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ROLE OF PROVENANCE TRIALS IN THE STUDY OF POPULATION DIFFERENTIATION

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ABSTRACT

Wide distribution of many Linnean species across climatic, edaphic and biotic barriers may be either due to a wide ecological amplitude of the species as such, or to the presence of a number of distinct local populations, well adapted to the respective ecoclimates. These populations may show continuous variation along an environmental gradient (ecocline) or discontinuous variation (ecotypes). The morphological or physiological adaptive characters are genetically fixed. Although a number of genecological techniques have been developed in the recent past, to differentiate such ecologic populations, the provenance trial technique has gained much importance. In the present communication the technique as well as some important results obtained through its use, both within and outside India, have been briefly discussed.

POPULATION

The term population refers to an aggregate of individuals considered together because of some characteristic(s) which are common to all of them. For example, the habitation of a given area at a given time by the individuals of a species may be the common feature. The significance of cohabitation of an aggregate of sexually reproducing individuals lies in the fact that they can exchange genes. Such populations are often termed Mendelian and are defined by Dobzhansky (1950) as, "a reproductive community of sexual and cross-fertilising individuals which share in a common gene pool". Hence in widest sense the Mendelian population corresponds to the "biological" species, because grouping above the species level, e.g., subgenus, genus, etc., do not usually interbreed. According to Major (1958), "Population is a concept which bridges between the study of individual plants and of vegetation. A population is an interbreeding group of individuals which behaves in this way because of a temporal-spatial, and ecological, relationship between the individuals". From statistical view point Mather (1946) defines a population as, "the hypothetically infinitely large series of observations or individuals of which those observations or individuals actually obtained form a sample."

Populations are liable to change in respect of different parameters, e.g., size, composition, area, etc. A population consists of one or more biotypes. The latter term refers to the assemblage of individuals with the same or nearly same genotype. The population of autogamous species (inbreeders) which is more or less homozygotic, consists essentially of one biotype. However, in allogamous species (outbreeders) every individual of the population may represent a different biotype.

POPULATION DIFFERENTIATION

Howsoever uniform the population may appear to be, the individuals constituting it, invariably show small variations amongst themselves. The variations between individuals may be due to the following reasons:

(a) modifications imposed by varying environmental factors,

(b) variations arising due to random gene mutation, and

(c) genetic recombinations, especially in populations consisting of cross-fertilizing individuals.

Phenotypic variations amongst individuals, even at the same stage of development, are imposed by slight differences in the magnitude of various environmental factors. Such variations tend to be continuous and follow a normal distributional curve. Genetic variations within populations are caused by mutations, which may occur at gene, chromosome or genome levels. Further, the gene segregation and recombination, occurring in cross-fertilised populations, produce newer variants.

The importance of variation in evolution had been realised by nineteenth century botanists such as Jordan, Kerner, Bonnier and Darwin. However, the pioneer work of Turesson (1922*a*, 1923, 1925, 1930) for the first time established the genetical basis of ecological differentiation occurring within populations of a species inhabiting different habitats. Such study of infra-specific variation of plants in relation to environment was termed genecology. To quote Turesson (1922*b*), "The mass of genetically distinct forms which make up the Linnaean species do not distribute themselves indiscriminately over an area comprising different types of localities, but, on the contrary, are found in nature to be grouped into different types, each confined to a definite habitat. Further, these 'ecotypes' do not originate through sporadic variation preserved by chance isolation; they are, on the contrary, to be considered as products arising through the sorting and controlling effect of the habitat-factors upon the heterogenous species-population." However in contrast to the concept of ecotypes, which is based on variational discontinuity within the populations, the researches of Gregor and his associates (*Gregor* 1938, 1939; *Gregor et al.* 1936, 1950) clearly demonstrated the occurrence of continuous inter-population variation, which was correlated with the graded variation of habitat factors. The correlated gradient in measurable characters and habitat factors was termed as the ecocline by Gregor, who believed the ecotype to be merely a particular range of variation on an ecocline. Continuing the discussion of continuous or discontinuous population differentiation, the work of Carnegie School (*Clausen et al.* 1940, 1948; *Clausen & Hiesey* 1958) showed the existence of morphological distinguishable climatic ecotypes, which occurred along a graded altitudinal series.

Ecotypes or distinct ecological populations are essentially adapted to diverse habitats and thus increase the distribution area of the species. In other words, ecotypic differentiation equips the species to tide over climatic, edaphic or biotic barriers. Further, if certain plant communities inhabiting widely different ecological environments are found to possess many species in common to them, the resulting degree of similarity would be highly misleading if those plants belong to different ecotypes (*Curtis* 1959). McMillan (1959*b*) has rightly given emphasis to this aspect of ecotypic differentiation in community function. As will be clear by now, ecotypes express ecologically important adaptation which according to Daubenmire (1959) is, "any feature of an organism or its parts which is of definite value in allowing that organism to exist under the conditions of its habitat". Physiological adaptations which may not be apparent visually, must also be included herein.

GENECOLOGICAL TECHNIQUES

The problem of distinguishing genecological differentiation has two important attributes: Firstly, the separation of adaptive from random inter-population variation, and secondly the separation of adaptive variation into genetic and non-genetic components (*Heslop-Harrison* 1964). Thus, much attention has been drawn recently to the problems of sampling populations for genecological studies (*Clausen* 1960; *Harberd* 1957, 1958; *Wilkins* 1959).

The techniques used in genecological studies can be summarised as given below:

(1) The transplant technique originally used by Turesson involves cultivation of plants collected from different habitats in a neutral medium. Persistence of difference between populations in a neutral medium indicates genetical variability. On the other hand, the environmentally induced phenotypic modifications, giving rise to ecads and ecophenes, tend to disappear in a neutral medium. However, this method has the disadvantage of being able to provide a poor representation of the variability within a population. This difficulty may be encountered to certain extent by employing reciprocal transplant methods to test survival capacity or controlled environments to measure response to individual habitat factors (*Heslop-Harrison* 1964).

(2) Clonal method involves the testing of ramets of a single genotype under different environmental conditions. Seed clones can be obtained in apomictic plants; for example, as has been done in *Poa* spp. (*Watson & Clausen* 1961).

(3) Seed sampling procedure has been advocated by Clausen (1960) to be the best method for obtaining a wide perspective of the variability within a population. It is generally agreed that the minimum seed sample obtained should be from at least 60-100 plants of a population. Further, the number of seedlings raised from each seed sample may vary between 300-600, out of which about 120 seedlings may be picked up for study. It is in this context that Clausen (1960) remarks that, "sound sampling is more important than using refined statistical techniques on poorly sampled material." Cultivation of plants through seeds or ramets

obtained from widely separated areas and habitats under uniform culture conditions is termed provenance trial technique.

SOME EXAMPLES OF POPULATION DIFFERENTIATION AS ELUCIDATED BY PROVENANCE TRIAL

The variation of Scots pine (*Pinus sylvestris*) throughout Europe has evoked much controversy (*Langlet* 1934, 1936, 1959, 1963; *Wright & Baldwin* 1957). In 1938 large scale experimentation was undertaken by Langlet in which 52 provenances were grown near Stockholm. Out of several morphological and physiological features taken into account, percentage of dry matter in the needles of 2-4 year seedlings in the late autumn showed close relationship with the length of the growing season, assessed as the number of days with an average temperature of 6°C or more, in the native habitats. A better curvilinear correlation was obtained ($r > +0.98$) between dry matter content and the length of the first day of the year with an average temperature greater than 6°C. In contrast to clinal variation established in Scots pine by Langlet the studies of Wright and Baldwin (1957), which involved 46 of the 52 provenances used by Langlet, suggested discontinuous variation in the species, resulting in the recognition of a number of regional ecotypes. Some of these ecotypes distributed along latitudinal belts showed clinal relations, whereas others bore no relationship with latitudinal or climatic zones. These findings were strongly criticised by Langlet (1959), who, by replotting the data of Baldwin and Wright, was able to demonstrate clearly the correlation between tree height and day length.

Population differentiation in tree species in response to day length has been investigated by a number of workers (*Sylven* 1940, *Pauley* 1950, *Pauley & Perry* 1954, *Hoffman* 1953, *Vaartaja* 1954, *Wassink & Wiersma* 1955, *McMillan* 1967). In a considerably detailed work, Vaartaja (1959) has provided ample evidence for the occurrence of photoperiodic ecotypes in a number of forest trees. This study was concerned with the response of northern and southern races in several tree species tested for growth rate and onset of dormancy under various photoperiods. Plants were raised from seeds, collected from different latitudes, under different photoperiods (12-18 hrs.) obtained in greenhouses and observed for characters like duration of elongation, amount of growth and lateral development. These greenhouses differed with respect to photoperiod but received the same amount of light from sun and fluorescent tubes. It was concluded that the northern races showed growth inhibition in longer photoperiods than those from southern sources. Vaartaja suggests that the photoperiodic ecotypes have evolved as an indirect mechanism in the adaptation of trees to various seasonally changing climatic factors.

Clones of three species of *Populus*, collected from widely separated localities, were grown near Boston by Pauley and Perry (1954). These authors were able to correlate the time of termination of stem elongation with latitude of the locality. Discussing his studies of *Prosopis* from India, Sudan and America, McMillan (1957) has pointed out that "Although the India and Sudan seed collections were derived from plants growing over a latitudinal range from 30° to 40°N latitude, they did not show the response gradient of North American plants from a comparable range of latitude (Austin, Texas 30°N to Tecamachalco, Puebla 18°N). The similarity of response and the uniformity of their morphology suggested that the India and Sudan plants may have diverged little in the nearby hundred years since introduction. Their behaviour and morphology most closely resembles that of plants that have been studied from Northern Arizona." It may be mentioned here that seeds of *Prosopis* were first obtained for India from Mohave desert and later perhaps from Mexico also. Sudan material may have also originated from the same seed source as that of India (*McMillan* loc. cit.).

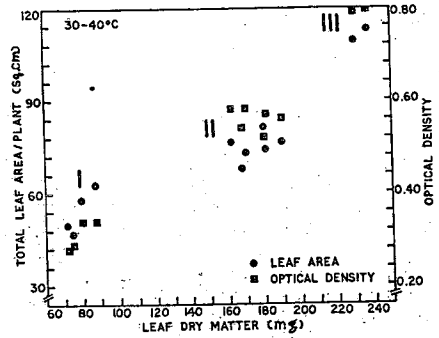
Data on ecotypic differentiation in grassland species have been gathered through the last three decades (*Riegel* 1940, *Olmsted* 1944, *Cornelius* 1945, *Larsen* 1947, *McMillan* 1956*a*, *b*, 1957, 1959*b*) which indicate that owing to climatic selection northern grassland communities of America are composed of individuals capable of growing under long photoperiods and short frost free periods. Southern communities, on the other hand, have individuals adapted to reverse conditions. These studies have elucidated the genetic basis for latitudinal differentiation and have thus demonstrated the presence of genetic gradients in a number of species. During a very thorough and competent study, McMillan (1959*b*) grew a number of species in a transplant garden at Lincoln, Nebraska, and in the greenhouse under various light periods from clones collected in a longitudinal and latitudinal grid extending through central United States and adjacent Saskatchewan and Manitoba. He (*McMillan* loc. cit.) also compared the growth and flowering behaviour of these transplants with those of the plants in their native habitat. From this study it is apparent that the differences in flowering time of several grass species are more of a hereditary nature (*i.e.*, genetic). It is further evident that the length of the photoperiod has greatly affected the latitudinal distribution of grassland vegetation and the yearly moisture variations have considerably affected selection of species. The author has rightly emphasized the role of ecotypic differentiation, "as the possible mechanism allowing the continuity of grassland vegetation over a broad geographic area."

Evidence of population differentiation within small study areas has been presented by Bradshaw (1959, 1960), Gregor and Watson (1954), Harberd (1961), Ehrendorfer (1953) and Cook (1962). Working with a grass species, *Agrostis tenuis*, in a small area of central Wales, Bradshaw collected samples from 36 colonies and compared their growth in standard and varied environments. Under standard cultivation these populations showed considerable physiological and morphological variability. This species is claimed by Bradshaw to show a mixture of continuous and discontinuous variations.

In India, the Varanasi School has been engaged with the problem of population differentiation since 1948, and on the basis of field observations, reciprocal transplantation, uniform culture and breeding experiments, a number of species have been shown to possess ecotypes. These studies have been recently reviewed by Misra (1967), Pandeya (1967) and Singh & Singh (1967 *a*, *b*). Based on similar studies and karyotypic analysis Pandeya (1967) has recognised 11 ecotypes in *Cenchrus ciliaris*. Singh J. S. (1962) has compared the growth, chemical composition and litter production of Teak (*Tectona grandis*) of different seed origins when planted on Gangetic alluvium. His observations clearly indicate differential response of trees originating from different localities. Through uniform culture of certain provenances of *Cassia tora* L. and *C. obtusifolia* L. and their seed germination, Singh J. S. (1968*a*, *b*) has supported the separation of these two as distinct taxa. Ramakrishnan (1965 *a*, *b*) has demonstrated the presence of edaphic ecotypes in *Euphorbia thymifolia* on the basis of soil calcium. Latitudinal differentiation in the populations of *C. tora* and *Anagallis arvensis* as revealed by provenance trials at Varanasi is briefly discussed here. The details of this study will be published elsewhere.

Cassia tora L.

Plants were raised under uniform culture conditions from seeds of seven different localities (Ahmedabad, Ujjain, Sagar, Varanasi, Patna, Shillong and Delhi) situated in six states of India and covering three latitudinal belts (from 23.20°N to 28.38°N latitudes) in 1965. Out of several characters examined, height of plants, root penetration, lateral spread of the root system, fresh weight of shoot, dry



Text fig. 1. Relationship of total leaf dry matter, total leaf area and optical density of chlorophyll extract in *Cassia tora* grown at high temperature.

weight of root, number of flowers, length of pod and number of seeds per pod exhibited statistically significant variation (*Singh, J. S.* 1966). In general, maximum values for these morphological characters were obtained for plants originating from 28.38° N latitude and minimum for those originating from localities in the vicinity of 25° N latitude. The plants originating from various latitudes exhibited significantly different values for the following characters when subjected to 't' test:

23° Vs 25° N lat. —Height of the plants and lateral spread of the root system.

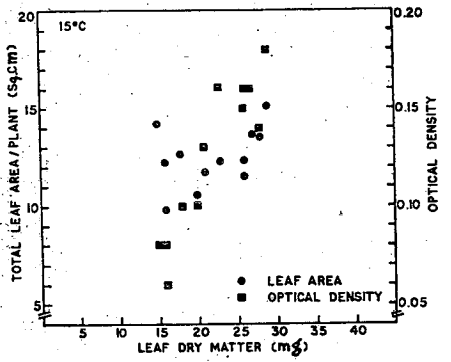
23° Vs 28° N lat. —Lateral spread of the root system.

25° Vs 28° N lat. —Height of the plants, root penetration, fresh weight of shoot, fresh weight of root and dry weight of root.

Lateral spread of the root system was further found to be positively related to latitude ($r = +0.581$) according to $y = -14.48 + 0.746\,x$, where y is the lateral spread of the root system and x is the

latitude of origin. Likewise dry weight of the root system was found to be positively related to latitude ($r = +0.635$) according to $y = -0.350 + 0.018\,x$, where y is the dry weight of the root system and x is the latitude of origin.

Later in 1966 plants were grown from seeds of the above cultivated provenances (I generation) and also from seeds of original collection at two different temperatures (15°C and 30-40°C). Dry matter yield and other characters of these populations

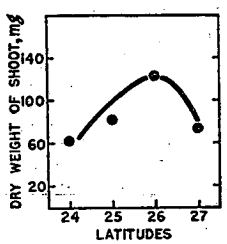


Text fig. 2. Relationship of total leaf dry matter, total leaf area and optical density of chlorophyll extract in *Cassia tora* grown at low temperature.

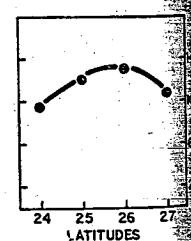
again exhibited differential response to uniform cultivation at both the temperatures. In general, plants originating from 28.38°N latitude maintained their superiority and the values for the plants originating in the vicinity of 25°N latitude were minimum (*Singh, J. S.* 1967 *a*). This study, thus, again supports the earlier findings. It was further brought out that when provenance trial is conducted at suboptimal environmental conditions, the segregation of populations may be masked to some extent. In the scatter diagram for optical density of the acetone extract of leaf, leaf area and leaf dry matter for plants grown at 30-40°C (Text fig. 1), three groups labelled as I, II and III corresponding respectively to 25°, 23° and 28°N latitudes of seed origin are segregated. On the other hand, no segregation is affected for plants raised at 15°C (Text fig. 2) pointing to the fact that segregation of the populations is best marked when provenances are grown at the temperature of their normal occurrence (*Singh, J. S.* 1967 *b*). Nevertheless, the interrelationships among these leaf characteristics remain similar (*Singh, Misra* 1968).

Anagallis arvensis L. var. **coerulia** Gren. et Godr.

Plants were raised in the weed garden of Banaras Hindu University from seeds collected from following places which are distributed between 24°N and 27°N latitudes. Kanpur (24°N); Allahabad, Banda, Varanasi, Jhansi (all within 25°N); Gwalior, Etawah (26°N); and Agra (27°N.) Analysis of variance carried out with reference to a number of morphological characters indicated statistically significant variation between different localities in the following (*Singh, K. P.* 1967): Shoot length, root length, number of nodes, number of leaves, dry weight of root, number of capsules (in all cases $p<0.001$), dry weight of shoot ($p<0.01$), and number of flowers ($p<0.05$). When the data of different localities are grouped into different latitudinal belts (24°, 25°, 26° and 27°N) the effect of latitude becomes perceptible in a number of characters, *e.g.* root length and dry weight of shoot (Text figs. 3 & 4). It is evident that in most of the characters, which are lowest in plants raised from seeds of 24°N latitudinal belt, tend to



Text fig. 3. Relationship between shoot dry weight and latitude of seed origin in *Anagallis arvensis*.



Text fig. 4. Relationship between root length and latitude of seed origin in *Anagallis arvensis*.

increase to maximum in plants of 25° or 26° origin. At still higher latitude (27°N) there is a decrease in most of the characters. In another study it has been shown that the seeds collected from these localities show significant differential behaviour with respect to duration of post harvest dormancy (*Singh, K. P.* 1968*a*). Recently evidence has been gathered to show that plants raised from these localities differ significantly with respect to rate of absorption of P^{32} (*Singh, K. P.* 1968*b*).

From the above observations it is, therefore, concluded that in both the species (*Cassia tora* and

Anagallis arvensis) distinct local populations are present which facilitate their distribution and tend to be latitudinally differentiated.

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