ISOZYME VARIATION IN *Colobanthus quitensis* (Kunth) Bartl.: METHODS AND PRELIMINARY ANALYSIS

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ABSTRACT. Isozyme analysis techniques were developed for testing individual seedlings of cool temperate populations of *Colobanthus quitensis* (Kunth) Bartl. Isozymes were resolved from six different enzymes, of which three (aspartate aminotransferase, malate dehydrogenase and peroxidase) were employed in an analysis of genetic variation within and between populations from Tierra del Fuego and West Falkland Island. No variation in 22 isozymes was observed in an analysis of 20 seedlings from each population. These preliminary results support the hypothesis that plants at high elevations and latitudes tend to be genetically uniform because of strong physical selection pressures.

EVOLUTIONARY processes in extreme environments have always fascinated biologists. This is certainly true for plants growing in polar and alpine regions, where two basic questions have been asked about the survival of these plants. The first is what physiological and biochemical adaptations have evolved that contribute to survival in a cold environment. The second question is what genetic and reproductive systems these plants have evolved that allow them to self-perpetuate under these extreme conditions.

In the Antarctic there are only two species of flowering plant, *Colobanthus quitensis* (Kunth) Bartl. and *Deschampsia antarctica* Desv., both of which are restricted to scattered localities on or near the Antarctic Peninsula and the island groups of the Scotia Ridge (Greene and Holtom, 1971). Both species are found on several sub-Antarctic islands (Greene and Greene, 1963), Falkland Islands, Tierra del Fuego and the southern Andes mountains, with *C. quitensis* occurring in scattered localities northward to Mexico (Moore, 1970). The taxonomic history of these plants has been provided by Skottsberg (1954) and Moore (1970), while accounts of research performed on these taxa in the Antarctic and sub-Antarctic have been provided by Holtom and Greene (1967) and by several authors in a series of papers published in this journal (see Greene, 1970). In brief, the following has been learned about *Colobanthus quitensis:* (1) the species commonly produces flowers but only occasionally develops viable seeds throughout its range, (2) it possesses specific physiological and morphological adaptations to the cold environment, and (3) although vegetative uniformity is seen at all localities, variation in flower and seed development occurs. Because of its interesting distribution and the research already completed, *C. quitensis* is an ideal species for investigating the basic questions raised above.

The recent development of isozyme analysis techniques as a means of assessing physiological responses and genetic variation in plants is well recognized (Shannon, 1968; Scandalios, 1969). Isozymes are multiple molecular forms of enzymes that can be rapidly separated through electrophoresis and specific staining techniques. The analysis of plant genetic variation using isozymes as markers has been performed almost exclusively on temperate populations (Marshall and Allard, 1970; Scogin, 1971; Solbrig, 1971; Payne and Fairbrothers, 1973). These studies indicate that there is extensive genetic variation within and between natural plant populations. This work has also revealed a significant percentage of heterozygous alleles in individuals of these populations. These results are similar to those obtained from tropical *Drosophila pseudoobscura* populations (Lewontin and Hubby, 1966). Although genetic variation in polar and alpine plants does not appear to have been studied in detail, it has been argued that they too should exhibit extensive variation. Dunbar (1968) cited the morphological variation observed in polar plankton as evidence for intra-specific variation. The existence of numerous taxonomic problems in arctic plant-species complexes would support this contention. In addition, Löve (1964) and Johnson and Packer (1965) have speculated that arctic plants should possess a high degree of genetic variation, this variation being maintained through polyploidy. However, the more prevalent view has been that plants at polar latitudes should be genetically uniform because of very strong physical selection pressure as well as reproductive specialization (Mosquin, 1966; Bliss, 1971).

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Isozyme analysis was performed on seedlings of *Colobanthus quitensis* to determine the extent of isozyme and thus genetic variation. Two populations were studied, one from West Falkland Island and one from Tierra del Fuego. The purpose of this paper is to describe the methods developed for the analysis of several different isozymes and also to present some preliminary data on variation in the two populations.

Materials and Methods

Source of plants

Seeds of *Colobanthus quitensis* were collected from plants growing at the two following locations:

i. Tierra del Fuego, on the bank of a stream flowing into Beagle Channel 1·5 km. south-east of Puerto Williams (lat. 54°56′S., long. 67°37′W.). Collected by D. M. Moore as seed, 2 January 1961.
ii. West Falkland Island, on a wet bank by a gentoo penguin colony near Hope Harbour (lat. 51°20′S., long. 60°38′W.). Collected by D. M. Moore as seed, 17 February 1964.

The seeds from each population were germinated and the plants grown at the University of Birmingham, England. The seeds used in this research were derived from these plants and were provided by S. W. Greene and J. A. Edwards.

Seed germination

The seeds from each population were germinated after stratification for 1 week on wet filter paper at 3° C at the Institute of Polar Studies, Ohio State University. Over 90 per cent of the seeds from both populations germinated. They were then transferred to a growth chamber and grown under a regime of 16 hr. of fluorescent light at 15° C and 8 hr. of darkness at 7° C. Seedlings were harvested when they were 1 cm. in length (after a minimum of 5 weeks germination). These seedlings were then used for the isozyme analysis.

Enzyme extraction

Individual seedlings (weighing approximately 10 mg. each) were placed in a small glass tissue grinder kept in an ice bath. The entire extraction process was performed at 3° C. The breakage buffer consisted of 20 per cent (w/v) sucrose and 0·1 per cent (v/v) β-mercaptoethanol in 0·2 M tris-HCl buffer at pH 8·3. After addition of 0·1 ml. of buffer and subsequent grinding, the resulting slurry was added to the electrophoresis gel (0·025 ml. sample).

Electrophoresis

The discontinuous chemical formulation of Davis (1964) was employed for all isozyme separations, using an Ortec slab gel apparatus. The separations took about 2 hr. (42 mA and 600 V) which gave a distance of 6 cm. from the sample origin to the position of the tracking dye. The polyacrylamide gel concentration was 7 per cent. Electrophoresis reagents were obtained from Distillation Products Industries; acrylamide and bis-acrylamide were re-crystallized prior to use (Loening, 1967).

Isoenzyme staining

The staining procedures were essentially those described by Lee and Dougall (1973) with the following modifications. The glucose-6-phosphate dehydrogenase and isocitrate dehydrogenase staining recipes were identical to malate dehydrogenase except that NADP was substituted for NAD, and the substrates were 60 mg. glucose-6-phosphate and 60 mg. isocitric acid per 10 ml. of buffer. The leucine aminopeptidase stain was identical to esterase except that the substrate was 3·0 mg. of β-leucyl naphthylamide. All staining reagents were obtained from Sigma. Nine enzyme activities were stained: malate dehydrogenase, glutamate dehydrogenase, glucose-6-phosphate dehydrogenase, isocitrate dehydrogenase, esterase, acid phosphatase, leucine aminopeptidase, aspartate aminotransferase and peroxidase. Each seedling could be analysed for four different enzymes. After the stains had developed, the gels were recorded by (1) photography, (2) diagramming and noting positions of bands on gels, and (3) scanning gels

in a Gilford No. 2400 spectrophotometer at 591 nm. for all dehydrogenases and 540 nm. for other enzymes. The peroxidase gels could not be scanned because the stain develops immediately and it is ephemeral.

Results

Most of the staining techniques described above were successful. However, only three enzymes (peroxidase, aspartate aminotransferase and malate dehydrogenase) produced patterns of several bands each and it was these which were employed in tests of seedling variation. Twenty individual seedlings from each population were analysed for isozyme variation of these three enzymes. The results were completely uniform within and between both populations and the patterns illustrated in Fig. 1 were observed in all seedlings analysed. The only differences observed between the two populations were the persistently different staining intensities of certain peroxidase isozymes. Even in this case, the staining intensities were uniform from seedlings within each population.

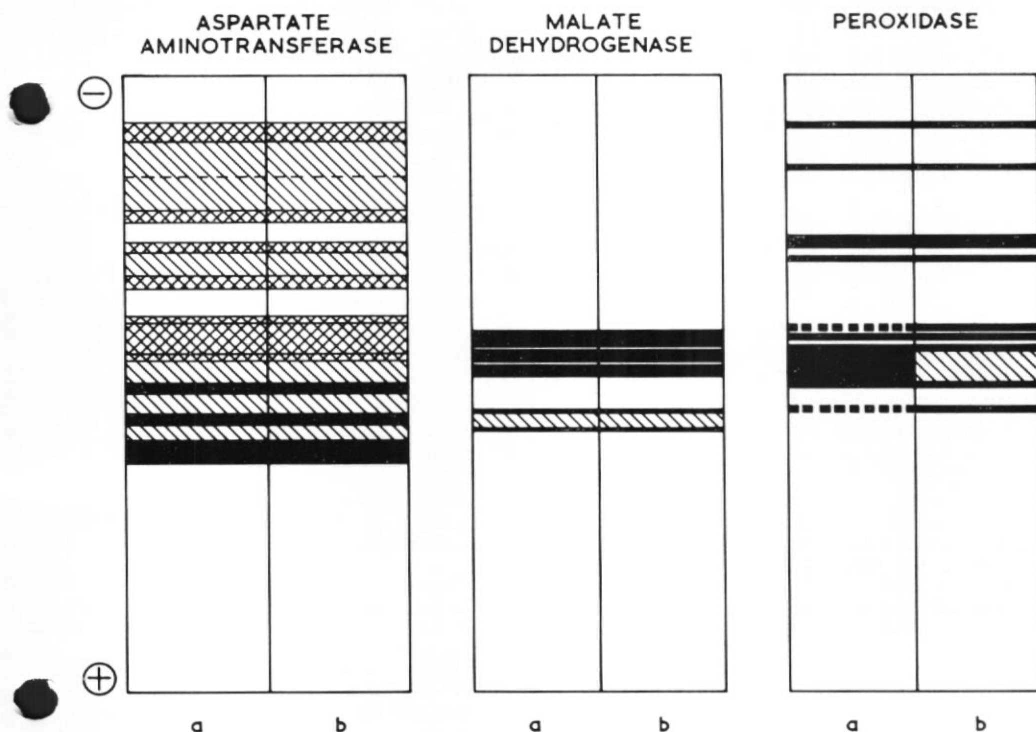

Fig. 1. Diagrams of isozyme stains in 5 cm. polyacrylamide gels for populations of *Colobanthus quitensis* from West Falkland Island (a) and Tierra del Fuego (b). Stains for aspartate aminotransferase and malate dehydrogenase were the same in both populations but those for peroxidase exhibited slight differences in staining intensities between the two populations. + and − symbols indicate the direction of the current.

Of further enzymes investigated in the tissues of *Colobanthus*, isocitrate dehydrogenase, leucine aminopeptidase and esterase could not be detected and acid phosphatase was only barely detectable. Glutamate dehydrogenase developed only one band of activity, with an rP (ratio of isozyme band distance to dye front) of 0·17, so this isozyme was not analysed in detail. Although the enzyme has not been successfully used in systematic or genetic analyses (Thurman and others, 1965), it may be important in future physiological investigations of *Colobanthus*. Two isozymes were observed for glucose-6-phosphate dehydrogenase with rP's

of 0·12 and 0·19. These were not analysed further in this investigation but may be helpful in future genetic and physiological studies.

Discussion and Conclusions

The results indicate that seedlings of *Colobanthus quitensis* are suitable material for isozyme analysis. In the tests it was possible to analyse each seedling simultaneously for four isozymes. The variety of isozyme stains used in this research should assist in future physiological and genetic analyses of *Colobanthus*. In addition, the preliminary analysis of three enzymes from 20 seedlings of each of the two populations indicates virtually no genetic variation for the traits examined (about 22 isozymes from all the three enzyme stains). These results are consistent with the hypothesis that strong physical selection pressures in high-latitude and high-altitude environments should contribute towards genetic uniformity in plants. Furthermore, the genetic uniformity demonstrated in these *Colobanthus* populations exists despite variation that would be expected as a result of sexual reproduction. There is every indication that these plants produce fertile flowers and seed by normal sexual means (personal communication from J. A. Edwards). In addition, high germination rates of over 90 per cent and careful horticultural methods should have preserved genetic variation in these plants if it existed. These results point towards a preliminary hypothesis that the *Colobanthus quitensis* populations are genetically uniform. This hypothesis, however, is amenable to more critical testing by analysing more seedlings, further populations from other localities within its geographical range and using additional enzyme stains.

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