DNA FROM HERBARIUM SPECIMENS: I. CORRELATION OF DNA SIZE WITH SPECIMEN AGE

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ABSTRACT

Comparisons are made of DNA [(mode, bp); range (max, min)] extracted from 50 herbarium specimens of *Juniperus* ranging from 1 to 80 years old. The size of DNA declined with age, but varied considerably for specimens less than 20 yrs. old. After about 20 yr. the size of the DNA appeared to asymptote at about 200 - 500 bp. Variation in the quality of DNA from recent specimens may be due to drying methods and storage conditions (humidity, temperature). Degradation in older specimens may be more influenced by oxidative processes. *Phytologia 92(3): 346-353 (December 1, 2010).*

KEY WORDS: DNA, herbarium specimens, degradation, *Juniperus*.

It is now considered standard procedure to collect specimens and put some leaves in silica gel for subsequent DNA extraction. Liston et al. (1990) seem to be the first to utilize silica gel in the field, although Doyle and Doyle (1987) suggested that drying appears to be effective in preserving DNA and Doyle and Dickson (1987) also found that drying was a good preservation method for DNA restriction analysis.. Later, Chase and Hills (1991) promoted silica gel for field preservation. However, with the ephemeral condition of some species, the increased restrictions on the transport of plant materials and the geopolitical problems encountered in collecting, obtaining DNA from herbarium materials is sometimes the only option.

In a seminal paper, Rogers and Bendich (1985) reported on the extraction of DNA, using the CTAB method, from herbarium and mummified plant materials. They concluded that the condition of the leaf DNA depended on the method of drying, and developmental stage. They obtained DNA that could be restricted from dried specimens ranging from 22 - 118 yr. old and mummified seeds up to 44K years old.

But, Nickrent (1994) cited problems in obtaining good DNA from specimens due to age and drying procedures as well as the use of chemicals such, as ethanol and formaldehyde, to combat fungal growth in moist, tropical areas.

Drabkova et al. (2002) compared 7 methods for DNA extraction from herbarium specimens of the Juncaceae and concluded that the Qiagen DNeasy Plant Kit and hot CTAB protocol generally gave the best results. They noted that air-dried specimens (up to 42ºC) generally gave suitable DNA.

However, the drying methods utilized in the past were subject to considerable variation among institutions. The purpose of this study is to establish some baseline values for DNA quality from *Juniperus* specimens of various ages.

MATERIALS AND METHODS

Plant herbarium specimens utilized:
BAYLU - *Juniperus virginiana* L., *Adams 2433*, 1977; *J. scopulorum* Sarg., *Adams 2512*, 1978. *J. coahuilensis* (Mart.) Gaussen ex R. P. Adams, *Adams 387*, 1970, *Adams 438*, 1970, *Adams 1474*, 1975, *Adams 1511*, 1975, *Adams 2522*, 1978, *Adams 2539*, 1978, *Adams 6829*, 1991, *Adams 10634*, 2005, *Adams 11849*, 2009, *J. ashei var. ovata* R. P. Adams, *Adams 98*, 1970,
OSC - *J. communis*, *OSC100300*, 1957, *OSC97657*, 1957, *OSC152071*, 1979, *OSC210646*, 1997, *OSC25127*, 1930, *OSC103501*, 1955, *OSC110375,* 1979, *OSC91155*, 1953, *OSC116403*, 1962, *OSC111301*, 1932, *OSC194044*, 1995, *OSC05533*, 1951, *OSC150461*, 1977,

OSC70268, 1948, *OSC70142*, 1947, *OSC100299*, 1957, *OSC97972*, 1955.
VPI - *J. virginiana*, *VPI 105320, Wright 8992*, 1999, *VPI105305, Wright 7730*, 1997, *VPI105314*, *Wright 7731*, 1997, *VPI105312*, *Wright 8745*, 1999, *VPI 105319, Wright 8986*, 1999, *VPI105309*, *Wright 8119*, 1998, *VPI105313, Wright 8120*, 1998, *VPI105317, Wright 8988, 1999*, *VPI105318*, Wright 7290, 1999, *VPI105315*, *Wright 7291, 1999*, *VPI105316*, *Wright 8917, 1999*, *VPI105310, Wright 7815*, 1997, *VPI105311*, *Wright 8012*, 1997, *VPI105308*, *Wright 7951*, 1997, *VPI105306*, *Wright 7947*, 1997, *VPI103759*, *Townsend 3702*, 2006, *VPI103702*, *Townsend 3710*, 2006, *VPI103703,* Townsend 3712, 2006, *VPI100283*, *Perkinson ns,* 2000, *VPI35799, Connell ns,* 1947, *VPI51546, Crutchfield 5604*, 1967, *VPI69443, Godfrey 70154*, 1971.

DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit as per manufacturer's instructions. Genomic DNA was visualized by agarose gel electrophoresis by mixing 3 µl DNA extract, 3 µl pGEM markers and 3 µl λ\HindIII, and loading 6 µl on a 0.6% agarose gel, then running at 100 v for 20 min. The images were captured on a Kodak Gel Logic 100 Imaging System, and profile analysis was used to determine the modal DNA size and range of DNA sizes. The DNA from some samples was subjected to PCR amplification. ITS (nrDNA) and petN-psbM amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN-psbM) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized.

RESULTS

Table 1 shows the sizes of DNAs and ranges for the 50 herbarium specimens analyzed. It seems unusual that even the most recent specimens (2009, 1 yr. old) had some degraded DNA, but this may be due to normal shearing during leaf grinding with a mortar and

pestle. The linear correlation between specimen ages and DNA modal sizes is -0.58 (33.6% of the variance). Graphing specimen ages vs. modal sizes is shown in Figure 1. Notice that a quasi-logarithmic curve shows that the trend is for the DNA sizes to rapidly decline to about 200-500 bp after 30 years. Paabo (1989) found little correlation between DNA sizes and ages in mummified animal samples. He found remarkably uniform genomic degraded DNAs from samples ranging in age from 4 to 5,000 years old (~70 bp - 500 bp, with a mode of ~200-400 bp).

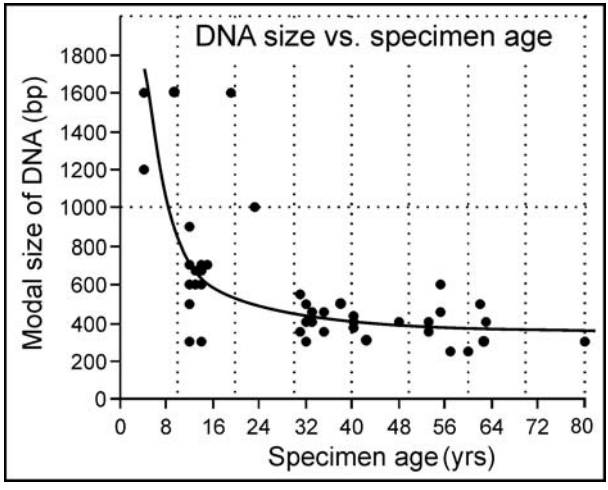

Figure 1. Plot of modal size of DNA vs. specimen age in years.

There are a number of outliers (Figure 1) that have much larger DNA sizes than average. It may be that these DNAs are contaminated with modern fungal/ and or bacterial DNA. Additional testing is being conducted to determine this. There are also a number of samples that have unusually degraded DNA from relative new specimens (Fig. 1). A large amount of variation in DNA sizes is particularly noticeable during the first 20 years (Fig. 1), after which, the sizes are much more uniform.

Several specimens of *Juniperus*, collected by the senior author and dried and stored in comparable conditions, provide a useful serial view of DNA degradation (Fig. 2). Profile analyses of progressively older specimens shows (Fig. 2) that large amounts of degradation are

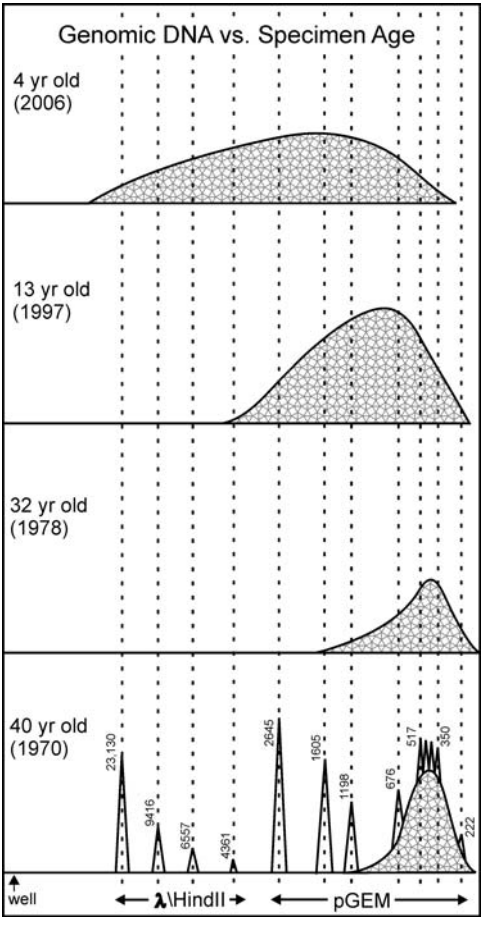

Figure 2. Comparison of genomic DNAs from 4, 13, 32, and 40 year old *Juniperus* specimens.

accumulated during the first 4 years and that the amounts of large DNA fragments (100K - 1000 bp) gradually declines with age in this set of specimens. Because these data are enhanced by the analysis of more concentrated extracts (to be able to see the DNA), it is not apparent from this series, but we have observed that both quantity and quality declines as specimens age. However, the decline in DNA yields is generally not as much of a concern as the quality, as nearly all studies utilize PCR to amplify gene regions of interest, and only minute amounts of DNA are needed for PCR.

Paabo (1989) concluded "the rapidity with which the body [*human in this case*] has been desiccated immediately after death is a major factor that determines the extent of size reduction of the DNA." Paabo (1989) further concluded that after initial desiccation, the DNA is severely damaged by oxidation. Although it is relatively easy, today, to curate herbarium specimens in low humdities by air conditioning, there does not seem to be a practical way to store herbarium specimens in an oxygen-free environment. It may be, that due to the large amounts of anti-oxidants in some plant tissues, oxidative degradation of DNA may not be as severe as in human tissue. Additional research is in progress to attempt to address the impact of oxidation on DNA size in herbarium specimens.

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Table 1. Size (modal value) and range of DNA extracted from 50 herbarium specimens. The 24K bp values were assigned to the genomic band that co-elutes with the 23,130 bp λ\HindIII band. These genomic bands likely range from >100,000 to about 20,000 bp (see Fig. 2), but are merely noted as 24K.

Specimen	Date	Mode(bp)	Range (bp)
BAYLU98	1970	430	1100-100
BAYLU387	1970	400	1000-100
BAYLU438	1970	370	1200-100
BAYLU1474	1975	350	1600-100
BAYLU1511	1975	450	1200-100
BAYLU2433	1977	400	9000-100
BAYLU2512	1978	300	2600-100
BAYLU2522	1978	500	1000-100
BAYLU2539	1978	400	1600-100
BAYLU6829	1991	1600	9400-150
BAYLU10634	2005	24K	24K-9400
BAYLU11849	2009	24K	24K-9400
OSU100300	1957	400	2700-100

Table 1. Size (modal value) and range of DNA (continued).

Specimen	Date	Mode(bp)	Range (bp)
OSU97657	1957	400	2600-100
OSU152071	1979	550	2645-150
OSU210646	1997	1000	2700-150
OSU25127	1930	300	600-100
OSU103501	1955	600	2700-150
OSU110375	1979	350	1600-100
OSU91155	1953	250	2600-100
OSU116403	1962	400	2600-100
OSU194044	1995	700	4300-150
OSU05533	1951	250	600-100
OSU150461	1977	450	4400-100
OSU70268	1948	500	800-100
OSU70142	1947	400	1600-100
OSU100299	1957	350	1000-100
OSU97972	1955	450	1200-100
VPI105320	1999	900	24K-150
VPI105305	1997	670	9500-150
VPI105314	1997	300	2600-100
VPI105312	1999	300	6500-150
VPI105319	1999	700	24K-150
VPI105309	1998	600	24K-150
VPI105313	1998	670	24K-150
VPI105317	1999	700	24K-150
VPI105318	1999	500	2600-100
VPI105315	1999	500	4300-100
VPI105316	1999	600	24K-100
VPI105310	1997	700	24K-150
VPI105311	1997	600	4400-150
VPI105308	1997	600	4300-150
VPI105306	1997	600	4300-150
VPI103759	2006	1200	24K-150
VPI103702	2006	1600	24K-150
VPI103703	2006	1600	24K-150
VPI100283	2000	1600	24K-150
VPI35799	1947	300	1600-100
VPI51546	1967	300	2600-100
VPI69443	1971	500	2600-100