PERGAMON Biochemical Systematics and Ecology 26 (1998) 371–377

biochemical systematics and ecology

Invasion of grasslands by *Juniperus ashei*: A new theory based on random amplified polymorphic DNAs (RAPDs)

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Received 23 June 1997; accepted 6 September 1997

Abstract

Random amplified polymorphic DNAs (RAPDs) were analyzed for three populations of *Juniperus ashei*: ancestral (Ozona, TX), a typical central Texas limestone substrate, and an adjacent population on deep black soil. A total of 175 RAPD bands were analyzed by principal coordinate analyses. As with the previous research based on morphology and volatile leaf oils, the Ozona plants clustered separately. In addition, there appears to be differentiation between the trees on limestone and those adjacent on blackland soil (separated by 30–50 m). It is hypothesized that new mutations and/ or allelic combinations have enabled the *J. ashei* to invade the tall grasses and deep soils. If true, this could help explain the recent (and continuing) invasion of abandoned farmlands by *Juniperus* in the United States.

Keywords: Grassland ecology; Juniperus; Cupressaceae; RAPDs, Genetic differentiation

1. Introduction

The invasion of range grasslands by *Juniperus* in the United States has now expanded to affect millions of acres. In fact, Juniper species affect over 21.5 million acres in Texas alone (Smith and Rechenthin, 1964). The principal species involved are *Juniperus ashei* Buch. (rock cedar or mountain cedar), *J. deppeana* Steudel (alligator

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0305-1978/98/$19.00
PII: S0305-1978(97)00132-4

bark juniper), *J. monsperma* (Engelm.) Sarg.(one-seeded juniper), *J. pinchotii* Sudw. (copper fruited juniper) and *Juniperus virginiana* L. (eastern red cedar). The common substrate inhabited by *Juniperus* (as a genus) is limestone (Adams, 1993). However, in the eastern United States one can find old trees of *J. virginiana* along fence rows in tall grasses and deep soils. This may be due to openings in the habitat due to fires or erosion or may be due to the sheer numbers of seeds "planted" by birds sitting on fence rows and the chance favorable germination and survival of a seedling in these conditions. In Texas, J. ashei, was thought to have been confined to rocky outcrops and steep slopes until recently (Owens, 1996).

Throughout the world, extensive juniper forests occur on limestone. These are often called cedar glades or cedar breaks. Only a few *Juniperus* species occur in mesic habitats (e.g. *J. barbadensis* L. var. *lucayana* (Britton) R.P. Adams in the Caribbean and *J. virginiana* L. var. *silicicola* along the coast in the southeastern United States (Adams, 1993, 1995).

Juniperus ashei occurs on limestone from northern Mexico to Oklahoma and Arkansas, with the bulk of the distribution found in the limestone hill country of Texas (Adams, 1977). Analyses based on volatile leaf terpenoids and morphology revealed the major geographical trend divided the populations into a recently derived group (Pleistocene) that was very uniform and the ancestral populations in northern Mexico, and far west Texas (Adams, 1977).

Juniperus ashei is now invading deep, blackland soils in central Texas. We recently discovered young (ca. 5–7 years old) *J. ashei* plants growing on the edge of an old field in deep, blackland soil in the Leon River flood plain of central Texas and a population of old (50–several hundred years) *J. ashei* tree growing on limestone about 30–50 m away. We hypothesize that some novel mutation(s) or gene combinations are involved in the success of *J. ashei* in invading this tall grass, deep blackland soil area.

In order to search for genetic differences between these two edaphically different sites, one must have genetic markers that are not environmental influenced. DNA fingerprints are just such genetic markers. For example, DNA polymorphisms have been detected by random amplified polymorphic DNAs (RAPDs) (Williams et al., 1990; Hu and Quiros, 1991; Demeke et al., 1992; Heusden and Bachmann, 1992). One of the earliest taxonomic use of RAPDs was for the analysis of the classical *Brassica* U triangle relationships (Demeke et al., 1992). They found that using about 100 RAPD bands gave very good agreement with the classical *Brassica* relationships. Heusden and Bachmann (1992) used RAPDs for systematics studies in *Microseris elegans* and found RAPDs to be complementary to isozymes and morphology.

Adams and Demeke (1993) found RAPDs to be useful in *Juniperus* at taxonomic levels ranging from the sub-generic to the varietal level. RAPDs appear ideal for taxonomic use because analyses are very quick, inexpensive and randomly distributed over the entire genome (Williams et al., 1990).

In this paper, we report on the use of RAPDs to detect genetic differences between *J. ashei* trees growing on limestone site and young, invading trees growing in the deep, black soils of central Texas, as well as differences between the ancestral and recent populations of *J. ashei* (Adams, 1977).

2. Materials and methods

Foliage samples were taken from *J. ashei* and placed on ice until frozen (later the same day). All of the plants sampled are vouchered at BAYLU! Vouchers (location, *R. P. Adams* collection numbers): Limestone (recent – Pleistocene) population: limestone hill, 25 km east of Gatesville, TX, 7423–7442; blackland (invading) population: approx. 30–50 m south of the limestone hill in deep black soil in the Leon River flood plain, 7443–7462; Ancestral population: on limestone, 5 km west of Ozona, TX, 7447–7462. Fig. 1 shows the study site where individuals were collected from typical limestone habitat and the area where junipers have invaded the deep, blackland soil on the Leon River flood plain.

The RAPDs analyses follow that of Adams and Demeke (1993). Leaves were transported fresh and frozen upon arrival. DNA was extracted from juniper leaves by the hot CTAB protocol (Doyle and Doyle, 1987) with 1% (w/v) PVP added to the extraction buffer. Ten-mer primers were purchased from the University of British Colombia (5′-3′): 123: GTA GAC GAG C; 131: GAA ACA GCG T; 134: AAC ACA CGA G; 153: GAG TCA CGA G; 184: CAA ACG GAC C; 204: TTC GGG CCG T; 212: GCT GCG TGA C; 218: CTC AGC CCA G; 227: CTA GAG GTC C; 232: CGG TGA CAT C; 237: CGA CCA GAG C; 250: CGA CAG TCC C; 265: CAG CTG TTC A; 268: AGG CCG CTT A; 347: TTG CTT GGC G;.

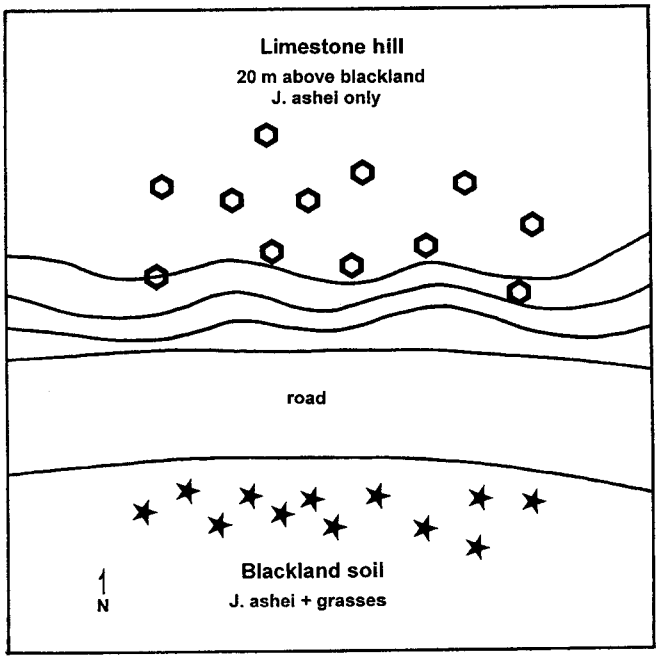

Fig. 1. Site map showing the locations of *J. ashei* individuals sampled near the Leon River in central Texas. Note the near proximity of the limestone hill plants to those across the road in the deep blackland soil.

PCR was performed in a volume of 12.5 μl containing 50 mM Tris-HCl (pH 9), 2.0 mM $MgCl_2$, 0.01% gelatin and 0.1% Triton X-100, 0.2 mM of each DNTPs, 0.36 μM primers, 0.25 ng genomic DNA, and 0.5 unit of Taq DNA polymerase (Promega). A control PCR tube containing all components, but no genomic DNA, was run with each primer to check for contamination. DNA amplification was performed in an MJ Programmable Thermal Cycler (MJ Research, Inc.). The thermal cycle was: 94°C (1.5 min) for initial strand separation, then 40 cycles of 38°C (2 min), 72°C (2 min), 91°C (1 min). Two additional steps were used: 38°C (2 min) and 72°C (5 min) for final extension. Bands were scored in four classes: very bright(= 6); medium bright(= 5), faint(= 4) and absent(= 0). See Adams and Demeke (1993) for details on electrophoresis and RAPD band scoring.

Similar measures were computed using absolute character state differences (Manhattan metric), divided by the maximum observed value for that character over all taxa(= Gower metric: Gower, 1971; Adams, 1975a,b). Principal coordinate analysis (PCO) of the similarity matrix follows Gower (1966). Program PCO3D is available for MS DOS IBM compatibles with a hard disk and math co-processor (correspond to RPA for distribution details).

3. Results and discussion

The invasion of *J. ashei* into sites not previously considered typical is shown in Fig. 1. The limestone hill site is typical for millions of acres of *J. ashei* habitat. It is in the limestone hills that one finds pure stands of *J. ashei* with no other terrestrial plant species present. The deep, blackland site (Fig. 1) is in the flood plain of the Leon River and is the corner of a field that has apparently not been cultivated in several years (the oldest junipers found at this site were 6 years old). The deep soil site is not typical of the habitat for *J. ashei.* The site is filled with competitive grasses, herbs and other species. Clearly, *J. ashei*, under these conditions is at a disadvantage during the first few years growth. This seemed a suitable site to examine the theory that the invading junipers have a new mutation enabling them to compete in this environment.

After screening over one hundred RAPD markers, we have yet to find a clear marker that has a 100% fidelity in separating the limestone based and blackland soil junipers. Primer 347 did, however, reveal some differences that generally, but not completely discriminated between the populations (Fig. 2).

However, if one subjects the data matrix to principal coordinate analysis to factor the matrix, one can examine the trends (if present) in the data. Although eleven eigenroots were larger than the average diagonal element, the eigenroots appear to asymptote after the first two roots and no biological patterns could be discerned in eigenroots 3–11. The first three eigenroots accounted for 11.4, 7.1, and 6.4% of the variance among the 29 individuals. This seems to indicate that there is not strong groupings among the individuals (i.e., subsets within the set). However, if we ordinate the 29 individuals onto the first three coordinates, we do see biological information (Fig. 3). Notice that the ancestral (Ozona) population is clearly separated from the other individuals on axis 1 (11.4% of the variance). This trend is congruent with the

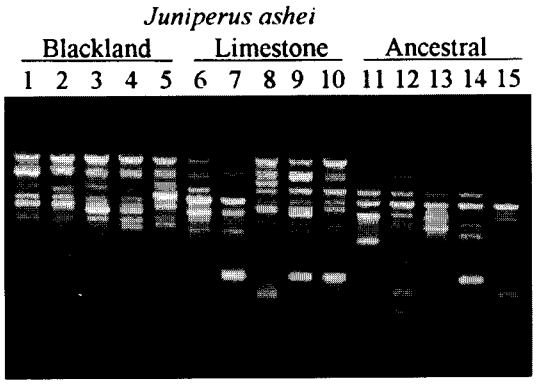

Fig. 2. Comparison of RAPDs for *J. ashei* individuals invading blackland soil (lanes 1–5), limestone hill nearby (lanes 6–10), and the ancestral population at Ozona (lanes 11–15). Note the bright fast running band in three of the five Limestone individuals and the lack of slow running bands in the blackland individuals.

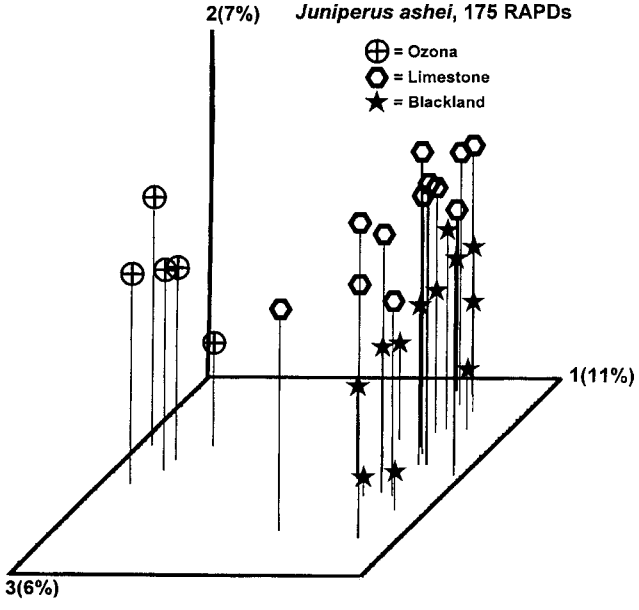

Fig. 3. Principal coordinate analysis and 3-D ordination of individuals based on 175 RAPD bands. Axis one (11% of the variation) separates the ancestral individuals (crossed circles) from the limestone (recent, open hexagons) and blackland (stars) junipers. Axis two (7%) separates the recent (Pleistocene) limestone substrate based individuals from the individuals invading the blackland soil.

previous work (Adams, 1977), which showed the Ozona (and northern Mexico and far west Texas) population to be different from the more recent (Pleistocene) populations in central Texas. There is also some separation between the limestone (open hexagons) and the blackland soil (stars) (Fig. 3).

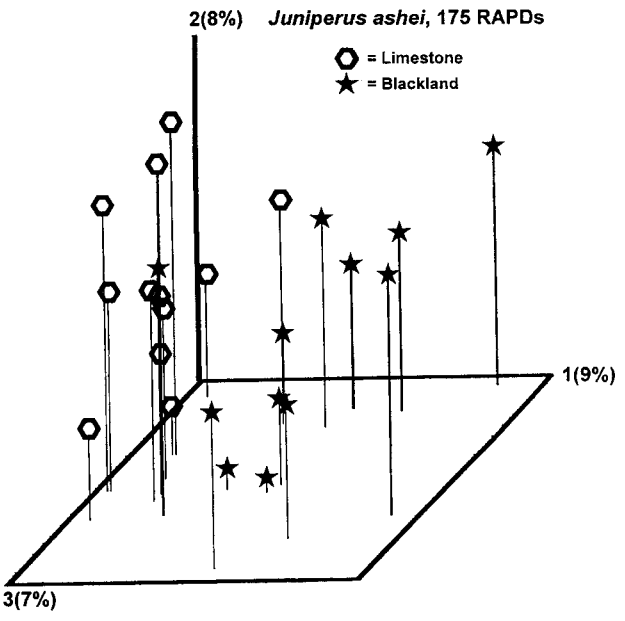

Fig. 4. Principal coordinate analysis and 3-D ordination of limestone and blackland growing junipers based on 175 RAPD bands. The blackland (stars) and limestone (open hexagons) are nearly separated. See text for discussion.

In order to further examine the separation between the limestone and blackland growing junipers, the five Ozona junipers were removed from the data set and the remaining 24 junipers (12 Limestone, 12 blackland) were subjected to PCO. Ordination shows the limestone and blackland junipers are nearly separated (Fig. 4). Notice the lone blackland juniper that clusters with the limestone junipers and one of the limestone junipers clusters with the blackland junipers. Clearly, these two populations are almost genetically identical. It is likely that they differ only in a few genes – but a very important difference, in that the junipers on the blackland have the unusual (for *J. ashei*) ability to compete with tall grasses while very young.

One should remember that the 3-D ordination in Fig. 4 only accounts for about 24% of the variation among these 15 trees and 76% of the variation is not shown. Much of this variation is due to individual polymorphisms. A second factor to consider is that we are only examining length and sequence polymorphisms for 175 sites in the genome. Although Williams et al. (1990) have shown RAPD bands to be randomly distributed across the chromosomes, we still have only a small portion of the total genome represented.

Is there a gene for "weediness" enabling these *J. ashei* to invade these deep soils with tall grasses? Although we have yet to a single band that clearly separated all of the "blackland" plants from the "limestone" plants, the ecological evidence is supportive of intense selection. In spite of the fact that these two sites are separated by only about 30–50 m and this juniper species is dioecious and wind pollinated, we have evidence of

differentiation across this short distance. Three of the largest junipers on the blackland were cut and found to have six growth rings, indicating that the population is very new. It is likely that the field edge was abandoned about 8–10 years ago and the junipers became established.

It is seems likely that the major hindrance in the establishment of juniper in grasslands is the relatively slow growth of juniper seedlings during the first few years. In tall grasses, the young seedling faces stiff competition for sunlight, moisture and nutrients. In order to further investigate this, we are currently attempting to clone plants from both the limestone and blackland sites. These cloned plants will then be used in common gardens to measure relative growth rates and also to set out some competitive test plots with grasses.

Acknowledgements

This research supported by funds from the USDA.

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