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ESTERASE ISOENZYMES IN POPULATIONS OF *AGROSTIS STOLONIFERA* L.

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Abstract

The genotypic and colonal structure of six populations of *Agrostis stolonifera* were revealed by esterase isoenzyme analysis. In the three copper refinery populations, despite the strong directional selectian created by copper pollution and the enormous power of vegetative propagation, considerable variation of esterase zymograms was detected for individual plants. In contrast, the Freshfield sand dune and Sefton Park populations only one and two types of esterase zymograms were detected. However, the existence of different esterase zymograms was revealed in the Cliff population. These results indicate that in areas where powerful selection is operoting many different genotypes are available for selection, and in some natural populations of *A. stolonifera* may vegetatively spread and covered a large area by a single genotype.

Introduction

Previous studies (Wu and Bradshaw, 1972; Wu, Bradshaw and Thurman, 1975) showed that substantial stores of genetic variability existed in the copper tolerance and morphological characters of the populations of *Agrostis stolonifera* L. that have been seriously contaminated by the air-born copper pollution. This variability is being strongly and rapidly altered by the copper pollution of the refineries. Despite this strong directional selection and the enormous power of vegetative propagation of *A. stolonifera*, a great deal of variability remains; there appears to be more variability in the highly selected refinery populations than in the normal populations.

There are clear limitations in the studies of genetic variability set by the use of morphological and physiological characters because they are difficult to quantify. This is obvious with the character of metal tolerance. However, these difficulties can be partly overcome by the use of the electrophoretic techniques which have been widely used in the areas of population genetics, evolutionary genetics, and systematics. A detailed introduction to the use of gel electrophoresis and its application to evolutionary studies has given by

Gottlieb (1972).

Enzyme polymophisms are known to be widespread in natural populations (Lewontin and Hubby, 1966; Burns and Johnson, 1967; Harris, 1969; Marshal and Jain, 1969). The role of selection in maintatining polymorphisms and the importance of specific environmental factors as selective forces can be determined by investigating the adaptive significance of individual protein polymorphisms (Kohen, 1969; Powell, 1971; McNaughton, 1972). The implication of polymorphism in the adaptive strategy of organisms on a metabolic basis has been discussed by Jonson (1971).

It would be useful if the proteins associated with the character of metal tolerance could be identified so that the selective relationships of metal tolerance could be established, as has been done for sickle cell anaemia. Unfortunately this is not yet possible. As an alternative the esterase isoenzymes of *A. stolonifera* were examined. Although no evidence of the adaptive significance of these isoenzymes as related to metal tolerance has been observed. The distribution of esterase alleles in *Avena brabata* Brot has been shown to be closely associated with environment both on macro-and micro-geogaphical scales (Allard, Kahler and Wier, 1972; Allard, Babbel, Clegg and Kaeler 1972). This study provide an opportunit of examine whether or not metal pollution can exert selective effects on these isoenzymes. Thus, it might offer an alternative to the physiological and morphological and morphological method for examining the variability and colonal structure of the populations.

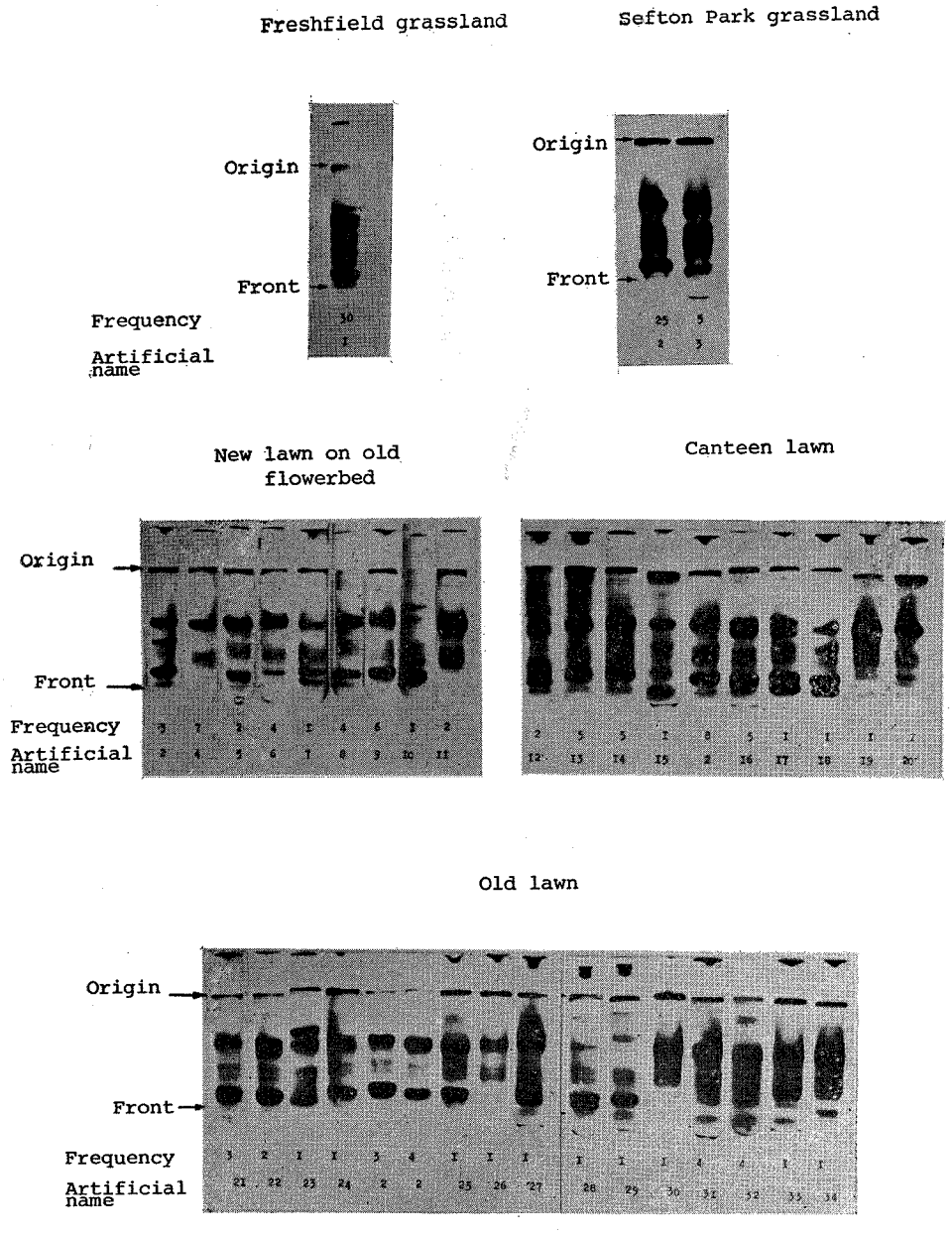
Materials and Methods

Preparation of enzyme extract

The present study was based on six populations of *A. stolonifera:* 1. the Freshfield sand dune, 2, the Sefton Park, populations existed in the area which is faraway from the industries and not being polluted by copper, 3. the Flowerbed new lawn, 4. the Canteen lawn and 5. the Old lawn, populations in an area of copper refinery industy where plants were contaminated by copper with different intensity and the duration of copper pollution. The detailed description of these populations were described in the previous work (Wu and Bradshaw, 1972). The plant materials of the sixth population was collected by Dr. I. McBean from the lower part of the Cliffs at Abram's Bosom which face the Irish sea and are extremely exposed to wind.

Plants from these six populations were transfered and grown from the separate tillers in plastic pots in a green house for three months before they were used. The leaf-blades of the two or three uppermost leavese were cut off in their entirety but without their ligules. The esterase patterns of various leaves of an individual plants were tested and were found to be identical for

Plate 1. Esterase Zymograms of Different Genotypes of *Agrostis Stolonifera*



any particnlar genotype. Crude enzyme extract were made by homogenizing 100 mg of fresh material at 4°C in a glass motar with 0.5 ml of 0.01 M tris-HCl buffer (pH 6.8), containing 5 per cent (w/v) sucrose. The homogenates were centrifuged at 20,000 g for 30 mins. The clear supernatants were used for enzyme assay immediately or stored in a deep freezer overnight.

Gel electrophoresis

The polyacrylamide disc electrophoresis was carried out essentially as described by Davis (1964). 50 μl of the enzyme extract was applied on each gel. After electrophoresis, the gel were removed and preincubated at 5°C in 0.5 M boric acid for 2 hours to lower the PH to approximately 6.5. They were then incubated for 1.5 to 2 hours at 25°C, in 10-ml tubes with 6 ml of 0.1 M phosphhate buffer at PH 6.5. The substrate and dye were added in the buffer as 2 mg α-naphthyl-acetate and 50 mg fast red per 100 ml buffer.

Results

In the Freshfield sand dune population, one type of esterase zymogram was found. In the Sefton Park population there were two. In the three refinery populations, 9, 10 and 15 types of esterase zymograms were found in the Flowerbed new lawn, Canteen lawn and Old lawn population respectively. The different types of zymograms were artifically named and their distribution in the five populations are shown in Plate 1. Thirty-four different esterase zymograms were found within the five populationstaken as a whole, but only one type of esterase zymogram (zymogram 2) was found in more than one population. This occurred in the Sefton Park population and the three refinery populations. It seemed that more genotypes were to be found in the Old lawn than in the Canteen lawn and Flowerbed new lawn populations. Apparently, no single genotype was dominat in the refinery populations.

In order to obtain a quantitative measure of the genetic variarion of esterase isoenzymes within each of the five populations, each individual plant was scored for the presence of esterase bands. If a particular band was present in all of the population, the corresponding locus was considered to be monomorphic in the particular population. However, if a band was present in some individuals but absent in others, the population was considered to be polymorphic for different forms of the enzyme. The bands appeared in each population which were scored in this manner are summerized diagrammatically in Table 1. It should be emphasized that there were several bands in some of the individuals which stained faintly and appeared inconsistently in different repeat assays; these were omitted in the present study.

Altogether, 12 esterase bands of different electophoretic mobilities were

Table 1. *Relative Frequency and Polymorphic index of Esterase Isoenzymes of five Populations of* ***Agrostis Stolonifera***

Popolation and mean index of copper tolerance	Artificial names	Polymorphic or monomorphic	Relative frequency	Polymorphic index
Freshfield dune population 0.07	E3	M	1.00	0.00
	E4	M	1.00	
	E5	M	1.00	
	E6	M	1.00	
	E7	M	1.00	
	E8	M	1.00	
	E9	M	1.00	
	E10	M	1.00	
Sefton Park grassland 0.06	E3	P	0.16	0.098
	E4	P	0.83	
	E5	P	0.16	
	E7	M	1.00	
	E8	M	1.00	
	E10	P	0.83	
	E12	P	0.16	
New lawn on old flowerbed 0.32	E2	P	0.03	0.171
	E3	P	0.50	
	E4	M	1.00	
	E5	P	0.06	
	E6	P	0.63	
	E7	P	0.53	
	E8	P	0.53	
	E9	P	0.30	
	E10	P	0.70	
	E11	P	0.10	
	E12	P	0.26	
Canteen lawn 0.42	E3	P	0.20	0.120
	E4	P	0.96	
	E5	P	0.03	
	E7	P	0.80	
	E8	P	0.73	
	E9	P	0.20	
	E10	P	0.93	
	E12	P	0.10	
Old lawn 0.53	E1	P	0.16	0.100
	E2	P	0.06	
	E3	P	0.16	
	E4	P	0.83	
	E5	P	0.03	
	E7	P	0.90	
	E8	P	0.90	
	E9	P	0.03	
	E10	P	0.83	
	E12	P	0.16	

found within the five populations. The distributien of these 12 isoenzyme bands can be classified into four categories according to thc frequency which they appeared in each population. a) Bands which appeared at high frequency and were universally distributed, such as the band E^4 (distribution frequency between 83% and 100%) and the band E10 (distribution frequency between 70% and 100%) in all of the five populations. b) Bands which appeared at high frequency (100%) in uncontaminated populations but lower frequency (50% to 90%) in the refinery populations such as the bands E7 and E8. c) Eands which appeared monomorphic in the Freshfield population but in low frequency in the other populations, such as E5 and E9. d) Rare or specifically distributed bands: the band E12 was not found in the Freshfield sand dune population but it existed as very low frequency in the rest of the four populations; the band E6 was found monomorphic in the Freshfield sand dune populations, at 6% frequency in the Flowerbed new lawn, but not found in the rest of the three populations; the band E2 existed in the Flowerbed new lawn and in the Old lawn; the band E1 was found only in the Old lawn; and the band E11 only in the Flowerbed lawn. A mathematical method which facilitates the comparisons of the degree of the polymorphism was introduced by Maeshall and Jain (1969),

$$PI=\sum_{i=1}^{N} Pi\ (1-Pi)/N$$

in which Pi is the frequency of the ith band and N is the number of bands for each enzyme system and population. This polymorphic index was calculated to indicate the degree of polymorphic to monomorphic bands and therefore, the degree of the heterogeneity of each population. This index can vary from

Table 2. *Relative Frequency and Polymorphic index of Esterase Isoenzymes of Cliff Population of* ***Agrostis Stolonifera***

Artificial Names of Esterase Bands	Polymorphic or Monomorphic	Relative Frequence	Polymorphic Index
E_3	P	0.75	
E_{13}	P	0.03	
E_4	P	0.64	
E_5	P	0.17	0.054
E_6	P	0.42	
E_8	P	0.82	
E_{10}	P	0.89	
E_{11}	P	0.17	
E_{12}	P	0.25	

0 to 0.25; the higher the index, the higher the heterogeneity. Values for the index are given in Table 1. The two uncontaminated populations have low values of the polymorphic index. Among the three refinery populations, the polymorphic index is higher in the Flowerbed new lawn (0.171) and lower in in the Canteen lawn (0.12) and Old lawn (0.100). All together nine esterase bands were found in the Cliff population and these bands were all polymorphic. The relative frequency of esterase bands are shown in Table 2. Except for the hand E13, which had a mobility between E13 and E4, rest of the bands were found in the previous studies of the five populations. The bands E8 and E10 have highest frequency (0.28 and 0.89 respectively) which both showed monomorphic or very high frequency in the Freshfield sand dune, the Sefton Park, the Canteen lawn and the Old lawn populations. The value of the polymorphic index of the esterase bands of the Cliff population is 0.054 and is lower than the three refinery populations.

Discussion

Present studies demonstrated that polyacrylamide disc gel electrophoresis offers a method for examing the genotypic and colonal structures of the populations of *A. stolonifera.*

The results indicate that the considerable variation of esterase zymograms were detected in the refinery populations. However, only one type (phenotype 2) of esterase zymogram was found commonly present in both Sefton Park population and the three refinery populations. These observations suggest that the populations have been isolated from each other and have evolved independently. Even though the refinery populations were found to locate in a similar environment, the Flowerbed new lawn was only about two meters from the Old lawn, the esterase pattern were quite different. However, the populations in the refinery area might have originated from different sources. Since they have been given little chance to flower and to produce seeds because of heavy mowing. Although occasionally some small panicles are found in these lawns, the populations would evolve independently of each other.

The same is true for individual esterase hands. There is no indication that any band is associated with any particular environment. Certainly there is no association of particular bands with the copper contaminated environment. The frequency of esterase band E7 and E5 was increased in the Old lawn, but both these two bands were monomorphic in the two uncontaminated populations. This suggests that esterase isoenzymes have no selective value in evolution against copper contaminated environments. However, selective values have proposed elsewhere as an explanation for esterase polymorphism

(Lewontin and Hubby, 1966; Burns and Jonson, 1967; and Harris, 1969), and for rapid change in esterase gene frequency in barley populations (Allard, Kahler and Weir, 1972).

However, the result suggests that there might be only one or two genotypes involved in the Freshfield sand dune population and Sefton Park population samples. What is the significance of the uniformity of these two populations? It is known that the vegetative spread by a single genotype of perennil creeping species to cover a large area in natural environments has been demonstrated by many authsrs (Camp, 1949; Moldenke, 1975; Harberd, 1961, 1962, 1967; Harberd and Owen, 1967).

Conditions in the refinery populations of *Agrostis stolonifera* were similar in a manner to which the evolution of a populatisn dominated by one or a few genotpeys would be expected. At the outset, it was expected that the older lawns would be dominated by few well adapted individuals, but this was not found. It appeared that a great number of individuals existed in the refinery populations. This might be due to that a large number of individuals with appropriate copper tolerance were available for selection either in the original populations or by recombination subsequently, because the plants occasionally did produce seeds in the lawns where they were miss mowed.

The existence of different genotypes was revealed by the esterase zymograms in the Cliff population demonstrates that considerable genetic variability does exist in a natural population of *A. stolonifera*, which is not polluted by copper. Since the Cliff population was extremely exposed to wind considerably, only few genotypes would be adapted to this environment. The powerful selection for short stolons in the Cliff population was reported by Aston and Bradshaw (1966). The present sampling of this site was done at the lower part of the Cliffs, where some vegetative spread of well adapted genotypes could be expected because the stolons of the plants hang down from the upper part of the Cliff allowing easy spread of individual clones. Nevertheless, a considerable number of different genotypes was found in this area. This result suggests that in areas where powerful selection is operating many different genotypes are available for selection.

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不同匍匐剪股穎 (*Agrostis stolonifera* L.) 族羣中脂化酵素同位酵素之分析

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利用電泳分析法對脂化酵素同位酵的分析觀察匍匐剪股穎族羣遺傳因子與無性繁殖系統的結構。由脂化酵素同位酵素分離圖（zymogram）顯示在三個受銅污染的族羣雖然受到十分强的單方向的天擇力，與其本身無性繁殖的能力，而在這些族羣中仍包含了許多不同因子型的個體。此結果證明以同位酵素分析的方法來鑑定族羣中因子型的結構是一個十分方便的方法，可以補助以形態及生理分析方法的不足，並且顯示出植物族羣雖然在很强的天擇壓力下，許多不同因子型的個體可供選擇。