THE GENETICS AND EVOLUTION OF DIFFERENCES BETWEEN CLOSELY ADJACENT PLANT POPULATIONS WITH SPECIAL REFERENCE TO HEAVY METAL TOLERANCE

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by

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Frontispiece

THE CLOSELY ADJACENT POPULATIONS

To the left of the wall is uncontaminated soil on which grows the non-tolerant population.

To the right of the wall is mine soil which is contaminated with zinc and on which grows the tolerant population.

Trelogan Mine, Flintshire.

ACKNOWLEDGEMENTS

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ABSTRACT

The evolution of differences between adjacent populations is studied using populations of *Agrostis tenuis* and *Anthoxanthum odoratum* at the boundary of contaminated mine soil and normal pasture. The populations on the mine are tolerant whereas those on the pasture are non-tolerant to high concentrations of heavy metals.

This is considered as a situation that can throw light on the process of primary evolution. The tolerant and non-tolerant populations are in sufficient proximity for them to exchange genes freely. Selection will be operating to eliminate unadapted types formed as a result of gene transfer. The production of unadapted genotypes will produce pressures for factors limiting gene flow. And since genotypes from one habitat are entering the other habitat the genetic consequences of colonisation can be studied.

Studies on natural populations are combined with studies using computer models. The main conclusions to emerge from these studies are as follows:

(a) *The situation in nature*

(1) Highly tolerant individuals can be selected from non-tolerant populations in one generation.

(2) Selection for tolerance occurs in the early seedling stage. Selection is strong and has directional and stabilising components.

(3) There is considerable population turnover on mine soils. Tolerant populations are more vegetative than non-tolerant populations when grown as spaced plants.

(4) Tolerant and non-tolerant populations differ in many morphological characters. These characters form different clinal patterns across the mine/non-mine boundary.

(5) Tolerant and non-tolerant plants are cross compatible.

(6) Tolerant and non-tolerant plants are partially isolated by a difference in their flowering time. The difference is a result of selection for adaptation to local ecological conditions and selection

for mechanisms reducing the harmful effects of gene flow.

(7) Tolerant plants have a greater self-fertility than non-tolerant. There is a negative relation between selfing and distance of the tolerant population from the non-tolerant population: selfing may be acting as an isolating mechanism.

(8) Metal tolerance is inherited.

(b) The theoretical situation

(1) The consequences of seed flow (migration - selection - mating) are different from those of pollen flow (migration - mating - selection). The latter is considered in detail.

(2) Pollen flow maintains a gene in a population even if there is strong selection against that gene. It increases the heterozygosity of the population.

(3) Pollen flow imposes a genetic load on the population.

(4) A gene for selfing spreads through a population of its own accord and also assists the fixation of a favoured gene.

(5) Selfing counteracts pollen flow and reduces the genetic load on the population.

(6) The evolution of dominance and overdominance is possible under conditions of pollen flow and selection.

CONTENTS

Chapter V. THE PROCESS OF EVOLUTION : A COMPUTER MODEL

Chapter I

INTRODUCTION

Introduction

The unit of evolution is the population and it is within the population that we must look for the mechanism of evolution. Comparative morphology, palaeontology or even experimental taxonomy can provide only limited information about the factors which affect genetic change in populations and hence in species. These disciplines while documenting the course of evolution and the conditions under which it occurred, only provide problems for the population geneticist interested in the mechanisms of evolution.

The investigation of differences within species is the first stage in population studies. It is a valuable approach that has led to the recognition that species are differentiated into numerous distinct populations and that ~~therefore~~ natural selection is an ubiquitous and important factor. However, even though critical population comparisons distinguish the effects of the environment and the genotype, they are little more than an extension of the method of comparative morphology. They go little beyond Darwin in helping us to understand the units of evolution and their detailed behaviour.

A more rewarding study is to look at the process of genetic change within populations over time. This has been fruitful in helping to recognise different types of selection and their different consequences. A detailed study of such changes, the comparison of the young populations before selection with the parent populations after selection, and a study of genetic polymorphism, has led to the realisation that the selection pressures which must be operating in natural populations may often be very severe. The importance of breeding systems in plants, the reaction of an organism to its environment, gene expression, and many other phenomena have been brought into relation with natural selection through the study of changes within populations. Work on natural populations is however often slow and difficult, but considerable help has been obtained by using experimental populations and mathematical and computer models.

As a result of these studies natural selection is no longer a poorly documented phenomenon.

But there is a third angle of attack on evolution in natural populations which has received remarkably little attention: this is population differentiation over short distances. This is the subject of the thesis. Although it may seem a rather obscure and arbitrary topic for study it is important for two reasons.

Until a few years ago population differentiation over short distances was thought to be impossible, and when first noticed was quite unexpected. It had been thought that populations only a short distance apart could not remain distinct because they would interbreed and thereby annul the differentiating effects of natural selection. If such populations can be different then the initial premises about interbreeding and selection must be false. This alone would justify the investigation.

But there is more to the problem than this. Here is a situation which can throw light on the whole process of primary evolution. We can consider that there are two genetically distinct populations which are in sufficient proximity for them to exchange genes more or less freely. Selection must be operating to eliminate the unadapted types that are continually being formed as a result of gene transfer. The effect of gene transfer will nevertheless be to change the genetical architecture of the populations, and maintain their variability. The production of unadapted genotypes will produce pressures for factors limiting gene flow and promoting speciation.

In adjacent populations genotypes from one habitat are continually entering the other habitat and the genetic consequences of colonisation can be studied.

The study of changes within populations provides information about the process of directional evolution, whereas situations at the boundary of two populations throw light on branching and expansive evolution. Recently man's activities have disturbed natural habitats enormously: this suggests that expansive evolution may be a widespread process at the present time, and therefore of practical consequence. Since the origin of major taxa has been quickly followed by expansive evolution in newly available adaptive zones, it is a very important process from a more general standpoint.

For such a study it is necessary to have populations that are very distinct yet which are still in sufficient proximity to exchange genes. Such populations are to be expected wherever there is a sharp habitat change, such as from cliff to cliff-top pasture, from grazed to ungrazed pasture, between soils of different pH, between water and land, and so on. The discrete habitats chosen for this study were soils contaminated with large quantities of heavy metals as a result of tipping from old mine workings. The boundary between the contaminated mine soil and the normal pasture is often very sharp, the intermediate zone being usually only a few feet wide. Several species which grow on mine soil also occur in the adjacent pasture. Many mine soil plants have been shown to be tolerant to high levels of metal, whereas pasture plants are intolerant and die if planted into toxic soil. Because this character seemed reasonably clear cut, it was chosen for particular study.

Metal tolerance is an important character which has received much attention. But the aim of this study has been to elucidate evolutionary mechanisms using metal tolerance as a model, so little attention has been paid to tolerance _per se_. To set the background a general review of work on metal tolerance has been included in Appendix 1.

Because closely adjacent populations have rarely been studied in great detail, many of the problems associated with such populations remain undefined. This thesis is a survey of the types of problem that might be expected, rather than a detailed investigation into any one particular feature. The situation in nature is complex, and therefore practical investigations have been carried out in conjunction with theoretical studies using computer models. The practical study has been concentrated on two contrasting mines with two species, the grasses _Agrostis tenuis_ and _Anthoxanthum odoratum_.

The results from one example may be an accident or peculiar to the species or the mine. But two examples, if they show the same thing, point more clearly to general evolutionary situations. The computer study has used models developed largely with the results of the practical investigation in mind: it is hoped to show how the study of natural populations points to new situations amenable to mathematical and computer treatment. Until recently ecological genetics has bee the handmaiden of taxonomy, and population genetics has been the executive of mathematical theories. But these approaches together can show that evolution as a phenomenon of natural populations can be a discipline in its own right.

Chapter II

TECHNIQUES AND APPROACHES

1. THE MINES

The two mines chosen for the main part of this study differ in numerous respects and are contrasted in tabular form (Table 1). However they have the following in common:

(a) A sharp boundary between contaminated and non-contaminated areas with a small intermediate zone only about three feet wide.

(b) A difference in the tolerance of the populations that closely follows the pattern of contamination (Putwain, 1963; McNeilly, 1965).

(c) The possibility of gene flow between the populations.

The other mines used in this study are lised in Appendix 2.

The species studied on Trelogan mine was <u>Anthoxanthum odoratum</u> L., Sweet Vernal Grass. Plants were collected from eight positions on a transect across the boundary of the mine (see Figs. 1, 2a and Frontispiece). Each site was sampled from an area approximately five yards wide and two yards long. The sites on the mine were on an area of loose grindings which showed evidence of having been re-worked: the soil therefore probably came from the period of re-working, 1873-1898 (see Table 1). The sites off the mine came from a rough, lightly and sporadically grazed pasture.

The species studied at Drws-y-Coed mine was <u>Agrostis tenuis</u> Sibth., Common Bent Grass. Plants were collected again from eight positions across the boundary of the mine (see Fig. 2b). The transect used was the same as that of McNeilly (1965) except that an extra position at both extremities was included. The plants on the mine come from a silty area of grindings with a very high water table. The plants from the pasture come from a rocky hillside with a very thin soil layer that carries an upland pasture.

The individual sites along the transects could be termed separate populations but the term population will be restricted to the tolerant and non-tolerant types either side of the main boundary. When the two populations are being compared as a whole, the boundary site (number 5 at Trelogan and number 6 at Drws-y-Coed) is not included in the comparison.

Table 1. Comparison of general features of the Trelogan and Drws-y-Coed mines.

	Trelogan mine	Drws-y-coed mine
Location	Trelogan, Flintshire.	Drws-y-coed, near Rhyd-ddu, Caernarvonshire.
Grid reference	SJ123805	SH542535
Situation	Very exposed, near sea, area of open pastures. Winds westerly.	At bottom of a steep sided west facing valley. Winds blow up the valley.
Rainfall	30-35 ins	80-100 ins
pH of soil	6.1 - 7.6	4.2
Metal content Zn	24,000-80,000	280
Pb	2,600-3,600	100
Cu	100-500	2,600
Vegetation	Anthoxanthum odoratum* Festuca ovina Agrostis tenuis* Agrostis stolonifera* Agrostis tenuis × stolonifera* Rumex acetosa* Minuartia verna and towards edge of mine and areas of lesser contamination Euphrasia sp. Plantago lanceolata* Viola lutea Cerastium sp. (* Have shown to be tolerant to zinc).	Agrostis tenuis** Festuca ovina Rumex acetosa** Galium hercynicum (**Have shown to be tolerant to copper).
Mining history	(from Smith, 1921)	(from Dewey and Eastwood, 1925)
First published returns	1848	1855
Periods of working	1859-1873 2,594 tons Pb ore 7,542 tons Zn ore 1873-1898 Sporadic reworking of old waste heaps 1898-1909 2,400 tons Pb ore 15,346 tons Zn ore	1855-1909 8,696 tons of Cu ore

Fig. 1a. Map of Trelogan Mine to show position of transect.
(Scale 1 in. : 100 yds.)

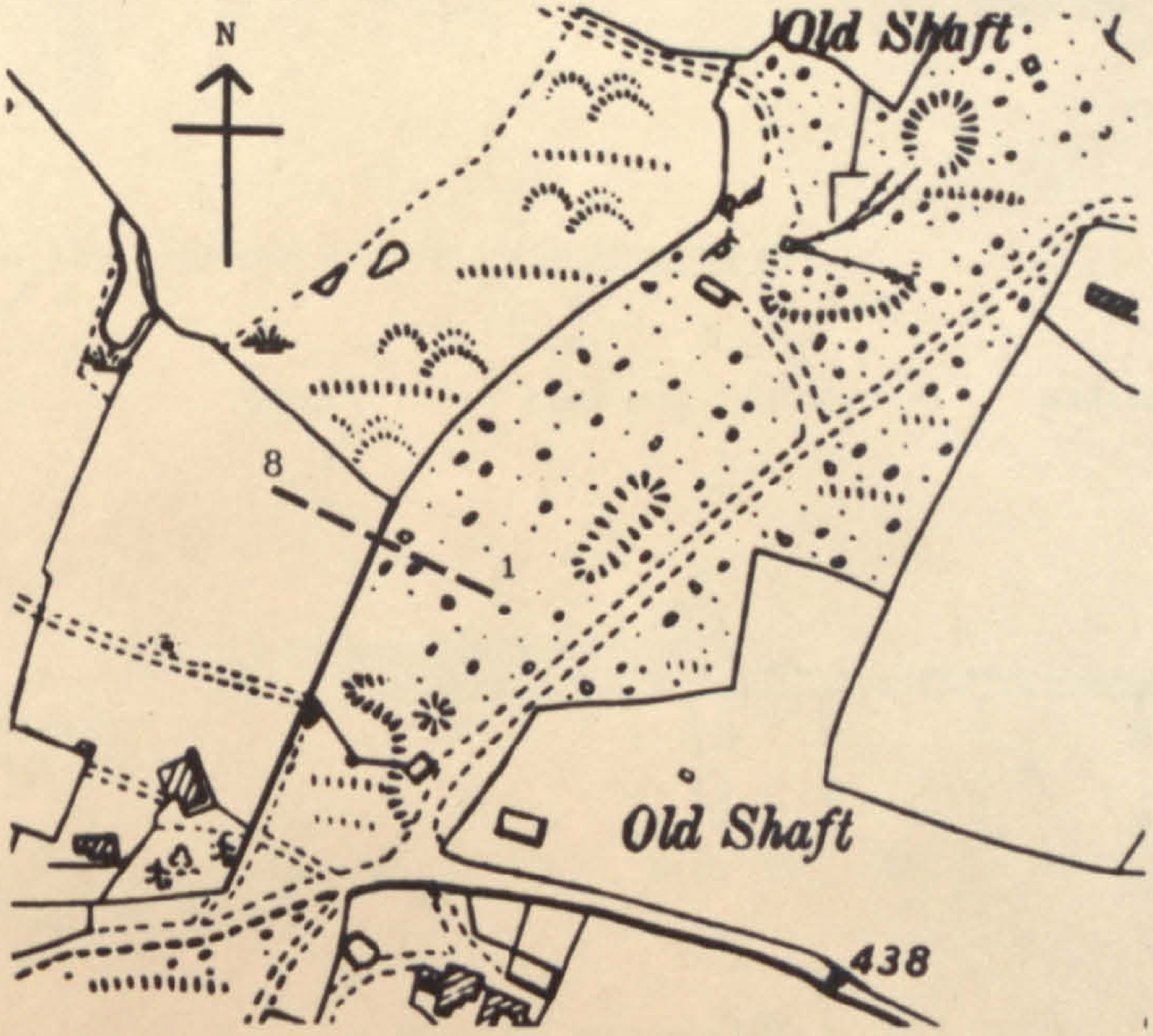


Fig. 1b. Photograph of the mine-pasture boundary at Trelogan.

Fig. 2a. The Trelogan transect (<u>Anthoxanthum</u>).

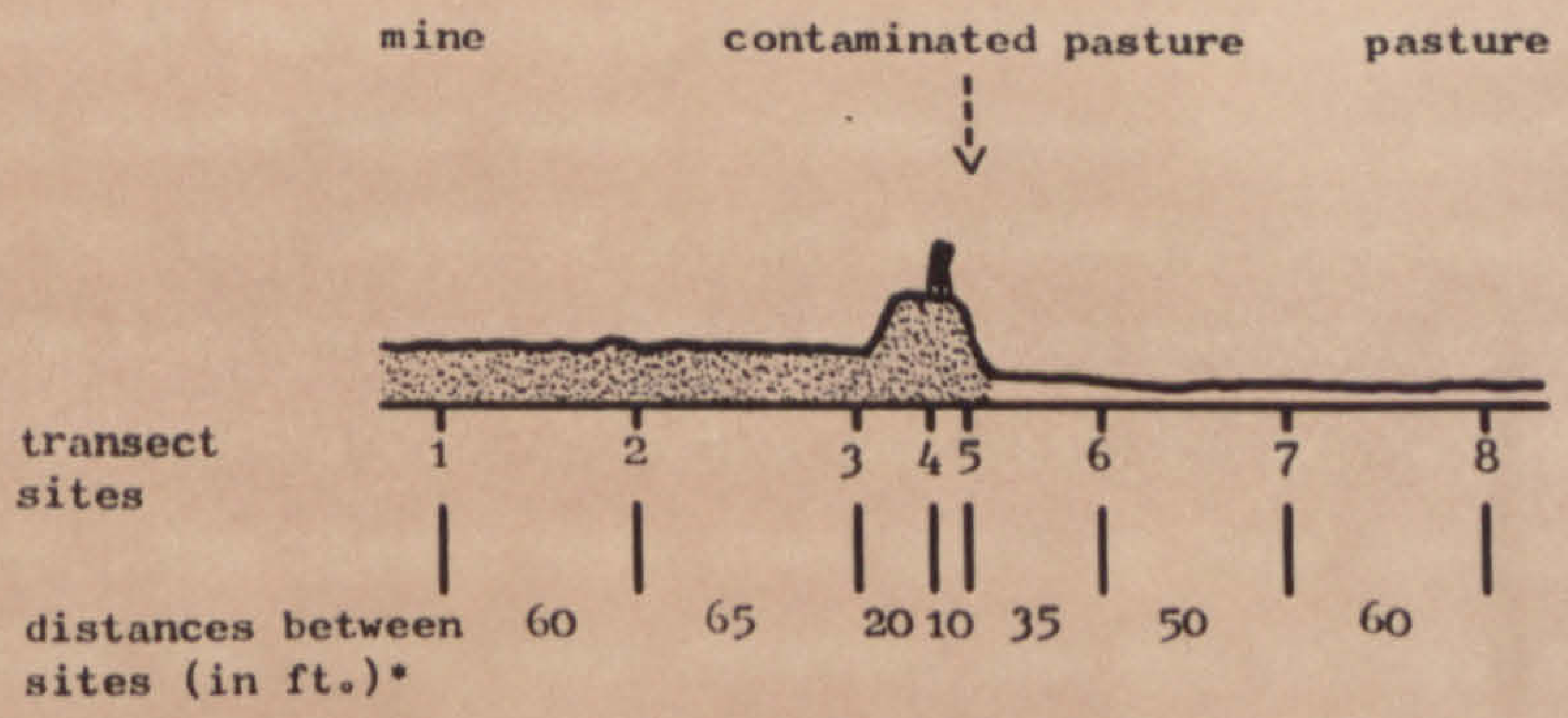
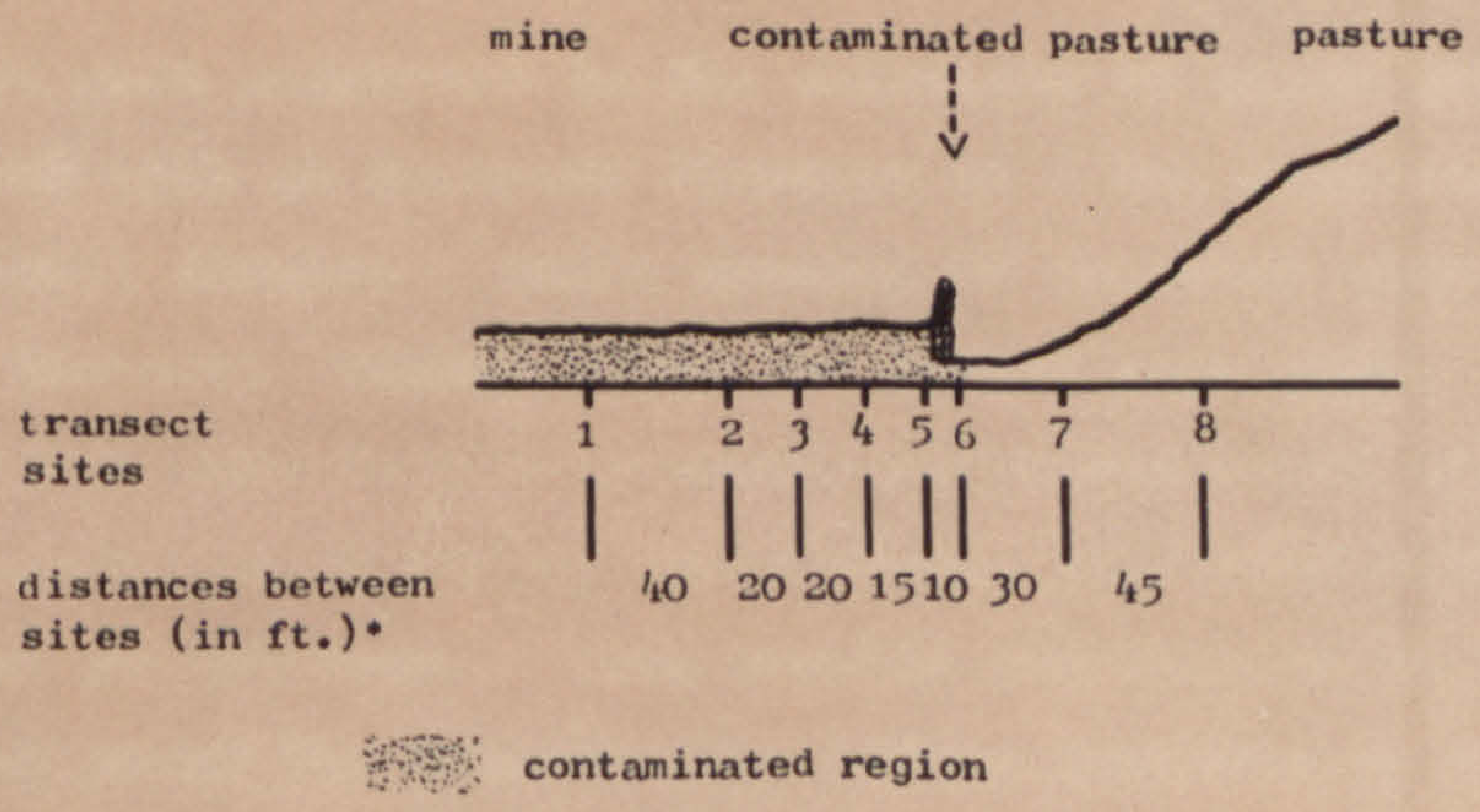


Fig. 2b. The Drws-y-Coed transect (<u>Agrostis</u>).



* distances given are those between the centre of sites.

2. THE TESTING OF TOLERANCE

The techniques used in the testing of tolerance have followed closely those of Jowett (1959), Gregory (1965) and McNeilly (1965) and involve a comparison of root growth in water (or simple nutrient solution) with the root growth in metal solution. Whereas both tolerant and non-tolerant individuals root readily in water, the metal solution inhibits the rooting of the non-tolerant, but not that of tolerant (Fig. 3).

(a) Adults

Three plants of the genotypes to be tested were put as single tillers or small plants into boxes of John Innes No. 1 potting compost and allowed to grow for 6–8 weeks in a warm greenhouse with supplementary lights to provide a 16 hr day. This period of preculture was essential both to standardise material and also to get numerous uniform healthy tillers. The plants were then split up into individual tillers from which the roots were removed, and the tillers were placed in the appropriate solutions. Calcium nitrate (0.5 gms/litre) was added to both metal solution and water treatments to ensure better rooting.

The tillers were supported in the solutions by glass tubing 3.5 ins. long and with an internal diameter of 4 mm, suspended from cobex (rigid vinyl) sheets. Small amounts of material were tested in polystyrene beakers: twenty tubes per beaker were used. Large amounts of material were tested in square polythene bowls or tanks: tubes were suspended using larger cobex sheets stiffened with 0.25 ins square strips of polystyrene. This enabled 360 tillers to be tested in one tank, and saved the considerable labour involved in changing solutions. In both systems, solutions were changed every two days, and ten days were allowed for each test. Twenty tillers of each genotype were placed in water and the same number in metal solution.

The index of tolerance was calculated as -

$$\text{Index of tolerance} = \frac{\text{Length of longest root in metal solution}}{\text{Length of longest root in water}} \times 100$$

The levels of metal used were those chosen by McNeilly (1965) and Gregory (1965) on the basis of rooting tests over a range of

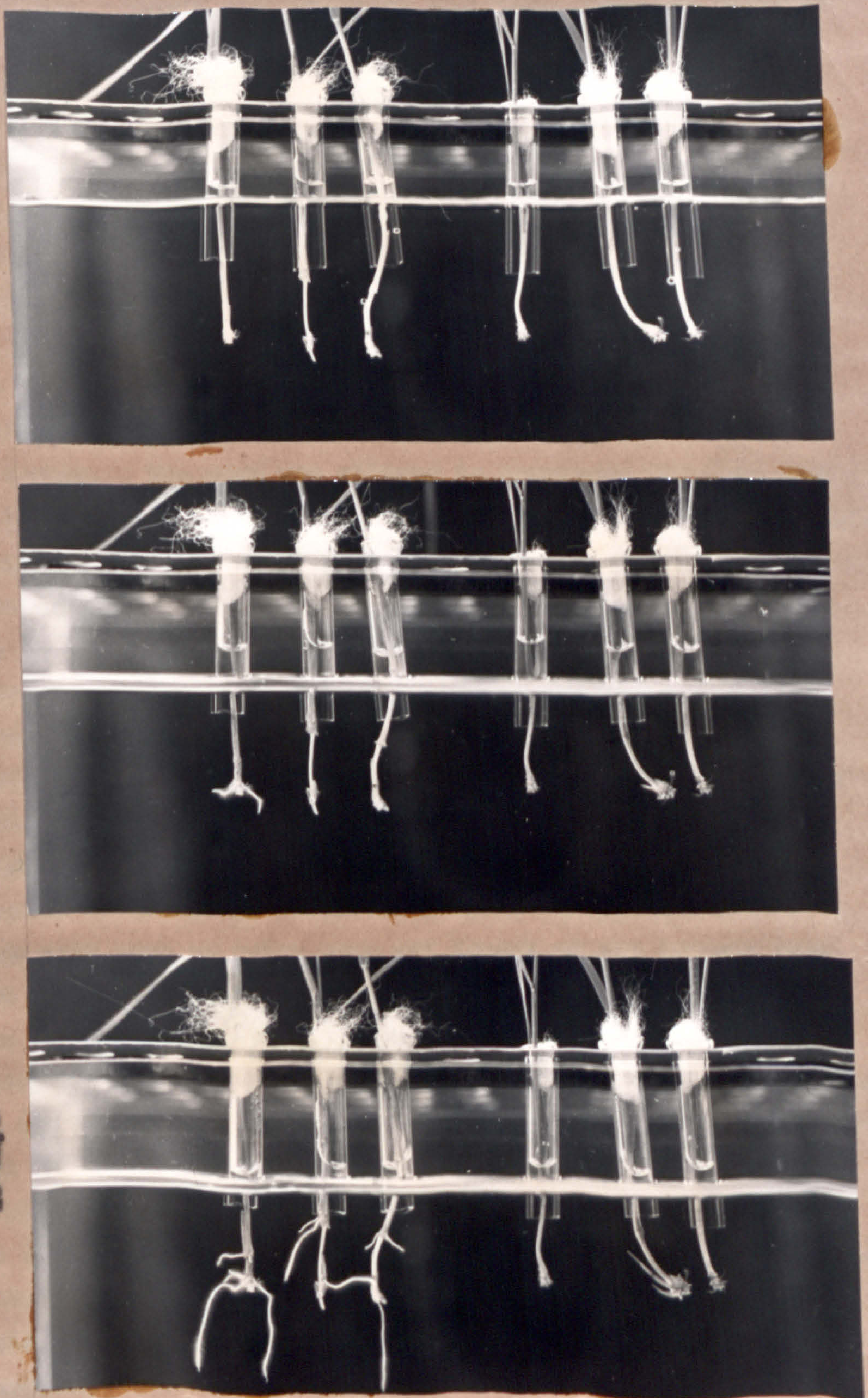


Fig. 3. Rooting of tillers of tolerant (left) and non-tolerant (right) *Agrostis* in copper solution.

0 days

5 days

10 days

concentrations - copper (as sulphate) 0.5 ppm., zinc (as sulphate) 15 ppm., lead (as nitrate) 12 ppm.

(b) Seedlings

Although seedling tests have not yet been used extensively in this investigation, attempts were made to develop a seedling test still simpler than that devised by McNeilly (1965) in order to facilitate the screening of non-tolerant populations for tolerant individuals and to test large numbers of seedlings resulting from crosses.

The method used by McNeilly involved 'sowing' seed on fine nylon mesh touching the surface of a solution contained in a plastic beaker. The seed was allowed to germinate and then growth rate of the root in water and growth rate in metal solution measured on a single seedling. The ratio of the two rates gave an index of tolerance: this correlated well with the tolerance of the same genotype tested as an adult.

However this technique involved the necessity of

(a) identifying and labelling individual seedlings

(b) making several measurements on each seedling to get an index

(c) handling the seedlings while they were still growing.

To eliminate these difficulties the absolute root growth of mine and non-mine seedlings in different metal solutions was examined. Seedlings were sown on nylon mesh and length of the longest root measured after three weeks.

The method distinguished clearly between tolerant and non-tolerant populations (Fig . 4) but when the length distribution is plotted (Fig. 4b) it is seen that a very large proportion of seedlings, although they germinate and produce a shoot, do not show any appreciable root growth, even when copper is not present.

To investigate this further, non-tolerant seedlings were grown under the following conditions -

(a) Cold water (20°C instead of 25°C)

(b) Aeration

Fig. 4a. Response of tolerant and non-tolerant seedlings to copper.

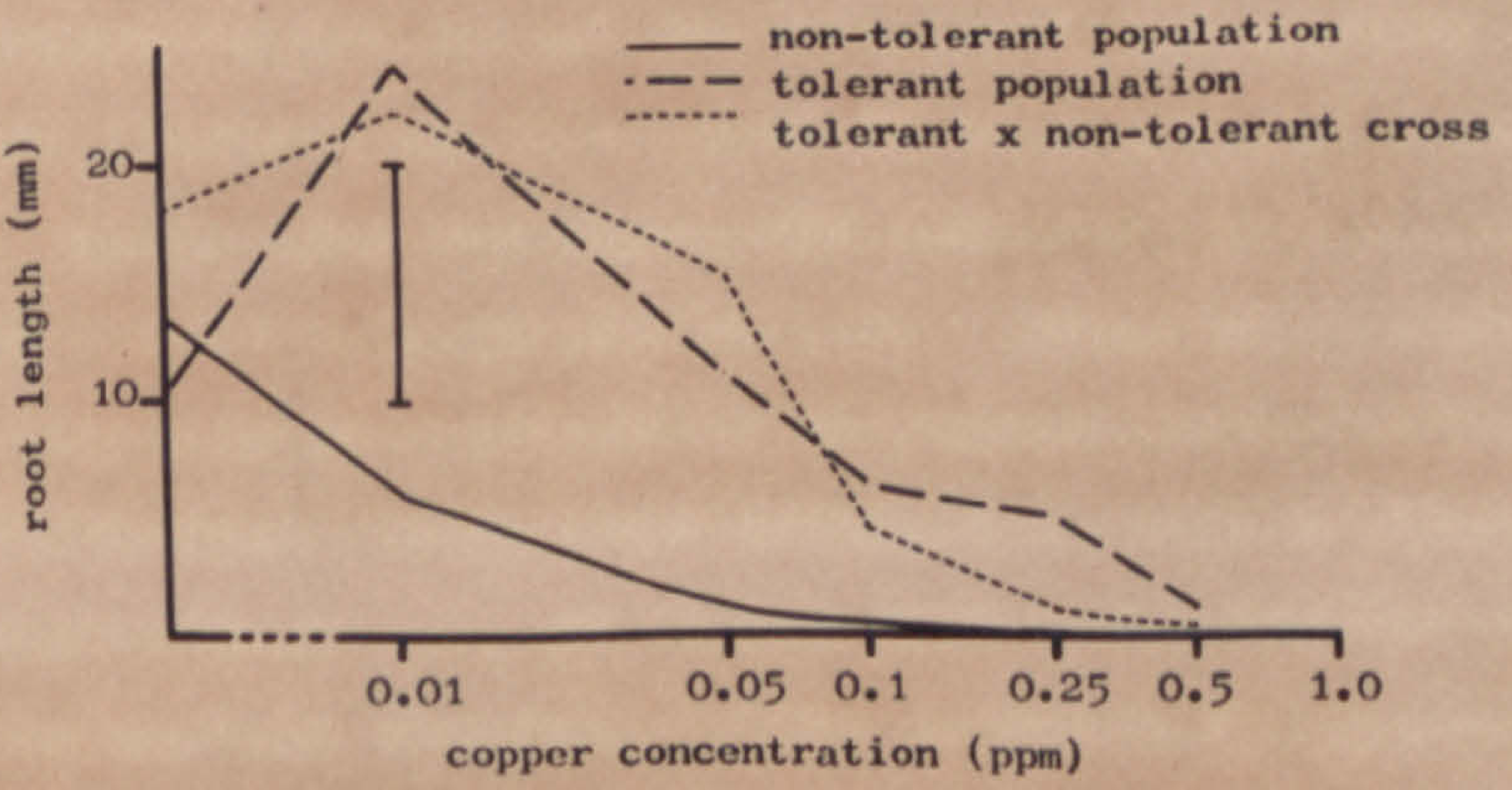
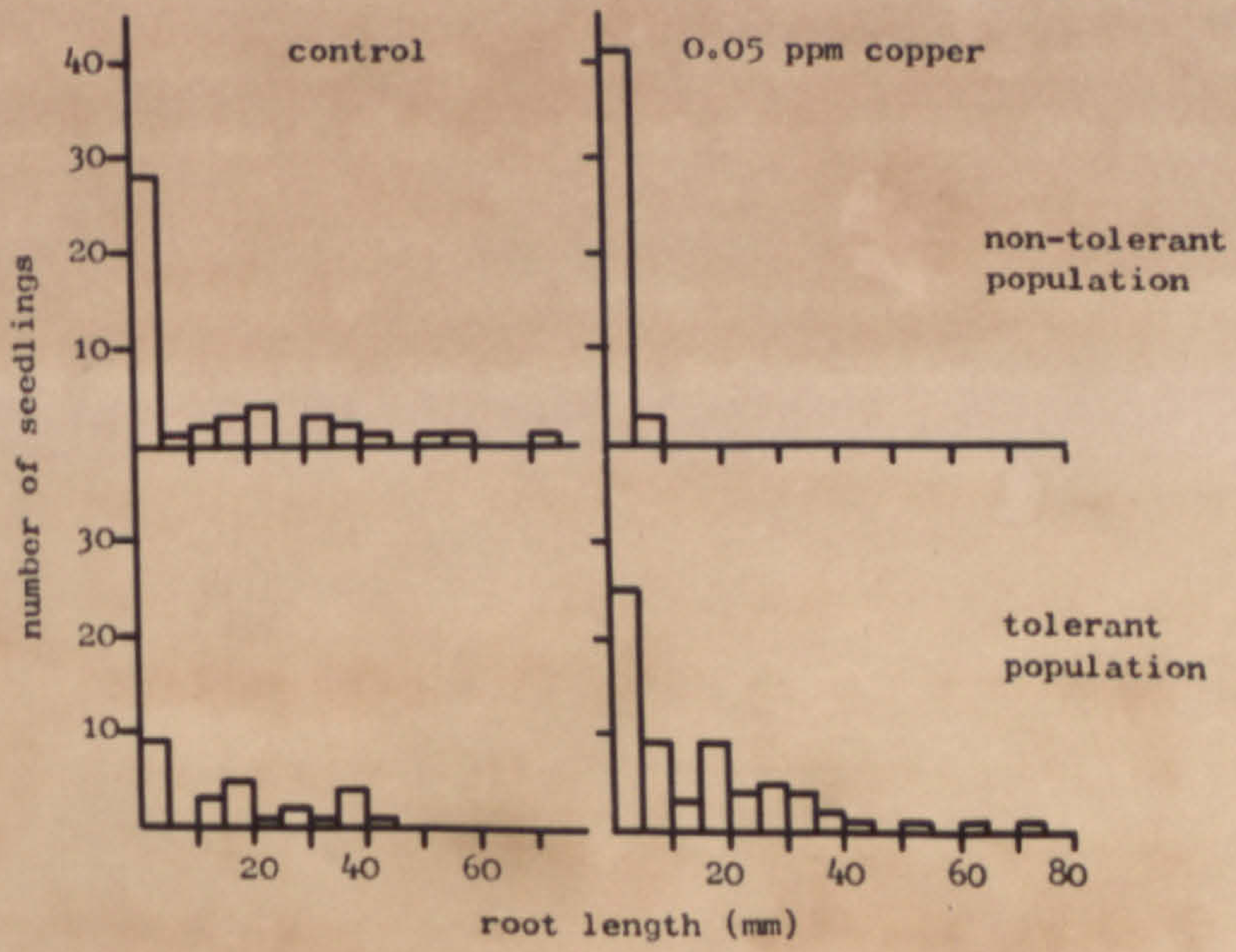


Fig. 4b. Root length distributions of tolerant and non-tolerant seedlings.



(c) Full nutrient solution

(d) Sand culture

Giving a full nutrient solution, growing at 20°C, and growing the seedlings on sand increased the proportion of rooting.

This technique was used to try and select tolerant seedlings from non-tolerant populations (see Chapter III.1.b). For this purpose a modification was developed to test a large number of seedlings. Nylon mesh was stretched over long rectangular holders made of 1/4" x 1/4" polystyrene strips. These were then floated by mean of polystyrene foam strips in plastic tanks containing the solution. The foam strips were adjusted in size so that the weight of the holders made the nylon just touch the water and be held there by surface tension. This method had the added advantage that air bubbles did not form between the nylon mesh and the solution, and there was no danger of the nylon mesh leaving the solution as a result of evaporation.

3. THE CONSTANT ENVIRONMENT CHAMBER

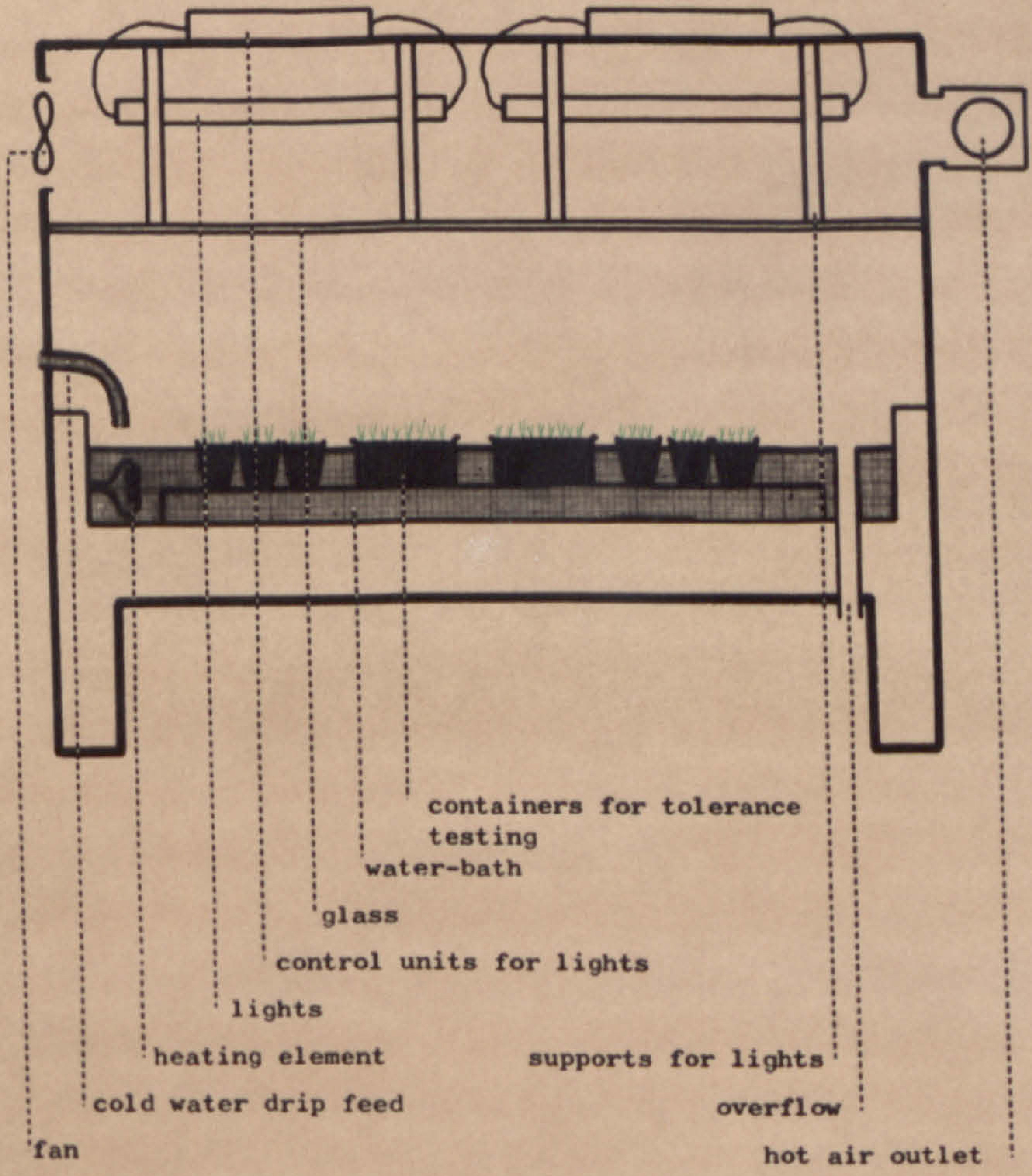
So that tests at different times could be compared with confidence a special constant environment chamber was constructed. All the tests of adults and seedlings were carried out in this chamber.

A diagram of the chamber showing relevant details is seen in Fig. 5. The temperature of the room in which the chamber was housed was kept constant at 20°C by means of a fan and heater working alternatively via a relay switch. The air cooling the lights was therefore at a constant temperature. The tanks and beakers used for tolerance testing were placed in the water bath which was kept at 25°C. The constant room temperature, the uniform cooling of the lights (with hot air ducted to the outside), and thermostated water bath ensured that the temperature in which the plants were grown did not fluctuate more than one or two degrees.

The water bath and lights were completely enclosed, so that all the lighting was artificial and therefore was not affected by diurnal and seasonal fluctuations. The lights were separated from the water bath by glass plates. This complete enclosure of the water bath also ensured a high humidity favourable for rooting of the tillers.

The light intensity at plant level was 1,500 foot candles and the plants were kept under continuous illumination.

Fig. 5. The constant enviroment chamber.



Chapter III

THE PROCESS OF SELECTION

Since mine populations are tolerant whereas normal pasture populations are not, we must presume that natural selection has occurred to cause this difference. McNeilly (1965) has produced evidence for very powerful selection pressures maintaining tolerance in mine populations. Indeed if non-tolerant seed is sown on mine soils it dies - selection pressures must be very high. Nevertheless certain aspects of the process of selection on mines remain unanswered. The following aspects were therefore investigated. 1. Speed of selection: is the evolution of highly tolerant types gradual or can it occur very rapidly? 2. Time and intensity of selection: at what stage in the life cycle does selection for tolerance occur and what is the intensity of selection? 3. Longevity: does the longevity of plants influence the pattern of genetic change, what is the population turnover, and do the tolerant and non-tolerant populations differ in this respect? 4. Changes in associated characters: are mine plants different from normal plants in characters other than tolerance?

1. SPEED OF SELECTION

Although evolution has taken place over millions of years, selection can produce rapid changes in populations. This has been repeatedly shown in many organisms. The potential variability available for selection that resides in an outbreeding grass population has been shown by Cooper (1959, 1961), where selection for date of ear emergence in Lolium produced types well outside the range of the original varieties in less than three generations.

It therefore seemed relevant to investigate the speed with which metal tolerance could be evolved. It is known that natural outcrops of metal ore produce marked effects on natural vegetation (Nicolls et al. 1965). Even on some mines in the North Wales area there is evidence that they were worked directly from the surface (e.g. Cwm Bychan Mine, Nr. Beddgelert, Caernarvonshire, Grid Ref. SH 475603), suggesting that high metal concentrations occur naturally in many areas. Apart from natural outcrops, metal working can be traced back to prehistoric times (Clark, 1952, pp. 183-99), and there is evidence that the Romans mined for copper at Parys Mountain, Anglesey.

Genes for tolerance could therefore have been established for a long time in natural populations.

The main flush of mining activity was in the early and middle part of the nineteenth century, and although the populations growing on contaminated spoil heaps are mostly less than a hundred years old, the genes responsible for the adaptation may well have been selected earlier.

However, it is difficult to see that this would be the case in the Swansea Valley area. The contaminated tips in this area are made of ore which has been sterilised during smelting, and there is no evidence of ore deposits or metal mining in this area. The tips are very toxic to normal plant growth (Sparke and Carr, 1962) and yet populations of Agrostis stolonifera can be found growing on them which have been shown to have a higher zinc tolerance than normal pasture populations (Gregory and Bradshaw, 1965). The smelting of metal ores in the Swansea area first began in 1717 and extensive spoil heaps must already have been present by the end of the century since in 1814 the Nant-Rhyd-y-Vilais works were built to extract copper and iron from slag left by previous works (Sparke and Carr, 1962). It therefore seems that metal tolerance in this area has developed within the last 150 years.

Even more rapid evolution is suggested by the work of Snaydon (1965) who showed that populations of Festuca ovina and Agrostis canina directly under galvanised fencing have a significantly higher zinc tolerance than those in the surrounding pasture. The fences were first erected in 1936 in the course of an ecological study and therefore the tolerance had developed within the last 30 years.

In spite of these long term estimates it seems quite likely that tolerance might be evolved much more rapidly, indeed in one generation. Several systems were therefore designed to look at this.

(a) Artificial mines

In order to look at the process of selection, plots of copper contaminated and non-contaminated soil were set up at the experimental station, Bangor. These artificial mines had overal dimensions of 6 ft x 3 ft and consisted of polythene lined pits 1 ft 6 ins deep in which the soil types were put. The contaminated soil came from Parys Mountain, Anglesey, and the non-contaminated soil was a fertile loam. Both soils were steam sterilised to make sure that no seeds survived.

In September, 1964, ten grams of Agrostis seed (equivalent to about 170,000 seeds) were scattered evenly on each mine. The tolerant seeds came from Drws-y-Coed Mine and the non-tolerant seeds were commercial Agrostis (New Zealand Brown Top).

The following types of mine were constructed

Mine 1. This consisted of equal patches of mine soil and loam soil; tolerant seeds were sown on both. This was to confirm that tolerant seeds grew on the mine soil and also to study selection against tolerance on the normal soil.

Mine 2. This consisted of equal patches of mine soil and loam soil; non-tolerant seeds were sown on both. This was to study the evolution of tolerance in the presence of an adjacent population of non-tolerant plants.

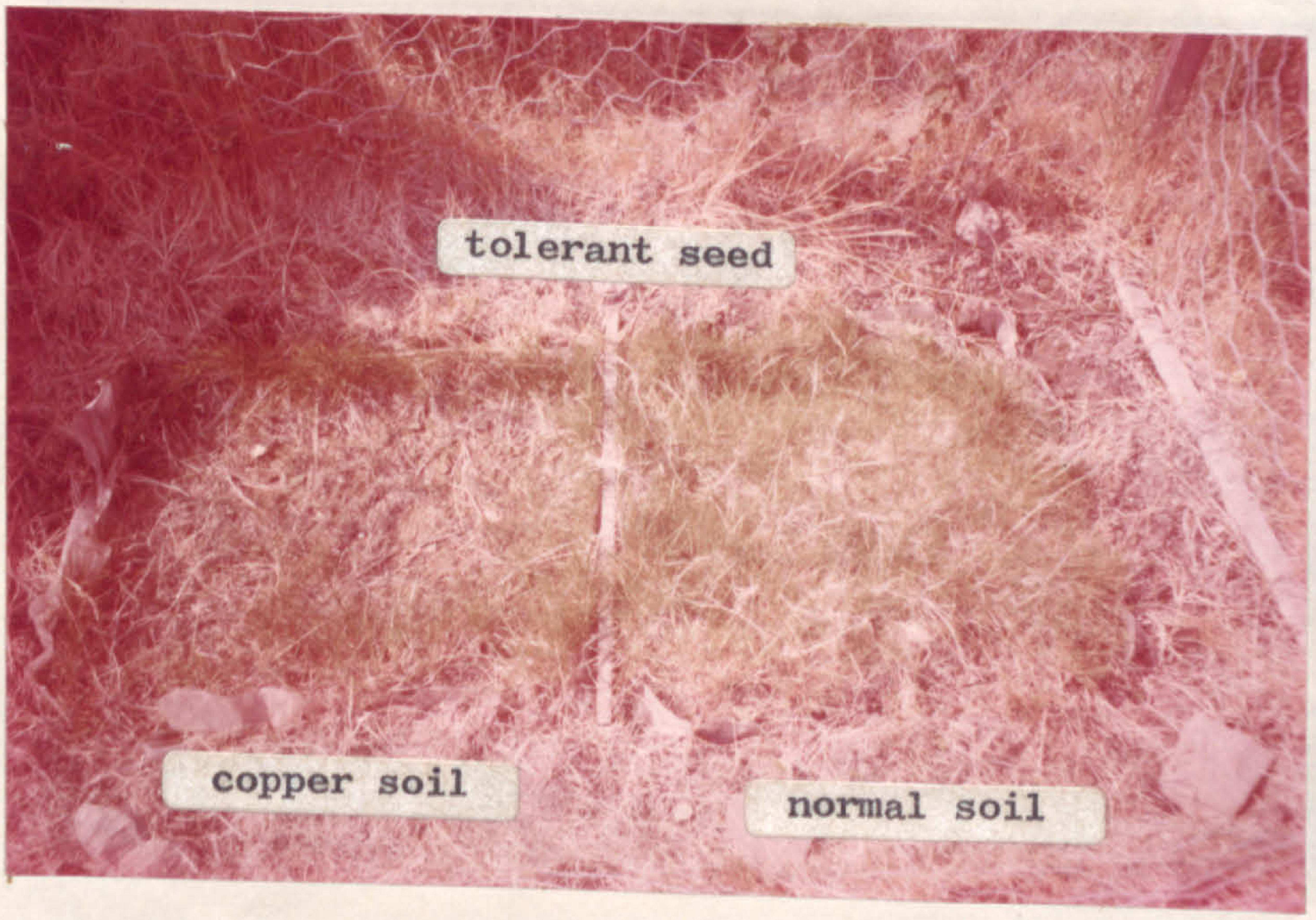
Mine 3. This consisted of mine soil only; it was sown with non-tolerant seeds. This was to study the evolution of tolerance in the absence of gene flow from adjacent non-tolerant types.

Mine 4. This consisted of three equal patches: a patch of mine soil, and a patch of loam soil, separated by a patch of mine and loam soil mixed in 50 : 50 proportions by volume. All three patches were sown with non-tolerant seeds. This was to study the effect of an 'intermediate zone' on the evolution of a tolerant population.

The state of the mines in July, 1966 (Fig. 6) shows that when tolerant seeds are sown onto mine soil, many plants survive (Fig. 6a). When non-tolerant seeds are sown on mine soil, although nearly all of the seed germinates, most of the seedlings are killed and very few adults survive (Figs. 6b, 6c and 6d). Ten individuals survived on Mine 2, one individual on Mine 3, but no individuals grew on the

Fig. 6. Artificial mine plots.

(a) Mine 1

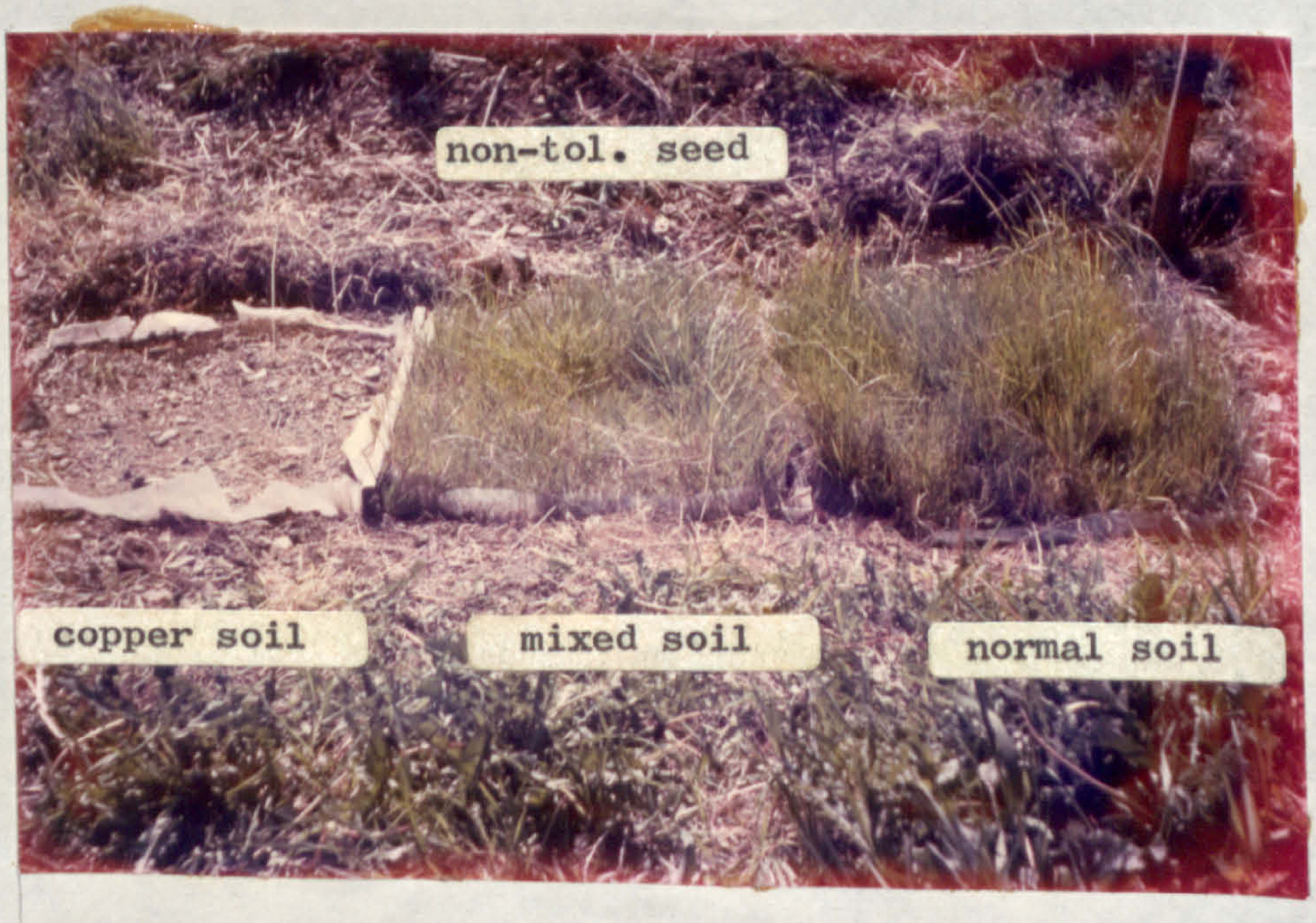


(b) Mine 2

Fig. 6. (cont.) Artificial mine plots.

(c) Mine 3

(d) Mine 4



pure copper soil of Mine 4. The reasons for the differences in survival of non-tolerant seeds on different patches of copper contaminated soil are not clear. Presumably the general conditions at Mine 3 and 4 were not as favourable to seed establishment as the conditions at Mine 2: this latter mine was at the bottom of a sloping field and considerably wetter than the others.

In July 1966 plants were taken from these mines and tested for tolerance. Unfortunately the material from Mine 1 was lost and could not be tested in time. The results are shown in Table 2. The plants growing on the copper contaminated soil of Mine 2 are not more tolerant than those growing on normal soil. This seems anomalous but can be explained firstly by the wetter nature of this site and secondly by the fact that most of the plants from this mine were taken from the edge of the plot where there was considerable humus enrichment by vegetation falling from the surrounds. On the Mines 3 and 4 however the plants growing on the undiluted mine soil, and on mine soil diluted with loam, show a greater tolerance than the non-tolerant controls. Although the tolerance is not as great as in the tolerant material, these results illustrate the possibility of selecting plants from normal populations which show an enhanced tolerance. The evolution of tolerance is therefore not necessarily a long term process and can occur in one or two generations.

Table 2. Tolerance of Agrostis plants taken from artificial mines, sown with non-tolerant commercial seed

Source of plants	Mean index of tolerance	Individual tolerances			
Tolerant seed from Drws-y-Coed	41	47	46	36	33
Commercial seed	9	12	9	6	7
Copper soil Mine 2	9	9	11	10	5
Normal soil Mine 2	11	10	5	11	17
Copper soil Mine 3	26	26			
Copper/normal soil Mine 4	20	25	18	27	9
Normal soil Mine 4	13	31	3	7	12

(b) Selection in solution

Seeds were sown on nylon mesh floated (see Chapter II. 2) on solutions containing various copper levels. A preliminary experiment showed that at 0.1 ppm many tolerant genotypes showed root growth but no roots were produced by the non-tolerant type. This level was therefore chosen for screening a non-tolerant population for tolerant individuals. One thousand seeds, of a commercial sample, were sown on nylon mesh and allowed to germinate and grow for three weeks. Nearly all the seed germinated and produced a shoot but only two individuals of the non-tolerant populations produced plants with roots (6 mm and 20mm). Out of 82 seeds that germinated from a tolerant population 32 individuals produced roots longer than 5 mm.

The two selected individuals were then grown to adults and their tolerance tested. The results obtained were as follows:

Plants	Tolerance	
Control pasture	3.9 ± 0.2	(mean pop. value 8.9)
Selected A	9.8 ± 1.4	
Selected B	18.3 ± 0.5	
Control mine	62.5 ± 1.4	(mean pop. value 40.5)

It is seen that one of the selected types shows a slightly greater tolerance than the control, yet its index of tolerance by no means approaches that of the fully tolerant mine type. Again we have here the possibility of selecting material that shows a slightly enhanced tolerance by simple screening of normal material.

(c) Selection on soil

Abbott and Misir (1960) showed that if non-tolerant seeds were sown on copper soil (again from Parys Mountain, Site 2) diluted with different amounts of John Innes No. 1 compost, then the individuals showed a marked differential survival with the numbers surviving increasing in relation to the amount of dilution (Table 3).

Table 3. Survival of non-tolerant commercial Agrostis on mine soil diluted with normal soil.

Dilution Copper soil : Normal soil	Survival from c. 7,000 seeds
48 - 1	0
24 - 1	7
12 - 1	33
6 - 1	54

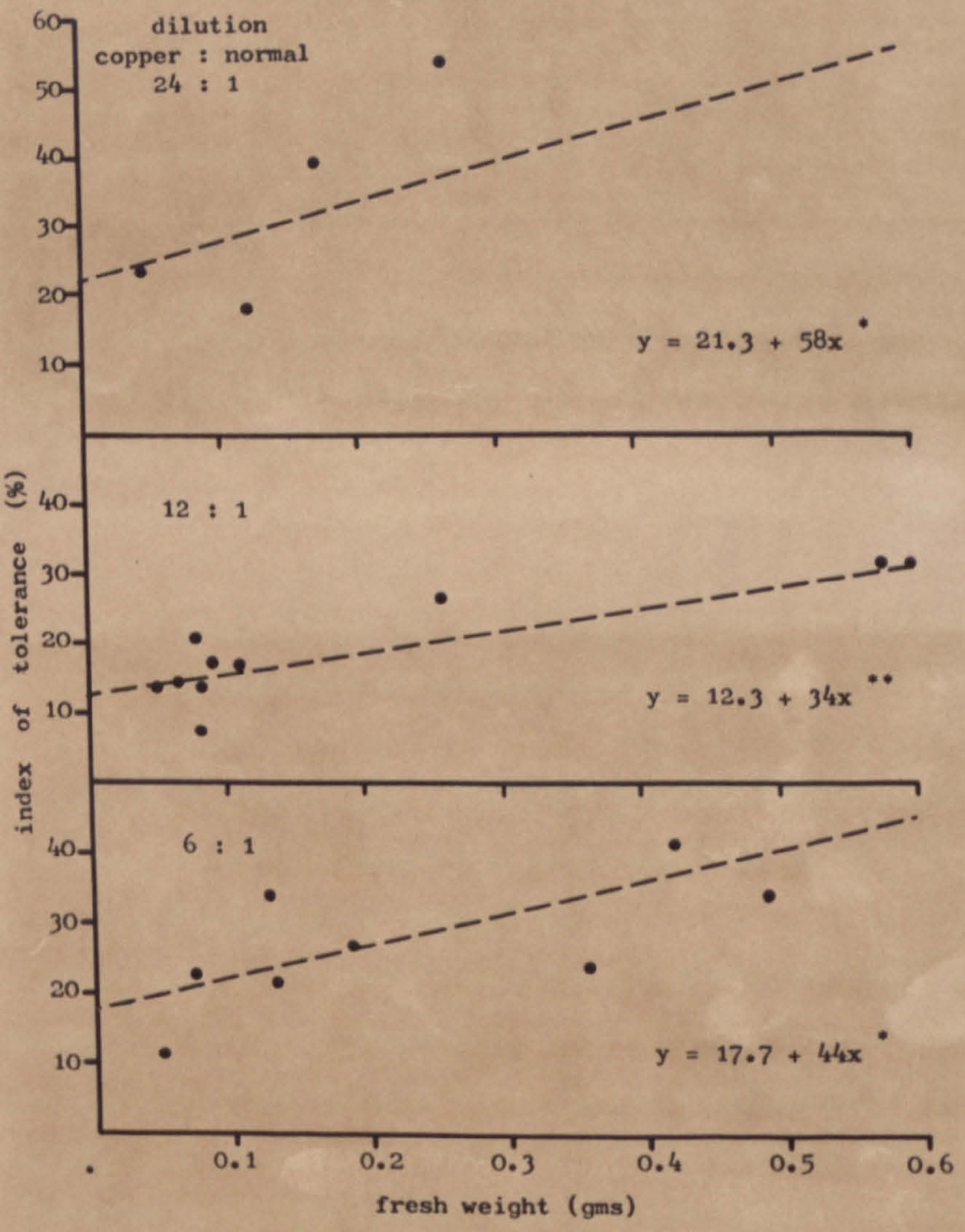
The survivors from this experiment were examined further. The fresh weight of the surviving material was taken, and the plants were grown to adults and tested for tolerance. Plants that grew better (visually and by fresh weight estimates) not only showed a higher index of tolerance (Fig. 7), but the mean tolerance of the survivors was above that of the base population. When the copper soil was only slightly diluted with normal soi, one type with an index tolerance of over 50 was selected. Moreover out of 22 plants tested, only one had an index of tolerance below 10. Selection can therefore be extremely effective on contaminated soil, and more effective than in solution.

(d) Discussion

Selection for tolerance can be a very rapid process and individuals highly tolerant to copper can be selected from a normal population by screening numerous seedlings. There is every reason to believe that these individuals will produce tolerant offspring.

The presence of extreme variants in normal populations has important implications. Many species, although often present in the vicinity of metal mines, seem to be incapable of evolving tolerance. In general terms one could say that these species do not have the available tolerant variants, but this is really a

Fig. 7. Relationship between index of tolerance and fresh weight of healthy plants growing on copper soil diluted with normal soil and sown with non-tolerant seed of *Agrostis*.



re-statement of the facts. The physiological and ecological properties of a species, such as Agrostis tenuis that enable it to evolve tolerance have been little examined and it is all the more interesting therefore that tolerance appears already to be present in normal populations.

What are these tolerant individuals doing in normal populations? What is their origin and the reason for their maintenance? These questions cannot yet be answered but have great bearing on the problem of pre-adaptation. This subject has been dealt with from a grosser evolutionary standpoint by Simpson (1953, pp. 188-98) who distinguishes nine types of situation to which the term pre-adaptation has been applied.

The more relevant are the following (Simpson's numbering is used):

"4. A specific adaptation in one ecological relationship may, without change, be requisite in another."

Are these tolerant individuals adapted to a certain niche in normal habitats?

"5. A specific adaptation in one situation may by intensification become adaptive to another situation more rigorously selective in the same direction."

Are the tolerant individuals relics of ancient metal outcrops? Does the frequency of tolerant types in non-tolerant populations increase in the vicinity of deposits?

"6. An adaptive (structural) modification may without essential change serve a different function in a different adaptive relationship."

Metal tolerance may be very similar physiologically to adaptation to low fertility and be maintained in a normal population because of this. However McNeilly (1965) looking for increased tolerance in normal populations growing on poor soils found no evidence for this.

"9. A character non-adaptive or inadaptive in an ancestral group may be adaptive in a descendant group."

Metal tolerance may have no role to play in normal populations and may in fact be inadaptive and not detectable in adult populations. In other words it could be an unwanted segregant.

Pre-adaptation and the various phenomena associated with this has attracted very few population geneticists. Gibson and Thoday (1962) traced back the genes responsible for the response to disruptive selection for chaeta number in *Drosophila* to the original population and showed that this population was "cryptically polymorphic for chaeta-number genes". Bennett (1960) using sib-selection (where the selected types were never exposed to DDT) found that resistance to this chemical still increased, showing that "a single population can contain the genetic factors for either sensitivity or resistance to an environmental agent and that those factors can be selected and concentrated without contact with the agent". The existence of tolerant types in normal populations opens up a field which none of the above workers considered, namely, Simpson's various hypotheses to explain pre-adaptation.

The results of the screening outlined above allow another important conclusion. If a single mutant type of standard tolerance was responsible for tolerance, then the number of tolerant individuals appearing would be independent of the concentration. But this is not so. In the experiments of Abbott and Misir, the number of genotypes succeeding varied with the concentration. It is *not* an all or nothing effect. And in the selection in solution experiment only slight tolerance was achieved suggesting different low tolerance genes are involved. As McNeilly (1965) showed, similar continuous variation in tolerance is found in established populations. The character must be determined by a number of genes.

As a field of study, the origin of tolerance *de novo* is likely to prove rewarding. Not only should it throw light on the population genetics of pre-adaptation, but by using artificial selection any pleiotropic effects of tolerance will be clearly distinguished from differences between tolerant and non-tolerant plants brought about by independent selection.

2. TIME AND INTENSITY OF SELECTION

The experiments outlined in the previous section show that selection occurs in the early seedling stages. An opportunity to investigate the process of selection further arose when a group of <u>Holcus lanatus</u> plants growing on lead mine soil at Hafna Mine, Llanrwst, were found surrounded by a mass of seedlings. The group of plants covered about a square metre and no other <u>Holcus</u> plants were in the immediate vicinity (50 yds).

Samples of the seedlings were taken in February and May 1964. In the later sample seedlings showing very poor growth, with several small dead needle like leaves, were kept separate from healthier, larger and greener, seedlings. The adults and the seeds they produced were sampled in the late summer of 1964. The material was grown up to maturity and tested for lead tolerance in 1965.

(a) <u>Time of selection</u>

The results (Fig. 8) show that most of the selection for tolerance does indeed occur at the early seedling stages, since the seed population shows a significantly ($P < 0.1\%$) lower mean tolerance than the seedlings, whereas the seedlings collected in February and May do not differ significantly in tolerance. Although the "poor" seedlings of the later sample show a slightly lower tolerance than the "good" seedlings, this difference is not significant. Selection is occurring at this stage for factors other than tolerance <u>per se</u> (resistance to exposure, drought, low nutrient levels, etc) especially since the difference between "poor" and "good" seedlings is very marked. The evidence therefore is that the process of selection involves an initial selection for tolerant plants followed by selection for other factors important in survival in the seedling stage.

(b) <u>Intensity of selection</u>

Using the techniques described by Van Valen (1965) the selection pressure (or intensity) acting on the <u>Holcus</u> population described above was calculated. The results are shown in Table 4.

Fig. 8. The process of selection in a small population of <u>Holcus</u> growing on a lead mine.

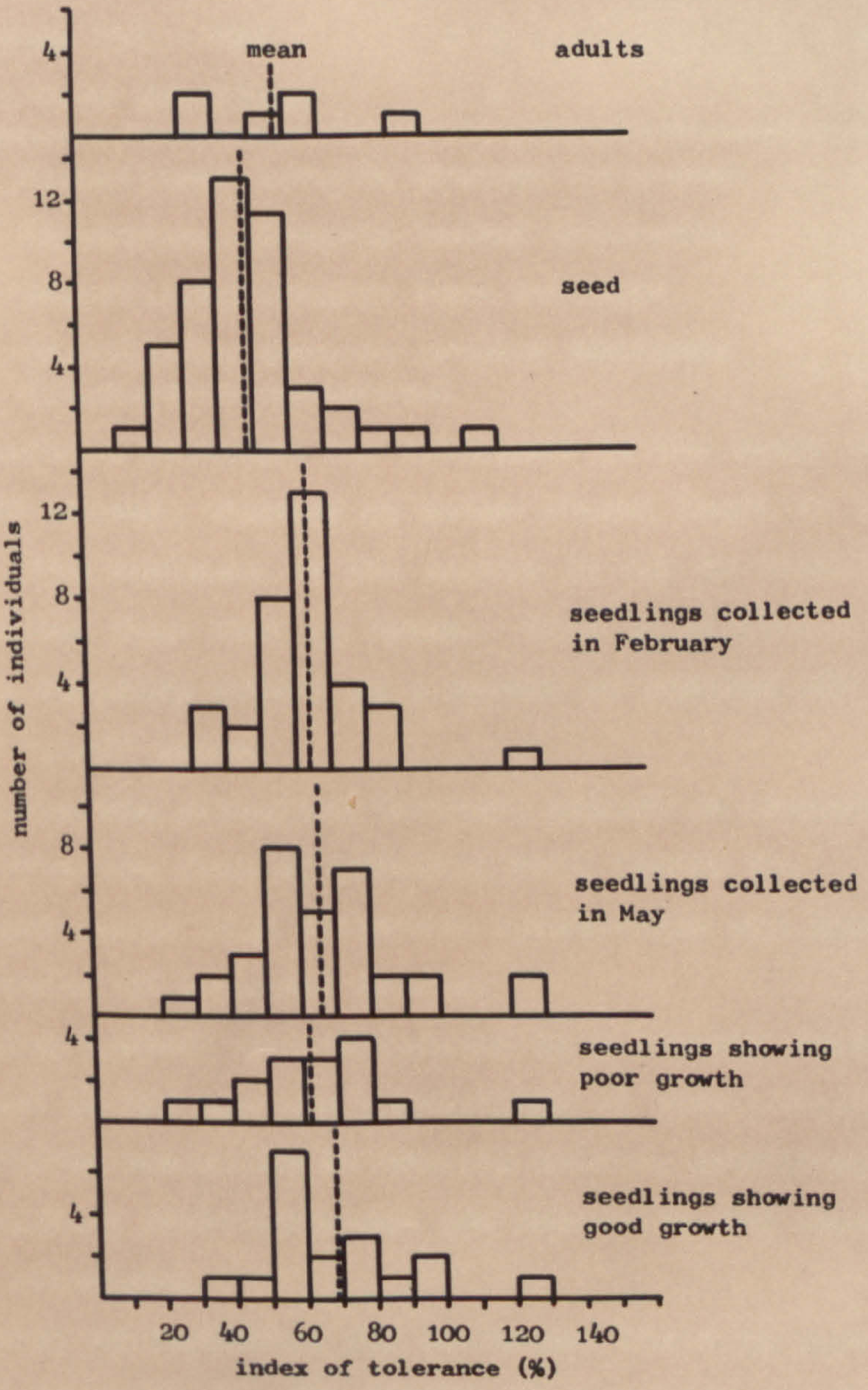


Table 4. Intensities of selection in a tolerant *Holcus* population

	Selection intensity (%)	
	Directional	Stabilising
Seed - Seedlings	+ 46***[1]	+ 1
Seedlings Feb - May	+ 3	- 26*
Poor - Good Seedlings	+ 7	+ 3

Note: Directional towards greater tolerance = +
Directional towards lesser tolerance = -
Stabilising towards less variance = +
Stabilising towards greater variance (disruptive selection) = -

The selection pressure for tolerance in the early seedling stage is very strong, whereas at later stages there is little selection. The variance of the sample in May is significantly (P < 5%) greater than that in March: this is difficult to understand and may be a sampling effect as a result of picking out extreme "poor" and "good" types.

To examine the situation further, selection pressures were calculated from the data of McNeilly, 1965 (Table 5). It is seen that here again high selection intensities are involved. Several features of interest emerge:

(a) Selection against over-tolerance is a regular feature at Parys Mountain, and in the pasture populations of Drws-y-Coed, Transect 2.

(b) Stabilising selection is equally common, providing evidence of selection against over-tolerance and selection for characters other than tolerance.

(c) The level of variation for tolerance found in normal populations can be used to calculate the amount of selection needed to get from

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[1] Footnote: probability levels throughout this thesis as follows:

* P < 5%, ** P < 1%, *** P < 0.1%

Table 5. Intensities of selection in normal and copper tolerant populations of *Agrostis* based on adult/seed comparisons. (data from McNeilly, 1965)

	Selection intensity (%)	
	Directional	Stabilising
Pasture		
Llandegfan	-2	+5
Mynedd Llandegai	-14	+18
Mine		
Parys Mt. 1.	-10	+13
Parys Mt. 2.	-2	+13
Parys Mt. 2. (isolation)	-31	+20
Mine/pasture transects at Drws-y-coed		
Transect 1.		
Site 1 (2)	+65	+27
2 (3)	+62	+28
3 (4)	+59	+11
4 (5)	+54	+7
5 (6)	+31	+6
6 (7)	+0	+4
(Figures in brackets are numbering of sites as used in this thesis)		
Transect 2.		
Mine A	+43	+5
Pasture B	+26	+1
Pasture C	-27	+8
Pasture D	-45	+12
Pasture E	-62	+10
Estimated selection to get from pasture (Mean I.T. = 13.7) to Mine (Mean I.T. = 55.3) in one generation.	+99.91	-

Note: Directional towards greater tolerance = +
Directional towards lesser tolerance = -
Stabilising towards less variance = +
Stabilising towards greater variance (disruptive) = -

a fully non-tolerant to tolerant population. This is 99.9%, i.e., 1 in 1,000 seedlings, assuming a high heritability. This is of the same order as detected in the screening/selection experiments.

(e) Discussion

Strong selection pressures are acting to maintain the *status quo* of the mine populations and of the surrounding pasture populations. This selection is not only powerful, but it is also complex. On the mine there is directional selection for tolerance. On the pasture there is directional selection against tolerance: tolerance is here disadvantageous, presumably because the adjustment needed to achieve tolerance lowers the fitness of the plants (see Chapter IV.4.d.). As well as the forces of directional selection, there are those of stabilising selection: these too can be very strong. The apparent stabilising selection in pasture populations is probably a result of the directional selection against a few tolerant segregants. In mine populations there is clear evidence that the stabilising selection results not only from selection against non-tolerant segregants but also from selection against over-tolerance. Directional selection against over-tolerance is a regular feature at Parys Mountain: even on contaminated soils too much tolerance can be a disadvantage.

Attributes other than tolerance *per se* must therefore be important for survival on mine soil. This is confirmed by the studies on *Holcus* which show that seedlings showing great differences in vigour on mine soil do not show corresponding differences in tolerance. Differences between tolerant and non-tolerant plants in many characters other than tolerance are discussed in later sections of this thesis and the evidence presented here suggest that such differences could well come about by selection that is independent of selection for tolerance.

3. LONGEVITY, POPULATION TURNOVER AND GENERATION TIME

(a) Theoretical considerations

The longevity of the members of a population can have a considerable effect on its evolution. It is important from two points of view: it will affect both the rate of colonisation and the rate of genetic change. These two processes will obviously interact since if the colonisation requires specific adaptation to the new environment, then the rate of genetic change will affect the rate of colonisation.

Nevertheless, these two phenomena are to some extent distinct and will be first considered separately.

The effect on colonisation can be understood if we assume that two organisms are similar in every other respect (e.g., time to reach maturity, fertility, etc.) but one is long lived and the other short lived. Then the long lived one will colonise an area more rapidly since each year will include not only individuals established in that year but also all those established in previous years.

The effect on genetic change can best be examined using a computer model. The model is described more fully in Chapter V.1.a: essentially the model is of an idealised population (no random effects) of infinite size. The results of the computer simulation are similar to what might be expected intuitively (Fig. 9). Perennial populations change more slowly than the annual but the final equilibrium frequencies are the same in both annual and perennial.

The slower genetic response of a perennial population can have interesting consequences. Firstly, since adaptation is slower it implies that the genetic load in a perennial population is greater than in an annual under circumstances where the population is approaching an equilibrium (Fig. 9a). Secondly, the lag of the perennial population behind the annual is reflected in the genotype frequency. Fig. 9c. shows the change in number of heterozygotes in a population with 0.2 pollen flow and complete selection against non-tolerant.

Fig. 9a. Effect of perenniality on response to selection.
(selection pressure = 1, favoured gene dominant)

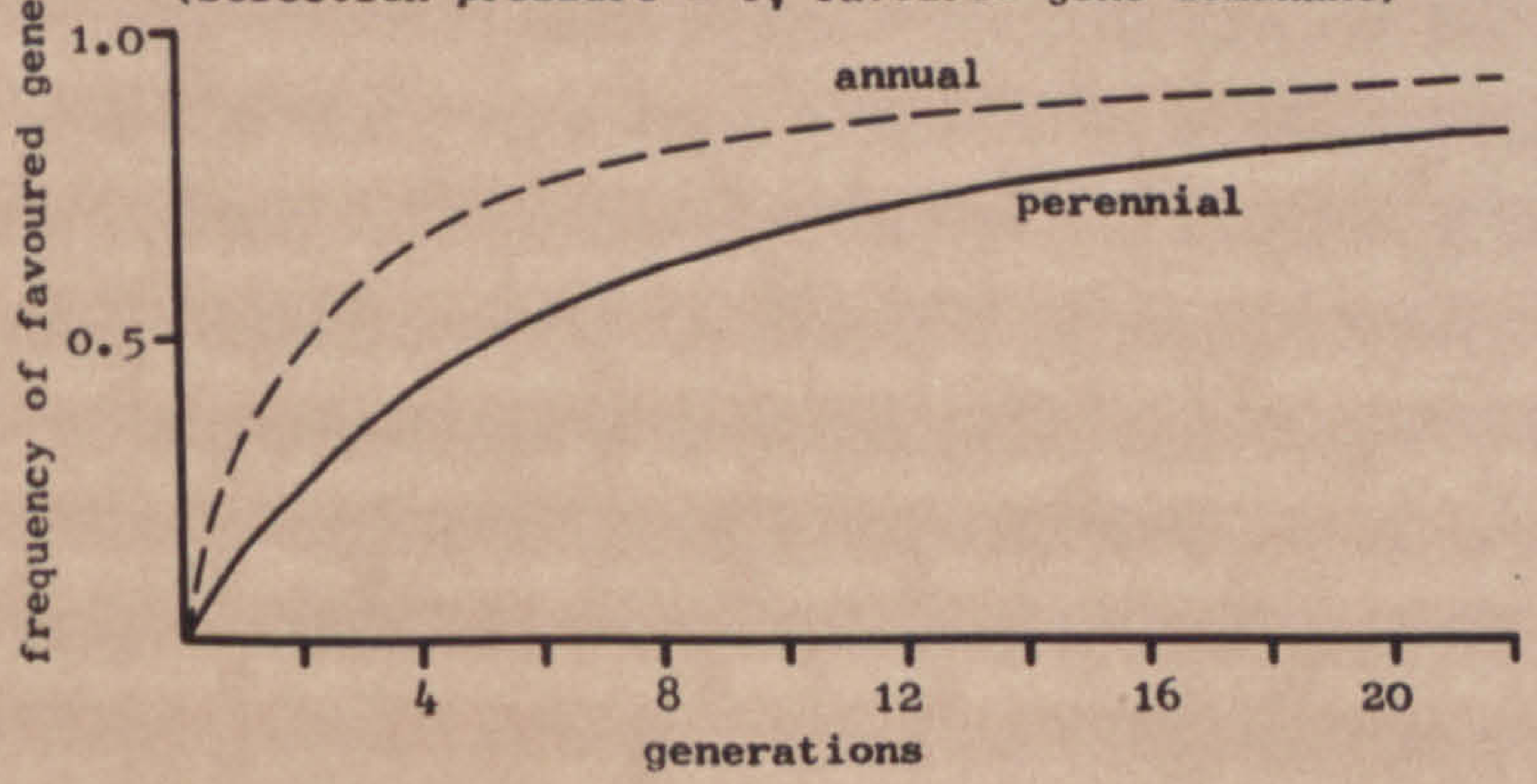


Fig. 9b. Effect of perenniality on response to selection with gene flow.
(population as above, gene (pollen) flow = 0.2)

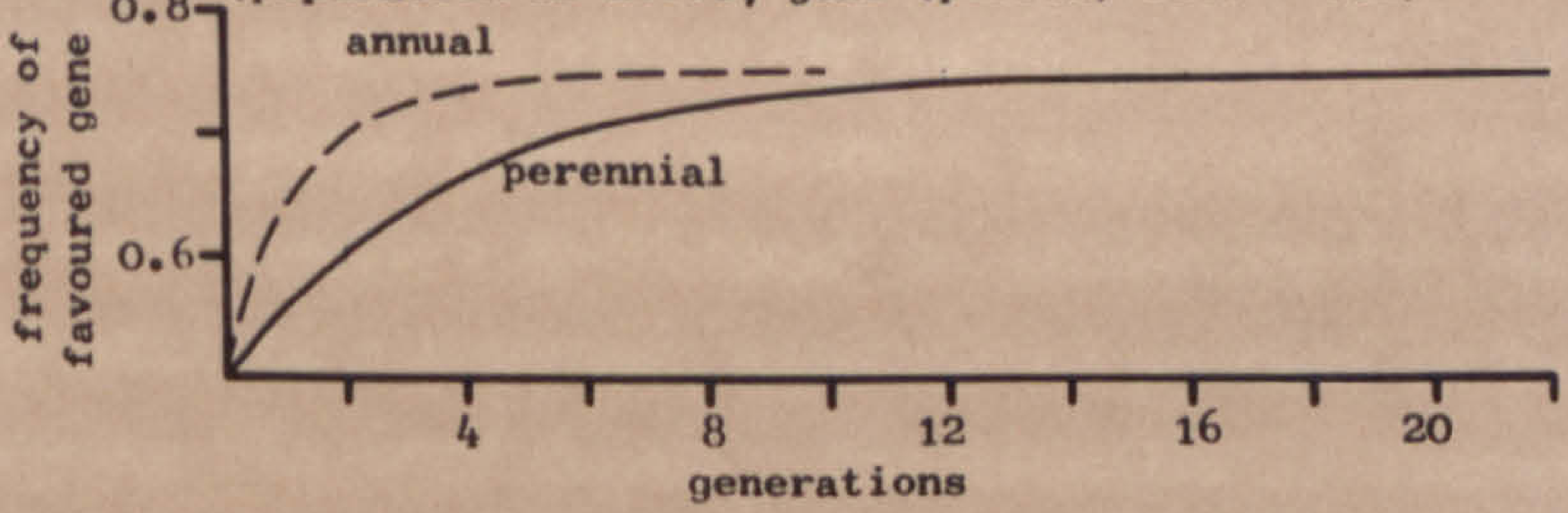
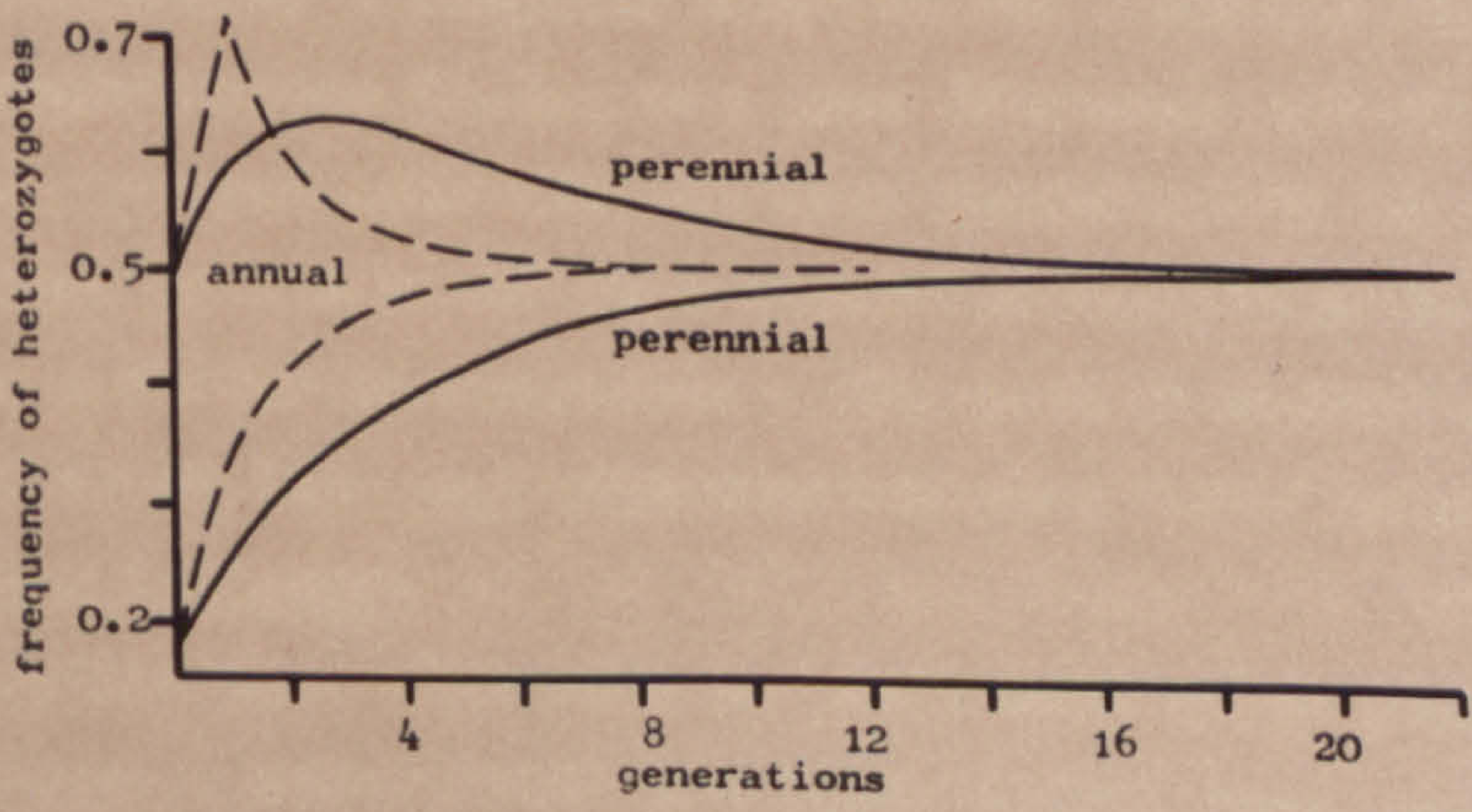


Fig. 9c. Effect of perenniality on heterozygosity.
(population and gene flow as above, initial frequencies = 0.5 and 0.9)



It is seen that if at the beginning the number of heterozygotes is below the equilibrium frequency, then the number increases more slowly in the perennial. The converse is true if we start with a high initial frequency: here the perennial population is more heterozygous than the annual.

If colonisation involves genetic change a greater longevity will have two opposite effects: it will increase the genetic load on a population (and thereby result in greater mortality) because of its slower adaptation, yet at the same time there will be more colonisation (and fewer deaths per year because of the increased perenniality). The balance between these two forces may have interesting consequences, for example in studies on the evolution of the annual or perennial habit.

The evolution of differences over short distances, such as are being considered in this thesis involve evolution and colonisation in the presence of a swamping effect by foreign pollen. If the swamping effect is serious then a perennial habit will obviously be favoured since once a mature individual is established it will live for a long time and will in subsequent generations provide an extra source of genes conferring the adaptation. Colonisation is therefore likely to be more rapid in a perennial than in an annual if the population is suffering from a serious migrational or gene-flow load.

This is confirmed by a computer simulation. If the first coloniser is a heterozygote (i.e., the gene frequency = 0.5), then frequency of the favoured gene will be less depressed by an initially high gene flow load if the plant is a perennial (see Fig. 42 and Chapter V.2. for a fuller discussion). The gene flow load is therefore likely to be less <u>and</u> colonisation more rapid in a perennial.

(b) The situation in nature

Very few studies have been made on the longevity of plants in natural populations. Tamm (1948) and Sagar (1960) have mapped individual plants of a range of species and followed their progress over several years. Tamm showed periods of seedling establishment followed by long periods during which only a few individuals died. The more intensive studies of Sagar, on *Plantago lanceolata* growing in permanent grassland gave a population turnover of 17.5% per year, with only about 30% of the individuals living for more than two years.

No data is available for perennial grasses but the work of Harberd (1961) showed that *Festuca rubra* in natural grassland has very little population turnover: the populations consist instead of a few dominant individuals which spread by vegetative propagation.

In view of the importance of longevity and the paucity of data on this subject, it seemed very relevant to investigate the situation in mine populations. The techniques used by Harberd (1961) and Sagar (1960) were both very tedious, the former involving extensive genecological trials and sampling, and the latter, detailed pantograph mapping. A simpler, but less accurate, method was therefore used to estimate the population flux in mine populations.

The investigation was carried out on *Anthoxanthum odoratum* at Trelogan mine. This plant has a tufted habit and occurs on the mine as scattered individuals, which can be easily distinguished. The plants were mapped by marking their position on line transects: a plant was recorded if it touched or came directly below a tape measure. The deficiencies of this method were mainly two-fold. Firstly, since the tape measure was often stretched over uneven ground, parallax errors could occur. Secondly, since a line transect was used plant death could not be distinguished from plant movement off the transect. However the results show that little movement along the transect occurs, and although it cannot be excluded, especially since considerable 'frost-erosion' is seen during the winter, it is likely to be fairly negligible. The transects were all about ten yards from normal pasture, all radiated from one point and were on a patch where there were numerous *Anthoxanthum* plants.

The results are shown in Figs. 10, 11 and Tables 6 and 7. The salient points to emerge are as follows:

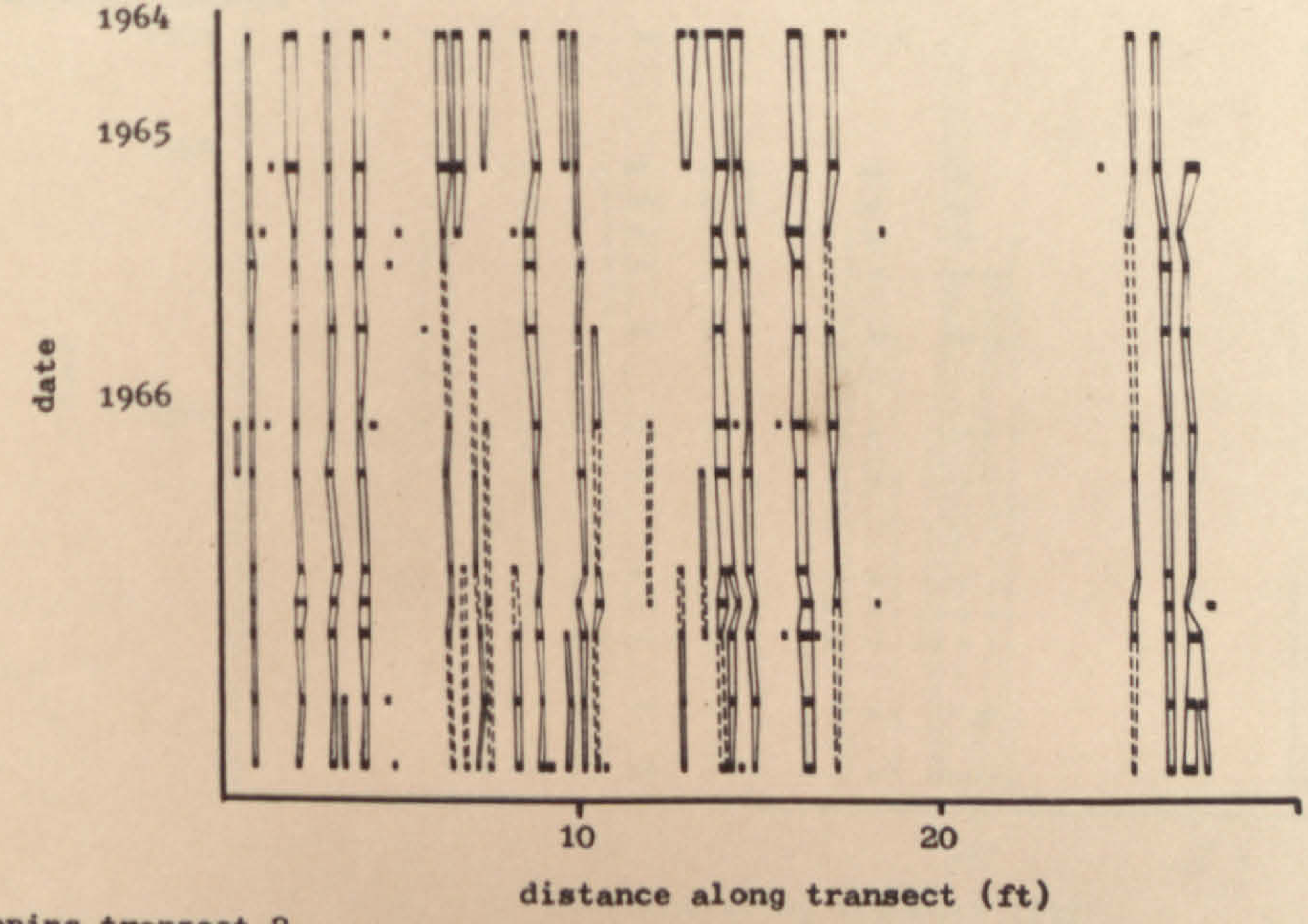
Fig. 10. Longevity of individuals of *Anthoxanthum* at Trelogan

plant record

continuous record

discontinuous record

Mapping transect 1



Mapping transect 2

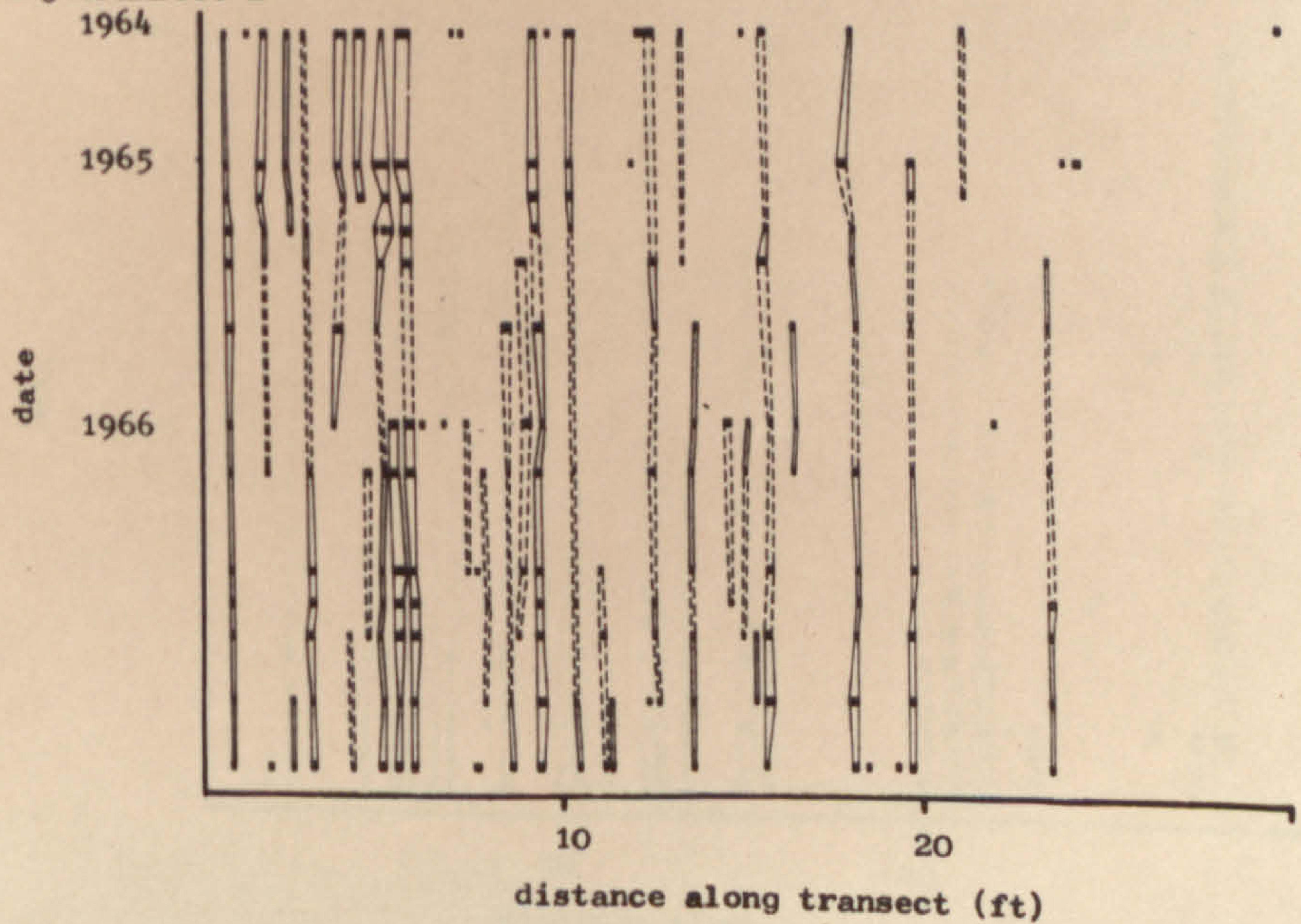
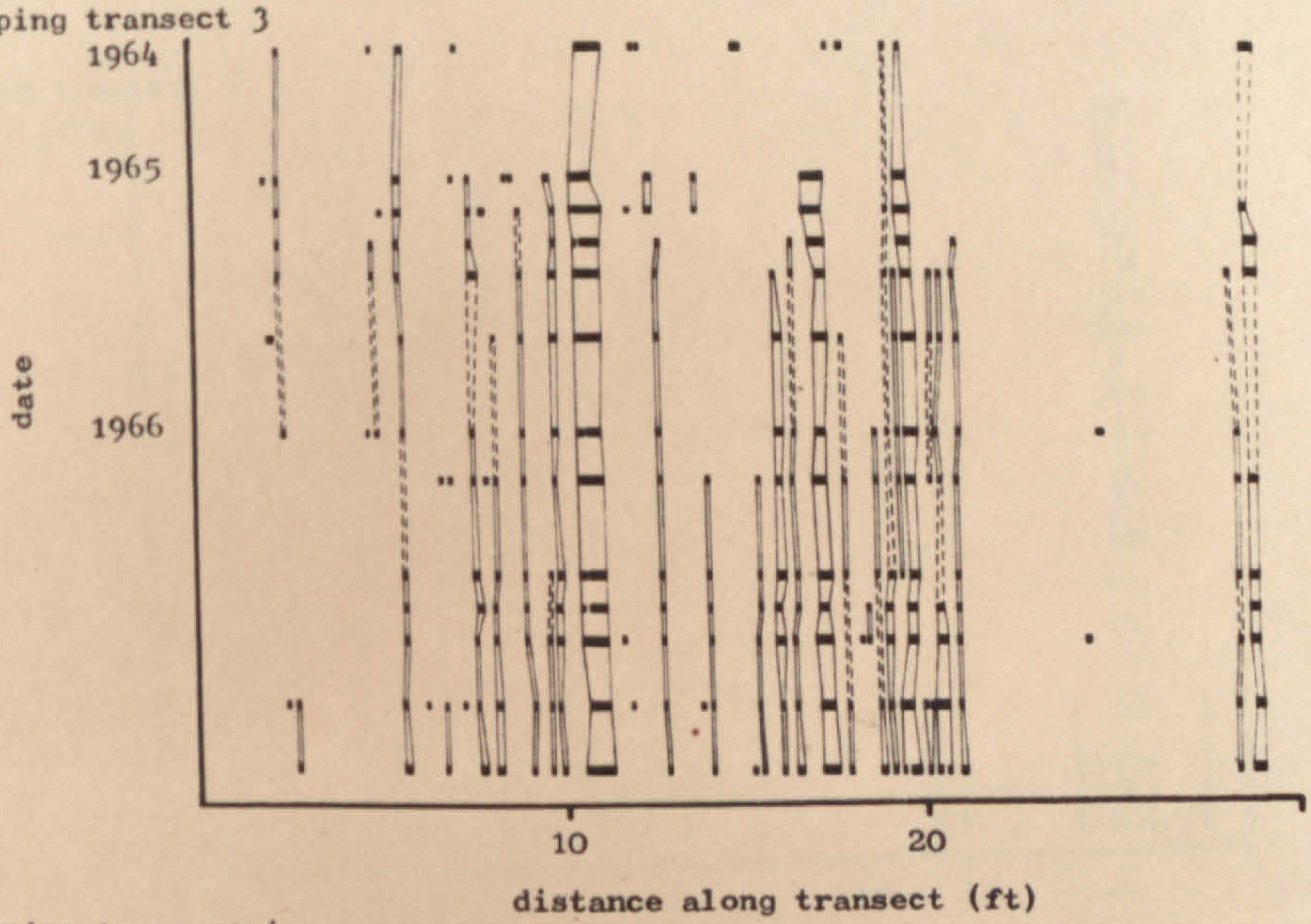


Fig. 10. (cond.) Longevity of individuals of __Anthoxanthum__ at Trelogan

Mapping transect 3



Mapping transect 4

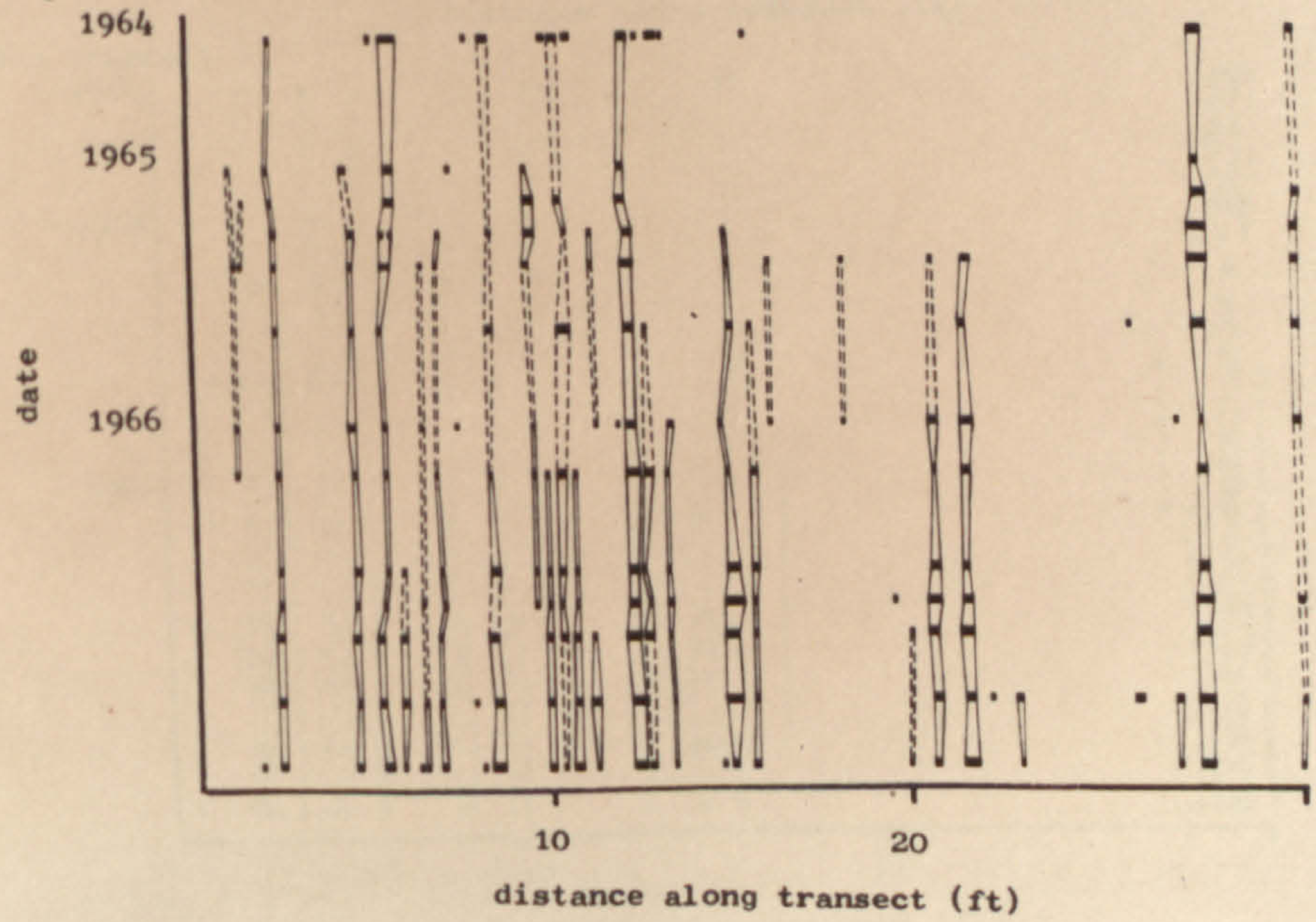
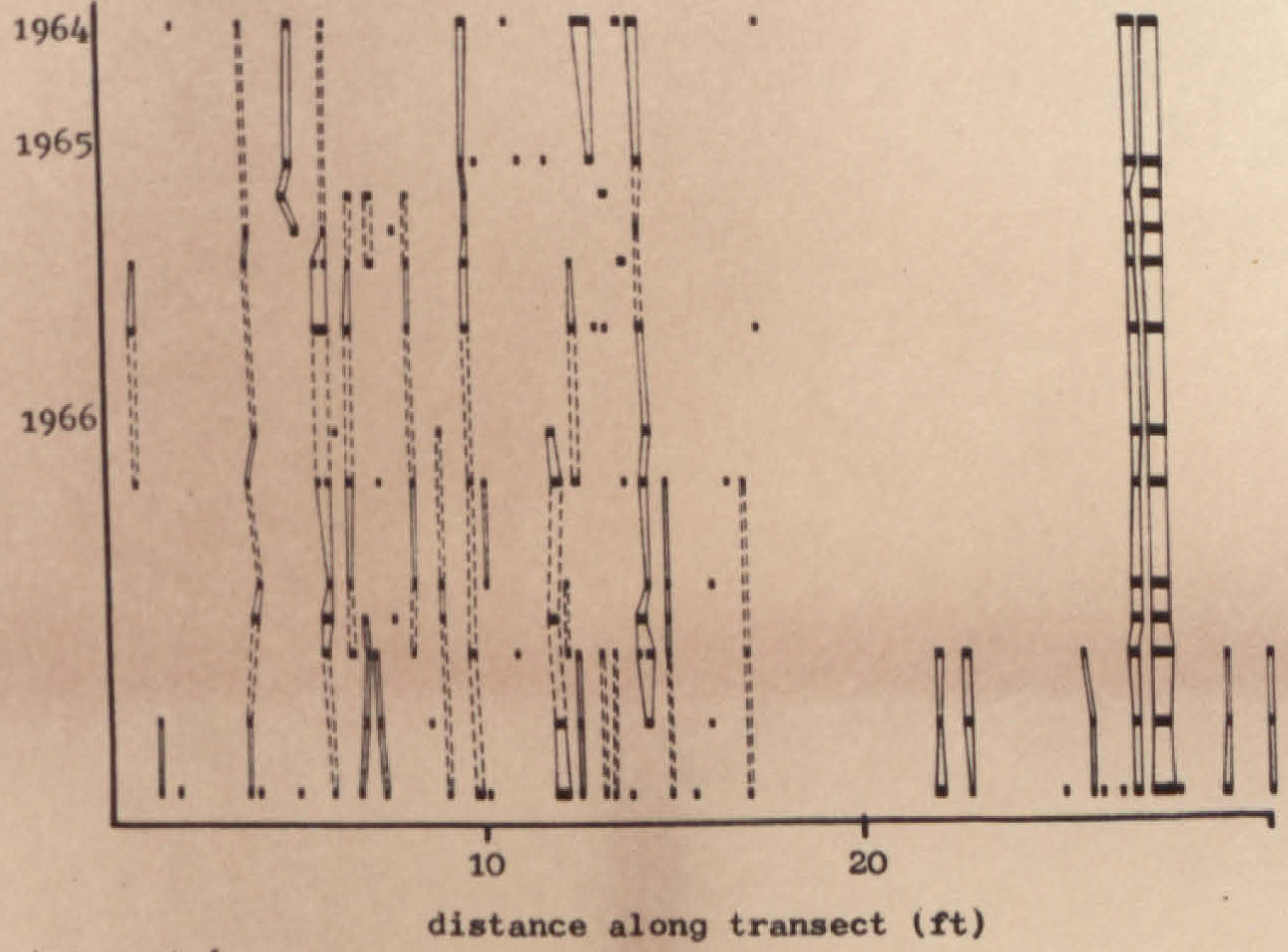


Fig. 10. (cond.) Longevity of individuals of Anthoxanthum at Trelogan

Mapping transect 5



Mapping transect 6

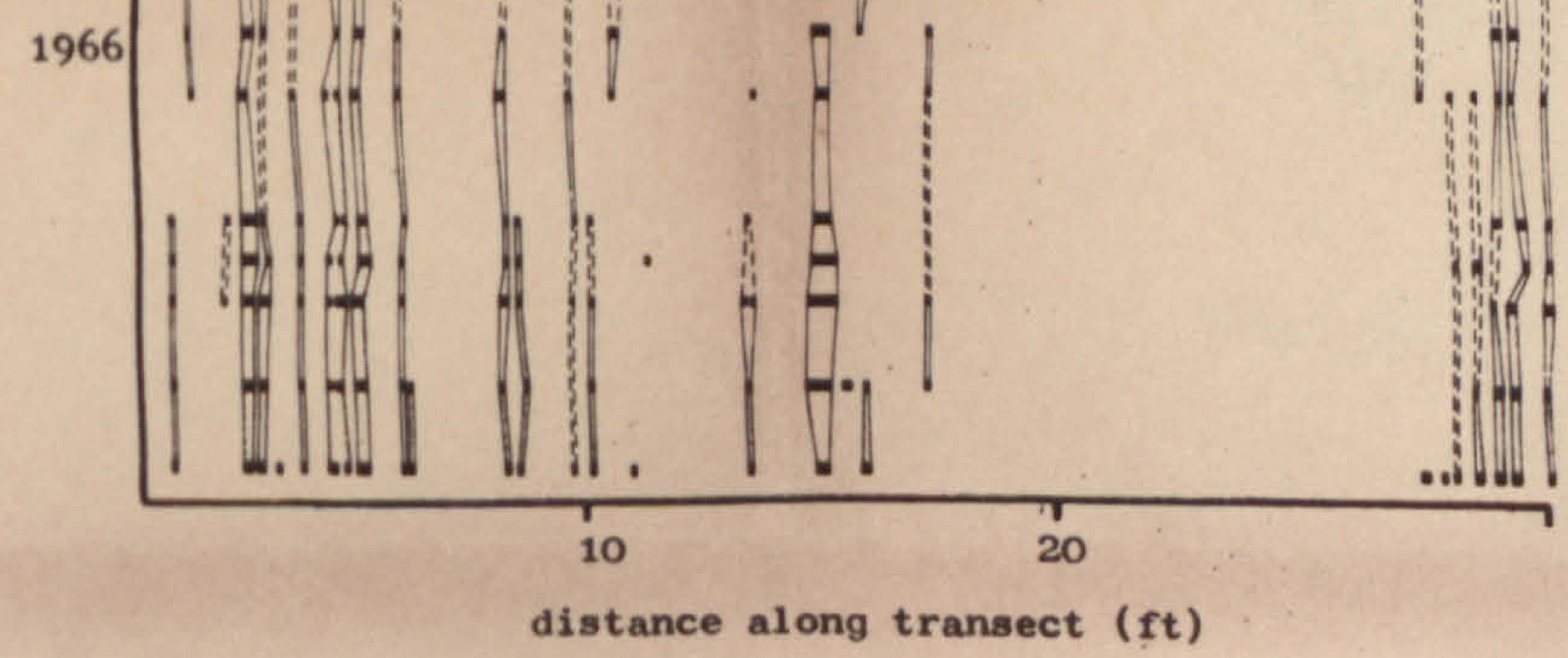


Fig. 11. Change in number of mapped individuals of *Anthoxanthum* in mine population at Trelogan.

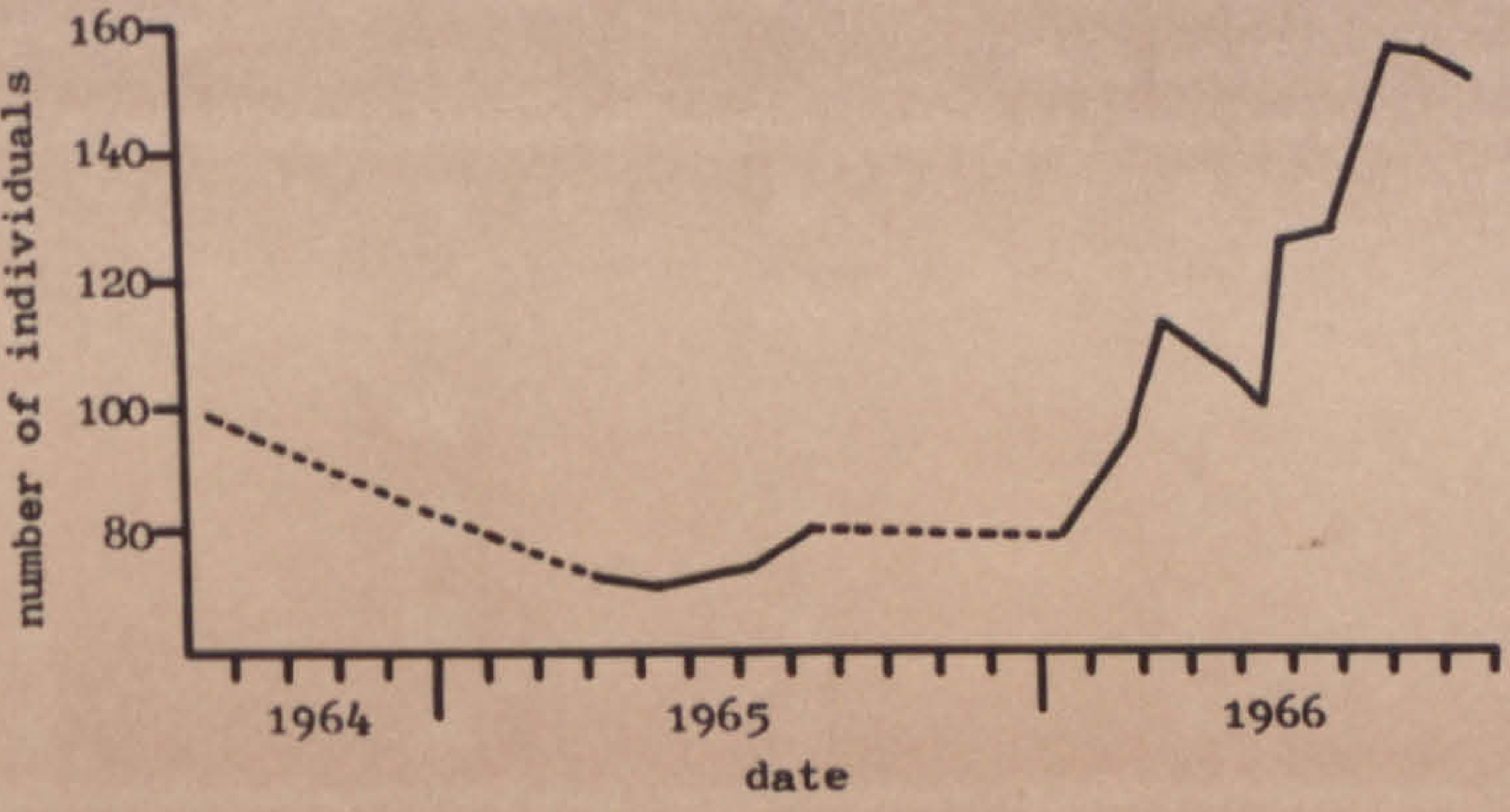


Fig. 12. Photographs showing dead or dying individuals in mine population at Trelogan.

Selection : longevity

Table 6. Survival of individuals of Anthoxanthum at six transects on Trelogan mine over a period of two years.

	number of individuals				
Transect	Through Survivors	Arrivals	Deaths	Arrivals and Deaths	Sporadic [1] Appearances
1	13	9	4	3	19
2	8	9	5	7	18
3	5	19	1	6	29
4	7	16	0	6	21
5	5	15	3	7	30
6	4	15	3	5	16
Total	42	83	16	34	133

1. Individuals recorded on only one date.

(i) There is considerable turnover of individuals: the percentage turnover of individuals present in both 1964 and early 1965 is 19%, but many of the plants which survive for less than twelve months flower and produce seed, so the turnover of these more temporary plants (= 100% per year) is also important from the standpoint of evolutionary change.

(ii) The degree of population turnover is less on transects (1) and (2). These transects cross a much lower, wetter part of the area investigated and this suggests that drought either directly, or by raising the concentrations of ions in soil solution, is important in survival of mine plants.

(iii) The population size shows an overall increase from 1965 to 1966 (Fig. 11), but since no increase is observed in 1965 over 1964, no definite trend can be established. The population numbers could well be fluctuating about an equilibrium.

(iv) Larger plants on the whole show better survival, suggesting that longevity is associated with increase in size of the individual.

Table 7. Population parameters for the mine population of *Anthoxanthum* at Trelogan

Parameter	Value
Total number of individuals observed	308
Number present on 21/8/64	99
" " " 27/7/66	158
Net gain	59
Percentage increase over two years	59.6%
Total arrivals	250
Total lost	183
Potential rate of increase/yr.	126%
Number present present in 1964 and early 1965 (established individuals)	61
Numbers lost from established individuals	16
Numbers surviving through period	42
Survival of established individuals	72.4%
Population turnover per year of established individuals	19%
Percentage mortality per year of all individuals observed	52.4%
Age distribution of plants that died or arrived during period of observation	
dying in less than 12 months	157
living for 12-24 months	10
living more than 24 months	42

results are supported by general observation. On most mines there is evidence of dead and dying vegetation. The photographs (Fig. 12) show that large individuals, and not just seedlings, do die and have therefore a limited life span.

In view of this population turnover, there must be dynamic forces maintaining the mine populations. Selection must be continuously operating, and genetic change is possible in many characters other than tolerance. Whether this turnover is peculiar to Anthoxanthum on Trelogan mine or whether it is general to other grasses in different habitats was not established. Since the longevity of the plants at Trelogan varied even from site to site, it is likely that longevity is specific for given populations under given conditions.

(c) Experimental evidence

To investigate the problem of longevity further, and to make comparisons between tolerant and non-tolerant populations, measurements were made on Anthoxanthum plants growing in a field trial in the experimental garden. The experiment was primarily designed to investigate morphological differences between tolerant and non-tolerant types and is fully described in Chapter III.4.

During the course of this experiment two features were noted.

(1) Survival

The individuals were planted as spaced plants in August 1965 and any that failed to establish were replaced in early October.

The number of individuals that died were noted (Table 8). No obvious differences between the populations are seen, even though the mortality is to some degree clone specific. The distribution of mortality deviates significantly, $P < 1\%$, from the Poisson.

Table 8. Number of deaths in single plant trial of *Anthoxanthum*.

(Brackets denote a clone, and numbers inside brackets show number of individuals dying out of a total of 8 in that clone)

	Number of deaths			
Transect sites	Before 30th May 1966	During flowering	After flowering	Total
1	(3) (1) (1) (1)			6
2	(1) (2) (5)			8
3	(1)	(1)		2
4	(2) (1) (1)	(1)		5
5	(2)		(1)	3
6	(1) (1) (1) (1) (1) (3)			8
7	(1)			1
8	(1) (4)		(1)	6

(ii) Vegetativeness.

The plants were assessed for degree of vegetativeness by the ratio of the number of vegetative tillers to number of reproductive tillers. Since in general the vegetative tillers carry the plants over to the next season, whereas the reproductive tillers die after flowering, this was considered also to give some measure of perenniality. Where very low ratios were obtained, with none or a few vegetative tillers, the plants had all the appearance of annuals and some in fact died after a strong burst of flowering. The ratio has been plotted in Fig. 18 (see p. 32) on a log transformation since plotting log a/b (= log a - log b) removes the skew distribution. Essentially similar results, but with wider scatter of the points, were obtained using untransformed data. It is seen that the tolerant population is on the whole (except a few plants at Site 2) more vegetative than the non-tolerant (the difference is highly significant, see Table 9), suggesting that the mine populations are more perennial than the pasture populations.

Evidence has already been presented from a theoretical analysis which suggests that perenniality is likely to be favoured under conditions of gene-flow load such as occur on mine habitats. However the data can be interpreted in other ways.

Firstly, growing the plants as spaced individuals in an experimental garden may not reflect their behaviour in the field.

Secondly, differences in vegetativeness and longevity can come about by direct adaptation to local conditions (Bradshaw, 1959, Bradshaw, unpublished data on Anthoxanthum, and Cook, 1962).

Thirdly, the whole problem of perenniality is coupled (for full review, see Cole, 1954) and interrelated with many other aspects of life-cycle strategies.

There are therefore many reasons why populations should differ in their perenniality. There is evidence from crop plants that the longevity of a plant is easily changed by selection (Schwanitz, 1957, p. 38, Helbaek, 1959, Kihara, 1959, Hutchinson, 1962, and Khush, 1962). Differences between tolerant and non-tolerant populations with respect to this character are nevertheless interesting since rarely have such differences been noted between populations of wild species. Although the data presented here is inadequate to draw firm conclusions about the causes of the difference in perenniality, it does emphasise the possibility of studying such life cycle characteristics at a population level.

4. CHANGES IN ASSOCIATED CHARACTERS

(a) Introduction

Previous work on metal tolerant plants has shown that they differ from normal plants in several features other than tolerance. Differences in calcium and phosphate response are recorded by Jowett (1959) and McNeilly (1965), and Turner (1966) has shown differences in sulphur response. Schwanitz and Hahn (1954a, 1954b) grew the tolerant and non-tolerant populations of a range of species under standard conditions and showed that in general tolerant plants had smaller flowers, smaller leaves and thinner stems. These differences were reflected in the size of the epidermal cells of the leaves, but using other non-tolerant variants they showed that small cell size did not go hand in hand with tolerance. They concluded that there must be independent selection for morphology as well as tolerance. This conclusion was confirmed in *Silene inflata* by Broker (196[illegible]) : in F2 progeny of tolerant × non-tolerant crosses, tolerance was not associated with morphology. Similar but less startling differences have been detected in *Agrostis tenuis* on lead mines (Bradshaw, 1959, Jowett, 1964) and again the data suggested a complex adaptation of the populations to local factors rather than an effect of tolerance on overall size and yield. Only slight differences in morphology between copper tolerant and non-tolerant plants were detected by McNeilly (1965).

The situation with regard to morphological characters was re-investigated using *Anthoxanthum* from Trelogan with four main points in view.

(i) It has not yet been established if tolerant *Anthoxanthum* differs morphologically from non-tolerant.

(ii) The intensity of selection is likely to be different for different characters, and although the boundary between mine and non-mine is just as clear cut in factors like exposure, dryness, and associated vegetation as it is in tolerance, the different selection pressures should lead to clines of a different steepness (Jain and Bradshaw, 1966).

(iii) The strong selection for tolerance in mine populations is analogous to the strong selection pressure used in many artificial selection experiments. It therefore seemed worthwhile to look for changes in "secondary characters" such as are characteristic of artificial selection programmes. Work on *Drosophila* has frequently shown that strong selection for one character leads to correlated responses in other characters. Such experiments often show an increase in the variance of these other characters (Mather and Harrison, 1949, Clayton, *et al.*, 1957). Similar changes have been investigated in *Lolium perenne* (Cooper, 1960) where correlated responses occurred in various floral (morphological) characters as a result of selection for date of ear emergence.

(iv) The evolution of tolerance is permissive in the sense that it allows colonisation of an open habitat, and an increase in the numbers of plants in that habitat. Ford (1962, p. 11) has argued on the basis of earlier investigations (Ford and Ford, 1930) that in an expanding population selection is relaxed, and that this therefore permits the survival of numerous variants. In other words stabilising selection is relaxed. However on theoretical grounds, changes in variability will only be concomitant with changes in population size if the controlling factor is the same for both: selection controls the variability but it is unlikely that the controlling factor of population size is selection against extreme variants. The situation on metal mines is an obvious model to test these two alternative hypotheses.

(b) Method

Ten plants from each of the eight sites along the Trelogan transect were grown for two months in John Innes No.1 compost. Bunches of two large or three smaller tillers were then planted at nine inch spacing in the experimental garden in a single plant trial according to the following design. There were four blocks and within each block the design was hierarchical. Two plants per genotype were grown at each point. The design was therefore:

2 populations (mine and pasture)
8 sites (four per population)
80 genotypes (ten per site)
160 plants (two per genotype)
4 replicates

This design enabled comparisons to be made between populations, sites, and genotypes, as well as comparisons of the within sites variation.

The experiment was set up in August 1965 and any plants that did not establish were replaced a month later. The following characters were measured in the spring and summer of 1966.

(i) Flowering time (see Chapter IV.3.b. for full discussion)
(ii) Plant height (on 25/6/66)
(iii) Number of vegetative tillers (on 1/8/66)
(iv) Number of reproductive tillers (= inflorescences) (on 1/7/66)
(v) Width of flag leaf (mean of eight leaves) (on 3/6/66)
(vi) Length of flag leaf (mean of eight leaves) (on 3/6/66)

From these measurements the following further parameters were calculated

(vii) Total number of tillers
(viii) Ratio of vegetative to reproductive tillers (vegetativeness) (See Chapter II.3. for full discussion)
(ix) Coefficient of within plant variation in flag leaf length.

The general layout of the experiment is shown in Fig. 13 which also illustrates the obvious size difference between tolerant and non-tolerant plants.

(c) Results

(i) Differences between populations, transect sites, and genotypes (Table 9). Differences are found between tolerant and non-tolerant populations in several characters. Considerable differences are also found between sites within populations, suggesting considerable local heterogeneity. Thus within the tolerant population, site 2 behaves anomalously for several characters, whereas in the non-

Fig. 13. Photograph illustrating the general size difference between tolerant and non-tolerant *Anthoxanthum* in a spaced plant trial.
(non-tolerant material indicated by white surrounds)

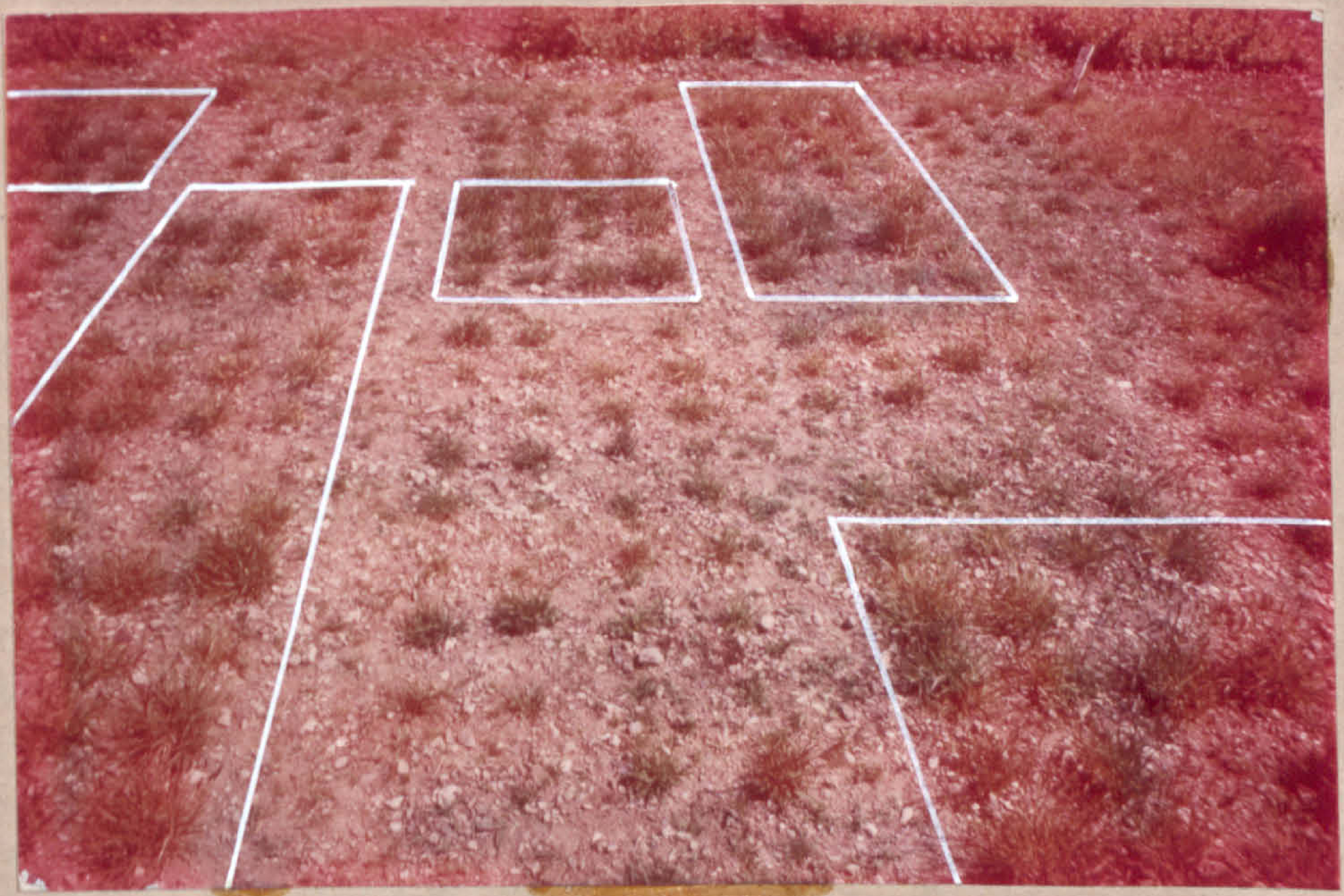


Table 9. Differences between tolerant and non-tolerant plants of *Anthoxanthum*.

Character	Mean of tolerant populations	Mean of non-tol. populations	Significance of various comparisons	
			Between populations	Between sites
Flowering time				
Stage 1	2.9	8.6	***	***
Stage 2	11.3	17.8	***	***
Stage 3	17.2	21.9	***	***
Stage 4	32.1	28.0	***	***
Plant height	38.7	56.1	***	***
Veg. tiller number	37.0	40.9	N.S.	*
Reprod. tiller number	47.9	79.9	***	***
Width of flag leaf	5.3	5.4	N.S.	**
Length of flag leaf	25.8	33.9	***	***
Vegetativeness (log)	1.902	1.627	***	***
Within plant variability	62.4	61.4	N.S.	N.S.

Differences between individual genotypes all significant (*** level).

tolerant population site 8 behaves rather like the tolerant population with respect to plant height, length of flag leaf and flowering time (see Figs. 14-22). However, the index of tolerance of the plants from site 8 is no higher than in the other pasture sites (Fig. 14), but the region here is slightly raised above the rest of the pasture and is probably slightly drier.

These points emphasise the possibility of very local differentiation and are warnings against considering either tolerant or non-tolerant areas as ecologically uniform: this is particularly important when considering population variability.

(ii) The clinal pattern - means

The clines obtained for the different characters are shown in Figs. 14-22. It is seen that for some characters no clinal pattern emerges (e.g. flag leaf width, Fig. 21, within plant variation, Fig. 22) whereas with other characters clines of different shape are obtained (e.g. plant height, Fig. 15, versus flag leaf length, Fig. 20). The existence of such different clinal patterns across the same ecological boundary can have several explanations.

Firstly, characters could be subjected to selection by different environmental parameters each of which changes gradually in a specified way which matches the distribution of the character across the boundary. Kimura (1958) found that the distance over which the selective advantage changes determines the steepness of the cline.

Secondly, the selective pressures across a boundary may <u>not</u> match the distribution of the character but may change sharply at this boundary. The different selection pressures on the various characters lead to clines of different steepness. The shape of the cline will also depend on the gene flow (Jain and Bradshaw, 1966), but in a given ecological situation and for a given species, gene flow is at a certain level for all characters.

There are therefore two alternative hypotheses for explaining the different clinal patterns. The latter explanation is the most probable in the case of the clines at Trelogan since it is difficult to see what selective forces could change gradually over such a

Fig. 14. Zinc tolerance along Trelogan transect.
(in this and subsequent set of figures, the points indicate individual genotypes)

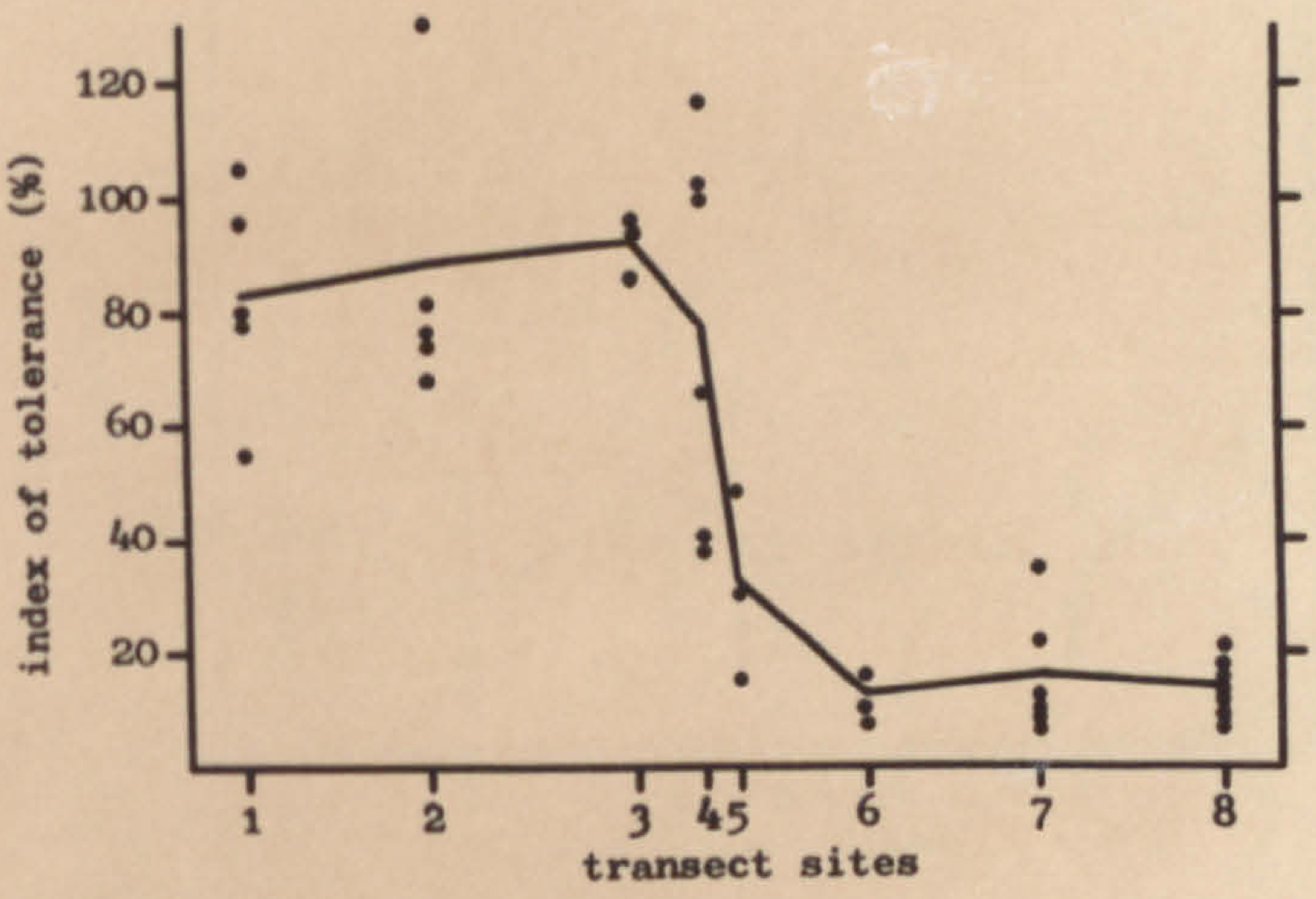


Fig. 15. Plant height along Trelogan transect.

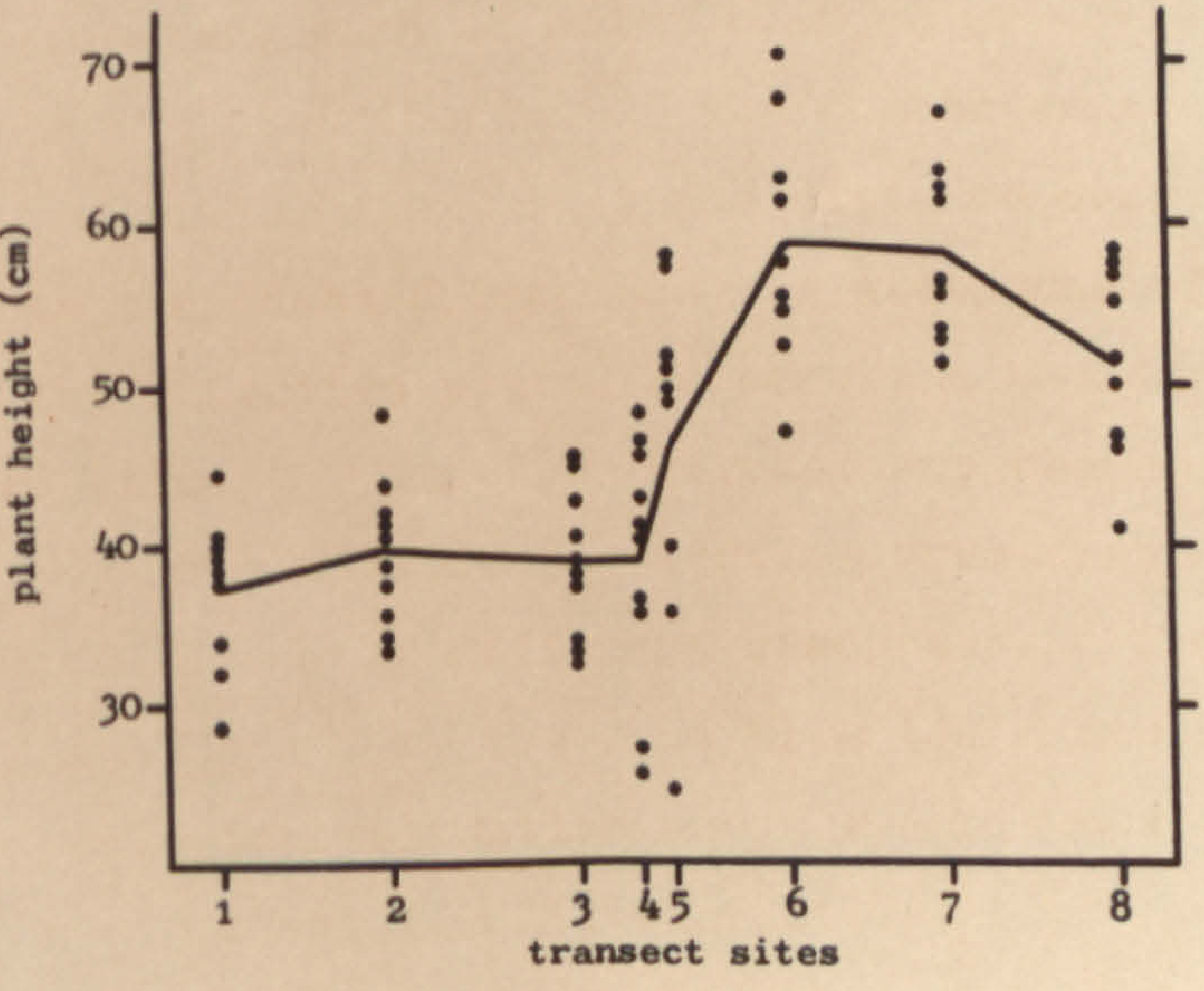


Fig. 16. Number of vegetative tillers along Trelogan transect.

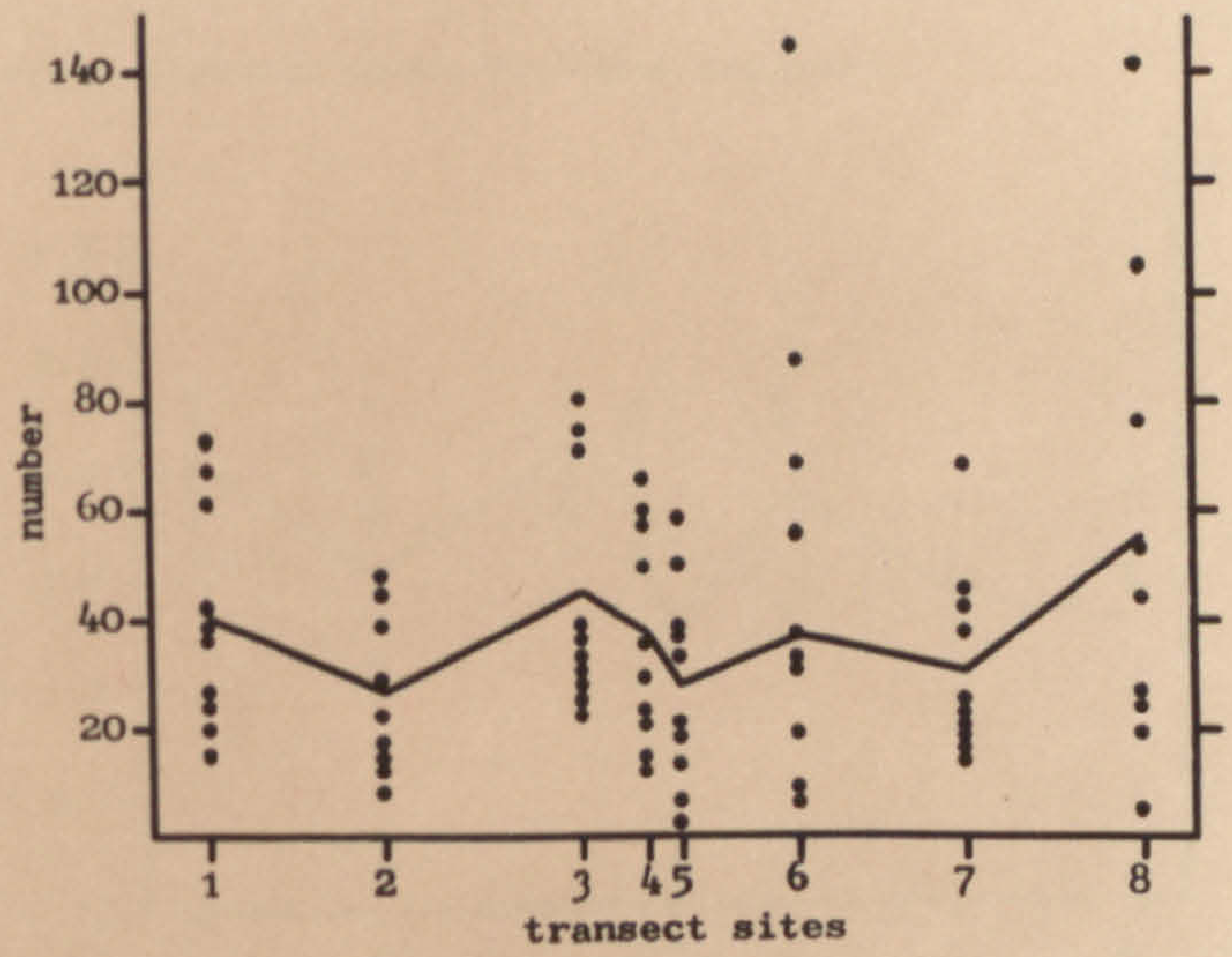


Fig. 17. Number of reproductive tillers along Trelogan transect.

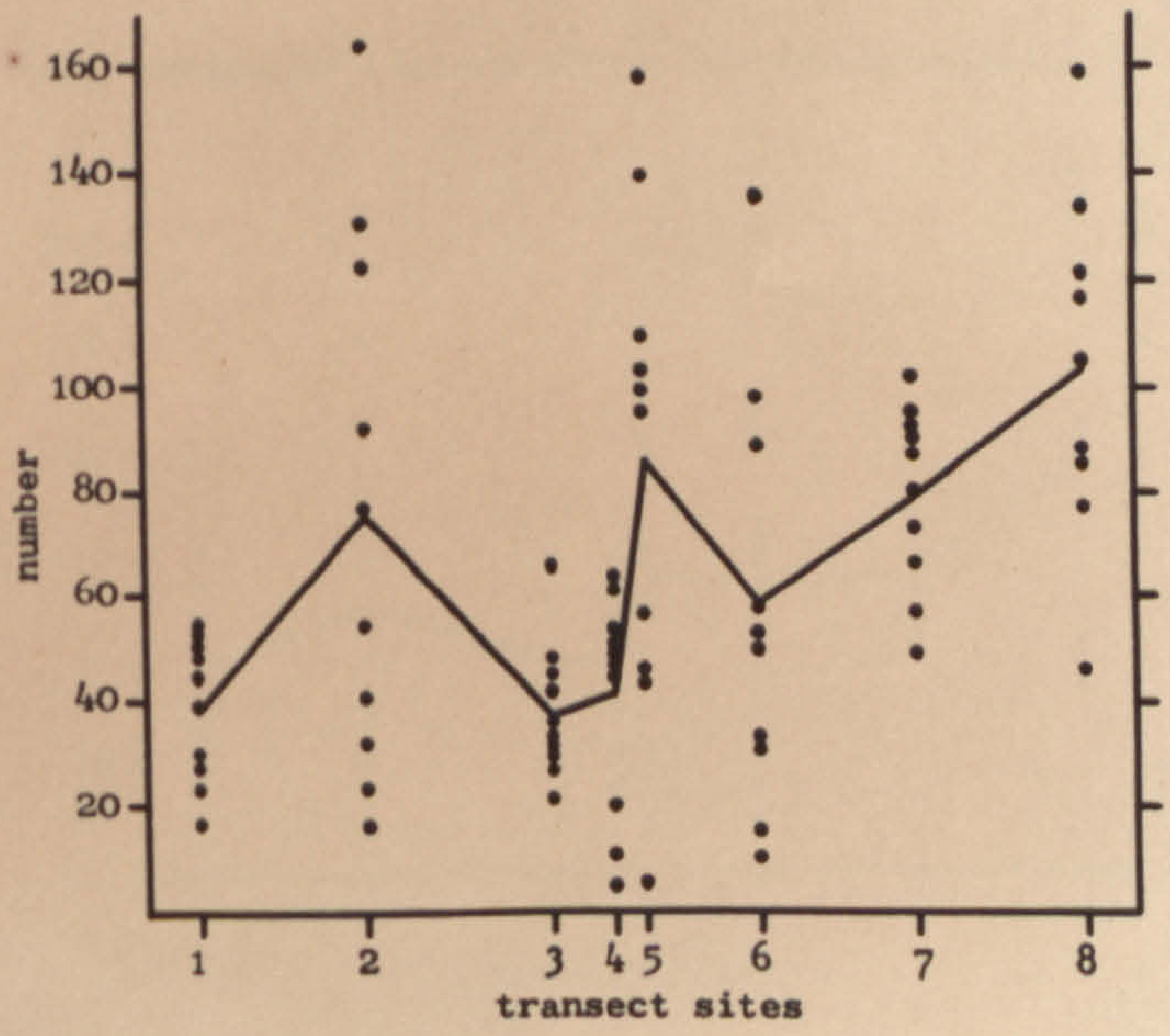


Fig. 18. Vegetativeness along Trelogan transect.

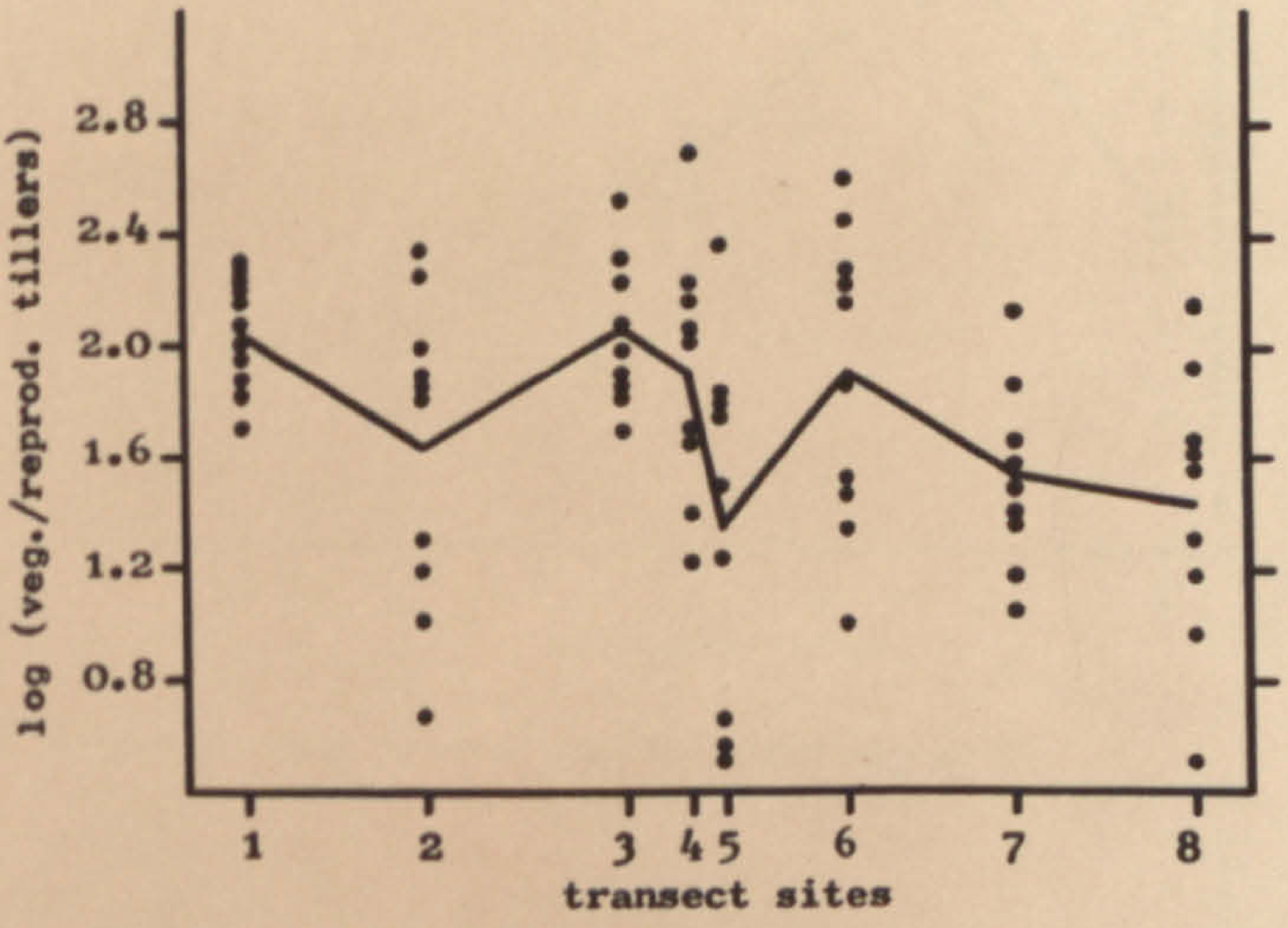


Fig. 19. Total number of tillers along Trelogan transect.

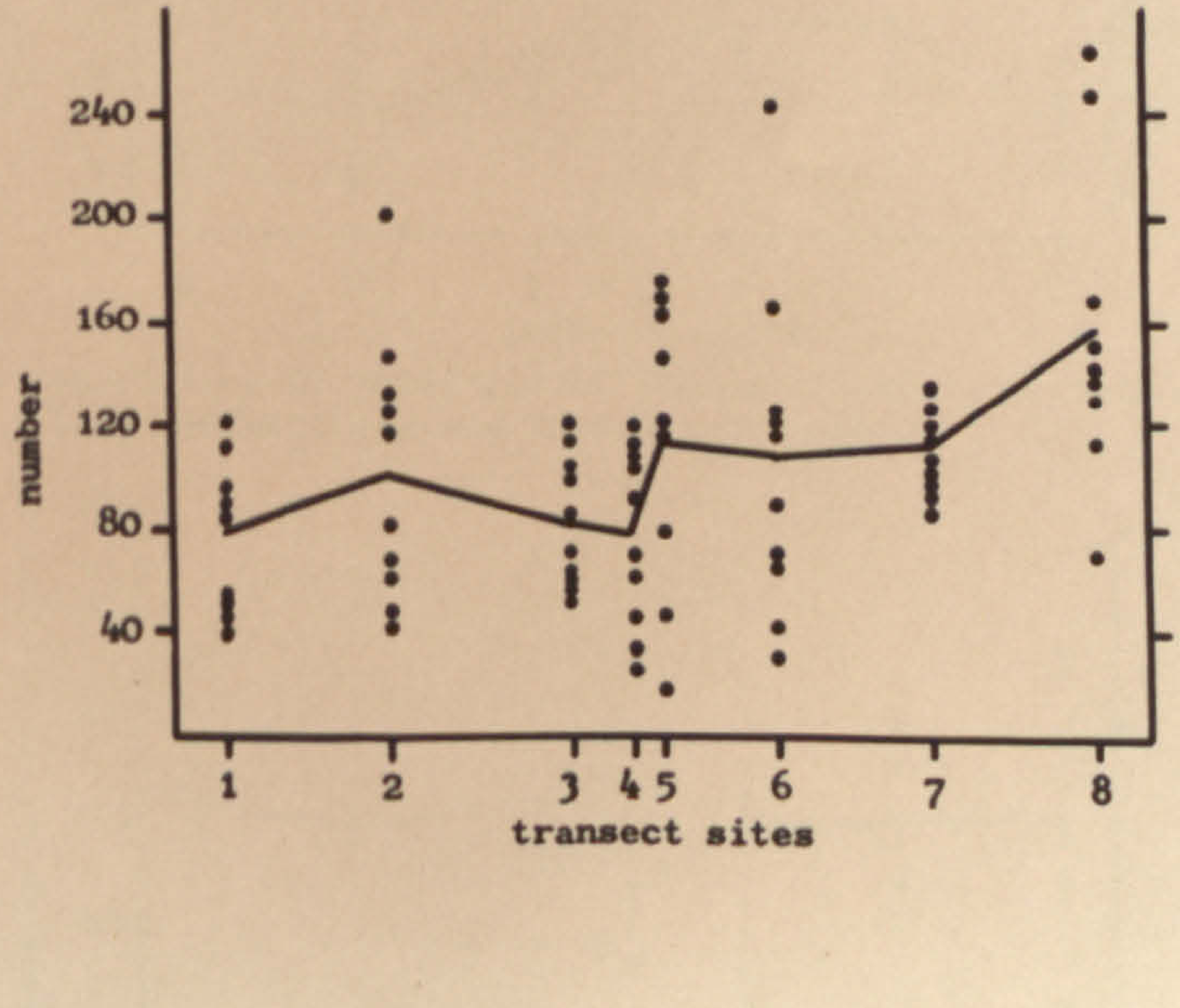


Fig. 20. Length of flag leaf along Trelogan transect.

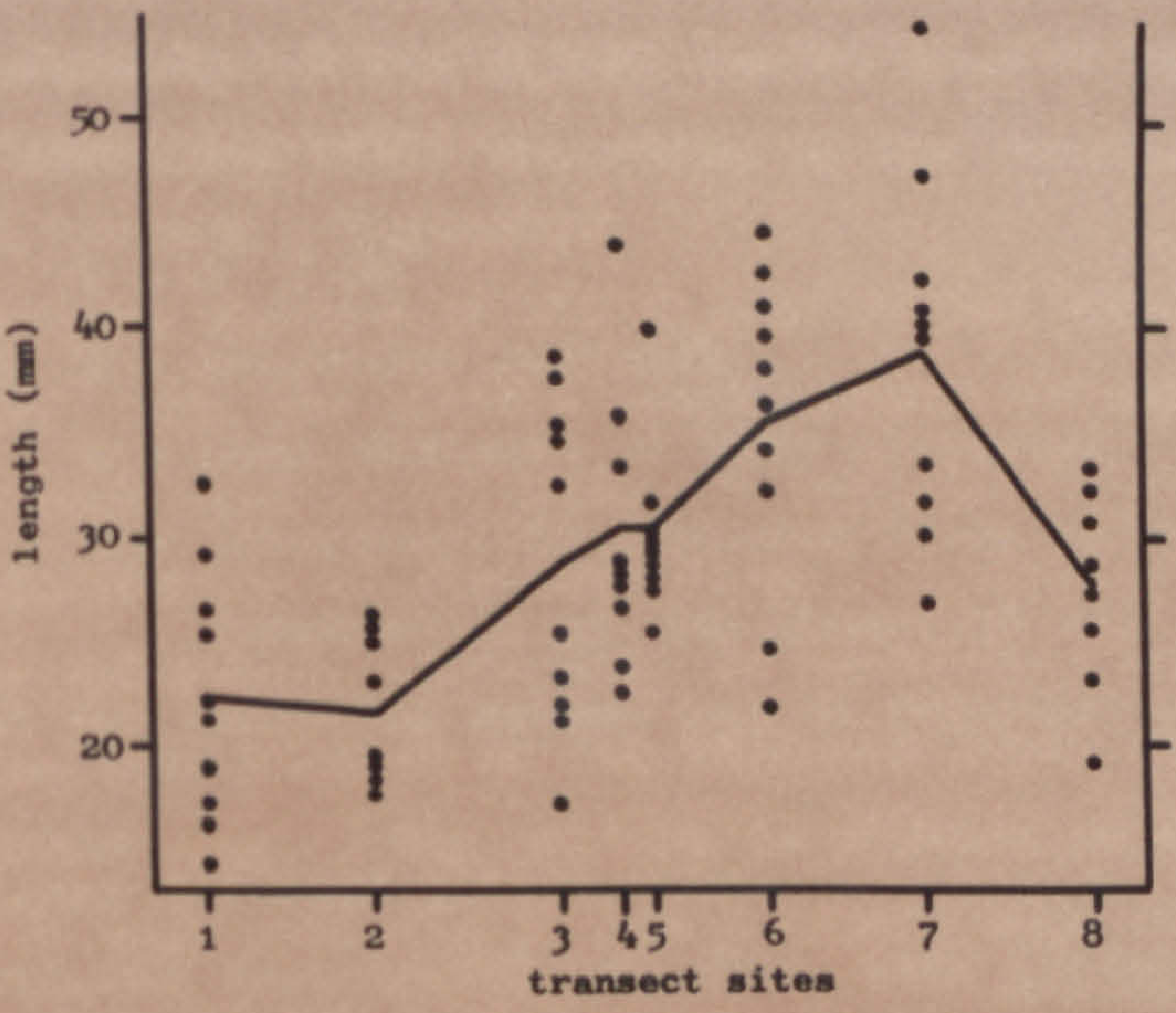


Fig. 21. Width of flag leaf along Trelogan transect.

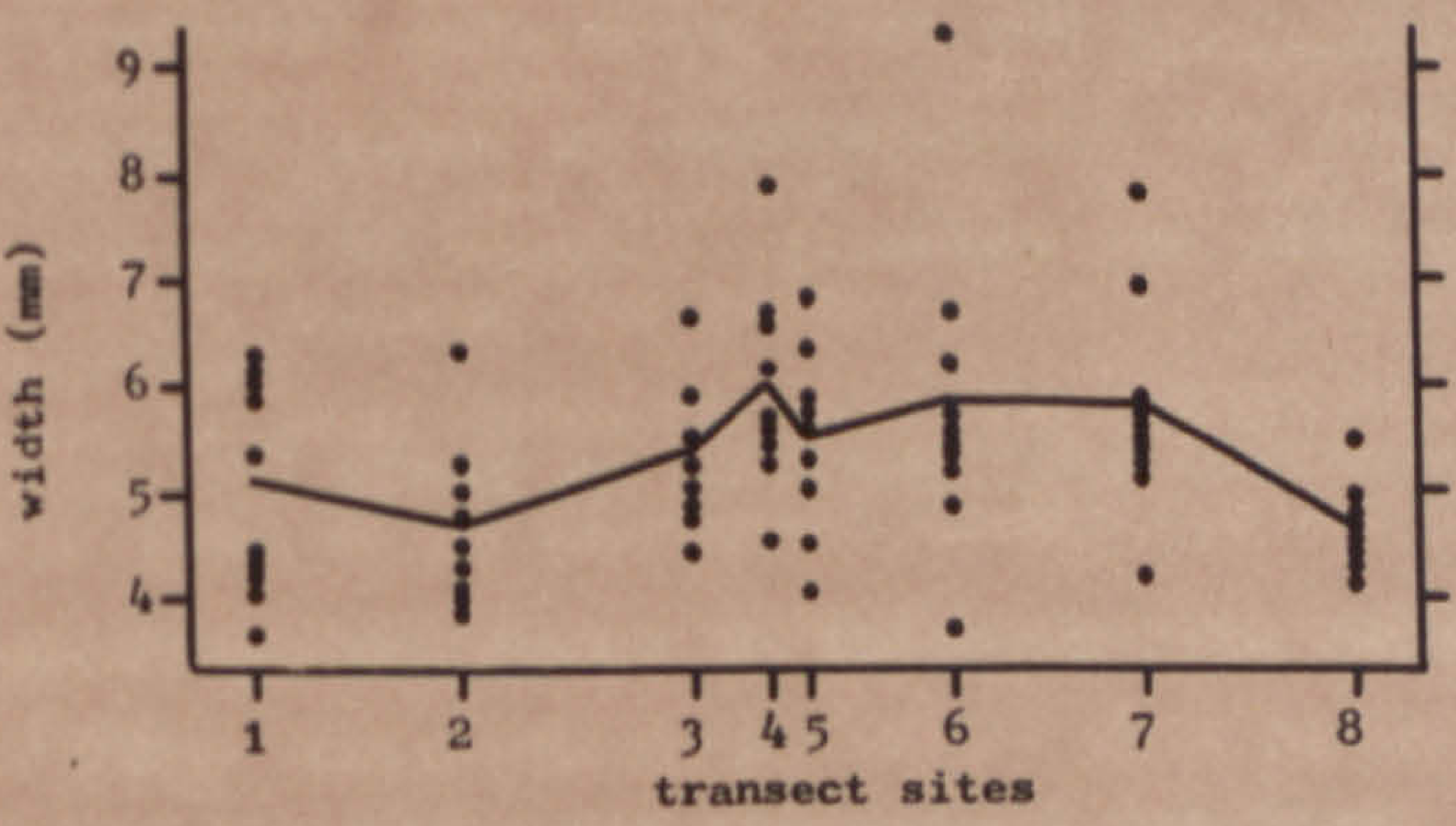
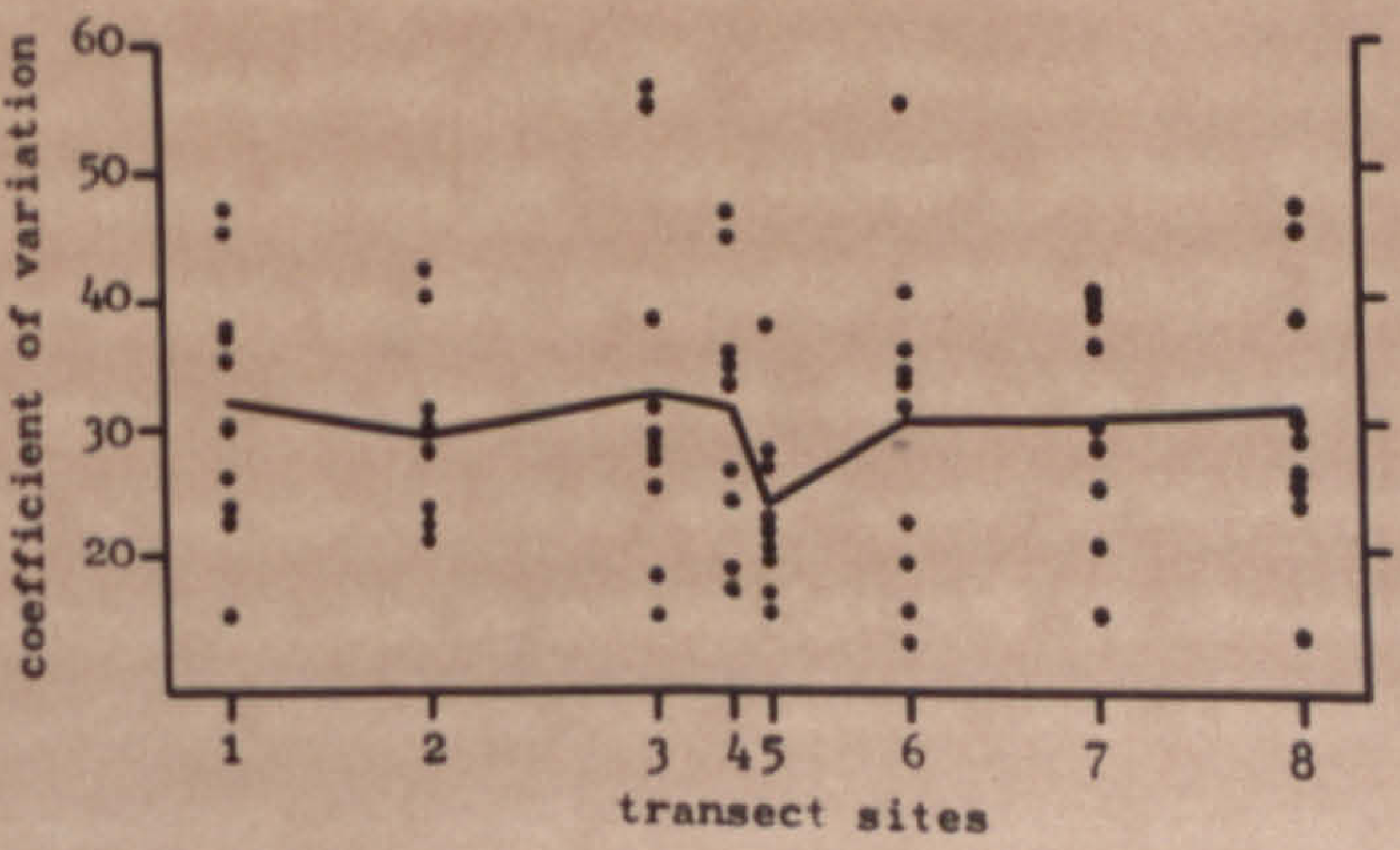


Fig. 22. Coefficient of variation of flag leaf length (within plant variation) along Trelogan transect.



clear cut and distinct boundary.

If we accept that the selective forces change abruptly across the mine boundary, steepness of the clines can be used to estimate selection pressures (Haldane, 1948) according to the formula

$$K = m^2 / 2d^2$$

where, K = selection pressure
m = mean distance of gene dispersal
d = interquartile distance (i.e. between where the frequencies of one phenotype are 25% and 75%).

For mine populations the following values can be regarded as reasonable estimates.

Mean dispersal distance for wind pollination

(a) 8.2 feet (calculated from Griffiths, 1952, with *Lolium perenne*)
(b) 7-11 feet (McNeilly, 1965, using Pine and *Lycopodium* pollen)
(c) 11.3-14.2 feet (personal data using Pine and *Lycopodium* pollen)

This gives a mean square distance of approximately 100 feet.

Interquartile distances can be roughly estimated for some of the more clear cut clines described.

Plant height (Fig. 15) gives an interquartile distance of approx. 30 feet.

Flag leaf length (Fig. 20) gives an interquartile distance of approx. 100 feet.

The selective pressures for these two characters are then

Plant height	5.5%
Flag leaf length	0.5%

The value for plant height might be much higher, since the steepness of the cline may be determined by the intensity with which the sampling along it is carried out. In the present study the sites on either side of the boundary site are 45 feet apart, and even though the character may change very abruptly at the boundary (as may be the case for plant height) the interquartile distance will appear much larger. Intensive sampling would be essential to such a study, since in the case of plant height, if the interquartile distance was 10 feet, the selection pressure would be 50%.

A full scale investigation of clinal patterns across mine boundaries could provide a powerful technique for studying selection pressures on different characters.

(iii) The clinal pattern - variances

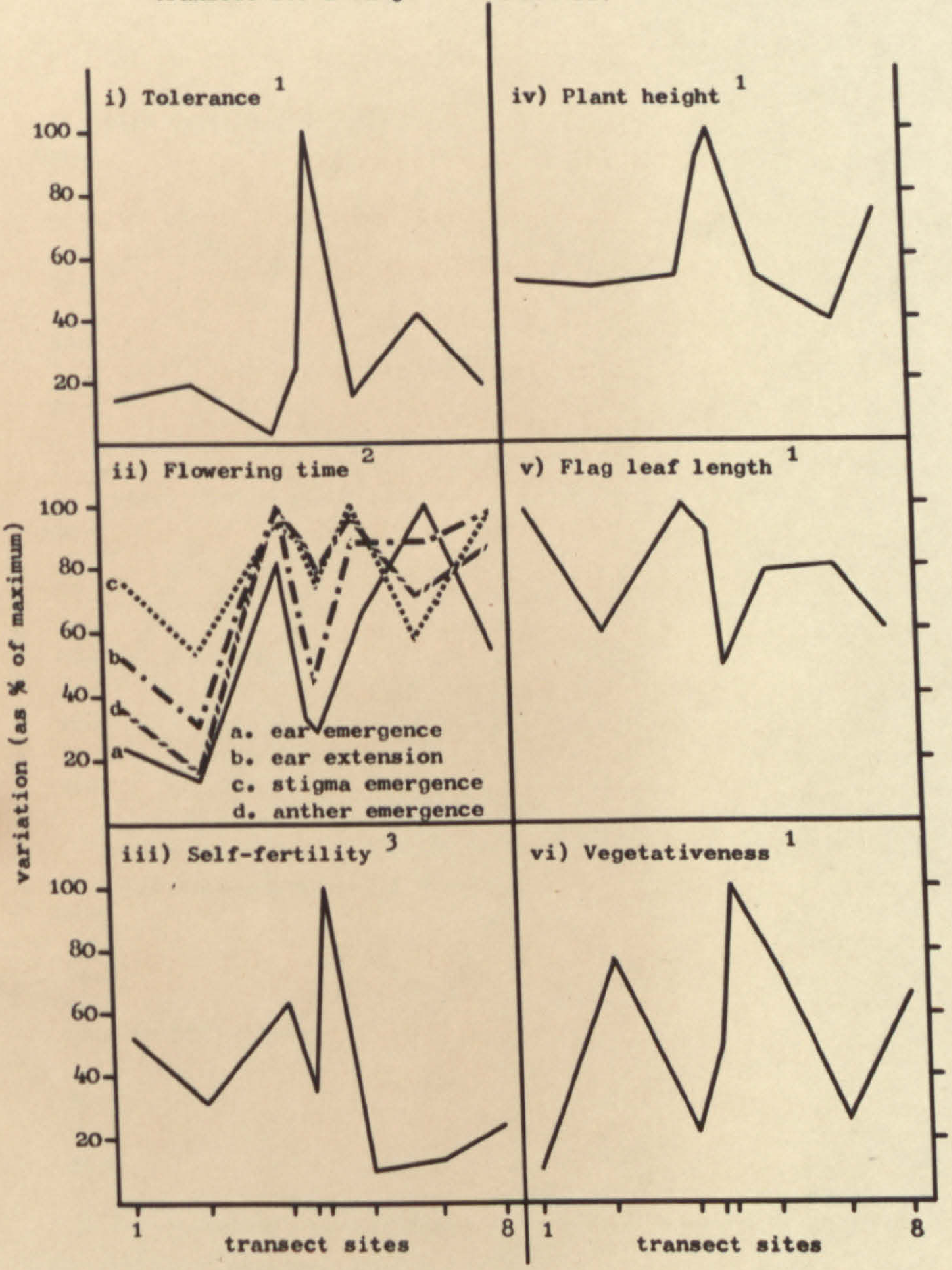
Another important feature of the clines is the between plant variability (Fig. 23). For the morphological characters the coefficient of variation has been used to estimate the variability since it eliminates the effects of differences in absolute size. A similar estimate has been used for tolerance, since this is based on a growth phenomenon. Flowering time has been plotted as the variance since it is taken from an arbitrarily fixed date. The selfing is plotted as the variance over mean since at each position it has a highly skew distribution the variance is proportional to the mean.

In many of the clines it is seen that the between plant variability increased at the boundary (Figs. 23a). Similar results have been obtained by McNeilly (1965) and Snaydon (see Jain and Bradshaw, 1966). There are two possible reasons for this.

Firstly, there is much evidence that the selective processes along a cline will lead to an increased population variability, at a boundary. Holgate (1964) gives a formula for the variance of gene frequencies in a local sub-population (site) on a cline, from which one can conclude that the variance will be greater the more equal the gene frequencies, and the greater the selection. We should therefore expect the variance to be greater at the boundary since here the gene frequencies are more equal. Moreover greater selection pressures will be acting when the clines are steeper so the variance at the boundary should also be greater if the cline is steeper.

Secondly, in a boundary zone there will be the maximum amount of gene exchange and therefore selection will be disruptive rather than

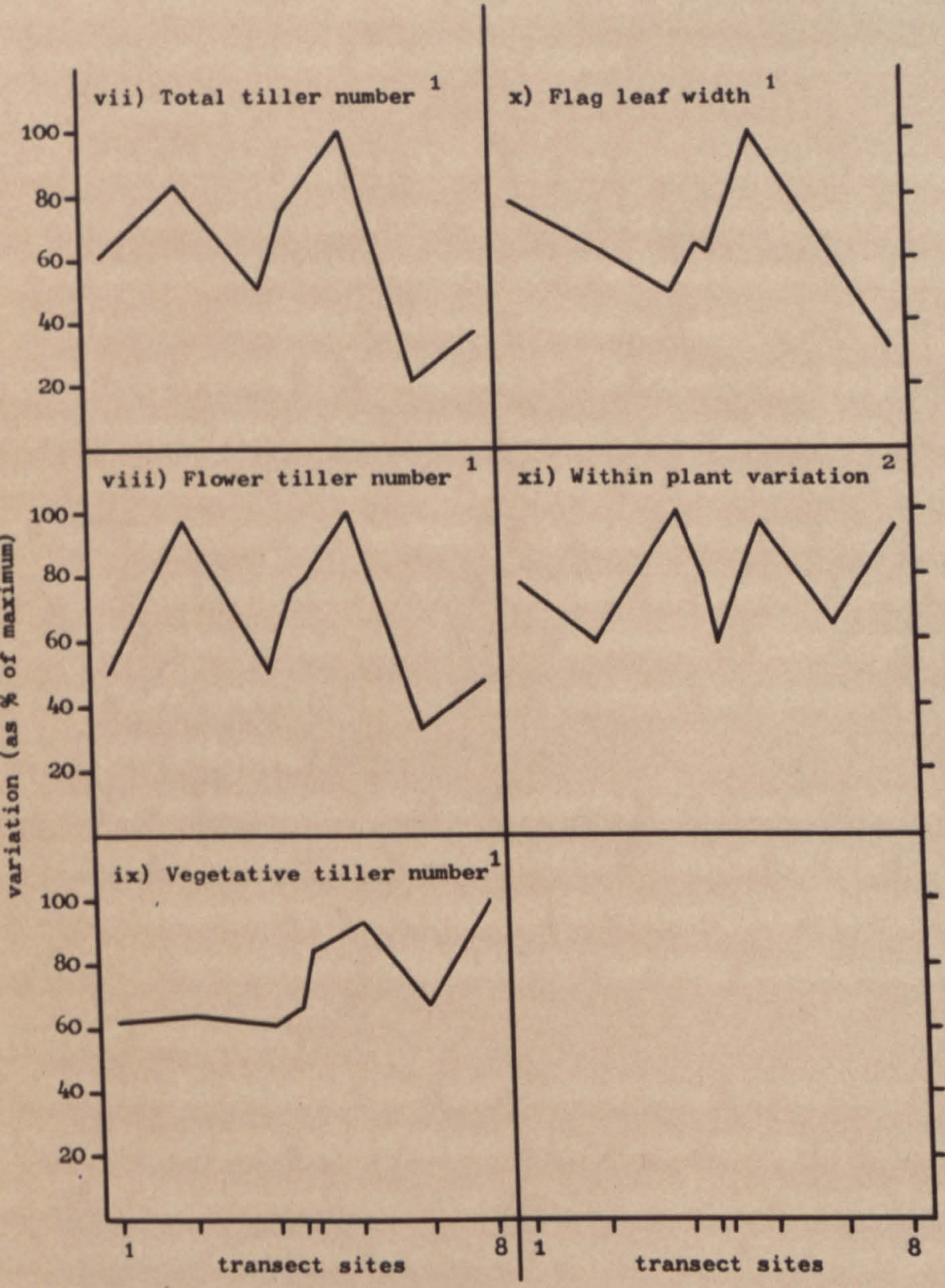
Fig. 23a. Variation between genotypes, within sites, along Trelogan transect for a range of characters.



Note: 1. coefficient of variation
2. variance
3. variance/mean

(for explanation, see text)

Fig. 23b. Variation between genotypes, within sites, along Trelogan transect for a range of characters.



Note: 1. coefficient of variation
2. variance

(for explanation, see text)

bi-directional, especially if the boundary is itself a heterogeneous habitat. The importance of disruptive selection in maintaining variability has frequently been demonstrated (Thoday, 1959, Gibson and Thoday, 1964, and Clarke and Sheppard, 1962). Disruptive selection is more effective again if the selection pressures are stronger.

No firm conclusions can be drawn as to the relative roles played by disruptive selection and general boundary effects in clines, in increasing the variability at the boundary. Undoubtedly both are important. However several features emerge. The consistency of the effect is remarkable: it recurs in many different characters for which there is specific selection. Moreover, if the clines are classified in order of decreasing steepness (they have been arranged and numbered in this order in Fig. 23), it is seen that the variance at the boundary is greater when the cline is steeper, i.e. when selection is more intense. This supports the conclusion that variability at the boundary is greater when selection is more intense.

Since the mine boundary can be regarded as a model for a heterogeneous environment (two environments scanned by one population), the maintenance of variability is remarkable and emphasises the importance of disruptive selection in natural populations.

(iv) Correlated response.

The differences between the two populations in various morphological characters could either have come about by independent selection or by correlated response. The evidence presented here and in the previous section, suggests that strong independent selection is occurring for these characters.

However, since there is considerable variation in tolerance on the mine we should expect, if correlated responses exist, significant relationships between tolerance and the characters measured. This could result either from pleiotropy or linkage. Quadratic regressions were therefore fitted to test for correlations between some of the relevant associated characters and tolerance, but no significant relationship was found (Table 10).

Table 10. Relationship between tolerance and other characters in *Anthoxanthum*.

Character	Regression coefficients		Expected sign of relationship	Probability
	x	x^2		
Flowering time (stigma emergence)	-0.119	+0.0006	-	> 10%
Plant height	-0.115	+0.0007	-	> 10%
Veg. tiller no.	+0.515	-0.0026	-	> 10%
Reprod. tiller no.	-1.357	-0.0101	-	> 10%
Vegetativeness	+0.037	-0.0002	+	< 10%
Within plant variability	+1.008	-0.0007	+	< 10%

Tolerance therefore appears to be a physiological character that does not produce associated changes in morphology.

(v) Population variability.

The two populations do not differ greatly in variability (Table 11) and no trends can be established. Moreover there is no difference in the within plant variability (Fig. 22). Selection for tolerance under natural circumstances even though it is very severe has therefore few of the characteristics of artificial selection: it leads neither to an increased variance of associated characters (cf. Mather and Harrison, 1949, Clayton *et al.*, 1957) nor to an increase of the within-individual

Table 11. Differences in variation of characters of tolerant and non-tolerant plants of Anthoxanthum in the single plant trial.

Character	Variability between plants within sites		
	Tolerant		Non-tolerant
Flowering time (i)	11.4	<	21.5
(ii)	18.4	<	28.3
(iii)	17.0	<	18.5
(iv)	16.5	<	23.1
Plant height*	13.7	>	12.4
Veg. tiller no.	51.5	<	70.3
Reprod. tiller no.*	47.2	>	41.4
Width of flag leaf*	16.2	<	16.4
Length of flag leaf*	22.9	>	19.6
Total tiller number			
Vegetativeness	0.146	<	0.213
Within plant var.*	17.0	<	18.4

*coefficient of variation.

variance (cf. Thoday, 1958b). Nor can any evidence be obtained for Ford's hypothesis (Ford and Ford, 1930) that new variants will be permitted in a population that is increasing in size. Selection in nature is a precise process which does not allow the survival of variants which deviate widely from the norm.

Chapter IV.

THE PROCESS OF ISOLATION

1. INTRODUCTION

The importance of isolation in promoting population divergence and speciation has long been recognised and fully documented (e.g., Mayr, 1942, Dobzhansky, 1941, and Baker, 1959). Isolation was considered a prerequisite for population divergence until Thoday (1958a) showed that disruptive selection could effect such divergence in the absence of isolation. Recently, the occurrence of divergence in nature by disruptive selection has been shown in *Papilio dardanus* (Clarke and Sheppard, 1962) in *Maniola jurtina* (Creed *et al.*, 1959) and in various grass species (Jain and Bradshaw, 1966, Aston and Bradshaw, 1966).

The existence of population differences in the face of gene flow illustrates the power of natural selection in keeping populations distinct. However gene flow is not without effect. Generally it slows down population divergence (but see Millicent and Thoday, 1961, and Streams and Pimentel, 1961) and produces unadapted genotypes from the crossing of two adapted types. In such situations we might expect the evolution of barriers to gene exchange (gene flow). Evidence for the development of breeding barriers when two previously separate species meet has been discussed by Dobzhansky, 1941. Hybrid zones between *Corvus corone* and *C. corvix*, two species of crow, are narrowest where the two types have been together longest. Similarly breeding barriers between species are often greatest where two types meet: evidence for this has been found by Ehrman (1965) and others in *Drosophila paulistorum*. The process has also been demonstrated experimentally (Knight *et al.*, 1956) and theoretically (Crosby, 1964). However Bigelow (1965) states that "the evolution of mechanisms to inhibit interbreeding appears to have taken place as a direct result of selection in a narrow zone of contact rather infrequently" and considers that since the perfection of such mechanisms presumes the production of "poorly adapted genotypes" by hybridisation, there must have been prior divergence and hence isolation.

Nevertheless, Thoday and Gibson (1962) have shown that not only can divergence occur without prior isolation but also that evolution of crossing barriers can take place under disruptive selection in Drosophila. Disruptive selection, by definition, creates "poorly adapted genotypes".

Mechanisms reducing the harmful effects of gene flow were investigated in closely adjacent populations as occur at mine boundaries since this is a situation in which barriers to gene flow might be expected. Several possible barriers to gene exchange will be considered in turn.

2. INCOMPATIBILITY BARRIERS

There would be evidence of an incompatibility barrier (as opposed to, for example, flowering time or geographical barrier) between the two populations if crosses between mine and pasture plants were less successful than crosses within the populations. However evidence from previous work suggests that if such barriers are present they are not very powerful. Evidence for gene-flow (McNeilly, 1965) is itself evidence for the absence of breeding barriers. Moreover Wilkins (1960) and Broker (1963) found no difficulty in crossing tolerant and non-tolerant races of Festuca ovina and Silene inflata respectively. To investigate this more thoroughly, the success of crosses made during a genetical analysis of metal tolerance was measured.

(a) Method

Crosses were made by enclosing inflorescences of the plants to be crossed in glassine bags. The plants were matched for flowering time, and the crosses were made in an unheated greenhouse. Compatibility in Anthoxanthum was measured as the number of seeds per inflorescence. In Agrostis the seed is small and difficult to count so that seed set was indicated by the "success" (setting of at least ten seeds) or "failure" (setting less than ten seeds) of a cross. No differences in seed viability were found between different crosses and the viability was generally high.

(b) Results

The results (Tables 12a and 12b) have been pooled for the separate sites along the transects (the intermediate site is not included) to give four types of crosses.

1. tolerant x tolerant
2. tolerant (female) x non-tolerant (male)
3. non-tolerant (female) x tolerant (male)
4. non-tolerant x non-tolerant.

In Agrostis in both 1964 and 1965 and in Athoxanthum in 1964 there is no significant difference between the success of crosses within populations and crosses between populations, thus giving no

Table 12a. Success of crosses between and within tolerant and non-tolerant populations of *Agrostis*.

	Success of Cross	Tol. × Tol.	Tol. × Non-tol.	Non-tol. × Tol.	Non-tol. × Non-tol.
1964	Failure	19	6	14	12
	Success	15	4	7	7
1965	Failure	-	12	11	14
	Success	-	20	21	27

CONTINGENCY χ^2

Within populations/between populations 1964 = 0.298 n.s.

1965 = 0.468 n.s.

Within tolerant/within non-tolerant 1964 = 0.266 n.s.

Reciprocals 1964 = 0.132 n.s.

Reciprocals 1965 = 0.068 n.s.

Table 12b. Seed set in crosses between and within tolerant and non-tolerant populations of Anthoxanthum

	Seed per Inflorescence	Tol. x Tol.	Tol. x Non-tol.	Non-tol. x tol.	Non-tol. x Non-tol.
1964	0	22	23	33	16
	1-5	17	19	21	7
	6-10	10	8	5	2
	11-100	3	9	1	3
1965	0	-	33	37	17
	1-5	-	33	58	19
	6-10	-	20	10	16
	11-100	-	23	7	8

CONTINGENCY χ^2 on classes 0-5 and 5-100

Within populations/between populations	1964	=	0.294 n.s.
Within non-tolerant/between population	1965	=	4.375 *
Within non-tolerant/tolerant x non-tolerant	1965	=	0.005 n.s.
Within tolerant/within non-tolerant	1964	=	0.533 n.s.
Reciprocals 1964		=	6.753 **
Reciprocals 1965		=	16.453 ***

evidence of the evolution of breeding barriers. However, in 1965 in Anthoxanthum the between population crosses yields significantly less than the within non-tolerant population crosses. This difference can be completely accounted for by the difference in the success of the reciprocal crosses which is observed both in 1964 and 1965. There the difference was startling and the cross of tolerant x non-tolerant yields far fewer seed when the non-tolerant was used as the female parent.

(c) Discussion

Apart from the startling differences in the reciprocal crosses of Anthoxanthum, there is no evidence of clear cut incompatability barriers between the two populations. However these crosses were made in isolation and the results might be different if, for example, a tolerant parent was offered simultaneously pollen from both tolerant and non-tolerant plants. Competition in the style between two types of pollen may be important (Darlington and Mather, 1949, p. 253). This has not been investigated. The origin of incompatibility barriers between species of Gilia growing in the same area, but not between species widely separated on islands, has been recently shown by Grant (1966).

Differences in the success of reciprocal crosses are well known in plants and may indicate the beginnings of an incompatibility barrier. This phenomenon is seen, for example, between the two sub-species of rice (Chandraratna, 1964, p. 20). However, the least successful cross in the present study is non-tolerant female × tolerant male, i.e. only gene flow off the mine onto the pasture appears to be hindered. This seems surprising because selection pressure against tolerance on the pasture is likely to be less than selection for tolerance on the mine and it will be shown later (Section V.2.c) that gene flow load is more serious when selection intensities are high.

Tolerant plants have been shown to have a markedly greater self-fertility than non-tolerant plants (see Section IV.4.b). The difference in self-fertility between tolerant and non-tolerant Anthoxanthum is itself too small to account for the difference in seed set of the reciprocals (Table 13). However, it is known that crosses between a self-incompatible species and self-compatible species are less successful if the self-compatible species is used as the female parent. Grun and Radlow (1961) considered this to be the result of the deleterious effect of self-compatibility genes entering the self-incompatible species and causing inbreeding depression in the population. In Solanum such a reciprocal barrier is only present where self-incompatible and self-compatible species come into contact.

Another possible reason for the reciprocal difference is that the mine plants are generally shorter (Chapter III.4.c) than non-mine

Table 13. Comparison of differences between seed set by reciprocal crosses and by selfing in Anthoxanthum

	Selfing		Reciprocals Female Parent		Difference between Tol. non-tol.	
	Tol.	Non-tol.	Tol.	Non-tol.	Selfing	Reciprocals
1964:	0.571	0.065	3.912	1.239	0.506	2.673
1965:	0.576	0.084	5.059	2.735	0.492	2.324

plants: they would therefore tend to come lower in the pollination bag and receive falling pollen. The non-tolerant however would receive very little pollen, and then only when the bag was agitated. However when crosses were made inflorescences were matched for height as well as flowering time so this is not likely to be a serious error.

The evidence for the occurrence of isolating barriers in Anthoxanthum in the direction of preventing the spread of tolerant genes into non-tolerant populations is therefore considerable. However because it is rather unexpected further work is necessary to confirm whether it is a real phenomenon under field conditions.

3. FLOWERING TIME

One of the simplest methods of reducing gene flow between populations is for them to differ in their flowering time. Such seasonal isolation is common between related species, and it therefore seemed obvious to investigate differences in flowering time of mine and non-mine populations.

(a) Flowering in the field

Observations on the flowering time of the populations of *Agrostis* and *Anthoxanthum* were made in the field in 1964 and 1965.

(i) Agrostis

Flowering time was assessed from the number of inflorescences at different stages of development found in 50 cm. quadrats at a given time. The quadrats were thrown randomly on the sites along the Drws-y-Coed transect already described (Chapter II.1).

The flowering stages were numbered and scored as follows:

Stage 1. Inflorescence enclosed within the sheath, flag leaf conspicuous, tiller swollen.

2. Inflorescence head showing but culm not visible.
3. Culm beneath inflorescence visible, panicle not expanded.
4. Panicle open and spread widely.
5. Anthers and/or stigmas exposed on part or whole of inflorescence.
6. Flowers closed, glumes brown.

The results (Fig. 24a) show that plants on the mine flower earlier than those on the adjacent pasture, and that the isolation is more pronounced on the boundary. The difference in stages of flowering can be used to estimate theequivalent isolation in terms of days if estimates are made on two dates. The time taken to pass through a certain number of stages at a given site can be related to the differences between sites. Independent estimates of the duration of the stages are obtained from different sites. The values estimated in this way gave the following:

$$7 \text{ days} \equiv 0.511 \pm 0.085 \text{ stages}$$

This gave the number of days isolation as:

Fig. 24a. Flowering time in field, along Drws-y-Coed transect.

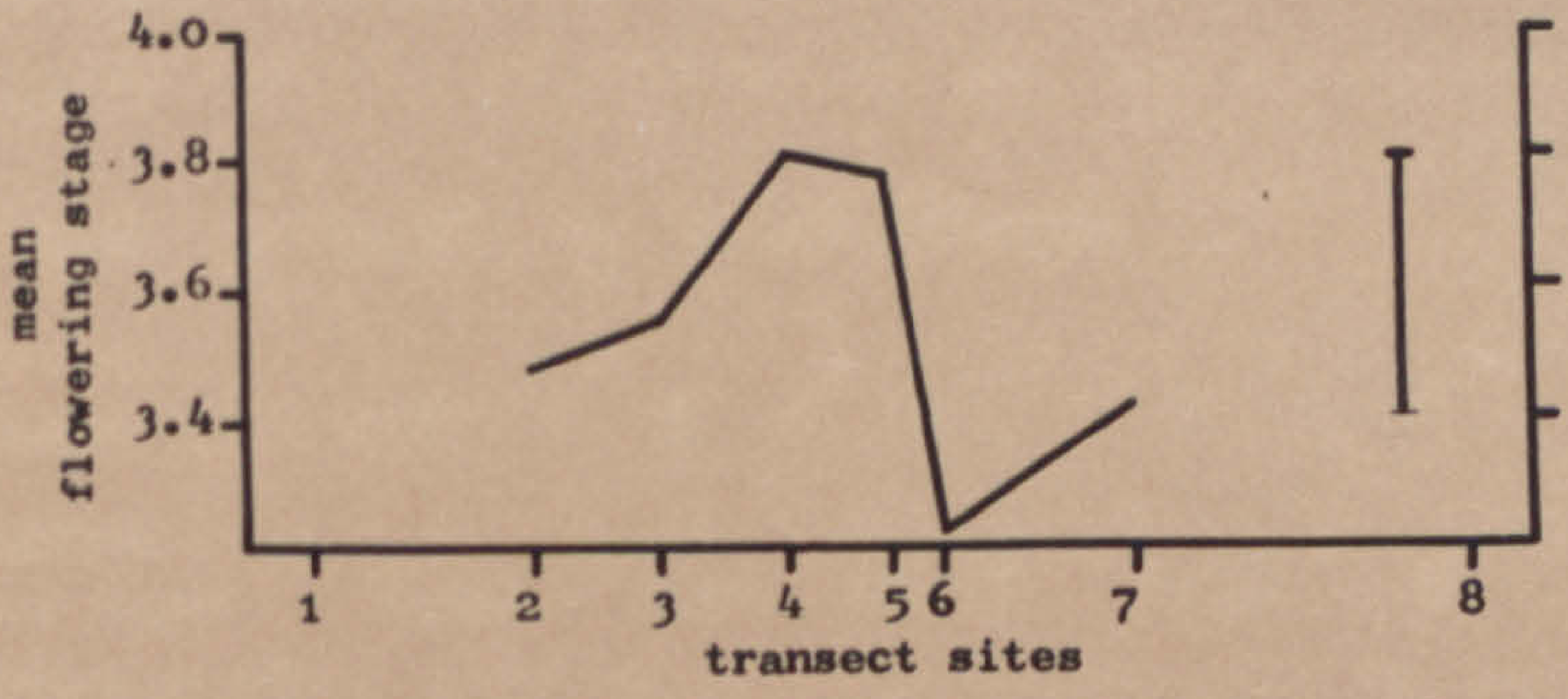


Fig. 24b. Flowering time in greenhouse, along Drws-y-Coed transect.

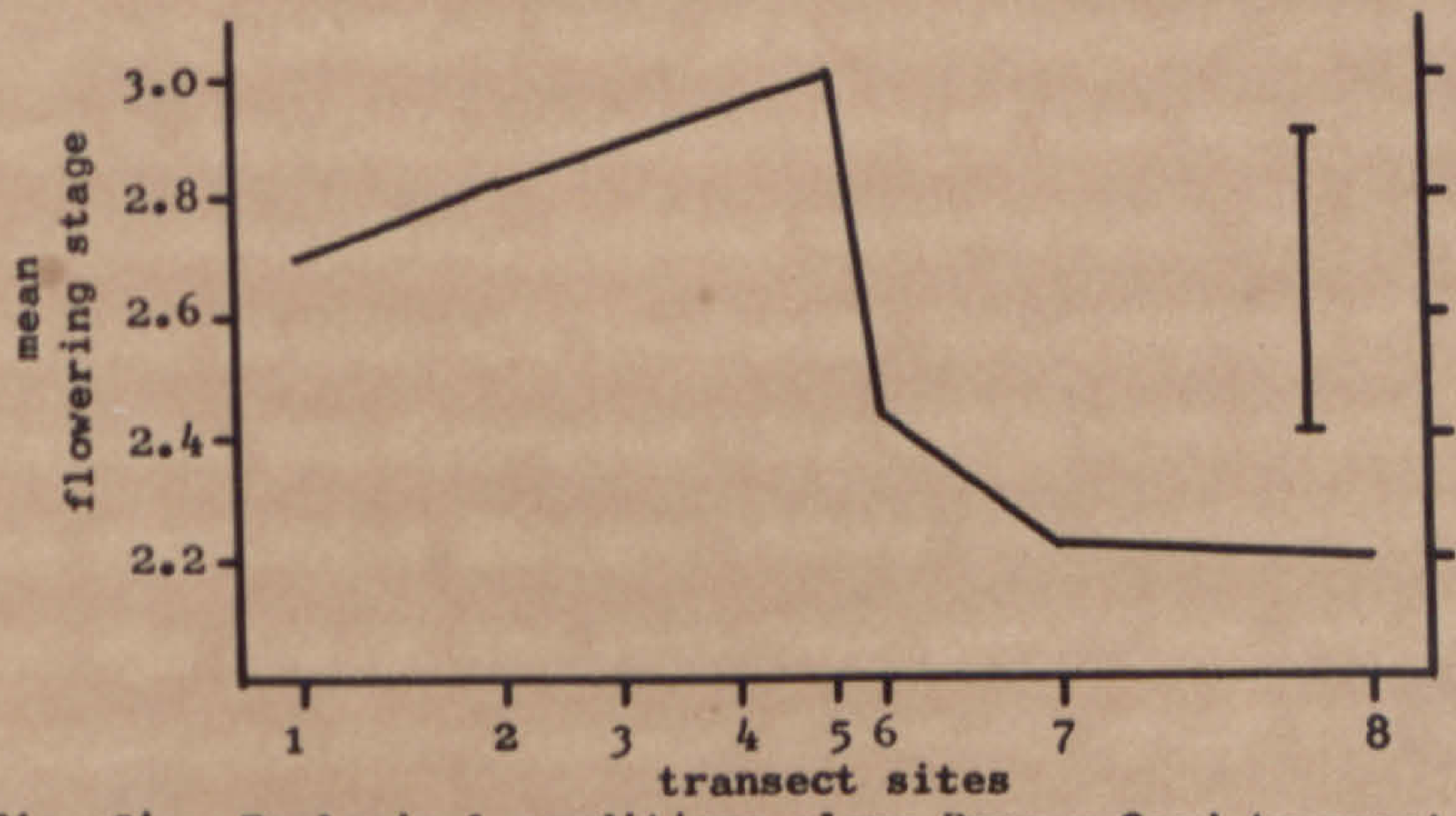
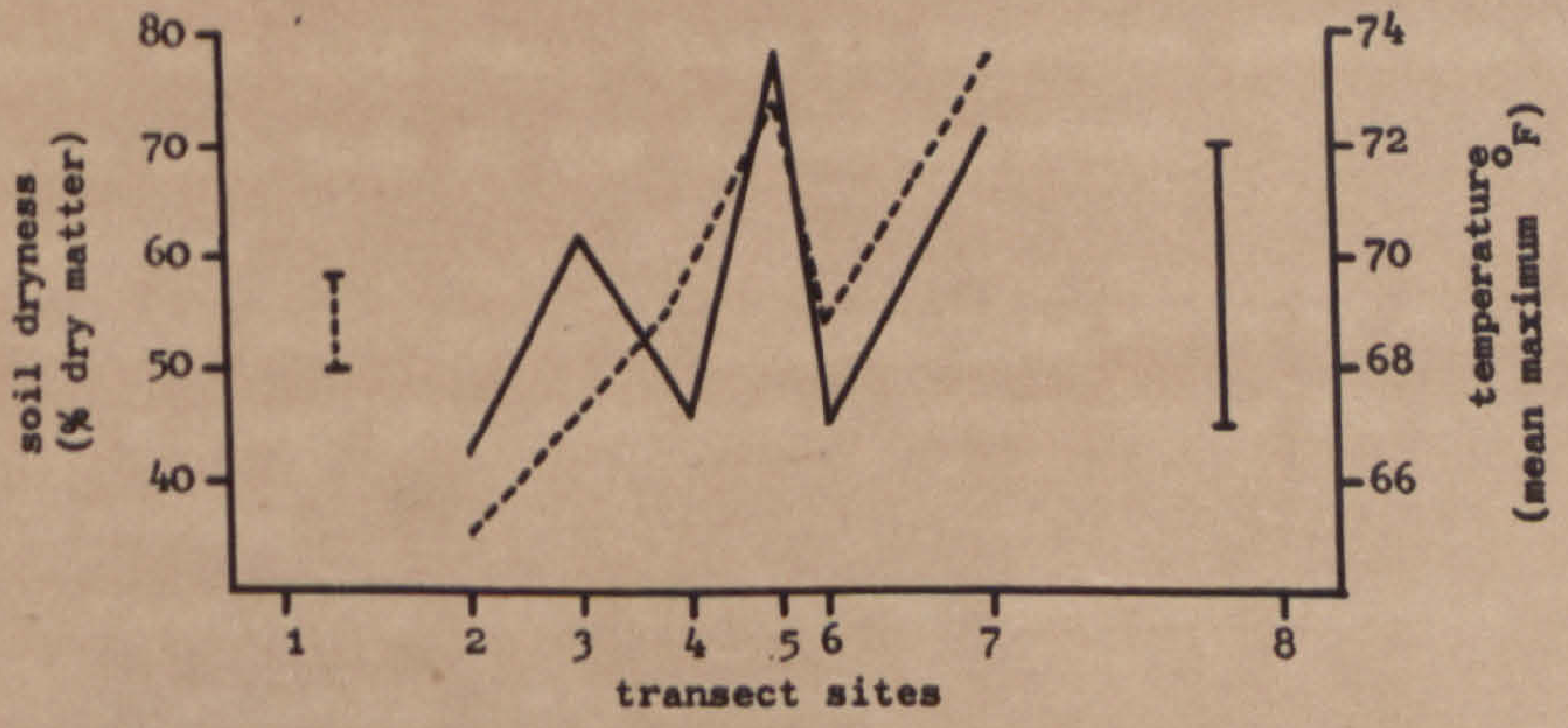


Fig. 24c. Ecological conditions along Drws-y-Coed transect.



Site number	2	3	4	5	6	7
Flowering score converted to days (earliest site = 0)	3.73	4.86	8.49	7.85	0	2.79

Similar data on flowering stages was obtained by McNeilly (1965) studying the same transect in 1964 and in terms of days isolation his results were:

$$7 \text{ days} \equiv 0.563 \pm 0.024 \text{ stages}$$

Site number	2	3	4	5	6	7
Flowering score converted to days (earliest site = 0)	6.09	5.96	12.17	7.95	0	3.23

The results of McNeilly (1965) agree well with the results of the present study, showing that the isolation is consistent over years.

Although a detailed investigation of the duration of the flowering period was not undertaken, regular observations at Drws-y-Coed show that most of the plants are in flower for a period of 3-5 weeks. In effect therefore, the flowering time difference means that a quarter of the tolerant population flowers before the non-tolerant. The situation at the end of the flowering period has not been examined to see if the non-tolerant continue flowering after the tolerant have stopped. Observations on plants in the greenhouse and experimental garden suggest that they do. There is therefore isolation between populations at both ends of the flowering period.

(ii) Anthoxanthum

Since single individuals of *Anthoxanthum* could be distinguished, the plants in a given area were scored individually for the following stages of flowering.

Stage 1. Inflorescence head showing but culm not visible.
2. Inflorescence fully exposed.
3. Stigmas extruded: Anthoxanthum is markedly protogynous.
4. Stamens extruded.

Preliminary results obtained in 1964 were from a transect about twenty yards away from the main transect studied in 1965. The results (Fig. 25a) show that plants on the mines flower earlier than those on the pasture and that the plants from the positions nearer the mine boundary are the earliest to flower. The differences between the sites are highly significant in both years ($P < 0.1\%$). The Isolation in terms of days was calculated using the method outlined above for *Agrostis*. The results of the estimates are as follows:

7 days = 0.761 ± 0.099 stages

Site number		1	2	3	4	5	6	7	8
Days isolation (earliest site = 0)	1964	4.60		7.17		2.94		0	
	1965	2.21	5.79	5.70	3.13	1.56	2.21	0	0.74

To calculate the flowering time difference in terms of days more accurately a modification of the above method was used in the case of *Anthoxanthum*. Here the duration of the stages was measured on 30 inflorescences of 4 plants taken from the mine in one foot square boxes (with as little disturbance of the soil as possible), and brought back to the garden. Knowing the duration of each stage and the number of each stage at the transect sites a precise estimate of the differences between the sites could be made.

The results from estimating lengths of individual stages are

Stage 1 = 11.59 ± 0.20 days
2 = 2.22 ± 0.12 days
3 = 7.78 ± 0.25 days
4 = 9.19 ± 0.45 days

These estimates give the following values for flowering time in terms of days.

Site number	1	2	3	4	5	6	7	8	
Flowering stage converted to days (earliest site = 0)	4.14		6.55		3.23		0		1964
	1.97	5.03	4.96	2.70	1.38	2.01	0	0.62	1965

The estimates using this method are slightly lower than those obtained

Fig. 25a. Flowering time in field, along Trelogan transect.

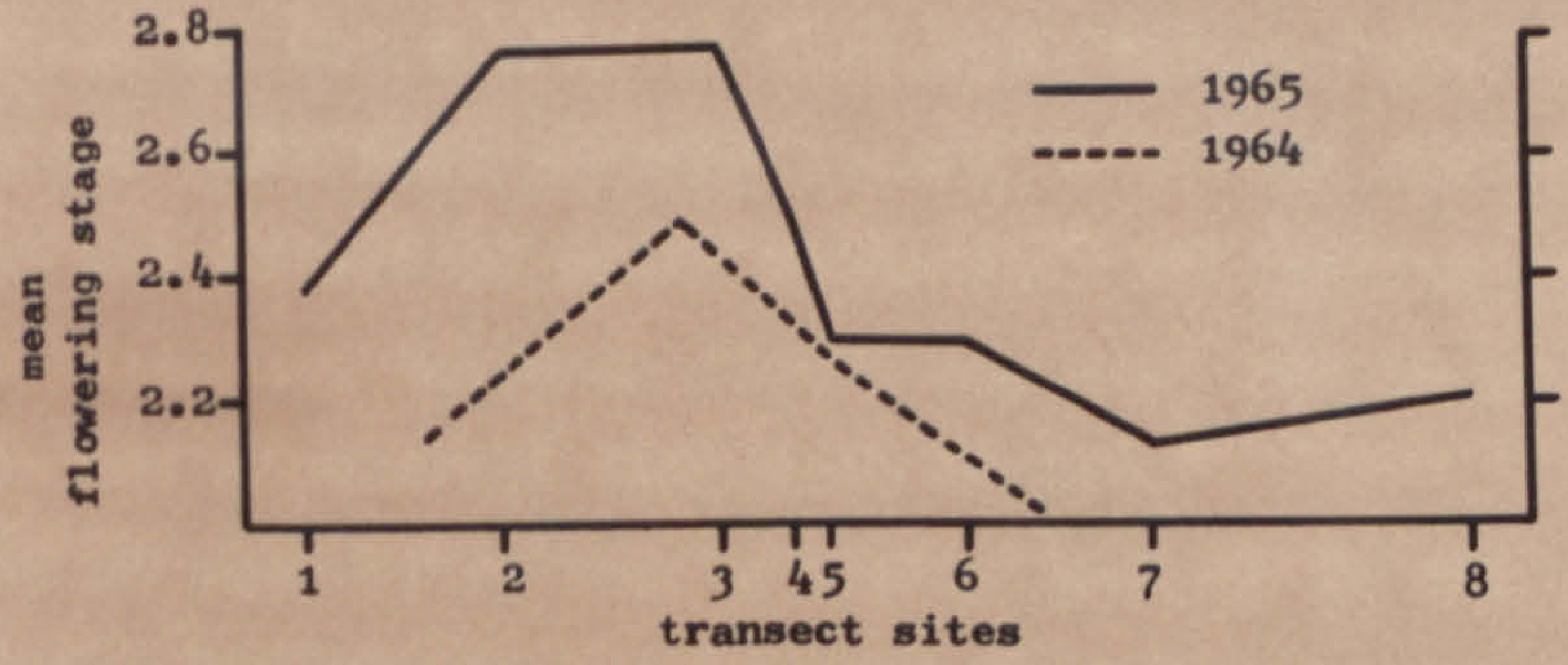


Fig. 25b. Flowering time in garden, along Trelogan transect.

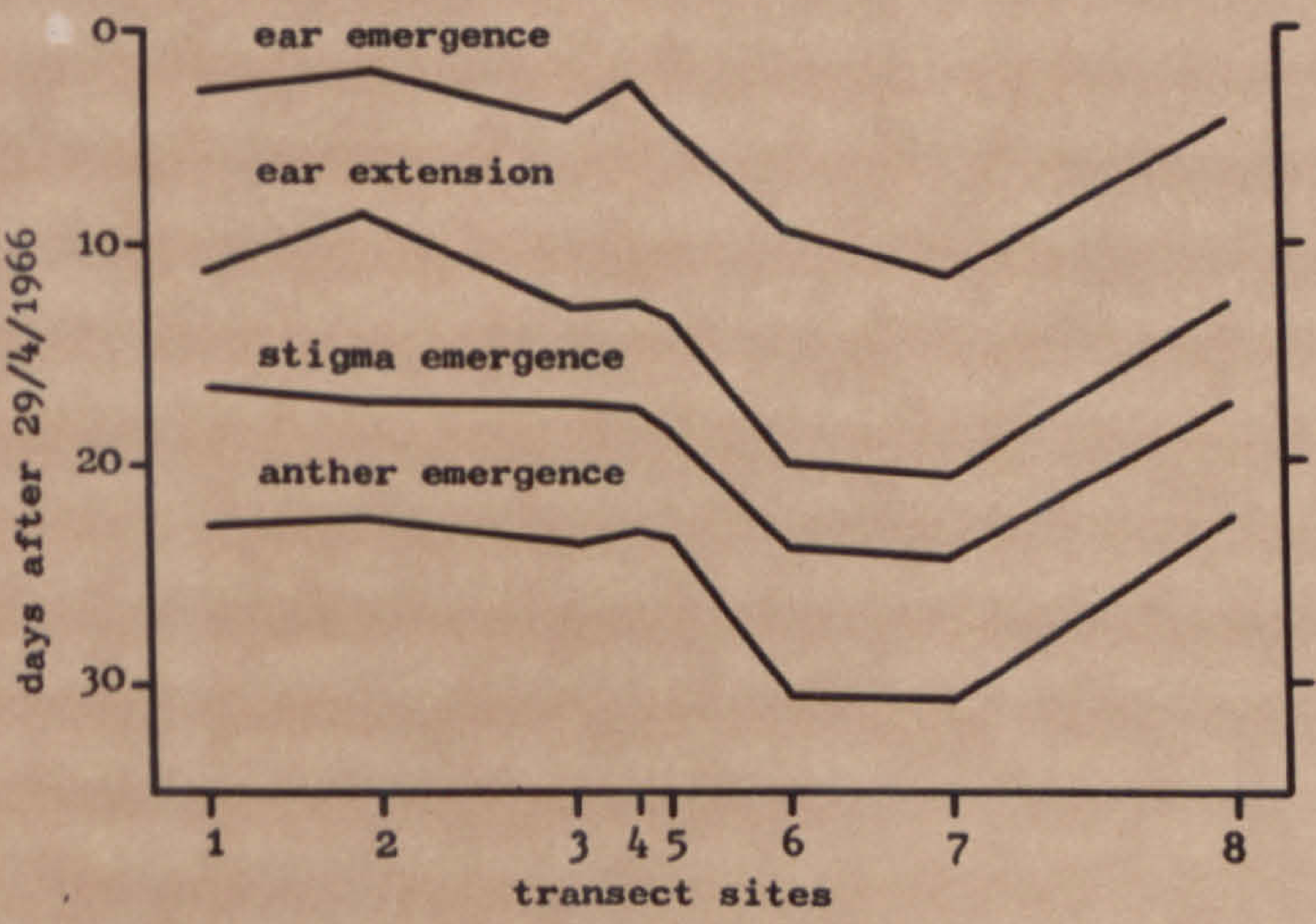
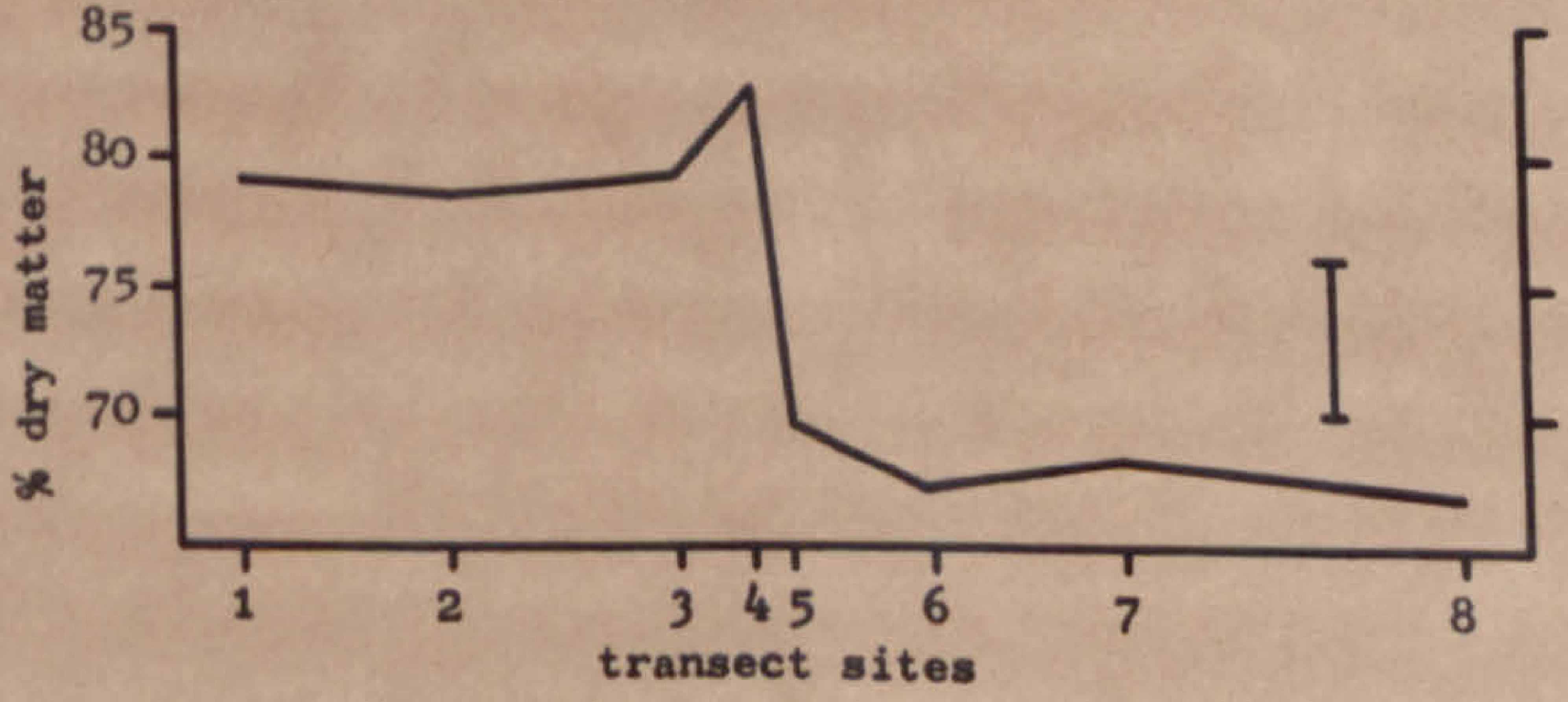


Fig. 25c. Soil dryness along Trelogan transect.



above, but the general pattern is the same.

The duration of the flowering period in Anthoxanthum is about 3-4 weeks, and therefore the isolation is of the same order as at Drws-y-Coed.

These figures of the degree of isolation between the two populations do not take into account losses of pollen due to the effects of distance and the effect of plant density. The effect of distance is discussed by Jain and Bradshaw (1966) and need not be elaborated here: distance has a powerful isolating effect.

The effect of density is that a higher density at the source of pollen will give a greater amount of gene flow into another population. In the early stages of colonisation of contaminated soils, plant density on the mine would have been much less than in the surrounding areas and the early colonisers would have been subject to considerable gene flow. At such a stage isolation caused by any means would have been of great importance, and selection pressures to produce differences in flowering time would consequently have been higher than later on. At the present time the density of plants on the mine is considerably greater than off the mine (since the species studied are dominant members of the mine communities) and isolation through flowering time is reinforced to some degree by "isolation through density".

(b) Flowering time in cultivation

The differences seen in flowering time in the field could either be environmentally induced, or genetically determined and therefore brought about by evolutionary processes. To test this plants were grown under standard conditions in the experimental garden and differences in flowering time studied.

(i) Agrostis

The following material was scored for flowering in 1965.

(a) Plants growing outside in 7" pots in John Innes compost. Collected in Autumn 1963.

(b) Material the same as above, but cloned in December 1964 and planted in 4" pots in John Innes compost, and kept in an unheated greenhouse.

Isolation : flowering time

It is seen (Fig. 24b) that the pattern of flowering parallels that found in the field. Moreover the regression of genotypes in small pots against the same genotypes in large pots is significant (Fig. 26a), showing that the genetic component is not obscured by environmental effects. Similar data were obtained by McNeilly (1965) on plants growing in water culture.

(ii) Anthoxanthum

The flowering time of plants along the Trelogan transect was recorded during the course of a spaced plant experiment described more fully in Chapter III.4.

The results (Fig. 2[illegible]b) indicate that the pattern of flowering is here again similar to that in nature. The differences between tolerant and non-tolerant populations are of the same order as in the field. Site 8 is anomalous, since here the plants are considerably earlier than in the field. The reason for this is not clear.

In the previous year, the date of ear emergence had been recorded on the same genotypes, but which were growing unreplicated and unrandomised as normal stock material. There is a good correlation between date of flowering in 1965 and 1966 (Fig. 26b). The difference in flowering is therefore consistent over years.

In both Agrostis and Anthoxanthum therefore, the evidence shows that the differences recorded in the field are genetically determined. Although extensive results are available only for these two contrasting mines, Jowett (1964) noted that lead mine populations of Agrostis in cultivation flower on average about four days earlier than pasture populations, and Bradshaw (1959) found that a single lead mine population again of Agrostis flowered a week earlier than the pasture population adjacent to it. Broker (1963) reported that the prostrate tolerant ecotype of Silene inflata flowers several weeks earlier in water culture than the normal form.

It can therefore be concluded that selection has produced differences in flowering time that are not only important as an

Fig. 26a. Relationship between flowering time of *Agrostis* in two contrasting conditions.

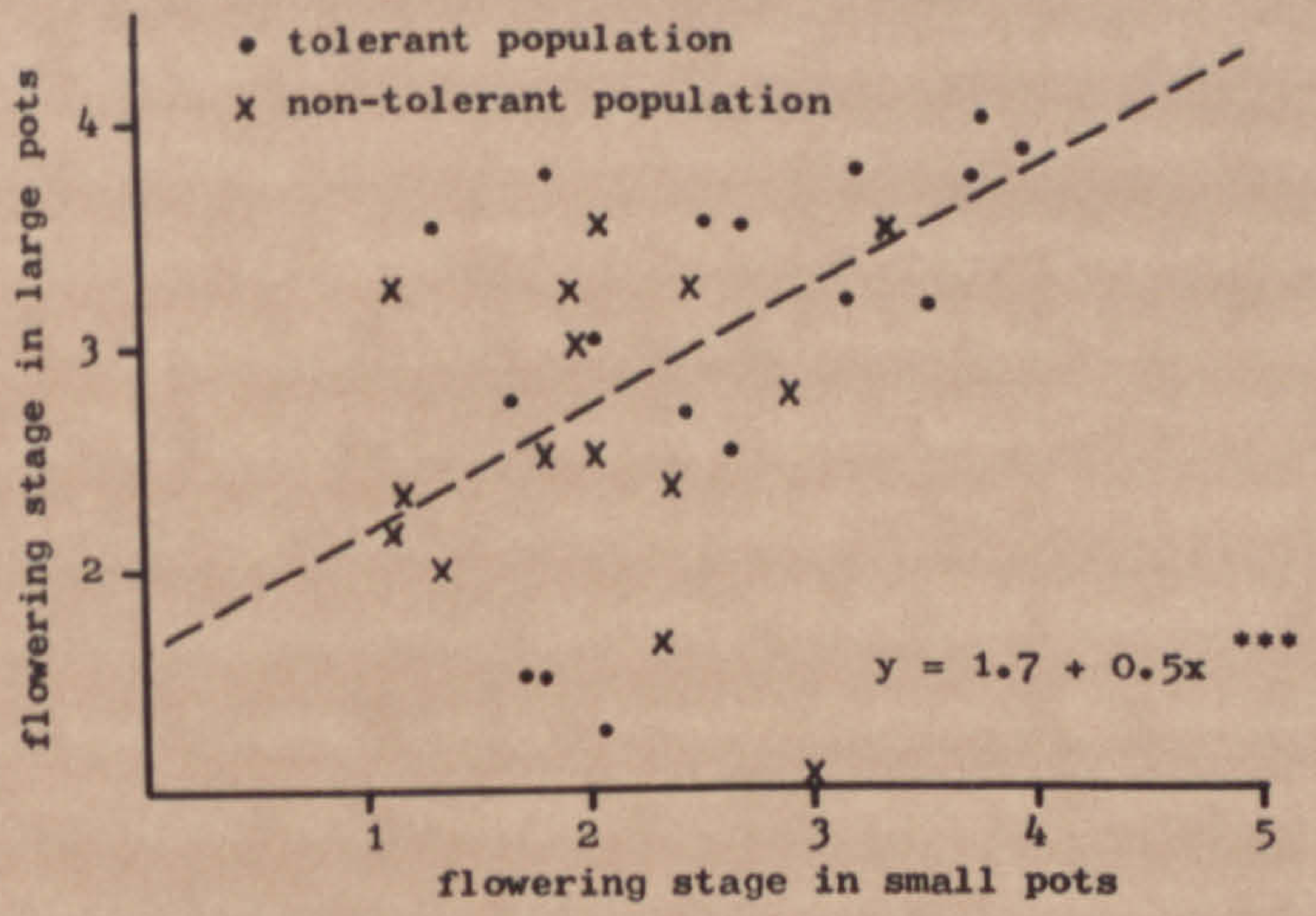
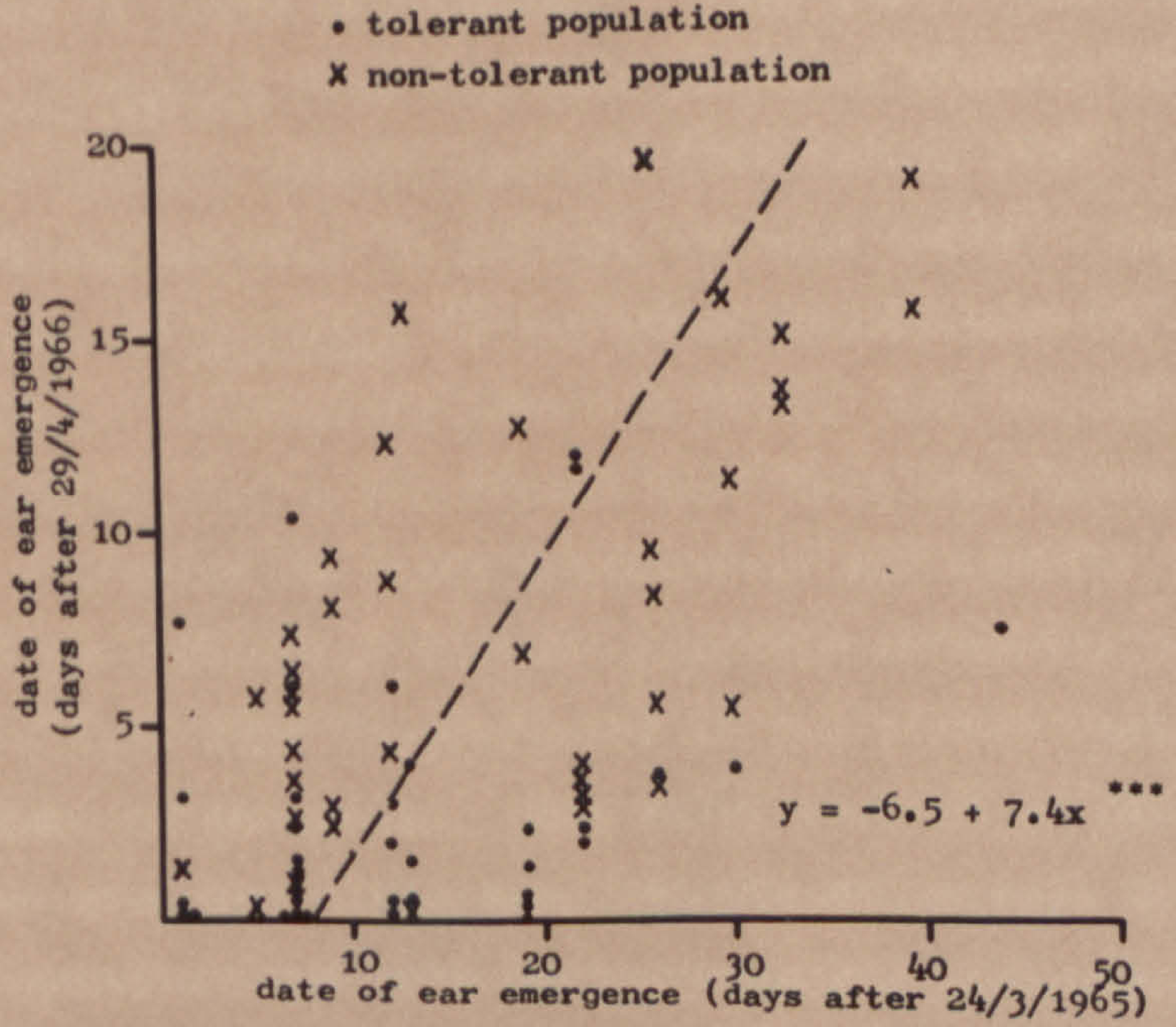


Fig. 26b. Relationship between flowering time of *Anthoxanthum* in 1964 and 1965.



isolating mechanism, but surprising in view of the short distances over which they occur.

(c) The origin of the flowering time differences

The differences in flowering time recorded in the previous section may either be the result of adaptation to local ecological conditions, nothing to do with gene flow, or they may have evolved as a consequence of gene flow, i.e., specifically as an isolating mechanism. Although the fact of isolation is indisputable, it is important to keep these causes distinct.

(i) Adaptation to local conditions.

The mine environment differs from the pasture in many factors. Apart from the higher metal concentration, it usually has a lower fertility, higher pH, coarser soil texture, and generally there is less competition from other plants. Changes in several associated characters have already been discussed (Chapter III.4) and it is possible that flowering time is another example of adaptation to local conditions. Mine populations are ungrazed: Dactylis glomerata from ungrazed pastures flowers earlier than that from heavily grazed pasture (Stapledon, 1926). Mine soils are sometimes similar in texture to sand dunes: sand dune populations of Agrostis flower considerably earlier than pasture populations. Moreover, Lewontin (1965) has argued that in a species colonising a relatively bare area and expanding rapidly, there will be a premium on rapid development and earlier flowering.

Two environmental factors possibly selecting for early flowering time were investigated in the field: water content and temperature of the soil. The water content of the soil was estimated from the difference between wet weight and air-dry weight of soil samples from both Drws-y-Coed and Trelogan. Soil temperature was measured using maximum and minimum thermometers placed five centimetres below the soil surface. Readings were taken at weekly intervals for two months prior to flowering. This was done only for the Drws-y-Coed transect.

The results (Fig. 24c and 25c) show that there are distinct correlations between flowering time and soil dryness, as well as between flowering time and soil temperature. These correlations are in the expected direction; warmer drier soils have the earlier flowering types. However there are some interesting exceptions to this pattern. At Drws-y-Coed, although on the mine earlier flowering is seen at warmer, drier sites, site 7 (off the mine) has much later flowering than site 5 (on the mine) in spite of similar ecological conditions. Again at Trelogan sites 1 and 8 are exceptional in that they do not correlate with the ecological pattern.

The ecological factors that have been measured could therefore to some extent determine the flowering time pattern. Other ecological factors could also be involved, but to identify these in any detail would require further study. There is another way in which flowering time may be altered by selection: it may be linked (physiologically or genetically) to some other physiological or morphological character being selected on their own account: flowering time will then itself change indirectly. Clausen and Hiesey (1958) have shown that in *Potentilla* flowering time is linked to many morphological characters and day length is known to affect flowering as well as morphology (e.g. White, 1960, p. 176). However no significant relationship between flowering time and tolerance was found within the tolerant population (Table 11), showing the characters to be unlinked in any way.

(ii) Restriction of gene flow

Evidence that differences in flowering time can arise as a result of selection for a mechanism restricting gene flow has been demonstrated in very few instances. Partial but pronounced temporal isolation occurs between neighbouring races of pines in Europe

Table 14. Differences in flowering time of various ecotypes

Species	'Ecotype'	Flowering time	Author
Gilia capitata	normal form	earlier	
	sand dune form	later	Grant (1952)
Madia elegans	vernalis (lowland)	spring*	
	aestivalis (mid-alt.)	summer	
	densifolia (lowland)	autumn	Clausen (1951)
Layia platyglossa	normal form	earlier*	
	maritime form	later	Clausen (1958)
Hemizonia citrina		April*	
lutescens		August-September	
luzulaefolia		April	
rudis		August-September	Babcock (1924)
Lactuca graminifolia		early spring*	
canadensis		summer	Whittaker (1944)
Ixeris denticulata	ssp. typica	spring	
	ssp. sonchifolia	autumn	
	ssp. elegans	summer	Stebbins (1950)
Pinus attenuata	inland	later*	
radiata	coastal	earlier	Stebbins (1950)
Lamium amplexicaule	normal form	summer*	
	vernal race	spring	Bernstrom (1952)
Viola tricolor	normal form	earlier	
	coastal sand dune	later	Clausen (1926)
Silene cucubalis	normal	earlier	
maritima	prostrate coastal	later	Marsden-Jones (1928)
Geranium robertianum	normal	earlier	
	shingle beaches	later	Bocher (1947)
Mimulus guttatus	coastal	late	
	mountain	latest	
	valley and foothills	early	Vickery (1953)
Silene vulgaris	normal form	earlier	
	calamine form	later	Broker (1963)
Geum urbane	normal	later*	
rivale	wet habitats	earlier	Clausen (1958)
Succisa pratensis	northern race	earlier	
Ranunculus acer	alpine	earlier	
Solidago virgaurea	alpine and coastal	earlier	
Rumex acetosa	alpine	earlier	
Leontodon autumnale	coastal	earlier	Turesson (1925)
Agrostis canina	var. fascicularis	fifth June*	
	var. arida	sixth June	
tenuis		ninth June	
stolonifera		fifteenth June	
gigantea		tenth June	Davies (1953)
Clarkia xantiana	normal race	later*	
	Self compatible race	earlier	Moore (1965)
Salvia mellifera		early spring	
apiana		late spring	Grant (1964)

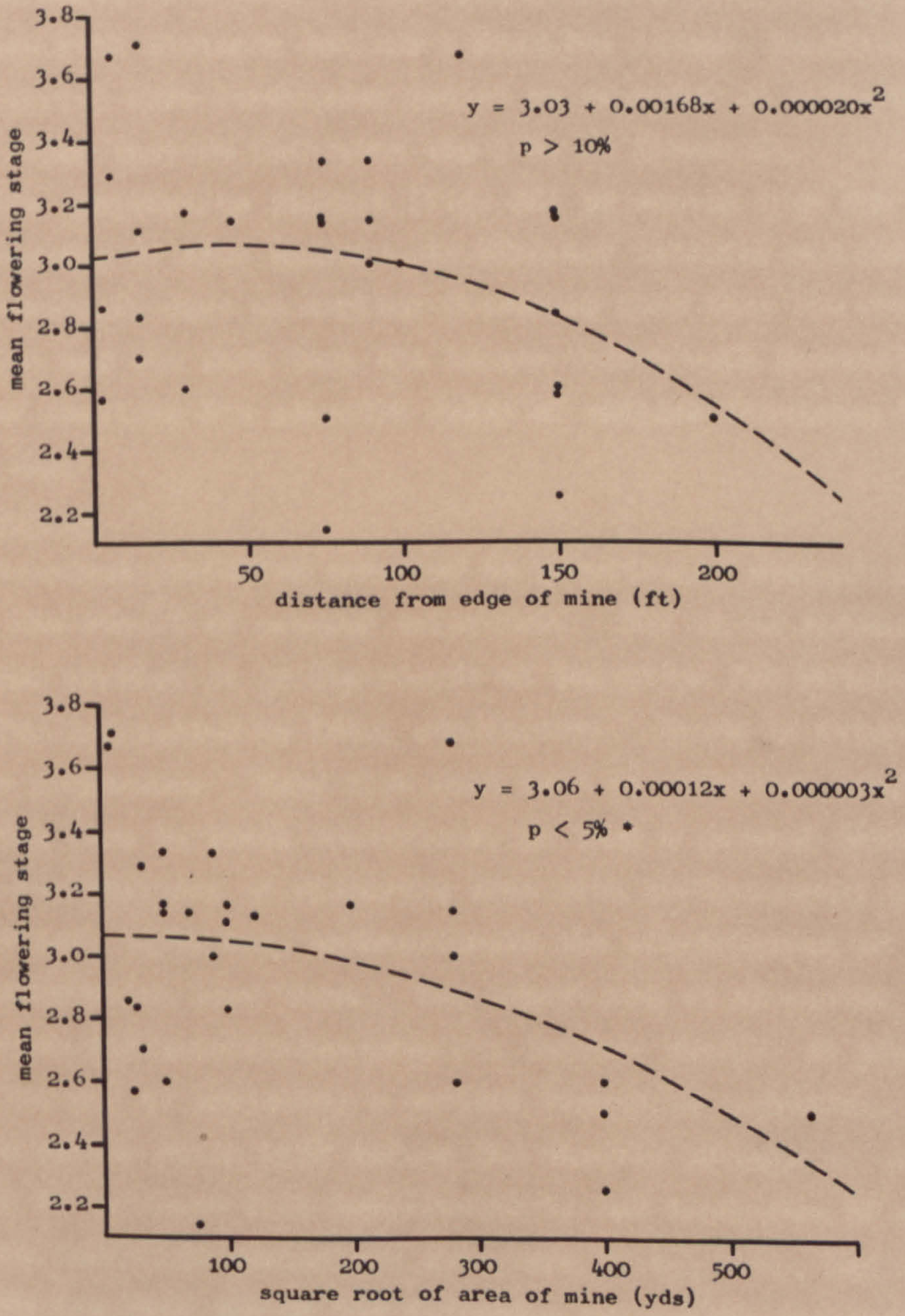
*evidence that
'ecotypes'
closely
adjacent

(Wettstein and Onno, 1948); a similar situation occurs in related species of pines on the California coast (Stebbins, 1950). And it is perhaps relevant that ecotype formation very often goes hand in hand with differences in flowering time (Table 14), but this can equally be interpreted in terms of adaptation to local conditions.

Although natural selection for adaptation to local conditions can explain in part the differences in flowering time reported here, this cannot be the complete explanation. The earlier flowering towards the boundary in the mine populations of both Agrostis and Anthoxanthum is present on mines that are ecologically very different and it can only partly be explained on the basis of local adaptation to water content and soil temperature. This suggests that boundary populations flower earlier possibly as a result of a selection for a mechanism to prevent gene flow; the evidence from this data is however by no means clear. It can be visualised that gene flow will reinforce local adaptation in flowering time, since the plants that do flower earlier will be pollinated by similar earlier flowering adapted types and therefore set more of the appropriate type of seed. In this way gene flow will assist in the build up of earlier flowering types if such types are an advantage in terms of local ecological adaptation.

To investigate the matter further a series of populations were collected in 1965 from lead mines in Cardiganshire (Appendix 2 and see also Chapter IV.4.6). The area of the mines and the distance of the populations from the edge of the mines was recorded when the plants were collected. These were taken as measures of the prximity of the tolerant populations to the non-tolerant. The populations were planted in normal potting compost, and scored for flowering time in the summer of 1966. The results (Fig. 27) show that the flowering time is significantly earlier the smaller the area of the mine but not significantly so in relation to the distance from the edge of the mines. The same trend is nevertheless present

Fig. 27. Relationship between flowering stage of tolerant *Agrostis* populations from Cardiganshire and distance from nearest non-tolerant plants.



in both data.

This data therefore provides evidence for the evolution of earlier flowering as a means of preventing gene flow. However because of the low significance of the fitted regression and because the curve is a positive linear, negative quadratic (pollen distribution with distance follows a negative linear, positive quadratic curve) this conclusion cannot be regarded as definite: certainly other factors influence the time of flowering.

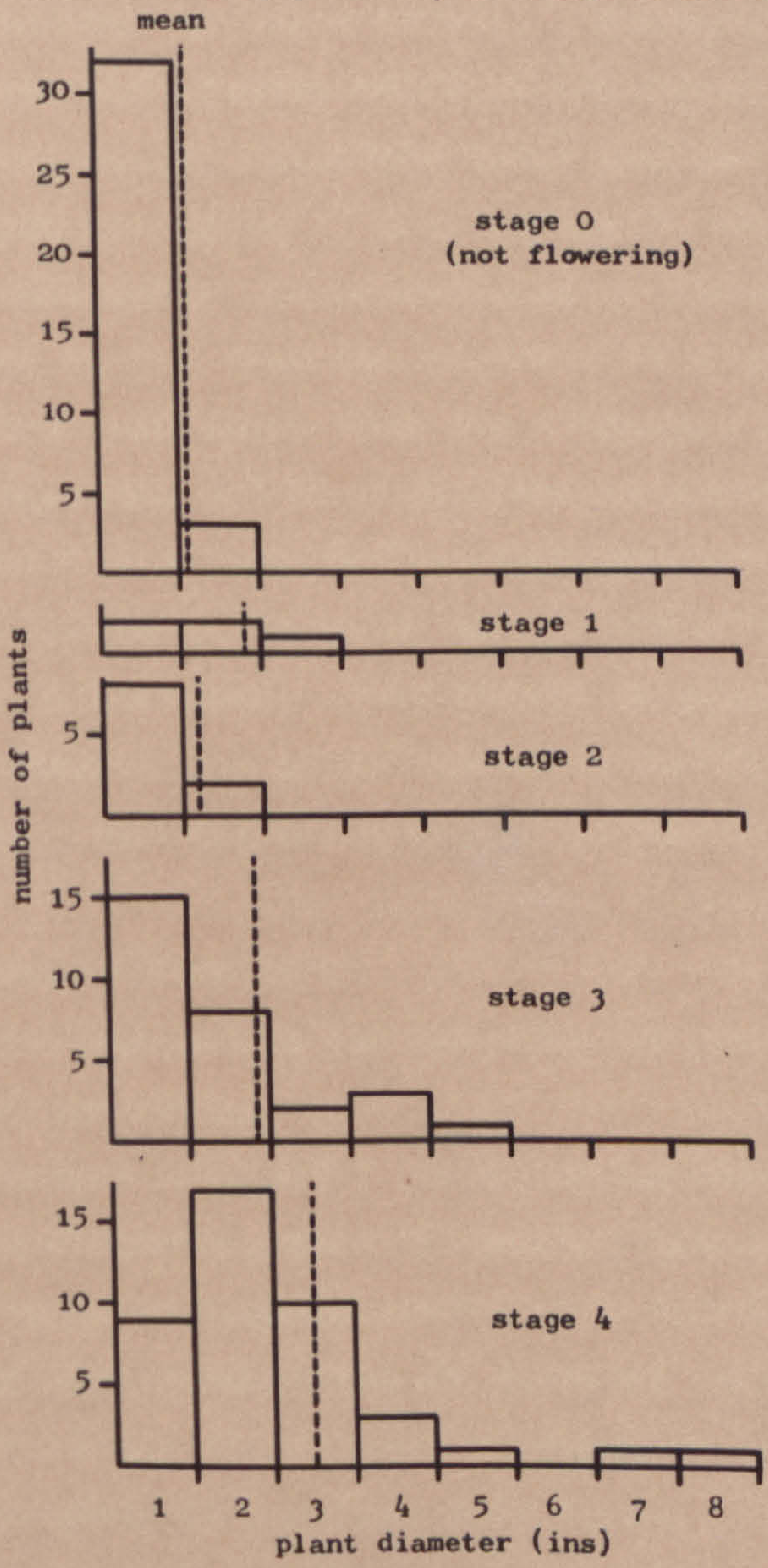
Another interesting feature (Fig. 28) to emerge from the detailed mapping of mine individuals (Chapter III.3.b) was that larger individuals have an earlier flowering time than the smaller ones. This and the fact that the larger individuals generally live longer confirms the adaptive value of earlier flowering in a startling way.

(d) Discussion

The temporal isolation observed is genetically controlled and therefore the result of natural selection. But it is difficult to determine whether it is the result of selection for reduced gene flow, or for adaptation to local conditions. There is evidence for both these processes. Gene flow will tend to reinforce adaptation of flowering time to local conditions, so that a general study involving correlations of flowering time with environmental factors (e.g. Bradshaw, 1959) many underestimate the significance of flowering time in restricting gene flow. The importance of flowering time in population differentiation requires further study: it seems an effective way of achieving a certain degree of isolation, and seems a flexible system which will reduce the drastic effects of gene flow.

There is evidence that self-fertility and dominance of genes can also be isolating mechanisms (Chapters IV.4., V.4., and VI.2). We can then ask if the isolating mechanisms observed in mine

Fig. 28. Relationship between size of plants of *Anthoxanthum* from the mine population, and stage of flowering.



populations are related to speciation: are we seeing the beginnings of this process? The formation of breeding barriers in this instance assists selection in promoting divergence, but is not an integral part of the process. The evolution of breeding barriers is here therefore very different from the process of 'catastrophic selection' described by Lewis (1962). Raven (1964), in discussing edaphic endemics and species which at the edges of their ranges have peculiar habitats, considers these species to be the products of 'catastrophic selection': i.e. selection of a few specially adapted, 'automatically' (chromosomally or through inbreeding) isolated types, rather than the products of a gradual process.

The present work shows that the processes leading to reproductive barriers and morphological or physiological differences are independent; the reproductive barriers can therefore begin gradually and become quite major, as can the morphological and physiological differences. The result is progressive speciation which can be seen at any stage depending on the particular circumstances. The following stages of speciation might be expected:

(a) continuous uniformity.

(b) continuous population (no breeding barriers), but divergence of two or more sections.

(c) discontinuous population (breeding barriers), with divergence

(d) very discontinuous, with increasing divergence.

Several workers (Schwickerath, 1931 and Auquier, 1964) have in fact regarded mine populations as distinct taxa which are relicts of a formerly widely distributed species, or as endemics (and therefore isolated) to peculiar soil types. Serpentine species have also been viewed in this light (Kruckeberg, 1957). There is here a problem of terminology. Many of the so-called separate taxa found on special soils should most probably not be regarded as true "biological species" (Grant, 1957) and may not have reached the more advanced stages of speciation ((c) and (d) above).

Colonisation of mine habitats not only requires the evolution of tolerance but can include changes in morphological and other physiological characters. Distinct types may therefore be the products of <ins>recent</ins> evolution: they may also not be reproductively isolated from the parental types. Parallel independent evolution of similar morphological ecotypes (cf. Turesson, 1922) may give a pattern that would erroneously be interpreted as a formerly widespread 'species' with a present-day disjunct distribution.

It also seems unlikely that populations adapted to peculiar conditions at the edges of a species range could only occur when the populations of this species are widely separated with little gene flow between them (Raven, 1964, after Mayr, 1959). These and the results of Thoday (1958) show that sympatric (or parapatric) divergence is a reality and can lead to reproductive isolation.

Selection for reproductive barriers is largely secondary to selection for directly adaptive characters, but the potential for permanent isolation is there. The present work suggests that the processes of divergence, directional change, colonisation, and speciation are inextricably linked and that forces promoting speciation are common in adjacent natural populations, even if these populations never reach the stage where we can definitely say that they are two species. Evolution begins at the population level and we need not look for the processes causing speciation only in taxa which are already highly distinctive.

4. SELF-FERTILITY

(a) Introduction

The role of breeding systems in plant evolution has been frequently discussed. The problem has considered mainly the function of inbreeding; on the basis of simple theory, inbreeding should lead to homozygosity and loss of variability, whereas outbreeding should conserve this variability. The problem is that of inbreeding as a "blind alley of evolution": inbreeding is generally regarded as a retrogressive step which eventually leads to extinction. Stebbins (1957) states that since few large genera of angiosperms consist entirely of self-fertilising species, and self-fertilisers are invariably derived from cross-fertilisers, "one can safely make the assumption, therefore, that self-fertilisation slows down evolutionary progress in flowering plants" but qualifies this statement with the remark, "the adoption of predominant self-fertilisation is by no means the prelude to evolutionary extinction, since some self-fertilising groups appear to have existed for several geological epochs, and may even have outlasted many of their cross-fertilising relatives".

Recently it has been shown that the amount of variability present in inbred populations has generally been underestimated (for discussion see Imam and Allard, 1965, and Allard, 1965). Variability is preserved because heterozygotes are often superior to homozygotes in fitness and is released to a surprising degree by only occasional out-crossing.

The evolution of breeding systems has been generally considered to occur by a process of selection between populations with different strategies rather than direct selection of individuals with certain attributes within populations. The process has been called "interdeme" selection, and seems a slower process than selection of a more direct kind. In view of the breakdown of the distinction between the value of different breeding systems and in the absence of any direct evidence for interdeme selection, it is pertinent to look for the direct adaptive value of inbreeding rather than inquire why it has developed "faut de mieux".

Several possible reasons have been discussed by, among others, Stebbins (1957) and Baker (1959). They may be listed as follows:

(i) The certainty of fertilisation.

Several species self-pollinate under conditions unfavourable to cross-pollination. Good examples, apart from those quoted by Stebbins (1957), come from crop plants where the transport of a crop away from its source of pollinators has often led to the evolution of self-fertility. In California, the tomato is largely selfed since little outcrossing is possible because of the absence of the insect vectors native to Peru, the original home of the tomato (Rick, 1950).

(ii) Establishment after long distance dispersal.

Baker (1955) has given evidence that species at the margins of the range of a genus (or isolated on islands) show monomorphic and not dimorphic or dioecious flowers. Stebbins (1957) gave similar instances for *Bromus*, *Hordeum* and *Secale*. Bannister (1965) working on *Pinus radiata* showed that "colonisation is likely to be accompanied at first by an increase in the degree of inbreeding, but outbreeding will tend to be restored as the population density increases". The absence of other plants, after establishment following long distance dispersal, is given by all these workers as the reason for the greater self-fertility of marginal and colonising populations.

(iii) Prevention of gene flow.

During the colonisation of a new and fairly uniform habitat, a high selective advantage is given to a genetic type which can quickly build up a large population of well adapted individuals from the progeny of a few initial colonizers. It is advantageous therefore that the descendants of an initial coloniser should resemble that coloniser as closely as possible. There are two main aspects to this. Firstly, it is advantageous for the original coloniser to be homozygous. Stebbins (1957) quotes the case of several self-incompatible annuals who may keep their genetic constancy through having reduced chromosome numbers and/or chiasma frequencies. Secondly, crossing with extraneous pollen would lead to a dilution of the new adaptive character by the parent character (Baker, 1959). It has been suggested (Jain and Bradshaw, 1966) that selection pressures acting on perennials are considerably higher than those on annuals, since the selection pressures acting on a perennial population must be summed over many years. Therefore one might expect gene flow to have less drastic effects on perennials than on annuals, as annuals in particular would then have to evolve some mechanism of avoiding the deleterious effects of gene flow. The relative high frequency of selfing annuals as opposed to perennials (Stebbins, 1950) is therefore interesting from this standpoint.

(iv) Exposure of recessives.

During the colonisation of a new habitat, new genes may be required. If these are in a recessive condition they will more easily be unmasked by inbreeding. Moore and Lewis (1965) describe a derived self-fertile population of Clarkia, which has white petals (a recessive character) whereas most of the species has lavender-pink flowers.

(v) Earliness of flowering.

Individuals of a cross-pollinating species that flower first may produce no seeds because there are few other plants to pollinate them. Self-pollination in plants that flower early would therefore be of selective advantage. This is one of the main reasons advocated by Moore and Lewis (1964) for the self-fertility of populations of Clarkia. Here there is a premium on earliness of flowering because the growing seasons is often truncated by drought; the degree of selfing and flowering time are also correlated. The two would reinforce each other to prevent gene flow and indeed little evidence for the occurrence of gene exchange can be found in these populations.

(vi) Density of plants.

Plants growing in a dense stand have more chance of being pollinated than those growing far apart and widely scattered. This factor has been considered indirectly by Baker (1953) who showed that in tropical forests, where the climax vegetation is a mixture of many species, there are far more hermaphrodite species than in temperate forests which are more or less dense stands of one or a few species. This is related to factors (i) and (ii).

There is therefore evidence for a wide range of factors which might be important in putting self-fertilisation at a premium. However, the processes of selection whereby these factors produce self-fertilisation have been little examined. Most of the evidence is circumstantial and comes from comparisons between species. Baker (1953) remarks that "despite the relative ease with which outbreeding may give way to inbreeding, it seems that the natural existance of self-incompatible and self-compatible races within the same species is rather unusual".

Work by Julen (1948), Beddows (1931) and Jenkin (1931) on herbage plants has shown that there is considerable natural variability in the level of self-compatibility in legumes and grasses. In some instances, the highly self-fertile plants also have highly self-fertile progenies (Jenkin , 1931, and Thomas, 1955). Although in general, progeny selfed over several generations show evidence of inbreeding depressions, in some species, e.g., *Phleum pratense* and *Festuca rubra*, occasional lines show apparently normal vitality (Julen, 1948). More direct evidence for the inheritance of self-fertility comes from a diallel analysis of this character in *Vicia* (Rowlands, 1960); the genes responsible show dominance and non-additivity of action. Selection for self-fertility in this plant is reasonably effective (Rowlands, 1961).

Selection for self-compatibility therefore seems quite possible and there seems very little reason why it should not occur under the right conditions in natural populations.

In previous sections of this thesis situations were described where there was sharp differentiation between populations only a few metres apart. These situations seemed ideal for examining the evolution of breeding systems: fairly wide seed dispersal is often necessary to colonise the mine soil, there is considerable gene flow between the populations, the mine populations flower earlier, and there is a wide range of plant density on different mines.

The study was divided into four sections: the establishment of differences in self-compatibility of plants from adjacent populations, an examination of differences between various tolerant populations, an investigation into the effects of self-fertility on vigour and a computer simulation of the process. The computer simulation is presented in Chapter V.

(b) Self-fertility of adjacent populations

(i) Method

The self-fertility of Agrostis and Anthoxanthum plants collected from the transects described in Chapter II.1. was estimated by enclosing about five inflorescences from one genotype inside a glassine bag, and counting the seed set. Plants were collected as one or a few tillers, to make sure that only a single genotype was sampled. The seed of Agrostis was counted using an illuminated background and viability confirmed by germination tests on selected samples.

(ii) Results

The results were startling (Figs. 29, 30): the self-fertility of tolerant populations was far in excess of the self-fertility of the adjacent non-tolerant populations. Not only were the mean differences considerable, but the pattern of distribution of self-fertility within the populations (Fig. 29) showed that while all the individuals in the non-tolerant population had a low self-fertility, the tolerant population contained some individuals with a very high self-fertility.

Fig. 29a. Self-fertility of tolerant and non-tolerant populations of Agrostis from Drws-y-Coed.

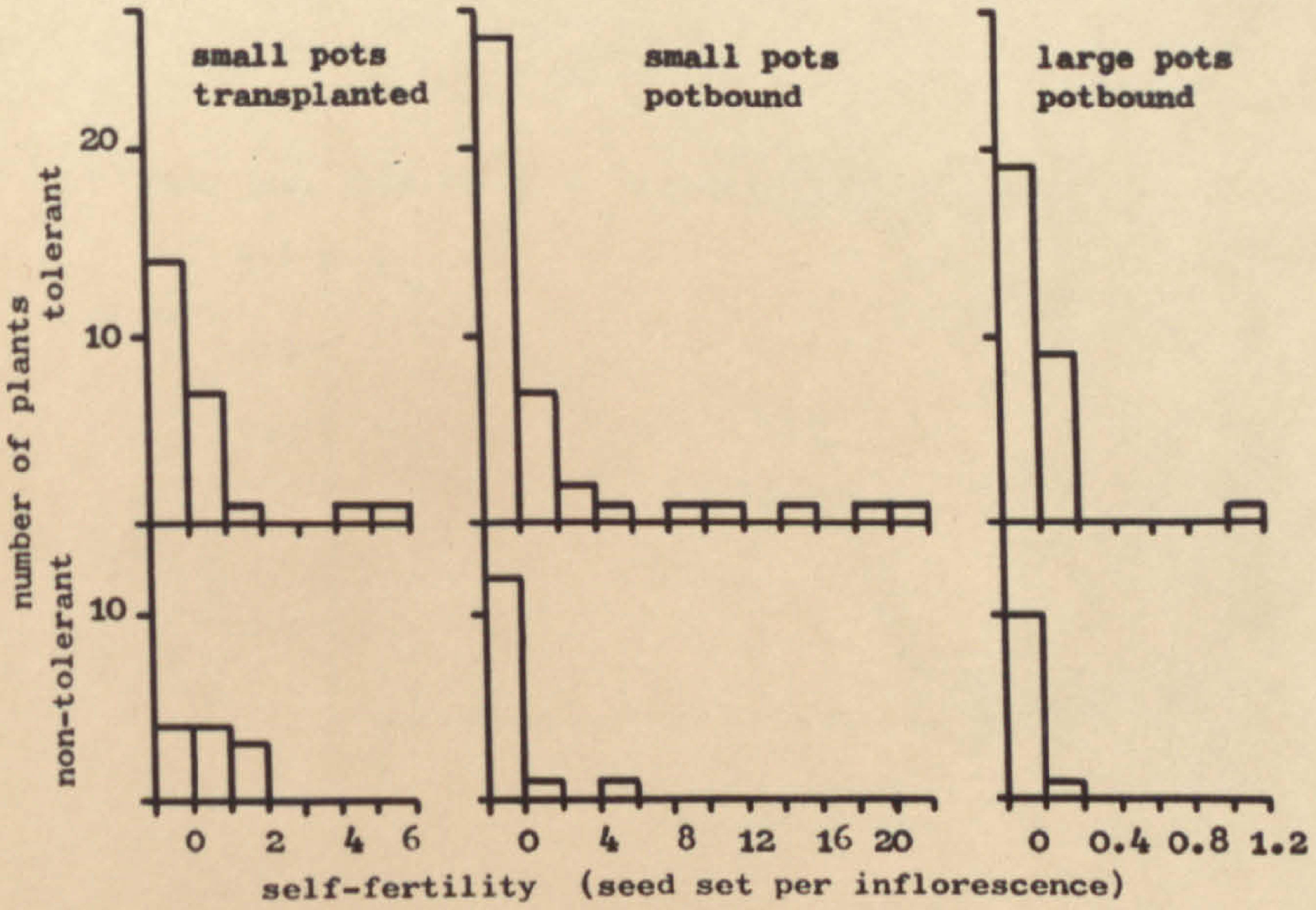


Fig. 29b. Self-fertility of tolerant and non-tolerant populations of Anthoxanthum from Trelogan.

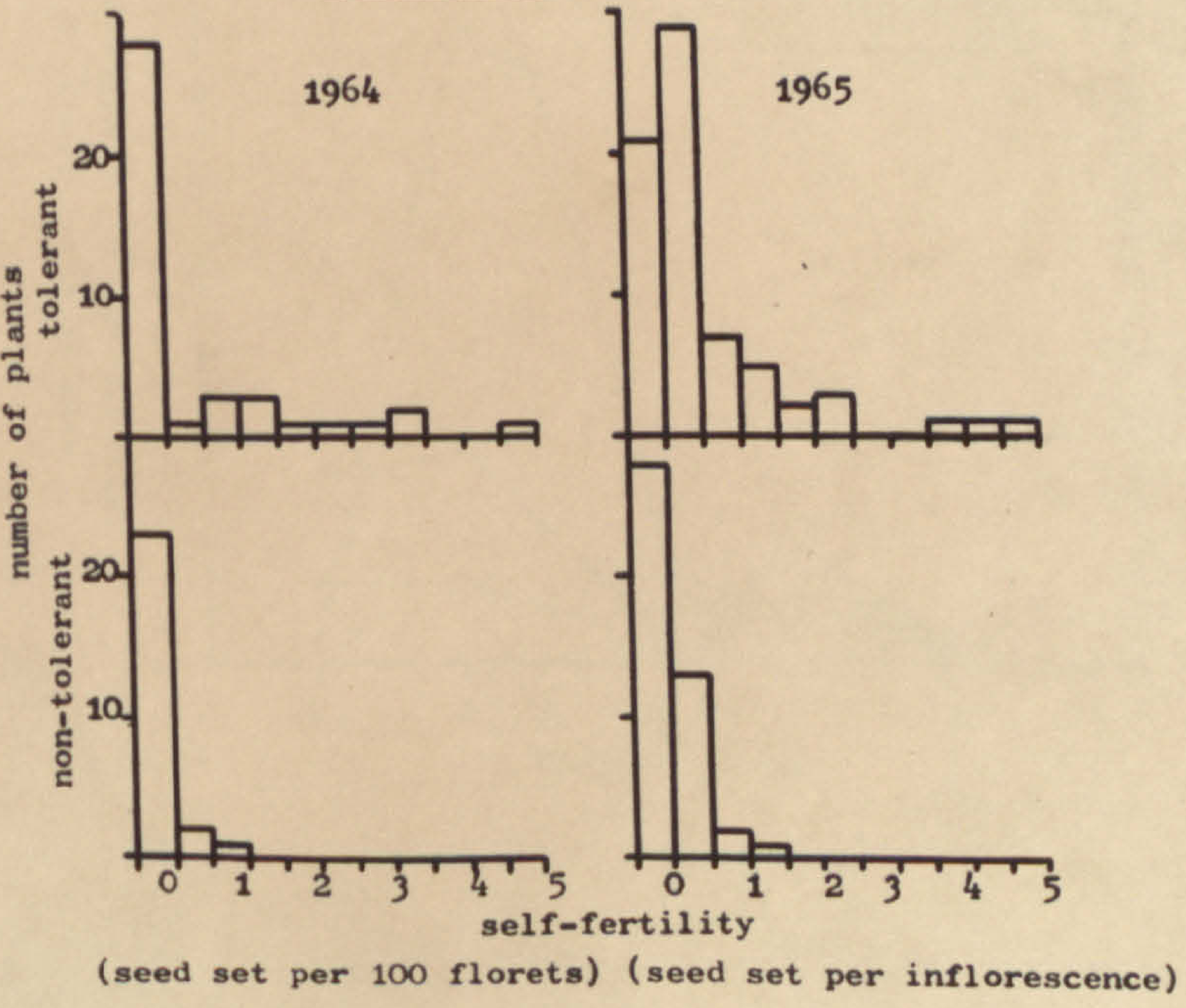


Fig. 30a. Self-fertility along Trelogan transect.

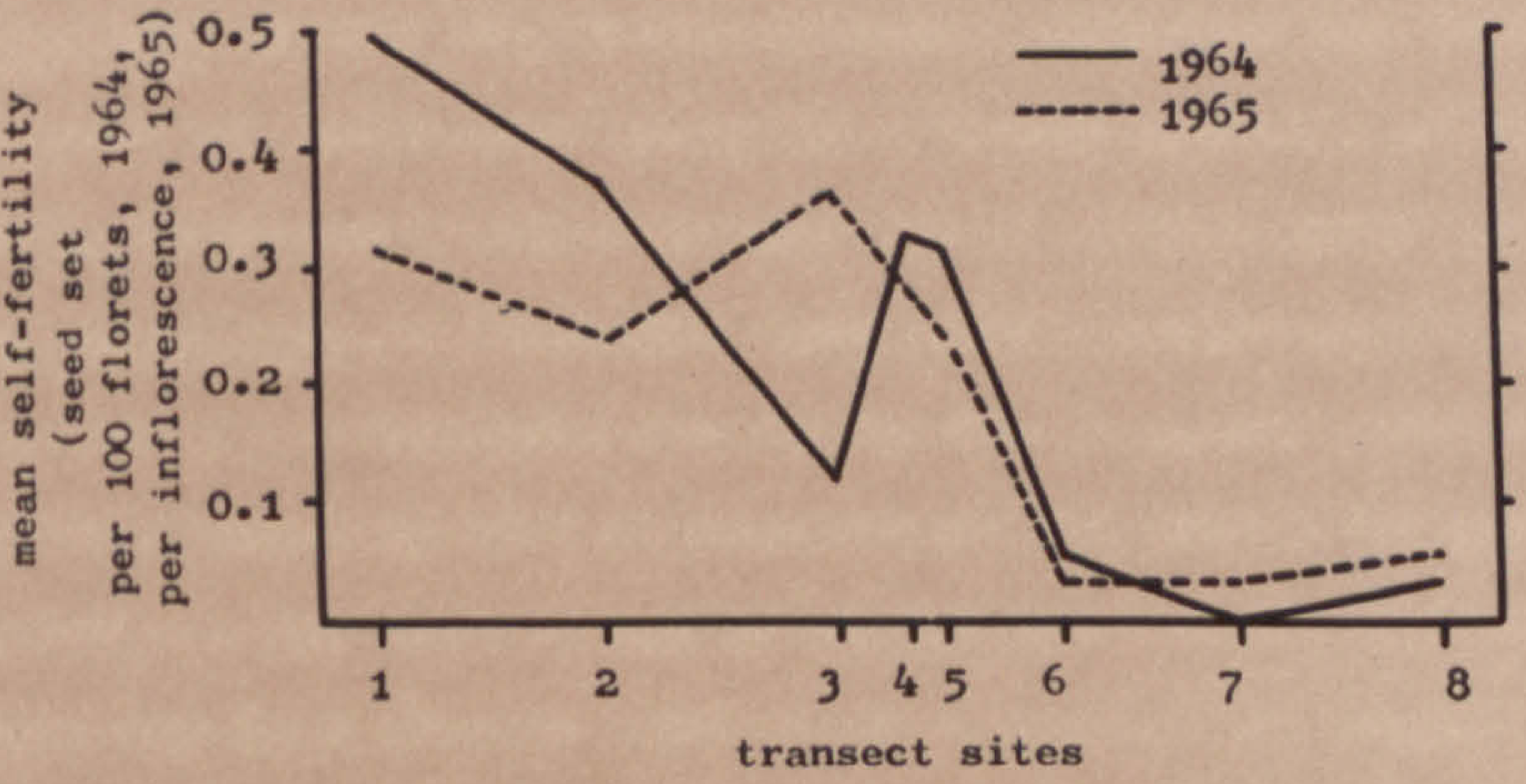
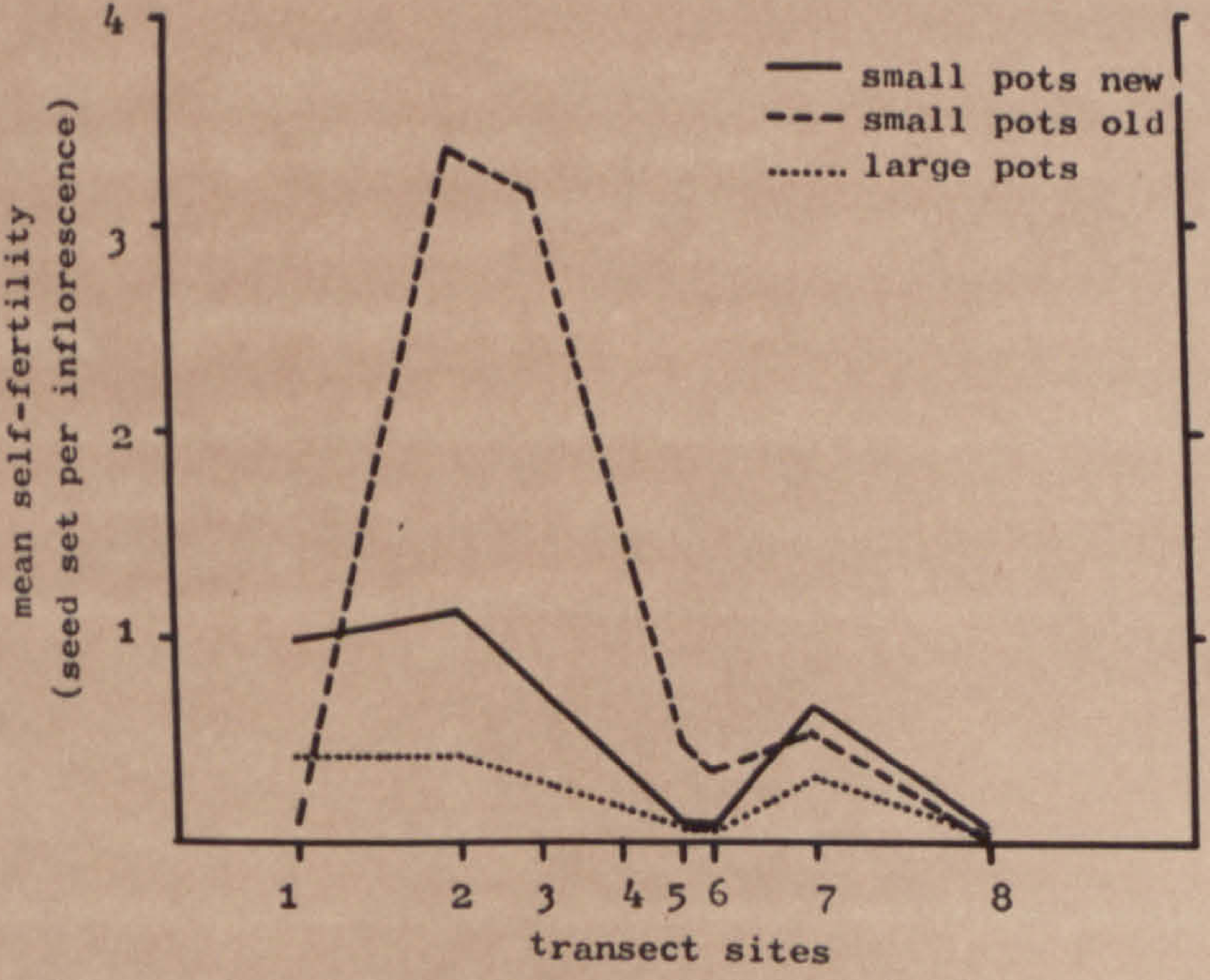


Fig. 30b. Self-fertility along Drws-y-Coed transect.



The mean values therefore tend to underemphasise the differences between the populations.

Equally startling was the sharp difference at the population boundary (Fig. 30); it is another example of population differentiation over short distances. Even for two species and two contrasting mines, the pattern was very similar.

The reality of the differences was confirmed by the following:

(a) The degree of self-fertility is characteristic of the genotype.

In 1965, replicate selfs (separate plants and/or bags) were made in *Anthoxanthum*. From the analysis of variance (Table 15a) it was possible to calculate the relative genetic contribution to the overall variance in the character of selfing ("broad-sense heritability"). It gave a value of 55.3%.

Similar results (Table 15b) from a series of mines in Cardiganshire (see next section, Chapter IV.4.c) and where the replicate selfs were separate bags, gave the value 59.1%.

Table 15. Analysis of variance on degree of self-fertility in different tolerant genotypes

(a) *Anthoxanthum* (Trelogan)

Source	Sums of Squares	d.f.	Mean Square	F	Expectation
Total	875.46	178			
Genotypes	658.23	48	13.71	8.21 ***	$6V_a + V_e$
Error	217.23	130	1.67		V_e

(b) *Agrostis* (Cardiganshire)

Source	Sums of Squares	d.f.	Mean Square	F	Expectation
Total	7908.0	81			
Genotypes	6260.3	40	1565.1	3.89 ***	$2V_a + V_e$
Error	1647.7	41	401.9		V_e

There is therefore a strong genetic component for this character.

This is further reflected in *Anthoxanthum* by the fact that the degree of fertility is correlated between genotypes over years (Fig. 31a) within the tolerant population. (Not enough data was available for non-tolerant plants to reach any firm conclusion).

In *Agrostis* there was a significant correlation between the self-fertility of different genotypes grown under a range of preculture conditions (Fig. 29a and 31c). The preculture conditions were: plants cloned from stock material in December 1964 and growing in the greenhouse in small pots: plants collected early in 1964, growing in the greenhouse in small 4" pots and seriously pot-bound: and stock material collected in Autumn 1963, growing outside in large 7" pots.

(b) The degree of self-fertility is inherited.

Seed produced by selfing was grown up and the self-fertility of the mature plants tested. This was done for the tolerant *Anthoxanthum* population and various plants of *Agrostis* from the Cardiganshire mine populations. In *Anthoxanthum* a significant parent-offspring regression was obtained (Fig. 31b). This regression is not a true heritability since the offspring are products of selfing and not crossing:

$$\text{regression coefficient} = \frac{\text{covariance } (S_n + S_{n+1})}{\text{variance } (S_n)} \quad \text{where } S_n = \text{n'th generation of selfing}$$

$$= \frac{{}^1\!/_2 D + ({}^1\!/_2)^n H}{{}^1\!/_2 D + ({}^1\!/_2)^n H + E}$$

$$\text{cf. } h^2 = \frac{{}^1\!/_2 D}{{}^1\!/_2 D + {}^1\!/_4 H + E}$$

The results for *Agrostis* were less clear cut because many of the plants (43 plants, 14 families) failed to set any seed on selfing. Only plants from one mine (Cwm Rheidol) set seed at all successfully (10 plants, 5 families). Plants from this mine showed a significant parent offspring regression (Fig. 31d) when an extreme

Fig. 31a. Relationship between self-fertility of tolerant genotypes of Anthoxanthum in 1964 and 1965. (seed set per inflorescence)

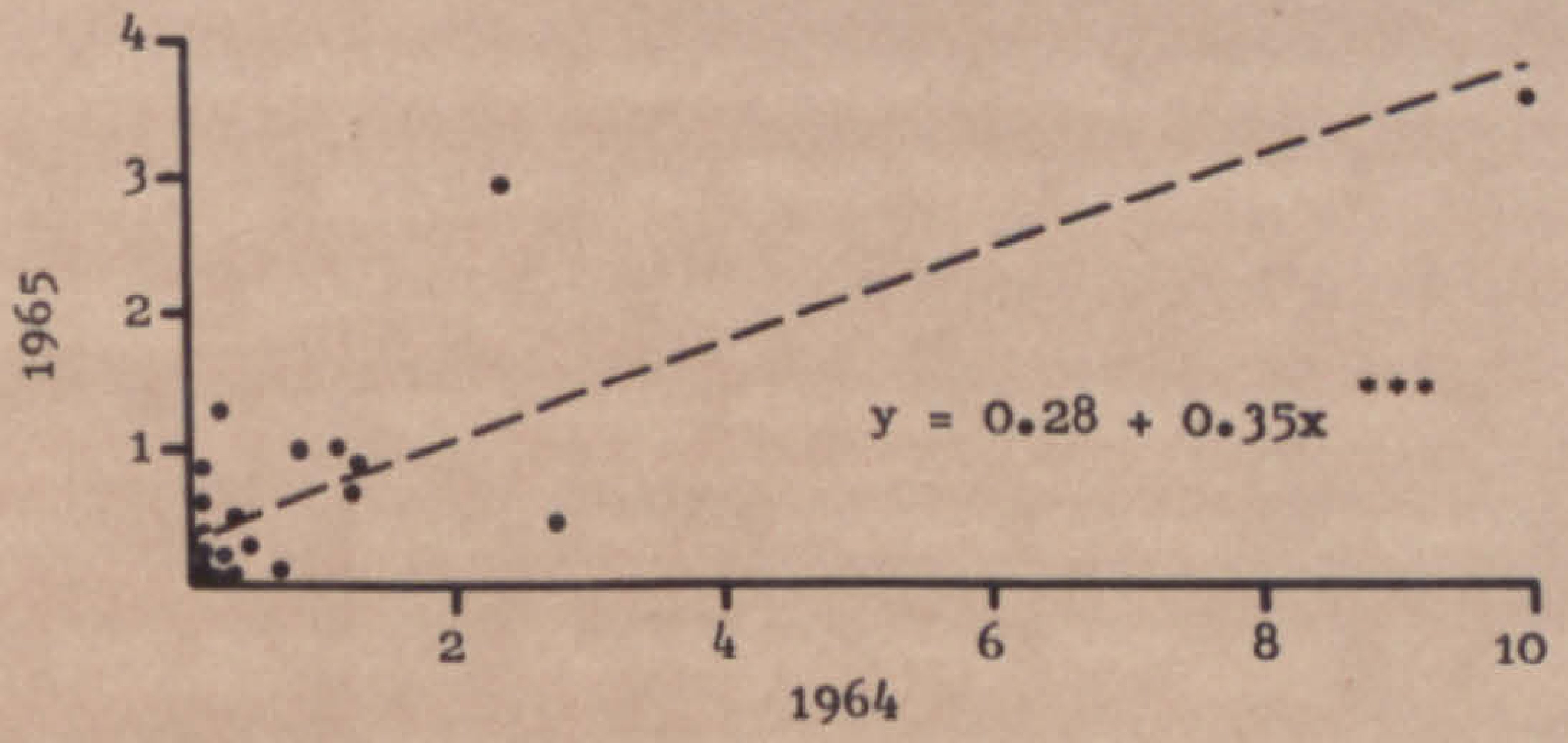


Fig. 31b. Relationship between self-fertility of parents and offspring (produced by selfing) in tolerant Anthoxanthum. (seed set per inflorescence)

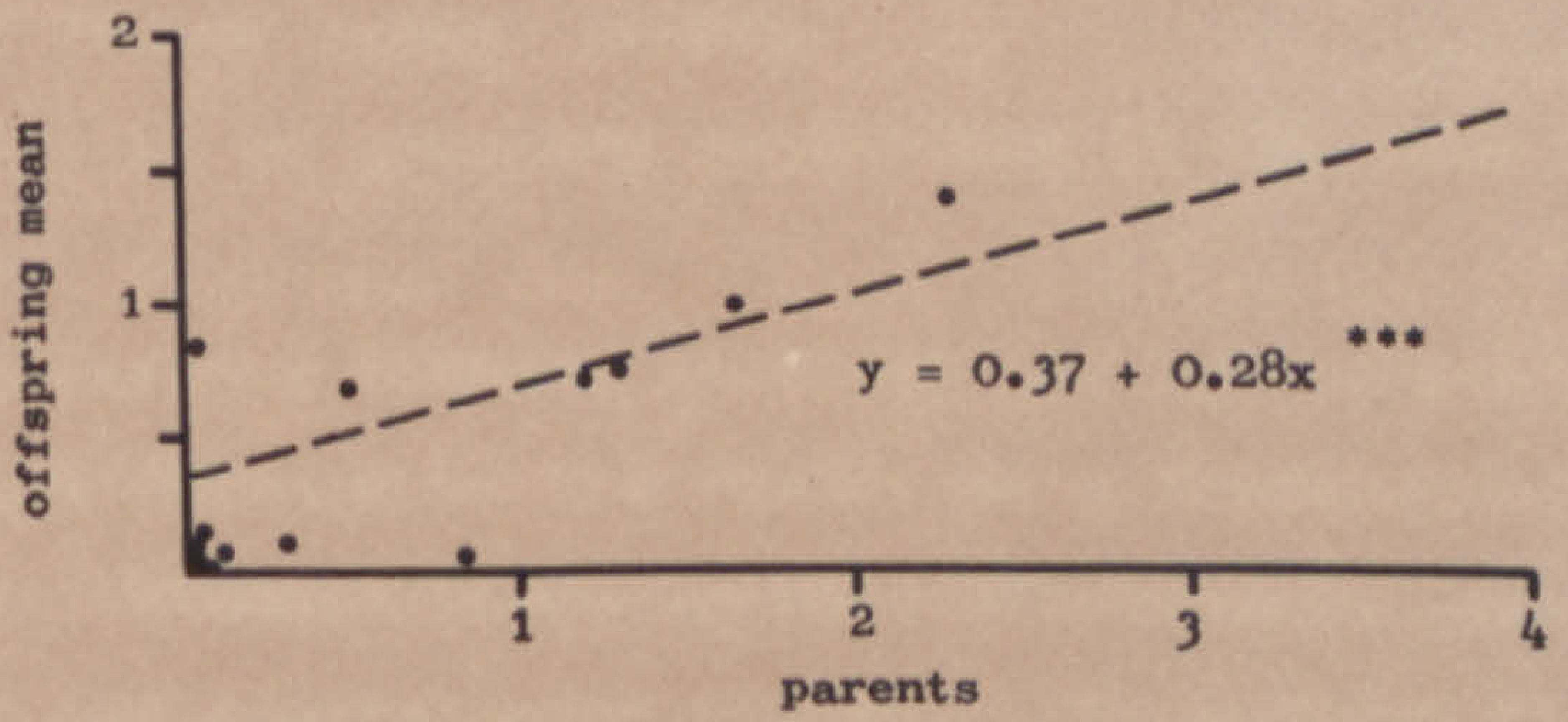


Fig. 31c. Relationship between self-fertility of tolerant genotypes of Agrostis in two contrasting conditions. (seed set per inflorescence)

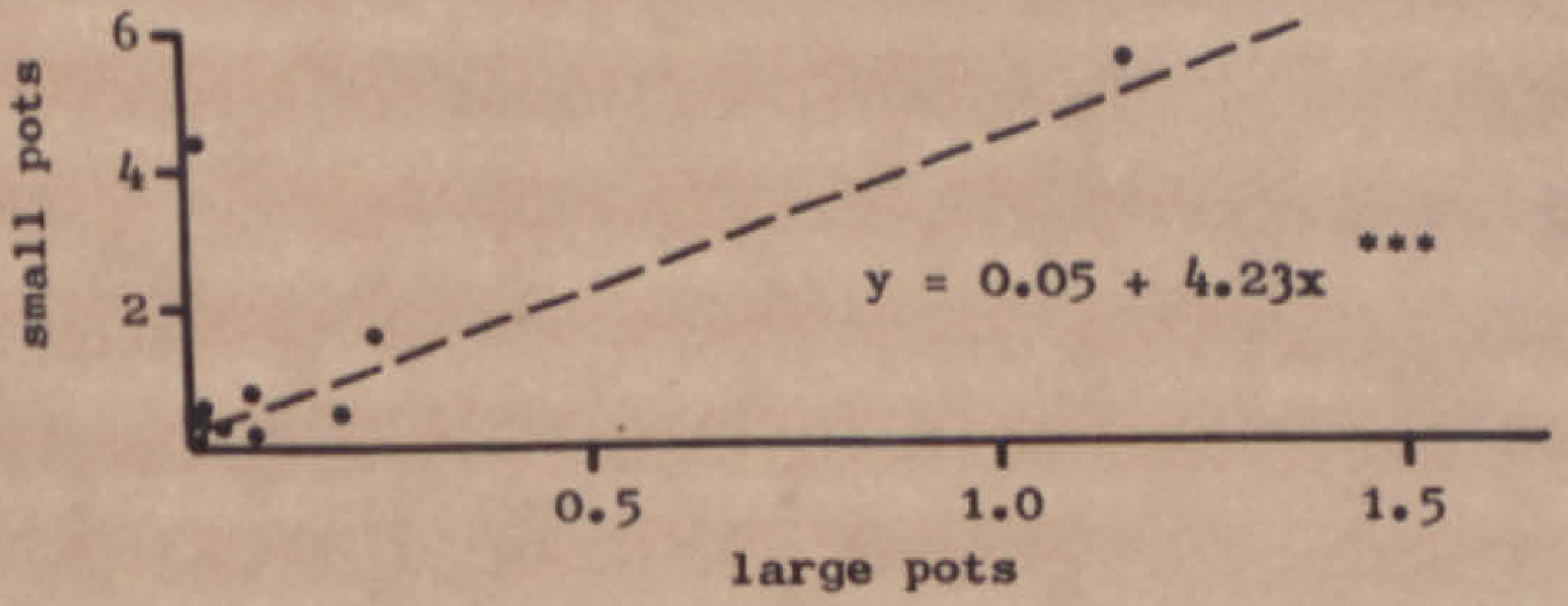
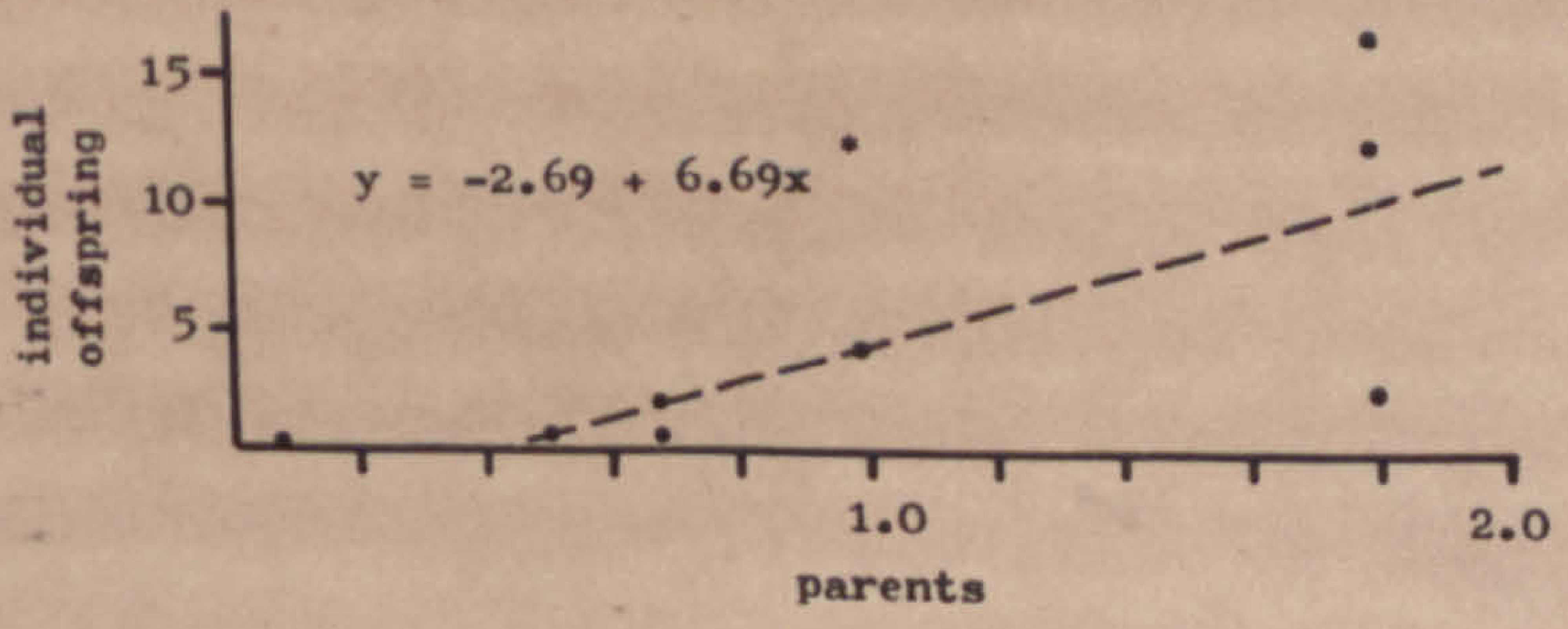


Fig. 31d. Relationship between self-fertility of parents and offspring (produced by selfing) in a population of lead tolerant Agrostis from Cardiganshire. (seed set per inflorescence)



individual which produced sixty seeds per inflorescence and which came from the highest selfing parent is removed from the calculation. Even with this extreme individual the regression has a slope greater than unity suggesting that the progeny produced by selfing have an improved ability to self.

It is not clear why none of the other plants of Agrostis produced seed. It could be a direct consequence of inbreeding depression, but equally it could have been due to the fact that the plants were planted as seedlings rather late in the season (January) and therefore did not have the full photoperiod. They did in fact flower rather poorly.

(c) Sensitivity to crossing conditions is the same in both populations.

The difference between tolerant and non-tolerant in seed set on selfing might perhaps be the result of a differential sensitivity to bagging. However there is no difference in seed set in crosses of tolerant and non-tolerant plants. In Section IV.2, on incompatibility barriers, it was noted that there were no significant differences between tolerant and non-tolerant plants when crosses were made between genotypes within each population. Moreover the mean seed set (Table 16) shows that in 1964 the non-tolerant set more seed, whereas in 1965 the tolerant did so. No trend therefore emerges and it can be concluded that the two populations do not differ to any detectable extent in their sensitivity to bagging.

(iii) Discussion

The greater self-fertility of tolerant populations is interesting in view of the rarity with which differences in breeding systems have been demonstrated within a species. The work of Moore and Lewis (1965) is the only other clear cut case described.

Although one can speculate about the possible reasons for the greater self-fertility in the tolerant populations at Trelogan and Drws-y-Coed, the data does not provide any clear cut answers.

Table 16. Seed set data for crossing and selfing in *Anthoxanthum* and *Agrostis* (expressed as seed set per inflorescence, unless otherwise stated)

Population	mean seed set for selfing	maximum seed set for selfing	mean seed set for crossing	Conditions of selfing and crossing
Anthoxanthum				
non-tolerant (1964)	0.065 per 100 florets	0.85 per 100 florets	3.20	bagging
(1966)	0.084	1.04	4.48	bagging
(after Beddows, 1931)	0.79	-	-	open isolation
(after Borrill, 1963)	0.030 per 100 florets	-	67 per 100 florets	limited period of bagging
tolerant (1964)	0.571	4.85	2.71	bagging
(1965)	0.576	4.82	5.23	bagging
Agrostis				
non-tolerant (1965)	0.24	5.00	17.48	bagging
(after Beddows, 1931)	6.22	-	-	open isolation
(after Davies, 1953)	2.97	30	100	bagging
tolerant (1965, Drws-y-Coed)	0.54	24	17.48	bagging
(1965, Cardiganshire	1.17	37.6	-	bagging

The transects do not show any marked "inverse cline" with regard to selfing: plants nearer the boundary do not clearly show more selfing even though at both mines there are trends in this direction (Fig. 30).

The earlier flowering of the mine populations (Chapter IV.2) suggests another reason for the greater self-fertility of mine populations; perhaps the earlier genotypes tend to self more because they have fewer other plants with which to cross pollinate. The time of flowering was therefore plotted against the degree of selfing for plants from the mine populations.

The results of the regression analyses are as follows:

Anthoxanthum: selfing 1965 / date of stigma emergence 1966.

$y = 2.24 - 0.012x + 0.000065x^2$ $P > 10\%$

Agrostis: selfing 1965 / stage of flowering 1965.

$y = 1.11 - 0.28x$ $P > 10\%$ (a non-linear regression was not calculated)

Neither of these regressions approaches significance, showing that flowering time is not important in promoting selfing in mine populations.

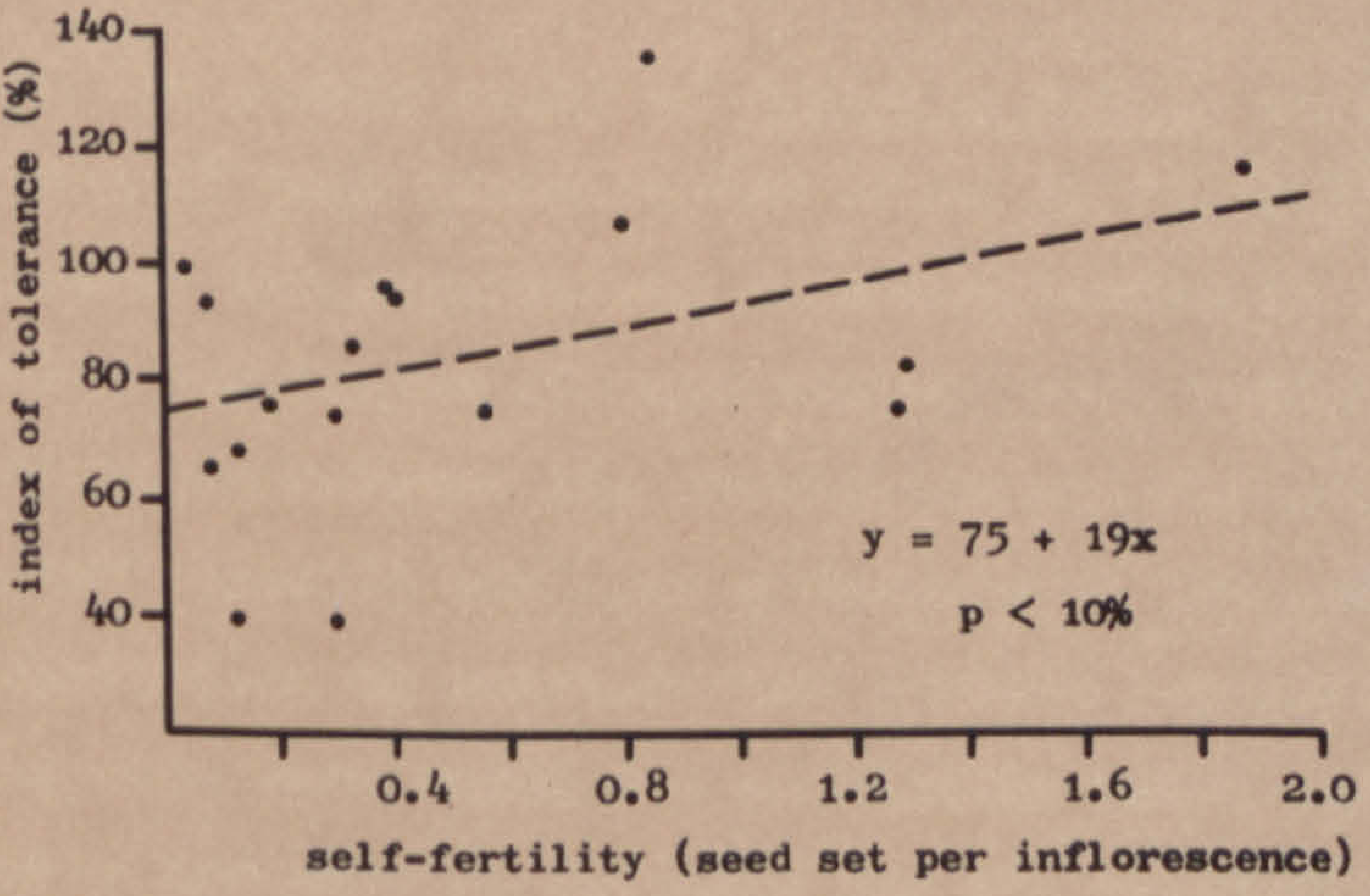
Another reason for the greater self-fertility of tolerant populations may be that the tolerance mechanism has such far reaching effects on the plants metabolism, that the self-incompatibility mechanism is upset. The tolerance of plants of Anthoxanthum on the mine at Trelogan was therefore plotted against their self-fertility (Fig. 32). A highly suggestive relationship is obtained. However apart from 'physiological upset' two other reasons might lead us to expect a relationship between tolerance and selfing.

Firstly, selfing could be a method of avoiding gene flow and a means of helping selection to fix the tolerant gene. Then the more an individual is selfed the more tolerant it should be, since it will probably be the progeny of a selfed parent.

Secondly, if selfing does promote the evolution of tolerance, then it will be more effective if it is linked to a gene for tolerance.

Simple correlations are inadequate in distinguishing between

Fig. 32. Relationship between tolerance and self-fertility of tolerant population of *Anthoxanthum* from Trelogan



these possibilities. They are therefore explored theoretically in Chapter V.4.

Since the results presented here establish a clear difference in self-fertility between the two populations, but provide little information about the possible causes of such a difference, a further investigation was undertaken.

(c) Self-fertility in a range of tolerant populations.

The relationship between self-fertility and other population characteristics were studied on a range of populations quite unrelated to the previous.

(i) Method.

Populations (10 plants each) of *Agrostis tenuis* were collected in April 1965 from 29 lead contaminated areas in Cardiganshire. The mines from which the populations came are listed in Appendix 2.

The following population characteristics were recorded:

(a) Shortest distance between the population and the edge of the mine: this was estimated visually and gave a measure of the proximity of the tolerant populations to non-tolerant pasture populations.

(b) Area of the mine from which the population came: this was also estimated visually and gave an estimate for the proximity of the mine populations to pasture populations. Area is in some ways a better estimate than distance, since if for example the wind direction over the distance measured is off the mine then the population is from a gene flow standpoint 'far' from this edge.

(c) Plant density: the density of the individuals in the area of collection was estimated on the following scale:

1. isolated
2. widely scattered
3. sparsely distributed

4. frequent
5. close
6. individuals not easily distinguished and forming a sward

(d) Age of the mine: this was obtained from Jones (1922)

(e) Flowering time: the flowering stage of the individuals was noted in July, 1966. This has already been discussed (Chapter IV.3).

The plants were grown in standard greenhouse conditions for several months and tested for self-fertility. In as many cases as possible two replicate selfs were made and there is good agreement between degree of selfing in replicates, as already discussed (Table 15b). Ten plants from each population were tested and the analysis of variance (Table 17) shows significant (P = approx. 1%) differences between populations.

Table 17. Analysis of variance on degree of self-fertility in different mine populations of *Agrostis* from Cardiganshire

Source	Sums of squares	d.f.	mean square	F
Total	24.857	271		
Populations	3.855	27	1.428	1.659 *
Error	21.002	244	0.861	$F_{27,244}$ = 1.814 at $P < 1\%$

A log transformation has been used because there was a marked skewness within populations in the direction of low selfing.

(ii) Results

The multiple non-linear regression of selfing on four features, namely distance, area, density and age is not significant and selective regression techniques fail to pick out any particular relationship as significant even on a log transformation. (Flowering time, which only became available later, could not be included in this analysis). This probably lay in the fact that many of the variables were themselves correlated. This has the effect of reducing the contribution to the regression since a negative covariance term is present in the regression constants. Individual regressions on the separate variables (Figs. 33-35 and Table 18) confirmed that they were correlated.

(a) Distance and area.

Apart from the main collection of populations from Cardiganshire in 1965, plants were also collected from a few mines in 1964. These mines were different from those investigated in 1965, and only their area could be estimated (from 2.5 inch: 1 mile maps) since when these plants were collected the higher self-fertility of mine populations was not known.

The degree of selfing has been plotted against the distance of the population from the edge of the mine, and against the square root of the area of the mine (Fig. 33). The square root of the area has been used so that the size of the mine can be considered in terms of "distance" i.e. on a linear scale. The graphs are difficult to interpret. It is seen that selfing is generally greater where there is greatest gene flow, i.e. towards the edge of the mine or on smaller mines. Such trends are seen clearly in the case of selfing/area in both 1964 and 1965. All the regressions are moreover positive quadratic and negative linear: the approximate pattern of pollen distribution from a source. If four exceptional populations are not considered in the calculations (shown as points above the dotted line in Figs. 33 and 34) then the regressions of selfing on distance and selfing on square root of area (1965) are significant (Table 18b).

The exclusion of four populations from the calculation is obviously questionable. However several reasons suggest that this may not be wholly invalid:

1. The same trends are seen even if the four points are included in the calculation.
2. The four populations are the populations with the highest degree of self-fertility: they may have become 'adapted' to inbreeding and genes for self-fertility are spreading through the population

Fig. 33. Relationship between self-fertility and distance from non-tolerant plants in tolerant populations of *Agrostis* from Cardiganshire.
(in this and subsequent set of figures:
the points indicate individual populations,
points above horizontal line not included in fitted regressions)

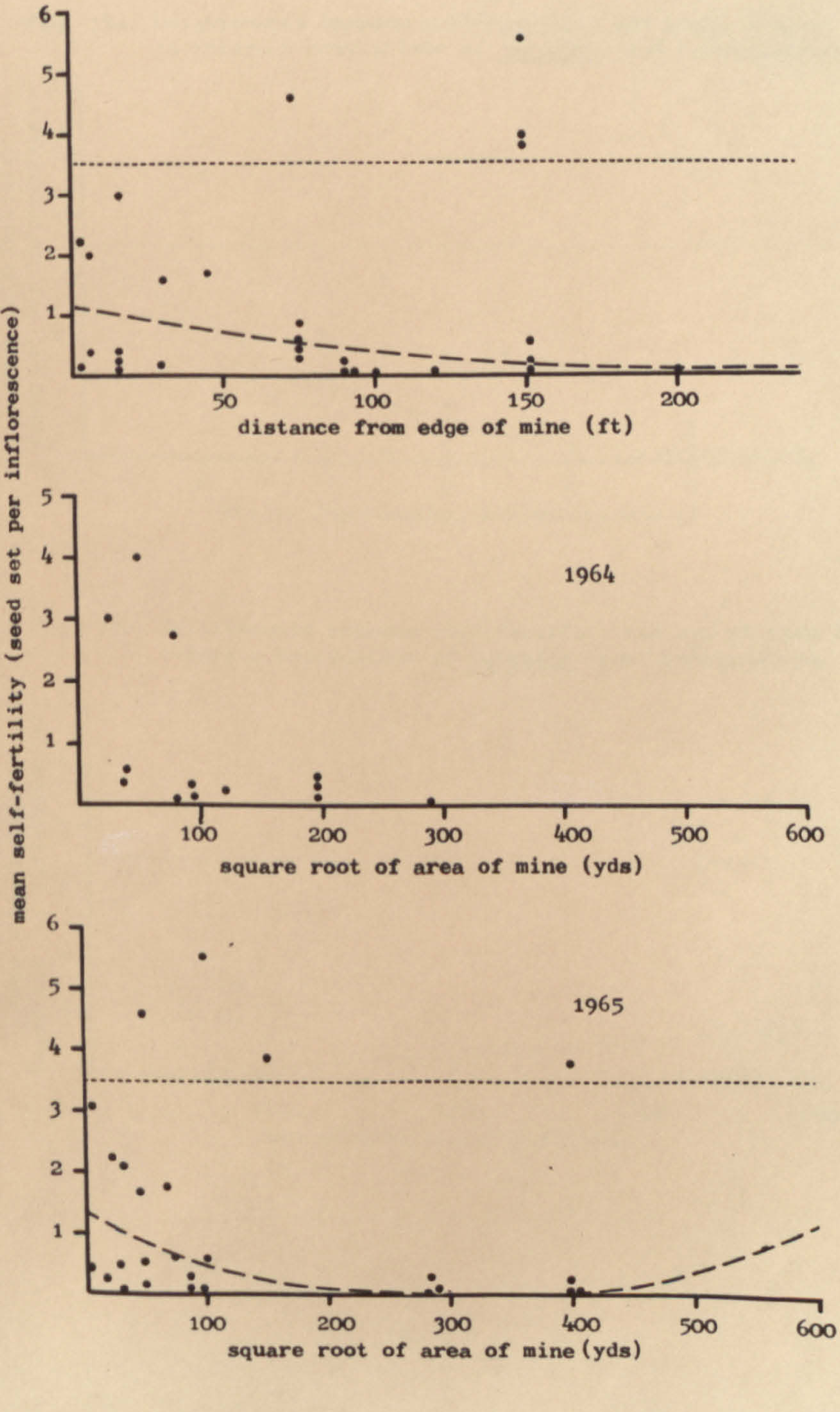


Fig. 34a. Relationship between self-fertility and plant density in tolerant populations of *Agrostis* from Cardiganshire.

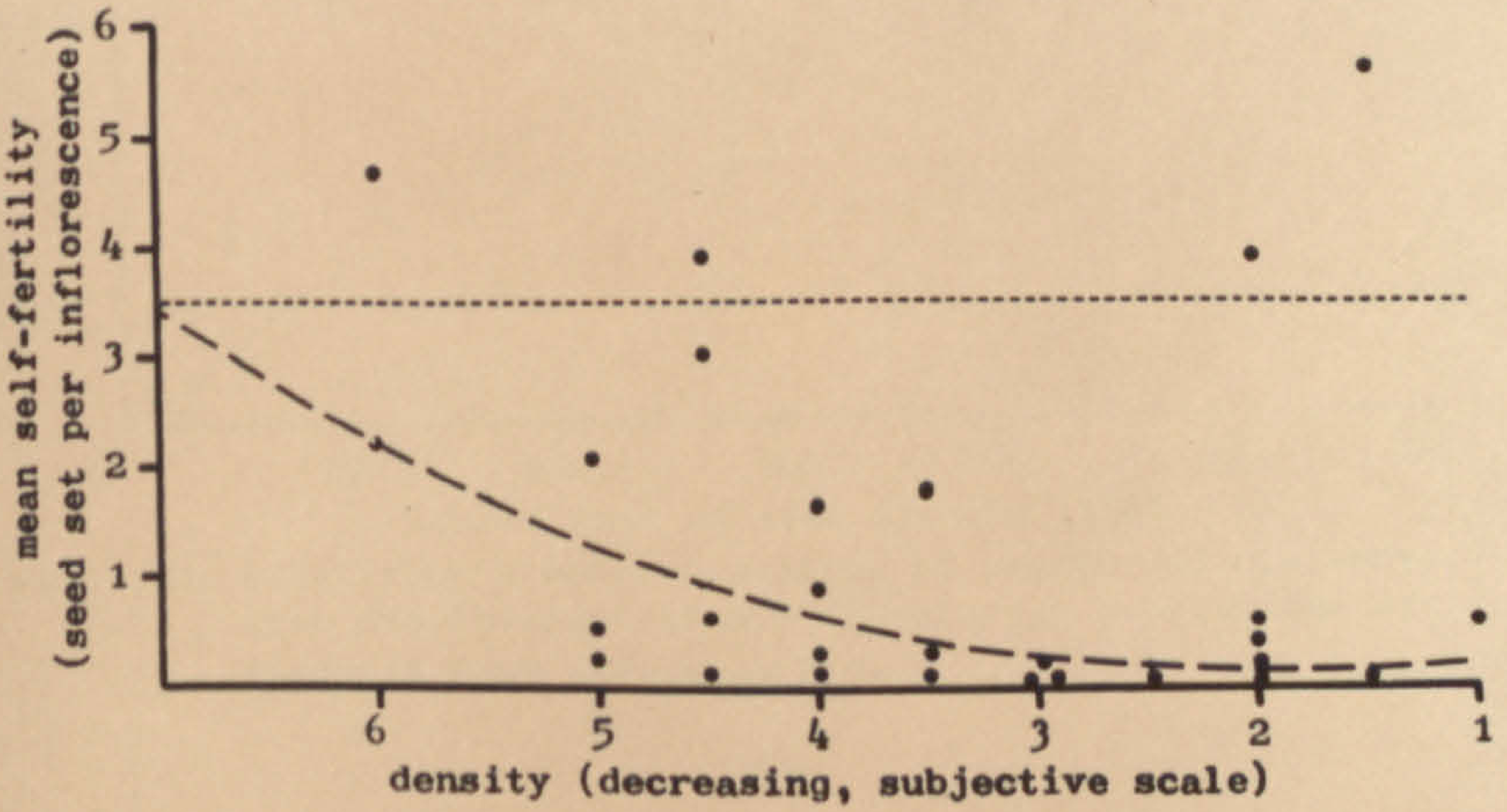


Fig. 34b. Relationship between self-fertility and age of mine in tolerant populations of *Agrostis* from Cardiganshire.

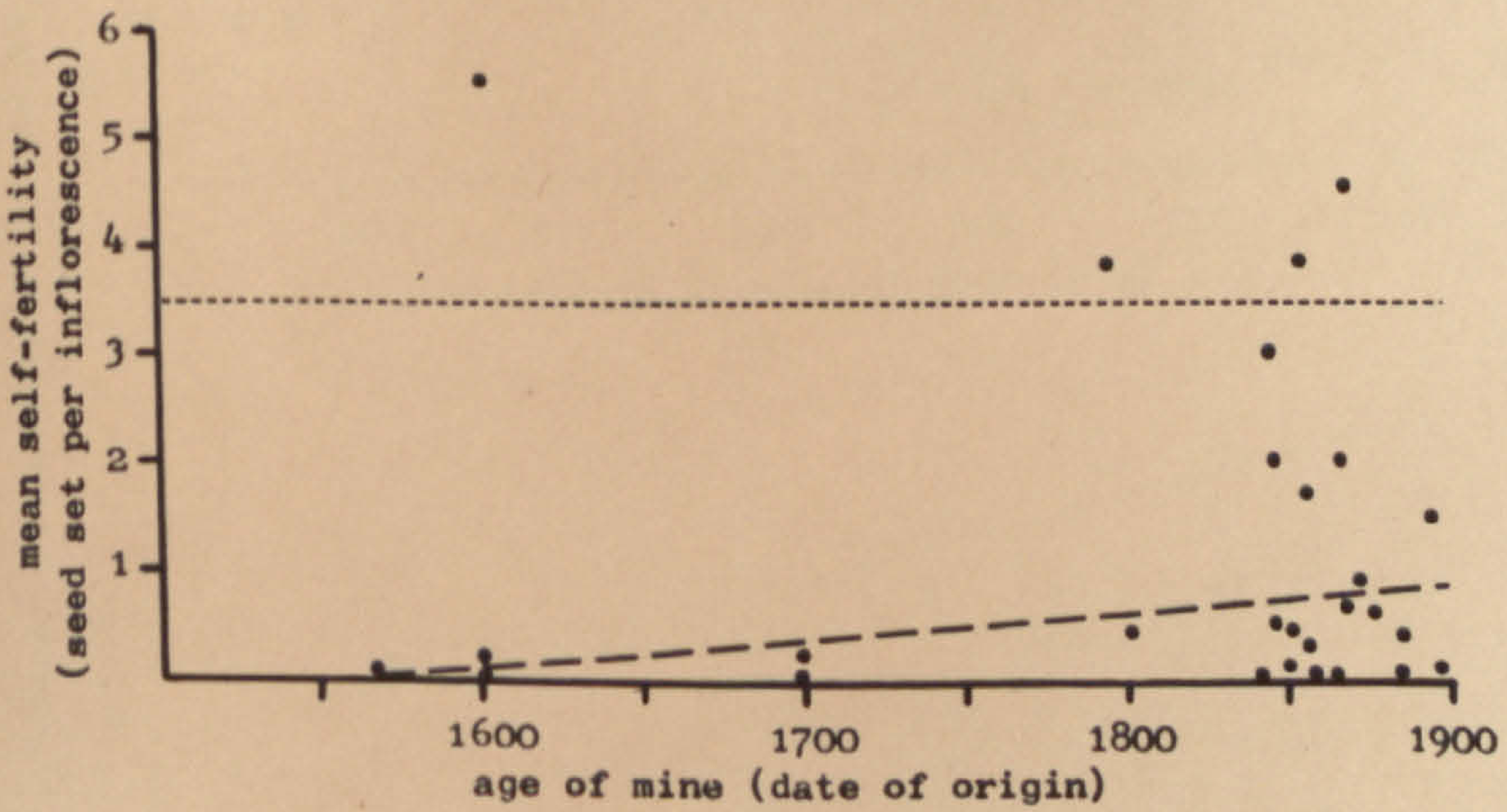


Fig. 35a. Relationship between distance of tolerant populations from edge of mine and area of mine in tolerant populations of *Agrostis* from Cardiganshire.

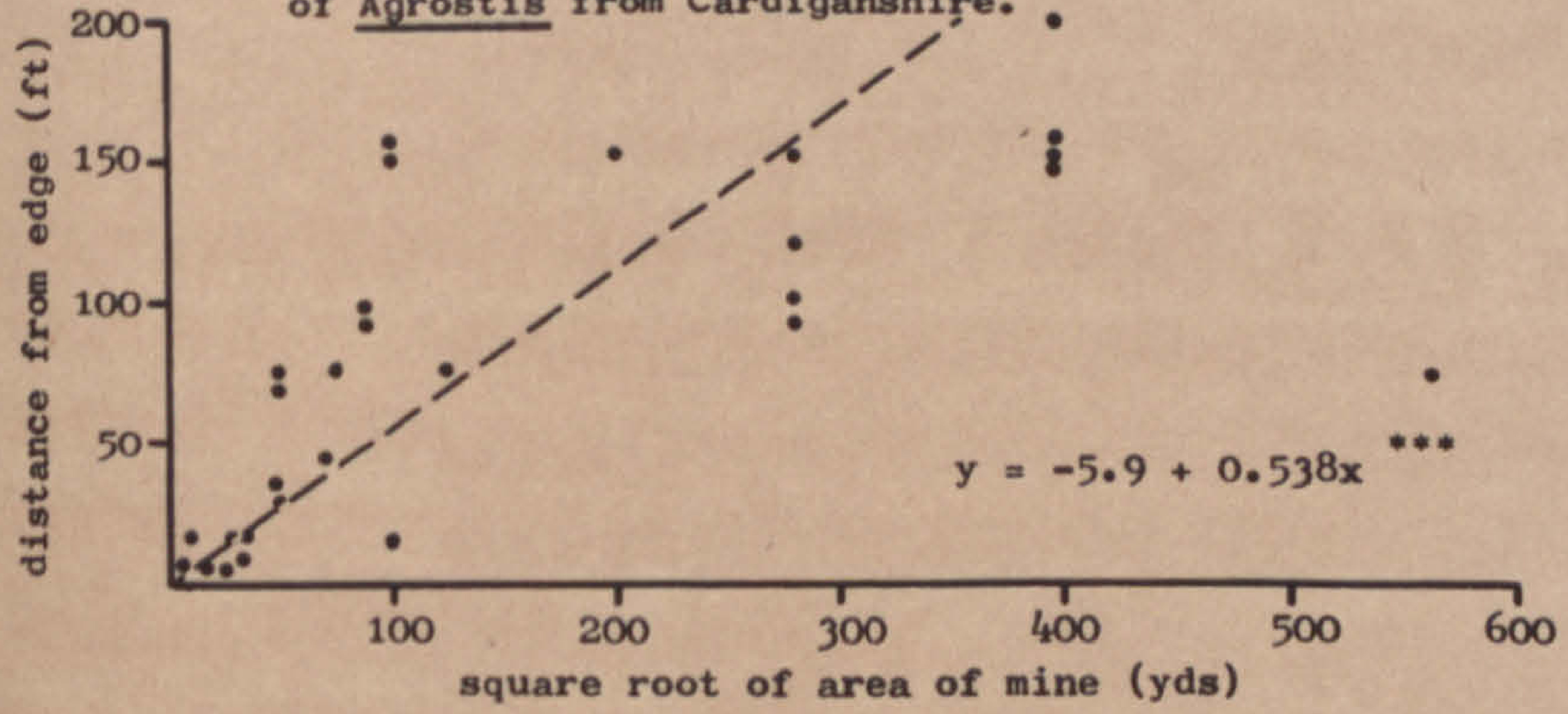


Fig. 35b. Relationship between density of plants and distance from non-tolerant plants in tolerant populations of *Agrostis* from Cardiganshire.

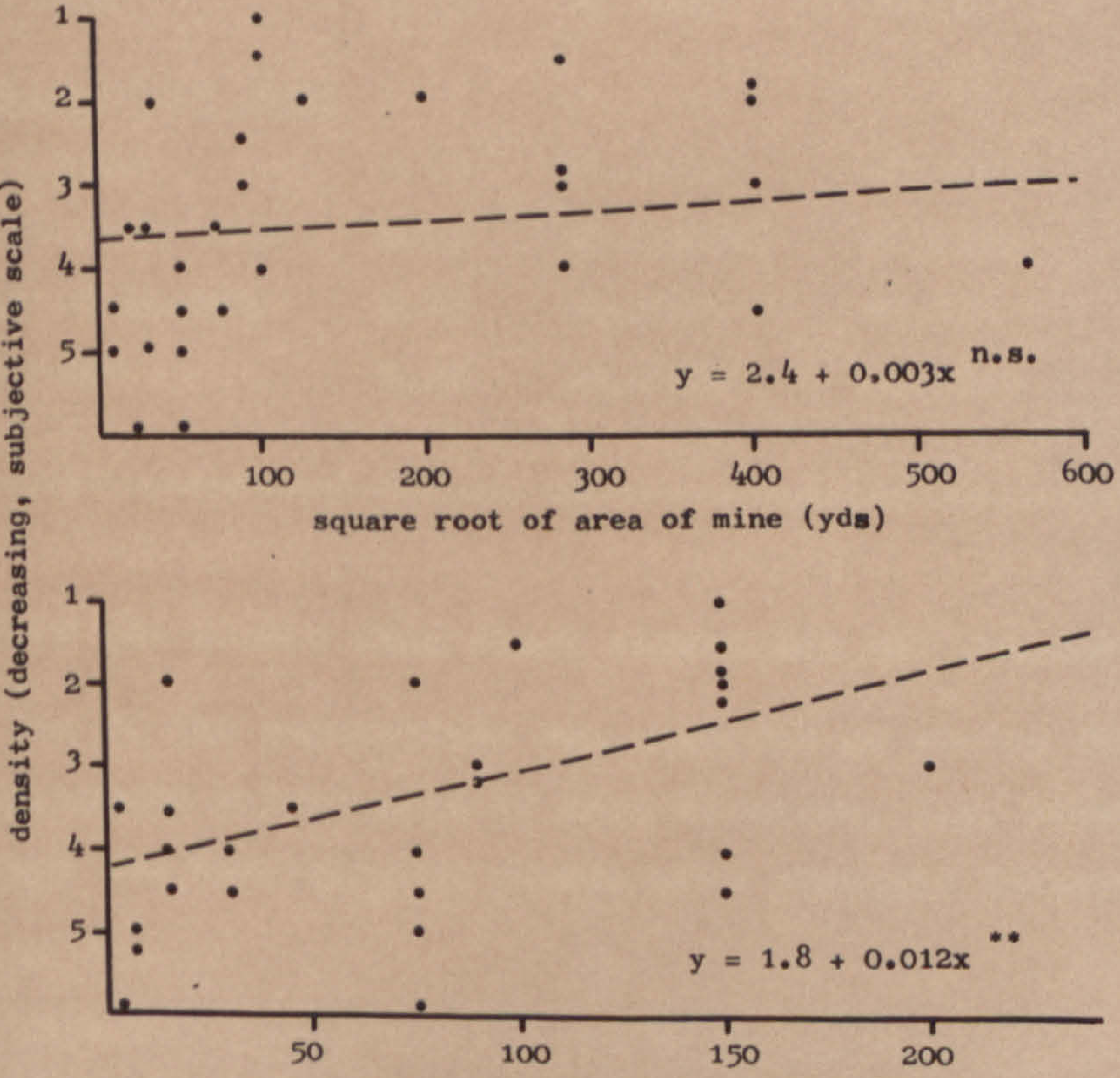


Table 18a. A relationship between self-fertility and various population features of Agrostis mine populations from Cardiganshire.

	Regression parameters and their significance			Significance of regression
	Constant	Coefficient of x	coefficient of $x^2 \times 10^4$	F
<u>All points</u>				$F_{2,26} = 3.37$ at $P = 5\%$
<u>untransformed data</u>				
area of mine	2.35	-0.0077	+0.22	0.33 n.s.
distance from edge	2.25	-0.014	+1.1	0.52 n.s.
density of plants	6.70	-0.27*	+35*	3.66 *
age of mine	1.79	+0.0048	-0.11	0.03 n.s.
<u>log transformed data</u>				
area of mine	0.13	-0.00032	-0.00056	0.53 n.s.
distance from edge	0.12	+0.000010	-0.0026	0.04 n.s.
density of plants	0.46	-0.020*	+2.6*	3.82 *
age of mine	0.07	-0.00026	-0.0034	0.09 n.s.

of their own accord (see final discussion and Chapter V.3).

3. One of the populations was recorded as coming from a large but extremely patchy area of contamination, i.e. pockets of tolerant and non-tolerant plants were probably intermingled.

Table 18b. A relationship between self-fertility and various population features of <u>Agrostis</u> mine populations from Cardiganshire.

	Regression parameters and their significance			Significance of regression
	Constant	Coefficient of x	Coefficient of $x^2 \times 10^4$	F
minus four highest selfing points				$F_{2,22}=3.44$ at P=5
untransformed data				
√area of mine	1.30	-0.0092*	+15*	4.49*
area of mine	1.89	-0.013	+0.40	2.85
distance from edge	2.14	-0.012*	+0.37	2.34
density of plants	4.37	-0.13**	+13	5.59*
age of mine	0.63	+0.0044	-0.035	1.18
log transformed data				
area of mine	0.11	-0.0015**	+0.032**	7.99**
distance from edge	0.13	-0.00092**	+0.016	5.13*
density of plants	0.28	-0.0088*	+0.83	4.48*
age of mine	-0.0054	-0.00011	+0.0048*	2.92
relationship between various population features				
area/distance	-4.11	0.61**	4.0	4.95*
area/density	-73.42	6.84	8400	1.00
area/age	104.97	-0.56	11	0.14
distance/density	[illegible]	5.3	2500**	5.11*
distance/age	141.21	-0.44	7.0	0.53
density/age	5.54	-0.16	2.9	0.74

(b) Density.

The regression of selfing on plant density is significant (Table 18 and Fig. 34a). However the negative relationship between the two is unexpected; whereas low density could well lead to an increase in selection for self-fertility, it is difficult to see that a high density would do so.

However plant density is related to distance (Table 18 and Fig. 35b) and some of the most dense populations come from the smallest mines (Fig. 35c). In other words, the relationship between selfing and density might be a consequence of the relation between selfing and area and distance. From the available data it is impossible to decide if this is the case.

The relationship between selfing and area is itself interesting, and suggests either that the ecological conditions at the edge of the mines are more favourable for colonisation (i.e. less toxic) or that there are towards the edge of the mines more seeds available for colonisation.

(c) Age.

No clear relationship between selfing and age emerges (Fig. 34b and Table 18). A significant regression is obtained only on a log transformation and when the four exceptional populations are not considered: selfing is greater if the mine is younger.

(d) Flowering time.

Flowering time does not appear to be related to selfing. No significant relationship was obtained, and the trends do not show any particular features.

$$y = 2.95 + 0.000014x - 13 \times 10^{-8} x^2 \quad P > 10\%$$

(iii) Discussion.

This investigation illustrates the inadequacy of general regression techniques in picking out causative factors of population differences. A more intensive study on fewer mines (measuring degree of metal contamination, wind direction, density of plants in adjoining pasture etc.) might have been more effective, especially since the results presented here show that selfing must be determined by a range of factors, and no one population characteristic emerges as important.

The possible reasons for an increase in the self-fertility of a population discussed earlier, will be considered in turn in the light of the above work on metal mines.

(a) Certainty of fertilisation.

This is an unlikely reason since pollination on the mine should not be any harder than on the pasture. Both populations of both species are wind-pollinated and their anthers shed readily in the wild.

(b) Establishment after long distance dispersal.

The mine populations are normally surrounded by members of the same species, but plants are often found near the centre of large areas of contamination and here establishment may present problems. However, the relationship between selfing and distance is, if anything, negative, although some of the highest selfing points ("exceptional populations") are among the furthest from the edge of the mine.

(c) Prevention of gene flow.

Distinct trends are seen that suggest selfing is to some extent related to gene flow, being more where there is likely to be more gene flow.

(d) Exposure of recessives.

The genetic analysis of tolerance (Chapter VI) indicates that this character is dominant. Selfing is therefore not likely to have been important in preadapting populations by revealing tolerant recessives.

(e) Earliness of flowering.

No relationship between selfing and flowering time is detectable. Presumably flowering time is so well regulated that it is unusual for individuals to be as precocious as to remain unpollinated.

(f) Density of plants.

The relationship between selfing and plant density is in the opposite direction to that expected. Possible reasons for this have already been discussed.

(d) Self-fertility and vigour.

Studies here were all done on Anthoxanthum. Normal populations of this plant are strongly outbred (see above and Borrill, 1963) and the plants show marked protogyny. It might therefore be expected that the greater self-fertility of the mine populations leads to inbreeding depression. This was investigated by measuring the morphology and self-fertility of different genotypes, and by a competition experiment.

(i) Morphology of selfed plants.

Since the self-fertility of Anthoxanthum was measured (see Chapter IV.4.b) on the same genotypes that were used in the spaced plant trial (Chapter II.4), the relationship between morphology and selfing within the tolerant population was investigated (Table 19) using regression methods. There is no evidence that plants that self more are in any way less 'fit' than plants that self less. This is perhaps not surprising because

(a) they were grown as spaced plants

(b) they were already the result of selection in the mine population: any non-vigorous individuals would have been eliminated.

Because no effect of selfing on fitness could be detected in the spaced plant trial the performance of individuals grown from seed produced by selfing was studied in dense pure stands and in competition with seed produced by crossing.

(ii) Competitive performance of selfed material.

(a) Method.

In order to assess the competitive performance of selfed material, the following types were grown in 50 : 50 mixture and pure stands, after the techniques of De Wit (1960).

Tolerant crosses / Non-tolerant crosses
Tolerant crosses / Tolerant selfs

Table 19. Relationship between self-fertility and morphological characters in the tolerand population of *Anthoxanthum*

Character(x)	Constant	Regression coefficients x	 x^2	Expected direction of relationship	Significance P
Height	39.2	-0.00017	-0.000024	-	> 10%
Number of vegetative tillers	41.0	-0.097	+0.0018	-	> 10%
Number of flowering tillers	30.1	+0.0047	[illegible]0.00011	-	> 10%
Vegetativeness (Log)	1.934	-0.0027	-0.0000065	?	> 10%
Within plant variation	63.4	-0.014	+0.000036	+	> 10%

Tolerant crosses / Tolerant x Non-tolerant crosses.
Non-tolerant crosses/ Tolerant x Non-tolerant crosses.

The first combination was used as a control to look at the general competitive performance of tolerant material; the second was to look for any inbreeding depression in competitive performance and the last two to look for any hybrid vigour between the populations.

Pure stands at half-density were also included so that the percentage reduction by a competitor ("selection pressure" due to the other type) could be assessed (Seaton and Antonovics, 1966).

Seed from selfs and from artificial crosses was used in this experiment. This eliminated any effects of gene flow (c.f. natural seed) and also meant that seed could be chosen from crosses where the parents were known not to self. However because the amount of seed was limited the experiment was on a small scale (two replicat

The plants were sown as seedlings (previously germinated in damp filter paper in petri-dishes) at two inch spacing in a grid pattern in wooden tomato boxes, filled with sterilised loam. Loam is preferable to John Innes in showing competitive relations (McNeilly, 1965). In the competition "plots" the types were arranged alternately. Twenty-four plants were put in each box as well as a guard row of the appropriate types. The material was sown in October 1965 and assessed in April and August 1966.

The parent source of the seed produced by selfing was noted so that a comparison of the performance of seed produced by genotypes with different amounts of selfing could also be made.

(b) Results.

Because of seed shortage there was inadequate replication and the results cannot be considered as conclusive. They are presented in Tables 20 and 21 and as Replacement Series Graphs (De Wit, 1960) in Fig. 36. Because the material was required for further experimentation, only tiller number was measured.

Behaviour as pure stands (Table 20):

It can be seen that progeny produced by selfing of tolerant

Table [illegible]. Yields (tiller number) of pure stands and mixtures of types of *Anthoxanthum*.

-x	non-tolerant cross
+x	tolerant cross
-s	non-tolerant self
+s	tolerant self
±x	tolerant x non-tolerant cross

	Mixture	April 1966			August 1966		
		Rep. 1	Rep. 2	Mean (1)	Rep. 1	Rep. 2	Mean (1)
yield of pure stand at full density	+x/+x	213	191	202	199	165	182
	-x/-x	243	260	252	389	257	270
	±x/±x	223	192	212	254	180	217
	+s/+s	161	196	183(91)	193	197	195(107)
	-s/-s	217		317(86)	194		194(72)
yield of pure stand at half density	+x/+x	146	231	188	183	284	236
	-x/-x	184	201	192	218	229	224
	±x/±x	128	94	111	161	179	170
	+s/+s	178	85	131(70)	193	117	155(66)
yield of mixtures	[illegible]	115/160	67/72	91/116	123/206	70/130	94/169
	+s/+x	84/89	83/146	83/117	74/111	73/198	74/155
	+x/±x	120/148		120/148	106/113		106/113
	-x/±x	102/149	128/132	115/143	130/154	87/174	112/164

(1) Figures in brackets refer to yield of selfs as % of yield of cross.

Fig. 36a. Yield (tiller number) of progeny of tolerant crosses and non-tolerant crosses in mixtures and pure stands.

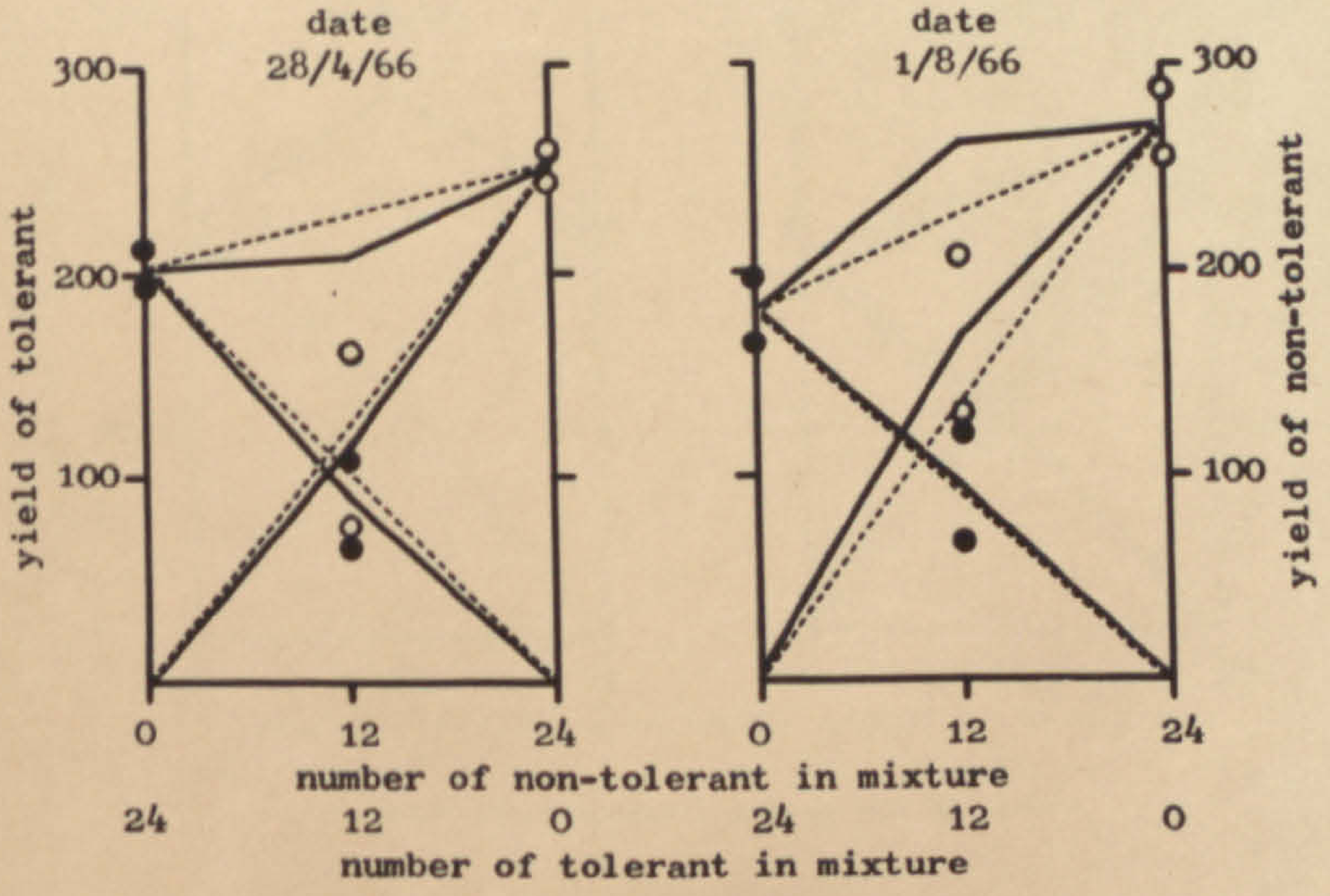


Fig. 36b. Yield (tiller number) of progeny of tolerant selfs and tolerant crosses in mixtures and pure stands.

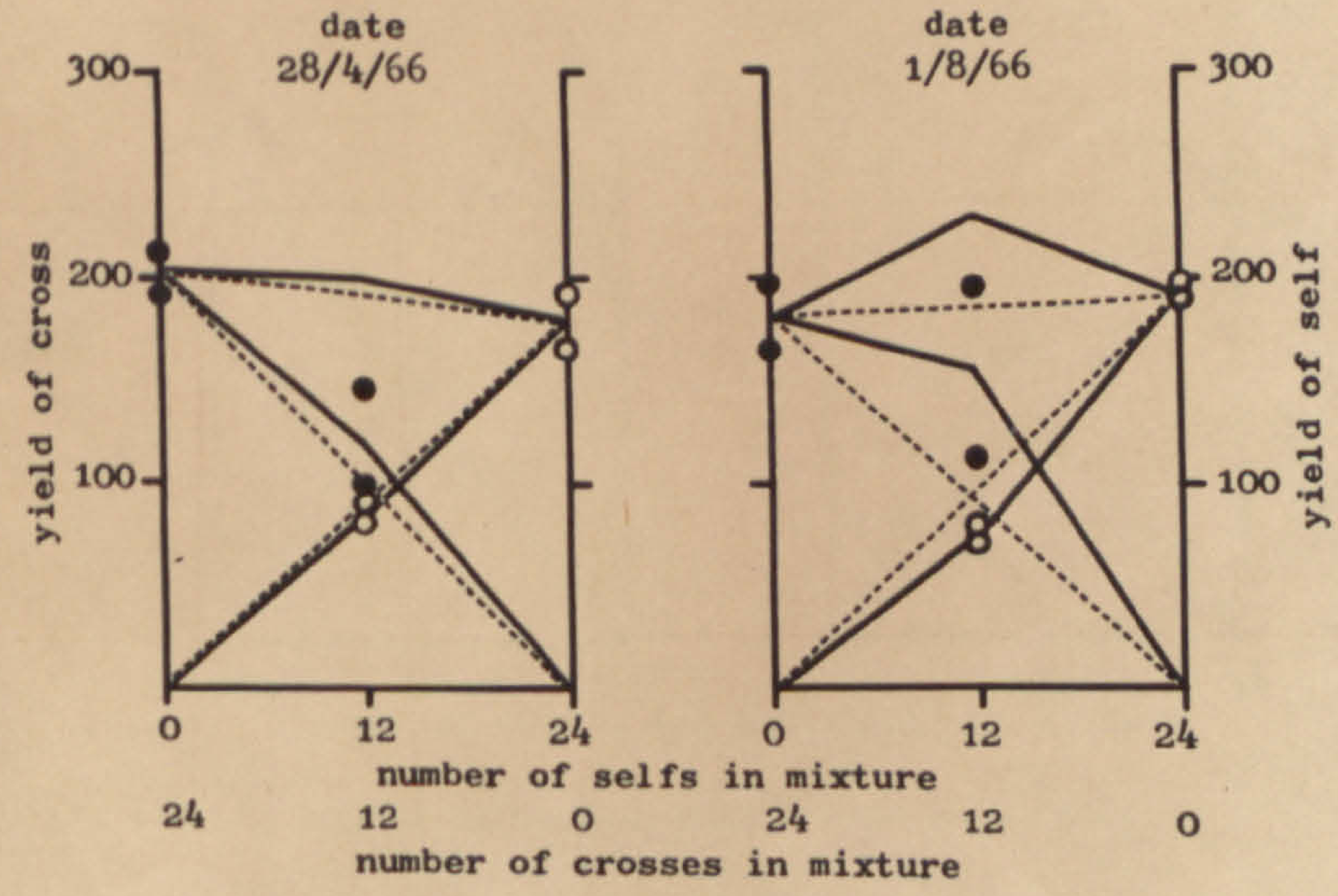


Fig. 36c. Yield (tiller number) of progeny of tolerant crosses and tolerant x non-tolerant crosses in mixtures and pure stands.

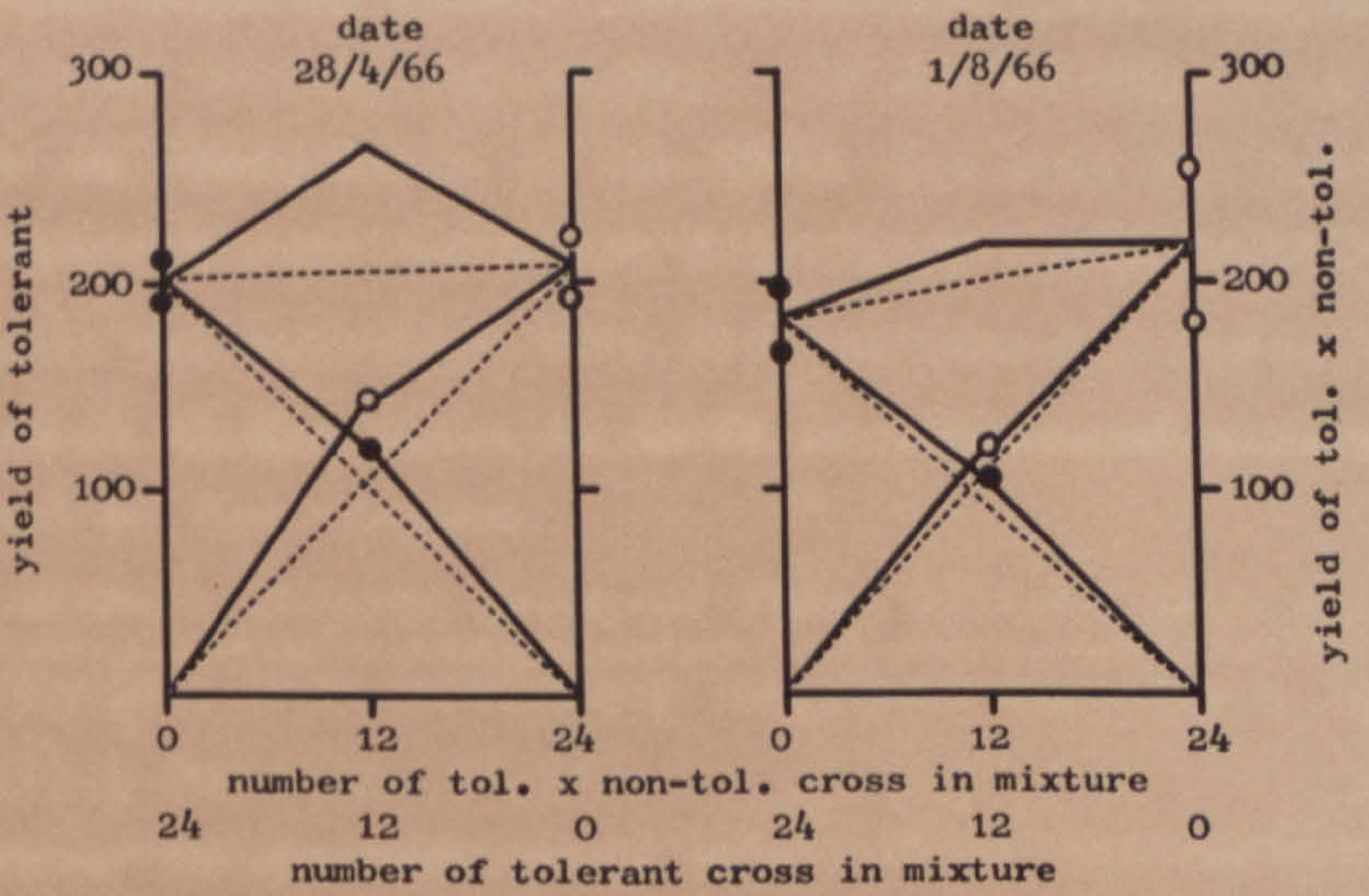
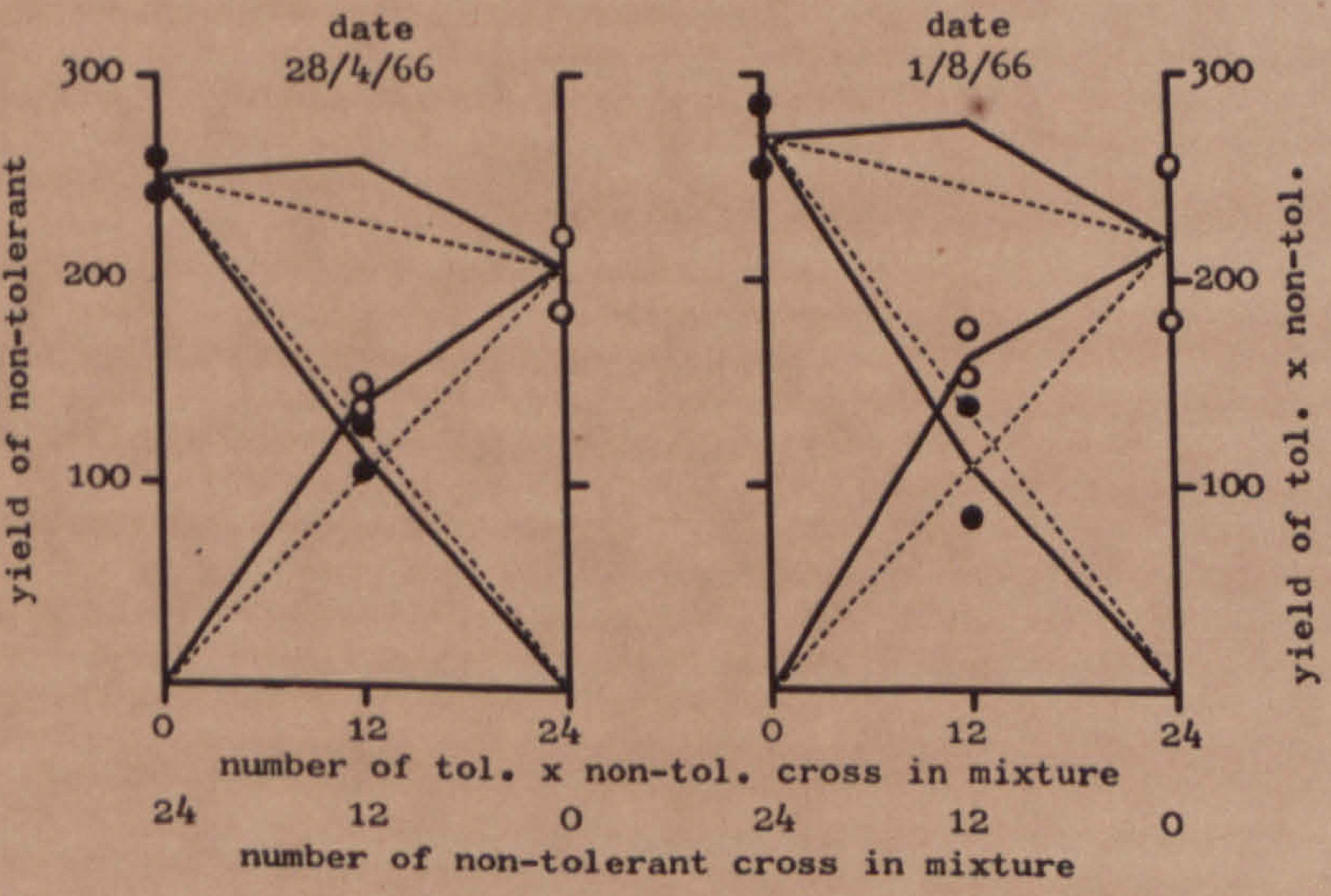


Fig. 36d. Yield (tiller number) of progeny of non-tolerant crosses and tolerant x non-tolerant crosses in mixtures and pure stands.



plants do not do much worse than those produced by crosses when pure stands are considered. Thus at full density the agreement between replicates is reasonable and in April 1966 the selfs yielded 91% as much as the crosses. In August they yielded more than the crosses, 107%. The data from half density showed great variation between replicates: in one replicate no great difference was seen, while in the other the self did far worse.

When non-tolerant selfs are considered (unfortunately only one replicate was possible) they did considerably worse than the tolerant selfs if the yield of selfs is considered a percentage of the yield of the crosses.

The tolerant crosses produced fewer tillers than the non-tolerant crosses, thus confirming the field experiment data (Chapter III.4 and Figs. 16,17).

Behaviour in mixtures (Fig. 36):

The results show that non-tolerant plants contribute more to the yield of a mixture than tolerant, and that tolerant crosses yield more in a mixture than tolerant selfs. These results are confirmed if we look at the percentage reduction from pure stands at half density (Table 21). The selection pressures are strongest on the tolerant type in the tolerant/non-tolerant mixture, and on the selfed type in the self/cross mixture.

This is confirmation that tolerant types are competitively inferior to non-tolerant (McNeilly, 1965, Putwain, personal communication). It also suggests that inbreeding depression does occur in mine plants as a result of selfing, but not to a very marked degree.

The results of competing tolerant x non-tolerant crosses against crosses within populations are also interesting since they show the between population crosses to be rather better in competition. This is particularly so when the non-tolerant type is involved. "Hybrid vigour" therefore seems to occur when the two types are crossed. If this effect is real then it has quite

Table 21. Percentage reduction in yield (tiller number) due to the other component in the mixture (yield at half density compared with yield in 50 : 50 mixture).

Types in mixture	% reduction	
	April	August
+ x	52	60
- x	40	25
+ x	38	34
+ s	37	52
+ x	36	55
± x	-33	34
- x	40	59
± x	-29	4

important consequences in considering the effects of gene flow.

(c) Behaviour of plants produced by genotypes with different amounts of selfing(Fig. 37).

Since the individual genotypes used in this experiment were noted, it was possible to investigate the relationship between degree of selfing and performance in pure stand under high density and in competition with the crosses. The results both show the same pattern, and the relationship is highly significant in the case of genotypes from competition with material from crosses: the better yielders (tiller number) are both low selfers and very high selfers, with intermediate types yielding the worst.

(iii) Discussion.

The results of this small experiment are extremely interesting. Not only is the poorer competitive performance of tolerant types (cf. non-tolerant) confirmed but inbreeding depression effects

Fig. 37a. Yield (tiller number) of progeny from selfs of parents with different degrees of self-fertility, in crowded pure stands.

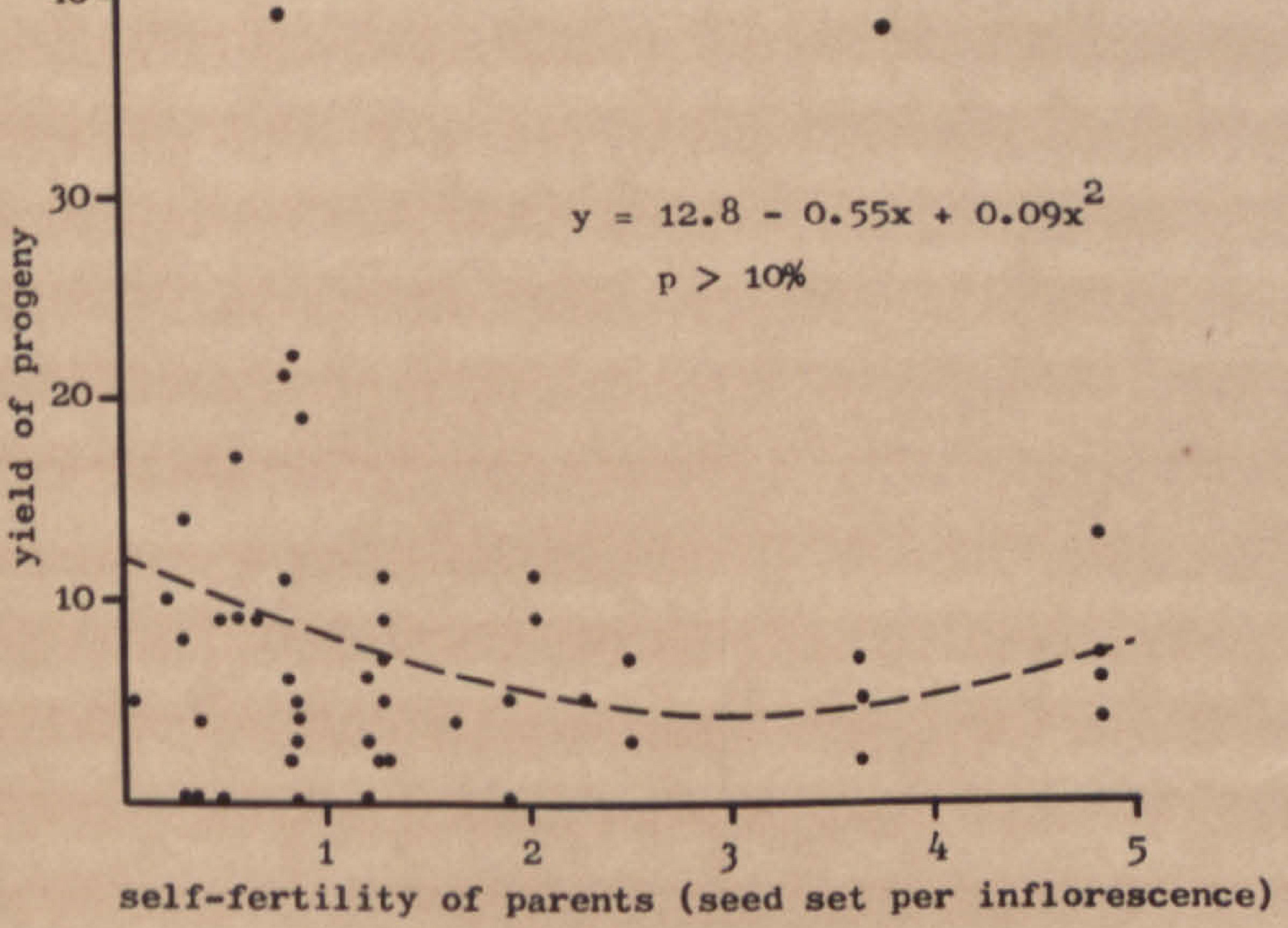
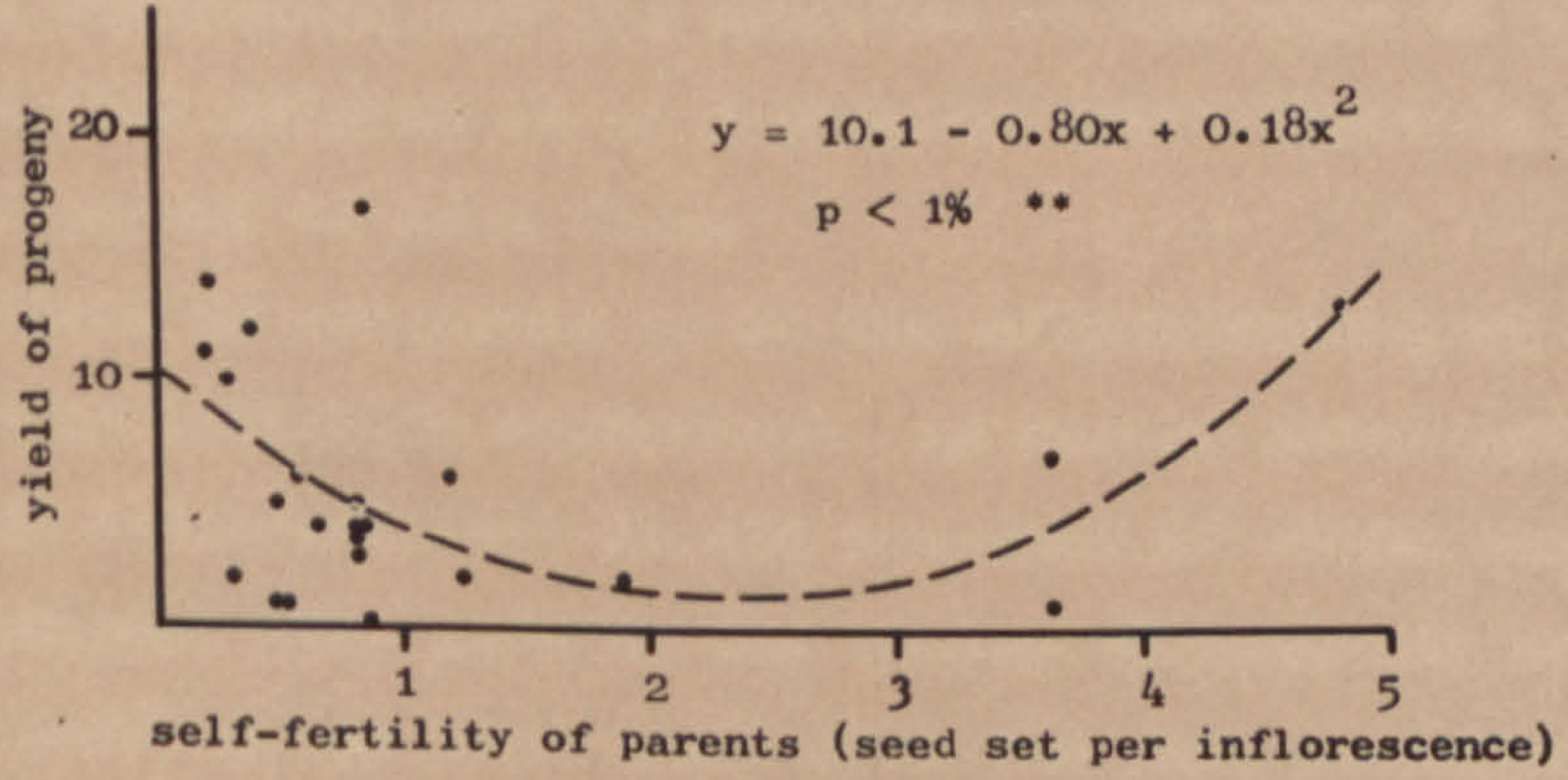


Fig. 37b. Yield (tiller number) of progeny from selfs of parents with different degrees of self-fertility, in mixtures with progeny from crosses.



become apparent in mixtures of selfed and crossed material. However, the inbreeding depression is only seen clearly in competition and the depression seems less than in the non-tolerant population. This suggests that the tolerant population has become adapted to inbreeding, a hypothesis which is confirmed by the individual performance of genotypes in competition: the high selfing types yield as much as the low selfing types, while the intermediate selfers do worst. The high selfing types are likely to have had a history of inbreeding and therefore could have become adapted to it. In the intermediate selfers inbreeding may be more recent or may have been eliminated largely by outcrossing and they are therefore not adapted to inbreeding.

(e) General discussion.

The existence of populations with different degrees of self-fertility opens up the possibility of investigating the adaptive significance of different breeding systems. A preliminary investigation presented here has shown that no one selective factor can be considered to have determined the breeding system, but that a fairly consistent relationship exists between the degree of selfing of a population and its nearness to the edge of the mine. This implies that selfing is at a premium where there is a considerable amount of gene flow.

No evidence is available as to the effectiveness of self-fertility as an isolating mechanism: no marker genes are available and self-fertility in the presence of other genotypes cannot be tested. The gain in the number of tolerant seeds that result from selfing may seem small from the data presented here, but two factors must be remembered.

Firstly, although the mean amount of selfing is low, tolerant populations do contain individuals with a very high self-fertility, often at values approaching normal cross fertility (Table 16).

These individuals are likely to be more successful than the rest of the population, when gene flow load is serious.

Secondly, the amount of selfing is measured at Drws-y-Coed and Trelogan at a stage when evolution has already progressed for some time. In the earlier stages of colonisation, the density of plants on the mine was probably far lower than the density ~~on~~ the pasture: in other words the effects of gene flow were more serious then than they are now (see Chapter V.1.a for theoretical analysis). We expect a similar situation to that described by Bannister (1965), namely an initial increase in the amount of selfing followed by a decline. It is therefore interesting that the relationship of (log) selfing on the age of the mine from which the population came gave a small but significant regression: the later the date of the mine (the younger the mine), the greater the amount of selfing.

Another factor which could promote selfing on mines is inbreeding amongst a few founder individuals. In the face of gene flow, the successful plants under these conditions are most likely to be 'sib-matings' between the founders. This would lead to an initial readjustment of the gene complexes to preadapt them to the more violent effects of inbreeding depression that would otherwise result from selfing. Such a system is suggested by Wexelsen (1952) and Rolands (1961) for achieving successful self-fertile lines in legumes. Breese (1956) in an experimental and theoretical analysis of assortative mating (mating of like with like) has said that such mating "is the initial and most important step towards the establishment of facultative inbreeding in hitherto obligate outbreeding species".

It is also possibly one of the reasons why selfing does not occur off the mine populations: these non-mine habitats have been long colonised and the populations have never suffered from serious inbreeding. Another reason for the absence of selfing in non-mine populations (which also suffer from gene flow) is the fact that gene flow load is greater, the higher the selection pressure (Chapter V.2.c): it is unlikely that the selection pressure in the pasture is anywhere near the same order as that on the mine (McNeilly, 1965).

Tentative evidence for the mine populations having become 'adapted' to selfing (and presumably inbreeding) is presented, and this may be a very important element in understanding the change to inbreeding. This is particularly so in relation to the results of the computer simulation (Chapter V.3) which shows that a gene for self-fertility will spread through the population of its own accord, if there is no selection against it. However the efficiency of gene flow in maintaining heterozygosity (Chapter V.2.d) suggests again that inbreeding depression may not be serious in mine populations: heavy selection for tolerance can retain this character in the population despite gene flow from non-tolerant plants, but this gene flow will also cause other characters to be highly heterozygous. The roles of selfing in mine populations are discussed later, after a presentation of the results of the computer simulation (Chapter V).

The conclusions arrived at here are applicable to any colonising species moving into new ground by evolutionary advance; and the higher self-fertility of mine populations may show the beginnings of the processes that have made so many colonising species self-fertile.

Chapter V

THE PROCESS OF EVOLUTION

A COMPUTER MODEL

1. THE BASIC MODELS

Before considering the results of the computer simulation a fairly detailed account of the model that has been used is necessary, since the conclusions to be drawn from such an investigation frequently depend on the premises on which it is based.

(a) Single gene model

The model used here starts with a single random breeding population, consisting of the genotypes AA, AB and BB. The fate of the population over subsequent generations is obtained as follows:

Genotypes	AA	AB	BB
Genotype frequencies	u	v	w

Assuming mating to be at random the frequencies of the different matings are

	AA	AB	BB
AA	u^2	uv	uw
AB	uv	v^2	vw
BB	uw	vw	w^2

Knowing the frequency of each type of mating we can calculate the genotype frequencies in the next generation, since on the basis of simple Mendelian laws, the products of these matings are known.

The equations giving the genotype frequencies in the following generation are known as recurrence equations since if the frequency in any n'th generation is known, then the genotype frequencies in the (n + 1)'th generation can be calculated. The gene frequencies over a series of generations can thus be calculated to find rates of change and equilibrium positions (when no more change is apparent). Here the recurrence equations were worked out by the computer, and by inserting a loop in the programme, the progress of the genes and genotypes over generations was calculated.

The model therefore is of an idealised population, infinitely large, with no random effects.

Various influences were then put on this population to investigate their effects.

(i) Selection

At each generation the genotypes are subjected to selection pressures as follows:

Genotype	AA	AB	BB
Selection	s_1	s_2	s_3
Fitness	$(1-s_1)$	$(1-s_2)$	$(1-s_3)$

which can be noted as s_u, s_v, s_w respectively.

In subsequent generations the genotype 'frequencies' become

$u' = u \times s_u$ where u', v', w' = frequency in the following generation.

$v' = v \times s_v$

$w' = w \times s_w$

but since $u' + v' + w'$ are now not equal to one, they are converted to true frequencies by dividing by T, where $T = u' + v' + w'$.

(ii) Gene Flow.

Gene flow is imposed on the population by the addition of BB genotypes. If a certain proportion of these genotypes, g, enters the population, the other genotypes are reduced to a frequency of 1 - g, i.e. gene flow is measured as the frequency of incoming genotypes. Two types of gene flow are studied and for the sake of convenience they will be termed pollen flow and seed flow.

(a) Pollen flow.

Selection occurs after the incoming genotypes have mated with the remainder of the population.

The model here is as follows:

	AA	AB	BB	incoming genotype BB
Genotype frequencies males	$(1-g)u$	$(1-g)v$	$(1-g)w$	g
Genotype frequencies females	u	v	w	

Matings take place in the following frequency:

	AA	AB	BB
AA	$(1-g)u^2$	$(1-g)uv$	$(1-g)uw$
AB	$(1-g)vu$	$(1-g)v^2$	$(1-g)vw$
BB	$(1-g)wu$	$(1-g)wv$	$(1-g)w^2$
BB	gu	gv	gw

Genotype frequencies in the next generation are calculated as before and selection is imposed on these.

(b) Seed flow

Selection occurs both on the pre-existing population and on the incoming genotypes, before they mate. Here the extra genotypes do not enter the mating scheme till the genotype frequencies for the next generation (and before selection) are calculated. Then:

$u' = u(1-g)$ where u', v', w' =
$v' = v(1-g)$ frequencies in the following
$w' = w(1-g)$ generation

followed by selection on these genotypes.

(iii) Changing gene flow

It has been stressed previously that in the early stages of colonisation, the density of individuals in an area will probably be low and therefore the gene flow from outside high. As colonisation proceeds, the density increases and therefore the gene flow decreases. To investigate the genetic changes that are likely to occur during early colonisation, a model of changing gene-flow was developed.

The formula for population increase under "limited resources" is as follows

$$N = \frac{K}{1 - e^{n-rt}}$$

where N = number at given time

K = number at end

n = constant to define number at beginning, given by

$$N = \frac{K}{1 - e^{n}}$$

r = constant to determine rate of population increase with time

t = time.

This gives the well known sigmoid curve, where an initial exponential phase is followed by a slowing down to reach a constant number when resources become limiting.

It seemed reasonable to assume that the decrease of pollen flow due to increase in population numbers in a given area (density) would follow a similar pattern. The equation developed for this was as follows:

$$N = (1 - g)(1 - \frac{1}{1 + e^{n-rt}}) + g$$

where

N = gene flow at a given generation

g = final gene flow

n = constant to determine initial amount of gene flow

r = constant to determine rate of decrease of pollen flow with generation

t = generation.

Using this formula the amount of pollen flow at each generation could be defined from an initially high to a final low value.

(iv) Self-fertility

Different degrees of self-fertility are imposed on the genotypes AA and AB, in the following way.

Genotype	AA	AB	
Self-fertility	a_1	a_2	females

If, say, the genotype AA selfs to a degree a_1, then the proportion a_1 of AA females produce offspring without the involvement of males. The remainder of the females (in a frequency $1 - a_1$) breed at random. The frequencies of the different matings are then

	AA		AB		BB
AA	$a_1 u +$	$(1 - a_1)u^2$		$(1 - a_2)uv$	uw
AB		$(1 - a_1)vu$	$a_2 v +$	$(1 - a_2)v^2$	vw
BB		$(1 - a_1)wu$		$(1 - a_2)wv$	w^2
	self	random	self	random	

Recurrence equations are calculated as before. In this model the genotype BB, is <u>not</u> given any self-fertility. This is important. The reason for this is that the model for selfing was developed in conjunction with a model for gene flow where it was desired that the incoming genes BB should not self. To fix the selfing of any genotype <u>also</u> subjected to selection implies that the gene for selfing is linked to the gene that is selected, i.e. the model here is of a gene for selfing completely <u>linked</u> to AA and dominant or recessive according to the value of a_2. An unlinked two gene model is described later.

(v) Perenniality

This feature was imposed on the population by including the genotypes of the previous generation in those of the present one

Thus	AA	AB	BB
Frequency in generation n	u_n	v_n	w_n
Frequency in generation n + 1 after mating, selection, geneflow	$= u'_{n+1}$	v'_{n+1}	w'_{n+1}
Then 'frequency' in new generation	$= u_n + u'_{n+1}$	$v_n + v'_{n+1}$	$w_n + w'_{n+1}$
	$= u_{n+1}$	v_{n+1}	w_{n+1}

after correction for change in total frequency.

In other words we are dealing here not with gene frequencies at each generation separately, but with the <u>cumulative</u> gene frequency.

(vi) Summary

The whole model can be summarised in terms of the situation existing at the boundary of metal contaminated areas. The tolerant population carries a gene for tolerance, A, whereas the pasture population carries, B. There is selection for tolerance on the mine, and non-tolerant genes are continually entering and tending to dilute the tolerance. The tolerant genotypes AA, and the heterozygote, can self to varying degrees, and can be annual or perennial. Selection and gene-flow can also vary, the latter between populations or over generations as colonisation proceeds. The model therefore assumes no two way flow across a boundary. The model is also quite general for any habitat where colonisation and selection from a neighbouring source is occurring.

(b) <u>Two gene model</u>

This was developed to study conclusions from the one gene model further. Essentially it did not differ from the one gene model, except that the 'extra' gene was used to impose dominance (see Chapter VI) and selfing on the population.

Instead of regarding selfing as an 'automatic attribute' of certain genotypes, a gene determining this character was introduced into this model. The gene for this character was unlinked to the other gene, on which selection and gene-flow were imposed in a similar way to that described for the one gene model. The gene for selfing was given the property of "incomplete penetrance": when present in a homozygous state with a genotype of the other gene, a certain proportion a_1 of those genotypes selfed. When present in the heterozygous state, a proportion a_2 selfed. By varying a_2 in relation to a_1 the gene for selfing could be given different degrees of dominance.

(c) The recurrence equations and programmes

Recurrence equations for the one gene and two gene models, when there is pollen flow, and selfing are given in Appendix 3.

The programmes are also given in this Appendix together with a brief description of the method of presenting the data for the programmes and a description of the form of the print out. Only a few selected programmes are included. Modifications are made using the methods described above.

2. THE EFFECTS OF GENE FLOW

(a) Pollen flow

If pollen flow is the only influence on the population, then the incoming type, BB, rapidly spreads through the population. However if there is selection against BB, the result will depend on the intensity of selection and the amount of pollen flow: with low selection pressures and a high degree of pollen flow, the incoming type wins, otherwise an equilibrium is set up with both genes in the population. The result also depends on the degree of dominance of the favoured gene.

Several important features emerge from the data.

(i) Pollen flow is remarkably effective in maintaining a gene in a population in spite of selection against that gene (Fig. 38). For example, when dominance is present, only 0.1 pollen flow will reduce the frequency of the favoured gene to 0.68, when the selection against the incoming gene is 0.4.

(ii) Pollen flow is very effective in maintaining heterozygosity (Fig. 39). When there is dominance of the favoured gene, an incoming recessive gene will be sheltered in the heterozygous state, and this leads to an excess of heterozygotes over random expectation. This may give the impression, from looking at genotype frequencies, that there is selection in favour of the heterozygote. Moreover there is also an excess of heterozygotes when no dominance is present: here the selection against the heterozygote, AB is half that against BB. When the favoured gene is recessive, the frequency of heterozygotes is slightly below random expectation, but not very much so in spite of strong selection against heterozygotes.

The relative number of heterozygotes is also greater, the greater the selection pressure against BB. In other words, on a metal mine where selection pressures are high the number of heterozygotes in the population will be large. The amount of segregation observed in seedlings from mine populations (McNeilly, 1965) supports this.

(iii) The number of generations required to reach equilibrium shows some interesting features. In this model, the equilibrium position

Fig. 38. Effect of selection and pollen flow on gene frequency at equilibrium.
(selection coefficients indicated on graphs, degrees of dominance are of favoured gene)

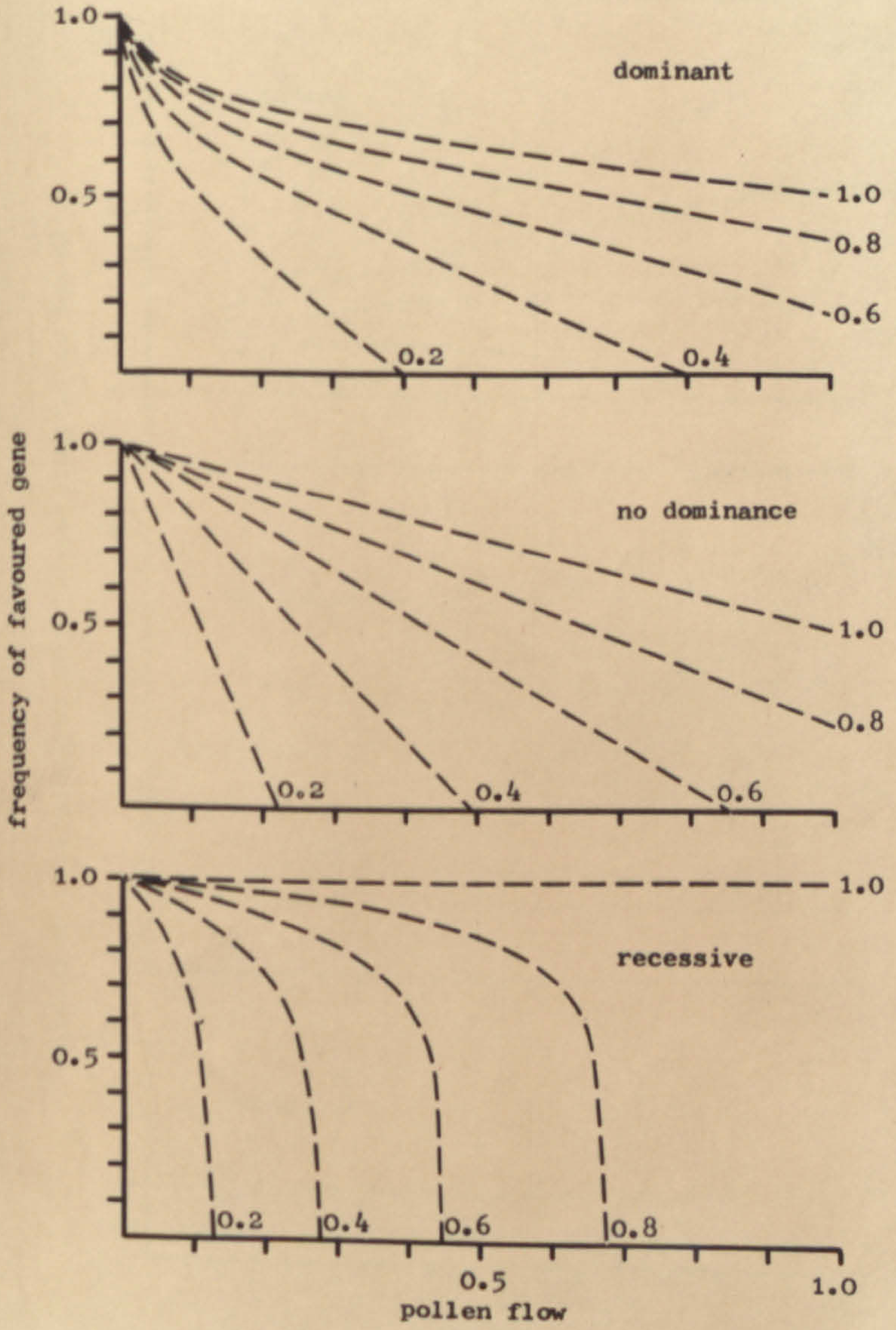
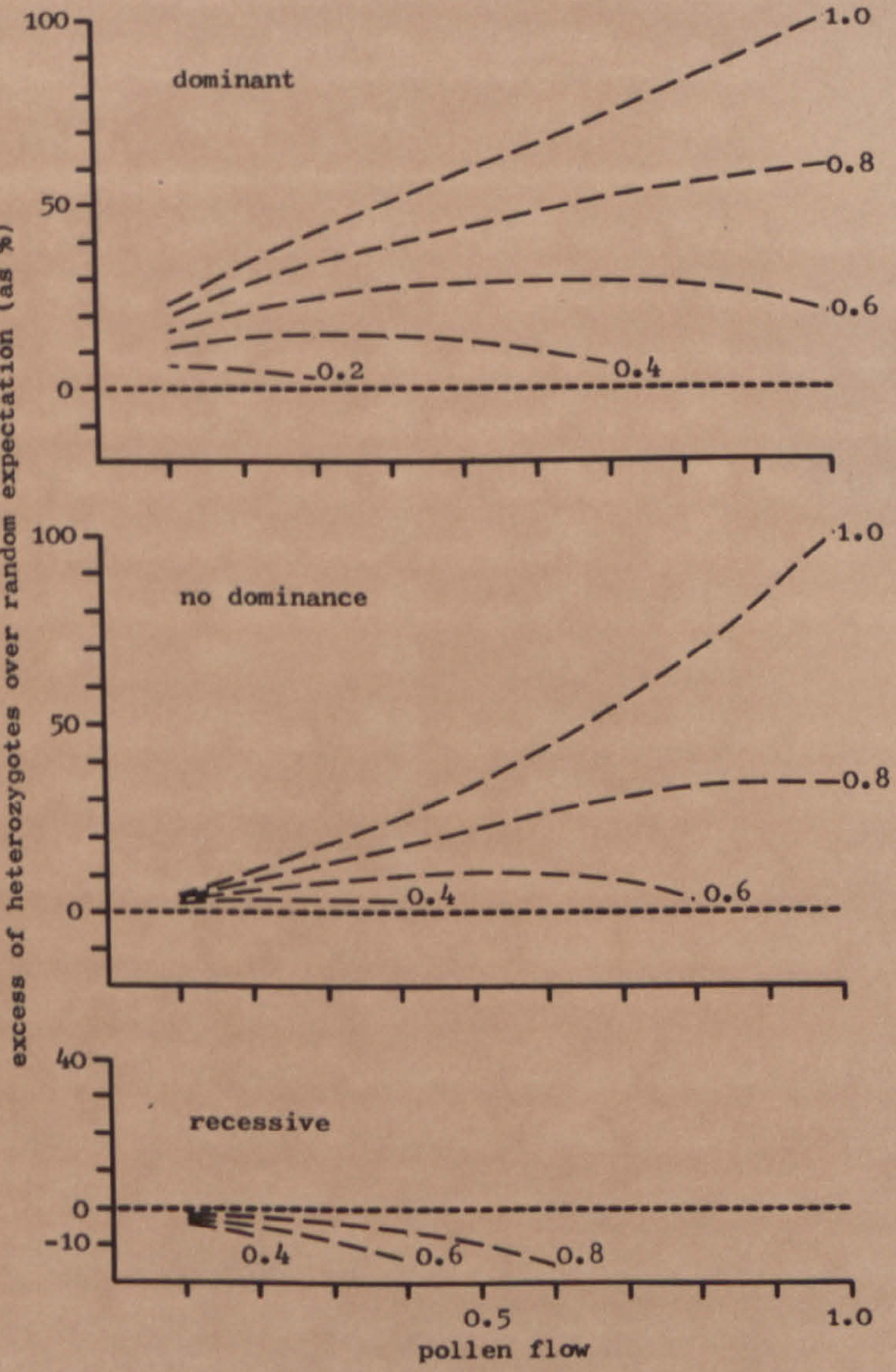


Fig. 39. Effect of selection and pollen flow on heterozygosity at equilibrium.
(selection coefficients indicated on graphs, degrees of dominance are of favoured gene)



was chosen when the gene frequencies did not change by more than 0.000001 between generations. Three general features emerge.

Table 22. Number of generations to equilibrium with different initial gene frequencies and different amounts of pollen flow. (selection coefficient = 0.4, dominance of favoured gene)

	Initial frequency			
Pollen flow	0.01	0.1	0.5	0.9
0	501	501	501	501
0.1	63	58	50	51
0.2	57	50	38	45
0.3	57	49	37	45
0.4	61	52	44	48
0.5	71	58	52	55
0.6	90	70	69	71
0.7	143	63	113	115
0.8	501	501	501	501
0.9	78	98	104	106
1.0	42	53	58	50

Firstly, the rate to equilibrium does not depend greatly on the initial frequency (e.g. Table 22).

Secondly, the rate also depends on the degree of perenniality. In a perennial equilibrium is reached more slowly. (See Chapter III.3).

Thirdly, when dominance is present pollen flow considerably hastens the approach to equilibrium, particularly so when there is a small amount of pollen flow and strong selection (e.g. Table 22). Mine populations are in fact quite likely to be in equilibrium, since with dominance and complete selection against the incoming type, equilibrium is reached after 24 generations with only 0.1 pollen flow.

Equilibrium was also reached very rapidly in the models of Jain and Bradshaw (1966). Evolution on mines is theoretically (as well as in practice) a rapid process.

(iv) The pattern of progress to equilibrium was quite straightforward, with a rapid approach in the initial stages, followed by a slowing down as equilibrium is approached (Fig. 40). Because of the extreme choice of the equilibrium definition, the effective equilibrium values are actually approached in much fewer generations than is suggested above.

(b) Changing pollen flow

In the previous section, the effects of a high degree of pollen flow were shown to be startling. It therefore seemed relevant to investigate the effects of changing from a high degree of pollen flow in the early generations, as would happen at the beginning of colonisation, to a low degree of gene flow, as would be the case in the later stages of colonisation when population density had increased. Only the situation when the favoured gene was dominant has been investigated.

The results confirm that this initial phase of intense pollen flow can have serious consequences for the population. Evolution in a colonising species is a dynamic process. The high initial gene flow lowers the frequency of the favourable gene considerably and the degree to which this happens depends on several features.

(i) The speed of colonisation (Fig. 41): if colonisation is slower and the amount of gene flow decreases more slowly then the effects of pollen flow are more serious. The rate of colonisation will of course depend on the rate of evolution but this particular "feed-back" model has not been considered.

(ii) The selection pressure against the incoming type (Fig. 42): if the selection pressure is great, then the effects of this gene flow will not be so serious as when the pressures are of a lower order. Here the incoming genes can swamp the favoured gene completely in this initial phase.

Fig. 40. Change in gene frequency under influence of selection and pollen flow.
(selection coefficients indicated on graph,
pollen flow = 0.2)

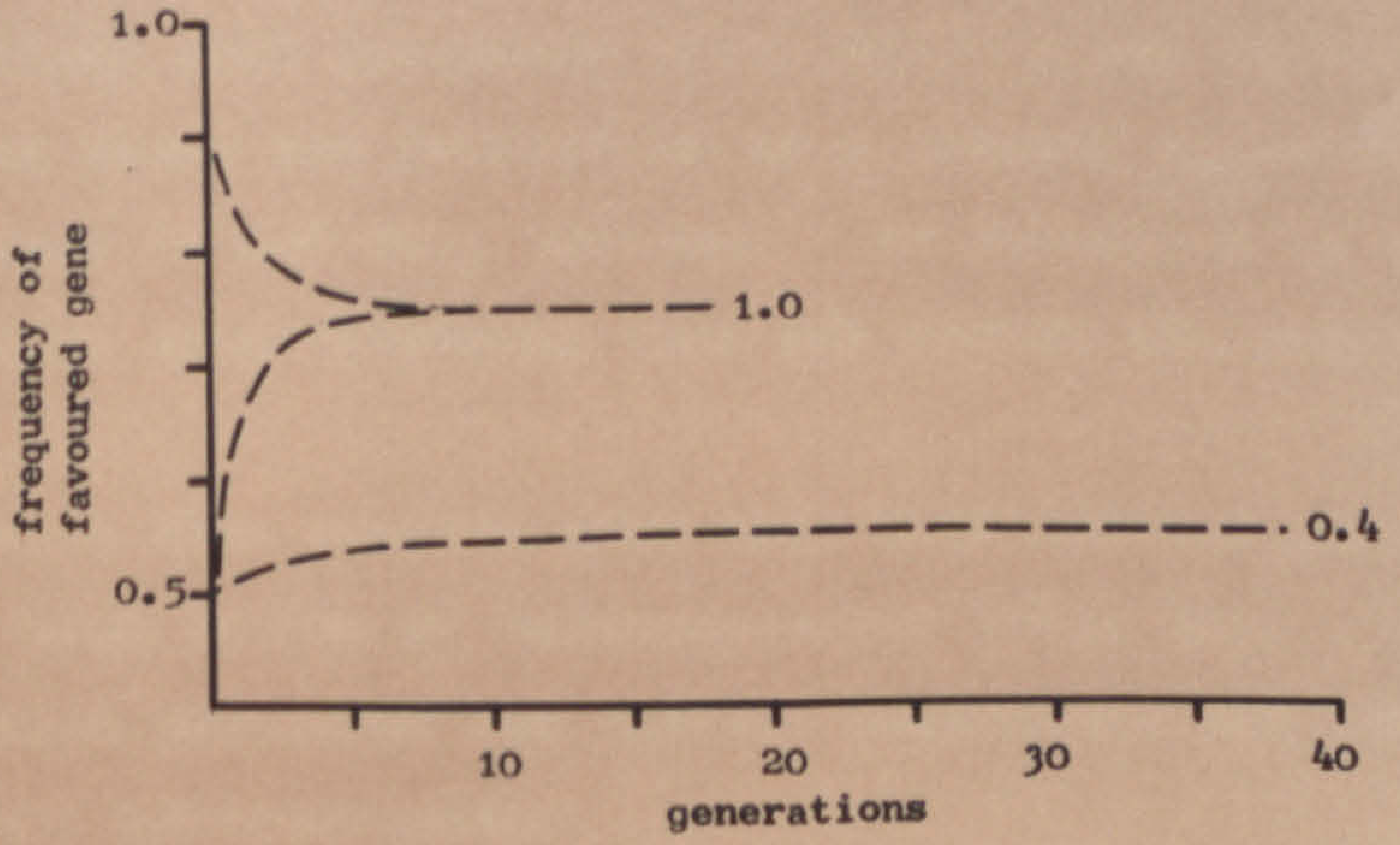
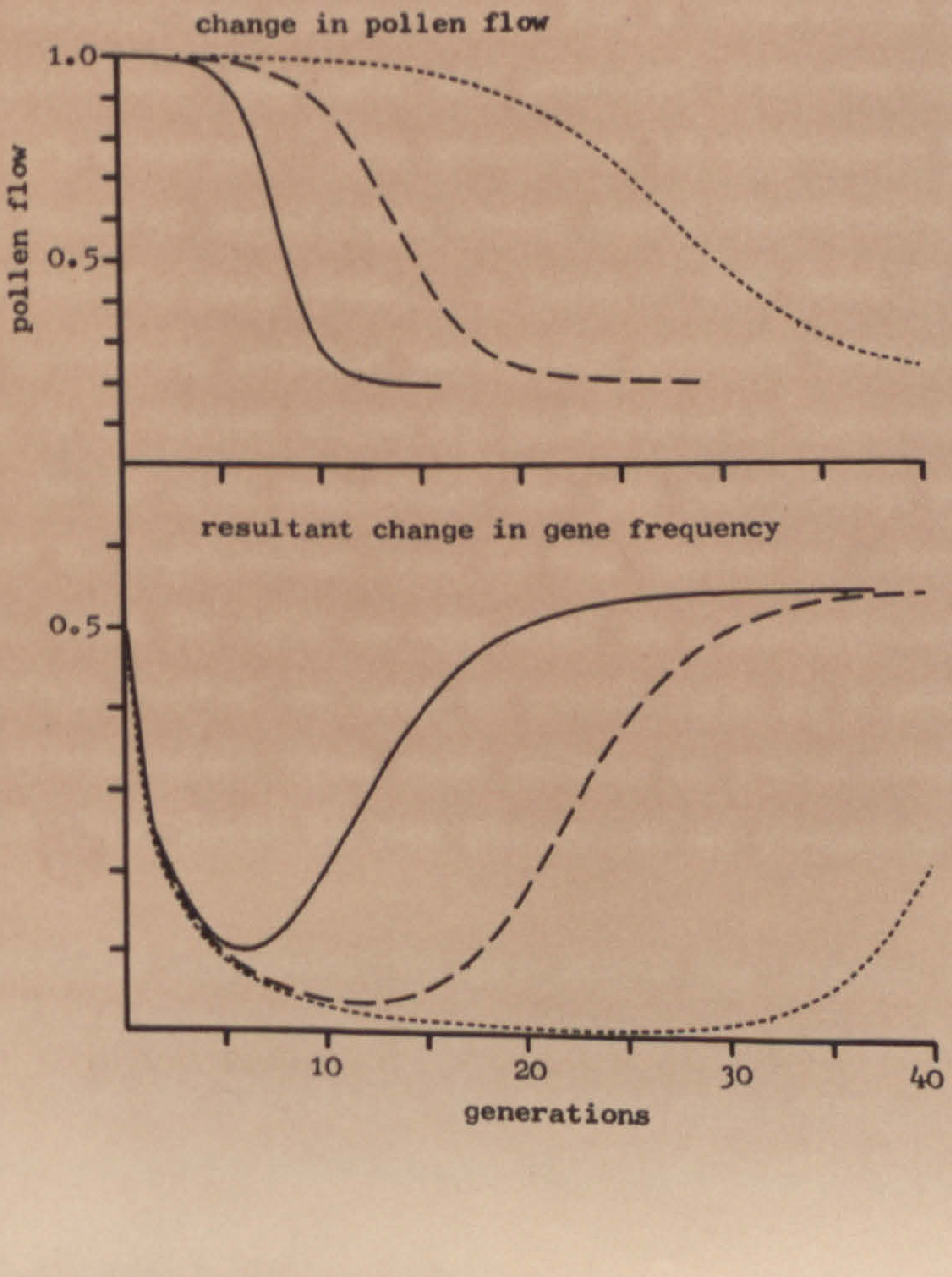


Fig. 41. Effect of different rates of changing pollen flow on gene frequency.
(selection coefficient = 0.4,
favoured gene dominant)



(iii) The perenniality of the colonising species (Fig. 42): a perennial suffers a far lower reduction in the frequency of the favoured gene in this initial stage. It is also slower to recover from this initial reduction but by this time the situation is less serious.

(iv) The initial gene frequency (Fig. 43): a low initial gene frequency does place the population at a greater disadvantage but again the effect is not very serious. If selection is very high then the initial gene frequency does not affect the population at all.

The results also confirm that this high initial pollen flow means a very high degree of heterozygosity (Fig. 44). With very strong selection pressures the initial colonisers are likely to be practically all heterozygotes.

(c) Pollen flow load.

It has been repeatedly stressed in previous sections that gene flow can have deleterious consequences for a population striving to achieve adaptation: the gene and genotype frequencies of the adapted types are lowered. The situation is analogous to a load resulting from harmful mutations, where again favourable genes are substituted by deleterious ones. A genetic and gene-flow load has the effect of producing greater mortality amongst members of a population. This is a factor which is particularly important in human populations. It is also important for any population where the limiting factor to population size is the number of adapted individuals it can produce, such as will be the case in a colonising species where the limiting factor is rate of adaptation to a peculiar habitat. Moreover apart from this effect on population size, genetic changes will occur in the direction of reducing the gene-flow load.

It is therefore important to calculate the degree of mortality that a population suffers from the introduction of unadapted types by gene flow. This is difficult to assess intuitively: thus if we have a dominant gene the population will have many incoming harmful

Fig. 42. Effect of changing pollen flow on gene frequency with different amounts of selection. (selection coefficients indicated on graphs, favoured gene dominant)

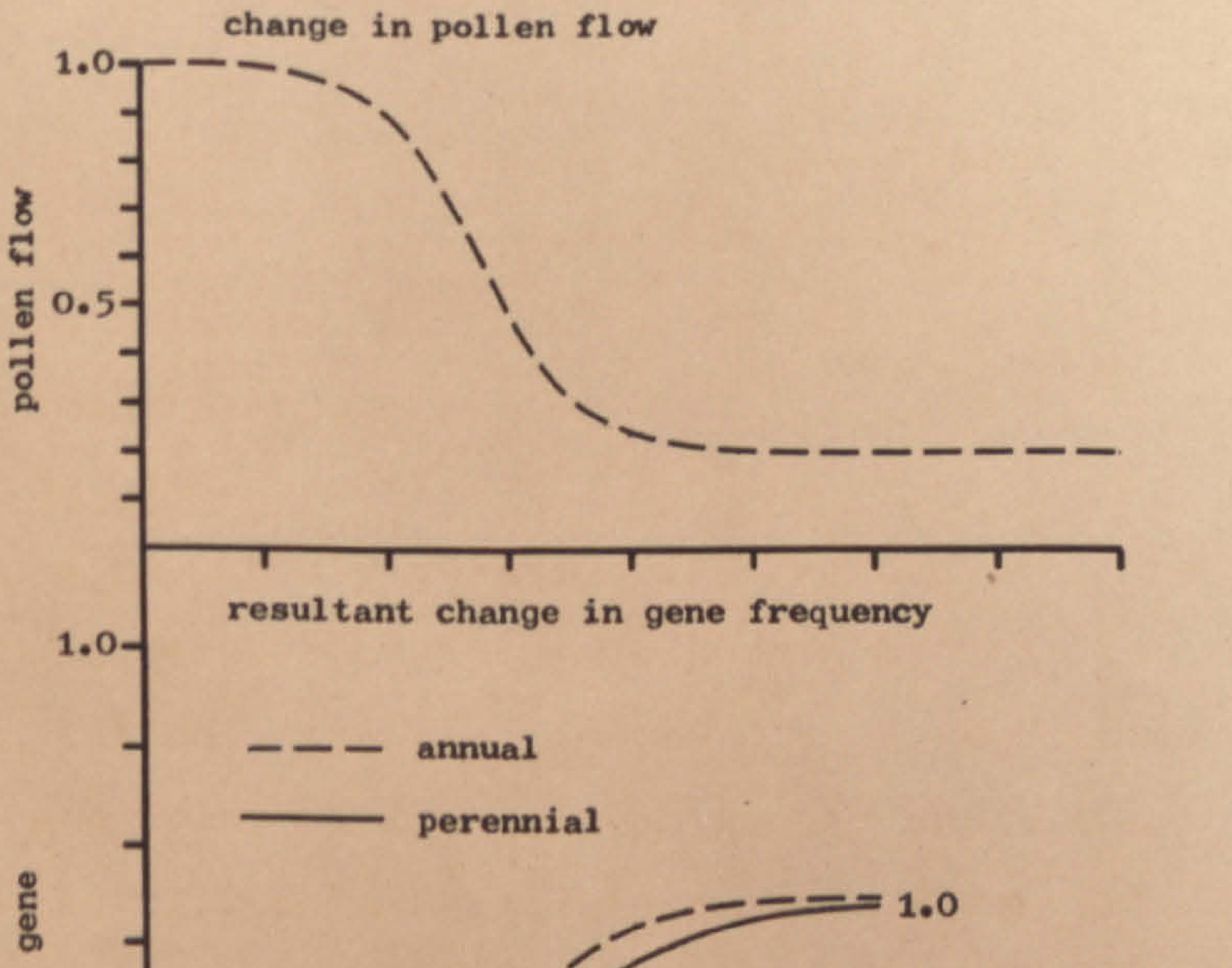


Fig. 43. Effect of changing pollen flow on gene frequency with different initial gene frequencies.
(selection coefficients indicated on graphs,
favoured gene dominant,
initial gene frequencies = 0.1, 0.5, and 0.9)

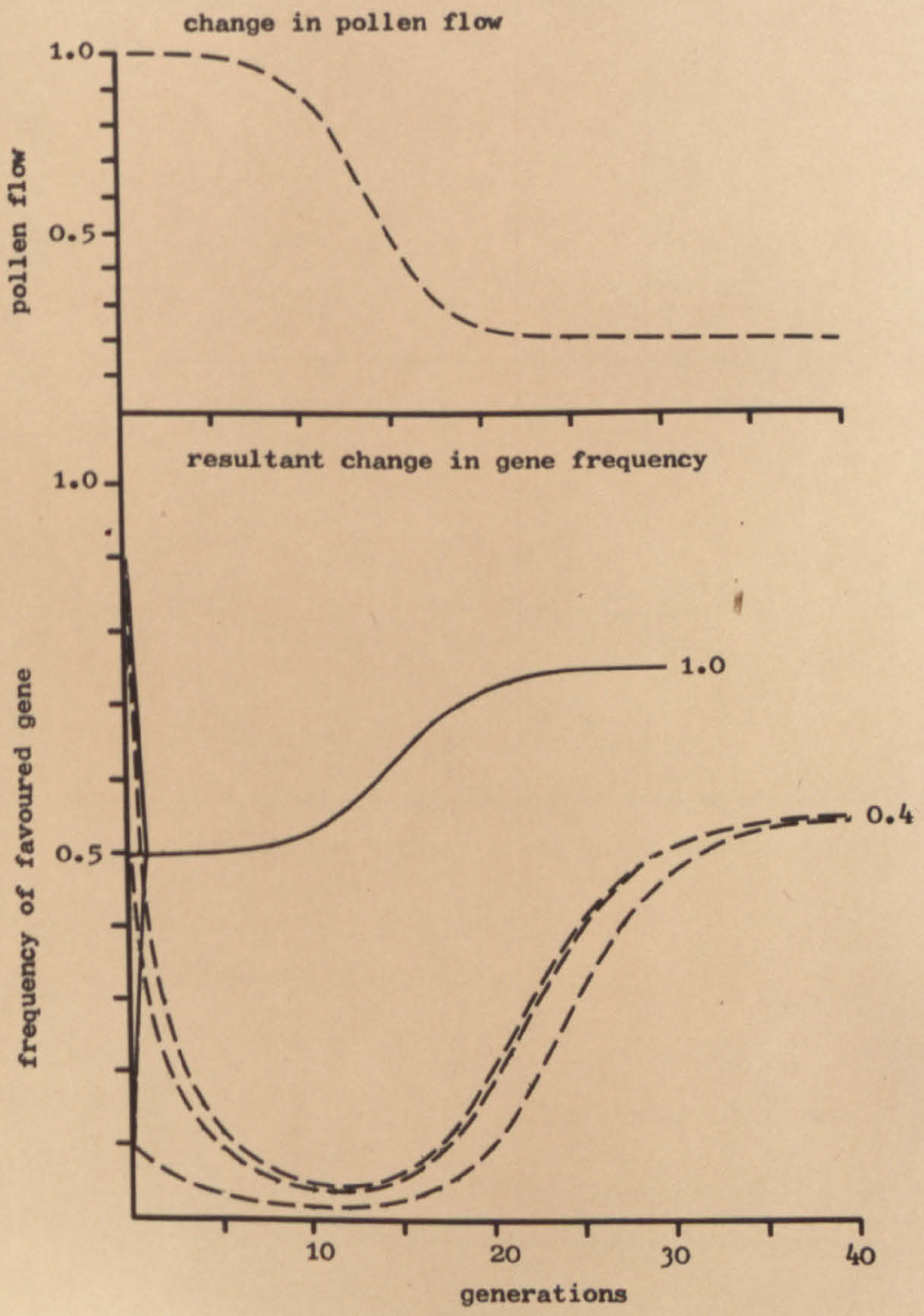
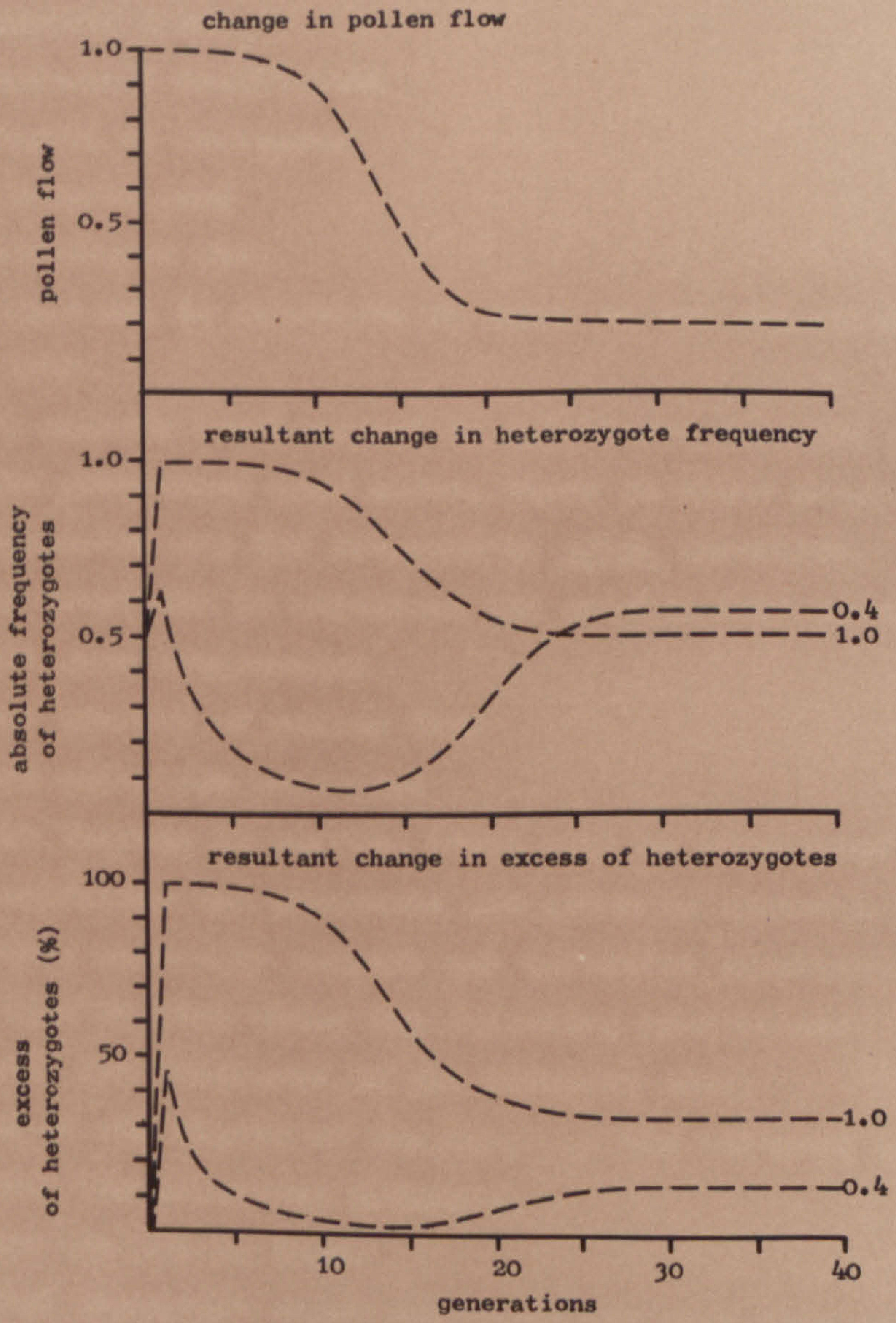


Fig. 44. Effect of changing pollen flow on frequency of heterozygotes.
(selection coefficients indicated on graphs,
favoured gene dominant,
initial gene frequency = 0.5)



change in pollen flow

resultant change in heterozygote frequency

resultant change in excess of heterozygotes genes present and sheltered in the heterozygous state, but these heterozygotes have a fitness equal to the homozygotes; however the presence of these genes could be serious.

The genetic load on a population under selection is given by the formula

$$I = s(BB + (1-h)AB)$$

where I = genetic load
s = selection coefficient
h = degree of dominance
A = favoured gene
B = unfavoured gene

The genetic load due to selection is equal to the selection intensity (Van Valen, 1965).

However, genetic loads cannot be calculated directly from the results of the programmes used above, since in these programmes the genotype frequencies are the frequencies after selection. A programme was therefore developed to calculate the genetic load under:

different gene frequencies
different amounts of pollen flow
different selection pressures
different degrees of selfing (this will be considered later).

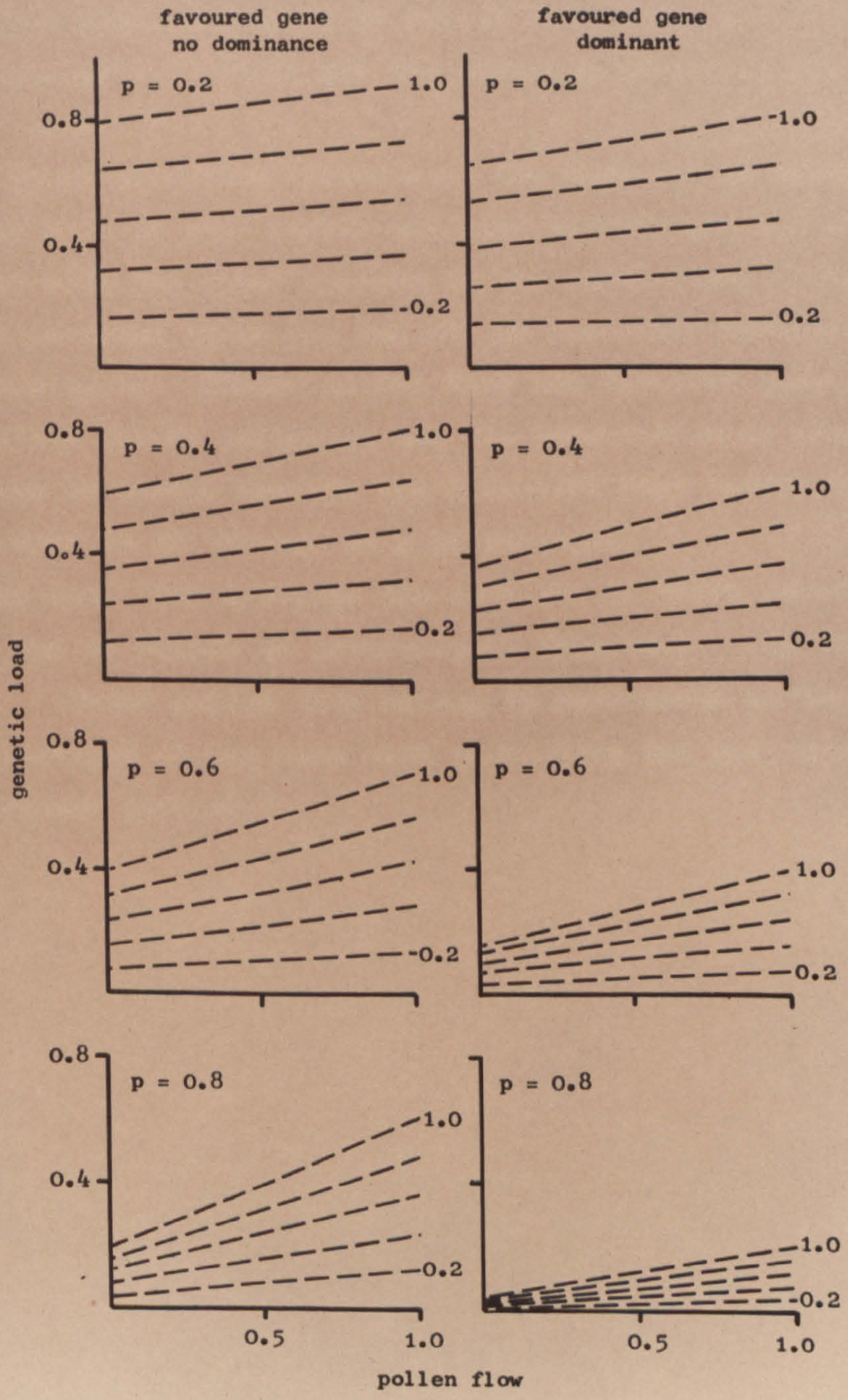
The general equations and programme are given in Appendix 3.

The results generally support our intuitive expectations (Fig. 45). The genetic load is less under the following conditions:

(i) the higher the frequency of the favoured gene,
(ii) the greater the degree of dominance of the favoured gene,
(iii) the lower the selection pressure,
(iv) the lower the pollen flow.

Any factor therefore which decreases the gene flow or increases the dominance of the favoured gene will be selected. A high pollen flow will however be more serious if the gene frequency of the favoured gene is high and if the selection is high; both of these conditions occur in mine populations.

Fig. 45. Genetic load with different amounts of selection and pollen flow, at various gene frequencies. (selection coefficients indicated on graphs, gene frequencies, indicated by 'p', are of favoured gene)



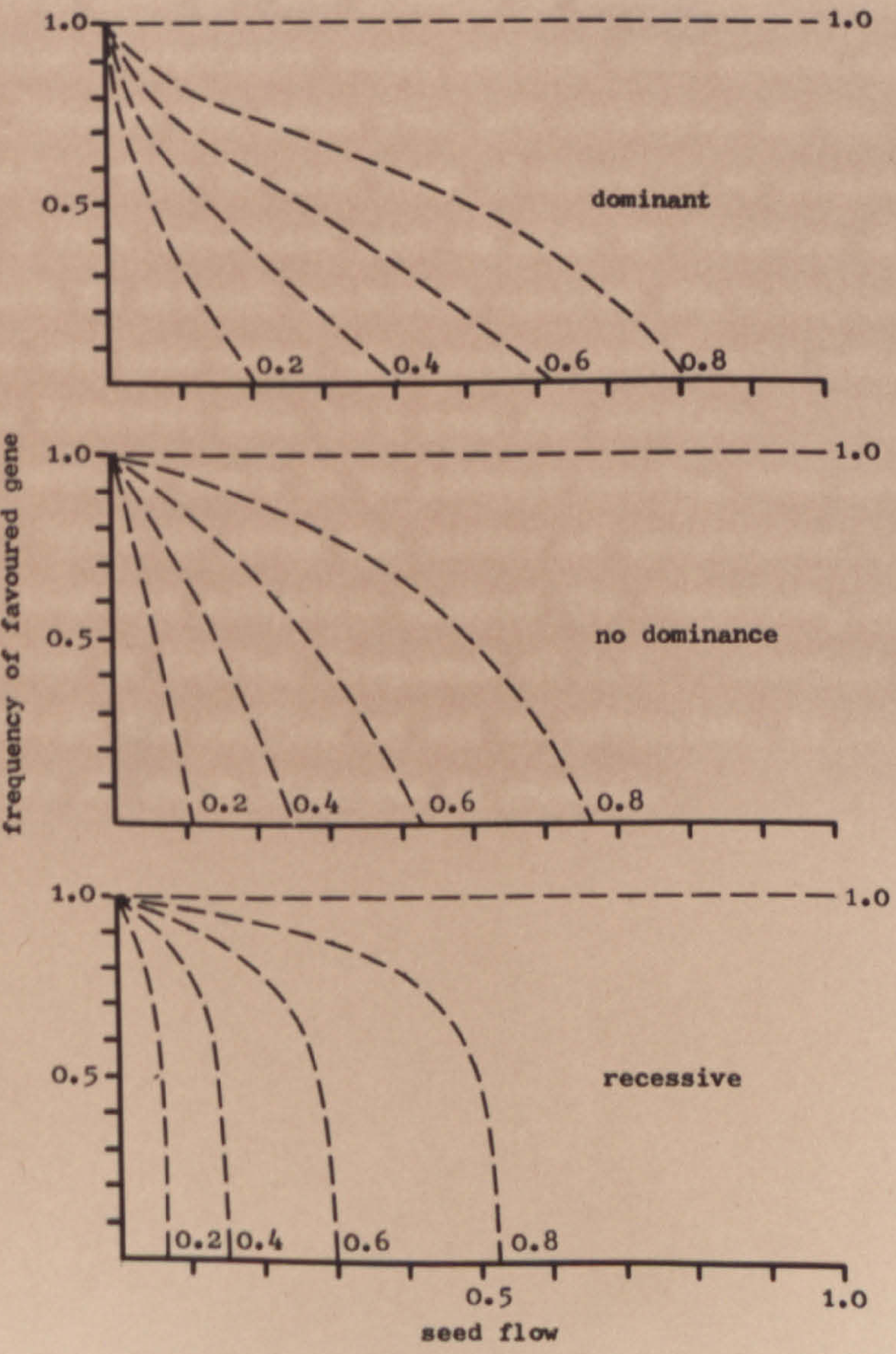
(d) Seed flow

The effects of seed flow were investigated only briefly since seed flow did not seem as important a factor as pollen flow in mine situations. Seed probably does not travel as far, and under very high selection intensities, such as occur in mines, the harmful effects of seed flow were less than those of pollen flow (Fig. 46). Under high selection intensities nearly all the incoming genotypes are killed before they mate.

Under lower selection intensities the opposite is true. Here the incoming seed is only partly selected against and the remainder are left to mate with the other members of the population. The frequency of both the favoured types (AA and AB) is therefore reduced by the extra BB genotypes. (Under pollen flow the favoured genotypes mate with incoming pollen before selection and the favoured genes are not so drastically reduced in number because the reduction that occurs in AA is offset by the increase in AB through AA × BB matings.)

Because seed flow involves the addition of extra genotypes before mating, it has no effect on heterozygote frequency: all the members mate at random. (Under pollen flow mating is non-random in that the incoming genotypes do not mate with themselves but only with the (female) genotype present already.)

Fig. 46. Effect of selection and seed flow on gene frequency at equilibrium.
(selection coefficients indicated on graphs, degrees of dominance are of favoured gene)



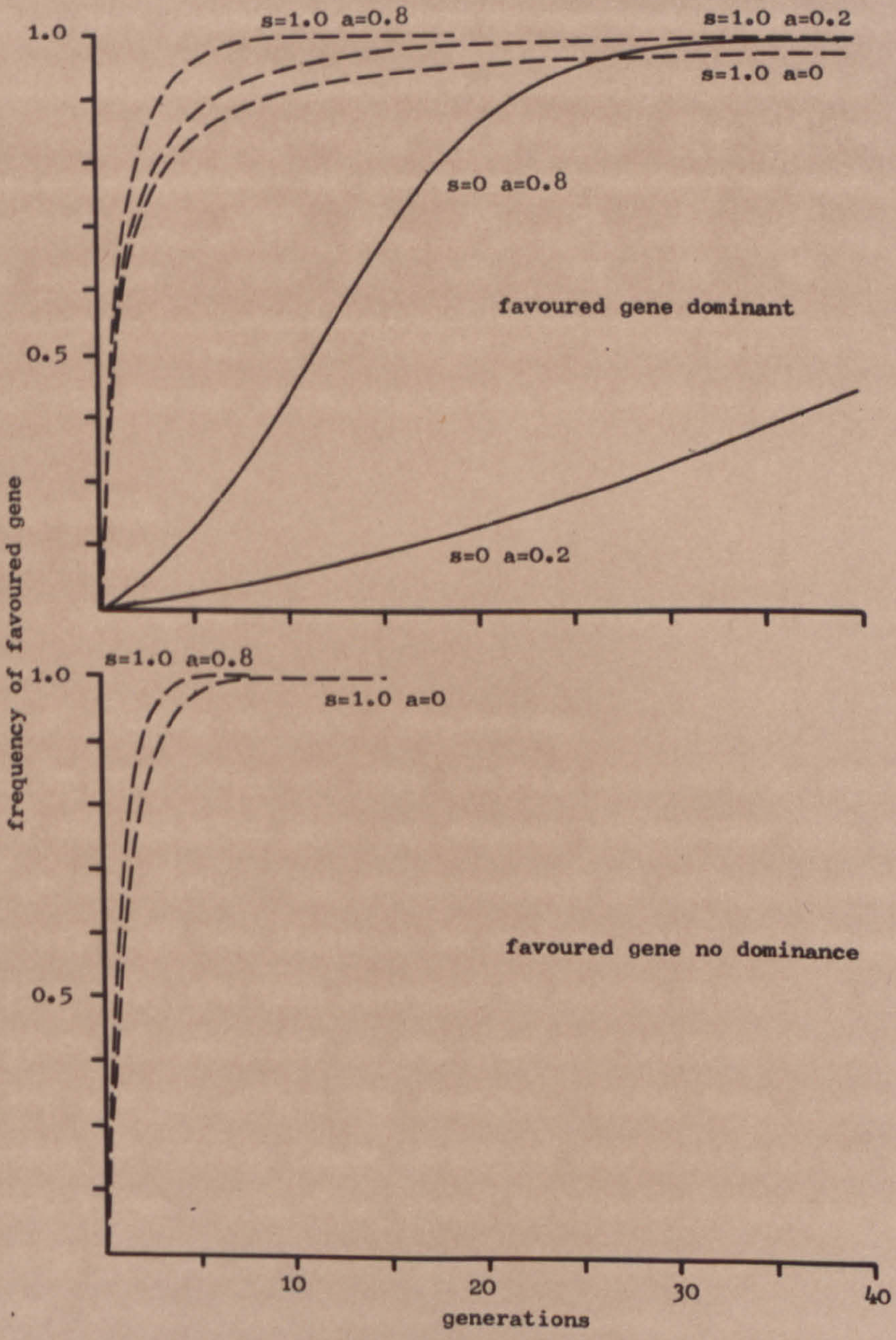
3. THE EFFECTS OF SELFING

Selfing has a considerable effect on the spread of genes through populations. A gene for selfing will itself spread through a population if there is nothing to oppose it. Crosby (1949) showed that the homostyle self-fertile type of the Primrose will spread through the population and reach fixation if no viability effects oppose it. This is further illustrated in Fig. 47, in the instances where selection = 0.

Selfing will also aid the process of selection. Thus if there is selection against a recessive gene, selfing will assist in the elimination of this gene, because the genes that are sheltered in the heterozygous state will be exposed. This is also true if there is no dominance, but here selfing will have a less important part to play in fixation of the favoured gene since there is already selection against the heterozygote (Fig. 47). This is on the one gene model and assumes pleiotropic effects or linkage of the favoured gene to a gene for selfing.

However, even if there is no linkage selfing does speed the rate to fixation. Table 23 gives the number of generations to fixation when there is complete selection against the recessive, and when the favoured gene has an initial frequency of 0.1. Although in the absence of linkage the selfing is not so effective, it still considerably hastens the rate at which fixation is reached.

Fig. 47. Change in gene frequency under the influence of selfing, selection, and selfing plus selection, in the absence of gene flow.
(selection coefficients indicated by 's',
self-fertility indicated by 'a',
selfing gene no dominance,
selfing gene completely linked to favoured gene)



Evolution : selfing

Table 23. Effect of linkage of gene for selfing to favoured gene on rate of fixation of the favoured gene in the population.

(selection coefficient = 1.0, dominance of favoured gene, no dominance of selfing gene, initial frequency of both genes = 0.1).

	Selfing					
	0	0.2	0.4	0.6	0.8	1.0
Linkage	>500	89	50	34	26	20
No linkage	>500	261	136	93	70	56

4. THE EFFECTS OF SELFING PLUS GENE FLOW

(a) No selection

In the previous sections it has been shown that gene flow acts against selection while selfing acts in the direction of selection. To understand how all these three features interact, the effects of gene flow and selfing with no selection will be considered first.

The results, presented in a simplified form, (Fig. 48), show that gene flow is a very potent force that is not easily counteracted by selfing. The incoming genotype wins unless there is a high degree of selfing. Pollen flow is less potent than seed flow and can be counteracted more easily. If the gene for selfing is dominant (i.e. the heterozygote also selfs), then it is much more effective in counteracting gene flow.

A gene for selfing, if it imposes sufficient self-fertility, will therefore spread through a population in the face of gene flow opposing it.

(b) With selection

(i) One gene model.

This and subsequent models were only investigated in the case of pollen flow.

(a) Effect of selfing on gene frequencies.

It can be seen (Fig. 49) that selfing considerably increases the frequency of the selected gene and thereby alleviates some of the effects of pollen flow. The effectiveness of selfing increases disproportionately with the increase in selfing, with the decrease in selection pressure, and with decrease in pollen flow.

The degree of dominance of the gene for selfing or of the favoured gene does not greatly affect the result. Under high amounts of pollen flow dominance of selfing leads to better resistance to pollen flow, whereas under lower pollen flow there is little difference evident.

(b) Effect of selfing on heterozygote frequencies.

Selfing, as expected, reduces the frequency of heterozygotes in the population (Fig. 50). However, when dominance is present, and under strong selection pressures against the incoming gene, even a very high

Fig. 48. Effect of gene flow and selfing in the absence of selection.

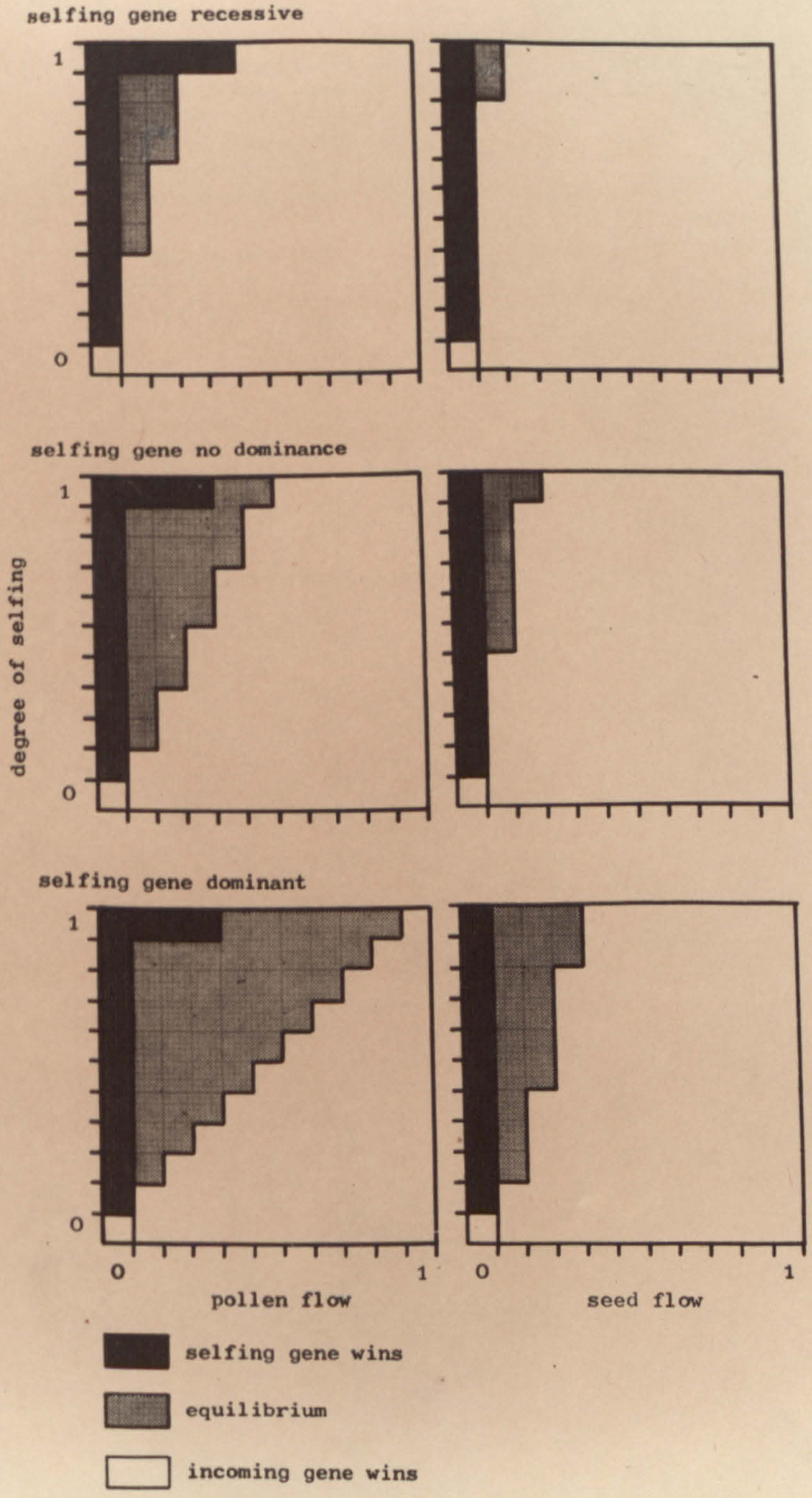


Fig. 49. Effect of selfing on gene frequency at equilibrium with different amounts of selection and pollen flow. (self-fertility indicated on graphs, selfing gene no dominance, favoured gene dominant, selfing gene completely linked to favoured gene)

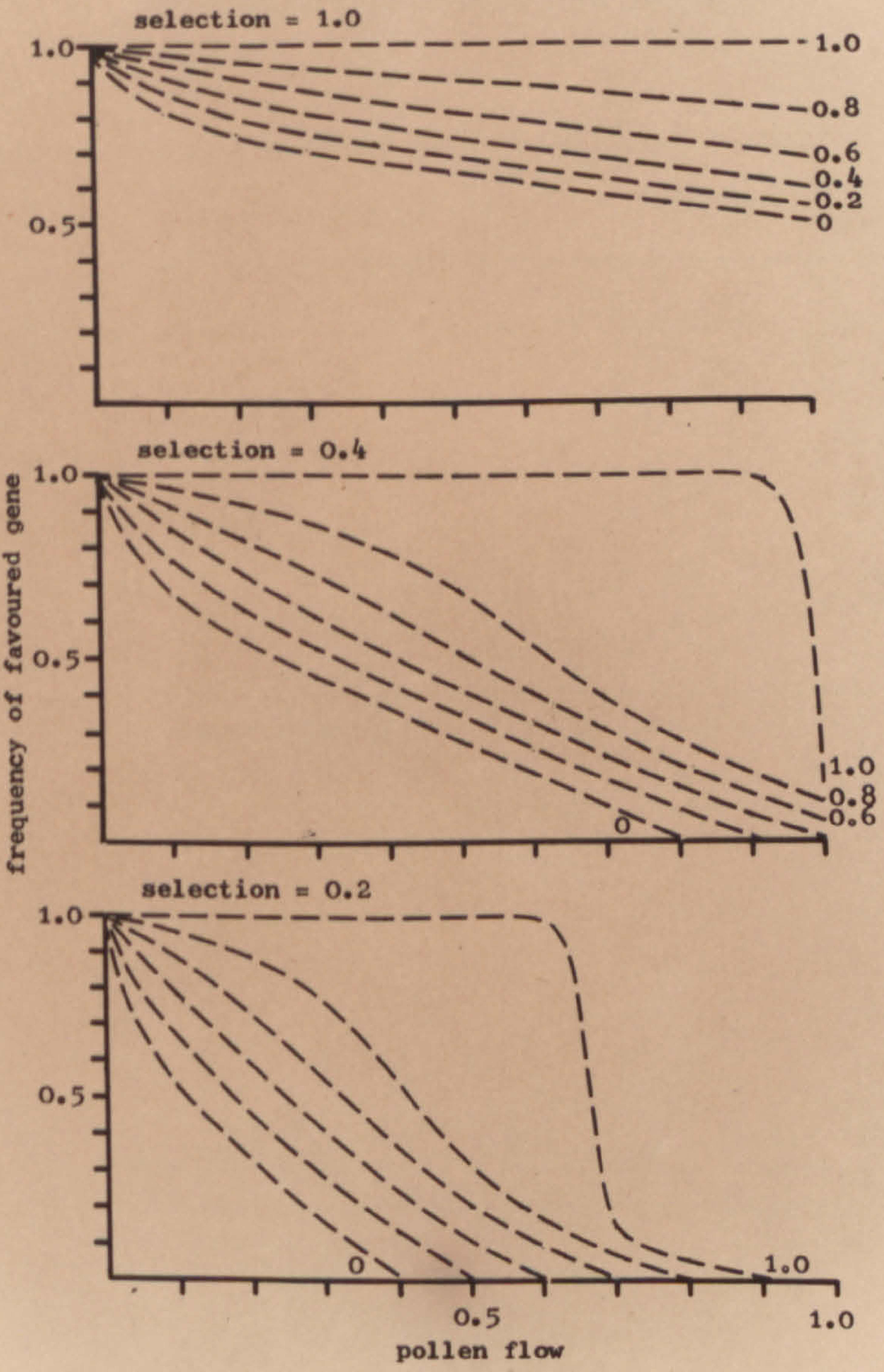
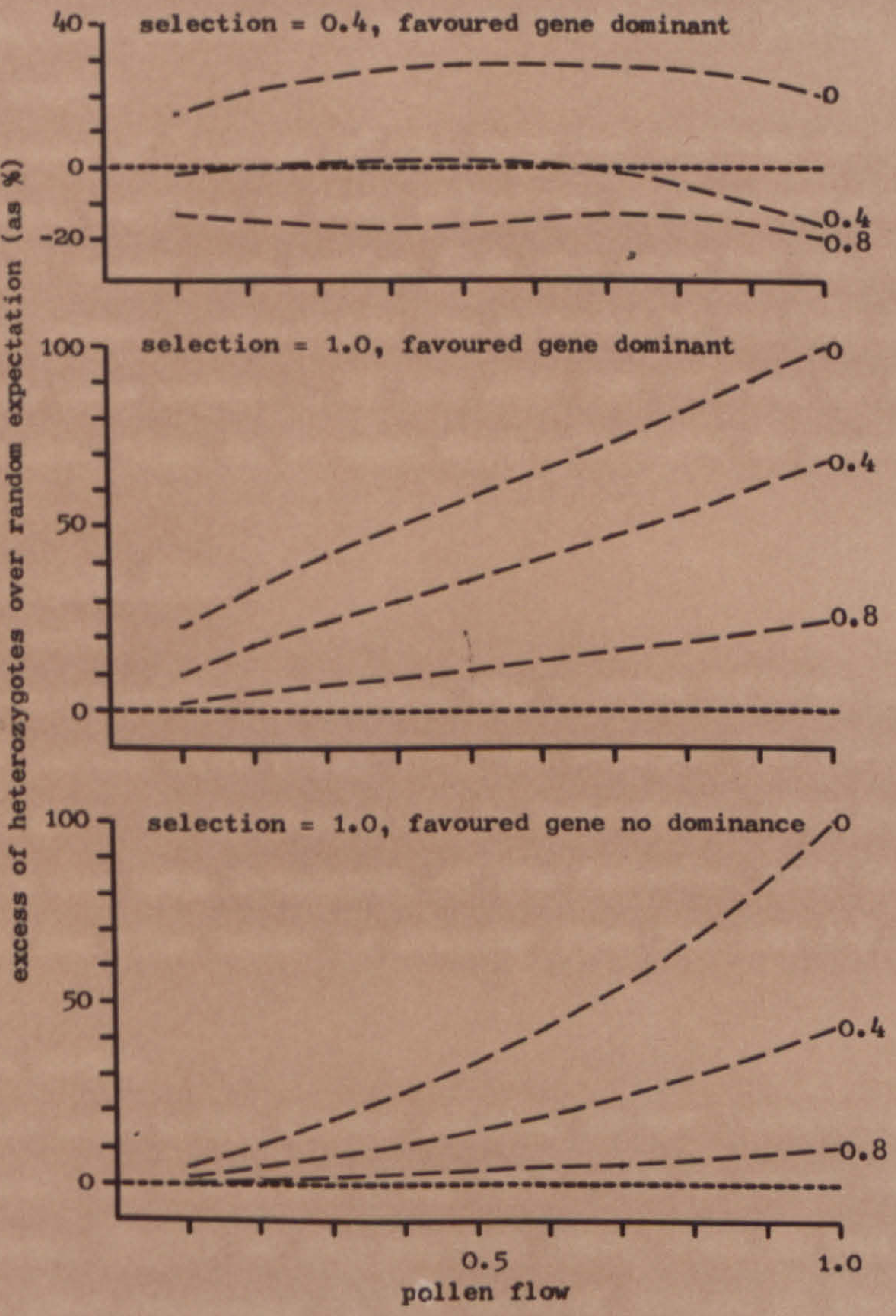


Fig. 50. Effect of selfing on heterozygosity at equilibrium with different amounts of selection and pollen flow. (self-fertility indicated on graphs, selfing gene no dominance, selfing gene completely linked to favoured gene)



degree of selfing does not reduce the frequency of heterozygotes below the random breeding expectation: pollen flow maintains heterozygosity to a remarkable degree, even in the face of selfing.

(c) Number of generations to equilibrium.

Selfing acts in the same direction as selection in opposing pollen flow and therefore the pattern of the time taken to reach equilibrium is the same as with selection and pollen flow. Thus with a low degree of pollen flow, selfing hastens the rate at which equilibrium is reached.

(ii) Two gene model.

In the absence of linkage of the gene for selfing to the gene that is being selected, selfing is less effective at counteracting pollen flow (Fig. 51). Under high pollen flow the gene for selfing is swamped by the incoming genes. Otherwise the effects of selfing are similar to those in the one gene model. If the gene for selfing is linked to the gene for tolerance, then it is much more effective in counteracting pollen flow.

(c) Changing pollen flow

(i) One gene model.

Selfing considerably alleviates the harmful effects of an initially high degree of pollen flow (Fig. 52), and hastens the time needed to reach equilibrium. If perenniality is introduced into the model (Fig. 52), then the gene frequency of the favoured type is depressed even less, but the time taken to recover from the initial depression is longer.

(ii) Two gene model.

In the two gene model (where selfing is not linked to the favourable gene), the result is different (Fig. 53). If we start with a low initial frequency (0.01) of the selfing gene, then it has no appreciable effect on the gene frequency in the initial stages of colonisation, and the gene for selfing is still depressed further in its frequency by the incoming genes. The gene for selfing only starts to spread through the population after about 50 generations, long after the population has overcome the high degree of pollen flow in the initial

Fig. 51. Effect of selfing on gene frequency at equilibrium
with different amounts of selection and pollen flow,
when selfing gene not linked to favoured gene.
(self-fertility indicated on graphs,
selfing gene no dominance,
favoured gene dominant)

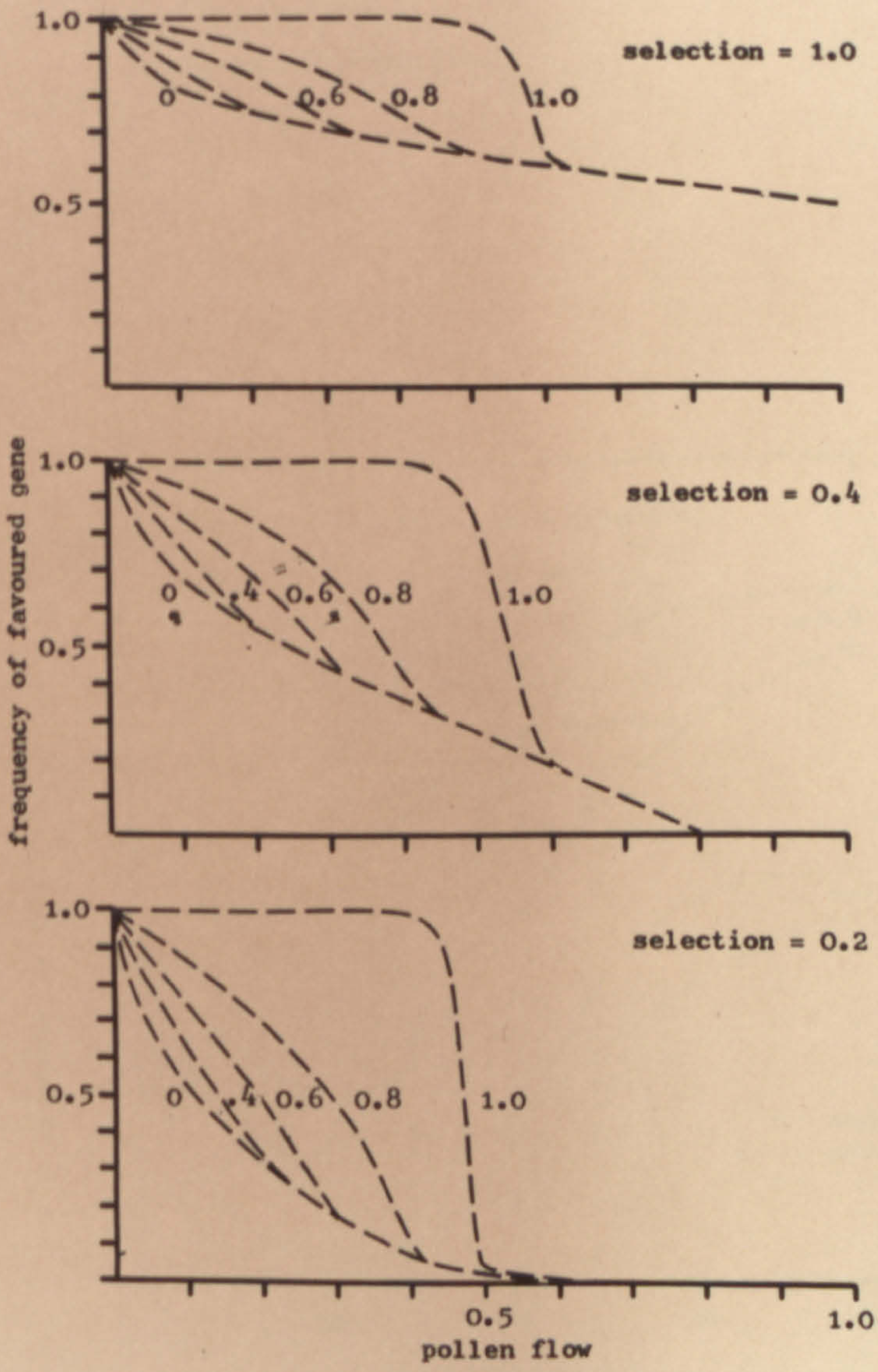


Fig. 52. Effect of selfing on gene frequency under conditions of changing pollen flow.
(selection coefficients indicated by 's',
self-fertility indicated by 'a',
selfing gene no dominance,
favoured gene dominant)

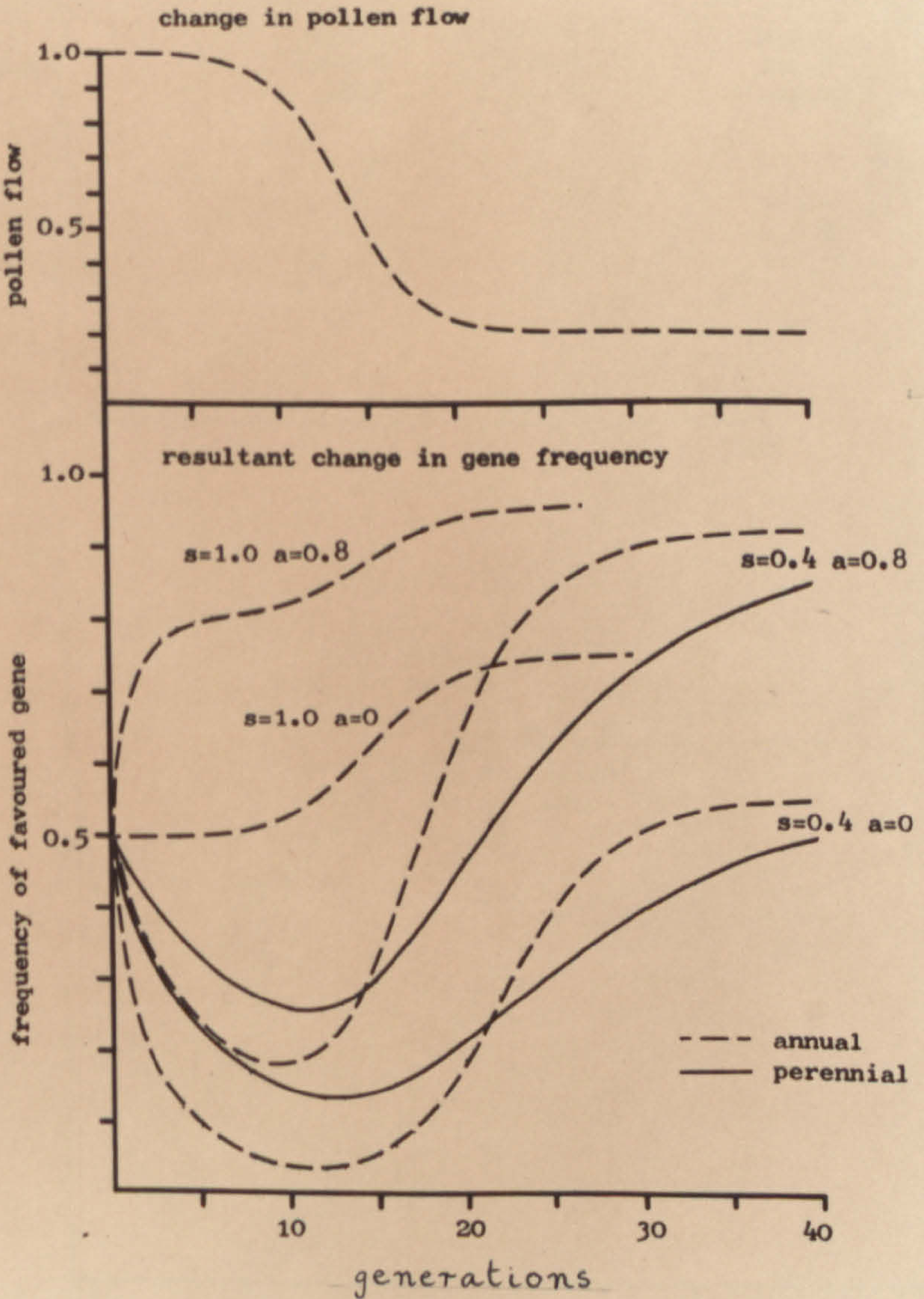
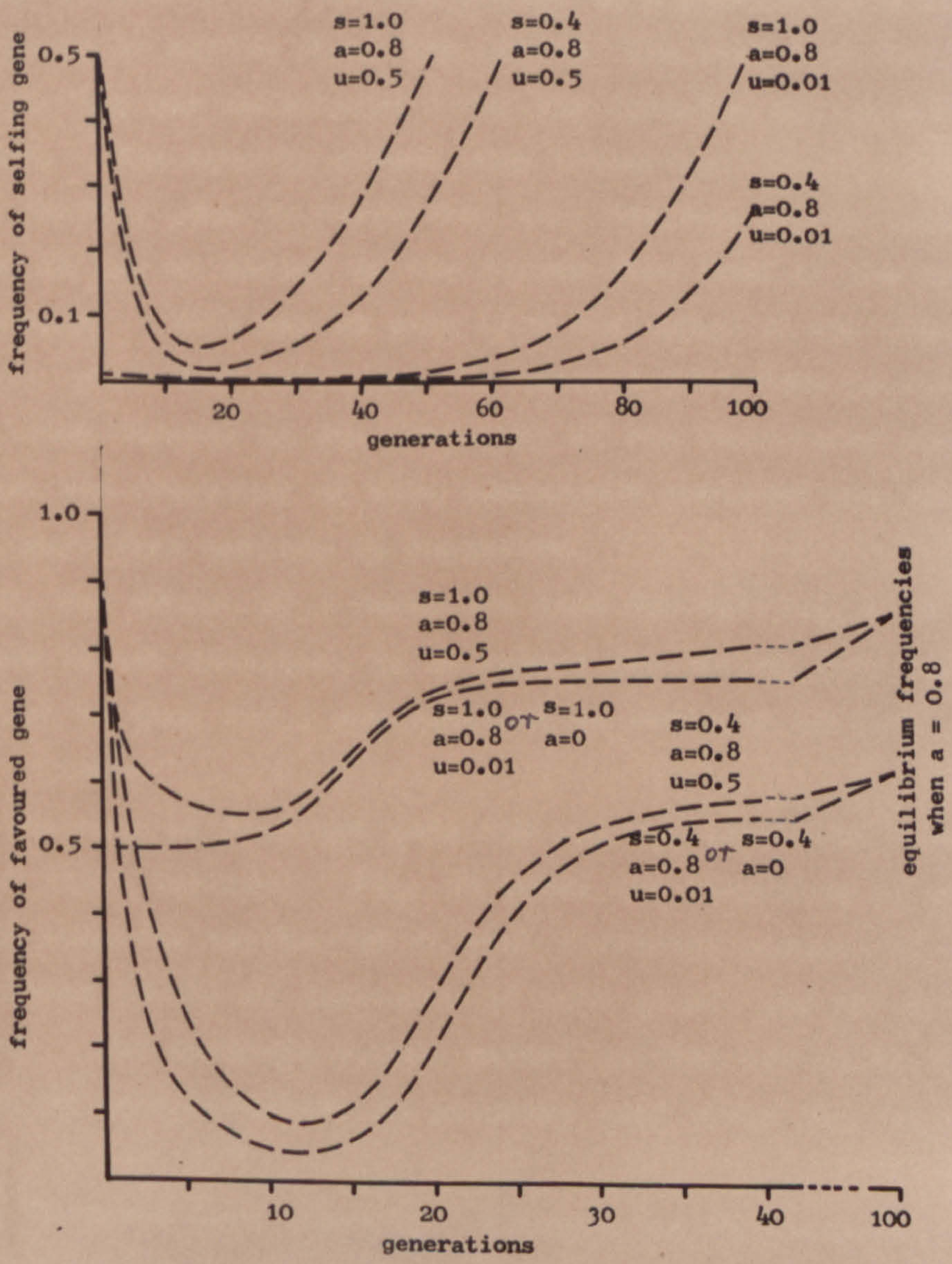


Fig. 53. Change in frequency of selfing gene and favoured gene under conditions of changing pollen flow, and with selfing gene not linked to favoured gene.
(selection coefficients indicated by 's',
self-fertility indicated by 'a',
initial frequencies of selfing gene indicated by 'u',
selfing gene no dominance,
favoured gene dominant,
change in pollen flow as before)



stages.

With a higher initial frequency (0.5) the selfing gene is effective in reducing the effects of gene flow and itself spreads through the population more rapidly. However the effect is not so marked as in the model where there is complete linkage of selfing gene to favoured gene.

(d) Selfing and pollen flow load.

In order to assess the precise effect on a population suffering from the effects of pollen flow, the pollen flow load with different degrees of selfing was calculated using the formulae discussed in Chapter V.2.c. The results proved very revealing.

If the selfing gene is recessive, i.e. the homozygote only is selfed, then it has no effect on the pollen flow load if the favoured gene is dominant. This can be seen from the equation for genetic load (Appendix 3): with full dominance the heterozygote term vanishes and the genetic load depends on the frequency of the unfavoured homozygote. The value for the frequency of this homozygote contains the selfing term and is zero when selfing is recessive.

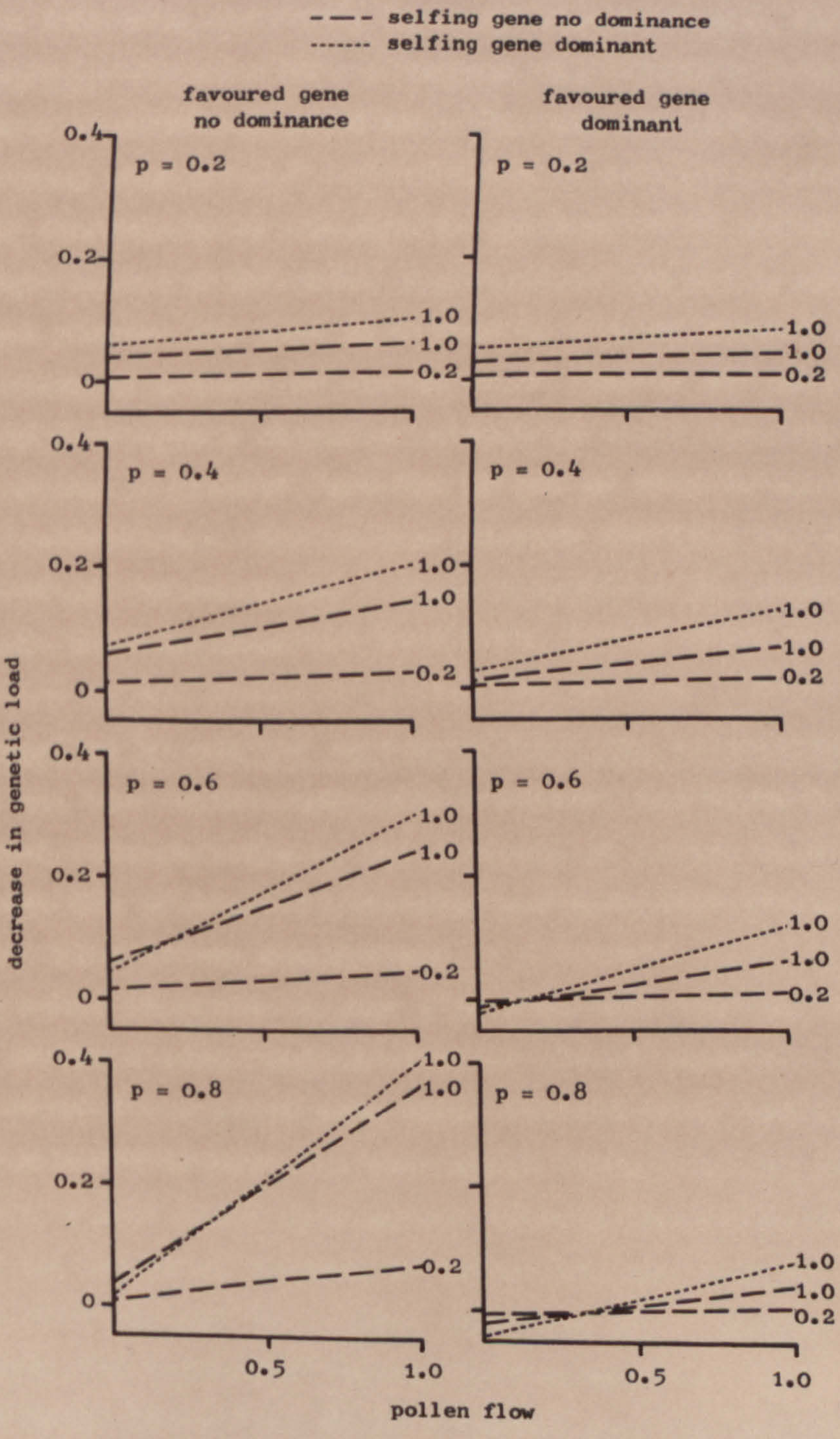
However, the effect of a recessive gene for selfing, then the favoured gene is not dominant, is to reduce the pollen flow load in a manner similar to that for a non-dominant or dominant selfing gene, but to a lesser extent.

A non dominant or dominant gene for selfing generally reduces the genetic load still further. Fig. 54 shows the reduction in pollen flow load by complete selfing of AA with different amounts of gene flow, at various levels of dominance of both genes, and at various levels of selection. The absolute values before selfing are as in Fig. 45.

Selfing is more effective in reducing load under the following conditions:

(i) if the favoured gene shows no dominance (or is recessive) rather than if it is not dominant. In other words self-fertility is likely to spread through a tolerant population if the tolerance is non-dominant or recessive. However, the absolute pollen flow load is reduced if

Fig. 54. Decrease in genetic load due to complete selfing (self-fertility = 1.0, as against 0) with different amounts of selection and pollen flow, and at various gene frequencies.
(selection coefficients indicated on graphs,
gene frequencies, indicated by 'p', are of favoured gene,
selfing gene completely linked to favoured gene)



tolerance is dominant (Fig. 45). There are therefore two processes acting.

(ii) if the gene for selfing is itself dominant.

(iii) if the pollen flow is greater. In the absence of pollen-flow and at a high frequency of a dominant favoured gene, selfing does in fact increase the load on the population. This is presumably because it unmasks deleterious genes which otherwise would be hidden in the heterozygous state.

(iv) if the selection pressure is greater.

Again here the intuitive expectations are borne out. Selfing on the whole reduces the gene flow load on a population. However, other interesting features emerge which lead us to expect self-fertility to follow a definite pattern in the field: greater if the selection is greater, greater if pollen flow is greater, greater if the favoured character is non-dominant and also the gene for selfing should be dominant. These hypotheses are amenable to practical investigation.

(e) Spread of a gene for selfing through the population.

It has been shown that selfing confers two types of advantage to a population evolving in the presence of pollen flow. Firstly it hastens the process of selection and secondly it alleviates the gene flow load on the population.

We would therefore expect a gene for selfing that is unlinked to the favoured gene (and on which no direct selection is acting) to spread through the population under conditions of selection and gene flow. However it has also been shown (this Chapter, section 4 a) that such a gene would also spread through the population of its own accord, in the total absence of selection, and even in the presence of gene flow against it. Does selection on another unlinked gene (e.g. for tolerance) hasten this process?

Although the effect of an unlinked gene for selfing on pollen flow load was not calculated, the spread of such a gene through the population was investigated on a two gene model under different selection pressures on the favoured gene.

The results are interesting (Fig. 55). If the gene for selfing is not dominant, then selection for the other gene only slightly increases its final frequency compared with the frequency when there is no selection. However it also hastens the rate at which equilibrium is reached. In this case therefore the spread of a selfing gene through a population is largely because of its inherent tendency to do so, as well as because of pollen flow plus selection (pollen flow load).

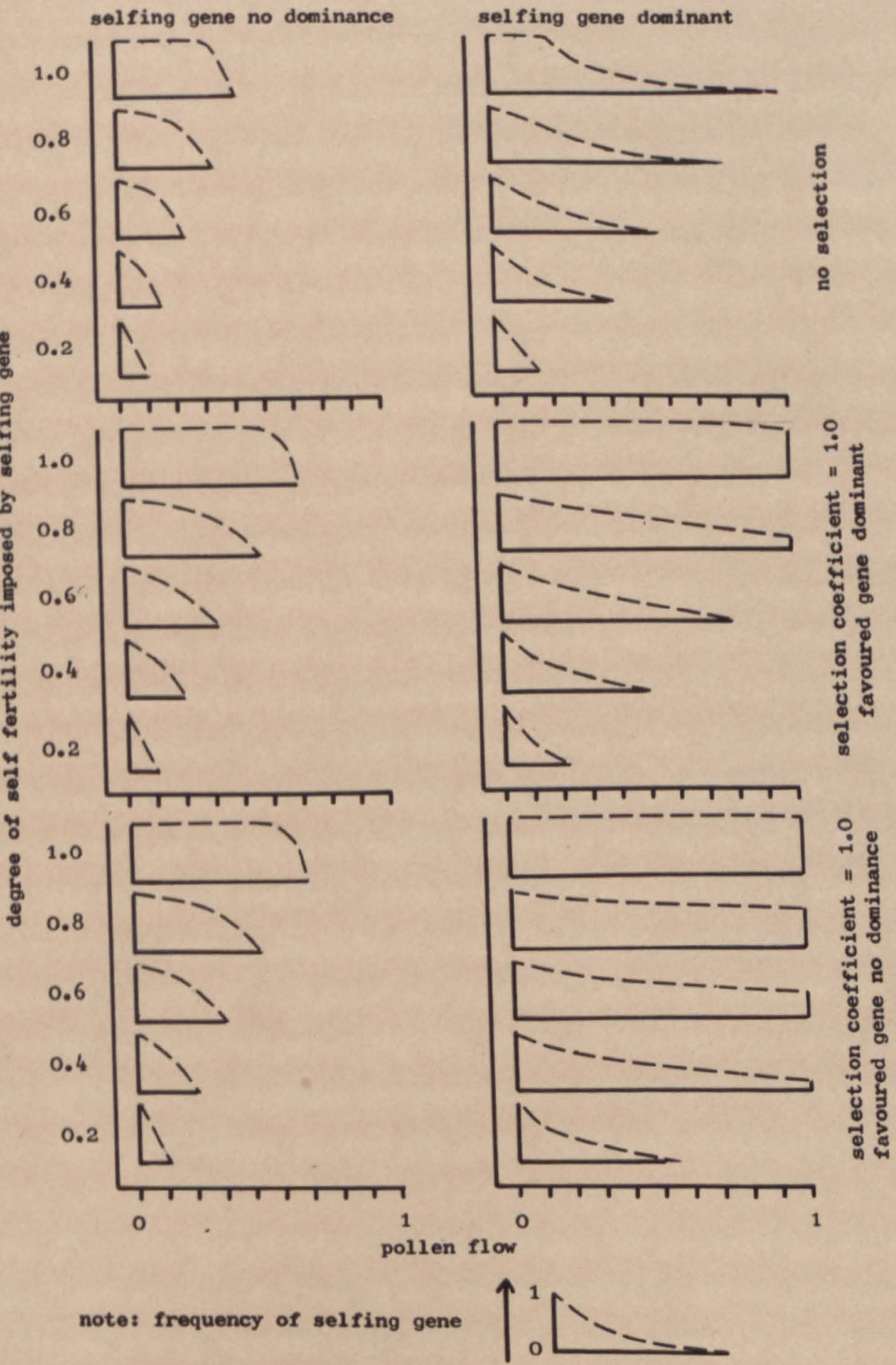
When the selfing gene is dominant, then it spreads through the population less because of its automatic spread and more because of its favourable effect on pollen flow load. If the gene for selfing imposes a high fertility then its spread is considerably more when there is selection. This spread is even greater, if the favoured gene shows no dominance.

These results therefore show three forces which may be very determinant in the spread of a gene for selfing. Firstly, given the population is adapted to inbreeding, i.e. suffers no inbreeding depression, the selfing gene will increase in frequency in the population automatically.

Secondly, if there is selection, but no gene flow, then the rate at which the selfing gene spreads is increased.

Thirdly, if there is gene flow this will tend to oppose the spread of the selfing gene if there is no selection. But if there is selection the selfing gene will increase more rapidly than under any other circumstance.

Fig. 55. The frequency of the selfing gene at equilibrium, with different amounts of selection on the favoured gene, and with various degrees of self-fertility and pollen flow.
(selfing gene unlinked to favoured gene)



5. DISCUSSION

One of the most remarkable features to emerge from this computer simulation is the effectiveness of gene flow as a force determining the genetic structure of populations. This effectiveness is a reflection of the former belief that differentiation over short distances (sympatric differentiation) is impossible (Mayr, 1947).

Gene flow can, if it is sufficiently powerful and if selection is weak, completely obliterate the favoured gene. Even where selection is very strong and genotype frequencies are not greatly affected, it can impose a serious gene flow load on the population. This gene flow load is the inverse of population fitness since load measures the frequency of mortality in the population. Gene flow load can have serious consequences from two points of view.

Firstly, it may affect the rate of colonisation if this is dependent on having a sufficient supply of adapted variants. The supply of an adequate number of variants adapted to the particular habitat may not seem a great problem in view of the large amounts of seed that can be produced by, for example, one individual of *Agrostis*. However selection pressures are multiplicative, and returning to the example of mine populations, even if numerous tolerant individuals are produced, they must *also* carry genes adapting them physiologically and morphologically to other features of the mine habitats. The rate of genetic adaptation to a complex of factors could well be important in determining the rate of colonisation.

Secondly, even if the population size is at a maximum or there is some other factor determining the rate of increase, gene flow load will still have genetic consequences; there will be constant selection for mechanisms to reduce gene flow and increase population fitness.

Gene flow is important also as a means of preserving heterozygosity. Even wihh very little pollen flow the proportion of heterozygotes in the population is above random expectations, particularly so when the incoming genes are recessive. Strong selection against them increases the proportion and therefore even with such selection incoming genes are maintained in the population. The pattern of pollen distribution is leptokurtic which implies that while a considerable amount of pollen lands a short distance from the source, an appreciable amount travels very long distances. This "background rain" of pollen is probably a very important factor in dispersing genes over a wide distance since even if there is selection against such genes they will be maintained in the population.

The extreme heterozygosity has probably another important consequence. It might well be a factor permitting the initial spread of genes for self-fertility or it may swamp some of the otherwise harmful consequences of in-breeding among a few founder members of the population in its early stages. Whereas the population may tend to become homozygous for the favoured (tolerant) gene because of *selection and selfing*, the other genes will effectively still resemble the outside population, since many of them will *tend* to be homozygous because of *selfing* alone; and it has been shown that gene flow counteracts this. There is a balance here between several processes.

The problem of self-fertility is in general not a simple one; and this is most powerfully illustrated in the above computer simulation. The spread of genes for self-fertility may be expected for several reasons: they may spread of their own accord, they may favour the process of selection, or they may act as an isolating mechanism. The latter has been investigated in some detail and the results suggest that whereas a gene for selfing will reduce the gene-flow load on a population, the selection for such genes (in the face of genes for cross fertility entering the population) is not very effective. However, the linkage of genes for self-fertility and the favoured gene is an effective way of assisting the spread of self-fertility with its consequent "beneficial" effects.

Although the computer results do not support any one theory about selfing unequivocally, certain predictions amenable to experimental examination can be made. Linkage of the gene for selfing to the gene for tolerance, greater selfing where the tolerance is not dominant and other such features are discussed earlier.

The models discussed in this chapter show, above all, the complexity of small scale evolution and illustrate the interrelationships of the numerous factors influencing this evolution.

Chapter VI

THE GENETICS OF HEAVY METAL TOLERANCE

1. REVIEW

There has in fact been very little work done on the genetics of heavy metal tolerance. Wilkins (1960) working on lead tolerance in Festuca ovina states that "in spite of the amount of effort devoted to refining the measurement of tolerance, the nature of the genetic mechanism controlling it has not been established with certainty". He nevertheless found that tolerance (whether high or medium range) was dominant and that a major gene with just two alleles was an inadequate model to explain the results. Whether these several alleles were at one locus or at more than one, was not established.

Broker (1963) again found dominant inheritance of zinc tolerance in Silene inflata. However, the F2 data (from selfing F1's) allowed one to conclude very little since only ten plants per family were tested: it was therefore again not decided whether segregation was continuous or discontinuous.

That many genes are involved in the determination of tolerance is supported by Jowett (1959) and McNeilly (1965), and by the present work which shows tolerance to be not an all or nothing effect but continuously variable in natural populations.

More specific studies of Jowett (1959) on the genetics of tolerance showed indications that lead tolerance in Agrostis tended to be partly recessive but Jowett suggests that this could be an artefact of pre-culture conditions which were different in parents and progeny. Nevertheless his data provide evidence for continuous variation in the character and also considerable segregation, suggesting quite marked heterozygosity of the parents.

McNeilly (1965) compared the copper tolerance of seed and adults of different populations, and found a high correlation (r = 0.983) between the two. This suggests that the character of tolerance has a high heritability.

Further evidence of the genetic control of metal tolerance comes from a study of the phenomenon in yeasts. Seno (1962) found that when yeast strains were grown in high copper concentrations they produced two levels of resistance. Both levels were controlled by dominant genes which were very closely linked. Similar results have been obtained by Antoine (1965): here four alleles at one locus were considered to be responsible for copper resistance in yeast. Again the alleles for copper resistance were dominant to the non-resistance allele. It is interesting that evidence was also presented for a remarkable effectiveness of copper ions in mutating the gene for copper resistance to alleles of higher resistance : in this instance mutation appears to be directed. General mutagenic activity of copper ions has been demonstrated by Von Rosen (1964) and may therefore be important in the evolution of metal tolerance itself. However there is no evidence for this in higher plants.

There is therefore considerable evidence that metal tolerance is genetically determined, but apart from indications that it has a high heritability and is often dominant, very few details of its inheritance in higher plants are available.

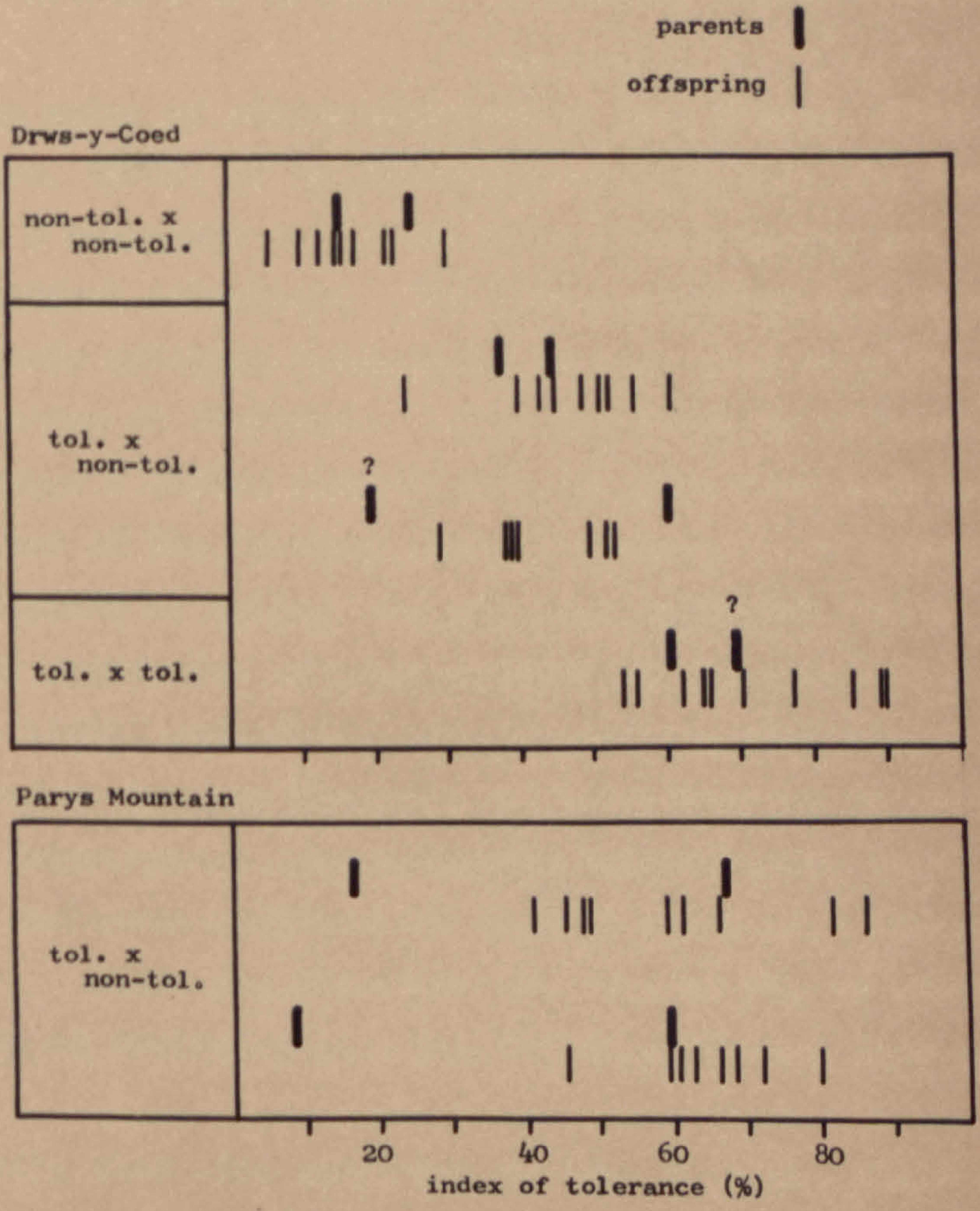
2. INVESTIGATIONS

In view of the scarcity of information on the inheritance of metal tolerance in plants, a genetic investigation involving several hundred crosses was undertaken with a view to elucidating the following:

What is the dominance of the character?
Is it determined by one or many genes?
Is it determined differently on different mines?
What is the relationship between tolerances to different metals?
What is the degree of heterozygosity of the genes for tolerance in the populations?

Although the crosses have been made (seed set data has already been discussed in Chapter [illegible]), most of the material has not yet been assessed for tolerance. However, some preliminary data for copper tolerance are available (Fig. 56). The character is clearly inherited. But there is a possibility that the genetics of tolerance varies from mine to mine. Thus in crosses where plants from Drws-y-Coed are involved, copper tolerance seems to show no dominance, whereas at Parys Mountain it shows dominance. The dominance of copper tolerance on Parys Mountain is confirmed in the study reported in Chapter II.2.b. and Fig. 4a (the cross involved tolerant plants from this region). The analysis of further crosses will confirm or disprove these findings.

Fig. 56. Genetic analysis of copper tolerance in *Agrostis*.
(parents and F1 progeny of preliminary crosses)



note: ? estimated value

3. EVOLUTION OF DOMINANCE

It is usually impossible to predict without genetic analysis whether a character will be dominant, recessive or show no dominance. On biochemical grounds a mutant resulting in an enzyme deficiency will tend to be recessive (or show no dominance) whereas one conferring a new enzyme will tend to be dominant (or show no dominance). In this sense we might well expect tolerance to be dominant or to show no dominance, rather than be recessive.

However, in spite of recent controversy (see Crosby, 1963, and Sheppard and Ford, 1966), it is generally accepted that the degree of dominance of a character is not an inflexible property but can be altered by natural selection. The situation occurring at the boundary of closely adjacent populations is such that we might well expect the evolution of dominance. Thus the computer simulation outlined in Chapter V showed firstly that the gene flow load on a population is less if the favoured gene is dominant, and secondly that under conditions of pollen flow and high selection there was a very large proportion of heterozygotes in the population. One of Crosby's (1963) main objections to the idea that dominance cannot be evolved is the low frequency of heterozygotes in natural populations. Polymorphism also maintains heterozygotes in a population and dominance modification is well known here (Sheppard and Ford, 1966). There are therefore two reasons why we should expect evolution of dominance in a population suffering from gene flow load.

Unfortunately this idea could not be tested in the field except that the indications are that tolerance is frequently though perhaps by no means invariably dominant; this latter suggests that dominance may not be an inherent property of tolerance genes.

A computer model was therefore developed to investigate the phenomenon further.

(a) <u>The model</u>.

The model was a modification of the two gene case described earlier (Chapter V.1.b). One gene was considered a dominance modifier of the other unlinked gene which determines the tolerance. The dominance relations of the gene tolerance were changed according to which modifier genotype was present. An example is given in the table and shows the fitnesses of the different genotypes, when the initial gene has no dominance and one homozygote a fitness of zero.

Genotypes subjected to direct selection

	AA	Aa	aa	
BB	1	1	0	dominance
Bb	1	0.75	0	partial dominance
bb	1	0.5	0	no dominance

(b) Results

The results show that the dominance modifier does not spread through the population to any appreciable extent in the absence of pollen flow.

In the presence of pollen flow however the outcome (Table 24) depends on several factors. The spread, not only of dominance modifiers, but also of modifiers producing overdominance is possible.

The model is one of the modifier being not linked to the gene for tolerance. With linkage, the spread of the modifier will obviously be more effective.

Considering the general situation, the modifier reaches a higher gene frequency,

(i) if the selection is greater.

The spread of the modifier is greatest with very high selection pressures as would occur on a mine.

(ii) if the pollen flow is less.

The effect of pollen flow is complex. Although a greater final equilibrium is reached if the pollen flow is less, the time taken to reach equilibrium is longer. Presumably, with no pollen flow the time taken to equilibrium is so long and the approach so slow that it registers in the model as the <u>defined</u> equilibrium value (in this case gene frequency change is less than 0.0001).

Table 24a. Equilibrium frequency (%) of a dominance modifier in a population subjected to selection and pollen flow

(Figures in brackets refer to number of generations to equilibrium,
initial frequency of modifier gene = 1%
initial frequency of favoured gene = 90%).

Selection	pollen flow					
	0	0.1	0.2	0.3	0.4	0.5
Modifier no dominance						
1.0	1.1	39	26	16	7	1
	(11)	(652)	(413)	(351)	(342)	(8)
0.8	1.1	1	1	1	0	0
	(13)	(12)	(13)	(14)	(38)	(30)
0.6	1.1	1	1	0	0	0
	(18)	(13)	(23)	(36)	(29)	(29)
0.4	1.1	1	0	0	0	0
	(26)	(30)	(44)	(51)	(76)	(173)
Modifier dominant						
1.0	1.2	54	48	40	35	30
	(11)	(171)	(108)	(84)	(72)	(65)
0.8	1.2	48	38	30	23	15
	(14)	(188)	(125)	(105)	(100)	(107)

Table 24b. Equilibrium frequency (%) of an over-dominance modifier in a population subjected to selection and pollen flow.

(Figures in brackets refer to number of generation to equilibrium,
initial frequency of modifier gene = 1%
initial frequency of favoured gene = 90%
selection coefficient = 1.0).

Degree of over-dominance			pollen flow					
AA	AB	BB	0	0.1	0.2	0.3	0.4	0.5
<u>Modifier no dominance</u>								
0.8	1	0	1.2	72	56	44	35	27
			(11)	(238)	(163)	(133)	(117)	(111)
0.4	1	0	1.5	90	81	73	66	58
			(11)	(55)	(43)	(38)	(35)	(32)
<u>Modifier dominant</u>								
0.8	1	0	1.3	65	56	50	45	41
			(11)	(97)	(65)	(53)	(45)	(41)

(iii) if there is greater dominance of the modifier.

With no dominance the modifier only spreads under high selection.

(iv) if the dominance modification is greater.

Genes for over-dominance reach a higher frequency than genes for dominance.

(c) Discussion

An obvious corollary to these results is the expectation that a gene will show different directions of dominance at the opposite ends of a cline (assuming the selection is sufficiently strong at both ends). There is very little evidence for this in the literature, but it is a very real possibility that has yet to be examined. Some evidence comes from the work of O'Donald and Davis (1959) who provide evidence for the dark-phase in the colouration of the Arctic Skua being more dominant where it is more frequent; the light phase is conversely less recessive where it is more frequent. O'Donald and Davis however consider that such evolution of dominance "can only occur once the population has become isolated from the migration taking place within the cline: the introduction of alien gene complexes must continually break down the modifier balance". The present data suggest that such isolation may not only be unnecessary, but positively detrimental to the evolution of dominance within a cline.

Chapter VII

GENERAL CONCLUSIONS

GENERAL CONCLUSIONS

The evolution of differences between closely adjacent populations is a complex process and reflects the complexity of genetic adaptation in natural habitats.

In the particular case which has been the subject of these investigations, the differences that have been found are very extensive. Tolerant and non-tolerant populations differ in their response to nutrients, morphology, longevity, flowering time, and self-fertility. The exact causes of these various differences between the populations have only been briefly examined: but it seems clear that while many are direct adaptations to the local conditions of the habitats, others such as flowering time and self-fertility appear to be adaptations to the particular genetic conditions in adjacent diverging populations.

This has significant bearing on the general problem of expansive evolution. The evolution of a species may be necessitated by changing conditions; but in other cases a species extends its range and enters a new habitat by the evolution of genotypes adapted to this new environment. This is so in the case of species entering metal contaminated regions.

The essential prerequisite for this is that the basic population or species should carry variants that confer adaptation to the new habitat. This adaptation may be gradual and involve the occupation of intermediate zones, or, as has been shown to be a possibility in Agrostis, it may be immediate because of the presence in the base population of extreme variants confering adaptation in one generation. Once the primary requirements of the new habitat have been fulfilled in this way, the adaptation can become precise by more specific selection.

The factors determining such evolutionary change are complex, but the general principles have been frequently discussed. While it is generally accepted and can be shown on theoretical grounds that complete inbreeding leads to homozygosity and loss of variability, a completely panmictic population is probably not the most effective in promoting the origin of infraspecific novelties. Wright (1931) first proposed that a complex of semi-isolated populations is the most effective system for evolutionary change, and this is reflected in the idea that selfing with occasional outcrossing is a very effective breeding system. It is also analogous to a population, such as Anthoxanthum on Trelogan mine, consisting of a few dominant individuals around which there is a high turnover of smaller individuals which must be a source of, occasionally highly adapted, segregants. In all these systems there is room for favourable gene complexes to become established and not be destroyed by random interbreeding.

In the case of metal-tolerance the precise source of the adaptation is not known: tolerant plants in normal populations either have some alternative adaptive role or are unwanted segregants.

Once the available variation is to hand the populations will tend to enter a new adaptive peak. This in itself will create problems since any large scale interbreeding of the locally differentiated population with the base population tends to be deleterious. Previous work on metal mines has shown that natural seed produced by tolerant plants has a lower tolerance than seed produced by the same plants in isolation: there is intercrossing with the non-tolerant populations in the natural habitats (McNeilly, 1965) and this dilutes the tolerance.

On such metal mines the forces of disruptive selection seem in themselves sufficient to effect divergence: gene flow is not an impossible burden. But there are nevertheless pressures to evolve breeding barriers and so reduce the gene flow load on the population.

The breeding barriers may be themselves very subtle. The earlier flowering time and greater self-fertility of the tolerant populations are by no means a simple response to selection for mechanisms to reduce the gene flow. They are related to environmental factors of the mine habitat and general genetic strategies of colonising species (in the absence of gene flow). Other changes such as greater longevity are similarly of wide implication. But whatever their causes, they have a marked effect on gene flow.

Another 'barrier' to gene flow is the evolution of dominance which has been shown in this study to be a theoretical possibility. An increased dominance of the genes conferring adaptation in the new habitat reduces the gene flow load and shelters the incoming genes in a recessive form. At the same time as the effects of gene flow are minimised, there is increased heterozygosity and increased variability of the new population.

Gene flow in small doses can have directly beneficial effects: its deleterious effects can be easily held in check by natural selection. Firstly, the colonising species may suffer from inbreeding amongst a few initial colonisers: crossing with the parent population may offset some of the serious effects. Secondly, the old population may continue to provide useful variants for the new population, helping it to adapt to local conditions: this is the principle of recurrent back-crossing in plant breeding. Greater divergence under disruptive selection with a limited gene flow than under bi-directional selection (no gene flow) has been found by experimental selection in <u>Drosophila</u> (Millicent and Thoday, 1961). The precise result of gene flow under a given set of conditions can only be defined by experimentation; this definition would take the form of a relationship between the basic variability of a given character, the selection pressure, and the amount of gene flow needed to annul progress to selection.

There is a third beneficial effect of gene flow. A species which occupies a number of contrasting habitats becomes broken up into several differently adapted populations by disruptive selection. Each population, subject as it is to its own directional and stabilising selection, is not very variable. But if gene flow is imposed on such a system of populations, each population shares the variability of all others. This problem has been considered on a theoretical level by Levins (1965) who suggests that the optimum level for gene flow increases if there is a variation of the environment with time, but is decreased if there is stability of the environmental differences. This stability will be less if the environment is patchy and the organism has little opportunity of escaping from where it first establishes: it then lands in different conditions in different generations. Where the organism has the ability to choose its own uniform niches in a heterogeneous environment, then it is effectively in a stable situation and the optimum gene flow is much lower. The work of Levins shows therefore that in any given environmental pattern, there is a gene flow strategy which is the optimum for any species. The balance between variability and genetic load demands an optimum gene flow.

In view of these considerations, the tentative nature of the isolating mechanisms reported here may not be surprising. One might expect these mechanisms to proceed only to a certain degree but there is as yet insufficient knowledge about the genetic architecture of tolerant and non-tolerant populations to decide whether such an optimum has been approached.

Whatever the precise balance in mine populations, it is clear that forces leading to isolation are present and that this isolation has already proceeded to some degree. The evolution of breeding barriers can therefore occur within a unit that is more or less panmictic. The former belief that allopatric divergence is an essential prerequisite to the evolution of breeding barriers (Mayr, 1947) is therefore brought into question. The adjacent populations because they are in different areas are perhaps by definition not sympatric, and therefore it could be said that this example does not show the beginnings of sympatric speciation. (The term 'parapatric' is a useful alternative since these situations are clearly distinct from gross allopatry).

Whatever definition is given to this process it illustrates two features about speciation. Firstly, isolation is not a prerequisite to divergence: adjacent populations can be as different from each other as populations hundreds of miles apart. Secondly, expansive evolution inevitably leads to geographical isolation. At the beginnings of this process specific isolating mechanisms may have arisen between adjacent populations which have subsequently become separated. The study of two already separated groups may lead to the hypothesis that the divergence and isolating mechanisms have developed purely in isolation, when in fact they might have arisen before geographical separation. After all it is at the initial stages of divergence that isolating mechanisms would be the most useful.

Closely adjacent populations therefore provide a powerful tool for the study of evolution and this thesis has indicated the several ways in which such populations can help us understand the processes of selection, colonisation, and speciation. It also has repercussions on subjects as diverse as evolution of gene expression and of life cycle strategies. General studies of already distinctive races or closely related species have been invaluable in indicating the types of evolutionary change that might be expected at the population level. However, they have diverted attention from the primary factors leading to population change, divergence and expansion. Adjacent populations show, above all, that evolution has a beginning.

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NOTE

Abbreviations are from:

World List of Scientific Periodicals
Brown, P., and Stratton, G.B.(1964) 4th Edition.

except

Heredity, Lond.	is abbreviated	as		Heredity
Genetics, Princeton	"	"	"	Genetics
Evolution, Lancaster, Pa.	"	"	"	Evolution
Nature, Lond.	"	"	"	Nature

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APPENDIX

Appendix 1

PLANTS GROWING ON AREAS OF HEAVY METAL CONTAMINATION: A REVIEW

CONTENTS

This review deals mainly with higher plants but does not contain a detailed reappraisal of the work of Jowett (1959), McNeilly (1965) and Gregory (1965) carried out in the Department of Agricultural Botany, University College of North Wales, Bangor.

Nor does it consider the ecological, physiological and evolutionary problems associated with serpentine soils and with aluminium and manganese toxicity in plants.

1. ECOLOGICAL INVESTIGATIONS

(a) Species present on contaminated soil

The occurrence of plants on metal contaminated soil has been long recognised. The earliest record is probably that of Thalius (quoted by Ernst, 1965) who noted *Minuartia verna* as an indicator of metals, in 1588. Even before 1900 there was considerable work in this field: for example Baumann (1885) and Jensch (1894) quote lists of species which are found on zinc contaminated soils. And early works on mining (e.g. Foster, 1894) also frequently mention indicator plants.

An attempt to classify these plants into various types was made by Lambinon and Auquier (1964), who recognised the following:

Metallophytes - taxa uniquely found on metal contaminated soil.

Absolute metallophytes - found only on metal contaminated soil over all their distribution e.g. *Viola calaminaria*, *Thlaspi alpestre ssp. calaminare*.

Local metallophytes - only occur on contaminated soil in a given region, e.g. *Armeria maritima*, *Stereocaulon nanodes* (lichen).

Pseudometallophytes - taxa occurring also on normal soil.

Elective pseudometallophytes - abundant and often more vigorous on contaminated soil e.g. *Campanula rotundifolia*, *Polygala vulgaris*, *Thymus pulegioides*, *Agrostis tenuis*, *Weisia controversa* (moss).

Indifferent pseudometallophytes - live on contaminated soil but neither show abundance nor particular vitality e.g. *Plantago lanceolata*, *Avena pubescens*, *Genista tinctoria*, *Cladonia* spp. (lichens).

Accidental pseudometallophytes - usually weeds and ruderals appearing sporadically and showing reduced vigour on contaminated soils.

A similar but less extensive study was made by Schwickerath (1931). He showed very sharp changes in species composition across the boundary between contaminated and normal pasture soil: within a transect length of 13 metres, 11 species were confined to the mine, 13 were more or less common to both habitats, and 6 were confined to the pasture and did not appear on the mine. Nicolls et. al. (1965) term such sharp vegetation changes, "cut-outs" and discuss their use in biochemical prospecting.

Species of mosses restricted to copper contaminated areas are well known and some (often belonging to the genera Mercoya and Meliochhoferia) have been given the name of "copper mosses" (Persson, 1948, 1956, Schatz, 1955, Noguchi, 1956, and Noguchi and Furuta, 1956).

These classifications are a reflection of the fact that the species found on metal contaminated soils are often very characteristic either in that they are largely restricted to such soils or in that only a few species (out of a whole range of species in the background vegetation) can colonise them.

The species found on the mine soils are various and obviously differ according to the geographical area and local ecological conditions. However no very clear cut taxonomic pattern emerges. If we look at the list given by Schwickerath, of plants growing on more or less contaminated zinc soil in Germany, the following families are represented:

Graminae	5	species
Papilionaceae	4	"
Compositae	3	"
Caryophyllaceae	2	"
Rosaceae	2	"

and 16 other families are singly represented. Few other comprehensive lists are available from other areas.

Most of the species found on mine soils are herbs (with a few low shrubs such as Calluna vulgaris) and the majority are perennials. This may be a reflection of the fact that such mines are normally surrounded by pasture communities, or may in some other way be related to the ecological conditions.

Although space does not permit the inclusion of lists of species growing on contaminated soils, examples of the commonest recur frequently in this review. Other records (not mentioned subsequently) of plants growing on contaminated soils are frequent. Thus Andrae (1882) quoted by Schultz (1912) mentions Arabis halleri as an indicator of zinc; Bailey (1898) dealt with "copper plants"; Stutzer (1907), Simon (1909) and Batemann and Wells (1917) considered several species found on copper contaminated soils; Linstow (1929) and Dorn (1937) gave further examples of indicator plants; Nemec et al. (1936) quoted Equisetum arvense as a gold accumulator; Robinson et al. (1947) considered several species growing on zinc soil; Rune (1953) listed plants found on naturally occurring toxic soils in Sweden; and more recently lead from exhaust fumes and orchard sprays has been shown to have profound effects on vegetation by Warren and Delavautt (1960).

Plants growing on metal contaminated soil are therefore well known; the phenomenon is by no means rare. The colonisation of toxic areas is a regular feature, and whereas the colonisation of some is sporadic, other areas carry regular communities.

(b) Community studies on contaminated soils

Several workers mostly in Germany, have examined in detail the plant communities found on metal contaminated soil.

Schwickerath (1931) studied the association on twenty lead and zinc mine tips and found that they could be subdivided into vegetation types representing different phases of colonisation. The early colonisers and species characteristic of all the vegetation types were Viola lutea, Armeria elongata, Minuartia verna, Thlaspi alpestre.

Koch (1932) also gave a brief account of plant communities on zinc soil, in particular the Alsine verna - Thlaspi alpestre association.

The most detailed ecological investigation on mine populations as yet carried out is that by Ernst (1965). He reconsidered the classifications of the mine plant communities by other workers

(Schwickerath, 1931, Heimans, 1936, Koch, 1932, and Schubert, 1952, on lead, and Tüxen, 1937 and Lebrun et al., 1949, on copper) and regarded the communities growing on lead and zinc soils in mid-Europe as all belonging to one association which he termed the Violetea calaminariae, being characterised by Viola calaminaria, Silene cucubalis var. humulis and Minuartia verna ssp. hercynica. Within this general class he recognised three groups: the Armerion halleri in drier habitats in mid-Germany, the Thlaspeion calaminariae of mid-Europe and the Galio-Minuartion vernae of the alpine regions.

This work confirms that mine communities carry characteristic species and can be identified as forming distinct associations. Although extensive classifications of mine communities have been rarely carried out elsewhere other than in Germany, it is clearly evident that they are very different from those of the surrounding vegetation and that communities on different contaminated areas are similar. This fact is used in biogeochemical prospecting (e.g. Nicolls et al. 1965) and is therefore important.

Species and communities characteristic of contaminated areas are therefore well known. Several workers have considered the factors which influence the distribution of species in the mine communities.

(c) Factors determining plant distribution on contaminated soil

(i) Metal concentration and type.

The overriding characteristic of these contaminated soils, whether they are natural outcrops or waste tips, is the high metal concentrations in the soil. However although the area of contamination is frequently recognisable by a flora different from that in the surrounding areas, very few workers have studied precisely to what extent metal concentration and type of metal are important in determining the distribution of mine plants.

Jensch (1894) showed that soil taken from around the roots of calamine plants, had a slightly lower concentration of zinc than soil taken from bare areas (no estimate of significance can be made from the data), suggesting that the plants colonised areas of lower concentration and therefore presumably lower toxicity.

Schwickerath (1931) found that his vegetation types, representing increasing colonisation, were correlated with both lead and zinc concentration of the soil: increasing colonisation was correlated with lower concentrations of the metals. Whether the colonisation ameliorated the soil or whether the soil concentration determined the colonisation was not examined in detail. However the evidence was that colonisation depended on the presence of other species (therefore on some ameliorative effect) and not just on soil type: five detailed quadrats showed that, with increasing colonisation, new species were distinctly associated with the *Festuca* sward.

Ernst (1965) recognised three initial phases in the colonisation of zinc contaminated areas: and the last of these phases, which contained several species, had on the whole a lower concentration than the first.

Both of these studies are open to the criticism that neither of them followed a particular community over a period of time. The different, apparently sequential, phases may simply reflect a decreasing metal concentration in the original soil. Certainly other factors are involved but whether a marked successional process occurs on mines cannot be regarded as finally established.

The study of Nicolls et al. (1965) emphasised in a subtle way, the controlling effect of metals on the vegetation. As expected, sharp changes in the vegetation coincided with changes in total contamination. However, changes in the relative amounts of lead, copper and zinc were also important. Of the various species considered, *Eriachne mucronata* seemed to tolerate high concentrations of all three metals, *Bulbostylis barbata* and *Polycarpaea glabra* were found in areas of high copper, while *Tephrosia sp. nov.* was found where the copper concentration was lower. In general, changes in lead and copper were more determinant than changes in zinc.

These various studies re-emphasise the obvious importance of metal contamination and show that it can have a precise effect on species distribution. However other factors are also important.

(ii) Factors other than metal contamination.

The importance of factors other than metal concentration in determining the vegetation growing on mine soil is often very clear cut: mines on acid soils differ markedly from those with a higher pH, having generally fewer species and being typified by Agrostis tenuis as the dominant component. However there is evidence from other sources that additional factors can be critical.

Schwickerath (1931) first noted that elements in the soil, other than metals, may be important for survival of plants in contaminated areas. Thus his vegetation types, mentioned earlier, although they correlated with total lead and zinc in the soil seemed to correlate better with the calcium/zinc ratio. Since calcium alleviates the uptake and toxic effects of zinc, and more so of lead, this ratio is probably a good measure of zinc availability. Lead was perhaps completely unavailable to plants in this instance.

The importance of other soil factors was further pointed out by Ernst (1965). He studied the ecological conditions on mines characterised by the three initial stages of colonisation, namely the Silene cucubalis var. humilis stage, the Minuartia verna ss hercynicium stage and the Euphrasia spp. stage. The Silene and Minuartia stages grew on soils differing mostly in their water capacity. This was a reflection of the soil texture. Silene grew on soils with a coarse texture, low water capacity, and (because of the high conductivity of large soil particles) low surface temperature. Minuartia on the other hand grew on soils with finer texture, high water capacity, but (because of a low conductivity of heat from surface because of numerous small air spaces) high surface temperature. The habits of these plants, deep rooting Silene with narrower leaves than the normal forms, and the shallow rooting but tufted habit and needle like leaves of Minuartia adapted them to their respective habitats. The Euphrasia phase was considered to be a succession from the above two phases, but also characteristic of soils of even higher water capacity and, as mentioned already, lower metal content. Ernst therefore provides very clear cut evidence for the importance of factors other than metal content to plants growing on metal contaminated soils.

Nicolls et al. (1965) found that while calcium and phosphorus level in the soil exerted an important effect on the composition of the vegetation on metal outcrops, physical factors such as relief, drainage and soil texture, seemed relatively unimportant.

These studies on the factors determining plant distribution in contaminated areas have been inadequate from several standpoints.

Firstly, they have rarely been done in conjunction with physiological studies to determine the tolerance of the various species to different metal concentrations. As a consequence, it is not know whether the mine plants under consideration are tolerant or not to different levels of metal in the soil, or whether other factors must be controlling their distribution.

Secondly, evidence will be presented in the following section which shows that the ability to colonise mine areas, requires the ability to evolve tolerance. The evolutionary factor in plant distribution on metal contaminated areas is important. The classification of metal soil plants into metallophytes and pseudometallophytes (Lambinon and Auquier, 1964) outlined earlier may reflect not simply an ability to tolerate high contamination but an ability to evolve such tolerance.

2. EVOLUTIONARY INVESTIGATIONS

The existence of plants on metal contaminated soils immediately raises the question of whether these plants belong to species which are for some reason inherently tolerant to metals, or whether they are plants that have evolved a special tolerance not possessed by the remainder of the species.

(a) Evidence for tolerant races

The first comparative study of mine and non-mine populations comes from Prat (1934). He found *Melandrium silvestre* growing on soil containing 0.8% - 1.8% copper by dry weight. Seed from *Melandrium silvestre* on the mine, and seed of the same species from a botanical garden were compared for growth on garden soil with varying quantities of copper carbonate. Plants from the contaminated soil grew far better at the higher concentrations of copper than those from the uncontaminated and at the highest concentrations the plants from uncontaminated areas died in the seedlings stage. On normal soil the plants from the mine were quite healthy and therefore had no absolute need for copper. Prat attributed the increased resistance of plants growing on copper mines to the action of natural selection.

This investigation was the only one of its kind, till the 1950's when studies of mine and non-mine populations were resumed, independently, in Great Britain and in Germany.

Bradshaw (1952) reported populations of *Agrostis tenuis* tolerant to mine soil; plants of the same species from a neighbouring pasture did not grow on the mine soil. Following this Wilkins (1957, 1960) developed a rooting technique which showed *Festuca ovina* to be tolerant of lead, and demonstrated a correlation between tolerance and amount of extractable lead in the soil. Using this technique subsequent workers showed numerous other species to be tolerant, often to several metals (depending on the type of metal contamination). These results are summarised in the following table.

Metal tolerance : evolution

Species	Metal	Author
Agrostis tenuis	Pb,Zn,Ni,Cu	Jowett (1958), Gregory and Bradshaw (1965)
Agrostis stolonifera	Pb	Jowett (1958)
	Zn	Gregory and Bradshaw (1965) Archer (1964)
A. tenuis x stolonifera	Zn	Gregory and Bradshaw (1965) Archer (1964)
Agrostis canina	Zn	Gregory and Bradshaw (1965)
Festuca rubra	Zn	Gregory and Bradshaw (1965)
Festuca ovina	Zn	Gregory and Bradshaw (1965)
Holcus lanatus	Zn	Jenkins and Winfield (1964)
Anthoxanthum odoratum	Zn	Gregory and Bradshaw (1965), Putwain (1963)
Rumex acetosa	Zn	Spilling and Thomas (1964)
	Cu	Coackley and Dawson (1966)
Plantago lanceolata	Zn	Williams and Morgan (1964)

Minuartia was investigated by Humphreys and Farnworth (1964): plants from a zinc/lead mine were shown to grow satisfactorily on soil from the mine while Lawrence (1964, personal communication) reports that Minuartia from normal soils is not tolerant to metals.

Work in Germany has also produced an impressive list of plants showing tolerant races. Thus Schwanitz and Hahn (1954a, 1954b) recording death (or not) in water culture with different amounts of zinc, showed the following to be tolerant if taken from contaminated soil: Viola lutea, Alsine verna, Silene inflata, Linum catharticum, Campanula rotundifolia, Plantago lanceolata, Rumex acetosa. They also showed that Silene inflata produced a copper tolerant race.

Further work by Baumeister (1954), Baumeister and Burghardt (1956)& Wachsmann (1961) recording rate of photosynthesis and general growth and Broker (1962) recording dry wt in water culture, has confirmed that Silene inflata forms races specifically tolerant to zinc and to copper.

Repp (1963) measured the cellular resistance to metals by finding the concentration that produced death of epidermal cells (as recognised by failure of the cell to plasmolyse in strong sugar solution). This technique showed that Taraxacum officinale as well as Tussilago farfara could evolve races more tolerant to copper than the normal ones.

Url (1956) using a similar technique showed that species of mosses from copper contaminated regions had a far higher resistance to copper than species from normal soils. This work was however not extended to the intra specific level.

From all these findings we can say that the evolution of metal tolerance is a very general phenomenon and characteristic of a wide range of species. Is there any evidence that some species are inherently tolerant to metals (even when not growing on metal contaminated soil) and are thereby able to colonise contaminated areas?

(b) Evidence for general tolerance of the species

The only work which suggests that inherent tolerance may be important in colonising metal mines, comes from the work of Repp (1963). She showed that the cellular resistance of Silene inflata from normal soil was just as high as the resistance of Silene from mine soils. This finding is however in contrast to the whole plant investigations of Wachsmann (1961) and Schwanitz and Hahn (1954a). It implies either that the technique of Repp was too insensitive to pick up a difference, or that copper tolerant Silene has some exclusion mechanism which prevents the copper reaching the cells.

(c) The 'need' for metals by tolerant plants

Several workers in the literature on plants growing on metal contaminated soils have suggested that these plants have a positive need for the metal and are for this reason restricted to such areas (e.g. Schatz, 1955). The findings on tolerant races suggest that this is not the case: tolerant plants (and plants from contaminated areas generally) grow well in normal soil. However there is some evidence that tolerant plants are stimulated in their growth by levels of metal considerably above the normal micro-nutrient (trace) levels.

The first evidence for this was produced by Baumeister (1954), and Baumeister and Burghardt (1956), who grew plants of *Silene inflata* in different levels of zinc and measured dry-mass production as well as the rate of assimilation. In a preliminary experiment where zinc was added to pots of soil, it was shown to be stimulating in its effect and resulted in a greater fresh weight and a greater rate of carbon dioxide assimilation of the tolerant type.

The results were extended to sand culture experiments. Plants from the mine showed a slight stimulation in their rate of carbon dioxide assimilation by the addition of 10 mg and 100 mg zinc sulphate per litre of culture solution whereas control plants at 100 mg showed a lower rate of carbon dioxide assimilation. Repeat experiments in water culture, where 10 mg and 100 mg zinc sulphate per litre were added to the culture solution, gave a much more clear cut effect of the zinc: and the higher concentration increased the rate of carbon dioxide assimilation of tolerant plants but led to the death of the non-tolerant.

These results were confirmed by a more extensive investigation (Baumeister and Burghardt, 1956). The stimulating effect of the zinc on the rate of carbon dioxide assimilation was parallel~~l~~ed by similar effects on the chlorophyll contents of the leaves.

Further evidence comes from Jowett (1964) on *Agrostis tenuis* with lead, Putwain (1963) on *Anthoxanthum* with zinc, and Barker (1966, personal communication) on *Agrostis tenuis* with lead, copper and zinc, and McNeilly (1965) on *Agrostis tenuis* with copper. All these authors found indices of tolerance greater than 100% i.e. there was more root growth in metal than in water. Again Jenkins and Winfield (1964) found a very clear cut stimulatory effect of zinc on *Holcus*. In none of these cases did the control plants show stimulated growth with increasing metal concentration.

Although Ernst (1965b) did not use normal control populations, 5 out of 6 species from zinc soils produced a greater percentage germination and rate of germination in 50 ppm zinc than in 1 ppm. *Epilobium angustifolium*, a species from a normal area did not show this effect.

There is therefore considerable evidence that tolerant plants are stimulated in their growth by small amounts of metal. This could be interpreted as a definite need for metal in these plants but a fairer interpretation is probably that, because of the efficiency of the tolerance mechanism in inactivating the metals, the normal trace element requirement is rather higher.

Again it has been shown in this thesis and elsewhere (McNeilly, 1965, Putwain, 1963, personal communication) that metal tolerant plants are generally competitively inferior to normal plants when grown on normal soil. This again may be a reflection of a 'need' for metal by tolerant plants. Or again it may indicate that the normal metabolism is in some way upset by the tolerance mechanism.

3. GEOGRAPHICAL INVESTIGATIONS

Metal contaminated areas, either naturally occurring or man-made, are very widely distributed. They also, as has been previously shown, carry a very distinct type of vegetation which often contains species largely or wholly restricted to such areas. The geographical distribution of these species is therefore highly disjunct and this has attracted the attention of several workers.

Schultz (1912) looked at plants growing on metal mines from a geographical standpoint and came to some interesting conclusions which however cannot go unchallenged. He argued that since both *Viola lutea* and *Minuartia verna*, two plants commonly found on contaminated soil, have their main distribution in the alpine regions of Europe (and *Minuartia* also in the arctic regions and Asiatic mountains) and show elsewhere in Europe a highly disjunct distribution (being largely limited to metal contaminated areas), they must have been widely distributed throughout Europe at one time when conditions were favourable. These conditions would have been cool summers which allowed little forestation and may have been encountered, he suggests, in the fourth ice age. Since then conditions have been much warmer and he argues that their distribution has been limited to mine tips where they do not suffer from the additional effects of competition. He also considered some other famous heavy metal plants: *Thlaspi alpestre* and *Arabis halleri* he suggested are also of a formerly wide distribution but *Armeria halleri* and *Silene vulgaris* do not show a clear cut picture.

Similar arguments to those developed by Schultz (1912) have been used in connection with a study of *Festuca spp.* growing on contaminated areas in and around Belgium (Auquier, 1964). Auquier came to the following conclusions. The Fescues belong to several taxa but none of these taxa are completely restricted to calamine soils, their relatives being often found in upland areas. However, in Belgium itself these plants are strictly confined to calamine (zinc contaminated) soils, and seem to be absent from neighbouring normal soils. Auquier infers from this that these taxa represent remains of plants which once had a wide distribution during the arctic-alpine climate of the last ice age.

However several of the species mentioned by Schutz (1912) and the *Festuca spp.* have been shown (previous section) to evolve tolerant races. It is probable that the other species have also done so. Moreover there is no reason to believe that this process takes a particularly long time (see this thesis). This leads to several explanations of the disjunct distributions.

Firstly, these so-called species are not distinct and reproductivity isolated from their relatives, but are products of parallel evolution on different mines from these relatives in the neighbouring pastures. This may be the case with Auquier (1964) where the taxa are by no means very distinct.

Secondly, these species are distinct (do not have any near relatives in the vicinity) and have in the past evolved tolerance which now enables them to escape competition from normal plants. This is a modification of the explanation of Schultz and Auquier.

Thirdly, contaminated soils are commonly associated with man's activities, and may simply indicate the efficiency of dispersal through human agency. Many of the mines are in mountainous regions and dispersal of alpine members such as *Viola lutea*, *Minuartia verna* and *Thlaspi alpestre* from upland to lowland mining areas could easily have occurred.

Fourthly, many metal mines are recent and in no way correlated with natural metal outcrops. Arguments about plant distribution on them, cannot involve factors present more than a few hundred years ago.

There are therefore several explanations for the disjunct distribution of mine species and no-one has yet considered these possibilities fully.

4. TAXONOMIC INVESTIGATIONS

The difference in morphology of plants growing on metal contaminated areas, from plants in normal areas was noted by Baumann (1885) and Jensch (1894). Numerous workers have since then remarked on this feature of tolerant populations.

It is therefore not surprising that taxonomists have attempted to classify these plants as separate taxa. Floras and the literature are strewn with subspecific and varietal epithets (e.g. see Ernst, 1965) referring to mine taxa.

Two genera have received considerable taxonomic attention. Heimans (1960) considered taxonomic problems associated with the type Viola calaminaria. Chromosome counts on this type from the zinc contaminated areas of the Aachen district gave the chromosome number of $2n = 52$, whereas counts on its relative, Viola lutea from normal regions gave $2n = 48$. On this basis Heimans removed Viola calaminaria (sometimes previously termed, Viola lutea var. calaminaria) from the Viola lutea group of the genus and re-established it as a distinct species. This work is interesting in that it suggests that perhaps some of the mine races may have been formed by a process similar to catastrophic selection (involving drastic chromosomal adjustments of a specially adapted type, leading to isolation) described by Lewis (1962). However no attempt to cross these species have been reported.

Auquier (1964a) investigated the genus Festuca, growing on metal contaminated soils in and around Belgium and classified the genus into two main species, one of which they divide further into subspecies, varieties and forms. This work can however be criticised from several standpoints. Firstly, material used in this study was collected directly from the field: no attempt was made to grow it under standard conditions. The taxa include the fine delimitations of variety, sub-variety and form; these could easily be environmental modifications. Secondly, even if the differences between the taxa are inherited they could easily have been acheived by parallel evolution on different mines since the characters that were measured (length of floral parts, anatomy of tillers, hairiness, vein number, leaf diameter) could easily have been affected by selection for local adaptation.

The taxonomic investigations on mine plants are either too cursory or unreliable. Types adapted to extreme conditions must be classified with the help of experimental taxonomy if any reliable picture is to emerge, and no experimental taxonomy has so far been done.

5. PHYSIOLOGICAL INVESTIGATIONS

Physiological investigations on plants growing on contaminated soil have been largely limited to measuring the levels of the various metals in the plants.

(a) Zinc

Baumann (1885) quotes the several early works in this field. Risse found the following values for plants growing on zinc contaminated soil.

	% ZnO in ash	% ZnO of dry wt.
Thlaspi alpestre	21.30	1.32
Viola tricolor var. *calaminaria*	4.28	0.37
Armeria vulgaris	6.27	0.37
Silene inflata	2.66	0.44

He also quotes Braun as finding considerable quantities in *Viola tricolor*, and Krauch who found unhealthy grasses growing near a zinc tip to contain 0.242% ZnO (of dry wt.) and unhealthy rye in the same area, 0.076% ZnO. Jensch (1894) showed that plants growing on heavily contaminated zinc soil (percentage by dry wt.) of $ZnCO_3$ = 14.25 - 17.75, and of $ZnO.\ SiO_2.\ 2H_2O$ = 0.81 - 3.73) contained fairly large quantities of zinc, but that the quantities varied according to the plant organ investigated.

	% Zn in ash Root	Petiole	Stems	Leaves
Tussilago	2.5 - 3.3	1.8 - 1.6		2.8 - 2.9
Polygonum	1.8 - 1.9	-	2.3 - 2.9	1.2 - 1.5

Further studies on calamine plants are quoted by König (1899), Emmerling and Kolkwitz (1914), Bertrand and Audreitcheva (1933), Javillier (1908), Laband (1901) and Macquinay et al. (1961) on lichens.

Ernst (1965) confirmed that zinc was taken into plants growing on contaminated soil and extended these studies further. Most of the zinc was found in the roots and leaves (five species investigated), less in the stems, and least of all in the flowers. Moreover the quantity of zinc in the plants increased (by 113.3% in *Cardaminopsis* *halleri*) during the growing season (June-October). Different plants were also shown to accumulate different amounts of zinc, the average values for the more common members of calamine soils being:

	Zinc content in ppm dry wt.	Number of sites investigated
Thlaspi alpestre ssp. calaminaria	7,757	7
Armeria maritima ssp. halleri	3,328	5
Minuartia verna ssp. hercynica	3,007	17
Armeria maritima ssp. calaminaria	1,895	2
Silene cucubalis var. humulis	1,719	37
Viola calaminaria	686	4

Thlaspi alpestre should perhaps be considered as an accumulator plant since in several instances it contained a higher concentration than the concentration of available zinc in the soil.

Although this may to some extent reflect the quantity of zinc in the soil on which these plants were living, this order was consistently maintained even if the plants were taken from the same contaminated region. Ernst quotes the work on plants growing on normal soil and here the zinc values range from 4-104 ppm.

Ernst studied the relation between available zinc (Scharrem and Munk, 1956) and the zinc in the plant and found that in general the higher the zinc in the soil, the higher the quantity in the plant. Although the absolute amount taken up varied from region to region, the correlation was always good within any one area and for all the species studied. This was in contrast with the results of Macquinay and Ramaut (1960) who, measuring the total zinc content, only found a good relation with *Silene cucubalis* var. *humilis*.

Nicolls *et al*. (1965) studied the metal content of plants and soil at natural metal outcrops. All the species studied took up zinc when growing on rocks with a high zinc content, and again as with the results of Ernst the species showed differing zinc accumulation, and moreover some organs accumulated more than others. The precise pattern of zinc accumulation within the plant varied with the species, some species having a higher accumulation in the flower heads (*Bulbostylis barbata*), some showing no definite differences (*Tephrosia* sp. nov.) while in others the pattern depended on the metal concentration in the soil (*Polycarpaea glabra*). Again there was a marked difference in the uptake of zinc by *Trioidea pungens* growing on normal and contaminated soil. In all the species examined there was a clear cut linear relation between zinc concentration in the plant and that in the soil.

Other investigations on the uptake of zinc include those Tooms and Jay (1964), Vogt et al. (1943) and Robinson et al. (1947).

Zinc therefore is readily taken in by plants growing on zinc contaminated soil and nowhere in the literature is there any evidence of these plants having an exclusion mechanism, enabling them to survive on contaminated soils. The tolerance mechanism of zinc must be internal.

(b) Copper

Studies on copper uptake have been fewer than those on zinc uptake.

The earliest reference is probably that of Bateman and Wells (1917) who found appreciable quantities of copper in plants (both living and dead) on copper contaminated soils. Figures of 2,000 - 6,000 ppm were obtained for *Plantago*, *Agropyron* and *Dasiophora*, while lower values were found in *Medicago*, *Equisetum* and *Trifolium*. Dead vegetation on the whole contained more copper than living plants. Prat and Komarek (1934) found that plants of *Agrostis alba* (*stolonifera*) and *Melandrium silvestre* growing on soils rich in copper (1% - 39% Cu) contained 0.2% - 3.25% copper in the ash. Persson (1956) again found that "copper mosses" do in fact take up this element. Similar results have been obtained by Tooms and Jay (1964), Clarke (1953) and Duvigneaud and Denaeyer-de Smet (1963).

The only detailed investigation (Nicolls et al. 1965) has produced some very interesting results. Here a very peculiar pattern was seen for all species: copper uptake stayed low and constant with increasing soil copper till a certain level was reached at which this 'resistance' to uptake seemed to break down. Above this level the quantity in the plant increased abruptly and at higher levels in the soil, no plants were found. Species differ both in the copper content at low soil levels and in the level at which the sudden increase in copper is seen. These differing reactions to copper in the soil suggest that the copper does not just become available to the plant at a given total soil level but that a genuine exclusion mechanism is in action. This is supported by the finding that values of copper even a little above the level at which there is greatly increasing uptake are lethal. Moreover the species found in the most toxic areas, Polycarpaea glabra, takes up very little copper (a maximum of 20 ppm in the leaves) on soils containing 10,000 ppm.

There is therefore evidence that the mechanism of copper uptake is different from the uptake of zinc. This is further supported by Vogt et al. (1943): analyses of plants growing over an exposure of copper ore showed that they did not differ in copper content from those growing off the ore. However there were marked differences in zinc content. These results are supported by investigations on copper and zinc in plants growing on normal soil. McHargue and Roy (1932) found that there was little variation in copper content of tree leaves over the growing season, but that zinc showed considerable variations. Holmes (1944) noted that whereas copper in plants rarely varies more than 5-15 ppm. the zinc content can vary from 20 ppm - 10,200 ppm.

However the interpretation of studies relating soil content to plant content is difficult, since even high soil copper contents may go hand in hand with low copper availability (Dykeman and DeSousa, 1966). Clear interpretation is only possible if such ecological investigations are coupled with experiments using culture solutions of known composition. The ability to restrict copper uptake must also be peculiar to a few species since the absence of high copper in plants is by no means universal.

(c) Lead

Studies on the uptake of lead have been very few. Jensch (1894) showed that whereas *Tussilago* and *Polygonum* on contaminated soils contained zinc, no lead was detectable, even though the soil contained 0.72- .06% of this element. These results are similar to those found by Nobbe, Bressler and Will (quoted in Schwickerath, 1931, but no reference given): if equal quantities of lead and zinc are given to a plant, then a smaller quantity of lead than zinc is taken up.

Nicolls *et al.* (1965) also deal with lead uptake. The pattern here resembles that of copper rather than zinc, in that the uptake is constant with increasing levels of soil lead, till a certain point is reached when uptake becomes unrestricted, and rises abruptly. The species also are rarely present when the soil lead value is above 1,000 ppm - the level at which there is a sudden increase in lead uptake. Again *Polycarpaea glabra* seems to have a higher level than the other species: this again suggests affinities with copper. The levels of lead in the plants are always much below the levels of copper or zinc.

Further studies are needed on the uptake of lead by plants growing on lead contaminated soil.

(d) The mechanism of tolerance

There have been no attempts to investigate the mechanism of heavy metal tolerance in higher plants (apart from the recent work of Gregory, 1965). It is a subject that has generally been overlooked, but is likely to prove physiologically and biochemically revealing.

6. CONCLUSIONS

Plants growing on metal contaminated soil have generally become adapted to high metal levels by natural selection: normal populations cannot colonise the mine habitats. This is confirmed by the physiological studies: plants on mine habitats generally take up the metal and high metal concentrations are well known to be toxic to normal cells. The evolution of tolerance is therefore a critical factor in considering distribution on metal contaminated areas. This has repercussions in other areas of the subject. Classifications of species according to their frequency on contaminated soil is a reflection of their ability to evolve tolerance. The structure of the various communities may be determined in a similar way. Adaptation to local conditions suggests that related factors other than tolerance are involved: these lead to changes in morphology and associated confusions in geographical and taxonomic interpretations.

Evolution is an important process and has repercussions on other botanical aspects of mine plants. Similar problems are undoubtedly present in other species adapted to extreme environments, but they may not have come to light. Plants growing on mine habitats are conspicuous and have been studied extensively.

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Appendix 2

LIST OF MINES

(a) North Wales Mines

Parys Mountain, Anglesey. Grid reference SH 438907. Mined for copper.
Hafna Mine, Llanrwst, Caernarvonshire. Grid reference SH 784603.
Mined for lead and zinc.

(b) Cardiganshire and Montgomeryshire Mines

An account of these mines is given by Jones (1922). This account includes a map showing the location of the mines, and gives details of the mining history of the different mines. These details are therefore not included below.
The numbering used is that of Jones, p. 187.
Montgomeryshire mines are indicated by (M) .

(i) Mines from which collections were made in 1964.

1\. Abbey Consols.
3\. Aberystwyth Bwlchgwyn.
18\. Caegynon.
22\. Castell.
25\. Cwmbrwyno.
30\. Cwmsymlog West.
31\. Cwmsymlog North.
43\. Great West Van.
47\. Frongoch.
70\. Llywernog.
87\. Rheidol United.

(ii) Mines from which collections were made in 1965.

4\. Alltycrib.
9\. Bronfloyd.
10\. Brynarian.
15\. Bwlch.

16. Bwlchglas.
23. Cefngwyn.
27. Cwmerfin.
35. Daren.
36. Daren East.
37. Daren South.
41. Elgar.
76. Mynyddgorddu.
80. Pengraigddu.
81. Penpontbren.
83. Penycefn.
85. Pwll Roman.
95. Ystrad Einion.
4. (M) Cae Conroy.
16. (M) Gorn.
17. (M) Llanerchyraur.
23. (M) Penyclun.
26. (M) Rhoswydol.

(iii) Other mines visited but which show : no clearly contaminated area.

11. Bryndyfi.
63. Llancynfelyn.
66. Llechweddhen.
72. Loveden.
74. Moelglomen.
94. Ynys.
3. (M) Brynyfedwen.
6. (M) Ceulan.
30. (M) Van East.

Appendix 3

RECURRENCE EQUATIONS AND COMPUTER PROGRAMMES

CONTENTS

1. ONE GENE MODEL: NOTATION

a_1	self-fertility of AA
a_2	" " " AB
g	gene (pollen or seed) flow of BB
p	frequency of A
q	" " B
u	frequency of AA
v	" " AB
w	" " BB

2. ONE GENE MODEL: EQUATIONS

(a) Recurrence equations for a population subjected to pollen flow and selfing.

notation:

AA' frequency of AA in the next generation

AB' " " AB " " " "

BB' " " BB " " " "

$$AA' = a_1u + (p - a_1u) \quad 1-(pg + q) \quad + {}^1/4\, a_2v \quad (q-p) - 2gp$$

$$AB' = q + \quad (p - a_1u) - q \quad (pg + q)$$

$$BB' = q\ (pg + q) - {}^1/4 a_2v \quad (q-p) + 2gp$$

(b) Equation for genetic load

Notation:

AB', BB' refer to recurrence equations in previous section

s selection coefficient

h degree of dominance of favoured gene (= 1 for full dominance)

Genetic load (I) = s BB' - (1-h) AB'

3. ONE GENE MODEL: PROGRAMMES

Note: adjustable parameters which have been built into the programmes are underlined on the programme sheets.

(a) Pollen flow selfing: equilibrium positions

Input data as series of numbers:

x_1	a_1 takes the value x_1 in steps of x_2 until x_3 .
x_2	
x_3	
x_4	degree of dominance of selfing gene, i.e. $a_2 = a_1 . x_4$.
x_5	g takes the value x_5 in steps of x_6 until x_7 .
x_6	
x_7	
x_8	initial frequency of p .
x_9	fitness of AA (= 1 - selection pressure on AA)
x_{10}	" " AB
x_{11}	" " BB

Output data as series of numbers in columns:

Column 1. number of generations to equilibrium
2. a_1
3. a_2
4. g
5. u
6. v
7. w
8. p
9. q

Programme adjustable for:

definition of equilibrium point
maximum number of generations

```
ANTONOVICS POLLEN FLOW SELFING - PFS(1)'
BEGIN SWITCH S:= AGAIN'
REAL A, B, BO, C, U, V, W, PO, SU, SV, SW, T, P, P1, P2,
Q,
 D, G, H, J, L, E1, E2, I1, I2, F1, F2'
INTEGER CO'
PRINT ££L4??,
£COUNT   A     AB     LAM     UU      VV      WW     P       Q?,
££L??'
 READ E1, I1, F1, BO, E2, I2, F2, PO, SU, SV, SW'
FOR A:= E1 STEP I1 UNTIL F1 DO
FOR C:= E2 STEP I2 UNTIL F2 DO
BEGIN
CO:= 0'
 := A*BO'
P1:= PO'
AGAIN:
CO:=CO+1'
P:=P1'
 :=1-P'
 :=Q-I'
 :=C*P'
H:=2* '
U:= + '
  := - '
 :=0.25*B'
 :=2*P*Q'
L:= * '
J:= *J'
  :=D+Q'
U:=P*P'
W:= *Q'
 :=A*U'
 :=P-I'
W:= * '
W:= - '
  :=P-Q'
  :=V*D'
 V:=V+Q'
 :=1-D'
 U:=U*  '
  :=U+T'
 H:=  * '
 U:=U+  '
 U:=U*SU'
  V:= *SV'
 W:=W*SW'
 T:=U+V+W'
```

```
T:=1/[illegible]'
U:=U*T'
V:=V*T'
W:=W*T'
P:=0.5*V'
P2:=U+P'
Q:= W+P'
IF (ABS(P1-P2) LESS 0.000001 OR CO GR 500)
THEN PRINT ££L??, DIGITS(3), CO, PREFIX(££S1??), FREEPOINT
(3),
A, B, C, FREEPOINT(4), U, V, W, P2,Q
ELSE BEGIN P1:=P2'
GOTO AGAIN
END'
END'
```

(b) Seed flow selfing: equilibrium positions.

Input, output and adjustable parameters same as in (a) Pollen flow selfing: equilibrium positions. Parameter defining pollen flow now defines seed flow.

```
ANTONOVICS  SEED FLOW SELFING - SFS (1).'
BEGIN SWITCH S:= AGAIN'
REAL A, B, BO, C, U, V, W, UO, VO, WO, SU, SV, SW ,
 T, P, P1, PO,
 P2, Q, R, D, E1, E3, I1,  I3, F1,  F3'
INTEGER CO'
PRINT  ££L4??,
 COUNT  AA     AB     LAM     UU      VV      WW      P       Q
?,
££L??'
READ  E1, I1, F1, BO, E3, I3, F3,  PO, SU,
   SV, SW'
FOR A:=E1 STEP I1 UNTIL F1 DO
FOR C:=E3 STEP I3 UNTIL F3 DO
BEGIN
B:= A*BO'
 CO:=0'

 P1:=PO'
 AGAIN:
 CO:=CO+1'

 P:=P1'
 Q:=1-P'
 U:=P*P'
 V:=2*P*Q'
 W:=Q*Q'
 T:=A*U*Q'
 R:=0.25*B*V*(Q-P)'
 U:=U+T+R'
 V:=V-T'
 W:=W-R'
 D:=1-C'
 T:=U+V+W'
 T:=1/T'
 U:=U*T*D'
 V:=V*T*D'
 W:=W*T*D'
 W:=W+C'
 U:=U*SU'
 V:=V*SV'
 W:=W*SW'
 T:=U+V+W'
 T:=1/T'
 U:=U*T'
 V:=V*T'
```

```
W:=W*T'
P2:=0.5*V+U'
Q:=0.5*V+ '

IF (ABS(P1-P2) LESS 0.000001 OR CO GR 500)
THEN  PRINT ££L??, DIGITS(3),CO, PREFIX(££S1??), FREEPOINT
(3),
A, B, C, FREEPOINT(4), U, V, W, P2, Q
ELSE  BEGIN  P1:=P2'
GOTO AGAIN
END'
END'
END'
```

(c) Pollen flow selfing: progress to equilibrium.

Input data as series of numbers:

x_1	initial frequency of p
x_2	fitness of AA
x_3	" " AB
x_4	" " BB
x_5	a_1
x_6	a_2
x_7	g

Output data as series of numbers in columns:

Column 1. generation
2. u
3. v
4. w
5. p
6. q

and when equilibrium is reached, also, as a series of numbers a_1, a_2, g.

Programme adjustable for:

definition of equilibrium point
maximum number of generations

```
ANTONOVICS RATE TO EQUILIBRIUM POLLEN FLOW SELFING
 - PFS(2)'

BEGIN  SWITCH S:=AGAIN'
REAL  A, B, C, U, V, W, SU, SW, SV, T, P, P1, P2, Q,
D, P0,
 G, H, J, L'
INTEGER  CO'
PRINT   £L2?
COUNT  UU      VV      WW      P       Q?'
READ   P0,  SU, SV, SW, A, B, C'

CO:=0'
P1:=P0'
Q:=1-P1'
U:=P1*P1'
V:=2*P1*Q'
W:=Q*Q'

AGAIN:
PRINT  £L£L??, DIGITS(3), CO, PREFIX(££S1??), FREEPOINT
(4),
U, V, W, P1, Q'

CO:=CO+1'
P:=P1'
Q:=1-P'
G:=Q-P'
D:=C*P'
H:=2*D'
J:=H+G'
H:=G-H'
L:=0.25*B'
V:=2*P*Q'
U:=P*P'
W:=Q*Q'
L:=L*V'
J:=L*J'
D:=D+Q'
T:=A*U'
P:=P-T'
W:=Q*D'
W:=W-J'
V:=P-Q'
V:=V*D'
V:=V+Q'
U:=1-D'
U:=U*P'
U:=U+T'
H:=L*H'
U:=U+H'
```

```
U:=U*SU'
V:=V*SV'
W:=W*SW'
T:=U+V+W'
T:=1/T'
U:=U*T'
V:=V*T'
W:=W*T'
P:=0.5*V'
P2:=U+P'
Q:=W+P'
IF (ABS(P1-P2) LESS 0.000001 OR CO GR 500)
THEN  PRINT ££L??, DIGITS(3),CO, PREFIX(££S1??), FREEPOINT
(4),
 U, V, W, P2, Q,
££L??, FREEPOINT(3), A, B, C
ELSE  BEGIN  P1:=P2'
GOTO  AGAIN
END'
END'
```

(d) Pollen flow selfing in a perennial: progress to equilibrium.

Input, output and adjustable parameters exactly as in (c) Pollen flow selfing: progress to equilibrium.

```
ANTONOVICS RATE TO EQUILIBRIUM POLLEN FLOW SELFING
 PERENNIAL
- PFSP(2)'

BEGIN  SWITCH S:=AGAIN'
REAL  A, B, C, U, V, W, SU, SW, SV, T, P, P1, P2, Q,
D, P0,
 G, H, J, L, Q1, Q2, U1, U2, V1, V2, W1, W2'
INTEGER  CO'
PRINT  ££L2?
COUNT  UU      VV      WW      P       Q?'
READ   P ,  SU, SV, SW, A, B, C'

CO:=0'
P1:=P0'
Q1:=1-P1'
U1:=P1*P1'
V1:=2*P1*Q1'
W1:=Q1*Q1'

AGAIN:
PRINT  ££L??, DIGITS(3), CO, PREFIX(££S1??), FREEPOINT
(4),
U1, V1, W1, P1, Q1'

CO:=CO+1'
P:=P1'

Q:=1-P'
 :=Q-P'
 :=C*P'
H:=2* '
J:= + '
H:= - '
L:=0.25*B'
 :=2*P*Q'
 := * '
 := *  '
 :=D+Q'
U:=P*P'
W:=Q*Q'
 := *U'
P:=P- '
W:=Q*D'
W:=W-J'
V:=P-Q'
```

```
  [illegible]:=V•[illegible]'
V:=V+Q'
U:=1-[illegible]'
[illegible]:=U•P'
[illegible]:=U+[illegible]'
[illegible]:=L•H'
U:=[illegible]+[illegible]'
U:=U•SU'
 V:=V•SV'
W:=[illegible]•SW'
U:=U+U1'
[illegible]:=[illegible]+V1'
  [illegible]:=W+W1'
T:=U+V+W'
T:=1/T'
U2:=U•T'
V2:=V•T'
W2:=W•T'
P2:=U2+0.5•V2'
Q2:=1-P2'

IF (ABS(P1-P2) LESS 0.000001 OR CO GR 500)
THEN PRINT ££L??, DIGITS(3), CO, PREFIX(££S1??), FREEPOINT
(3),
A, B, C, FREEPOINT(4), U2, V2, W2, P2, Q2
ELSE BEGIN P1:=P2'
Q1:=Q2'
V1:=V2'
W1:=W2'
U1:=U2'
GOTO AGAIN
END'
END'
END'
```

(e) Changing pollen flow selfing: progress to equilibrium.

Input data as series of numbers:

x_1	initial frequency of p
x_2	fitness of AA
x_3	" " AB
x_4	" " BB
x_5	a_1
x_6	a_2
x_7	final pollen flow, g
x_8	number determining rate of change of pollen flow defined as r in the basic equation (Chapter V.1) e.g. when r = 1 , and initial pollen flow = 0.999, then final pollen flow reached in approx. 15 generations; when r = 0.5 , final pollen flow reached in approx. 30 generations

Output data as series of numbers in columns:

Column 1. generation
2. pollen flow at a given generation
3. u
4. v
5. w
6. p
7. q

and when equilibrium is reached, also, as a series of numbers a_1, a_2, g

Programme adjustable for:

maximum number of generations

definition of initial pollen flow by n , where, initial flow $= (1 - g)(1 - {}^{1}/_{e}n) + g$

(note: e = exponential)

```
ANTONOVICS RATE TO EQUILIBRIUM CHANGINGPOLLEN FLOW
 SELFING - CPFS(2)'

BEGIN  SWITCH S:=AGAIN'
REAL  A, B, C, U, V, W, SU, SW, SV, T, P, P1, P2, Q,
 D, PO,
 G, H, J, L, R, LA'
INTEGER  CO'
PRINT  ££L2?
COUNT  UU      VV      WW      P       Q?'
READ   PO,  SU, SV, SW, A, B, LA, R'


CO:=0'
P1:=PO'
Q:=1-P1'
U:=P1*P1'
V:=2*P1*Q'
W:=Q*Q'
C:=1+EXP(6.9078-R*CO)'
C:=1-1/C'
C:=(1-LA)*C'
C:=C+LA'

 AGAIN:
 PRINT  ££L??, DIGITS(3), CO, PREFIX(££S1??), FREEPOINT
 (3), C,
 U, V, W, P1, Q'

 CO:=CO+1'
 C:=1+EXP(6.9078-R*CO)'
 C:=1-1/C'
 C:=(1-LA)*C'
 C:=C+LA'
 P:=P1'
 Q:=1-P'
 G:=Q-P'
 D:=C*P'
 H:=2*D'
 J:=H+G'
 H:=G-H'
 L:=0.25*B'
 V:=2*P*Q'
 U:=P*P'
```

```
W:=Q*Q'
L:=A*V'
C:=L*J'
D:=B+C'
T:=A*U'
P:=P-T'
W:=Q*U'
W:=W-U'
V:=P-Q'
V:=V*U'
V:=V+Q'
U:=1-D'
U:=U*P'
U:=U+T'
H:=L*H'
C:=C+H'
U:=U*SU'
V:=V*SV'
W:=W*SW'
T:=U+V+W'
T:=1/T'
U:=U*T'
V:=V*T'
W:=W*T'
P:=0.5*VV'
P2:=U+P'
Q:=W+P'
IF  CO GR  100
THEN  PRINT ££L??, DIGITS(3),CO, PREFIX(££S1??),
FREEPOINT(3),
 U, V, W, P2, Q,
££L??, FREEPOINT(3), A, B, C
ELSE  BEGIN  P1:=P2'
GOTO  AGAIN
END'
END'
```

(f) Genetic load.

Input data as series of numbers:

x_1	p takes the value x_1 in steps of x_2 until x_3
x_2	
x_3	
x_4	selection coefficient takes the value x_4 in steps of x_5 until x_6
x_5	
x_6	
x_7	g takes the value x_7 in steps of x_8 until x_9
x_8	
x_9	
x_{10}	a_1 takes the value x_{10} in steps of x_{11} until x_{12}
x_{11}	
x_{12}	
x_{13}	degree of dominance of the selfing gene, i.e. $a_2 = a_1 . x_{13}$
x_{14}	1 - h, where h is degree of dominance of favoured gene

Output data as series of numbers in columns:

Column 1. p
2. selection coefficient
3. g
4. a_1
5. genetic load

```
ANTONOVICS GENETIC LOAD PFS  - GL'
BEGIN
REAL A, AA,  B, C, P, Q, I, S, LA, AB, ABO, H, E1,
 I1, F1, E2,
I2, F2, E3, F3, I3, E4, I4, F4'
READ E1, I1, F1, E2, I2, F2, E3, I3, F3,  E4, I4, F4,
 ABO, H'
FOR P:=E1 STEP I1 UNTIL F1 DO
FOR S:= E2 STEP I2 UNTIL F2 DO
FOR LA:=E3 STEP I3 UNTIL F3 DO
FOR AA:=E4 STEP I4 UNTIL F3 DO
BEGIN
AB:=AA*ABO'
Q:=1-P'
A:=LA*P'
B:=A+Q'
A:=2*A'
C:=Q-P'
I:=H*(Q-(C+AA*P*P)*B)'
I:=I-0.5*AB*P*Q*(C+A)'
I:=I+Q*B'
I:= S*I'
PRINT ££L??, PREFIX(££S1??), FREEPOINT(3), P, S, LA,
 AA, I'
END'
END'
```

4. TWO GENE MODEL: NOTATION

a_1	self-fertility imposed by AA
a_2	" " " " Aa
b_1	$1 - a_1$
b_2	$1 - a_3$
g	pollen flow (aabb)
h	$1 - g$
p	frequency of A
q	" " a
u	" " B
v	" " b

5. TWO GENE MODEL: EQUATIONS

Recurrence equations for a population subjected to pollen flow and with selfing determined by an unlinked gene.

notation:

AABB'	frequency of AABB in next generation
AABb'	" " AABb " " "
etc.	etc.

$$AABB' = hp^2u^2(b_1p + b_2q) + pu(u + 0.5v)(a_1p + 0.5a_2q)$$

$$AABb' = 2hp^2uv(b_1p + b_2q) + puv(a_1p + 0.5a_2q)$$

$$AAbb' = hp^2v^2(b_1p + b_2q) + pv(v + 0.5u)(a_1p + 0.5a_2q)$$

$$AaBB' = hpqu^2(b_1p + b_2 + q) + pqu\ (u + 0.5v)\ a_2$$

$$AaBb' = 2hpquv\ (b_1p + b_2 + q) + pquva_2 + pug\ (b_1p + b_2q)$$

$$Aabb' = hpqv^2\ (b_1p + b_2 + q) + pqv\ (v + 0.5u)\ a_2 + pvg\ (b_1p + b_2q)$$

$$aaBB' = hq^2u^2\ (b_2p + q) + 0.5\ pqu\ (u + 0.5v)\ a_2$$

$$aaBb' = 2hq^2uv\ (b_2p + q) + 0.5pquva_2 + qug\ (b_2p + q)$$

$$aabb' = hq^2v^2\ (b_2p + q) + 0.5pqv\ (v + 0.5u)\ a_2 + qvg\ (b_2p + q)$$

6. TWO GENE MODEL: PROGRAMMES

(a) Two gene pollen flow selfing: equilibrium positions.

Input data as series of numbers:

x_1	a_1 takes the value x_1 in steps of x_2 until x_3
x_2	
x_3	
x_4	degree of dominance of selfing gene, i.e. $a_2 = a_1 \cdot x_4$
x_5	g takes the value x_5 in steps of x_6 until x_7
x_6	
x_7	
x_8	initial frequency of selfing gene p
x_9	" " " favoured " u
x_{10}	fitness of AABB
x_{11}	" " AABb
.	
.	
.	
x_{17}	" " aaBb
x_{18}	" " aabb

Output data as series of numbers in columns on two lines:

Line 1. Column 1. number of generations to equilibrium

2. a_1
3. a_2
4. g
5. p
6. q
7. u
8. v

Line 2. Column 1. frequency of AABB

2. " " AABb

.

.

.

8. " " aaBb

9. " " aabb

Programme adjustable for:

definition of equilibrium point

whether overall equilibrium, or equilibrium of p, or equilibrium of u

maximum number of generations

```
ANTONOVICS TWO GENE POLLEN FLOW SELFING - TPFS(1)'
BEGIN  SWITCH  S:= AGAIN'
REAL  A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, R,
 P,
Q, U, V, AA, AB, BA, BB, GA, LA, RR, SS, TT, MM, NN, WW,
 XX, YY,
ZZ, E1, E3, I1, I3, F1, F3, PO, UO,
S1, S2, S3, S4, S5, S6, S7, S8, S9,  P1, P2, U1, U2, T, Q2,
 V2,
ABO'
INTEGER  CO'

PRINT  ££L4??,
£COUNT AA    AB    LAH    P      Q      U      V?,
££L?   RR    SS    TT     MM     NN     WW     XX
    YY    ZZ
?,
££L??'
READ  E1, I1, F1, ABO,  E3, I3, F3, PO, UO,
S1, S2, S3, S4, S5, S6, S7, S8, S9'

FOR  AA:=E1  STEP  I1  UNTIL  F1  DO
FOR  LA:=E3  STEP  I3  UNTIL  F3  DO
BEGIN
AB:=AA*ABO'
CO:= 0'

P1:=PO'
U1:=UO'

AGAIN:
CO:=CO+1'
P:=P1'
Q:=1-P'
U:=U1'
V:=1-U'

GA:=1-LA'
BA:=1-AA'
BB:=1-AB'
A:=P*P'
B:=P*Q'
C:=Q*Q'
D:=U*U'
E:=U*V'
```

```
F:=V*V'
G:=GA*D'
H:=GA*2*E'
I:=GA*F'
J:=BA*P+BB*Q'
K:=BA*P+BB+Q'
L:=BB*P+Q'
M:=B*AB'
N:=A*AA+0.5*M'
O:=D+0.5*E'
R:=F+0.5*E'
U:=P*LA*J'
K:=B*K'
MM:=G*K+M*O'
NN:=H*K+M*E+U*D'
WW:=I*K+M*R+V*D'
J:=A*J'
RR:=G*J+N*O'
SS:=H*J+N*E'
TT:=I*J+N*R'
A:=Q*LA*L'
L:=C*L'
M:=0.5*M'
XX:=G*L+M*O'
YY:=H*L+M*E+U*A'
ZZ:=I*L+M*R+V*A'

RR:=RR*S1'
SS:=SS*S2'
TT:=TT*S3'
MM:=MM*S4'
NN:=NN*S5'
WW:=WW*S6'
XX:=XX*S7'
YY:=YY*S8'
ZZ:=ZZ*S9'

T:=RR+SS+TT+MM+NN+WW+XX+YY+ZZ'
T:=1/T'

RR:=RR*T'
SS:=SS*T'
TT:=TT*T'
MM:=MM*T'
```

```
NN:=NN*T'
WW:=WW*T'
XX:=XX*T'
YY:=YY*T'
ZZ:=ZZ*T'

P2:= RR +SS+TT+0.5*(MM+NN+WW)'
U2:=RR+MM+XX+0.5*(SS+NN+YY)'
Q2:=1-P2'
V2:=1-U2'

IF (ABS(P1-P2)+ABS(U1-U2)  LESS  0.00001  OR CO GR 500)
THEN
PRINT  ££L??, DIGITS(3), CO, PREFIX(££S1??), FREEPOINT
(3),
  AA, AB, LA, FREEPOINT(4), P2, Q2, U2, V2, ££L??, PREFIX
(££S1??),
FREEPOINT(4),     RR, SS, TT, MM, NN, WW, XX,
  YY, ZZ

            ELSE  BEGIN
P1:=P2'
U1:=U2'
GOTO   AGAIN
END'
END'
END'
```

(b) Two gene changing pollen flow selfing in a perennial: progress to equilibrium.

Input data as series of numbers:

x_1	a_1
x_2	a_2
x_3	final pollen flow g
x_4	number determining rate of change of pollen flow (see One Gene Model, changing pollen flow)
x_5	initial frequency of selfing gene p
x_6	" " " favoured " u
x_7	fitness of AABB
x_8	" " AABb
.	
.	
.	
x_{14}	" " aaBb
x_{15}	" " aabb
x_{16}	initial frequency of AABB
x_{17}	" " " AABb
.	
.	
.	
x_{23}	" " " aaBb
x_{24}	" " " aabb

Output data as series of numbers in columns on two lines:

Line 1. Column 1. generation

2. g

3. p

4. q

5. u

6. v

Line 2. Column 1. frequency of AABB
2. " " AABb
.
.
.
8. " " aaBb
9. " " aabb

and when equilibrium is reached, also, as series of numbers a_1, a_2

Programme adjustable for:

maximum number of generations
definition of initial pollen flow by n, where,

$$\text{initial flow} = (1 - g)(1 - {}^{1}/_{e}n) + g$$

(note: e = exponential)

```
ANTONOVICS TWO GENE RATE TO EQUILIBRIUM CHANGING POLLEN
 FLOW
SELFING - TCPFS(2)'
BEGIN  SWITCH  S:= AGAIN'
REAL  A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, R, P,
RO, LAF,
Q, U, V, AA, AB, BA, BB, GA, LA, RR, SS, TT, MM, NN, WW,
XX, YY,
ZZ,  PO, UO,
RRO, SSO, TTO, MMO, NNO, WWO, XXO, YYO, ZZO,
S1, S2, S3, S4, S5, S6, S7, S8, S9,  P1, P2, U1, U2, T,
Q2, V2,
Q1,  V1'

 INTEGER  CO'

 PRINT  ££L4??,
 £COUNT     LAM      P         Q         U         V?,
 ££L?     RR        SS        TT        MN        NN        WW        XX
       YY       ZZ
 ?,
 ££L??'
 READ  AA, AB, LAF, RO,  PO, UO,
 S1, S2, S3, S4, S5, S6, S7, S8, S9,
 RRO, SSO, TTO, MMO, NNO, WWO, XXO, YYO, ZZO'

 CO:= 0'
 LA:=1+EXP(6.9078-RO*CO)'
 LA:=1-1/LA'
 LA:=(1-LAF)*LA'
 LA:=LA+LAF'

 P1:=PO'
 U1:=UO'
 Q1:=1-P1'

              V1:=1-U1'
 Q2:=1-P1'
 V2:=1-U1'
 RR:=RRO'
 SS:=SSO'
 TT:=TTO'
 MM:=MMO'
 NN:=NNO'
```

```
WW:=WWO'
XX:=XXO'
YY:=YYO'
ZZ:=ZZO'

AGAIN:
PRINT ££L??, DIGITS(3), CO, PREFIX(££S1??), FREEPOINT
(3), LA,
FREEPOINT(4), P1, Q2, U1, V2, ££L??, PREFIX(££S1??),

FREEPOINT(4), RR, SS, TT, MM, NN, WW, XX, YY, ZZ'
CO:=CO+1'
LA:=1+EXP(6.9078-RO*CO)'
LA:=1-1/LA'
LA:=(1-LAF)*LA'
LA:=LA+LAF'

P:=P1'
Q:=1-P'
U:=U1'
V:=1-U'

GA:=1-LA'
BA:=1-AA'
BB:=1-AB'
A:=P*P'
B:=P*Q'
C:=Q*Q'
D:=U*U'
E:=U*V'
F:=V*V'
G:=GA*D'
H:=GA*2*E'
I:=GA*F'
J:=BA*P+BB*Q'
K:=BA*P+BB+Q'
L:=BB*P+Q'
M:=B*AB'
N:=A*AA+0.5*M'
O:=D+0.5*E'
Q:=F+0.5*E'
D:=P*LA*J'
K:=B*K'
MM:=G*K+M*O'
```

```
NN:=H•K+M•E+U•D'
WW:=I•K+H•R+V•D'
J:=A•J'
RR:=G•G+[illegible]•O'
SS:=[illegible]•G+[illegible]•E'
TT:=I•[illegible]+[illegible]•[illegible]'
A:=Q•LA•L'
L:=[illegible]•L'
M:=0.5•[illegible]'
XX:=G•[illegible]+M•O'
YY:=H•[illegible]+[illegible]•L+U•A'
ZZ:=I•[illegible]+[illegible]•H+V•A'

RR:=RR•S1'
SS:=SS•S2'
TT:=TT•S3'
MM:=MM•S4'
NN:=NN•S5'
WW:=WW•S6'
XX:=XX•S7'
YY:=YY•S8'
ZZ:=ZZ•S9'

T:=RR+SS+TT+MM+NN+WW+XX+YY+ZZ'
T:=1/T'

RR:=RR•T'
SS:=SS•T'
TT:=TT•T'
MM:=MM•T'
 NN:=NN•T'
WW:=WW•T'
XX:=XX•T'
YY:=YY•T'
ZZ:=ZZ•T'

P2:= RR +SS+TT+0.5•(MM+NN+WW)'
U2:=RR+MM+XX+0.5•(SS+NN+YY)'
Q2:=1-P2'
V2:=1-U2'

P2:=P2+P1'
Q2:=Q2+Q1'
U2:=U2+U1'
V2:=V2+V1'

T:=P2+Q2'
```

```
T:=1/T'

P2:=P2*T'
Q2:=Q2*T'

T:=U2+V2'
T:=1/T'

U2:=U2*T'
V2:=V2*T'

 IF ( CO GR  50 )
  THEN
 PRINT  ££L??, DIGITS(3), CO, PREFIX(££S1??), FREEPOINT
 (3),
   AA, AB, LA, FREEPOINT(4), P2, Q2, U2, V2, ££L??, PREFIX
 (££S1??),
 FREEPOINT(4),     RR, SS, TT, MM, NN, WW, XX,
   YY, ZZ

             ELSE  BEGIN
 P1:=P2'
 U1:=U2'
 Q1:=Q2'
 V1:=V2'
 GOTO   AGAIN
 END'
 END'
 END'
```

(c) <u>Two gene pollen flow evolution of dominance: equilibrium positions.</u>

Input data as series of numbers:

x_1	g takes the value x_1 in steps of x_2 until x_3
x_2	
x_3	
x_4	initial frequency of dominance modifier p
x_5	" " " favoured gene u
x_6	fitness of AABB
x_7	" " AABb
.	
.	
.	
x_{13}	" " aaBb
x_{14}	" " aabb

Output data as series of numbers in columns on two lines:

Line 1. Column 1. number of generations to equilibrium
2. g
3. p
4. q
5. u
6. v

Line 2. Column 1. frequency of AABB
2. " " AABb
.
.
.
8. " " aaBb
9. " " aabb

Programme adjustable for:

definition of equilibrium point

whether overall equilibrium, or equilibrium of p, or equilibrium of u

maximum number of generations

```
ANTONOVICS TWO GENE POLLEN FLOW SELFING - TPFS(1)'
BEGIN  SWITCH  S:= AGAIN'
REAL  A, B, C, D, E, F, G, H, I, P,
Q, U, V, GA, LA, RR, SS, TT, MM, NN, WW, XX, YY,
ZZ,  E3,  I3,  F3, PO, UO,
S1, S2, S3, S4, S5, S6, S7, S8, S9,  P1, P2, U1,
 U2, T, Q2, V2,
ABO'
INTEGER  CO'

PRINT  ££L4??,
£COUNT     LAM      P       Q       U       V?,
££L?    RR      SS      TT      MM      NN      WW      XX
     YY      ZZ
?,
££L??'
READ  E3, I3, F3, PO, UO,
S1, S2, S3, S4, S5, S6, S7, S8, S9'
FOR  LA:=E3  STEP  I3  UNTIL  F3  DO
BEGIN
CO:= 0'

 P1:=PO'
 U1:=UO'

 AGAIN:
 CO:=CO+1'
 P:=P1'
 Q:=1-P'
 U:=U1'
 V:=1-U'

  GA:=1-LA'
  A:=P*P'
  B:=2*P*Q'
  C:=Q*Q'
  D:=U*U'
  E:=U*V'
  F:=V*V'
  G:=GA*D'
  H:=GA*2*E'
  I:=GA*F'
  RR:= G*A*S1'
  SS:=H*A*S2'
```

```
TT:=I*A*S3'
MM:=G*B*S4'
A:=P*LA'
NN:=H*B+A*U'
WW:=I*B+A*V'
XX:=G*C*S7'
B:=Q*LA'
YY:=H*C+B*U'
ZZ:=I*C+B*V'
 NN:= NN*S5'
WW:=WW*S6'
YY:=YY*S8'
ZZ:=ZZ*S9'

T:=RR+SS+TT+MM+NN+WW+XX+YY+ZZ'
T:=1/T'

RR:=RR*T'
SS:=SS*T'
TT:=TT*T'
MM:=MM*T'
NN:=NN*T'
WW:=WW*T'
XX:=XX*T'
YY:=YY*T'
ZZ:=ZZ*T'

 P2:= RR +SS+TT+0.5*(MM+NN+WW)'
U2:=RR+MM+XX+0.5*(SS+NNYY)'
 Q2:=1-P2'
  V2:=1-U2'

 IF (ABS(P1-P2)+ABS(U1-U2)  LESS  0.0001  OR CO GR 5000 )
 THEN
 PRINT  ££L??, DIGITS(4), CO, PREFIX(££S1??), FREEPOINT
 (3),
  LA, FREEPOINT(4), P2, Q2, U2, V2, ££L??, PREFIX(££S1??),

 FREEPOINT(4),    RR, SS, TT, MM, NN, WW, XX,
   YY, ZZ

 ELSE  BEGIN
 P1:=P2'
 U1:=U2'
 GOTO L AGAIN
 END'
 END'
 END'
```