Genotype, soil type, and locale effects on reciprocal transplant vigor, endophyte growth, and microbial functional diversity of a narrow sagebrush hybrid zone in Salt Creek Canyon, Utah[1]

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When addressing the nature of ecological adaptation and environmental factors limiting population ranges and contributing to speciation, it is important to consider not only the plant's genotype and its response to the environment, but also any close interactions that it has with other organisms, specifically, symbiotic microorganisms. To investigate this, soils and seedlings were reciprocally transplanted into common gardens of the big sagebrush hybrid zone in Salt Creek Canyon, Utah, to determine location and edaphic effects on the fitness of parental and hybrid plants. Endophytic symbionts and functional microbial diversity of indigenous and transplanted soils and sagebrush plants were also examined. Strong selection occurred against the parental genotypes in the middle hybrid zone garden in middle hybrid zone soil; F_1 hybrids had the highest fitness under these conditions. Neither of the parental genotypes had superior fitness in their indigenous soils and habitats; rather F_1 hybrids with the nonindigenous maternal parent were superiorly fit. Significant garden-by-soil type interactions indicate adaptation of both plant and soil microorganisms to their indigenous soils and habitats, most notably in the middle hybrid zone garden in middle hybrid zone soil. Contrasting performances of F_1 hybrids suggest asymmetrical gene flow with mountain, rather than basin, big sagebrush acting as the maternal parent. We showed that the microbial community impacted the performance of parental and hybrid plants in different soils, likely limiting the ranges of the different genotypes.

Key words: Asteraceae; big sagebrush; endophyte; fitness; functional microbial diversity; hybrid zone; reciprocal transplant experiment; Salt Creek Canyon.

Stable hybrid zones are important, not only to evolutionary biologists because they contradict prevailing speciation theories and provide insight into key evolutionary processes, but also to biologists, in general, because they can show the nature of ecological adaptation that limits species' ranges and potentially provide understanding of how species arise and are maintained when ecological rather than reproductive isolating barriers exist. Stable hybrid zones are puzzling because of their persistence (many are thousands to millions of years old; e.g., Jackson, 1973; Eckenwalder, 1984) despite gene exchange between divergent taxa. Such zones neither contract nor expand, as adaptive speciation theory predicts (e.g., Dobzhansky, 1940; Mayr, 1942; Stebbins, 1950; Grant, 1963).

In our ongoing studies of the big sagebrush (*Artemisia tridentata* Nutt. subsp. *tridentata* × *A. tridentata* Nutt. subsp. *vaseyana*: Asteraceae; i.e., basin × mountain big sagebrush) hybrid zone in Salt Creek Canyon, Utah (citations in Appendix S1, see Supplemental Data with online version of this article), we have tested several assumptions of two models proposed to account for stable hybrid zones: the dynamic equilibrium model (Barton, 1979a, b; Barton and Hewitt, 1985, 1989) and the bounded hybrid superiority model (Moore, 1977). One key assumption that differs between the two models is the kind of selection operating to maintain the hybrid zone. The dynamic equilibrium model assumes that selection is endogenous, with the fitness of hybrids less than that of the parental taxa regardless of environmental conditions; whereas the bounded hybrid superiority model assumes that selection is exogenous, with hybrids more fit within the boundaries of the hybrid zone but less fit outside it due to varying ecological conditions. Results of these prior studies indicated that hybrids have superior growth and reproduction within, but not outside the hybrid zone and that exogenous selection is operating against nonindigenous parental and hybrid genotypes (Wang et al., 1997; Freeman et al., 1999).

The exact ecological factors separating the parental big sagebrush taxa and those to which the different genotypes have become adapted are not known. Subspecies of big sagebrush have distributions delimited by moisture–elevational gradients, soil property differences and seasonal moisture trends (McArthur, 1994; Wang et al., 1998; Freeman et al., 1999). Any one or a combination of these or other factors possibly maintain the integrity of basin and mountain big sagebrush when their populations are in contact.

Temperature and soil properties are two ecological factors that could maintain taxonomic differences between the parental

taxa. Coincident with the elevational gradient is a temperature gradient, to which parental and hybrid genotypes might adapt. Wang et al. (1998) showed that soils vary by site across the hybrid zone; soil properties (depth, pH, and elemental composition) in the parental (i.e., basin and mountain) habitats not only differed significantly from each other, but also from those found in the middle of the hybrid zone. Hybrid zone soils are more variable than parental habitat soils and have unique characteristics; in fact, soil differences are so pronounced that they can be seen (Appendix S2, see Supplemental Data with online version of this article) and texturally felt (K. Miglia, personal observation). These soil property differences may also contribute to differences in the ecological requirements of the parental taxa and hybrids and the microorganisms associated with them and/or their habitat soils (e.g., Allen et al. [1995] found that *A. tridentata* subsp. *tridentata* is symbiotic with at least 48 different arbuscular mycorrhizal fungal taxa.).

Many plant–soil interactions, which are thought to be major determinants of plant community structure (e.g., see Grime et al., 1987; van der Heijden et al., 1998a; Clay and Holah, 1999; Hartnett and Wilson, 1999), are mediated through the soil microbial community. A plant's response to its environment, including plant–soil interactions, is consequently the result of a community response rather than simply reflecting the plant's genotype. Redman et al. (2002), for example, showed greater heat tolerance in both the indigenous perennial grass *Dichanthelium lanuginosum* and its endophytic fungal counterpart (a single *Curvularia* species) when grown symbiotically in the field or laboratory. The adaptation of both plant and fungus to geothermal soils was due to the mutualistic relationship between them.

Stable hybrid zone models are best tested using reciprocal transplant experiments, which can detect whether selection is occurring and if so, whether it is endogenous or exogenous. Plant hybrid zones make excellent model systems because plants are relatively easy to manipulate and track over time (Levin and Schmidt, 1985; Schmidt and Levin, 1985; Harrison, 1990; Freeman et al., 1991; Wang et al., 1997). Relatively few reciprocal transplant experiments have been conducted with plants to test stable hybrid zone models. Those by Wang et al. (1997) and Campbell and Waser (2001) looked for significant genotype-by-environment interactions using parental and hybrid seed either collected directly from the hybrid zone or generated in the laboratory from manipulated crosses between field parental plants, respectively. Both studies, and that by Emms and Arnold (1997), looked at elemental concentrations in soils across their study site and found soil chemistry to be significantly different among the parental and hybrid zone locations; however, none sorted out the confounding effects of soil properties and location.

We took the experiments of Wang et al. (1997, 1998, 1999) a step further by reciprocally transferring not only parental and middle hybrid zone plants, but also parental and middle hybrid zone soils, into gardens at the Salt Creek Canyon study site. This allowed us to sort out the effects of location (in our case, temperature differences due to an elevational gradient), genotype, and soil composition. Specifically, we looked at the effect these parameters might have on the growth and reproductive performance of parental and hybrid big sagebrush in reciprocal transplant gardens. We also performed controlled crosses at the parental and middle hybrid zone locations to generate putative parental and hybrid seed that included both F_1 and indigenous (i.e., later generation) hybrid seed.

Additionally, we examined the functional microbial diversity (diversity based on the role of microorganisms in the process of decomposition and biogeochemical cycling) of both indigenous and transplanted soils to ascertain how the microbial community responded to the different locations and surveyed the endophytic symbionts of both indigenous and transplant sagebrush plants across the hybrid zone. We were particularly concerned with interactions among the three factors (garden, soil type, and genotype) because they can potentially reveal aspects of the environment to which a particular genotype and/or its microbial community (i.e., soil–garden interactions) are or are not adapted.

MATERIALS AND METHODS

Study site and organisms—The study site is located in Salt Creek Canyon, Utah, where basin (*Artemisia tridentata* subsp. *tridentata*) and mountain big sagebrush (*A. tridentata* subsp. *vaseyana*) are parapatrically distributed, with the basin subspecies occurring below 1790 m a.s.l. and the mountain subspecies at elevations ranging from 1850 m a.s.l. to timberline on Mt. Nebo (Graham et al., 1995). The hybrid zone is a narrow band, approximately 380 m wide (Miglia, 2003), situated between the two parental populations along the mountainside. Three fenced common gardens were established across the hybrid zone in October 1994: one in each of the parental populations and one in the middle of the hybrid zone (Wang, 1996). The parental taxa differ in a number of genetically based traits (Appendix S3, see Supplemental Data with online version of this article), including AFLP markers, which have recently been identified in preliminary analyses (Miglia, 2003; K. Miglia, unpublished data).

Temperature—Five battery-operated HOBO Weather Station Data Loggers (Onset Computer Corp., Bourne, Massachusetts, USA) were installed by Smith et al. (2002): one in each garden, one at the near-basin site, and one at the top of the ridge, directly above the mountain population (1890 m a.s.l.). Hourly temperatures were measured from 12 September 2000 until 10 July 2001.

Due to autocorrelation of the data, hourly and daily average temperatures were analyzed using a Box-Jenkins time series analysis with the statistical software NCSS (Hintze, 2001). The same model, which was applied to data from all five locations, included four seasons, one autoregression term, one seasonal regression term and a moving average. Means were compared using an ANOVA, followed by a Student Newman Keuls test to determine which pairs of means differed significantly from each other.

Plant and soil transplant experiment—Putative reciprocal F_1, parental and indigenous hybrid seed (B × M, M × B, B × B, M × M, and H × H, respectively, with the first letter in each cross representing the maternal parent, either basin or mountain sagebrush) were made in the field following the protocol of McArthur et al. (1988). Even with controlled pollination techniques, some self-pollination may have occurred (McArthur et al., 1988). In May 1999, seeds were germinated and subsequent seedlings raised in randomly arranged pots in the USDA Forest Service Shrub Sciences Laboratory greenhouse in Provo, Utah, as described by Miglia (2003). Five replicates of each genotype were planted in each of three soil types (basin, mountain, and middle-hybrid) collected at sites immediately adjacent to the three common gardens.

Three trenches (approximately 60 cm wide, 90 cm deep, and 12 m long) were excavated with a backhoe in each garden in May 2000, lined with 4-mil polyethylene sheeting and then filled with one of the same three soil types used in the greenhouse (i.e., each of the three soil types was represented in each garden, including that indigenous for a given garden). One-year-old seedlings were transplanted at random in the soil-filled trenches, with the choice of trench corresponding to the soil type in which a seedling was raised in the greenhouse. There were 25 transplants per trench, giving a total of 75 transplants per garden. Each plant was marked with a tagged rebar and watered weekly until watering was tapered to once every other week in mid-August 2000 and then stopped completely in mid-September 2000.

Measurements were taken in August 2001 and 2002 for height, crown diameters 1 and 2, average inflorescence length, and total number of inflorescences. Plant volume, total inflorescence length, and inflorescence length per unit of volume (L) were estimated for each plant. (Appendix S4

TABLE 1. Coefficients for the Box-Jenkins time series analysis of the temperature data collected at each of the three gardens and two additional sites (see Temperature subsection in the Materials and Methods for details) across the Salt Creek Canyon sagebrush (*Artemisia tridentata*) hybrid zone. The AR coefficient for each site differed significantly from the AR coefficient for each of the other sites, but for SAR and MA, their coefficients did not differ among the sites.

| Coefficient | Temperature data collection sites | | | | |
	Basin garden	Near basin garden	Middle hybrid zone garden	Mountain garden	Top of ridge
AR	0.485	0.539	0.564	0.510	0.507
SAR	−0.009	−0.038	−0.001	0.018	0.021
MA	−0.111	0.070	−0.154	−0.138	0.061

Notes: AR = auto regressive, SAR = seasonal auto regressive, MA = moving average.

provides details regarding these morphological measurements and estimates, see Supplemental Data with online version of this article.) Here we only report on the last three summary measurements. To estimate relative fitness, we took the product of the probability of a genotype surviving in each treatment × the probability of its flowering × its total inflorescence length. To convert this product to a relative value, we divided it by the same product calculated for the genotype indigenous to a given site when raised in its indigenous soil.

Soil microbial functional diversity—Five soil samples (100 g) were collected in each garden: one from each of the three experimental trenches and two from the middle of the garden in undisturbed soil. Four other soil samples (100 g) were collected immediately outside of each garden around the perimeter, equidistantly spaced. These last four samples, as well as the two samples collected within the center of each garden, provided controls to determine the effects of transplantation on the soil microbial community. Bacterial and fungal functional diversity of the soils was assessed using the Biolog identification system (Biolog, Inc., Hayward, California, USA) and procedures by Zak et al. (1994) and Sobek and Zak (2003), respectively. These methods evaluate the ability, if any, of a bacterium or fungus to grow using one or more of the 95 different carbon compounds contained within the growth medium of the microtiter plate (see Zak et al., 1994; and Sobek and Zak, 2003, respectively, for details). For assessment of bacterial functional diversity, the plates were incubated at 25°C and read on a plate reader every 12 h for 72 h, beginning just after the initial 24 h of incubation. Analysis of fungal functional diversity differed in that the plates were read every 24 h for 120 h. These readings allowed us to calculate two different measures of functional diversity: (1) total substrate activity, a measure of the amount of carbon utilized by the microorganisms for each of 95 different carbon substrates, calculated by summing the optical densities of all 95 substrates per microplate; and (2) substrate richness, the total number of 95 compounds used per microplate by the microorganisms, measured as the number of carbon compounds having an optical density of greater than 0.1. This approach determines the functional abilities of soil microorganisms to carry out mineralization and decomposition based on their abilities to use 95 different carbon compounds as energy sources, which in turn has an effect on soil productivity.

Endophyte assessment of indigenous plants and garden transplants—Five whole indigenous plants were collected from each parental population and from three locations in the center of the hybrid zone (near-basin, middle hybrid zone, and near-mountain), whereas in the gardens, samples consisted only of shoots, 10 cm in length, collected from all transplants. Plants and shoots were submersed in 2.0% (v/v) sodium hypochlorite for 20–30 min with moderate agitation and rinsed with 10–20 volumes of sterile distilled water. Rinsed plants and shoots were aseptically cut into sections representing the lower, middle, and upper stem sections, the roots (whole plants only) and leaves. The sections were then plated onto 0.1× PDA medium (Difco Medium, Becton Dickinson Microbiology Systems, Franklin Lakes, New Jersey, USA) and incubated at room temperature under cool fluorescent lights for 5–7 d to allow fungal growth. Endophyte identification was verified microscopically after formation of conidia (i.e., spores).

Data analyses of morphological and microbial assessment data—All morphological and microbial assessment data were analyzed using a multivariate analysis of variance (MANOVA) (Zar, 1998). Cases involving equal variances were followed by Bonferroni post hoc tests. Data violating the assumption of equal variances were square-root transformed. In those cases in which data transformation did not yield equal variances, Dunnett's T3 post hoc tests were performed. All statistical tests were performed using SPSS (SPSS for Windows, Rel. 14.0.0, 5 Sept. 2005, Chicago: SPSS Inc.) statistical software, except for the Box-Jenkins time series analysis (described earlier).

RESULTS

Temperature—The autoregression coefficients are listed in Table 1. The middle hybrid zone garden had the highest autocorrelation coefficient, while the top of the ridge site had the highest seasonal autocorrelation coefficient. Daily average temperatures differed significantly among the sites ($F_{2,329} =$ 171.95, $P < 0.001$), and the Student Newman Keuls analysis showed that all five means differed significantly from each other at the $P < 0.05$ level. The middle hybrid zone garden had the lowest average daily temperature (Fig. 1A). The basin garden had the lowest average daily minimum temperature, and the near basin and top of the ridge sites had higher daily minimum temperatures than the other locations (Fig. 1B). The middle hybrid zone garden site had the highest average maximum temperature and widest average daily range of temperatures (Fig. 1C and D), while the near basin site had the lowest average maximum temperature and narrowest range of temperatures, followed by the top of the ridge site for both measures.

Plant and soil transplant experiment—*Main effects*— Because our focus was on significant genotype-by-environment interactions and composite relative fitness, results for main effects will not be included here. Details of these results are in Appendix S4 (see Supplemental Data with online version of this article).

Interactions—The bounded hybrid superiority model requires specific genotype-by-environment interactions with superior fitness occurring for the parental genotypes in their indigenous habitats and the hybrid genotypes in the middle of the hybrid zone. Therefore, we should expect either significant genotype-by-soil type or genotype-by-garden interactions with the indigenous taxa and hybrids performing best in their respective habitats if the bounded hybrid superiority model is to be supported. However, for both years, the significant interactions we observed were garden-by-soil type, except for a significant genotype-by-soil type interaction for inflorescence length per unit of volume in 2001. Because plants with the same maternal genotype behaved similarly in 2001 for garden-by-soil type and genotype-by-soil type interactions, we pooled the data for B × B and B × M into one group and the M × M and M × B into another group.

Genotype-by-soil type—In 2001, both groups of maternal genotypes generally produced equal total inflorescence length and inflorescence length per unit of plant volume in all soils. However, these parameters declined for indigenous hybrids going from basin to mountain to middle hybrid zone soil, suggesting that the indigenous hybrids are not as well adapted to their indigenous soil (data not shown, see Miglia, 2003).
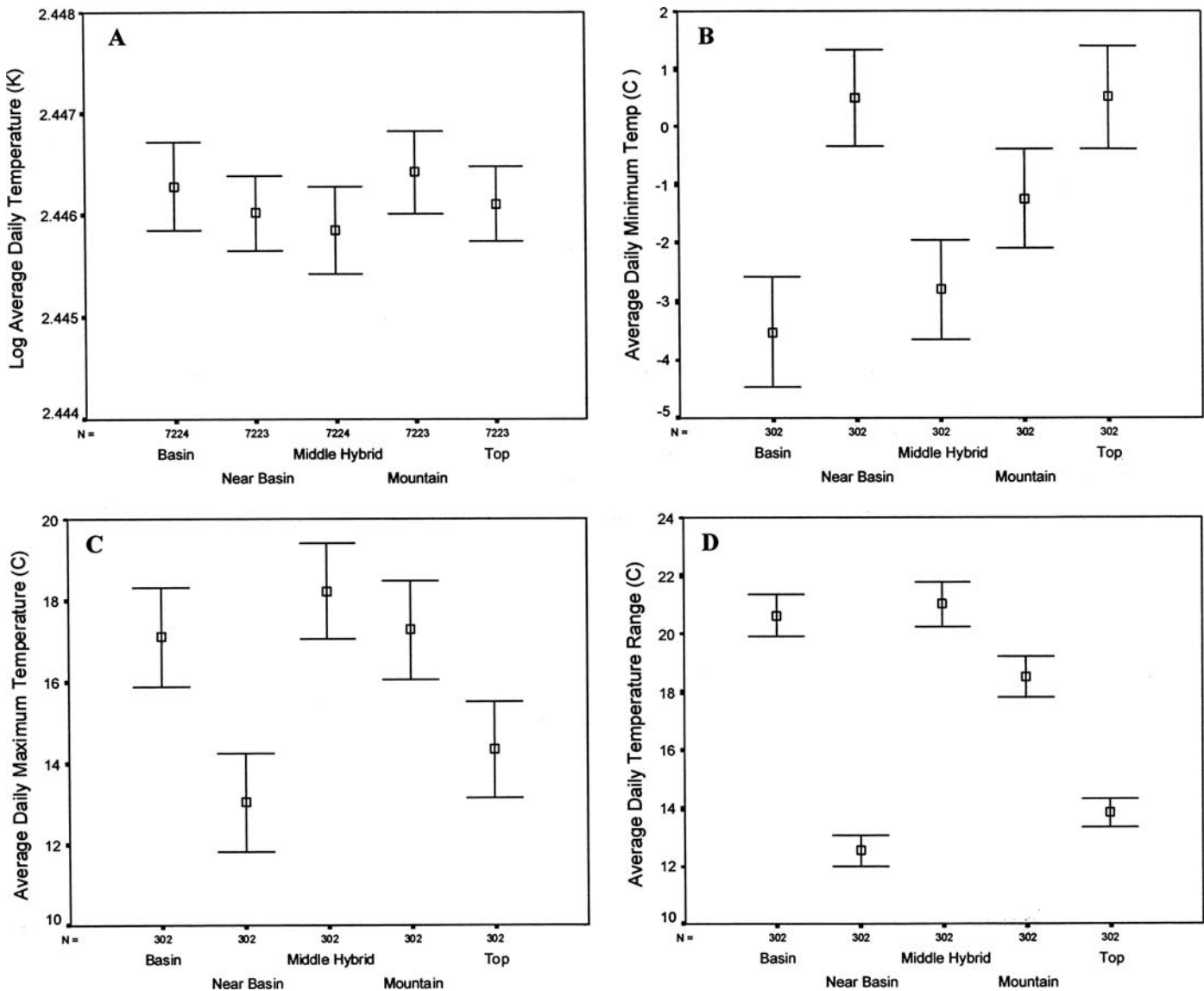
Fig. 1.   Daily averages at five locations (basin, middle hybrid, and mountain gardens; near basin site; and the top of the ridge, directly above the mountain garden site) in or near the Salt Creek Canyon sagebrush (*Artemisia tridentata*) hybrid zone for (A) log temperature in K, (B) minimum temperature in °C, (C) maximum temperature in °C, and (D) temperature range in °C. Error bars represent the 95% confidence intervals for the means.

There were no significant interactions in 2002 for any of the summary variables.

Genotype-by-garden—There were no significant interactions overall in either year. Orthogonal contrasts of 2001 data indicated that the hybrid maternal parent in the middle hybrid zone garden produced slightly more plant volume ($P < 0.11$) and significantly more total inflorescence length ($P < 0.001$) and inflorescence length per unit of volume ($P < 0.003$) than did the basin or mountain parental genotypes in the same garden (Fig. 2). These results indicate that the indigenous hybrids may be best adapted to their indigenous location.

Garden-by-soil type—There were no significant interactions for data collected in 2001, even when data were pooled by

maternal genotype. In 2002, plant volume was greatest among plants in the middle hybrid zone soil in the middle hybrid zone garden (Fig. 3A). In the basin and mountain gardens, plant volume was greatest among plants in the basin soil, whereas plant volume was least among plants in the mountain garden in mountain soil, although not significant. Thus in two gardens (basin and middle hybrid zone), all genotypes grew best in the garden's indigenous soil. For plants in the middle hybrid zone soil, total inflorescence length was greatest in the middle hybrid zone garden and least in the basin and mountain gardens when averaged over all genotypes (Fig. 3B). Plants in the basin and mountain soils produced statistically equal total inflorescence lengths regardless of the garden in which they were grown. Results from both years' data suggest that the microbial communities of the basin and middle hybrid zone soils may be adapted to their indigenous locations.
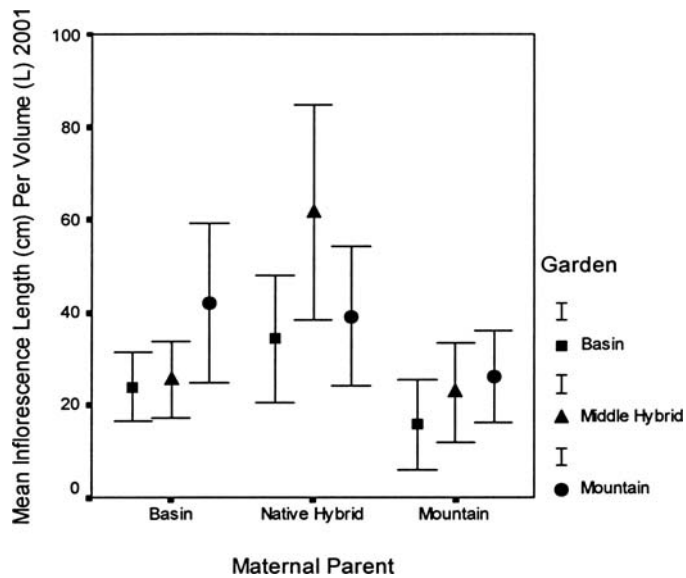
Fig. 2.   Mean 2001 inflorescence length per unit of plant volume in each garden for sagebrush (*Artemisia tridentata*) plants with either basin (B × B and B × M), mountain (M × M and M × B), or indigenous hybrid (H × H) as the maternal parent. This same trend occurred for plant volume and total inflorescence length; thus, graphs for these are not shown here. Error bars represent the 95% confidence intervals for the means.

Relative fitness—Table 2 summarizes the composite fitness values of all genotypes in each garden for plants grown in soil indigenous to a given garden; fitness was based relative to the fitness of the genotype indigenous to a given location, grown in its indigenous soil. Both garden and soil type greatly influenced a genotype's relative fitness (hereafter referred to as just fitness). In the basin garden, the basin genotype was more fit than the mountain genotype and the F_1 hybrid with basin as the maternal parent, but less fit than the F_1 hybrid with mountain as the maternal parent and the indigenous hybrid genotype. In the mountain garden, the mountain genotype was more fit than the F_1 hybrid with mountain as the maternal parent and the indigenous hybrid genotype, but less fit than the F_1 hybrid with basin as the maternal parent and the basin genotype. In the middle hybrid zone garden, the indigenous hybrid genotype was more fit than both parental genotypes and the F_1 hybrid with basin as the maternal genotype.

Averaged over all soil types—None of the indigenous genotypes had the highest relative fitness in their indigenous garden. H × H hybrids had the highest fitness in the basin garden; in fact, their fitness almost doubled that of the indigenous B × B plants. M × B hybrids had the highest fitness in the middle hybrid zone garden, whereas the B × B plants had the highest fitness in the mountain garden. In the gardens overall, the lowest fitness occurred in the mountain garden among the M × B hybrids, whereas the highest fitness occurred among the same hybrids in the middle hybrid zone garden. These data indicate that neither the parental taxa nor the hybrids are particularly adapted to their indigenous location.

Averaged over all gardens—The best performance occurred in the basin soil among the B × M hybrids, whereas the worst performance occurred in the mountain soil among the B × B
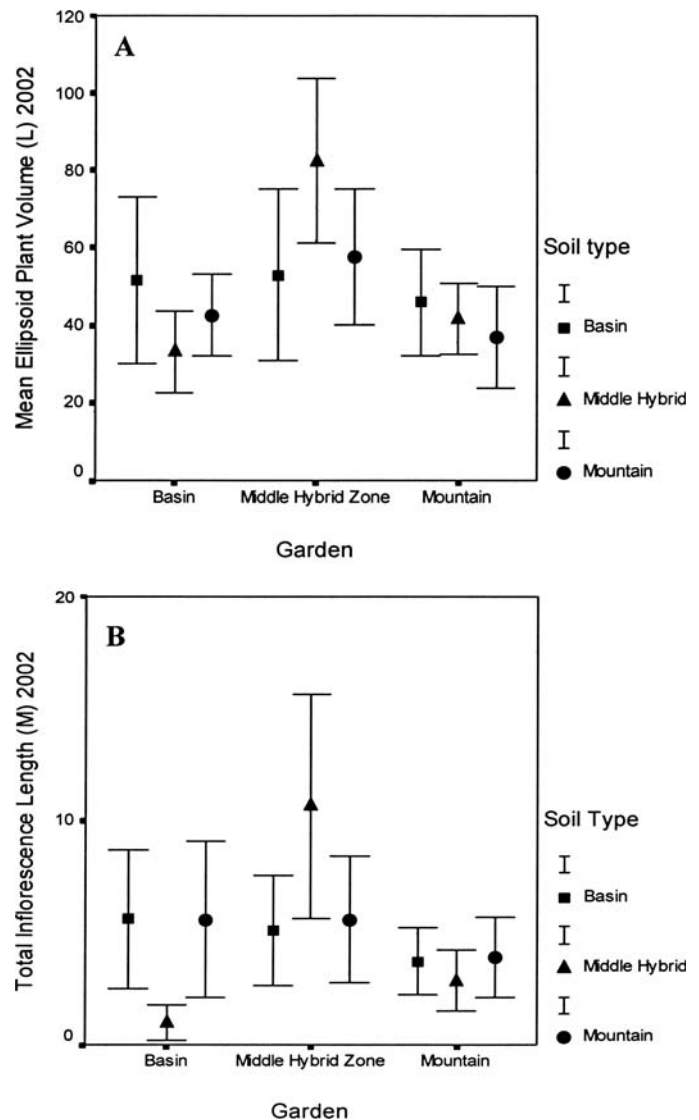

Fig. 3.   Mean (A) plant volume and (B) total inflorescence length of sagebrush (*Artemisia tridentata*) reciprocal transplants for each of the three soil types in each garden in 2002, summed over all genotypes. Error bars represent the 95% confidence intervals for the means. Dead plants were included.

plants. In the hybrid soil, the B × M hybrids had the greatest fitness, whereas the indigenous H × H hybrids had the lowest. The H × H hybrids had the lowest fitness in all three soils, except in the mountain soil where both H × H and M × B hybrids had the lowest fitness. These results suggest that both the parental taxa and hybrids do not appear to be adapted to their indigenous soil.

Averaged over all genotypes—The best and worst performances overall occurred in the middle hybrid zone and mountain gardens, respectively. The highest fitness occurred in the mountain soil for both the basin and middle hybrid zone gardens, while that for the mountain garden occurred in the basin soil. The lowest fitness occurred in the basin soil for both the basin and middle hybrid zone gardens, whereas middle

TABLE 2. Fitness of each genotype at each location in the Salt Creek Canyon sagebrush (*Artemisia tridentata*) hybrid zone, relative to the fitness of the indigenous genotype grown in its indigenous soil at its indigenous location. (See Relative fitness subsection in the Materials and Methods for how composite relative fitness was calculated.)

Location of garden	Elevation (m a.s.l.)	Genotype	Composite relative fitness
Mountain population	1850 to timberline	M × M*	1.00
		M × B	0.24
		H × H	0.60
		B × M	1.08
		B × B	1.36
Middle hybrid zone population	1790–1830	M × M	0.74
		M × B	1.26
		H × H*	1.00
		B × M	0.74
		B × B	0.90
Basin population	Below 1790	M × M	0.78
		M × B	1.24
		H × H	1.72
		B × M	0.64
		B × B*	1.00

Notes: * = Indigenous genotype of one of the three locations.

hybrid zone soil in the mountain garden yielded plants with the lowest fitness.

While relative fitness is a function of the total reproductive output and thus dependent to some extent upon plant size, it is also useful to examine how reproductive output, corrected for size, varies with soil type and garden (Fig. 4). Clearly all of the genotypes are sensitive to changes in soil type. For example, the reproductive output per unit of plant volume varied by more than two orders of magnitude for basin plants in the basin garden, depending on soil type (from 0.04 to 8.1 cm of inflorescence length per liter of plant volume). Similarly, garden also had a profound impact. Inflorescence length per liter of plant volume for the M × B hybrids, for example, ranged from an average of 2.91 cm/L in the basin garden in middle hybrid zone soil to 26.05 cm/L in the middle hybrid zone garden in middle hybrid zone soil. On a per unit of volume basis, gene flow via seed dispersal should be fairly unidirectional from the mountain population to the middle of the hybrid zone because the F_1 hybrid with mountain as the maternal parent performed well in the middle hybrid zone soil in the middle hybrid zone garden. However, the reciprocal F_1 hybrid with basin as the maternal parent performed relatively poorly in that garden. There also appears to be some general heterosis as the F_1 hybrids outperformed both parental taxa in some, if not most environments. However, the indigenous hybrids had lower size-corrected reproductive output than the mountain parent in the mountain environment.

Soil microbial functional diversity—Neither soil type nor garden into which the soils were transplanted influenced either fungal total substrate activity or fungal substrate richness. For
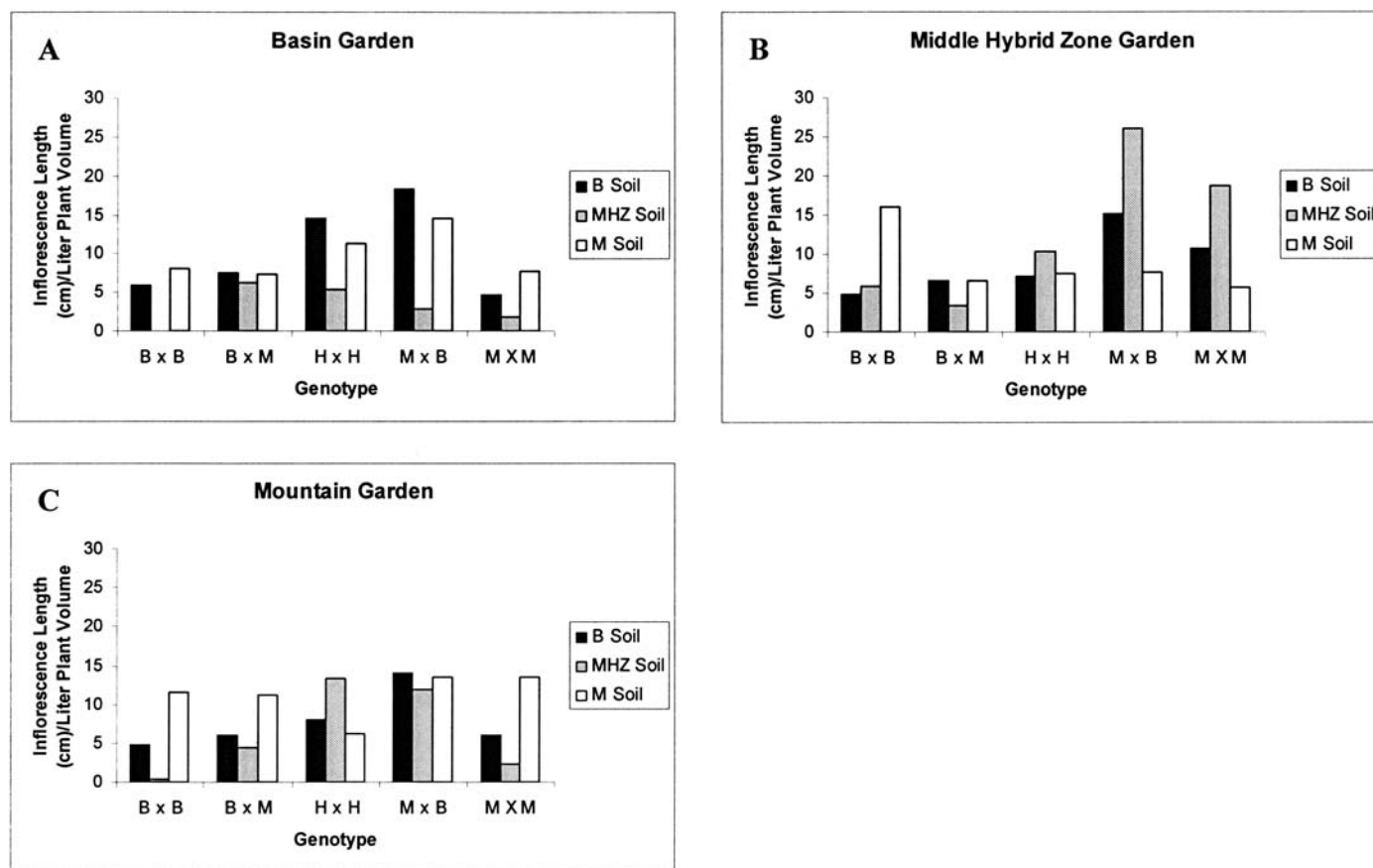

Fig. 4. Size-corrected reproductive output for total inflorescence length per liter of plant volume for each sagebrush (*Artemisia tridentata*) transplant genotype in each soil type in (A) basin garden, (B) middle hybrid zone garden, and (C) mountain garden. (B = basin, MHZ = middle hybrid zone, and M = mountain).

TABLE 3. Endophytes present within indigenous plants sampled across the Salt Creek Canyon sagebrush (*Artemisia tridentata*) hybrid zone in each of three populations (both parental and middle hybrid zone).

| Habitat | Plant # | Endophytic fungal morphotype | | | | |
		M1	M2	M3	M4	M5
Basin	1		S/L	R	R	
	2		S/L	R	R	
	3		S/L	R	R	
	4		S/L	R	R	
	5		S/L	R	R	
Hybrid	1		S/L	R	R	S/L
	2		S/L	R	R	S/L
	3		S/L	R	R	S/L
	4		S/L	R		S/L
	5		S/L	R		S/L
Mountain	1	R	S/L	R		S/L
	2	R	S/L	R		S/L
	3	R	S/L	R		S/L
	4	R	S/L	R		S/L
	5	R	S/L	R		S/L

Notes: R = root, S = stem, L = leaf.

TABLE 4. Percentage of big sagebrush (*Artemisia tridentata*) transplants with 0–5 endophytes, summed over all gardens at the Salt Creek Canyon study site.

Number of endophytes	Percentage
0	51.9
1	33.6
2	12.1
3	0.9
4	0.9
5	0.5

the soil bacteria, however, both total substrate activity and substrate richness depended upon the soil type ($F_{3,44} = 3.20$, $P < 0.05$; $F_{3,44} = 2.57$, $P < 0.066$, respectively), and there was also a significant garden-by-soil type interaction for total substrate activity and substrate richness ($F_{6,44} = 2.82$, $P < 0.02$; $F_{6,44} = 2.29$, $P < 0.052$, respectively). The middle hybrid zone soil had both the greatest bacterial total substrate activity and substrate richness when placed in the middle hybrid zone garden. Similarly, the basin soil had the greatest bacterial total substrate activity and substrate richness when placed in the basin garden. In the mountain garden, the mountain soil performed poorly for both measures and did not differ from the other soils (data not shown).

There was no significant difference between the control and transplanted basin soils in the basin garden for either bacterial total substrate activity or substrate richness. The same was true for the middle hybrid zone control and transplanted soils in the middle hybrid zone garden. However, transplanted mountain soil had a much lower bacterial functional diversity, as measured by both parameters, when placed in the mountain garden. The mountain soil's peak bacterial functional diversity occurred in the basin garden (data not shown).

Endophyte assessment—Indigenous plants—Endophytes were isolated from the roots, stems, and leaves of indigenous mountain, basin, and hybrid plants. All plants were colonized with up to four of the five fungal species found (Table 3). Although these endophytes have not yet been characterized, either morphologically or at the genomic level, conidiophore (specialized hypha bearing asexual spores or conidia) and conidial morphology indicate that there are five distinct species. Plants from both parental and middle hybrid zone habitats could be differentiated based on fungal endophytic colonization patterns. For example, morph 1 was only isolated from the roots of the mountain plants, while morph 4 was isolated from the roots of all basin and most hybrid plants. Morph 5 was isolated from the leaves and stems of both hybrid and mountain, but not basin plants.

Garden transplants—Endophytes were also isolated from the shoots (stem and leaves) of plants from almost all

experimental treatment combinations in the gardens (Appendix S5, see Supplemental Data with online version of this article). Whereas every plant in the indigenous populations had at least three or four endophytes, we were unable to culture any endophytes from over half the plants in the transplant gardens. Roughly 33% of the plants in the transplant gardens had one endophyte, and 14.4% had two or more endophytes (Table 4). This paucity of endophytes among the transplants may be due to culturing technique, but the same techniques were employed as for the indigenous plants. We therefore believe that the results are real and, if so, indicate that seed germination and initial growth in a greenhouse and subsequent transplantation of seedlings into disturbed soils in the gardens may have influenced the infection of plants by endophytes.

Our data show no effect of genotype; however, sample size was small. We thus combined the data by genotype and examined the influence of the number of endophytes on inflorescence length by either garden or soil type, summed over all genotypes. The result was no significant effect in either parental garden or either parental soil type. In the middle hybrid zone garden, total inflorescence length tended to increase as the number of endophyte morphs infecting a plant increased (Fig. 5A). An orthogonal contrast indicated that the log of the total inflorescence length was significantly greater for plants with two endophyte morphs than those with no endophytes ($t = 2.05$, $P < 0.05$). While plants with two endophyte morphs produced longer inflorescences than plants with one morph, the difference was not significant.

In middle hybrid zone soil, total inflorescence length increased as the number of endophytes infecting a plant increased (Fig. 5B). Orthogonal contrasts showed that plants infected with two morphs produced significantly greater total inflorescence length than plants infected with 0 or 1 morph ($t = 2.22$ and 2.36, respectively; $P < 0.05$ in both cases). Taken together, these results suggest that the microbial community may play an important role in determining a genotype's fitness and thus potentially its distribution.

DISCUSSION

Long-term stability of hybrid zones is believed to result from a balance between gene flow and selection. For the Salt Creek Canyon hybrid zone, our results indicated that within the hybrid zone exogenous selection should occur against both parental genotypes, in keeping with the bounded hybrid superiority model. While the indigenous hybrids were not superior in fitness within the boundaries of the hybrid zone, they were more fit than either parental taxon. The F_1 hybrid with mountain as the maternal parent had the greatest fitness,

which might also indicate some directionality in gene flow. This trend occurred whether or not we corrected for plant size.

F_1 hybrids with basin as the maternal parent were only 64% as fit as indigenous B × B plants in basin soil in the basin garden. This should limit long-range gene flow, via pollen dispersal, from the mountain to the basin population, despite the fact that pollen flow in this direction is physically feasible (i.e., big sagebrush is wind-pollinated and produces copious amounts of pollen that should easily disperse downhill). Pollen flow in the opposite direction would be more difficult but still likely occurs. However, F_1 hybrids with mountain as the maternal parent were only 24% as fit as the indigenous M × M plants in mountain soil in the mountain garden. Thus, long-distance gene flow via pollen dispersal from either parent into the other parental population would most likely produce offspring that are strongly selected against.

Remarkably, in both parental-habitat gardens, F_1 hybrids with the nonindigenous maternal genotype had greater fitness than the indigenous parental genotype, which means that relatively long-distance dispersal of F_1 hybrid seed from one parental population to the other, while highly unlikely, could be very successful. Hybrids with mountain as the maternal parent were 124% as fit as indigenous B × B plants in basin soil in the basin garden, while F_1 hybrids with basin as the maternal parent were 108% as fit as indigenous M × M plants in mountain soil in the mountain garden, although most of the increased fitness of the B × M genotype in the mountain environment was simply due to the fact that these plants were taller. For gene flow to occur this way, hybrid seed would have to form in one parental population and then be transported to the other parental habitat. This two-stage scenario is extremely unlikely because big sagebrush seed, although propagated by wind, is relatively poorly dispersed. About 85–90% of big sagebrush seed disperse within a distance of 1 m from the canopy edge, while some seed disperse as far as 30 m away (Young and Evans, 1989; Wagstaff and Welch, 1990). The distance between the basin and mountain gardens is 774 m, far greater than 30 m. Thus, for gene flow to be successful, hybrid plants would need to colonize the area between the parental populations; i.e., the location of the present hybrid zone.

Within the hybrid zone, strong selection occurs against both parental genotypes (i.e., both parental genotypes had lower fitness than the indigenous hybrids when raised in the middle hybrid zone garden in middle hybrid zone soil, even when plant-size corrected). When grown in middle hybrid zone soil in the middle hybrid zone garden, plants with the B × B genotype were only 90% as fit as plants with the indigenous H × H genotype (67% as fit on a size-corrected basis), while plants with the M × M genotype had only 74% of the fitness of the indigenous hybrids. Plants with basin as the maternal parent were only 74% as fit as the indigenous H × H hybrids in middle hybrid zone soil in the middle hybrid zone garden. However, F_1 M × B hybrids were 126% as fit as the indigenous H × H hybrids, which means that they should be very successful if found in the middle of the hybrid zone. Accordingly, we expect asymmetrical gene flow within this hybrid zone from the mountain population, with the mountain subspecies serving as the seed parent.

The fitness differences reported here might be due to the response of the soil microbial community and related interactions with the plant genotypes, rather than being solely dependent upon the genotype of the plant. This hypothesis is based upon the following observations: (1) Our results
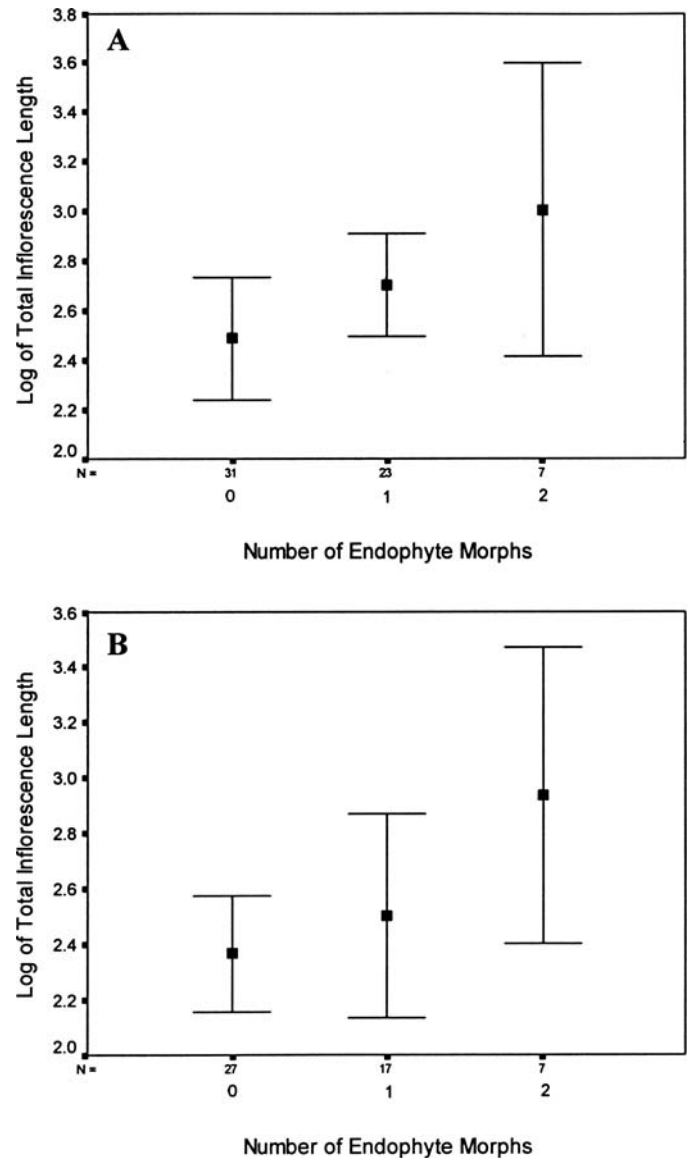

Fig. 5. Log of mean total inflorescence length of sagebrush (*Artemisia tridentata*) reciprocal transplants vs. the number of endophytes for (A) middle hybrid zone garden, summed over all soil types; and (B) middle hybrid zone soils, summed over all gardens. Error bars represent the 95% confidence intervals for the means.

indicated a strong garden-by-soil type interaction for growth and some fitness components (even on a per-unit size basis); (2) there was a tendency for total inflorescence length to increase in either the middle hybrid zone garden or middle hybrid zone soil as the number of endophytes in the shoot increased; (3) bacterial functional diversity of the basin and middle hybrid zone soils was greatest for bacteria in their indigenous gardens, and all genotypes performed better under these conditions than when raised in nonindigenous soils; and (4) results of a separate greenhouse experiment (Miglia, 2003) in which big sagebrush plants [same five seed types (B × B, B × M, H × H, M × M, and M × B), using the same pollination protocol as the experiment reported here] were grown in one of three sterilized soils (i.e., basin, middle hybrid zone and mountain) inoculated with one of the same three soils (not
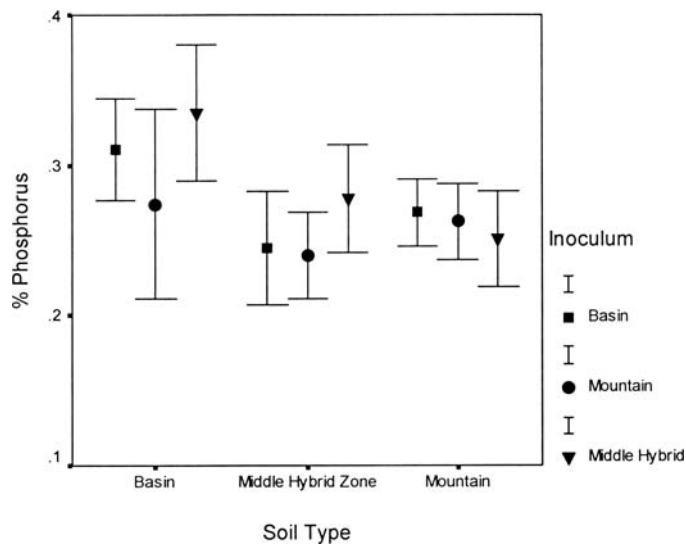
Fig. 6. Results from a separate greenhouse experiment for sagebrush (*Artemisia tridentata*) leaf elemental concentrations of percentage phosphorus in each soil type with one of three inocula, averaged over all genotypes. (See Discussion for details.) Error bars represent the 95% confidence intervals for the means.

sterilized) indicated that reciprocal F_1 hybrids always grew best when inoculated with microorganisms from the soils nonindigenous to the maternal genotype, regardless of sterilized soil type. These results support the data reported here. (5) Results of the separate greenhouse experiment also indicated that the middle hybrid zone inoculum proved most effective at providing plants with phosphorus in the middle hybrid zone soil; this was especially true of indigenous H × H hybrids (Fig. 6) and (6) functional bacterial diversity greatly differed between control (undisturbed) and transplanted mountain soils. Perhaps disruption of soil structure and aeration of the soil, inherent in transplanting it, negatively impacted the microbial dynamics of soils at the mountain location (see second from last paragraph of Discussion). Our findings suggest that the interactions among soil type and garden, as mediated by the microbial plant and/or soil communities, may set the upper and lower limits of the Salt Creek Canyon hybrid zone by affecting the distribution of the parental taxa and their hybrids. However, further experiments directly testing this hypothesis are required to determine whether such a cause and effect actually exists and if so, which mechanisms are responsible. The remaining issues concern what happens inside the hybrid zone.

Soil properties across the Salt Creek Canyon hybrid zone vary significantly in pH, depth, and chemical composition (Wang et al., 1999) and, as we have shown here, in bacterial functional diversity, all of which impact soil productivity. The middle hybrid zone soil was the most productive soil type for all genotypes when located in the middle hybrid zone garden for all three summary variables in 2002. Moreover, basin was the most productive soil type in the basin garden. The fact that mountain was not the most productive soil type in the mountain garden may be due to the effect of transplantation reducing the functional diversity of mountain soil in the mountain garden. Although when we examined reproduction on a per unit of plant volume basis, mountain soil was the most productive soil in the mountain garden. It may well be that the plant's adaptation is as much to accommodate the local microbial

community as it is to the physical and chemical environment of the soils. The separation of these two factors is complex because both plant growth and the activities of soil microorganisms determine soil nutrient dynamics and other edaphic characteristics that, in turn, affect the distribution of plant roots and microorganisms (Paul and Clark, 1996).

Variation in root morphology and distribution in sagebrush is, in part, determined genetically, most likely resulting from different life history strategies. Basin and mountain big sagebrush have root systems that differ in growth rate and morphology. Roots of basin big sagebrush, which elongate vertically, grow faster and reach greater maximum length than the horizontally elongated roots of mountain big sagebrush (Welch and Jacobson, 1988). These differences are thought to be adaptive responses to soil property differences between the basin and mountain habitats (Meyer, 1994). By examining the response of plants to more-or-less homogenized soils in trenches, we were unable to ascertain if these differences in root structure are adaptive. A comparison of our results with the experimental plants (still growing at the time of our experiment in the same gardens) of Wang et al. (1997; see also Miglia et al., 2005), in which soil structure was not greatly disrupted, suggests that root morphology and soil structure and aeration may also be important because the 2-yr-old plants we report on here were nearly as large and fecund as the 9-yr-old plants that Wang et al. originally reported on in 1997. Moreover, the transplants of Wang et al. after 2 yr of growth were much smaller and much less fecund than our 2-yr-old plants. The root structure of the hybrid genotypes is unknown. Further research is needed comparing the root morphology and growth of both parental taxa and hybrids.

Numerous studies have shown the importance of mycorrhizal fungi to plant performance (e.g., Sanders and Tinker, 1971; Smith and Read, 1997; Read, 1999; Bever et al., 2001). More recent work indicates that plant adaptations may also result from endophytic fungus– and bacterium–plant interactions (e.g., van der Heijden et al., 1998b; Redman et al., 2002). These factors should therefore be considered when studying any system involving plants, including hybrid zones. The indigenous hybrids had the greatest relative fitness at their indigenous location in their indigenous soil. This supports part of the pattern predicted by the bounded hybrid superiority model (Moore, 1977), which assumes that each parental taxon is most fit in its indigenous habitat and indigenous hybrids are most fit within the boundaries of the hybrid zone. The parental taxa in our experiment did not have superior fitness at their indigenous locations in their indigenous soils, however. In every case, the F_1 hybrid with the nonindigenous maternal parent had greater fitness than the indigenous parent, though the indigenous hybrids did have lower fitness than the native parent. Because of the distance required for dispersal, we suspect that seed produced by these plants would rarely if ever be dispersed into the parental habitats. Moreover, because the F_1 hybrid with the indigenous parental genotype as the maternal parent was always less fit than the indigenous parent, there should be strong selection against hybrids formed via long distance pollen dispersal.

According to Moore and Koenig (1986), hybrid superiority is delimited geographically by distinct ecological communities and thus "bounded." This includes soil microbial communities. However, hybrid zone literature commonly considers only the genotype of an organism and its response to the environment. In the case of the sagebrush hybrid zone, however, one must

also consider the close associations that plants have with their microbial communities and the adaptation of those microorganisms to both the soils and other physical factors in their indigenous habitats. We found that the microbial community in the middle of the hybrid zone appeared to be strongly adapted to its location, and when the results of these studies are combined with those of our greenhouse experiment (Miglia, 2003), the microbial community of the middle hybrid zone location is also adapted to its own soils. Taken together, our results strongly suggest that the microbial community may have played a role in the development and maintenance of the stable hybrid zone between basin and mountain big sagebrush in Salt Creek Canyon.

We observed a strong garden-by-soil type interaction for both plant growth and reproduction. Moreover, the number of substrates used by bacteria differed significantly by garden with the greatest functional diversity occurring in the middle hybrid zone garden. Our results indicated that the average daily temperatures differed among the sites, with the basin garden site having the lowest daily minimum temperature. The temperature differences we observed most likely would not be dramatic enough to affect the chemical or physical properties of the soils, but they certainly could affect the biological properties, specifically, the performance of the soil microorganisms, in terms of species occurrence, density and activity for each habitat across the hybrid zone. If the soil microorganisms are unable to adapt to changes in soil temperatures when moved to the nonindigenous gardens, then performance of plants could be adversely affected as well. Redman et al. (2002) showed that the symbiotic relationship between a perennial grass and an endophytic fungal species provided the plant thermal protection, which was enhanced as soil temperatures increased. In other examples, reviewed by Entry et al. (2002), growth of mycorrhizae were either negatively or positively affected by adverse soil conditions such as flooding, drought, pH, temperature extremes, depending, in some cases, on the specific host–fungus association and life stage of the plant symbiont.

Not all of the microbial interactions can be considered positive. In this and our greenhouse experiment (Miglia, 2003), we found that sagebrush plants actually performed better when grown in foreign soils or with foreign inocula, respectively. Here, this was especially true of the reciprocal F_1 hybrids, which always performed poorly when grown in both the indigenous soil and garden of their maternal parent, but did exceedingly well when grown in both the indigenous soil and garden of the paternal parent. Similarly, in the greenhouse experiment (Miglia, 2003), all genotypes performed better when grown with foreign inoculum. This suggests that some members of the microbial community may be pathogenic to the indigenous maternal genotype, adapted to the maternal sagebrush and its habitat. This finding reiterates the importance of the microbial community, its adaptation to a specific soil type and location, and potential important role in the structuring and maintenance of plant hybrid zones. Equally important is the possible role of plant–microbe interactions in the distribution of plant populations, in general, and the limiting of plant species' ranges. However, in the case of the sagebrush hybrid zone, further experiments are needed to elucidate and confirm, if real, the role of microorganisms in structuring and maintaining this hybrid zone.

We examined the effects of only a single garden at each site across the Salt Creek Canyon hybrid zone; consequently, as a reviewer correctly noted, our experimental design reflects pseudoreplication, which may mean that our results do not represent what typically occurs across the whole of the hybrid zone. Logistically, however, it would have been extraordinarily difficult to replicate the gardens at each of the three sites. Despite this, we anticipate that the variance among replicate gardens within a site would be low, based on previous data collected for this hybrid zone, which is extensive (Appendix S1, see Supplemental Data with online version of this article). Nonetheless, we cannot definitively know that our results are typical, and thus, our inferences are somewhat limited.

Conclusions—Using a three-factor reciprocal transplant experiment involving five big sagebrush genotypes (basin, mountain, reciprocal F_1, and indigenous hybrids), three transplant gardens and soils from each parental population and the middle of the hybrid zone, we explored the nature of the adaptation of the parental taxa and indigenous hybrids to their respective habitats. The indigenous hybrids had the greatest fitness when grown at their indigenous location in their indigenous soil. This result partly affirmed the findings of Wang et al. (1997) and the premise of the bounded hybrid superiority model (Moore, 1977) that exogenous selection stabilizes the big sagebrush hybrid zone. The parental taxa in our experiment, however, did not have the highest fitness in their indigenous habitats. This difference in results may be due to the disruption of soil structure, which occurred when we transplanted the soils into trenches in the gardens. The microorganisms that inhabit both the soils and the plants themselves appear to be adapted, with those in the middle of the hybrid zone having the strongest adaptation to their soil and habitat. We also found that gene flow should be asymmetrical with mountain big sagebrush acting as the maternal parent because these F_1 hybrids had much higher relative fitness in the middle hybrid zone habitat than F_1 hybrids with basin as the maternal parent. Finally, both types of F_1 hybrids performed relatively poorly in the habitat of the maternal parent but were superior in fitness to the paternal parent in its indigenous habitat.

Our results further suggest that one should not only consider the genotype of an organism and its response to the environment, but also the close association that organism has with other organisms, such as herbivores, parasites, and symbiotic soil microorganisms. Whitham (1989) and Whitham et al. (1994), for example, found the hybrid zones of two cottonwood and two eucalypt species to have a greater concentration of aphids and of insect and fungal species, respectively. They argued that these findings have important consequences in terms of pest management, biodiversity and biological conservation issues. Here we propose that symbiotic relationships, particularly those involving mycorrhizal and endophytic fungi, are also important and could significantly influence the structure of plant hybrid zones and the distribution of plant populations and species' ranges.

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