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Strong spatial genetic structure in peripheral but not core populations of Sitka spruce [*Picea sitchensis* (Bong.) Carr.]

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Abstract

We examined spatial genetic structure within eight populations of Sitka spruce classified as core or peripheral based on ecological niche, and continuous or disjunct based on species distribution. In each population, 200 trees were spatially mapped and genotyped for eight cDNA-based sequence tagged site (STS) codominant markers. Spatial autocorrelation was assessed by estimating p_{ij}, the average co-ancestry coefficient, between individuals within distance intervals. The distribution of alleles and genotypes within core populations was almost random, with nonsignificant co-ancestry values among trees as close as 50 m in core populations. In contrast, the distribution of alleles and genotypes within peripheral populations revealed an aggregation of similar multilocus genotypes, with co-ancestry values greater than 0.20 among trees up to 50 m apart and significant, positive values between trees up to 500 m. The relatively high density of reproductive adults in core populations may lead to highly overlapping seed shadows that limit development of spatial genetic structure. However, in peripheral populations with a lower density of adults, the distribution of alleles and genotypes was highly structured, likely due to offspring establishment near maternal trees and subsequent biparental inbreeding, as well as more recent population establishment at the leading edge of post-Pleistocene range expansion. Conserving genetic diversity in peripheral populations may require larger reserves for *in situ* conservation than required in core populations. These data on spatial genetic structure can be used to provide guidance for sampling strategies for both *ex situ* conservation and research collections.

Keywords: co-ancestry, gene conservation, isolation by distance, peripheral populations, Sitka spruce, spatial genetic structure

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Introduction

Spatial genetic structure is the distribution of genotypes over two-dimensional space (Epperson 1992). It can be assessed through joint analysis of physical and genetic relationships between individuals. Spatial genetic structure is present when the distribution of genetic variation among individuals within populations is nonrandom (McCauley 1997). Many evolutionary and ecological factors can affect the development of genetic structure within plant populations, including seed dispersal patterns and degree of seed shadow overlap (Wright 1943; Bacilieri *et al*. 1994), adult density (Knowles *et al*. 1992; Hamrick & Nason 1996), colonization and disturbance history (Epperson & Chung 2001; Parker *et al*. 2001), spatial and temporal patterns of seedling establishment (Schnabel *et al*. 1998), and micro-environmental selection (Slatkin & Arter 1991). Under restricted dispersal at the population level, genetic drift will lead to local spatial genetic structure, meaning that genetic similarity is higher among neighbours than among more distant individuals.

Spatial genetic structure may also be influenced by the spatial variation of selection through environmental heterogeneity (e.g. Linhart & Grant 1996). Several studies on plant populations using neutral markers have revealed fine-scale, intrapopulation genetic structure that is associated with habitat variation, e.g. edaphic factors for the wind-pollinated *Festuca ovina* (e.g. Prentice *et al*. 2000), and plant community composition for the outcrossing *Gypsophila fastigiata* (Lonn *et al*. 1996). Genetic adaptation to heterogeneous environments may result in the formation of distinct ecotypes, populations of which may or may not continue to exchange effective migrants.

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Studies in temperate tree species have predominantly revealed weak or no within-population spatial genetic structure (Berg & Hamrick 1995; Leonardi & Menozzi 1996; Parker *et al*. 2001). Conifers have often shown random or only weakly autocorrelated spatial distributions of genotypes, a pattern typical of primarily outcrossing species with both pollination and seed dispersal by wind (Xie & Knowles 1991). For example, spatial genetic variation in lodgepole pine [*Pinus contorta* ssp. *latifolia* (Douglas ex Loudon); Epperson & Allard 1989] and black spruce [*Picea mariana* (Mill) B.S.P.; Knowles 1991] indicated a near-random distribution of genotypes and weak short-distance genetic structure. The general observations of weak genetic structure among tree species could be the result of common life history traits including greater longevity, high pollen and seed dispersal, and higher density of adults with overlapping seed shadows than in most other plants (Hamrick *et al*. 1979, 1981; Young & Merriam 1994).

In contrast to the general trend of weak spatial structure, tree species in which seed dispersal is limited have shown stronger genetic clustering within populations. *Quercus* species, in which large seeds are dispersed by gravity, are typical. For example, a continuous old-growth stand of *Quercus laevis* (Walt) showed one of the highest autocorrelations recorded (+0.25) over scales of 10 m or less, presumably because of short-distance seed dispersal by gravity (Berg & Hamrick 1995). With such high values, it is possible that clones may have been included in the old-growth stand of *Quercus laevis*. However, the degree of autocorrelation observed was not as strong as that predicted by the authors' simulations given the expected isolation by distance. The authors suggested that pollen flow and bird-cached seed may have important effects on the genetic structure of the stand. Population history can also modify spatial structure. A study of spatial structure in *Larix laricina* (Du Roi) by Knowles *et al*. (1992) revealed moderate genetic structure resulting from identified historical disturbances.

Investigating within-population structure can help quantify major evolutionary forces affecting a particular population, and provide information needed to select natural populations or individuals within them for conservation or as candidates for breeding programs. Family structure should also be taken into account in order to maximize diversity in a conservation sample of fixed size, and to avoid making erroneous estimates of population diversity (Epperson 1989). Information on genetic processes in natural populations derived from knowledge of such within-population genetic structure can inform the spatial scale for optimal sampling of genotypes for *ex situ* gene conservation.

Gapare *et al*. (2005) examined the genetic structure, population diversity and evolutionary history of Sitka spruce at eight sequence tagged site (STS) loci, by comparing genetic diversity in core vs. peripheral populations (in terms of ecological niche), and continuous vs. disjunct populations (in terms of species distribution). While all classes of populations had similar expected heterozygosities, core populations (both continuous and disjunct) showed significantly higher levels of observed heterozygosity than peripheral populations. There was also substantial inbreeding in peripheral but not core populations. Furthermore, the Cornuet & Luikart (1996) tests provided stronger evidence of past bottlenecks in peripheral, disjunct populations than in core, continuous populations. We speculate that these core and peripheral populations are structured differently at a microspatial scale due to differences in both population density and history.

In this study, we focus on the distribution of the approximately 97% of the genetic variation harboured within populations of Sitka spruce ($G_{ST} \approx 0.03$) (Gapare *et al*. 2005), to determine whether there is spatial genetic structure, and if so, to compare spatial patterns in core, continuous (CC); core, disjunct (CD); peripheral, continuous (PC); and peripheral, disjunct (PD) populations. The information can be utilized to infer evolutionary processes at a microgeographical scale, and contribute to the debate on the value of peripheral and disjunct populations for conservation (e.g. Lesica & Allendorf 1995). Analysis of spatial genetic structure can be used to provide guidance for reserve design and sampling strategies for either *ex situ* conservation or research collections. An understanding of the genetic processes operating in natural populations will enhance the quality and efficiency of conservation efforts, improve management of genetic resources, and reduce the risk of genetic deterioration in ecologically and economically important widespread species.

Materials and methods

Sampling locations and technique

A two-way scheme was devised in which populations of Sitka spruce were classified as either core or peripheral based on environmental conditions relative to those of the centre of the species' ecological niche, and continuous or disjunct based on geographical distribution (proximity of nearest populations) for a total of four classes (Table 1). Peripheral populations are found at the margins of species' range, and experience different abiotic and biotic environments than those occupying environments in the centre of the species' ecological niche. Disjunct populations are physically separated from continuous populations, but may or may not experience similar environments.

In each population, fresh foliage from the current year's growth was collected from 200 mature trees along several east–west transects, each approximately 100 m wide. Sampled trees were separated by a minimum of 30 m. Mean distance between adult trees in all populations

Table 1 Two-way classification scheme of Sitka spruce distribution according to ecological and geographical distribution within its range and sampling sites

	Core populations	Peripheral populations
Continuous	Port McNeill, BC	Brookings, OR
	Prince Rupert, BC	Seward, AL
Disjunct	Qualicum, BC	Fort Bragg, CA
	Queen Charlotte Islands, BC	Kodiak Island, AL

BC, British Columbia; OR, Oregon; AL, Alaska; CA, California.

was approximately 5 m except for the Fort Bragg and Qualicum populations where the mean distance was above 10 m. The overall area sampled for each population was approximately 550 ha (3200 m × 1700 m) except for the Fort Bragg and Qualicum populations, which each covered well over 800 ha due to the lower density and patchy distribution of Sitka spruce in those locations.

Total genomic DNA was extracted from 0.3 to 0.5 g of fresh frozen needle tissue following a modified CTAB procedure (Doyle & Doyle 1990). DNA samples were subjected to polymerase chain reactions (PCR) using specific primers revealing codominant alleles for eight polymorphic STS loci (Sb16, Sb17, Sb21, Sb29, Sb32, Sb49, Sb60 and Sb62) developed for *Picea mariana* and previously characterized in *Picea glauca* and *Picea sitchensis* (Perry & Bousquet 1998a, b). Seven of the loci reveal indel polymorphisms in introns, while Sb29 alleles result from exon indel mutations.

Spatial autocorrelation analysis

Individual tree locations were identified by a coordinate grid system, first by recording tree position using a hand-held global positioning system instrument (GPS Garmin Model 12XL) during sampling, then mapping the location of each tree on north–south and east–west (x, y, respectively) axes. Intertree distance matrices were then used in the spatial autocorrelation analysis.

To visually assess spatial distributions, figures were constructed showing the locations of specific alleles and genotypes within populations for each locus and population (see Fig. 1a–d). Sb16 and Sb17 were the most variable loci with six and four alleles, respectively (Gapare *et al*. 2005), thus were the most useful for visual inspection of nonrandom patterns.

We estimated co-ancestry coefficients (ρ_{ij}) to test for spatial population structure by combining information on spatial variation across alleles and loci. This approach provides a powerful test of spatial population genetic structure (Smouse & Peakall 1999) and has been used in a number of studies (e.g. Loiselle *et al*. 1995; Kalisz *et al*. 2001; Parker *et al*. 2001; Chung *et al*. 2002). The software program Spatial Pattern Analysis of Genetic Diversity (SPAGEDI) 1.1 (Hardy & Vekemans 2002; http://www.ulb.ac.be/sciences/lagev) provides several statistics designed for pairwise estimates of co-ancestry coefficients, ρ_{ij}, including those based on Loiselle *et al*. (1995) and Ritland (1996). These two estimators differ mainly in the way data from different alleles and different loci are combined to provide average single-locus or multilocus estimates (Hardy & Vekemans 2002). Ritland's (1996) estimator weighs allele distributions by the inverse of allele frequency, giving more weight to rare alleles. This approach results in lower sampling variance, hence it is more powerful for detecting genetic structure. However, Ritland's (1996) estimator is biased downward by the sampling properties of low-frequency alleles (< 0.05). The estimator described by Loiselle *et al*. (1995) weighs allele frequencies by $p_i(1 - p_i)$, where p_i is the frequency of allele i, thus is not biased by the presence of low-frequency alleles. Because low-frequency alleles were detected in the populations (Gapare *et al*. 2005), we used the estimator by Loiselle *et al*. (1995).

Spatial autocorrelation analysis compares all pairs of trees within a specified distance interval and asks whether the pairs exhibit the same alleles more often than expected by chance under a random spatial arrangement. The process is repeated for all pairs falling within each distance class. Thirteen distance classes were used for the spatial autocorrelation analysis for all populations except Fort Bragg and Qualicum. These were 0–50 m, 50–100 m, 100–300 m, and ten 300 m intervals up to 3300 m. Fort Bragg and Qualicum populations had 23 distance classes: 0–50 m, 50–100 m, 100–300 m, and twenty 300 m intervals up to 6300 m. These two disjunct populations have a more scattered distribution than the other six. The first three distance classes were set at smaller intervals (50–200 m) and the rest at 300 m intervals as a compromise between resolution (having distance classes small enough to detect spatial structure over short distances) and power (having enough pairs within each distance class). This was based on the rule of thumb that over 50% of all individuals should be represented at least once in each distance class (Hardy & Vekemans 2002).

Co-ancestry has an expected value of $\rho_{ij} = 0$ if there is no genetic correlation between the alleles in individuals at the spatial scale of interest, $\rho_{ij} > 0$ if individuals in a given pairwise distance class are more similar than expected by chance, and $\rho_{ij} < 0$ if individuals within a given distance class are less similar than expected by chance. Randomization procedures were used to test the significance of the

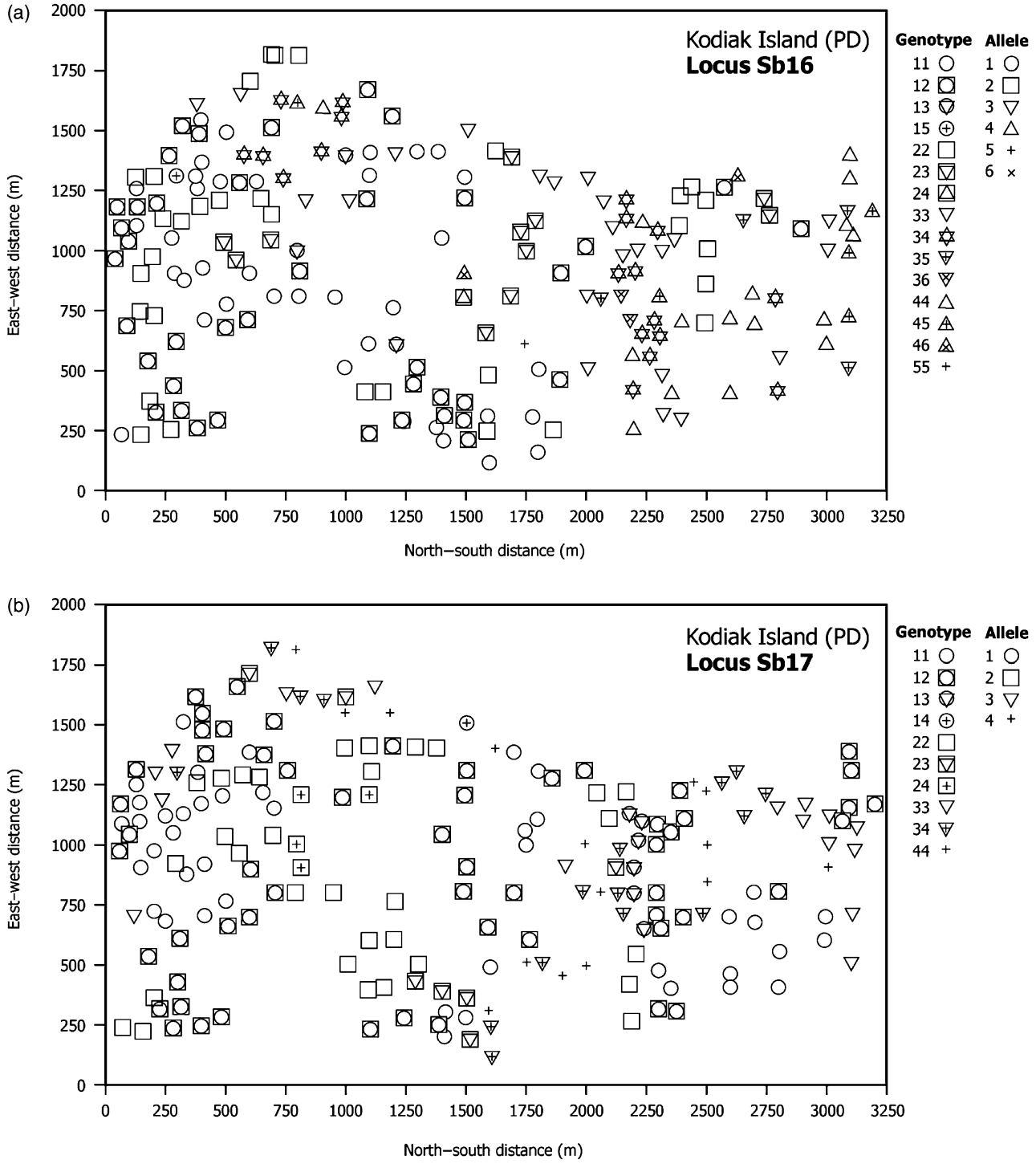

Fig. 1 Spatial distribution of STS loci polymorphisms (Sb16 and Sb17) in 550-ha area of Sitka spruce located on Kodiak Island (a and b) (peripheral, disjunct population) and Port McNeill (c and d) (core, continuous population). Symbol locations indicate mapped tree location and genotype at loci Sb16 and Sb17.

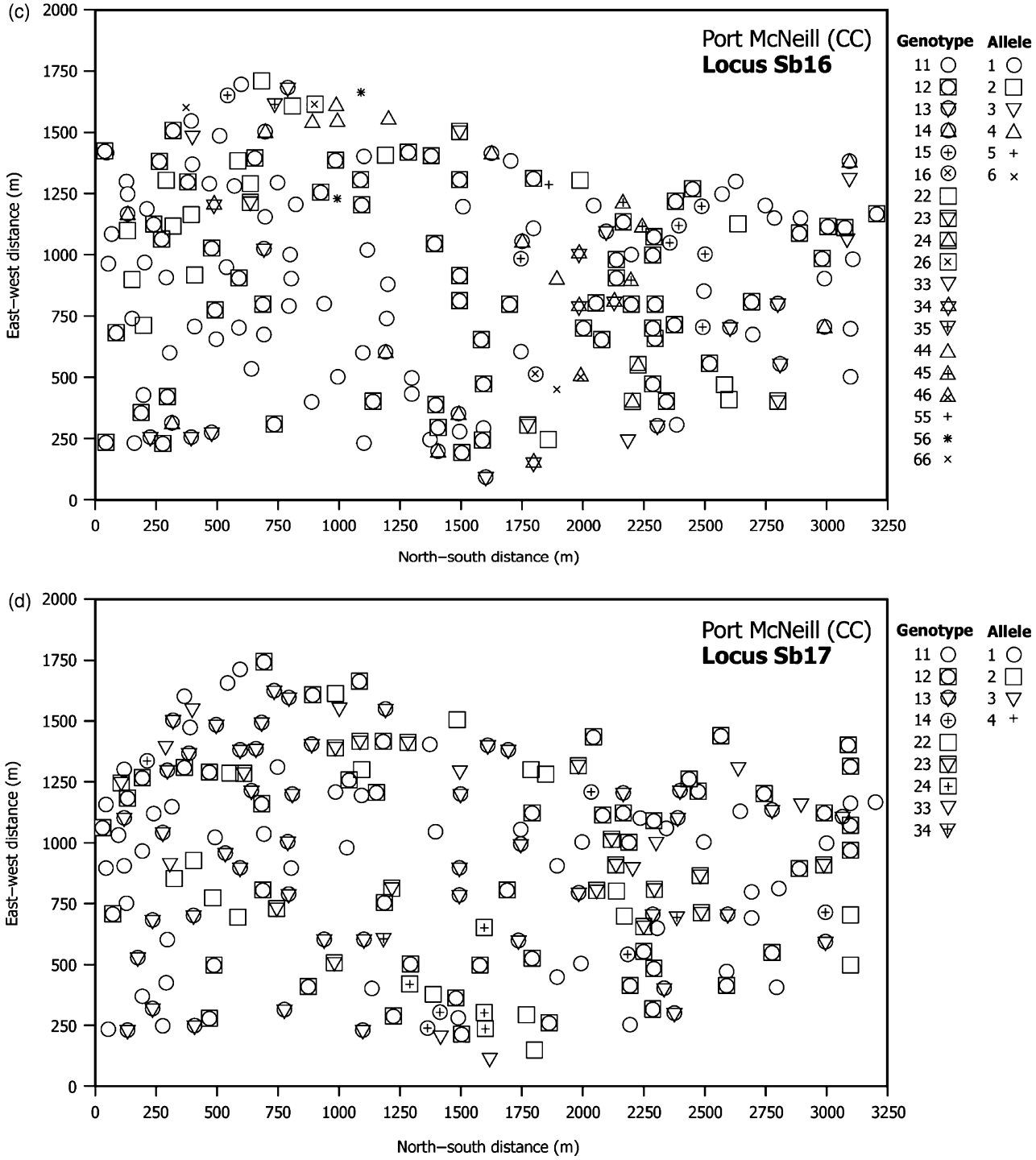

Fig. 1 *Continued*

estimated ρ_{ij} values by constructing a confidence envelope about the null hypothesis of no spatial structure: H_O: $\rho_{ij} = 0$ (Slatkin & Arter 1991). In this procedure, map locations occupied by individuals within a population are reassigned by randomly drawing locations with replacement from the set of observed locations. For a given distance class, the values of ρ_{ij} from the $N - 1$ simulation trials are ranked $\rho_{(1)}, \rho_{(2)}, \ldots, \rho_{(N-1)}$, where $\rho_{(1)}$ is the highest and $\rho_{(N-1)}$ the lowest simulated value. The null hypothesis that there is no spatial genetic structure of the sample population is rejected if a co-ancestry coefficient (the Nth estimate), is greater than $\rho_{(1-\alpha/2)N}$ or less than $\rho_{(\alpha/2)N}$. In this study we

conducted $N - 1 = 499$ simulation trials with $\alpha = 0.05$. Thus $\rho_{(488)}$ and $\rho_{(12)}$ represent the upper and lower limits, respectively, of a 95% confidence interval on the distribution of simulated genetic structure statistics assuming no spatial genetic structure. A ρ_{ij} estimate falling outside this confidence limit was considered significant. If genetic structure exists, then we expect a pattern of significant positive values at shorter distance classes with ρ_{ij} becoming nonsignificant or negative with increasing distance.

Results

Maps of the distribution of alleles and genotypes for the most variable marker loci genotyped (Sb16 and Sb17) show relatively strong genetic clustering in a peripheral, disjunct (PD) population (Kodiak) but a relatively random distribution of genotypes in a core, continuous (CC) population (Port McNeill) (Fig. 1a–d). These patterns were typical of those observed for other populations in the same population classes. Visual inspection of the genotypic and allelic scores for Kodiak (Fig. 1a, b) reveals more clustering of some genotypes than others. For example, at locus SB17, homozygotes 11, 22 and 33 tended to cluster whereas the heterozygous genotype 23 has a nearly random distribution. All genotypes appear to be relatively randomly distributed in the Port McNeill population (Fig. 1c–d).

Multilocus estimates of co-ancestry for all population classes are presented in Fig. 2(a–h), in the form of correlograms. Peripheral populations, both continuous and disjunct (Brookings, Seward, and Kodiak), had high co-ancestry coefficients in the first four distance classes. For example, an average co-ancestry value of 0.197 within 100 m suggest that a high proportion of genotypes at 100 m distance class are more likely to be as genetically similar as full-sibs or parents and their offspring (coefficient of relatedness of 0.25) than more distant relatives such as half-sibs or first cousins (0.125). The coefficients were positive and significantly different from zero (alpha = 0.05) for the four shortest distances classes (30–50 m, 50–100 m, 100–300 m, and 300–600 m), up to approximately 500 m (ln distance 6.2 = 500 m) then became negative and nonsignificant for the subsequent eight distance classes (up to 3300 m; Fig. 2a–c). The same pattern was observed in Fort Bragg, a peripheral, disjunct population and Qualicum, a continuous, disjunct population, where coefficients were positive and significantly different from zero (alpha = 0.05) at four distances classes up to 500 m, then becoming negative and significantly different up to 6100 m (Fig. 2d, f). In contrast, co-ancestry coefficients in core, continuous (CC) populations (Fig. 2g, h) (Port McNeill and Prince Rupert), were not significantly different from zero for any distance interval. Average co-ancestry values < 0.05 suggest that genotypes within 50 m of each other are likely to be only distantly or unrelated. The core, disjunct Queen Charlotte Islands (Fig. 2e) and Qualicum (Fig. 2f) populations both had positive, significant correlations similar to those of all peripheral populations (both continuous and disjunct).

Discussion

Both visual inspection and co-ancestry estimates revealed striking differences between core, continuous populations and other population classes in terms of spatial genetic structuring. The distribution of alleles and genotypes within core, continuous populations (Fig. 2g–h) was almost random, suggesting no spatial structure. However, we detected a significant and strong pattern of fine-scale spatial genetic structure in core, disjunct; peripheral, continuous; and peripheral, disjunct populations that is consistent with isolation by distance: individuals separated by short distances are more likely to be genetically related than those that are further apart (Fig. 2a–f).

The co-ancestry coefficients were higher than expected in the first four distance classes up to 500 m. Several other studies have shown a more abrupt drop in co-ancestry values between trees at scales of less than a tree height apart in various species. For example, mean genetic relatedness in an adult population of mountain hemlock dropped to 0.03 at 5 m distance interval and to −0.0025 at 20 m (Ally 2001). Miyamoto *et al*. (2002) found nonsignificant co-ancestry values at distances less than 10 m in four populations of *Alnus trabeculosa*. Ueno *et al*. (2000) detected strong genetic structure in *Camellia japonica* L. within 5 m, but relatedness declined as the distance intervals increased to 10 m.

Identifying the causes of fine-scale genetic structuring or lack thereof in populations is often difficult because of the diverse influences on this genetic structure throughout the species range. The lack of spatial genetic structure in core, continuous populations is a logical outcome of two phenomena. First, core populations usually have higher densities with a more continuous distribution of mature trees than peripheral populations, where Sitka spruce is a relatively minor stand component (particularly in southern peripheral populations). The higher stand densities in core populations could lead to many overlapping seed shadows that would limit the development of genetic structure (Fig. 2g–h). For example, Young & Merriam (1994) suggested that adults in denser populations of *Acer saccharum* have overlapping seed shadows limiting the development of spatial genetic structure. Vekemans & Hardy (2004) reviewed patterns of spatial genetic structure among plant populations differing in adult density. They showed that density is a major determinant of spatial genetic structure as it affects the strength of local genetic drift. Our results are also consistent with those of Epperson & Allard (1989) and Knowles (1991) who reported lack of spatial genetic structure in lodgepole pine and black spruce populations, respectively. It is

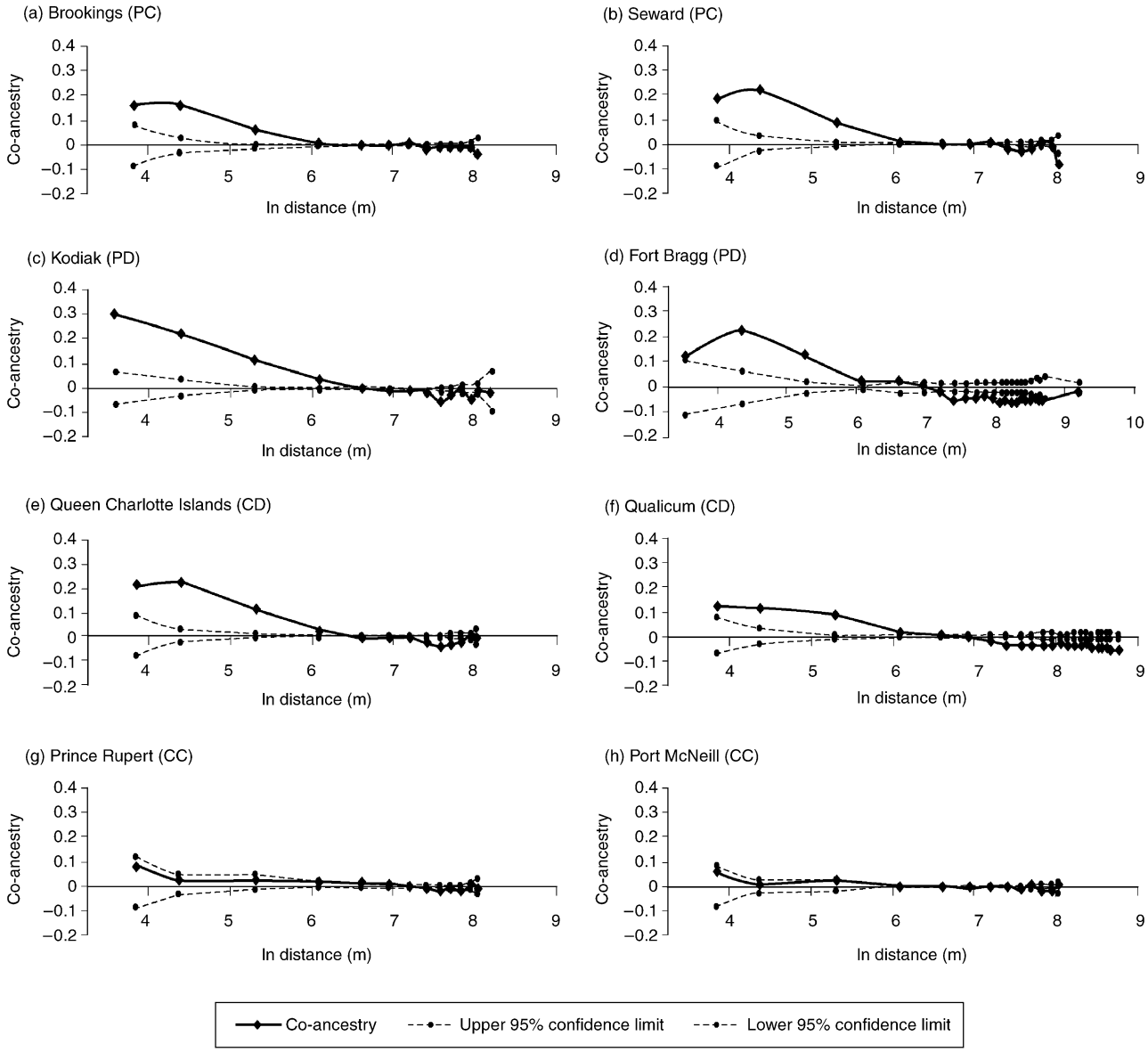

Fig. 2(a–h) Spatial correlograms of co-ancestry coefficients (ρ_{ij}) for core and continuous (CC), core and disjunct (CD), peripheral and continuous (PC), and peripheral and disjunct (PD) populations of Sitka spruce. Dashed lines represent upper and lower 95% confidence limits for ρ_{ij} under the null hypothesis that genotypes are randomly distributed.

possible that Epperson & Allard (1989) may have missed any genetic structure that existed in the high-density lodgepole pine because they sampled on a 50-m grid. However, our work was sampled on a 30-m grid and did not detect spatial structure in core populations. These species generally occur in dense, monotypic stands and dispersal of both seed and pollen is generally high. Second, gene flow was indirectly estimated to be higher ($Nm = 9$) in core Sitka spruce populations than in peripheral populations ($Nm = 3$) using Slatkin's (1985) rare allele method in the populations sampled in the current study (Gapare *et al*. 2005).

Little overlap in seed shadows could initiate spatial genetic structure, and subsequent biparental inbreeding among siblings, parents and offspring, or other relatives would reinforce this structure over successive generations. The mean F_{IS} (0.17) for core, disjunct; peripheral, continuous; and peripheral, disjunct populations (Gapare *et al*. 2005) is close to the average co-ancestry coefficient between individuals within 100 m (0.197), and may reflect biparental inbreeding in addition to selfing (e.g. Vekemans & Hardy 2004). For example, Kodiak, a peripheral and disjunct population has ρ_{ij} value of around 0.30, which is higher than expected for half sibs, which is indicative of some level of inbreeding which increases the relatedness values.

Nonrandom associations of alleles within distances up to 500 m in peripheral populations could also be attributed to local genetic drift if only a few dominant, reproductive trees contributed to the next generation's gene pool but this would likely also be reflected in lower levels of genetic diversity (i.e. expected heterozygosity, allelic richness) which was not observed (Gapare *et al*. 2005). This pattern could also be due to historical founder effects that established a pattern of genetic relatedness that has been retained in subsequent generations.

Studies by Leonardi & Menozzi (1996) and Parker *et al*. (2001) have suggested that stand history or homogeneity of seed sources may explain some of the causes of fine-scale genetic structure. For example, adult density influences the distribution of genotypes in young cohorts because the arrangement of adults, along with environmental factors, dictates patterns of pollen and seed dispersal (Parker *et al*. 2001). Recent demographic events in the newly founded populations may also result in neighbourhood structure. For example, Kodiak, which has strong evidence of spatial structure, is the youngest population at the northwestern migration tip of the species' range, having arrived on Kodiak Island about 400 years ago (Griggs 1934). The spatial structure in this population may reflect seed shadows around sparsely distributed population founders, and it is unlikely to have reached equilibrium.

Thus low density can be invoked to explain the genetic structure in most of the peripheral and disjunct populations, and a recent founder event has likely left a pronounced signature in the Kodiak population. It is more difficult to hypothesize a cause of the strong spatial structure in the Queen Charlotte Islands population, however. The Queen Charlotte Islands are thought to have been a glacial refugium for numerous species at the last glacial maximum, and pollen evidence of subsequent rapid recolonization of Sitka spruce in coastal British Columbia supports this hypothesis (e.g. Soltis *et al*. 1997). The Queen Charlotte Islands also have a relatively high current population density, comparable to the core, continuous populations, with nearly pure Sitka spruce in some places.

The striking contrast in spatial genetic structure between core, continuous and peripheral populations has not previously been documented in a widespread species. There may be several explanations for this. First, large population sizes with hundreds of individuals sampled over hundreds or thousands of hectares were needed to detect these patterns. Second, multiple populations must be sampled in each population class for true replication, and species distribution dictates the opportunities for such natural experimental designs. Finally, most genetic studies focus on core, continuous populations as species, particularly common species of economic importance, tend to receive the most research focus in the core of their range.

If these patterns are more general for widespread species, they have substantial implications for both gene conservation strategies and optimal sampling for research collections. While core, continuous populations had comparable levels of genetic diversity to peripheral and disjunct populations based on either expected heterozygosity or allelic richness (Gapare *et al*. 2005), the striking differences among population classes in how that genetic variation is distributed has implications for: (i) size and location of *in situ* reserves; (ii) sampling strategies for *ex situ* conservation; and (iii) sampling strategies for research collections. Small reserves or collections made over a small area are likely to capture a higher portion of current standing genetic variation in core, continuous populations than in peripheral or disjunct populations due to the strong spatial structuring in the latter. If genetic diversity is estimated based on research collections made from only a small portion of stands, it will appear that core, continuous stands have more genetic diversity than peripheral or disjunct stands. This apparent difference will be a function of contrasting genetic structure rather than overall genetic diversity. Sampling strategy for research or gene conservation purposes will be relatively unimportant in core, continuous populations but may be more critical for peripheral or disjunct populations.

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This work comprises a portion of the PhD dissertation of Washington Gapare on genetic diversity and spatial population structure of Sitka spruce and implications for conservation of widespread species. The study was conducted at the Centre for Forest Gene Conservation at the University of British Columbia in the laboratory of Dr Sally Aitken whose interests lie in ecological genetics, conservation genetics and adaptation to climate change of conifer populations. This work reflects the interests of the authors to gain a better understanding of microevolutionary processes at a microgeographical scale in tree species and to contribute to the debate on the value of peripheral and disjunct populations for conservation. Washington Gapare is both an evolutionary and quantitative geneticist currently working with CSIRO-Forestry and Forests Products in Canberra, Australia.
