, the genus ranges from valleys and foothills to sub-alpine habitats. Douglas' dustymaiden blooms from early to midsummer, after most spring bloom in its plant communities has withered. It also readily establishes in disturbed sites

(Tilley et al. 2010). As a consequence of its ecological versatility, *C. douglasii* has promise for rangeland revegetation and restoration. Wildland seed production is erratic and prohibitively expensive to harvest, particularly from the typically sparse wild populations of *C. douglasii*. However, the species' upright stature, abundant bloom, and vigorous growth in cultivation make it suitable for row crop production (Tilley et al. 2010).

Farming seed crops often requires pollinator supplementation. To evaluate pollination needs, a plant species' breeding biology must first be understood, but there are no published

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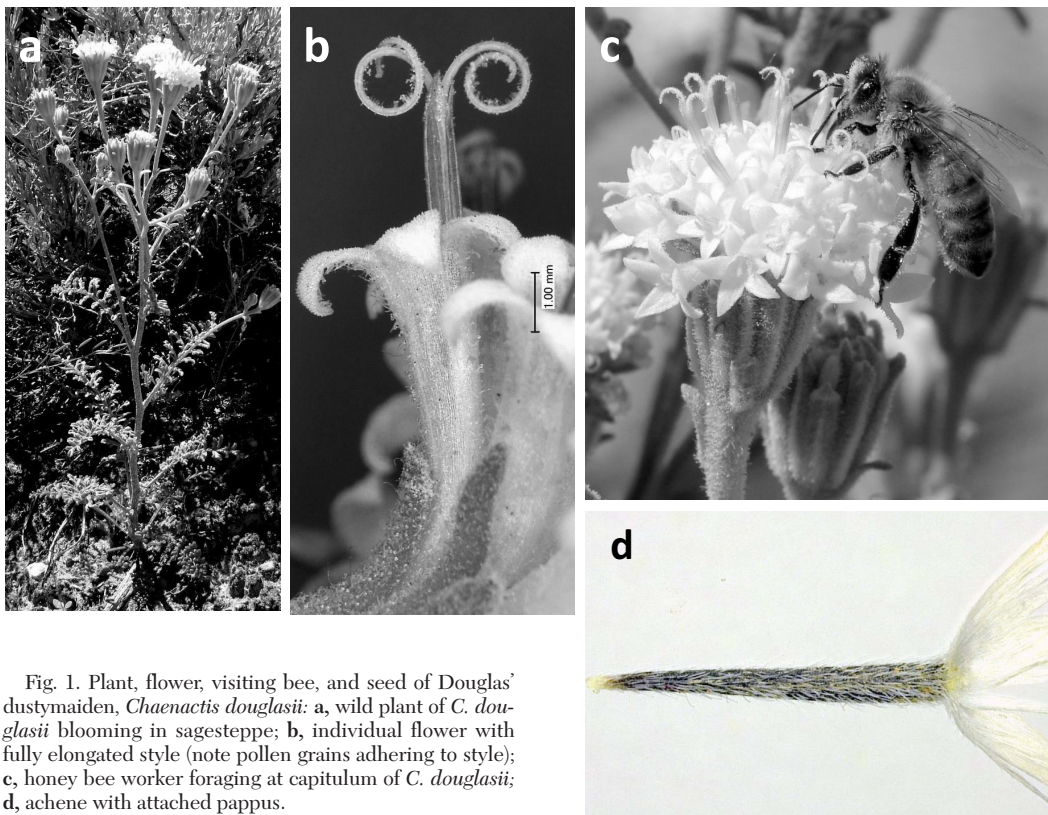


Fig. 1. Plant, flower, visiting bee, and seed of Douglas' dustymaiden, *Chaenactis douglasii*: a, wild plant of *C. douglasii* blooming in sagesteppe; b, individual flower with fully elongated style (note pollen grains adhering to style); c, honey bee worker foraging at capitulum of *C. douglasii*; d, achene with attached pappus.

accounts for any species in the tribe Chaenactideae. This study's 3 objectives with regard to *C. douglasii* were to (1) experimentally define, through manual pollinations, its breeding biology; (2) characterize its guild of floral visitors in terms of abundance, specialization, and diversity; and (3) propose pollinators that are practical to manage for its pollination on seed farms.

METHODS

Pollination

At the Pollinating Insect Research Unit (PIRU) in Logan, Utah, seed of *C. douglasii* was planted under the snow in March 2009, because cold, moist stratification promotes germination. To limit weed competition, seed was planted into holes spaced 30 cm apart in a weed barrier. The clay loam soil had been augmented with compost to improve drainage. The seed was sourced from a single population 22 km southwest of Murphy in Owyhee County, Idaho. Although the species contains diploid, tetraploid, and hexaploid elements,

populations typically consist of a single ploidy level (Mooring 1980).

To exclude pollinators, the array of plants was enclosed in a walk-in field cage ($7 \times 7 \times 2$ m) made of Lumite screening (Synthetic Industries, Chicopee, GA). Individual pollinator exclusion bags were not used because the dense capitulum (flowerhead; Fig. 1a), when rubbed against the bag's mesh, seemed prone to inadvertently transfer pollen between flowers (Fig 1b).

On each of 15 plants, 9–12 capitulae in the caged plot were tagged before any of their flowers had opened. Once bloom commenced, 6 of the capitulae on each plant (9 on one) were manually pollinated thrice weekly, with 3 of these 6 capitulae always receiving self pollen from a different capitulum on the same plant (geitonogamy) and the other 3 receiving pollen from a different plant (xenogamy or outcrossing). The 3 additional capitulae per plant remained untouched throughout bloom to test for autogamy (unassisted autopollination). On some robust plants, an additional set of capitulae was tagged to be used as replacements if

needed. Pollen transfer was effected by rubbing the furry thoracic dorsum of a dead honey bee against dehiscing anthers of an extra (untagged) capitulum and then against the floral styles of a recipient capitulum. The short dense pile of the thoracic dorsum is ideal for pollen pickup and delivery, and bees could be discarded after each use to avoid risks of cross-contamination that would accompany use of a brush. In all, 150 capitulae were treated between 12 July and 8 August 2010.

A sample of freely visited capitulae was taken on 12 July 2011 from a wild population found near Juniper, Box Elder County, Utah. Twenty mature capitulae were collected individually from as many plants, with care taken to harvest only those capitulae that had not yet shed any achenes (dry fruits that each contain a single seed). The indented sockets of 10 receptacles were later counted and compared with achene counts to confirm retention. In the lab, achenes were removed, counted, and x-rayed to judge their endosperm fill.

Seed Production

Once achenes were mature, but before they were shed, seed heads were individually bagged, clipped, and air-dried indoors for 2 weeks. Visibly plump achenes and shrunken achenes (Fig. 1d) were sorted and tallied for each seed head. Endosperm content was visualized by digital X-rays (Faxitron MX-20, 25 KV, 30 s). To assess our visual sorting of achenes, a sample of x-rayed achenes was also sliced open to check for a seed filled with endosperm.

The 3 pollination treatments were compared both for the median numbers of flowers per capitulum treated and for the median percentage of filled achenes produced, both by Kruskal–Wallis tests. When the overall test was significant, treatments were compared in a pairwise manner using Dunn's method.

Pollinator Fauna

Floral visitors were collected from 3 populations of *C. douglasii* growing in sagesteppe habitats of northern Nevada, southern Idaho, and northwestern Utah. Flowering individuals were examined haphazardly over several hectares and counted, and their visitors were collected, counted, and later identified. In addition, floral visitors were collected at a 2-year-old, 0.12-ha seed-increase plot of *C. douglasii* grown at the NRCS Plant Materials Center

outside Aberdeen, Idaho. At this site, one 48-hole drilled wooden nesting block with paper-straw inserts was placed in a shelter at the field edge and populated with overwintered nests of the bee *Osmia californica* Cresson (Megachilidae). At a wildland population of *C. douglasii* amid sparse junipers near Snowville, Utah, overwintered nests of *O. californica* and prepared drilled wooden nesting blocks were similarly deployed. This solitary bee is a broad pollen specialist (mesolege) for the Asteraceae and is a known pollinator of *Balsamorhiza* (Asteraceae) (Cane 2005, 2011).

RESULTS AND DISCUSSION

First-year plants of *C. douglasii* grown in the common garden produced capitulae with an average of 46.8 (SD = 12.5) disk flowers (range 19–85 flowers per capitulum; $n = 300$ capitulae). A given capitulum produced new flowers for 7–9 (up to 12) days. Capitulae from the 3 pollination treatments had equivalent numbers of flowers ($H = 2.1$, $P = 0.35$). Hence, we avoided systematic size bias in assigning capitulae to the different pollination treatments.

Endosperm fill of achenes was visually scored from digital X-radiographs. Of 42 achenes dissected, the 32 achenes found to contain endosperm matched exactly the X-ray images that we judged to show endosperm (pale fill in the negatives). Achenes that lacked endosperm were thin-walled, flimsy, and usually pale in color; and, in the X-rays, their contents were uniformly dark in the negative images. Guided by the X-ray differences, we could with confidence visually sort filled achenes from unfilled achenes (Fig. 1d).

The pollination treatments differed in the fraction of achenes produced that were filled with endosperm ($H = 66.7$, $P < 0.001$). Fully 97% of the 7013 flowers set an achene. Like other Asteraceae, pollen presentation is secondary. As the bifurcate styles elongate, their tips curl full circle, facilitating autopolination (Fig. 1b). However, flowers that were cross-pollinated with pollen from a different individual produced 4-fold more filled achenes ($Q > 7$, $P < 0.05$) than those that were selfed or left to autopolinate (57% vs. 15% of achenes per capitulum filled with endosperm) (Fig. 2). Individual plants that have newly colonized a site apparently can set some viable achenes, even in the absence of pollinators. However,

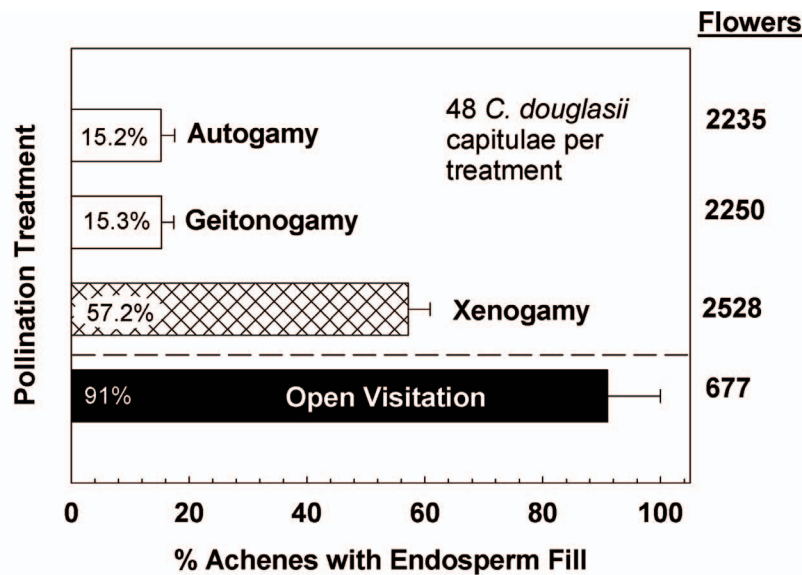


Fig. 2. Comparison of average proportions of filled achenes produced per capitulum (flowerhead) among three pollination treatments plus openly pollinated flowers of *Chaenactis douglasii*. Xenogamy was statistically superior to autogamy or geitonogamy in viable achene set ($P < 0.05$). The freely visited flowers came from a wild population and were not part of the experiment. Means and 95% confidence intervals (whisker bars) are presented. Numbers of flowers for the 15 plants and 150 capitulae are given in the column to the right of the graph used.

plants will be far more successful reproductively if they are cross-pollinated by a pollinator.

Only bees were found visiting flowers of *C. douglasii*, both in the wild and under cultivation. The bee taxa that we collected by net at *C. douglasii* were as follows: ANDRENIDAE: *Andrena* spp. APIDAE: *Bombus huntii* Greene; *Ceratina* (*Zadontomerus*) *nanula* Ckll., *C. neomexicana* Ckll., and *C. pacifica* Smith. HALICTIDAE: *Agapostemon angelicus/texanus* Ckll. and *A. femoratus* Crawford; *Dialictus* spp.; *Halictus ligatus* Say and *H. tripartitus* Ckll.; *Lasioglossum* spp. MEGACHILIDAE: *Megachile* spp.; *Osmia* (including the *Cephalosmia* species *O. californica*, *O. marginipennis* Cresson, and *O. montana* Cresson). Visitors were rather sparse, averaging one bee per 14 plants surveyed. At the cultivated plot of *C. douglasii* near Aberdeen, Idaho, floral visitors were also all bees, mostly honey bees (Fig. 1c) from nearby hives, plus *H. ligatus* and *Micranthophora flexipes* (Cresson) that were found nesting amid the rows.

The guild of bee species that we sampled should mostly persist after fire. Only the 3 species each of *Osmia* and *Ceratina* are known cavity nesters; the other bees nest underground. Nest cells of species in these ground-

nesting genera lie deeper than the penetration of lethal heat that is expected from fire in sagesteppe habitats (Cane and Neff 2011). Even the twig-nesting *Ceratina* and wood-nesting *Osmia* might soon recolonize after fire. For example, we collected several of the small-bodied *Ceratina* visiting *C. douglasii* 3 km inside the fire line of the Hepworth fire, which had burned 3 years earlier. This persistence after fire has relevance for sexual reproduction of *C. douglasii*, whether the plants survive the heat of burning or seedlings establish after the fire. In both cases, bloom can be expected from survivors the year following fire. The species will greatly benefit from cross-pollination provided by the surviving guild of ground-nesting generalist bees, as well as from the minority of cavity-nesters that may colonize in subsequent years.

Given our limited collections, a surprisingly rich bee fauna labeled as having been collected from *Chaenactis* was found in the extensive bee collections at PIRU. These bees were taken mostly during intensive bee community surveys in California and southern Utah at *C. douglasii*, *C. fremontii* A. Gray, *C. stevioides* Hook. & Arn., and *C. glabriuscula* DC. The latter 3 species are closely related

through aneuploidy (Kyhos 1965). These species of *Chaenactis* were visited for pollen and nectar primarily by nonsocial univoltine native bees, which are undoubtedly these species' primary pollinators. The 1167 bee specimens represent 175 native species from 39 genera and all 6 families of North American bees. Half of the individuals are species of *Anthophora* (162 bees), *Lasioglossum* (118), *Osmia* (102), and *Perdita* (236, three-fourths were *P. fallugiae*). Four genera contributed one-third of the bee species at *Chaenactis*: *Megachile* (15 species), *Lasioglossum* (16), *Osmia* (24), and *Perdita* (13). Only 40 of the bee specimens were kleptoparasites. Most all of the 175 bee species from *Chaenactis* are floral generalists, but a few are restricted to the Asteraceae for pollen (e.g., *O. californica*, *O. montana*, *Dufourea malacothricis* Timberlake). The only candidate oligolege (pollen specialist) for *Chaenactis* is the bee *Micralictoides chaenactidis* Bohart and Griswold, which has been reported only from flowers of *Chaenactis* and *Eriophyllum* (Bohart and Griswold 1987).

The lack of specialist bees associated with *Chaenactis* contrasts with guilds of bees at flowers of Asteraceae in other biomes of temperate zones, which often contain abundant and diverse oligolectic bees (Hurd et al. 1980, Neff and Simpson 1997, Müller and Kuhlmann 2008). The Great Basin sagesteppe receives most of its soil moisture from winter precipitation—a pattern that likely disfavors most other bee-pollinated Asteraceae that would bloom in the late spring and summer when *Chaenactis* flowers. By way of contrast, the Great Plains receives periodic summer rains that foster numerous summer-flowering composites (Anderson and Schelfhout 1980) that host diverse oligolectic bees (Marlin and LaBerge 2001).

From the nesting blocks and released population of *O. californica* placed in sagesteppe habitat where *Chaenactis* was part of the flora, most (24) of the 32 uneaten provision masses sampled from as many nests consisted entirely of pale composite pollen closely resembling that of *Chaenactis*; 3 others were mixed and 5 consisted of orange asteraceous pollen. Paradoxically, very few progeny resulted from an identical nesting block, also with *O. californica*, placed with the plot of *C. douglasii* grown at the Aberdeen Plant Materials Center in Idaho. This *Osmia* bee is a pollen specialist on the Asteraceae. It also is known to pollinate

and provision with pollen from earlier-flowering *Balsamorhiza* and cultivated *Eriophyllum lanatum* (Pursh) Forbes. For 2 years, we have used this bee to pollinate a seed-increase plot of *E. lanatum* at the NRCS–Plant Materials Center farm in Corvallis, Oregon, where the managed population of *O. californica* has prospered as well. This bee or the honey bee (Fig. 1c) will be the most practical, manageable bees to pollinate seed crops of *C. douglasii*.

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