# Nine-year reciprocal transplant experiment in the gardens of the basin and mountain big sagebrush (*Artemisia tridentata*: Asteraceae) hybrid zone of Salt Creek Canyon: the importance of multiple-year tracking of fitness

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We have studied reciprocal transplant gardens involving the hybrid zone between basin and mountain big sagebrush (*Artemisia tridentata*) in Salt Creek Canyon, Utah, for 9 years. Previously, we showed that the parental taxa and hybrids had superior reproductive and vegetative performance in their native garden. These earlier data supported the Bounded Hybrid Superiority model. Now, after 9 years, we find that the mountain seed source plants have greater relative fitness than middle hybrid zone seed source plants in the middle hybrid zone garden. These results may be due to plant density and climatic factors more conducive to mountain seed source growth than that of either basin or middle hybrid zone seed source plants. On the other hand, these fitness estimates do not take into account the timing of reproduction, which together with the age-specific survival rate, can profoundly affect lifetime fitness. The intrinsic rate of increase ($r$) takes both of these factors into account, providing another estimate of fitness. Middle hybrid zone seed source plants had the greatest rate of increase in both the middle hybrid zone and mountain gardens and a greater rate of increase than either parent in the basin garden. This is most likely due to the greater reproductive performance of middle hybrid zone plants earlier in life than either parental taxon. These results partly support the Bounded Hybrid Superiority model and show the importance of long-term studies of hybrid fitness. © 2005 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2005, **86**, 213–225.

ADDITIONAL KEYWORDS: Bounded Hybrid Superiority model – Dynamic Equilibrium model – endogenous selection – Environmental Gradient model – exogenous selection – genotype–by–environment interaction – intrinsic rate of increase – stable hybrid zone.

## INTRODUCTION

Reciprocal transplant gardens were established in 1994 across the *Artemisia tridentata* hybrid zone in Salt Creek Canyon, Utah, by Wang *et al*. (1997). Vegetative and reproductive performance of reciprocally transplanted parental and hybrid seedlings have been monitored ever since. After 2 years of growth in the gardens, both parental taxa, as well as the hybrids, had superior performance in their native garden. Estimates of composite relative fitness showed the same trend (Wang *et al*., 1997). Performance of the transplanted seedlings was again assessed after 5 years of growth in the gardens (Freeman *et al*., 1999). Both the garden in which the seedlings were raised, and the population from which the seedlings originated, significantly influenced vegetative and reproductive

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performance. Generally, native-source seedlings had superior performance, especially hybrids in the middle hybrid zone garden.

The purpose of this experiment was to test predictions of three stable hybrid zone theories: the Dynamic Equilibrium, Environmental Gradient and Bounded Hybrid Superiority models. Stable hybrid zones are those that neither expand nor contract geographically over time, despite gene flow between genetically divergent taxa. The big sagebrush hybrid zone at Salt Creek Canyon is believed to be stable; its width ($\approx$ 380 m; Miglia, 2003) has not changed in 20 years. During this 20-year period, this hybrid zone should have increased by at least 80 m in width (Freeman *et al*., 1995) because sagebrush pollen and seed are both wind dispersed and produced in abundance (McArthur, Welch & Sanderson, 1988; Young, Evans & Palmquist, 1989). Current patterns of distribution for big sagebrush and their contact zones were probably established about 12 000 years ago after the retreat of the Wisconsin glacier (Freeman *et al*., 1991 and references therein); therefore, this hybrid zone has likely been stable for thousands of years, maintained by dispersal and strong exogenous selection; Wang (1996) estimated a selection coefficient on the order of 0.1.

All three models assume that both natural selection and dispersal structure and maintain hybrid zones: parental genotypes disperse into the hybrid zone but are counterbalanced by either endogenous and/or exogenous selection. This continual process serves to maintain the hybrid zone for long periods of time. The Dynamic Equilibrium model (Barton, 1979a, b; Barton & Hewitt, 1985, 1989) assumes that hybrids are universally unfit, due to endogenous selection against inferior genotypes resulting from the disruption of coadapted parental genomes. The parental taxa experience neither endogenous nor exogenous selection, performing equally well in all environments. Such zones are believed to move until they become trapped in an area of low population density. In contrast, the Environmental Gradient model (Endler, 1973, 1977) assumes that exogenous selection operates to structure stable hybrid zones. Here, the hybrid zone is a continuous environmental gradient; fitness varies as a function of position along the gradient with exogenous selection occurring against genotypes found in suboptimal environments. Hybrids are always less fit than one of the parental taxa depending on their location along the gradient, except in the middle of the hybrid zone where the fitness of the hybrids equals that of the parental taxa.

The Bounded Hybrid Superiority model (Moore, 1977) also assumes that the environment plays the central role in hybrid zone structure and maintenance. This model is a special case of the Environmental Gradient model (Endler, 1977) where hybrids have superior fitness at the centre of the hybrid zone. The

hybrids and parental taxa are each adapted to their native habitat; exogenous selection occurs when genotypes are found in non-native habitats. Endogenous selection does not occur against hybrids because genetic divergence between the parental taxa is not great enough to disrupt coadapted genomes. Interestingly, Anderson & Stebbins (1954) presented this possibility as one explanation for adaptive radiation. Viable, fertile $F_2$ and later-generation hybrids formed between genetically distinct populations could lead to the relatively rapid appearance of new variant forms that occupy new ecological niches.

A number of different factors can give rise to the same hybrid zone structure; therefore, it may be difficult to infer the exact process or combination of processes involved (Endler, 1977; Harrison, 1990). Recently, Ross & Harrison (2002) reported that habitat preference, premating isolation and fertility selection might be acting in combination with either exogenous and/or endogenous selection to maintain the cricket hybrid zone that they studied. Jiggins *et al*. (1996) proposed five different selective forces that collectively might be acting to structure and maintain a narrow hybrid zone between the butterfly species *Heliconius erato* and *H. himera*: exogenous selection, competitive exclusion, frequency-dependent selection, assortative mating and intrinsic hybrid inferiority.

Reciprocal transplant experiments allow one to directly test whether the selection occurring within a stable hybrid zone is endogenous or exogenous. Hybrid unfitness in both the hybrid zone and parental habitats would be direct empirical evidence for endogenous selection, thereby supporting the assumption of the Dynamic Equilibrium model. Alternatively, significant genotype–by–environment interactions would support either the Environmental Gradient or Bounded Hybrid Superiority model. If hybrids have superior fitness within the hybrid zone but lower fitness outside it, then this would support the assumption that ecological selection (along with dispersal) stabilizes the hybrid zone. The most feasible reciprocal transplant experiments are those involving plants. Linhart & Grant (1997) noted that the benefits of using plants in manipulative, transplant experiments were known long before Darwin's time. These authors reported on the interesting early history of the use of common gardens to compare the different morphologies of intraspecific plants from widely diverse, usually continent-wide environments; their examples date back to the late eighteenth century.

Few plant hybrid zone studies have incorporated the use of reciprocal transplant experiments. One of the earliest was that of Levin & Schmidt (1985), involving the *Phlox drummondii* ssp. *drummondii* × *P. d. mcallisteri* hybrid zone. Although parental and hybrid seeds were sown at both parental and hybrid zone

locations, each genotype was raised separately in its own quadrat, which most likely added a confounding source of variation. The authors concluded that neither parental taxon nor hybrids were disadvantaged in terms of fitness; however, superior relative fitness was generally not observed for site-indigenous plants, a factor that was not addressed but would definitely contribute to the structure of the hybrid zone by limiting introgression. Other reciprocal transplant experiments testing stable hybrid zone theories include those of Wang *et al*. (1997), Emms & Arnold (1997), Fritsche & Kaltz (2000) and Campbell & Waser (2001). All of these experiments provided empirical evidence for environment-dependent selection, where parental and hybrid plants displayed differential vegetative and/or reproductive performance across parental and hybrid zone reciprocal-transplant sites. Only the results of Wang *et al*. (1997), however, specifically supported the Bounded Hybrid Superiority model, which showed hybrids and parental seed source plants to each have the highest fitness in the garden of their indigenous habitat. Two other reciprocal transplant experiments involving a Pacific iris hybrid (Young, 1996) zone and Australian flax hybrid zone (Carlsson-Granér, Burdon & Thrall, 1999) were also conducted, but these experiments were not designed to specifically test stable hybrid zone theories. Consequently, reciprocal transplant sites occurred only in pure parental stands but not within the hybrid zone.

Here, we provide a 9-year update for the experiment initially reported by Wang *et al*. (1997). To our knowledge, this is the longest running reciprocal transplant experiment of its kind. We wanted to determine the current status of hybrid and parental seed source plants in the reciprocal transplant gardens, to see if the fitness and performance trends reported in earlier years still existed. We specifically wanted to test the main predictions of the above three stable hybrid zone models. Superior fitness of the parental taxa coupled with hybrid unfitness in all of the gardens would support the Dynamic Equilibrium model. Alternatively, significant genotype–by–environment interactions would support either the Environmental Gradient or Bounded Hybrid Superiority models, depending on the level of fitness of hybrids within the middle of the hybrid zone. Hybrids with fitness values equalling those of the parental taxa would support the Environmental Gradient model and hybrid superiority would support the Bounded Hybrid Superiority model.

## MATERIAL AND METHODS

### STUDY SITE

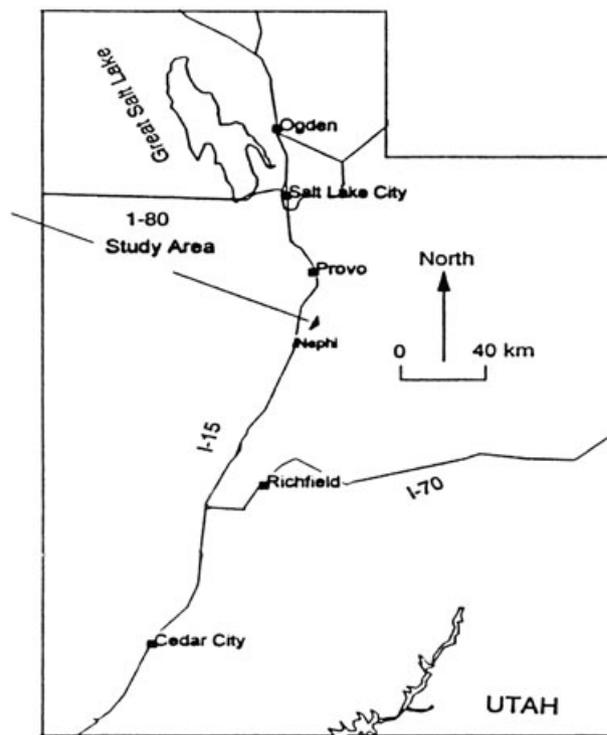The hybrid zone is located in Salt Creek Canyon, near Nephi, Utah (Fig. 1). Here, basin and mountain big



**Figure 1.** Map of the study site in central Utah.

sagebrush are parapatrically distributed, with the parental and hybrid populations situated along an elevational–moisture gradient (McArthur, 1994). Basin big sagebrush occurs below 1790 m in elevation, while mountain big sagebrush occurs at elevations ranging from 1850 m to timberline on nearby Mt. Nebo; the horizontal distance between the parental populations is approximately 774 m (Miglia, 2003). The hybrid zone is a narrow elevational band situated between the parental populations at elevations ranging from about 1790 m to 1830 m. Three reciprocal transplant gardens were established in 1994: one in each parental population and one in the centre of the hybrid zone (Wang, 1996; Wang *et al*., 1997).

### EXPERIMENTAL PROCEDURE AND STATISTICAL ANALYSES

Seeds were collected from five source populations (basin, near-basin, middle hybrid zone, near-mountain and mountain) across the hybrid zone, germinated and resultant seedlings grown 1 year in a greenhouse, using sterilized soils collected from the habitats in which the seedlings would be transplanted. Seedlings were then transplanted into the three gardens; each garden contained 12 seedlings from each source population, giving a grand total of 180 transplants (see

Wang *et al*., 1997 for a more detailed account of the experiment). Height, crown diameter, number of inflorescences and inflorescence length have all been measured yearly except 1999. In later years when most plants were highly floriferous, only the lengths of three representative inflorescences were measured and their average calculated. Two crown diameters (crown 1 = greatest diameter of plant; crown 2 = diameter directly perpendicular to crown 1) instead of one were measured from 1998 to 2002 and then the average of the two was calculated.

Plant volume and composite relative fitness were estimated parameters. Plant volume was calculated as the product of plant height (1/2 crown diameter)$^2$ (or [1/2 average crown diameter]$^2$) and $\pi$, to give the volume of a cylinder. This differed from Wang *et al*. (1997) in that they calculated plant size (i.e. height × crown diameter) rather than volume. Composite relative fitness was calculated for each source genotype in each garden as the product of survivorship through 2002, the probability of flowering, estimated total inflorescence length, seed germination and estimated number of seed produced per seedling. To convert this product to a relative value in each garden, we divided it by the same product calculated for the source genotype native to a given garden. We used the seed germination and estimated number of seed-per-seedling data of Wang *et al*. (1997), which they collected once in 1995 using the transplanted seedlings in the gardens. For descriptions of the experimental procedures and how these data were collected and analysed see Wang (1996) and Wang *et al*. (1997).

Big sagebrush reproduces early in life and has high seedling mortality (Meyer, 1994 and references therein). Because early reproduction in this case is far more advantageous in terms of lifetime fitness than later reproduction (Cole, 1954), the effect of the age of first reproduction on relative fitness was taken into account by calculating the intrinsic rate of increase ($r$) as another estimator of relative fitness using Euler's equation ($\Sigma l_x m_x e^{-r} = 1$). The intrinsic rate of increase is one of two ways to estimate potential growth of a population. It assumes environmental resources are unlimited and depends on two determinants of relative fitness: survival and fecundity (Ricklefs, 1990).

To analyse survivorship and flowering over the measurement period, these data were recoded and then grouped into the number of years that plants both survived and flowered per garden-source treatment. A MANOVA procedure was used to analyse these data. Proportion of plants that flowered during the measurement period was also calculated and analysed using a univariate ANOVA.

We wanted to see if evapotranspiration varied by year and if so, whether or not this variation was correlated with yearly differences in reproductive growth. This measure reflects the effect of several meteorological parameters collectively, including solar energy, air temperature and humidity and wind speed. Evapotranspiration measurements were obtained from the Utah Climate Center (Utah State University, 4825 Old Main Hill, Logan, Utah, 84322–4825) for the 8 years of data collection reported here. These measurements took place at meteorological observing station U14 (three letter FAA station identifier) located in Nephi, Utah, approximately 6 miles away from the study site. The data were analysed using a univariate ANOVA.

To determine if plant density differed across the hybrid zone study site, interplant big sagebrush distances were measured in both parental populations (basin and mountain), and within the hybrid zone at three locations (near-basin, middle hybrid zone, and near-mountain). Distances were measured between the centres of the plants. We looked at this parameter to determine whether or not the spacing of the transplants in the gardens favoured the densities of any of the genotypes in their indigenous habitats. Twenty plants from each site were chosen at random for the distance measurements, giving a total of 100 plants. The distance of each randomly chosen plant from each of five nearest-neighbour big sagebrush plants was measured at each site. These interplant distances were averaged per plant and the averages analysed using a univariate ANOVA. Variance in interplant distances for the basin, middle hybrid zone and mountain sites were compared using an *F*-ratio test (Zar, 1999) for each pairwise comparison.

Any of the above data not meeting the assumption of equal variances were square-root or log transformed. Significant ANOVA tests were followed by post hoc Bonferroni tests for non-transformed data and Dunnett's T3 tests for transformed data. All statistical tests, including those post hoc, were done using SPSS v. 11.0. Moreover, because the experiment as a whole was not replicated, variances could not be assigned to either composite relative fitness or the intrinsic rate of increase, thereby precluding significance testing for both.

## RESULTS

For clarity of presentation and to omit redundancy due to their incorporation in estimating composite relative fitness and the intrinsic rate of increase, results for plant volume and total inflorescence length will not be considered any further here. Details for these results can be found in Miglia (2003). Results for survivorship and proportion flowering are considered, however, because of their importance in parts of the discussion.

## COMPOSITE RELATIVE FITNESS

*Year 2002*

Similar to the presentation of results by Wang *et al.* (1997), we show the relative fitness values for only the parental taxa and middle hybrid zone plants from each combination of seed source and garden in 2002 (Fig. 2). Basin and mountain seed source plants had the greatest relative fitness in the garden of their native habitat. Mountain seed source plants also had the greatest relative fitness in the middle hybrid zone garden. Basin source plants had the lowest relative fitness in both the middle hybrid zone and mountain gardens; in fact, their fitness decreased by 62% in the middle hybrid zone garden and 97% in the mountain garden, compared to their fitness in the basin garden. When looking at the fitness of all seed source plants in all of the gardens, mountain seed source plants had the greatest fitness overall in the middle hybrid zone garden.

*All years*

Relative to each other, parental seed source plants had the greatest fitness in their native garden each year the plants were measured; there was always great disparity between the fitness of parental native and parental non-native plants. Middle hybrid zone seed source plants had the greatest relative fitness in the middle hybrid zone garden from 1994 through 1997; thereafter, however, mountain seed source plants exceeded them in fitness, notably so after 1998. Basin seed source plants always had the lowest fitness in the middle hybrid zone and mountain gardens.
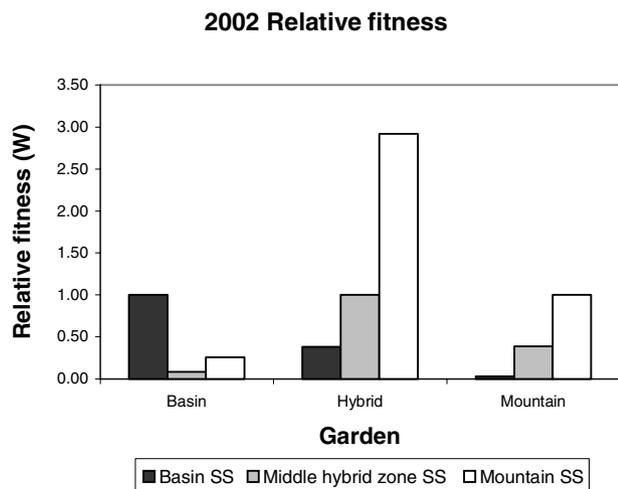
**2002 Relative fitness**



**Figure 2.** Relative composite fitness for each of the source plants in each of the three gardens (see text for calculations). SS, seed source.

## INTRINSIC RATE OF INCREASE

Estimates of the intrinsic rate of increase were calculated based on several different scenarios, including limiting the life span to an average of 12 years or allowing it to range up to 100 years. In each case we used the fecundity data observed in 2002. The results showed that the value of *r* was invariant to at least three decimal places in all cases. Middle hybrid zone seed source plants had the greatest intrinsic rate of increase in both the middle hybrid zone and mountain gardens, whereas in the basin garden the greatest rate was seen for near mountain seed source plants, followed by the middle hybrid zone seed source plants. When comparing the basin and mountain seed source plants, each had a greater intrinsic rate of increase in the garden of their indigenous habitat compared to the other (Fig. 3).

## SURVIVORSHIP AND FLOWERING

Results of the MANOVA showed that garden, seed source and garden–by–source interaction did not significantly affect survivorship. Garden and seed source, however, significantly affected the occurrence of flowering over the 8-year measurement period ($F_{2,165} = 7.742$, $P < 0.001$; $F_{4,165} = 2.523$, $P < 0.043$, respectively). There was no significant garden–by–source interaction for number of years flowered. Survivorship was least in the basin garden but greatest in the mountain garden, when averaged over all seed sources; however, the opposite was true for the mean number of years that a plant flowered (Fig. 4A, B). Plants in the mountain garden flowered significantly fewer years than those in the basin garden. Near-mountain and mountain seed source plants flowered more years than basin seed source plants, which statistically equaled that of near-basin and middle hybrid zone seed source plants (Fig. 4C).

## PROPORTION FLOWERING

Results of the univariate ANOVA showed that garden had a significant effect on the proportion of plants that flowered ($F_{2,150} = 27.593$, $P < 0.0001$). A greater number of plants growing in the basin garden flowered more often during the 8-year measurement period than in the mountain garden, when averaged over all seed sources (Fig. 5). There were no significant effects due to seed source or garden–by–seed source interaction.

## EVAPOTRANSPIRATION

Measurements did not meet the assumption of equal variances and therefore were square-root transformed. Transformation greatly improved the homogeneity; however, variances were still unequal. Results
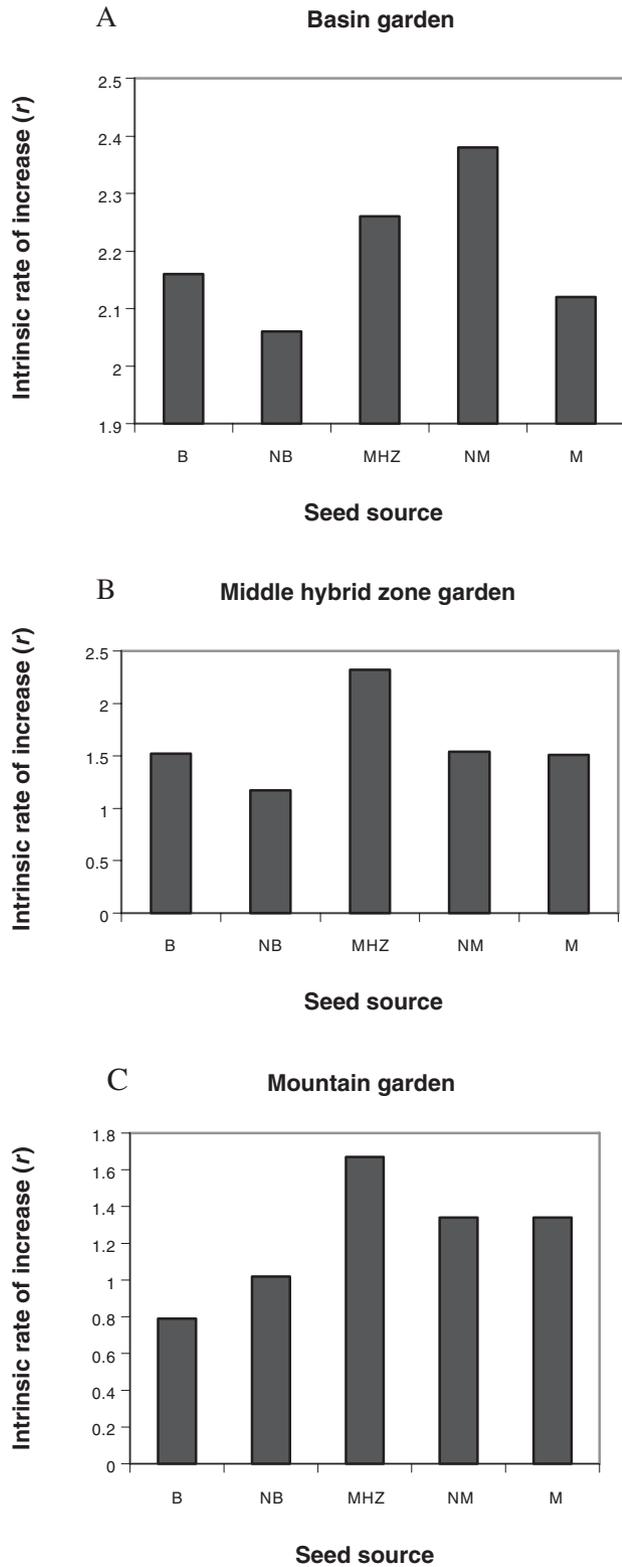
**Figure 3.** Intrinsic rate of increase for each genotype in each of the three gardens (see text for how this was calculated). Seed source populations: B, basin; NB, near basin; MHZ, middle hybrid zone; NM, near mountain; M, mountain.
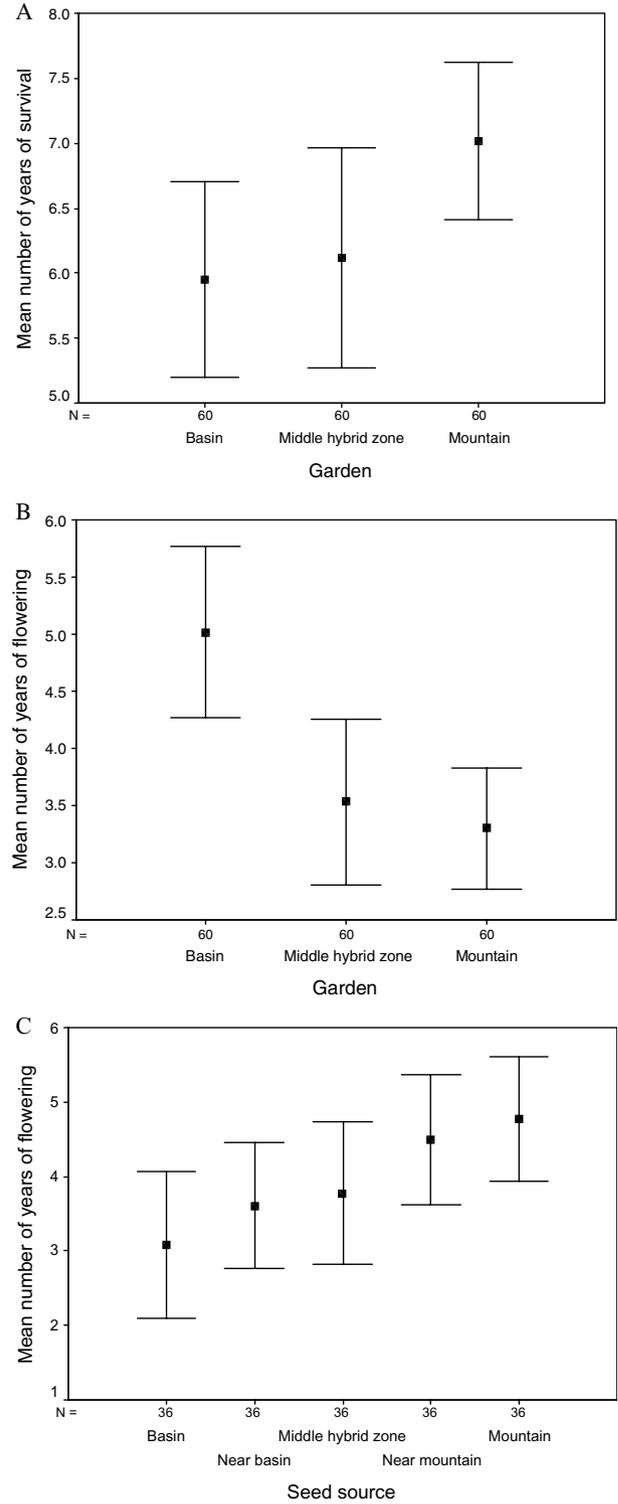


**Figure 4.** Mean number of years plants survived in each garden (A), flowered in each garden (B) and flowered for each seed source (C). Error bars represent 95% confidence intervals for the means.
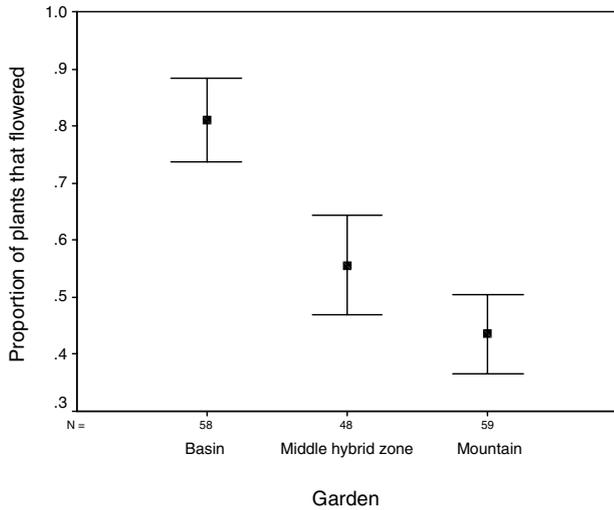
**Figure 5.** The proportion of surviving plants that flowered during the 8-year measurement period when averaged over all seed sources. Error bars represent 95% confidence intervals for the means.
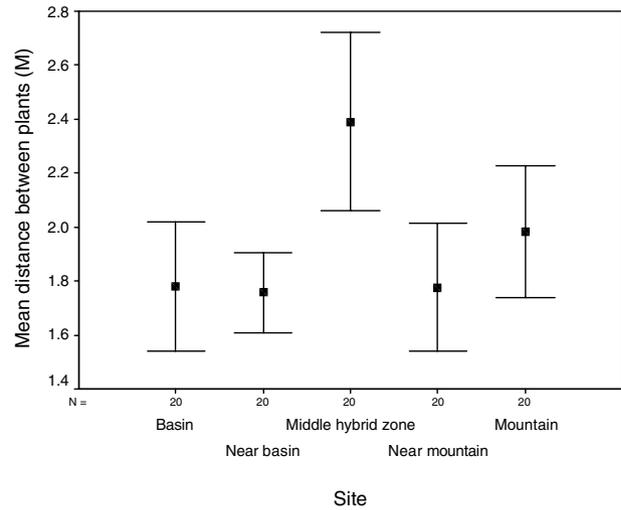


**Figure 7.** Densities (average distances among neighbouring plants) of big sagebrush plants at each of five sampling sites across the Salt Creek Canyon study site. Error bars represent 95% confidence intervals for the means.
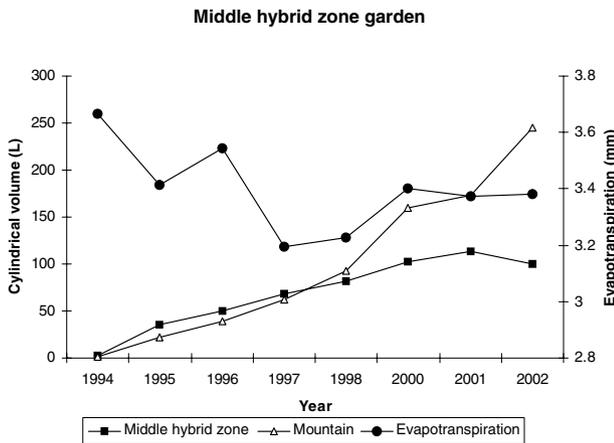


**Figure 6.** Mean cylindrical volume for middle hybrid and mountain seed source plants and average daily evapotranspiration in Nephi, Utah, during the years that the morphological data were collected from transplants in the gardens.

of the univariate ANOVA showed that evapotranspiration varied significantly by year ($F_{7,2822} = 17.54$; $P < 0.001$) (Fig. 6). Evapotranspiration was greatest in 1994 through 1996 and then dropped significantly in 1997 and 1998; thereafter it increased slightly.

### INTERPLANT DISTANCE

Big sagebrush plant density differed among the sites ($F_{4,95} = 5.187$, $P = 0.001$); it was lowest at the middle hybrid zone site and highest at the basin population site (Fig. 7). Bonferroni post hoc tests showed that

plant density separated into two groups: middle hybrid zone site densities sorted into the first group, basin population, near-basin, near-mountain and mountain site densities into the second. The density of mountain population site sagebrush plants was less than that at the basin population site, but not significantly so. The $F$-ratio tests showed that the variance in interplant distances at the middle hybrid zone site was significantly greater than that of both the basin ($F_{99,99} = 2.856$, $P = 0.0001$) and mountain ($F_{99,99} = 1.797$, $P = 0.002$) population sites. Variance in interplant distances also differed significantly between the basin and mountain population sites ($F_{99,99} = 1.590$, $P = 0.01$).

### DISCUSSION

The results of the later years differed from those of Wang *et al.* (1997) who only measured the transplants during the first two years of growth. The fitness of mountain plants was greater (rather than less) than middle hybrid zone plants in later years in the hybrid garden. There are two possibilities why this might be the case. First, the interspacing of plants in the gardens may be more conducive to the growth and reproduction of mountain vs. middle hybrid zone plants. The density of indigenous plants was significantly less in the middle of the hybrid zone than that of indigenous plants in either the mountain or basin habitats across the Salt Creek Canyon hybrid zone. Density was also greater for transplants in each of the three gardens compared to natural plant densities. If middle hybrid zone plants are adapted to greater interplant

spacing, then their vegetative and/or reproductive growth would be more adversely affected over time than that of the parental seed source plants, as the gardens became more crowded.

For vegetative growth between years 2001 and 2002 and reproductive output between years 2000 and 2001 this trend was observed (Miglia, 2003), but not for any of the other years of measurement. All plants had similar vegetative growth rates from 1994 through 1997, whereas both vegetative and reproductive output was far greater for mountain plants in the middle hybrid zone garden compared to middle hybrid zone, as well basin plants after 1998. Perhaps interspacing became a selective force in later years as plant density increased, such that hybrid growth rates diminished relative to those of the mountain plants. To test this, the growth rates of naturally occurring seedlings outside the gardens vs. those of seedlings growing inside the gardens would have to be tracked and compared over time.

A second possibility explaining the disparity in productivity between mountain and middle hybrid zone plants in the middle hybrid zone garden during the later years is that the climate may have varied by year, such that environmental conditions in later years were more conducive to the growth of mountain plants, but less so in earlier years. Growth rate of big sagebrush has been shown to decrease under suboptimal conditions (DePuit & Caldwell, 1973), with dieback and even death occurring if conditions are too unfavourable (Ferguson, 1964, referenced by McArthur & Welch, 1982). To explore this hypothesis, we looked at evapotranspiration during the years of measurement reported here. Evapotranspiration is the transfer of moisture to the atmosphere from both the soils through evaporation and plant surfaces through transpiration. We chose this variable because it reflects several meteorological parameters simultaneously, including radiation, air temperature, humidity and wind speed.

Results showed that evapotranspiration varied significantly by year; cooler, wetter climates (i.e. lower evapotranspiration) occurred from 1997 through 2002, which generally corresponded to the dramatic increase in the vegetative and reproductive growth rates of mountain plants in the middle hybrid zone garden (Fig. 6). Middle hybrid zone plants did not show the same dramatic response to this change in climate. Mountain population plants live at higher elevations than basin population or indigenous middle hybrid zone plants (McArthur & Plummer, 1978) and are thought to have a different life history strategy than basin big sagebrush (see below). At these higher elevations, the soils are moister and the temperatures cooler (McArthur *et al.*, 1988; McArthur, 1994). It is quite plausible that the cooler, wetter climates in later

years fell within the optimal ranges of growth for mountain big sagebrush, thereby facilitating faster vegetative and reproductive growth rates compared to the middle hybrid zone plants or their own growth rates in earlier years.

Basin plants had the lowest fitness all years in the mountain garden, while mountain plants had lower fitness than basin plants all years in the basin garden. This trend was most likely due to their adaptation to different environmental conditions, which was evidenced by the significant seed source–by–garden and year–by–seed source–by–garden interactions observed for both total inflorescence length and plant volume. The dramatic growth response observed for mountain plants in the middle hybrid zone and mountain gardens was probably not observed in the basin garden because of the genotype–by–environment interactions. As for the basin and middle hybrid zone plants, there was generally not a dramatic response to a change in evapotranspiration in any of the gardens. The different life history strategies and morphological structures of mountain and basin big sagebrush may also explain the general disparity in response of the parental and middle hybrid zone plants to the varied climatic conditions.

Bonham, Cottrell & Mitchell (1991; see also Meyer, 1994) proposed different life-history strategies for basin and mountain big sagebrush, based on populations growing in western Colorado. Basin big sagebrush should have a colonizing strategy, due to the prolific production of smaller seed and faster growth rates at early seedling, as well as later life stages (McArthur & Welch, 1982; Meyer, 1994 and references therein). Basin big sagebrush often occurs in the frequently disturbed, relatively fertile floodplains (McArthur *et al.*, 1979; McArthur, 1994). Seedlings that establish themselves quickly will lock up nutrients, thereby preventing other seedlings from becoming established. Moreover, they also have a greater chance of reproducing earlier in life and in doing so, increase their fitness. Alternatively, mountain big sagebrush should have a competition-based strategy, because it produces a regular, but not always large seed crop, and has slower growth rates.

Such a strategy seems appropriate for plants that live in resource-limited environments where competition is more intense, such as that of mountain big sagebrush, which is generally found in shallow, less fertile and more mesic soils on mountain slopes (McArthur *et al.*, 1979; McArthur, 1994; Meyer, 1994; and references therein). Greater energy should be put into vegetative growth, rather than reproduction, in order to compete successfully (see Meyer, 1994 for a complete discussion). In years when resources are more abundant, a greater allocation to seed production would increase the fitness of mountain plants, but

not in years where resources are limiting. In the present study, mountain seed source plants experienced slower vegetative and reproductive growth rates in the first four years; this was most likely due to their intrinsically slower growth rates and greater allocation of energy to vegetative growth rather than reproduction during the years when the climate was drier.

The life-history strategy of middle hybrid zone hybrids has not been thoroughly examined. Their lack of response to evapotranspiration changes suggests that it differs from the competition-based strategy proposed for mountain big sagebrush. Because their habitat has characteristics distinct from those of either parental population, it is likely that they have a life-history strategy that differs from that of basin big sagebrush as well. More complete studies of seed production, seed size and vegetative and reproductive growth rates of middle hybrid zone hybrids are needed to determine their life-history strategy.

Overall, our results support the more generalized Environmental Gradient model (Endler, 1977) and seem to be at odds with the Bounded Hybrid Superiority model (Moore, 1977). Although we observed significant seed source by environment interactions, as well as superior fitness of parental plants all years of study in their native gardens, there was an absence of hybrid superiority during the last four years in the hybrid garden. During this time, the fitness of mountain seed source plants exceeded that of middle hybrid zone seed source plants. However, the fitness estimates above did not take into account the timing of reproduction, which coupled with the age-specific reproduction and survival rates, can greatly affect lifetime fitness. Plant species with high seedling mortality, like big sagebrush, should have greater relative fitness if reproduction begins early in life. Big sagebrush reproduces relatively early in life and continues to be highly floriferous into later years (Meyer, 1994).

The intrinsic capacity for population increase ($r$) takes both the timing of reproduction and age-specific survival rate into account, providing a broader-based estimate of relative fitness (Stearns, 1976; Krebs, 2001). One drawback in using this estimator is the assumption that environmental conditions remain unchanged during the lifespan of the organism. Nonetheless, it provides a useful projection of population growth. Our estimates of the intrinsic rate of increase showed that the middle hybrid zone plants had the greatest rate of increase in both the middle hybrid zone and mountain gardens and a greater rate of increase than either parent in the basin garden. This is undoubtedly due to the greater reproductive performance of middle hybrid zone plants earlier in life. Near basin plants had the greatest rate of increase in

the basin garden. In comparing the parental taxa, each had the greatest rate of increase in their native garden. These results partly support the Bounded Hybrid Superiority model in that the middle hybrid zone plants experienced no hybrid inferiority in any of the gardens compared to the parental plants. The fact that middle hybrid zone plants had a greater rate of increase, and presumably greater fitness, than either parent in all three gardens shows the importance of not only reproducing earlier in life, but also the importance of greater allocation to reproduction at this early life stage, in long-lived species with high juvenile mortality.

These results point out the importance of measuring fitness at more than just one point in time. Fitness is relative, not absolute; it therefore depends on both the set of environmental conditions and the genotypes present at a given time in a given population (Futuyma, 1998; Krebs, 2001). As summarized by Niklas (1997), the fitness of a flowering plant involves many factors, including successful seed germination, subsequent seedling growth to maturation and reproduction. Flowers produced must be successfully pollinated and viable seeds produced. Big sagebrush has the potential for high fitness in that it is long-lived, iteroparous and produces copious amounts of both pollen and seed. Moreover, two-year-old plants can be as fecund as relatively old, 100-year-old plants (Young *et al.*, 1989). However, a number of factors can limit seed production and the establishment and growth of seedlings. As mentioned above, genetic differences between the subspecies, habitat and year-to-year rainfall differences can affect seed set (Young *et al.*, 1989), whereas seed germination and establishment can be affected by non-persistent seed banks, drought, late-spring frosts, both intra- and interspecific competition, timing and amount of snowfall and grazing (Meyer, 1994 and references therein). Because of these factors, big sagebrush is a good example of the importance of tracking estimators of fitness multiple times in different seasons and years, especially when testing stable hybrid zone theories, which assume either universal hybrid unfitness or parental and hybrid superiority in their unique, indigenous environments.

Interestingly, plants in the basin garden survived the fewest number of years, but produced flowers the greatest number of years during the entire measurement period (Fig. 4), with both findings statistically significant. The proportion of surviving plants that flowered (Fig. 5) and total inflorescence length (data not shown here, see Miglia, 2003) was also the greatest in the basin garden over the same period. The opposite was true for plants growing in the mountain garden for all four variables. These results coincide with mean plant volume, where plants in the basin

garden had significantly the greatest volume and plants in the mountain garden the least (data not shown here, see Miglia, 2003). Although interplant distances in all gardens were less than those occurring outside the gardens, plants in the basin garden were most likely more stressed than plants in the mountain garden, due to their significantly larger plant sizes. It is possible that increased amount and incidence of flowering for basin garden plants was due to this additional stress.

Research has shown that plants under stress can flower earlier and/or allocate more energy to reproductive than to vegetative structures (but see Meekins & McCarthy, 2002). For example, one study of reproductive allocation in cotton (Sadras, Bange & Milroy, 1997) showed that plants grown under severe stress (no fertilization and high plant density) had a significantly greater allometric coefficient (i.e. the ratio or reproductive growth rate vs. shoot growth rate) than non-stressed plants. Desclaux & Rournet (1996) showed that stress due to drought during the vegetative stage caused the earlier emergence of flowers in two soybean cultivars at the expense of delaying node appearance. In fact, each reproductive stage was shortened for plants under stress, which also led to earlier senescence. Lu & Chacko (2000) also showed that a water deficit promoted earlier and greater flowering in two cultivars of mango. Other studies have shown an increase in seed and/or fruit yields with increased plant density (e.g. increased seed yield in watermelon: Edelstein & Nerson, 2002; increased fruit yield in coffee: Arias-Basulto *et al*., 2002; increased fruit yield in both the early and late seasons: Lopez-Medina *et al*., 2001).

Interplant distances were significantly greater and more variable for indigenous sagebrush plants growing at the middle hybrid zone site. These findings suggest that the hybrid zone proper is situated in a habitat distinct from that of either parental population, which coincides with results from earlier studies of the Salt Creek Canyon study site. Wang, McArthur & Freeman (1999) found that soil properties (pH, depth and elemental composition) differed significantly among the soils of both parental population and middle hybrid zone sites. Middle hybrid zone soils were not only chemically distinct from the parental population soils but also spatially more variable, especially in the proportions of the ten elements assayed. Middle hybrid zone plants have also been shown to differentially use available resources (Wang *et al*., 1999), display greater plant-to-plant variation in their biological absorption coefficients (the ratios of elemental leaf to soil concentrations) and leaf chemistry (Freeman *et al*., 2001) and produce novel terpenes compared to the parental taxa (Byrd *et al*., 1999).

More recent work (Miglia, 2003) found that: (1) soils in the middle hybrid zone garden had the greatest bacterial substrate activity and substrate richness (i.e. greater bacterial functional diversity); (2) middle hybrid zone plants had unique fungal endophytic colonization patterns, from both parental population plants, which also differed from each other; and (3) the middle hybrid zone site had the widest average daily range of temperatures and highest average daily maximum temperature during the 2 years surveyed. The middle hybrid zone habitat is not only unique but also heterogeneous. Because the hybrids have more successfully occupied this heterogeneous environment than the parental taxa (Freeman *et al*., 1995, 1999; Graham, Freeman & McArthur, 1995; Wang *et al*., 1997; Graham, McArthur & Freeman, 2001), the environment must be important in structuring the Salt Creek Canyon hybrid zone, providing evidence for the environment-dependent Environmental Gradient and Bounded Hybrid Superiority models.

Significant genotype–by–environment interactions have been observed in previous Salt Creek Canyon studies (Wang *et al*., 1997, 1999; Byrd *et al*., 1999; Freeman *et al*., 1999) for a number of morphological and chemical parameters and for the morphological variables in the update reported here. This, coupled with the greater plant-to-plant variation outlined above for middle hybrid zone plants, not only shows that there is a genetic basis underlying the differential performance of parental and hybrid plants across the hybrid zone, but also reflects the greater genetic variability of hybrid plants. As Wang *et al*. (1998, 1999) have argued, hybridization may provide big sagebrush hybrids with the genetic variability necessary to successfully occupy the highly heterogeneous environment of the middle hybrid zone site.

## CONCLUSION

Our results clearly show that ecological selection structures the Salt Creek Canyon hybrid zone, with hybrids having the highest intrinsic rate of increase, not only in their native garden, but in the parental gardens as well. These results partly support the Bounded Hybrid Superiority model, a specialized case of the Environmental Gradient model. It should be noted, however, that the two types of models presented here (i.e. the two environment-dependent gradient models vs. the environment-independent Dynamic Equilibrium model) are not mutually exclusive. It is possible to have both hybrid superiority and hybrid unfitness with an Environmental Gradient model. One would expect such variation in fitness among hybrids within the hybrid zone due to both endogenous and exogenous factors. Among hybrids most intrinsically fit, there would then be ecological selec-

tion both in space and over time. The data of later years in the sagebrush hybrid zone, when the mountain plants outperformed the middle hybrid zone plants in the hybrid garden, do not allow us to exclude this possibility.

The results of this study show the importance of long-term measures of fitness for long-lived taxa with complex life histories in hybrid zones such as basin and mountain big sagebrush. Multi-life-stage comparisons, using the intrinsic rate of increase, provide more comprehensive estimates of fitness than composite relative fitness within any given year, which does not take into account the timing of reproduction or age-specific fecundity and survival rates. By incorporating these two factors, the intrinsic rate of increase integrates fitness across the entire lifespan of an organism, taking into account the variations in fitness that occur over time due to various genetic and environmental factors. Our results exemplify this important difference between these two estimates of fitness. Moreover, the fact that hybrid fitness varied by year and presumably by life stage, reiterates the importance of multiple-year tracking of fitness.

Several evolutionary factors can often yield the same pattern. In such cases, the factors receiving the most attention in the study may reflect the investigator's bias. We have tried to minimize these interpretative problems by conducting manipulative experiments; yet, despite our efforts, weather conditions and age of plant have been somewhat confounded. Moreover, the present study also confounded edaphic and elevational differences. Consequently, we have yet to identify all aspects of the environment to which each of the big sagebrush genotypes have become adapted.

Most studies of hybrid zones are conducted by population geneticists that naturally seek answers among allele frequency differences and genetic interactions. Alternatively, we have adopted an ecological approach (Harper, 1967), using manipulative experiments in the field. Our work shows that each parental taxon and their hybrids are adapted to their own unique environment, which is in agreement with Linhart & Grant (1997) – that strong local selection structures the Salt Creek Canyon hybrid zone.

## ACKNOWLEDGEMENTS

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