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Lack of allozymic variation in disjunct Newfoundland populations of red pine (*Pinus resinosa*)

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Disjunct, geographically isolated populations of red pine (*Pinus resinosa* Ait.) on the island of Newfoundland were investigated by enzyme electrophoresis to determine if these populations were genetically distinct from a range-wide sample of mainland populations. Genetic variation at 23 putative gene loci from 12 enzyme systems was assayed by cellulose acetate gel electrophoresis. Each of the 96 trees sampled was monomorphic for all enzyme gene loci assayed, and no genetic differentiation between Newfoundland and mainland populations was detected. The striking lack of genetic variation at enzyme gene loci in red pine has been confirmed for the most isolated populations at the extreme northeastern margins of its range.

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Des populations disjointes de pins rouges (*Pinus resinosa* Ait.) de l'île de Terre-Neuve, géographiquement isolées les unes des autres, ont été analysées par électrophorèse de certaines enzymes afin de déterminer si elles se démarquaient génétiquement d'échantillons provenant de l'ensemble de l'aire d'extension du continent. On a procédé à l'électrophorèse en gel d'acétate de cellulose de 23 loci de gènes censés codés pour 12 systèmes enzymatiques en vue d'y découvrir des variations génotypiques. Chacun des 96 arbres étudiés s'est révélé monomorphe pour tous les loci de gènes d'enzymes analysés, et aucune variation génotypique n'a été décelée entre les populations de Terre-Neuve et celles du continent. Cette absence remarquable de variation génotypique des loci de gènes d'enzymes a même été confirmée pour les populations de pins rouges les plus isolées, aux limites nord-est de son territoire.

Introduction

Genetic studies indicate that red pine (*Pinus resinosa* Ait.) lacks genetic variation at enzyme loci (Fowler and Morris 1977; Allendorf *et al.* 1982; Simon *et al.* 1986) and is unusually uniform in its quantitative traits (Rudolf 1957; Fowler 1964; Fowler and Lester 1970). In a review of the impact of Pleistocene glaciation on North American conifers, Critchfield (1984) believed that red pine was probably eliminated from all or nearly all of its present range during the last glacial advance, and it may have undergone a population bottleneck following glaciation as suggested by Fowler and Morris (1977). The genetic structure of populations of red pine on the island of Newfoundland is of special interest because these populations are disjunct and geographically isolated from the mainland range of the species.

A genetic comparison between a range-wide sample of red pine and the most disjunct populations of this species would be useful in confirming the genetic uniformity of this species throughout its natural range. The electrophoretic studies that have established the genetic uniformity of red pine were based on small samples of loci or few populations (Fowler and Morris (1977), five enzyme systems assayed from five populations; Simon *et al.* (1986), four enzyme systems assayed from six stands at Lake Duparquet, Quebec; Allendorf *et al.* (1982), 27 enzyme loci assayed from two stands in Minnesota) and did not include samples from the

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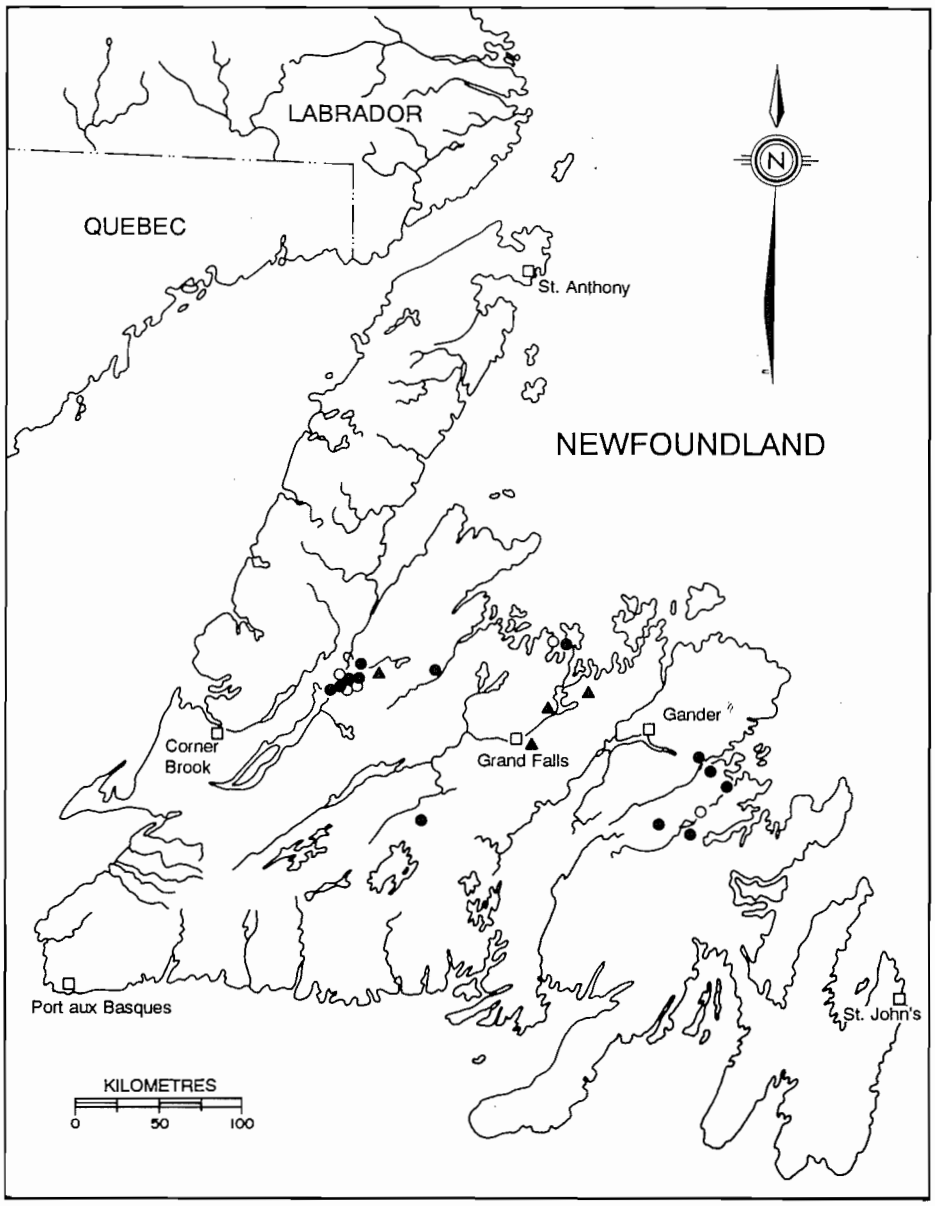

FIG. 1. Extant red pine populations of Newfoundland (○), populations from which seed was collected (●), and isolated individual trees (▲) from which cones were collected in 1989 (adapted from Roberts 1985).

most disjunct, marginal populations of the species. From their electrophoretic study of genetic variation in some of the most northerly populations of red pine in the Abitibi region of Quebec, Simon *et al.* (1986) suggested that further studies of red pine lying outside regions glaciated during the Pleistocene would be needed to verify the apparent genetic uniformity of this species over its entire range.

Populations of red pine on the island of Newfoundland exist at the northeastern extreme of the geographical range of the species and have been isolated from mainland populations by the Gulf of St. Lawrence for 8000 to 10 000 years, at least since the last glacial retreat of the Wisconsin ice age (Macpherson 1981). There is evidence that ice-free areas existed on the island during the last glaciation (Grant 1977; Ives 1978; Brookes 1982). Rogerson (1981) has compiled a map of Newfoundland that delineates these ice-free areas. While the present distribution of Newfoundland's red pine populations (Fig. 1) suggests that they may have predated the last glaciation as opposed to having re-entered the island following the last glacial retreat, there is no unequivocal evidence that red pine survived the last glacial period in refugia on the island. Our objective was to determine if disjunct populations of red pine in Newfoundland could be genetically differentiated from mainland populations by using a larger sample of individuals, populations, and loci, and to determine if the effects of geographical isolation were reflected in the genetic structure of Newfoundland populations.

Methods and materials

In 1989, seed was collected from a range-wide sample of 27 red pine trees established near Maple, Ontario (44°N, 80°W). These 27 trees originated throughout the mainland range of red pine in

Ontario (Lake Abitibi (6),[1] Chapleau (1), Hearst (4), Cochrane (4), Mattagami (5), and the Thousand Islands (1)), Nova Scotia (Lower Seabright (1) and Stanley (3)) and near Madison, Wisconsin (2). Seed from 69 trees native to Newfoundland (6 trees from each of 11 of the 18 extant red pine stands in Newfoundland, and from 3 isolated individual trees) was also screened by gel electrophoresis for an estimate of genetic variation. Seeds were extracted from cones following artificial cone ripening under cool, dry conditions in a seed extractory.

Freshly collected seed was used in our study, and enzyme resolution was not improved when seeds were soaked prior to megagametophyte extraction. The megagametophyte tissue from six seeds per tree was excised, bulked, and macerated in three drops of extraction buffer (0.1 M Tris–HCl, pH 8.0, with 0.5 μL/mL of mercaptoethanol) to screen for electrophoretically detectable genetic variation. Enzymes were separated by electrophoresis on a cellulose acetate gel medium in a Tris–glycine buffer at pH 8.5 (Easteal and Boussy 1987; Hebert and Beaton 1989; Ringius and Innes 1989; Innes and Ringius 1990). The prefabricated cellulose acetate gel plates (manufactured by Helena Laboratories, Beaumont, TX) measure 7.5 cm^2. The front on these gels advanced at least 6.0 cm within a running time of 20 min. Enzyme banding patterns were resolved using standard staining procedures (described by Cheliak and Pitel 1984; Hebert and Beaton 1989).

Results and discussion

Our zymogram patterns (Fig. 2) differed somewhat from those of previous studies. Using starch gel electrophoresis, Allendorf *et al.* (1982) reported three putative loci for diaphorase, three loci for aspartate amino transferase, and one locus for alcohol dehydrogenase, whereas we observed one locus (zone of activity), four loci, and four loci, respectively (Fig. 2). Allendorf *et al.* (1982) also reported four loci for malate dehydrogenase. Our zymograms, for malate dehydrogenase and glutamate dehydrogenase, consistently revealed only the four zones of activity characteristic of the alcohol dehydrogenase enzyme system, and therefore these enzymes were disregarded in our analysis. Several other enzyme systems (sorbitol dehydrogenase, phosphoglucomutase, and mannose-6-phosphate isomerase) also revealed the banding pattern of alcohol dehydrogenase along with their own unique zones of activity. Fowler and Morris (1977) observed two loci for esterase, and Simon *et al.* (1986) were able to resolve nine zones of activity for this enzyme. We were able to resolve only one locus for esterase, and only when β-naphthyl acetate was used as a substrate.

No allelic variation was detected in 23 putative loci from the 12 enzyme systems assayed, and each of the 96 trees sampled from both mainland and Newfoundland populations were identical in their enzyme banding patterns (Fig. 2). The electrophoretic evidence from this study confirms the genetic uniformity of red pine based on allozyme variation (Fowler and Morris 1977; Simon *et al.* 1986) and further illustrates the contrast between red pine and the relatively high levels of heterozygosity found in most other conifers (reviewed by Hamrick 1983; Cheliak *et al.* 1985; Yeh *et al.* 1985; Strauss 1986). While electrophoretically detectable genetic variation may not adequately describe variation in the genome (Lewontin 1974; Nevo 1978; Giles 1984), our inability to detect any variation indicates that Newfoundland populations of red pine are undifferentiated from mainland populations.

[1]Numbers in parentheses indicate the number of trees assayed from each population.

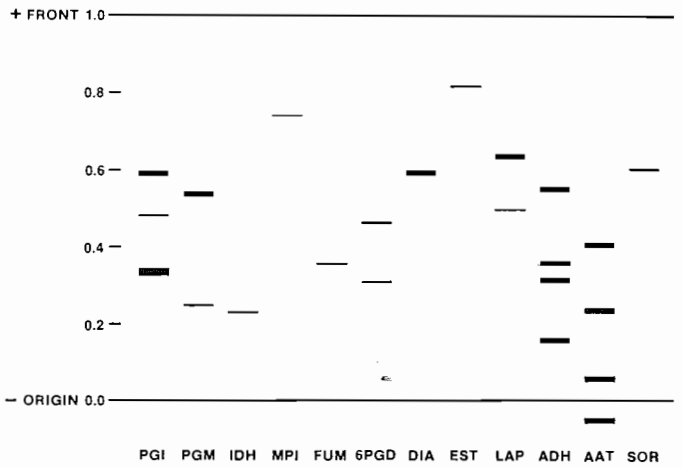

FIG. 2. Monomorphic banding patterns from red pine megagametophytes for the following enzyme systems: alcohol dehydrogenase (ADH), aspartate aminotransferase (AAT), diaphorase (DIA), esterase (EST), fumarase (FUM), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), mannose-6-phosphate isomerase (MPI), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (6PGD), and sorbitol dehydrogenase (SOR).

Morphological mutations in branch angle have been observed in red pine from Newfoundland and in the range-wide collection of red pine established near Maple, Ontario, from which seed was collected for the present study. It is reasonable to assume that other mutations have accumulated in red pine that remain undetected. Perhaps a more direct analysis of variation in DNA will provide the genetic markers necessary to infer genetic structure in red pine. Until such investigations have been completed, the absence of genetic variation in red pine may be adequately explained by the population bottleneck hypothesis put forward by Fowler and Morris (1977).

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Sprouting ability of aerial and underground dormant basal buds of *Betula pendula*

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The effect of ground level on the bursting and development of primary basal buds, formation of secondary basal buds, and survival of sprouts was studied with 2-year-old pot plants of silver birch (*Betula pendula* Roth) in an experiment in which the ground level was changed. Both intact and decapitated seedlings were manipulated. Raising the ground level reduced the bursting of dormant basal buds, while lowering it promoted both the bursting of buds and survival of the sprouts, especially in the decapitated plants. Many new basal buds developed regardless of the ground level. The intact plants also formed secondary basal buds, but these mostly remained dormant, like old, primary buds. In addition, the concentration of new bud clusters in the uncovered part of the stem suggested that although the plant's internal condition is important for the development and bursting of its basal buds, environmental factors are also involved. Ground-level changes on drained mires and at afforestation sites may significantly affect the success of sprouting.

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