GENE FREQUENCIES IN WILD POPULATIONS OF *TRIFOLIUM REPENS* L.

III. WORLD DISTRIBUTION

HUNOR DADAY
Division of Plant Industry, C.S.I.R.O., Canberra, Australia

1. INTRODUCTION

ALTHOUGH there have been a great number of publications describing morphological and cytological adaptation, Dobzhansky (1951) has stated that " the extent of the diversity of physiological and biochemical traits in living beings is still quite imperfectly known ". The aim of this investigation was to establish the pattern of gene controlled chemical variation in world populations of *Trifolium repens*.

It is already known that *T. repens* possesses cyanogenetic glucosides consisting of 80 per cent. lotaustralin and 20 per cent. linamarin (Melville and Doak, 1940) and their hydrolysing enzyme linamarase (Coop, 1940). One of the products of hydrolysis is HCN. This was believed to cause bloat or poisoning effects in ruminants but Corkill (1952) was unable to demonstrate deleterious effects when sheep were grazed on high cyanogenetic glucoside lines of *T. repens*.

The presence of these cyanogenetic glucosides and their hydrolysing enzyme in *T. repens* is determined by independent single genes (Corkill, 1942 ; Atwood and Sullivan, 1943) while modifying genes determine the quantity of cyanogenetic glucosides produced (Corkill, 1942). In the following pages the term " lotaustralin " is used instead of cyanogenetic glucosides and " linamarase " refers to the hydrolysing enzyme. Previous investigations (Daday, 1954a) demonstrated that the frequencies of the lotaustralin and linamarase genes varied from 0 to 100 per cent. in European and Near Eastern wild populations of *T. repens*. A similar gene frequency cline was observed in Alpine populations (Daday, 1954b). Winter temperature was considered to be the major factor responsible for the formation of these gene frequency clines in wild populations.

The major part of this investigation was carried out at the Welsh Plant Breeding Station, Aberystwyth, Great Britain and completed at C.S.I.R.O., Canberra.

2. METHODS

The presence of lotaustralin and linamarase in plants was shown by the Guinard picric acid paper test (Corkill, 1940) with certain modifications (Daday, 1954a). According to their chemical constituents the plants were classified into four chemical phenotypes : (1) Lotaustralin and linamarase *(AcLi)* ; (2) Lotaustralin only *(Acli)* ; (3) Linamarase only *(acLi)* ; (4) Neither lotaustralin nor linamarase *(acli)*. (Appendix I).

The gene frequencies were calculated by the Hardy-Weinberg formula.

The collection of seed samples was organised by the British Council, the United States Department of Agriculture, and Universities and Agricultural Experiment Stations of various countries. The technique of collection has been described in a previous paper (Daday, 1954a).

3. GEOGRAPHICAL DISTRIBUTION OF T. REPENS

T. repens is a typical species of the temperate regions of the world. However, the high adaptability of the species enabled it to extend to the Arctic regions (Murmansk, Kanin Peninsula, U.S.S.R.) and to the sub-tropical regions as in Queensland (Australia). The geographical distribution of T. repens is presented in fig. 1.
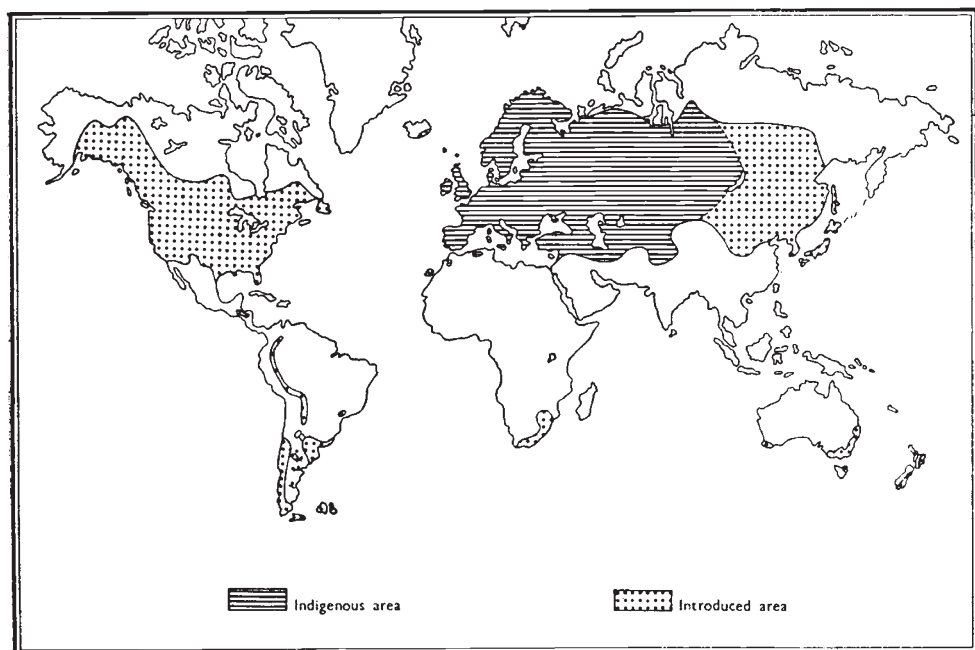

Indigenous area   Introduced area

FIG. 1.—Geographical distribution of Trifolium repens L.

The Mediterranean region is considered by Vavilov (1951) to be the centre of origin of T. repens. This species has spread all over Europe and has also invaded the larger part of Asia by means of animal, human and spontaneous distribution. Its occurrence is recorded on the Asiatic mainland (Komarovii, 1945) as follows : Turkey, Lebanon, Israel, Iraq, Persia, Pakistan, India (Himalaya Range up to 20,000 feet), U.S.S.R., China, Mongolia and Korea. It is considered that the indigenous area consists of the whole European and the western half of the Asiatic distribution area, whilst east from the Baikal Lake is the recently occupied region. Personal information obtained from Japan indicates that the first sample of T. repens was carried by a Dutch vessel which arrived at Nagasaki in 1846. This is considered as one of the ancestral sources of the Japanese T. repens.

This species is indigenous to North Africa. It occurs both in low

land and at high elevations (Atlas Mountains) in Morocco and Tunisia wherever sufficient soil moisture is available. One collection came from an altitude of 7500 feet. The plant was introduced into South Africa, establishing itself in Cape Province and in cool mountain areas where the rainfall is evenly distributed and along stream banks in Natal, Transvaal, and Pretoria. Botanical surveys indicate the presence of the species in natural vegetation on the Canary Islands.

T. repens was introduced into North America by the earliest settlers and now it is extremely abundant in most of the settled areas. It is one of the most widespread species and extends northward to Canada and Alaska. It has invaded the whole U.S.A. but between the Cascade, Sierra Nevada mountains and the 100° parallel, grows along rivers, around habitations and irrigated fields. According to a personal communication, *T. repens* was carried to Alaska in about 1920.

The distribution of introduced *T. repens* is restricted to the temperate regions in Central and South America. It occurs in the high altitudes of the Andes and other mountainous regions in Guatemala, Costa Rica, Venezuela (up to 9000 feet), Columbia, Peru, and Bolivia. Comparatively recent introduction started the spread of this species in Brazil (Rio Grande do Sul, Minas Gerais). It is more common in Chile, and in the humid parts of Argentina and some fertile regions of Uruguay.

T. repens is not indigenous in Australia and New Zealand. The spontaneous spread of this species is mainly restricted to humid coastal regions of Australia, but is often seen along streams, and under irrigation in the drier areas. This species was introduced as a pasture plant during the early history of New Zealand and was used to oversow cleared forest areas, bush burns, and *tussock* grassland areas. Now it has become the most important legume component of the grassland vegetation but it also appears naturally along roadsides and where the soil has been disturbed.

4. GENE FREQUENCIES IN WILD POPULATIONS OF THE WORLD

A survey of lotaustralin and linamarase gene frequencies (Daday, 1954a) in European and Near Eastern *T. repens* populations disclosed a cline of dominant gene frequency from the warm winter region, where high frequencies predominate, to low frequencies which are most common in Northern Europe. This species is considered to have spread by natural means to the largest part of the Asiatic distribution area.

Four samples were tested (Appendix I) from the mainland of Asia. The southern populations exhibited a higher frequency (Iran, Karaj river area, *Ac* 100·0, *Li* 40·6 per cent. ; India, Almora, *Ac* 67·1, *Li* 73·2) than those collections derived from U.S.S.R. (Turgatuj and Novosibirsk, *Ac* 0·0, *Li* 0·0).

Seven samples represent the Japanese *T. repens* populations. Four of these originated from the southern warm winter regions and three from the cold winter regions. The warm winter region collections are from the South Pacific Coast Region (Miyazaki and Kochi), Seto-Inland—Sea Coast Region (Zentsuji) and the Kanito region with a dry winter climate (Konosu collection). They represent rather mixed gene frequencies (*Ac* 14·7-52·3, *Li* 18·8-42·3 per cent.). The remaining three samples are from the Sea Coast Region in a cool winter area of Japan, Shindo-Mura (*Ac* 54·7, *Li* 21·5 per cent.) ; the cold area of the Pacific Coast Tohoku (North Eastern) Region represented by the Shimo-Kuriyagawa collection (*Ac* 26·6 ; *Li* 28·0 per cent.) ; and the coldest winter region of Japan, Hokkaido in the north, where *Ac* 15·0, *Li* 23·2 exhibit the lowest frequencies in the Tsukissapau sample. The Japanese samples may thus be divided into those from the southern warm winter region with mixed gene frequencies, and those from the northern colder winter area with decreasing gene frequencies of *Ac*.

T. repens is indigenous to North Africa, where it shows some indication of altitudinal gene frequency clines (Rabat, near sea level, *Ac* 100·0, *Li* 100·0 ; Tirsal, at 4500 feet, *Ac* 100·0, *Li* 100·0 ; Oukaiemden, at 7500 feet, *Ac* 59·7 and *Li* 69·5 per cent.). All the samples tested from South Africa varied between 51·7 and 91·0 per cent. for *Ac*, and 43·0 and 70·2 per cent. for *Li*. There is no evidence for gene frequency cline formation in South Africa.

The U.S.A. and Canadian populations are represented by considerable numbers of *T. repens* samples from Louisiana to Alaska. A high mean lotaustralin gene frequency was found in most of the samples above the 40° F. January isotherm (Baton Rouge 71·9, New Orleans 75·8, San Francisco 61·4, San Francisco 84·3, Rio Monte 100·0 for *Ac* gene frequencies). Between the 40° and 0° F. isotherm, there are collections from Oregon, Ohio, Nebraska, Illinois, Minnesota, Alaska, Quebec and Alberta which exhibit rather mixed gene frequencies with respect to the *Ac* gene. However, 32·6 to 2·2 per cent. frequencies were found below the 0° F. line (Brandon, Fairbanks). This relationship between the January mean temperature of localities and gene frequencies (fig. 2) is highly significant (P<0·001).

The regression slope indicates that the change in the *Ac* frequency is 0·681 per cent. per 1° F. mean temperature. The relationship between *Li* gene frequencies and temperature change is not significant. Natural selection seems to have occurred in North America and a frequency cline for the *Ac* gene has already formed. The occurrence of a *Li* frequency cline in North America does not seem to have any statistical support.

South American collections were obtained from Peru, Brazil and Argentina. Gene frequencies vary considerably, 7·9-100·0 per cent. for the *Ac* gene and 13·0-100·0 per cent. for the *Li* gene. The lowest *Ac* frequency (7·9 per cent.) was found at Pedro Leopoldo in Minas

Gerais, Brazil, where the winter temperature is characterised by being the highest (60° F.) among the sampled areas. High *Ac* frequencies were established at Esquel, Argentina, where the mean temperature is the lowest among the samples (30° F.). The mean July temperatures of these South American localities are comparatively high (30-60° F.) and no collection was obtained from regions having July mean temperatures below 30° F. The absence of a gene frequency cline might reflect the absence of a large range in winter temperature in the parts of South America studied.
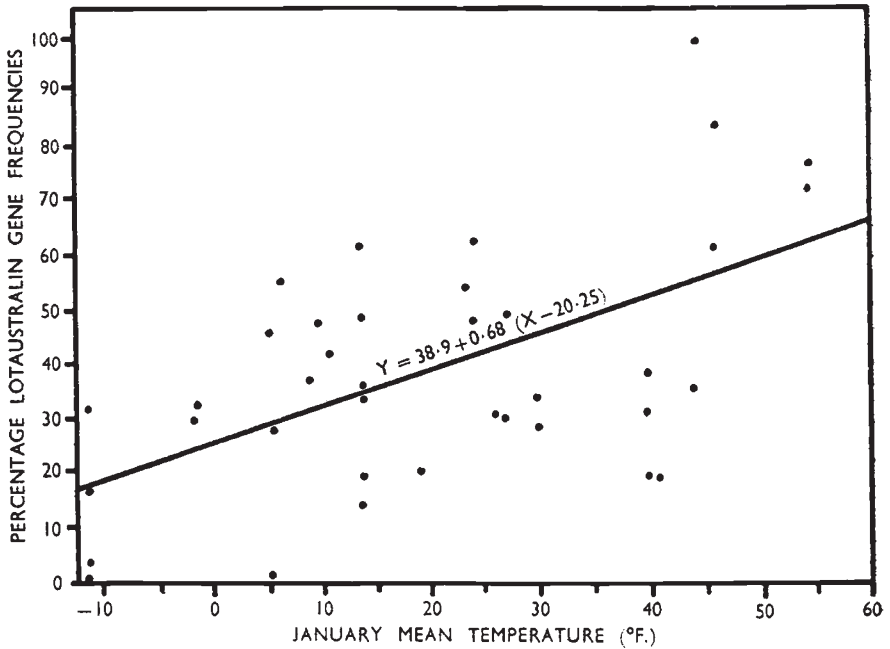

FIG. 2.—Correlation between lotaustralin gene (*Ac*) frequencies and January mean temperature in North American wild populations of *T. repens.*

Australian populations were represented by collections from old established pastures of New South Wales, Victoria, Tasmania, South Australia, and the Australian Capital Territory. Apart from the low land collections, altitudinal samples (Mt. Kosciusko, Mt. Gingera) were also tested. The frequencies of *Ac* range from 0·0 to 75·1 per cent. and those of *Li* from 6·1 to 78·8 per cent. in low land populations. There was no pattern or indication of cline formation. Mt. Kosciusko is in New South Wales and has the coldest winter in Australia. The highest elevations are covered by snow for 6 to 8 months of the year. Collections were made at altitudes from 3000 to 5800 feet. *Ac* frequencies were uniformly low (3·2 to 8·2 per cent.) but variable proportions of *Li* frequencies (28·6 to 51·2 per cent.) were found in this region.

The North and South Islands of New Zealand were represented by four collections. Hastings exhibited 100·0 per cent. frequency for the *Ac* gene (*Li* 88·0), while the lowest frequency, 37·7 per cent. for *Ac* (*Li* 70·0 per cent.), was at Winston. Other *Ac* values varied between these extremes.

The samples collected in Japan, South Africa, South America, Australia and New Zealand appear to be rather heterogeneous. The χ^2 test for heterogeneity has been carried out for samples of each of the above geographical areas. The heterogeneity test proved to be highly significant for all the samples with the exception of *Li* containing plants in New Zealand.

The geographical distribution of the lotaustralin gene frequencies in *T. repens* populations of the world is illustrated in fig. 3. Both Europe and the mainland of Asia show a 100-0 per cent. decrease. This gradual diminishing in gene frequencies occurs across the January isotherms. Above the 50° F. isotherm all populations have 100·0 per cent. dominant *Ac* frequency. Below the 50° F. isotherm the gene frequencies fall from 100·0 to 5·0 per cent. at the 30° F. isotherm. The rest of the area has 5 per cent. or lower frequencies. In North America, 100·0 to 60·0 per cent. was the most common frequency above the 40° F. isotherm, whilst below the 0° F. isotherm, dominant gene frequencies below 30·0 per cent. prevailed. *Ac* gene frequencies in northern Japanese populations also show a tendency to diminish with decreasing winter temperature. With the exception of mountainous regions with cool winters, mixed gene frequencies have been found all over the southern hemisphere.

Linamarase gene frequencies are presented in fig. 4. As with *Ac*, the *Li* frequency variation occurred within a range of 100 per cent. in Europe and Asia. More variable than *Ac*, but still high, were the frequencies of *Li* above 50° F. The decrease at lower temperatures was not as sharp as with the *Ac* gene. The mean frequency of *Li* is 15 per cent. at the 30° F. isotherm and then it gradually falls to 0 per cent. No cline has yet been found in recently colonised areas of the northern hemisphere. Mixed populations have also been found in the southern hemisphere.

The quantity of lotaustralin and linamarase produced by plants varies considerably. Corkill (1942) showed that modifying genes determined the amount of lotaustralin synthesised by plants. The relationship between the *Ac* gene frequencies and the mean rating of lotaustralin produced by world *T. repens* populations is presented in fig. 5.

Association between *Ac* frequencies and mean rating was examined by classifying the data into a 3 × 3 contingency table with the boundaries defined by dividing the range of each variable into thirds. χ^2 is 40·41 with 4 df (P < 0·001) so that an association clearly exists.
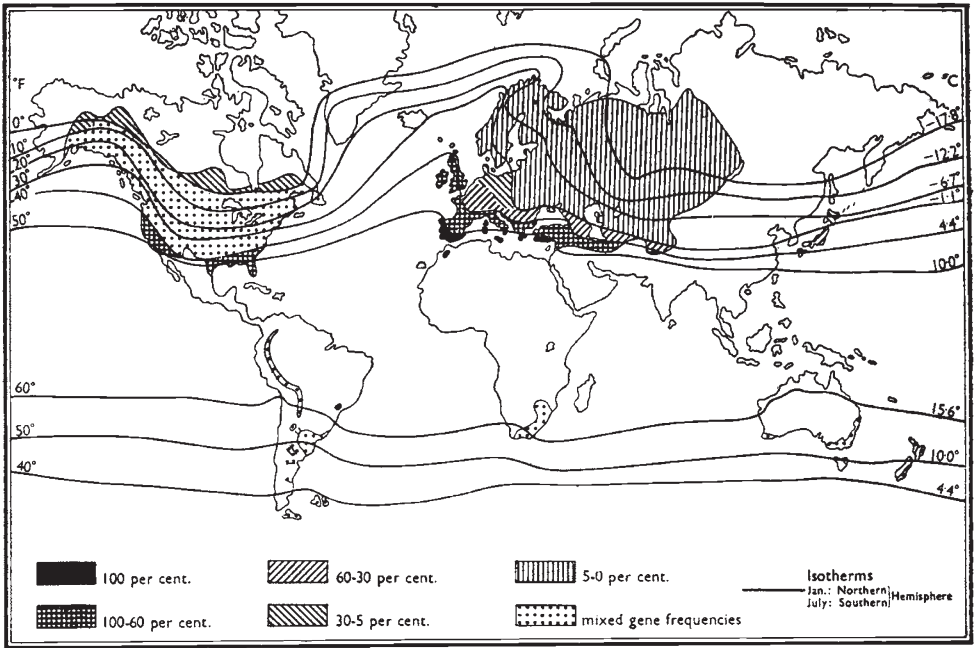
Fig. 3.—Distribution of lotaustralin gene frequencies in world
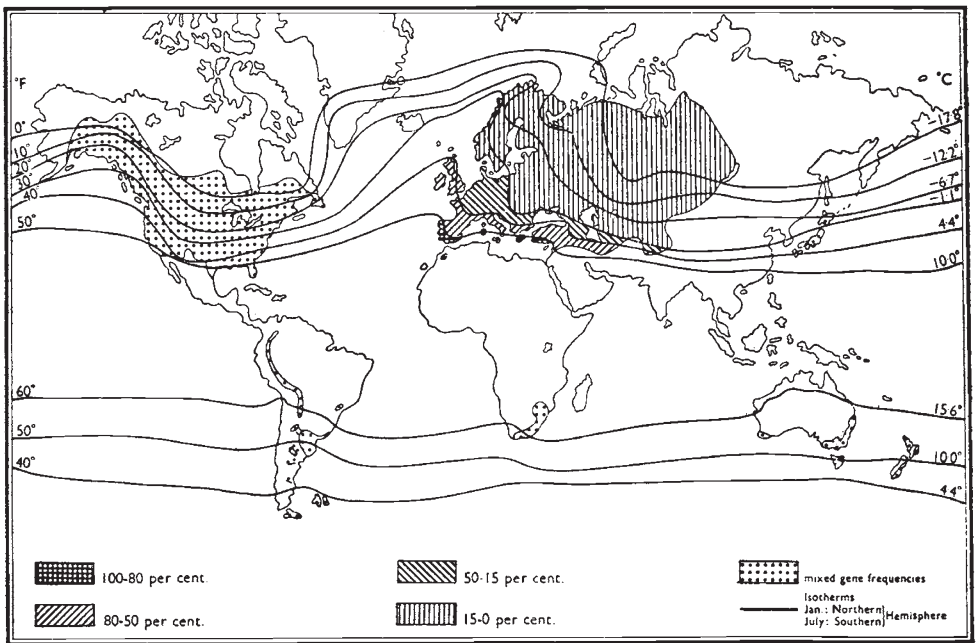populations of *Trifolium repens* L.

Fig. 4.—Distribution of linamarase gene frequencies in world
populations of *Trifolium repens* L.

5. DISCUSSION

Huxley (1955) has emphasised the evolutionary significance of intraspecific differentiation. He stated that genic differences between populations may enable the species to occupy areas which environmentally are sharply distinct. If there is adaptation to a continuous range of environments, the formation of a ratio-cline will be apparent where a balanced equilibrium exists between the adaptive morphism
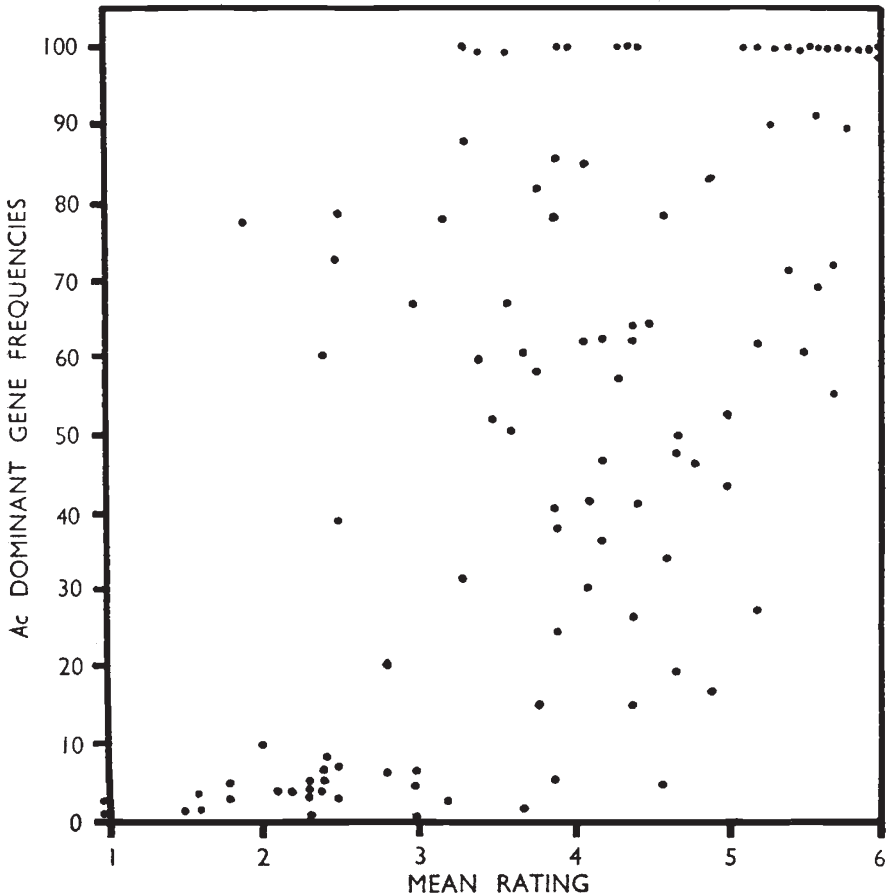

Fig. 5.—Correlation between lotaustralin (*Ac*) gene frequencies and mean rating of produced lotaustralin in world populations of *T. repens*.

and the environment. The investigation of ratio-clines may lead to the exploration of selective forces. A typical example of a ratio-cline has been demonstrated in *T. repens*. The dominant lotaustralin and linamarase gene frequencies decrease from 100 to 0 per cent. as the mean winter temperature decreases. Therefore, winter temperature is considered as the major selective force in this example. It can also be seen that the gene frequency cline is more evident in those regions where the coldest month of the year has a temperature range below 40° F. This range of required winter temperature can

be found in the northern hemisphere but not in the southern hemi-
sphere with the exception of such cold winter mountainous regions as
Mt. Kosciusko.

The occurrence and spread of a mutant gene can influence con-
siderably the selective advantage or disadvantage of a population, as
is well illustrated in *Pisum arvense* L. (Scheibe, 1955). A pair of genes
governs the presence or absence of a waxy layer on the leaves of
P. arvense. A thick layer of wax is found on plants of dry climatic
regions such as Asia Minor and the European Continent while
populations have been found in the British Isles with thin waxy
layers. Scheibe studied the behaviour of genotypes differing only in
the waxy character under competition in a low rainfall area in
Germany where a mixture consisting of 50 per cent. of each genotype
was grown for four years. The waxy genotype increased to a frequency
of 80·9 per cent. while the non-waxy genotype was reduced to 19·1
per cent. of the original population. The non-waxy phenotype was
associated with a higher rate of transpiration which in turn reduced
seed number and seed weight. This example shows that single gene
mutants can contribute considerably to the selective potential of a
species.

The distribution of allelic frequencies in populations according
to selection pressure is well demonstrated in *Ricinus communis* L. and
species of *Eucalyptus*. A single dominant allele *B* produces waxy bloom
on the stem of *R. communis* (Harland, 1947). The survey of *B* gene
frequencies in populations of Peru shows a gradual increase in the
dominant allele with altitude. Plants carrying the *B* gene failed to
fruit except when growing in dry sunny conditions, but the *bb* genotype
showed normal fruiting under coastal, foggy conditions during the
winter. This ratio-cline clearly indicates that the *B* gene or some
closely linked genes have some physiological disadvantage under humid
conditions. Similar clinal variation was disclosed in *Eucalyptus gigantea*,
E. gunnii and *E. coccifera* by Barber (1955). The appearance of
glaucous stems and leaves shows a considerable clinal variation in
Eucalyptus along a mountain transect from Golden Valley at 1200 feet
to Great Lake at 3500 feet in Central North Tasmania. Within a
species, the non-glaucous type is most common at low elevations,
while increased glaucousness frequencies are found at higher elevation.
According to Barber, the abundance of glaucous trees is correlated
with changes in frost activity.

If one of the alleles becomes disadvantageous in a population,
natural selection will rapidly reduce its frequency. This rapidity of
natural selection is well illustrated in moths (Lepidoptera) (Ford,
1955). The dominant melanic allele of the moth has rarely been found
in moth species of non-industrial areas. However, a number of
species have become fully, and other species considerably, darker
melanics during the last 100 years in the industrial areas of Great
Britain and Western Europe. The influence of natural selection is

also indicated in *T. repens* populations of North America and Japan. The introduced *T. repens* is slowly adapting to these new environments by forming gene frequency clines. Comparison can be made between the gene frequency clines of indigenous (European, Near Eastern) and introduced (North American) *T. repens* populations through their regression lines (fig. 6). The increase of gene frequencies per 1° F. of January mean temperature is 4·23 per cent. for *Ac* and 3·15 per cent. for *Li* in indigenous populations, while the value for *Ac* falls to 0·68 per cent. in North American populations. In the European and Asiatic (mainland) regions, which are the oldest distribution areas, it can be considered that there is a balanced equilibrium
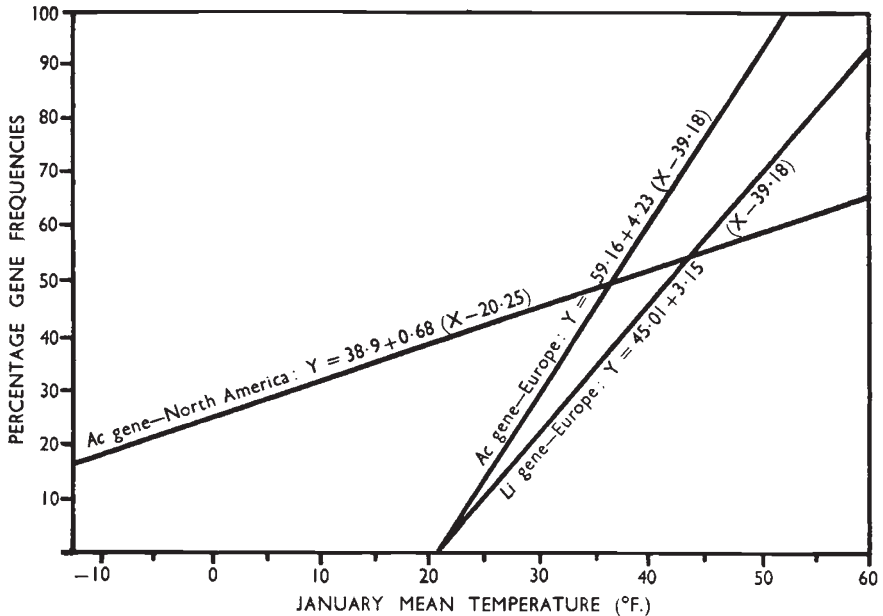

Fig. 6.—Comparison between the regression lines of gene frequencies in European and North American wild populations of *T. repens* L.

between gene frequencies and January mean temperature. On the other hand, gene frequencies and January mean temperature have not yet reached an equilibrium in the North American population. It is postulated that the American gene cline is gradually moving under selection pressure and its regression line will occupy the same position as the European regression line when equilibrium is reached during the course of evolution. The postulate that natural selection is acting to form gene frequency clines in *T. repens* populations in North America has also been supported by the evidence of Portz and Jackobs (1955). Four thousand four hundred and fourteen plants of *T. repens* cultivated Ladino, have been tested from a geographical range between Washington and California. The frequencies of plants containing lotaustralin varied from 67 to 0 per cent. between seed lots of certified Ladino strains. A typical cline from south to north

(high-low lotaustralin plant frequencies) has been disclosed.   The original Ladino strain was found to contain $5 \cdot 2 \pm 1 \cdot 95$ per cent. lotaustralin genes (Daday, 1954c) and this increase is partly due to natural selection in U.S.A.

In spite of the fact that several single gene controlled enzymes are known in micro-organisms, no single gene controlled enzyme has been reported in flowering plants other than linamarase in *T. repens*. Six different quantities of enzyme production have been indicated (Daday, 1954b).   It is still unknown whether the different quantities of linamarase are controlled by a series of alleles or by modifying genes.

The presence together of the products of the genes *Ac* and *Li* determine the *in vitro* production of HCN ; but, as suggested to the author by his colleague Dr F. H. W. Morley, it cannot be assumed that it is the product of this chemical reaction which is recognised by the forces of natural selection.   The presence of several populations in which *Ac* is at low frequency and *Li* at high frequency and vice versa (*e.g.* in North America, Fairbanks : *Ac* 4·2, *Li* 38·6 per cent. ; Brandon : *Ac* 29·3, *Li* 81·1 per cent. ; and also the fact that in Europe *Ac* and *Li* regression coefficients are significantly different, '(86) = 2·206, P = 0·05-0·02) suggests that selection acts on these genes or their products independently.   The presence of frequency clines in the European populations (Daday, 1954a) indicates that both genes are subject to agents of selection associated with temperature, but in the absence of further information, nothing more may be deduced.

These findings underline an important principle.   Although the particular action of a gene product may be known, it is not necessarily this action which is the subject of selection under natural conditions.

The old naturalists (*e.g.* Kerner *et al.*, 1894) emphasised the protective function of alkaloids and other substances in flowering plants.   The presence of lotaustralin in *T. repens* may afford protection against the feeding of such animal predators as rabbits and snails, but probably the major role is exerted through a physiological process as yet not understood.

The present investigation has shown that *T. repens* has formed a balanced gene frequency cline in its indigenous distribution areas, and that natural selection is still acting to establish equilibrated gene frequency clines in the areas of introduced populations wherever the selective force, low winter temperature range, exists.

6. SUMMARY

1. The distributions of lotaustralin (*Ac*) and linamarase (*Li*) gene frequencies in wild populations of *T. repens* are presented.

2. Gene distribution :

    (*a*) In Europe, North Africa and the mainland of Asia, there are 100 to 0 per cent. gene frequency clines.

(*b*) In North America and Japan, decreases in *Ac* gene frequencies exist from South to North.

(*c*) In South America, South Africa, Australia and New Zealand, gene frequencies exhibit an irregular pattern.

3. The decreasing gene frequencies were found to be highly correlated with decrease in mean winter temperature.

4. There is a relationship between gene frequency and quantity of gene products.

5. It is apparent that natural selection has already produced a balanced equilibrium in Europe and the mainland of Asia, and that natural selection is still in progress in North America and Japan.

6. Formation of *Ac*, *Li* gene frequency clines are not expected in the southern hemisphere with the exception of mountainous regions.

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[Appendix

APPENDIX I

Phenotypes and gene frequencies in wild populations of
Trifolium repens

Localities	Phenotypes No. of plants				Genes Percentages		January * mean temperature (° F.)
	AcLi	*Acli*	*acLi*	*acli*	*Ac*	*Li*	
ASIA							
India							
Almora . . .	94	5	9	3	67·1	73·2	54·0
Iran							
Karaj River area . .	101	56	0	0	100·0	40·6	40·0
U.S.S.R.							
Turgatuj Baikal Region .	0	...	...	68	0·0	0·0	−4·6
Novosibirsk . . .	0	...	...	58	0·0	0·0	−2·9
Japan							
Miyazaki . . .	16	24	34	73	14·7	18·8	44·2
Kochi	56	46	14	16	52·3	31·5	43·0
Zentsuji . . .	23	29	42	73	17·0	21·8	42·8
Konosu . . .	75	26	25	24	42·8	42·3	39·6
Shindo-Mura .	49	75	11	21	54·7	21·5	34·5
Shimo-Kuriyagawa .	27	38	41	35	26·6	28·0	28·2
Tsukissapau . . .	22	18	37	67	15·0	23·2	20·7
AFRICA							
Morocco							
Rabat	124	0	0	0	100·0	100·0	54·5
Tirsal (4500 feet) . .	42	0	0	0	100·0	100·0	...
Oukaiemden (7500 feet) .	67	5	11	3	59·7	69·5	...
							July mean temperature (° F.)
Union of South Africa							
George, Cape . .	79	13	2	0	85·4	62·8	55·0
Humensdrop, Cape .	49	9	8	3	60·1	58·3	55·0
Escourt, Natal . .	43	3	10	4	51·7	65·8	55·0
Bloemfontein, Orange Free State	60	10	8	5	60·4	57·6	50·0
Pretoria, Transvaal .	83	39	0	1	91·0	43·0	50·0
Potchefstroom, Transvaal	62	4	10	3	59·4	70·2	50·0
							January mean temperature (° F.)
NORTH AMERICA							
U.S.A.							
Baton Rouge, La. . .	104	1	8	1	71·9	86·8	54·6
New Orleans, La. . .	112	0	7	0	75·8	100·0	54·6

* Data obtained from individual meteorological stations and also from C.S.I.R. (1933), Clayton (1927).

APPENDIX 1—*continued*

Localities	Phenotypes				Genes		January mean temperature (° F.)
	No. of plants				Percentages		
	AcLi	*Acli*	*acLi*	*acli*	*Ac*	*Li*	
San Francisco, Cal. .	87	10	12	5	61·4	63·7	47·2
San Francisco, Cal. .	72	7	2	0	84·3	70·6	47·2
Guerneville, Cal. . .	62	1	40	6	35·0	74·7	43·7
Rio Monte, Cal. . .	119	0	0	0	100·0	100·0	44·0
Woodburn, Ore. . .	14	5	18	18	19·1	35·3	40·0
Albany, Ore. . .	3	18	5	34	19·4	6·9	40·0
Shedd, Ore. . .	18	4	11	9	31·0	44·4	39·2
Hubbard, Ore. .	33	8	19	6	38·4	54·0	39·2
N.E. Oregon, Ore. .	38	27	19	32	33·7	28·7	30·0
N.E. Oregon, Ore. .	18	1	16	5	27·5	61·3	30·0
Union, Ore. . .	39	1	30	8	30·2	66·0	26·0
Lat. 41° 14′ 30″ . Long. 81° 18′ 0″ Ohio	43	41	9	21	48·7	26·2	26·9
Lat. 41° 14′ 30″ . Long. 81° 28′ 0″ Ohio	18	36	10	42	30·0	14·2	26·9
Lat. 40° 55′ Nebr. . Long. 96° 10′ Nebr.	35	62	5	10	63·4	19·8	23·7
Chicago, Ill. . .	71	24	11	14	54·4	43·7	23·6
St Paul, Minn. . .	5	16	10	50	13·9	9·7	13·1
St Paul, Minn. . .	15	19	19	47	18·8	18·8	13·1
Mandam, N. Dak. .	38	33	28	18	37·3	34·0	9·0
Homer, Alaska . .	31	13	11	6	47·2	44·2	23·7
Homer, Alaska . .	11	10	15	21	20·5	26·2	19·0
Matanuska Valley, Alaska	23	15	18	10	34·9	38·4	13·0
Palmer, Alaska . .	22	7	13	11	32·7	41·7	13·0
Fairbanks, Alaska . .	21	21	21	15	32·1	32·1	−11·0
Fairbanks, Alaska . .	3	2	35	21	4·2	38·6	−11·0
Fairbanks, Alaska . .	11	1	22	4	17·3	63·7	−11·0
Fairbanks, Alaska . .	3	1	42	46	2·2	28·5	−11·0
Canada							
Lennoxville, Que. . .	90	2	31	3	48·1	80·1	14·0
Lennoxville, Que. . .	96	2	17	0	61·6	86·8	14·0
St Anne de la Pocatire, Que.	71	10	26	7	46·2	61·4	10·0
Grandes Bergeronnes, Alb.	71	8	32	9	41·5	62·4	11·8
Lacombe, Alb. . .	69	9	18	1	55·7	67·9	7·4
Lacombe, Alb. . .	21	2	20	5	27·8	61·8	7·4
Peace River area, Alb. .	53	29	15	19	45·9	35·7	6·0
Peace River area, Alb. .	0	1	30	87	0·4	13·6	6·0
Brandon, Man. . .	27	9	21	9	32·6	47·8	−1·6
Brandon, Man. . .	13	1	14	0	29·3	81·1	−1·6
							July mean temperature (° F.)
SOUTH AMERICA *Peru* Cuzco	165	0	0	0	100·0	100·0	30·0
Brazil Curitiba . . .	27	84	0	0	100·0	13·0	54·3
Pedro Leopoldo. area Minas Gerais	11	4	48	36	7·9	36·4	60·0

APPENDIX 1—*continued*

Localities	Phenotypes				Genes		July mean temperature (° F.)
	No. of plants				Percentages		
	AcLi	*Acli*	*acLi*	*acli*	*Ac*	*Li*	
Argentina							
Monte, Prov. Buenos Aires	76	7	17	4	55·1	67·5	48·9
Las Rosas, Prov. Santa Fe	122	12	13	7	64·0	64·9	50·8
Esquel, Gov. of Chubut	46	45	7	7	63·5	29·6	30·0
Sarmiento, Gov. Comorodo Rivadavia	21	28	30	61	19·4	20·3	37·8
AUSTRALIA							
Bateman's Bay, N.S.W. .	6	2	72	23	4·0	50·7	53·0
Bodalla, N.S.W. . .	78	3	14	3	58·4	75·3	51·9
Central Tilba, N.S.W. .	1	0	78	22	0·5	53·3	51·9
Central Tilba, N.S.W. .	1	0	84	4	0·6	78·8	51·9
Borogo, N.S.W. . .	59	4	24	16	37·7	55·9	48·7
Bega, N.S.W. . .	37	7	21	2	41·4	63·4	48·7
Bega, N.S.W. . .	0	0	29	44	0·0	22·4	48·7
Bega, N.S.W. . .	10	0	76	21	4·8	55·7	48·7
Tathra, N.S.W. . .	12	0	44	51	5·8	31·0	48·7
Nimmitabel, N.S.W. .	0	1	37	35	0·7	29·8	36·7
Butlers, N.S.W. . .	7	0	51	44	3·5	43·3	...
Big-Bog, N.S.W. . .	8	2	60	35	4·9	40·6	...
Melbourne, Vic. . .	61	8	15	61	27·6	31·0	50·3
Somers, Vic. . .	19	9	11	7	37·4	41·0	48·8
Pomborneit, Vic. .	8	12	6	92	8·9	6·1	50·0
Mt. Gambier, S.A. .	44	9	36	33	24·8	41·3	49·1
Mt. Gambier, S.A. .	16	22	20	56	18·3	17·3	49·1
Hd. Benara, S.A. .	99	7	5	2	75·1	71·8	...
Hobart, Tas. . .	84	6	5	3	71·4	69·7	46·6
Ranelagh, Tas. . .	85	1	30	7	45·1	74·5	43·0
Mt. Kosciusko Region							
Maffra, 3000 feet . .	4	2	43	47	3·2	28·6	...
Jindabyne, 3000 feet .	7	6	84	39	4·9	42·5	...
Hotel Kosciusko, 5200 feet	8	1	78	30	3·9	48·5	31·9
Munyang, 4600 feet .	6	2	92	42	2·9	44·3	...
The Smiggin Holes, 5500 feet	12	2	84	28	5·7	51·2	...
Spencer's Creek, 5700 feet	2	4	20	12	8·2	35·1	...
The Chalet, 5800 feet .	11	5	84	53	5·4	38·4	26·5
Mt. Gingera							
Mt. Gingera, A.C.T., 5973 feet	0	0	83	2	0·0	84·7	...
Mt. Gingera, A.C.T., 5973 feet	2	0	16	39	1·8	17·3	...
NEW ZEALAND							
Winston . . .	70	4	41	7	37·3	70·0	48·8
Hastings . . .	68	1	0	0	100·0	88·0	55·1
Nelson	44	3	3	3	66·4	66·4	54·2
Palmerston North . .	30	1	7	4	48·8	65·5	53·1