Plant Species Biology (2001) 16, 219–230

Demographic genetics of the American beech, *Fagus grandifolia*. II. Genet substructure of populations for the Blue Ridge, Piedmont and the Great Smoky Mountains

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Abstract

Populations of American beech in Virginia and the Great Smoky Mountain National Park in Tennessee and North Carolina were investigated for demographic genetic substructurings. Two Virginia populations, one on the Blue Ridge (WG1) and the other on the Piedmont (WG2) occur over an elevational gradient of several hundreds meters. One of the Great Smoky Mountain populations (GS1) was in a 'beech gap' and the other (GS2) in a 'cove forest' along a creek. The populations in the Great Smoky Mountain National Park were only separated by a few hundred meters in elevation, but both on the same physiographic province. The populations had two growth forms. Trees produced extensive root suckers at WG1, GS1 and GS2, but WG2 had no root suckers and all individuals had obviously been established from seeds. A total of 1335 shoots were mapped at the four sites, their size measured [diameter at breast height (DBH) or diameter at ground height (DGH)], and genotypes were determined for each locus using allozyme analysis. F_{IS} among five different size-classes revealed an excess of homozygotes in WG1, GS1 and GS2, and an excess of heterozygotes in WG2. The offshoot formation from root suckers obviously contributed to the abundance of intermediate size-classes in WG1, GS1 and GS2. Exceedingly localized patchiness of different multilocus genotypes reveals genetic clustering of shoots that have obviously originated from root suckers in WG1, GS1 and GS2. The Piedmont population (WG2), on the other hand, showed loose localization of genetically related trees at a scale of 35–40 m in area, suggesting broader ranges of pollen and seed dispersal. The data are discussed in the light of the differences in growth form and mode of reproduction, and also in relation to the post-glacial migration and the current geographic distribution of the species.

Keywords: allozyme, American beech, clonal diversity, *Fagus grandifolia*, *F*-statistics, root sucker.

Received 1 April 2001; revision received 17 October 2001; accepted 20 October 2001

Introduction

Differentiation in growth forms usually reflects environmental constraints, interactions with other plant and animal species within communities, and the origin and evolutionary history of a given species. Genetically fixed growth forms within a species represent the consequences of adaptation to environmental regimes of the habitat for that particular species. Direct effects of environmental constraints are expressed in varying phenotypic expressions of a specific genotype (Bradshaw 1965), and thus genotype–environment interaction is a prime factor in the evolution of a specific growth form.

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In a previous study, we reported the results of demographic genetic analyses on northern populations of *Fagus grandifolia*, the American beech (Kitamura *et al*. 2000). The northern half of the North American continent was entirely covered by huge glaciers during the Pleistocene Ice Age, and vegetation including deciduous hardwood forests had completely retreated (Davis 1983; Delcourt & Delcourt 1987). Thus contemporary vegetation in glaciated areas are all consequences of geological successions from the southern refugia, including the Apalachicola region of Florida. Northern populations of the American beech, which extend over shallow soil layers in the glaciated areas, are known to produce root suckers (Ward 1961). This was confirmed in our earlier studies (Kitamura *et al*. 2000; Kitamura & Kawano 2001), in which we showed a dominance of clonally produced plant forming populations, having different multilocus genotypes intermingling with one another and forming complex patches. However, we also found that the recruitment by sexual reproduction was also prevalent in northern populations of American beech (Kitamura *et al*. 2000).

In Virginia, American beech (*Fagus grandifolia* Ehrh.) is distributed over an altitudinal gradient from the Piedmont (ca. 300 m in elevation) to the Blue Ridge (ca. 500–700 m in elevation). It is an interesting question whether or not beech stands that developed over the altitudinal gradient in Virginia share a similar life history as well as genetic structures. A morphological study of American beech populations in North Carolina (Cooper & Mercer 1977) indicated the existence of two distinct varieties; a root sucker type at higher elevations and a non-root sucker type at lower elevations, just as found in Piedmont-Coastal Plain groups (Kitamura *et al*. 2000).

Further south from the Blue Ridge, American beech populations are scattered throughout the Appalachian Mountains, although they do not form continuous populations. In the Great Smoky Mountains, there are isolated populations of American beech called 'beech gaps' at higher elevations and isolated remnant populations in 'cove forests' that occur in valleys along streams at lower elevations (Russell 1953; Peterson & Jones 1997; Busing 1998). It is also interesting to see whether or not root sucker formation is as prominent as a regeneration form for American beech populations in the southern Appalachians.

The purpose of this study is to evaluate the demographic genetic substructurings for the central–southern ridge and valley populations of American beech. The size-class structures and fine-scale spatial structures of allozyme variations were investigated to reveal whether or not there are any different spatio-temporal genetic substructurings in populations in the Blue Ridge, Piedmont and southern Appalachians.

Materials and methods

Study sites and sample collections

We chose four study sites (Table 1). Transects were established in two distinct populations in the Wintergreen Resort area, Virginia; one in the Shamokin Springs Nature Reserve on the Blue Ridge (WG1, 10 m × 50 m), and the other in the Stoney Creek Park located at the upper marginal of the Piedmont (WG2, 10 m × 100 m). Two additional transects were established in the Great Smoky Mountains; one was located at a typical 'beech gap' (Russel 1953) on the ridge (GS1), and the other in a remnant 'cove forest' (Whittaker 1966) in the valley (GS2).

The ridge population at Wintergreen (WG1) has a high density of *Fagus* with extensive root sucker formation. The study site was on a moist fluvial terrace along the small creek with a shallow soil layer. Seedlings were seldom observed at WG1.

In the Piedmont population (WG2), American beech was mixed with *Tsuga canadensis*, and seedlings were abundant on the forest floor that was covered with a rich floodplain soil layer. Root sucker formation was not significant in the Piedmont population. The two Wintergreen sites were very close, only ca. 10 km apart and a couple of hundred meters in elevation.

Two study sites were established in the Great Smoky Mountains (GS1 and GS2), Tennessee and North Carolina,

Table 1 Study sites for *Fagus grandifolia* in Virginia and Great Smoky Mountains

Study plot	Location	Elevation	Plot size	No. shoots	PL	MLG
Wintergreen, VA						
WG1	Blue Ridge	500–700 m	10 m × 50 m	501	4	11
WG2	Piedmont	ca. 300 m	10 m × 100 m	327	13	169
Great Smoky Mts, TN & NC						
GS1	Beech gap, NC	1300 m	30 m × 40 m	323	2	4
GS2	Cove forest, TN	730 m	35 m × 15 m	184	3	3

PL, number of polymorphic loci; MLG, number of multilocus genotypes.

which are referred to as southern Appalachian forest type (Russel 1953). The ridge site GS1 (30 m × 40 m) was established in a beech gap at a higher elevation in the Great Smoky Mountains; 4.8 km from New Found Gap on Clingmans Dome Road. The shrubby beech shoots occurred in very dense stands or 'islands' in the spruce-fir forest (*Picea rubens–Abies fraseri*) (Russell 1953).

Site GS2 (35 m × 15 m) was established in a cove forest at a lower elevation along Porter's Creek. Here, American beech was mixed with some other deciduous tree species such as *Acer saccharum* and *Aesculus octandra*.

All shoots of the American beech within transects and mature trees in the surroundings of the study plots were mapped and their diameters at ground height (DGH) or at breast height (DBH) were measured. A total of 1335 shoots (501 in WG1, 327 in WG2, 323 in GS1 and 184 in GS2) were measured and their leaves collected for enzyme electrophoreses. Sampling was carried out in May 1997 in WG1 and WG2, and July 1998 in GS1 and GS2. Enzyme extractions, electrophoreses and detections of allozymes follows the same procedures as described in Kitamura and Kawano (2001). Genotypes of 17 allozyme loci from 13 enzyme systems (Table 2) were critically evaluated for subpopulational differences.

Data analyses

Genetic differentiations in size-classes Each shoot was placed into one of five size-classes according to DGH or DBH: class 1 = DGH < 5 mm; class 2 = DGH between 5 and 10 mm; class 3 = DGH between 10 and 25 mm; class 4 = DGH between 25 and 300 mm; and class 5 = DBH over 300 mm.

Wright's *F*-statistics (Wright 1943, 1951, 1965) were calculated according to Weir and Cockerham (1984) using the computer program FSTAT (Goudet 1995) for different size classes of four study plots.

Clonal diversity for each size-class was estimated by Simpson's index (*D*: Pielou 1966). For calculations, we identified a multilocus genotype as a clone.

Spatial genetic differentiations Shoot genotypes were plotted on maps with the relative size of shoots shown for all polymorphic loci. A combined genotype of all polymorphic loci for shoots can be regarded as a multilocus genotype for each shoot. Thus, different multilocus genotypes are considered to represent different clones.

Spatial substructurings were analyzed by spatial autocorrelations (Sokal & Oden 1978a,b): Moran's *I* (Moran 1950; Duncan & Stewart 1991). Ranges of genetic patchiness and levels of genetic clusters were compared by distrograms based on the estimated values and the point at which the value changes from significantly positive to negative. For diallelic loci, either of the two alleles were used for calculations and represented its locus. In order to avoid inaccurate estimations biased by a small number of allele frequencies, rare alleles whose frequency was less than 0.05, and loci whose common allele exceeded 0.95 in frequency were excluded from the calculations.

Spatial autocorrelations were calculated based on the distance class of 5 m-intervals (*I*(5)), and estimated up to 50 m distance in WG1 and WG2, and up to 30 m distance in GS1.

Table 2 Allozyme loci used in this study

Enzyme (abbreviation)	Locus
6-Phosphoglucose dehydrogenase (6PGDH)	*6Pgdh1, 6Pgdh2, 6Pgdh3*
Acid phosphatase (ACP)	*Acp*
Aconitase (ACO)	*Aco*
Alcohol dehydrogenase (ADH)	*Adh1, Adh2*
Amylase (AMY)	*Amy*
Diaphorase (DIA)	*Dia*
Fumarase (FUM)	*Fum*
Glutamate oxalomate transaminase (GOT)	*Got1, Got3*
Isocitric acid dehydrogenase (IDH)	*Idh*
Leucine aminopeptidase (LAP)	*Lap*
Malate dehydrogenase (MDH)	*Mdh*
Phosphoglucose isomerase (PGI)	*Pgi*
Phosphoglucomutase (PGM)	*Pgm*

Results

Differences in size-class genetic components

Size-class distributions of shoots for each study site are shown in Fig. 1. The number of shoots was largest in intermediate size-classes for WG1, GS1 and GS2, while WG2 showed a L-shaped distribution.

Allozyme polymorphisms were observed in four loci (*6Pgdh2*, *6Pgdh3*, *Lap* and *Pgi*) for WG1, 13 (*6Pgdh2*, *6Pgdh3*, *Acp2*, *Adh1*, *Amy2*, *Fum*, *Got1*, *Got2*, *Idh*, *Lap*, *Mdh*, *Pgi* and *Pgm*) for WG2, two (*Lap* and *6Pgdh3*) for GS1 and three (*Lap*, *Fum*, and *Acp*) for GS2. The number of shoots for each genotype in polymorphic loci is listed in Appendices I–IV. In general, genetic components did not differ among size-classes in WG1, GS1 and GS2. On the contrary, genetic components were different among size-classes in WG2.

In WG2, rare genotypes were observed only among smaller size-classes in eight of the 13 polymorphic loci: *Amy2*, *Got1*, *Got2*, *Idh*, *Lap*, *Mdh*, *Pgi* and *Pgm*. Rare genotypes and/or alleles of *6Pgdh3* were observed in size-classes 1 and 3. A rare allele *Adh1-a* was observed in size-classes 1 and 4.

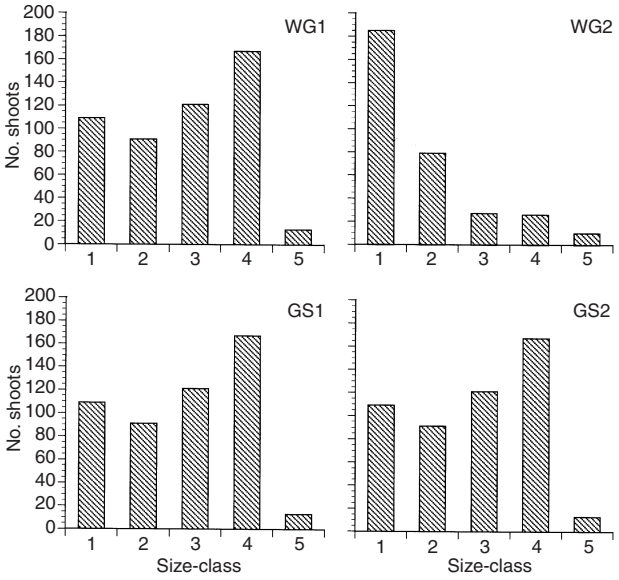

Fig. 1 Size-class distributions of four study sites. Five size-classes were discriminated according to DGH or DBH class 1 = DGH < 5 mm; class 2 = DGH between 5 and 10 mm; class 3 = DGH between 10 and 25 mm; class 4 = DGH between 25 and 300 m; and class 5 = DBH over 300 mm. WG1, Blue Ridge, Wintergreen, Virginia; WG2, Piedmont, Virginia; GS1, Beech gap, Great Smoky Mountains, North Carolina; GS2, Cove forest, Great Smoky Mountains, Tennessee.

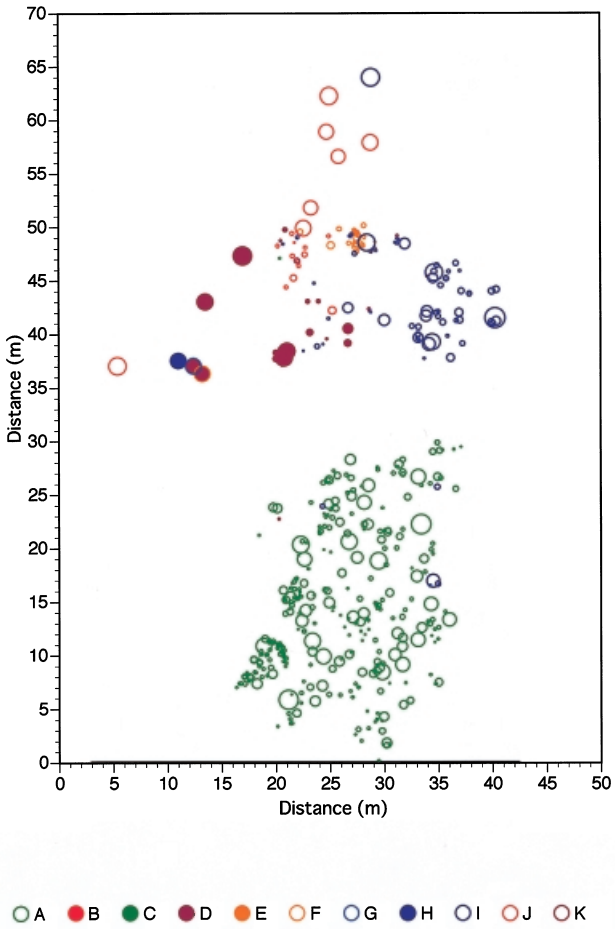

Fig. 2 Map of multilocus genotypes for individuals at WG1, Blue Ridge, Wintergreen, Virginia. Circle size is relative to the diameter of the shoot. Different color symbols refer to different multilocus genotypes.

Based on these polymorphic loci, the numbers of multilocus genotypes detected were 11, 169, 4 and 3, in WG1, WG2, GS1 and GS2, respectively. The most common multilocus genotype included 352 shoots in WG1, 240 in GS1 and 180 in GS2. It is notable for site GS2 that all but two shoots were fixed to one multilocus genotype.

Spatial distributions of genotypes

Distributions of genotypes in polymorphic loci demonstrate different genetic substructurings among populations, with specific patterns at each site.

Shoot density was extremely high in WG1 (ca. one shoot/sq. m), compared to the other three study sites (ca. 0.3 shoot/sq. m). In all four polymorphic loci (Appendix I), the patchiness of the genotypes is very clear for WG1. Various degrees of genotype patchiness were observed with each forming a tight clump with itself. Thus, there was not much overlapping of different genotypes at WG1. These patterns of genotype patches were similar to each other in all polymorphic loci in WG1.

A loose concentration of genotypes was observed in WG2 (Appendix II). Several loci such as *6Pgdh2*, *6Pgdh3*, *Pgi* and *Pgm* showed allelic patchiness in the larger shoot sizes. Genotypic polymorphism was maintained throughout the study plot, for example, as revealed in the spatial distributions of *Acp2*, *Fum* and *Lap*. Rare genotypes were scattered, mostly among small shoots, for example, in *Adh1*, *Amy2*, *Got1*, *Got2*, *Idh* and *Mdh*.

In GS1, genotype patchiness was clearly observed in two polymorphic loci (Appendix III). The study site was spatially split into two genotypes for *6Pgdh3*; *bc* heterozygote in the upper part of the slope and *bb* homozygote for lower part. The population was also spatially divided into three parts with three major genotypes for *Lap*. Genotype substructurings in this ridge population showed a somewhat similar pattern to the WG1 population; tight clumps of the same genotype and not much intermingling with different genotypes.

An extremely striking distribution of genotypes was observed in GS2 (Appendix IV). All sampled shoots were fixed to one genotype except for one small shoot and one intermediate size shoot for *Fum* and *Acp*, respectively. These two shoots also have different genotypes for *Lap* (Appendix IV). It was remarkable to see that only one single genotype for each polymorphic locus was thriving in a 10 m × 30 m plot (Fig. 5).

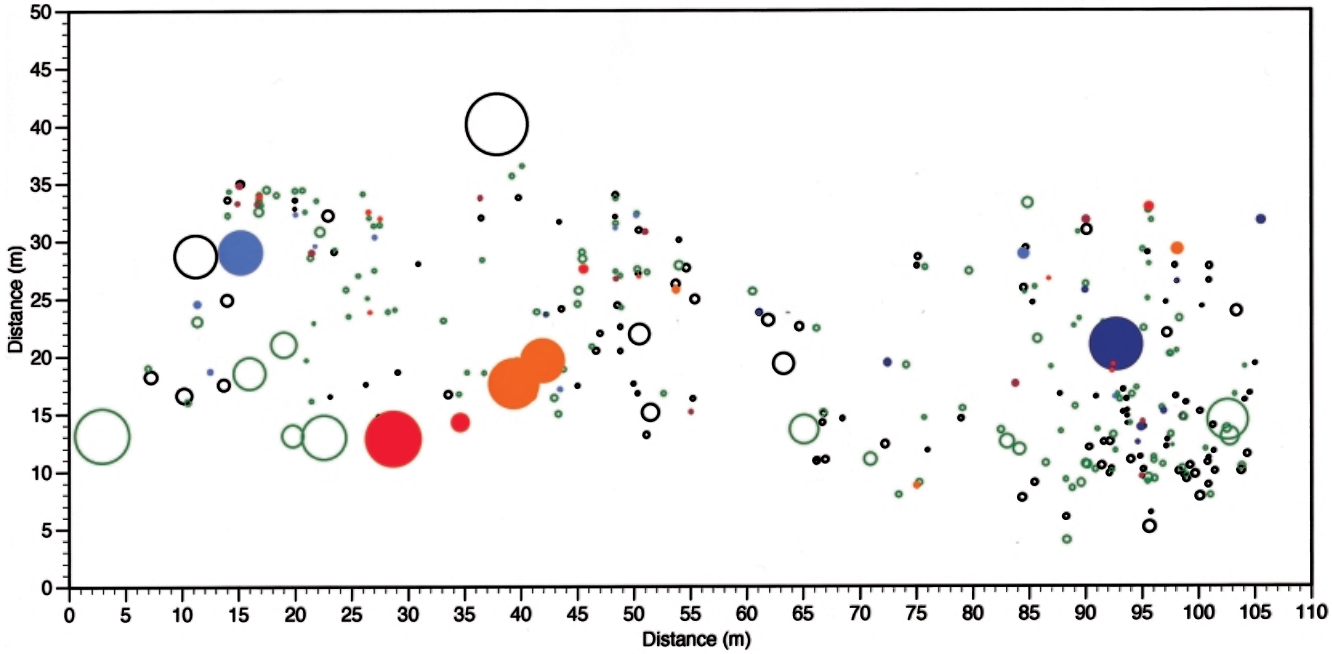

Fig. 3 Map of multilocus genotypes for individuals at WG2, Piedmont, Virginia. Circle size is relative to the diameter of the shoot. Different color symbols refer to different multilocus genotypes. Black circles represent shoots with unique multilocus genotypes.

Genotypes of all polymorphic loci were combined to show multilocus genotypes. The distributions of multilocus genotypes in relation to shoot size are shown in Figs 2–5 for WG1, WG2, GS1 and GS2, respectively. Each multilocus genotype is considered to represent a clone, and thus distributions of clonal structures are clearly shown.

In WG1, only 11 multilocus genotypes were observed among the 501 shoots investigated. A remarkable feature of WG1 is that one huge clone (A in Fig. 2) covers most parts of the transect without any other intermingling clones, reflecting extensive root sucker formation (Fig. 2). This largest multilocus genotype A, indeed, includes 352 shoots, and is expanding exclusively against other clones. The other major four genotypes (D, F, I, and J) also form compact clumps without intermingling with each other. Only a few shoots of D and I were established within a huge clone A, which are possibly of seedling origin.

On the contrary, a large number of multilocus genotypes (a total of 169 genotypes) were observed in the Piedmont population (WG2) (Fig. 3), of which 116 were unique multilocus genotypes. However, no conspicuous clonal patchiness was recognized in the WG2 population.

The distribution of multilocus genotypes in GS1 clearly showed clumping of different multilocus genotypes with a similar manner of spatial distributions. This is a good indication of clonal expansions (Fig. 4). Large-sized shoots were surrounded by intermediate-sized shoots of the same genotype, forming a clone substructure, which is extremely localized to the immediate neighbors.

An extreme case of clonal structure was observed in GS2 (Fig. 5). Here, only two shoots, which are possibly of seedling origin with different genotypes (B and C), were established within a huge clone of a single multilocus genotype (A). The expansion of genotype A was ubiquitous over the 10 m × 30 m plot. This evidence indicates that the potential for root sucker formation might be exceedingly high in the 'cove forest' of the Great Smoky Mountains.

Clonal diversity in four study sites

Figure 6 shows the clonal diversity estimated by Simpson's index (*D*) for each size-class and total population, using a multilocus genotype as a clone. This parameter represents the number of genotypes/shoot and is sensitive to common genotype changes.

The Piedmont population (WG2) showed a high clonal diversity in all size-classes, indicating that unique genotypes were abundant in all size-classes. Populations on the ridges (WG1 and GS1) showed lower values in the intermediate size-classes, high in larger size-classes, while size-class 1 are higher than intermediate size-classes. Higher values for size-class 1 in WG1 and GS2 indicate the introduction of new multilocus genotypes by seedling establishment. The cove forest of the Great Smoky Mountains (GS2) was almost zero throughout all size-classes, indicating practically no clonal variation in this relict population regardless of size-classes.

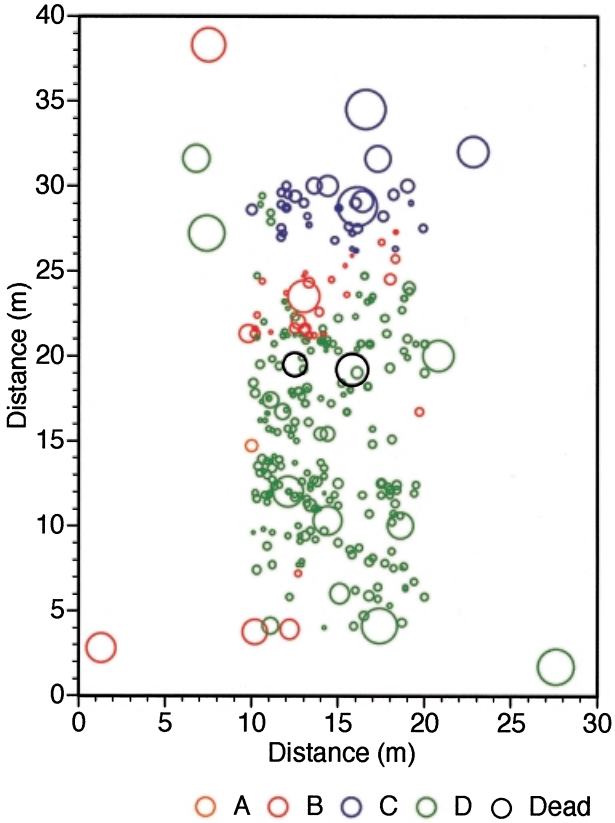

Fig. 4 Map of multilocus genotypes for individuals at GS1, Beech gap, Great Smoky Mountains, North Carolina. Circle size is relative to the diameter of the shoot. Different color symbols refer to different multilocus genotypes. Black circles represent dead shoots.

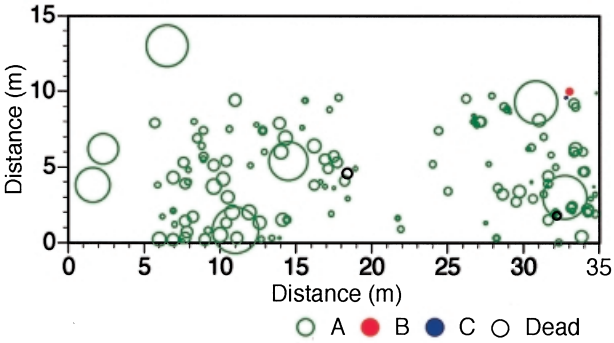

Fig. 5 Map of multilocus genotypes for individuals at GS2, Cove forest, Great Smoky Mountains, Tennessee. Circle size is relative to the diameter of the shoot. Different color symbols refer to different multilocus genotypes. Black circles represent dead shoots.

In light of Simpson's index for total population, WG2 had a value close to one, while WG1 and GS1 had intermediate values. GS2 showed a very low value of nearly zero for the clonal diversity. A similar value was found for clonal diversity in the two ridge populations, WG1 and GS1.

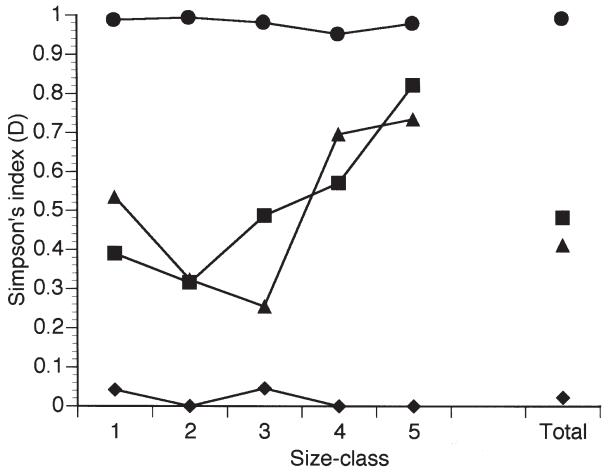

Fig. 6 Changes in Simpson's index for size-classes. ■, WG1; ●, WG2; ▲, GS1; ◆, GS2.

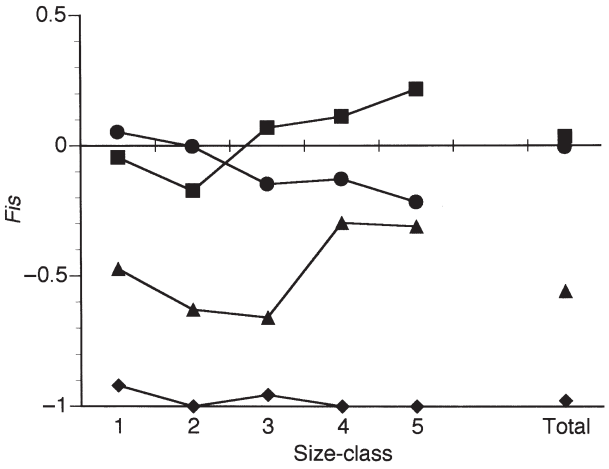

Fig. 7 Changes in inbreeding coefficient, F_{IS}., for size-classes. ■, WG1; ●, WG2; ▲, GS1; ◆, GS2.

F-statistics for size-classes and four study sites

Figure 7 shows F_{IS} for each size-class and for the total population. In WG1, the inbreeding coefficient (F_{IS}) was negative for size-classes 1 and 2, and positive for classes 3, 4 and 5. A negative F_{IS} was observed in WG2 for all size-classes except size-class 1. GS1 showed negative F_{IS} values in all size-classes; however, lower values in smaller size-classes and higher in larger classes. An extreme case is demonstrated in GS2, whose F_{IS} value is equal or nearly equal to –1, since this population is fixed to one heterozygote genotype except for two individuals.

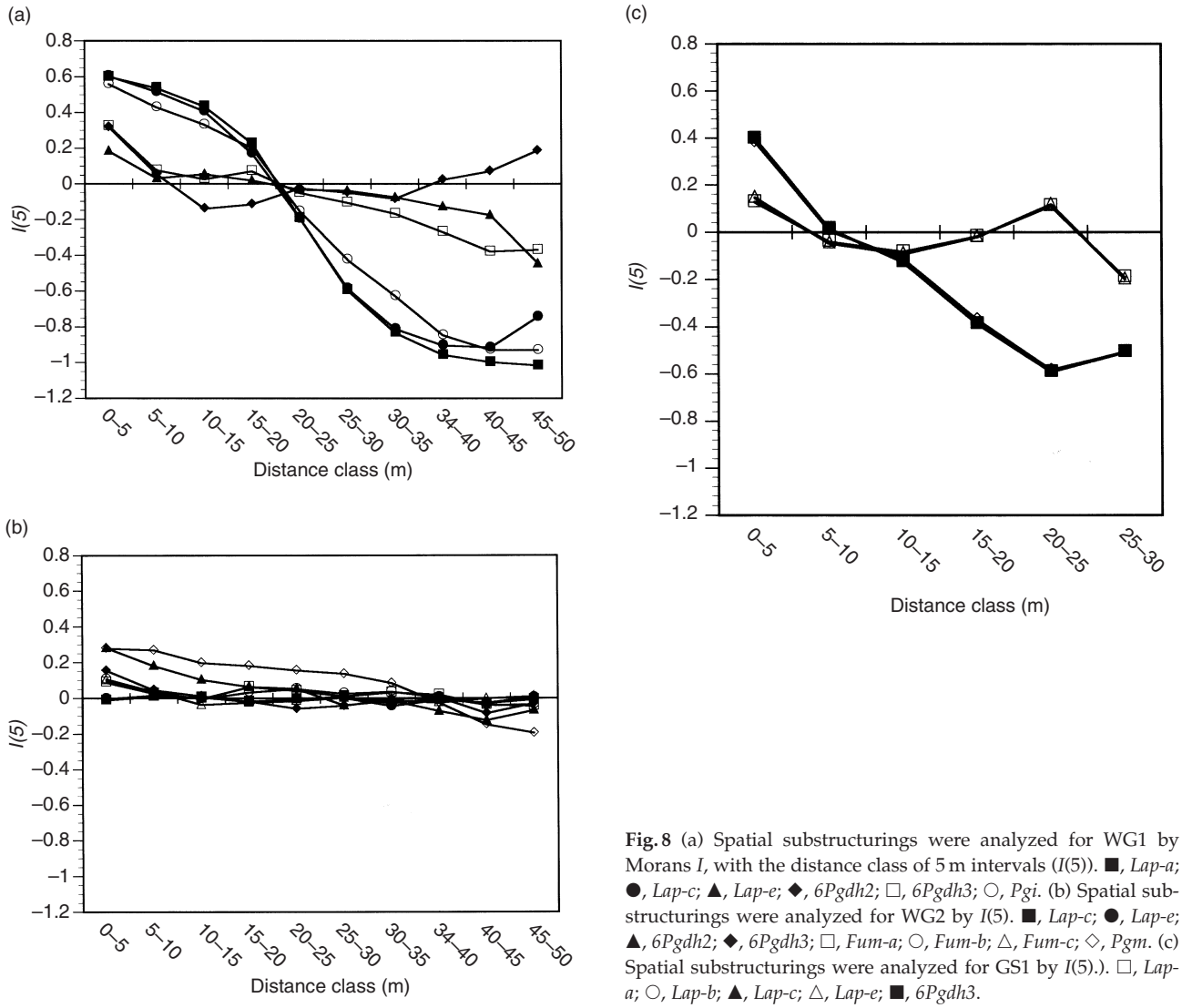

Fig. 8 (a) Spatial substructurings were analyzed for WG1 by Morans *I*, with the distance class of 5 m intervals (*I*(5)). ■, *Lap-a*; ●, *Lap-c*; ▲, *Lap-e*; ◆, *6Pgdh2*; □, *6Pgdh3*; ○, *Pgi*. (b) Spatial substructurings were analyzed for WG2 by *I*(5). ■, *Lap-c*; ●, *Lap-e*; ▲, *6Pgdh2*; ◆, *6Pgdh3*; □, *Fum-a*; ○, *Fum-b*; △, *Fum-c*; ◇, *Pgm*. (c) Spatial substructurings were analyzed for GS1 by *I*(5).). □, *Lap-a*; ○, *Lap-b*; ▲, *Lap-c*; △, *Lap-e*; ■, *6Pgdh3*.

Spatial autocorrelation analyses

The results of the spatial autocorrelation analyses are shown in distrograms of Moran's I for 5 m intervals *I*(5) (Fig. 8a–c). We did not calculate the spatial autocorrelation for GS2 since no genetic variation and localization were observed in this study plot.

In Virginia, contrasting patterns were demonstrated in two different distrograms of WG1 and WG2. Both distrograms were positive in the small distance classes and negative in large distance classes; however, the absolute values for WG1 were greater than WG2 in the small and large distance classes. The *I*(5) values for WG1 changed drastically from high positive values in small distance classes to large negative values in large distance classes, while *I*(5) values gently incline from relatively small values in small distance classes to slightly negative in large distance classes for WG2. Since the value indicates the levels of genetic relatedness, these results suggested a strong genetic relationship among near-neighbors in the WG1 site compared to WG2. This is obviously due to high concentrations of extensive root sucker formations.

In addition, distance classes at which the *I*(5) turns from positive to negative were different between the two study sites; 20–25 m in WG1 and 35–40 m in WG2. This result indicates the differences in ranges of genetic clustering of each population. Consequently, WG1 shows a small cluster of highly concentrated genetic structures within a local population. In contrast, WG2 shows genetically loose substructurings in rather large areas. All of the estimated values were significant in WG1, and about half of the values (37 estimates out of 80 calculations) were significant in WG2.

As mentioned earlier, there were only two polymorphic loci for GS1 whose distrogram was somewhat similar to WG1. However, the distance class, in which the index turns positive to negative value (5–10 m), was smaller than that of WG1. This result indicates that the extent of root sucker extension was more confined to the ridge population of the Great Smoky Mountains.

Discussion

The American beech populations that developed on the Blue Ridge are similar to the northern type, with extensive root sucker formation. On the other hand, populations in the upper Piedmont in Virginia represent a typical coastal plain type without any significant root sucker formation (Kitamura *et al*. unpublished data). These two extreme forest types occur over a relatively narrow altitudinal range of only a couple of hundreds meters. In the Great Smoky Mountains, both of the populations analyzed, one in beech gaps at higher elevation and the other in cove forests at middle to lower elevations (Whittaker 1966), had root sucker formation characteristic of the northern type of American beech.

We have demonstrated that the differences among the four stands are reflected in the demographic genetic substructures, which showed clonal and nonclonal patterns. The geographical as well as geohistorical backgrounds of such characteristic demographic genetic features of American beech populations with these two different growth forms were also demonstrated for 21 populations over the entire geographical range of the species (Kitamura & Kawano 2001).

We have recently evaluated the roles and origin of root sucker formation in northern beech stands in heavily glaciated areas such as Quebec, Ontario and the Great Lakes regions (Kitamura *et al*. 2000), as well as those that occur in the north to south-eastern Cordilleran Mountain System (i.e. the Allegheny Mountains, Appalachian Mountains and the Blue Ridges), and the Great Smoky Mountains in Tennessee at the southern end (Davis 1983; Delcourt & Delcourt 1987; Kitamura & Kawano 2001).

Earlier work (Ward 1961; Cooper & Mercer 1977) has previously described similarities in growth forms and other morphological characteristics of the American beech populations isolated along the southern moun-tains and ridges of the Appalachian Mountains, as well as northern populations extending over glaciated territories.

Difference in demographic genetic structures

Earlier genetic analyses on geographic variations of American beech, combined with data from these four populations suggest the different origins between populations with or without root sucker formation (Kitamura & Kawano 2001). A similar situation was also shown in some other deciduous tree species, such as yellow poplar (Parks *et al*. 1994). The size-class distributions of 'with' and 'without' root sucker populations (Fig. 1) clearly indicated the differences in their regeneration processes between these two growth forms. The WG1 and the GS1 populations mainly regenerate by root sucker formation, and seedling establishment seems to be rare. The extreme case of root sucker formation found in the GS2 population indicates that regeneration by sexual reproduction seldom occurs there. The very active root sucker formation obviously contributes to the development of shoots of intermediate size-classes (Sain & Blum 1981). These regeneration processes resulted in extreme size-class skewness with a large number of intermediate size-class shoots (Fig. 1). Since the American beech population in WG1 is established on a fluvial terrace with a shallow soil layer, that show signs of repeated flooding, seedling establishment would be difficult at this particular site.

American beech shoots at GS1 were small in stature and mostly occurred in the canopy gaps in the spruce-fir forest that was densely covered with saplings of maples. In GS2, the American beech showed extensive root sucker formation, which is well over 30 m, although root sucker shoots showed signs of repeated die backs due to shade from a dense, closed canopy of maples, horse chestnuts, yellow poplars as well as beeches. A high competitive interaction exists between new beech shoots regenerated from the root suckers and newly established seedlings.

On the other hand, seedling establishment is obviously prevalent in WG2, which had a typical L-shaped size-class distribution (Fig. 1). WG2 was established on the terrace along a creek, although this site is higher than a flood plain and the frequency of disturbance seems to be very low.

Size-class distributions of genetic components, for example genotype and allele frequencies, and the number of polymorphic loci in different size-classes, also differed among study sites. In WG1, GS1 and GS2, the numbers of polymorphic loci were fewer, and genetic components were homogeneous throughout all size-classes, while a much higher genetic polymorphism was detected in WG2, reflecting effective sexual reproduction (Appendices I–IV).

Differences in the size-class structures of local populations reflect their life history processes and regeneration strategies (Kawano & Kitamura 1997; Kitamura *et al*. 1997a,b; Kitamura *et al*. 2000; Kitamura & Kawano 2001). In the present study, a high level of similarity among size-class genetic components at WG1, GS1 and GS2 suggests that offshoot formation by root sucker plays a significant role in stand development and maintenance. The high percentage of genetically identical shoots in intermediate

size-classes is especially noteworthy. The production of root suckers not only allows the population to occupy empty patches more quickly, but it also plays a key role in the maintenance of a genetic reservoir for the entire local population. As shown in our previous studies (Kitamura *et al*. 2000; Kitamura & Kawano 2001), such circumstances are more obvious in northern populations.

Positive F_{IS} values obtained at WG1 are an indication of vegetative reproduction characteristic of this mountain population with an extensive root sucker formation (Kitamura *et al*. 2000). Positive F_{IS} values in size-classes 3 and 4 of the WG1 population indicate the consequences of overlapping generations provided by root sucker formation to the intermediate size-classes. On the other hand, positive to negative decline in F_{IS} values for WG2 size-classes demonstrates a mixed mating followed by natural selection preferring heterozygotes (Brown 1979). Similar observations were reported for gymnosperms such as Douglas-fir (Shaw & Allard 1985), *Pinus sylvestris* (Muona *et al*. 1987; Muona *et al*. 1988) and *Chamaecyparis obtusa* (Tang & Ide 1998).

However in the Great Smoky Mountains where the root sucker formations are very extensive, F_{IS} values are significantly negative; GS2 is nearly equal to –1. This suggests the fixation of heterozygotes is occurring in the southern Appalachian population. At present, however, we cannot conclude firmly with a small number of polymorphic loci which may bias our estimation.

The high heterogeneity in genetic components among different size-classes at WG2 reflects the consequences of present as well as past effective sexual reproduction, for example, in the number of mutilocus genotypes (MLG) (Table 1). The levels of genetic variation found in the size-class 1 is also direct evidence of genetic reshuffling among mother trees in the study plot. However, the presence of rare genotypes found in specific size-classes also suggests the occurrence of recent as well as past gene flows from mother trees within or outside the sampling plots or nearby patch populations. Likewise, unique variations in intermediate size-classes may represent remnants of past reproductive events (Appendix II). A similar situation was observed for *Fagus crenata* populations (Kawano & Kitamura 1997).

Differences in fine-scale spatial structures

As expected, contrasting patterns of spatial genetic structures between two growth forms were seen in American beech populations. Specific genotypes turned out to be localized exclusively in the WG1, GS1 and GS2 populations (Figs 2,4,5), representing a phalanx type of growth (Lovett Doust & Lovett Doust 1982). In northern populations with root sucker formation (Kitamura *et al*. 2000), different clones were often intermingling with each other and the boundaries among the clones were not sharp. Earlier studies by Jones and Raynal (1986, 1987, 1988) also reported the intermingling of various clones in the northern populations of the American beech. Comparing the clonal structure of these more southerly stands with northern populations, those that occur on the Great Smoky Mountains and Blue Ridge at higher elevations turned out to be similar to the northern stands, forming large clumps of single genets without intermingling with other genets (Figs 2–5).

The distance class at which $I(5)$ values shifted from positive to negative was 20–25 m at WG1 and 5–10 m at GS1, respectively, indicating the ranges of root sucker formation (Fig. 8a,c). The effective range of root sucker formation for WG1 revealed by spatial autocorrelation analyses is in agreement with clonal patterns of the northern populations (Kitamura *et al*. 2000). However, the levels of genetic concentrations or relatedness were much higher in the southern mountain ridge populations.

More diffuse clumps of differing genotypes were observed in WG2; in particular, a large number of unique multilocus genotypes were observed in the WG2 population, suggesting that this population is regenerating mainly by seedlings (Fig. 3). The localizations of genetic variations were, however, observed in several loci, large trees growing side by side with genetically similar small trees in their surroundings. The seed dispersal of the American beech is a typical barochore, although long-range dispersal by birds is also known (Johnson & Adkisson 1985), and thus these genetically similar juvenile trees are assumed to be progenies from neighboring reproductive trees. For populations with only sexual reproduction, the seed dispersal range would be a prime factor in determining the fine-scale spatial geneticstructures.

It is noteworthy that evidence was obtained by spatial autocorrelation analyses for WG2 that suggests the distance at which $I(5)$ significant values shifted from positive to negative was greater compared to populations with a root sucker formation (Fig. 8b). The distance where this shift occurred was 35–40 m, which indicates the seed dispersal ranges from the mature reproductive trees.

Loose clumps of genotypes were well reflected in the small values of spatial autocorrelations. A mixture of various kinds of genotypes that originated from sexual reproduction, makes the distrograms flat (Fig. 8b). Likewise, spatial as well as temporal overlappings of seed dispersal ranges from a good number of reproductive individuals would prevent highly concentrated establishments of genetically related seedlings.

Population differentiations in altitudinal gradient

Northern populations that predominantly spread by root sucker formation no doubt originated from higher eleva-

tion populations scattered in the southern mountain refugia during the Pleistocene Ice Age. The chains of mountains or mountain ranges, such as the Allegheny Mountains, Appalachian Mountains and the Blue Ridge, served as corridors for the American beech and several other plant species that have survived in the southern refugia. This incident is indicated by the similarity of genetic parameters between two populations at high altitude, that is, demographic genetic feature of clonal diversity (*D*; Fig. 6) (see also Kitamura & Kawano 2001). This consistency also indicates that the American beech populations in the Piedmont-Coastal Plain differentiated from their ancestral populations during or after the Ice Age (Kitamura & Kawano 2001), and rapidly expanded their ranges over the lowland habitats, which emerged in the post-glacial periods after the glacial retreat. These forest stands, one on the Blue Ridge and Great Smoky Mountains, and the other on the upper Piedmont, represent different origins and reflect different migratory histories during the post-glacial period. An exceptional feature of the demographic genetic substructuring for the relict population in the GS2 can be explained by the isolation from the ridge population and reduction in population size during the species' geohistorical changes of distribution.

Acknowledgements

We would like to thank Doug Coleman for his support at the Wintergreen study sites and Jerome E. Goudet for supplying the computer program. Thanks are also due to Daiju Hagiwara for his help in the field. This study was supported by Grants-in-Aid for International Scientific Research (Field Research; no. 05041090; no. 08041143 to Shoichi Kawano, Correspondence) from the Ministry of Education, Science and Culture, Japan.

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Appendix I The number of genotypes for polymorphic loci in WG1

		Observed no. shoots for each size-class					
Locus	Genotype	Class 1	Class 2	Class 3	Class 4	Class 5	Total
Lap	*aa*	84	75	85	104	4	352
	ac	7	5	4	13	2	31
	cc	17	10	24	41	5	97
	ce	1	1	8	9	2	21
6Pgdh2	*aa*	5	3	18	17	3	46
	ab	84	76	85	106	4	355
	bb	20	12	18	44	6	100
6Pgdh3	*bb*	106	88	112	157	12	475
	bc	3	3	9	10	1	26
Pgi	*bb*	90	80	89	117	6	382
	bd	19	11	32	50	7	119
Total		109	91	121	167	13	501

Appendix II The number of genotypes for polymorphic loci in WG2

		Observed no. shoots for each size-class					
Locus	Genotype	Class 1	Class 2	Class 3	Class 4	Class 5	Total
Lap	*ac*	3	1	0	0	0	4
	ae	10	0	1	0	0	11
	cc	54	23	4	3	1	85
	ce	86	38	19	19	9	171
	ee	32	17	3	4	0	56
6Pgdh2	*aa*	28	15	7	12	2	64
	ab	82	39	15	10	6	152
	bb	75	25	5	4	2	111
6Pgdh3	*ac*	1	0	1	0	0	2
	bb	142	46	11	15	6	220
	bc	36	30	12	9	3	90
	cc	6	3	3	2	1	15
Fum	*aa*	30	12	1	2	1	46
	ab	74	33	15	13	6	141
	ac	5	5	0	2	0	12

Appendix II *Continued*

Locus	Genotype	Observed no. shoots for each size-class					Total
		Class 1	Class 2	Class 3	Class 4	Class 5	
	bb	69	24	10	2	2	107
	bc	7	4	1	6	1	19
	cc	0	1	0	1	0	2
Pgm	*bb*	126	47	19	21	9	222
	bc	53	31	8	5	1	98
	cc	6	1	0	0	0	7
Pgi	*bb*	178	73	24	23	7	305
	bc	7	5	3	3	3	21
	cc	0	1	0	0	0	1
Adh1	*ab*	1	0	0	1	0	2
	bb	184	79	27	25	10	325
Got1	*ab*	1	0	0	0	0	1
	bb	184	79	27	26	10	326
Got2	*bb*	184	78	27	26	9	324
	bc	1	1	0	0	1	3
Mdh	*ab*	2	2	0	0	0	4
	bb	183	77	27	26	10	323
Idh	*ab*	1	0	0	0	0	1
	bb	184	79	27	26	10	326
Acp	*aa*	172	70	23	19	7	291
	ab	10	7	3	7	3	30
	bb	1	1	1	0	0	3
Amy2	*aa*	185	77	27	26	10	325
	ab	0	2	0	0	0	2
Total		185	79	27	26	10	327

Appendix III The number of genotypes for polymorphic loci in GS1

Locus	Genotype	Observed no. shoots for each size-class					Total
		Class 1	Class 2	Class 3	Class 4	Class 5	
Lap	*aa*	0	0	0	1	0	1
	ac	5	8	14	11	2	40
	ae	12	10	5	12	1	40
	ce	29	79	114	15	3	240
6Pgdh3	*bb*	41	89	119	28	4	281
	bc	5	8	14	11	2	40
Total		46	97	133	39	6	321

Appendix IV The number of genotypes for polymorphic loci in GS2

Locus	Genotype	Observed no. shoots for each size-class					Total
		Class 1	Class 2	Class 3	Class 4	Class 5	
Lap	*aa*	1	0	0	0	0	1
	ac	47	31	43	53	6	180
	cc	0	0	1	0	0	1
Fum	*ab*	1	0	0	0	0	1
	bb	47	31	44	53	6	181
Acp	*aa*	48	31	43	53	6	181
	ab	0	0	1	0	0	1
Total		48	31	44	53	6	182