EVOLUTION IN CLOSELY ADJACENT PLANT POPULATIONS

VI. AN ENTOMOPHILOUS SPECIES, *POTENTILLA* *ERECTA*, IN TWO CONTRASTING HABITATS

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1. INTRODUCTION

THE fact that the influence of the habitat upon the species is genetically based has been acknowledged for many years but it must be conceded that the mode of sampling has a profound effect upon the interpretation which is put upon the final analysis of the data obtained from trials under uniform conditions. The random sampling of widely separated populations, the method employed by Turesson, led him to the conclusion that discrete ecotypes may be delimited from specific habitats, that "they are to be considered as products arising through the sorting and controlling effect of the habitat factors upon the heterogeneous species-population (Turesson, 1922)". Clausen *et al.* sampled an extensive transect across central California and both in *Potentilla glandulosa* (1940) and in *Achillea millefolium* (1948) recognised large intra-specific groupings forming distinct ecotypic races, geographically separated and related to climatic changes. Though admitting that individual and population variation existed within these large "ecotypes", Clausen *et al.* considered it to be of secondary importance and stressed the separateness of each grouping to the extent of identifying each with taxonomic subspecies.

Gregor however, felt that "degrees of ecotypic discontinuity and divergence should not be assessed only from the evidence obtained from comparison of extreme habitat populations, but by an examination of differences exhibited by populations inhabiting the various habitats lying between the extremes" (1944) and sampled both extensively and intensively over a wide area. The data which was accumulated led him to an interpretation differing markedly from that reached by previous workers since he suggested that an "ecotype" should be thought of "as a certain range of variation on an ecotypic gradient selectively developed in response to a climatic, edaphic, biotic or for that matter any environmental gradient we may choose to examine" (1944). Because individual biotypes or populations may be at different points on many environmental clines running in all directions, this is a difficult concept and might even be confusing (Stebbins, 1950) but it has directed attention to the complex situation in plant species to the "micro" as well as the more obvious "macro" aspects of genecological change.

Later studies have indicated the possibility of abrupt changes occurring within clines and in his examination of a wind-pollinated species, *A. tenuis* in the Aberystwyth region, Bradshaw (1959) found that in this generally continuously distributed species "there are clines of varying slopes interspersed with rather sharp discontinuities corresponding to changes in the environment" (Jain and Bradshaw, 1966). Although no such remark can be made

about *A. tenuis* in the south of Scotland where 162 populations were sampled from five strongly contrasting habitats repeated throughout the area (Harberd, Watson and Wilkins, personal communication), there was evidence of abrupt differentiation between habitats in *Plantago lanceolata* (Gregor and Watson, 1961) and even more obviously, in *Potentilla erecta*. (L) Räusch. In particular, there were statistically highly significant differences between populations from the different habitats in several different characters. The observations that there were considerable plant size differences between populations from contrasting habitats which were less than 50 metres apart led to the present investigations.

2. MATERIAL AND METHODS

Potentilla erecta was collected in 1962 from four sites in Scotland chosen for sampling because a considerable area of closely grazed *Agrostis fescue* (Pasture) was immediately adjacent to a reasonably extensive area of very uniform *Molinia* dominated vegetation (Molinietum). Three sites were in the south of Scotland; (1) Loch o' the Lowes, roughly 14 miles over hills to (2) Smidhopeburn which is also about 14 miles over hills to (3) Wanlockhead. There was a mosaic distribution of the two habitats at the fourth site which was on the lower slopes of Ben Hope in Sutherlandshire and about 200 miles from the other sites. The boundary between Pasture and Molinietum was

TABLE 1
Mean pH values of original habitats

	Pasture	Molinietum
Loch o' the Lowes	4.8	4.1
Smidhopeburn	5.0	3.8
Wanlockhead	4.8	4.3
Ben Hope	5.0	4.6

most distinct at Smidhopeburn and populations were sampled along three transects crossing the whole area. Mean pH values for Pasture at the four sites are greater than those for Molinietum, the greatest difference being at Smidhopeburn where the mean for Pasture is 5.0, that for Molinietum 3.8 but at Ben Hope the difference is only 0.4.

Seed was also collected from the habitats at each site, often from the sample plants themselves. Germination was not uniformly good and a second collection of seed had to be made from the Wanlockhead site in the following year. The populations of the original and of the seedling samples were planted out in the field in adjacent but separate trials either in randomised complete blocks or in a split arrangement with populations as the main treatment. Plants were spaced 3 ft. apart with 3 ft. between rows.

Preliminary crossing experiments in the greenhouse proved that *P. erecta* is an outbreeding species setting seed in abundance either in the presence of honey bees or in response to hand pollination. A very few single achenes were harvested from the receptacles of flowers whose stigmas had been dusted with pollen from the same plant but were thought to be the result of accidental fertilisation by stray pollen grains during the pollination with foreign pollen of "crossed" flowers since individual flowers were not bagged, entire plants being completely isolated between pollinations under pollen proof cages.

3. RESULTS

(a) *The original plants*

The length of the longest undamaged stem and the length of its basal internode were recorded immediately after collection and analysis of variance calculations showed that while there were no significant effects due to sites, there was a highly significant difference between the values for Pasture and Molinietum plants for both characters. Since the measurements were made on plants taken straight from their natural habitats, it would have been easy to believe that the short stems of the plants from the Pasture areas ($\bar{x} = 8.2$ cm.) and the long stems of plants collected in Molinietum ($\bar{x} = 24.9$ cm.) were due to environmental effects. But similar calculations applied to measurements obtained from the same plants but now growing in the more uniform environment of the field trial gave very similar results to those calculated from the values obtained by measuring stem length after collection.

The diameter of the plant is probably a more reliable measurement of size than length of stem and though not obtainable from the material in the wild, was used in later calculations in preference to stem length with which it is highly correlated. Table 2 shows the high degree of statistical significance persisting between the diameters of plants originating from Pasture and those from Molinietum after two years in the field trial.

TABLE 2
Analysis of variance
Plant diameter of original plants in second year of trial

	d/f	S.S.	M.S.
Origins	7	16178.35	
Habitats	1		14155.24**
Sites	3		380.17
Habitats/Sites	3		307.53*
Error	247	20078.22	81.29
Total	254	36256.57	

Naturally within the populations from each habitat there was some variability in plant size but each year in the trial, as in the wild, the mean values for size characters were smaller for Pasture populations than those from Molinietum and this is well illustrated by the values obtained from the field trial in 1964 for stem length in the transect populations from the Smidhopeburn site (fig. 1).

A most noticeable point is the considerable difference which exists between means for Pasture and Molinietum populations growing only 2.7 metres on either side of the boundary under natural conditions and though this diminishes when the plants are not subject to differing environments, it does not disappear nor is any gradual change with increasing distance from the boundary in either direction more noticeable than it was in the material measured after collection.

First casual observations did not suggest that there were marked size differences in other characters but measurements were made in the 2nd year of trial and many significant differences became apparent (table 3 (a)). Further analysis of the values within sites showed even more significant differences between the generally small Pasture plants and the larger plants

from the adjacent *Molinietum* particularly at Loch o' the Lowes and Smidhopeburn (table 3 (b)).

FIG. 1.—Smidhopeburn transects.

Open circles: Measurements of stem length made on plants immediately after collection.
Closed circles: Measurements of stem length made on same plants after one year in field trial.

--- Mean value for "wild" measurements.

— Mean value for measurements in trial.

The statistical evidence so far presented supports the observations that there is a marked difference in size characters between plants from Pasture and those from *Molinietum* irrespective of geographical site, in spite of a certain amount of variation within all populations.

(b) *Seedling generation*

The distribution of sizes of the original plants, as measured by plant diameter, on the Smidhopeburn transects is given in fig. 2a together with the sizes of those plants grown from seed collected from the same populations. There are no obvious differences between the three transects and since the S generation is unfortunately small owing to the erratic germination already mentioned, the three transects have been condensed into one to give greater clarity. Even in 1964 a pattern similar to that obtaining with the original

TABLE 3

Mean habitat values for two characters measured immediately after collection and again in 1964 together with mean habitat values for all other characters measured in 1964.
Significance of differences obtained from analysis of variance calculations

(a) between Pasture and Moliniatum plants irrespective of geographical origin

Habitat { Pasture Moliniatum	Stem length (wild)	Length of Basal internode (wild)	Plant Diameter	Stem length	Length of Basal internode	Leaf length	Leaf breadth	Leaf length breadth	Number of leaf teeth	Number of stems	Weight of Rootstock (uncut)
	8.2	3.0	20.8	12.7	2.2	1.3	0.6	2.3	6.3	59.3	31.1
	24.9***	10.0***	28.7*	17.5**	3.2***	1.6	0.6	2.7*	7.2	64.2*	51.1***

Geographical Site

Habitat/Site Interaction

(b) between Pasture and Moliniatum populations within geographical sites

Loch o' the Lowes	{ Pasture Molinietum	9.5	3.9	22.7	13.5	2.3	1.4	0.6	2.5	6.1	64.7	35.2
		21.6***	9.0***	29.5***	17.3**	3.2***	1.6*	0.7*	2.4	7.8***	64.3	50.2*
Smidhopeburn	{ Pasture Molinietum	7.9	2.7	19.3	11.9	2.1	1.3	0.6	2.2	6.2	59.4	34.1
		27.7***	9.9***	29.9***	18.1***	3.2***	1.7***	0.7**	2.6**	7.1**	67.1	56.1***
Wanlockhead	{ Pasture Molinietum	5.1	2.2	22.1	13.1	1.8	1.3	0.6	2.2	6.3	54.8	22.7
		22.1***	10.1***	25.9**	16.7**	3.2***	1.6*	0.6	2.7***	6.6	66.1	37.2**
Ben Hope	{ Pasture Molinietum	8.3	2.6	21.5	13.8	2.4	1.5	0.6	2.6	7.1	56.0	31.5
		28.0***	11.1***	29.1***	17.7***	3.1*	1.7**	0.6	2.9*	7.1	59.1	51.8*

* Significance 0.05; ** Significance 0.01; *** Significance 0.001.

NS: Failed to reach 0.05; No entry: No significance at all.

plants can be traced but in 1966 when statistical significance is found in analysis of variance calculations (table 4), there is a clear difference in mean value between the S plants from Pasture and those from Molinietum.

The pattern at the Wanlockhead site (fig. 2*b*) where an elongated area of Molinietum was immediately adjacent to Pasture on the two long sides, is similar in both generations to that shown in fig. 2*a* for Smidhopeburn transects. With considerably greater numbers in the S generation from Wanlockhead, there can be little doubt about the validity of the distribution pattern sketched by the values for the small number in this generation at Smidhopeburn.

The values for some other characters were scrutinised in the same manner and it is interesting that again similar patterns occur at both Smidhopeburn and Wanlockhead, a fact which gives good grounds for the deduction that not all characters are selected by the environment in a like manner. Leaf length, plant diameter and stem length (not given because of its close association with the previous character) are obviously strongly selected by habitat conditions, yet number of teeth on the leaf which one might have expected to bear a close relationship to leaf length shows no material differentiation save in one instance at Loch o' the Lowes (table 3 (b)). Again leaf breadth is a character virtually unaffected by the prevailing selective pressures and while length of basal internode is differentiated, there is a greater scatter of values in this character than in any other examined.

There is no significant difference between Pasture and Molinietum in the important character of plant diameter in the seedling generation in 1964 but differences become significant in subsequent years (table 4). This absence of significance in 1964 might be due to differential establishment of the seedlings or to an increase in variability in this generation in both Pasture and Molinietum due to gene flow between the habitats. Both the distribution patterns in figs. 2 and 3 and the coefficients of variation for 1964 in table 5 on the whole suggest greater variability.

TABLE 4

*Significance of differences between Pasture and Molinietum
seedling generation within geographical sites:
plant diameter*

	1964	1965	1966
Loch o' the Lowes	NS	**	*
Smidhopeburn	NS	*	**
Ben Hope	NS	NS	NS
Wanlockhead	†	†	***

* $P = 0.05$; ** $P = 0.01$; *** $P = 0.001$.

† No measurements available.

TABLE 5

Coefficients of variation for original and seedling generations: plant diameter

	1964				1965			
	Pasture		Molinietum		Pasture		Molinietum	
	O	S	O	S	O	S	O	S
Loch o' the Lowes	13.4	26.7	19.9	29.1	27.9	24.3	15.8	23.3
Smidhopeburn	19.8	31.2	10.1	28.7	24.2	22.4	18.2	24.2
Ben Hope	16.0	23.8	19.4	23.3	22.3	22.3	19.0	13.5

O = Original; S = Seedling.

FIG. 2a.—Comparison of measurements of plant diameter in original and seedling generations at Smidhopebum transects.
Arrows indicate mean values.

FIG. 2*b*.—Comparison of measurements of plant diameter in original and seedling generations at Wanlockhead.
~ represents ten circles. Arrows indicate mean values.

FIG. 3a.—Comparison of measurements for three characters in original and seedling generations at Smidhopeburn transects.

FIG. 3b.—Comparison of measurements for three characters in original and seedling generations at Wanlockhead.

~ represents ten circles.

Arrows indicate mean values.

However, it is unwarranted to compare coefficients of variation unless an increase in the mean coincides with a proportional increase in the standard deviation and this is far from being the case in the present data, a fact which probably accounts for the anomalous coefficients of variation in 1965. The method of Day and Fisher (1937) was therefore used in which an allowance for differences in the means is "derived from the data themselves by means of the analysis of the covariance of the means and standard deviations in the populations observed". Table 6 gives the results of analyses for the years 1964 and 1965.

TABLE 6

Amount of variability in Pasture and Molinietum populations. Comparison between original and seedling generations: plant diameter

	Smidhopeburn, Loch o' the Lowes and Ben Hope Sites		Wanlockhead
	1964	1965	1966
Generations	***	NS	NS
Habitats within generations	NS	NS	NS

*** $P = 0.001$.

The Wanlockhead populations were analysed separately as they were not growing under similar conditions and also comprised two seedling generations as well as the original generation. Notwithstanding the lack of proportionality of increase in mean and standard deviation, the coefficients of variation in table 5 for 1964 seem to give a reasonable indication of the true situation as measured by the more accurate statistical analysis though this cannot be said of those calculated for 1965. It must be concluded, on the whole, that the initial variability in the seedling generation was due to different rates of establishments in the neutral environment of the field trial.

Time of flowering was also measured, but in the conditions of cultivation no differences between habitat populations or sites could be detected. Flowering commenced about the beginning of May and continued without interval until the stems died in the autumn.

It cannot be shown from the data available that the results from the seedling generation give incontrovertible proof of genetic differentiation occasioned solely by the selective effect of the habitat since some maternal effect could be an important factor, but there is a small piece of evidence which suggests that in this material, the effect of the maternal parent is not paramount.

The progeny from a reciprocal cross made under greenhouse conditions between a plant from Pasture and one from Molinietum at Loch o' the Lowes, were grown and measured for several characters. Plant diameter for the parent from Pasture was 8 cm. less than that for the parent from Molinietum when grown in the field trial, but the following results, were obtained from the progeny. Mean plant diameter for Pasture ♀ × Molinietum ♂ (44.1 cm.) was greater than the mean for Molinietum ♀ × Pasture ♂ (37.0 cm.) by 7.1 ± 3.49 cm. (95 per cent. fiducial limits 0.16 cm. and 14.05 cm.) so that the cross with the Pasture plant as maternal parent produced progeny with greater plant diameters than did the cross with the Molinietum plant as maternal parent.

4. DISCUSSION

The micro-environment which favours *Molinia caerulea* is very different from the habitat conditions which permit the growth of floristically rich *Agrostis fescue* grassland and though it is not a simple matter to define the differences in their entirety, there is no doubt about their ecological effectiveness. Nevertheless when each includes a widely occurring species such as *Potentilla erecta* it could be argued that this is a flexible species which is tolerant of a variety of conditions and capable of considerable phenotypic plasticity. The fact that the difference between the stem lengths of plants from Pasture habitats and those from adjacent *Molinia*-dominated areas are generally much greater when measured after sampling from the wild than those obtained in later years from the same plants grown in uniform conditions is indicative of an ability to modify growth in response perhaps to light requirement. However, statistical analyses on material in cultivation show that the populations from those contrasting habitats, in spite of a certain degree of overlap, are indeed differentiated and furthermore, remain so after a period of three years in uniform trial conditions, strongly suggesting that this is a genetic difference and not a carry-over effect from the original environment. Differentiation is not confined to length of stem but is repeated in a number of other size characters including the weight of the rootstock itself. Grime (1963) reported larger, heavier rootstocks of *P. erecta* growing in a plateau area with relatively fertile conditions compared with poor rootstock development on an adjacent rather waterlogged slope but in our material all the Pasture areas sampled at the four sites, produced rootstocks which were on average smaller and lighter than those from the adjacent Molinietum. The small rootstock size in the grazed areas might be attributable to the tendency noted by Grime and occasionally observed during sampling, for the rootstock to grow horizontally and to decay distally, but this was not found in the field trial although it may occur over a longer period.

It has been considered impossible for sympatric differentiation to occur in the absence of some form of isolation (Mayr, 1947) but Blair (1947) found that populations of *Peromyscus eremicus* in an area of dark and light soil types are differentiated by pelage colours corresponding to their natural background and concluded that selection pressure exercised by soil colour must exceed the "homogenising effect of interbreeding between the several adjacent local populations". More recently, experiments with *Drosophila* have served to emphasise the powerful long-term effect of selection. For example, Waddington (1953) demonstrated that it was possible by applying a high temperature shock to induce a wing venation defect in otherwise normal flies and further that in a line selected for this characteristic, some flies bearing the defect appeared after the fourteenth generation even without receiving the heat treatment in pupation. Again in *Drosophila*, Thoday and Boam (1959) proved that with disruptive selection "two components of a population can diverge without any isolation limiting gene flow between them". It is, therefore, not difficult to understand the physiological differentiation without isolation of plant populations in response to the powerfully selective effect of a heavy metal in the edaphic environment (Kruckeberg, 1951; Wilkins, 1960) but it is also of considerable interest to discover the amount of gene flow which can be present.

McNeilly (1968) examined adult (tiller) and seed generations of a wind pollinated species *Agrostis tenuis* from adjacent but edaphically contrasting habitats and found good evidence of a high rate of gene flow following the direction of the prevailing wind. The selection pressures exerted by the toxic soil of the mine are strong enough, however, to eliminate non-tolerant genotypes almost entirely so that in the adult generation, divergence between the habitat populations was quite obvious though more tolerant genotypes survived in the "normal" habitat. For the purposes of the present investigation it is perhaps most instructive to examine examples in literature in which all the habitats could be termed "normal".

In a wind-pollinated species, *Plantago maritima*, since it was not possible to transplant successfully from the wild into a field trial (Gregor *et al.*, 1936), large seedling populations from habitats ranging from waterlogged mud to drained meadow were used and Gregor (1944) found a graded ecocline of continuous variation in several hereditary characters but also marked differentiation between two populations separated only by a stream. There was in this case a sharp difference in mean values for plant height, for example, but each population included some genotypes characteristic of the other. In another wind-pollinated species, *Agrostis stolonifera*, Aston and Bradshaw (1967) using tillers collected in the natural habitats, found that the effect of a sudden change in local environment notably with regard to exposure, was sufficient to cause divergence in stolon length between two populations a few metres apart and that the discontinuity was abrupt. When a greater number of populations occurring over an area of more gradual environmental change was considered, an ecocline gradient was apparent. In a second trial using the same tillers together with a seedling generation, the population means remained very distinct but there was evidence of some overlap in the stolon lengths of the seedling generation as indeed there was in the tiller generation in this second trial suggesting that the appearance of discontinuity might have been deceptive. The pattern of differentiation with regard to a major size characteristic seems to be very similar in these examples of wind-pollinated species, the selective pressure of the environment being sufficient to maintain population divergence in the face of considerable gene flow. Further it does not appear to differ markedly from that deduced from the entomophilous *P. erecta* under consideration.

There are no obvious isolating barriers between the habitats at any site and *P. erecta* being a self incompatible outcrossing species with a prolonged flowering period, theoretically there are unlimited opportunities for panmixia and consequent free gene flow between the populations though even a small amount of maternal effect could mask the true situation. However, there is no evidence of such an effect from the present data. An ethological isolation such as that mentioned by Grant (1949) when he emphasised the role played by the "constancy of pollinators to one kind of flower" might be postulated were it not that the flowers from the two habitats are indistinguishable from one another. Heslop Harrison (1964) suggested that in certain situations "an ecological limitation of the vectors may be so strong as effectively to exclude gene exchange between neighbouring concentrations of individuals" but though limited, there is some gene flow between Pasture and Molinietum.

Similar differentiation has occurred under slightly dissimilar physical conditions at the four sites. For example, there is a sharp visible division

between the Pasture and Molinietum habitats at two of the sites but it is most marked at Smidhopeburn, where also there was the greatest difference in mean pH values as well as the greatest between mean size characters. This might seem to support Heslop Harrison's suggestion but the situation is different at the Ben Hope site, where there is a mosaic distribution of the two habitats without visibly sharp divisions and mostly the patches are only a few metres square, with minor differences between pH values. At this site there is an absence of significant differentiation in the seedling generation between Pasture and Molinietum, even after three years' growth, which argues considerable gene flow, yet divergence is as obvious in the original generation as it is at the other sites.

Bateman (1947a) pointed out that in his experiments, entomophilous species showed "substantially similar relationships between contamination and isolation distance" to those obtaining in wind pollinated species. In other words, contamination is greatly reduced in ten to twenty feet (9-18 m.) from the source but thereafter the rate of decrease is quite slow for a considerable distance. Though the amount of data available from the seedling generation is less than would have been desirable, there is no evidence of a pattern of contamination of this type.

It is not known whether one, or more, species of insect acts as vectors in nature but honey bees are efficient pollinators of *P. erecta* under greenhouse conditions and Bateman (1947b) has remarked upon the tendency for foraging bees to concentrate on areas which will give the maximum returns of nectar and pollen for energy expended. It is pertinent to remember that the Pasture areas have a greater profusion of flowers at any one time than the Molinietum possibly because the tall dense growth of *Molinia* limits the number of flowers per stem and *P. erecta* is, in any case, more sparse in this habitat.

Therefore, even through the habits of the insect pollinators have some effect upon the spread of genes from one habitat to another, it does not appear to be solely because they are ecologically restricted to one habitat and there is still evidence of the selection pressure exercised by the environmental conditions of the habitat. Indeed the fact that the original plants invariably maintain a significant difference between Pasture and Molinietum habitats whether the boundaries are sharp or blurred argues strongly for the powerful selective effect of the vegetation. That there is gene flow between the habitats is strongly suggested by the results for the generation grown from seed collected in the wild, for there is a wider range of variability in the seedlings than in the original plants though this does not reach statistical significance in the material examined after the first year.

The seed, being relatively heavy, falls very close to the mother plant as was frequently observed in the field trial, and since the pollinators tend to be restricted within a more floriferous area by supply, it might be expected that there would be less variation in Pasture than in Molinietum where pollination might be more random. There is, however, no statistically significant difference between the variabilities in the habitats within generations but while *Molinia caerulea* is little grazed except in spring before *P. erecta* stems are elongating appreciably, the vegetation height in Pasture will fluctuate according to the intensity of stocking in the area and it is in the latter habitat, that genetic diversity could be most advantageous even in a species possessing considerable phenotypic plasticity.

In the first year of growth there is greater variability in the generation from seed produced under natural conditions and collected within the habitats than in the original generation and also a lack of significant differences between seedling populations from the habitats. Subsequently, the differences between the variabilities of original and seedling generations disappear between the variabilities of original and seedling generations disappear and the habitat seedling populations give indications of being differentiated in a similar manner to the original populations. It has been suggested that the variability in the first year could be the result of a failure to "settle down" but this in itself is good evidence of considerable genetical diversity in developmental growth which is no doubt a powerful factor in promoting the success of the species but not necessarily fully expressed in a neutral environment. As Iman and Allard (1965) have said in discussing genetic variability in natural populations of wild oats in California, "populations composed of mixtures of genotypes differing in morphology, physiological requirements and growth rhythms may exploit the available moisture, light and nutrients more efficiently than uniform populations" and, it can be added, adapt to changing circumstances such as varying vegetation height caused by the fluctuations in numbers of grazing animals. Further, should the seed be dispersed by animals, as must frequently be the case, there must be a considerable reservoir of genotypes potentially capable of colonising new habitats.

5. SUMMARY

1. The entomophilous and self-incompatible species, *Potentilla erecta* (L.) Rätisch was collected from two ecologically contrasting but adjacent habitats (Pasture and Molinietum) in four geographically isolated sites.

2. Differences occurred in the distribution of the habitats at the different sites but in spite of possessing a considerable degree of phenotypic plasticity, *P. erecta* was found to be similarly genetically differentiated in respect of size characters at each site. All populations show intra-habitat variation but Pasture and Molinietum populations had small and large mean values respectively and there was no evidence of topoclinal differentiation.

3. Populations were grown from seed produced under natural conditions and while there is no evidence of maternal effect there appears to be gene flow between habitats, this gene flow is greatest at the site where boundaries are confused and the habitats distributed in small patches. Where habitat areas are relatively large and boundaries between them sharply defined, gene flow is reduced and this could be due to two factors

- (a) There are a greater number of flowers in Pasture than in Molinietum and honey bees tend to be restricted by supply.
- (b) if, as is not known, *P. erecta* is pollinated also by insects other than honey bees, these vectors may be ecologically limited.

4. That the selective effect of the different environments is strong whatever the distribution of the habitats, is shown by the differentiation in the adult (original) generation.

5. In the first year of growth (1964) of the seedling generation variabilities were greater in the seedling than in the adult generation in the same year.

6. The disappearance of differences between variabilities in adult and seedling generations in subsequent years could be due to the absence of

selective habitat effects in the neutral environment of the trial. Variability in the first year of the seedling generation could be the expression of cryptic genetic variability which normally contributes to the success of this widespread species in colonising new habitats.

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