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Latitudinal variation in cold hardiness in introduced *Tamarix* and native *Populus*

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Abstract

To investigate the evolution of clinal variation in an invasive plant, we compared cold hardiness in the introduced saltcedar (*Tamarix ramosissima*, *Tamarix chinensis*, and hybrids) and the native plains cottonwood (*Populus deltoides* subsp. *monilifera*). In a shadehouse in Colorado (41°N), we grew plants collected along a latitudinal gradient in the central United States (29–48°N). On 17 occasions between September 2005 and June 2006, we determined killing temperatures using freeze-induced electrolyte leakage and direct observation. In midwinter, cottonwood survived cooling to −70°C, while saltcedar was killed at −33 to −47°C. Frost sensitivity, therefore, may limit northward expansion of saltcedar in North America. Both species demonstrated inherited latitudinal variation in cold hardiness. For example, from September through January killing temperatures for saltcedar from 29.18°N were 5–21°C higher than those for saltcedar from 47.60°N, and on September 26 and October 11, killing temperatures for cottonwood from 33.06°N were >43°C higher than those for cottonwood from 47.60°N. Analysis of nine microsatellite loci showed that southern saltcedars are more closely related to *T. chinensis* while northern plants are more closely related to *T. ramosissima*. Hybridization may have introduced the genetic variability necessary for rapid evolution of the cline in saltcedar cold hardiness.

Introduction

Native woody species often demonstrate inherited latitudinal variation in the timing of growth cessation and cold hardiness in response to the latitudinal gradient in temperature (Pauley and Perry 1954; Howe et al. 1995). Can an introduced species develop such a pattern in a century or two (Weber and Schmid 1998)? Development of clinal variation could be accelerated by multiple introductions of populations from different latitudes (Novak and Mack 2001), by hybridization between closely related species from different climates (Hurka et al. 2003), or by epigenetic inheritance of cold hardiness (Saxe et al. 2001). This question can be addressed by studies comparing clinal variation in native and introduced species.

The diploid genus *Tamarix* was introduced to North America in the mid 1800s to control erosion and to serve as a drought-tolerant ornamental flowering shrub or tree (Robinson 1965). Although there are now several *Tamarix* species in the United States (USA), *Tamarix chinensis* Lour. and *Tamarix ramosissima* Ledeb. are by far the most abundant (Gaskin and Schaal 2003). *Tamarix chinensis* is native to China, Korea, and Japan at latitude 23–42°N and longitude 79–110°E, whereas *T. ramosissima* occurs more widely across temperate Asia at latitude 30–53°N and longitude 42–127°E (Baum 1978). Although these two species are morphologically distinct in Asia (Baum 1978), North American specimens cannot always be readily distinguished (Crins 1989). Hereafter, we refer to the complex of *T. ramosissima*, *T. chinensis*, and their hybrids as saltcedar. In a study of a single nuclear locus in saltcedar, Gaskin and Schaal (2002) found that the most common genotype in North America is a heterozygote containing one allele that in Asia is found only in

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T. ramosissima and another allele that in Asia is found only in *T. chinensis*. In other words, these North American heterozygotes have a hybrid genotype that has not been found in Asia. Although homozygotes for either of the two alleles are also common in North America, it is not possible using a single locus to determine whether they are true *T. chinensis* and *T. ramosissima* or cryptic hybrids. For this reason, there is a strong need for analysis of additional loci to characterize the taxonomic status of North American saltcedar.

Saltcedar is now the second most abnndant riparian woody plant in the interior western USA (Friedman et al. 2005), occupying 470 000–650 000 ha (Zavaleta 2000). Because replacement of native cottonwood-willow (*Populus-Salix*) communities by saltcedar can be associated with degradation of habitat for vertebrates and increased water loss from evapotranspiration (Shafroth et al. 2005), there is an intense interest in controlling its spread (Hart et al. 2005). To inform such efforts, it is important to know what factors limit saltcedar distribution and to understand how that distribution would be altered by changing temperatures. Although the cold hardiness of saltcedar is unknown, there is evidence that cold temperatures may limit its expansion in North America. In the US north of about 39°N latitude, saltcedar is relatively scarce, individuals are small, and dieback is frequent (Lesica and Miles 2001; Friedman et al. 2005), suggesting that cold sensitivity may limit northward expansion. On the other hand, some populations of saltcedar in Montana are growing rapidly (Pearce and Smith 2003; Sexton et al. 2006), suggesting that there may be genetic variabiliry in cold hardiness. Furthermore, growtb chamber experiments comparing northern and southern saltcedar have demonstrated inherited temperature-dependent differences in root–shoot ratio (Sexton et al. 2002). Genotypic differences among *T. chinensis*, *T. ramosissima*, and their hybrids could cause variation in cold hardiness across North America.

If a brief residence in North America has limited development of clinal variation in saltcedar, we might expect a stronger cline in a comparable native species. The native riparian tree plains cottonwood [*Populus deltoides* Marshall subsp. *monilifera* (Aiton) Eckenwalder] is an important competitor of saltcedar ranging from northern Texas to southern Manitoba, Saskatchewan, and Alberta at latitude 30–55°N and longitude 96–114°W (Van Haverbeke 1990; Friedman et al. 2005). *Populus deltoides* is known to be cold hardy; buds and stems collected in winter can survive chilling to −80°C (Sakai and Weiser 1973). Common-garden studies of cottonwood including *P. deltoides* bave shown inherited latitudinal variation in the timing of fall growth cessation (Howe et al. 1999). When grown in Massachusetts, *P. deltoides* from 43° to 46°N ceased growing an average of 1 month earlier than *P. deltoides* from 30° to 32°N (Pauley and Perry 1954). Northern cottonwood, therefore, may develop cold resistance earlier in the fall than southern cottonwood.

We used a common-garden approach to compare cold hardiness in plains cottonwood and saltcedar collected along a latitudinal gradient in the central USA. We hypothesized that plains cottonwood is more cold tolerant than saltcedar, and that the physiological limits imposed by cold temperatures are consistent with the northern limit to saltcedar distribution in the USA. We expected to find strong latitudinal variation in cold hardiness in plains cottonwood, but not in saltcedar, because there has been little time for evolution of such variation in this introduced species. Finally, we used microsatellite analysis in plains cottonwood and saltcedar to investigate genetic variation along rhe latitudinal gradient. In saltcedar, it was necessary to develop the microsatellite primers to perform the analysis (Gaskin et al. 2006). This work is especially important in saltcedar, a widespread dominant tree in North America whose taxonomic status is still uncerrain (Gaskin and Scbaal 2002). We hoped to determine to what extent saltcedar in North America behaves as a single population as opposed to two or more isolated populations.

Methods

We collected plains cottonwood and saltcedar from 15 sites along a latitudinal gradient from 29° to 48°N in the central USA (Table 1). In February and March of 2005, we collected samples from 25 (or more) distinct genetic individuals of each species at each site, except for the three southernmost sites where plains cottonwood was scarce or absent (Table 1). The sampled populations occurred as bands 2–200 m wide along the shore of a river or reservoir. We walked along the band and sampled the first 25 plants that had young, rapidly growing stems suitable for vegetative propagation. Collections were stem cuttings 25 cm long and about 1 cm in diameter. We stored cnttings in plastic bags in a refrigeraror. On March 17, 2005 we planted the cuttings in pots (top diameter = 7 cm, depth = 25 cm) filled with Fafard F2-SV growing medium with 25% perlite and no added fertilizer or rooting hormone. We rooted the cuttings under mist in a greenhouse in Fort Collins, CO. On May 31, we moved the plants to a shadehouse in Fort Collins at latitude 40.58°N and longitude 105.14°W. The shadehouse is an outdoor space with no walls and a roof of widely spaced boards to reduce direct sun. We provided supplemental water two to three times per week to eliminate drought stress.

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Table 1. Collection locations for plains cottonwoods and saltcedars grown in the Fort Collins shadehouse. Also shown are the number of individuals collected at each location and the number tested for cold hardiness on April 17, 2006.

Site name	Latitude (°N)	Longitude (°W)	Elevation (m)	Individuals collected		Individuals tested	
				Saltcedar	Cottonwood	Saltcedar	Cottonwood
Fort Peck Reservoir, Hell Creek Arm, MT	47.60	106.90	683	25	25	3	9
Musselshell River near Roundup, MT	46.45	108.53	962	25	25	20	3
Powder River near Broadus, MT	45.43	105.41	927	25	25	9	8
Keyhole Reservoir, WY	44.37	104.79	1254	25	28	20	18
Boysen Reservoir, WY	43.22	108.18	1379	25	25	19	18
Lake McConaughy, Cedar Vue Campground, NE	41.29	101.93	988	25	25	18	20
Cache la Poudre River, Fort Collins, CO	40.56	105.01	1504	32	25	23	15
Bonny Reservoir, CO	39.62	102.19	1120	25	25	5	18
Arkansas River, Grenada State Wildlife Area, CO	38.09	102.29	1053	25	25	14	22
Cimarron River, OK	37.12	101.89	1036	25	25	14	18
Lake Meredith, Mullinaw Creek, TX	35.53	101.77	896	25	25	9	14
Buffalo Lake National Wildlife Refuge, TX	34.90	102.12	1105	25	25	17	18
Lake Alan Henry, TX	33.05	101.04	649	25	12	10	6
Colorado River west of Silver, TX	32.02	100.74	581	25	0	10	0
Tornillo Creek, Big Bend National Park, TX	29.18	103.01	568	25	0	1	0

We determined the change in cold hardiness over time by periodically testing plants from four species–latitude combinations: northern and southern plains cottonwood and saltcedar. To conduct a test, we selected a single individual from each of the four species–latitude combinations; different individuals were selected for each test. We performed the test 17 times from September 13, 2005 to June 5, 2006. For the first eight tests, ending with January 23, 2006, the selected species–latitude combinations were plains cottonwood from latitudes 33.06° and 47.60°N and saltcedar from latitudes 29.18° and 47.60°N; for the final nine tests, beginning with February 6, 2006, we used plains cottonwood and saltcedar from latitudes 34.90° and 45.43°N. The change in latitudes was necessary to provide sufficient stem material to perform the tests. We measured cold hardiness of the selected individuals using freeze-induced electrolyte leakage (Burr et al. 1990; Calkins and Swanson 1990; Lenmartsson and Ögren 2002). This method is based on the fact that freezing of stem tissues causes leakage of electrolytes, which can be quantified by measurement of electrical conductivity. To perform a test, we collected a 36-cm piece of current-year stem from each of the four selected individuals. We cut each piece into 1-cm samples and placed each sample in a vial (or test tube) with 1 mL of deionized water and a piece of lead shot to promote nucleation of ice crystals. The 36 samples from each selected individual were divided into six sets of six tubes (or vials). We placed one set (controls) in a refrigerated water bath (fall) or an ice water bath (spring). We placed the remaining five sets in a programmable temperature chamber (TestEquity Model 115, Thousand Oaks, CA), held them at −2°C (fall) or −4°C (spring) overnight to ensure that all were frozen, and then exposed them to gradually declining temperatures (5°C per hour). At five different temperatures, we removed a set of the samples and temporarily stored them at 4°C until all were removed from the chamber. The five temperatures varied seasonally and were chosen to be close to the warmest lethal temperatures for all of the selected individuals. After adding 10 mL (vials) or 4 mL (tubes) of deionized water, we agitated samples for 20 h on a shaker table, measured the conductivity of each vial or tube using an Amber Science, conductivity meter Model 1056 (Amber Science Inc., Eugene, OR), boiled samples for 20 min, agitated again for 20 h and remeasured the conductivity. The index of injury (Flint et al. 1967) for a frozen 1-cm sample was

$$I_t = 100(L_t/L_k - L_0/L_d)/(1 - L_0/L_d),$$

where I_t is index of injury resulting from exposure to temperature t, L_t is specific conductance of leachate from sample frozen at temperature t, L_k is specific conductance of leachate from sample frozen at temperature t and then heat-killed, L_0 is specific conductance of leachate from unfrozen control sample, and L_d is specific conductance of leachate from unfrozen sample, heat killed.

The index of injury is designed to correct for error introduced by variation between samples in the quantity of electrolytes available for leaching following injury (Flint et al. 1967). For an individual plant on a given day, this parameter displayed a sigmoid (s-shaped) relationship with temperature. It approached a lower asymptote of 0 at warm temperatures that did not harm the sample, and approached an upper asymptote between 0 and 1 at cold temperatures that killed the sample. Comparison to

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frozen stems held for 2 weeks at room temperature for direct observation of cold-induced mortality indicated that stem samples died when I_t reached a point midway between the low and high asymptotes. We designated the temperature at this point as the killing temperature. For each of the four individuals selected in a test, we determined the killing temperature using a nonlinear sigmoid regression of the form

$$I_t = a/(1 + be^{-ct}),$$

where t is temperature; a, b, and c are estimated parameters, such that a is the upper asymptote and $(\ln(b))/c$, the inflection point of the curve, is the killing temperature.

We determined 95% confidence intervals using inverse prediction. We estimated these intervals for the nonlinear model following the format for linear models where an inverse prediction interval for a new value of the independent variable (here temperature, t) takes the form of a prediction interval for a new observation of the dependent variable (here index of injury, I_t) divided by the slope estimate (Neter et al. 1996:167–169). Because the nonlinear functional form prohibited this simple algebraic manipulation, we estimated 95% prediction intervals for a new observation of I_t in the nonlinear model for an interval of values of t and determined the smallest and largest values of t that had 95% prediction intervals for I_t that included the estimated killing temperature.

We measured the variation in cold hardiness over latitude by comparing all individuals with ≥35 cm of stem (cottonwood, $n = 187$; saltcedar, $n = 192$) on April 17, 2006. The numbers of individuals tested by species and latitude are shown in Table 1. We cut a 35-cm stem piece from each individual, cut each piece into seven 5-cm samples and distributed the samples into zip-lock plastic bags. For each latitude, there were seven plastic bags, each containing one 5-cm sample from each of the selected individuals from that latitude. We placed one bag from each latitude (control) in an ice water bath and the remaining bags in the temperature chamber at 20°C. We reduced the temperature to −3.3°C in 15 min and held the chamber at that temperature for 14.5 h to ensure that all twigs were frozen. We then lowered the temperature 5°C per hour to −71.1°C and held this temperature for 11.5 h. We removed one bag from each latitude at −8.5, −20.3, −29.4, −41.9, −55.4, and −71.1°C (at the end of the 11.5-h hold). We then placed samples upright in moist sand at room temperature for 2 weeks before determining survival by visual observation (Calkins and Swanson 1990). Live samples had moist bright green inner bark, and often grew new roots and leaves. Dead samples had brown–green inner bark, which was often dry, and grew no roots or leaves. For a given species at a given latitude, there was a sigmoid relationship between temperature and percent survival similar to the sigmoid relationship observed in the conductivity trials. This allowed us to use an analogous approach designed for categorical (live or dead) data, in which we defined the killing temperature as the temperature at which half of stem samples were killed. For each species–latitude combination, we used PROC PROBIT in SAS (SAS Institute, Inc., Cary, NC) to calculate the killing temperature ($n = 3$–23 individuals, depending on species and latitude, Table 1). Finally, for each species, we performed a multivariate probit analysis on all individuals combined using temperature and latitude as independent variables. This allowed us to test the significance of the latitudinal effect for each species.

We assessed genetic similarity among plants using neighbor-joining analysis of microsatellite loci for each species. Extraction of DNAs, PCR amplications, and fragment analysis were performed as in Gaskin et al. (2006). For saltcedar, we used the nine microsatellites from Gaskin et al. (2006), and for plains cottonwood we used the nine microsatellites: PMGC 61, PMGC 223, PMGC 667, PMGC 2060, PMGC 2088, PMGC 2105 and PMGC 2573 (from International *Populus* Genome Consortium SSR Resource 2007, http://www.ornl.gov/sci/ipgc/ssr_resource.htm); WPMS14 (Smulders et al. 2001); and PTR2 (Dayanandan et al. 1998). For each species, we calculated chord distances (Cavalli-Sforza and Edwards 1967) among laritudes and used these to prepare an unrooted dendrogram. The saltcedar dendrogram included 15 populations (369 individuals) from North America and two populations (12 individuals) from Asia. The 369 North American individuals were all the saltcedar introduced to the shadehouse. The 12 individuals collected in Asia (six *T. ramosissima* and six *T. chinensis*) were never introduced to the shadehouse or used for cold-hardiness testing, but were included in this analysis to explore genetic relatedness between North American and Asian saltcedar. The plains cottonwood dendrogram included 13 populations (all 304 cottonwood individuals introduced to the shadehouse). All calculations were carried out using the programs SEQBOOT, GENDIST, NEIGHBOR, and CONSENSE from the PHYLIP ver. 3.65 package (http://evolution.genetics.washington.edu/phylip.html; Felsenstein 2004). Analyses of molecular variance (AMOVA) were carried out for both species using the distance method (Weir and Cockerham 1984; Excoffier et al. 1992; Weir 1996).

As a dioecious species, plains cottonwood is an obligatory out-crosser (Van Haverbeke 1990). Saltcedar has perfect flowers and has been categorized as self-compatible (Brotherson and Winkel 1986), but Stevens (1989) in Arizona, found no seeds produced by flowers enclosed in

mesh bags to exclude pollinators. Using some of our shadehouse individuals planted in a nearby garden, in Fort Collins, Colorado, we also observed no seeds produced by bagged flowers even when we attempted to promote self-pollination using bees on a stick (A. Blair, Colorado State University, personal communication, 2007). Therefore, both plains cottonwood and saltcedar appear to be obligatory out-crossers.

Results

Both plains cottonwood and saltcedar were more resistant to cold temperatures in the winter than in the summer, but this seasonal cycle was more extreme in cottonwood (Fig. 1). Although saltcedar was slightly more cold hardy in the early fall, cottonwood hardened off more rapidly and deeply (Fig. 1A). In early September, northern and southern individuals of both species were killed by temperatures ranging from −4 to −19°C. By mid October, both northern and southern cottonwood survived cooling to −70°C, the coldest temperature attainable in our temperature chamber. Even when we extended the coldest treatment by holding the chamber at −70°C for 13 h, we observed no cold damage to cottonwood during the winter (November through February, data not shown). Freezing temperatures warmer than −70°C did not kill cottonwood again until March (Fig. 1B). In contrast, even in mid-winter saltcedar was killed at temperatures ranging from −33 to −47°C.

From September through January, there was latitudinal variation in cold hardiness of saltcedar (Fig. 1A). During this period, the killing temperature for saltcedar from 29.18°N latitude was 5–21°C higher than that for saltcedar from 47.60°N. Latitudinal variation in saltcedar was not apparent in the time sequence of cold hardiness measurements from February to May (Fig. 1B). This may be because of weaker latitudinal variation in the spring or because the latitudes tested in the spring were not as extreme as those tested in the fall (Fig. 1). Although latitudinal variation was not apparent in the spring at this coarse scale (Fig. 1), the detailed data from April 17 showed latitudinal variation for saltcedar (Fig. 2), and multivariate probit analysis demonstrated that this latitudinal effect was significant ($P < 0.0001$, Table 2).

There was strong latitudinal variation in cold hardiness of cottonwood during the fall and spring (Fig. 1). For example, on September 26 and October 11, killing temperatures for cottonwood from 33.06°N were >53 and >43°C higher than those for cottonwood from 47.60°N. From November 11 to March 13, however, cottonwood from all latitudes survived temperatures down to −70°C, the coldest temperature attainable in our temperature chamber, which prevented observations of latitudinal variation during this period (Fig. 1). Multivariate probit analysis of the detailed data set from plants collected on April 17 showed a significant effect ($P < 0.0001$) of

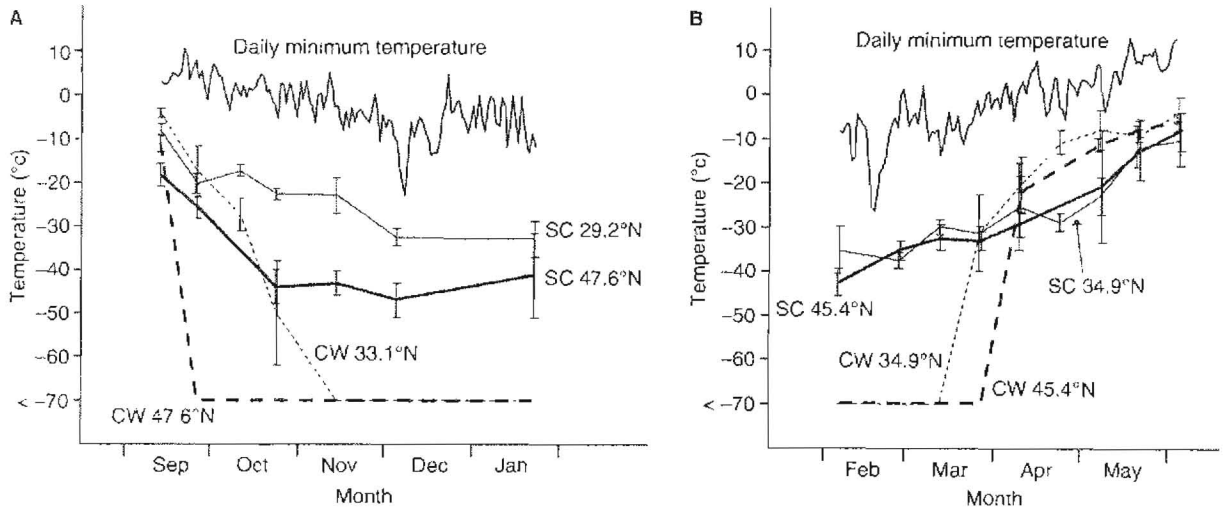

Figure 1 Fall (A) and spring (B) temporal variation in killing temperature for twigs of northern and southern plains cottonwood (*Populus deltoides* subsp. *monilifera*) and saltcedar (*Tamarix ramosissima*, *Tamarix chinensis*, and hybrids) grown in Fort Collins, Colorado. Latitude of origin is given in decimal degrees. Saltcedar (SC) data are solid lines, and cottonwood (CW) data are dashed lines. Northern (southern) populations are indicated by thick (thin) lines. Different individuals were used on each sampling date. Note that different latitudes were used in the spring and fall. Daily minimum temperature data are from the Christman Field Weather Station (Colorado Climate Center) about 1 km from the shadehouse. Error bars are 95% confidence intervals for an individual sample.

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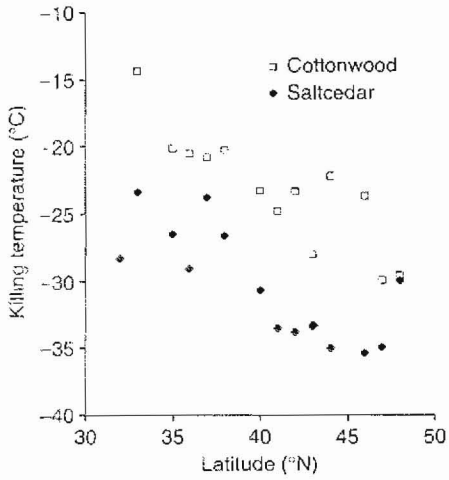

Figure 2 Killing temperature for twigs of plains cottonwood (*Populus deltoides* subsp. *monilifera*) and saltcedar (*Tamarix ramosissima*, *Tamarix chinensis*, and hybrids) as a function of latitude of origin on April 17, 2006. The number of individuals included is shown by latitude in Table 1. For saltcedar at latitude 29.18°N, there was insufficient data to calculate the killing temperature.

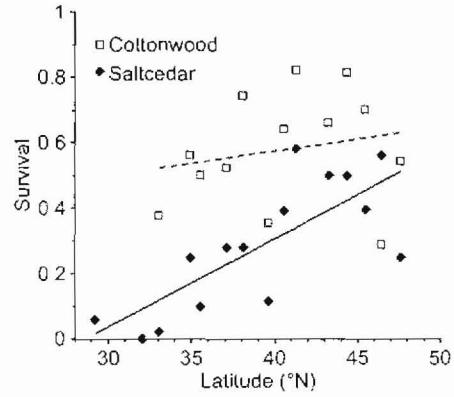

Figure 3 Overwinter survival for whole plants of plains cottonwood (*Populus deltoides* subsp. *monilifera*) and saltcedar (*Tamarix ramosissima*, *Tamarix chinensis*, and hybrids) in the shadehouse as a function of latitude of origin. Survival is the proportion of plants alive on June 1, 2006. Survival was correlated to latitude in saltcedar ($P = 0.0009$, $R^2 = 0.58$), but not in plains cottonwood ($P = 0.51$, $R^2 = 0.04$).

latitude of origin on the killing temperature for cottonwood (Fig. 2, Table 2).

Overwinter survival of whole saltcedar plants in the shadehouse was mostly below 0.4 and strongly correlated with latitude of origin ($R^2 = 0.58$, $P = 0.0009$, Fig. 3), which is consistent with the hypotheses that frost damage is an important source of mortality in saltcedar, and that southern plants are more susceptible to cold than northern plants. In contrast, overwinter survival of whole cottonwood plants in the shadehouse was mostly above 0.4 and uncorrelated to latitude ($P = 0.51$, Fig. 3), suggesting that frost damage was relatively unimportant in plains cottonwood in the winter of 2005–2006. Heavy winter mortality of southern saltcedar is inconsistent with the fact that temperatures from September 2005 through May 2006 were always above the killing temperatures we measured for twigs of these species (Fig. 1), suggesting either that saltcedar roots are more sensitive to cold than stems, that prolonged exposure to cold is more damaging than the brief exposures in our freezing trials, or that our application of freeze-induced electrolyte leakage underestimated killing temperatures for saltcedar.

Microsatellite analysis demonstrated that neither saltcedar nor cottonwood have strong barriers to gene flow in the central USA. Fixation index (F_{ST}) was low for both species (0.049 for plains cottonwood and 0.075 for saltcedar), indicating that genetic variation within populations is large compared to variation between populations (Table 3). The dendrogram for saltcedar shows a gradual north–south genetic gradient with the Eurasian parent populations as approximate end members (Fig. 4A). Northern saltcedar is most similar to *T. ramosissima* and southern saltcedar is most similar to *T. chinensis*. There is no evidence of distinct populations in the USA. The

Table 2. Analysis of parameter estimates for multivariate probit analysis of the effects of temperature and latitude on survival of stems of plains cottonwood (187 individuals) and saltcedar (192 individuals) on April 17, 2006

Species	Parameter	Estimate	Standard error	Confidence limits (95%)		χ^2	Probability
Cottonwood	Intercept	0.96	0.788	−0.58	2.51	1.5	0.22
	Temperature	0.18	0.013	0.15	0.20	204.6	<0.0001
	Latitude	−0.13	0.021	−0.17	−0.09	37.6	<0.0001
Saltcedar	Intercept	0.44	0.563	−0.66	1.55	0.6	0.43
	Temperature	0.13	0.008	0.11	0.14	290.3	<0.0001
	Latitude	−0.11	0.015	−0.14	−0.08	51.2	<0.0001

Numbers of individuals by species and latitude are shown in Table 1.

Table 3. Analysis of molecular variance (AMOVA) calculated using the distance method for plains cottonwood (*Populus deltoides* subsp. *monilifera*, 304 individuals) and saltcedar (*Tamarix ramosissima*, *Tamarix chinensis*, and hybrids, 369 individuals) collected along a latitudinal gradient in the central USA

Species	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Cottonwood	Among populations	12	129.056	0.16298	4.90
	Within populations	595	1881.365	3.16196	95.10
	Total	607	2010.421	3.32494	
Saltcedar	Among populations	14	200.899	0.23357	7.54
	Within populations	723	2071.795	2.86555	92.46
	Total	737	2272.694	3.09912	

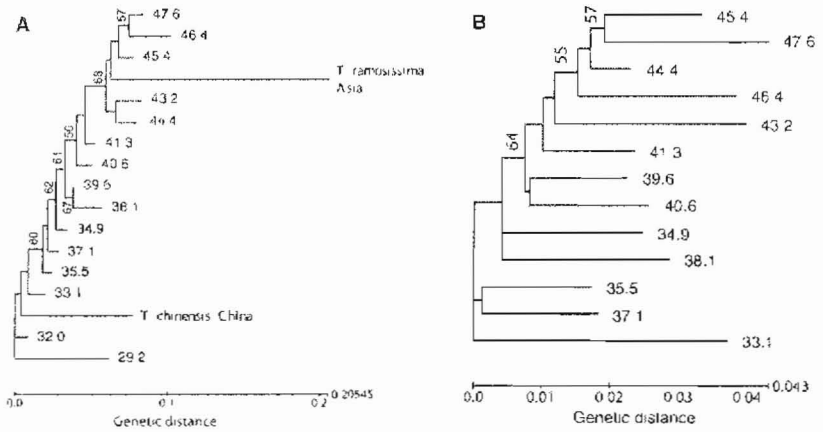

Figure 4 Unrooted dendrogram of chord distances (Cavalli-Sforza and Edwards 1967) inferred from neighbor-joining analysis of microsatellite loci data. Bootstrap values above 50% (vertically oriented) are shown to the left of the respective nodes, and were derived from a consensus of 1000 trees. Ultimate dendrogram branches are populations identified by latitude (Table 1). (A) Dendrogram of 15 US populations (369 plants) of saltcedar (*Tamarix chinensis*, *Tamarix ramosissima*, and hybrids) based on nine microsatellite loci (Gaskin et al. 2006). Populations of six *T. chinensis* from China and six *T. ramosissima* from Asia were also included in the analysis for comparison. (B) Dendrogram of 13 US populations (304 plants) of plains cottonwood (*Populus deltoides* subsp. *monilifera*) based on nine microsatellite loci (International Populus Genome Consortium SSR Resource 2007, http://www.ornl.gov/sci/ipgc/ssr_resource.htm, Smulders et al. 2001, and Dayanandan et al. 1998)

cottonwood dendrogram shows a similar gradual north–south trend with no distinct breaks (Fig. 4B).

Discussion

All of our plains cottonwoods survived colder temperatures than are known to occur in the range of this species, suggesting that short-term exposure to extreme cold in mid-winter is not an important mortality factor. Other studies have reported extreme cold hardiness in *Populus* (Sakai and Weiser 1973). Cold-related mortality is still possible in cottonwood when plants that are not completely hardened off encounter sudden extreme drops in temperature. In fact, plains cottonwood may be more susceptible to cold damage than saltcedar in early fall and late spring (Fig. 1).

Saltcedar, even when completely hardened off, did not survive below −33 to −47°C, which is within the temperature range of the northern Great Plains. In addition, there was heavy mortality in the shadehouse of southern saltcedar (but not cottonwood) during the winter of 2005–2006 (Fig. 3). Whereas saltcedar grows to large size in the southern USA, northern stems are small in size and often older below ground than above (Tesica and Miles 2001), suggesting that winter die-back may be common in the north. Finally, saltcedar is much more abundant in the south than in the north, and its occurrence is strongly correlated to mild winter temperatures (Friedman et al. 2005). We conclude that winter cold is a significant factor influencing distribution of saltcedar in the USA. Increases in winter low temperatures could promote northward spread of saltcedar in the future.

We observed latitudinal variation in cold hardiness in both plains cottonwood and saltcedar. This variation was expected for the native cottonwood, which is known to exhibit inherited latitudinal variation in the

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timing of growth cessation (Pauley and Perry 1954; Kaszkurewicz and Fogg 1967; Howe et al. 1995, 1999; Dunlap and Stettler 1996), but not for saltcedar, a woody species that has been present in North America for only about 150 years (Robinson 1965). Such latitudinal variation in an introduced species may be a result of multiple introductions of genotypically and phenotypically distinct individuals or populations, or of rapid evolution after introduction (Maron et al. 2004). The dominance in the North American invasion of hybrids (*T. ramosissima* × *T. chinensis*) that have not been found in Eurasia (Gaskin and Schaal 2002), the absence of genetic isolation in the North American population (Fig. 4), and the fact that North American *Tamarix* are more similar to each other than to Eurasian *Tamarix* (Fig. 4) support the hypothesis that the observed latitudinal gradient in North America evolved after introduction. Hybridization involving *T. ramosissima*, *T. chinensis* and possibly other species may have accelerated this process by producing a population that was more heterogeneous with respect to cold hardiness than any of the originally introduced populations.

An alternative explanation for the observed latitudinal gradient in saltcedar cold hardiness is a carryover effect. Cuttings might have a chemical memory of the climate of the source plant that influences the phenology and cold hardiness of the clones grown in our Colorado shadehouse (Van Zandt and Mopper 2002; Heide 2003). If a carryover effect exists, it could be expected to fade over time as the plants are influenced by the Colorado climate. There is a need for multi-year common-garden studies of cold hardiness to investigate whether the observed latitudinal variation fades over time. Development of clinal variation in saltcedar cold hardiness could be accelerated by maternal effects (Galloway and Etterson 2007). For example, in *Picea abies* (Norway spruce) temperature and photoperiod experienced by a tree during seed production can affect the cold hardiness of progeny (Saxe et al. 2001; Johnsen et al. 2005).

Spread of introduced species across long environmental gradients is commonly attributed to phenotypic plasticity and multiple introductions (Neuffer and Hurka 1999; Novak and Mack 2001). The results of this and other studies, however, suggest that invasion can also be facilitated by rapid evolution of clinal variation (Weber and Schmid 1998; Sexton et al. 2002; Maron et al. 2004). Our microsatellite data show a lack of strong genetic isolation among *Tamarix* populations in the central USA, and a gradual trend from individuals that resemble Asian *T. chinensis* in the south to individuals that resemble Asian *T. ramosissima* in the north. This result argues against the possibility that the observed latitudinal variation in cold hardiness reflects multiple introductions of reproductively isolated taxa, and against the possibility that hybrid *Tamarix* in North America are reproductively isolated from sympatric populations of either parent. Our results are consistent with the possibility of adaptive radiation from a single, mostly hybrid population, and with the possibility of hybridization and backcrossing following introduction of *T. ramosissima* in the north and *T. chinensis* in the south. Therefore, some of the latitudinal gradient in saltcedar cold hardiness may be a result of patterns of introduction as opposed to natural selection. Future genetic work should focus first on analyzing additional Asian samples of *T. chinensis* and *T. ramosissima* and other *Tamarix* species to pinpoint the locations of origin and genotypes of material brought to North America, second on developing additional genetic loci to further quantify the genetic structure of the saltcedar population in North America, and third on characterization of specific genes related to cold hardiness (McKay and Latta 2002).

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