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Creosote bush (*Larrea tridentata*) ploidy history along its diploid-tetraploid boundary in southeastern Arizona-southwestern New Mexico, USA

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ABSTRACT

Creosote bush (*Larrea tridentata*) is a dominant shrub in the warm deserts of North America and also a classic example of an autopolyploid complex. We determined ploidy levels for creosote leaves preserved in ancient packrat middens from the Peloncillo Mountains, AZ to better understand the history of ploidy race distribution along its diploid-tetraploid boundary. We also measured modern creosote ploidy level at several sites spanning the AZ-NM border to augment sampling in this large geographic area. Modern plants were mostly diploids, with tetraploids only observed in our northernmost sites. Ancient creosote from the Peloncillo middens (3170-145 cal yr BP) were all diploids. Modern creosote at the Peloncillo site is also diploid, but with significantly larger guard cells areas that may be a response to increasingly hot, dry conditions.

The lack of tetraploids in the midden fossils suggests the arrival of tetraploids at their eastern margin may have occurred only recently, mirroring the late arrivals of other Sonoran Desert plants (e.g., *Simmondsia chinensis*) along the AZ-NM border.

1. Introduction

Creosote bush (*Larrea tridentata*) is the most abundant and wide-ranging shrub in the warm deserts of North America. It is extremely tolerant of high temperatures and xerophytic conditions and ranges from sea level up to ~1600 m and frequently occurs as pure or nearly pure stands, especially on sandy or gravelly substrates along hillslopes, valley bottoms, and plains (Benson and Darrow, 1981; Turner et al., 1995). Creosote bush is a classic example of an autopolyploid complex, in which whole genome duplication within a species results in organisms with more than two complete sets of chromosomes (Ramsey and Schemske, 1998; Soltis et al., 2009). Since its arrival in North America sometime during the late Pliocene or Pleistocene (Laport et al., 2012), creosote bush evolved to include diploids ($2n = 2x = 26$), tetraploids ($2n = 4x = 52$), and hexaploids ($2n = 6x = 78$). These ploidy races exhibit an allopatric distribution with diploids primarily found in the Chihuahuan Desert, tetraploids in the Sonoran Desert, and hexaploids in the Mojave and western Sonoran Deserts (Hunter et al., 2001; Yang, 1970; Yang and Lowe, 1968). This allopatric distribution is similar to several other polyploid taxa in which ploidy races are segregated geographically along environmental gradients (Levin, 2002; Manzaneda et al., 2012; Parisod et al., 2010; Ramsey et al., 2008; Raven et al., 1968; Sutherland and Galloway, 2017). Because of a strong southeast to northwest increase in summer aridity and heat load in the warm North American deserts, creosote bush ploidy race distributions are hypothesized to be associated with trait variations that allowed for adaptation along this gradient (Barbour, 1969; Hunter et al., 2001; Hunziker et al., 1977; Yang, 1970).

Polyploids often exhibit traits that vary from those of their diploid progenitors. Trait variations observed in polyploids include increased cell size; changes in the size, number and/or architecture of vegetative structures, flowers, and/or seeds; changes in phenology; greater rates of self-fertilization and asexual reproduction; increased resistance to pathogens and herbivores; and increased heat tolerance (Barbour, 1969; Barringer, 2007; Laport and Ramsey, 2015; Levin, 1983; Masterson, 1994; Ramsey, 2011; Ramsey and Schemske, 2002). The extent to which novel traits observed in natural polyploid populations are derived from genome duplication versus subsequent evolutionary change is difficult to assess, but immediate phenotypic effects of genome duplication are well-documented in experimentally-created polyploids (Husband et al., 2008; Maherali et al., 2009; Ramsey, 2011). Such trait variations may contribute to altered or broader ecological niches in polyploids, thus “pre-adapting” them to exploit new habitats and conditions (Brochmann et al., 2004; Glennon et al., 2014; Levin, 1983;

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Martin and Husband, 2009; McIntyre, 2012; Pandit et al., 2011). In the case of creosote bush, recent ecological niche modeling supports the hypothesis that climatic adaptation contributed to the current allopatric distribution of its ploidy races (Laport et al., 2013).

Determination of ploidy races from fossil creosote bush leaves, however, shows that their geographic distributions have shifted through time and that today's pattern is of relatively recent origin (Hunter et al., 2001). Because one immediate result of genome duplication is an increase in cell size to accommodate additional chromosome sets (Johansen and van Bothmer, 1994; Stebbins, 1971), ploidy level can be determined from leaf guard cell area in both modern and fossil leaves (Masterson, 1994). Hunter et al. (2001) used such differences in guard cell areas to map changes in creosote bush ploidy distributions since the Last Glacial Maximum (LGM, ~21,000 years ago) from modern plants as well as fossil leaves preserved in ancient packrat middens. Packrat middens are amalgamations of plant remains, fecal pellets, insects, and bones collected by members of the genus *Neotoma* and encased in crystalized urine, which allows for preservation of materials for up to tens of thousands of years. Midden series can be collected and materials washed, radiocarbon dated, and identified to provide "snapshots" of vegetation change through time, as well as materials for morphological and chemical analyses (Betancourt et al., 1990). Midden radiocarbon ages from Hunter et al. (2001) discussed herein have been calibrated using the Calib 7.1 IntCal 13 curve (Stuiver et al., 2018; http://calib.org), and are reported as the midpoint of the 2-σ age range.

In their study, Hunter et al. (2001) found both diploid and tetraploid populations present in the lower Colorado River Basin by the LGM, an area where diploids are rare today, as well as a separate area of diploids in the central Chihuahuan Desert by late glacial times. Creosote bush slowly expanded during the postglacial period as climate became warmer and drier, with hexaploids first appearing ~9400 cal yr BP in the lower Colorado River Basin and then spreading northward into southern Nevada. Tetraploids spread into southern Nevada too, while also largely replacing diploids in the lower Colorado River Basin by ~5500 cal yr BP. Tetraploids spread eastward more slowly, not appearing in the eastern Sonoran Desert at Camp Verde, AZ until ~1550 cal yr BP. Here they encountered diploids, which were present in middens from the site dating to 2540, 1450, 1080, 630, and 250 cal yr BP (Hunter et al., 2001). The Verde Valley, where the dominant substrate is limestone (like the Chihuahuan Desert), remains a rare area of sympatry between tetraploids and diploids today. Much remains unknown, however, about the history of creosote ploidy races along the diploid-tetraploid transitional region in southeastern Arizona and southwestern New Mexico.

Although both ploidy races occur in the transitional region today, Hunter et al. (2001) identified only diploids outside of the Verde Valley in midden fossils. This pattern, however, is based on a relatively small number of fossils from the area, with diploids measured in two middens from the Waterman Mountains, AZ (7080 and 1240 cal yr BP), one from Rough Canyon, NM (3410 cal yr BP), five from Sevilleta, NM (2930-0 cal yr BP) and one from Sentinel Butte, NM (810 cal yr BP) (Fig. 1). If confirmed, this pattern suggests the arrival of tetraploids at their eastern margin may have occurred only recently, mirroring the late arrivals of other Sonoran Desert plants (e.g., *Simmondsia chinensis*) along the AZ-NM border (Holmgren et al., 2006). To further assess the history of creosote ploidy race distribution along the diploid-tetraploid boundary, we determined ploidy levels for fossil creosote leaves obtained from ancient packrat middens from the Peloncillo Mountains, AZ that had been previously collected and radiocarbon dated (Holmgren et al., 2006), expanding the number of fossils analyzed from this area. In addition, we measured modern creosote ploidy level at several sites spanning the AZ-NM border to augment sampling in this large geographic area.

2. Materials and methods

2.1. Modern creosote bush

To determine ploidy races in modern creosote populations, we collected branchlets from 20 plants at each of 10 sites spanning the southern Arizona-New Mexico border region (Fig. 1). We collected plants along approximately straight-line transects and selected plants growing at least 10 m apart to avoid repeated sampling of ring clones. At the Double Adobe site, we were limited to 10 plants based on the small areal extent covered by creosote growing there. At the Peloncillo Mountains and Camp Verde sites with ancient midden records, we sampled creosote at three elevations (high, middle, low) along the hillslope-to-valley gradient to see if microsite differences in elevation affected ploidy level. For the Peloncillo Mountains these elevations were at 1407, 1284, and 1175 m and for Camp Verde at 1186, 1066, and 994 m. We used a handheld GPS unit to record latitude, longitude, and elevation at each sampling site.

In the laboratory, we measured guard cells area following Hunter et al. (2001). We placed a few (3–5) leaves from each plant into shallow dishes, covered them with water to remove detritus and soften for ~30 min, then pressed the leaves between blotting paper to flatten and dry. We then painted the leaves with clear fingernail polish and allowed them to dry for 12 + hours. We peeled off the dried polish with a razor blade, placed the peel between a microscope slide and coverslip, and measured the lengths and widths of 10 guard cell impressions at 400X magnification on a compound light microscope equipped with an eyepiece reticule. Representative guard cells with sharp, well-defined boundaries were chosen for measurement. We calculated guard cell area using the formula for an ellipse: $\pi(L/2)\ (W/2)$, where L is the length and W is the width of a guard cell. We then used the average of the 10 guard cell measurements to determine the plant's ploidy level.

We assigned ploidy races based on published values in Hunter et al. (2001). In that study, ploidy levels were assigned based on guard cell sizes from central regions of deserts. For diploids they used the Chihuahuan Desert mean ± 2SD (166.6–292.2 μm^2) and for tetraploids the Sonoran Desert mean −2/+1 SD (288.1–428.5 μm^2). To deal with the small overlap of diploids and tetraploids at 2 SD (288.1–292.2 μm^2) we chose 290 μm^2 for the upper limit of diploids/lower limit of tetraploids, although only four leaves (out of the 303 in our study) fell within the range of overlap.

2.2. Fossil creosote

We also determined ploidy level for 53 fossil creosote leaves from 20 middens ranging in age from 3170 to 145 cal yr BP from rock shelters in volcanic rocks in the northern Peloncillo Mountains, AZ (just north of Interstate Highway 10, a few miles west of the AZ-NM border). These 20 middens containing creosote bush are a subset of a larger series of 55 middens from the site spanning 36,000 years that was previously analyzed for macrofossils and pollen (Holmgren et al., 2006). Radiocarbon dates from associated midden materials have been calibrated using the Calib 7.1 IntCal 13 curve (Stuiver et al., 2018; http://calib.org) and are reported as the midpoint of the 2-σ age range (Table 1). Depending on the amount of fossil material available, we analyzed between one to four leaves per midden, following the same procedures as for modern leaves. Because of the limited number of leaves available, however, we measured the area of 20 guard cells per leaf (versus 10 for modern leaves) and used their average area to determine ploidy level.

3. Results

3.1. Modern creosote bush

Modern plant guard cell areas varied from 199 to 423 μ2 (Fig. 2a),

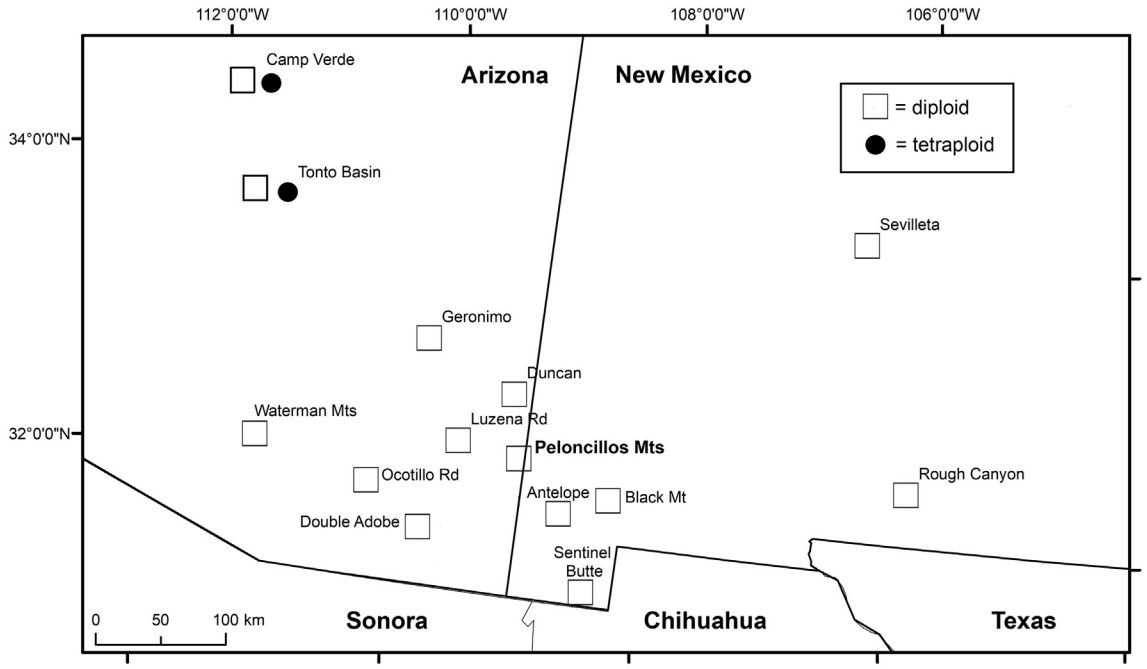

Fig. 1. Map showing location of fossil *L. tridentata* polyploids from Hunter et al., (2001) (Camp Verde, Rough Canyon, Sentinel Butte, Sevilleta, Waterman Mts) and modern *L. tridentata* polyploids as found in this study (all other sites). For the Peloncillo Mts, modern and fossil *L. tridentata* were all diploid.

with most values falling in the diploid (2x) range. The box plot to the right of the distribution histogram shows the interquartile range (IQR), with whiskers drawn to the furthest points falling within 1.5*IQR. The whiskers encompass all 2x values in our ploidy divisions, whereas the upper whisker value of 312 μ^2 coincides fairly closely with our diploid (2x) – tetraploid (4x) boundary of 290 μ^2. Accordingly, the individual points above the top whisker indicating outliers all fall within our 4x range. The close correspondence between the upper 1.5*IQR whisker in our measurements and the 2x – 4x boundary based on the work Hunter et al. (2001) suggests that a reliable transition between ploidy levels exists and supports the use of guard cell area in their diagnosis.

The only modern sites with tetraploids present were Camp Verde and Tonto Basin (Fig. 1). Both Camp Verde and Tonto Basin sites have a mix of both 4x and 2x plants present. Tetraploids make up 18% of the plants measured at Camp Verde and 85% of the plants measured at Tonto Basin. For all other sites, plants have guard cell areas with 2x values (Fig. 1). At the Camp Verde and Peloncillo Mountains sites, where we sampled creosote at three elevations (high, middle, low), we found no significant relationships between guard cell area and elevation.

3.2. Modern versus fossil creosote bush

We did, however, find differences in guard cell areas between modern and fossil leaves from the Peloncillo Mountains (Fig. 2b and c). Modern guard cell areas range from 217 to 274 μ^2 while fossil areas range from 168 to 236 μ^2. Furthermore, results from a *t*-test indicate mean guard cell areas of modern leaves are significantly larger than those of the fossil leaves (mean difference = 55.9 μ^2, $t_{105} = 20.0$, $P < 0.0001$). Despite these differences, all modern and fossil leaves fall

Table 1
Site locations and radiocarbon dates for the Peloncillo Mountains, AZ packrat middens containing creosote bush. The midden designation code WDC indicates West Doubtful Canyon within the Peloncillo Mountains Wilderness Area.

Midden designation	Latitude (°N)	Longitude (°W)	^{14}C age (yr BP)	SD	Calib. (2σ) age range (yr BP)	Midpoint	Guard cell area (μm^2) (all leaves 2x)
WDC 4B	32.312	109.091	170	30	0–290	145	179.5
WDC 16	32.310	109.093	180	50	0–303	150	181.2
WDC4A	32.312	109.091	395	30	322–511	470	180.4
WDC 37B	32.312	109.093	925	30	769–924	850	207.7
WDC 9B	32.310	109.097	1130	30	962–1172	1065	181.5
WDC 9D	32.310	109.097	1140	35	968–1173	1070	180.7
WDC 66A	32.299	109.099	1194	35	968–1240	1120	182.7
WDC 38C	32.313	109.092	1265	40	1081–1285	1185	200.25
WDC 40A	32.313	109.092	1280	50	1078–1294	1185	194.1
WDC 40C	32.313	109.092	1310	35	1181–1295	1240	181.6
WDC 14B	32.313	109.093	1485	35	1303–1516	1410	191.5
WDC 14C	32.313	109.093	1485	35	1303–1516	1410	223.8
WDC 14A	32.313	109.093	1495	40	1305–1520	1415	198.8
WDC 38A	32.313	109.092	1555	45	1353–1540	1445	218.4
WDC 34C	32.312	109.106	2070	40	1934–2143	2040	193.6
WDC 10A	32.300	109.109	2100	70	1901–2307	2105	211.9
WDC 38B	32.313	109.092	2235	45	2149–2342	2245	212.6
WDC 42	32.303	109.106	2530	70	2379–2755	2065	201.8
WDC 9A	32.310	109.096	2530	40	2489–2748	2620	189.3
WDC 15G	32.313	109.093	2985	40	3006–3329	3170	187.4

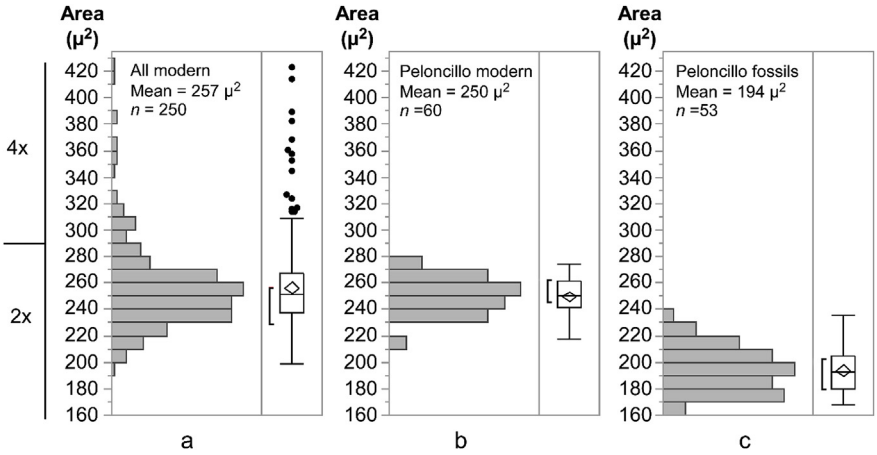

Fig. 2. Distribution of *L. tridentata* guard cell areas. Box plot shows the interquartile range (IQR), with whiskers drawn to the furthest points within 1.5 x the IQR. (a) Modern plants - all sites, (b) Modern plants - Peloncillo Mts only, (c) Fossil plants in middens - Peloncillo Mts.

within the diploid range of guard cell values.

4. Discussion

The absence of tetraploids outside of the Verde Valley in middens, including those from the Peloncillo Mountains, suggests co-occurrence of ploidy races in this contact zone is quite recent. It is possible that tetraploids arose recently in an independent polyploidization event here in response to environmental stress rather than migrating into the region. On the other hand, the late appearance of tetraploids mirrors the recent arrivals of other Sonoran Desert plants along the AZ-NM border. For example, jojoba (*Simmondsia chinensis*) is found as a disjunct population ~40 km east of its nearest populations at the Peloncillo Mountains site today but is absent in the midden record (Holmgren et al., 2006). This area in southeastern AZ-southwestern NM also corresponds to one of the suture zones, or areas where multiple hybrid zones exist, identified by Remington (1968) and later confirmed as a contact zone hot spot for birds, mammals, and phylogeographic breaks (Swenson and Howard, 2005). It has been surmised that the clustering of hybrid zones, contact zones, and phylogeographic breaks reflects post-glacial expansion out of refugia as climatic barriers were eliminated, followed by contact of populations (Remington, 1968; Swenson and Howard, 2005). These clusters are also strongly associated with mountain chains including the Peloncillo Mountains (Swenson and Howard, 2005), which act as barriers to migration and gene flow. Long-term vegetation records from middens are consistent with these ideas. Creosote would have been excluded from the area during the last ice age due to cooler, wetter conditions that supported pinyon-juniper woodland and desert grassland, and it was not until warming and drying in the Holocene that conditions become favorable for the species (Holmgren et al., 2003, 2006; 2007), with diploids first appearing in middens at 3170 cal yr BP. The absence of creosote with tetraploid-sized guard cells in middens, as well as the apparently recent arrival of jojoba, suggests ongoing expansion of Sonoran Desert elements into this area.

The increase in guard cell area from fossils to diploids and modern plants growing today in the Peloncillo Mountains is intriguing. An obvious question is whether smaller fossil guard cell areas are a result of aging and drying of leaf material. While we cannot absolutely rule this out, measurements of fresh versus older, pressed and dried materials in prior studies (Hunter et al., 2001; Masterson, 1994) indicate preservation and environment do not affect guard cell size. Furthermore, linear regression indicates no relationship between midden age and midden average guard cell area in the Peloncillo record ($R^2 = 0.15$, $P = 0.095$).

Thus, assuming preservation is not an issue, then the presence of larger guard cell sizes in the Peloncillo Mountains appears to be a very recent phenomenon. No guard cell sizes approaching the modern mean are seen in midden fossils. The youngest middens containing creosote date to 150 and 145 cal yr BP (Table 1), suggesting establishment of larger guard cells occurred since then.

One explanation is that larger modern guard cells reflect a response to hotter, drier conditions associated with recent anthropogenic climate change. Nordihydroguaiaretic acid (NDGA), a secondary phenolic compound produced by creosote bush that is particularly concentrated in its leaves, is thought to provide resistance to drought, UV radiation, and herbivory (Rhoades, 1977; Hyder et al., 2002). A study of creosote polyploids found that higher ploidy levels produced significantly higher NDGA concentrations (Zuravnsky, 2014). The study also documented variation in NDGA concentration in individuals growing in the Chihuahuan, Sonoran, and Mojave Deserts that successfully predicted the current ploidy distribution of creosote bush, an effect explained by interactions between temperature, precipitation, and solar radiation in a principal component analysis. Furthermore, diploid individuals growing in the field had significantly higher NDGA concentrations than those grown in greenhouse conditions with access to additional water. Combined, these factors suggest NDGA confers an advantage in hot, dry settings and that changes in concentrations may be driven by long-term changes in environmental conditions (Zuravnksy, 2014). The need to accommodate additional NDGA in response to warming over the past century may thus explain the larger guard cells seen in modern creosote at the Peloncillo Mountains. Although beyond the scope of this study, comparisons between fossil and modern leaves from additional midden series may provide additional evidence for such changes.

Another possible explanation for larger modern guard cell sizes is that they represent triploid plants resulting from natural crosses between diploids and tetraploids in the transitional region between deserts where the two ploidy types intermingle and, presumably, intermix (Hunter et al., 2001). The presence of triploids presumes the presence of tetraploids in the area. Although tetraploids were not found in the modern creosote populations we sampled southeast of Tonto Basin (Fig. 1), sympatric occurrences of diploids and tetraploids are fairly rare and transitions between populations are often abrupt (Laport et al., 2016). As such, our sampling could easily have missed tetraploids in the region. Indeed, tetraploids have been documented in southeastern Arizona and southwestern New Mexico by others (Hunter et al., 2001; Laport and Ramsey, 2015; Laport et al., 2016), confirming their presence in the region today.

Finally, it is also possible that larger guard cells are a result of an expansion of the genome over time, perhaps due to the activity of transposable elements. While we can only speculate as to which of these explanations is correct based on our data, they suggest several avenues for future research. The use of flow cytometry, while beyond the scope

of this study, would provide direct measurements of the c-value of modern creosote plants and allow for additional inferences about variation in genome size variability and potential changes over time. In addition, future work at the site to discover whether triploids are present would help assess whether their presence might account for larger modern guard cell sizes.

Our study provides new insight into the long-term history of creosote ploidy races along the diploid-tetraploid boundary in southeastern Arizona and southwestern New Mexico. As seen in Hunter et al. (2001), the geographic distribution of creosote bush is complex and many of today's patterns of are very recent origin. Additional studies have confirmed the complexity of modern creosote cytotype distributions (Laport et al., 2012), and many questions remain, especially about the dynamics along their contact zones. The apparently recent arrival of tetraploids in SE Arizona-SW New Mexico suggests this will remain a fruitful area for studying polyploid formation, migration, and evolution. Understanding these dynamics may prove critical to predicting future responses to ongoing anthropogenic climate change, so continued research into both past and modern patterns is warranted.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaridenv.2019.02.002.

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