GENETIC VARIATION AND CLONAL DISTRIBUTION OF QUAKING ASPEN IN THE CENTRAL SIERRA NEVADA

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ABSTRACT: Resource managers have become increasingly concerned over the apparent decline of Quaking Aspen (*Populus tremuloides* Michx.) in the western United States. To aid in the development of conservation and restoration strategies for aspen, we investigated genetic variability, clonal diversity, levels of differentiation, and patterns of geographic variation as measured by allozymes among 663 aspen individuals located in 82 stands from 8 watersheds throughout the western slope of the central Sierra Nevada, California. As is usual for the species, these aspens are highly diverse genetically (82% polymorphic loci, 3.1 alleles/locus). Stands are much less variable, and are genetically different from each other, with 46.5% of all genetic variation measured being the differences among stands. Clonal diversity was 30% with 198 of the 663 individuals sampled containing unique clonal genotypes. Fifty-six percent of the stands studied contain two or more clones. Monoclonal stands are usually small in size, and vary from 0.1 to 5 acres, averaging 0.8 acres. All watersheds contain more than one clone. In ten cases, a single clone extended across what are now classified as two or more stands. Although there are some general trends of increased genetic similarity with geographic proximity, stands that are in close proximity are not necessarily genetically similar, and can be, in fact, quite genetically distinct. Genetic variation detected by the allozyme data can be used to delineate genetic restoration units and to prioritize conservation efforts.

Key words: allozymes, California, clonal distribution, genetic variation, *Populus tremuloide*s, quaking aspen

TRANSACTIONS OF THE WESTERN SECTION OF THE WILDLIFE SOCIETY 40:32-44

Successful conservation management is dependent on the understanding of a species genetic structure. Quaking Aspen (*Populus tremuloides* Michx.) regularly reproduces by vegetative, or clonal, means (Mitton and Grant 1996), especially in the arid west (Kemperman and Barnes 1976). Occasional sexual reproduction by seed has been noted, and is important for spreading aspen into new areas and introducing new genetic variants into the gene pool (Ellison 1943, Barnes 1966, Yeh et al. 1995, Mitton and Grant 1996, Romme et al. 1997).

Resource managers have become increasingly aware and concerned over the apparent decline of aspen throughout the western United States. Several factors appear to be leading to these vegetative changes in aspen populations, including fire suppression, livestock grazing, wild ungulate browsing, conifer succession, and perhaps climate change. High elevation and boreal species are more likely to be adversely affected by a warming and drying climate. Drought, thaw-freeze events, insect defoliation, fungal pathogens, and wood-boring insects together have probably contributed to aspen dieback and mortality in western Canada (Hogg et al. 2002).

An important aim of conservation biology is the preservation of the evolutionary potential of species by maintaining or enhancing natural levels of adaptive genetic diversity (Hamrick et al. 1991). This begins with conducting a reliable genetic inventory of natural populations. Genetic inventories can serve many purposes, including: (a) forming a baseline for evaluating the effects of management practices on biodiversity, (b) reflecting environmental changes across the landscape, and (c) helping to describe and classify ecological units for management and protection. Understanding quaking aspen clone structure is crucial to preserving aspen genetic diversity. If genetic diversity is associated with elevation (temperature and moisture gradients), then climate change could reduce the evolutionary potential of aspen. Through effectively applied management efforts, species conservation and restoration goals can be achieved efficiently and economically.

The Eldorado National Forest, located along the western slope of the central Sierra Nevada, has been conducting inventories to determine the distribution and condition of quaking aspen stands. On-site surveys have been conducted in 230 of the 276 sites located to date. Aspen stands ranging in size from an individual stem up to 30 acres have been inventoried, some in clusters of as

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many as 17 stands and others located miles from the nearest known adjacent stand. Stands have been found in 14 different drainages of the South Fork of the American River and four drainages of the Rubicon River. Nearly all the stands in the inventory are located on soils related to glacial moraines, outwashes, or alluvial flows. The 1999-2000 field survey results for aspen stands in the Eldorado National Forest have been summarized (Burton, unpublished report). This summary indicates that a significant number of stands are declining in condition and lack evidence of significant regeneration.

We used starch-gel electrophoresis (allozymes) to assess the genetic structure of aspen located in the central Sierra Nevada, California. Specifically, this study used genetic data to identify the number of clones per stand, assess levels of genetic diversity, and detect geographic patterns of variation.

METHODS

Utility of Allozymes

The measurement of allozyme variation in plants helps in the interpretation of genetic diversity in natural populations. Allozymes are specific enzymes with discrete Mendelian inheritance. Since they are also codominant and free of environmental effects in their expression, they are used to directly calculate allele and genotype frequencies.

While not directly associated with adaptive traits, this laboratory analysis provides quick, inexpensive quantitative measures of genetic structure (amount and pattern of variation among and within populations), genetic diversity (heterozygosity), and mating systems (outcrossing rate). In addition, these parameters can be directly compared across species and can be related to species life-history traits to interpret genetic systems, survival "strategies," and historical lineages of different species. Complex, statistically significant geographic patterns have been revealed in some species using multilocus allozyme analyses (Westfall and Conkle 1992).

Sampling

Leaf tissue was collected from 663 aspen individuals (saplings, mature trees, or root suckers) in the spring of 2001 by David Burton, USDA Forest Service, Region 5 Aspen Delineation Project. Samples were collected from 82 stands, ranging in elevation from 5300 to 7800 feet, located on the Pacific, Placerville, and Amador Ranger Districts, Eldorado National Forest, Eldorado County, California (Table 1). Stand habitats included meadow fringes, riparian areas, forest openings, and boulder fields (Burton, unpublished report). Samples were collected at somewhat even spacing throughout major aspen clusters as well as from small, outlier stands. Sample/stand location maps were drawn for each site (Figure 1) by D. Burton and are available upon request. Leaf tissue was transported on ice to the USDA Forest Service National Forest Genetics Laboratory (NFGEL), Placerville, California.

Enzyme Electrophoresis

Enzyme extracts were prepared from 50mg leaf tissue per tree in a Tris buffer pH 7.5 (Gottlieb 1981), and were resolved on 11% starch gels using three buffer systems (Conkle et al. 1982). Six loci were resolved on a lithium borate (pH 8.3) buffer system (leucine aminopeptidase (LAP-1 and LAP-2), malic enzyme (ME7), phosphoglucose isomerase (PGI), and phosphoglucomutase (PGM-1 and PGM-2)); four loci on a sodium borate (pH 8.0) buffer system (glutamate-oxaloacetate transaminase (GOT), catalase (CAT), and uridine diphosphoglucose pyrophosphorylase (UGPP-1 and UGPP-2)); and seven loci were resolved on a morpholine citrate (pH 8.0) buffer system (diaphorase (DIA), phosphogluconate dehydrogenase (6PGD-1 and 6PGD-2), shikimate dehydrogenase (SKD), and malate dehydrogenase (MDH-1, MDH-2, and MDH-3)). As per NFGEL quality control standards, all gels were scored independently by two people, and 10% of the individuals were run twice. A total of 17 loci were scored using genetic interpretations that were inferred directly from isozyme phenotypes (Gottlieb 1981, 1982; Weeden and Wendel 1989), and that have been shown to be inherited in a non-linked, single-gene Mendelian manner in *Populus tremuloides* (Hyun et al. 1987a, Liu and Furnier 1993a).

Data Analysis

Estimates of genetic diversity were obtained from a set of 210 clonal multilocus genotypes. All samples with the same multilocus genotype within a stand were considered ramets of the same clone. Each clonal multilocus genotype per stand was represented once in the dataset. The chance of a random match among clonal genotypes was calculated as the product of the allele frequencies in that multilocus genotype. Identical allozyme genotypes were assumed to be different clones when geographic separation among genotypes was relatively great and when probability of the same genotypes occurring by chance recombination was relatively small. A total of 198 genotypes exist in the study when genotypes shared between stands are removed from the dataset.

Allozyme analysis was used to: (a) define genotypes to infer clonal composition of stands, and (b) quantify the amount and describe the pattern of geographic variation. Standard genetic diversity statistics were calculated using Popgene version 1.21 (Yeh et al. 1997), and

Table 1.*Populus tremuloides* stands sampled from the Eldorado National Forest, central Sierra Nevada, California. Stand area is in acres.

Watershed	Stand Code	Stand #	Stand Area	UTME	UTMN	N	G
Caples Creek						113	35
	AM-AA	31	6	748344	4287659	21	11
	AM-AA	32	0.3	748921	4287823	3	1
	AM-AA	33	0.1	747940	4287400	3	1
	AM-AA	34	1.5	749278	4288118	12	2
	PL-AA	19	0.3	745672	4286900	2	1
	PL-AA	20	0.5	745884	4287104	5	1
	PL-AA	21	0.1	746073	4287260	1	1
	PL-AA	24	2	746205	4287210	12	1
	PL-AA	25	5	746350	4287466	14	4
	PL-AA	27	1.2	747782	4288303	13	4
	PL-AA	28	1	747852	4288196	10	3
	PL-SB	39	0.2	751950	4290604	2	1
	PL-SB	39A	5	752130	4290510	3	1
	PL-SB	39B	0.2	751650	4290640	3	1
	PL-SB	44	1.2	750350	4290750	9	3
Lower Jones Fork Silver Creek						16	3
	PA-IH	20	1.5	727680	4302020	8	1
	PA-IH	30	0.5	735760	2402550	8	2
Sayles Canyon						110	34
	PL-ST	11	0.2	751515	4297437	10	3
	PL-ST	12	0.2	751579	4297343	4	2
	PL-ST	13	0.1	751700	4297010	4	2
	PL-ST	14	0.1	751895	4297020	6	2
	PL-ST	16	1	751976	4296940	6	3
	PL-ST	16A	1	751976	4296940	8	3
	PL-ST	22	4	752709	4296589	14	1
	PL-ST	23	0.1	753426	4296179	1	1
	PL-ST	25	10	752855	4296635	14	4
	PL-ST	27	6	752791	4296733	17	8
	PL-ST	29	0.2	751250	4297790	4	1
	PL-ST	31	0.3	753605	4296123	4	1
	PL-ST	32	4	753738	4296123	12	3
	PL-ST	33	0.2	753829	4295882	2	1
	PL-ST	38	0.1	751311	4297718	4	1
South Fork American River-Forni Creek						81	21
	PL-CS	13	2.6	750303	4298443	13	3
	PL-CS	14	0.3	749175	4298354	5	1
	PL-CS	15	0.1	749310	4298728	5	1
	PL-CS	16	0.4	749215	4298807	6	2
	PL-CS	17	0.2	749613	4298946	3	1
	PL-CS	18	0.2	749500	4298999	2	1
	PL-CS	18A	0.2	749500	4298999	1	1
	PL-CS	20	6	749280	4298985	16	4
	PL-CS	22	0.2	749869	298689	2	1
	PL-CS	23	0.5	750089	4298459	6	2
	PL-SB	45	0.5	747678	4297563	6	1
	PL-SB	49	2	747375	4297410	14	4
	PL-SB	50	0.2	747526	4297458	2	1

Table 1. *Populus tremuloides* stands sampled from the Eldorado National Forest, central Sierra Nevada, California. Stand area is in acres. UTM coordinates were established using 1927 North American datum (NAD 27) and are located in Zone 10S. N = number samples (stems) analyzed per stand or watershed. G = number of multilocus genotypes per stand or watershed based on allozyme data.

Watershed	Stand Code	Stand #	Stand Area	7UTME	UTMN	N	G
South Fork Silver Creek-Junction Reservoir						39	2
	PA-IH	10A	0.1	27522	4299083	3	1
	PA-IH	10B	0.4	727483	4298970	10	1
	PA-IH	10C	0.5	727587	4298963	14	1
	PA-IH	10D	1.2	727685	4298850	12	1
South Fork Silver Creek-Wrights Lake						116	44
	PA-LC	18	0.3	743050	4300250	9	6
	PA-LC	46	28	742150	4300398	22	6
	PA-LC	48	0.15	742820	4300180	4	1
	PA-LC	49	13	742546	4300194	18	10
	PA-LC	51	5	742770	4300521	15	6
	PA-SF	10	0.2	739940	4300840	4	2
	PA-SF	11	0.2	740020	4301100	4	1
	PA-WL	15	0.5	740803	4303550	5	2
	PA-WL	24	0.5	741375	4303920	7	1
	PA-WL	25	1	741127	4303875	9	4
	PA-WL	26	0.5	741037	4303759	5	2
	PA-WL	27	0.2	740986	4303632	4	2
	PA-WL	28	0.1	740848	4303578	1	1
	PA-WL	30	0.25	741305	4304100	9	1
Strawberry Creek						84	19
	PL-SB	13	5	749523	4295893	15	1
	PL-SB	22	2	751720	4292650	12	3
	PL-SB	27	0.5	751729	4292259	9	3
	PL-SB	27A	1	751680	4292240	8	1
	PL-SB	28	0.75	751700	4292160	11	2
	PL-SB	37	0.75	751448	4291257	10	5
	PL-SB	42	1.5	751400	4290800	13	5
	PL-SB	42B	1.5	751400	4290800	6	2
Upper South Fork Rubicon River						104	40
	PA-TC	14	1	733831	4315283	11	2
	PA-TC	15	1	733689	4315385	10	1
	PA-TC	16	0.6	733560	4315395	5	1
	PA-TC	17	1.4	733463	4315400	6	1
	PA-TC	18	5	733303	4315446	14	9
	PA-TC	18A	0.3	733617	4315518	2	1
	PA-TC	21A	0.75	731912	4314823	14	6
	PA-TC	21C	0.5	731703	4314802	7	2
	PA-TC	36	4	732968	4315205	16	10
	PA-TC	41	3	731350	4315150	3	1
	PA-TC	42	11	731495	4315189	16	7
Total by Stand						663	210
Total by Watershed						663	198

include: unbiased genetic similarities (Nei 1978), effective number of alleles per locus (Kimura and Crow 1964), expected heterozygosity (Nei 1973), gene flow (Slatkin and Barton 1989), and fixation indices (Weir 1990).

A canonical correlation analysis (CCA) was used to determine if a significant geographic pattern existed in allozyme loci of aspen across its range on the Eldorado National Forest. The objective was to build a model that would best represent the geographic pattern of allozyme genotypes in aspen. This part of the study used a subset of the total allozyme diversity sampled, and includes only that allozyme diversity that is associated with geography and may imply adaptive significance. The 198 unique allozyme genotypes distributed across the Eldorado National Forest formed the base for the CCA between allozymes (genes) and their geographic locations (latitude, longitude, and elevation). Due to missing data, 129 clones from 73 stands were used to build the model. CCA is the multivariate equivalent of multiple regression, but with more than one dependent variable (e.g. many allozymes). The first step in the statistical process was to transform allele presence or absence in the diploid genotypes to an additive score (Smouse and Williams 1982) to achieve normality for analyses. The three geographic variables were expressed as a 2nd order polynomial, creating nine geographic terms. In developing the CCA model, allozymes that contributed negligibly were dropped from the model (Westfall and Conkle 1992). Canonical scores for the first three highly significant vectors were regressed on the nine geographic terms to create predicted scores.

Predicted stand scores for each of the three vectors were subdivided in halves (above and below the mean) to create eight genetic classes (A to H). Minimum differences between multi-locus genotypes were predicted to be within classes. Maximum differences were predicted between A and H (which differ in all three vectors). The final step was to compute the average probability (p) of a genetic mismatch (and perhaps of genetic maladaptation) within delineated groups, a statistical concept known as transfer risk. Mismatch statistics were computed using (Westfall and Conkle 1992) formulae; SAS code: dev=((pv1-mv1)**2)/ems1 + ((pv2-mv2)**2)/ems2 + ((pv3-mv3)**2)/ems3; d=sqrt(dev)/2; p=2*(probnorm(d)-0.5); predicted (pv1—pv3) and group-mean (mv1—mv3) scores, pure-error mean squares (ems1—ems3), and probability distribution followed by PROC UNIVARIATE on variable "p". "P" is the probability that a clone's multivariate predicted scores (pv1—pv3) do not match the mean scores (mv1—mv3) for the genetic-geographic class that the clone was associated with. Smaller probabilities indicate a closer match; larger values indicate greater mismatch.

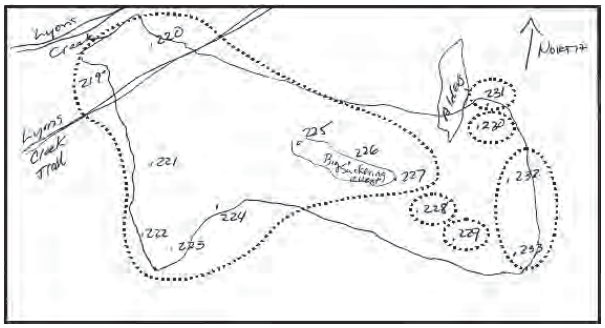

Figure 1. Sampling map of aspen stand PA-LC 51. Fifteen aspen individuals, indicated by three digit numbers, were sampled for genetic analysis. Clonal distribution based on allozyme data is indicated by dashed lines. Maps of the additional 81 sampled stands are available upon request.

RESULTS

Clonal Identification

The 663 aspen samples analyzed were located in 82 stands from eight watersheds on the Eldorado National Forest. Stand area ranged from 0.1 to 28 acres, with an average stand size of 2.0 acres (s.d. = 3.8). The number of stems sampled within a stand ranged from 1 to 22 (average = 8.1, s.d. = 5.1). Four stands consist of only a single sampled individual (Table 1).

A total of 198 multilocus genotypes (clones, or genetic individuals) were identified among the 663 sampled individuals (Table 1). The number of clones ranged from 1 to 11 within stand (average = 2.6). Forty (49%) of the 82 stands are monoclonal (containing only one multilocus genotype). However, only one individual was sampled in each of four stands (PA-WL 28, PL-AA 21, PL-CS 18A, and PL-ST 23). When these stands are excluded, 44% of stands are monoclonal. Watersheds contain between 2 to 44 clones (average = 24.8), and therefore, no watershed is monoclonal. The extent of clones in each stand was mapped (Fig. 1).

Ten genotypes are duplicated in adjacent stands within single watersheds (Table 2). These duplications were treated as clones that extended between stands. Most duplicated clones are separated by no more than 1,000 meters. The average probability of occurrence for a multilocus genotype within this study is 1.4×10^{-5} (s.d. 4.8×10^{-5}), with a range of 5.0×10^{-4} to 1.2×10^{-14}. Of these genotype pairs repeated within the same watersheds, the probabilities of these matches occurring by chance vary between 1.3×10^{-4} and 1.2×10^{-14}. Stands that consist of a single clone vary in size from 0.1 to 5 acres (Table 1).

Table 2. Matching aspen genotypes found in different stands but treated as single clones. G = the number of different genotypes in stand. R = the number of samples with the matching genotype/total number of samples in the stand. P = the probability that the genotype would occur, based on the frequency of alleles in the genotype. Separation = distance between stands, in meters. Multilocus genotypes are presented in this order: LAP1, LAP2, PGM1, PGM2, ME7, PGI2, UGPP1, UGPP2 CAT, GOT, SKD, MDH1, MDH2, MDH3, 6PGD1, 6PGD2, DIA. 0 = missing data.

Watershed	Stand Code	Stand #	G	R	P	Separation (meters) 160 - 340	Genotype
S. Fork American River-Forni Creek	PL-SB	45	1	6/6	2.1×10^{-8}	775	11 11 11 12 11 79 11 12 11 22 11 11 11 12 11 11 15
	PL-SB	49	4	8/14			
	PL-SB	50	1	2/2			
Sayles Canyon	PL-ST	14	2	2/6	1.1×10^{-6}	190	11 11 14 11 11 33 11 22 11 12 11 11 11 11 11 11 15
	PL-ST	16A	3	1/8			
South Fork Silver Creek-Wrights Lake	PA-WL	24	1	7/7	1.8×10^{-8}	105 - 235	11 16 11 33 11 23 11 22 11 12 11 11 11 23 12 11 15
	PA-WL	30	1	9/9			
South Fork Silver Creek-Junction Reservoir	PA-IH	10B	1	10/10	1.5×10^{-6}	1070	11 16 11 33 11 77 11 22 11 22 11 11 11 12 12 11 15
	PA-IH	10C	1	14/14			
	PA-IH	10D	1	12/12			
Caples Creek	AM-AA	32	1	3/3	2.1×10^{-5}	0	11 00 11 11 11 33 11 12 11 00 11 11 11 12 12 11 55
	AM-AA	33	1	3/3			
Strawberry Creek	PL-SB	42	5	3/13	1.2×10^{-14}	0	22 47 11 11 11 77 11 12 11 13 13 11 11 12 30 00 55
	PL-SB	42B	2	5/6			
Sayles Canyon	PL-ST	16	3	3/6	3.4×10^{-10}	320	14 16 11 11 11 27 11 22 11 16 13 11 11 12 12 11 15
	PL-ST	16A	3	4/8			
Upper South Fork Rubicon River	PA-TC	18A	1	2/2	1.3×10^{-4}	50	11 11 11 11 11 33 11 22 11 22 11 11 11 12 11 11 11
	PA-TC	18	9	3/14			
Strawberry Creek	PL-SB	27	3	2/9	4.9×10^{-5}	3920	11 11 11 11 11 33 11 22 11 22 11 11 11 12 12 11 11
	PL-SB	27A	1	8/8			
Strawberry Creek	PL-SB	13	1	15/15	8.8×10^{-5}		11 11 11 11 11 77 11 22 11 00 11 11 11 12 11 11 55
	PL-SB	22	3	8/12			

Monoclonal stands averaged smaller in size than stands with two or more clones (0.8 and 3.1 acres, respectively; $R^2 = 0.7122$).

Genetic Diversity

Individual aspen stands consist of only one to 11 genetic individuals, or clones. Stands contain low to moderate levels of diversity and average 33.3% polymorphic loci with 1.4 alleles per locus (Table 3). Watersheds, on average, contain more diversity than do individual stands (He = 0.261 and 0.229, respectively). The aspens as a whole contain a substantial level of variation as measured by percent polymorphic loci, alleles per locus, and expected heterozygosity (82.4, 3.1, and 0.279, respectively; Table 3).

Of the total variation measured, 46.5% was found among stands, indicating that stands are very differentiated, or genetically different from each other (Table 3). Watersheds are much less differentiated with only 10% of the total variation measured found among watersheds, indicating shared genetic diversity among the watersheds. The mean percent variation found among stands within a watershed is 39.4% (Table 3). Genetic similarities among aspen stands were highly variable and often low, averaging 85.8% (similarities range from 100% to 61.8%). Similarities among watersheds average 95.8%. Genetic similarities of outlying stands to the cluster they were considered outliers of averaged 85.1%, and genetic similarity of stands within watersheds averaged 89.8%.

Geographic Patterning

The best fitting model to explain geographic patterning of the data related a subset of 27 allozymes from 12 loci with all nine geographic variables. These allozymes in combination varied in frequency in a geographic pattern. Geographic patterning was relatively strong and complex. In the 1st canonical vector, the geographic model accounted for 64% (adjusted=54%) of the new allozyme variable. Elevation and its interaction terms were primary components of the 1st vector and longitude was secondary. The 2nd vector was composed

Table 3. Genetic diversity of *Populus tremuloides* using one sample per clone per stand. #Std = number of stands. Abbreviations are: N = sample size, %P = percent polymorphic loci, A = mean number of alleles per locus, Ae = effective number of alleles per locus, Ho = observed heterozygosity, He = expected heterozygosity, F = fixation index: (He-Ho)/He, Fst = differentiation of stands within region, and Nm = calculated gene flow among stands. Standard deviation are in parentheses. Diversity statistics for the 82 individual stands are available upon request.

Region	#Std	N	%P	A	Ae	Ho	He	F	Fst	Nm
Entire study	82	407	82.4	3.1 (1.5)	1.5 (0.5)	0.229 (0.264)	0.279 (0.236)	0.179	0.465	0.287
Stand (mean)	—-	5	33.3	1.4	1.3	0.226	0.229	0.011	———-	———-
Watershed (mean)	10	50	62.9	2.0	1.5	0.233	0.261	0.102	0.394	0.427
Caples Creek	15	66	72.2	2.1 (1.0)	1.5 (0.5)	0.212 (0.267)	0.267 (0.225)	0.206	0.547	0.207
Jones Fork Silver Creek-Forni Creek	2	6	52.9	1.8 (0.9)	1.5 (0.7)	0.275 (0.377)	0.267 (0.302)	-0.030	0.216	0.908
Sayles Canyon	15	69	70.6	2.3 (1.0)	1.5 (0.5)	0.231 (0.241)	0.287 (0.229)	0.195	0.464	0.288
South Fork American River-Forni Creek	13	45	70.6	2.2 (1.1)	1.5 (0.5)	0.242 (0.285)	0.251 (0.225)	0.036	0.397	0.380
South Fork Silver Creek-Junction Reservoir	4	8	50.0	1.6 (0.7)	1.4 (0.5)	0.250 (0.393)	0.232 (0.259)	-0.078	0.385	0.400
South Fork Silver Creek-Wrights Lake	14	84	66.7	2.1 (1.0)	1.5 (0.6)	0.233 (0.287)	0.258 (0.252)	0.097	0.348	0.469
Strawberry Creek	8	38	55.6	2.1 (1.2)	1.5 (0.5)	0.189 (0.281)	0.258 (0.249)	0.267	0.389	0.392
Upper South Fork Rubicon River	11	80	64.7	2.1 (1.1)	1.5 (0.6)	0.235 (0.280)	0.269 (0.246)	0.126	0.403	0.371

of latitude and latitude-longitude interaction. The 1st three vectors were highly significant and accounted for 72% of the original allozyme variation. These three vectors were used to form genetic-geographic classes having similar predicted gene (allozyme) frequencies (Table 4). Sequential pairs (e.g. A vs B, C vs D, E vs F, G vs H) differ only in the 3rd vector. A + C vs B + D and E + G vs F + H differ only in the 2nd vector. A+B+C+D vs E+F+G+H differ only in the 1st vector.

Probability of Genetic Mismatch

The average probability of genetic mismatch within watersheds varied from 18 to 47%, and within genetic class from 11 - 43% (Table 5). The Upper SF Rubicon River had the highest probability of genetic mismatch. This watershed contains 40 clones in 11 stands and three genetic classes ('C', 'D', and 'F'). The Rubicon 'F's are genetically similar (in predicted values) to the Rubicon 'D's, and similarly, the Caples Creek 'A's can be considered practically as Caples Creek 'B's. The Lower Jones Fork Silver Creek watershed followed with 37%, but the sample was small (only three clones and two stands: 'C' and 'D') and widely separated by five miles. Well-sampled watersheds had the lowest probabilities of genetic mismatch. The South Fork Silver Creek-Wrights Lake watershed with 44 clones and three genetic classes had an 18% average probability of genetic mismatch, followed by Strawberry Creek (20%, all 'G's). Sayles Canyon was uniform in terms of genetic class (all 'H's), but it was relatively diverse with 34 clones in 15 stands and 25% average probability of genetic mismatch. The genetic diversity of Caples Creek watershed was greatly increased by inclusion of two very high (>7600 ft) elevation stands (Buck Pasture), which were nearer geographically and genetically to upper Strawberry Creek stands (Figs. 2, 3).

DISCUSSION

Clonal Structure and Genetic Diversity

The clonal structure of aspen stands in the central Sierra Nevada combine traits of aspens studied elsewhere in North America. As in northeast North America, the monoclonal Sierra Nevada stands observed in this study

Table 4. Pattern classification of genetic-geographic variation for *Populus tremuloides* samples. Data are plotted in Figures 2 and 3.

Genetic Class	Stand Code	Stand #	# Clones	Ave He
A	AM-AA	32,33,34	30	0.198
	PA-LC	46,49,51		
	PA-WL	15,27,28		
B	AM-AA	31		
	PL-CS	15,16,17,18,18A,20,22	41	0.236
	PL-SB	45,49,50		
	PL-AA	19,20,21,24,25,27,28		
C	PA-IH	30		
	PA-TC	21A,21C,36,41,42	31	0.234
	PA-SF	10,11		
D	PA-TC	16,17,18,18A		
	PA-IH	10A,10B,10C,10D,20	14	0.194
E	PA-LC	18,48		
	PA-WL	24,25,26,30	17	0.197
	PL-SB	44		
F	PA-TC	14,15	3	0.183
G	PL-SB	13,22,27,27A,28,37,39,39A,39B,42,42B	22	0.228
H	PL-CS	13,14,23		
	PL-ST	11,12,13,14,16,16A,22,23,25,27,29,31,32,33,38	40	0.234

are often small, averaging 0.8 ha (Barnes 1966; Kemperman and Barnes 1976). However, much like those stands in the intermountain west, a large proportion of the stands in the central Sierra Nevada (44%) are monoclonal, and stands arising from seed establishment may be rare (Mitton and Grant 1996).

Despite the clonal structure of aspen, the species contains a surprisingly high level of measurable genetic diversity. The 82% polymorphic loci observed in this study is similar to the values observed elsewhere (Cheliak

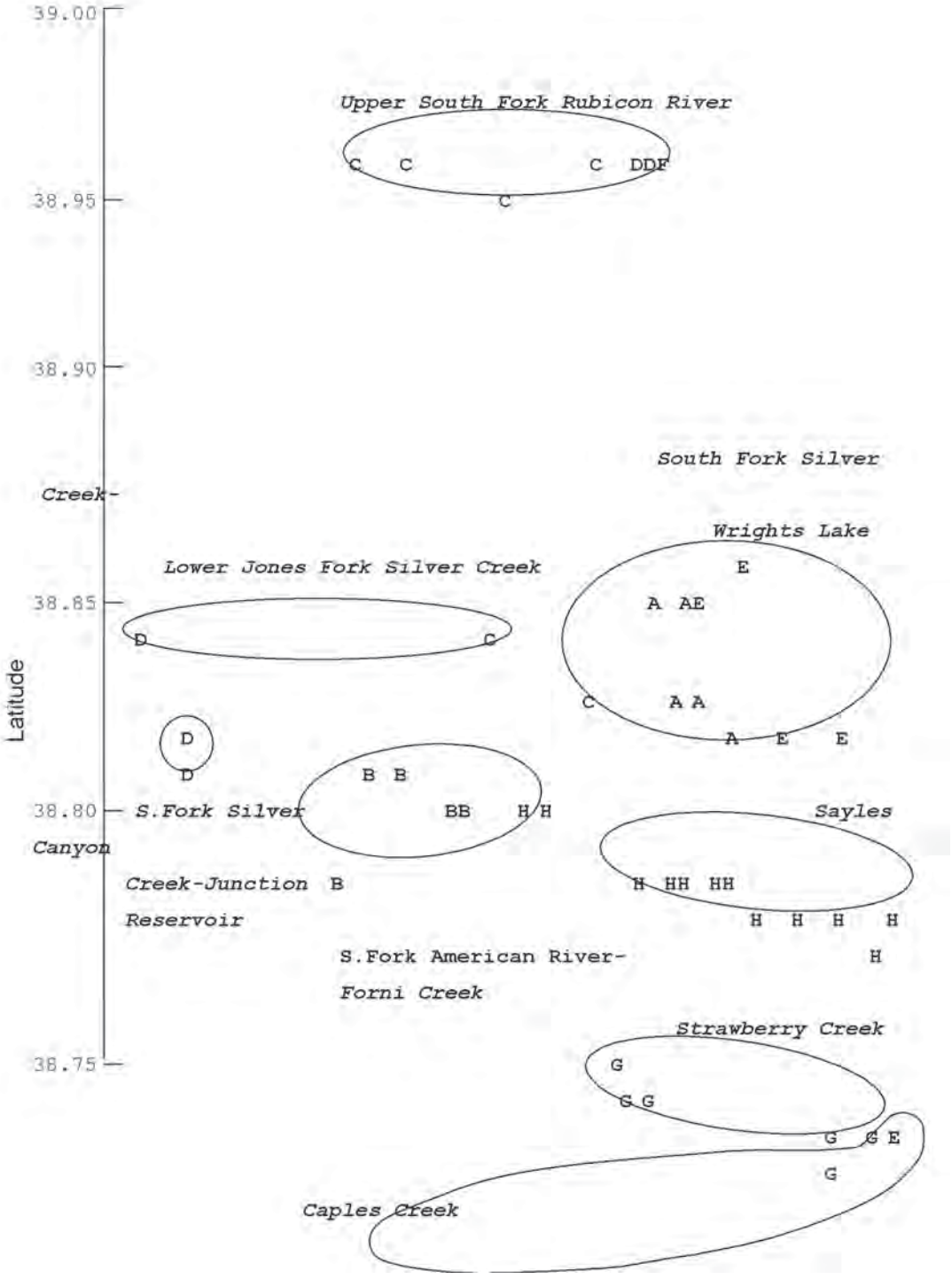

Figure 2. Geographic variation pattern for aspen clones, based on first three canonical vectors. Watersheds are outlined with a solid line, and named in italics. Letters are genetic pattern classifications found in Table 4. Note: some observations are hidden.

and Dancik 1982, Cheliak and Pitel 1984, Hyun et al. 1987b, Jelinski and Cheliak 1992, Lund et al. 1992, Liu and Furnier 1993b). Watersheds contain similar levels of genetic diversity (Table 3). The low percent polymorphic loci within stands reflects the monoclonal nature of nearly half the stands sampled. The monoclonal nature of many of the stands, together with the spread of some clones between stands, suggests a previously broader range of aspen than its current size. Levels of genetic diversity per cluster of stands generally increase as the number of clones increase. This is reflected in several diversity measures (percent polymorphic loci, R^2=0.776 and alleles per locus, R^2=0.837).

Adaptation and Geographic Variation

Table 5. Probability of genetic mismatch within Genetic Class and within watershed, which includes coefficient of variation (CV) and the extreme genetically divergent stands. The abbreviation P % = probability of genetic mismatch.

	P %	CV %	Number of:		Highest probability of genetic mismatch		Genetic Classes in watershed
			Stands	Clones	Stand ID	P %	
Genetic Class							
A	11	78	9	30	AM-AA 33,34	42, 32	——
B	20	76	18	41	PL-AA 19,21,24 PL-CS 15,22	44, 38, 39 45, 44	——
C	43	41	8	31	PA-TC 41,42	75, 60	——
D	31	64	9	14	PA-IH 10A,10C,20	64, 64, 71	——
E	29	25	7	17	PL-SB 44	41	——
F	2	43	2	3	n/a		——
G	19	29	11	22	PL-SB 22	29	——
H	33	58	18	40	PL-CS 13,14,23	63, 70, 68	——
Watershed							
Caples Creek	32	77	15	35	PL-SB 39A,39,39B,44	80—73	A, B, G, E
Lower Jones Fork Silver Creek	37	39	2	3	n/a		C, D
Sayles Canyon	25	56	15	34	PL-ST 38,32,11	49,47,44	H
South Fork American River-FC	24	64	13	21	PL-SB 49, PL-CS 13	41, 40	B, H
Upper South Fork Rubicon River	47	39	11	40	PA-TC 41, 42	85, 74	C, D, F
South Fork Silver Creek-JR	1	0	4	2	PA-IH 10A,C	78	D
South Fork Silver Creek-WL	18	85	14	44	PA-SF, PA-LC 18	47, 46	A, C, E
Strawberry Creek	20	20	8	19	PL-SB 22	27	G

The distribution of species, populations, and genotypes of individual plants (i.e. genetic variation) across the landscape is often associated with geography (i.e. latitude, longitude, and elevation). Geographic patterns associated with climatic variables (e.g. temperature and moisture) suggest natural selection for adaptation of plant populations to different climates. However, recent changes in climate may cause current populations to be sub-optimal ("adaptational-lag"). Also, migration (gene flow) routes and/or genetic drift (in small, reproductively-isolated populations) may also influence genetic-geographic variation patterns and delay adaptation to local environments.

Although single-locus correlations with geography have been found for allozymes, adaptation of an individual is likely controlled by alleles (genes) at many loci, with small effects. Some genes convey a selective advantage or disadvantage to the individual, depending on the adaptive traits and the environment involved. For example, highly competitive environments may induce ("turn-on") specific genes and exert strong selective pressure for growth (e.g. rate and phenology) traits. In contrast, highly stressful environments may induce a different set of genes and exert strong pressure for defensive traits (e.g. stress-tolerance, injury-repair).

The cumulative effects of small differences in allelic frequencies, when summed across multiple loci can increase detection of differentiation among populations and allow reliable grouping based on genetic similarity of multi-locus genotypes (Smouse et al. 1982). Multi-variate statistical analyses have been successfully employed to reveal geographic patterns in trees (Guries 1984; Yeh et al. 1985). Therefore, multi-locus allozyme variation based on multi-variate analytical methods may detect underlying adaptive variation patterns.

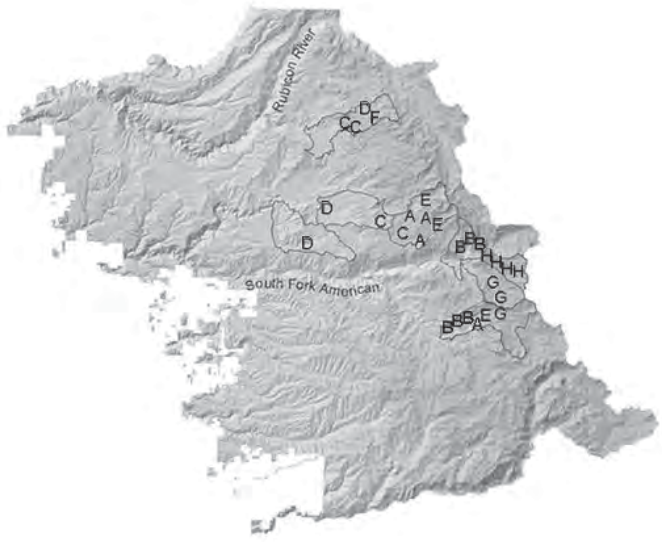

Figure 3. Genetic-Geographic classes of Aspen stands on the Eldorado National Forest. Eight HUC 7 watershed boundaries are indicated by black lines. Letters are genetic pattern classifications found in Table 4. Note: some observations are hidden.

Geographic structuring exists among the aspen stands on the Eldorado National Forest. West of the 120.24 longitude and ranging from 5200 to 6800 ft elevation, stands share a high degree of genetic similarity. Most of these stands belong to genetic class 'C' and 'D'. East of 120.12 longitude and ranging from 6400 to 7700 ft elevation, stands belong to classes 'G' and 'H'. Stands along Sayles Canyon (PL-ST) share genetic similarity and all 15 belong to class 'H'. Strawberry Creek (PL-SB) is comprised entirely of genetic class 'G'. Stands along Caples Creek divide into two elevation groups: low (<6500ft) belonging to two classes: 'A' and 'B'; and high (>7500ft) belonging to 'E' and 'G'. The 'E' genetic group is one of the more geographically diverse groups and extends from Caples Creek at 7800 ft north into Lyons Creek (PA-LC) at ca 7500 ft, and encompasses half of the PA-WL stands near Wright's Lake.

In this study, the greatest divergence was between the low elevation northwestern 'C' plus 'D' classes versus the high elevation southeastern classes 'G' plus 'H' (Figures 2, 3). Certainly gene flow has been greatly restricted and climates are very different between these two geographic populations. The 'A' and 'B' classes are southern low elevation populations that are intermediate both genetically and geographically to the 'C' - 'D' and 'G'-'H' extremes, and may have exchanged genes in the past from both groups. 'A' is more similar to 'C' than it is to 'D', and 'B' is more similar to 'H' than it is to 'G'. Also, the 'A's in Caples Creek are very close genetically to the geographically adjacent 'B's. Similarly, the 'F's in the South Fork Rubicon River watershed are very close genetically to 'D's. There could have been two major migration routes from lower to higher elevation as glaciers receded, one southern route out of the American drainage involving class 'B', and one northern route moving out of the Rubicon including classes 'C'-'D'.

Are these genetic-geographic differences associated with different climates, fire, or grazing regimes and therefore subject to strong natural selection? In the broadest extremes, the answer is probably yes. First, natural selection modifies growth and phenology of populations toward better harmony (or adaptedness) with local environmental conditions. Second, growth rate of aspen decreases with increased elevation, steepness of slope, age, and exposure to wind. In this study, aspen stands range from 5100 ft to 7800 ft elevation, a span of 2700 ft. Third,

by Hopkins bioclimatic law, a phenological event occurs 1 day later for every 100 ft increase in elevation. Thus, 2700 ft translates to a difference of 27 days in flowering and flushing. If females remain receptive and pollen sheds for 2 weeks at low elevation then even 15 days would prevent gene exchange between the high stands and the low elevation stands. A strong climatic gradient associated with elevation coupled with restricted gene flow due to difference in flowering phenology could explain the divergence we found between the low elevation northwestern populations ('C' plus 'D') and the high elevation southeastern populations ('G' plus 'H').

The most genetically diverse watersheds are the Upper SF Rubicon River and Sayles Canyon, and the least diverse are SF American River-FC and the SF Silver Creek-Wrights Lake (based on expected heterozygosity, number of clones, and probability of genetic mismatch; Tables 1, 3, 5). The most genetically diverse classes are 'C' and 'H', and the least diverse is 'A' (Tables 4, 5).

This genetic-geographic classification may be used as a guide for delineating tentative gene management units, for prioritizing gene conservation strategies, and for further genecological study (e.g. previous migration routes, adaptive gene-environment relationships). The additional concept of genetic mismatch has practical application to the transfer of genes or genotypes within groups through management practices such as breeding efforts and restoration plantings. If a high probability of a genetic mismatch is due to adaptation to different environments via selection, the transfer may have a high risk of maladaptation of transferred genetic material to its new environment. Our analysis identifies those extreme individual genotypes and stands with the highest and lowest probability of mismatch within their group. In our case, group categories are: a) watershed, and b) genetic class. A watershed with high probability of genetic mismatch is genetically more variable than one with a low value. Conversely, a genetic class that has low probability of mismatch is genetically more uniform than one with a high value. If high probability of genetic mismatch of stands does not involve adaptation to different environments via selection, then this probability statistic may simply reflect a previously large local breeding population with high genetic mixing. Such population "centers of genetic diversity" represent compact genecological units that can be efficiently managed for preserving the evolutionary potential of aspen.

MANAGEMENT IMPLICATIONS

The geographic patterning of genetic variation detected by the allozyme data was relatively strong and complex. Some of the eight genetic classes corresponded closely with the eight watersheds. The two most genetically distinct groups were those north of the South Fork of the American River below 6800 ft elevation versus those south of the American River above 6800 ft. These two groups may be adapted to different climates and probably should remain as separate genetic entities. Between these most distinct populations there is considerable commonality in genes and in climate, and there appears to be much less risk of maladaptation from transferring or mixing genes. Therefore, we propose a simple delineation of four tentative genecological units or local gene pools with splits at the American River and at ca. 6800 feet elevation. The North-Low group includes 'C', 'D', and 'F', North-High includes 'A' and 'E', South-Low includes 'B', and South-High includes 'H' (Fig. 3). Within these four broad tentative gene management units, the more refined genetic classifications can guide conservation efforts. For example, if restoration objectives for monoclonal stands require additional clonal diversity, priorities for clonal introductions from other stands may follow transfers within genetic classes or between similar genetic classes, while also considering clonal and gene diversity. Clone maps (Fig. 1) may also be used at the stand level to help guide fine-scale management decisions with the goal of aspen conservation and restoration.

ACKNOWLEDGMENTS

We thank Suellen Carroll, Patricia Guge, and Randy Meyer at the National Forest Genetics Laboratory for technical support, and David Burton for sample collection. We thank Vicky Erickson and other anonymous reviewers for helpful comment on the manuscript.

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