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Genetic variation in postfire aspen seedlings in Yellowstone National Park

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Abstract

A rare episode of regeneration of aspen (*Populus tremuloides* Michx.) by seeds occurred in Yellowstone National Park (YNP), Wyoming, USA, following extensive fires that occurred in 1988. In 1997, we sampled 410 aspen seedlings from 23 local populations distributed widely across YNP to determine how genetic diversity varies with elevation, substrate, plant competition, ungulate browsing, and geographical location. We employed 132 randomly amplified polymorphic DNA (RAPD) markers based on six primers to show genetic relationships within and among the postfire aspen seedling populations. Measures of genetic variation, including estimates of percentage polymorphic loci, expected heterozygosity, and Nei's F_{ST}, indicated that most of the variation occurred within rather than among local populations. There was no indication of geographical differentiation among sampled populations based on hierarchal estimates of Nei's F_{ST}, neighbour-joining, or correlations between genetic distance and geographical distance. Even genetically distant populations shared nearly 90% of the same markers. Within plots, the amount of genetic variation decreased slightly in response to increased percentage vegetative cover, mean seedling basal diameter, and mean seedling height. Geological substrate, density of lodgepole pine (*Pinus contorta* var. *latifolia* Dougl.) seedlings, browsing intensity, and elevation were not significantly related to levels of genetic variation within the seedling plots. These data suggest that genetic variation and geographical structure among seedling populations may occur over time as the transition from seedling-dominated stands to clone-dominated stands occurs.

Keywords: genetic variation, *Populus tremuloides*, *RAPD*, seedling regeneration, vegetative cover

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Introduction

Aspen (*Populus tremuloides* Michx.) is the most widely distributed tree species in North America, occurring from the Tropic of Capricorn to north of the Arctic Circle (Jones 1985; Perala 1990; Barnes & Han 1993). It is also an important component of landscapes of the western United States and Canada. Aspen is dioecious, wind-pollinated, and produces large quantities of viable wind-dispersed seeds. Regeneration of aspen by seeds is common in the moist environments in eastern North America (Mitton & Grant 1980). However, throughout the Rocky Mountain region, aspen is primarily a clonal species which reproduces almost exclusively via root sprouting and produces large stands composed of genetically identical stems (Barnes 1966; McDonough 1985). For example, a single aspen clone in Utah covers 43 ha, includes 47 000 stems, and is estimated to weigh 6×10^6 kg (Grant *et al*. 1992).

Some forest biologists have asserted that broad-scale establishment of aspen by seed has not occurred in the Rocky Mountain region since the last glaciation, some 10 000 years ago (e.g. Einspahr & Winton 1976; Cook 1983). However, studies of genetic heterozygosity in aspen clones in Alberta, Canada suggest that rare episodes of seedling recruitment must have occurred (Jelinski & Cheliak 1992). The few instances of successful seedling recruitment

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reported in the literature suggest there may be brief 'windows of opportunity' characterized by favourable moisture conditions, an absence of competition, the availability of suitable substrates (e.g. burned sites), or a combination of these conditions (Olmstead 1979; Jelinski & Cheliak 1992; Kay 1993; Romme *et al*. 1997). Such a window occurred following the extensive 1988 fires in Yellowstone National Park (YNP).

The 1988 Yellowstone fires burned nearly 700 000 ha in and around YNP. Aspen seedlings established in 1989 and 1990 throughout the burned forests and in places beyond the species' prefire range (Kay 1993; Romme *et al*. 1997). Little or no seedling establishment has occurred since 1990. The wide expanses of open canopy and exposed mineral soil, combined with a subsequent spring and summer of above average precipitation, provided conditions suitable for seed germination and establishment. These postfire aspen seedlings may have a marked and lasting effect on the YNP landscape, particularly given their potential size and longevity. Prior to the 1988 fires, aspen covered only ≈ 2% of YNP and was most common at elevations < 2100 m (Despain 1990). Aspen is YNP's only native upland deciduous tree, and aspen stands support a diversity of breeding birds and other animals (DeByle 1985). Mature aspen stands have also been reported to be deteriorating in YNP and other parts of the Rocky Mountain region due to interacting factors of disease, fire suppression, and browsing by ungulates (Romme *et al*. 1995). The extreme longevity of some clonal plants suggests that long-lived clones may not be adapted to current conditions (Eriksson 1992). The extreme longevity of aspen (Grant 1993) suggests that extant stands are comprised of individuals which may no longer be adapted to current conditions. This may provide a genetic explanation for the decline of aspen in YNP. The postfire aspen seedlings may represent an important influx of genetic recombination into the aspen community (Tuskan *et al*. 1996).

The objectives of this study were to characterize the genetic structure of small but widely dispersed populations of seedling aspen in YNP using randomly amplified polymorphic DNA (RAPD) markers and to determine how the genetic variability of these populations is affected by ecological characteristics including elevation, soil substrate, density of potential competitors, including lodgepole pine seedlings (*Pinus contorta* var. *latifolia* Dougl.) and herbaceous vegetation, ungulate browsing intensity, and geographical distance between populations. The postfire aspen seedlings in Yellowstone are at an early stage of development, and are located in close proximity to one another. The fine scale of this study allows for tight characterization of a plot's environmental attributes without confounding factors of habitat differences and/or genetic drift (Jelinski & Cheliak 1992).

We hypothesized that genetic variability would indicate that the aspen seedling populations that were geographically proximal to each other would be more closely related than populations that were geographically distant. We also hypothesized a decline in genetic variability in the aspen seedling populations with increasing elevation. The growth rate of aspen declines with elevation (Jelinski 1993), and aspen seedling densities have already declined at high elevations (> 2100 m) (Romme *et al*. 1997). We further hypothesized less genetic variation in plots which were heavily browsed, exhibited high densities of lodgepole pine seedlings, or had high total vegetative cover; and we hypothesized greater genetic variation on more fertile substrates (e.g. detrital deposits as compared with the infertile rhyolite substrates) (Stratton 1994).

Materials and methods

Study area

YNP is a high plateau that straddles the Continental Divide in northwest Wyoming. Elevations range from 1500 m in the north to 3500 m in the east. The Yellowstone subalpine plateau which occupies the majority of the park is between 2000 m and 3000 m in elevation. Approximately 80% of the park is dominated by lodgepole pine (Despain 1990). The forested subalpine plateau is characterized by dry, infertile, rhyolite substrates with more mesic and fertile andesite lake-bottom substrates. The climate is generally cool and dry with a mean January temperature of –11.4 °C and a mean July temperature of 10.8 °C. The mean annual precipitation is 56.3 cm (Dirks & Martner 1982). Much of the yearly precipitation comes as snow during long winters.

Field methods

Twenty-three aspen seedling study plots were established along 13 trails in YNP during the summer of 1996 (Table 1, Fig. 1). Plots were located between 2.5 m and 100 m (mean of 33 m) from trails where aspen seedlings were relatively abundant. Plot size was varied to encompass at least 20 aspen seedlings within an area of relatively homogeneous environmental conditions. The average plot size was 30 m^2, but ranged from 4 m^2 to 180 m^2 depending on local density of aspen seedlings. In 1997, aspen seedling density per plot averaged 1.3 m^{-2} but ranged from 0.1 m^{-2} to 4.7 m^{-2}. Plot elevations ranged from 2145 m to 2505 m (Table 1). To provide broad spatial coverage, plots on the same trail were positioned at least 1.4 km apart. The largest geographical distance between plots was 74.3 km, but two plots, each at the head of separate trails, were only 0.2 km apart. The 23 aspen study plots clustered into four geographical regions:

Table 1 Site characteristics of plots in which postfire aspen seedlings were sampled in Yellowstone National Park in 1997 and descriptive variables based on RAPD markers. Plots in relatively close proximity (Fig. 1) are grouped by geographical region

Plot name	Abbreviation	Elevation (m)	Vegetative cover (1997) (%)	No. of individual aspen sampled	Mean bands per individual	Polymorphic loci (%)	Weighted average expected heterozygosity
Northern Region							
Mount Holmes	MH1	2330	43.1	15	31 ± 3.4	63.7	0.18
Harlequin Lake	QL1	2145	84.6	15	41 + 2.8	71.3	0.22
Old Faithful Region							
Fern Cascades	FC1	2300	39.7	17	49 + 1.0	74.3	0.24
Firehole Meadow no. 1	FM1	2375	15.9	14	50 + 1.2	57.6	0.20
Firehole Meadow no. 2	FM2	2505	37.6	13	48 + 1.0	68.2	0.21
Mallard Creek no. 1	MK1	2160	82.2	20	45 + 2.4	68.2	0.20
Mallard Creek no. 2	MK2	2265	29.7	17	43 + 2.9	70.5	0.22
Mallard Creek no. 3	MK3	2370	64.5	14	41 + 2.2	66.0	0.20
Mallard Creek no. 4	MK4	2390	53.0	19	48 + 1.3	69.0	0.22
Mallard Lake no. 1	ML1	2290	95.0	17	44 + 1.0	62.9	0.21
Mallard Lake no. 2	ML2	2370	31.4	11	44 + 1.9	68.2	0.22
Summit Lake no. 1	SM1	2220	32.0	22	48 + 0.8	78.8	0.23
Summit Lake no. 2	SM2	2420	38.4	23	47 + 1.1	75.0	0.22
Heart Lake Region							
Heart Lake no. 1	HE1	2390	70.2	15	42 + 1.3	67.5	0.20
Heart Lake no. 2	HE2	2455	36.8	16	44 + 1.3	68.2	0.22
Heart Lake no. 3	HE3	2390	84.5	42	46 + 0.7	79.6	0.23
Heart Lake no. 4	HE4	2335	82.3	20	47 + 0.8	69.0	0.21
Lewis River Channel	LR1	2374	77.2	17	48 + 1.1	69.7	0.23
Shoshone Lake	SL1	2417	50.4	18	45 + 1.7	73.5	0.22
Yellowstone Lake Region							
Outlet Creek	OK1	2380	87.0	19	52 + 1.1	72.8	0.24
Trail Creek no. 1	TK1	2385	86.3	17	48 + 1.1	67.5	0.21
Trail Creek no. 2	TK2	2415	85.8	13	50 + 2.2	74.3	0.24
Two Ocean Plateau	TO1	2385	85.2	16	48 + 0.8	73.5	0.23

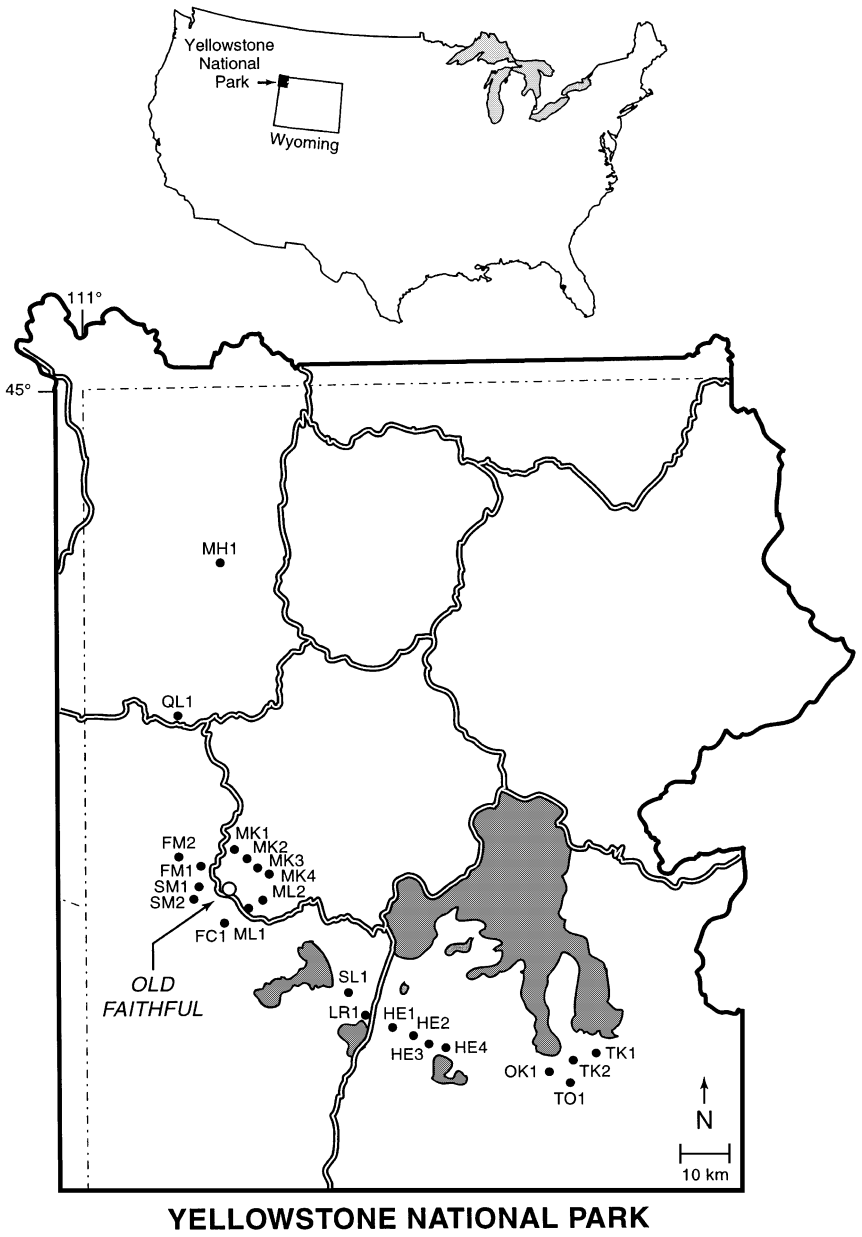

Fig. 1 Geographic locations of the 23 postfire aspen seedling plots sampled within Yellowstone National Park, WY, USA. Park roads are shown in double lines, state boundaries in dashed lines, and major lakes are shaded grey. See Table 1 for plot abbreviations and descriptions.

Northern, Heart Lake, Old Faithful, and Yellowstone Lake areas. The Northern region is only comprised of two plots because aspen seedling regeneration was sparse there.

Plots were established and permanently marked with rock cairns in 1996, as part of a broad study of distribution and persistence of postfire aspen seedlings in the Yellowstone landscape (M. G. Turner, W. H. Romme, unpublished data). All aspen seedlings occurring within the plots were measured in 1996 and 1997. Measurements included height (length of the main stem), basal diameter, and whether the plant had been browsed by ungulates. In 1997, we also collected genetic samples from each individual within the plots (described below). The 23 plots contained an average of 18 aspen seedlings in 1997 (range = 11–42, n = 410 individuals). For each plot, we estimated percentage vegetative cover (all vascular plant species combined) using a line-intercept method along a representative 10 m along the central axis of the plot. In plots that were less than 10 m in length, vegetative cover was determined along a 10-m transect centred on the plot. The density of lodgepole pine seedlings was obtained by counting the number of such seedlings in two 1-m belts along the 10-m transect. Elevation and universal transverse mercator coordinates of each plot were obtained from 7.5′ topographic maps, and geological substrate was obtained from detailed geological maps of the Yellowstone area.

We sampled leaf tissue for genetic analysis in 1997 by collecting one to four of the newest fully expanded leaves from each individual. The leaf tissue was then placed in separate microcentrifuge tubes containing 1 mL of 1× TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) (Tuskan *et al*. 1996). The leaf tissue was kept under ice while in the field and air mailed the next day in insulated containers to University of Wisconsin-Madison for storage at −80 °C. Leaves from three to four adults growing in six different plots at 8-km intervals along Wisconsin State Highways 22 and 60 between Madison and Poynette, WI were collected to serve as an outgroup. These leaves were transported fresh and stored at −80 °C in plastic freezer bags.

Laboratory methods

We thawed 10–20 mg of the frozen leaf tissue, transferred this into new microcentrifuge tubes, and performed a modified CTAB DNA extraction procedure based on Doyle & Doyle (1987). Specifically, leaf tissue was snap frozen in liquid nitrogen and ground into a fine powder with mini-pestles. Further grinding was done after adding CTAB isolation buffer (containing 2% (w/v) CTAB (hexadecyltrimethylammonium bromide), 100 mM 1 M Tris (pH 8.0), 20 mM 0.5 M EDTA (pH 8.0), 1.4 M NaCl, and 1% (w/v) polyvinylpolypyrrolidone (PVPP2)) and 0.2% (v/v) β-mercaptoethanol. The leaf tissue–buffer mixture was incubated at 60 °C for 30 min and extracted using an equal volume of chloroform–isoamyl alcohol (24:1). Following centrifugation, the aqueous portion was transferred into new tubes. We added RNAse to 10 µg/mL and incubated the tubes at 37 °C for 30 min.

Genomic DNA was precipitated with two-thirds volume of 0 °C isopropanol and samples were centrifuged at 11 000 *g* for 30 min. The isopropanol was removed and the DNA pellet was washed with 75% (v/v) ethanol containing 10 mM ammonium acetate and centrifuged again at 11 000 *g* for 15 min. The supernatant was discarded, and the pellet was air-dried for 15 min and then placed under a vacuum for an additional 15 min. The pellet was resuspended in 100 mL of 1× TE buffer (pH 8.0) for storage at 4 °C. Total DNA was quantified using a TKO fluorometer (Hoefer Scientific), and samples were diluted to 4 ng/mL using sterilized water and 0.5 mM tartrazine (Nepokroeff 1997).

A PCR (polymerase chain reaction) method was used to characterize DNA variation via RAPD markers (Williams *et al*. 1990). The RAPD reactions were conducted in a 96-well Thermolyne 'Amplitron II' thermocycler (Dubuque, IA) with a 35-cycle protocol involving 1 min at 94 °C denaturation, 1 min at 36 °C annealing, and 2 min at 72 °C polymerization steps within each cycle, followed by a 5-min final polymerization step and a 4 °C hold until samples could be transferred to a 20 °C freezer (Tuskan *et al*. 1996). Each reaction contained 50 mM Tris (pH 8.5), 10 mM KCl, 2 mM $MgCl_2$, 500 µg/mL bovine serum albumin, 0.01% (w/v) xylene cyanole, 1.5% (w/v) Ficoll, 2.5 mM dNTPs, 10 mM primer, 20 ng of total DNA, and 0.6 units of *Taq* polymerase (Promega Corporation, Madison, WI, USA), to a final volume of 10 mL (Nepokroeff 1997).

Control reactions containing all PCR components except the DNA template were included with each set of reactions to verify the lack of foreign DNA. Multiple runs of individual templates from selected plots were conducted to ensure repeatability. Amplified PCR products were transferred into wells in 1.5% (w/v) agarose 0.5× TBE gels containing 0.1 mg/mL ethidium bromide under constant voltage (100 V) for 2.5 h. A 100-bp DNA standard (Gibco-BRL, Grand Island, NY, USA) was used to assign molecular weights to individual RAPD markers. Each gel was recorded using Polaroid ISO 3000 B & W film (Cambridge, MA, USA) and a UV light source (254 nm).

Data analysis

Data regarding RAPD markers were recorded as (1) = band present (0) = band absent, and (.) = missing information. These data were initially entered into Microsoft Excel which allowed them to be imported into a suite of FORTRAN programs for RAPD data analysis. RAPDPLOT was used to calculate genetic distance between individuals using both a similarity and matching index for each pairwise comparison of genotypes (Kambhampati *et al*. 1992; available from William C. Black IV). The similarity index is based on Nei & Li's (1985) formula: $S = 2N_{AB}/(N_A + N_B)$, where N_{AB} is the number of bands that individuals A and B share, and N_A is the number of bands in individual A, and N_B is the number of bands in individual B. The matching index is based on the formula $M = N_{AB}/N_T$ where N_{AB} is the total number of markers, either present or absent in individuals A and B, and N_T is a fixed number of loci in the study (Apostol *et al*. 1993).

Because the output from both the similarity and matching matrices were comparable (data not shown), only the similarity matrix was used as an input file in the NEIGHBOUR program of PHYLIP 3.5c (Felsenstein 1993). NEIGHBOUR is based on the neighbour-joining function of Saitou & Nei (1987), constructing successive linkages without the assumption of a molecular clock (Felsenstein 1993). Individuals were placed in the analysis using the jumble option to avoid input-order bias.

RAPDDIST was used to calculate the genetic distance between plots by performing 100 bootstraps using Nei's (1972) genetic distance measure which provides an estimate of the mean number of mutations separating the genes from two populations. The RAPDDIST matrix was used to produce a consensus tree to determine support for the

genetic relationship among plots. The nonalgorithmic program FITCH with CONSENSE (Felsenstein 1993) was used in a similar manner. RAPDFST, which assumes that subpopulations are in Hardy–Weinberg equilibrium and that RAPD markers are dominant at each locus, was used in conjunction with BIOSYS-1 (Swofford & Selander 1981) to calculate Wright's F_{ST} (1931) and Weir & Cockerham's F_{ST} (1984), an F_{ST} calculation corrected for small and unequal sample sizes. RAPDBIOS was used to calculate marker frequencies and expected heterozygosity.

Within-plot mean genetic distance and variance were regressed against ecological factors recorded in 1997. These included slope, aspect, elevation, percentage aspen seedlings browsed, percentage total vegetative cover, density of lodgepole pine seedlings, and percentage mortality of aspen seedlings between 1996 and 1997. To verify that relationships between genetic distance and environmental factors were not artefacts of the sampling design (e.g. variation in sample size per plot), the mean and variance of within-plot genetic distance were also regressed against both the average density and total number of aspen seedlings per plot. The mean within-plot genetic distance was also regressed against mean height and basal diameter per plot. To determine the relationship between geographical and genetic distance, 253 pairwise comparisons of genetic distance among plots were regressed against 253 pairwise comparisons of geographical distance (d.f. = 1251).

Results

Genetic structure

The average number of RAPD markers present per individual varied among the plots from 31 ± 3.4 to 52 ± 1.1, out of a total of 132 scored markers. The percentage polymorphic loci per plot ranged from 57.6% to 78.8%. The weighted average expected heterozygosity (Nei 1978) ranged from 0.18 to 0.24 (Table 1). Of the markers examined in this study, there were 17 RAPD markers unique to the Madison, Wisconsin outgroup and one marker unique to the Yellowstone population.

Wright's F_{ST} was 0.34 ± 0.31 (standard deviation) and θ was 0.18 ± 0.32. Both values indicate that most of the genetic variation in the total population can be attributed to differences among individuals within plots. θ values comparing variation among the four regions showed that 49% of the variation in the total sample can be attributed to variation among regions. Within the Northern region, 84.9% of the variation can be explained by variation among individuals within the Harlequin Lake (QL1) and Mount Holmes (MH1) plots. Within the Old Faithful region, 81.2% of the variation can be explained by variation within the region's plots. In the Yellowstone Lake region, variation among individuals within plots accounts for 83.7% of the variation; and in the Heart Lake region, this estimate was 83.6%.

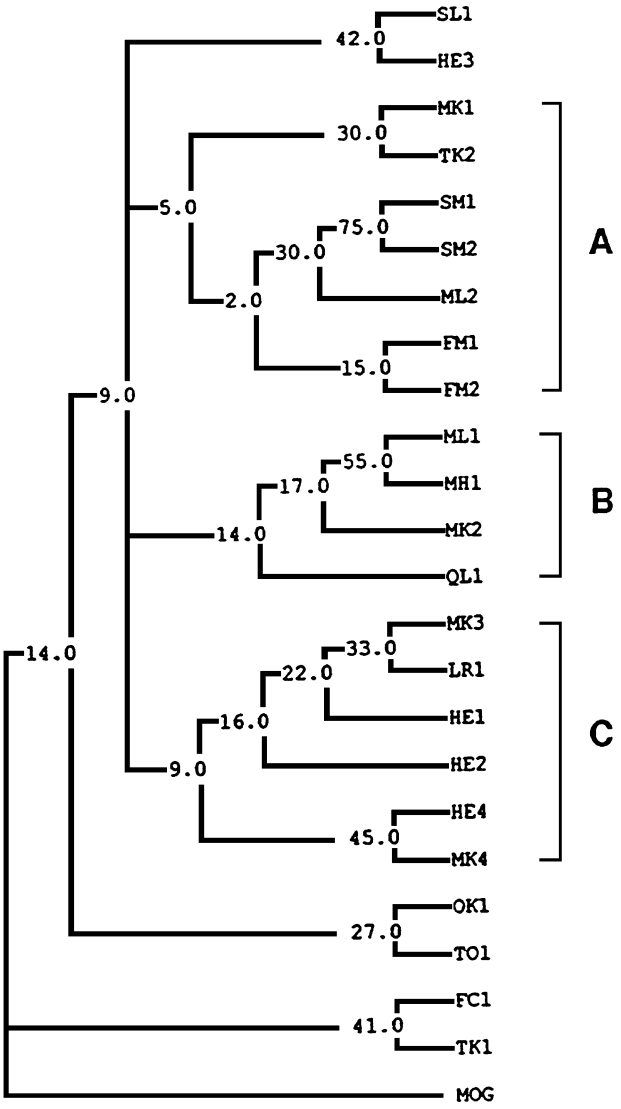

Fig. 2 Consensus tree of 100 bootstraps of genetic distances using the neighbour-joining algorithm showing clustering among the 23 postfire Yellowstone aspen seedling study plots and the adult Madison, Wisconsin outgroup (MOG). The numbers at the forks indicate the number of times the group consisting of the sites to the right occurred out of 100 trees. The three largest subdivisions within the consensus tree are indicated with brackets and labelled A, B, and C. The outgroup is depicted as an unresolved trichotomy.

The neighbour-joining phenogram of all individuals showed some population structure, but in most cases one to several individuals at any given plot clustered with individuals from an alternate plot. The neighbour-joining consensus tree reveals that some plots that are geographically proximal clustered together (Fig. 2). For example,

Summit Lake no. 1 (SM1) and no. 2 (SM2), separated by 1.4 km, clustered together 75% of the time in bootstrap analysis. Little Firehole Meadow no. 1 (FM1) and no. 2 (FM2) are also geographically close (3.2 km) and clustered together, although only in 15% of occasions. Outlet Creek (OK1) and Two Ocean Plateau (TO1) plots, separated by 3.6 km, clustered together in 27% of occasions.

In other comparisons, however, plots that were geographically distant and in two separate regions clustered with some degree of consistency. For example, Fern Cascades Loop (FC1) in the Old Faithful region clustered with Trail Creek no. 1 (TK1) in the Yellowstone Lake region 41% of the time. Similarly, Mallard Creek no. 1 (MK1) and Trail Creek no. 2 (TK2) clustered in 30% of the estimated trees. The two plots in the Northern region (QL1 and MH1) grouped together in the same largest subdivision (B) of the consensus tree, yet they also clustered with two plots in the Old Faithful region (ML1 and MK2) as well. Most of the branches depicted in the consensus tree are supported by low bootstrap values. The average frequency per pairwise bootstrap cluster was 26.4%.

Of the three largest subdivisions in the consensus tree, 'A' contained six out of the 11 Old Faithful plots and one representative (Trail Creek no. 2) from the Yellowstone Lake region. 'B' contained representatives from the Northern and Old Faithful regions only, while 'C' contained representatives from two regions, Old Faithful and Heart Lake. Three smaller clusters were comprised of only two plots each. One cluster contained two plots from the Heart Lake region, another contained two plots from the Yellowstone Lake region, while another had one plot from the Yellowstone Lake Group and one from the Old Faithful region. This last group clustered with the Madison, WI area outgroup in 14 out of 100 trees (Fig. 2). The consensus tree derived from the nonalgorithmic Fitch method showed similar subdivisions and low bootstrap values. The average frequency per pairwise bootstrap cluster was 20.5%. Correlation analysis revealed no significant correlation between geographical distance (in km) and genetic distance between plots, with genetic distance between plots averaged over 100 bootstraps.

Genetic variation and ecological characteristics

Vegetative cover in the 23 plots ranged from 15.9% to 95.0% and averaged 59.0% (Table 1). Lodgepole pine seedling densities ranged from 0.1 m^{-2} to 15.4 m^{-2} and averaged 2.3 m^{-2}. All of the plot geological substrates were classified as rhyolites, although 10 out of the 23 plots were found on detrital deposits.

Regression analysis demonstrated no relationship between mean within-plot genetic distance and slope, aspect, elevation, percentage mortality between 1996 and 1997, percentage browsed, percentage vegetative cover, density of lodgepole pine seedlings, or density of aspen seedling ($P > 0.05$). However, the variance of within-plot genetic distance was negatively related to percentage total vegetative cover ($P = 0.02$, $R^2 = 0.22$), suggesting that within-plot genetic variance was greater in plots containing less vegetative cover (Fig. 3). To ensure that these results were not an artefact attributable to differences in sample size among stands, we tested whether the mean and variance of within-site genetic distance was related to sample size but found no significant effect.

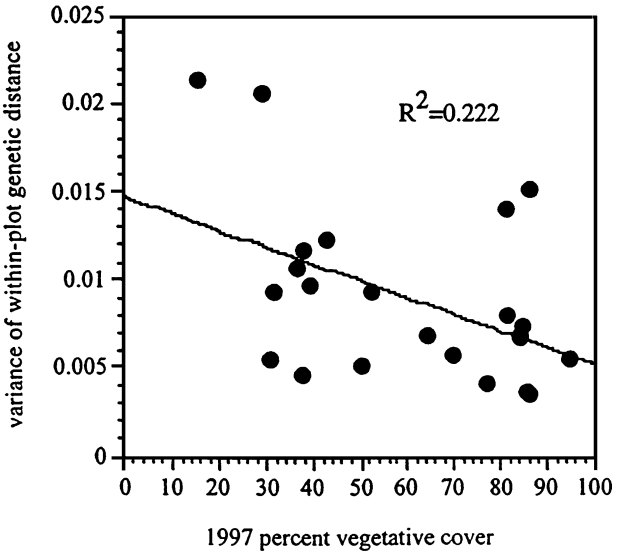

Fig. 3 Results of regressions showing negative correlation between variance of within-plot genetic distance and the 1997 percentage vegetative cover.

Plots with a higher average aspen basal diameter also had a lower mean within-plot genetic distance ($P = 0.02$, $R^2 = 0.21$) (Fig. 4a). Similarly, mean within-plot genetic distance declined slightly with average aspen height within plots ($P = 0.09$, $R^2 = 0.13$, Fig. 4b). As expected, mean height and mean basal diameters were positively correlated ($P = 0.0009$, $R^2 = 0.42$). Although there was no significant relationship between average aspen basal diameter and their density, average aspen height declined slightly in the more dense plots ($P = 0.10$, $R^2 = 0.12$).

Discussion

Genetic structure

Genetic variation often is not randomly distributed among individuals (Young 1995). The percentage polymorphic loci per plot, a measure of genetic variation, ranged from 57.6% to 78.8%, which is intermediate with values reported for aspen by Tuskan *et al.* (1996) and colleagues

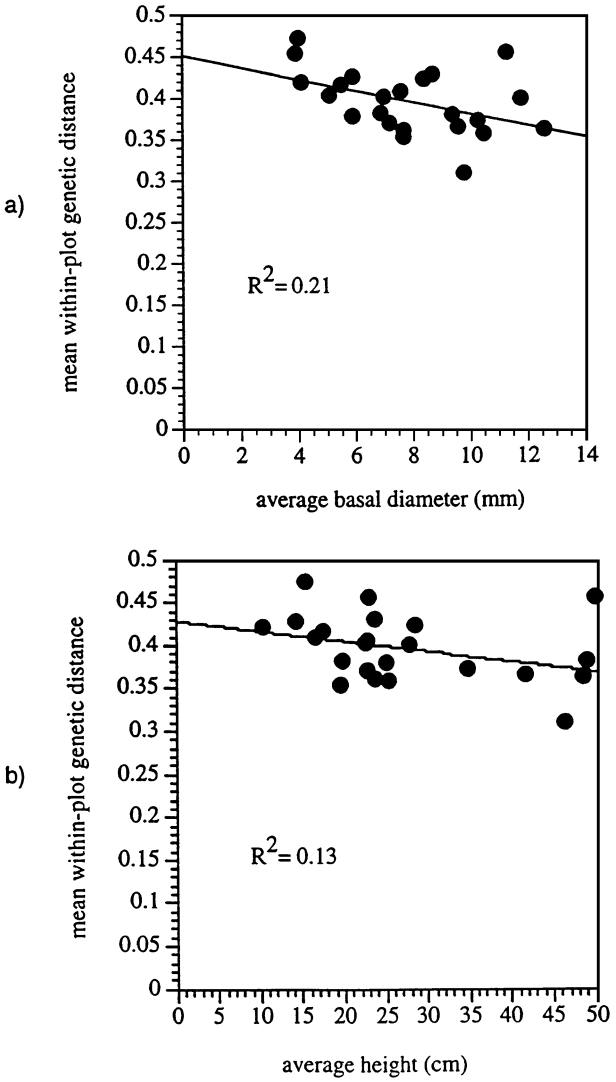

Fig. 4 Results of regressions showing negative correlation between mean within-plot genetic distance and (a) average basal diameter of aspen seedlings, and (b) average height of aspen seedlings.

(Liu & Furnier 1993; Yeh *et al*. 1995). Our values are slightly higher but consistent with the 52% to 69% range reported in Tuskan *et al*. (1996). Fewer RAPD markers per individual (132) were used in this study than by Tuskan *et al*. (1996) who used 194 markers. Liu & Furnier (1993) used 61 RAPD markers and showed 100% polymorphic loci, while Yeh *et al*. (1995) used 28 RAPD markers and observed 90.2% polymorphic markers. High levels of genetic variation are often associated with species such as aspen that have large geographical ranges spanning extreme environments (Hamrick *et al*. 1979; Hamrick & Loveless 1989; Mitton & Grant 1996).

The level of expected heterozygosity, also a measure of genetic variation, in the current study was 0.22, similar to those values found by Hyun *et al*. (1987) and Lund *et al*. (1992) using isozymes in aspen populations in the Great Lakes region (0.24 and 0.22, respectively). In comparing molecular markers, Liu & Furnier (1993) reported levels of expected heterozygosity of 0.25 with allozymes, 0.25 with RFLPs, and 0.30 with RAPDs in aspen from the Great Lakes region. Two isozyme studies on aspen in Alberta by Jelinski & Cheliak (1992) and Cheliak & Dancik (1982) reported expected levels of heterozygosity of 0.32 and 0.42, respectively. Jelinski & Cheliak (1992) suggested that the higher levels of expected heterozygosity in the drier western portion of aspen's range were due to the accumulation of mutations in populations with very limited recruitment and where clones are considered to be of great age. When sexual reproduction does occur in western North America, the expected heterozygosity in the seedling populations more closely mirrors levels of expected heterozygosity in the moister environs of the Great Lakes region.

The average percentage polymorphic loci (70.0%) and the average expected heterozygosity (0.22) for this study are similar to averages reported for coniferous trees (67.7% and 0.21) but higher than reported for dicots (31.2% and 0.11) (Hamrick & Loveless 1989). This may reflect the wind-pollinated and wind-dispersed nature of both aspen and most coniferous trees. Hamrick *et al*. (1979) note that tree species contain more variation than herbaceous dicots. In a survey of predominately outcrossing species, Loveless & Hamrick (1984) reported a mean diversity within populations (expected heterozygosity) of 0.21. Average expected heterozygosity for the 81 studies of wind-pollinated species and 48 studies of species with wind-dispersed seeds were 0.15 and 0.20, respectively (Loveless & Hamrick 1984).

Most genetic variation in the postfire Yellowstone aspen seedling plots is distributed within rather than among sites, as evidenced by the F_{ST} and θ values found in this study. Loveless & Hamrick (1984) reported an average G_{ST} of 0.32 for 81 plants with wind-dispersed pollen, similar to the F_{ST} value of 0.34 reported here and the 0.31 reported by Tuskan *et al*. (1996) for the postfire Yellowstone aspen seedlings.

The observation that most of the variation in the postfire Yellowstone aspen seedling populations is found within rather than among plots may be related to several biological characteristics of aspen. As previously mentioned, aspen normally reproduces vegetatively but, because it is dioecious, when sexual reproduction does occur, outcrossing is obligate except in the rare cases of perfect flowers (Lester 1963). Predominately outcrossed, dioecious species show high within-population variation and reduced divergence among populations due to increased gene flow. The same pattern is observed in species with winged or plumose wind-dispersed seeds (Loveless & Hamrick 1984). In fact, long-distance seed

dispersal may limit divergence among populations more than long-distance pollen transfer because seeds carry twice the genetic material of pollen.

Using isozymes, Hyun *et al*. (1987) reported that 93.2% of the genetic variability in aspen stands in Ontario could be attributed to within-population differentiation, and Lund *et al*. (1992) also found most variation (99.7%) within stands in Minnesota. Using RAPDs, Yeh *et al*. (1995) reported that 97.4% of the total variation in Alberta aspen was due to within-population differences.

The lack of a significant relationship between geographical and genetic distance was not unexpected, because Hyun *et al*. (1987) also reported no significant correlation between genetic and geographical distance in the study of clonal aspen populations in Ontario. The fact that many of the geographically distant aspen study plots are genetically similar could reflect a common genetic origin of the seedlings' parents. As regeneration from seed is rare, individuals established in the previous episode of sexual reproduction may be close genetic relatives. Additionally, the lack of genetic structure among seedling plots may be due to long-distance travel of both the wind-dispersed pollen and seeds (Stoeckeler 1960). In fact, all postfire aspen seedling plots showed limited genetic differentiation. The most genetically distant seedling populations differed by fewer than 12% of the markers sampled. This supports the suggestion by Tuskan *et al*. (1996) that the current seedling populations were progeny of mature aspen that themselves share many markers.

Genetic analyses conducted by Tuskan *et al*. (1996) on four seedling populations separated by approximately 10 km in a linear northwesterly transect on the Yellowstone Plateau revealed that each seedling represents a unique genotype, that individuals within a stand are highly variable, and that individuals within plots form discrete clusters. Thus, seedling genotypes within plots may share a common parental genotype (probably maternal due to the larger dispersal potential of pollen; Tuskan *et al*. 1996). In the current study, individuals are more variable and do not form consistent, discrete clusters at the population level. This may reflect how seedling populations were sampled. Many of the plots in the current study are closer than 10 km and are scattered across regions. Alternatively, differences between the Tuskan *et al*. (1996) study and the current study may be attributed to the average number of markers scored per primer (14 vs. 22, respectively). The higher the number of recorded markers per primer, the higher the probability of scoring artefacts. Finally, the highly variable nature of the individual seedlings within each population may also help explain why the bootstrap values on the consensus tree are low. As genotypes within plots were quite similar, it is difficult to assess relationships among plots with a high degree of consistency. Higher variation among the plots than among the regions explains the lack of substantial regional clustering in the consensus trees, as well as the lack of significant correlation between geographical and genetic distance.

Genetic variation and ecological characteristics

Mature aspen stands are often comprised of one to a few genotypes. As each of the individuals sampled in this study represents a unique genotype, mortality presumably eliminates certain genotypes over time. One of the mechanisms eliminating genotypes may be plant competition. It is interesting to note that it is the biotic rather than the abiotic factors within plots which are significantly correlated with reduced within-plot genetic variation. Increased vegetative cover in study plots acts to reduce variance in genetic distance between individuals (Fig. 3). High levels of plant competition may eliminate extreme genotypes, reducing the genetic variance at a plot. The density of lodgepole pine seedlings does not currently affect the mean or variance of within-plot genetic distance in the aspen seedling populations. However, this factor may become more important as the pines increase in height and overtop and shade the shrubby, shade-intolerant aspen. These effects appear to be real rather than an artefact of rarefaction in that variation in genetic distance is not related to sample size within plots.

Elevation is not significantly correlated with the mean or variance of within-plot genetic distance. The range of elevation in this study is 360 m (2145 m to 2505 m), and may not be sufficient to detect genetic differences due to elevation. Mitton & Grant (1980) showed that frequencies of polymorphic proteins revealed little differentiation with elevation in aspen, although significant differences in isozyme frequency have been detected in sugar maple with elevation (Ledig & Korbobo 1983). Elevation may become more important in the future as extreme years bring cold temperatures and/or deep snowfalls that prove fatal for some of the seedling cohort. Kay (1993) reported 78% mortality between 1989 and 1991 after initial postfire aspen seedling recruitment in Yellowstone. Between 1996 and 1997, we observed a much lower mortality rate (9.1%) in the aspen seedlings (M. G. Turner, W. H. Romme, unpublished). In most plant populations, mortality rates after initial establishment are much higher than in older seedlings (Harper 1977). At the time of this study, geological substrate did not affect within-plot genetic diversity, but this physical attribute may become more important in the future through indirect effects. For example, geological substrate may prove to favour lodgepole pine seedlings or herbaceous cover. Xie & Knowles (1992) found that certain allozymes in Ontario jack pine correlated with both high and low levels of soil nutrients and elements. It is important to note that although the geological substrates

differ with respect to detrital origin, they are all classified as rhyolites and may not adequately represent a range of possible geological conditions which may influence aspen genetics.

Plots with higher average basal diameters and with higher average aspen heights had lower mean within-plot genetic distances (Fig. 4). This may indicate that as the postfire aspen seedlings reach more adult-like proportions, the genetic diversity within each plot will more closely mirror the monoclonal nature of mature stands, i.e. plots with larger trees have lower estimates of genetic variation. Both random (rarefaction) and nonrandom (selective) forces may be responsible for the elimination of individual aspen seedlings, and the concomitant loss of genetic variability within stands.

Conclusions

The large-scale 'natural experiment' of the 1988 Yellowstone fires provided an unusual opportunity to understand the processes of seedling recruitment and establishment of new genetic individuals within a clonal species, aspen, that usually exhibits only vegetative reproduction in the Rocky Mountain region. Aspen seedlings became established in the first 2 years after the fires, and by 1997 were still widely distributed throughout the burned forests of YNP. Genetic variability among seedlings in 1997, determined by RAPD analysis of 410 individuals distributed among 23 plots within four geographical regions of the Yellowstone Plateau, was comparable to what has been measured in other aspen populations in North America. Patterns of genetic variability and structure with respect to physical features of the environment (elevation, topographic characteristics, geographical location, and proximity of seedlings to one another), were relatively weak or absent. The paucity of strong spatial patterns probably reflects high genetic similarity among parental plants, widespread and long-distance dispersal of aspen pollen and seeds, and the brief length of time since seedling establishment during which selection has had time to operate to eliminate poorly adapted genotypes. Our study also encompassed a relatively narrow elevational zone. However, local genetic variability of aspen seedlings (at the scale of individual plots) was negatively correlated with total vegetative cover and with mean basal diameter of aspen seedlings, suggesting that intra- and interspecific competitive interactions may speed the loss of aspen genotypes less adapted to local site conditions. If this kind of selection continues, then the poorly structured spatial pattern of aspen seedling genotypes across the landscape that we documented in 1997 may develop into stronger patterns of genetic composition and variability over the next decade. A better understanding of the spatial and temporal patterns in aspen seedling establishment and genetic structure following the 1988 Yellowstone fires can provide insights into population dynamics of long-lived clonal plant species and may provide information on potential responses of aspen in the northern Rocky Mountains to global climate change.

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