GENE FREQUENCIES IN WILD POPULATIONS OF *TRIFOLIUM REPENS*

II. DISTRIBUTION BY ALTITUDE

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1. INTRODUCTION

A previous investigation (Daday, 1954) was concerned with the distribution of the glucoside lotaustralin and enzyme linamarase gene frequencies in *Trifolium repens* populations derived from locations at low altitudes in Europe and the Near East. As the location of origin of populations moved from the Mediterranean region to north-eastern Europe, a progressive diminution in the frequency of the dominant alleles was found. This diminution ranged from 100 per cent. to nil. A correlation was also found to exist between decreasing gene frequencies and decreasing January mean temperatures. It is known that the presence and absence of glucoside and enzyme is determined by two separate pairs of genes—*Ac-ac* and *Li-li* respectively (Corkill, 1942). The phenotypic frequency was calculated from the proportions of plants having the four chemical phenotypes : (1) Glucoside and enzyme (*AcLi*) ; (2) Glucoside only (*Acli*) ; (3) Enzyme only (*acLi*) ; (4) Neither glucoside nor enzyme (*acli*). The genotypic structure was determined by means of the Hardy-Weinberg formula ($p^2+2pq+q^2 = 1$). The aim of the present investigation was to study the genetical structure of *T. repens* populations derived from different altitudes in the Central European Alps. For this purpose a collection of wild *T. repens* seed was obtained from Switzerland, together with a sample from Austria.

2. METHOD

The modified Guignard picric acid test was used to distinguish between the four phenotypes of *T. repens*. This method described by Corkill (1940) was applied, together with an additional test. The seed was sown in boxes in a greenhouse, and the picric acid test was carried out when the plants were two to three months old, using solutions of linamarase (prepared according to the method of Coop, 1940) and a standardised solution of isolated lotaustralin (Melville and Doak, 1940). The presence and intensity of the colour reaction was recorded and graded into six groups.

The method described by Corkill (1940) was extended by making an additional test on the *Acli* phenotype. Plants of the *Acli* phenotype were further tested with lotaustralin. Two drops of solution with chloroform were added to freshly collected leaf samples in a test tube.

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After 18-20 hours' incubation many plants showed a colour reaction on the picric acid paper. Plants of the *Acli* phenotype which reacted positively were classified as *AcLi* phenotype, while plants which gave no reaction to this test remained in the *Acli* phenotype group. These reactions in most cases were very weak, but they indicated that many plants designated *Acli* phenotype by the first test may also contain a small amount of enzyme, the quantity being so small as to be insufficient to hydrolise the cyanogenetic glucoside in the plants.

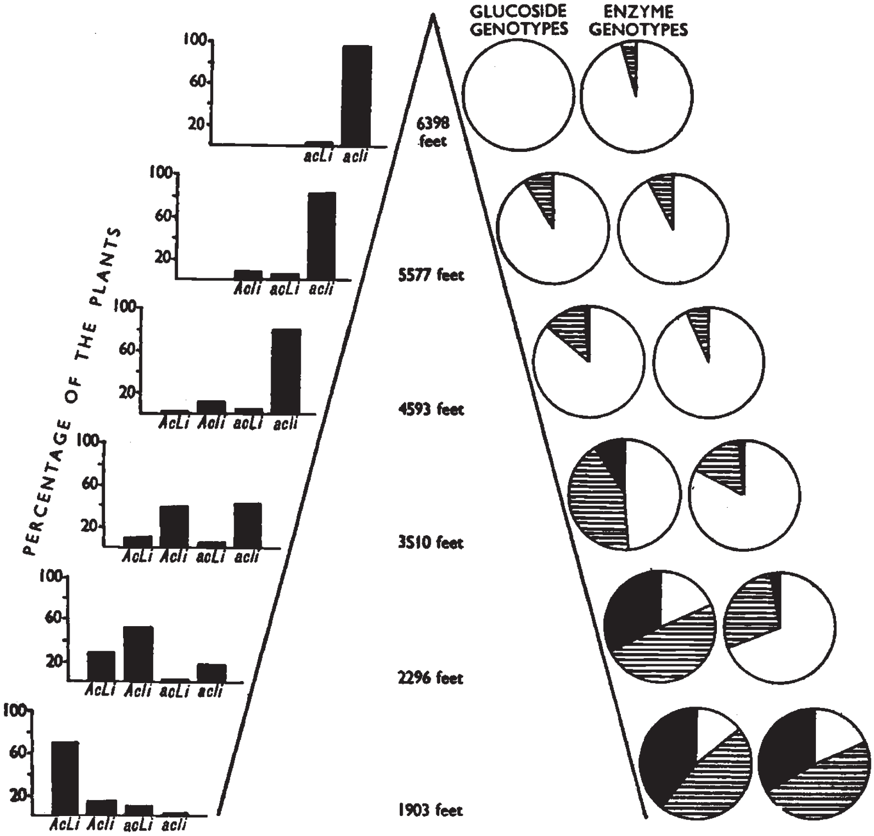

FIG. 1.—Phenotypic and genotypic frequencies in wild populations of *Trifolium repens* from different altitudes.

Phenotypes (left) :
AcLi = glucoside and enzyme
Acli = glucoside only
acLi = enzyme only
acli = neither glucoside nor enzyme

Genotypes (right) :
Black section = dominant homozygotes
Lined section = heterozygotes
White section = recessive homozygotes

Variation in enzyme gene action suggests the presence of a gene with very low enzyme output, which could not be identified without applying this additional test. The picric acid test was found to be suitable for estimating the enzyme output by using a standard solution of isolated lotaustralin. In some samples the proportion of this type of plant exceeded 20 per cent.

3. GENETICAL STRUCTURE OF THE WILD POPULATIONS

The first indication of differences between the gene frequencies of populations from high altitudes and those from low altitudes was observed after testing a small sample of *T. repens* collected by Beddows (1949) from the Pyrenees (Gavarnie—altitude about 5000 feet). Tests of this sample showed the presence of a preponderance of the *acli* phenotype as compared with French samples from low altitudes, which consisted mainly of plants of the *AcLi* phenotype.

In the present investigations, *T. repens* samples were collected from regions of different altitudes in the Central European Alps. The results of analyses of the phenotypic and genotypic structure of these samples are presented in tables 1 and 2 and in fig. 1. Lausanne, situated beside Lake Geneva at 1903 feet a.s.l., was the lowest location from which samples were collected.

TABLE 1

Frequency (per cent.) of the four phenotypes in wild populations of Trifolium repens *from different altitudes*

Localities	Altitude in feet	*AcLi*	*Acli*	*acLi*	*acli*	No. of plants
Lausanne . .	1903	70·65	15·22	10·87	3·26	184
Naters . . .	2296	28·00	53·00	2·00	17·00	100
Fiesch . . .	3510	10·91	40·00	6·36	42·73	110
Alpage de Crausey .	4593	1·11	12·22	4·45	82·22	90
Kreuz . . .	5577	0·00	8·51	7·45	84·04	94
Gross Glockner .	6398	0·00	0·00	4·04	95·96	99

Collecting points at higher altitudes were as follows :—Naters (2296 feet), Fiesch (3510 feet), Alpage de Crausey in Canton Valais (4593 feet), Kreuz in Canton Grison (5577 feet) and Gross Glockner in Salzburg (6398 feet). The Lausanne sample was typical of the Swiss lowlands, and no altitudinal effects were observed. Over two-thirds of this population consisted of the *AcLi* phenotype ; the *Acli* and *acLi* phenotypes were present in the small proportion of about 15·22 and 10·87 per cent., while the *acli* phenotype constituted only 3·26 per cent. of this population. If the proportions of the four phenotypes in the populations from the six different altitudes are compared, marked differences are apparent. In the sample from Naters (2296 feet) the percentage of the *AcLi* phenotype drops to less than one half of that in the population from Lausanne (1903 feet). At 3510 feet (Fiesch) and 4593 feet (Alpage de Crausey) the proportions of the *AcLi* phenotype in the populations are 10·91 and 1·11 per cent. respectively. The populations at the two highest altitudes contain no *AcLi* phenotype.

The distribution of the *Acli* phenotype in Alpine populations may be summarised as follows. In contrast to the low frequency of *Acli* at 1903 feet, 53·00 per cent. of the sample from 2296 feet was of this phenotype. As the altitude increases—Fiesch (3510 feet), Alpage de Crausey (4593 feet), Kreuz (5577 feet)—the percentage of *Acli* again declines ; at 6398 feet this phenotype is completely absent. There is little variation in the proportion of the *acLi* phenotype, which is present in a low percentage in all the Alpine populations studied.

TABLE 2

Frequency of the glucoside and enzyme genotypes in wild populations of Trifolium repens *from different altitudes*

Localities	Glucoside genotypes			Enzyme genotypes		
	p^2	$2pq$	q^2	p^2	$2pq$	q^2
	AcAc	*Acac*	*acac*	*LiLi*	*Lili*	*lili*
Lausanne	0·390	0·469	0·141	0·325	0·490	0·185
Naters	0·318	0·492	0·190	0·027	0·273	0·700
Fiesch	0·089	0·420	0·491	0·008	0·165	0·827
Alpage de Crausey	0·005	0·128	0·867	0·001	0·055	0·944
Kreuz	0·002	0·083	0·915	0·001	0·073	0·926
Gross Glockner	0·000	0·000	1·000	0·0004	0·0400	0·9596

As altitude increases, there is a corresponding increase in the percentage of the *acli* phenotype from 3·26 at Lausanne to 95·96 per cent. at Gross Glockner. As previously reported in the lowland populations of Europe there is here further evidence of a change in frequency of the different phenotypes with changing environmental conditions.

TABLE 3

Frequency of the glucoside lotaustralin and enzyme linamarase genes in populations of Trifolium repens *from different altitudes*

Localities	Altitude in feet	Glucoside gene		Enzyme gene		January mean temperature °F.
		Ac	*ac*	*Li*	*li*	
Lausanne	1903	0·6241	0·3759	0·5701	0·4299	30·10
Naters	2296	0·5642	0·4358	0·1633	0·8367	29·30
Fiesch	3510	0·2994	0·7006	0·0904	0·9096	25·70
Alpage de Crausey	4593	0·0690	0·9310	0·0282	0·9718	28·40
Kreuz	5577	0·0435	0·9565	0·0380	0·9620	24·80
Gross Glockner	6398	0·0000	1·0000	0·0204	0·9796	20·30

The genotypic structure of the populations is presented in table 2. The frequencies of the genotypes *AcAc*, *Acac*, *acac*, and *LiLi*, *Lili*, *lili* were calculated by means of the Hardy-Weinberg formula. About one-third of the population from Lausanne has the *AcAc*, *LiLi*, genotypes, and nearly half of the sample appears to be heterozygous (*Acac*, *Lili*), while *acac*, *lili* occur at a frequency of 0·141 and 0·185 respectively. The frequency of both the dominant homozygous genotypes decreases progressively throughout the whole series as altitude increases. Simultaneously the proportion of the *acac* and *lili* genotypes increases until plants of populations existing at the Gross Glockner location (6398 feet) are entirely *acac*, and the *lili* genotype shows a frequency of 0·9596. These findings provide evidence of the changes occurring in phenotypes and genotypes of wild populations of *T. repens* at different altitudes.

Table 3 and fig. 2 represent the allele frequencies in wild *T. repens* populations of the Central European Alps. A gradual decrease was found in the proportion of dominant glucoside lotaustralin (*Ac*) and enzyme linamarase (*Li*) alleles. Consequently both recessive alleles

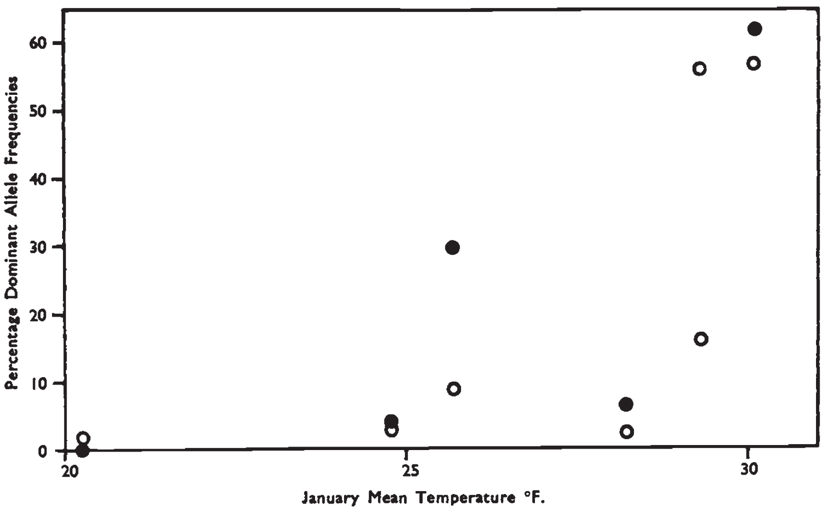

FIG. 2.—Relation between the January mean temperature and both the frequencies of the dominant glucoside lotaustralin (*Ac*) and enzyme linamarase (*Li*) alleles in populations of *Trifolium repens* from different altitudes.
● = dominant glucoside allele. ○ = dominant enzyme allele.

(*ac*, *li*) increased as altitude increased and as the January mean temperature decreased.

Melville *et al.* (1940) established that the picric acid test is not only qualitative but also provides a rough quantitative measure. The six grades of colour reaction were found to correspond to an increase from about 0·006 to 0·0335 per cent. HCN content in the fresh leaf material. The frequency of the six reaction groups are given in table 4.

From this table it is apparent that the largest proportion of plants from Lausanne, Naters and Fiesch populations fall into reaction groups 4, 5 and 6, the greatest proportion falling into group 5, though the enzyme reaction of the Fiesch population is an exception. A large majority of plants from the other populations fall into the first three reaction groups. High frequency of occurrence of the dominant alleles appears to be correlated with a high output of glucoside enzyme products at low altitudes. In contrast, *T. repens* populations from higher slopes and which exhibit lower dominant gene frequencies have, in general, a lower output of gene products.

4. DISCUSSION

Variability within the species in response to diverse environmental conditions at different altitudes was established in *Potentilla glandulosa* (Clausen *et al.*, 1940). On the basis of morphological and ecological

TABLE 4

Frequency of the picric acid paper reactions in populations (in per cent. of the plants bearing glucoside or enzyme) from different altitudes

Localities	Altitude in feet	No. of plants bearing glucoside	Glucoside reaction					
			1	2	3	4	5	6
Lausanne	1903	158	1·9	8·2	12·7	8·9	*56·3*	12·0
Naters	2296	81	5·0	7·4	11·1	11·1	*33·3*	32·1
Fiesch	3510	56	1·8	0·0	10·7	17·8	*50·0*	19·7
Alpage de Crausey	4593	12	0·0	25·0	*58·3*	16·7	0·0	0·0
Kreuz	5577	8	25·0	0·0	*62·5*	0·0	12·5	0·0
Gross Glockner	6398	0	0·0	0·0	0·0	0·0	0·0	0·0

Localities	No. of plants bearing enzyme	Enzyme reaction						No. of tested plants
		1	2	3	4	5	6	
Lausanne	150	4·6	6·0	12·7	5·3	*46·7*	24·7	184
Naters	30	13·4	26·6	3·3	10·0	*30·0*	16·7	100
Fiesch	19	*37·0*	21·1	15·7	15·7	10·5	0·0	110
Alpage de Crausey	5	20·0	*60·0*	20·0	0·0	0·0	0·0	90
Kreuz	7	14·3	28·6	*42·8*	0·0	14·3	0·0	94
Gross Glockner	4	*75·0*	0·0	0·0	0·0	25·0	0·0	99

characters, four subspecies were distinguished in that species originating from the central Californian transect. Within each subspecies further differentiation into climatic races was observed. Other work of Clausen *et al.* (1948) described the altitudinal climatic races of *Achillea* from a west to east transect across central California. Dobzhansky (1948) found that differences in the gene arrangement of the third chromosome occurred in populations of *Drosophila pseudoobscura* and *Drosophila persimilis* from Sierra Nevada, California. Out of the seven different gene arrangements, the Standard is the most frequent at low altitudes, but becomes progressively less frequent at higher altitudes of the mountain range. The Arrowhead gene arrangement is most frequent at the subalpine zone and decreases in frequency down the slope. Six gene arrangements were found in the third chromosomes of *Drosophila persimilis*. The Whitney gene arrangement was more common in populations at low than at high altitudes; other gene arrangements show a reverse trend. The results obtained from *T. repens* also show a change in genetical composition with elevation. Both dominant glucoside lotaustralin and enzyme linamarase allele frequencies decrease with increasing altitude from 1903 to 6398 feet in the Central European Alps. This gene frequency cline shows a corresponding gradation with the January mean temperature (table 3). Detailed analyses of European populations indicated a significant relationship between changes in gene frequency and decreasing January mean temperature (Daday, 1954). A correlation between gene frequency and altitude is therefore not unexpected.

Some explanation is necessary in respect of the relation between January mean temperature and gene frequency at Alpage de Crausey. This place is situated at a considerably higher altitude (4593 feet) than Fiesch (3510 feet), but the January mean temperature is higher, being 28·40° F., compared with 25·70° F. at 3510 feet. In general, temperature declines with increasing altitude, and this reversal of temperature conditions at Alpage de Crausey must be attributed to local geographical conditions. In spite of this, however, the dominant allele frequency remains lower at 4593 feet than at 3510 feet; this may be due to the microclimate of Alpage de Crausey where the sample was collected.

A divergence in the action of genes has also been shown by Raper (1927) in investigations on colour differences in mammalian hair. The pigmentation of hair is due to the presence of melanin, which is derived from tyrosine by means of its oxidation into dihydroxyphenylalanine, this being then converted into pigment in the presence of an enzyme. Other studies (Russell, 1939) indicate that gene replacement at other loci influences the quantity of pigment by affecting the activity of the enzyme system, thus producing various degrees of hair colour. The interaction of modifying genes which control the output of cyanogenetic glucoside in *T. repens* was suggested by Williams (1939). A similar mechanism may also govern enzyme production. It can be assumed on the basis of the present investigation that these modifying factors have also been subjected to natural selection.

It has been shown (Daday, 1954) that in its spread over Europe and the Near East *T. repens* has undergone changes in gene frequencies in response to the January mean temperatures. It may be concluded that a similar response occurs in populations at different altitudes.

was shown to be correlated with the decreasing January temperature at high altitudes.

2. Variation was observed in the amounts of enzyme and glucoside gene products, and this appears to be positively correlated with dominant gene frequencies in the populations.

3. The quantity of enzyme linamarase in plants appears to be governed by modifying genes in a manner similar to the glucoside.

4. Both the differences in gene frequency and gene output are considered to be due to natural selection.

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