

Genetic Variation and the Natural History of Quaking Aspen

The ways in which aspen reproduces underlie its great geographic range, high levels of genetic variability, and persistence

Jeffrey B. Mitton and Michael C. Grant

In the fall, sightseers take to the highways of North America to enjoy the brilliant colors that are revealed as deciduous trees recycle the dominant greens of chlorophyll. In the western United States and Canada, the most colorful tree in the fall is the quaking aspen, *Populus tremuloides*. Brilliant yellows, rich golds, and shimmering shades of red shine, contrasting with the various green shades of the conifers.

Quaking aspen earns its name for the distinctive fluttering of its leaves, even in the most gentle breezes. Early French-Canadian trappers called the tree an aspen because of its similarity to *Populus tremula*, a closely related species in Europe and Asia. One of the legends attached to the aspen reflects Judeo-Christian influences. The aspen quake in fear today, according to folklore, because Jesus Christ was crucified on a cross of aspen. In addition to the esthetics of quaking leaves and brilliant fall colors, extraordinary features of the natural history and genetics of aspen lend it special appeal for naturalists and laboratory scientists.

Quaking aspen merits a variety of superlatives: It is North America's most widely distributed native tree species and the second most widely distributed in the world (Barnes and

The most massive known organism, the 6-million-kilogram quaking aspen clone we call Pando, highlights remarkable features of the species

Han 1993, Jones 1985). The world's most massive individual organism is a quaking aspen (Grant et al. 1992). Individuals may reach ages in excess of 1 million years (Barnes 1966, Grant 1993, Kemperman and Barnes 1976). And quaking aspen may also be the most genetically variable plant species studied to date (Cheliak and Dancik 1982).

Quaking aspen is an intriguingly multifaceted species, varying from a twisted dwarf bush with a height of less than 1 m to straight stems reaching 30 m tall and 60 cm in diameter. Although aspen commonly exists as an early successional species, sprouting profusely after a fire or an avalanche, it is a climax species in some environments.

Broad geographic and environmental range

Quaking aspen can be found from the mountains of Mexico to northern Alaska, from the Atlantic to the Pacific (Figure 1), from sea level to

3700 m. It thrives in a variety of plant communities, especially those subject to major disturbances, from the spruce-fir forests of the Rocky Mountains to the deciduous forests of New England. In the eastern and central parts of North America, aspen tends to be distributed nearly continuously, occurring in many different plant associations (Graham 1963). In the much more arid West, aspen tends to be restricted to high plateaus and mountain sides. At the lower limit of its elevational range (approximately 2200 m in the Rocky Mountains of Colorado), aspen occasionally grows on north-facing slopes with ponderosa pine, *Pinus ponderosa*, and Douglas-fir, *Pseudotsuga menziesii*. More commonly, aspen grows in the lodgepole pine, *Pinus contorta*, elevational range. At the highest elevations, aspen is observed in scattered stands within spruce-fir forests of Engelmann spruce, *Picea engelmannii*, and subalpine fir, *Abies lasiocarpa*. In rare locations (e.g., at the University of Colorado's Mountain Research Station in the Front Range), aspen grows at tree line (elevation of 3600 m) in association with stunted Engelmann spruce, subalpine fir, and limber pine, *Pinus flexilis*. The aspens at this elevation are short, being twisted and pruned by winter winds.

Life history

The quaking aspen is a member of the willow family (Salicaceae) and is closely related to various poplars such as cottonwoods. One other as-

Jeffrey B. Mitton and Michael C. Grant are professors in the Department of Environmental, Population, and Organismic Biology at the University of Colorado, Boulder, CO 80309. © 1996 American Institute of Biological Sciences.

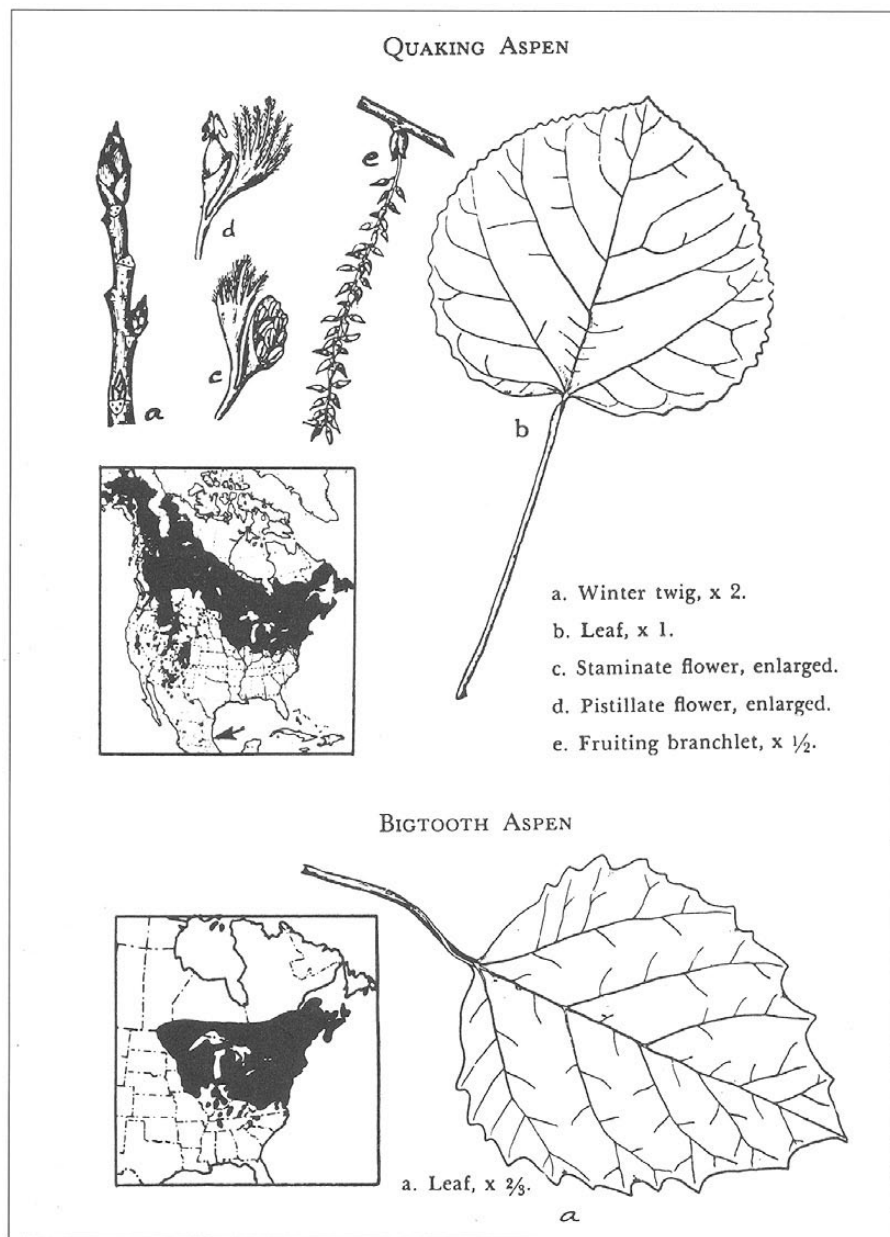


Figure 1. Morphological detail and distributions of quaking aspen (*Populus tremuloides*) and biotooth aspen (*Populus grandidentata*). From Preston 1976.

pen species is found in North America, the bigtooth aspen, *Populus grandidentata*. As the name implies, the leaf margins of these trees show regular, easily recognizable serrations in contrast to the finely dentate, nearly smooth leaf margins of *P. tremuloides* (Figures 1 and 2). Aspen flowers are small, relatively inconspicuous and produced in strings called catkins. The entire flowering sequence, including seed maturation, is completed in the early spring before the tree leaves out. In the Front Range of Colorado, we have observed that most

females produce seeds each year.

Seed production appears to be positively correlated with age and size of the stem. Old stems have been known to produce 54 million seeds in a single season (Schopmeyer 1974), and seed viability generally exceeds 90% in Colorado.¹ The mature seeds are widely scattered as self-contained, windblown dispersal units with the easily observed parachute-like structures that give the related cottonwoods their common name. Their ability to reproduce

¹MC Grant, unpublished data.

sexually (via seeds) partly underlies the remarkable levels of genetic variability in this species.

Despite enormous crops of viable seeds, successful seedling establishment appears to be a rare event in the semiarid West (Kemperman and Barnes 1976, Romme 1982, Romme and Despain 1989), but the establishment of new trees from seeds appears to be common in the moist, humid forests of New England. Although most of the seeds shed by aspen are capable of germinating, several factors, singly and in combination, dramatically limit successful establishment. In fact, many botanists argue that widespread quaking aspen establishment from seeds probably has not occurred in the western United States since the last glaciation, some 10,000 years ago (Einspahr and Winton 1976, McDonough 1985), although some local patches have certainly arisen more recently. In these regions, the seedlings usually wither and die before their roots reach an abundant and reliable source of water, or they do not receive adequate sunlight. In addition, aspen seedling establishment can be dramatically reduced by ungulate browsing (Beetle 1974, Olmstead 1979). The availability of sites suitable for new colonization, particularly following fires (DeByle and Winokur 1985, Jelinski and Cheliak 1992, Jones 1974, Kay 1993), plays a major role in determining seedling establishment.

At present, we know of no direct way to test the hypothesis that the majority of the quaking aspen individuals in the Rocky Mountains were established several thousand years ago, when those environments were substantially wetter than they are today. However, we do now have clear evidence of one major disturbance in the Rocky Mountains that has produced thousands of new aspen seedlings—the 1988 fire in Yellowstone National Park. Following the severe fire of 1988 (Kay 1993, Turner and Romme 1994), large regions of Yellowstone were successfully colonized by young seedlings in regions previously devoid of aspen. Apparently, the last previous episode of this scale occurred nearly 300 years ago (Romme 1982, Romme and Despain 1989). Al-

though these episodes qualify as rare on a human time scale, they are sufficiently frequent to influence the ecological genetics of quaking aspen (Jelinski and Cheliak 1992). If seedling success is so rare, why are aspen so abundant and widespread in the semiarid West? One important answer to this question lies in aspen's ability to reproduce asexually.

Asexual reproduction

Unusual, but not unique among forest tree species, quaking aspen regularly reproduces via a process called suckering. An individual stem can send out lateral roots that, under the right conditions, send up other erect stems; from all aboveground appearances the new stems look just like individual trees. The process is repeated until a whole stand, of what appear to be individual trees, forms. This collection of multiple stems, called ramets, all form one single, genetic individual, usually termed a *clone*. Observers usually fail to appreciate the critical underground portion of the aspen clone responsible for asexual reproduction—the root system.

A search through a lodgepole pine or spruce-fir forest may detect just a few, widely spaced, suppressed stems of aspen beneath a dense canopy of conifers. But when a fire or an avalanche clears out the standing crop of conifers, the true expanse of the aspen root system may be revealed by a vigorous production of abundant new ramets throughout the disturbed area, testimony that the root system cannot be assessed by simply counting aboveground stems.

Persistent root systems allow aspen to colonize, occupy, and even prefer disturbed sites, justifying their general characterization as an early successional species. After a fire has removed the conifers, the ramets that sprout from a healthy, mature root system may grow vertically as much as a meter in a single summer season (Bailey et al. 1990, Crouch 1981), while the density of new shoots may exceed 400,000 per acre (Schier et al. 1985). The root system of aspen grows aggressively; adjacent stems can be spaced more than 30 m apart (DeByle and Winokur

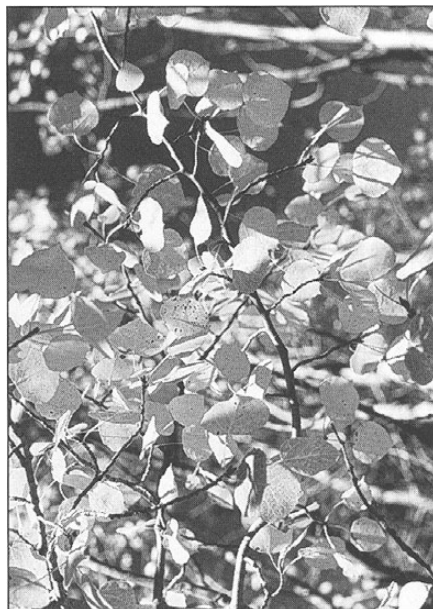


Figure 2. A close-up of quaking aspen leaves.

1985). During spring growth and when stems have been damaged (e.g., by fire), the hormonal suppression of root suckering diminishes, and many new stems may be produced from the relatively undamaged roots. At the same time, aspen clones tend to be intolerant of shade (Jones and DeByle 1985) and typically succumb to succession by conifer species in a few hundred years if left undisturbed (e.g., by fire, avalanche, mud slide, or logging).

Quaking aspen clone and stand size influence the biology of several associated species. For example, the species diversity of insectivorous birds increases with the size of aspen stands (Johns 1993).

Immense and ancient

The quaking aspen has demonstrated a remarkable ability to spread and persist in a given location via the process of root suckering. In the most spectacular example for which detailed information is available (Barnes 1966, Kemperman and Barnes 1976), the clonal growth of a single quaking aspen clone covers 43 hectares and contains more than 47,000 individual stems—a sizable forest in and of itself. Largely in response to the attention given to a giant fungus, *Armillaria bulbosa*, in a Michigan forest (Gould 1992,

Smith et al. 1992), we resurrected the data on this enormous aspen clone showing it to be, by far, the most massive living organism known, weighing more than 6 million kg (Grant et al. 1992). This mass (estimated for both above- and belowground parts) more than triples that of its nearest rival, the giant sequoia (*Sequoiadendron giganteum*) named General Sherman.

We have nicknamed the giant aspen clone Pando (Grant 1993), a Latin word meaning *I spread*. Pando, an exceptionally beautiful male clone, is located in the Wasatch Mountains of south-central Utah, straddling the highway to Fish Lake (Figure 4). The spectacular size of this clone appears to reflect a rare balance of the frequency and intensity of environmental disturbances, particularly fire, that encourages the persistence of quaking aspen. The fire frequency has been sufficient to prevent conifer succession, yet the local environment has been stable enough to allow healthy, vigorous growth and asexual reproduction for perhaps 1 million years.

Over the last several decades, space for a US Forest Service campground and several privately owned cabins has been cut out of this natural wonder. We are concerned that this colonization of Pando by humans dramatically reduces the likelihood of periodic cleansing by natural fires. These changes may mark the beginning of the end for Pando.

The true age of aspen clones at present can only be inferred from indirect means and, therefore, remains something of a mystery. While the age of most temperate forest trees can be estimated by counting the annual rings in a core extracted from the trunk, cores extracted from an aspen stem mark the age of that ramet, not the age of the clone. In a study of aspen in the Front Range, we selected and cored five of the largest ramets from each of 104 clones. The average age was 65 years; the oldest ramets were approximately 120 years old.

But biologists most familiar with natural aspen dynamics propose that these western clones are ancient, perhaps on the order of 10,000 years (Kemperman and Barnes 1976), and conceivably as much as 1 million



Figure 3. A clone of aspens in winter.

years (Barnes 1966, 1975), and potentially immortal. Part of the rationale behind current age estimates for aspen clones is that sexual reproduction is effectively frustrated by the rarity of a favorable suite of conditions in semiarid environments. Clonal age, in the strictest sense, truly applies only to the individual genome, which is the single element of clone identity that would be continuous across such time spans. No physical tissue such as root or stem or leaf presently in existence would have been in existence from the original seedling. Perhaps DNA sequence data from various parts of a clone could be used to estimate age from the accumulation of mutations. Surely somatic mutation (Keim et al. 1989) would have occurred frequently enough that different areas of the clone would retain different mutations.

Extreme longevity is not limited to aspen, but it appears to be a common attribute of clonal species. In a survey of clonal plants, Cook (1983) listed bracken fern (*Pteridium aquilinum*), red fescue (*Festuca rubra*), sheep fescue (*Festuca ovina*), and velvet grass (*Holcus mollis*) all as having clones older than 1000 years. Aspen is listed as having clones in excess of 10,000 years of age, while creosote bush (*Larrea tridentata*) and huckleberry (*Gaylussacia brachycerium*) are credited with ages in excess of 11,000 years and 13,000 years, respectively.

Patterns of sex ratio

Individual clones of quaking aspen are either male or female (i.e., dioe-

cious), in contrast to the much more common situation in which individual forest trees produce both ovules and pollen. This separation of sexes among different individuals, the standard in vertebrates, leads to some interesting patterns in plants. For example, sex ratio varies with environmental conditions in several dioecious species; male individuals generally predominate in environments imposing moisture stress while females tend to predominate in moist areas (Freeman et al. 1976).

The sex ratio of aspen may vary among environments. In particular, males and females show dramatically different distributions along elevational gradients in the Rocky Mountains. At low elevations in the Front Range, females are more common than males. However, the proportion of females declines with elevation, and above 3200 m, more than 90% are male (Grant and Mitton 1979). The mode of sex-determination remains a mystery, but it seems that the primary ratio is 1:1 (Grant and Mitton 1979). If, as our observations indicate, sex is determined strictly by a genetic mechanism, then these observed gradients in sex ratio necessarily derive from differential establishment and survival. Differential establishment, survival, and growth can be best demonstrated by studies of genetic variation.

Genetic variation

Genetic variation in and among populations has been measured most commonly with electrophoretic sur-

veys of protein variation. Proteins from leaves or buds are separated by size and charge in a starch gel subjected to an electric current. Genetic variation is revealed as different rates of migration of proteins through the gel, and the pattern of the proteins on the gel reveals the genotypes.

Quaking aspen is a diploid species and, therefore, has two genes, or alleles, coding for each protein; heterozygotes have two different alleles, and homozygotes have two identical alleles. The level of genetic variation in a population is usually presented as the average heterozygosity, H , for the proteins in the survey. The distribution of the genotypes in a population is compared with the distribution expected in a large, randomly mating population, at equilibrium, undisturbed by natural selection. The deviation of a genotypic distribution from the expected distribution is measured with the inbreeding coefficient, F , which varies from -1.0 to 1.0. A perfect fit to the expected distribution of genotypes produces a value F of 0.0, while values of -1.0 and 1.0 indicate extreme excesses and deficiencies of heterozygotes, respectively.

Population genetics theory predicts that genetic variation maintained by a species will increase with the environmental variation experienced by a species, with its population size, and with the size of the geographic range. All of these variables predict high genetic variation for quaking aspen, and the prediction is accurate. Quaking aspen appears to be the most genetically variable species of plant investigated to date.

An electrophoretic survey of protein variation revealed that more than 90% of the enzymes analyzed were genetically variable (Cheliak and Dancik 1982), in comparison with the average of 50% for all plant species that have been analyzed (Hamrick and Godt 1989). Surveys of DNA markers are consistent with the survey of proteins, indicating high levels of genetic variation (Chong et al. 1994, in press, Liu and Furnier 1993a, b, Rogstad et al. 1991).

Of the aspen measured, those in Alberta, Canada, have the highest levels of genetic variation and large

excesses of heterozygotes (Cheliak and Dancik 1982). Genetic variation at 26 polymorphic enzyme loci was studied in 222 clones sampled from seven localities in Alberta. The observed proportion of heterozygotes exceeded the expected proportions in every one of the sample localities. The average heterozygosity of clones was expected to be 42%, but the observed heterozygosity was 52%. The inbreeding coefficient from this survey was $F = -0.24$, indicating a substantial excess of heterozygotes. Similarly, excesses of heterozygotes were observed in a survey of six prairie and montane populations in Waterton Lakes National Park, Alberta ($F = -0.10$; Jelinski and Cheliak 1992).

In contrast to the studies in Alberta, substantially lower levels of genetic variation were reported in electrophoretic surveys of aspen in Minnesota ($H = 22\%$; Lund et al. 1992) and Ontario ($H = 25\%$; Hyun et al. 1987). Observed genotypic proportions fit expected levels in Minnesota (Lund et al. 1992) and Colorado (Mitton and Grant 1980), but a strong deficiency of heterozygotes was reported for the populations in Ontario ($F = 0.46$; Hyun et al. 1987).

The level of genetic variation in populations of aspen varies with the environmental conditions. High levels of genetic variation and excesses of heterozygotes are found in semi-arid environments, while much less genetic variation and deficiencies of heterozygotes are found in the relatively moist eastern forests.

We hypothesize that these regional differences in genetic variation reflect the aridity of the climate, the propensity for clonal reproduction, the longevity of the clones, and the predominant modes of natural selection. Clonal reproduction is more common in arid environments, and clones may become larger and older in more arid climates (Barnes 1966, Kemperman and Barnes 1976). Heterozygotes often exhibit superior longevity in forest trees (Mitton and Jeffers 1989), and the extreme ages of clones may amplify the advantages of heterozygotes. Natural selection favoring heterozygous genotypes maintains high levels of genetic variation.

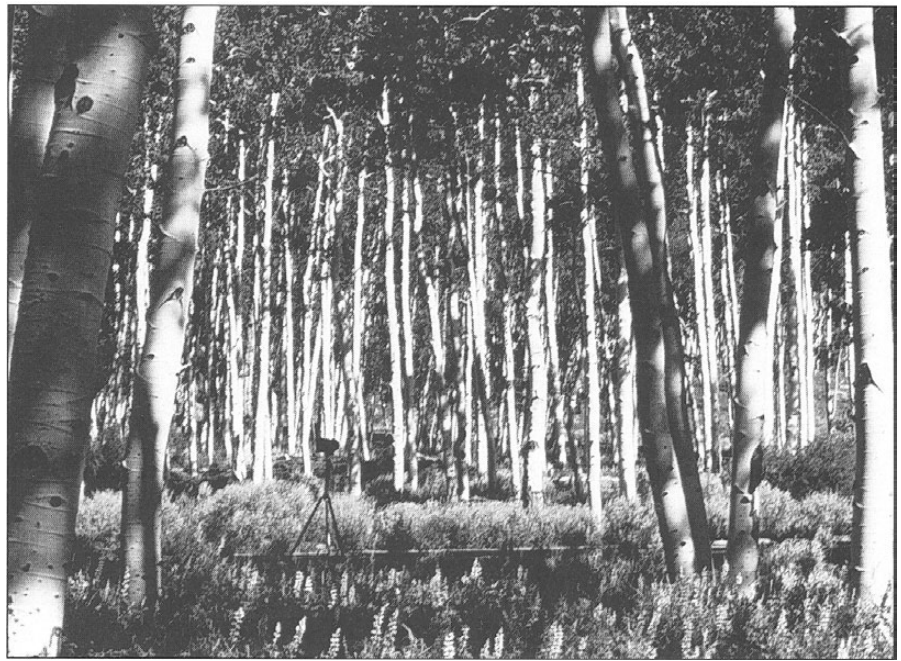


Figure 4. Some of the beautiful silvery-white trunks of Pando, the giant aspen clone.

Given that high levels of genetic variation and excesses of heterozygosity in aspen are associated with environmental variation, we would expect the frequencies of alleles also to vary among environments. General support for this hypothesis is found in the study of genetic variation in 156 clones in six natural populations in ecologically diverse habitats in Waterton Lakes National Park (Jelinski and Cheliak 1992). These populations were chosen for their environmental range—some are situated on the prairie, others are montane. Although the populations were, on average, only 6.7 km distant from one another, substantial genetic differentiation in allelic frequencies was observed among populations. If the differentiation among populations is produced by natural selection, we should be able to measure differences among genotypes in some trait or set of traits, such as survival or growth rate, that directly influences clonal fitness.

Growth rate

The growth rate of aspen is intimately tied to its environment (Grant and Mitton 1979, Jelinski 1993). Ideally, one would measure growth of entire clones by measuring their biomass including the extensive root systems, but this approach is not

feasible. Pragmatically, one measure of growth rate of aspen can be taken from the annual increment in the radius of a stem. Traditionally, foresters removed a core from a tree and counted the rings to estimate age and to measure the widths of annual rings to estimate growth rate and the variability of growth rate. One study in Waterton Lakes National Park used multiple regression to predict growth rates of aspen as a function of elevation, slope position, age, and exposure to the wind (Jelinski and Cheliak 1992). This study revealed that growth rate of aspen declines with elevation, steepness of the slope, age of the ramet, and exposure to wind. These variables explained 56% of the variation among individuals (Jelinski 1993). Similarly, a study of 104 clones in the Front Range revealed that growth rate decreased dramatically with elevation (Grant and Mitton 1979).

The growth rate of aspen also differs between the sexes. In an elevational transect that ranged from below 1900 m to above 3000 m in the Front Range, the growth rates of both males and females declined with elevation (Grant and Mitton 1979). At all elevations, the growth rate of females surpassed that of males by approximately 12%. Sakai and Burris (1985) compared measure-

ments taken in 1956 and 1981 to study the growth of 23 clones of quaking aspen growing in field trials in lower Michigan. This comparison did not reveal any difference between the sexes of the average annual ring width, but the growth of the clones differed. Over the 25 years of this study, the area occupied by female clones increased by 292%, while the area of male clones increased by only 219%. The basal area of females (area covered by the ramets at ground level) and the number of ramets (205 for females, 129 for males) also exceeded those in males.

A study of growth in male and female clones of bigtooth aspen revealed trends consistent with the differences found in quaking aspen (Sakai and Sharik 1988). Seven male and seven female clones were measured for size, number of ramets, and basal area at the University of Michigan Biological Station near Hickory Corners. The researchers reported that the area of female clones was 41% greater than males, the number of female ramets 52% greater, and the basal area of females 56% greater. Furthermore, over a 29-year period between measurements, the increase in female clonal area was 23% greater than in males, and the increase in female basal area was 32% greater than in males (Sakai and Sharik 1988).

These studies of sex-dependent growth rates revealed consistent growth advantages of females over males, data apparently inconsistent with the widely held notion that higher energetic investment by females in sexual reproduction reduces their growth rates. Furthermore, the superior growth rate of females at all elevations appears to be inconsistent with the strong predominance of males seen at higher elevations. However, the actual energy expenditures of female clones versus male clones have yet to be measured.

At least some of the variation in growth rates among clones is influenced by genetic variation, because two studies of growth rate have reported the radial growth of ramets to increase with enzyme heterozygosity. The first of these studies examined 104 clones in the Front Range (Mitton and Grant 1980).

Cores were taken from five of the largest ramets in each clone, and the genotype of each clone was identified at three genes coding for enzymes. Growth rate decreased with elevation and with the age of the ramet, but it increased with heterozygosity. Similar results were obtained in a study of growth rates of aspen in Waterton Lakes National Park (Jelinski 1993). From each of 156 clones, cores were extracted from five of the largest ramets, and genotypes were identified at 14 polymorphic enzyme loci. The growth rates of the most heterozygous clones were approximately 35% higher than those of the most homozygous clones.

Although population geneticists have usually presumed genes coding for enzymes to be a random sample of the genome, this assumption may not be correct for all genes, in all species, under all environmental conditions. Enzymes catalyze metabolic reactions, and genetic variation of a few enzymes has been demonstrated to influence flux through known metabolic pathways (Koehn et al. 1983, Powers et al. 1993). Consequently, this genetic variation might be related to differences in physiology, growth, and survival among genotypes.

Currently, geneticists debate whether protein polymorphisms contribute directly to differences in physiology and life history or whether they are neutral genetic markers that detect differences in performance attributable to other loci (Mitton and Grant 1984). If enzyme polymorphisms are simply convenient, neutral markers, then any genetic marker should reveal the associations with fitness that have been reported for protein polymorphisms. But if the associations are attributable to biochemical differences among protein genotypes, then heterozygosity at a different set of genetic markers, such as DNA markers in the form of restriction fragment length polymorphisms (RFLP) or variable numbers of tandem repeats (VNTR), should not be correlated with components of fitness.

Even looking beyond the quaking aspen, we know of only one study that used both protein genetic varia-

tion and DNA markers to determine whether correlations with fitness are found with both sets of markers. Pogson and Zouros (1994) employed this rationale in a study of the scallop, *Placopecten magellanicus*. Shell height was used to estimate growth rate, and heterozygosity was estimated with genes coding for seven enzymes and eight DNA markers. The correlation between heterozygosity at the enzymes and shell height was positive and significant, while the correlation between the DNA markers and shell height was not significantly different from zero. At the least, we can conclude that genes coding for enzymes and DNA markers provided different insights into fitness differentials in this population. More strongly, we conjecture that enzymes themselves periodically fall under the direct influence of natural selection.

Conclusions

The extreme size and, perhaps, age of the clone we called Pando serves to highlight some of the remarkable features of the beautiful forest tree species, quaking aspen. Many years of ecological genetic studies of aspen biology have revealed several general patterns. The ability for aspen to reproduce and disperse via seeds, even if such reproduction and dispersal only occurs on a widespread basis on intervals of several hundred or several thousand years, clearly underlies its great geographic range and its remarkably high levels of interclonal genetic variability. Further, aspen's ability to reproduce and regenerate asexually following major disturbances contributes to its ability to persist in a given region for long periods of time. This same vegetative capability also contributes to its ability to persist across long spans of time when circumstances are not favorable for seedling establishment. The ability to span both spatial and temporal environmental heterogeneity is a critical characteristic of this extraordinary species.

References cited

- Barnes BV. 1966. The clonal growth habit of American aspens. *Ecology* 47: 439-447.
_____. 1975. Phenotypic variation of trem-

- bling aspen of western North America. *Forest Science* 221: 319–328.
- Barnes BV, Han F. 1993. Phenotypic variation of Chinese aspens and their relationships to similar taxa in Europe and North America. *Canadian Journal of Botany* 71: 799–815.
- Bailey AW, Irving BD, Fitzgerald RD. 1990. Regeneration of woody species following burning and grazing in aspen parkland. *Journal of Range Management* 43: 212–215.
- Beetle AA. 1974. Range survey in Teton County, Wyoming. Part 4—quaking aspen. Agricultural Experimental Station SM-27. Laramie (WY): University of Wyoming.
- Cheliak WM, Dancik BP. 1982. Genetic diversity of natural populations of a clone forming tree *Populus tremuloides*. *Canadian Journal of Genetics and Cytology* 24: 611–616.
- Chong DKX, Yang R-C, Yeh FC. 1994. Nucleotide divergence between populations of trembling aspen (*Populus tremuloides*) estimated with RAPDs. *Current Genetics* 26: 374–376.
- Chong DKX, Yeh FC, Yang R-C. In press. RAPD variation within and among natural populations of trembling aspen (*Populus tremuloides* Michx.) from Alberta. *Journal of Heredity*.
- Cook RE. 1983. Clonal plant populations. *American Scientist* 71: 244–253.
- Crouch GL. 1981. Regeneration on aspen clearcuts in northwestern Colorado. US Department of Agriculture Forest Service Research Note RM-407.
- DeByle NV, Winokur RP, eds. 1985. Aspen: ecology and management in the western United States. US Department of Agriculture Forest Service General Technical Report RM-119. Ft. Collins (CO): US Forest Service.
- Einspahr DW, Winton LL. 1976. Genetics of quaking aspen. US Department of Agriculture Forest Service Research Paper WO-25. Washington (DC): USDA.
- Freeman DC, Klikoff IC, Harper KT. 1976. Differential resource utilization by the sexes of dioecious plants. *Science* 193: 597–599.
- Gould SJ. 1992. A humongous fungus among us. *Natural History* (July): 10–16.
- Graham SA. 1963. Aspens, phoenix trees of the Great Lakes region. Ann Arbor (MI): University of Michigan Press.
- Grant MC. 1993. The trembling giant. *Discover* (October): 83–88.
- Grant MC, Mitton JB. 1979. Elevational gradients in adult sex ratios and sexual differentiation in vegetative growth rates of *Populus tremuloides* Michx. *Evolution* 33: 914–918.
- Grant MC, Mitton JB, Linhart YB. 1992. Even larger organisms. *Nature* 360: 216.
- Hamrick JL, Godt MJW. 1989. Allozyme diversity in plant species. Pages 43–63 in Brown AHD, Clegg MT, Kahler AL, Weir BS. *Plant population genetics: breeding and genetic resources*. Sunderland (MA): Sinauer Publishers.
- Hyun JO, Rajora OP, Zsuffa L. 1987. Genetic variation in trembling aspen in Ontario based on isozyme studies. *Canadian Journal of Forest Research* 17: 1134–1138.
- Jelinski DE. 1993. Associations between environmental heterogeneity, heterozygosity, and growth rates of *Populus tremuloides* in a Cordilleran landscape. *Arctic and Alpine Research* 25: 183–188.
- Jelinski DE, Cheliak WM. 1992. Genetic diversity and spatial subdivision of *Populus tremuloides* (Salicaceae) in a heterogeneous landscape. *American Journal of Botany* 79: 728–736.
- Johns BW. 1993. The influence of grove size on bird species richness in aspen parklands. *Wilson Bulletin* 105: 256–264.
- Jones JR. 1974. Silviculture of southwestern mixed conifers and aspen: the status of our knowledge. US Department of Agriculture Forest Service Research Paper RM-122. Fort Collins (CO): Rocky Mountain Forest and Range Experiment Station.
- _____. 1985. Distribution. Pages 9–10 in DeByle NV, Winokur RP, eds. *Aspen: ecology and management of the western United States*. US Department of Agriculture Forest Service General Technical Report RM-119. Fort Collins (CO): Rocky Mountain Forest and Range Experiment.
- Jones JR, DeByle NV. 1985. Other physical factors. In *Aspen: ecology and management in the western United States*. US Department of Agriculture Forest Service General Technical Report RM-119. Ft. Collins (CO): US Forest Service.
- Kay C. 1993. Aspen seedlings in recently burned areas of Grand Teton and Yellowstone National Parks. *Northwest Science* 67: 94–104.
- Keim P, Paige KN, Whitham TG, Lark KG. 1989. Genetic analysis of an interspecific hybrid swarm of *Populus*: occurrence of unidirectional introgression. *Genetics* 123: 557–565.
- Kemperman JA, Barnes BV. 1976. Clone size in American aspens. *Canadian Journal of Botany* 54: 2603–2607.
- Koehn RK, Zera AJ, Hall JG. 1983. Enzyme polymorphism and natural selection. Pages 115–136 in Nei M, Koehn RK, eds. *Evolution of genes and proteins*. Sunderland (MA): Sinauer Associates.
- Liu Z, Furnier GR. 1993a. Comparison of allozyme, RFLP, and RAPD markers for revealing genetic variation within and between trembling aspen and bigtooth aspen. *Theoretical and Applied Genetics* 87: 97–105.
- _____. 1993b. Inheritance and linkage of allozymes and restriction fragment length polymorphisms in trembling aspen. *Journal of Heredity* 87: 97–105.
- Lund ST, Furnier GR, Mohn CA. 1992. Isozyme variation in quaking aspen in Minnesota. *Canadian Journal of Forest Research* 22: 521.
- McDonough WT. 1985. Sexual reproduction, seeds and seedlings. In DeByle NV, Winokur RP, eds. *Aspen: ecology and management in the western United States*. US Department of Agriculture Forest Service General Technical Report RM-119. Ft. Collins (CO): US Forest Service.
- Mitton JB, Grant MC. 1980. Observations on the ecology and evolution of quaking aspen, *Populus tremuloides*, in the Colorado Front Range. *American Journal of Botany* 67: 202–209.
- _____. 1984. Relationships among protein heterozygosity, growth rate, and developmental stability. *Annual Review of Ecology and Systematics* 15: 479–499.
- Mitton JB, Jeffers RM. 1989. The genetic consequences of mass selection for growth rate in Engelmann spruce. *Silvae Genetica* 38: 6–12.
- Pogson GH, Zouros E. 1994. Allozyme and RFLP heterozygosities as correlates of growth in the scallop *Placopecten magellanicus*: a test of the associative overdominance hypothesis. *Genetics* 137: 221–231.
- Powers DA, Smith M, Gonzalez-Villasenor I, DiMichele L, Crawford D, Bernardi G, Lauerman T. 1993. A multidisciplinary approach to the selection/neutralist controversy using the model teleost *Fundulus heteroclitus*. Pages 82–157 in Futuyma D, Antonovics J, eds. *Oxford surveys in evolutionary biology*. Vol. 9. Oxford (UK): Oxford University Press.
- Preston RJ Jr. 1976. Page 122 in *North American trees*. 3rd ed. Ames (IA): Iowa University Press.
- Rogstad SH, Nybom H, Schaal BA. 1991. The tetrapod “DNA fingerprinting” M13 repeat probe reveals genetic diversity and clonal growth in quaking aspen (*Populus tremuloides*, Salicaceae). *Plant Systematics and Evolution* 175: 115.
- Romme WH. 1982. Fire and landscape diversity in subalpine forests of Yellowstone National Park. *Ecological Monographs* 52: 199–221.
- Romme WH, Despain DG. 1989. Historical perspective on the Yellowstone fires of 1988. *BioScience* 39: 695–699.
- Sakai AK, Burris TA. 1985. Growth in male and female aspen: a twenty-five year longitudinal study. *Ecology* 66: 1921–1927.
- Sakai AK, Sharik TL. 1988. Clonal growth of male and female bigtooth aspen, *Populus grandidentata*. *Ecology* 69: 2031–2033.
- Schier GA, Jones JR, Winokur RP. 1985. Vegetative regeneration. In DeByle NV, Winokur RP, eds. *Aspen: ecology and management in the western United States*. US Department of Agriculture Forest Service General Technical Report RM-119. Ft. Collins (CO): US Forest Service.
- Schopmeyer CS. 1974. Seeds of woody plants in the United States. *Agricultural Handbook* Nr. 450. Washington (DC): US Department of Agriculture Forest Service.
- Smith ML, Bruhn JN, Anderson JP. 1992. The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. *Nature* 356: 428–431.
- Turner MG, Romme WH. 1994. Landscape dynamics in crown fire ecosystems. *Landscape Ecology* 9: 59–77.