

## COTTONWOOD HYBRIDS GAIN FITNESS TRAITS OF BOTH PARENTS: A MECHANISM FOR THEIR LONG-TERM PERSISTENCE?<sup>1</sup>

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Using surveys of natural populations, experimental crosses, and common garden trials, we tested the hypothesis that hybrid cottonwoods (*Populus fremontii* × *P. angustifolia*) from the Weber River in northern Utah would produce as many viable offspring as their parental species. We found that both F<sub>1</sub> generations and backcross generations can be just as fit as the parent taxa. First, F<sub>1</sub> hybrids produced as many viable seed as *P. angustifolia* (but less than *P. fremontii*), and backcross genotypes produced as many viable seeds as both parent taxa. Second, hybrids produced nearly two times as many ramets from root sprouts as *P. angustifolia* and four times as many ramets as *P. fremontii*. Third, the high mortality of germinated seedlings of all tree types (i.e., >90%) and very low mortality of asexually derived ramets provide hybrids with equal sexual reproduction and enhanced asexual reproduction, especially since backcross hybrids exhibit transgressive segregation in ramet production. Our findings suggest that the introgression of *P. fremontii* seed traits into the hybrid genome is responsible for their equivalent performance (at least to one parent) in sexual reproduction, while the contributions of asexual traits from *P. angustifolia* results in hybrids having equal or greater fitness.

**Key words:** hybrid fitness; introgression; *Populus*; ramets; seed viability; sexual and asexual reproduction; transgressive segregation.

Hybrids are important evolutionarily only if they persist in time and space by demonstrating some level of fitness, thus avoiding the label of “botanical mules” (Roberts, 1929). Both endogenous and exogenous factors can play a part, individually or together, in selection against a hybrid individual or population (Shaw et al., 1993; Rieseberg, 1995, 1997; Arnold, 1997; Rieseberg and Carney, 1998). Three major models have been developed to explain the role of endogenous (genetic traits) vs. exogenous (environmental) selection in hybrid zone evolution (Moore, 1977; Barton and Hewitt, 1985; Rand and Harrison, 1989). Of these, the tension zone (Barton and Hewitt, 1985) and mosaic models (Rand and Harrison, 1989) of hybrid stability both assume an inherent lack of fitness for the hybrids (see Arnold, 1997, for a complete review). However, many empirical studies examining plants fail to demonstrate hybrid inferiority (Arnold, 1997; Rieseberg and Carney, 1998; Arnold et al., 1999), though the localized occurrence and limited distributions of many hybrid zones indicate that a combination of factors act to limit their populations.

In an attempt to better account for this complexity, Arnold (1997) proposed a new model (Hybrid Novelty Model) of hybrid zone dynamics. An important contribution of Arnold's model is that it emphasizes the importance of examining the fitness of different genotypic classes of hybrids. Hybrid genotypes are extremely variable with respect to fitness, both within and between hybrid classes (Rieseberg and Carney, 1998). Generally, however, F<sub>1</sub> or intermediate hybrids are low-

est in fitness, while advanced generation hybrids that resemble one or the other parental species are higher in fitness (Arnold and Hodges, 1995).

Understanding the factors that contribute to fitness could help us understand how hybridization plays a role in plant evolution (e.g., Rieseberg et al., 1996). Such studies may be especially important in plant groups that have a widespread and long evolutionary history of hybridization, such as in cottonwoods (*Populus* spp.). Considering that intersectional hybridization in poplars has been recorded as common in the fossil record of the Tertiary throughout western North America (Eckenwalder, 1984b) and that hybrid speciation is thought to have played a role in the evolution of poplars (Smith and Sytsma, 1990), it is important to understand the mechanisms of hybrid zone persistence. Furthermore, because most studies of hybrid fitness have concentrated on animals or herbaceous plants, it is novel and relevant to broaden the perspective to long-lived species, such as trees that dominate specific habitat types, have significant economic and conservation value, and are known to support a large dependent community of organisms (e.g., Whitham et al., 1999).

Here we examine sexual and asexual reproduction of the intersectional hybrids between two species of poplar (*P. fremontii* L. and *P. angustifolia* James). Although the importance of sexual reproduction, in general, has been extensively studied, much less is known about the ecology and evolution of asexual reproduction. Thompson and Stewart (1981), Buss (1985), Eriksson (1992), and Gardner and Mangel (1997) argue that clones can extend the genet by allowing it to persist in space and time, facilitate introgression, and contribute to the overall fitness of hybrids. Cloning, as an evolutionary strategy, is thought to have evolved as a mechanism to reduce the risk of mortality as well as a mechanism to exploit heterogeneous habitats, due to the interconnections of the genet (Cook, 1979, 1985; Sutherland and Stillman, 1988; de Kroon and

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Schieving, 1990). Clonal growth occurs in all 11 classes of vascular plants indicating its importance as a mode of reproduction (Mogie and Hutchings, 1990). The formation of ramets (asexual units that may become independent) can contribute to plant fitness by extending or maximizing the expected time to extinction of the genet (sensu Cooper, 1984).

We examined a suite of reproductive traits of hybrid cottonwoods relative to the parental taxa to determine which parameters contribute to their persistence. To determine whether sexual and asexual reproductive traits of hybrid cottonwoods are less than, equal to, or greater than either or both of the parent species in the short term, we initiated a 3-yr observational and experimental study that compared both sexual (seed number, mass and viability up to 3 yr in both wild and synthetic populations) and asexual reproduction (ramet production and survival in the wild and in common gardens).

*Populus fremontii* and *P. angustifolia* naturally hybridize whenever the two species come in contact, creating hybrid zones at the edges of their elevational boundaries (Eckenwalder, 1984a). Depending upon the river system, hybrid zones range in size from a few kilometers to over 100 km in length (Floate, Martinsen, and Whitham, 1997). Because these species only demonstrate unidirectional introgression (Keim et al., 1989), we used the results of molecular studies to trace the introgression of nuclear markers from one parent (Fremont cottonwood) to different hybrid classes (Martinsen et al., 2001) and correlate their effect on specific reproductive traits. We conducted a series of field surveys of known female genotypes of both pure species as well as two hybrid classes to examine the relationship between level of introgression and seed and ramet traits. We also experimentally crossed known genotypes in the greenhouse to generate both pure parents,  $F_1$ , backcross one ( $BC_1$ ) and backcross two ( $BC_2$ ) generations of known pedigree. With these observations and experiments we addressed the following question: Do hybrids exhibit inherently lower fitness in sexual or asexual reproduction than either parental species?

## MATERIALS AND METHODS

**Natural surveys**—Field surveys of each pure species and hybrid class were performed in the summer of 1997 and in 1999 to assess reproductive parameters of both seeds and ramets. For both years we conducted field surveys of reproductive traits of trees of known genotype along the Weber River near Ogden, Utah to assess the effects of hybridization on female reproductive capacity, seed traits, and seedling mortality. These field genotypes were previously identified by restriction fragment length polymorphism (RFLP) analyses in which the number of Fremont alleles (of 35 markers) was quantified for each tree. For example, pure Fremont cottonwood is characterized by 70 Fremont alleles, pure narrowleaf cottonwood is characterized by having 0 Fremont alleles,  $F_1$  hybrids as 35, and backcross hybrids were any score between 0 and 35 (see Martinsen et al., 2001 for specific methods).

To assess seed production, we collected 15–20 open-pollinated catkins from 20 to 25 Fremont,  $F_1$ , and backcross hybrids and narrowleaf cottonwood trees from their respective zones. For five randomly chosen catkins from each tree we counted the number of capsules per catkin, and from those we quantified the number of seeds per catkin from ten randomly chosen capsules to estimate the number of seeds per catkin. Subsampling was necessary due to the large number of seeds produced and their small size. Furthermore, the consistency of seed traits from the large sample sizes in our experimental crosses and initial field surveys indicated that subsampling was appropriate.

High seedling mortality is commonly reported for cottonwoods in both managed and unmanaged systems, because they require specific geomorphic niches and moist conditions to persist until the roots of the seedlings are long

enough to reach the water table (Schreiner, 1974; Fenner, Brady, and Patton, 1985; Asplund and Gooch, 1988; Siegel and Brock, 1990; Shafroth, Auble, and Scott, 1995; Mahoney and Rood, 1998). Because of this and the high rates of mortality in the greenhouse, in 1999 we assessed the mortality of seedlings in the field by establishing 75 0.25 m<sup>2</sup> field plots (25 plots in each zone) in which seedlings had germinated but were still at the cotyledon stage. To quantify natural mortality we counted the number of seedlings in each plot and resurveyed after 1 mo.

In addition to measuring seed number per capsule and capsules per catkin, in 1999 we quantified the seed mass and viability of 15 randomly chosen seeds from 25 Fremont, 25 narrowleaf, 5  $F_1$  hybrids, and 25 backcross hybrid cottonwoods of known genotype. The small number of  $F_1$ s is due to their natural low frequency in the wild. Quantifying the percentage of seed germination assessed seed viability. Within 3 d of collection, seeds were placed on filter paper in petri dishes that were moistened with distilled water every 12 h. Viable seeds generally germinated in less than 24 h though assessment was quantified over 48 h. A successful germinant had a cotyledon and a radicle.

To assess the relative female reproductive effort between species and crosstypes (i.e., hybrid class) in the field we quantified the total number of seeds and ramets produced by each individual tree. We estimated total catkin production by counting the number of catkins on a large branch and estimating the number of branches on each mature tree. From this estimate we combined the number of seeds produced per catkin to estimate the total number of seeds produced by each tree (see methods in Moran and Whitham, 1990). Additionally, for each tree we also counted the total number of ramets that likely shared a common rootstock. Synchronous phenology by trees and their ramets (Gom and Rood, 1999a), separate sexes, similar morphology of ramets within a clone, the spatial separation of many clones, as well as digging to expose a few connected root systems gave us confidence in our ability to accurately assess clonal affiliations.

Each of the response variables from the natural surveys were analyzed within each year with ANOVA to compare the differences between the species and crosstypes, covaried by tree size (measured as diameter at breast height [dbh]). Post hoc tests were conducted with Tukey's HSD (honestly significant difference) test, corrected with sequential Bonferroni adjustments when pairwise comparisons were made. The data were square-root transformed when assumptions of normality and homogeneity of variance were not met.

**Common garden surveys**—Clonal reproduction is an important mechanism for regeneration in many cottonwood species (Moss, 1938). Fremont cottonwood, along with other members of the section *Aeigerios*, only occasionally clone or send up root suckers (Irvine and West, 1979; Howe and Knopf, 1991), while members of the *Tacamahaca* section, which include narrowleaf cottonwood, clone prolifically (Schier and Campbell, 1976; Rood et al., 1994; Braatne, Rood, and Heilman, 1996; Eckenwalder, 1996). We used a common garden that was planted in 1991 to experimentally assess clonal traits between the species and hybrid classes in a common environment. These trees are just becoming mature and are highly variable in catkin production, making analysis of seed traits difficult at this time. To quantify ramet mortality we surveyed the number of ramets that had been produced by the pure species and hybrid classes ( $F_1$  and  $BC$ 's) from trees in the common garden that were the same age and of similar dbh. The average number of ramets produced by individual Fremont, narrowleaf,  $F_1$ , and backcross hybrids were assessed in 1998, and the same trees were resurveyed in 1999 to quantify mortality.

We compared the number of ramets that were produced by individual genotypes of each hybrid class and parent taxa ( $N = 20$ – $40$  trees of each tree type) with ANOVA. We used a Tukey's HSD test to determine differences between groups in ramet production. Because of unequal variances, the tree categories were assigned ranks before running the ANOVA. Differences in mortality among tree types were compared using chi-square analysis on percent mortality for each tree type.

**Experimental crosses**—To assess various reproductive traits of trees with known pedigree, we experimentally crossed genotypes of male and female cottonwoods to create seeds of known hybrid class. We cut branches with

large numbers of flower buds from known genotypes along the Weber River in northern Utah in early spring. The genotypes were previously identified by RFLP analyses in which the number of Fremont markers (of 35) were quantified from >500 individual trees along the length of the Weber River drainage to identify hybrid status (Martinsen et al., 2001). Branches with male flower buds were placed in 18.93-L (5-gallon) buckets filled with water and an aerator while female branches were placed in 10 × by 28 cm polyvinyl chloride (PVC) tubes with a potting mix and then placed in a 18.93-L bucket with water and an aerator (Stanton and Villar, 1996). To make each specific cross, the branches were placed in separate greenhouses to prevent accidental pollination. After the male flowers emerged, pollen was collected daily and stored in plastic vials until the female flowers emerged, which generally occurred within 1 wk. The pollen was dusted daily onto each open female flower with separate paintbrushes. The following crosses were made with single female and pollen donors in an effort to assess general traits of each species and cross type that naturally occur in the field: Pure Fremont cottonwood was established by crossing *P. fremontii* × *P. fremontii*, pure narrowleaf was established by crossing *P. angustifolia* × *P. angustifolia*, F<sub>1</sub> hybrid was established by crossing *P. fremontii* × *P. angustifolia*, BC<sub>1</sub> hybrid was established by crossing an F<sub>1</sub> hybrid × *P. angustifolia* and BC<sub>2</sub> was made by crossing a BC<sub>1</sub> × *P. angustifolia*. For each cross we assessed the following variables: number of seeds per catkin, seed mass, seed germination, and viability in the laboratory and greenhouse as well as long-term seedling survival ( $N = 500$ – $1000$  seeds/cross). Morphological data were collected on seedlings that survived 3 yr. We measured seedling height, collar diameter, and the length of the longest internode from the current year's growth as measures of performance.

We compared the mean mass of seeds as well as the performance measurements from the hybrid cross types and parent taxa with ANOVA, using Tukey's HSD test as a post hoc test, and long-term survival, after 3 yr, with chi-square. We used logistic regression to assess the relationship of seed mass to germination for each specific cross type from the experimental crosses (Hassler, Sinclair, and Kallio, 1986; Agresti, 1996; Nicotra, 1998; Fritsche and Kaltz, 2000).

**Introgression**—To better assess the relationship between various parameters of fitness and the hybrid status of individual trees, we computed a hybrid score (identification index based on the number of Fremont markers/the total number of markers surveyed) for each genotype. We then regressed this score on specific seed and ramet traits, using a linear model. Because introgression in this system is unidirectional (from Fremont to narrowleaf cottonwood), hybrid status was quantified based on presence/absence of Fremont alleles (35 RFLP markers described above; also see Martinsen et al., 2001). In addition to illustrating the importance of introgression to hybrid fitness, hybrid indices (for examples see Rand and Harrison, 1989; Nason, Ellstrand, and Arnold, 1992) may circumvent the difficulties of classifying hybrid phenotypes into discrete groups based on character data (Boecklen and Howard, 1997; Rieseberg and Linder, 1999).

## RESULTS

**Seed traits**—Hybrids allocate as much or more biomass to seeds and germinate equally well as one or both of the parental species. From both the natural surveys and experimental crosses we found that hybrid seeds weigh as much or more than at least one parent. Open-pollinated F<sub>1</sub> genotypes in the field produce seed that is equivalent in mass to Fremont seeds and almost two times heavier than narrowleaf genotypes while backcross hybrid seeds were 28.7% heavier than narrowleaf seeds (Fig. 1A;  $F_{3,76} = 21.721$ ,  $P < 0.0001$ ). Hybrid seed from the experimental crosses demonstrated intermediacy, with both hybrid classes weighing more than narrowleaf seeds but less than Fremont seeds (Fig. 1B;  $F_{4,36} = 12.114$ ,  $P < 0.0001$ ). Similar to other published accounts (Braatne, Rood, and Heilman, 1996) Fremont seeds in the field and from the experi-

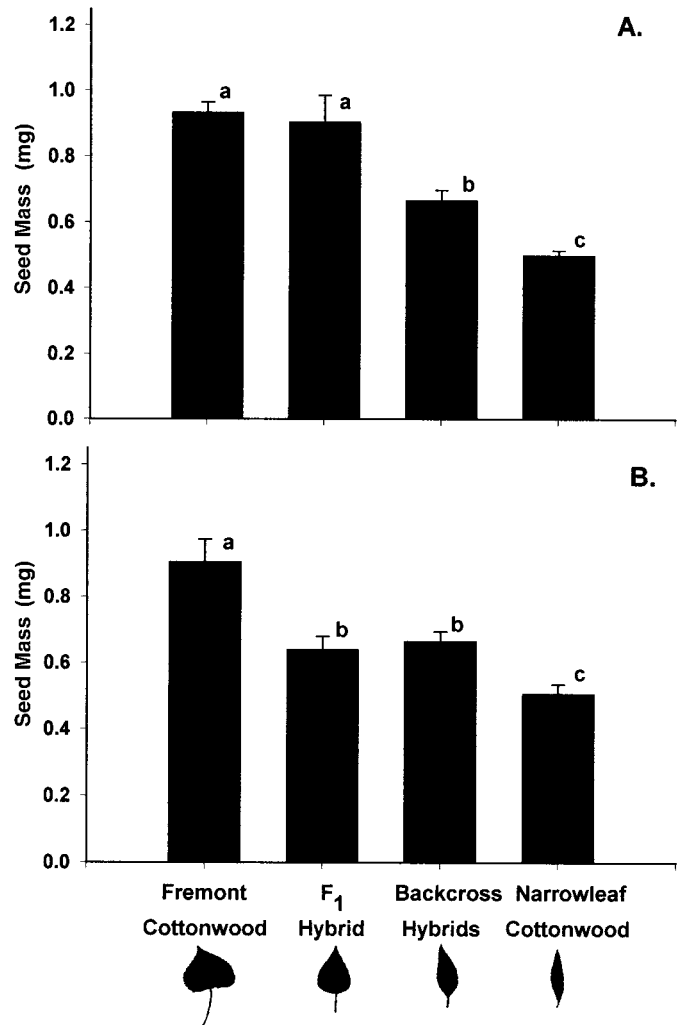


Fig. 1. Mean seed mass for each *Populus* species or cross type. The seeds of hybrid cross types weigh as much or more than the parent trees. In the field (A), seeds from both F<sub>1</sub> and backcross genotypes (BC<sub>1</sub> and BC<sub>2</sub> combined) weigh more than narrowleaf seeds, while F<sub>1</sub> seeds are equivalent in mass to Fremont seeds ( $F_{3,76} = 21.721$ ,  $P < 0.0001$ ). The experimental crosses (B) demonstrate that the hybrid classes are intermediate to both parents in the size of their seeds ( $F_{4,36} = 12.114$ ,  $P < 0.0001$ ). Error bars represent 1 SE of the mean.

mental crosses weighed nearly twice as much as narrowleaf seed.

We found that >80% of open-pollinated seeds from genotypes in the field germinated, regardless of species or cross type ( $F_{3,75} = 2.079$ ,  $P = 0.111$ ). All seeds germinated in less than 12 h and had roots and leaves after 24 h. High germination rate and rapid growth are commonly reported for *Populus* as a strategy for establishing along exposed point bars after flooding (Fenner, Brady, and Patton, 1985; Shafroth, Auble, and Scott, 1995; Braatne, Rood, and Heilman, 1996; Mahoney and Rood, 1998), and the hybrid genotypes performed no differently.

When we compared the relationship of the mass of seeds from the experimental crosses with germination in the greenhouse, we found that seed mass was important for germination for all tree types except Fremont cottonwood. Bigger seeds increased the probability of germination for F<sub>1</sub> hybrids, back-

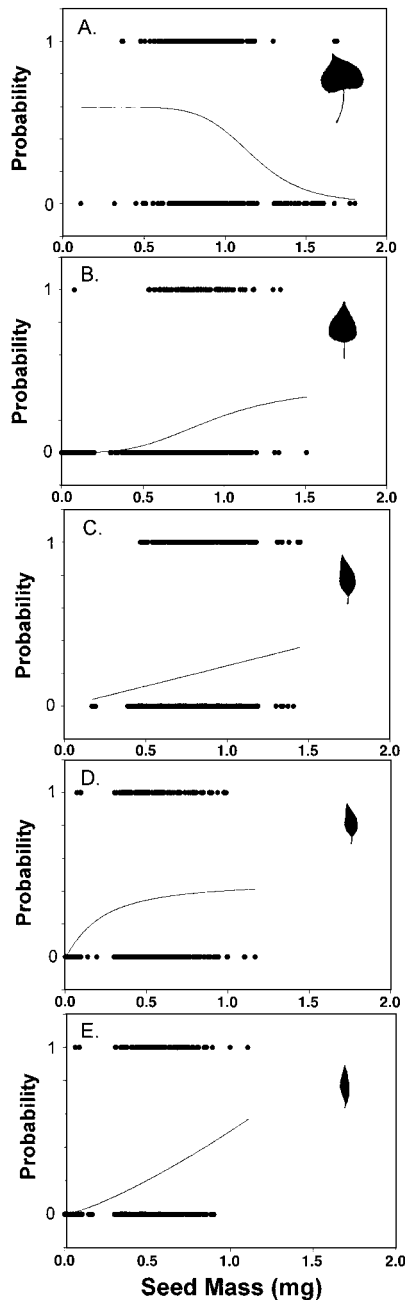


Fig. 2. Probability of germination for each *Populus* species and cross type regressed against seed mass. There are significant positive relationships between seed mass and probability of germination for  $F_1$  hybrids (B),  $BC_1$  hybrids (C),  $BC_2$  hybrids (D), and narrowleaf cottonwood (E). Larger seeds do not enhance the odds of germination for Fremont cottonwood (A; see Table 1 for statistics).

cross hybrids ( $BC_1$  and  $BC_2$ ), and narrowleaf cottonwood but reduced the probability of germination for Fremont cottonwood (Fig. 2A–E). All tree types represent significant logistic regressions where seed mass impacts the probability of germination (Table 1). These data also show that Fremont cottonwood has the largest seeds, narrowleaf cottonwood has the smallest seeds, and  $F_1$  and backcross hybrids are intermediate in size. Larger seeds in Fremont do not germinate at higher rates, indicating that the larger investment in seed biomass has

TABLE 1. Results of logistic regression comparing the relationship of seed mass to germination, for all species and cross types.

Species or cross type	Wald $\chi^2$	$\beta$	$P$
Fremont cottonwood	23.8113	0.1125	<0.001
$F_1$ hybrid	47.0134	32.5679	<0.001
$BC_1$ hybrid	12.1440	4.2930	0.005
$BC_2$ hybrid	7.1703	4.0792	0.007
Narrowleaf cottonwood	22.8446	3.2398	<0.001

no obvious benefit to Fremont genotypes. In contrast, larger seeds in hybrids probably benefit from introgression of Fremont genes, which increases the probability of germination and survival.

In our 2 yr of field surveys we found that hybrid genotypes produced just as many viable seeds in a single year as either of the parent taxa (Fig. 3). By combining the number of seeds per catkin, the number of catkins per tree, and average germination rate from our field surveys, we estimate that, on average, the hybrid genotypes can produce 150 000–475 000 viable seeds per year which is intermediate to our estimates of Fremont and narrowleaf cottonwood ( $F_{3,77} = 2.848$ ,  $P = 0.043$ ). Backcross hybrids produced as many viable seeds as either the Fremont and narrowleaf genotypes, and  $F_1$  genotypes produced as many viable seeds as the narrowleaf cottonwood. Both Fremont and narrowleaf cottonwood are known to produce high seed crops annually (Schreiner, 1974), but to our knowledge these are the first data to provide estimates of annual viable seed production for these species or their hybrids.

Because a few individuals may account for most of the viable reproduction, we compared the success of the five top producers in each tree type, following plant-breeding examples (Stoskopf, Tomes, and Christie, 1993; Hansen and Rouland, 1998). We found that the top five producing genotypes (top

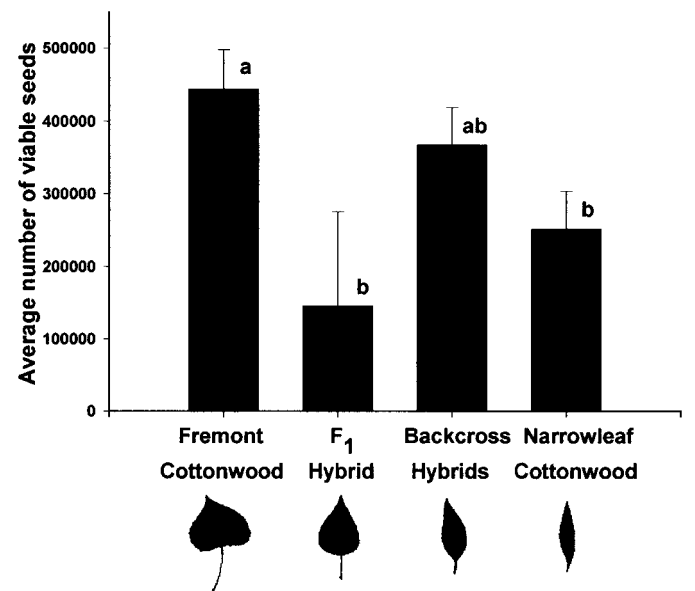


Fig. 3. Mean viable seed production for each *Populus* species and cross-type from the field surveys. The  $F_1$  hybrids produced as many viable seeds as *P. angustifolia* (but less than *P. fremontii*) and backcross genotypes produced as many viable seeds as both parent taxa ( $F_{3,77} = 2.848$ ,  $P = 0.043$ ). Error bars represent 1 SE of the mean.



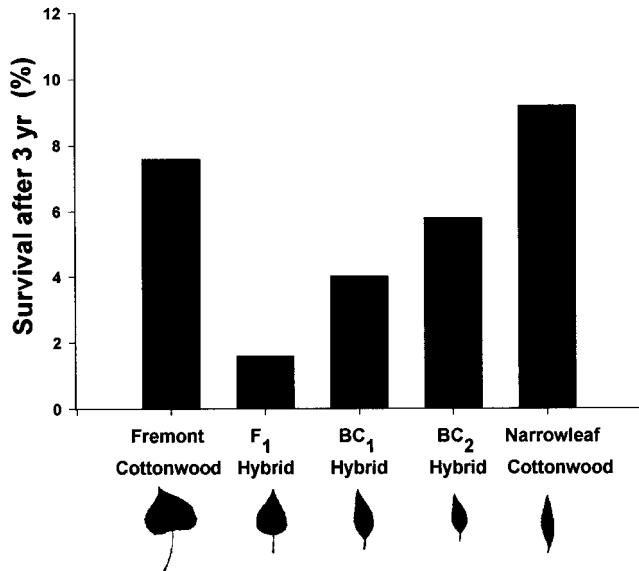


Fig. 4. Seedling survival for each *Populus* species and crosstype from the experimental crosses. Three-year survival of seedlings in the greenhouse varies significantly among cross types ( $\chi^2 = 54.793$ ,  $P < 0.0001$ ). Early hybrid generations (F<sub>1</sub> and BC<sub>1</sub>) survived 19.6–68.7% less than expected values would predict. The parent species and BC<sub>2</sub> generation survived 13.5–45.9% more than expected.

20% of the studied trees) within each tree type produced equal numbers of viable seed (ANOVA;  $F_{3,18} = 1.503$ ,  $P = 0.260$ ). Thus, for the best genotypes, seed production is equivalent across the species and the hybrid classes, a different pattern than we observed with the population-level contrasts (e.g., Fig. 3).

**Seedling survival and performance**—Field and greenhouse trials showed similar patterns of high mortality. From our 75 field plots (25 in each zone), originally containing 5845 naturally germinated seedlings, we found that similar to other published accounts about 90% of seedlings died quickly (within the first month;  $F_{2,57} = 0.360$ ,  $P = 0.699$ ; Moss, 1938; McBride and Strahan, 1984; Rood, Kalischuk, and Mahoney, 1998). None of the 5845 surveyed seedlings survived a full year in the field.

Although mortality was also high in the greenhouse with our experimental crosses, after 3 yr of monitoring under ideal conditions, we found differential survival among the species and cross types (Fig. 4;  $\chi^2 = 54.793$ ,  $df = 4$ ,  $P < 0.0001$ ). Thirty-eight Fremont seedlings survived from 500 planted seeds (7.6% survival), while 14 of 900 (1.6%), 40 of 1000 (4%), 46 of 800 (5.8%), and 46 of 500 (9.2%) seedlings survived from F<sub>1</sub> hybrids, backcross (BC<sub>1</sub> and BC<sub>2</sub>) hybrids, and narrowleaf cottonwood, respectively. We found that both pure species and the most advanced generation (BC<sub>2</sub>) had more seedlings survive than expected; 34% more Fremont seedlings survived than expected, 45.9% more narrowleaf seedlings survived than expected, and 13.5% more BC<sub>2</sub> seedlings survived than expected. In contrast, the early generations (F<sub>1</sub> and BC<sub>1</sub>) survived at much lower rates than expected (68.7 and 19.6%, respectively). These data support the findings of other studies that the generations most resembling one or the other parent taxa have the highest fitness, at least in terms of seedling survival (Arnold and Hodges, 1995). Though it is well established

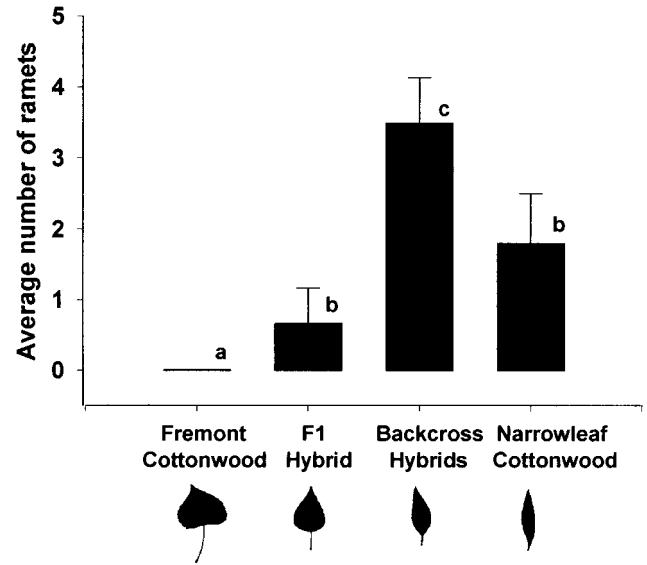


Fig. 5. Mean number of ramets produced by each *Populus* species or crosstype. In a common garden, backcross hybrids produce the most ramets ( $F_{3,92} = 16.515$ ,  $P < 0.0001$ ). Backcrosses genotypes produce four times as many ramets as Fremont and two times as many as narrowleaf cottonwood. Error bars represent 1 SE of the mean.

that cottonwood recruitment is episodic and seedling survival in the field is often low to nonexistent (Baker, 1990; Mahoney and Rood, 1998; Rood, Kalischuk, and Mahoney, 1998), these data indicate that under certain circumstances some hybrid crosstypes may survive as well as the parent species.

After 3 yr of growth in the greenhouse we found that the surviving hybrids performed at least as well as their parent taxa. From our experimental crosses, we found no difference in seedling height or collar diameter between all hybrid classes and the parent species ( $F_{4,169} = 1.985$ ,  $P = 0.099$  and  $F_{4,169} = 1.103$ ,  $P = 0.357$ , respectively). However, when we examined the length of the longest internode (i.e., stem elongation), we found that the tree types demonstrated significant differences ( $F_{4,169} = 6.779$ ,  $P < 0.000$ ). The differences were the result of greater internode lengths in the narrowleaf and BC<sub>2</sub> hybrids, which grew 17.4–35% more than the other types.

**Asexual traits**—The intersectional hybridization of Fremont and narrowleaf cottonwood results in hybrid genotypes that vary significantly in ramet production from the parent taxa (Fig. 5). From our survey of same-aged trees of known genotype in a common garden we found that F<sub>1</sub> hybrids can produce as many ramets as narrowleaf cottonwood but backcross hybrids produce more than any other group. Backcross genotypes produced nearly 4 times as many ramets as Fremont cottonwood and twice as many as narrowleaf cottonwood ( $F_{3,92} = 16.515$ ,  $P < 0.0001$ ).

Furthermore if only the “best” genotypes persist in a population, when we examined the best five ramet producers for each tree type we found significant differences in the mean number of ramets that were produced (ANOVA;  $F_{3,17} = 8.260$ ,  $P = 0.002$ ). Though confirming the population-level pattern, the best backcross genotypes produce up to 5 times more ramets than the best narrowleaf genotypes and 7.5 times more than the best F<sub>1</sub> genotypes.

When we compared the total seed production from our field

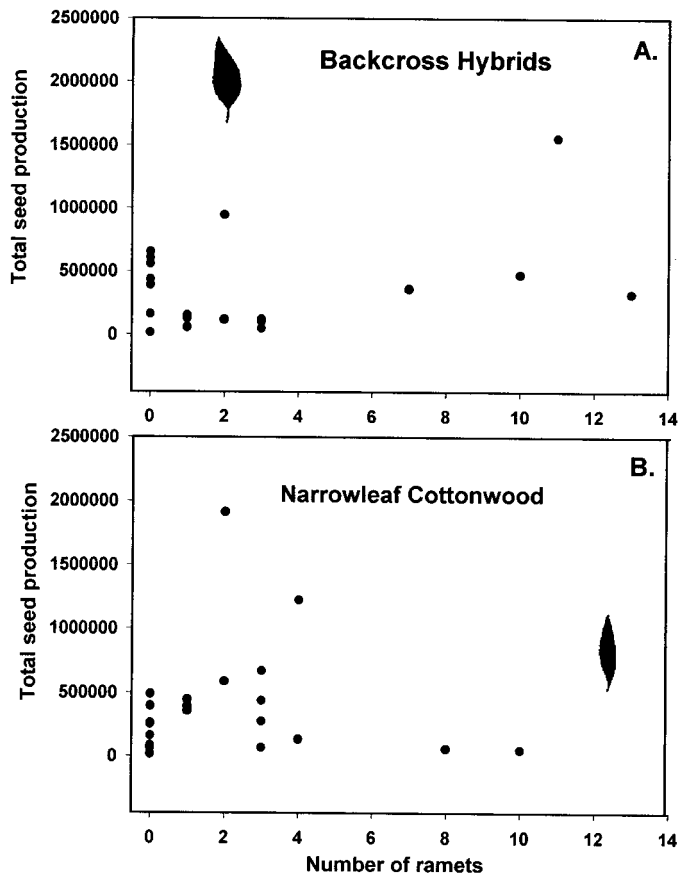


Fig. 6. Field comparison of seed to ramet production for backcross hybrids and narrowleaf cottonwood. There is no apparent relationship between the number of seeds produced and ramet production for either backcross hybrids (A;  $R^2 = 0.027$ ,  $P = 0.447$ ) or for narrowleaf cottonwood (B;  $R^2 = 0.028$ ,  $P = 0.456$ ), indicating that there is no immediate cost to supporting ramets, at least in the short term. We did not make this comparison for Fremont cottonwood or  $F_1$  hybrids because Fremont cottonwood generally do not clone and we have identified few  $F_1$  hybrids in the field.

survey trees in 1999 to the number of ramets that those same trees produced and supported, we found no significant relationship that might suggest a trade-off between sexual and asexual reproduction. For both backcross hybrids (Fig. 6A;  $R^2 = 0.027$ ,  $P = 0.447$ ) and narrowleaf genotypes (Fig. 6B;  $R^2 = 0.028$ ,  $P = 0.456$ ), there was no relationship between seed production and the support of clonal ramets, indicating no immediate trade-offs between these modes of reproduction, at least in the short term. Fremont cottonwood was not included because they do not produce ramets, and  $F_1$  hybrids were not included because of their low sample size.

In contrast to the high mortality of seedlings, ramets that remain connected to the parent tree suffer low rates of mortality, at least in the short term. There was no significant difference between hybrid and narrowleaf ramet mortality as about 8% of each tree type died across years in the common garden ( $\chi^2 = 0.15$ ,  $P > 0.05$ ). These data suggest that relative to noncloning Fremont cottonwood, extensive clonal reproduction could be very important to the overall fitness and persistence of hybrid genotypes and populations.

**Introgression and reproductive traits**—We found significant relationships between species or hybrid identity, deter-

mined by molecular marker score, and several seed and ramet traits from our field surveys. These data more clearly examine the potential importance of introgression (i.e., the incorporation of genes from one population to another) for hybrid fitness than examining lumped categories of hybrids (e.g.,  $F_1$ ,  $BC_1$ ,  $BC_2$ ). As Rieseberg and Linder (1999, p. 361), argue, "It is the actual genetic constitution of hybrids, not the pedigree, that is most predictive of their characteristics or behavior." Using field trees of known genotype in their natural setting, we found significant, positive relationships between species identity and two fitness traits: seed mass and number of seeds per catkin (Fig. 7A, B). For example, Fremont cottonwood had the heaviest seeds while narrowleaf trees (0 Fremont markers) had the lightest seeds with the  $F_1$  and backcross hybrids intermediate in mass ( $R^2 = 0.756$ ,  $P < 0.0001$ ). These data suggest that marker number explained about 76% of the variation in seed mass. The number of seeds produced per catkin followed a similar pattern, with species identity explaining nearly 60% of the variation in seeds produced per catkin ( $R^2 = 0.576$ ,  $P < 0.0001$ ).

When we examined the relationship of species identity with ramet production using the >200 trees in the 9-yr-old common garden, we found a significant negative relationship (Fig. 7C). As the marker score number increased (more Fremont markers), the number of ramets produced per tree decreased ( $R^2 = 0.119$ ,  $P = 0.004$ ), reflecting the reduced cloning ability of Fremont cottonwood and high propensity of narrowleaf cottonwood to clone (Braatne, Rood, and Heilman, 1996; Gom and Rood, 1999b).

## DISCUSSION

**Hybrid equality**—The major findings of this study were: (1) cottonwood hybrids are at least as fit as one of the parent taxa in sexual reproduction, and (2) hybrids are 2–4 times greater than their parent taxa in asexual reproduction. Viable hybrid seeds were produced in equal numbers to at least one of the parents and germinated at equal rates to both parent taxa, which does not indicate inherent sterility or inferiority. Although we found significant differences in survival over 3 yr, the surviving hybrids of all crosstypes performed (in terms of growth) equally well or better than the parent species. Furthermore, the high production of asexual ramets by hybrid genotypes may extend the lifetime of the genet and increases the likelihood that hybrid genets will reproduce sexually. The low survival rates of seedlings from all tree types (especially the early hybrid generations) and the high survival rate of ramets indicated that this mode of reproduction could be very important to the overall fitness of hybrid cottonwoods. Though we did not examine the specific exogenous or environmental factors that act to limit cottonwood hybrid zones in the field, similar to the findings of other studies (see below), our findings argue that hybrid cottonwoods have the potential to persist and/or expand due to their high level of fitness relative to the parent taxa.

Contrary to the tension zone (Barton and Hewitt, 1985) and mosaic models (Rand and Harrison, 1989) of hybrid zone stability, Arnold and Hodges (1995) and Arnold (1997) concluded that many hybrid animals and plants are not universally or inherently unfit relative to their parental species. Studies focused specifically on plants have also shown that hybrids are not always limited by inherent inferiority in fitness traits. Equivalent or better fitness of hybrids, relative to the parent

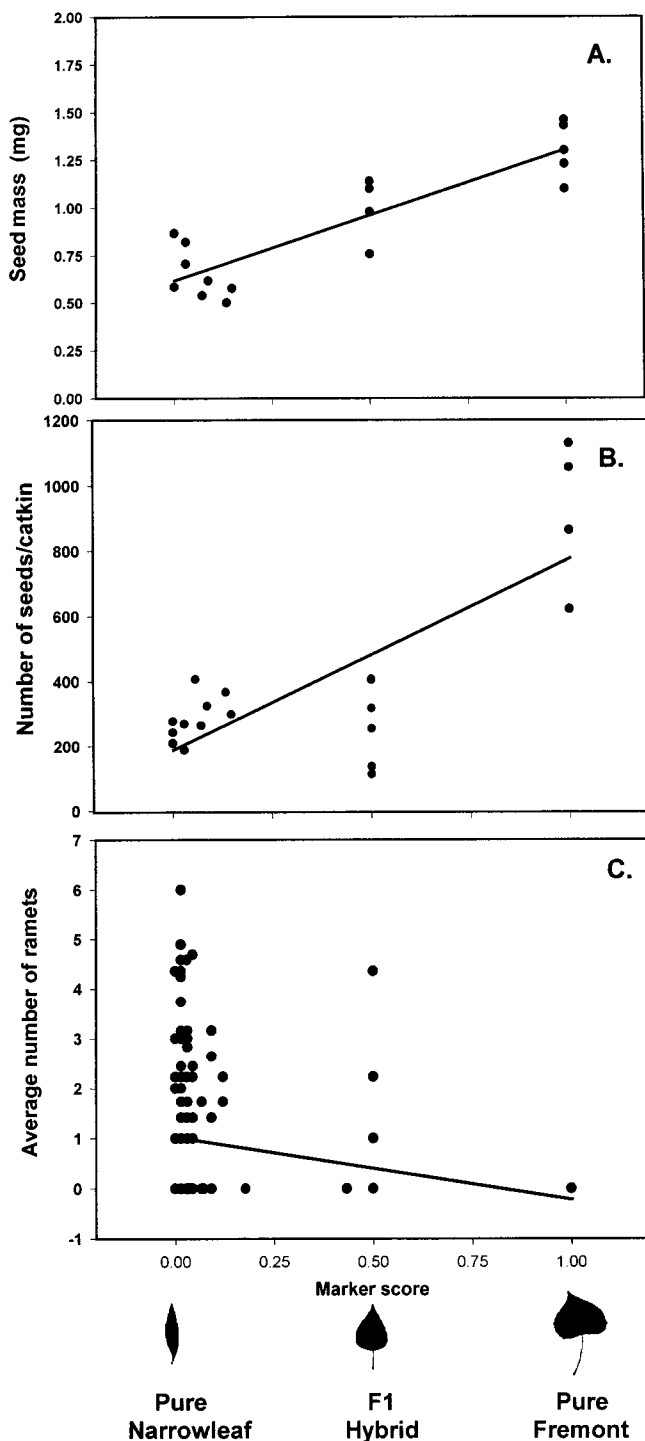


Fig. 7. The introgression of Fremont genes has significant effects on hybrid reproductive traits such that marker score is related to both seed and ramet production. There is a positive relationship between the proportion of Fremont marker alleles and seed mass (A;  $R^2 = 0.756$ ,  $P < 0.0001$ ,  $N = 5-10$ /tree type), as well as with the number of seeds per catkin (B;  $R^2 = 0.576$ ,  $P < 0.0001$ ,  $N = 5-10$ /tree type). There is a negative relationship between Fremont marker alleles and ramet production, as Fremont cottonwoods generally do not clone (C;  $R^2 = 0.119$ ,  $P = 0.004$ ,  $N = 20-100$ /tree type).

taxa, has been demonstrated in many plant hybrid complexes including *Carpobrotus* spp. (Vila and D'Antonio, 1998a, b), *Artemisia* spp. (Graham, Freeman, and McArthur, 1995; Wang et al., 1997), *Phlox* spp. (Levin and Schmidt, 1985), *Eucalyptus* spp. (Drake, 1981a, b), *Oryza* spp. (Langevin, Clay, and Grace, 1990), *Ipomopsis* spp. (Campbell, Waser, and Wolf, 1998; Campbell and Waser, 2001), and *Iris* spp. (Emms and Arnold, 1997; Burke, Carney, and Arnold, 1998).

**Advantages of cloning**—The ability to produce asexual clones or ramets vegetatively is recognized as an important attribute of many species and their hybrids (Buss, 1985; Hutchings, 1988; Ellstrand, Whitkus, and Rieseberg, 1996). Hybrid plants have been observed to clone at disproportionately higher rates than nonhybrid plants (Ellstrand, Whitkus, and Rieseberg, 1996; Orians, 2000). Of the ten most important hybrid genera in the British Isles, nine of them have the ability to clone; this ability is thought to stabilize hybrid populations (Eriksson, 1992; Ellstrand, Whitkus, and Rieseberg, 1996). Studies with *Oryza* spp. (Langevin, Clay, and Grace, 1990), *Iris* (Emms and Arnold, 1997), and *Prunella* spp. (Fritsche and Kaltz, 2000) have shown that hybrids can produce as many or more tillers, rhizomes, or ramets as the parent taxa.

There is only one study that we are aware of that compares the contributions of cloning vs. sexual reproduction to the overall reproduction of the hybrid offspring. Burke et al. (1998) found that  $F_1$  *Iris* hybrids demonstrated a high propensity to clone in addition to high seed production even though one of the parent taxa seldom produced seed. This study indicated that hybrids exhibit dominant inheritance of seed production, which could make significant contributions to hybrid persistence. Emms and Arnold (1997) found that  $F_1$  *Iris* hybrids could produce vegetative rhizomes at higher rates that subsequently grew faster than rhizomes of the parent taxa. They hypothesized that this was a mechanism for hybrids to persist and introgression to occur because cloning allowed long-term survival of the  $F_1$  genet despite its relative rarity in the population. High rates of cloning in many species of cottonwood have been hypothesized to permit forest reestablishment in the absence of seedling establishment (Shaw, 1976; Rood et al., 1994), indicating the advantage this form of reproduction can confer to hybrid genotypes of *P. fremontii* and *P. angustifolia*.

**Best genotypes**—Because our field and greenhouse data demonstrated high seedling mortality and some ramet mortality, we examined the top five producers of viable seed and ramets (following the methods of plant breeders) and found very different patterns. By examining the top producing genotypes we saw the variability in traits within each hybrid class as well as tested for transgressive segregation. With regard to viable seed we found that the top five genotypes of each tree type produced equivalent numbers of viable seed, while the mean of 25 genotypes of each tree type showed significant differences between the pure and hybrid types. This disparity indicates high variability in this trait. While the top seed producers produce the same amount of viable seed, the top genotypes in ramet production confirm the mean pattern. However, the ramet data illustrate another level of variation: the best backcross genotype cloners are much better than the average of the backcross genotypes (a fivefold difference vs. only a twofold difference between the same groups). These patterns indicate the range of variability of reproductive traits

within hybrid genotypes is extensive and support the idea that some hybrid genotypes (within any hybrid class) can be highly fit and have the potential to contribute to the maintenance and long-term persistence of the hybrid zone (Arnold, 1997).

**Introgression**—A strength of this study is the molecular data that allowed us to both confidently discriminate between naturally occurring hybrid classes in the field and show a significant relationship between hybrid index (marker proportion) and specific reproductive traits. The data indicated that introgression of Fremont traits into the hybrid genome may contribute to the allocation of resources to the seeds, in which heavier hybrid seeds are predicted by Fremont marker number. This is important because heavier  $F_1$  and backcross seeds germinated at higher rates than lighter seeds and seed mass is an important indicator of germination and survival rate in many plant species (Saverimuttu and Westoby, 1996; Simons and Johnston, 2000; Susko and Lovett-Doust, 2000).

The introgression of Fremont seed traits into hybrid lineages seems to be advantageous to hybrid genotypes even though these same traits are not advantageous to Fremont cottonwood. Despite allocating the most resources to their seeds and producing the most seed, Fremont seedlings survived or performed no better than advanced generation hybrids or narrowleaf cottonwood. While having large seeds benefited both narrowleaf and hybrid cottonwoods, it was not advantageous to Fremont seeds.

High ramet production is characteristic of narrowleaf and backcross genotypes with few Fremont markers. Other studies of hybrids that have one parent species that clones and another that does not support the idea that cloning is under tight genetic control. For example, crosses between cloning and non-cloning species of *Festuca*, *Poa*, and *Viola* have hybrid offspring that are intermediate to the parents in cloning ability indicating the heritability and additive inheritance of this trait (Tutin et al., 1964; Stace, 1975; Tutin et al., 1980).

Furthermore, cloning in backcross genotypes appears to be a transgressive characteristic (Rieseberg and Carney, 1998) as they produce nearly two times more ramets than narrowleaf cottonwood, which commonly clones (Schier and Campbell, 1976; Rood et al., 1994). Transgressive segregation occurs only in segregating hybrid generations, (i.e., backcross generations) and results in individuals whose phenotypes exceed the parental phenotypes. It is different than hybrid vigor or heterosis, which commonly occurs in first-generation hybrids, whose phenotypic traits positively exceed the parental phenotypes (de Vincente and Tanksley, 1993; Rieseberg, Baird, and Gardner, 2000). It is thought that transgressive characteristics, such as higher rates of cloning than either parent, allows transgressive individuals to better compete in their environments (de Vincente and Tanksley, 1993).

Lastly, the data on both seed and ramet production for these species of cottonwood indicate that the use of specific markers or other genetic data to trace introgression levels are important to discriminate between species and hybrid classes to assess their relative fitness. By using the marker data we could accurately discriminate between the pure and hybrid cross types to address hypotheses regarding hybrid status (Figs. 1, 3, 4, 5), and we could also examine the effects of percent Fremont cottonwood markers as a continuous variable (Fig. 7). For example, complex backcross hybrids and narrowleaf cottonwood co-occur in the field, are very difficult to separate based on morphological traits, and differ by only a few Fremont cot-

tonwood molecular markers, yet the two groups perform very differently in both sexual and asexual reproductive traits. Similarly,  $F_1$  leaf morphology can be very similar to first-generation backcrosses ( $BC_1$ ) but backcross genotypes, on average, produced twice as much viable seed and four times as many ramets as  $F_1$  genotypes. Without the marker data we could have come to very different conclusions about the relative fitness of hybrids and the parent species in the field, emphasizing the need for studies examining hybrid fitness to confidently discriminate between the species and hybrid categories.

Although lifetime reproduction is very difficult to quantify in long-lived species such as trees, it is possible to assess parameters of fitness that could contribute to the success of hybrid offspring at early stages of their life cycle when selective pressures are strong. High seedling mortality is a characteristic of trees and shrubs and may be an evolutionary strategy for species that reproduce more than once (Bell, 1976; Fenner, 1987). High seedling mortality is especially common in cottonwood species that are adapted to habitats that are prone to high disturbance levels and that require specific environmental conditions for recruitment to occur (Baker, 1990; Shafroth, Auble, and Scott, 1995; Braatne, Rood, and Heilman, 1996). This makes the high production and survival of ramets very important to the survival and persistence of these hybrid genotypes and populations.

In summary, we found that cottonwood hybrids along the Weber River (especially later backcross generations) are at least as fit as one of the parent species in both sexual and asexual reproductive parameters. It appears that hybrids benefit from both the introgression of Fremont traits for larger seed size and narrowleaf traits, for increased asexual reproduction. Larger seeds and high rates of cloning may allow hybrid genotypes to survive, which could help explain the common occurrence and persistence of hybrid populations along riparian areas of the Weber River and potentially across the western United States.

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