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# Importance of Outcrossing for Fruit Production in Slickspot Peppergrass, *Lepidium Papilliferum* L. (Brassicaceae)

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## IMPORTANCE OF OUTCROSSING FOR FRUIT PRODUCTION IN SLICKSPOT PEPPERGRASS, *LEPIDIUM PAPILLIFERUM* L. (BRASSICACEAE)

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*Key words:* slickspot peppergrass, *Lepidium papilliferum*, outcrossing, self-pollination.

Plants with insect-mediated pollination are often assumed to be obligate outcrossers; i.e., pollen must be supplied from flowers of other individuals for pollination and subsequent fruit production. Indeed, many flowers with insect-mediated pollination exhibit incompatibility to their own pollen or have a separation in time between pollen production and maturation of the stigma on a given flower (Proctor et al. 1996). However, because the breeding systems of plants are diverse and include varying levels of outcrossing and selfing, experiments are required to determine whether pollination in a particular species occurs via outcrossing, self-pollination, or both. Here I report the results of such a study on slickspot peppergrass, *Lepidium papilliferum* L. (Brassicaceae), a rare mustard endemic to sagebrush-steppe habitat in southwestern Idaho.

Within sagebrush areas, *L. papilliferum* is restricted to microsites known as “slick spots,” which are characterized by their high levels of clay or salt and higher soil water retention than surrounding areas (Meyer 1995). Currently, there are approximately 60 known sites in Idaho with slick spots that contain *L. papilliferum* populations (Moseley 1994, U.S. Fish and Wildlife Service 2002). General degradation of sagebrush-steppe habitat from sources such as wildfire, livestock grazing, irrigated agriculture, exotic species invasions, and urban development has contributed to the plant’s rapid decline and increasingly fragmented distribution over the past century (Moseley 1994). In July 2002, *L. papilliferum* was proposed for listing as an endangered species by the United States Fish and Wildlife Service. Uncertainty surrounding long-term viability of *L. papilliferum* makes it imperative that biologists gain

greater understanding of this species’ life history, including its breeding system, so that sound management decisions can be made.

Flowering in *L. papilliferum* extends from early May to mid-July. Reaching 5–40 cm in height, the plant has numerous, multi-flowered inflorescences that terminate at the branches. The small flowers have white petals, and filaments of the anthers are covered with club-shaped hairs. By the end of the flowering period, large amounts of orbicular, flattened seed about 3 mm in length are produced. Insects, including species from several families of Hymenoptera (Apidae, Halictidae, Colletidae, Vespidae, Sphecidae), Coleoptera (Melyridae, Dermestidae), and Diptera (Syrphidae), frequent *L. papilliferum* flowers and are necessary for pollination and fruit production (Robertson 2002, Robertson and Klemash 2003). However, it is not clear whether the plant relies on outcrossed pollination, self-pollination, or both. Making this distinction for *L. papilliferum* is important from a conservation perspective because continued fragmentation of the plant’s habitat will likely reduce the plant’s opportunity for outcrossing and in the process lead to a loss of genetic diversity within populations.

I conducted an experiment to determine the breeding system of *L. papilliferum* from early May to mid-July 2002 at 3 sites in southwestern Idaho. The sites, which are being used as part of a longer-term study on the pollination biology of *L. papilliferum*, are separated from one another by at least 20 km that includes areas of irrigated agriculture and rangeland.

At the onset of the experiment, 48 similarly sized plants with unopened (i.e., virgin) flowers were enclosed individually within cylindrical,

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insect-proof cages (5–15 cm diameter, 10–20 cm height) made from 10-mm hardware cloth covered with fine bridal veil (0.25-mm mesh). Cages were fixed securely to the substrate with small pegs to minimize the possibility that insects could enter at the base. A previous study (Robertson and Klemash 2003) showed that cages do not reduce plant survival or inhibit fruit production.

The caged plants were divided randomly and in equal number into 1 of 3 groups: control, self-pollination treatment, and cross-pollination treatment. All 3 groups were represented at each of the 3 sites. Experimental manipulation began once a plant was in full bloom. For the self-pollination treatment, I snipped a small inflorescence of opened flowers from the plant and then brushed it gently over other opened flowers on the same plant. The cross-pollination experiment was similar in technique except that the inflorescence used for brush pollination came from a different plant growing at least 2 m away. As a sham operation, control and cross-pollination plants had a small inflorescence snipped off and discarded. The manipulations were repeated within a week. In early to mid-July, once plants had ceased flowering, I determined percent fruit set for each plant by collecting 1–3 inflorescences and counting the number of wilting flower pedicels (i.e., unpollinated flowers) and seed-bearing fruits.

Two plants in the cross-pollination treatment and 3 control plants wilted and died before the end of the experiment. This frequency of die-off is typical for *L. papilliferum* in the field (Robertson unpublished data). Nonparametric statistics were used in the analysis of fruit production because percent fruit sets within the control and self-pollination experiments were not normally distributed. Median percent fruit set in control plants and self-pollination plants was 0% and 5%, respectively. In contrast, median percent fruit set in cross-pollinated plants was 40%. The overall difference in percent fruit set between groups was statistically significant and caused by the higher percent fruit set in the cross-pollination treatment (Fig. 1; Kruskal-Wallis test,  $H = 17.3$ ,  $df = 2$ ,  $P < 0.001$ ); there was no significant difference in percent fruit set between the control and self-pollination treatments (Mann-Whitney U test,  $Z = 1.3$ ,  $P = 0.2$ ). Although median percent fruit set was low in both the control and

self-pollination treatments, several individuals in both groups did produce fruits. Distributions of percent fruit set in the control and self-pollination treatments were highly skewed (Fig. 2).

Insect visitations are crucial for pollination and fruit production in *L. papilliferum* (Robertson and Klemash 2003). A wide variety of insects are commonly observed flying from plant to plant both within and between slick spots at a site, contacting many flowers in the process (Robertson and Klemash 2003). Although the rate and distribution of pollen dispersal among flowers and between populations is not known for *L. papilliferum*, it is clear from the present study that most, if not all, fruit production is achieved via outcrossing.

The presence of mature fruit on some plants in the self-pollination treatment suggests either that self-pollination is possible in *L. papilliferum*, at least to a small extent, or that insects found their way into a few cages and cross-pollinated some of the flowers. The latter explanation seems more likely given that 25% of the plants in the treatment produced no fruit, more than half of the plants produced less than 5% fruit, and none had fruit sets as high as the median for the cross-pollination treatment. If *L. papilliferum* had a general ability to self-pollinate, a higher and more-or-less normal distribution of percent fruit sets would be expected rather than the low and highly skewed distribution that was observed. Nevertheless, it is worth noting that the genus *Lepidium* includes a number of species known to be self-compatible; however, these species, unlike *L. papilliferum*, tend to have reduced floral structures (J.L. Bowman, University of California, Davis, personal communication).

The production of small fruit sets in less than half of the control plants may again suggest insect contamination within cages as the cause. The possibility of wind-mediated self- or cross-pollination is remote given that the structures of *L. papilliferum* flowers and pollen grains are not consistent with those of anemophilous species, which generally produce copious amounts of smooth-surfaced pollen and have an exposed stigma and long stamens with exposed anthers (Proctor et al. 1996). Reproductive structures of *L. papilliferum* are relatively protected within the flower's corolla, and pollen production in this species could not be described as copious (Robertson personal observation).

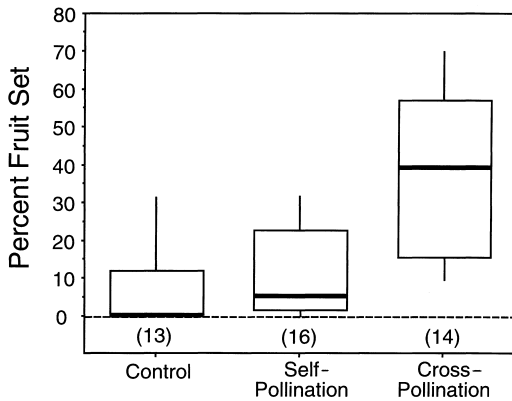


Fig. 1. Box plot chart showing results of the hand-pollination experiment. Top, middle, and bottom horizontal lines of a box show the 75th, 50th (median in bold), and 25th percentiles, respectively. Vertical lines extend from the 10th to the 90th percentiles. Sample sizes are given in parentheses below the dashed line.

The apparent reliance of *L. papilliferum* on insect-mediated cross-pollination has implications for management and conservation of the species. Over the past century populations of the plant have become increasingly fragmented due to both human activity and altered habitat resulting from wildfire and exotic species invasions (Moseley 1994). A population of *L. papilliferum* that becomes isolated from other populations risks losing genetic diversity that is normally maintained via outcrossing. Adequate levels of genetic diversity and gene flow are generally viewed as critical to the survival of threatened or endangered species because they help to ensure individual fitness through a maintenance of heterozygosity, and they provide for the long-term viability of a species in the face of environmental change (Haig 1998). For a species that relies on outcrossing, loss of genetic diversity and gene flow may lead to reductions in pollination success and even infertility. Thus, management efforts for *L. papilliferum* should consider the effects of land use on the movement of insects between populations. Unfortunately, documenting long-distance insect movement and pollen transfer has proven difficult in pollination studies (Proctor et al. 1996). An alternative approach for *L. papilliferum* would be to examine the current amount of genetic variability within and between populations and to relate this information to population size, both current and his-

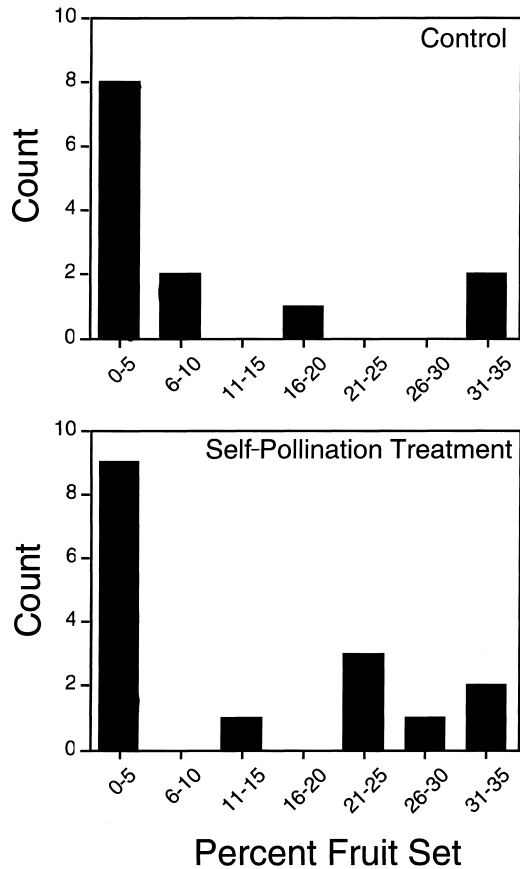


Fig. 2. Frequency distribution of percent fruit set in the control and self-pollination treatment.

torical, and distance to the nearest population. One could also examine the effects of genetic variability within populations on individual pollination success, fruit production, and offspring vigor. Thus, understanding the population genetics of *L. papilliferum* may provide valuable insight into the effects of habitat fragmentation on outcrossing and gene flow between populations.

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