RESEARCH ARTICLE

Ploidy race distributions since the Last Glacial Maximum in the North American desert shrub, *Larrea tridentata*

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ABSTRACT

1 A classic biogeographic pattern is the alignment of diploid, tetraploid and hexaploid races of creosote bush (*Larrea tridentata*) across the Chihuahuan, Sonoran and Mohave Deserts of western North America. We used statistically robust differences in guard cell size of modern plants and fossil leaves from packrat middens to map current and past distributions of these ploidy races since the Last Glacial Maximum (LGM).
2 Glacial/early Holocene (26–10 ^{14}C kyr BP or thousands of radiocarbon years before present) populations included diploids along the lower Rio Grande of west Texas, 650 km removed from sympatric diploids and tetraploids in the lower Colorado River Basin of south-eastern California/ south-western Arizona. Diploids migrated slowly from lower Rio Grande refugia with expansion into the northern Chihuahuan Desert sites forestalled until after ~4.0 ^{14}C kyr BP. Tetraploids expanded from the lower Colorado River Basin into the northern limits of the Sonoran Desert in central Arizona by 6.4 ^{14}C kyr BP. Hexaploids appeared by 8.5 ^{14}C kyr BP in the lower Colorado River Basin, reaching their northernmost limits (~37°N) in the Mohave Desert between 5.6 and 3.9 ^{14}C kyr BP.
3 Modern diploid isolates may have resulted from both vicariant and dispersal events. In central Baja California and the lower Colorado River Basin, modern diploids probably originated from relict populations near glacial refugia. Founder events in the middle and late Holocene established diploid outposts on isolated limestone outcrops in areas of central and southern Arizona dominated by tetraploid populations.
4 Geographic alignment of the three ploidy races along the modern gradient of increasingly drier and hotter summers is clearly a postglacial phenomenon, but evolution of both higher ploidy races must have happened before the Holocene. The exact timing and mechanism of polyploidy evolution in creosote bush remains a matter of conjecture.

Key words amphitropical, autopolyploidy, creosote bush; deserts, guard cell size, Holocene, *Larrea tridentata*, North America, packrat middens, Pleistocene.

INTRODUCTION

Polyploidy is a very important mechanism of adaptation and speciation in plants, but pathways,

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mechanisms and rates of polyploidy formation are poorly understood (Grant, 1971; Levin, 1983; Thompson & Lumaret, 1992; Soltis & Soltis, 1993; Ramsey & Schemske, 1998). Polyploidy is common in higher plants, with up to 70% of all angiosperm species having at least chromosome doubling (Grant, 1971; Masterson, 1994). The frequent occurrence of polyploids has

important evolutionary implications, including reproductive isolation and subsequent differentiation (Levin, 1983; Soltis & Soltis, 1993). Genetic evidence suggests that polyploids exhibit nuclear as well as cytoplasmic (chloroplast DNA) diversity, which may give a selective advantage to new polyploids. Polyploidy is prevalent at higher altitudes and latitudes and in deglaciated landscapes, and especially in monocots. Polyploid races often exhibit self-fertilization, asexual reproduction

and increased drought tolerance, enabling polyploid species to colonize new, more stressful habitats (Johnson *et al.*, 1965; Grant, 1971; Lewis, 1980a; Levin, 1983; Ramsey & Schemske, 1998). Early studies suggested that new polyploids become established under unstable environmental conditions (Ehrendorfer, 1980), with obvious opportunities available during the dramatic climatic shifts of the Quaternary period (e.g. Stutz, 1978). Reconstructing the timing of polyploid formation, distributional

Fig. 1 North American creosote bush, *Larrea tridentata*, in the desert of Baja California, Mexico (photograph by William H. Clark).

shifts and their correlation with environmental change is fundamental to understanding the dynamics of polyploidy in nature.

One approach to understanding the evolution of polyploidy is to study a particular lineage in transition across a well-defined environmental gradient, ideally through time. An example of such a gradient is the North American distribution of creosote bush (*Larrea tridentata* [Sesse & Moc. ex DC.] Cov.) (Fig. 1). Creosote bush is the most abundant and widely distributed shrub in the warm deserts of North America; in fact, its distribution is used to delineate the boundaries of the individual deserts (Fig. 2). Three morphologically cryptic ploidy levels have been identified: diploid ($2n = 26$) in the Chihuahuan Desert, tetraploid ($2n = 52$) in the Sonoran Desert and hexaploid ($2n = 78$) in the Mohave Desert (Yang, 1970). This analysis led to the generalization that a different ploidy level is associated with each desert, and that increasing ploidy levels are aligned along a south–east to north–west gradient of increasing summer aridity and heat load. A plausible hypothesis is that polyploidy evolved as creosote bush encountered areas with progressively drier/hotter summers as it migrated from
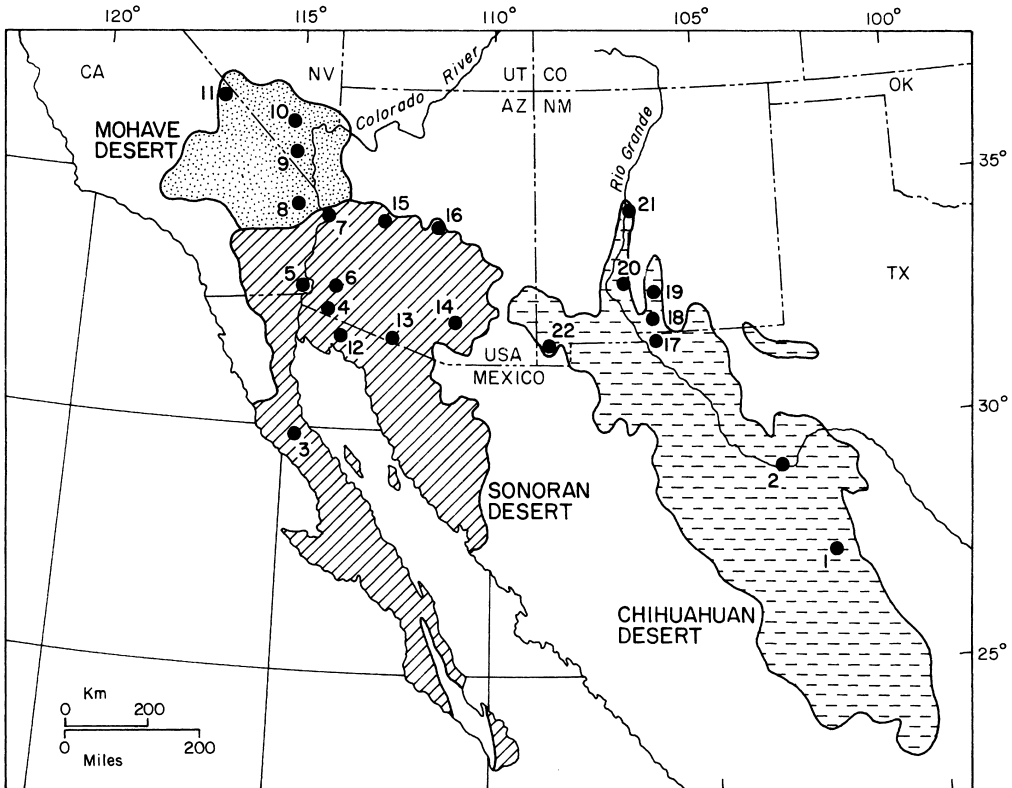

Fig. 2 Map of the Chihuahuan, Sonoran, and Mohave Deserts of North America. The distribution of *Larrea tridentata* generally coincides with the boundaries of all three hot deserts. Abbreviations for states are TX = Texas, NM = New Mexico, CO = Colorado, AZ = Arizona, NV = Nevada, CA = California. Midden sites mentioned in the text are keyed into the map: (1) Cañon de la Fragua/Cuatro Cienegas; (2) Big Bend National Monument; (3) Cataviña/San Fernando; (4) Tinajas Altas & Butler Mountains; (5) Picacho Peak; (6) Wellton Hills; (7) Whipple Mountains; (8) Marble Mountains; (9) McCullough Range; (10) River Mountains; (11) Eureka Valley; (12) Hornaday Mountains; (13) Puerto Blanco Mountains/Organ Pipe National Monument; (14) Waterman Mountains; (15) Eagle Eye Mountain; (16) Camp Verde, Verde Valley; (17) Hueco Mountains; (18) Otero Mesa escarpment; (19) Sacramento Mountains; (20) San Andres Mountains; (21) Sevilleta Long-Term Ecological Research Site; (22) Sentinel Butte.

summer-wet areas of Mexico into higher latitudes of the south-western United States.

Polyploid species may arise as part of a hybridization event between two species (allopolyploidy) or may result from somatic doubling or unreduced gametes in a single individual (autopolyploidy) (Lewis, 1980b). Autopolyploids commonly are morphologically indistinguishable from their diploid progenitors, as in *Larrea*. An immediate and consistent effect of increased ploidy is an increase in cell size (Stebbins, 1971). This increase is correlated with DNA content (Johansen & von Bothmer, 1994), allowing estimation of chromosome number from guard cell size in both modern and fossil leaves. Guard cell area, for example, has been used as a proxy for chromosome number or genome size in Tertiary (50 Ma) fossils (Masterson, 1994), but no studies to date have used this technique to determine regional ploidy changes through time. Here we use guard cell area in living and fossil leaves from ^{14}C-dated packrat middens to map polyploidy distributions in North American creosote bush since the Last Glacial Maximum (LGM).

LARREA SYSTEMATICS

The genus *Larrea* has four species (*L. divaricata, L. cuneifolia Cav., L. nitida Cav., L. ameghinoi* Speg.) in South America and one (*L. tridentata*) in North America. Taxonomically, the *Larrea* complex has been divided into two sections, *Larrea* (multifoliate leaves and small flowers) and *Bifolium* (bifoliate leaves and larger flowers) (Hunziker *et al.*, 1977). The *Larrea* section is composed of two diploid species, *L. nitida*, a shrub, and *L. ameghinoi*, a woody chamaephyte, with overlapping ranges in the Patagonian Desert of southern Argentina. The relationship between these species has been examined using chloroplast DNA and isozymes (Hunter, 1996; Lia *et al.*, 1999). The *Bifolium* section contains the remaining three species of *Larrea*, which all have a bushy growth form: *L. cuneifolia* ($2n = 26$), *L. divaricata* ($2n = 26$) and *L. tridentata* ($2n = 26$ [$2\times$], 52 [$4\times$], 78 [$6\times$]). *L. cuneifolia* and *L. divaricata* are dominant and often sympatric shrubs in the Monte Desert of Argentina. Natural hybridization of all four South American species has been documented in Patagonia (Hunziker *et al.*, 1977).

The taxonomic rank of *L. tridentata* remains controversial. The diploid ancestor to *L. tridentata* is assumed to be South American *L. divaricata* (Fig. 3), which is morphologically similar to and interfertile with the North American creosote bush (Hunziker *et al.*, 1977; for an opposite view see Turner, 1972; Porter, 1974). *L. divaricata* and diploid *L. tridentata* are considered to have homologous genomes and Hunziker *et al.* (1977) refer to them as amphitropical 'semispecies'. The only morphological difference is the orbicular stipules of the leaves of *L. divaricata* and the acuminate ones of *L. tridentata*. Hybrids of *L. divaricata* × *tridentata* and distant disjuncts of *L. divaricata* in Peru (near Chuquibamba and Aplao, Fig. 3) have intermediate stipule shapes, suggesting that the diploid form of *L. tridentata* evolved in South America. Claims for a sterility barrier between garden hybrids grown in Tucson, Arizona (Yang *et al.*, 1977) are questioned based on the lack of experimental control, the normal difficulty of germinating *Larrea* seeds and the presence of additional sterility barriers between ploidy levels (Felger, 2000). This has led some authors to adopt the use of *L. divaricata* Cav. ssp. *tridentata* (Sesse & Moc. ex de Candolle) Felger and Lowe to denote North American populations of creosote bush (Hunziker *et al.*, 1977; Felger, 2000). Work is ongoing to determine if *L. divaricata* and *L. tridentata* are distinct species, but their sister-group affinity has been supported by chloroplast DNA and isozyme data (Hunter, 1996; Cortes & Hunziker, 1997; Yang *et al.*, 2000).

Larrea divaricata, diploid throughout its range, experienced glacial–interglacial climatic variation without producing polyploids (Hunziker *et al.*, 1977; Markgraf *et al.*, 1995). Autopolyploidy and allopolyploidy events produced the two higher polyploids in North American *L. tridentata*. These events were postulated to be adaptive responses to increased summer aridity encountered as creosote bush migrated north-westward from southern Mexico, presumably the point of immigration for South American *L. divaricata* (Yang, 1970; Hunziker *et al.*, 1977; Wells, 1977).

These early hypotheses about polyploid formation and shifting distributions were inspired by packrat midden evidence that pinyon–juniper woodlands, and not creosote bush, covered what are now desert elevations (300–1500 m) during the last glacial period. Wells (1977) suggested current disjuncts ~200 km north of Mexico City as possible glacial refugia for creosote bush. He
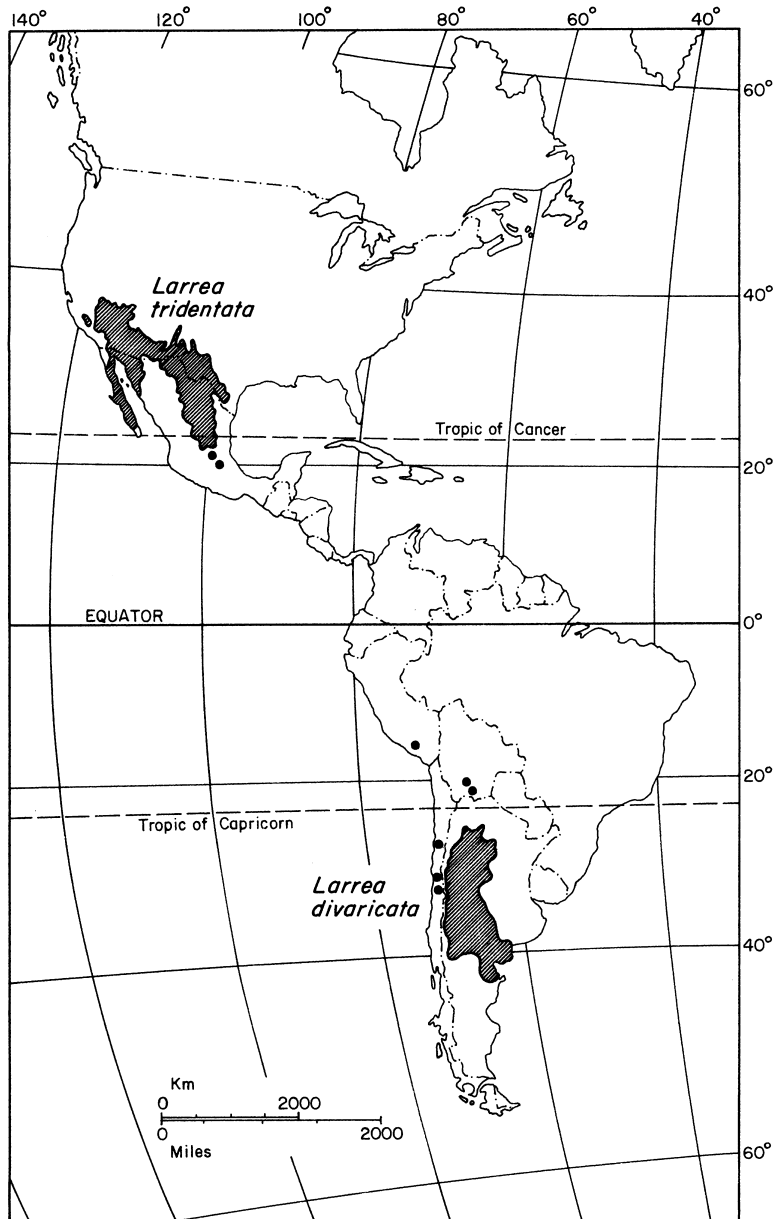
Fig. 3 Map showing disjunct amphitropical distributions of *Larrea tridentata* (North America) and *Larrea divaricata* (South America), assumed to be conspecific by many authors (distributions from Hunziker *et al.*, 1977).

speculated that, to the north, Holocene aridity opened: 'a vast and varied desert niche into which a burgeoning population of *Larrea* could have expanded and differentiated explosively' (Wells, 1977: 76). Van Devender (1990b) later obtained a direct AMS (Accelerator Mass Spectrometry) date (18.7 ^{14}C kyr BP) on creosote bush near the head of the Gulf of California, showing that creosote bush survived the LGM ~1750 km north of present disjuncts in Mexico. These glacial

populations of creosote bush grew with California juniper (*Juniperus californica* Carr.) and Joshua tree (*Yucca brevifolia* Engelm.), species characteristic of the winter-wet/summer-dry Mohave Desert. Van Devender (1990b) thus speculated that these glacial populations were probably hexaploids rather than the modern tetraploids present at the site today. The ability to infer ploidy level from guard cell size provides the opportunity to recast the biogeographic history of creosote bush and its ploidy races beyond the level of speculation.

MATERIALS AND METHODS

Modern plants

To determine if cell size could be used to diagnose ploidy, we first measured the guard cell area of 116 glasshouse-grown plants from 50 locations across the Chihuahuan, Sonoran and Mohave Deserts. Twenty seeds per location were planted since germination and successful establishment is low in this species. Three leaves from each of the plants were painted with clear fingernail polish and allowed to dry. The polish was peeled off, and lengths and widths of 30 guard cells per plant (10 per leaf) were measured (400×). Guard cell area was calculated using the formula of an ellipse: $\pi(L/2)(W/2)$, where L is length and W is width of a pair of guard cells. Forty-nine samples were selected from the centres of the deserts, to avoid any potential ploidy level transition zones between deserts. Data were analysed by ANOVA to determine if guard cell area differed between desert populations, and among the plants from a particular desert. The general linear models procedure of SAS (Version 5) (SAS Inst. 1985) was used with several multiple comparison tests. Ploidy level was assigned to each plant based on mean guard cell sizes for the central areas of the three deserts. For the diploid we used the Chihuahuan mean ± 2 SD, for the tetraploid the Sonoran mean –2/+1 SD, and for the hexaploid the Mohave mean –1/+2 SD (Table 1). The tetraploid (Sonoran) and the hexaploid (Mohave) guard cell area distributions overlapped at 1 SD.

Fossil plants

Fossil leaves of creosote bush were obtained from ^{14}C-dated packrat middens collected and archived

Table 1 Mean (± SE) guard cell area and sample size from the polyploids of *Larrea tridentata* in North America

Desert	N	Guard cell area (μm^2)	Ploidy
Chihuahuan	15	229.4 ± 8.1	2×
Sonoran	21	381.7 ± 10.2	4×
Mohave	13	482.6 ± 15.5	6×
Chihuahuan/Sonoran Transition	10	288.1 ± 12.8	
Mohave/Sonoran Transition	32	538.8 ± 17.7	
Baja, California	25	337.4 ± 18.6	
Total	116		

by several investigators during the past 40 years (for syntheses, see Van Devender *et al.*, 1987; Betancourt *et al.*, 1990; Thompson *et al.*, 1993). Radiocarbon dates are from associated material in the midden, except in a few cases where AMS dating of creosote bush leaves was prudent to discriminate between first arrivals, unusual associations and temporally mixed assemblages (Van Devender *et al.*, 1985; Betancourt *et al.*, 2001). We are confident that packrats collected the fossil leaves from plants growing in rocky environments within 100 m of the middens. By the same token, we recognize that the packrat midden record is biased against more open terrain, and thus may not fully represent the full range of habitats occupied by creosote bush in the past. In several midden studies where both macrofossils and pollen have been analysed, first appearances of southern species tend to be earlier in pollen than in macrofossil assemblages (King & Van Devender, 1977; Thompson, 1985; Davis, 1990; Anderson & Van Devender, 1991; Betancourt *et al.*, 1991, 2001). One explanation is common contamination by younger pollen in older middens as crystallized urine rehydrates, flows and cracks. Another explanation is that these species appear in the regional vegetation long before they become registered in the very local floras around midden sites. A high percentage (> 20%) of creosote bush pollen in a 14 ^{14}C kyr BP midden from Organ Pipe Cactus National Monument, southern Arizona, antedates first appearance of leaves by 6 kyr (Davis & Anderson, 1987; Davis, 1990). An AMS date of 18.7 ^{14}C kyr BP on creosote bush leaves from a

Table 2 Measured guard cell area and inferred ploidy of creosote bush leaves from fossil packrat middens across the North American deserts

Lat. (°N)	Long. (°W)	Location	Radiocarbon date (yr. BP ± SD)*	Guard cell area (µm²) (Ploidy)
37.22	117.50	Eureka View, CA	3930 ± 180 (Spaulding, 1980)	456 (6×)
37.22	117.50	Eureka View, CA	2625 ± 140 (Spaulding, 1980)	336 (4×)
37.22	117.50	Eureka View, CA	1580 ± 150 (Spaulding, 1980)	459 (6×)
35.45	115.10	McCullough Mountains, NV	6480 ± 90 (Spaulding, 1991)	536 (6×)
35.45	115.10	McCullough Mountains, NV	5060 ± 120 (Spaulding, 1991)	405 (4×)
35.45	115.10	McCullough Mountains, NV	modern (Spaulding, 1991)	378 (4×)
36.02	114.52	River Mountains, NV	8310 ± 55 (Spaulding, 1990)	359 (4×)
35.28	114.55	Searchlight, NV	970 ± 70 (Hunter & McAuliffe, 1994)	502 (6×)
34.40	115.35	Marble Mountains, CA	7930 ± 285 (Spaulding, 1980)	262 (2×)
34.40	115.35	Marble Mountains, CA	4475 ± 170 (Spaulding, 1980)	408 (4×)
34.40	115.35	Marble Mountains, CA	1680 ± 150 (Spaulding, 1980)	367 (4×)
34.13	114.22	Whipple Mountains, CA	11 015 ± 106 (Van Devender, 1990b)	255 (2×)
34.13	114.22	Whipple Mountains, CA	4240 ± 70 (Van Devender, 1990b)	466 (6×)
34.13	114.22	Whipple Mountains, CA	200 ± 70 (Van Devender, 1990b)	518 (6×)
32.58	114.50	Picacho Peak, CA	12 390 ± 340 (Cole, 1986)	217 (2×)
32.58	114.50	Picacho Peak, CA	10 640 ± 320 (Cole, 1986)	258 (2×)
32.58	114.50	Picacho Peak, CA	10 420 ± 110 (Cole, 1986)	165 (2×)
32.58	114.50	Picacho Peak, CA	8720 ± 110 (Cole, 1986)	340 (4×)
32.58	114.50	Picacho Peak, CA	8419 ± 275 (Cole, 1986)	503 (6×)
32.58	114.50	Picacho Peak, CA	8110 ± 160 (Cole, 1986)	453 (6×)
32.58	114.50	Picacho Peak, CA	7650 ± 120 (Cole, 1986)	334 (4×)
32.58	114.50	Picacho Peak, CA	7350 ± 90 (Cole, 1986)	542 (6×)
32.58	114.50	Picacho Peak, CA	4970 ± 80 (Cole, 1986)	436 (6×)
32.58	114.50	Picacho Peak, CA	630 ± 140 (Cole, 1986)	338 (4×)
32.58	114.50	Picacho Peak, CA	modern (Cole, 1986)	534 (6×)
32.36	114.08	Wellton Hills, AZ	10 580 ± 550 (Van Devender, 1990b)	308 (4×)
32.36	114.08	Wellton Hills, AZ	3520 ± 100 (Van Devender, 1990b)	332 (4×)
32.21	114.12	Butler Mountain, AZ	11 250 ± 410 (Van Devender *et al.*, 1985)	375 (4×)
32.21	114.12	Butler Mountain, AZ	8590 ± 570 (Van Devender *et al.*, 1985)	347 (4×)
32.21	114.12	Butler Mountain, AZ	610 ± 50 (Van Devender *et al.*, 1985)	373 (4×)
32.16	114.02	Tinajas Altas, AZ	21 300 ± 1250 (Van Devender, 1990b)	348 (4×)
32.20	111.27	Waterman Mountains, AZ	6195 ± 80 (Van Devender, 1990a)	255 (2×)
32.20	111.27	Waterman Mountains, AZ	1320 ± 45 (Van Devender, 1990a)	225 (2×)
31.58	112.47	Puerto Blanco Mountains, AZ	3400 ± 100 (Van Devender, 1987)	320 (4×)
31.58	112.47	Puerto Blanco Mountains, AZ	990 ± 50 (Van Devender, 1987)	296 (4×)
31.58	112.47	Puerto Blanco Mountains, AZ	130 ± 50 (Van Devender, 1987)	310 (4×)
34.58	111.83	Camp Verde, AZ	2510 ± 130 (Betancourt *et al.* in progress)	200 (2×)
34.53	111.80	Camp Verde, AZ	1655 ± 75 (Betancourt *et al.* in progress)	426 (4×)
34.58	111.84	Camp Verde, AZ	1560 ± 70 (Betancourt *et al.* in progress)	211 (2×)
34.53	111.80	Camp Verde, AZ	1135 ± 70 (Betancourt *et al.* in progress)	266 (2×)
34.58	111.84	Camp Verde, AZ	655 ± 80 (Betancourt *et al.* in progress)	204 (2×)
34.53	111.80	Camp Verde, AZ	300 ± 70 (Betancourt *et al.* in progress)	236 (2×)
31.58	113.36	Hornaday Mountains, Mexico	4430 ± 80 (Van Devender *et al.*, 1990)	312 (4×)
31.26	108.27	Sentinel Butte, NM	885 ± 115 (Betancourt *et al.* in progress)	221 (2×)
32.37	105.90	Rough Canyon, NM	3200 ± 70 (Betancourt *et al.*, 2001)	201 (2×)
34.05	106.44	Sevilleta, NM	2800 ± 65 (Betancourt *et al.* in progress)	226 (2×)
34.05	106.44	Sevilleta, NM	2290 ± 120 (Betancourt *et al.* in progress)	250 (2×)
34.05	106.44	Sevilleta, NM	1420 ± 60 (Betancourt *et al.* in progress)	153 (2×)
34.05	106.44	Sevilleta, NM	250 ± 60 (Betancourt *et al.* in progress)	252 (2×)
34.05	106.44	Sevilleta, NM	Modern (Betancourt *et al.* in progress)	244 (2×)
29.11	102.58	Big Bend National Park, TX	10 100 ± 90 (Van Devender, 1990a)	214 (2×)

* Radiocarbon dates published earlier.

midden at Tinajas Altas (Van Devender, 1990b), only 150 km to the west, makes the pollen interpretation seem plausible. In our synthesis of past creosote bush and ploidy race distributions, first appearance of creosote bush in the local midden record generally should be viewed as only minimum ages for first arrival in the region.

Fossil guard cell size was measured in multiple leaves from 51 different packrat middens at 18 locations across the south-western United States (Table 2). Fossil leaves were soaked in chloroform-isoamyl alcohol (24 : 1) for 5–12 h to remove leaf waxes, urine from the midden, and midden debris. The leaves were rinsed with water and pressed flat. Once dry, leaves were affixed to a microscope slide with fingernail polish to keep them stationary while a thin layer of polish was painted over the leaf. The polish was allowed to dry for 6–12 h, then the peel was removed and guard cell impressions in the peel were measured with a microscope. Twenty guard cells were measured per leaf, and in some cases only one leaf was available. To confirm that guard cell area was not affected by drying or environmental conditions, guard cell areas from five exemplar glasshouse plants were compared to the pressed and dried leaves from the seed source. The parent voucher material was 3–5 years old, and 20 guard cells were measured for each of three leaves. A *t*-test was used to compare the means.

RESULTS

The three ploidy levels in modern plants exhibited significantly distinct mean guard cell sizes ($F_{6,112} = 42.41$; $P < 0.0000$) (Table 1). Guard cell areas of the fresh and dried samples were statistically identical (mean difference = 4.4 μm^2; $t_4 = 0.59$; $P = 0.59$), confirming earlier evidence that preservation and environment does not confound cell size measurements (Masterson, 1994).

The present distribution of polyploids based on guard cell size (Fig. 4d) generally corroborates the original assumption that different polyploid populations were associated with different deserts (Yang, 1970), although there were a few notable exceptions. The transitional region between the Mohave (6×) and the Sonoran (4×) deserts showed substantial intermixing of the ploidy levels, and the mean guard cell area in this zone was higher than in the central Mohave. This differ-

ence could be a sampling artefact, and future work should be focused on this region. Sparse sampling in this relatively large geographical region prevents clear interpretation of this pattern. Overlap to a lesser degree occurred between the Sonoran (4×) and Chihuahuan (2×) deserts. Several diploid isolates were identified in regions dominated by tetraploids and hexaploids (Fig. 4d). All three ploidy levels are present in Baja California, where the hexaploids and tetraploids are separated by a region of diploids, and in the lower Colorado River Valley near Yuma, Arizona. Diploids and tetraploids intermingle in south-eastern New Mexico, and on limestone patches in southern and central Arizona.

Creosote bush was found in glacial-age middens from Cañon de la Fragua, Coahuila, Mexico; Big Bend National Monument, south-west Texas; central Baja California and the lower Colorado River Valley (Tinajas Altas Mountains, south-west AZ and Picacho Peak, south-east CA) near the boundary between the Mohave and Sonoran Deserts (locations of all midden sites are shown on Fig. 2). In the rainshadow of the Sierra Madre Oriental, Cañon de la Fragua represents one of the driest areas of the Chihuahuan Desert. Creosote bush was dated directly at 11.7 ^{14}C year BP (AA-4454, Van Devender, unpublished), but we did not measure guard cell sizes and thus cannot infer ploidy levels. We also did not directly date creosote bush or measure guard cell size in Big Bend middens that contain both creosote bush and honey mesquite (*Prosopis glandulosa* Torr.). The two middens were dated at 26.4 and 20.4 ^{14}C kyr BP, but the creosote bush fossils probably represent younger contaminants in older middens (Van Devender, 1990a). Today, Big Bend includes the hottest, driest habitats in the Chihuahuan Desert, an ideal setting for relics of diploid creosote bush populations during the last glacial period. This scenario is supported by a direct date on diploid creosote bush of 10.1 ^{14}C kyr BP from the Big Bend area (Fig. 4a) (Van Devender, 1990a).

About 650 km west of Big Bend, middens with glacial age or early Holocene diploid and tetraploid populations of creosote bush are from sites straddling the lower reaches of the Colorado River on the border between Arizona and California (Fig. 4a). Here high temperatures, high insolation and low rainfall (< 50 mm/yr) combine to produce the highest rates of pan evaporation
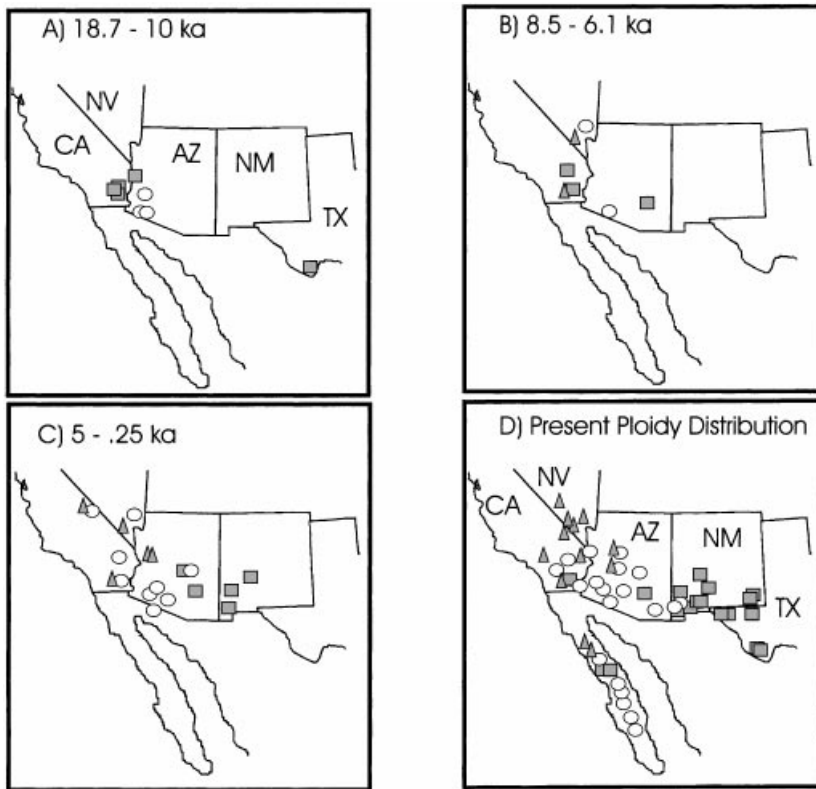
Fig. 4 Distribution of *L. tridentata* polyploids through time. The closed squares (■) are diploids, open circles (○) are tetraploids, and closed triangles (▲) are hexaploids. (a) Late Pleistocene/Early Holocene; (b) Mid-Holocene; (c) Late Holocene; (d) Modern distribution of polyploids.

(> 3650 mm/yr) on the North American continent. Tetraploid creosote bush fossils yielded ages of 18.7 ^{14}C kyr BP at Tinajas Altas, 11.3 ^{14}C kyr BP in the Butler Mountains, and 10.8 ^{14}C kyr BP in the Wellton Hills (Van Devender, 1990b). Diploid creosote bush yielded AMS dates of 12.3, 10.6, and 10.4 ^{14}C kyr BP at Picacho Peak (Cole, 1986) and 11.0 ^{14}C kyr BP in the Whipple Mountains, about 150 km to the north (Van Devender, 1990b). Creosote bush has also been identified from 21.7 ^{14}C kyr BP at Cataviña, Baja California, but its mixture with *Pinus juarezensis* Lanner, *Juniperus californica* and other chaparral elements suggests that creosote bush might be a younger contaminant. The creosote bush leaves in this sample were not directly dated with AMS, nor was guard cell size measured.

The postglacial history of North American creosote bush featured broadscale but surpris-ingly slow expansion into elevations and habitats vacated by pinyon–juniper–oak woodlands. In the lower Colorado River Valley, diploids expanded into the Marble Mountains, only 80 km west of the Whipple Mountains, by 9.5 ^{14}C kyr BP, becoming dominant by 7.9 ^{14}C kyr BP (Spaulding, 1990). Hexaploids first appear in the midden record at 8.4 ^{14}C kyr BP at low elevations at Picacho Peak (Cole, 1986) (Figs 2, 3b). Tetraploids expanded into southern Nevada, reaching the River Mountains by 8.3 ^{14}C kyr BP and the McCullough Range by 6.5 ^{14}C kyr BP (Spaulding, 1990). In both the Whipple and Marble Mountains, diploids were replaced by tetraploids by ~4.5 ^{14}C kyr BP (Spaulding, 1990; Van Devender, 1990b). Hexaploids continued to expand north into the northern Mohave Desert, reaching the modern northern limits of creosote bush at Eureka Valley between 5.6 and 3.9 ^{14}C kyr BP (Spaulding, 1990).

Postglacial expansion into the eastern Sonoran Desert was also gradual. In the Puerto Blanco Mountains, traces of creosote bush appear in a midden dated ~8.0 ^{14}C kyr BP, but tetraploids did not become dominant until after 3.4 ^{14}C kyr BP (Van Devender, 1987; 1990b). Tetraploids also appear in the Hornaday Mountains, Mexico, by 4.4 ^{14}C kyr BP. Diploid populations appear on limestone outcrops (rare for the Sonoran Desert) in the Waterman Mountains west of Tucson by 6.2 ^{14}C kyr BP (Van Devender, 1990b; Anderson & Van Devender, 1991). Creosote bush's modern, northern limits in the Sonoran Desert were established by at least 6.4 ^{14}C kyr BP (we did not measure guard cell size in this sample) (McAuliffe & Van Devender, 1998). Diploids and tetraploids, however, did not colonize upper elevation, limestone outcrops in the Verde Valley, central Arizona until after 2.5 ^{14}C kyr BP.

Diploids were slow to expand into the northern Chihuahuan Desert, arriving at the Hueco Mountains by 3.7 ^{14}C kyr BP, along Otero Mesa by 4.8 ^{14}C kyr BP, in the Sacramento Mountains by 3.3 ^{14}C kyr BP and the San Andres Mountains by 4.3 ^{14}C kyr BP (Van Devender, 1990a; Betancourt *et al.*, 2001). The first evidence of diploids at the Sevilleta Long-Term Ecological Research Site, at the northern limits of Chihuahuan Desert creosote bush, is for 2.8 ^{14}C kyr BP. Both diploid and tetraploid populations are now encroaching into desert grasslands along the Continental Divide that separate the Chihuahuan and Sonoran Deserts in south-western New Mexico/south-eastern Arizona. At Howell's Ridge Cave, located in desert grassland along the Continental Divide, reptile and amphibian fossils in a 12,000-year-old stratified deposit show that desert scrub expanded at the expense of desert grassland three times (3.9, 2.5, and 1.0 ^{14}C kyr BP) prior to European settlement (Van Devender, 1995). Thus, the range of creosote bush has probably expanded and contracted in south-western New Mexico several times in the late Holocene, with the latest invasion driven at least in part by overgrazing and fire suppression. Global warming may precipitate additional shifts in the distributions of all three ploidy races.

DISCUSSION

At present, there is insufficient evidence to determine the exact timing or climatic circumstances for creosote bush migration from South to North America. The most likely time for trans-tropic migration was during one or more Quaternary glaciations (the last 1.5 million years). During glaciations, the American tropics experienced drier episodes that probably closed the distance between semiarid habitats in the Andean highlands and Central American lowlands to less than 2000 km (e.g. Bradbury, 1997; Hooghiemstra & Van der Hammen, 1998; Harris & Mix, 1999). The most likely dispersal agents were migratory birds such as plovers (*Pluvialis dominica* Müller or *Bartramia longicauda* Bechstein) and birds of prey (*Falco peregrinus* Bonaparte or *Buteo swainsoni* Bonaparte) (Wells, 1977). Modern disjunct populations of creosote bush in southern Bolivia and Peru (Fig. 3) may represent either founder events from such dispersals or relicts from one or more northward expansions of Monte Desert elements into intermontane basins of the central and northern Andes.

Although we cannot date or place the arrival of creosote bush in North America, we do know that both the diploid and tetraploid races already inhabited the lower Colorado River Valley by the LGM, and diploids occupied sites in the central Chihuahuan Desert by late glacial times. Hence, during the glacial period, there were at least two distinct diploid populations east and west of the Sierra Madre Occidental, separated by ~650 km. One logical hypothesis is that both the tetraploid and hexaploid races were derived from the westernmost diploid populations. There is also a possibility that eastern diploids, western diploids and tetraploids overlapped in distribution during a previous interglacial.

The region where ploidy changed most dramatically was the lower Colorado River Valley of California (Cole, 1986). At this well-dated locality, six samples that ranged from 12 ^{14}C kyr BP to the present document a shift from diploids to tetraploids, and then from tetraploids to hexaploids (Fig. 2). Today, hexaploids are limited to summer-dry areas in Baja California and the Mohave Desert, and both the diploid and tetraploid races generally inhabit areas dominated by summer rainfall. Hypothetically, the hexaploid race could have evolved as creosote bush encountered hot, summer-dry areas at the north-western limits of its range during either a glacial or interglacial period. Summer-dry areas would have been most

extensive in Mexico and the south-western United States during glacial periods (Bradbury, 1997). Stable isotopic analysis of soil carbonates and megafaunal tooth enamel, however, indicate considerable C_4 grass biomass, and at least moderately wet summers, along the Mexico-United States borderlands during the last glacial (Liu *et al.*, 1996; Connin *et al.*, 1998; Monger *et al.*, 1998). Hence, if expansion into areas with dry summers drove the evolution of the hexaploid race, the most likely locus would have been one from central Baja California, the lower Colorado River basin and/or the present Mohave Desert.

The ploidy change from diploids eventually to hexaploids in North America can be explained by a shift in environmental conditions that would give the polyploids a selective advantage. A plausible mechanism for the success of higher ploidy races in North American *L. tridentata* is increased tolerance to aridity and/or higher temperature. Yang (1970) suggested increased aridity as the mechanism that produced the unique distribution of ploidy races seen in *L. tridentata.*

The physiological effects of polyploidy, either autopolyploidy or allopolyploidy, have been investigated in several crop species (Tal, 1980; McCoy & Bingham, 1988). Tetraploid tomatoes had larger but sparser stomata, reduced transpiration rates (84%), higher relative water contents and better water balance under high salinity in comparison with the diploid progenitor (Tal & Gardi, 1976). Polyploidization is thus a possible method of decreasing stomatal density rapidly because by increasing cell size the plant decreases stomatal density. A polyploid may have had a selective advantage over diploids due to decreased stomatal density and thus reduced water loss. It is plausible that the development of polyploids of *Larrea* both in North and South America is causally associated with climate change.

There is still the nagging question of why polyploidy evolved in North American *L. tridentata* but not *L. divaricata* in South America. One possibility is that the different species of *Larrea* in South America (*L. cuneifolia*, *L. nitida*, and *L. ameghinoi*) have prevented polyploids from becoming successful in nature by competitive exclusion. Equally possible is that the allotetraploid, *L. cuneifolia*, was formed under a similar selection gradient to that experienced in North America. *Larrea cuneifolia* occurs in the driest habitats in Argentina (Rossi *et al.*, 1999). Different evolutionary mechanisms, autopolyploidy and allopolyploidy, may have occurred due to the same selective regime.

Finally, fossil and modern guard cell sizes provide reliable inferences about the history and distribution of polyploidy, and could be analysed in many other polyploid complexes (e.g. the history of crop domestication). Even in the best of circumstances, however, the historical record is merely correlational. Although the historical record may imply specific mechanisms and pathways of polyploid evolution, it can seldom resolve them. Empirical and experimental studies are needed to investigate functional differences between polyploids and their progenitors. For creosote bush, new research could entail habitat differences in areas where two or more polyploid races co-occur, or differences in carbon allocation and water use efficiency among different ploidy levels grown under glacial to twice modern CO_2 levels.

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