Aspen Ecology in Rocky Mountain National Park: Age Distribution, Genetics, and the Effects of Elk Herbivory

By Linda C. Zeigenfuss, Dan Binkley, Gerald A. Tuskan, William H. Romme, Tongming Yin, Stephen DiFazio, and Francis J. Singer

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By Linda C. Zeigenfuss[1], Dan Binkley[2], Gerald A. Tuskan[3], William H. Romme[2], Tongming Yin[3], Stephen DiFazio[4], and Francis J. Singer[1]

Executive Summary

Lack of aspen (*Populus tremuloides*) recruitment and canopy replacement of aspen stands that grow on the edges of grasslands on the low-elevation elk (*Cervus elaphus*) winter range of Rocky Mountain National Park (RMNP) in Colorado has been a cause of concern for more than 70 years (Packard, 1942; Olmsted, 1979; Stevens, 1980; Hess, 1993; R.J. Monello, T.L. Johnson, and R.G. Wright, Rocky Mountain National Park, 2006, written commun.). These aspen stands are a significant resource since they are located close to the park's road system and thus are highly visible to park visitors. Aspen communities are integral to the ecological structure of montane and subalpine landscapes because they contain high native species richness of plants, birds, and butterflies (Chong and others, 2001; Simonson and others, 2001; Chong and Stohlgren, 2007). These low-elevation, winter range stands also represent a unique component of the park's plant community diversity since most (more than 95 percent) of the park's aspen stands grow in coniferous forest, often on sheltered slopes and at higher elevations, while these winter range stands are situated on the low-elevation ecotone between the winter range grasslands and some of the park's drier coniferous forests.

Several studies (Olmsted, 1979; C.E. Olmsted, University of Northern Colorado, 1997, written commun.; Baker and others, 1997; Suzuki and others, 1999) have documented the notable lack of regeneration on the low-elevation, eastern elk winter range of RMNP, especially since 1970, 2 years after the park's natural regulation, or "hands off" policy, of elk management went into effect (Baker and others, 1997). Similar results have been found at other low-elevation sites in the Colorado Front Range where chronic and intensive elk browsing occurs (Kashian and others, 2007). The findings of these studies contributed to a modeling effort to determine the history and future of aspen on the low-elevation, east side winter range of RMNP (M.B. Coughenour, Colorado State University, 2002, written commun.). The results of this modeling effort led to the speculation that these winter range aspen stands may have become established during the period when elk were rare or absent on the winter range from 1880 and 1920. When large predators, such as wolves and grizzly bears, still existed in the park, they may have contributed to aspen establishment or release by limiting elk numbers and concentrations.

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Currently, Rocky Mountain National Park is in the process of instituting a new elk management plan (National Park Service, 2007). Park managers have identified several critical needs in aspen research to more fully implement this management plan. As the park embarks upon this new elk management era, it will be important to answer several questions about winter range aspen stands in order to appropriately apply management actions and interpret their effects. Among the questions that park managers desired to have answered are the following:

- Are recent patterns in aspen recruitment unusual relative to long-term trends, or have periodic increases and decreases in aspen recruitment been common in the past?
- Did aspen stands on the low-elevation, east side elk winter range and in Kawuneechee Valley become established during a period of low elk density at the end of the 19th century, and therefore, are uncharacteristic on a landscape with large concentrations of elk?
- What is the degree of elk browsing currently occurring in these aspen stands and how are browsing levels and elk population size and density related to stem recruitment or plant growth rates? Previous studies of elk herbivory and aspen decline in the park have identified the connection between these variables (Olmsted, 1979; C.E. Olmsted, University of Northern Colorado, 1997, written commun.; Baker and others, 1997; Suzuki and others, 1999; Kaye and others, 2003, 2005); however, few of these studies have quantified these relationships.
- Is there a relationship between elk population trends and precipitation patterns that may have exacerbated the effects of intensive elk browsing on aspen? Increased snowfall may impact elk survival and thus lead to lowered population size and potentially less intense browsing on aspen. Greater precipitation may also lead to better growth of aspen and a better ability of aspen to withstand intense browsing.
- How much genetic variability exists in the park's aspen gene pool for natural selection to work on as changes take place in the climate, elk browsing pressures, and other environmental factors? Limited genetic diversity may constrain future adaptation to a changing environment.

The primary purpose of the investigations reported in this document was to determine when and under what conditions and estimated elk densities these low-elevation aspen stands established and under what conditions, and through what mechanisms, they may regenerate. To answer these research questions, three distinct research studies were conducted in RMNP by scientists specializing in (1) age distribution of aspen, (2) effects of elk herbivory on aspen, and (3) genetic characteristics of aspen. The Background section of this document describes the history of elk populations, aspen research, and human settlement, use, and management of the area, and it outlines the research objectives. Then each of the three studies is presented in its own separate section. The last section, Conclusions and Management Implications, provides a synthesis of all the findings and discusses management considerations for elk and aspen on the RMNP landscape.

We were particularly interested in determining whether a doubling of elk population (Lubow and others, 2002) following adoption of a predation-free ''natural regulation policy'' in the 1960s led to a substantial decline in numbers of young aspen trees. We know that most aspen stands have not successfully regenerated since the 1960s in areas with the highest elk use (Suzuki and others, 1999). However, a longer term context is needed to determine if recent patterns in aspen recruitment are unusual relative to long-term trends, or if periodic increases and decreases in aspen recruitment have been common in the past. Perhaps aspen recruitment was exceptionally high in the late 1800s and early 1900s, and a reduction in recruitment in the late 20th century might reflect a long-term legacy of an earlier pattern. Alternatively, aspen recruitment at a landscape scale might be fairly consistent, with little influence of historical changes in land use or legacies of previous recruitment success. We determined the age structure of aspen stems across the park, allowing us to evaluate whether any cohort was represented by substantially more or fewer stems than would be expected based on the long-term pattern. If aspen recruitment was consistent across the decades before the 1960s, then the unique event of very large populations of elk may be driving recent changes in aspen numbers.

The genetic makeup of a population reflects its evolutionary history, and provides insights into its potential for coping with future environmental changes. It is important to know how much genetic variability exists in the park's aspen gene pool as changes take place in the climate, elk browsing pressures, and other environmental factors. If genetic diversity is limited, then future adaptation to a changing environment may be seriously constrained. Our aspen genetics study in RMNP is one of the first in the world to critically test the potentially valuable method of using somatic mutation (that is, random changes in genes that are involved in functions other than reproduction) rates to reconstruct the age and history of long-lived clonal plants. Using this method, we will be able to date the establishment of the clone, not just the currently standing stems (ramets) that make up that clone. This will help further determine whether low-elevation, winter range stands became established during the period of low elk density at the end of the 19th century. Excessive heartrot in mature aspen stems on the winter range has prolonged the analysis required to obtain clone ages based on somatic mutation rates, and as a result, this information could not be included in the present (2008) document, but Dr. Gerald Tuskan of Oak Ridge National Laboratory continues his efforts to develop this method to age clones in Rocky Mountain National Park.

The density of aspen in RMNP averaged about 1,250 stems/km^2 for the low-elevation east side and 550 stems/km^2 for the rest of the park. A total of 651 trees were aged, the oldest of which dated to 1689 (with an estimated pith date of 1685, for an age of 321 years at breast height [i.e., 1.4 m high]), which we believe is the oldest reported age for an aspen tree. Aspen ages correlated moderately well with diameter. The distribution of aspen stem sizes showed an exponential increase with successive decades, as expected for a balanced, uneven age forest. The smallest trees (less than 5 cm in diameter at breast height [dbh]) accounted for a very low proportion (6 percent of sampled trees) of aspen basal area. The majority of sampled aspen were between 5 and 30 cm in dbh (86 percent of aged cores) and 8 percent of aged cores were larger than 30 cm in dbh.

Total annual offtake of aspen saplings (stems less than 2.5 m high) on the low-elevation, east side winter range ranged from 0.4 to 50.3 percent of current annual growth. Winter and summer offtake was greater in nonregenerating than regenerating stands. Nonregenerating stands were located in the areas where the heaviest concentrations of wintering elk have been observed during midwinter elk censuses conducted from 1994 to 2007. Mean elk density index was 6.5 times greater for nonregenerating stands than regenerating stands. None of the regenerating stands had a density index of greater than eight elk per square kilometer.

Aspen saplings were twice as tall in regenerating stands, and sapling height was negatively correlated to overwinter leader use ($p < 0.0001$, R = 0.77). The number of saplings that grew 1.5–2.5 m high was 46 times greater in the regenerating stands. No recruits greater than 1.0 m in height were found in the nonregenerating stands by spring. In regenerating stands where saplings $\leq$ 1.5 m in height were present, total offtake levels were $\leq$ 10 percent and leader use was $\leq$ 21 percent. Aspen leaders were not significantly longer prior to overwinter browsing in regenerating stands; however, aspen suckers in these stands did have an additional 17.3 cm of annual height growth ($p < 0.0001$). There were more than seven times as many small-diameter trees ($\leq$ 10-cm dbh and greater than 2.5 m in height) in the regenerating stands than in nonregenerating stands ($p = 0.0003$), but there was no difference in the number of larger diameter trees (greater than 10-cm dbh).

Total snowfall declined from 1931 through approximately 1968–70, after which time total snowfall increased ($p < 0.0001$, $r^2 = 0.58$). These low snow years coincided with the time period when the "natural regulation" policy of elk management was instituted in 1968 and could be related to a slowing of aspen growth and recruitment at the time when elk populations began to increase very rapidly.

The genetic variation of aspen populations in RMNP is high at the molecular level. Across the landscape as a whole, evidence of aspen decline is not obvious genetically. We expected to find that most patches of aspen in the park were composed of a single clone of genetically identical trees, but in fact we found just 7 percent of aspen patches consisted of a single clone. In the other 93 percent of sampled patches, we found two or more interdigitating clones that were genetically distinct from one another and often one or more genetically unique individual trees that were not related to any nearby clones

We discovered a surprisingly large frequency of polyploid genotypes (that is, aspen trees whose cells have three [triploid] or four [tetraploid] instead of the usual two [diploid] copies of each chromosome). Polyploidy actually is common in aspen, but the level of polyploidy in the aspen of RMNP was unexpectedly high. Of all the genotypes that we detected in RNMP, 31 percent were polyploids, with both triploids and tetraploids occurring in most stands. The percentage of polyploids varied significantly and accounted for 78 percent of the sampled trees on the east side compared with 23 percent on the west side.

The period from 1975 to 1995 at low elevation on the east side had 80–95 percent fewer aspen stems than would be expected based on the trend from 1855 through 1965. Across the rest of the park, no decades had significantly fewer than expected aspens, and 1965–75 had more aspen than expected. Prior to 1900, stand-replacing fires had a return interval on the order of 300–400 years across much of Rocky Mountain National Park (Buechling and Baker, 2004; Sibold and others, 2006). Fires were less extensive in the 20th century because of climate conditions and active fire suppression (Buechling and Baker, 2004), but Sibold and others (2006) found this low incidence of fire was not unique in comparison to the area's fire history from the previous 300 years. The current age distribution of aspen in the park does not show any marked legacy of a changed fire regime. Browsing effects on aspen recruitment and survival may have remained high even as elk populations declined, as a result of domestic livestock. In the early 20th century, elk were reintroduced and livestock were removed, with unknown effects on aspen. The age structure of aspen in the park indicates that the interacting effects of fires, elk population changes, and livestock grazing had more-or-less consistent effects on aspen from 1855 to 1965. Some periods of high or low recruitment and survival may have occurred during some decades, but these effects were not large enough to leave a large legacy in the current age structure. The post-1975 decrease in aspen numbers in the low-elevation, east side part of the park appears to be unprecedented in past the 140 years. The lack of a significant change in aspen numbers in recent decades in the higher elevation and west side parts of the park supports the idea that the extensive effects of elk browsing have been more important in reducing aspen numbers than climate conditions, fire incidence, or conifer in-growth (Kaye and others, 2005). Restoring the numbers of young aspen that would be characteristic of the low-elevation, east side of the park likely would require substantial reductions in elk browsing on aspen shoots, either by reducing elk numbers or by altering their patterns of landscape use.

Rates of offtake in RMNP increased with elk densities, and stands with greater levels of browsing had fewer, shorter aspen recruits. In Yellowstone National Park's northern elk winter range, Ripple and Beschta (2007) observed that young aspen did not grow beyond 150 cm in height until leader use dropped below 50 percent, and growth to more than 200 cm occurred when leader use dropped below 40 percent. The average leader-use values in our regenerating stands were well below this 40-percent threshold. Nonregenerating stands had leader-use levels of greater than 75 percent.

Elk browsing did not damage the potential of healthy suckers to continuously produce substantial annual leader growth. We believe this indicates the important potential of these nonregenerating stands to still recover and produce cohorts of stems that can recruit to the stand canopy and, thus, lead to stand regeneration, when protected from elk browsing. However, with a several-decades-long increase in temperatures, it is possible that protection from elk browsing may not be enough to preserve aspen stands on the elk winter range. If park managers desire to conserve the unique characteristics of these stands, it would be important to include some of the regenerating stands on the margins of the winter range in any management actions (fencing) as well as the nonregenerating stands on the winter range. Elk management actions (particularly redistribution activities) could lead to increased elk herbivory in these peripheral stands that are currently regenerating, and having protection measures in place would ensure continued stem recruitment and canopy replacement in these stands. Elk management actions would likely need to be targeted to bring total offtake levels down to 10 percent or less (leader use would likely need to be as low as 30 percent) to achieve aspen stand regeneration in stands that do not receive protective fencing in the winter range.

The patterns of spatial distribution of specific genotypes across RMNP further support the findings of tremendous genetic diversity within and between aspen patches. Generally, each "patch" (a visually distinctive group of aspen trees that all resemble each other morphologically but appear different from trees in other patches nearby) and each "stand" (a collection of patches, occupying a more-or-less discrete portion of the landscape, and separated from other stands by a kilometer or more) contained a more-or-less unique set of genotypes. Nearby patches often contained at least some ramets of the same clone, suggesting that the root systems of many clones are capable of considerable lateral expansion. However, each patch also contained other clones or genets not represented in the nearby patch. Moreover, we found no duplicate clones among different stands, indicating that each genotype occupies a somewhat limited resident space within the landscape rather than being distributed throughout the park. The relatively high variation among stands indicates divergent selection for genotypes leading to their fitness for the specific geographical environment.

Generally, stands from the west side of the park clustered together separately from stands from the east side when genetic makeup of all sampled stands was compared. Similarly, aspen populations in Yellowstone National Park clustered together and separately from the RMNP samples. However, there were single stands from all three geographic locations that were genetically distinct from each other and all other aspen stands. This finding further supports the interpretation of high overall genetic diversity in RMNP aspen, with populations in close geographic proximity to one another sharing some genetic similarity, but with each individual genotype being restricted to a relatively small area within the landscape.

The habitats in which aspen grows appear to be more varied on the east side as opposed to the west side of RMNP, and the east side generally receives less winter moisture, which may result in more stressful environments for east-side aspen. We hypothesize that genome doubling has been an important genetic mechanism of adaptation for aspen growing in the generally harsh environments of RMNP. Polyploidy is typically more prevalent in more stressful environments. The high percentage of polyploids detected in our samples suggests that these genotypes may be of special conservation significance and therefore may deserve special management attention. Moreover, because triploids are generally not capable of sexual reproduction, such genotypes can persist only through vegetative root suckering, which can expand the clone only a limited distance. Therefore, special care for triploids in RMNP may be called for. Moreover, the tetraploids are the usual resources for the generation of triploids. Thus, recruitment of triploid genotypes within the harsher environments that characterize portions of RMNP will require the maintenance of both diploid and tetraploid genotypes on the landscape.

Based on the frequency of the genetic markers used in this study, we conclude that asexual vegetative reproduction (root sprouting) is the dominant mode of aspen reproduction in RMNP. It has long been recognized that aspen throughout the Rocky Mountains reproduce primarily by root sprouting and that seedling establishment is very infrequent. However, the genetic findings from this study suggest that aspen seedling establishment has been far more frequent than commonly assumed.

We have been successful in detecting somatic mutations in RMNP aspen and therefore are confident that we will be able to estimate the absolute age of the sampled aspen clones in RMNP. However, this work is not yet complete owing to unexpected difficulties in determining the ages of sampled trees (ramets) because so many of the cores contained substantial heart rot. When we complete this work, we may be able to associate the occurrence of somatic mutations with specific stem ages. From this information we will estimate the absolute age of the clones in which we detected somatic mutations.

Long-range goals of aspen management in RMNP, related to genetic composition and diversity, might include (1) preserving the overall genetic diversity that now exists, (2) protecting genotypes conferring special resilience to the population as a whole, and (3) maintaining the ecological processes that create and maintain genetic diversity over the long term (centuries or millennia). The park currently plans an aspen fencing project that will contribute to all three of these goals, especially the first two. Regarding the second goal, protecting especially significant genotypes, we cannot yet link any specific aspen genotype to any particular ecological traits; to do so will require additional research beyond the scope of this project. However, because triploid and tetraploid individuals may be generally more capable of enduring environmental stresses than diploid individuals, it would be important to consider protecting triploid and tetraploid aspen genotypes from intensive elk browsing when selecting specific stands for fencing.

It would be somewhat easy to determine stands that could be fenced for purposes of preserving genetic diversity if large tracts of aspen having either high or low genetic diversity were located predictably within specific portions of the park landscape. Unfortunately, we found tremendous fine-scale spatial variability. If the park desires to preserve aspen genetic diversity, then it would be necessary to include some of the stands that were sampled in our genetics study among the aspen stands the park is planning to protect from elk browsing with fencing. This would ensure that at least some of the most genetically diverse and resilient stands in the park are protected from elk browsing. In addition, it would be instructive to resample the genetic makeup of these stands after several years of reduced browsing pressure to evaluate how the genetic composition of the stand responds to this change in the selective environment.

If park managers fence stands exhibiting the highest overall genetic diversity, that is, number of unique genotypes represented, this would likely conserve much of the aspen genetic resources found on the winter range. Some of the stands having the highest proportion of triploid and tetraploid individuals would also need protection. Because of overall higher genetic diversity of the aspen on the east side of the park and because the ongoing mountain pine beetle outbreak on the west side may have as yet unknown consequences for aspen regeneration in that area, managers may want to consider limiting the fencing of aspen stands to the east side of the park, at least initially. Based on our data, fencing aspen patches of approximately 2 acres would be sufficient in most cases to capture the necessary genetic diversity in the stand.

Based on the findings of all components of this study, the low-elevation, winter range aspen stands of Rocky Mountain National Park are without doubt a unique resource, but conservation efforts are necessary to protect them if park managers desire to increase their chances for continued viability. These stands appear to be highly resilient in the face of very intensive herbivory by elk and harsh environmental conditions. They have adapted through processes of natural selection to produce many genetically unique individuals, which may have a range of characteristics specifically selected to help them cope with the intense herbivory and xeric site conditions that characterize the low-elevation, east side elk winter range of RMNP. However, conservation efforts through fencing protection and reduced elk browsing pressure would enhance the continued survival of these aspen stands by providing conditions that encourage stem recruitment to the tree canopy. Monitoring of these low-elevation, winter range stands as new elk management actions are implemented and adapted would ensure that these stands are sustained on the landscape.

Background

The status of aspen (*Populus tremuloides*) forests across western North America has been debated by ecologists over the past half century. Some researchers have identified declines that could lead to aspen disappearance on many western landscapes (Packard, 1942; Krebill, 1972; Kay, 1997); others point to persisting or increasing stands (Romme and others, 2001; Manier and Laven, 2002; Kurzel, 2004) or argue that declines are apparent only at local, but not broader, landscape scales (Suzuki and others, 1999; Barnett and Stohlgren, 2001). The aspen of Rocky Mountain National Park (RMNP) in Colorado are no exception. Lack of aspen stand regeneration and, in some cases, the complete disappearance of aspen stands on the winter range of RMNP have been a cause of concern for nearly 70 years (Packard, 1942; Olmsted, 1979; Stevens, 1980; Hess, 1993). Aspen stands that grow on the edges of grasslands on the low-elevation elk (*Cervus elaphus*) winter range located on the eastern edge of RMNP are characterized by lack of successful recruitment and canopy replacement. In comparison to aspen in the rest of the park, these winter range, grassland aspen stands are not successional to conifers and do not require fire to regenerate, though fire may play a part in stand establishment through alteration of elk browsing patterns, plant competition, and release of nutrients that encourage aspen growth (R.J. Monello, T.L. Johnson, and R.G. Wright, Rocky Mountain National Park, 2006, written commun.). These stands are a significant resource since they are located close to the park's road system and thus are highly visible to park visitors. They also represent a unique component of the park's plant community diversity since most (more than 95 percent) of the park's aspen stands are located in coniferous forest, often on sheltered slopes and at higher elevations, while these winter range stands are situated on the low-elevation ecotone between the winter range grasslands and some of the park's drier coniferous forests. Furthermore, aspen stands in general have been found to be high in biodiversity, supporting rich and diverse populations of insects, mammals, plants, and birds (DeByle and Winokur, 1985; Chong and others, 2001; Simonson and others, 2001; Chong and Stohlgren, 2007).

Several surveys of aspen regeneration have been conducted in RMNP and the adjacent forest lands. Many of these studies focused only on the winter range (Packard, 1942; Olmsted, 1979; Baker and others, 1997), while others were conducted at the landscape scale (Suzuki and others, 1999; Kaye and others, 2003) or focused entirely outside the park (Kaye and others, 2005; Kashian and others, 2007). These studies found considerable regeneration of aspen stands located outside of the winter range and in the Kawuneechee Valley within RMNP, or on elk winter ranges on the adjacent Roosevelt National Forest, where elk are hunted and elk densities are lower. All of these studies indicated a notable lack of regeneration on the low-elevation, east side elk winter range of RMNP and in the Kawuneechee Valley (an area of low-elevation summer range on the west side of the park), especially since 1970, 2 years after the park's natural regulation or "hands off" policy of elk management went into effect (Baker and others, 1997), and at other low-elevation sites where elk browsing was chronic and extensive (Kashian and others, 2007). Baker and others (1997) identified three periods of cohort origins during the period 1930 through 1965—all periods coincided with periods when elk numbers were reduced by park management staff. The authors concluded elk numbers needed to be reduced by about one-third or more (that is, to about 600 elk) in order for aspen to regularly regenerate.

These conclusions have led to the speculation that RMNP winter range aspen stands may have become established during the period when elk were rare or absent on the winter range from 1875 to 1920. When large predators still existed in the park, they may have been a factor in aspen establishment or release. In the presence of wolves, elk may prefer open habitats and not dense tree stands, and they might avoid feeding in dense aspen stands (Dekker and others, 1996; Jedrzejewska and Jedrzejewski, 1998). Where wolf densities are high, in some cases elk apparently avoid the center of dense aspen thickets that are regenerating after a fire (White and others, 1998; White and Feller, 2001). Elk may use areas of winter range less where there are high densities of wolves, permitting aspen stands in those areas to regenerate (Ripple and Larsen, 2000). Wolves may also have the effect, when they are present, of redistributing elk into smaller groups (Creel and Winnie, 2005) and even changing their seasonal distribution (Mao and others, 2005). Such changes may lead, in turn, to smaller groups exerting less browse pressure on aspen stands.

Elk (*Cervus elaphus*) were extirpated from the area that now comprises RMNP by human exploitation in the late 1800s. In 1913 and 1914, elk were reintroduced to the park, and the population steadily increased until it reached an estimated 1,000 animals within the park boundaries in 1944 (Packard, 1947). Concerns over increasing elk numbers and potential effects on the park winter range (particularly aspen [*Populus tremuloides*] and willow [*Salix* spp.] communities) led to reduction of the park elk population from 1943 to 1968. During this period, a total of 1,664 elk were removed from the park with the goal of reducing the park herd to about 400–500 on the winter range on the eastern side of the park. In 1968, in concert with a National Park Service (NPS) change in management policy to one of natural regulation that occurred in Yellowstone National Park at the same time, elk were no longer controlled within the park's boundaries. After 1968, increasing reliance was placed on both regular and late season hunts outside of the park boundaries to limit the herd. A goal was set to harvest 500–600 elk each year with the intent to limit growth of the elk population. This goal of harvesting 500–600 elk was nearly achieved prior to 1987, when an average of 442±78 elk were harvested each year. Elk harvests declined to 302±36 from 1988 through 1996. Although the harvests may have slowed elk population growth, the desired limitation was never achieved and elk steadily increased both in the park since 1968 and in the adjacent town of Estes Park after elk pioneered the town area in the late 1970s. Habituation of elk to human activity in Estes Park has led to increasing use of the town area by elk. This habituation, combined with increased human development of the Estes Valley which resulted in diminished access to USDA Forest Service lands, has made elk much less accessible to sport hunters. In the early 1990s, concerns over large numbers of elk and decreased hunter access to public lands bordering the park prompted a reevaluation of park elk policy (F. J. Singer, National Biological Survey, 1993, written commun.) and led all the agencies to examine their interagency elk management efforts as well.

Human influences have occurred in the system that might confound the interpretation of the effects of the elk abundance alone. For example, climate change and fire suppression may have influenced aspen stand regeneration (Romme and others, 1995; Elliott and Baker, 2004). The presence of rapidly growing Estes Park, located within the winter range, has provided forage (highly productive, fertilized lawns, golf courses, and ornamental shrubs) for an increased wintering population of elk in town since the late 1970s (R.J. Monello, T.L. Johnson, and R.G. Wright, Rocky Mountain National Park, 2006, written commun.). This population summered in the park and during migration to and from summer range increased the duration of elk browsing on aspen each year and the concentration of elk in park aspen stands. The major predators of the system, wolves and grizzly bears, have long since been extirpated, and considerable debate and speculation surrounded what their effects might have been on ungulate populations in a pristine system (Wagner and others, 1995; Wright, 1996; Singer and others, 1998).

Rocky Mountain National Park managers are directed by NPS mandate to actively manage biological or physical processes altered in the past by human activities to restore them to a natural condition or to maintain the closest approximation of the natural condition when a truly natural system is no longer attainable (National Park Service, 2007a). One example of such a disruption might be potential overconcentrations of elk and the ecosystem effects of any overconcentrations that might be caused by human removals of natural, large predators, such as wolves or grizzly bears. RMNP elk are currently (2008) at levels approximating their Ecological Carrying Capacity within the park winter range (Lubow and others, 2002). The challenge to RMNP managers is that even at 25 percent to 50 percent fewer elk than there are at present, model scenarios suggest that aspen sucker escapement into the canopy on the elk winter range would be insufficient to replace the canopy of these stands (Weisberg and Coughenour, 2003). Unless elk populations are redistributed, model scenarios indicate that at high elk-use rates, even if the aspen stands are fenced for 30 years, would be insufficient for aspen sucker escapement unless climatic conditions were optimal for aspen growth during the fencing period (Weisberg and Coughenour, 2003). Under current xeric climatic conditions and current elk densities, aspen stands on the eastern elk winter range appear well on their way to becoming grasslands.

Currently, Rocky Mountain National Park is in the process of instituting a new elk management plan (National Park Service, 2007b). Park managers identified several critical needs in aspen research to more fully implement this management plan, which will include protection of several low-elevation, winter range aspen stands using elk-proof fencing and reductions of elk populations through lethal culling. The research described in this report was conducted in response to those needs. As the park embarks upon this new elk management era, it will be important to answer several questions about winter range aspen stands in order to appropriately apply management actions and interpret their effects. Among the questions that need to be answered are the following:

- Are recent patterns in aspen recruitment unusual relative to long-term trends, or have periodic increases and decreases in aspen recruitment been common in the past?
- Did aspen stands in the low-elevation, east side elk winter range and Kawuneechee Valley become established during a period of low elk density at the end of the 19th century and therefore are uncharacteristic on a landscape with large concentrations of elk?
- What is the degree of elk browsing currently occurring in these aspen stands, and how are browsing levels and elk population size and density related to stem recruitment or plant growth rates? Previous studies of elk herbivory and aspen decline in the park have identified the connection between these variables (Olmsted, 1979; C.E. Olmsted, University of Northern Colorado, 1997, written commun.; Baker and others, 1997; Suzuki and others, 1999; Kaye and others, 2003, 2005); however, few of these studies have quantified those relationships.
- Is there a relationship between elk population trends and precipitation patterns that indicates climate trends may have exacerbated the effects of intensive elk browsing on aspen? Increased snowfall may impact elk survival and thus lead to lowered population size and potentially less intense browsing on aspen. Greater precipitation

may also lead to better growth of aspen and a better ability of aspen to withstand intense browsing.

- How much genetic variability exists in the park's aspen gene pool for natural selection to work on as changes take place in the climate, elk browsing pressures, and other environmental factors? Limited genetic diversity may constrain future adaptation to a changing environment.

The primary purpose of this investigation was to determine when and under what conditions these low-elevation aspen stands in the park established, and under what conditions and through what mechanisms they may regenerate. We used a combination of traditional dendrochronology and genetic techniques to determine the establishment dates of stands in the winter range and Kawuneechee Valley as compared to higher elevation stands outside these areas and the genetic diversity within and between aspen stands. We looked at the characteristics of regenerating and nonregenerating stands on the low-elevation, east side elk winter range in order to link successful regeneration to various elk herbivory, climate, and topographic variables. We examined the genetic characteristics of winter range and Kawuneechee Valley aspen that may enable persistence of aspen stands under a variety of environmental conditions. Ultimately, this information can be useful to land and resource managers in determining how best to manage both elk and aspen to achieve desired conservation goals. Therefore, we summarize our conclusions and the management implications of our research findings at the close of this document.

Age Distribution of Aspen

By Dan Binkley

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Introduction

The forests of the Rocky Mountains are dominated by conifer species, with the notable exception of aspen (*Populus tremuloides* Michx.; Peet, 2000). Aspen forests contribute disproportionately to the diversity of plant and animal species in these landscapes, frequently containing twice the number of species per unit area as found in conifer forests (DeByle, 1985; Turchi and others, 1995; Stohlgren and others, 1997, 1999; Chong and others, 2001).

The establishment of new aspen stands in the central Rocky Mountains generally follows major disturbances, particularly forest fires, with new stems sprouting from surviving root systems and with rare establishment of new seedlings. The development of new stands then depends on a host of factors, particularly the level of browsing by wildlife and livestock and the extent of conifer establishment and competition. A reduction in landscape coverage of aspen could result from a reduction in stand-replacing fires and increases in browsing or conifer competition. All of these factors have operated throughout the long-term history of forests in the Rocky Mountains, but their influence on aspen stands may have changed in the 20th century as a result of fewer major fires, and increased browsing that may have resulted from extirpation of predators and introduction of exotic livestock.

Changes in the extent of aspen in Rocky Mountain National Park would have major implications for plant and animal species, as well as for tourism and overall goals of ecosystem protection. Does the park still have the amount of aspen that would have been typical prior to the mid-19th century? Previous studies have documented low recruitment of new stems in portions of the park with very extensive elk browsing (Olmsted, 1979; C.E. Olmsted, University of Northern Colorado, 1997, written commun.; Baker and others, 1997) but greater recruitment across the rest of the park. For example, only 20 percent of aspen stands in heavily browsed, low-elevation areas of the park contained stems younger than 30 years, compared with about 75 percent of aspen stands at higher elevations and in the surrounding national forest land (Suzuki and others, 1999). An aspen stand with a young cohort of stems may still have fewer stems than would develop in the absence of unusually intense browsing.

We were particularly interested in determining whether a doubling of elk population (Lubow and others, 2002) following adoption of a predation-free ‘‘natural regulation policy’’ in the 1960s led to a substantial decline in numbers of young aspen trees. Controlled experiments with browsing exclosures have clearly demonstrated that wildlife can reduce aspen regeneration, and we know that most aspen stands have not successfully regenerated since the 1960s in areas with the highest elk use (Suzuki and others, 1999). However, a longer term context is needed to know if recent patterns in aspen recruitment are unusual relative to long-term trends, or if periodic increases and decreases in aspen recruitment have been common in the past. Perhaps aspen recruitment was exceptionally high in the late 1800s and early 1900s, and a reduction in recruitment in the late 20th century might indicate a long-term legacy of an earlier pattern. Alternatively, aspen recruitment at a landscape scale might be fairly consistent, with little influence of historical changes in land use or legacies of previous recruitment success.

We determined the age structure of aspen stems across the park, which allowed us to evaluate whether any cohort was represented by substantially more or fewer stems than would be expected based on the long-term pattern. A period showing higher-than-expected numbers of aspen might indicate a period of high fire occurrence or small elk populations, whereas periods with fewer-than-expected aspen might indicate few fires, large populations of elk, or extensive conifer in-growth (Kaye and others, 2003, 2005). If aspen recruitment was consistent across the decades prior to the 1960s, then the unique event of the very high elk populations may be driving unique changes in aspen numbers.

Methods

The study area was the entire Rocky Mountain National Park from the lowest elevations (2,500 m) to the upper elevation range of aspen (3,200 m; Kaye and others, 2003). Average annual minimum and maximum temperatures at low elevation near Estes Park (105º83′00″W., 40º82′40″N.) at 2,400-m elevation are about 1.5 and 14.0°C. Growing season (May–October) temperatures average 4.0–21.0°C, with an average of 370 mm/year precipitation (http://cdo.ncdc.noaa.gov/). Forests at low elevations are typically dominated by ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.), shifting with increasing elevation to mixed conifer (ponderosa pine, Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco]), lodgepole pine (*Pinus contorta* Dougl.) to Engelmann spruce (*Picea engelmannii* Parry ex Engelm) and subalpine fir (*Abies lasiocarpa* Hook. Nutt.) forests. Aspen trees occur throughout this elevation range, with greatest numbers at middle elevations (near 2,800–3,000 m; Kaye and others, 2003).

Based on previous work, we expected aspen age structure might differ between the low-elevation (less than 2,800 m) winter range of elk on the east side of the park and the rest of the park. A 1-km × 1-km grid was established for the entire park for elevations below 3,200 m, and 150 grid points were chosen at random for sampling (fig. 1). Eight of these chosen grid points fell in the low-elevation, east side area that provides winter range for large numbers of elk. An additional 49 grid points were chosen at random from a 0.5-km × 0.5-km grid within the low-elevation, east side area, giving total sample sizes of 57 for low-elevation, east side and 142 for the rest of the park. Most of these random plots had no aspen stems, so the number of plots containing at least one prism-counted aspen was 22 for the low-elevation east side and 24 for the rest of the park.

Each chosen grid point was visited; if no aspen were present in the vicinity, no measurements were taken. If at least some aspen were present, a plot was established with a series of 12 prism points in a triangle (at 50-m intervals; fig. 2). Aspen stems were tallied with a 1.15-m^2/ha basal area factor prism, and each aspen was cored to determine age at 1.4 m height. Our focus was on the date when aspen stems would begin to exceed the major browsing height by elk. The basal area of conifers was also tallied in each plot, with a 4.59-m^2/ha basal area factor prism. Ages of conifers were not examined. Tree core dates were determined by standard dendrochronology techniques; about 2/3 of the cores could be cross-dated with confidence, and cross-dating ages were usually within 3 years of ring-count ages. We expect that aspen ages are accurate to within 0–3 years.

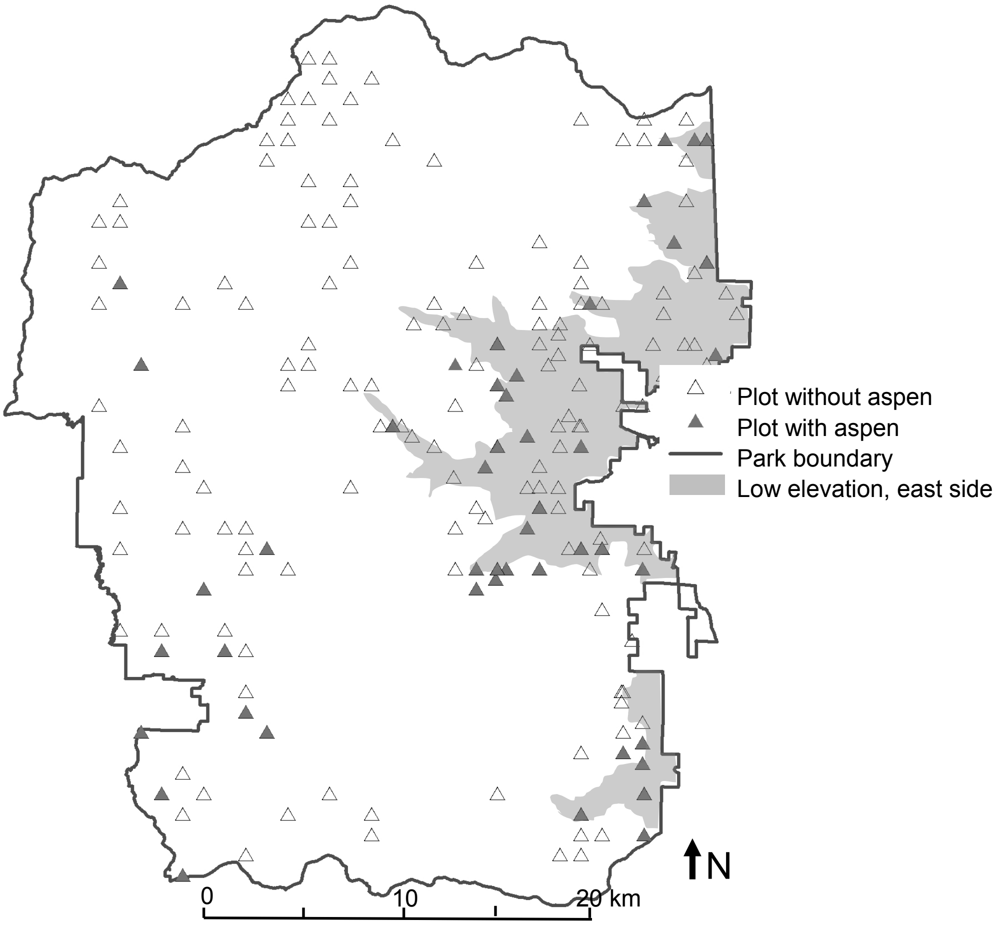


Figure 1. Random plot locations sampled from a 1-kilometer by 1-kilometer grid across Rocky Mountain National Park, supplemented by additional plots from a 0.5 –kilometer by 0.5-kilometer grid in the low-elevation, east side of the park.

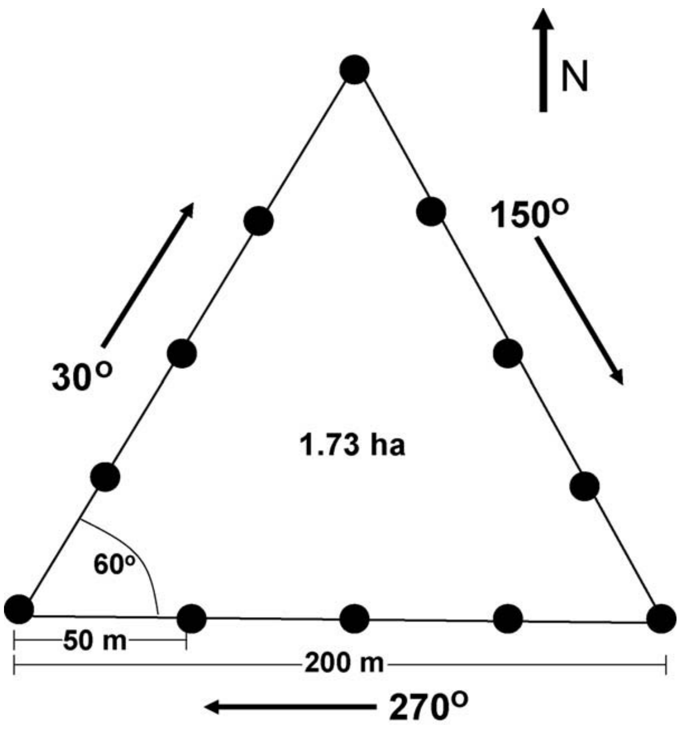


Figure 2. Twelve prism points were established at each plot, at 50-meter intervals in a regular triangle. All 12 prism points were averaged to provide a single estimate for each plot.

Typical prism cruises estimate tree size distributions by dividing the basal area factor of the prism by the basal area of each tree, and averaging across prism sample points. We converted this diameter distribution to an estimated age distribution by assuming each tree's age represented a cohort (of the calculated number) of trees of the same age (Binkley and others, 2006). For example, a 25-cm tree counted with a 1.15-m^2/ha factor prism would have a basal area of 490 cm^2 and would represent 23.4 trees of that size. If the age of the tree were 133 years, we would include 23.4 trees with an age of 133 years in the tally. The number of trees were summed across the entire area (low-elevation, east side or the rest of the park) and divided by the number of prism points to estimate the number of aspen trees in each 10-year age class. The number of stems in each decadal cohort was the product of 20–70 known core ages, extrapolated by using the prism's basal area factor.

We fit a linear trend to the logarithm of aspen numbers per decade from 1860 to 1960 (including years from 1855 to 1964). Any decade (including the three decades after the calibration period) that deviated from the line by more than two standard errors was defined as unusual.

The value of this prism-based approach to estimating age structure of the aspen population may be limited by two major assumptions. The processes leading to the death and recruitment of aspen stems would need to be generally consistent over the time period examined, as would be expected in a classic balanced, uneven-aged forest. If patterns of tree mortality and recruitment varied too greatly across decades, then patterns across many decades would be too noisy to detect decades with unusual numbers of aspen stems. The prism-based approach to determining the number of trees in each age class assumes that tree ages are sufficiently related to tree diameter to produce a reasonable representation of the age distribution across the landscape. Each of these factors could prevent detection of a real deviation in the overall trend in stem numbers and age classes. However, these potential problems would not lead to any false identification of unusual periods.

Age Distribution of Stands

The density of aspen in Rocky Mountain National Park averaged about 1,250 stems/km^2 for the low-elevation east side, and 550 stems/km^2 for the rest of the park (below 3,200 m); these estimates include locations where the prism cruise detected no aspens. Sample plots that included at least one aspen stem (among the 12 prism points) averaged 2.5 m^2/ha of aspen basal area and 19.4 m^2/ha basal area of conifers. Lodgepole pine trees accounted for about 64 percent of the conifer basal area, followed by about 20 percent each for Englemann spruce and for subalpine fir, with minor amounts of Douglas-fir, ponderosa pine, and limber pine. Aspen basal area declined weakly with increasing conifer basal area ($p = 0.02$, $r^2 = 0.13$).

Some aspen stems could not be aged because of heartrot. About 90 percent of trees less than 10 cm at 1.4 m were datable compared with 70 percent for larger diameter classes. A total of 651 trees were aged, and the oldest tree dated to 1689 (with an estimated pith date of 1685, for an age of 321 years at 1.4 m; P. Brown, Rocky Mountain Tree Ring Laboratory, written commun.), which we believe is the oldest reported age for an aspen tree. The next oldest tree was 205 years old, and 10 trees fell between 150 and 165 years.

Aspen ages correlated moderately well with diameter (fig. 3), with a standard error of the estimate of 23 years. The distribution of aspen stem sizes showed an exponential increase with successive decades (fig. 4), as expected for a balanced, uneven age forest.

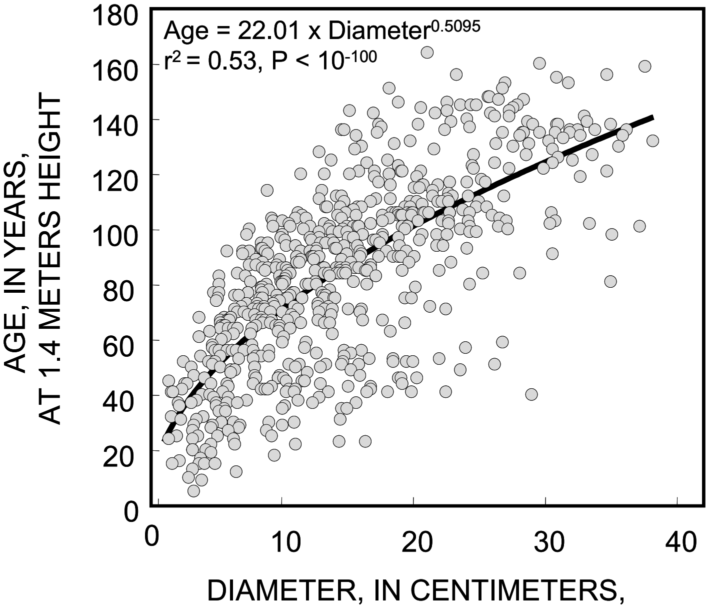


Figure 3. Relationship between tree diameter and age for 647 trees, omitting two trees older than 200 years and trees larger than 50 centimeters in diameter.

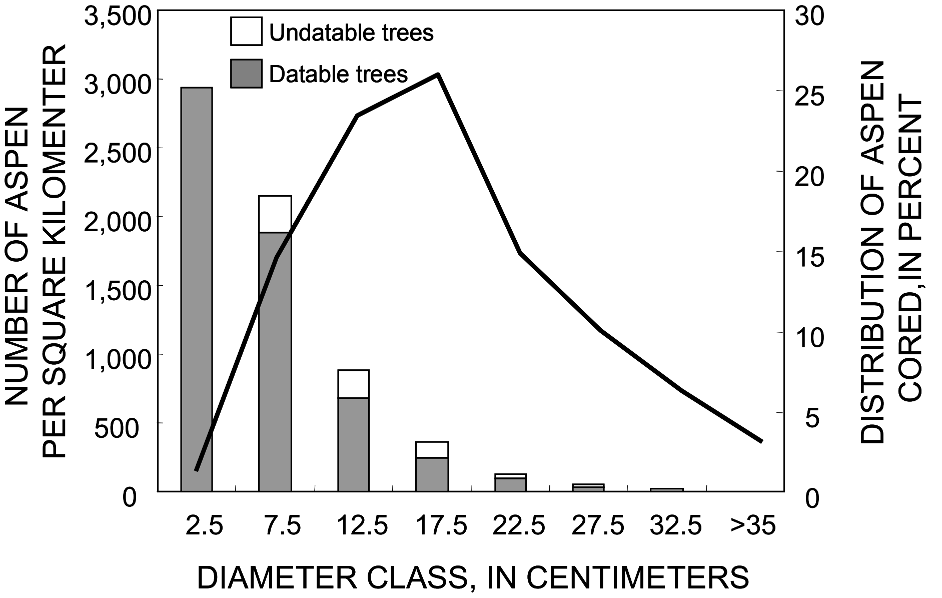


Figure 4. Across the whole park, the number of aspen per square kilometer (bars) declined exponentially with increasing tree size ($r^2 = 0.78$); undatable trees ranged from about 15 percent of trees between 5 and 15 centimeters in diameter, rising to about 30 percent for larger trees. The sample of dated aspen cores had the greatest representation from the 5- to 30-centimeter-diameter classes, with relatively few dates for trees smaller or larger.

The prism approach identified trees to be cored based on the proportion of size classes making up the total basal area of aspen. The smallest trees (less than 5 cm dbh) accounted for a very low proportion of aspen basal area and were represented by 44 sampled trees (6 percent of aged cores). The majority of sampled aspen were between 5 and 30 cm (86 percent of aged cores), with 8 percent of aged cores greater than 30 cm. If we had determined age structure from cores taken from trees in fixed-area plots, our sample would have been composed about equally of trees less than 5 cm and trees between 5 cm and 30 cm, with fewer than 0.05 percent of the cores coming from trees larger than 30 cm in diameter.

The period from 1975 to 1995 at low elevation on the east side had 80–95 percent fewer aspen than would be expected based on the trend from 1855 through 1965 (fig. 5). This finding is consistent with the low proportion of regenerating aspen stands (since the late 1960s) found by Baker and others (1997) and Suzuki and others (1999) for this portion of the park. Across the rest of the park, no decades had significantly fewer than expected aspens, and 1965–75 had more aspen than expected. Suzuki and others (1999) also found that most aspen stands contained young stems outside the low-elevation east side of the park, consistent with the density of young stems in our sampling.

A recent report noted that most aspen trees in Colorado are more than 120 years old (Colorado Forest Health Advisory Board, 2005), but only about 1 percent of aspen in Rocky Mountain National Park were older than 120 years (accounting for 10 percent of aspen basal area, fig. 6). About 90 percent of the trees and 50 percent of basal area were 80 years old and younger. Recent inventories of stand ages of aspen in Colorado showed that about 6 percent of aspen stands in Colorado are older than 120 years, half were over 75 years, and just 20 percent were younger

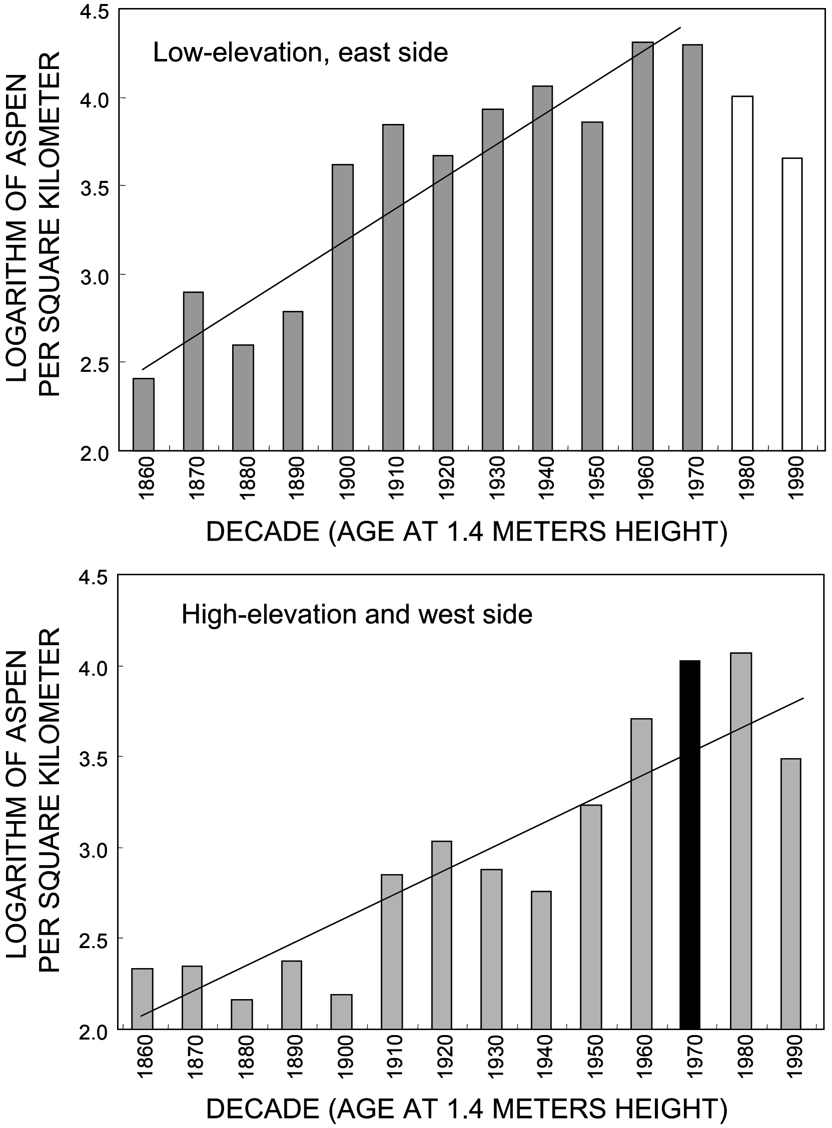


Figure 5. Logarithm of number of aspen stems per square kilometer by age class for the two portions of Rocky Mountain National park. Lines show the long-term trend from 1855 through 1965 (low-elevation, east side: log aspen = –31.432 + 0.018 x decade, $r^2 = 0.85$, $p < 0.0001$; rest of park: log aspen = –21.668 + 0.013 x decade, $r^2 = 0.76$, $p < 0.001$). Gray bars indicate aspen densities within two standard errors of the estimate from the long-term trend; solid bars are more than two standard errors above, and open bars are more than two standard errors below the trend.

than 50 years (data from USDA Forest Service Forest Inventory and Analysis; M.T. Thompson, unpub. data, March 2007). The aspen forests of Rocky Mountain National Park have greater proportions of younger stems, indicating either younger stands or a tendency in the statewide inventory data to represent older stems disproportionately to stem numbers.

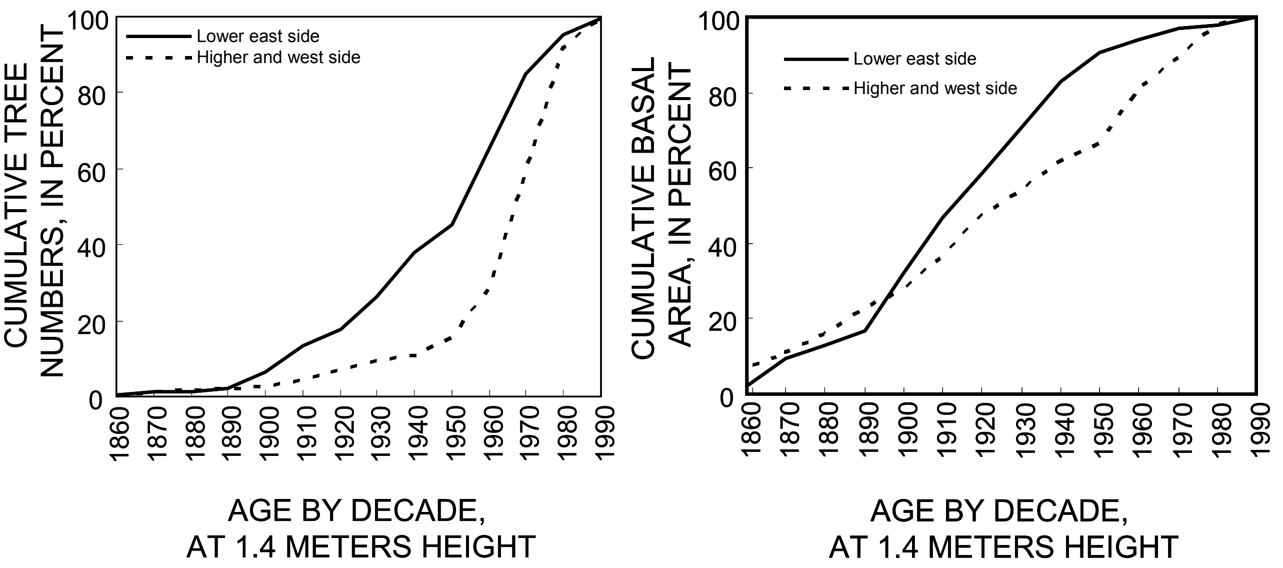


Figure 6. In the lower elevation, east side portion of the park, aspen trees dating after 1920 accounted for 83 percent of trees and 42 percent of basal area. For the rest of the park, 93 percent of trees and 53 percent basal area were accounted for by trees established after 1920.

Some decades may have differed substantially from the long-term trend of expected aspen densities, without being detected with confidence by our sampling approach. For example, the number of aspen stems dating to 1985–95 in the higher elevation and westside portions of the park fell about 45 percent below the long-term expectation, but this deviation was only one standard error below the trend line. The precision of our ability to determine anomalous periods depends on both the decade-to-decade variation among typical decades and the imprecision added by variation in the relationship between stem size (and probability of inclusion in the prism points) and tree age.

Prior to 1900, stand-replacing fires had a return interval on the order of 300 years across much of Rocky Mountain National Park (Buechling and Baker, 2004; Sibold and others, 2006). Fires were less extensive in the 20th century as a result of climate conditions and active fire suppression (Buechling and Baker, 2004). The current age distribution of aspen in the park does not show any marked legacy of a changed fire regime. This contrasts strongly with the Kaibab Plateau in northern Arizona, where introduction of livestock eliminated the frequent surface fire regime (Fulé and others, 2003) and led to tenfold increases in aspen numbers (Binkley and others, 2006). Surface fires were probably much less frequent in the elevation range of aspen in Rocky Mountain National Park than on the Kaibab Plateau, and any change in fire regimes in the late 19th century had too little effect on aspen to be detected by our survey.

The population of elk varied notably in the park in the 19th and 20th centuries. No records are available on elk population in the 19th century, but the elk were extirpated by the end of the century. Browsing effects on aspen recruitment and survival may have remained high even as elk populations declined owing to domestic livestock. In the early 20th century, elk were reintroduced and livestock were removed, with unknown effects on aspen. The elk population was managed with active population control (including culling and trapping and relocation) in the mid-20th century, before the policy of natural regulation (in the absence of major predators) was adopted in the late 1960s. The age structure of aspen in the park indicates that the interacting effects of fires, elk population changes, and livestock grazing had more or less consistent effects on aspen from 1855 to 1965. Some periods of high or low recruitment and survival may have occurred during some decades, but these effects were not large enough to leave a large legacy in the current age structure. The post-1975 decrease in aspen numbers in the low-elevation, east side portion of the park appears to be unprecedented in the past 140 years.

About two-thirds of all aspen stems occur within a matrix of conifer stands rather than in pure aspen stands (Kaye and others, 2003). Aspen regeneration is very low in stands with more than 10 m^2/ha of conifer basal area (Kaye and others, 2005). The basal area of conifers has likely increased across the park in recent decades, but any increase would not likely be more pronounced in the low-elevation, east side portion of the park.

The lack of a significant change in aspen numbers in recent decades in the higher elevation and westside portions (with the exception of the Kawuneechee Valley) of the park supports the idea that significant effects of elk browsing have been a larger factor in reducing aspen numbers than climate conditions, fire incidence, or conifer in-growth (Kaye and others, 2005). Restoring the numbers of young aspen that would be characteristic of the low-elevation, east side of the park will likely require substantial reductions in elk browsing effects on aspen shoots, either by reduced elk numbers or altered patterns of landscape use (cf. Ripple and Beschta, 2004).

Elk Herbivory Effects on Aspen Stand Regeneration and Growth Rates on Elk Winter Range

By Linda C. Zeigenfuss and Francis J. Singer

Introduction

The effects of elk (*Cervus elaphus*) herbivory on aspen (*Populus tremuloides*) throughout the Rocky Mountain region of North America have been widely studied and debated (Kay, 1993, 1997; Romme and others, 1995) and Rocky Mountain National Park (RMNP) in Colorado has been no exception (Olmsted, 1979; Baker and others, 1997; Suzuki and others, 1999; Kaye and others, 2003). These studies have identified elk browsing, along with fire suppression, as major factors in the decline of aspen on the low-elevation winter range of the park. Many studies throughout the Rocky Mountains also indicate that while aspen stand regeneration may be declining at a local level, at landscape levels aspen is generally persisting in the same area (Suzuki and others, 1999; Barnett and Stohlgren, 2001; Kaye and others, 2003; Kashian and others, 2007). Similar results have been found by Kashian and others (2007) for adjacent areas of the Colorado Front Range that do not experience the protection from elk hunting found in RMNP. These authors found that only 15 percent of the northern Front Range aspen stands they sampled were exhibiting dieback of large stems coupled with lack of recruitment of young trees into the stand canopy. These stands were most often associated with low-elevation locations where elk browsing was extensive and chronic and the effects of fire suppression have been most significant.

Currently (2008), RMNP is in the process of instituting a new elk management plan that will include protection of several low-elevation, winter range aspen stands using elk-proof fencing as well as culling and harassment to reduce and redistribute elk. Previous studies have identified the connection between elk herbivory and aspen decline in the park (Olmsted, 1979; C.E. Olmsted, University of Northern Colorado, 1997, written commun.; Baker and others, 1997; Suzuki and others, 1999; Kaye and others, 2003, 2005); however, few of these studies have quantified the degree of elk browsing occurring in these aspen stands or how elk browsing, population size, and density affect stem recruitment or plant growth rates. Most of what is known about these relationships has been inferred from either general trends in elk populations, by relating aspen stand changes to periods before and after 1968 when RMNP elk management policy changed from active management to natural regulation, or general observation and classification of browse levels as high or low. Further, few studies have tried to relate elk population trends and precipitation patterns that may have exacerbated the effects of intensive elk browsing on aspen.

Our objectives were to identify at what levels of elk herbivory aspen stands on the winter range become suppressed or are able to sustain stem recruitment beyond the height of browsing, thus allowing stand regeneration to take place. We also attempted to determine climatic or elk population and(or) density conditions that might allow for height release of aspen. Finally, we attempted to characterize variables other than elk herbivory that might account for success or failure of stands on the winter range to achieve stem recruitment levels that would lead to continued stand regeneration.

Study Area

The low-elevation elk winter range of RMNP encompasses approximately 11,000 ha in the upper montane zone along the eastern boundary of the park and the adjacent Estes Valley (fig. 7). Elk are free to move between protected national park land and the mix of public and private land in the Estes Valley. Glacial moraines running east-west divide the body of the park area into four major valleys, and the town of Estes Park lies in the middle of a broad park-like valley. Elevations in the study area range from 2,300 m at the lower end of the Estes Valley to 2,800 m on moraine ridgetops. Mean annual precipitation is 41 cm, most of it occurring as wet spring snows. Pure aspen stands occupy less than 1.5 percent of the area of the elk winter range within RMNP.

Methods

We selected 18 aspen stands from 53 identified on a park vegetation covertype map as being within the area defined as elk winter range at the outset of this study. Since then, the area defined as elk winter range has been altered such that four of our stands now fall out of the area that park managers presently consider elk winter range. A new park vegetation map was completed in 2006 that identifies a larger number of aspen stands. We visited the 53 stands in a random order

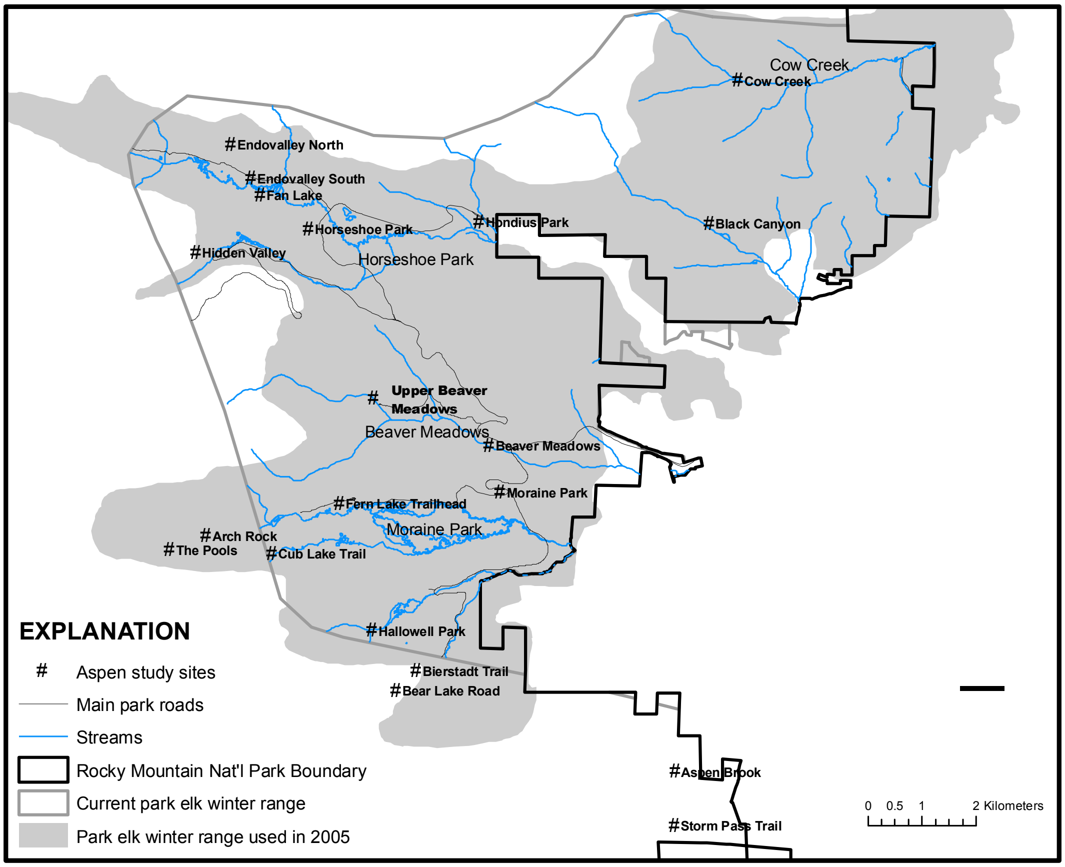


Figure 7. Location of aspen stands that were sampled for offtake, recruitment and regeneration, and browse production in 2005 and 2006 in Rocky Mountain National Park.

until we had identified 18 stands that were predominantly aspen, not conifer mixed stands. Ten of these had little or no stem recruitment (small diameter aspen stems less than 2.5 m in height) and 8 more showed signs of stem recruitment into the stand canopy. Due to difficulties identifying additional stands that showed stem recruitment and were within the winter range boundary, we selected two more stands slightly south of the winter range boundary that had been identified as fitting our criteria by field crews working on landscape-scale aspen age dynamics. Going slightly beyond the area designated by the park as elk winter range was necessary to obtain a sample of stands that actually had aspen stems that appeared to be recruiting to the tree canopy. However, no sampled stand was more than 2 km beyond the edge of the area currently designated as primary elk winter range, and all stands had signs of winter elk use based on observation of animals on the site or presence of scat, tracks, and(or) shed antlers.

Our final field sample consisted of a total of 10 stands on the elk winter range that were classified as nonregenerating (NR) stands because these stands had few or no suckers or saplings reaching a height that would allow the stand to continue to regenerate. The other 10 stands were classified as regenerating (R) stands (fig. 8). These stands all had a cohort of saplings that were in the 1.5- to 2.5-m-height range.

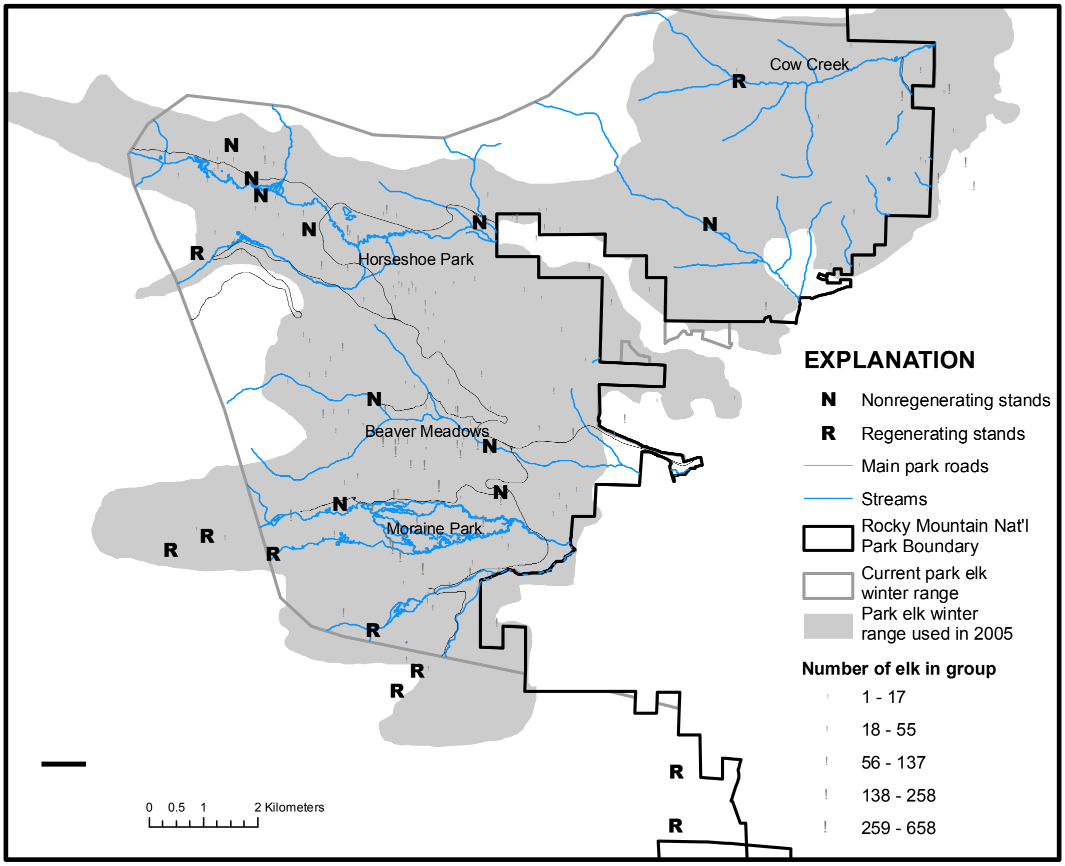


Figure 8. Location of regenerating and nonregenerating aspen stands in relation to elk groups observed during annual aerial elk population surveys conducted 1994–2007 in Rocky Mountain National Park.

We measured winter offtake at all 20 stands in spring 2005 and 2006, and summer offtake and browse production in all stands in 2005 and 18 stands in 2006. Offtake is the average percentage of current year's total shoot biomass that was removed (between 0 and 2.5 m in height). At each stand we measured 2–6 (depending on the size of the stand) randomly selected 10-m^2 fixed-radius circular plots. We sampled elk use patterns on all aspen saplings (stems ≤ 2.5 m maximum height) by estimating the shoot length and biomass removed by elk based on regressions of basal diameters of annual shoots and shoot diameters at point of browsing and by counting the number of browsed and unbrowsed shoots (Zeigenfuss and others, 2002). Percent leader use was determined using the equation: percentage of leader use = a/(a+b); where a = number of browsed shoots and b = number of unbrowsed shoots. Average percentage of shoot removed was determined using the methods of Jensen and Urness (1981) and Pitt and Schwab (1990) following the formula: percentage of shoot use = $100\ (D_p - D_t)/(D_b - D_t)$**;** where D_p = shoot diameter at point of browsing, D_t = diameter of a representative sample of shoot tips, and D_b = basal diameter of current year's shoot growth. Total offtake was determined by multiplying percentage of leader use by percentage of shoot use. We tallied aspen plants ≤ 2.5 m in height into classes of 5 cm (0–5 cm, 5–10 cm, 10–15 cm, 15–20 cm, 20+ cm) diameter at breast height (dbh). We visually estimated the percentage of the stem covered by old and recent bark scarring at heights of less than 2.5 m on these stems. Old scars were characterized as those which had healed, creating a black, thickened scar. Recent scars were those which been inflicted by browsing during the previous year and were characterized by exposure of the light colored cambium layer or sapwood over which a heavy scar had not yet formed.

To examine height growth over the past several years, each year we measured the height increment between bud scars of 10 young stems per stand. This method tends to be straightforward for the most recent 3–4 years but becomes increasingly difficult each year thereafter as older bud scars become increasingly difficult to see and identify. We verified our interpretation of nodes by removing a few stems per stand in 2005, cross-sectioning the stems, and counting annual growth rings. We then attempted to regress age from ring counts against our earlier visual interpretation of the estimated year each node was laid down.

Elk Populations

Elk populations in the park have been monitored by helicopter census once annually in mid-winter since 1994. In addition, a combination of methods was used to survey elk populations from 1965 to 1994. Raw population estimates were corrected using a model that takes into account sightability conditions during the elk census and weather covariates (Bruce Lubow, Colorado State University, unpub. data; Lubow and others, 2002). We used these corrected population estimates to examine trends in elk population size over time. Elk group location and group size data from aerial surveys conducted 1994–2007 (with the exception of 2 years) were plotted using ArcMap 9.2 GIS software (ESRI, 2006). Then density of animals per square kilometer was estimated using the kernel density function of ArcMap Spatial Analyst with a 1,000-m search radius between points. We estimated density for each year of survey data and then averaged all years to get an average elk density index across the winter range for the past 14 years. This density estimate is roughly comparable to that used to determine elk densities in previous studies at RMNP (Singer and others, 2001), but we caution that it should only be used as a means to determine relative elk density. We were limited in our ability to determine absolute elk density from the available data because of the surveys were conducted only once per year, providing only a single point-in-time estimate of elk density and distribution to represent density and distribution for an entire winter season.

Climatic and Topographic Conditions

We examined 76 years (1931–2006) of data from the Estes Park, Colorado, weather station (National Weather Service Coop station #52759) to look for trends in precipitation or temperature that might have affected growing conditions on elk winter range aspen stands in RMNP. Weather records at the Estes Park weather station were incomplete for many years, therefore data for these years were removed from the analysis. We used Digital Elevation Model (DEM) data to derive slope and aspect maps for the study area using ArcMap 9.2 Spatial Analyst GIS software (ESRI, 2006). We then compared these variables between our nonregenerating and regenerating stands to determine other site factors that may affect the determination of stand regeneration in addition to elk herbivory.

Statistical Analyses

All statistical analyses were conducted using SAS 9.1 statistical analysis software (SAS Institute, 2003). Where appropriate, based on normality of the data, t-tests were used to compare between regenerating and nonregenerating stands. When data were non-normal, a Kruskal-Wallis nonparametric test was used to compare the two stand types. We used simple regressions (using square root transformation of percentile data) to determine relationships between offtake and leader use and dependent variables such as aspen height and elk density. We tested for trends in weather and elk population data over time using linear and quadratic regressions. Differences between stand types at the $p = 0.05$ level were considered significant.

Results

Elk Herbivory

Total annual offtake ranged from 0.4 to 50.3 percent of current annual growth of shoots on aspen saplings (stems less than 2.5 m high, table 1). Patterns between nonregenerating and regenerating sites were consistent between the 2 years measured, so we averaged across years for each site. The heaviest offtake was over winter, but very high levels of offtake (more than 25 percent) were limited to only a few stands. However, the percentage of leaders browsed (percentage of leader use) was high on most of the nonregenerating stands that were located in the winter range. Winter and summer offtake was greater in nonregenerating than regenerating sites. Percentage of shoot removed was similar between types. Leader use (both in summer and winter) was much greater at nonregenerating sites compared to regenerating sites (table 1).

As was expected, nonregenerating sites were located in the areas where the heaviest concentrations of wintering elk have been observed during midwinter elk censuses conducted from 1994 to 2007 (fig. 8). Offtake was positively correlated with an index of average observed elk density based upon aerial surveys conducted from 1994 to 2007, but these correlations were not strong ($R \leq 0.57$). Mean elk density index was 6.5 times greater for nonregenerating stands than regenerating stands. No groups of greater than 20 elk were observed during the surveys closer than 500 m to a regenerating stand.

Aspen Growth

Average height of saplings (stems less than 2.5 m high) was two times greater in regenerating sites than in nonregenerating sites (fig. 9). Mean height of saplings was negatively correlated to the amount of overwinter leader use ($p < 0.0001$, R = 0.77). Overall, there were 1.5 times as many aspen stems less than 2.5 m in height in nonregenerating stands than regenerating stands (fig. 10a). However, the number of saplings that grew to a height of 1.5–2.5 m was 46 times greater in the regenerating stands (fig. 10b). No recruits greater than 1.0 m in height

Table 1. Winter and summer aspen offtake (consumption) rates on elk winter range of Rocky Mountain National Park. Offtake is the average percentage of current year's total shoot biomass that was removed (between 0 and 2.5 m in height). Leader use is the average percentage of shoots that are browsed (number of browsed shoots divided by total shoots).

Site name	Winter offtake (%)	Summer offtake (%)	Total offtake (%)	Winter leader use (%)	Summer leader use (%)
Regenerating Stands (Edge of winter range)					
Arch Rock	2.4	0.0	2.4	5.7	0.0
Aspen Brook	7.3	1.2	8.5	20.4	6.9
Bear Lake Road	9.5	4.3	13.8	49.7	15.7
Bierstadt Trail	4.7	0.1	7.6	14.3	0.6
Cow Creek	4.9	0.0	4.9	11.0	0.0
Cub Lake Trail	4.1	0.1	4.2	13.4	0.9
Hidden Valley	3.6	0.9	4.5	6.1	3.3
Hallowell Park	11.0	0.5	11.5	19.9	2.4
The Pools	0.4	0.0	0.4	1.4	0.0
Storm Pass Trail	1.2	0.1	1.3	7.0	0.2
Mean±s.e.	**4.9±1.1**	**0.7±0.4**	**5.9±1.4**	**14.9±4.3**	**3.0±1.6**
Nonregenerating Stands (Elk Winter Range)					
Beaver Meadows	38.8	11.5	50.3	69.6	27.4
Black Canyon	9.0	1.6	10.6	32.7	7.8
Fern Lake Trailhead	15.1	5.9	21.0	38.1	24.0
Fan Lake	34.0	10.1	44.1	85.5	47.8
Endovalley South	6.2	2.5	8.7	62.8	9.6
Hondius Park	25.9	4.8	30.7	73.0	26.2
Endovalley North	29.5	1.0	30.6	59.4	10.6
Horseshoe Park	19.1	5.4	24.4	52.9	27.3
Moraine Park	42.8	2.8	45.6	92.5	31.5
Up. Beaver Meadows	18.3	.	.	71.2	.
Mean±s.e.	**23.9±3.9**	**5.1±1.2**	**29.5±5.0**	**63.8±6.0**	**23.6±4.2**

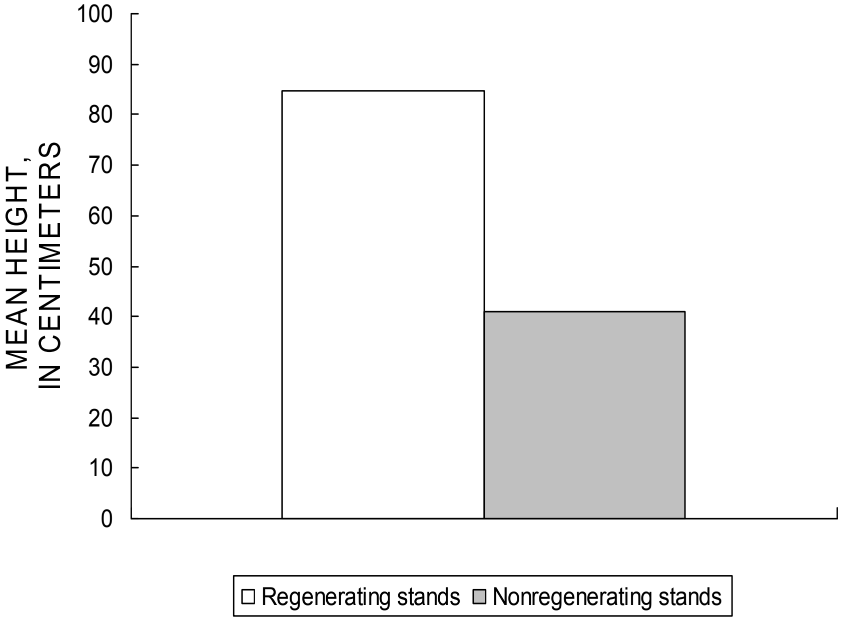


Figure 9. Mean height of aspen saplings (stems less than 2.5 meters height) in regenerating and non-regenerating aspen stands of elk winter range of Rocky Mountain National Park.

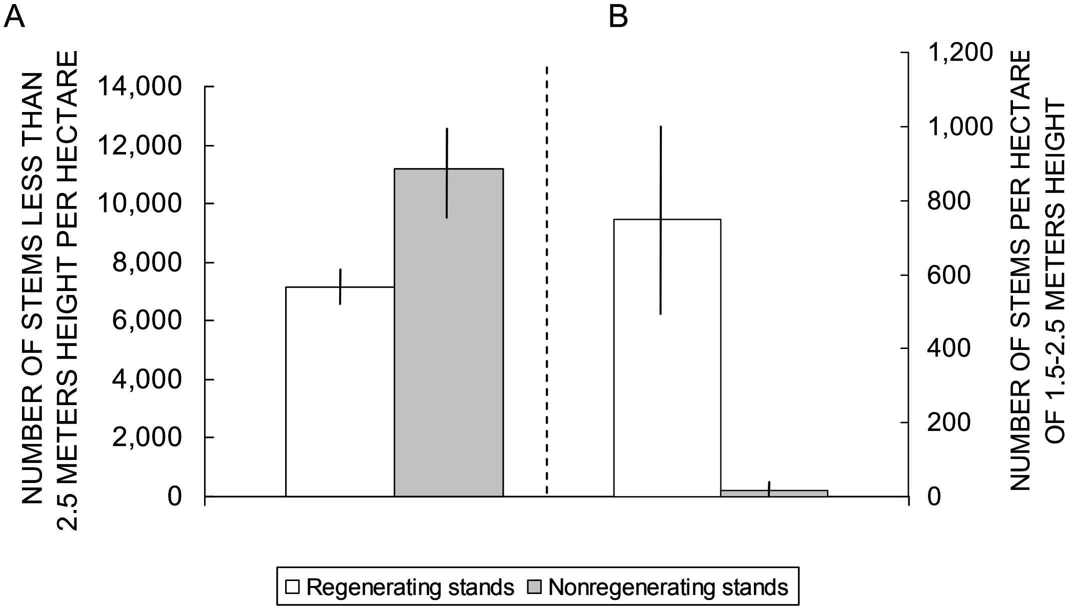


Figure 10. Density of aspen stems in regenerating and nonregenerating aspen stands of elk winter range of Rocky Mountain National Park for all stems less than 2.5 meters height (A) and stems between 1.5 and 2.5 m in height (B). Vertical lines denote standard errors.

were found in the non-regenerating stands by spring, and only half of measured nonregenerating stands had any recruits greater than 75 cm high in spring. In 80 percent of the regenerating stands, we measured saplings ≥ 1.5 m in height and all of these stands had total annual offtake levels of ≤10 percent (and ≤ 21 percent leader use). Density of these recruiting saplings was more than 2,000 stems/ha in 75 percent of the regenerating stands that experienced total offtake rates of less than 10 percent.

Annual leader lengths of aspen suckers in regenerating compared to nonregenerating stands were not significantly different prior to overwinter browsing. However, aspen suckers averaged an additional 7.9 cm of annual height growth (postbrowsing measures) in regenerating stands (17.2±1.6 cm) compared to nonregenerating stands (9.3±1.2 cm; $p < 0.0025$) in 2005 and an additional 17.3 cm of annual height growth in regenerating stands (28.9±2.5 cm) compared to non-regenerating stands (11.6±1.6 cm; $p < 0.0001$) in 2006. Growth internode lengths could only be poorly interpreted to age of stem. However, dating of a subsample of collected stems using growth annuli indicated that 28 percent of small-diameter stems less than 2.5 m in height from regenerating aspen stands dated earlier than 1999, while only 17 percent of stems from nonregenerating stands could be dated earlier than 1999.

There were more than seven times as many small-diameter trees (≤ 10 cm dbh, greater than 2.5 m high) in the regenerating stands than in nonregenerating stands ($p = 0.0003$), but there was no significant difference in the number of larger diameter trees (greater than 10 cm dbh). The number of trees (stems greater than 2.5 m high) that had bark scarring from elk browsing on only 25 percent or less of the trunk was 123 times greater in regenerating stands than nonregenerating stands ($p = 0.0012$). Four times as many trees in nonregenerating stands had bark scarring on 75–100 percent of their trunk surface than trees in regenerating stands, though this difference was not significant ($p = 0.1775$).

Total snowfall measurements (not corrected for density) at the nearby Estes Park weather station showed a decline from 1931 through approximately 1968–70, after which time total snowfall increased ($p = 0.003$, $r^2 = 0.26$, fig. 11). These years with little snowfall coincided with the time period when the “natural regulation” policy of elk management was instituted in 1968. Elk population estimates showed no relationship to total snowfall or precipitation patterns (fig. 12).

There were some differences in site characteristics between regenerating and non-regenerating stands. Regenerating stands were at slightly higher elevations (2,668±45 m, $p = 0.08$) compared to nonregenerating stands (mean = 2559±36 m). Slope was also greater (34.3±5.0 percent) in regenerating stands than in nonregenerating stands (14.6±4.2 percent, $p = 0.008$).

Effects of Elk Herbivory on Aspen Regeneration

The connection between extensive elk herbivory and declines in aspen stand regeneration on elk winter range of Rocky Mountain National Park has been clearly demonstrated through a variety of methods, including those presented here. Rates of offtake increased with elk densities and stands with high levels of browsing had fewer, shorter aspen recruits. In Yellowstone National Park’s northern elk winter range, Ripple and Beschta (2007) observed that young aspen height growth reaching beyond 150 cm did not occur until leader use dropped below 50 percent, and growth to more than 200 cm occurred when leader use dropped below 40 percent. The average leader use values in our regenerating stands were well below this 40 percent threshold while nonregenerating stands had leader use levels of greater than 75 percent.

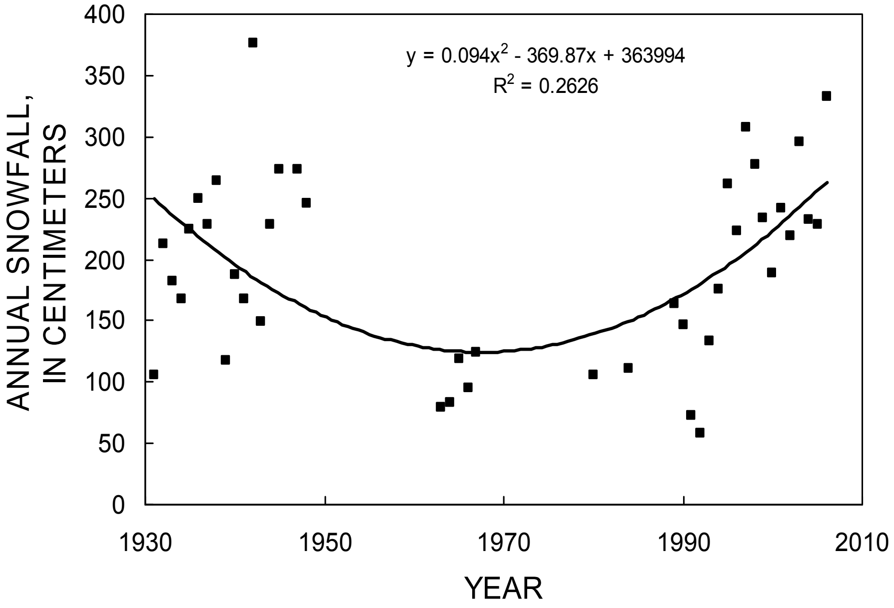


Figure 11. Total snowfall (in inches) at the Estes Park, Colorado weather station 1931–2006.

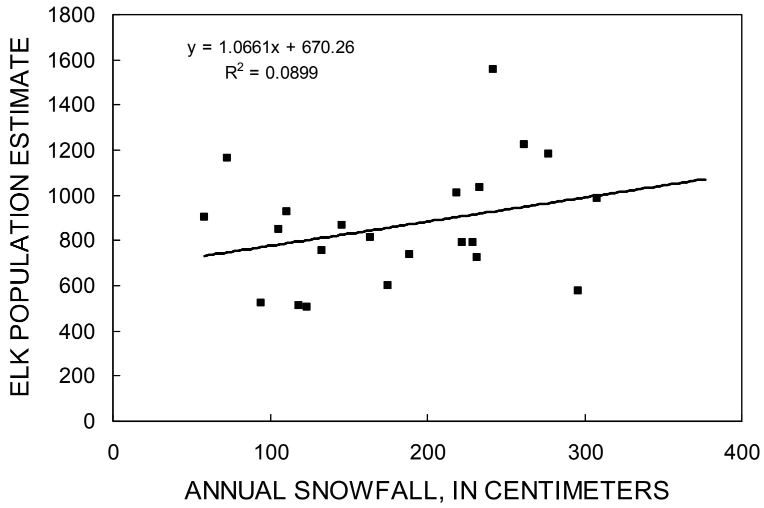


Figure 12. The relationship between Rocky Mountain National Park winter elk population and annual snowfall from 1965 to 2006 at the Estes Park, Colorado weather station.

Elk herbivory can also have secondary effects on young aspen growth. Binkley and others (2003) found that soil pH in grazed aspen stands was about 0.7 units higher than under aspen inside the exclosures. They also noted that nitrogen fertilization doubled the production of grasses and shrubs that could potentially outcompete aspen suckers. Mature aspen stems had greater rates of fungal infection in stands that experienced heavy browsing on the northern Colorado Front Range

(Kashian and others, 2007). We found that high levels of bark browsing were characteristic of nonregenerating stands, which might place mature stems in these stands at greater risk for mortality due to infection entering the bark wounds

It was evident that elk browsing did not damage the potential of healthy suckers to continuously produce substantial annual leader growth. Even after 40 years of what has been considered intensive elk browsing on this winter range, leader lengths in nonregenerating stands did not differ significantly from those in regenerating stands. In Yellowstone National Park, Ripple and Beschta (2007) found similar results for the current annual growth of aspen leaders in both riparian aspen stands that were experiencing lower browse levels and increased regeneration following wolf reintroduction and upland stands that were still intensely browsed and showing poor regeneration. We believe this indicates the important potential of these nonregenerating stands to recover and produce cohorts of stems that can recruit to the stand canopy and, thus, lead to stand regeneration, if management steps are taken to reduce levels of elk browsing and if other factors, such as a drier and(or) warmer climate and continued fire suppression, do not negate the positive effects of reduced herbivory.

Despite the large amount of evidence pointing to elk herbivory as the primary factor in aspen decline on this winter range, we cannot ignore the potential role that fire suppression, individual site characteristics, and climate variables may contribute to exacerbating the effects of elk herbivory (Romme and others, 1995; Kaye and others, 2003). Several studies have indicated that higher elevation stands, both within and near the park, have greater regeneration than those on the low-elevation elk winter range (Kaye and others, 2005; Kashian and others, 2007). However, most of these study stands have been a considerable distance from the park's primary elk winter range and, thus, benefited from lower levels of elk herbivory. Our results indicate that even at slightly higher elevation aspen exhibit significantly greater regeneration and lower levels of browsing. Furthermore, higher elevation stands on the winter range tend to grow on steeper slopes, whereas the low-elevation stands tend to be on valley floors or gentle slopes. This combination of higher elevation and steeper slopes appears to result in less use by elk.

A decline in total snowfall in the years preceding and immediately following the establishment of the "natural regulation" policy of elk management in 1968 may have contributed to reduced aspen establishment at a time when elk populations and likely browsing pressure were increasing. Aspen on the RMNP elk winter range generally grow in xeric conditions, so even a slight decline in snow cover and its associated soil moisture could have had an effect on the ability of plants to quickly grow beyond the height of elk browsing during those years.

Our results indicate that many nonregenerating stands on the elk winter range will likely have the ability to regenerate successfully when protected from elk browsing. However, with a several-decade-long increase in temperatures, it is possible that protection from elk browsing may not be enough to preserve aspen stands on the elk winter range. Therefore, it would be important to consider fencing some of these regenerating stands on the margins of the winter range as well as the nonregenerating stands on the winter range, if the park staff's goal is conservation of aspen on the winter range. The slightly higher elevation characteristics of these stands may contribute to better survival of aspen as a whole on the landscape of the east side of Rocky Mountain National Park. Also, if elk management actions (culling and redistribution activities) lead to increased elk herbivory in these margin stands that are currently regenerating, it would be important to have protection measures in place to ensure continued levels of regeneration in these stands. If stands are not protected with fences, then elk management actions would likely need to be targeted to bring total offtake levels down to 10 percent or less and elk densities to fewer than eight elk per square kilometer to achieve aspen stand regeneration in the heaviest elk-use areas of the winter range.

Genetic Diversity, Clone Ages, and Relatedness Within and Between Aspen Stands

By Gerald A. Tuskan, Tongming Yin, William H. Romme, and Steve P. DiFazio

Introduction

Although it covers only a small portion of the total area of Rocky Mountain National Park (RMNP), quaking aspen (*Populus tremuloides*) has long been a species of special interest and concern to managers and the public alike. There are at least two reasons for the attention given to aspen. First, aspen is the only major deciduous tree species in the park. Aspen forests thus contribute greatly to the esthetic experience of the park visitor, especially when the leaves turn color in the fall, and aspen forests also support unusually high numbers and diversity of other plant species and wildlife. Second, aspen appears to be declining in some portions of the park as a result of chronic and extensive browsing by elk, fire exclusion during most of the past century, and perhaps other factors such as recent climatic changes.

The genetic makeup of a population reflects its evolutionary history and provides insights into its potential for coping with future environmental changes. With respect to history, for example, an exciting idea among genetics researchers today is the possibility that we can determine the rate of somatic mutation (that is, random changes in genes that are involved in functions other than reproduction) in clonal organisms like aspen and thereby construct a "molecular clock" with which to estimate when a given clone became established as an original seedling, based on the number of somatic mutations accumulated throughout the clone's lifetime. Our aspen genetics study in RMNP is one of the first in the world to critically test this potentially valuable method for reconstructing the age and history of long-lived clonal plants. Looking to the future, it is important to know how much genetic variability exists in the park's aspen gene pool for natural selection to work on as changes take place in the climate, elk browsing pressures, and other environmental factors. If genetic diversity is limited, then future adaptation to a changing environment may be seriously constrained. Thus, the genetic studies that we report here nicely complement the other two components of this research project (the age structure of aspen stems throughout the park, and rates of elk browsing and biomass removal in selected locations).

For our study of aspen genetics, we asked two broad questions and addressed each one through a set of smaller questions:

1. What are the current patterns of genetic structure and genetic diversity in aspen populations throughout Rocky Mountain National Park?

1a. What is the clonal structure and overall amount of genetic diversity in the aspen populations of RMNP?

1b. What are the spatial patterns of genetic structure and relatedness, within and among aspen stands across the park?

1c. Are there particular aspen genotypes that might need special management attention?

2. Can we apply genetic techniques to estimate the absolute ages of aspen clones and to determine how important seedling recruitment has been in creating and maintaining genetic diversity in the park's aspen populations?

2a. Can we compute somatic mutation rates in RMNP aspen and thereby construct a "molecular clock" for this species?

2b. Is there genetic evidence of aspen seedling establishment occurring in the park during past centuries?

Overview of the Genetic Concepts and Methods Used in this Study

The field of molecular genetics has seen a veritable explosion of new concepts and techniques during the past two decades. These new techniques provide the foundation for the genetic analyses used in this study. Therefore, we provide here a very brief overview of the terms and concepts that will be used in this report. Additional explanations and illustrations can be found in almost any very recent college-level general biology textbook, or by searching the Web for the key words in italics below.

Molecular Genetics and DNA "Fingerprinting"

The genetic makeup (that is, the *genotype*) of each aspen tree that we sampled was determined using state-of-the-art techniques of DNA fingerprinting. By "aspen tree" we mean an individual stem, recognizing that this stem may be connected by its root system to other stems and therefore part of a clone. The most exciting thing about DNA fingerprinting techniques is that they allow us to recognize genetically unique individuals, either members of different clones or individuals that originated from a single seedling and are not part of any clone. The techniques also provide some information about the total genetic makeup of an individual, though we must point out two limitations of the techniques for this latter purpose. First, we are able to characterize a *sample* of the genes contained within an individual's cells, but not the entire genome (that is, not all of the thousands of genes present in a cell). Second, with these molecular analyses alone we cannot relate specific genes to specific functions (for example, control of growth or production of antiherbivore compounds). Nevertheless, these modern techniques of molecular genetics have vastly improved our ability to characterize and evaluate genetic diversity and clonal structure in populations of Rocky Mountain aspen, and RMNP is one of the first places in the country where this kind of research is being conducted.

The process of DNA fingerprinting is carried out as follows. Tissue samples are collected (aspen leaves in this case), and DNA is extracted from the cells. This initial extraction process is not trivial because most cells contain phenols and other compounds that quickly degrade the DNA once it is liberated from the chromosomes in the nucleus. Our research team had previously developed methods for safely extracting DNA from plants in the genus *Populus* (aspens and cottonwoods), and these methods were used in this study (Tuskan and others, 1996, 1999, 2004, 2006). Because the quantity of DNA obtained from a single sample (that is, a single leaf, or the DNA pooled together from several leaves collected from the same tree) is too small for molecular analysis, the next step is to *amplify* or duplicate the DNA contained in the sample to produce a suitable quantity for analysis, using a technique called *polymerase chain reaction* (*PCR*). It is not necessary (or feasible) to amplify all the DNA in a sample. Instead, small segments of the DNA (called *microsatellites*) are pulled out and amplified. The sample is gently heated to cause the two strands of DNA to separate (as they do in the normal replication process). Two *primers* are added, each of which is a length of DNA produced previously in the laboratory. These primers attach at two places on the sample DNA (places containing a complementary sequence of nucleotide bases), and the section of DNA between the two primers is the "microsatellite." An enzyme, *DNA polymerase*, is added, and this enzyme stimulates replication (production of an exact copy) of the microsatellite. If the proper chemical and physical environment for the reaction is maintained, the replication process will be repeated over and over until a large number of replicate copies of the microsatellite has been produced.

Multiple primers are added to a sample to produce multiple microsatellites. Once all these microsatellites have been amplified through the PCR procedure, the sample is placed on a gel with a mild electrical charge that causes the microsatellites to migrate through the gel at different rates, thereby separating them on the basis of very subtle differences in their makeup (that is, in their overall length and exact sequence of bases). This process is called *gel electrophoresis*. The locations within the gel of all the microsatellites are noted and compared between samples from different individuals. If the locations are identical for samples from two different aspen trees, then the two trees are genetically identical (at least with respect to the microsatellites that have been tested) and represent a single clone. If the locations are different, then the two individuals are not part of the same clone, but are distinct genotypes. Because microsatellites represent only a tiny portion of an individual's total genome, it is theoretically possible that two individuals could be identical within the segment of DNA represented by the microsatellites but different in other parts of the genome. However, by using numerous different primers to produce numerous different microsatellites, it is possible to compute the statistical probability of two individuals having different overall genotypes if their microsatellites are identical. If this probability is vanishingly low, then we can safely conclude that the genotypes are indeed distinct.

Somatic Mutations and the "Molecular Clock"

Somatic mutations are random, spontaneous changes in the genes of cells that are not involved with sexual reproduction (for example, somatic mutations may occur in buds of stems or leaves). Mutations also occur in the DNA of sex cells, and those mutations contribute to the genetic variability that is passed from one generation to the next. However, somatic mutations are not passed on to offspring. Microsatellites extracted from two stems or two branches that diverged long ago on a very old tree, may exhibit a very few genetic differences as a result of somatic mutations that occurred in one stem or branch (but not the other) at some time in its life. Similarly, two different trees of an aspen clone may show slight differences in their microsatellites because of somatic mutations that have occurred in the past, perhaps even before the extant stems sprouted from the clonal root system. The older a clone, the more somatic mutations it is likely to have accumulated among its component stems. If we can determine the rate at which somatic mutations accumulate within a long-lived clonal plant (that is, the average number of mutations per century), and if we then determine the number of somatic mutations now present within a given clone, we can estimate the absolute age of the clone (that is, the time since it was first established as a seedling) by dividing the mutation rate into the number of somatic mutations now present. Although this is relatively simple in theory, to actually calculate the somatic mutation rate is technically very challenging. Our research with aspen in RMNP is one of the first major efforts to attempt this.

Genets, Ramets, Ortets, and Clones

Special terminology has been developed to describe the population structure of clonal organisms like aspen. A *genet* is an individual that is genetically distinct from all other individuals, (that is, it has a unique genotype); a *ramet* is a stem or branch that is not unique but is genetically identical to all the other individuals composing a clone. An individual aspen tree could be either a unique genet, distinct from any clone, or a ramet that sprouted from the root system of a clone. In the latter case, the root system would represent the genet. An *ortet* is the original seedling of a potentially clonal species. An ortet may remain an individual organism throughout its life and eventually die without producing a clone. Or the ortet may produce multiple ramets, thereby developing a clonal structure. It generally is not possible to determine simply by looking at whether a given aspen tree is a genet or a ramet: molecular tests of the kind conducted in this study are the only means for conclusively making this determination.

Diploids, Polyploids, Tetraploids, and Triploids – Why Important?

Cells of *polyploid* individuals do not contain chromosomes in pairs, as is the usual condition in most species, but have chromosomes in threes or fours. Most plant and animal cells have two of every chromosome (that is, the chromosomes are in pairs) and are said to be *diploid*. These pairs of chromosomes separate during *meiotic* cell division (associated with sexual reproduction) to produce gametes (eggs and sperm), each containing only one of each chromosome, and then two gametes recombine to restore the pairs of chromosomes in the new individual, which has therefore regained the diploid condition. However, sometimes the pairs of chromosomes fail to separate, such that the gametes contain two copies of each chromosome rather than the usual one copy. When two of these gametes unite, the resulting cell contains not two but four of each chromosome—a condition referred to as *tetraploid*. Subsequent *mitotic* cell division (associated with ordinary growth or with asexual reproduction) results in an adult plant whose cells all contain four pairs of chromosomes rather than the usual two. Sometimes a gamete with two of each chromosome will combine with an ordinary gamete containing just one of each, to produce a cell with three copies of each chromosome, or a *triploid* cell, and this condition characterizes the resulting adult plant as well.

Polyploid genetic structure is important for two reasons. First, triploid and tetraploid plants may be better able to cope with unfavorable environmental conditions than diploid plants. This idea still needs more testing, but the mechanism may be that the extra chromosome(s) provides additional genetic material that allows for stronger and more diverse responses to local stresses and constraints on growth. These stresses and constraints might include such things as temperature extremes or climatic changes, lack of soil nutrients, and browsing by herbivores, although we must emphasize that we have not explicitly related the genetic structure of RMNP aspen to any specific environmental factors (to do so will require additional research beyond the scope of this present project). The second reason why polyploid genetic structure is significant is because triploid aspen plants are often incapable of sexual reproduction. A cell can cleanly separate either two (diploid) or four (tetraploid) chromosomes into gametes during meiosis, but the process often fails with three chromosomes (triploid). Triploid cells can undergo mitosis, which simply duplicates all of the chromosomes and thereby retains the original chromosome number, so triploid plants are capable of vegetative growth, including root sprouting. Indeed, root sprouting (which represents asexual reproduction) is the primary way that triploid plants reproduce.

Materials and Methods

Sample Collection

To characterize the spatial and geographical patterns in aspen genetics, we distributed our samples (1) between the east and west sides of the park; (2) within a northwest and a southwest segment on the west side, and within a central, northeast, and southeast segment on the east side (fig. 13); (3) within several "stands" in each segment, a "stand" being defined as a collection of several more-or-less discrete patches of aspen all clustered within an area of 1–100 ha (fig. 13); and (4) within several "patche*s*" within each stand, a patch being defined as a discrete group of aspen trees that appear to all have the same characteristics of bark color, height, and crown shape and that are separated from other patches by a distance of at least 15 m (fig. 14).

Within each patch we established a transect by running a meter tape through the longest axis of the patch, and stopped at fixed intervals (for example, every 10 m) along the transect to sample the canopy tree located closest to the point on the transect tape. Intervals between sampling points were shorter in small patches and longer in large patches to ensure that we would obtain approximately equal numbers of samples from every patch. From the tree at each sampling point, we collected leaves from five different locations within the crown, including upper, middle, and lower crown. We also collected an increment core from the stem for age determination.

In August 2005, 2,440 leaf samples were collected from 488 trees at the east side of RMNP. Trees were located in 11 different stands (fig. 13A), which included a total of 46 patches (for example, fig. 14). In May 2006, we collected 1,500 more leaf samples from 300 trees across 8 different stands on the west side of RMNP (fig. 13B), including 25 patches. GPS readings of the

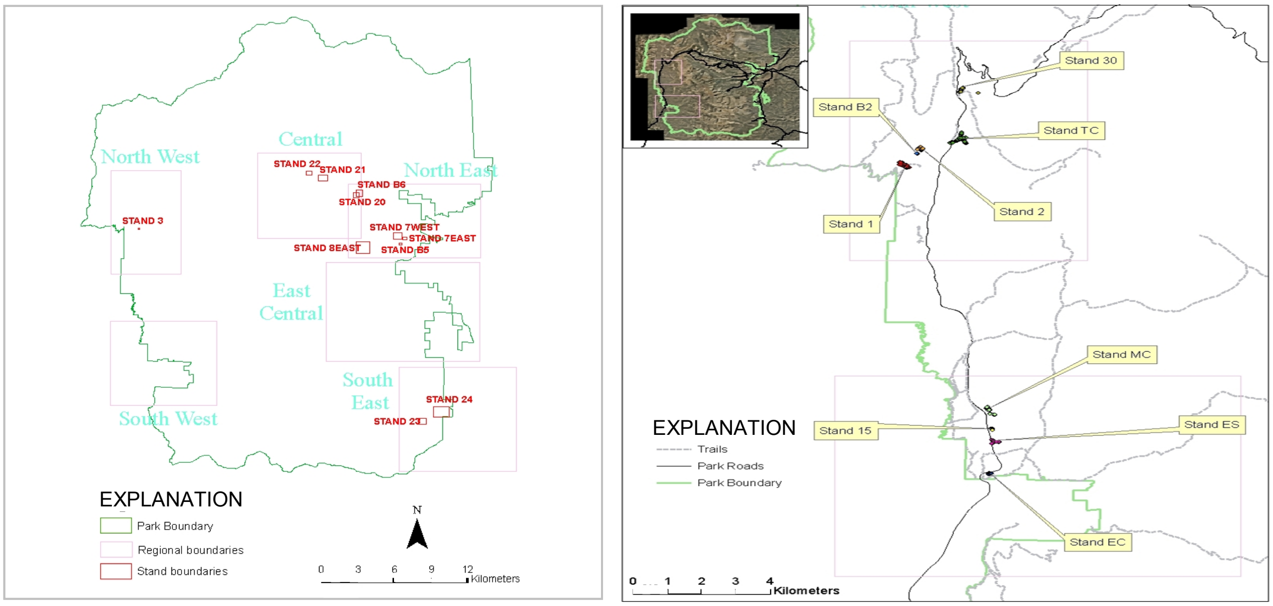


Figure 13. Locations of stands that were sampled in Rocky Mountain National Park for characterization of aspen genetics. A stand was defined as a collection of several more-or-less discrete patches of aspen, all clustered within an area of 1–100 hectares. Stands were sampled primarily on the east side of the park in 2005 (left) and on the west side in 2006 (right).

Figure 14. Patches at Stand B6 sampled in 2005. A patch is defined as a discrete group of aspen trees that appear to all have the same characteristics of bark color, height, and crown shape and that are separated from other patches by a distance of at least 15 meters. A stand is defined as a collection of several more-or-less discrete patches of aspen all clustered within an area of 1–100 hectares. In other words, stands represent different geographic locations across the park, and patches are smaller groups of aspen trees without any large gaps between trees. Red dots depict locations of ramets of the dominant genet within a patch; green dots represent other genets.

sampled trees and orientation from tree to tree were collected to spatially document sample distribution and for potential future relocation of trees.

Laboratory Methods

DNA was extracted from 3,590 samples by using an AutoGen DNA extraction robot coupled with manual tissue disruption. DNA was quantified using agarose gel electrophoresis and densitometry. For extractions with low yield or inability to amplify using PCR, DNA was reextracted manually to make sure every sample was extracted successfully. Substantial effort was devoted to optimizing extraction techniques to minimize the inhibitory and DNA degrading effects of the abundant phenolic compounds present in mature aspen foliage.

Extracted DNA was sorted according to sample location. We pooled four samples from each tree to derive a "consensus" genotype. The fifth sample was used to detect somatic mutation. We purified the DNA samples and checked the DNA quality using the Nanodrop spectrophotometer. Most DNAs had a 260/280 reading greater than 1.70, indicating a high level of quality. PCR products detected on an ABI 3730XL sequencer showed that the purified template provided robust amplification products with no artifacts.

A total of 864 SSR [single sequence repeat] new primer pairs were designed from the *Populus trichocarpa* genome sequence. Because this species is rather distantly related to *P. tremuloides* (more than 10 million years' divergence (Eckenwalder, 1996), we designed primers in exons flanking microsatellite repeats in introns. We compared these primers to a set of 767 primers designed from random BAC end sequences (Tuskan and others, 2004).

We screened the SSR primers strategy. In the first round, DNA template from two unrelated genotypes was used to identify primers with successful amplification in aspens. A total of 1,631 primers were screened in this round. Primers with successful amplification were then assessed using the following criteria: (1) size of PCR products within 80 to 300 bp, (2) successful amplification in both individuals, and (3) a clear amplification signal on the ABI3730XL.

We tested more than 1,600 primer pairs and identified 37 pairs that produced amplified PCR product with the most variable microsatellite loci within the genome. The average number of alleles revealed per primer pair was 12.5. These selected primer pairs were intensively screened, thus providing a valuable resource for population genetics studies in natural aspen stands. We genotyped 3,590 samples (one sample was amplified as consensus genotype control; the other four samples from the same tree were pooled to detect mutation) from 788 trees collected at 19 stands. Thirty-nine microsatellite loci produced informative markers.

Results and Interpretations of Genetic Studies

Question #1 — Genetic structure and diversity in RMNP aspen populations

1a. Clonal structure and genetic diversity: It is commonly thought that a patch of aspen trees in which all individuals are similar with respect to bark color, height, crown shape, and phenology (for example, timing of spring bud break and autumn leaf fall) represents a single clone of genetically identical stems (that is, the stems are all ramets produced by a root system that comprises a single genet). Indeed, we observed similar morphology and phenology among all the stems within several of the aspen patches that we sampled in this study. Therefore, we expected to find that most patches of aspen in the park were composed of a single clone of genetically identical trees.

To our surprise, we found just the opposite result: only 6 out of 86 aspen patches (7 percent) consisted of a single clone. In the other 93 percent of patches that we sampled, we found two or more interdigitating clones that were genetically distinct from one another, and often one or more genetically unique individual trees that were not related to any nearby clones. Most patches and stands did contain one dominant clone, that is, a genotype represented by more stems than any other genotype (see fig. 14); nevertheless, the genetic diversity of the trees within a homogeneous-appearing patch or stand was surprisingly high.

Overall, about two-thirds of the aspen trees that we sampled were ramets, (that is, genetically identical members of a clone), and about one-third were genetically unique individuals. At the scale of a stand (a collection of several more-or-less discrete patches of aspen, all clustered within an area of 1–100 ha), we found from 1 to 57 distinct genotypes within samples of 4–101 trees (table 2). The greatest number of ramets that we sampled in any patch was 28 trees. It is important to remember that, because we sampled trees along linear transects, our samples did not include all of the trees in any patch. Consequently, the actual total number of unique genotypes (genets) and the total number of ramets per genet could not be determined. Nevertheless, the amount of genetic diversity represented in these relatively small samples indicates that the overall level of genetic diversity throughout the aspen populations of RMNP is quite high.

How is it that a patch of aspen that visually appears to be a single clone can be so genetically diverse? We propose that two mechanisms may be at work. First, the ortets (the original seedlings) that gave rise to the separate clones within a single aspen patch may have been siblings from the same parent tree and thus had similar (though not identical) genetic makeup. Such a pattern has been documented in Yellowstone National Park (Tuskan and others, 1996). Second, being in such close proximity to one another, all of the original ortets must have been subjected to natural selection by very similar environmental factors. This combination of genetic relatedness and similarity of selection pressures may therefore account for the morphological and phenological similarity of the trees in aspen patches that are actually composed of more than one clone.

1b. Spatial patterns of genetic structure and relatedness: The patterns of spatial distribution of specific genotypes across RMNP further support the findings previously described of tremendous genetic diversity within and between aspen patches. Generally, each patch and each stand contained a more-or-less unique set of genotypes. Nearby patches often contained at least some ramets of the same clone, suggesting that the root systems of many clones are capable of considerable lateral expansion (for example, fig. 14). However, each patch also contained other clones or genets not represented in the nearby patch. Moreover, we found no duplicate clones among different stands (stands being our larger, more widely dispersed sampling units, each stand consisting of multiple smaller patches). This finding indicates that each genotype occupies a somewhat limited resident space within the landscape rather than being distributed throughout the park.

Putting this into the language of molecular biology, a high level of genetic variation and heterozygosity was detected in the aspen populations with mean Na=15.8, Ne=5.3, He=0.76 where Na is observed number of alleles, Ne is expected number of alleles, and He is expected heterozygosity. The distributions and frequencies of alleles of all loci were significantly different among the stands, and there are some rare alleles specific to aspens from different locations. The population differentiation coefficient (Fst) was 0.17; that is, 17 percent variation is accounted for in the differentiation of different stands. The results of AMOVA analysis indicate that 82.7 percent of variation exists within the stands, 13.1 percent is among the stands, and 4.2 percent is east and west side locations (table 3). Therefore, the variation among locations is much less than that among the different stands. The relatively high variation among stands indicates that there is divergent selection for genotypes leading to their fitness for the specific geographical environment.

Table 2. Summary of number of genotypes detected in different aspen stands. See figure 1 for locations of stands. Stands sampled in 2005 are mostly on the east side of Rocky Mountain National Park; those sampled in 2006 are mostly on the west side.

Year	Stand	Number of genotypes	Number of sampled trees	Proportion of clonal trees
2005	8E	9	42	0.79
	18E	3	4	0.25
	20	13	45	0.71
	21	16	47	0.66
	22	18	50	0.64
	23	16	49	0.67
	24	13	50	0.74
	3	9	49	0.82
	7E	6	24	0.75
	7W	11	29	0.62
	B5	5	44	0.89
	B6	23	55	0.58
	Total	**142**	**488**	**0.71**
2006	1	20	51	0.61
	15	2	10	0.80
	30	1	20	0.95
	B2	8	24	0.67
	EC	8	15	0.47
	ES	4	30	0.87
	MC	15	49	0.69
	TC	57	101	0.44
	Total	**115**	**300**	**0.62**

Table 3. Global AMOVA results as a weighted average over all loci.

Source of variation	Sum of squares	Variance components	Percentage variation
Among groups	539.842	0.61719	4.24166
Among populations within groups	1,558.013	1.90012	13.05873
Within populations	10,147.702	12.03329	82.69961
Total	12,245.557	14.55060	.

To produce a final picture of spatial patterns in the genetics of RMNP aspen, we plotted the Nei's genetic identity between the pair-wised genotypes (fig. 15). The maximum identity is 0.77 and the average was approximately 38 percent, with differences detected at least at seven loci. Using these estimates of genetic distance, we clustered stands across geographic collection sites. Generally, stands from the west side of the park clustered together separately from stands from the east side (fig. 16). Similarly, aspen populations in Yellowstone National Park (which we have recently sampled in a similar manner) clustered together and separately from the RMNP samples. However, there were single stands from all three geographic locations that were genetically distinct from each other and all other aspen stands, that is, stands 15 (west side), B5 (east side), and GT (Yellowstone) (fig. 16). The analysis depicted in fig. 16 further supports the interpretation of high overall genetic diversity in RMNP aspen, with populations in close geographic proximity to one another sharing some genetic similarity, but with each individual genotype being restricted to a relatively small area within the landscape.

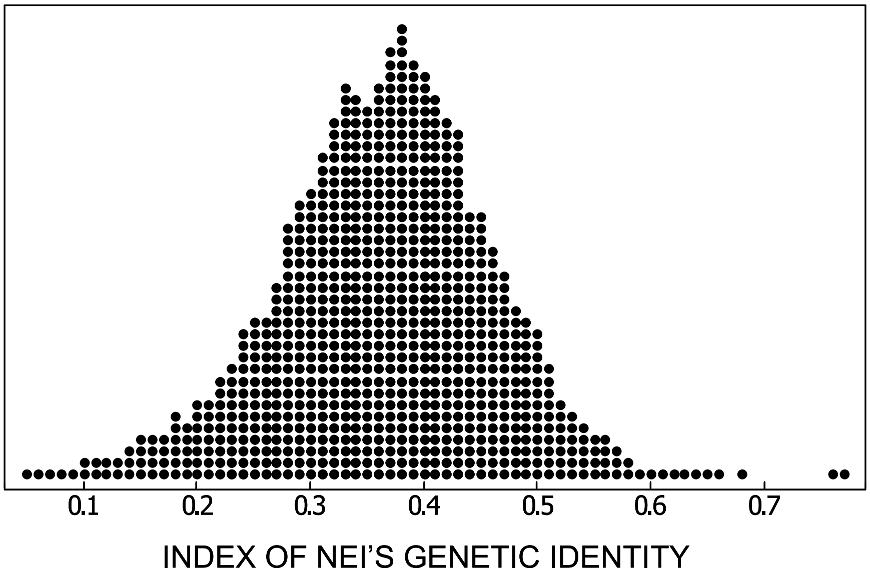
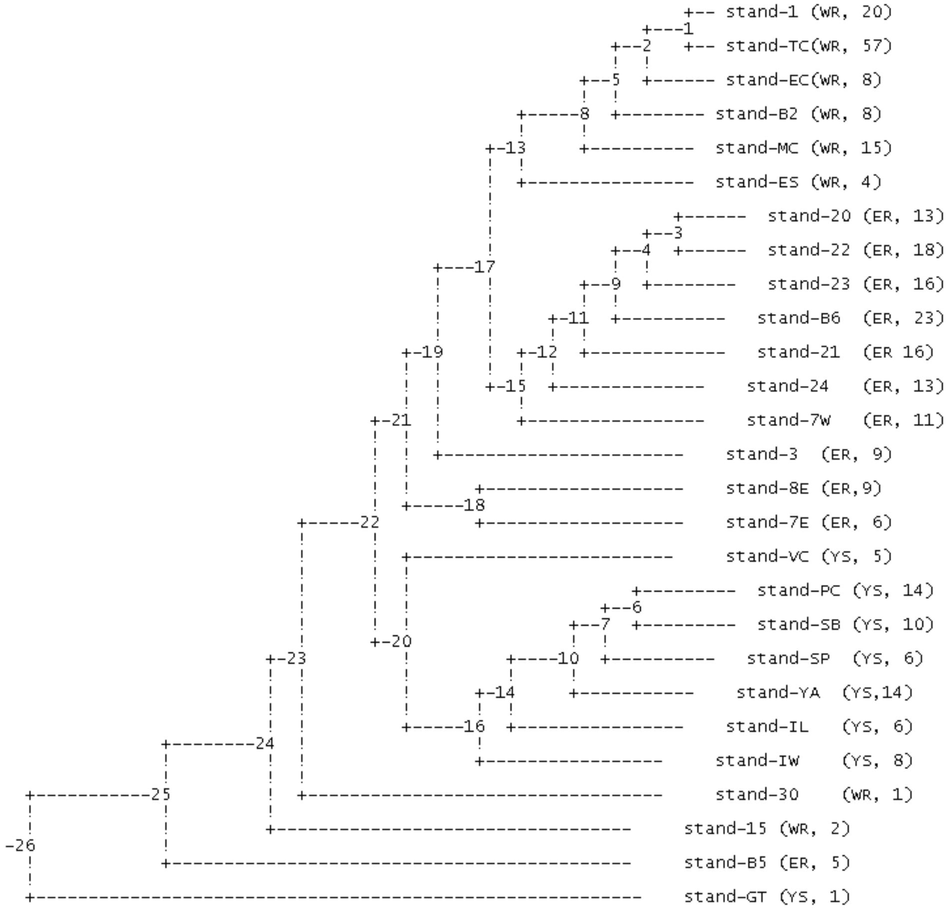


Figure 15. Dot plot of Nei's genetic identity among aspen sampled. Each symbol represents up to 18 observations.



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                                                                 +-- stand-1 (WR, 20)
                                                             +---1
                                                          +--2   +-- stand-TC(WR, 57)
                                                          !  !
                                                       +--5  +------ stand-EC(WR, 8)
                                                       !  !
                                                 +-----8  +-------- stand-B2 (WR, 8)
                                                 !     !
                                              +-13     +----------  stand-MC (WR, 15)
                                              !  !
                                              !  +----------------  stand-ES (WR, 4)
                                              !
                                              !                 +------  stand-20 (ER, 13)
                                              !              +--3
                                              !           +--4  +------  stand-22 (ER, 18)
                                         +---17           !  !
                                         !    !        +--9  +--------   stand-23 (ER, 16)
                                         !    !        !  !
                                         !    !     +-11  +----------   stand-B6  (ER, 23)
                                         !    !     !  !
                                      +-19    !  +-12  +-------------   stand-21  (ER 16)
                                      !  !    !  !  !
                                      !  !    +-15  +--------------    stand-24   (ER, 13)
                                      !  !       !
                                   +-21  !       +-----------------    stand-7W   (ER, 11)
                                   !  !  !
                                   !  !  +-----------------------    stand-3  (ER, 9)
                                   !  !
                                   !  !      +-------------------    stand-8E (ER,9)
                                   !  +-----18
                            +-----22         +-------------------    stand-7E (ER, 6)
                            !      !
                            !      !  +-------------------------     stand-VC (YS, 5)
                            !      !  !
                            !      !  !                     +---------  stand-PC (YS, 14)
                            !      !  !                  +--6
                            !      !  !               +--7  +---------  stand-SB (YS, 10)
                            !      +-20               !  !
                         +-23         !         +----10  +----------   stand-SP  (YS, 6)
                         !  !         !         !     !
                         !  !         !      +-14     +-----------    stand-YA  (YS,14)
                         !  !         !      !  !
                         !  !         +-----16  +----------------    stand-IL   (YS, 6)
               +--------24  !                !
               !         !  !                +-----------------     stand-IW    (YS, 8)
               !         !  !
  +-----------25         !  +----------------------------------     stand-30    (WR, 1)
  !            !         !
  !            !         +----------------------------------     stand-15 (WR, 2)
-26            !
  !            +--------------------------------------------     stand-B5 (ER, 5)
  !
  +----------------------------------------------------------    stand-GT (YS, 1)
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Figure 16. Dendrogram based on Nei's (1978) genetic distance: Method = UPGMA [unweighted pair group method with arithmetic averaging]. *Interpretation*: All of the sampled stands are listed from top to bottom on the right-hand side of the figure. WR = west side of Rocky Mountain National Park (RMNP); ER = east side of RMNP; and YS = aspen sampled in Yellowstone National Park. The numbers following the commas in the parentheses are the number of genotypes in that stand. See figure 13 for stand locations in RMNP. Note that every stand is connected to every other stand through the horizontal and vertical lines. If two stands are connected at a point close to the right side of the figure (for example, stands 1 and TC at the top), this means that the trees within both stands have relatively similar genetic composition. But if two stands are connected at a point far to the left, then the trees within those stands share very few genotypes; for example, the bottommost stand (GT, from Yellowstone Park) bears almost no genetic resemblance to any of the other stands listed in the figure. Observe that the top six stands (all from the west side of RMNP) cluster together with a moderate level of similarity, and the next seven stands (all from the east side) cluster together at a similar level of similarity. However, because these two clusters are joined at a point farther to the left in the figure, the degree of similarity between the two clusters is less than the similarity of any two stands within one of the clusters.

1c. Particular aspen genotypes that might need special management attention: We discovered a surprisingly large frequency of polyploid genotypes (that is, aspen trees whose cells have three [triploid] or four [tetraploid] instead of the usual two [diploid] copies of each chromosome) across all sampled stands and locations (table 4, fig. 17). Polyploidy actually is common in aspen and has contributed to speciation of the genus *Populus* (Tuskan and others, 2006), but the level of polyploidy in the aspen of RMNP was unexpectedly high.

Table 4. Summary of polyploids detected in different aspen stands in Rocky Mountain National Park (RMNP). See figure 13 for locations of stands. Stands sampled in 2005 are mostly on the east side of RMNP; those sampled in 2006 are mostly on the west side.

Year	Stand	Diploid	Triploid	Tetraploid	Proportion diploid	Proportion triploid	Proportion tetraploid
2005	8E	12	5	25	0.29	0.12	0.60
	18E	0	2	2	0.00	0.50	0.50
	20	8	6	31	0.18	0.13	0.69
	21	12	9	26	0.26	0.19	0.55
	22	5	19	26	0.10	0.38	0.52
	23	11	8	30	0.22	0.16	0.61
	24	0	16	34	0.00	0.32	0.68
	3	12	35	2	0.24	0.71	0.04
	7E	2	22	0	0.08	0.92	0.00
	7W	15	5	9	0.52	0.17	0.31
	B5	0	25	19	0.00	0.57	0.43
	B6	31	13	11	0.56	0.24	0.20
	Total	**108**	**165**	**215**	**0.22**	**0.34**	**0.44**
2006	1	50	1	0	0.98	0.02	0.00
	15	10	0	0	1.00	0.00	0.00
	30	0	0	20	0.00	0.00	1.00
	TC	83	17	1	0.82	0.17	0.01
	B2	24	0	0	1.00	0.00	0.00
	EC	15	0	0	1.00	0.00	0.00
	ES	21	9	0	0.70	0.30	0.00
	MC	27	22	0	0.55	0.45	0.00
	Total	**230**	**49**	**21**	**0.77**	**0.16**	**0.07**

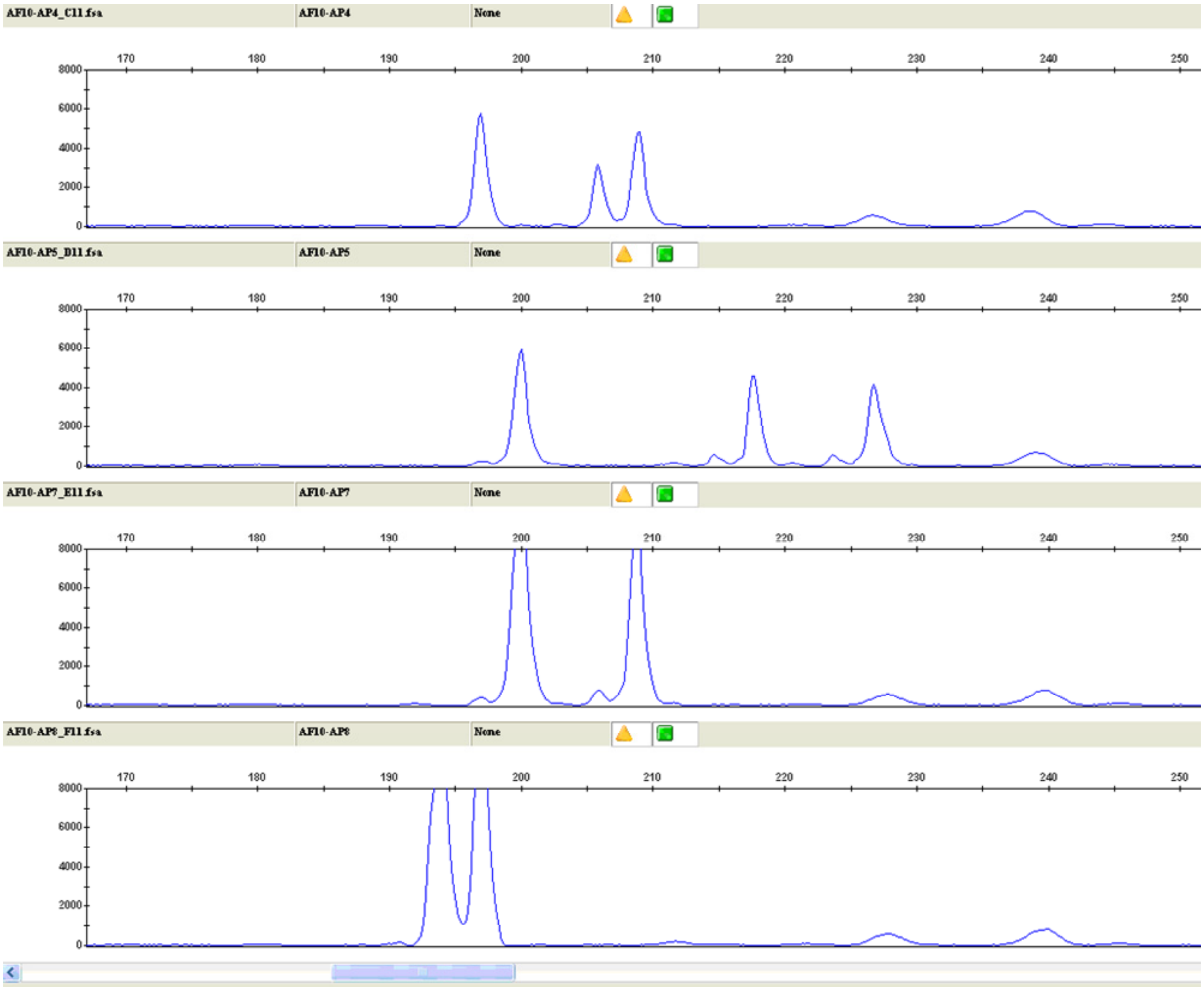

Figure 17. Genotyping profile for SSR [single sequence repeat] primer for locus GCPM2637. *Interpretation*: Each of the four boxes stacked upon each other in the figure represents the migration of microsatellites within the gel following PCR amplification of the DNA contained within the leaves of a single aspen tree. The vertical blips are the locations of the microsatellites within the gel. Where the blips from two different trees line up vertically, those two trees share the same DNA structure in that section of their chromosomes. Blips that appear in one box but not others represent DNA structures that are unique to that individual tree. Note, in this example, GCPM2637 revealed seven alleles (that is, seven different DNA structures) in four individuals. The first two trees were triploids and the last two trees were diploids. Locus GCPM2637 was heterozygous in these four individuals.

Of all the genotypes that we detected in RNMP, 31 percent were polyploids, with both triploids and tetraploids occurring in most stands (table 4). The proportions varied greatly from stand to stand, but on average, triploid accounted for 27 percent, and tetraploid accounted for 18 percent of the sampled trees; the remaining 55 percent were the usual diploid condition. The percentage of polyploids varied significantly between the east and west sides of RMNP. On the east side, the polyploids accounted for 78 percent of the sampled trees compared with 23 percent on the west side. The habitats in which aspen grow appear to be more varied on the east side than on the west side, and the east side generally receives less winter moisture, which may result in more stressful environments for east-side aspen.

It should be noted that whether these trees are complete triploids and tetraploids remains unclear, as they may be simply be aneuploids (that is, they may have an extra copy of only one or a few chromosomes, not a duplication of the entire genome). In the language of molecular biology, the evidence for triploids varies from 1 locus to 22 loci out of 36 loci, whereas that for tetraploids varies from 1 to 2 loci out of 36 loci. We cannot tell whether the genotype of a locus is AAB/ABB or diploidy AB. Thus, cytogenetic evidence will be required to make a definitive conclusion.

The high percentage of polyploids detected in our samples suggests that these genotypes may be of special conservation significance and therefore may deserve special management attention. We propose that genome doubling has been an important genetic mechanism of adaptation for aspen growing in the generally harsh environments of RMNP (we mean environments that are harsh for aspen, especially on the winter-dry east side, though these environments are not necessarily harsh for other species such as lodgepole pine). Polyploidy is typically more prevalent in more stressful environments. Moreover, because triploids are generally not capable of sexual reproduction, such genotypes can persist only by way of vegetative root suckering, which can expand the clone only a limited distance. Therefore, special care for triploids in RMNP may be called for. Moreover, the tetraploids are the usual resources for the generation of triploids. Thus, recruitment of triploid genotypes within the harsher environments that characterize portions of RMNP will require the maintenance of both diploid and tetraploid genotypes on the landscape.

In a previous report to Rocky Mountain National Park, we ranked our sampled stands by level of polyploidy and provided GPS coordinates to help locate individual stands that park manager’s may consider for protection from elk browsing or other potential stresses if they desire to manage in a manner that would maintain aspen genetic diversity on the winter range (table 5).

Table 5. Summary and ranking of genetic diversity (number of unique genotypes detected) and proportion of triploid and tetraploid individuals in 10 aspen stands sampled in 2005 within elk winter range on the east side of Rocky Mountain National Park. Note that the "stands" referred to in this table are larger units than the "patches" referred to in the text. The trees within a patch all appear very similar with respect to bark characteristics, tree height, and overall growth form. A stand as described in this table is a spatially distinct aspen grove that is separated from other aspen groves by areas of fundamentally different vegetation (for example, meadows or dense conifer forests). Each stand may be composed of two or more contiguous but distinctive-appearing patches.

Stand	Overall combined ranking[1]	Ranking: number of genotypes[2]	Ranking: proportion triploids and tetraploids[3]	General Location within the park	Latitude	Longitude
22	1	3	4	West of Horseshoe Park	40.41805 – 40.42045	105.67818 – 105.68299
24	2	7	1	Wild Basin trailhead	40.21040 – 40.21825	105.53652 – 105.55167
20	3	6	5	South side of Horseshoe Park	40.39965 – 40.40257	105.63003 – 105.63411
23	4	5	6	Wild Basin trailhead	40.20388 – 40.20865	105.55954 – 105.56696
7E	5	8	3	East end of Beaver Meadows	40.36312 – 40.36449	105.36312 – 105.36449
B6	6	1	10	South side of Horseshoe Park	40.40001 – 40.40489	105.62727 – 105.63217
7W	7	2	9	East end of Beaver Meadows	40.36433 – 40.36716	105.58535 – 105.59313
21	8	4	7	West of Horseshoe Park	40.41470 – 40.41612	105.66247 – 105.67087
B5	9	10	2	Northeast corner Moraine Park	40.35806 – 40.35949	105.58722 – 105.58789
8E	10	9	8	West end of Moraine Park	40.35511 – 40.35719	105.61923 – 105.63049

[1] Determined by combining the two other ranking systems (number of genotypes and proportion of triploids and tetraploids) into a single ranking: 1 = highest ranking, that is, greatest overall genetic diversity; 10 = lowest ranking, that is, least genetic diversity.

[2] Number of unique genotypes detected among the trees sampled within the stand (corrected for sampling intensity, that is, the total number of trees sampled). 1 = highest ranking, that is, greatest number of genotypes detected relative to sampling intensity; 10 = lowest ranking, that is, least number of genotypes detected relative to sampling intensity.

[3] Proportion of sampled trees that were either triploids or tetraploids. 1 = highest ranking, that is, greatest proportion of triploids and tetraploids combined; 10 = lowest ranking, that is, least proportion of triploids and tetraploids combined.

Question #2 — Techniques for Evaluating Clone Ages and Seedling Recruitment

2a. Computing somatic mutation rates and constructing a "molecular clock" for aspen clones: We have been successful in detecting somatic mutations (as described herein), and therefore are confident that we will be able construct a "molecular clock" and to estimate the absolute age of the sampled aspen clones in RMNP. However, this work is not yet complete, because we ran into an unexpected difficulty in determining the ages of the trees (the ramets) that were sampled (details below). Therefore, we cannot yet provide any of the specific answers to the question of how long ago the aspen clones in RMNP became established from original seedlings (ortets). However, we expect to resolve the tree-aging issue soon and if successful will send the park a separate report on this topic as soon as it is completed. Although it is disappointing not to be able to submit at this time the final results of this very interesting aspect of our genetics research in RMNP, we are heartened by the fact that we regarded this as a high-risk objective from the outset (given the technical challenges involved, and the fact that nobody previously had been able to do this).

When we grouped the different trees into genotypes, any two ramets that differed at 2 or more loci out of 37 loci were deemed mutants of the same genotype. Altogether, we detected nine mutation events within single ramets using the 37 SSR primers throughout the sampled trees. These mutants have been verified by repetitive PCR amplification. Thus, we conclude that it is feasible to set up the molecular clock to derive the age of the consensus root sucker (that is, the time that has elapsed since the original ortet became established).

The obstacle is in obtaining an accurate measure of the ages of the aspen stems that were sampled (fig. 18). We are using a new technique for counting the annual rings on increment cores, called WoodCAT (Tuskan and others, 1999). This technique has worked well in previous studies with *Populus* spp. but, because so many of the cores from RMNP contain substantial heart rot, we have to rely on an associated reconstruction technique for predicting stem age (fig. 19). When we complete this work we will be able to associate the occurrence of somatic mutations with specific stem ages. From this information we will estimate the absolute age of the clones in which we detected somatic mutations.

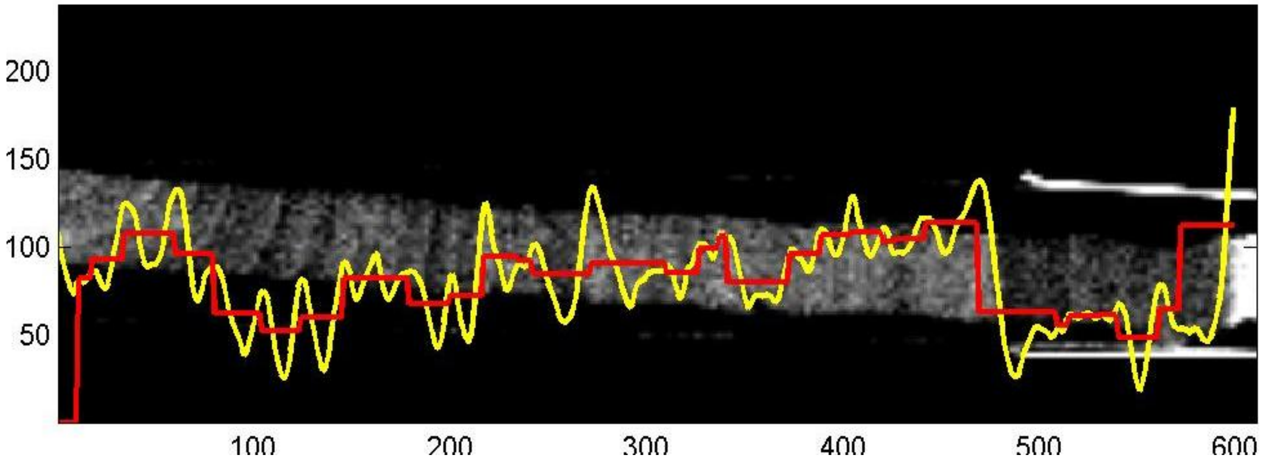

Figure 18. Preliminary automated wood sample annular ring counting (yellow plot) and relative density (red plot) analysis results; scale is pixel number along the transverse (x-axis) and radial (y-axis) planes.

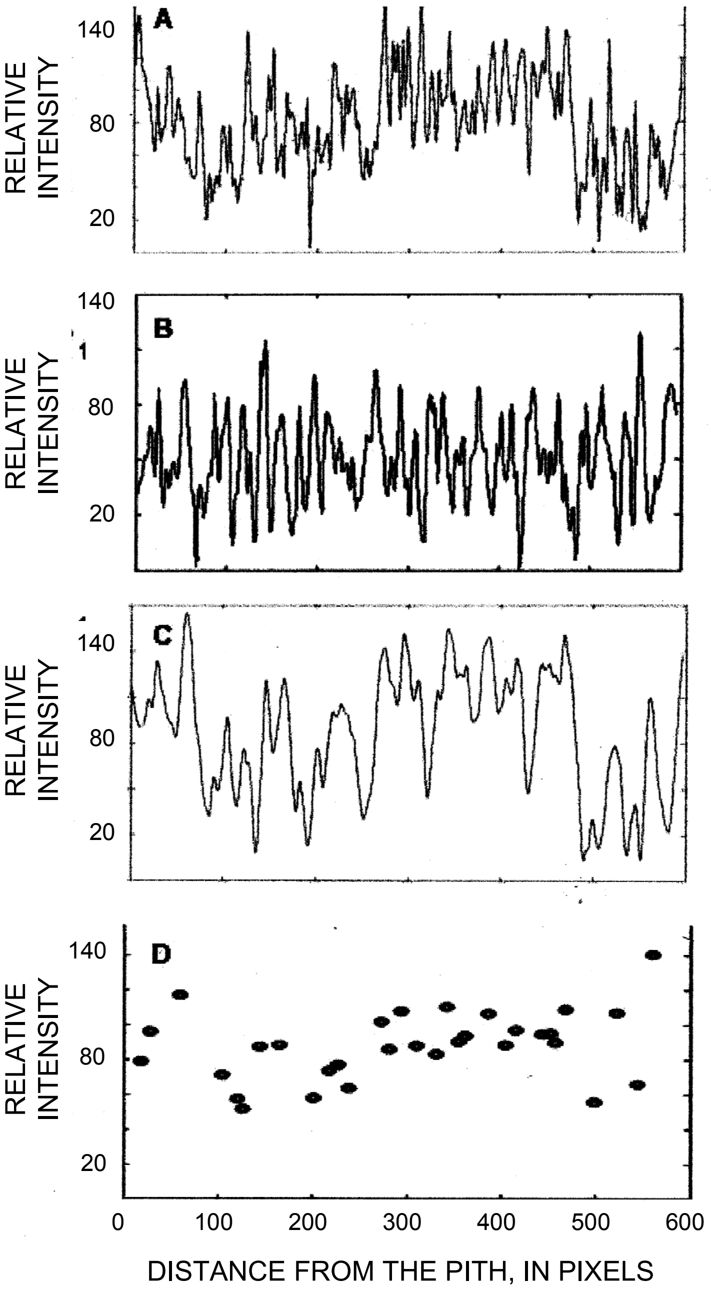

Figure 19. Intensity plot of the wood core image (see Figure 18) along the length (black line) (A). First derivative of the raw intensity data (B). Smoothening of the raw intensity data (C). Final peaks (D). The threshold was set at a level of 70 and only the peaks above the threshold were counted. These final peaks correspond to the number of rings in the wood core data. The total number of rings equals 31.

2b. Genetic evidence of aspen seedling establishment: Based on the frequency of the genetic markers used in this study, we conclude that asexual vegetative reproduction (root sprouting) is the dominant mode of aspen reproduction in RMNP. Overall, more than 70 percent of trees were found to be the result of vegetative reproduction from single genets. This is hardly surprising: it has long been recognized that aspen throughout the Rocky Mountains reproduce primarily by way of root sprouting and that seedling establishment is very infrequent. However, the genetic findings from this study suggest that aspen seedling establishment has been far less infrequent than commonly assumed. The fact that almost every aspen patch is composed of multiple genotypes, including many genotypes represented by a single individual stem, strongly suggests that seedlings have periodically become established and have added genetic diversity to these patches. The high overall genetic diversity throughout the park and the fact that nearby aspen patches and stands have more-or-less unique sets of aspen genotypes also support the idea of periodic seedling establishment.

More research is needed to fully understand the role of seedling recruitment in the broad-scale and long-term population genetics of RMNP aspen. A large cohort of aspen seedlings was documented after the 1988 Yellowstone fires (Romme and others, 1997, 2005), and it was suggested from those studies that aspen seedlings may become established primarily in the immediate aftermath of high-severity fires. Aspen seedlings also have been reported from the Kawuneechee Valley in RMNP. However, rigorous studies have not yet been conducted in RMNP to determine whether these seedlings in Kawuneechee Valley are unusual or are typical of the park as a whole.

Conclusions and Management Considerations

Based on the findings of all components of this study, the low-elevation, winter range aspen stands of Rocky Mountain National Park are without doubt a unique resource, and conservation efforts to protect them will likely increase their chances for continued viability. These stands appear to be highly resilient in the face of very intense herbivory by elk and harsh environmental conditions. They have adapted through processes of natural selection to produce many genetically unique individuals that may have a range of characteristics specifically selected to help them cope with the intensive herbivory and xeric site conditions that characterize the low-elevation, east side elk winter range of RMNP. Conservation efforts through fencing protection and decreased elk browsing pressure are already being planned as part of the park's new elk management plan. If these efforts are undertaken, conditions that encourage stem recruitment to the tree canopy will likely result and the continued survival of these aspen stands will be enhanced.

Based on the age structure of aspen in the park, it appears that the interacting effects of fires, elk population changes, and livestock grazing had relatively consistent effects on aspen from 1855 to 1965. Even without the somatic mutation rate data to determine date of clonal establishment for low-elevation, east side winter range aspen stands, the age structure data lead to the conclusion that these stands are not artifacts of the low levels of herbivory that would have accompanied elk extirpation or predation influences in the late 1800s. Thus far, our data appear to negate the argument that these stands only had the opportunity to establish under conditions of low elk densities and therefore are an anomaly and do not deserve special protection because an "un-natural" condition led to their existence on the landscape. Somatic mutation rate age data could further identify the exact timing of aspen clone establishment and provide some basis for correlating establishment with any documented events of fire, human habitation, and so forth.

Based on our current findings, if the park includes in its aspen fencing plan at least some of those stands listed as being of high genetic significance (see table 5), then a large component of aspen genetic diversity will be preserved. The current regeneration status of stands listed in table 5 that are outside the winter range but still on the east side of the park was not determined by this study. However, these stands could be surveyed with minimal effort to determine whether they would benefit from fencing protection to enhance their long-term sustainability and thus conserve their unique genetic qualities. Fenced areas that are at least 2 acres in size and encompass areas along stand boundaries that contain few or no large stems, as well as large sections of existing stands, would likely be adequate to create conditions for stand regeneration and expansion beyond current stand footprint.

Periodic surveys could be conducted in nonfenced stands to assess the effectiveness of fencing and elk reduction efforts in achieving aspen stand regeneration. A vegetation monitoring plan currently being developed by RMNP and USGS targets monitoring the effects of elk management on key vegetation communities that are strongly affected by elk browsing, including aspen stands. If the park wishes to determine whether changes in relative elk density appear to be occurring under the new management plan, then analysis of future elk survey data could be conducted to attempt to correlate elk density or population changes to any observed changes in aspen stand regeneration. Aspen offtake levels also might be used as a means to determine whether stands that were previously experiencing low levels of browsing are subject to more intense browsing and lower rates of regeneration following elk management actions.

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