EVOLUTION IN CLOSELY ADJACENT PLANT POPULATIONS

III. *AGROSTIS TENUIS* ON A SMALL COPPER MINE

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1. Introduction

Changes in population characteristics over short distances in several plant species have been reported in the two previous papers in this series (Jain and Bradshaw, 1966; Aston and Bradshaw, 1967). From such data it is possible to assess values for selection and gene flow in natural situations. Comparison of parents and natural progeny suggested the occurrence of gene flow between adjacent populations. Population divergence appeared to be maintained by strong selection pressures. However, this data did not show what influence considerable gene flow would have in situations where selection pressures are weak.

Agrostis tenuis Sibth. is one of several species of higher plants which are able to colonise derelict heavy metal mine workings in the British Isles. The colonising plants are able to withstand the toxic amounts of the metals present in the workings. They retain the character of heavy metal tolerance in cultivation, and grow normally on ordinary soils. Tolerance has a heritability of 0·7 obtained from polycross data (see McNeilly and Bradshaw, 1967). Plants taken from ordinary soil however, are rapidly killed when planted on metal contaminated soils. Heavy metals in sub-lethal concentrations severely retard root growth. The degree of metal tolerance can be measured by the root growth made in a standard time in the presence of a given amount of metal (McNeilly, 1966; McNeilly and Bradshaw, 1967).

Small mines can be found (2-5 hectares) which have tolerant populations growing on them. These small tolerant populations must be subject to gene flow from closely adjacent non-tolerant populations (McNeilly and Bradshaw, 1967). These are ideal situations for studying evolution in small populations and the interplay of selection and gene flow.

At Drws y Coed in Caernarvonshire there is a small mine some 300 × 100 sq. metres with a copper-tolerant population of *Agrostis tenuis*. The mine boundary is sharp to the north, east and west but is diffuse to the south. It lies on the floor of a steep-sided typical " U " shaped glaciated valley running in an east-west direction about 150 metres above sea-level and is entirely surrounded by normal pasture containing *A. tenuis*. The valley shape and direction have a marked effect upon the local wind direction

The local wind is highly polarised, shown by a comparison of the records for the nearest weather station (fig. 1, a (i)), and that for Drws y Coed over the same period (fig. 1, a (ii)). The wind over this period was very variable in direction; more normal records are given by fig. 1, a (iii)). This would suggest a wind very predominantly from the west.

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The precise age of the mine is not known, but it is recorded as having been worked in the thirteenth century. Mining activity continued until early this century.

Experiments were carried out to examine (i) the general distribution of copper tolerance across the mine boundary along two transects on the upwind and downwind sides of the mine; (ii) the amount of gene flow occurring between populations along the transects, and its possible influence upon the outcome of natural selection; (iii) the extent to which natural selection would be able to maintain population divergence.

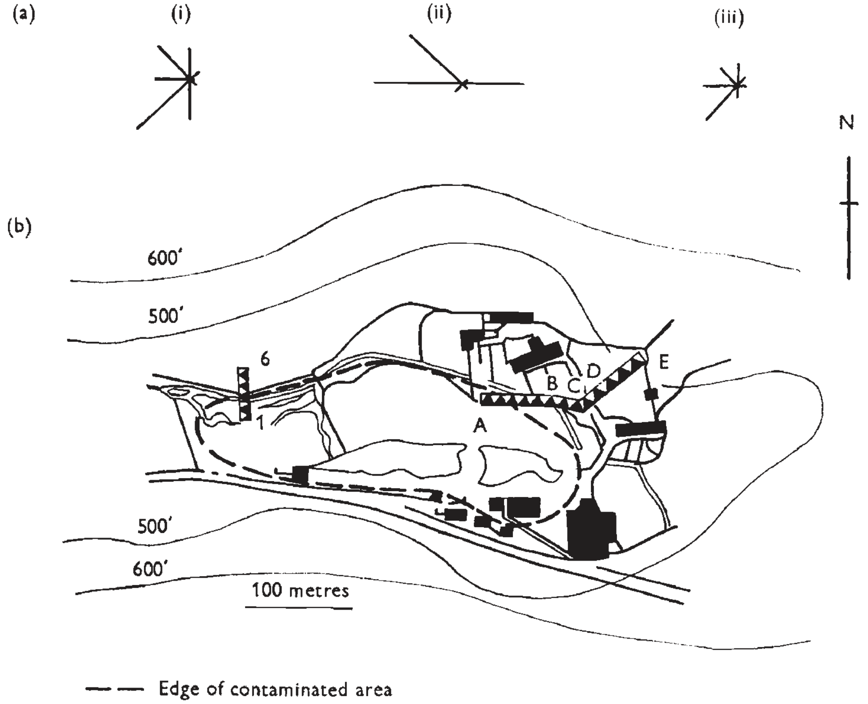

FIG. 1.—(a) (i) Wind rose 9 a.m. daily. Valley, Anglesey, June-September 1965.
(ii) Wind rose 9 a.m. daily. Drws y Coed, June-September 1965.
(iii) Mean daily wind direction. Valley, Anglesey, June and July 1962.
(b) Map of the mine showing transects 1, 1-6; and 2, A-D.

2. THE PATTERN OF POPULATION DIFFERENTIATION AND GENE FLOW

The populations were in two transects. One was directly downwind; the other was at the upwind end of the mine, but because of a sown pasture directly upwind, had to be taken transversely. Both passed from copper-contaminated soils on to uncontaminated soils. Their positions relative to each other are shown on the map (fig. 1).

Copper tolerance was measured for six sites at the upwind end and five sites downwind of the mine. Each site was approximately 15 × 2 sq. metres. For each site 15 tiller plants were tested as the adult sample, and 30 seedling plants obtained from seed produced in the field by the 15 adult plants were tested as the seed sample. Plants were grown in standard environment for three months before testing. The populations from each transect site are listed below together with mean total soil copper.

Transect 1

1. Centre mine. Pure stand *A. tenuis* continually water-logged by seepage from mine tips. Cu 2700 p.p.m. Toxic.
2. On mine, 4 metres from 1. As site 1 but drier. Cu 2600 p.p.m. Toxic.
3. Near mine edge, 3 metres from 2. Sheep walk much drier than 1 or 2. Cu 900 p.p.m. Toxic.
4. On mine edge, 4 metres from 3. Separated from 3 by narrow fast flowing stream. Dry. Cu 680 p.p.m. Toxic.
5. Just off mine, 1 metre from 4. Mixed vegetation: *Pteridium, Anthoxanthum, Festuca.* Soil with much organic matter. Presence of other species suggests non-toxic, although Cu 680 p.p.m.
6. Off the mine, 18 metres from site 5. Thin stony soil. Typical poor upland grassland. Cu 156 p.p.m. Certainly non-toxic.

Transect 2

A. Just on mine. *A. tenuis* in lush pure stand. Cu 720 p.p.m. Toxic.
B. Off the mine. *A. tenuis, Lolium, Galium* and other species. Soil with much organic matter. Cu 300 p.p.m. Variety of species and copper level suggests non-toxic.
C. Off the mine. 20 metres from site B. Stony dry soil. Typical *Agrostis/Festuca* upland pasture. Cu 136 p.p.m. Non-toxic.
D. Off the mine. 10 metres from site C. Soil and vegetation as C. Cu 180 p.p.m. Non-toxic.
E. Off the mine. 65 metres east of D. Soil damp with considerable organic matter. Mixed vegetation similar to site 6 transect 1. Cu 52 p.p.m. Non-toxic.

Total soil copper will not necessarily reflect available soil copper. The presence of considerable amounts of soil organic matter may chelate a large proportion of the total soil copper and render it unavailable to plants (Dykeman and De Sousa, 1966). This may explain the clear lack of toxicity implied by the vegetation on sites 5, B and D, despite moderate copper levels.

The tolerance of individual plants in the populations is presented as histograms in fig. 2, and diagrammatically with their spatial arrangements in fig. 3.

On transect 1 the adults show that the mine populations are all tolerant and that tolerant plants are confined to toxic soils. An abrupt change in the occurrence of copper tolerant plants is found between populations 4 and 5, although a distance of only one metre separates these two populations. Population 6, 18 metres away from population 5, shows no sign of tolerant individuals.

The seed samples have mean tolerances which are different from those of the adults. These differences may be due to gene flow. Their amount and direction show that while gene flow from tolerant to normal populations is low, gene flow from normal populations into tolerant populations is considerable, for the mine seed samples all have markedly lower mean tolerance than the adults. This is not unexpected because of the small size of the mine, and because the transect is at the upwind end of the mine.

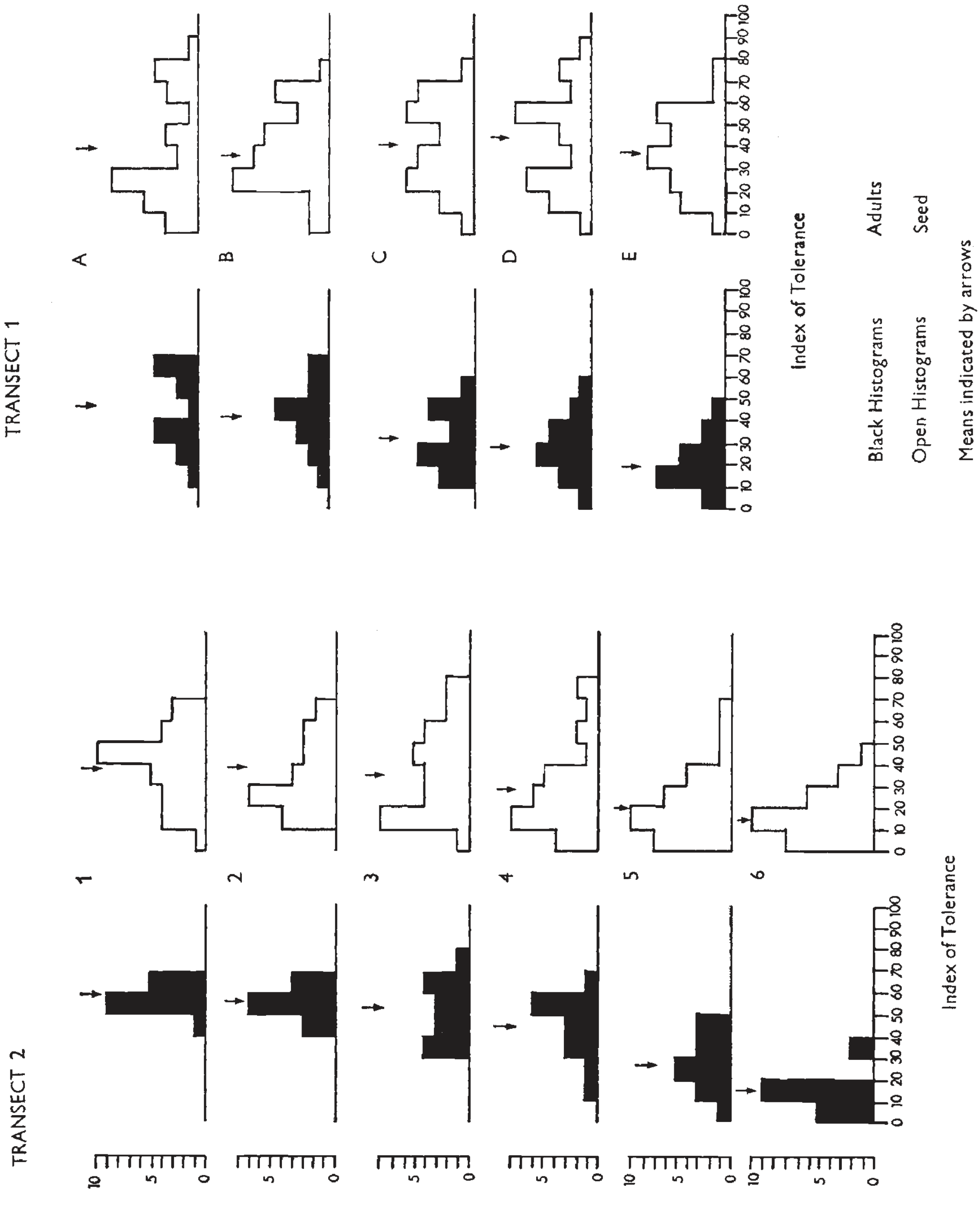

FIG. 2.—Index of copper tolerance of populations.

On transect 2, although only population A was from toxic soil, the results for the adults show that the character of copper tolerance has spread into populations B, C and even D, all growing on normal soil, a long way from the mine boundary. This contrasts sharply with transect 1 data. Further, no abrupt change in occurrence of copper-tolerant individuals was recorded. The seed samples are also different from those on transect 1. All those outside the mine show a higher mean index of tolerance than the corresponding adult populations. Gene flow along this transect is thus seen to be considerable, and carries genes from tolerant populations on the mine into non-tolerant populations on normal soil. At the same time there is some suggestion that populations A and B are receiving gene flow from non-tolerant populations similar to that shown in transect 1.

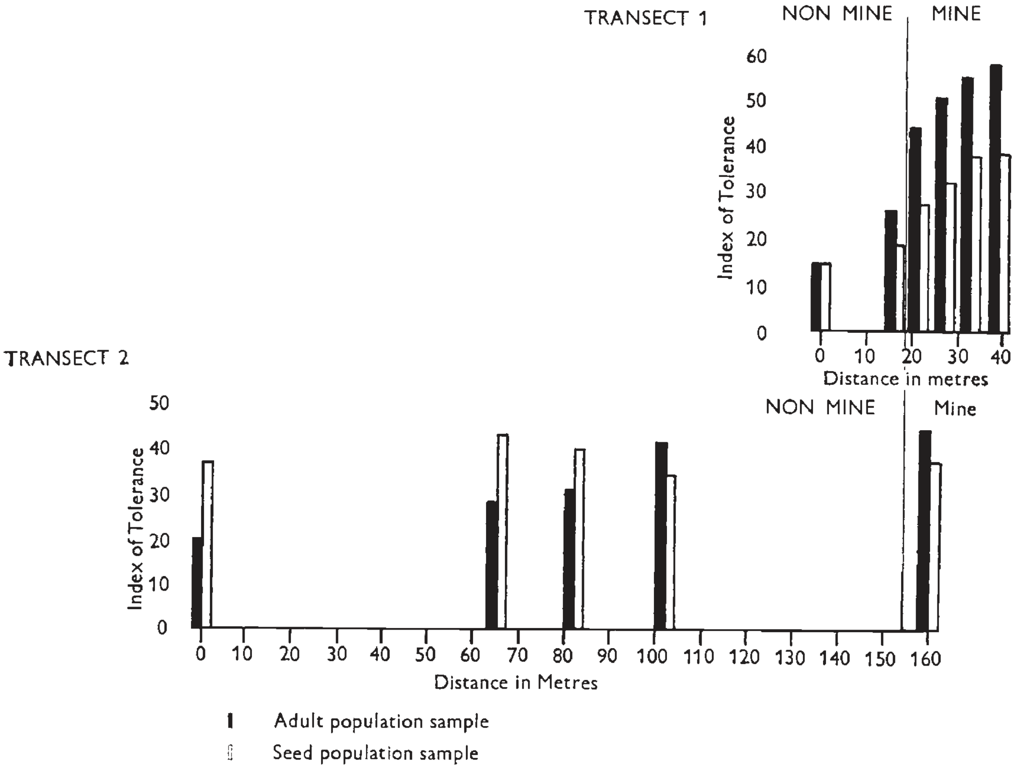

FIG. 3.—Mean index of copper tolerance of populations with their spatial arrangement.

The difference pattern in of distribution of copper tolerance along these two transects is most readily explained by the suggestion that pollen dispersal and hence gene flow follows the prevailing westerly wind direction. In transect 1 at the upwind end of the mine, the wind would transport pollen downwind from non-tolerant plants outside the mine into both mine and non-mine populations, but in transect 2 at the downwind end of the mine the wind would carry pollen from the tolerant mine plants out from the mine into the non-tolerant populations.

Since the *status quo* is maintained despite all this gene flow, we must presume the action of selection. On mine soils selection favours tolerant genotypes strongly. On normal soils selection must favour normal genotypes, but less strongly. Selection on normal and toxic soils was investigated to test this.

3. Possible selective forces

(i) *Spaced plants on normal soils*

In simple experiments using spaced plants, normal and copper-tolerant ecotypes were not found to differ significantly in the growth parameters, leaf area, leaf number, tiller number, dry weight and net assimilation rate. However, spaced plants may not reflect the behaviour of a population in the field where they are growing in high density and in the presence of other species. The ecotypes were, therefore, compared in dense mixtures.

(ii) *Dense mixtures on normal soils*

Pure stands and 50/50 mixtures of seed samples from tolerant and non-tolerant populations of *Agrostis tenuis* were grown at constant density using the method of de Wit (1960). Two soil types were used, John Innes No. 1 potting compost, and Mynydd Llandegai, a low nutrient soil approximating to the soil surrounding the mine at Drws y Coed. Week-old seedlings were spaced regularly, 42 per seven-inch pot, in three replicates and harvested after 12 weeks' growth.

When grown in pure stand no significant differences were found between the ecotypes on either soil type for dry weight or tiller number (fig. 4). When grown in 50/50 mixture, however, both dry weight and tiller number were significantly greater ($P = 0{\cdot}05$) for non-tolerant than for tolerant plants on both soil types. In a perennial grass species it seems reasonable to equate fitness and ultimate survival with tiller number and dry weight. Data of Bradshaw (1959) and Jowett (1959) suggest that density of vegetative tillers and density of reproductive tillers are closely correlated in metal tolerant and normal populations of *A. tenuis*. On this basis the coefficient of selection against tolerance on normal soils is 0·53.

(iii) *Seedlings on toxic soil*

Toxic soils are well known to have marked effects on non-tolerant plants. The effect of the mine soil on tolerant and non-tolerant seedlings was examined to ascertain the general features of germination and survival on such a soil.

Germination and subsequent growth of seed samples of two normal and one copper-tolerant populations were examined on two normal and one toxic soil. Twenty-five seeds of each population were sown on each soil type in four replicates.

Percentage survival of the populations after seven weeks differed very significantly (table 1). Only tolerant seed survived on toxic soil, whereas all survived on the non-toxic soils. No significant differences were found between the performance of populations on the two normal soils.

In view of this degree of difference between the populations, and because of the known effect of copper on root growth, in a second experiment early root growth was examined for one normal and one tolerant seed sample on one normal and one toxic soil in petri dishes.

The results (table 2) show that the effect of toxicity in non-tolerant individuals begins at radicle emergence. In the tolerant population growth of the radicle continues in the majority of cases. In non-tolerant seed, growth of the radicle ceases when contact is made with toxic soil. Both populations behave normally on non-toxic soil.

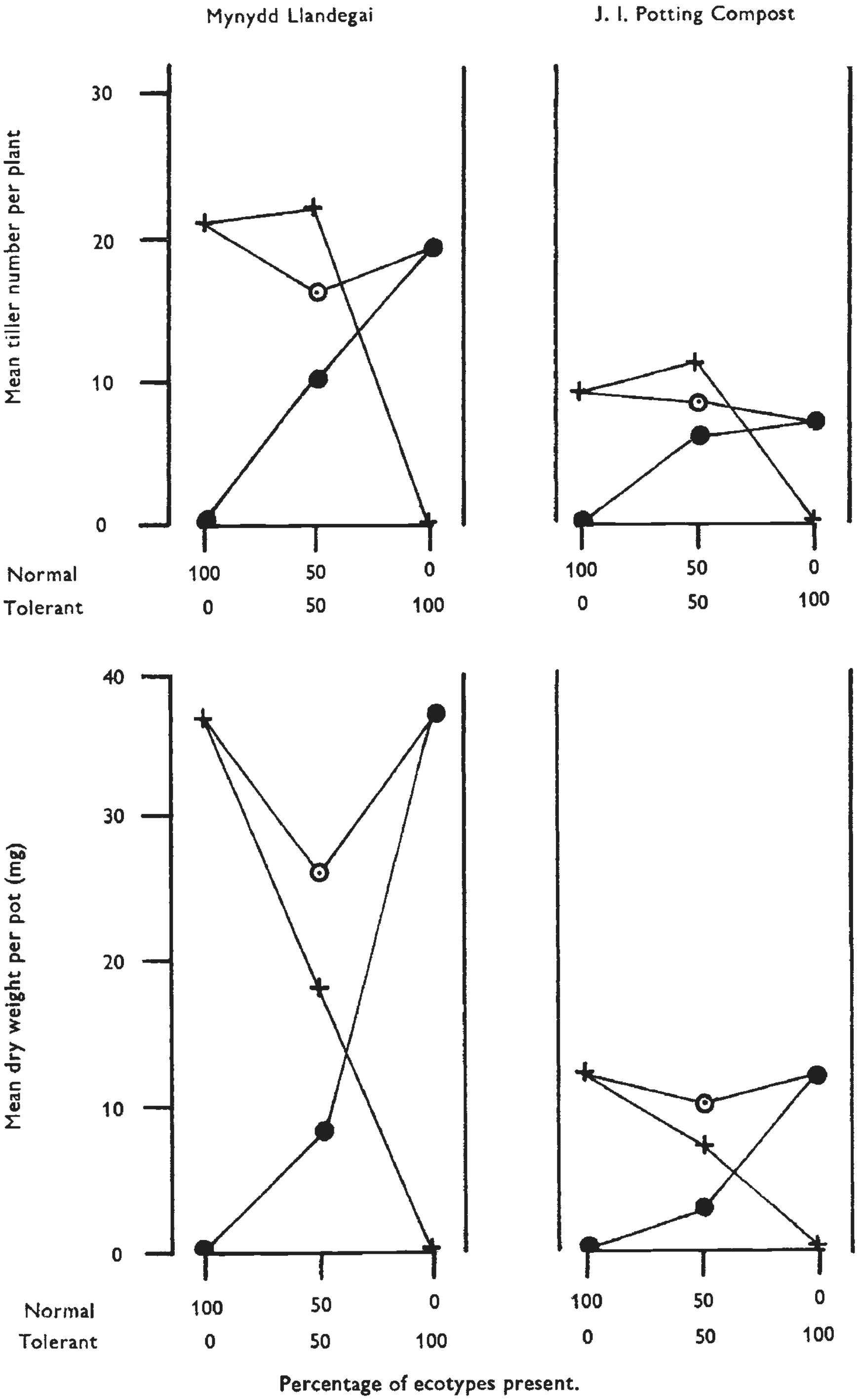

FIG. 4.—Performance of mixtures of tolerant and normal *A. tenuis* on two normal soils. ⊙ Yield of mixtures.

4. Discussion

(a) *Selection pressures*

The results of the effects of interference between two individuals or populations may become more apparent in mature stages than in juvenile stages (Harper, 1961) and, subsequently, more in the filial generation than in the parental generation. On normal soil small adjustments in dry weight and tiller number such as have been reported for the copper-tolerant ecotype of *Agrostis tenuis* in competition with the normal ecotype, may therefore be reflected in later life by a marked depression of growth, and in subsequent generations by a decrease in the number of copper-tolerant individuals occurring in mixed populations. This process would lead eventually to the elimination of copper-tolerant plants from mixtures of normal and copper-tolerant ecotypes. It seems clear, therefore, that interference from normal ecotypes may be instrumental in selection against copper-tolerant individuals on uncontaminated soils, although the selective pressure involved may be small. However, its effectiveness in eliminating any copper-tolerant ecotypes is indicated by the sparsity of such individuals growing on uncontaminated soils adjacent to mines. There is an indication from sand culture experiments (McNeilly, 1966) that a differential response to added phosphate may operate in favour of normal individuals on uncontaminated soils. Work to elucidate this point further is in progress at present.

Table 1

Survival of normal and tolerant populations on different soil types after seven weeks (percentage of total seedlings per soil)

	Soil		
	Normal		Toxic
Seed sample	J.I. potting compost	Mynydd Llandegai	Parys Mountain
Normal—Llandegfan Common	41	33	0
—New Zealand Commercial	24	26	0
Tolerant—Parys Mountain	35	41	100

L.S.D. 5 per cent. = 9·9.

Table 2

Germination of normal and copper-tolerant seed on different soil types

	Soil			
Seed sample	Normal	Potting compost	Toxic	Parys Mountain
Normal—New Zealand Commercial	92	(92)	88	(0)
Tolerant—Parys Mountain	97	(96)	92	(68)

Figures for number of radicles meerging per 100 seeds shown.
Figures in parentheses for number of radicles continuing growth after seven days.

On toxic soils selection pressures in the reverse direction are very high, since only those individuals possessing tolerance can survive. The rest are eliminated at the seedling stage.

Selection is seen to be of two types in the two environments. On normal soils the biotic environment is selective through interference. On toxic soils the physical environment is the selective agent, and all selection is achieved before interference can have any additional effects.

(b) *Selection and gene flow*

Localised patterns of differentiation between closely adjacent (parapatric) populations have been shown to be the resultant of the interacting forces of natural variation, gene flow and natural selection (Jain and Bradshaw, 1966; Aston and Bradshaw, 1967). The occurrence of sharp boundaries between such populations may be a reflection of high selection pressures or low gene flow, or a combination of both. Thoday and Boam (1959) and Jain and Bradshaw (*op. cit.*) have shown that populations continue to diverge in response to high selection pressures in spite of gene exchange between them. It is clear that the amount of gene flow permissable between closely adjacent divergent populations without arresting or reversing divergence is dependent upon the magnitude of the selective pressures maintaining population divergence.

The data presented here show the occurrence of both sharp *and* diffuse boundaries between the same two contrasting ecotypes under conditions of polarised gene flow. Selection pressures must be virtually the same in both cases, but the gene flow is vastly different. On the mine gene flow tending to dilute copper tolerance is considerable. This does not have an effect on population divergence, however, since selection pressures on the mine are also considerable and can overcome the swamping effects of gene flow. On normal soil, selection pressures are weak. In such a situation where gene flow from copper-tolerant to normal plants is low (transect 1), the forces of selection are sufficient to maintain population divergence. However, where gene flow from copper-tolerant to normal plants is high (transect 2), selection is unable to maintain a clear pattern of population divergence.

5. SUMMARY

1. Populations of *Agrostis tenuis* Sibth. in the vicinity of a small isolated copper mine in North Wales subject to markedly polarised wind direction were examined for copper tolerance along two transects passing from toxic to normal soils at the upwind end, and downwind from the mine.

2. Adult populations from the transect at the upwind end of the mine showed a sudden change from predominantly tolerant to predominantly non-tolerant individuals over a distance of one metre corresponding precisely with the mine boundary. Populations sampled downwind from normal soils remained with a high proportion of tolerant individuals 150 metres distant from the mine boundary.

3. Seed population samples showed that gene flow followed the prevailing westerly winds. This carries genes from normal populations upwind of the mine into tolerant mine populations, and genes downwind from tolerant populations into normal populations.

4. Selection pressures on toxic soils are strong and favour tolerant

genotypes; selection pressures on normal soils are weak but favour normal individuals.

5. Tolerant populations maintain their identity in spite of high gene flow because of strong selection pressures favouring tolerance. At the same time selection pressures in non-tolerant populations are not sufficient to maintain population divergence in the face of high gene flow.

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