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# Conservation and reintroduction of the endangered Willamette daisy

EFFECTS OF POPULATION SIZE ON SEED VIABILITY  
AND THE INFLUENCE OF LOCAL ADAPTATION

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## ABSTRACT

Our research suggests that habitat fragmentation and reduced population size in Willamette daisy has led to loss of fitness in some populations and to population differentiation. In outcrossing species, habitat fragmentation and reduced population size can lead to increased genetic drift, local adaptation, and (or) inbreeding. We explored some of these issues for the endangered forb Willamette daisy (*Erigeron decumbens* Nutt. [Asteraceae]). This species is currently restricted to approximately 40 sites in the Willamette Valley, Oregon; more than half of the known populations have fewer than 100 individuals. We found a positive relationship between seed viability (as measured by the percentage of filled seeds) and population size. In populations with 20 or fewer individuals, seed viability dropped to less than 2.5%. In modified reciprocal transplant and common-garden experiments, we found that although there were some differences in survival, growth, and reproduction in transplants from 2 source populations, the effect of source varied depending on response variable, year of planting, year of monitoring, and habitat. Conservation of Willamette daisy will benefit from increasing the size and genetic diversity of existing populations and from reintroducing genetically diverse populations within the historic range of the species. Knowledge of genetic diversity of populations can be critical for making appropriate management decisions for rare species, including determining if population augmentation is necessary and, if so, from which source populations.

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## KEY WORDS

reintroduction, Willamette Valley, prairie, endangered species, allee effect, *Erigeron decumbens*, Asteraceae

## NOMENCLATURE

Plants: USDA NRCS (2011)

Insects: ITIS (2011)

Small population size and habitat fragmentation can have a number of negative effects on populations (Saunders and others 1991). Knowledge of genetic diversity of populations can be critical for management decisions, particularly when determining if population augmentation is necessary and, if so, from which source populations.

In outcrossing plant species, reduced gene flow due to habitat fragmentation may decrease plant fitness through increased inbreeding and (or) genetic drift (Ellstrand and Elam 1993; Husband and Schemske 1996). Small, isolated populations are also at greater risk of pollen limitation owing to reduced pollinator efficacy (Agren 1996; Lennartsson 2002). Population size is positively correlated with seed set and progeny fitness in a number of plant species (Menges 1991; Morgan 1999; Kéry and others 2000). For example, Morgan (1999) found that in 2 of 3 study years, small populations (< 30 plants) of the endangered forb button wrinklewort (*Rutidosia leptorrhynchoides* F. Muell. [Asteraceae]) produced significantly fewer seeds per capitulum than were produced in large populations (> 500 plants).

Loss of gene flow between populations may also result in genetic differentiation between populations through local adaptation and random genetic drift (Linhart and Grant 1996). For example, firecracker penstemon (*Penstemon eatonii* A. Gray [Scrophulariaceae]) seeds from high-elevation populations required longer chilling for seed germination than did seeds from low-elevation populations (Meyer and others 1995). If population differentiation is adaptive, the success of reintroductions will be greater if seed is from sites with similar ecological conditions (Hufford and Mazer 2003). (Throughout this article, “reintroduction” will be used to include the introduction of plants to areas both with and without historical populations [Kaye 2006].)

Willamette daisy (*Erigeron decumbens* Nutt. [Asteraceae]) is listed by the US Fish and Wildlife Service (USFWS) and Oregon Department of Agriculture as an endangered species (OR-BIC 2010) and considered a Special Status Species by the USDI Bureau of Land Management. This species is endemic to prairies in the Willamette Valley in western Oregon. Widespread loss of native prairie habitat to agricultural and urban development is the primary threat to Willamette daisy (USFWS 2010). Although historically found throughout the Willamette Valley, it is now currently found in approximately 40 sites in 4 counties (USFWS 2010). The species has been extirpated from the 3 most northern counties in which it historically occurred. There is little climatic or ecological variation between the remaining occurrences and those in which it has been extirpated. It is likely that the loss of this species in the northern extent of its range is due to the much higher levels of habitat loss and the agricultural and urban development in these counties. The species also faces encroachment of prairie habitat by trees, shrubs, and invasive weeds, and possibly inbreeding depression arising from small population sizes.

Willamette daisy is an herbaceous perennial that primarily reproduces by seeds. Plants form clumps of basal leaves and produce one or more flowering stems. Although vegetative spread is possible, it is localized (< 10 cm [4 in]) around the parental plant. Willamette daisy attracts numerous insect species that are thought to act as pollinators, including field crescent (*Phyciodes pulchella pulchella* Boisduval [Lepidoptera: Nymphalidae] synonym = *P. campestris*), sweat bees [Hymenoptera: Halictidae], and a syrphid fly (*Toxomerus occidentalis* Curran [Diptera: Syrphidae]) (Jackson 1996). Seed dispersal has been estimated at an average distance of 94 cm (37 in) (Jackson 1996). Population size may fluctuate substantially between years (Thorpe and Kaye 2007); however, a putative decline in population size may be attributable in part to the difficulty in counting the inconspicuous vegetative plants (Clark and others 1993).

Reproductive failure in small populations of Willamette daisy is a serious concern for the conservation of this species. We have observed that very small populations ( $\leq 5$  individuals) lack seed production and in a study of several sites, Clark and others (1995) found that < 20% of achenes were filled with embryos. Although the breeding system of Willamette daisy is poorly documented, several closely related species are self-incompatible (Allphin and others 2002; Hiscock and Tabah 2003). As greater than half the known remaining populations contain < 100 individuals (Thompson 2010), understanding the relationship between population size and reproductive success is extremely important for informing management decisions.

An additional challenge in restoration of Willamette daisy populations is the potential for adaptive population differentiation. This species occurs in prairie habitats ranging from wet grassland prairies to well-drained soils in upland prairies (Kagan and Yamamoto 1987; Clark and others 1993), increasing the chance that populations may exhibit local adaptation to soil and hydrologic conditions (that is, ecotypic differentiation). If the species exhibits ecotypic differentiation, reintroductions might need to consider the habitat type of the source and the recipient populations during recovery actions. Currently, the guidelines for recovery are specific only to movement of genotypes within designated Recovery Zones (USFWS 2010).

Here, we report on 3 studies to explore the genetic characteristics of Willamette daisy that may affect conservation and restoration of this species. In the first study, we assessed the relationship between population size and seed viability in a representative sample of remaining Willamette daisy populations. We used the percentage of filled seeds as a measure of seed viability. Because of the extreme rarity of this species, we could not test for viability using destructive sampling methods (for example, tetrazolium and germination tests). In the second study, we used a common-garden approach to compare the success of outplanted individuals from 2 different ecotypes at

one site. Finally, we conducted a variation of a reciprocal transplant experiment to test for ecotypic variation. This type of information is critical to land managers engaged in recovery actions for this species, as outlined in the *Recovery plan for prairie species of southwestern Washington and western Oregon* (USFWS 2010).

## METHODS

All research and seed collection activities were conducted under permit from US Fish and Wildlife Service and Oregon Department of Agriculture.

### Seed Viability

In 2005, we identified all known populations of Willamette daisy and selected 18 populations for sampling. Populations were selected from throughout the range of the species (Table 1) and population sizes varied from less than 10 to more than 4000 individuals (Table 2). Two additional populations were visited in 2006. For each population, we determined the number of flowering individuals, collected mature capitula (flower heads), and determined the percentage of achenes (single-seeded fruits, hereafter referred to as seeds) filled with an embryo. Population size information for Muddy Creek, Balboa, Fisher Butte, Greenhill Ash Grove, Oxbow West, Vinci, and Willow Creek was obtained from existing monitoring programs (Messinger 2005; Nuckols 2005; Villegas 2005; Thorpe and Kaye 2006). In 2005, we counted the number of flowering plants at the remainder of the sites. Similar methods were used to obtain flowering plant numbers in 2006 at all sites except East Coyote, Balboa, Greenhill Ash Grove, Vinci, and Fisher Butte. For these sites, the population size in 2005 was used to determine the relationship between population size and filled seeds. Although vegetative spread is possible in this species, it is unlikely to have significantly affected our population size estimates. Most field investigators assume that plants separated by less than 7 cm (3 in) are the same individual (USFWS 2010) and connections between plants are frequently visible. In both 2005 and 2006, at sites with more than 30 individuals, we used a randomization method to locate and flag 30 to 40 individuals for seed collection. In populations with fewer than 30 individuals, we flagged all plants for seed collection.

Each population was visited several times in July to collect mature capitula. One or 2 capitula that had finished flowering but not yet dehisced their seeds were collected from each flagged plant. In populations with more than 30 flowering plants, one capitulum from 30 plants was examined. In populations with fewer than 30 flowering plants, we examined capitula from all flowering individuals in the population (with multiple capitula from some plants). For each sampled capitulum, we determined the numbers of filled and empty ovules by observing the seeds through a dissecting microscope. In populations with

fewer than 30 individuals, average seed set per individual was calculated based on all capitula sampled for each plant.

For each year, we used a linear regression (SPSS 17.0 [2008], IBM Corporation, Armonk, New York) to test for the effect of population size on percent filled seeds. Population size data were log transformed to meet assumptions of normality.

### Common-Garden Experiment

We conducted a common-garden experiment at Bald Hill Natural Area, managed by the City of Corvallis. Located in the Corvallis West Recovery Zone (USFWS 2010), this site contains a mosaic of upland prairie and oak savanna habitats. Historically, at least 400 individuals were located across 3 distinct patches. By 2006, the natural population at Bald Hill had declined to 32 individuals, with only 1.1% filled seeds. This site was selected due to the presence of a relatively high-quality prairie and the desire to augment the existing population (historical populations occurred 0.5 to 2 km [0.3 to 1.2 mi] from the planting area).

TABLE 1

*Ownership and county of sampled Willamette daisy populations.*

Site	Ownership	County
Bald Hill North Prairie	City of Corvallis	Benton
Mill Creek	Oregon Department of Transportation	Polk
Bald Hill South	City of Corvallis	Benton
Bald Hill North Trail	Greenbelt Land Trust	Benton
Baskett Butte Area 4	US Fish and Wildlife Service	Polk
Baskett Butte Area 10	US Fish and Wildlife Service	Polk
East Coyote	Army Corps of Engineers	Lane
Baskett Butte Area 3	US Fish and Wildlife Service	Polk
Kingston Prairie East	The Nature Conservancy	Lane
Sublimity Grasslands	Private	Marion
Bailey Hill <sup>2</sup>	The Nature Conservancy	Lane
Balboa	Bureau of Land Management	Lane
Greenhill Ash Grove	Bureau of Land Management	Lane
Lanel Substation	Railroad	Lane
Allen and Allen Farm	Private	Benton
Vinci	Bureau of Land Management	Lane
Holley <sup>2</sup>	Private	Lane
Willow Creek	The Nature Conservancy	Lane
Oxbow West	Bureau of Land Management	Lane
Fisher Butte	Army Corps of Engineers	Lane

<sup>2</sup>Populations included only in 2006.

TABLE 2

Percentage of filled seeds and population sizes for sampled Willamette daisy populations.

Site	2005		2006	
	Population size	Filled seeds (%)	Population size	Filled seeds (%)
Bald Hill North Prairie	3	0.00	7	2.14
Mill Creek	7	0.33	14	0.10
Bald Hill South	10	1.22	13	0.67
Bald Hill North Trail	12	1.28	12	1.11
Baskett Butte Area 4	20	0.08	75	1.32
Baskett Butte Area 10	27	9.74	43	13.19
East Coyote <sup>y</sup>	36	5.14	—	1.47
Baskett Butte Area 3	52	7.13	141	11.19
Kingston Prairie East	52	9.46	100	19.25
Sublimity Grasslands	58	23.55	55	35.86
Bailey Hill <sup>z</sup>	—	—	78	5.01
Balboa <sup>y</sup>	80	1.82	—	8.90
Greenhill Ash Grove <sup>y</sup>	86	5.57	—	14.96
Lanel Substation	89	10.47	89	6.41
Muddy Creek	131	20.23	135	10.34
Vinci <sup>y</sup>	526	9.87	—	10.16
Holley <sup>z</sup>	—	—	551	7.99
Willow Creek	597	9.51	846	19.61
Oxbow West	1480	4.85	3092	20.79
Fisher Butte	4631	11.40	—	38.75

<sup>z</sup>Populations included only in 2006.

<sup>y</sup>Population size estimates were unavailable in 2006; 2005 estimates were used in analyses.

Three seed sources (populations) were selected for reintroduction at Bald Hill. Muddy Creek is the largest population located in the Corvallis West Recovery Zone; approximately 130 flowering plants were observed in 2005 and 2006 (Table 1). At this site, the Willamette daisy population is located in wet prairie habitat. Baskett Butte, located in the Salem West Recovery Zone, had approximately 100 flowering plants in 2005 and nearly 250 in 2006 distributed in 3 patches in upland prairie habitat. The third source was the remnant Bald Hill population. In 2007, we planted 306 plants from Muddy Creek and 294 from Baskett Butte. We did not plant any Bald Hill plants in 2007 due to poor germination and survival in the greenhouse. In 2008, we planted 236 plants from Muddy Creek, 268 from Baskett Butte, and 33 from Bald Hill.

Similar grow-out procedures were used in 2007 and 2008.

The November prior to outplanting, we placed 1000 seeds each from Baskett Butte and Muddy Creek on moistened blotting paper in germination boxes. Seeds were previously collected from multiple individuals throughout each population in order to maximize the number of individuals sampled while collecting fewer than 25% of the mature capitula. Seeds from Baskett Butte and Muddy Creek were put into grow-out at the USDA Plant Materials Center in Corvallis, Oregon. All seeds from these populations were F<sub>1</sub> generation, thus minimizing selection during production. In 2008, we also used all available seeds from Bald Hill, which had been collected from the remaining individuals at Bald Hill as described in the seed viability study (above). Seeds were stratified at 4 °C (39 °F) in continuous dark for 16 wk to break dormancy (Kaye and Kuykendall 2001). Boxes were checked weekly and misted with deionized water to maintain moist conditions or with dilute hydrogen peroxide if mold was observed.

After 16 wk, boxes were placed in a room with a 25 °C day/15 °C night (77 °F/59 °F) temperature cycle and 8 h day/16 h night lighting cycle. After 1 wk in the warm, seedlings were planted in 5.08 cm × 5.08 cm × 12.7 cm (2 in × 2 in × 5 in) pots filled with Gardner's Gold potting soil that had been topped with approximately 1 cm (0.4 in) of EB Stone Seedling Starter soil. Pots were placed in a greenhouse maintained at 21 °C (70 °F) during the day and 13 °C (55.5 °F) at night with 14 h daily artificial light provided by Sun System 3 – 400 HPS bulbs. Pots were bottom-watered 3 d per week for approximately one mo, after which they were watered by misting. Plants were fertilized once a week with Scotts Miracle Gro (8N:7P<sub>2</sub>O<sub>5</sub>:6K<sub>2</sub>O; The Scotts Company, Marysville, Ohio). One week prior to outplanting, all pots were placed outside to harden-off.

Outplanting occurred in mid-April of 2007 and 2008. An area of high-quality prairie that provided similar habitat characteristics as the local wild populations was selected for reintroductions. In 2007, we established two 15 m × 20 m (49 ft × 66 ft) plots; in 2008, we established two 14 m × 18 m (46 ft × 59 ft) plots. Plots were divided into 1-m<sup>2</sup> cells, and plants were planted near the origin of each cell. Individuals from each population were randomly assigned cells in each plot, with the exception of the Bald Hill plants, which were planted in one row on the outside of one of the 2008 plots.

All plots were monitored in late May to early June of 2007, 2008, and 2009. Because little mortality was observed in the same year as the planting, we report data from only 2008 (for 2007 planting) and 2009 (2007 and 2008 plantings). For each individual, we determined presence/absence, measured the widest diameter of a plant (the outermost part of an individual, including flowers), the diameter perpendicular to the widest diameter, height, and number of capitula. We did not observe any new seedlings nor clonal growth during this study. The

shape of each plant approximates an oval, and the maximum diameter and perpendicular diameter were used to calculate elliptical crown cover (cm<sup>2</sup>) for each plant using this equation:

$$\text{Elliptical crown cover} = (0.5 \times \text{widest diameter}) \times (0.5 \times \text{perpendicular diameter}) \times \pi$$

Plant height was measured as the distance from the base of the plant to the outer tip of its longest part. For reproductive plants, this was the outer edge of the flower on the tallest stem. For vegetative plants, this was the length of the longest leaf. We used plant cover and height as response variables as they can vary in response to both genetic and environmental (for example, competitive environment) characteristics and are relatively efficient measurements to take. This monitoring protocol is also used in multiple natural populations of Willamette daisy, thus facilitating comparisons.

We analyzed each combination of planting and monitoring year separately. Differences in survival between populations were tested using a Pearson chi-square test. The effect of source population on the number of inflorescences was analyzed using a Generalized Linear Model with a negative binomial distribution. A General Linear Model was used to test for differences in height and cover. Cover data were log-transformed to meet assumptions of normality. All analyses were completed using SPSS 17.0.

### Reciprocal Transplant Experiment

We conducted a modified reciprocal transplant experiment at Finley National Wildlife Refuge (Finley). Finley is located approximately 16 km (10 mi) south of Corvallis, Oregon, in the Benton West Recovery Zone (USFWS 2010). Historical records indicate that this refuge once hosted a Willamette daisy population, but the population has not been observed in 20 y. No

Willamette daisy had been recorded in the specific fields used in this experiment.

In 2007 and 2008, we outplanted individuals grown from wetland (Muddy Creek) and upland (Baskett Butte) sources into wet (Field 1, n<sub>2007</sub> = 97; Field 31, n<sub>2008</sub> = 79), intermediate (Pigeon Butte; n<sub>2007</sub> = 89, n<sub>2008</sub> = 84), and upland (Field 29; n<sub>2007</sub> = 90, n<sub>2008</sub> = 84) prairies sites. Pigeon Butte is a prairie remnant; all other sites had undergone several years of intense restoration to reestablish a native plant community. In 2007, we observed high mortality, likely due to very high cover of a native grass, tufted hairgrass (*Deschampsia cespitosa* (L.) P. Beauv. [Poaceae]), and to flooding in the wet prairie site, Field 1. Thus, we used a different site, Field 31, during the 2008 planting. For the other sites, the 2008 plot was established adjacent to the 2007 plot. Grow-out and monitoring of plants was as described above for the common-garden experiment. We did not observe any new seedlings nor clonal growth during this study.

We analyzed each combination of planting and monitoring year separately. Differences in survival between populations were tested using a Pearson chi-square. The effect of source population on the number of inflorescences was analyzed using a Generalized Linear Model with a negative binomial distribution. A General Linear Model was used to test for differences in height and cover. Cover data were log-transformed to meet assumptions of normality. All analyses were completed using SPSS 17.0.

## RESULTS

### Seed Viability

The percentage of filled seeds increased with population size in both years of the study, though this pattern was stronger in 2006 than 2005 (Figure 1; 2005, R<sup>2</sup> = 0.189, P = 0.071; 2006,

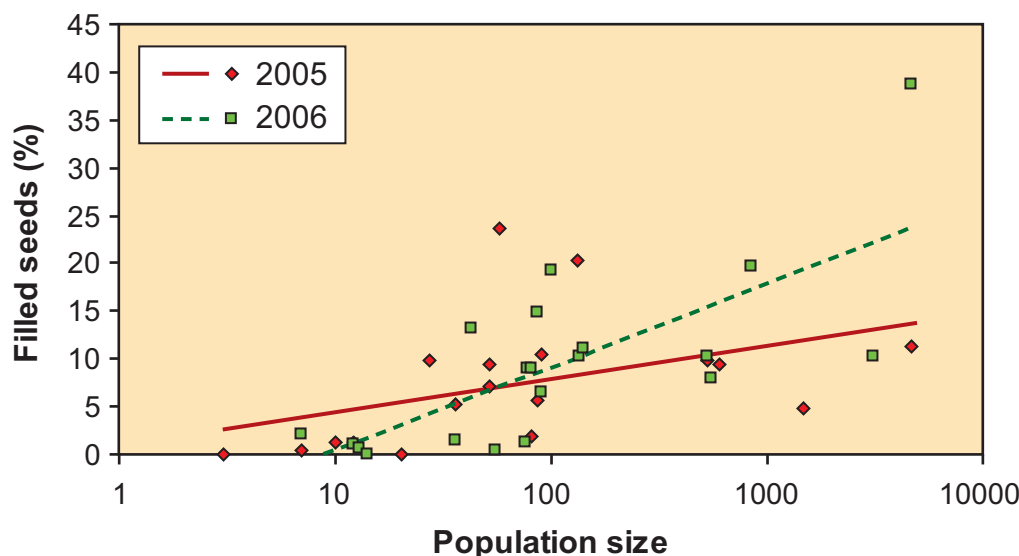


Figure 1. The relationship between percentage of filled seeds and population size in Willamette daisy populations. 2005: P < 0.071, n = 18, R<sup>2</sup> = 0.189; 2006: P < 0.0005, n = 20, R<sup>2</sup> = 0.520. Note the x-axis is on a log scale

TABLE 3

Survival (%), cover (mean  $\pm$  1 S.E.), height (mean  $\pm$  1 S.E.), and number of inflorescences (mean  $\pm$  1 S.E.) of Willamette daisy at the Bald Hill common garden from 3 source populations.

Year planted	Year monitored	Source		
		Muddy Creek	Baskett Butte	Bald Hill <sup>z</sup>
<b>Survival (%)</b>				
2007	2008	67 (306) <sup>a</sup> <sup>y</sup>	59 (294) <sup>b</sup>	—
	2009	62 (306)	54 (294)	—
2008	2009	69 (236)	74 (268)	77 (30)
<b>Cover (cm<sup>2</sup>)</b>				
2007	2008	42.2 $\pm$ 3.2	37.2 $\pm$ 2.8	—
	2009	32.2 $\pm$ 2.3	32.7 $\pm$ 2.3	—
2008	2009	75.8 $\pm$ 5.2 <sup>a</sup>	58.9 $\pm$ 3.7 <sup>b</sup>	58.7 $\pm$ 11.0 <sup>ab</sup>
<b>Height (cm)</b>				
2007	2008	13.0 $\pm$ 0.4 <sup>a</sup>	11.3 $\pm$ 0.4 <sup>b</sup>	—
	2009	9.63 $\pm$ 0.2	9.4 $\pm$ 0.3	—
2008	2009	13.9 $\pm$ 0.4 <sup>a</sup>	11.4 $\pm$ 0.3 <sup>b</sup>	12.7 $\pm$ 1.0 <sup>ab</sup>
<b>Inflorescences (n)</b>				
2007	2008	2.1 $\pm$ 1.9 <sup>a</sup>	1.1 $\pm$ 0.1 <sup>b</sup>	—
	2009	1.1 $\pm$ 0.1 <sup>a</sup>	0.5 $\pm$ 0.1 <sup>b</sup>	—
2008	2009	6.8 $\pm$ 0.5 <sup>a</sup>	5.3 $\pm$ 0.43 <sup>b</sup>	4.7 $\pm$ 1.2 <sup>ab</sup>

<sup>z</sup>Plugs were planted at Bald Hill in 2008 only.

<sup>y</sup>Different letters denote significant differences ( $P < 0.05$ ) between populations within each year planted and monitored.

$R^2 = 0.520$ ,  $P < 0.0005$ ). In populations with  $< 20$  individuals, the percentage of filled seeds was  $\leq 2.1\%$  (Table 2). Seed viability in populations with 20 to 100 individuals ranged from 1.8% (Balboa, 2005) to 23.6% (Sublimity, 2005). In most populations, seed viability varied considerably between years. For example, at Sublimity, plants produced 23.6% filled seeds in 2005, but only 0.5% in 2006.

### Common-Garden Experiment

At Bald Hill, Muddy Creek plants tended to perform better than did plants from Baskett Butte. This pattern, however, was not consistent between metrics or years (Table 3). In the 2007 planting, Muddy Creek plants had slightly higher survivorship, though this difference was significant only one y after planting ( $P_{2008} = 0.031$ ;  $P_{2009} = 0.147$ ). Population (seed source) had no effect on cover for the 2007 planting ( $F_{2008} = 1.44$ ,  $P_{2008} = 0.231$ ;  $F_{2009} = 0.03$ ,  $P_{2009} = 0.864$ ). Muddy Creek plants were 15% taller than Baskett Butte plants one y after the 2007 planting

( $F_{2008} = 8.57$ ,  $P_{2008} = 0.004$ ), but no difference was observed 2 y after planting ( $F_{2009} = 0.35$ ,  $P_{2009} = 0.555$ ). In both monitoring years, Muddy Creek plants had twice as many inflorescences as Baskett Butte plants ( $F_{2008} = 22.2$ ,  $P_{2008} < 0.0005$ ;  $F_{2009} = 22.2$ ,  $P_{2009} < 0.0005$ ).

In the 2008 planting, survivorship was unaffected by population. Although Baskett Butte plants were 13% larger than Muddy Creek plants ( $F = 4.20$ ,  $P = 0.016$ ), Muddy Creek plants were 22% taller than Baskett Butte plants ( $F = 12.29$ ,  $P < 0.0005$ ). Muddy Creek plants also tended to have more inflorescences than Baskett Butte plants ( $F = 5.88$ ,  $P = 0.053$ ). We observed no differences between the Bald Hill plants and the other populations in any of the variables ( $P > 0.050$ ), possibly because of the low number of Bald Hill plants available for comparison.

### Reciprocal Transplant Experiment

At Finley, the effect of source varied depending on response variable, year of planting, year of monitoring, and habitat (Table 4). Across all planting and monitoring years, populations did not differ in survival in the dry and intermediate habitats; however, survival of both populations in the wet habitat was less than 25% that observed in the intermediate and dry habitats ( $P < 0.0005$  in all years).

In both years of monitoring the 2007 planting, there was a significant population  $\times$  habitat interaction for Willamette daisy cover ( $F_{2008} = 4.17$ ,  $P_{2008} = 0.016$ ;  $F_{2009} = 5.13$ ,  $P_{2009} = 0.006$ ). In 2008, Baskett Butte plants were larger than Muddy Creek plants in the intermediate and dry habitats; this pattern continued in 2009 in the intermediate habitat only. Habitat had a significant effect on size only one y after the 2007 planting; plants in the wet habitat were larger than plants in the dry habitat ( $F = 5.14$ ,  $P = 0.006$ ). In contrast, after the 2008 planting, plants were larger in the dry habitat compared with the wet habitat ( $F = 4.78$ ,  $P = 0.009$ ).

One year after the 2007 planting, plants in the intermediate habitat were approximately 4 cm (1.5 in) taller than plants in either of the other habitats ( $F = 50.33$ ,  $P < 0.0005$ ). Neither habitat nor population affected height in the 2008 planting.

In the 2009 monitoring of the 2007 planting, the number of inflorescences was significantly affected by the interaction between population and habitat ( $F = 9.38$ ,  $P = 0.002$ ). Muddy Creek plants produced nearly twice the number of inflorescences as Baskett Butte plants in the intermediate habitat, but there was no difference in either the dry or wet habitats (none of the surviving individuals flowered in the wet habitat). In the 2009 monitoring of both planting years, plants in the dry habitat flowered more than plants in the intermediate and wet habitats ( $F_{2007} = 27.25$ ,  $P_{2007} < 0.0005$ ;  $F_{2008} = 38.09$ ,  $P_{2008} < 0.0005$ ). All other comparisons were not statistically significant ( $P > 0.050$ ).

TABLE 4

Survival, growth, and reproduction of Willamette daisy from 2 source populations in 3 habitat types<sup>z</sup> at Finley National Wildlife Refuge (reciprocal transplant experiment).

Year planted	Year monitored	Habitat <sup>x</sup>	Source <sup>y</sup>		Year planted	Year monitored	Habitat <sup>x</sup>	Source <sup>y</sup>	
			Muddy Creek (wet)	Baskett Butte (dry)				Muddy Creek (wet)	Baskett Butte (dry)
<b>Survival</b>					<b>Height</b>				
2007	2008	Wet <sup>a</sup>	15%	18%	2007	2008	Wet <sup>a</sup>	9.4 ± 1.1	6.2 ± 0.6
		Intermediate <sup>b</sup>	85%	92%			Intermediate <sup>b</sup>	12.6 ± 0.7	14.1 ± 0.6
		Dry <sup>b</sup>	83%	83%			Dry <sup>a</sup>	8.0 ± 0.4	8.3 ± 0.5
2007	2009	Wet <sup>a</sup>	3%	2%	2007	2009	Wet	6.7 ± 1.3	7.5 ± 0.5
		Intermediate <sup>b</sup>	82%	84%			Intermediate	9.5 ± 0.3	10.4 ± 0.3
		Dry <sup>b</sup>	80%	82%			Dry	10.8 ± 0.4	10.0 ± 0.4
2008	2009	Wet <sup>a</sup>	33%	30%	2008	2009	Wet	10.6 ± 1.0	11.5 ± 1.0
		Intermediate <sup>b</sup>	76%	75%			Intermediate	12.6 ± 0.5	12.3 ± 0.4
		Dry <sup>b</sup>	71%	70%			Dry	12.4 ± 0.5	13.0 ± 0.6
<b>Cover</b>					<b>Inflorescences</b>				
2007	2008	Wet	44.3 ± 17.4a	21.2 ± 6.2ac	2007	2008	Wet <sup>ab</sup>	1.36 ± 0.11	0
		Intermediate	23.4 ± 3.3ac	36.3 ± 3.0bc			Intermediate <sup>a</sup>	0.75 ± 0.13	0.70 ± 0.11
		Dry	17.3 ± 2.4bc	24.7 ± 2.8ac			Dry <sup>b</sup>	0.74 ± 0.21	0.71 ± 0.15
2007	2009	Wet	31.0 ± 30.4a	5.4 ± 7.1ac	2007	2009	Wet	0	0
		Intermediate	19.2 ± 2.4bc	26.0 ± 2.8a			Intermediate <sup>w</sup>	0.49 ± 0.13	0.70 ± 0.113
		Dry	30.9 ± 3.5a	24.2 ± 2.9a			Dry	0.78 ± 0.24	1.14 ± 2.65
2008	2009	Wet <sup>a</sup>	28.6 ± 7.9	35.8 ± 10.4	2008	2009	Wet <sup>a</sup>	1.92 ± 0.52	2.70 ± 0.79
		Intermediate <sup>b</sup>	47.1 ± 5.0	47.1 ± 6.3			Intermediate <sup>ab</sup>	2.09 ± 0.37	2.08 ± 0.33
		Dry <sup>b</sup>	42.6 ± 6.4	67.2 ± 7.6			Dry <sup>b</sup>	4.50 ± 0.817	5.39 ± 0.75

<sup>z</sup>The wet habitat site was changed to a new location after 2007 due to very poor plant survival the first year. All other sites remained the same between planting years.

<sup>y</sup>Within the source columns, different letters denote significant differences between population–habitat combinations (two-way interaction) at  $P < 0.05$ .

<sup>x</sup>Within the habitat column, superscript letters a and b denote significant differences among habitats (main effect) at  $P < 0.05$ .

<sup>w</sup>Denotes significant differences between populations (main effect) within each year planted and monitored at  $P < 0.05$ .

## DISCUSSION

Our studies suggest that habitat loss and fragmentation may affect characteristics of Willamette daisy that are important to the successful recovery of this endangered species. We found a positive relationship between seed viability and population size. Although we found some evidence for population differences in survival, growth, and reproduction, these differences were inconsistent between sites, planting years, and time since planting, providing little evidence for ecotypic differentiation in the populations examined.

The observed decline in seed viability associated with decreased population size may be attributable to inbreeding (Heschel and Paige 1995; Husband and Schemske 1996), loss of compatible mating types, pollen limitation (Agren 1996;

Lennartsson 2002), or maternal effects (Montalvo 1994). Inbreeding between related individuals (including self-pollination) in small populations is a well-known cause of reduced fitness (Allphin and others 2002; Hiscock and Tabah 2003). Also, species that require genetically compatible mating types for successful fertilization (such as in sporophytic breeding systems) may experience reduced seed production when compatible mating types become rare, either as a result of a population bottleneck or genetic drift. For example, both inbreeding depression and loss of compatible mating types caused reduced fitness and reproductive failure in the rare Lakeside daisy (*Hymenoxys acaulis* var. *glabra* [Asteraceae]) (Demauro 1993). Habitat quality and competitive environments did not explain variation in seed set in the populations included in this study (data not shown), decreasing the possibility that maternal



effects played a large role in our observed patterns. (As used here, maternal effects are when the condition of the maternal plant influences the offspring, such as through reduced allocation of resources to seeds.)

Pollinator abundance tends to be lower in smaller habitat fragments (Kearns and others 1998; Lennartsson 2002). While the pollination of Willamette daisy in small populations may be limited by infrequent pollination service by insects (low pollen quantity), we suggest that this species may also suffer from stigma contamination with pollen from other plant species by generalist pollinators (low pollen quality). The chance of generalist pollinators visiting and transferring pollen from different species increases in small populations (Feinsinger and others 1986; Klinkhamer and de Jong 1990). Finally, we sampled both small and large populations from throughout the range of this species, thus it is unlikely that observed effects are due to being near the edge of this species' range (Sagarin and others 2006).

The relationship between population size and seed viability in Willamette daisy has numerous conservation implications. Actual seed viability is expected to be lower than the values we reported based on percentage of filled seeds. As most populations contain fewer than 100 individuals, it is likely that population growth in many of these populations is limited by low seed viability. The introduction of novel genotypes has been observed to increase fitness of small populations in other species (Newman and Tallmon 2001; Paschke and others 2002) and may be necessary to ensure long-term survival of these populations. In addition, few populations are large enough to support seed collection for restoration, and most collected seeds are likely to be nonviable.

Habitat fragmentation, such as that experienced by Willamette daisy, may result in loss of gene flow and, subsequently, loss of genetic differentiation between populations through local adaptation and (or) random genetic drift (Linhart and Grant 1996). We observed some population differentiation in survival and growth. In the common-garden experiment, plants from Muddy Creek tended to be larger, taller, and to produce more inflorescences than those from Baskett Butte, suggesting that these plants may have greater competitive ability in this environment. However, we saw few population differences in the reciprocal transplant experiment. In the 2007 planting in the wet habitat, plants from the Muddy Creek (wet site) were larger and produced more inflorescences in 2008, suggesting potential ecotypic differentiation. Yet, these patterns were not evident in any of the other comparisons.

Several limitations suggest that caution in broad interpretations of the common-garden and reciprocal transplant experiments is appropriate. We used only 3 populations in these studies; different results may have been observed if a greater number of species and sites were used. Furthermore, population differentiation may be apparent in a longer-term study and

(or) in life stages not examined in this study (for example, seed production and viability).

Based on our results, we recommend that seed collection efforts for reintroduction occur for several years and that these seeds be put into a seed increase program (as described by Ward and others 2008) structured around the Recovery Zones identified in the *Recovery plan for prairie species of western Oregon and southwestern Washington* (USFWS 2010). This may improve the viability of reintroduced populations because populations from multiple ecotypes can show increased long-term stability compared with single-line populations (Marshall and Brown 1973; Martins and Jain 1979).

Willamette daisy exhibits interannual variability in a number of characteristics. Here, we found significant year-to-year variations in population size, seed viability, plant size, and flowering. Previous investigations have also observed large changes in population and plant size between years (Thorpe and Kaye 2007). As there have been few other changes to these populations between years, it is likely that this variability is due to climatic factors. Recent reports have projected significant climate change in the range of the Willamette daisy (Climate Leadership Initiative and the National Center for Conservation Science and Policy 2009; Doppelt and others 2009). Thus, we recommend that future research projects explore the role of climate factors on this species and potential population differentiation in response to climate.

Reduced population size and increased habitat fragmentation appear to have had negative impacts on Willamette daisy. Our observations have important implications for meeting recovery goals for this species as outlined in its recovery plan (USFWS 2010). First, small populations experience very little to no viable seed production and may have low long-term viability without augmentation from other populations. Second, while populations may differ in survival, growth, and reproduction, these differences do not appear to be consistent with local adaptation to different habitats but instead may be idiosyncratic and thus not a limitation to reintroduction efforts. Third, the interannual variability that we observed in all studies suggests that seed collection and reintroduction efforts should be continued for multiple years. We recommend further evaluations of population differentiation, breeding system, pollinator efficacy, and effects of climate change to improve our ability to recover this species.

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