

EVOLUTION IN CLOSELY ADJACENT PLANT POPULATIONS

IV. BARRIERS TO GENE FLOW

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Received 1.vii.67

1. INTRODUCTION

THE importance of isolation in promoting population divergence and speciation has long been recognised (*e.g.* Mayr, 1942; Dobzhansky, 1941; Baker, 1959). Isolation was considered a prerequisite for population divergence until Thoday (1958) showed that disruptive selection could effect such divergence in the absence of isolation. Recently the occurrence of divergence in nature in the face of gene flow has been shown in *Papilio dardanus* (Clarke and Sheppard, 1962), *Maniola jurtina* (Creed *et al.*, 1959) and various grasses (Jain and Bradshaw, 1966; Aston and Bradshaw, 1966; McNeilly, 1967).

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 ill-adapted genotypes from the crossing of two adapted types. In such situations we might expect the evolution of mechanisms to restrict gene flow. Evidence for the development of breeding barriers between adjacent (parapatric), or sympatric populations, and their absence between allopatric populations of the same species or group of species, has been presented in *Drosophila* (Dobzhansky and Koller, 1938; King, 1947; Ehrman, 1965), cotton (Stephens, 1946), *Streptanthus* (Kruckeberg, 1957), *Solanum* (Grun and Radlow, 1961) and *Gilia* (Grant, 1966). The process has also been demonstrated experimentally (Knight *et al.*, 1956) and theoretically (Crosby, 1964).

In all these instances there is evidence that breeding barriers have been developed between populations that have undergone prior allopatric divergence and which have subsequently met. However, Thoday and Gibson (1962) have shown that in *Drosophila* divergence and the evolution of breeding barriers can occur without isolation under disruptive selection. Since no evidence has been presented for the origin of breeding barriers under disruptive selection in natural populations, the occurrence of mechanisms reducing gene flow were investigated in closely adjacent plant populations at metal mine boundaries. The evidence suggests that considerable gene flow occurs in such situations (McNeilly, 1967; McNeilly and Bradshaw, 1967) and that mine populations are the product of recent evolution by disruptive selection (McNeilly, 1967; Antonovics, 1966).

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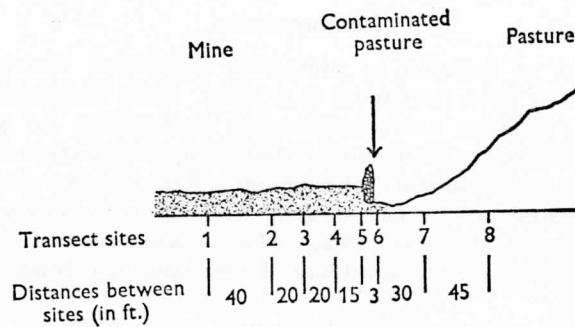
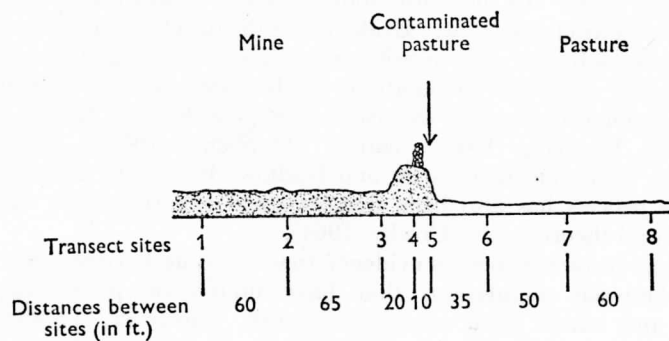
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2. THE POPULATIONS

Populations of two common grass species were taken from transects across the boundary between soil contaminated with toxic levels of heavy metals and uncontaminated pasture soil (fig. 1). The two species on two contrasting mines were:

(a) *Agrostis tenuis* Sibth. from the copper mine, Drws-y-Coed, Caernarvonshire (Grid Ref. SH542535).

(b) *Anthoxanthum odoratum* L. from the lead and zinc mine, Trelogan, Flintshire (Grid Ref. SJ123805).

The Drws-y-coed transect (*Agrostis*)The Trelogan transect (*Anthoxanthum*)


 Soil contaminated with metals and plants tolerant

FIG. 1.—Transects across the boundaries between mine and pasture showing positions of sites from which plants were sampled.

Metal tolerance of mine populations and non-tolerance of pasture populations has been confirmed by Jain and Bradshaw (1966), McNeilly (1967) and Antonovics (1966).

3. INCOMPATIBILITY BARRIERS

If crosses within mine and within pasture populations were more successful than crosses between these populations, the existence of incompatibility barriers would be suggested. Wilkins (1960) and Broker (1963) found no difficulty in crossing metal tolerant and non-tolerant races of *Festuca ovina* and *Silene inflata* respectively. To investigate incompatibility more thoroughly, the success of crosses made during a genetical analysis of metal tolerance was measured.

(a) Method

Crosses were made in an unheated greenhouse by enclosing inflorescences of the plants to be crossed in glassine bags. Plants were matched for flowering time. Compatibility in *Anthoxanthum* was measured as number of seeds set per inflorescence. In *Agrostis* the seeds are difficult to count and therefore 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 viability was generally high.

(b) Results

The results (tables 1a and 1b) have been pooled to give four types of crosses:

- (i) tolerant \times tolerant;
- (ii) tolerant (female) \times non-tolerant (male);
- (iii) non-tolerant (female) \times tolerant (male);
- (iv) non-tolerant \times non-tolerant.

In 1964 and 1965 in *Agrostis*, and in 1964 in *Anthoxanthum*, within and between population crosses were equally successful, thus giving no evidence of the evolution of breeding barriers. However, in 1965 in *Anthoxanthum* between-population crosses produced significantly less seed than crosses within non-tolerant populations. This difference can be accounted for by the difference in the success of reciprocal crosses observed both in 1964 and particularly in 1965. The cross of tolerant \times non-tolerant yields far fewer seed when non-tolerant was used as female parent.

(c) Discussion

There is no evidence of incompatibility barriers between tolerant and non-tolerant populations, apart from the considerable difference between reciprocal *Anthoxanthum* crosses. However, these crosses were made in isolation and the results might be different if, for example, a tolerant parent was simultaneously offered 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).

Differences in the success of reciprocal crosses are well known in plants, and may indicate the beginnings of an incompatibility barrier (Stebbins, 1958). The least successful cross is in the direction of hindering gene flow off the mine on to the pasture. This is difficult to explain because selection pressure against tolerance on the pasture is less than selection for tolerance on the mine (Jain and Bradshaw, 1966; McNeilly, 1967). Tolerant plants

have a greater self-fertility than non-tolerant plants (Antonovics, 1966), but this difference is too small to account for the difference in seed set of the reciprocals.

TABLE 1

Success of crosses between and within tolerant and non-tolerant populations

(a) *Agrostis*

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

CONTINGENCY χ^2

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

(b) *Anthoxanthum*

Seed per inflorescence	Tol.	Tol.	Non-tol.	Non-tol.
	× Tol.	× Non-tol.	× Tol.	× Non-tol.
0	22	23	33	16
1-5	17	19	21	7
6-10	10	8	5	2
11-100	3	9	1	3
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 6-100

Within populations/between populations	1964 = 0.294 n.s.
Within non-tolerant/between populations	1965 = 4.375 *
Within non-tolerant/tolerant × 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 ***

4. FLOWERING TIME

One of the simplest mechanisms of reducing gene flow between populations is a difference in flowering time.

(a) *Flowering in the field*(i) *Agrostis*

Flowering time was assessed from the number of inflorescences at various stages of development, within 50 cm. quadrats at a given date (Bradshaw,

1959), along the Drws-y-Coed transect. The flowering stages were scored as follows:

1. Inflorescence enclosed within the sheath.
2. Inflorescence head just visible.
3. Inflorescence completely visible but not expanded.
4. Inflorescence open and spread widely.
5. Anthers and stigmas exposed.
6. Flowers closed, glumes brown.

Plants on the mine flower earlier than those on the adjacent pasture and this difference is more pronounced on the boundary (fig. 2a). The difference in stages of flowering can be used to estimate the equivalent isolation in terms of days if estimates are made on two dates. The values estimated in this way gave the following times in terms of days (earliest site = 0).

1964: 7 days = 0.563 ± 0.024 stages
 1965: 7 days = 0.511 ± 0.085 stages

Site number	Flowering time	Tolerant			Intermediate	Non-tolerant	
		2	3	4	5	6	7
1964	6.09	5.96	12.17	7.95	0	3.23	
	3.73	4.86	8.49	7.85	0	2.79	

Regular observations at Drws-y-Coed show that the flowering period lasts for 3-5 weeks. In effect therefore, the flowering time difference means that a quarter of the tolerant population flowers before the non-tolerant. Observations on experimental material show that the non-tolerant plants continue flowering after tolerant plants have stopped. There is therefore the possibility of isolation between non-tolerant and tolerant populations at the beginning and end 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:

1. Inflorescence just visible.
2. Inflorescence fully exposed.
3. Stigmas extruded.
4. Stamens extruded.

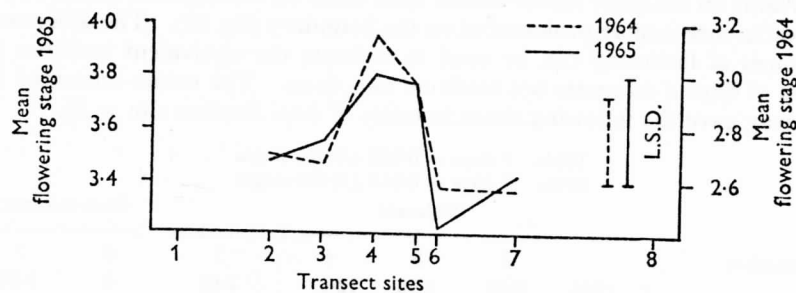
Results obtained in 1964 were from a transect about twenty yards away from the main transect studied in 1965. Plants on the mine flower earlier than those on the pasture and plants from the positions nearer the mine boundary are the earliest to flower (fig. 3a). The differences between the sites are highly significant in both years ($P < 0.1$ per cent.). The isolation in terms of days was again calculated.

1964: 7 days = 0.761 ± 0.099 stages
 1965: 7 days = 0.910 ± 0.033 stages

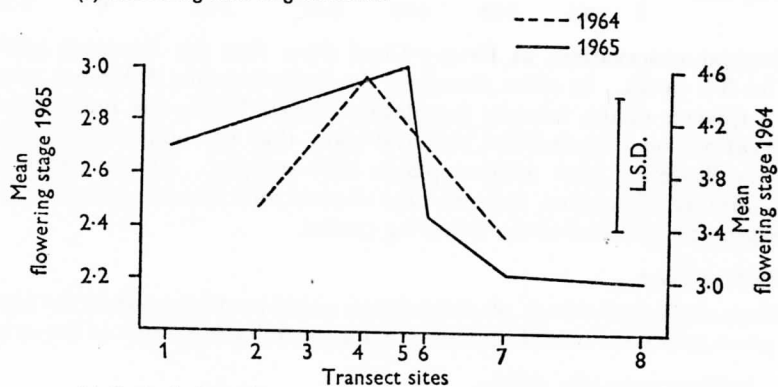
Site number	Flowering time	Tolerant				Intermediate	Non-tolerant		
		1	2	3	4	5	6	7	8
1964	4.60	—	7.17	—	2.94	—	0	—	
	1.85	4.85	4.77	2.62	1.31	1.85	0	0.62	

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

(a) Flowering time in field.



(b) Flowering time in greenhouse



(c) Ecological conditions

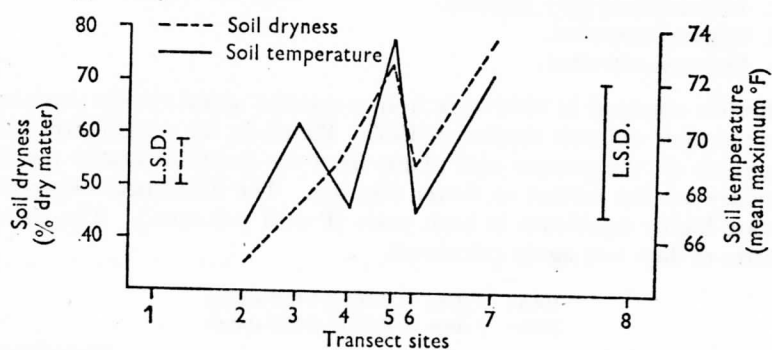
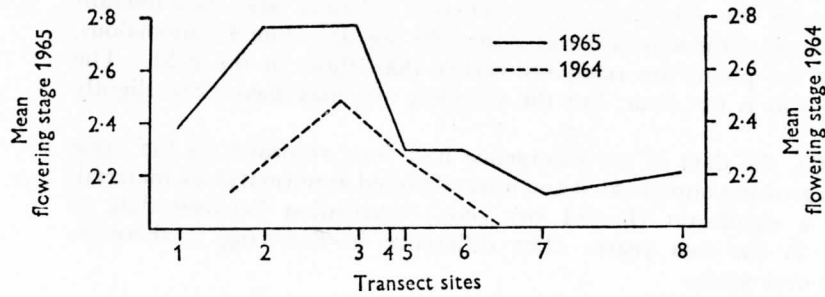


FIG. 2.—Flowering time and ecological conditions along the Drws-y-Coed (*Agrostis*) transect.

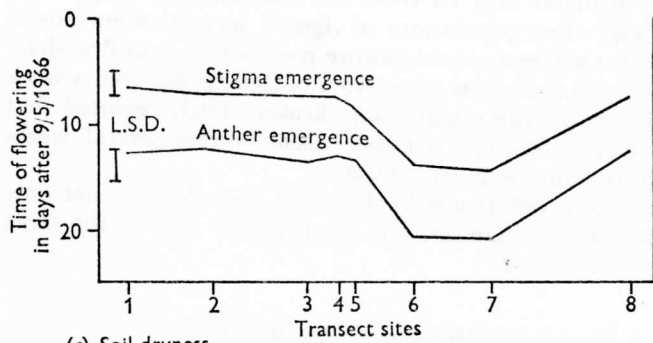
(b) Flowering time in cultivation

The differences in flowering time in the field could be environmentally induced, or genetically determined and the product of evolutionary processes promoting isolation. To test this plants growing under standard experimental conditions were scored for flowering time.

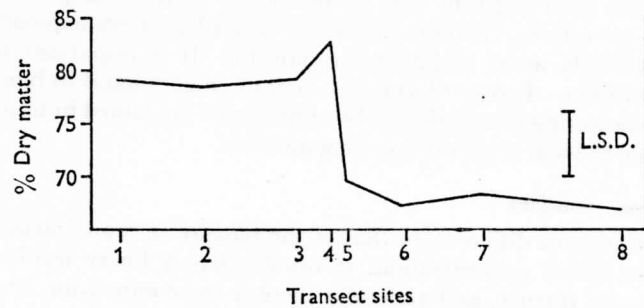
(a) Flowering time in field



(b) Flowering time in garden



(c) Soil dryness



and ecological conditions along the Trelogan (*Anthoxanthum*) transect.

(i) *Agrostis*

The pattern of flowering both in 1964 and 1965 parallels that found in the field (fig. 2*b*). In 1965 each genotype was grown in pots of two sizes and the regression of genotypes in small pots against the same genotypes in large pots is significant ($P < 0.1$ per cent.), showing that the genetic component is not obscured by environmental effects.

(ii) *Anthoxanthum*

Plants were recorded during the course of a spaced plant trial in an experimental garden.

Differences in flowering time between tolerant and non-tolerant populations are of the same order as in the field (fig. 3*b*). Site 8 is anomalous, since here the plants are relatively earlier than those in the field. The reason for this is not clear, but the sampling site may have been slightly different.

In 1965, the date of ear emergence had been recorded on the same genotypes growing unreplicated and unrandomised as normal stock material. There is a significant ($P < 0.1$ per cent.) correlation between date of flowering in the two years. The difference in flowering is therefore consistent over years.

(iii) *Conclusion*

In both *Agrostis* and *Anthoxanthum*, the evidence strongly suggests 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 about four days earlier on average than pasture populations, and Bradshaw (1959) found that a single lead mine population of *Agrostis* flowered a week earlier than an adjacent pasture population. Broker (1963) reported that the prostrate zinc-tolerant ecotype of *Silene inflata* flowers several weeks earlier in water culture than the normal form.

Selection has produced differences in flowering time that are not only important as an 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 may simply be the result of adaptation to local ecological conditions, or they may have evolved as a consequence of gene flow, specifically as an isolating mechanism. It is important to keep these causes distinct. Ecotype formation very often goes hand in hand with differences in flowering time (table 2) but this can be explained in terms of adaptation either to local conditions or to gene flow.

(i) *Adaptation to local conditions*

The mine environment differs from that of the pasture in many factors. Apart from 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 characters other than tolerance have been recorded (McNeilly, 1965; Antonovics, 1966), and it is possible that flowering time is another example of adaptation to local conditions in the same way that sand dune populations of *Agrostis* flower considerably earlier than pasture populations (Bradshaw, 1959).

Soil water content and temperature possibly select for early flowering time and they were therefore investigated in the field. The water content of the soil was estimated for both Drws-y-Coed and Trelogan. Soil temperature was measured using maximum thermometers, only for the Drws-y-Coed transect.

TABLE 2

Differences in flowering time of various ecotypes or closely related species

Species	"Ecotype"	Flowering time (compared with "normal" ecotype)	Author
<i>Gilia capitata</i>	sand dune	later	Grant (1952)
* <i>Madia elegans</i>	ssp. <i>vernalis</i> ssp. <i>aestivalis</i> ssp. <i>densifolia</i>	spring summer autumn	Clausen (1951)
* <i>Layia platyglossa</i>	maritime	later	Clausen <i>et al.</i> (1958)
* <i>Hemizonia citrina</i>		April	Babcock <i>et al.</i> (1924)
* <i>Hemizonia lutescens</i>		Aug.-Sept.	
* <i>Hemizonia luzulaefolia</i>		April	
* <i>Hemizonia rudis</i>		Aug.-Sept.	
* <i>Lactuca graminifolia</i>		early spring	Whitaker (1944)
* <i>Lactuca canadensis</i>		summer	
<i>Ixeris denticulata</i>	ssp. <i>typica</i> ssp. <i>sonchifolia</i> ssp. <i>elegans</i>	spring autumn summer	Stebbins (1950)
* <i>Pinus attenuata</i>		later	Stebbins (1950)
<i>Pinus radiata</i>		earlier	
* <i>Lamium amplexicaule</i>	vernal race	earlier	Bernstrom (1952)
<i>Viola tricolor</i>	sand dune	later	Clausen (1926)
<i>Silene cucubalis</i>		earlier	Marsden-Jones <i>et al.</i> (1928)
<i>Silene maritima</i>		later	
<i>Geranium robertianum</i>	shingle beach	later	Böcher (1947)
<i>Mimulus guttatus</i>	coastal mountain valley and foothills	late latest early	Vickery (1953)
* <i>Geum urbane</i>		later	Clausen <i>et al.</i> (1958)
* <i>Geum rivale</i>		earlier	
<i>Succisa pratensis</i>	northern race	earlier	Turesson (1925)
<i>Ranunculus acer</i>	alpine	earlier	
<i>Solidago virgaurea</i>	alpine and coastal	earlier	
<i>Rumex acetosa</i>	alpine	earlier	
<i>Leontodon autumnale</i>	coastal	earlier	
* <i>Clarkia xantiana</i>	self compatible race	earlier	Moore <i>et al.</i> (1965)
<i>Salvia mellifera</i>		early spring	Grant <i>et al.</i> (1964)
<i>Salvia apiana</i>		late spring	

* Some evidence given by author that "ecotypes" closely adjacent.

There is a distinct relationship between flowering time and soil temperature (figs. 2c and 3c). 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, sites 5 (mine) and

7 (non-mine) are both in dry warm sites, but site 7 has a much later flowering time than site 5. At Trelogan sites 1 (in the field) and 8 (in the garden) do not correlate with the ecological pattern. No significant relationship between flowering time and tolerance was found within the tolerant population.

(ii) *Adaptation to restrict gene flow*

Differences in flowering time could be a result of selection for a mechanism restricting gene flow. It would be very difficult to obtain direct evidence from purely ecological investigations, but any divergence in flowering time which cannot be explained ecologically may well have arisen by this process. If this hypothesis is true greater isolation should exist at the ecological boundary where gene flow is likely to be greatest, and in general the (figs. 2 and 3) flowering difference is greater in the boundary populations of both *Agrostis* and *Anthoxanthum*. The mines studied are ecologically very different (Antonovics, 1966), yet the same pattern is seen in both. This suggests that boundary populations flower earlier as a result of a selection for a mechanism to restrict gene flow.

A series of populations were collected in early 1965 from 28 lead mines in Cardiganshire. 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 proximity of the tolerant populations to non-tolerant and, hence, as measures of the intensity of gene flow. The populations were grown in pots and scored in the summer of 1966. 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 (fig. 4). The same trend is nevertheless present in both data. This is evidence of evolution of earlier flowering as a means of restricting gene flow. However, because of the low significance of the fitted regression and since the curve is a positive linear negative quadratic (pollen distribution with distance follows a negative linear, positive quadratic curve), there is evidence that factors other than gene flow also influence the time of flowering.

5. DISCUSSION

Evidence for the existence and mode of origin of isolating mechanisms has in the past been obtained mainly from studies of taxa that are already distinct. It is therefore not surprising that the process of speciation has been regarded as occurring by allopatric divergence followed by the development of breeding barriers either allopatrically (and therefore being chance by-products of the process of divergence) or sympatrically after the two divergent races meet. There is now considerable evidence (see Jain and Bradshaw, 1966) that divergence does not require prior isolation, and similarly it is pertinent to ask whether, as Thoday and Gibson (1962) have demonstrated, isolating mechanisms arise during this process of sympatric divergence.

The mine populations reported here must be the products of recent evolution. The copper mine at Drws-y-Coed and the lead/zinc mine at Trelogan were first worked in the thirteenth century (extensively after 1855) and in 1848 respectively, and the areas of contamination from which the plants were taken probably date from the latter part of the 19th century. The time scale of the evolution and colonisation reported here is therefore very

short. Moreover, the size of the mines is such that even if a race did develop by immigration from another geographical area, these initial colonies would have been subjected to intense gene flow from surrounding areas; the mine

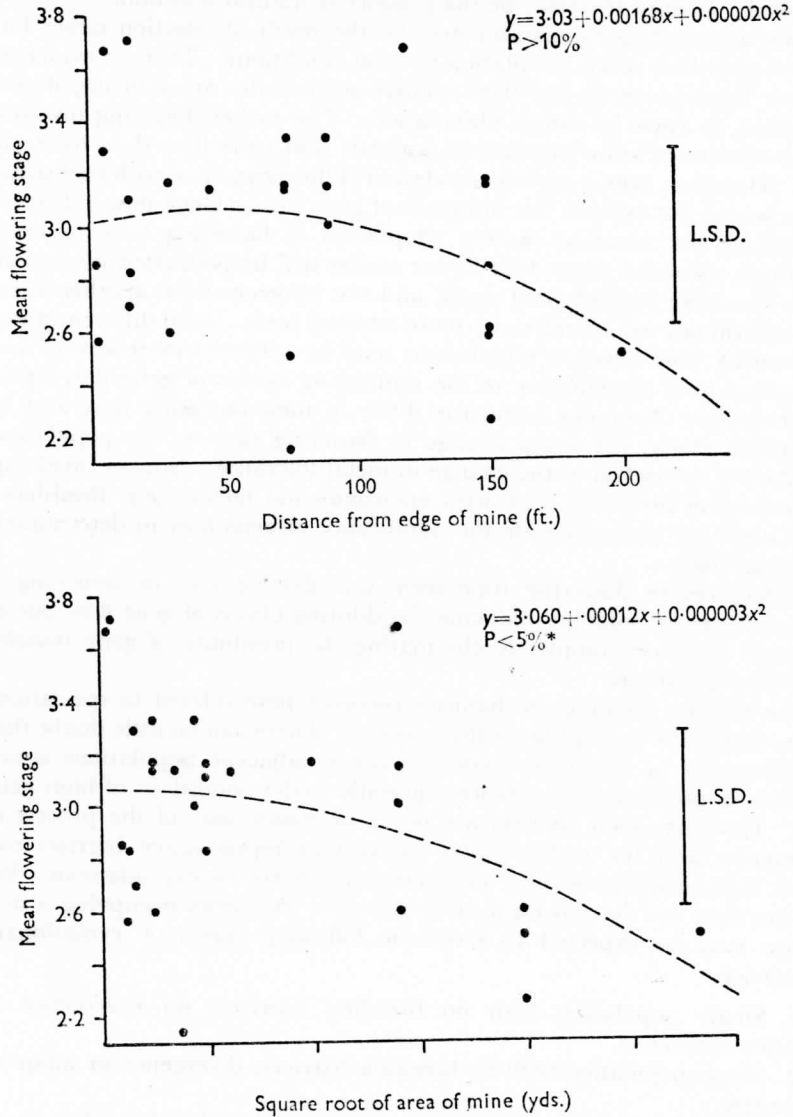


FIG. 4.—Relationship between flowering stage of tolerant *Agrostis* populations and their distance from nearest non-tolerant plants.

and pasture populations must have been more or less continuous right from the outset. There is also evidence that evolution of tolerance *de novo* from non-tolerant populations can be a rapid process (Antonovics, 1966). For these reasons, the evolution of mine populations cannot have been through allopatric divergence.

In the present study isolation between closely adjacent populations has

been demonstrated. Isolation by incompatibility barriers seems to be partially present in one population, but requires further investigation. Temporal isolation on the other hand is considerable and is genetically controlled. It must therefore be the product of natural selection.

Such an isolating mechanism may be the result of selection either for reduced gene flow or for adaptation to local conditions. There is evidence for both these processes but their relative magnitudes are difficult, if not impossible, to assess by simple observation. The earlier flowering towards the boundaries of mine populations suggests that gene flow does have an effect. However, even a perfect correlation of flowering time with ecological factors would not exclude the influence of gene flow. Gene flow will tend to reinforce the selection causing adaptation of flowering time to local conditions, since the plants that flower earlier will be pollinated by similar earlier flowering and adapted types, and not by genes from another area. Such individuals will therefore set more adapted seed. Local differentiation in flowering time between populations may be achieved in relatively few generations as a consequence of the reinforcing effects of gene flow upon this character. Ecotypes frequently differ in their flowering time and in the present study, the sharp change in flowering time at the population boundary is as marked as the change in metal tolerance. Studies involving correlations of flowering time with environmental factors (*e.g.* Bradshaw, 1959) may well underestimate the significance of gene flow in determining flowering time.

Differences in flowering time seem an effective way of achieving a certain degree of isolation, reducing the diluting effects of gene flow but at the same time not completely eliminating the possibility of gene transfer between populations.

Are the the isolating mechanisms reported here related to speciation: are we seeing the beginnings of this process? There can be little doubt that the formation of breeding barriers between adjacent populations assists selection in promoting divergence especially under conditions of high gene flow. However, such barriers are not a necessary part of the process of divergence, and the present work shows that reproductive barriers can evolve independently of other characters which are clearly adaptive. We can therefore see the process as a continuum. A species occupying a new habitat may be expected to show the following stages of evolutionary divergence.

1. Single population with no breeding barriers; no divergence in adaptive characters.
2. Single population with no breeding barriers; divergence in adaptive characters.
3. Discontinuous population with partial breeding barriers; divergence increasing.
4. Two isolated populations; independent of each other.

This final stage has not yet been reached in the mine populations studied here, but some serpentine species are isolated from what appear to be their immediate counterparts on normal soils, and the slight incompatibility between mine and pasture populations of *Anthoxanthum* may indicate the beginnings of a more radical isolating mechanism.

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 the populations in the present study are not yet two species. Evolution begins at the population level and the processes causing speciation should not be sought only in taxa which are already highly distinct.

6. SUMMARY

1. Reproductive isolation was studied in closely adjacent mine and pasture populations of *Agrostis tenuis* and of *Anthoxanthum odoratum*.

2. No clear-cut incompatibility barriers between the populations were found, but in *Anthoxanthum* crosses between mine and pasture plants set less seed when pasture plants were used as female parents than crosses within mine or within pasture populations.

3. Mine populations of both species flowered about a week earlier than pasture populations, this difference in flowering time being maintained under standard conditions. It appears to be genetically determined and the product of natural selection.

4. The difference in flowering time was interpreted as an adaptation to local ecological conditions, and also as a method whereby the deleterious effects of gene flow were reduced. Populations nearer the edge of the mine or from smaller mines generally flowered earlier.

5. Divergence of closely adjacent populations has been followed by reproductive isolation. The processes described are considered to show the beginnings of sympatric speciation in a natural situation.

Acknowledgments.—We are extremely indebted to Dr A. D. Bradshaw for his constant interest, advice and encouragement. J. A. would also like to acknowledge the tenure of a University of Wales I.C.I. Research Fellowship during part of this investigation.

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