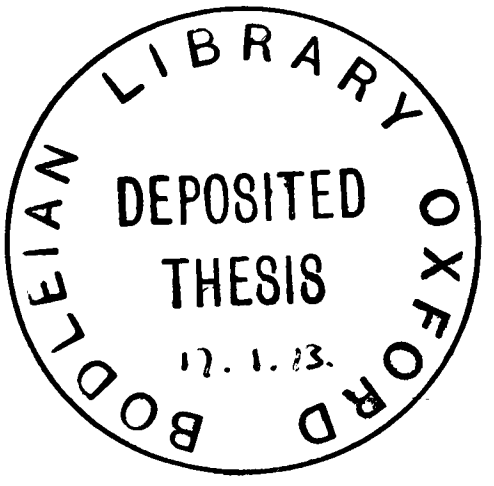
THE ECOLOGICAL GENETICS OF FLOWER COLOUR VARIATION IN CIRSIUM PALUSTRE

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ABSTRACT

The thistle Cirsium palustre exists over most of lowland Britain as a predominantly purple flowered species. However the populations of seacliffs and mountains exhibit a flower colour polymorphism, occurring as homozygous white morphs, homozygous and heterozygous intermediate morphs, and homozygous and heterozygous purple morphs.

The degree of polymorphism of the seacliff populations of the Gower Peninsula is correlated with population size, the larger populations being less polymorphic. This might be interpreted as indicating chance fixation of the white alleles. Such an explanation would gain support from the very small size of the more polymorphic populations and from the drastic fluctuations in population size which have been demonstrated as having occurred in these populations over a period of four consecutive seasons. However other explanations based on selective effects are possible. On seacliffs the frequency of the white morphs is inversely related to exposure, and decreases in population size are accompanied by differential survival of the purple morphs.

On mountains the distribution of the polymorphism is markedly correlated with altitude. For the mountains of southern Mid Wales, populations below 1000 ft. are strongly monomorphic

purple. Above 1000 ft. the degree of polymorphism increases abruptly, with white frequencies reaching over 80%. Morph frequencies among North Wales populations bear a similar relation to altitude but the increase in polymorphism occurs at about 1200 ft. In each case the general trend is that purple frequency declines with increase in white frequency, and that the frequency of intermediates shows a unimodal distribution with a quite precise peak. For both sets of populations this peak occurs at an altitude about 250 ft. higher than that at which the increase in white frequency occurs.

It is possible that the occurrence of the polymorphism on seacliffs and mountains may be related to a limitation of cross pollination consequent upon the exposure of seacliffs and the combined climatic characteristics of mountains, which include increase in exposure, mist and rain and decrease in temperature. Evidence on the levels of outbreeding in these populations was inconclusive but evidence in other species suggested that pollination might indeed be limited in these conditions.

An increase in homozygosity consequent upon inbreeding would promote the frequency of the white morphs. Moreover the white morphs were subject to preferential pollination and both this and certain forms of heterogeneity in morph distribution were likely to promote the frequency of inbreeding among white morphs. However in conditions of limited pollination the degree of

general outbreeding of the white morphs will be increased by preferential pollination and this may be assumed to be a fitness advantage which may be of particular importance in the maintenance of the polymorphism. In addition it is likely that the presence of white morphs within a population may result in the attraction of higher numbers of pollinators or encourage foraging for longer periods in which case the polymorphism may be said to be adaptive in the sense of Fisher (1930).

Other selective effects are also apparent. The occasional presence of highly polymorphic populations in valley bases and the regular occurrence of predominantly purple populations in mountain forests may both to some extent provide evidence for an effect of temperature other than upon pollination. Some evidence suggests that both exposure and moisture may also be of individual importance. Selection was apparent even by the arrangement of morph types within a population subject to no obvious environmental heterogeneity. It is possible that the polymorphism is maintained by a physiological heterozygous advantage and that this may be responsible for the maintenance of white and intermediate morphs in low frequency in the predominantly purple populations of inland lowland regions.

The maintenance of the polymorphism imposed a significant selective mortality upon the species. This was indicated by the above instance of selection within a uniform community and also in several instances in which intrapopulational selection occurred

between segments of population subject to differing exposure. However the growth in cultivation of seeds set in natural populations revealed that the complexity of the genetic system was sufficient to allow widely different morph frequencies to be maintained in different populations without the necessity of high selection in each generation.

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INTRODUCTION

1. The Polymorphism

2. The General Ecology of Flower Colour Variation

1. The Polymorphism

Genetic polymorphism has been defined by Ford (1940) as

> the occurrence together in the same locality of two or more discontinuous forms of a species in such proportion that the rarest of them cannot be maintained merely by recurrent mutation.

Work on the electrophoretic variation of enzyme and protein loci in *Drosophila* has indicated that thirty to fifty per cent of loci may be polymorphic (Harris, 1966; Johnson et al., 1966; Lewontin & Hubby, 1966). Since there is no reason to believe *Drosophila* to be atypical of organisms in general it would appear that polymorphism is a normal result of evolutionary processes; however despite this comparatively few studies have been conducted on the selective factors governing the maintenance of polymorphisms in natural populations. This is especially so among plants and accordingly it was considered of value to study the ecological genetics of the flower colour polymorphism exhibited by the Marsh Thistle, *Cirsium palustre* (L.) Scop. The species is typically purple flowered but in certain populations white and intermediate flowered types occur in high frequency.

Cirsium palustre is a fairly abundant species which is present in all the vice-counties of the British Isles (Druce, 1932). It occurs throughout a wide range of habitats on neutral or slightly acid soil. Grose (1957) records that in Wiltshire the habitats occupied comprise lowland meadows (48% of occurrences), woodland (15%), marshes (11%), downs and scrub (10%), bogs and heaths (8%), and the banks of streams and lakes (5%). It is common also on seacliffs. In South Wales its range extends from sea level along the coast to 785 m. on Pen y Fan, Brecon.

The species is biennial or perennial. In the first year of growth a flat rosette is produced from which an upright flowering stem emerges in the second year. The growth form of the species varies greatly between habitats; on the Gower Peninsula flowering stems vary in height from as little as 10 cm. on exposed seacliffs to 2 m. in shaded woodland clearings.

For the study of ecological genetics the species possesses several advantages. The discontinuity between the colour types is sufficient to allow their relative frequency to be scored with comparative ease. Since the species occupies a wide range of habitats the influence of a wide range of ecological conditions on the maintenance of the polymorphism may be determined. In addition, the species invariably appears sufficiently distinct for the recognition of populations from a distance. In many habitats, particularly those of seacliffs, the species forms very discrete populations thereby enabling population size to be estimated

Plates 1 & 2. Seacliff Populations of C. palustre.

Plate 1. A region of a very exposed population at Rhossili, Gower (SS 414875; popn. 11).

Plate 2. A relatively less exposed population at the rear of Oxwich Head, Gower (SS 508853; popn. 35).





Plates 3 & 4. Inland Populations of C. palustre.

Plate 3. A hillside population at Wentwood, Monmouthshire (ST 432932; popn. 82).

Plate 4. A woodland population at Pont Caniedydd, Brecon (SO 040245; popn. 106).





with accuracy and hence the influence of fluctuations in population size on the maintenance of the polymorphism to be studied. The disadvantages of the species are principally concerned with breeding (Ch. 1).

In the present work the previous literature on flower colour variation is reviewed. The distribution of the polymorphism is described and considered in relation to the influences of climatic conditions and population size. The intensity of selection at the intrapopulational level is then considered and the work is concluded with an account of the selective effects likely to result from the conditions in which the polymorphic populations occur.

2. The General Ecology of Flower Colour Variation

Intraspecific variation in flower colour is a phenomenon constantly encountered. In many cases this may be related to environmental conditions. Thus Weatherby (1925) has recorded colour changes in *Hepatica americana* following transplantation from woodland into garden conditions, while Anderson (1933) has recorded similar changes in *Hepatica acutiloba*. Eames has recorded inconstancy in the flower colour of *Viola pedata f. rosea* and *Rudbeckia hirta* (Weatherby, 1925). Klebs (1906) observed that white flowered plants of *Campanula Trachelium* became blue flowered when grown in cold conditions.

However in the majority of cases flower colour variation may be presumed to have a genetic component. In certain instances

such variation may be a consequence of hybridisation. Thus the presence of light blue and pink flowered variants in certain populations of the typically white flowered Oxytropis albiflora is indicative of hybridisation with the purple flowered Oxytropis lambertii (Anderson, 1953). Beeks (1962) has recorded a similar instance among hybridising populations of the Diplacus species complex in California. Hybrids occur between the red flowered D. puniceus and the orange flowered D. longiflorus, and between the latter species and the yellow flowered D. calycinus. In each case the hybrids display all grades of intermediacy in flower colour.

Flower colour variation may be characteristic of particular groups of species. Thus Lewis (1953) remarked that among Californian species of the genus Clarkia white flowered variants are encountered in C. biloba, C. cylindrica, C. unguiculata and C. xantiana, while the species C. epilobioides is characterised by white flowers. The genus Cirsium appears similarly variable; during the present work a survey of the literature attached to the European species revealed that of 22 species which were typically purple flowered, 11 were noted as producing occasional white forms. One section of the genus (section Ochroleuca) is composed of species which typically produce yellowish white flowers and it was of interest that two species of this section - C. oleraceum and C. eristhales - were noted as occasionally producing reddish flowers (Gremli, 1886; Clapham et al., 1962; Huxley, 1967; Polunin,

1969).

In a high proportion of cases in which rare flower colour variants occur it is likely that these variants are maintained solely by recurrent mutation and hence do not constitute polymorphisms by the definition of Ford (1940). Such mutants would be expected to be at a selective disadvantage and hence in most cases to disappear from populations containing them. Thus Lewis (1953) recorded that two white flowered plants of Clarkia cylindrica present in a population of the typical pink flowered type failed to set seed whereas the surrounding pink plants set seed freely. Consequently the type was eliminated from the population.

However as remarked by Ford (1964) it is constantly found that a rare mutant may become a polymorphic form in one or a few localities of a widespread species. The flower colour polymorphism in Cirsium palustre may be regarded as one such case if the white flowered plants present at low frequency in typical purple populations are assumed to be derived by mutation. In exceptional instances it is possible that chance fixation may result in high frequencies of the mutant type; thus Lewis (1953) observed a completely white population of the typically pink flowered Clarkia xantiana which seemed most likely to be derived from such cause.

Recognition of whether selective forces are involved in the maintenance of a polymorphism may prove difficult. Epling and Dobzhansky (1942) concluded from a study of the blue and white

flower colour polymorphism of Linanthus Parryae that no environmental influence on the frequency of the colour types was apparent. The distribution of the polymorphism among populations resembled that expected from the data of Wright (1931, 1937, 1940) on the distribution of gene frequencies to be expected from random fixation in effectively small populations. However Wright (1943) pointed out that the variation in gene frequency was somewhat larger than that expected from random fixation alone and that therefore selective processes may have been involved. Epling et al. (1960) concluded that genetic drift was likely to be of only minor importance in the maintenance of the polymorphism since the morph frequencies remained relatively constant over successive years and the capacity for seed dormancy appeared sufficient to reduce considerably the long term effects of annual fluctuations in morph frequency.

Relatively few studies have pursued in detail the ecology of flower colour variation. Clinal variation in flower colour frequency has been described in Linanthus Parryae (Epling et al., 1960) and also in Escholzia californica (Cook, 1962), Justicia simplex (Jain & Joshi, 1962; Joshi & Jain, 1964) and Lupinus succulentus (Harding & Mankinen, 1971). In the latter species it was established that the degree of polymorphism was promoted by habitat disturbance. Lewis (1962) recorded that a white flowered race of the Californian Clarkia xantiana appeared more successful than a race of the typical pink flowered form in conditions associated with a reduction in

rainfall. Weijer (1952) has recorded that in the Netherlands a white variety of the orchid Epipactis helleborine is relatively more common in sites exposed to seawinds than the typical red-violet veined type and an intermediate variety. Woodell, Mooney and Lewis (pers. comm.) have shown that in Linanthus androsaceus the purple flowered populations are associated with serpentine soils whereas white forms are found on soils derived from non-serpentine rocks.

Flower colour polymorphisms in Lupinus nanus and Phlox pilosa have been subject to more detailed study. Harding (1970) established that among populations of Lupinus nanus pink flowered morphs were subject to a higher mortality and a reduced seed set relative to the typical blue flowered morphs. He concluded that the polymorphism was maintained by a heterozygous advantage. In contrast Levin and Kerster (1970) demonstrated that the white flowered morphs which occur in high frequency in some populations of the typically pink flowered Phlox pilosa produced in most populations relatively higher seed yields than the pink morphs. However the mean percentage seed set per population was greater in populations containing higher proportions of the pink morphs, which was interpreted as indicating that the latter populations were relatively more attractive to pollinators. It was in addition found that the frequency of the white morphs was higher in the immediate presence of the pink flowered Phlox glaberrima (Levin & Kerster, 1967). This was demonstrated as promoting ethological barriers to pollen

exchange between the species. Change in the frequency of colour types is a mechanism likely to be of widespread importance in the reduction of hybridisation between the Phlox species of eastern North America (Levin & Kerster, 1967; Levin, 1969).

Preferential pollination of different colour types by visiting insects has been described in Lantana camera (Dronamraju, 1960) and Leavenworthia crassa (Lloyd, 1969). In the latter species the degree of polymorphism was higher in populations more adapted to cross pollination but the degree of preferential pollination was considered insufficient to be of importance in the maintenance of the polymorphism.

Preferential pollination may nevertheless be of widespread importance in the maintenance of flower colour polymorphisms. Hocking (1968) has commented that in a region of the Canadian Arctic in which pollinating insects occur in only limited number there occur white forms of Saxifraga, Erysimum, and Pedicularis species and that the majority of abundant plant species important to insects have yellow, cream or white flowers.

Anderson (1936) has remarked that in the Ozark woodlands certain species are more brightly coloured than in the more dense New England forests; thus Viola pedata occurs as bright bicoloured types in the Ozark woodlands but as only a self-coloured variety in the New England forests. Similarly the proportion of white flowered plants of Hepatica acutiloba and Dicentra cucullaria is relatively higher in the populations of the New England forests.

Anderson suggested that these effects may be due to the lighter flowers being more attractive to pollinators in dull light. This explanation gains support from the observation of Hulkkonen (1928) that at low light intensity bees visit white flowers in preference to coloured ones. It is relevant also that as remarked by Baker (1961) moth pollinated flowers, which are mainly night pollinated, are believed to be generally white or cream in colour.

Differences in flower colour have been frequently associated with altitude. Weevers (1952) has recorded from a study of available floras that whereas the proportion of different coloured species is relatively constant in most countries, in both Switzerland and Java the proportion of blue flowered species is higher among the plants of mountain regions than among the plants of these countries taken as a whole. Weevers concluded that this was probably due to selection but noted the suggestion of Bonnier (1896) that the stronger ultraviolet radiation at high altitudes might intensify the formation of blue pigment.

In the American composite Encelia farinosa the proportion of plants with brownish yellow florets relative to the proportion with yellow florets is greater at higher altitude (Khyos, 1971). Nijland has demonstrated that in Java an Ageratum species is predominantly white flowered in lowland regions but purple flowered on mountains (Weevers, 1952). In North America white flowered plants of Cypripedium acaule replace pink flowered plants on mountains (Anderson, 1936), an effect which Baldwin (1970) has

suggested may be induced by low temperatures.

During the present work it was noted that many of the species of Cirsium which produce occasional white forms are partly alpine in distribution. De Wit (1967) implies that white flowered Cirsium species are characteristic of alpine regions. No further information was available on this although it was notable that the extremely common alpine species Cirsium spinosissimum appeared exclusively white flowered. Viewed against this background the demonstration in the present work of an increase in the proportion of white flowered Cirsium palustre with increase in altitude is of especial interest. The relationship between the ecological genetics of this polymorphism and that of similar polymorphisms in other species of Cirsium is the subject of continuing research.

Chapter One

THE NATURE OF THE POLYMORPHISM

1. Expression
2. Taxonomy
3. Cytology
4. Genetics

1.1 Expression

The flower colour variation was regarded as comprising four phenotypic categories, which were defined as follows:

a. Purple — Stigma and corolla lobes dark reddish purple. Androecium dark bluish purple. Style and corolla "throat" normally tinged light reddish purple. Corolla lobe pigment especially intense at tips.

b. Intermediate — Stigma and corolla lobes pink-purple. Androecium light bluish purple. Style normally white, corolla "throat" normally only slightly tinged pink-purple. Corolla lobe pigmentation normally very uniform.

c. Purplish White — Stigma and corolla lobes only very lightly tinged pink-purple. Androecium with a very light bluish purple region beneath the tip. Style white, corolla "throat" normally white.

Corolla lobe pigment frequently restricted to immediately beneath tip.

d. White — No trace of colour anywhere within the floret. Stigma and androecium creamish white, remainder of floret white.

The colour types are illustrated in plates 5 and 6. It was apparent that the differences between the colour types were to a great extent differences in the amount of pigmentation, rather than in the distribution of pigment within the floret. The amount of floral pigmentation was to a considerable extent correlated with the amount of vegetative pigmentation of the stem and leaves but this association was by no means absolute and vegetative pigmentation was not considered in classification of the plants.

The degree of association between floral and vegetative pigmentation was assessed during the breeding programme by scoring each of the progeny plants for both colour and vegetative pigmentation. The latter was assessed subjectively using an arbitrary 0-10 scale, '0' indicating "no purple pigmentation" and '10' indicating "extreme purple pigmentation". The results are shown in figure 1.

Fig.1 Relationship between Colour Type and Vegetative Pigmentation for Plants raised during Breeding Programme

Colour Type	Purple	Intermediate	Purplish White	White
No. Samples	292	284	65	347
Vegetative Pigmentation Grading	6.14±0.29 ('p'=0.05)	4.67±0.23 ('p'=0.05)	6.17±0.67 ('p'=0.05)	2.91±0.30 ('p'=0.05)

Plates 5 & 6. Flower Colour Variation in C. palustre.

Inflorescences approximately twice natural size.

Plate 5.

Purple

Intermediate

Plate 6.

White

Purplish White





The significance of the observed differences in pigmentation were assessed using t-tests. The difference in the degree of pigmentation of the purple and the purplish white types was insignificant ('p' $\gtrsim$ 0.1). However the degree of pigmentation of the purple type was significantly greater than that of the intermediate type, while the degree of pigmentation of the latter type was significantly greater than that of the white type ('p' $<$ 0.001 in each case).

Population scorings were performed only upon fully opened fresh florets since neither the colour "in bud" nor the colour of fading florets provided a completely accurate indication of colour type. Similarly populations were not scored at low light intensities at which the colour differences appeared less distinct. However provided these precautions were observed the great majority of the plants encountered could be classified clearly. This was indicated also by the breeding programme since consistent results were obtained even though all the plants were classified without prior knowledge of parental colour type. The discontinuity between colour types was therefore considered sufficient for the types to be regarded as "morphs" in the sense of Ford (1940).

In fact however only the white category was phenotypically and genetically uniform. Each of the other categories displayed variation and occasional plants were encountered whose classification was uncertain. Certain members of the purplish white category were completely white except for slight purple pigmentation on the upper regions of the androecia and a hand lens was necessary

to confirm classification of these plants.

Several populations existed in which classification of the purple colour type was not clear, either because the "purple" flowers possessed a very low intensity of purple pigmentation, or because pigmentation was restricted to a high intensity dark purple colouration of the tips of the corolla lobes and the regions immediately beneath the tops of the androecia. Plants of the latter type were very uncommon, although one population was found (popn.191) in which these plants attained a high frequency. Several of these plants resembled the "purplish white" phenotype, a resemblance of particular interest in view of the apparent similarity between these types in the degree of vegetative pigmentation.

In conditions of cultivation no environmental influence on colour production became apparent. Even plants induced to flowering under artificial lighting in a greenhouse produced flowers of the completely normal colour types. As described later (Ch. 4) for each of two populations of very dissimilar morph frequency a close correspondence was obtained between the morph frequencies of the populations and the morph frequencies of plants grown in cultivation from seeds set in natural conditions in the populations. This suggested that even in natural conditions the degree of environmental modification of flower colour may be minimal.

1.2 Taxonomy

White flowered individuals of *Cirsium palustre* have been

recorded variously as varieties albiflora (De Wildeman & Durand, 1899), alba (Druce, 1930; Horwood & Noel, 1933) and ferox Dr. (Druce, 1911).

Druce described the variety ferox Dr. as possessing white or pale red flowers together with lighter green leaves, stronger and more numerous spines and a more condensed inflorescence than the type (plate 7). It is likely however that he did not regard flower colour as a definitive criterion of the variety since later (Druce, 1930) he recorded the species as possessing both varieties ferox Dr. and alba. Similarly Druce (1911) regarded the variety ferox Dr. as being likely to be equivalent to the Spanish variety spinosissimum Willkom. whereas flower colour is not listed as a character of that variety (Willkomm & Lange, 1870). During the present work examination of plants growing in a wide range of habitats has indicated that in seacliff and mountain habitats where the white morphs are most frequent the plants are indeed more compact and spinous than in other habitats, but no evidence is available that within a habitat the colour types differ in any respect other than floral and vegetative pigmentation.

1.3 Cytology

Examination of root tip preparations from several plants of each colour type taken from a population on the Gower Peninsula (popn. 57) revealed a constant chromosome number 2n = 34. Chromosome counts made by other workers on plants from the Continent have



Plate 7. Type specimens of variety <u>ferox</u> Dr. (deposited in the Fielding-Druce herbarium, Oxford).

also revealed only this value (Czapik, 1958; Gadella & Kliphuis, 1963, 1966; Lövkvist, 1962; Moore & Frankton, 1962; Poddubnaja, 1931; Rohweder, 1937; Tischler, 1934).

1.4 Genetics

Breeding experiments were performed on plants from two populations on the Gower Peninsula, the Clyne Common and Penmaen Marsh populations. The Clyne Common population (popn. 57) contained plants of all four colour types in high frequency. The Penmaen Marsh population (popn. 77) contained purple and white morphs in high frequency but an exceptionally low frequency of intermediate morphs. In addition as described later (Ch. 4) plants were grown in cultivation from seeds set in natural conditions in the Penmaen Marsh population and in a population in the Bishopston Valley. The latter population was also on the Gower Peninsula and was composed almost entirely of intermediate morphs.

The morph frequencies of each of these populations over four consecutive years of scoring are shown in figure 2. It may be seen that the morph frequencies remained fairly constant throughout this period. The only exception was the change in morph frequency at Clyne Common between 1968 and 1969 which was associated with destruction of part of the habitat by roadworks during the intervening period.

Plants from the Clyne Common and Penmaen Marsh populations were transplanted prior to flowering into greenhouse conditions

Fig. 2 Morph Frequencies of Populations used in the Breeding Programme

	Numbers Scored				Morph Percentages			
	P.	Int.	ppW.	Wh.	P.	Int.	ppW.	Wh.
Clyne Common								
1968	35	23	16	21	36.8	24.2	16.8	22.1
1969	106	16	3	12	77.4	11.7	2.2	8.7
1970	55	5	1	17	70.5	6.4	1.3	21.8
1971	59	8	0	18	69.4	9.4	0.0	21.2
Penmaen Marsh								
1968	- unscored -				- unscored -			
1969	126	4	2	103	53.6	1.7	0.8	43.8
1970	252	5	19	195	53.5	1.1	4.0	41.4
1971	88	1	1	65	56.8	0.6	0.6	41.9
Bishopston Valley								
1968	4	133	6	2	2.7	91.7	4.1	1.4
1969	2	46	2	0	4.0	92.0	4.0	0.0
1970	6	130	0	2	4.3	94.2	0.0	1.4
1971	13	89	3	6	11.7	80.2	2.7	5.4

during July 1968 and July 1969. Plants of the purple, intermediate and white types from Clyne Common were allowed to self pollinate, while cross pollinations were performed between purple and white plants from both habitats. Cross pollinations were effected by brushing together pollen laden inflorescences over consecutive days, transparent cellophane bags being used to protect the inflorescences from visiting insects. Due to the floral mechanism emasculation of the inflorescences was not possible, and since self pollinated inflorescences set seed freely (Ch. 5) it was likely that a proportion of the seed set in cross pollinated inflorescences was the result of self pollination. However comparison of the results obtained from self and cross pollinated white inflorescences suggested that this proportion was probably fairly small.

Most of the pollinations were performed during July and August 1969. The seeds were sown the following autumn. The seeds were germinated on moist filter paper and the seedlings grown in a greenhouse and finally in experimental beds. Most of the plants flowered out of doors during the following two seasons. A small proportion was induced to flower by extended lighting in a greenhouse during January 1971.

In conditions of cultivation mortality was very low and accordingly selection was considered negligible. However due to the length of the life cycle only one generation was possible, and due to the size of the plants and the consequent amount of garden space occupied investigations were limited to the crosses mentioned

above. Accordingly the results so far obtained are confined to the broad features of the genetic system.

The results are listed in Appendix Two. Self pollination of each of the four white plants from Clyne Common yielded exclusively white progeny thereby indicating that these plants were probably homozygous. Self pollination of purple plants from that habitat indicated that one parent (no.38) appeared possibly homozygous while two (nos. 7 and 10) represented heterozygous states and were perhaps themselves different ('p' = 0.06 using Fisher's exact test).

The two intermediate plants from Clyne Common (nos. 11 and 17) whose selfed progeny were raised in large numbers were both heterozygous but yielded significantly different progeny ratios ('p' < 0.001). The progeny of both plants were purple, intermediate and white but the lighter parent (no.17) yielded a significantly lower proportion of purples ('p' = 0.05). Intermediate plants from the Bishopston Valley under natural pollination yielded predominantly intermediate progeny. Since the progeny were presumably derived predominantly from intercrossing among intermediate plants it would appear probable that in this population the intermediate plants were mainly homozygous.

Intercrossings between purple and white plants from Clyne Common yielded purple and white progeny except in one instance where additionally purplish white progeny were present. Intercrossings between purple and white plants from Penmaen Marsh yielded

only purple and white progeny, a situation represented similarly by the progeny derived from natural pollination of these plants and to be expected in view of the low frequency of intermediate and purplish white morphs in this population.

It has therefore emerged that the white phenotype represents a homozygous condition, that the purple phenotype represents at least one heterozygous condition and possibly a homozygous condition, and that the intermediate phenotype represents at least one homozygous and two heterozygous conditions. It is clear that the polymorphism involves at least two gene pairs but the number of crosses so far available is insufficient for more precise analysis.

Chapter Two

THE DISTRIBUTION OF THE POLYMORPHISM AND ITS RELATIONSHIP WITH CLIMATIC FACTORS

1. The Distribution Pattern
2. The Relationship with Climatic Factors

2.1 The Distribution Pattern

White flowered forms of Cirsium palustre have been recorded from Britain, Belgium (De Wildeman & Durand, 1899), France (Rouy, 1905), Italy (Fiori, 1969), Portugal (Sampaio, 1946) and Switzerland (Schinz & Keller, 1909). In Britain the white forms have an extremely widespread distribution as may be seen from figure 3 which is a distribution map compiled from local floras and herbarium specimens from the Fielding-Druce herbarium at Oxford. In both the British and the Continental floras examined the white forms were invariably stated as being infrequent except for Salter (1928) who recorded that in Mid Wales white forms were very frequent at the upper altitude range of the species.

During the present work observations were conducted throughout a wide area of Wales and England and were concentrated in particular on the inland populations of North Wales and southern Mid Wales and the coastal populations of the Gower Peninsula and Pembrokeshire. Populations were scored using the four colour categories defined previously. In almost all cases at least one hundred plants per population were scored. In addition the position, altitude, size

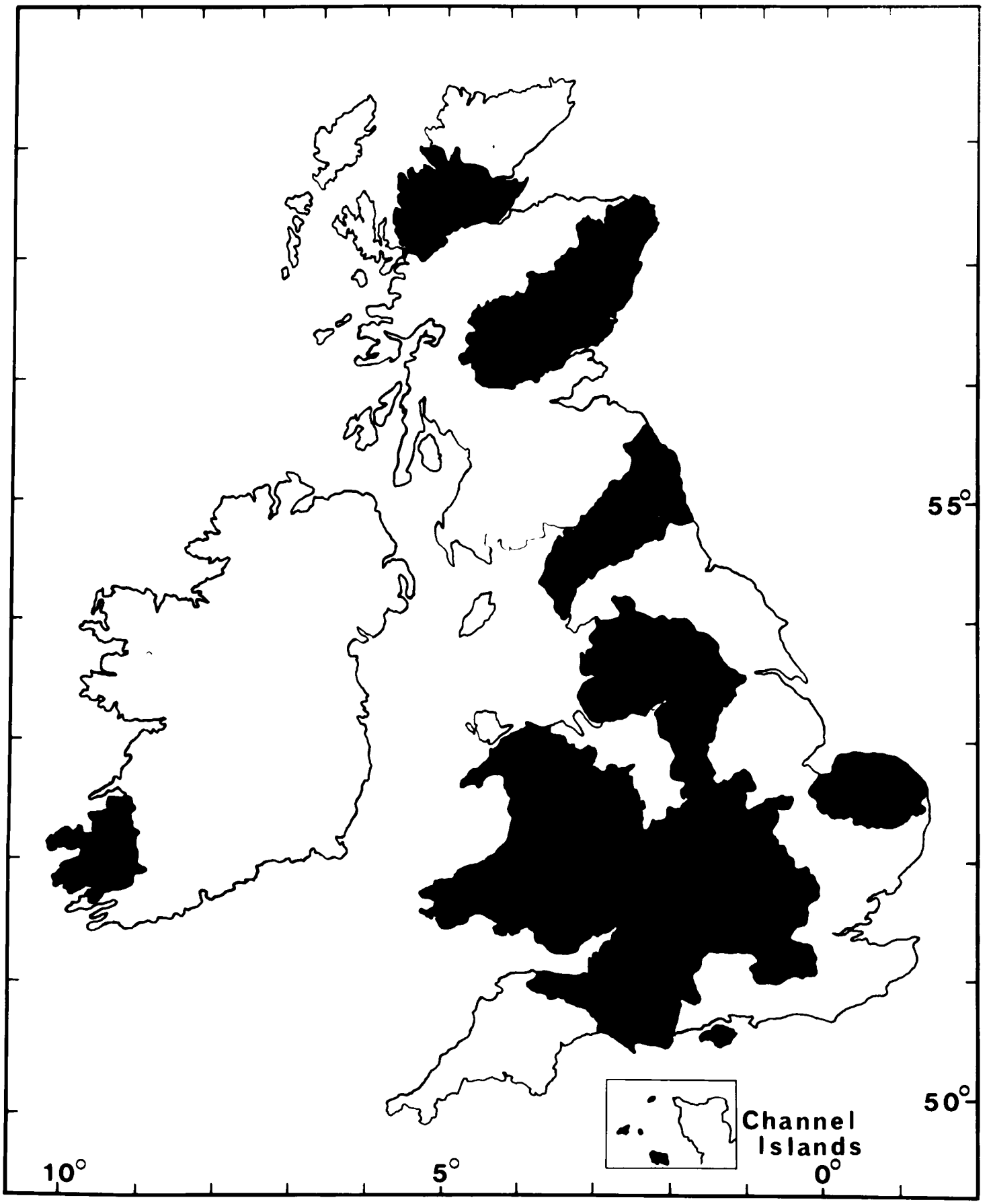


Fig. 3. County records of white flowered specimens in the British Isles, compiled from personal records, local floras and herbarium specimens. Black areas represent counties in which white flowered specimens have been recorded. Sources listed in Appendix 3.

and discreteness of each population were noted, and estimates of exposure and moisture made using a subjective 1-3 scale in each case. Populations varied greatly in discreteness; seacliff populations were generally very clearly defined but in particular the populations of mountain roadsides and lowland fields were often very diffuse. The morph frequencies of the populations of the Gower Peninsula are listed in Appendix Three and those of the remaining populations in Appendix Four.

Maps were prepared of the distribution of the polymorphism in Pembrokeshire (fig. 4), Gower (figs. 5 and 6), southern Mid Wales (figs. 7 and 8) and North Wales (figs.9 and 10). The relationships between height, exposure, moisture and white frequency were analysed by computer using multiple linear regression, this being performed separately for the populations of Mid Wales and North Wales and in each case being restricted to populations above 1000 ft.

The distribution pattern of the polymorphism may be summarised as follows. In the great majority of inland sites below 1000 ft. the species is uniformly almost completely purple flowered. Only 3 of the 90 such populations examined contained high proportions of the alternative colour types. Seacliff populations and inland populations above 1000 ft. were both highly polymorphic. The seacliff populations along the southern border of the Gower Peninsula showed some evidence of a cline in white frequency, those populations on the west of the peninsula containing lower

Figs. 4-10 Distribution of the Polymorphism

Explanation

Individual populations are represented by circles, related in size to population size and divided into sectors proportional in area to the frequency of the different morph types.

Population Size	Circle Size
150 or less	small
151-500 inclusive	medium
more than 500	large

Morph Type	Sector
white	white
purplish white	lightly stippled
intermediate	heavily stippled
purple	black

Dotted lines represent 1000' contour.

Forest populations denoted by 'f'.

Actual morph frequencies and years of scoring listed in appendices 3 and 4. Morph frequencies of the Gower populations calculated from the most recent of the scorings in which the number of plants scored exceeded 50.

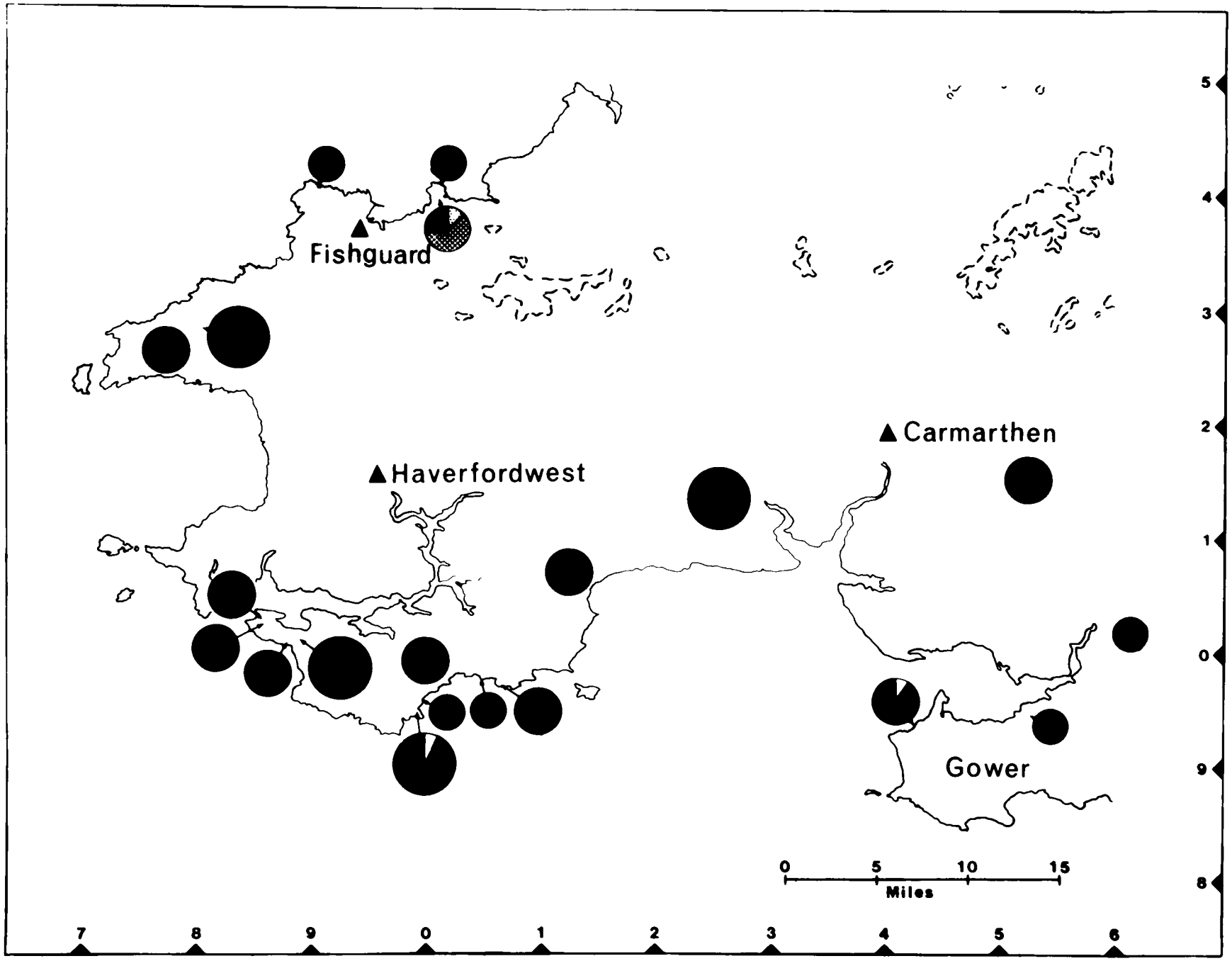


Fig. 4. Distribution of the polymorphism in Pembrokeshire.

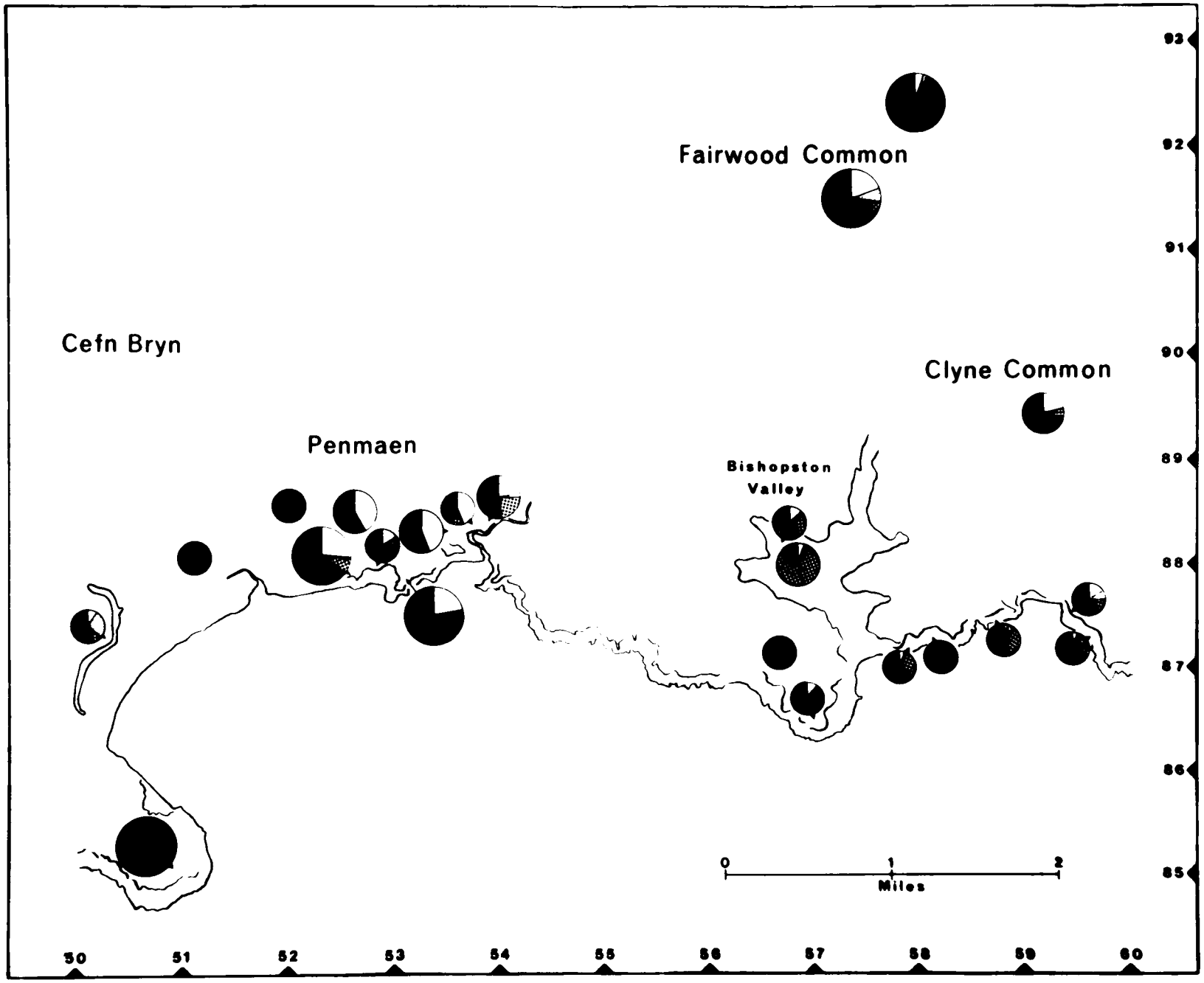


Fig. 5. Distribution of the polymorphism on the Gower Peninsula (eastern region of southern border).

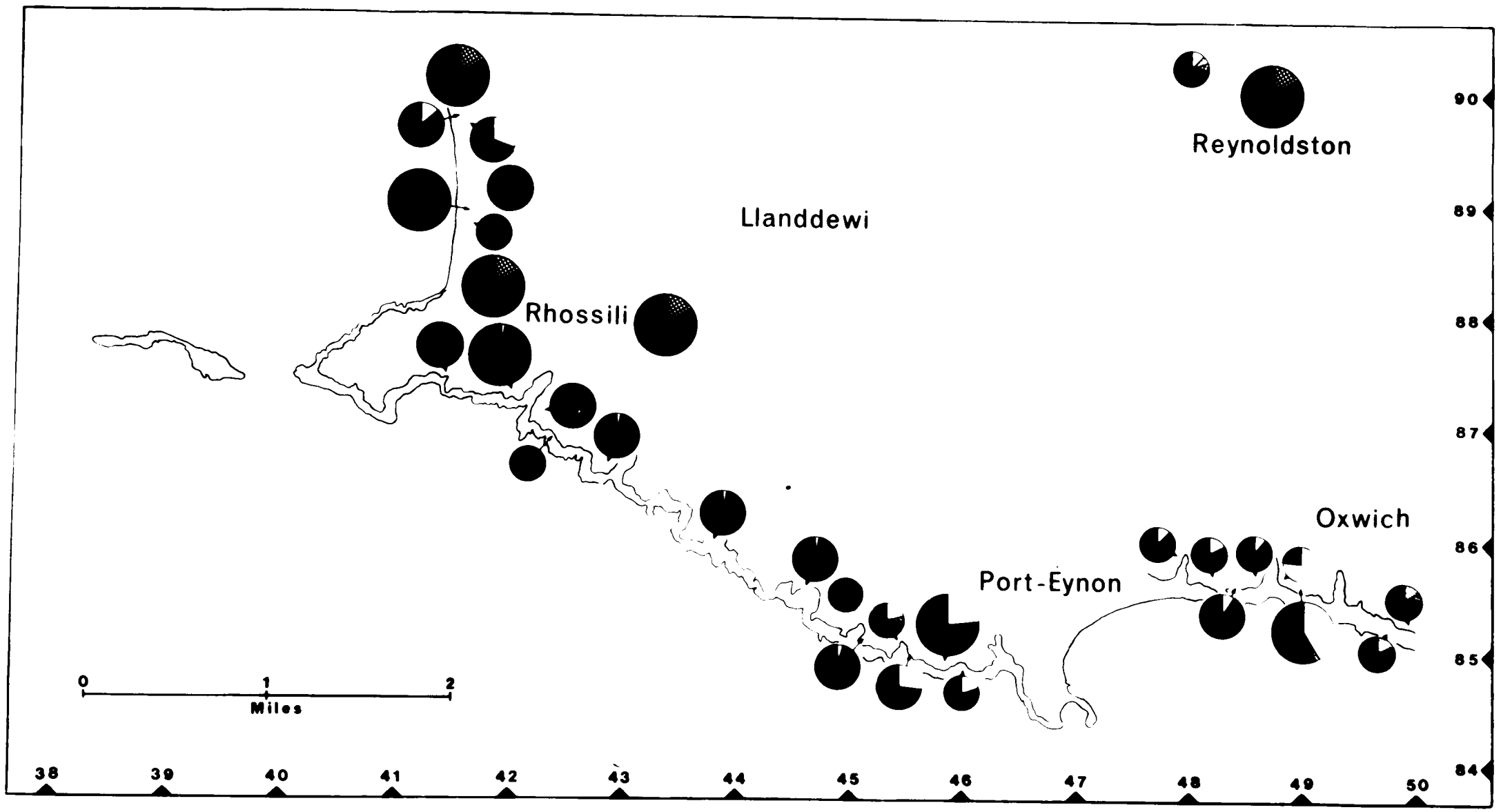


Fig. 6. Distribution of the polymorphism on the Gower Peninsula (western region of southern border).

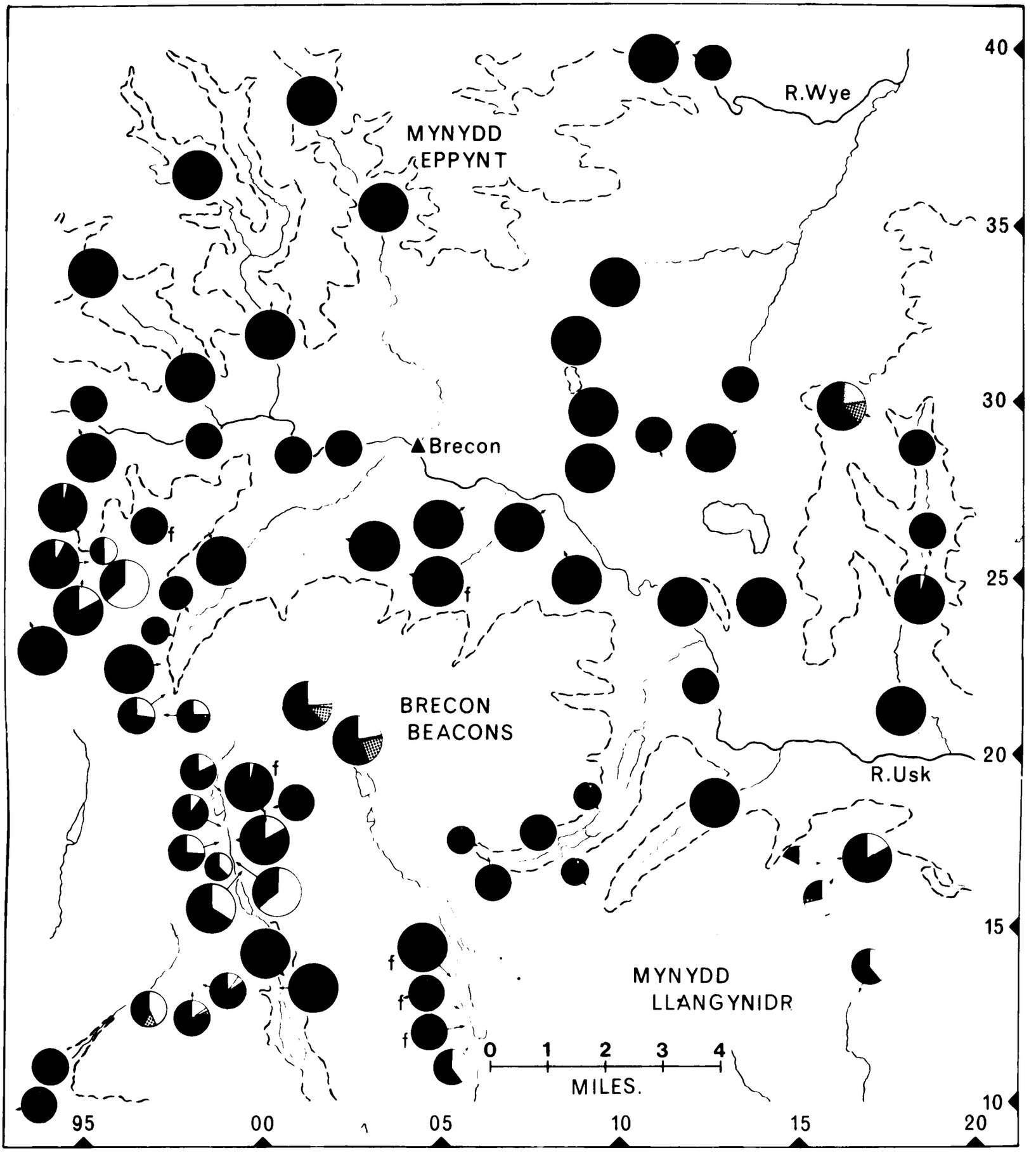


Fig. 7. Distribution of the polymorphism in southern Mid Wales (eastern region).

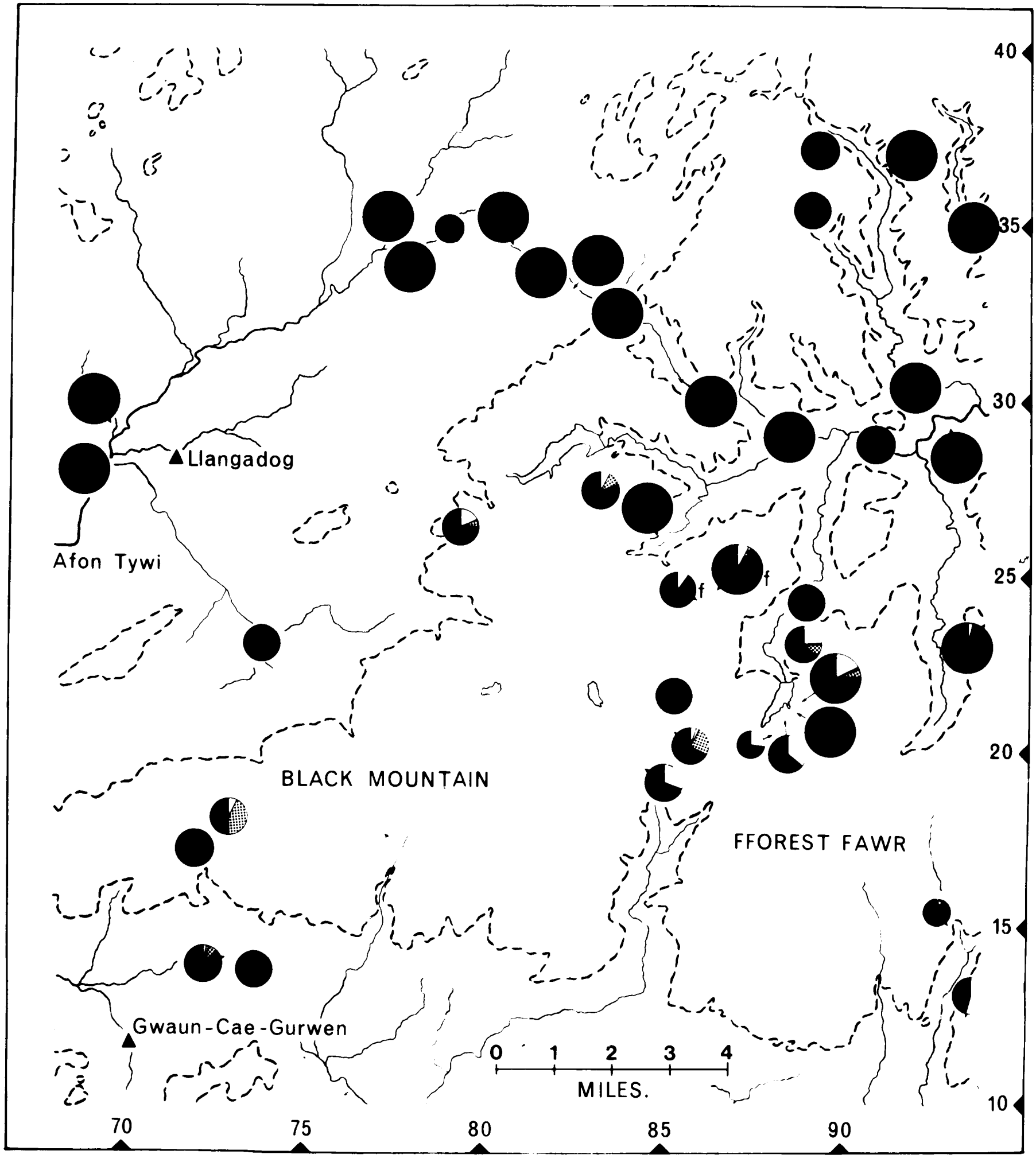


Fig. 8. Distribution of the polymorphism in southern Mid Wales (western region).

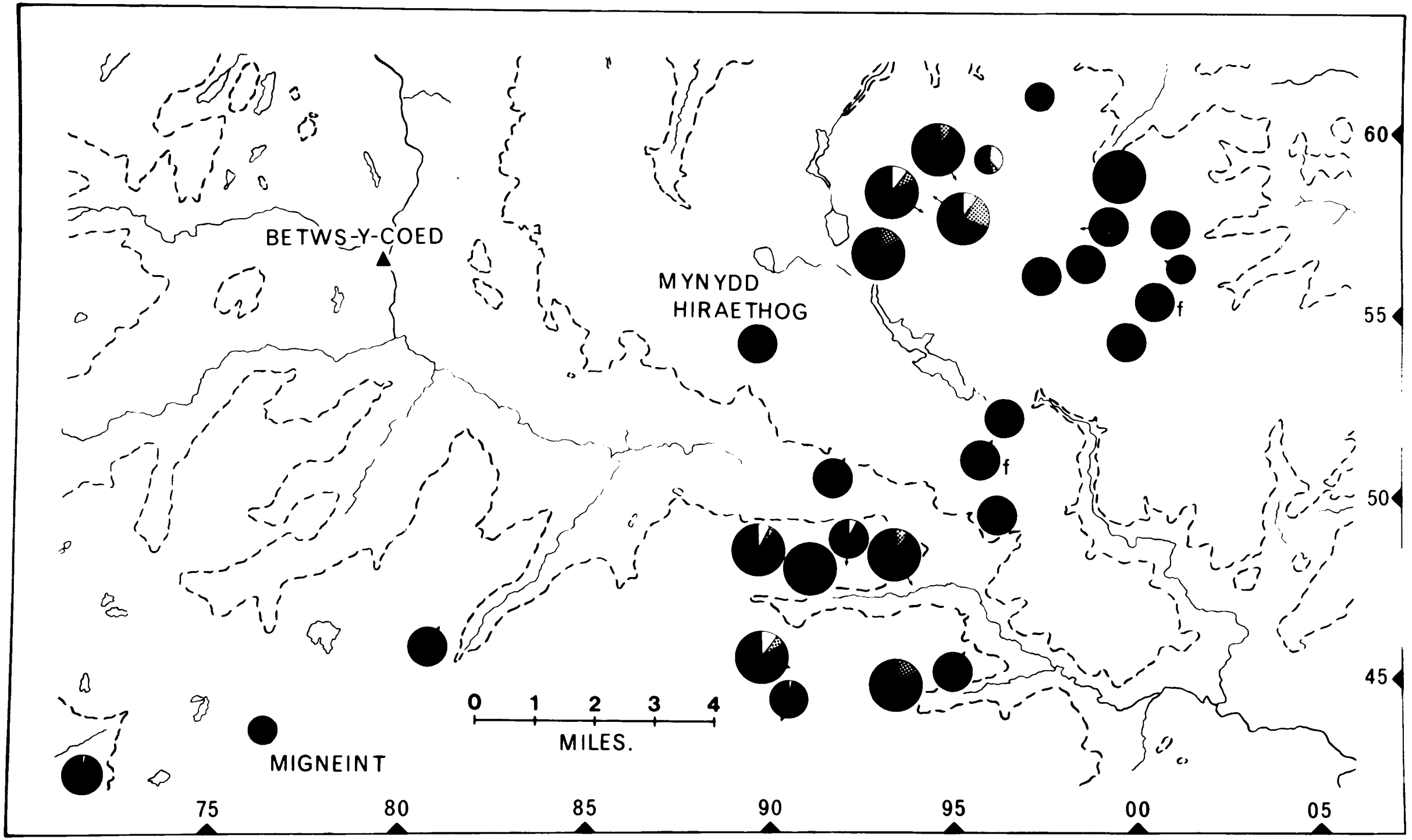


Fig. 9. Distribution of the polymorphism in North Wales (northern region).

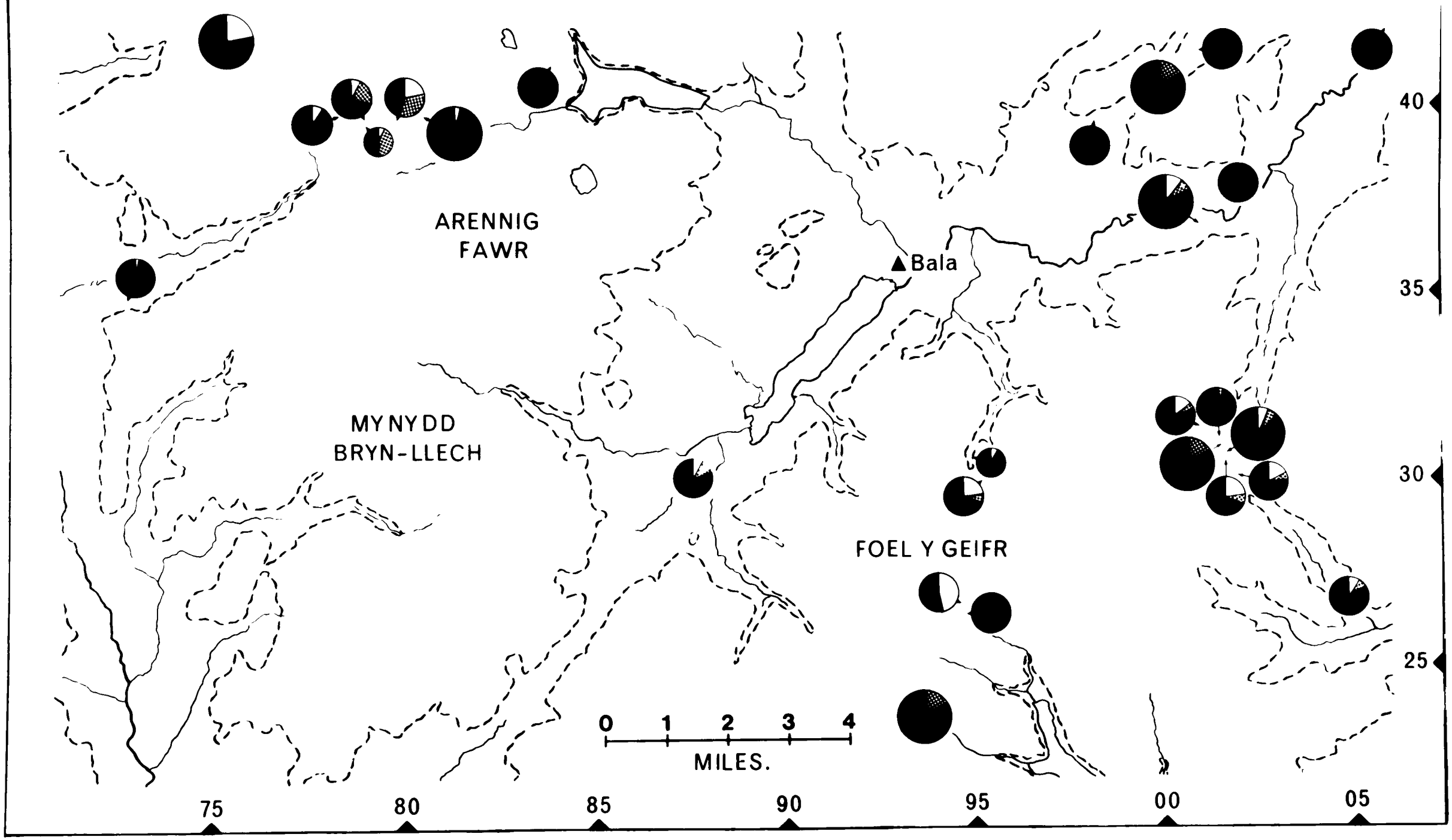


Fig. 10. Distribution of the polymorphism in North Wales (southern region).

proportions of whites than those on the east.

The relationships between morph frequency and height are displayed graphically in figures 11 and 12 for the populations of North Wales and in figures 13-15 for those of South and Mid Wales. Comparison of these graphs with the distribution maps revealed that the relationship between morph frequency and height was much more precise at the local level than for the total populations of each region considered together. Nevertheless the linear regression analyses indicated that the increase in white frequency with height was significant even for the populations above 1000 ft. in both regions.

In both regions the altitude at which high white frequencies were first encountered was quite precise. However whereas in Mid Wales the increase in white frequency occurred at about 1000 ft., in North Wales this transition occurred in the main at a higher level, at about 1,200 ft. In each case the general trend was that purple frequency declined with increase in white frequency, and that the frequency of intermediates showed a unimodal distribution with a quite precise peak. This peak occurred at ca. 1,225 ft. for the southern Mid Wales populations and at ca. 1,450 ft. for those of North Wales. In mountain populations the intermediate morphs were generally infrequent except around these altitude ranges, whereas in seacliff populations they were much more frequent and in three such populations (popns. 50, 51 and 271) were the predominant morphs.

Fig. 11.

North Wales Populations-Variation in the Frequencies of White Morphs with Altitude

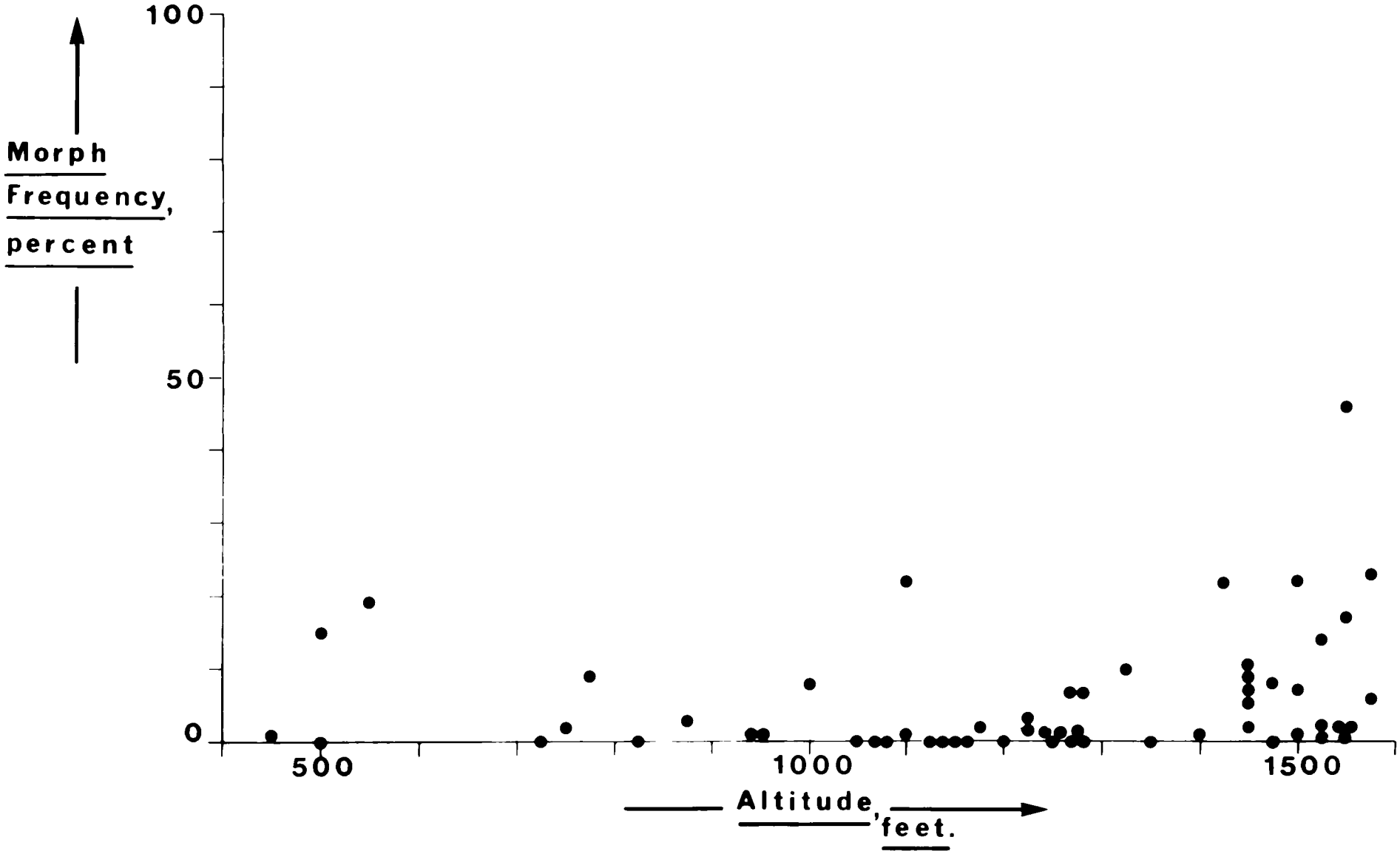


Fig. 12.

North Wales Populations—Variation in the Frequencies of Purple and Intermediate Morphs with Altitude

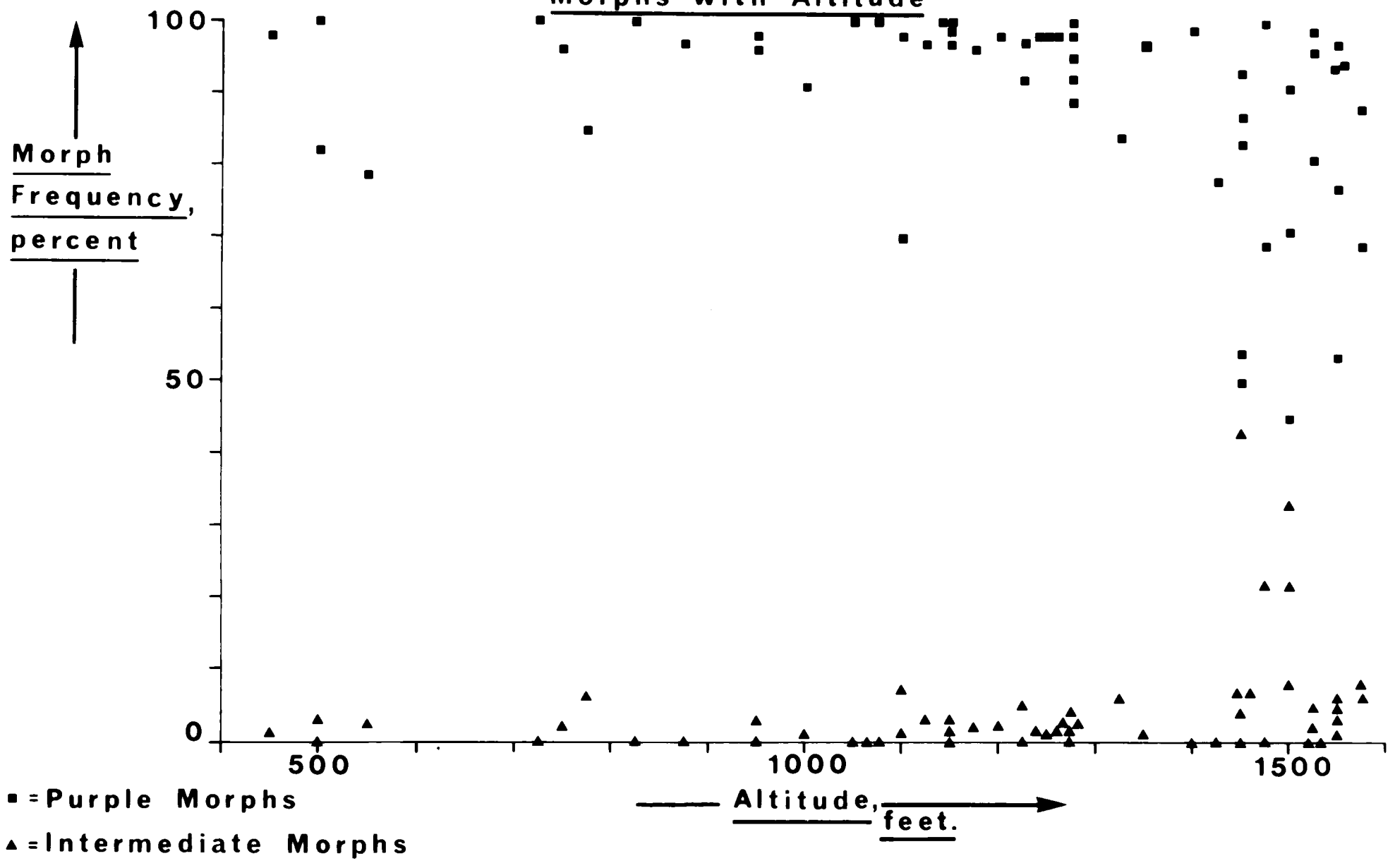


Fig. 13.

South and Mid Wales Populations – Variation in the Frequency of White Morphs with Altitude

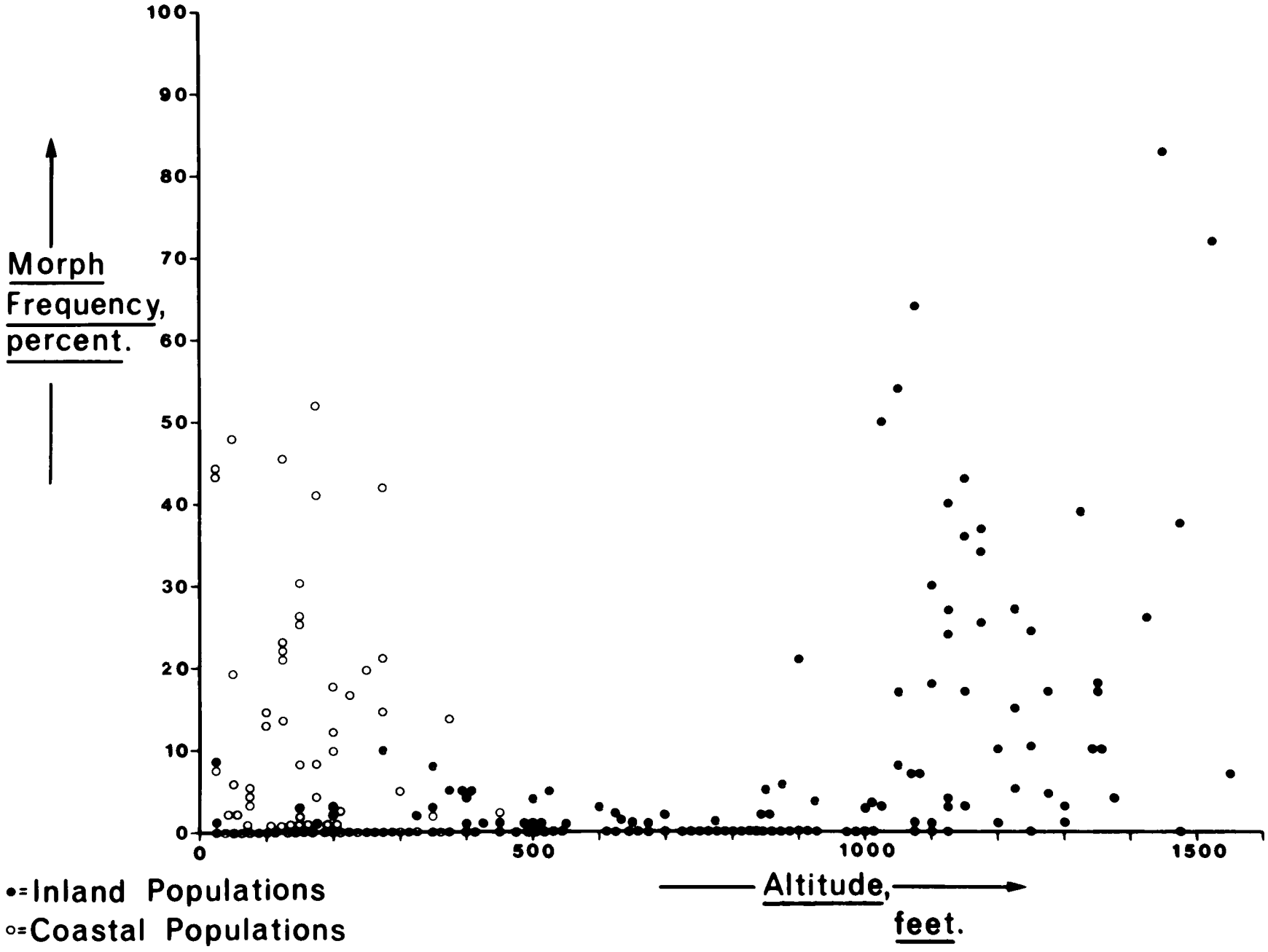


Fig. 14.

South and Mid Wales Populations — Variation in the Frequency of Intermediate Morphs with Altitude

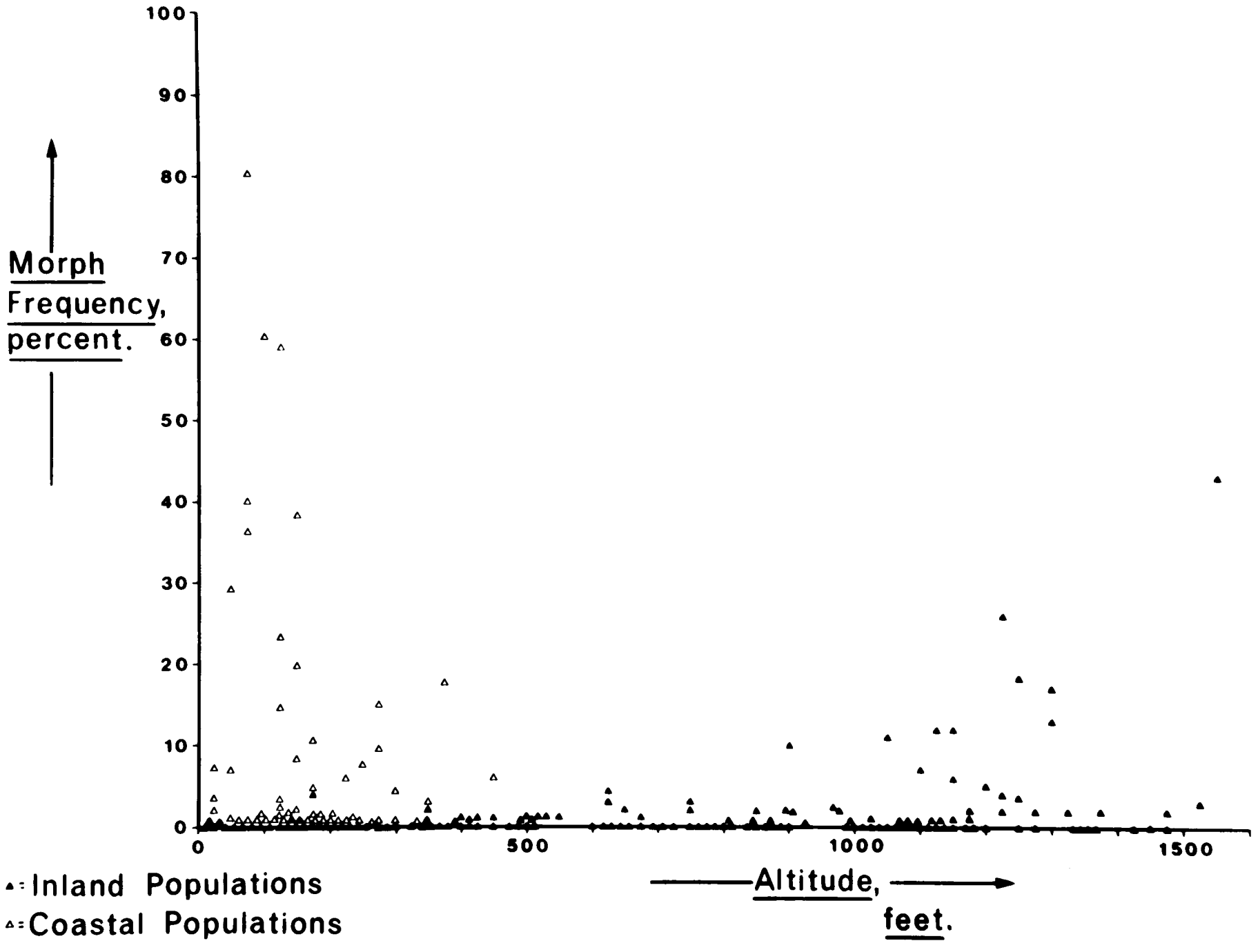
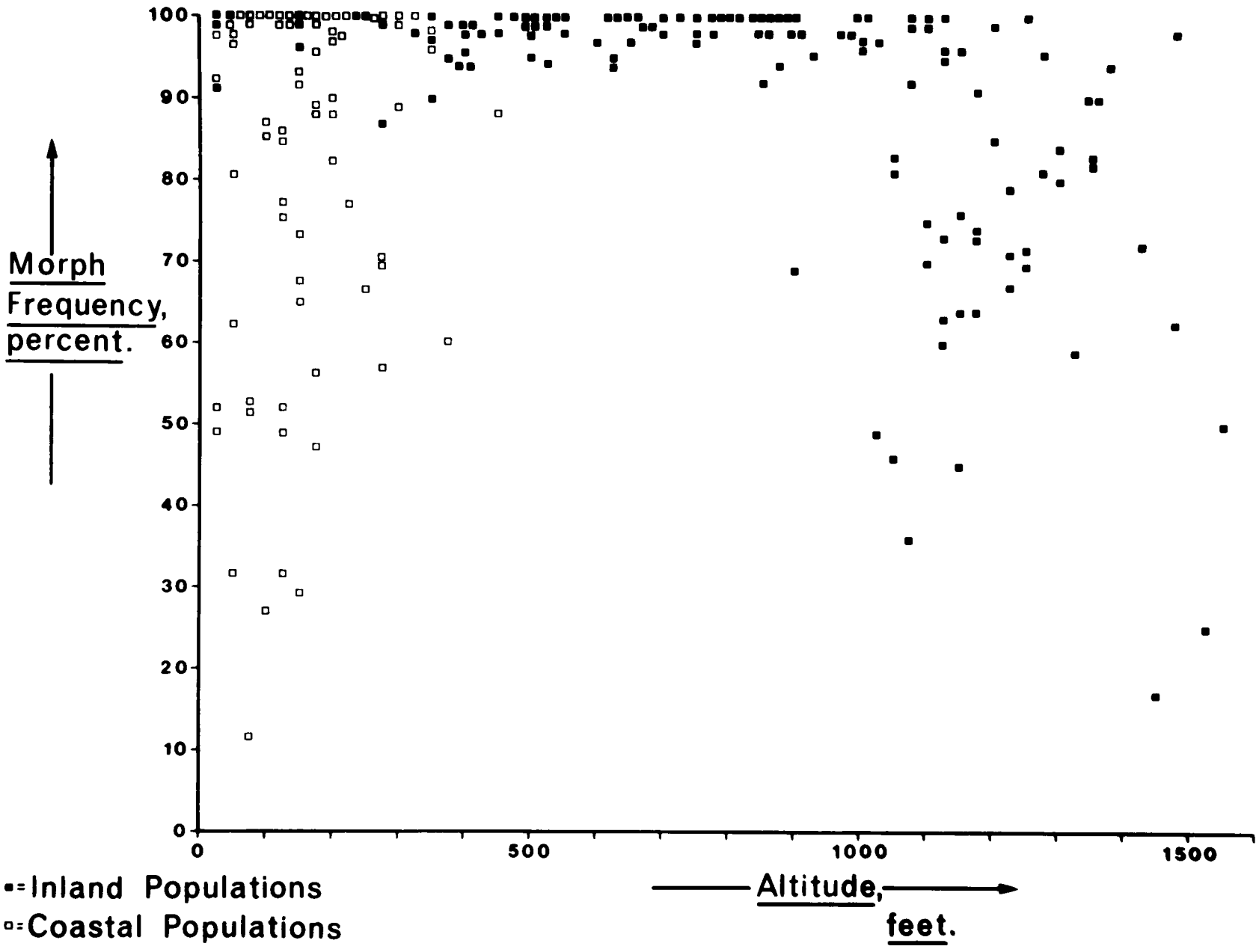


Fig. 15.

South and Mid Wales Populations – Variation in the Frequency of Purple Morphs with Altitude



2.2 The Relationship with Climatic Factors

The above facts may now be considered in relation to the climatic conditions characteristic of seacliffs and the upper regions of mountain. Discussion is reserved until later on the possible effects of the relatively high ultraviolet radiation at both sites (Ch.7) and of seaspray on drought resistance (Ch.3) and pollination (Ch.7).

Both seacliffs and mountains are climatically distinct from their surrounding regions. However both habitats are also climatically distinct from each other and exact comparison of individual factors at either site is confused by the close interrelationships between the factors. In general increase in altitude is associated with a lowering of temperature and higher degree of exposure, rainfall, mist and snow. In contrast seacliffs possess much shorter periods of exposure to frost and much smaller amounts of rain (Meteorological Office, 1952). The incidence of seafog on seacliffs is unlikely to produce a marked resemblance between seacliffs and mountains with respect to mist but the maintenance of high relative humidity on the cliff faces as a result of seaspray may possibly bear resemblance to that maintained on mountains during conditions of mist and rain (L.P. Smith, pers. comm.). However perhaps the only significant climatic resemblance between seacliffs and mountains is the high degree of exposure to which both are subject.

Further evidence for the importance of exposure as a selective factor derives from the apparent cline in the seacliff populations

of the Gower Peninsula mentioned above. The degree of exposure of populations along the peninsula may be considered as increasing both with cliff height and with proximity to the tip of the peninsula. The monomorphic purple populations at Rhossili were the most exposed by virtue of both these factors. The region of peninsula to the north of Rhossili was also very exposed but the more polymorphic populations in this region were situated at very low altitude. The region to the east of Rhossili was progressively more removed from the tip of the peninsula and many of the more polymorphic populations were also at low altitude.

The relationship between cliff height and morph frequency was treated quantitatively. The theoretical relationship between wind speed and height is given by the formula (Hess, 1959):

$$c^2 \propto (1 + e^{-2az} - 2e^{-az} \cos az)$$

where

c = wind speed, $cm.^2$ $sec.^{-1}$

z = height, cm., and

a = a constant which may be taken as 3.6×10^{-5}.

Values of wind speed for successive increments in height were modelled by computer and it was found that for heights of up to about 100 m. the relationship was approximately linear.

The relationship between cliff height and white frequency for all the Gower seacliff populations scored over a four year period which had a population size greater than 75 is shown in figure 16. Analysis of the relationship by linear regression indicated an

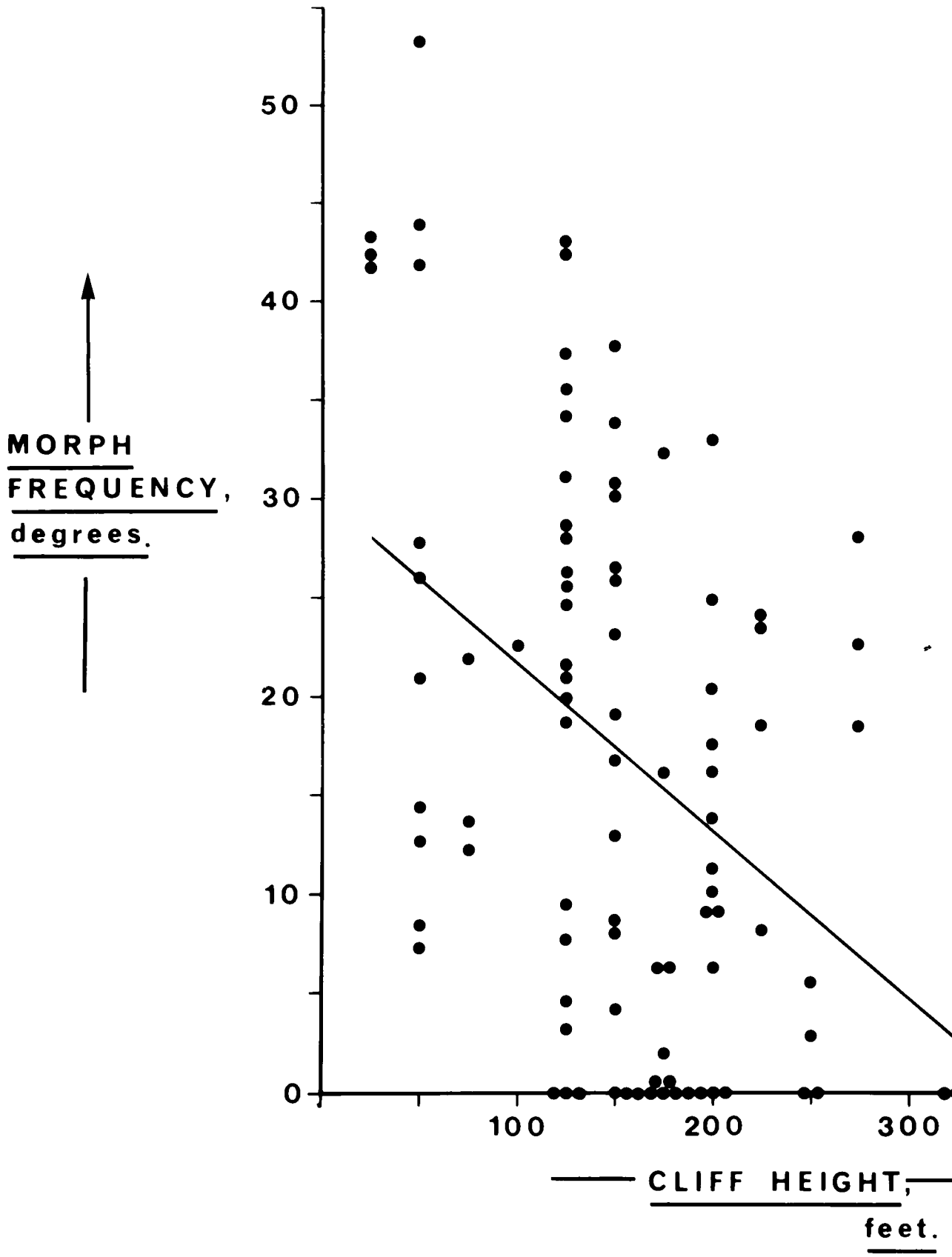


Fig. 16

Gower Seacliff Populations.—Variation in the Frequency of White Morphs with Cliff Height

Frequency values transformed by arcsine.

Values determined separately for each season at each population but restricted to populations larger than 75 in size.

inverse relationship which was significant at the 0.1% level (fig. 17). The square of the correlation coefficient indicated that 22% of the variation in white frequency could be attributed to variation in cliff height alone. If it is accepted that exposure is the principal factor likely to be correlated with cliff height then this would imply that exposure is an important factor in the determination of morph frequencies in these populations.

The influence of exposure was also apparent at the intra-populational level. Observations over consecutive seasons on three successively less exposed segments of a seacliff population at Overton Heath revealed that the proportion of white morphs was inversely related to exposure during each of the seasons in which the population size was at a significant level. Similar results were obtained for segments of seacliff populations at Oxwich Head and Stackpole Head, and for segments of a mountain population at Mynydd Troed (fig. 18). It may be noted however that the influence of exposure on seacliffs is not necessarily comparable to that on mountains since on seacliffs the influence of airborne seaspray may be of importance (Chs. 3 and 7).

As mentioned previously the influences of exposure and moisture on white frequency were examined for mountain populations above 1000 ft. by multiple linear regression. The results indicated for the Mid Wales populations a significant negative correlation between white frequency and moisture ('p' = 0.05 - 0.02). However the white frequency bore no apparent relationship to moisture in

Fig.17 Regression Analyses for Environmental Variables

Dependent variable = Percentage whites per population, transformed by arcsine.

Independent Variable	Coefficient	Standard Error	Significance 'p'
(a) Linear Regression for Gower Seacliff Population			
Sample size = 98 populations			
Constant	30.1509	2.9336	<0.001
Height, ft.	-0.0852	0.0164	<0.001
R^2 = 0.21998			
(b) Multiple Linear Regression for Mountain Populations above 1000 ft.			
(1) Mid Wales			
Sample size = 57 populations			
Constant	29.8372	12.4197	0.05-0.01
Height, ft.	0.0180	0.0087	0.05-0.01
Exposure, units	1.8096	3.1186	N.S.
Moisture, units	-8.2007	3.8918	0.05-0.01
R^2 = 0.14233			
(2) North Wales			
Sample size = 46 populations			
Constant	-8.6897	14.2351	N.S.
Height, ft.	0.0262	0.0080	0.01-0.001
Exposure, units	0.9185	3.8758	N.S.
Moisture, units	3.1070	2.7825	N.S.
R^2 = 0.23006			

Fig.18 Differences in the Frequency of White Morphs between Segments of Population subject to differing Exposure

Morph ratios presented as numbers scored per segment, figures in brackets representing percentage values. Significance values indicate the probability of the differences in white frequency between successively less exposed segments being significant; values calculated by Chi-squared, or, for ratios containing values of 5 or less, Fishers exact test.

(a) Overton Heath Population (Popn. 23).

Exposure of segment	(1) Very exposed				(2) Fairly exposed				(3) Sheltered				Significances (1) v. (2).	(2) v. (3)
Morph Ratio	P.	I.	ppW.	W.	P.	I.	ppW.	W.	P.	I.	ppW.	W.		
1968	63 (91)	0	0	6 (9)	68 (72)	0	0	26 (28)	67 (34)	0	0	130 (66)	***	***
1969	43 (90)	0	0	5 (10)	64 (76)	2 (2)	0	18 (21)	7 (33)	0	0	14 (67)	*	*
1970	21 (100)	0	0	0 (0)	28 (62)	0	0	17 (38)	5 (62)	0	0	3 (37)	***	N.S.
1971	126 (89)	0	0	16 (11)	310 (73)	0	0	113 (27)	99 (58)	0	0	71 (42)	**	**

(b) Oxwich Head Population (Popn. 35).

Exposure of segment	(1) Very exposed				(2) Fairly exposed				Significance (1) v. (2)
Morph Ratio	P.	I.	ppW.	W.	P.	I.	ppW.	W.	
1968	400 (98)	4 (1)	4 (1)	1 (0)	132 (67)	13 (7)	0	51 (26)	***
1969	314 (99)	1 (0)	0	3 (1)	138 (73)	11 (6)	0	40 (21)	***
1970	180 (100)	0	0	0	9 (82)	0	0	2 (18)	***

contd...

Fig. 18 (contd)

(b) Oxwich Head Population (Popn. 35) contd.

Exposure of segment	(1) Very exposed				(2) Fairly exposed				Significance (1) v. (2)
Morph Ratio	P.	I.	ppW.	W.	P.	I.	ppW.	W.	
1971	546 (99)	1 (0)	2 (0)	0	13 (87)	0	0	2 (13)	***

(c) Stackpole Head Population (Popn. 279).

Exposure of segment	(1) Very exposed				(2) Fairly exposed				Significance (1) v. (2)
Morph Ratio	P.	I.	ppW.	W.	P.	I.	ppW.	W.	
1971	100 (100)	0	0	0	87 (87)	0	0	13 (13)	***

(d) Mynydd Troed Population (Popn. 130).

Exposure of segment	(1) Very exposed				(2) Fairly sheltered				Significance (1) v. (2)
Morph Ratio	P.	I.	ppW.	W.	P.	I.	ppW.	W.	
1968	99 (70)	26 (18)	2 (1)	15 (11)	99 (51)	40 (21)	1 (1)	53 (27)	***

* = significant at 5% level;

** = significant at 2% level;

*** = significant at 1% level.

North Wales or to exposure in either Mid Wales or North Wales (fig. 17). The association between low white frequency and damp conditions was fully in accord with situations noted in the field. However little credence was applied to the other results since both exposure and moisture were graded on an arbitrary 1-3 scale which necessarily related more to local conditions at the time of visiting than to the influence of absolute height and the temporal variation in these factors.

The most conspicuous feature of the distribution of the colour types on mountains was the abruptness with which highly polymorphic populations appeared at a certain altitude. Such abruptness might in the first instance be assumed to imply a sudden climatic discontinuity at that altitude. However the demonstration by Creed et al. (1959) that different spot stabilisations in the butterfly Maniola jurtina may be maintained in similar habitats on either side of a hedge illustrates that marked ecological heterogeneity is not a precondition for biological divergence. Nevertheless it is of importance to the present work that altitudinal variation in climate is enormously greater than latitudinal variation and that accordingly even nearby sites on mountain may be subject to significant differences in climate.

The distribution pattern of the polymorphism on mountains suggests first that temperature may be of importance. Temperature undergoes an overall adiabatic decline of $1^{o}F$ per 300 ft. increase in altitude. The effect of temperature would be expected to be

most marked when the mean temperature approximates to the cardinal temperature for most physiological processes (Lundegardh, 1924; Wilson, 1957). Hunter and Grant (1971) in a demonstration that the effect of altitude on grass growth was quadratic maintained that this was consistent with temperature being the climatic variable of principal importance. Similarly Pearsall (1950) demonstrated that the effect of altitude on the growth of Juncus squarrosus was constant throughout regions of differing rainfall and was therefore most probably a consequence of temperature. Daday (1954) demonstrated that altitudinal variation in the frequency of genes controlling cyanogenesis in Trifolium repens was correlated with temperature.

It is of importance also that a hypothesis that low temperatures favour the existence of more polymorphic populations would be capable of explaining the two principal anomalies in the distribution of the polymorphism, namely the occasional presence of highly polymorphic populations at low altitude in valley bases (popns. 207 & 209), and the regular occurrence of predominantly purple populations in forest conditions at high altitude (populations marked 'f' in distribution maps). For in valley bases it would be expected that low night temperatures would be produced which might be more similar to those of mountains than to those of the immediate surrounds (Geiger, 1950); while in the enclosed conditions of upland forests comparatively high night temperatures might be maintained which would be relatively more similar to those of

lowland regions.

However it is equally possible that other factors may be of importance. Rainfall and mist increase with altitude and apart from other effects are likely together with temperature to be of particular importance in the limiting of pollination (Ch. 5). The occurrence of mist is of particular interest since the distribution of mist at higher altitudes is markedly different from that at lower altitudes due to the greater proportion at higher altitude which is caused by cloud (Meteorological Office, 1952). The limited data available does not provide precise information on the frequency distribution of cloud base heights (e.g. High Wycombe Terminal Forecast Facility, 1961; Narayanan, 1970). It would be of interest to determine whether the contribution of cloud to total mist on mountains is such as to produce substantial increase in the amount of mist at the heights at which the polymorphism becomes most frequent.

Interesting further evidence for a relationship between climatic conditions and the establishment of the polymorphism derives from the observation noted previously that in North Wales the increase in white frequency mostly occurs at an altitude about 200 ft. higher than in Mid Wales. Salter (1928) has noted that in the Eastern Highlands of Scotland the altitudinal limit of several species is much higher than in Mid Wales, an effect which he attributed to plants in the Eastern Highlands being covered by snow for a greater part of the year and so being protected from rainfall

and gales for a longer period. The difference in altitudinal limits is also in accord with the view of Burkill and Willis (1894) that in general the altitudinal limit of a species will be higher on mountain ranges of great height than on those of lesser height.

A possible third explanation derives from the sheltering effect of mountain ranges. Climatic differences may be expected on opposite sides of a mountain range in the presence of a prevailing wind as a consequence of the Foehn effect. Foehn winds are warm, dry winds descending in the lee of a mountain range (Brinkmann, 1971). The mechanism of their occurrence was explained by Hann (1866, 1885); warm air rising on the windward slopes of a mountain will undergo cooling and may as a consequence produce rain. On the subsequent descent whether or not precipitation has occurred the air stream will undergo adiabatic warming, and if precipitation has occurred will have additionally been warmed by the latent heat gained during the process.

Typical Foehn winds in North Wales have been described by Lockwood (1962). Since the mountain ranges of North Wales are higher and more extensive than those of Mid Wales it is possible that the Foehn effect may be more marked among the North Wales mountains and that consequently this area may possess relatively "lowland" conditions at higher levels than in Mid Wales. Both the "direction" of the difference observed between Mid and North Wales and the order of magnitude of this difference (200 ft.) are as would be expected as a consequence of the Foehn effect (L.P. Smith,

pers. comm.). However the evidence so far available is insufficient for assessment of the relative importance of this effect. For this purpose more complete climatic data would be necessary, together with a more detailed study of variation in morph distribution in relation to local topography.

Chapter Three

POPULATION SIZE EFFECTS

1. Genetic Drift
2. Selective Mortality
3. Conclusion

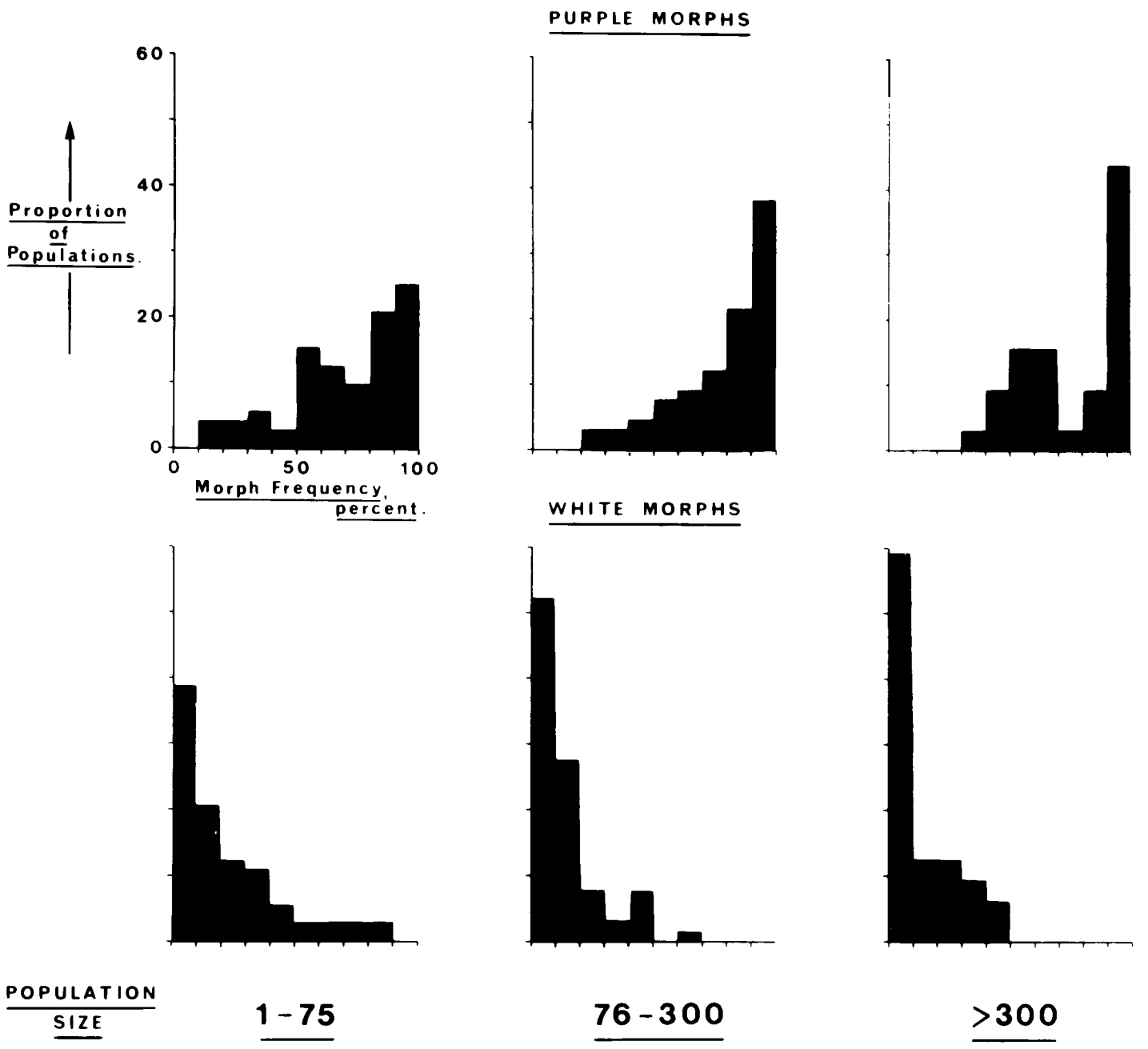
3.1 Genetic Drift

The importance of genetic drift in the maintenance of polymorphisms in nature is uncertain. In the present work the polymorphism under consideration has been shown to be distributed in a clinal manner on the Gower Peninsula and to bear a precise relationship to altitude, and to be subject to possible selective forces associated with exposure, fluctuation in population size (Ch.3.2) and discriminative pollination (Ch.6). Each such factor by implying the existence of selective forces reduces the probable importance of drift phenomena in the determination of morph frequency. However the morph frequencies of many populations were not as would be expected on the basis of the above factors, and the sizes of many populations were of the order of a few hundred plants or less, at which sizes drift phenomena might presumably be operative even in the presence of significant selective forces. Thus Dobzhansky and Pavlovsky (1957) have demonstrated that in laboratory populations of Drosophila pseudoobscura the frequencies assumed by a chromosomal inversion were determined not only by selection consequent upon a heterozygous advantage but also by drift phenomena dependent upon the numbers of founding individuals.

It was considered that drift could be of particular importance during periods in which population size was temporarily reduced to a low level. Such occurrence has been termed by Waddington (1957) "intermittent drift" and has been conceded by Ford (1964) as being possibly of some importance. In order to investigate this the population size and morph frequency of each of 45 seacliff populations on the Gower Peninsula was scored on each of the four seasons 1968-71. These populations were especially suited to such analysis since they were mainly very discrete, thereby enabling population sizes to be counted with accuracy.

The results obtained are listed in Appendix Three. The 170 pairs of values obtained for population size and morph frequency were divided into groups based on population sizes of 0 to 75, 75 to 300, and greater than 300. The distribution of morph frequency in the different size groups is illustrated in figure 19. Using angularly transformed values of morph frequency the mean frequency of each morph type in each size group was calculated (fig. 20) and the values obtained compared between size groups using t-tests. It was found that for both the intermediate and the purplish white morph types no significant differences existed between the mean morph frequencies in each of the size groups. However the mean frequency of the purple morph type was significantly lower in the 0-75 size group than in the 75-300 group ('p' $\simeq$ 0.05), and the mean frequency of the white morph type was significantly higher in the 0-75 group than in the 75-300 group and the 300+ group ('p' $\simeq$ 0.05 in each case).

Fig. 19



GOWER SEACLIFF POPULATIONS — DISTRIBUTION OF MORPH FREQUENCY VALUES IN DIFFERING CATEGORIES OF POPULATION SIZE

Fig. 20 Relationship of morph frequency parameters to population size for Gower seacliff populations

Morph frequency values transformed by arcsine

Morph Type	Population Size	Mean Frequency, degrees	Standard Error of Mean
Purple	0-75	60.734	2.311
	75-300	66.775	2.106
	300+	66.024	3.266
Intermediate	0-75	8.880	1.549
	75-300	9.514	1.381
	300+	11.742	2.517
Purplish White	0-75	4.023	0.925
	75-300	4.536	0.847
	300+	4.753	1.251
White	0-75	22.386	2.147
	75-300	17.087	1.671
	300+	15.277	2.605

The higher frequency of the white morph type in the smaller population size group might be taken as indicative of the operation of drift by the fixation of the white genotype at low values of population size. Lamotte (1951) has cited a similar demonstration as being indicative of a drift influence in the fixation of the bandless phenotype in the snail Cepaea nemoralis. However Cain and Sheppard (1954) pointed out in connection with the latter work that not only was there no evidence that selection was independent of population size, but that in addition smaller populations by occupying more homogeneous backgrounds would be likely as a result of selection alone to be the more diverse. A similar discussion has related to the observation of Dowdeswell and Ford (1953) that the patterns of wing spotting in the butterfly Maniola jurtina are more heterogeneous among the smaller populations of the Scilly Isles than among the larger populations of this archipelago. The authors attributed this to the more restricted conditions on the smaller islands favouring more diverse selection whereas Waddington (1957) and Dobzhansky and Pavlovsky (1957) favoured the involvement of a drift influence caused by the populations on the smaller islands being represented by only a few individuals at some stage in the past.

It is apparent that the above conflicts of view are as applicable to the present case as to Cepaea nemoralis and Maniola jurtina. However it is of importance that the populations studied in the present work were of much smaller size than those studied in either of these cases. In addition the C. palustre populations were subject

to extreme fluctuation in size; neglecting fluctuations involving population sizes of under 75 the mean annual fluctuation in size amounted to

$$82.8\% \pm 58.1\% \quad (\text{'p'} = 0.05)$$

Consequently even populations of several hundred plants were occasionally reduced to a few individuals.

However the magnitude of the fluctuations in population size does not by itself constitute evidence of drift. Creed et al. (1964) have demonstrated that the spot distribution characteristic of a Maniola jurtina population on the Scilly Isles was quickly regained by selection following a season in which the population was reduced to a few imagines. Indeed during the present work the morph frequencies of individual populations in almost all cases remained quite constant over successive seasons. Neglecting fluctuations involving population sizes of under 75 the mean annual fluctuation in frequency of the white morphs amounted to only

$$3.70\% \pm 1.17\% \quad (\text{'p'} = 0.05).$$

To at least some extent this may indicate the operation of strong selective forces.

The extent to which both selection and genetic drift will be manifest will be dependent upon the effect of gene flow between adjacent populations, and gene flow between successive generations of plants in a given population consequent on asynchrony in the timing of the developmental cycle of individual plants. Thus Epling et al. (1960) have remarked that in Linanthus parryae

variation in seed dormancy may produce a reduction in the response of populations to the selection and drift influences of individual seasons. During the present work it was indeed found that in most cases the morph frequencies of adjacent populations were broadly similar and those of a given population throughout successive seasons extremely similar, effects which it is possible may in part be a consequence of such gene flow.

The extent of gene flow between adjacent populations may have been moderate. The seeds appeared to possess an efficient mechanism for wind dispersal and since populations were commonly as close as 500 metres seed dispersal between populations may have been a common occurrence in the exposed conditions of the peninsula. Pollen transport by the bumblebee pollinators was possible between some populations; Free (Free & Butler, 1959) has demonstrated that Bombus lapidarius has a normal foraging range of up to 1300 yards from the nest while Rau (1924) has demonstrated that the flight range of bumblebees is potentially much larger. Many populations were in addition pollinated by honeybees, whose foraging range may extend to half a mile or more (Butler, 1954).

The extent of gene flow between successive generations of the same population would be dependent on the degree of asynchrony in the timing of the developmental cycle of individual plants. The latter was estimated from the data obtained. It would have been expected that if uniform germination and a strict biennial sequence of growth was apparent the number of instances in which the population

size during a given year deviated from the mean of the previous two years in the direction of the earlier of those years might have exceeded the number of deviations in the opposite direction. In practice the number of deviations of the former type amounted to 38 and those of the latter type to 43; this ratio did not differ significantly from unity (Chi-squared = 0.31, 'p' > 0.5). The corresponding ratio for deviations in white frequency amounted to 32:35, which also did not differ significantly from unity (Chi-squared = 0.13, 'p' > 0.5).

The absence of a clear biennial phasing in either population size or white frequency was as expected from the irregular growth cycle of the species. Seeds are shed from July to September and may germinate immediately or remain dormant at least until the following spring (Sempala-Ntege, 1969). Rosette plants require vernalisation before flowering but plants may spend up to two winters as sizeable rosettes before flowering. The plants are normally monocarpic but at least in experimental beds frequently survive to flower the following season.

Such reserves of variability may be of considerable effect in reduction of the influence of intermittent drift. It is probable also that they may be of a very general importance to the survival of the species in the presence of seasonal variation in the suitability of environmental conditions.

3.2 Selective Mortality

Fluctuation in the size of a population would be expected to be accompanied not only by the possible occurrence of intermittent drift but also by changed conditions of selection. These would include changes in the pattern of pollen flow consequent on any changes in morph distribution pattern (Ch. 6), together with increase in the general degree of pollen dispersal during years of low population size consequent on any reduction in the number of flowers per plant or the density of population (Appendix One). In addition in certain circumstances it might be expected that numerical increase in population size might be associated with a relaxation in selection for certain characters, and vice versa (Ford, 1964). Thus Ford and Ford (1930) noted an increase in the general variability of a population of the butterfly Melitaea aurinia during a period of rapid numerical increase and attributed this to relaxed selection during this period.

For a given increase or decrease in population size a morph type will possess an equal chance of increasing or decreasing in frequency if the factors which are associated with the change in population size do not exert a selective influence upon the morph types. If however the morph types differ in their responses then a given type may preponderate during a given change.

This possibility was tested using the present data. The direction of change (increase or decrease) in frequency of each morph type which accompanied the direction of a reduction in population size

was recorded for each successive two years for each population. For each morph type the number of instances in which a decrease in population size was accompanied by an increase in frequency was compared to that in which a decrease in population size was accompanied by a decrease in frequency. The results are shown in figure 21.

Fig. 21 Differential mortality analyses for Gower seacliff populations

Morph frequency changes indicate the number of changes of each type (increase or decrease) that accompanied the direction of a decrease in population size. Probability values (calculated from Chi-squared) indicate the significance of the departure of the increase:decrease ratio from unity.

Morph type	Morph frequency change		Probability value
	Increase	Decrease	
Purple	68	53	0.20-0.10
Intermediate	36	47	0.50-0.20
Purplish White	23	40	0.05-0.02
White	53	66	0.50-0.20
Non-Purple Morphs Combined	112	153	0.02-0.01

The results are rather inconclusive when considered for each morph type individually although they tend to suggest that a decrease in population size may be accompanied by an increase in the frequency of the purple morphs and decrease in that of each of the other types. When the results for the intermediate, purplish white and white morphs are combined then this trend is clearly significant. It may therefore be concluded that the purple morphs were subject to

differential survival in the conditions associated with a reduction in population size.

The direction of such selection is of course the converse of that which might have been expected in view of the more polymorphic nature of the smaller populations. Moreover the ecological conditions associated with the fluctuations in population size remain obscure, since in general the habitats appeared stable and undisturbed. With the exception of one instance in which ploughing occurred, human interference was limited to occasional gorse burning early in the season and the effects of this upon C. palustre did not appear drastic. It is possible however that in the thin exposed soils of seacliffs drought may be an important factor limiting the growth of the species. It may be noted that the direction of selective mortality is consistent with fluctuation in population size caused by drought if it is assumed that the purple morphs by occupying the most exposed sites (Ch. 2) are less subject to drought influences by virtue of a greater exposure to seaspray. However while the white morphs in mountain sites have been demonstrated as being most frequent in relatively drier conditions any such differences in sea-cliff populations remain unproven.

3.3 Conclusion

The degree of polymorphism of the seacliff populations of the Gower Peninsula is correlated with population size, the smaller populations being more polymorphic. While this may be interpreted as indicating chance fixation of the white alleles, other explanations

based on selective effects are possible. Nevertheless the influence of chance fixation is suggested also by the small size of the populations and by their high degree of size fluctuation; however morph frequencies remained very constant throughout successive seasons, and the probable occurrence of gene flow between populations and between different generations of the same population appeared likely to reduce the effects of drift. The polymorphism is subject to selective forces associated not only with exposure but also with fluctuations in population size.

Chapter Four

THE INTENSITY OF SELECTION

1. Introduction
2. Selection in the Intrapopulational Distribution of Morph Types
3. Selection in the Maintenance of Morph Frequencies

4.1 Introduction

The very high estimates available for the degree of biochemical polymorphism in higher organisms (Introduction) imply strongly that if mortality is not to be excessive the reproductive wastage consequent upon the existence of any one balanced polymorphism in a natural population must be minimal. Selective mortality is implied by any situation in which populations with dissimilar morph frequency occur within the range of gene flow between them. As noted previously (Ch. 3) such situations were frequently evident among C. palustre populations. Similarly Barber and Jackson (1957) during a study of the genes controlling glaucousness in the Australian Eucalyptus urnigera observed that clines are maintained in this characteristic despite pollen dispersal over considerable distances by birds.

Selective mortality is implied in particular by intrapopulational heterogeneity in a genetic character. In the past it has been frequently argued that marked genetic divergence between segments of population subject to divergent selection pressures is unlikely to

occur in the absence of a barrier to gene flow between the segments (e.g. Dowdeswell & Ford, 1953; Mayr, 1963). However experiments on disruptive selection in artificial populations of Drosophila have indicated that such divergence is possible without barriers to gene flow (Thoday & Boam, 1959; Streams & Pimental, 1961; Thoday & Gibson, 1962). Moreover evidence is accumulating for the occurrence of such divergence in natural populations; intrapopulational heterogeneity in genetic characters has been described in the growth form of Plantago maritima (Gregor, 1930), the patterns of shell banding in Cepaea nemoralis (Diver, 1940; Sheppard, 1952), the spot distributions of Maniola jurtina (Creed et al., 1959), stolon length in Agrostis stolonifera (Alston & Bradshaw, 1966) and heavy metal tolerance in Agrostis tenuis and other grasses (Bradshaw, 1960; Bradshaw et al., 1965; Jain & Bradshaw, 1966). Jain and Bradshaw (1966) calculated that selection of the degree indicated by studies on the metal tolerance of grasses would be sufficient to maintain intrapopulational differentiation even between sites subject to 50-60% pollen interchange.

In the present work four instances have been described in which intrapopulational selection was apparent from differences in morph frequency between segments of population subject to differing exposure (Ch. 2). However it was considered that even populations with no marked heterogeneity in morph frequency or environmental conditions might provide evidence of intrapopulational selection if morph arrangement was studied in detail. In addition it was considered that marked differences between apparently outbreeding

populations in the frequency of a given morph type might imply that the maintenance of some such population types involved a high degree of selection. These two possibilities are examined in this chapter.

4.2 Selection in the Intrapopulational Distribution of Morph Types

It was apparent that in most populations of C. palustre the species as a whole was distributed in a clumped manner. A population at Fairwood Common near Swansea (popn. 60) was scored in 1968 by Hopkins' method (Hopkins, 1954) which indicated a highly significant degree of clumping ('p' < 0.001 using the significance test of Hopkins). However for the purpose of determining whether selective differences were apparent in the distribution of the morph types it was necessary not only to demonstrate clumping at the level of the species or morph type but to demonstrate significant differences between morph types in their degrees of clumping. Whereas morph types could be clumped relative to each other on a basis of seed dispersal alone, significant differences between the degrees of clumping of the morph types could be explained only on a basis of differences between morphs which may be assumed to be selective differences.

For this purpose the distributions of morph types within a population were measured and compared using the method and significance test of Pielou (1959). The populations examined were at Cantref (popn. 269) and Forest Lodge (popn. 266), both being mountain

populations near Brecon. Both populations grew in grazed pasture and appeared extremely uniform ecologically. Each population was of the dimorphic purple and white type.

At each population rectangular areas of respectively 525 and 760 sq.m. were marked off and random points within these areas determined by measurements along two adjoining sides using random number tables. For each of 100 random points at each population the distance from the point to the nearest plant of each morph type was measured. The total number of plants of each morph type within each area was counted and density values thereby obtained for each morph type at each population.

Using this method the pattern of a plant type is given by the formula

$$\alpha = \frac{\pi D \Sigma r^2}{N - 1}$$

where

r = point to plant distance,

D = density, and

N = number of sample distances determined.

Values for α of <1, 1 or >1 are indicative of regular, random or clumped distributions respectively.

The α values obtained for the purple and white morphs were 3.82 and 1.68 respectively at Cantref, and 3.63 and 4.41 respectively at Forest Lodge. The values indicated that each morph type at each habitat showed a very significant degree of clumping ('p' <0.01 in each case). The difference between the degrees of clumping of the

morph types was significant at Cantref ('p' = 0.01-0.001) but not at Forest Lodge ('p' $>$0.1).

While the presence of a significant difference in the degrees of clumping of two morph types is necessarily indicative of a difference between their distributions in a population, the converse is not necessarily true since two morph types may possess different distributions within a population while retaining identical degrees of clumping. The results therefore indicate that at Cantref the distributions of the two morph types were indeed different, but that at Forest Lodge any difference in the distributions which may have existed was not reflected by a difference in the degrees of clumping.

It was therefore concluded that at least at Cantref the morph types appeared subject to intrapopulational selective forces despite the apparent ecological uniformity of this population.

4.3 Selection in the Maintenance of Morph Frequencies

The populations chosen for this analysis were two populations from the Gower Peninsula which were of highly dissimilar morph frequency; one population was at the Bishopston Valley (popn. 51) and was composed almost entirely of intermediate morphs, and the other population was an Penmaen Marsh (popn. 77) and was composed predominantly of purple and white morphs. The morph frequencies of each of these populations are shown in figure 2. The degree of selection involved in the maintenance of each population type was assessed by comparison of the morph frequencies in the natural populations with the morph frequencies of the progeny of seeds set

naturally in these populations.

Natural seed sets from the purple, intermediate and white morphs at each habitat were harvested during July 1969. The seeds were germinated the following autumn and the plants raised as described in Chapter One. In these conditions mortality was very low and accordingly selection during development was considered negligible.

The morph frequencies of the progeny are listed in Appendix Two. It was found that the intermediate morphs in the Bishopston Valley, which constituted the great majority of plants in this population, gave rise to predominantly intermediate progeny. Similarly the purple and white morphs at Penmaen Marsh, which constituted the great majority in that population, gave rise almost exclusively to purple and white progeny.

The progeny of the intermediate morphs in the Bishopston Valley population suggested that these morphs were predominantly homozygous, as contrasted with those at the nearby Clyne Common population which were predominantly heterozygous (Ch. 1).

The results from Penmaen Marsh were in accord with the results of cross pollinations between purple and white morphs from this population and from that at Clyne Common. The presence of purple morphs in the natural progeny of white morphs indicated that at least some outbreeding had taken place but knowledge of the genetics of the polymorphism was insufficient for estimation of its extent.

It was therefore concluded that the genetics of the polymorphism

was such as to allow extremely dissimilar morph frequencies to be maintained in the two populations without necessarily the involvement of high selection in either case.

Chapter Five

MECHANISMS OF SELECTION

1. COMPATIBILITY

1. Introduction
2. Seed Production in Greenhouse Conditions
3. Seed Production in Natural Populations

5.1 Introduction

It was considered of particular significance to the distribution of the polymorphism that both the polymorphic populations of seacliffs and those of mountains were subject to ecological conditions likely to bring about a reduction in insect pollination. Both regions were subject to high exposure while the mountain habitats were in addition subject to low temperatures and large amounts of mist and rain.

In the populations examined the species appeared to be pollinated principally by bumblebees (Ch. 6). Direct information on the level of bumblebee activity in seacliff and mountain conditions was lacking. However Wilson (1929) while recording that bumblebee activity may continue even during extremely unfavourable conditions noted that under such conditions foraging proceeded at a slower rate. Similarly Miyamoto (1960) and Heinrich (1972) have demonstrated that under moderate temperature conditions bumblebee foraging is reduced by decrease in

temperature. However this cannot necessarily be extrapolated to imply that total bumblebee activity is less in colder habitats and indeed Bruggemann (1958) has recorded bumblebee flights at an air temperature of -3.6°C in the high arctic. Nevertheless Hagerup (1950, 1951) has noted that in the Faroe Islands, which are subject to extremely high exposure and rainfall, pollinating insects are extremely scarce and that associated with this the flora of these islands includes a high proportion of autogamous species. Similarly Eisikowitch and Galil (1971) demonstrated that high wind completely inhibited the pollinating activity of hawkmoths among a seashore population of Pancratium maritimum in Israel.

Since C. palustre is a self compatible species any marked reduction in insect pollination would be expected to promote the frequency of inbreeding. The consequent increase in homozygosity would be likely to increase the frequency of the white morphs since as demonstrated previously these are apparently homozygous. Additionally however any morph type with a higher self compatibility would produce higher seed yields which might confer a selective advantage despite the potentially deleterious effects of inbreeding. Conversely any morph type with a higher attractiveness to pollinators might be at a selective advantage because of the production of a relatively higher proportion of outbred progeny. In either case the degree of selective advantage would be

determined both by the overall level of pollination and by the fitness of the progeny produced by selfing relative to that produced by outcrossing.

In this chapter experiments are described which were designed to compare the self compatibility of the different morph types. The relative effectiveness of selfing and outcrossing in the production of viable seed is described together with the effect of inbreeding on the capacity for seed production. In addition the levels of seed set in natural populations are compared.

5.2 Seed Production in Greenhouse Conditions

These determinations were conducted in 1968 on plants from Wentwood in Monmouthshire and Clyne Common in Gower, and in 1971 on plants derived from pollinations performed in 1969 on plants from the latter population. For each set of plants selfings and crossings were performed in the manner described previously (Ch. 1), and the percentage viability of the achenes measured. Firm fully developed achenes were clearly separable from the shrivelled aborted achenes and since the former gave almost complete germination on moist filter paper they were assumed to be fully viable.

The results are shown in figure 22. In both the Clyne and Wentwood plants tested in 1968 the proportion of viable seed set by selfing was significantly lower than that set by crossing. The number of Clyne plants selfed during 1968 was not large but

Fig. 22 Seed viability in greenhouse pollinations

Morph Type	Pollination	Population	Sample Size	Viability, degrees Mean	Viability, degrees Standard Error
Total Morphs	Selfing	Clyne 1968	6	35.48	6.11
Total Morphs	Crossing	Clyne 1968	28	53.10	2.53
Total Morphs	Selfing	Wentwood 1968	18	22.35	3.41
Total Morphs	Crossing	Wentwood 1968	21	46.92	3.44
Purple	Selfing	Clyne 1969 progeny	31	34.08	1.98
White	Selfing	Clyne 1969 progeny	22	32.77	3.31
Total Morphs (progeny of selfing)	Selfing	Clyne 1969 progeny	40	34.94	2.48
Total Morphs (progeny of crossing)	Selfing	Clyne 1969 progeny	23	29.06	2.24

"Sample Size" refers to the number of different parental types of selfing or crossing performed. Viability values are calculated from the mean viability values for each parental type of selfing or crossing performed, and are presented as proportions transformed by arcsine.

it nevertheless appeared probable that neither proportion was significantly different between populations. The 1971 results indicated that the purple and white morph types did not differ significantly in self compatibility. The self compatibility of the 1971 plants derived by selfing of parental plants did not differ significantly from that of those derived by crossing and did not appear to differ from that of the parental population tested in 1968.

It was therefore concluded that selfing was less efficient than outcrossing in the production of viable seed. The capacity for selfing did not appear to differ significantly between the populations and morph types tested and did not appear significantly altered after one generation of inbreeding.

5.3 Seed Production in Natural Populations

Determinations were conducted on four populations on the Gower Peninsula. These comprised two moderately exposed and highly polymorphic seacliff populations (Three Cliff Bay and Great Tor), one very exposed and relatively monomorphic seacliff population (Oxwich Point) and one marsh population (Oxwich Marsh). Mature inflorescences on the point of releasing seed were gathered from purple and white morphs at each population on July 23, 1968. The inflorescences were gathered from as large as possible a number of different plants at each population, but since in most populations the number of plants with suitable inflorescences

was limited several inflorescences were frequently gathered from the same plant. Using the technique described above the percentage viability of the seeds and the number of seeds per inflorescence were ascertained for a minimum of 1000 ovules from each morph type at each population.

The results are shown in figure 23. The percentage viability of the seeds was uniformly high in all the populations, and did not differ appreciably between morph types within populations. Such differences as existed between populations in seed viability appeared to be positively correlated with the number of ovules per inflorescence thereby suggesting that both might be a reflection of the general vigour of the plants. The differences in seed production between populations appeared to be related to the frequency of the purple morphs but the data was insufficient for conclusions on this.

It was noted that although the seeds were of high viability evidence suggested that they may have been derived from pollination in unfavourable conditions. Thus each of the populations, particularly that at Oxwich Point, were situated in exposed conditions. In addition the seeds were harvested about three weeks subsequent to a period of about two weeks during which the peninsula was subject to exceptional storms. Since in outdoor cultivation the seeds ripen about three weeks from flowering it is likely that a proportion of the seeds was derived from pollination in the unfavourable period.

Fig. 23 Natural seed set parameters for Gower populations

Morph Frequency percent. P. : I. : ppW. : W.	Morph Type	Total Number of Ovules Mean		S.E.		Viability, degrees Mean		S.E.	
(a) Three Cliff Bay (Popn. 47)									
32.2 : 24.4 : 16.7 : 26.7	Purple	36.61	36.66	1.53	0.95	70.25	68.78	1.99	1.48
	White	36.71		1.16		67.31		2.18	
(b) Oxwich Marsh (Popn. 38)									
48.5 : 5.3 : 0.0 : 46.2	Purple	39.42	39.27	2.10	1.67	69.47	73.49	1.72	1.53
	White	39.12		2.64		77.51		2.31	
(c) Great Tor (Popn. 43)									
56.7 : 2.7 : 0.0 : 40.5	Purple	44.83	46.42	1.34	1.11	74.57	73.89	1.35	1.07
	White	48.09		1.74		73.18		1.69	
(d) Oxwich Point (Popn. 35)									
97.8 : 1.0 : 1.0 : 0.2	Purple	48.43	51.26	3.00	1.93	78.06	75.81	1.93	1.55
	White	53.64		2.45		73.93		2.31	

Values underlined represent mean values per population for purple and white morphs combined. Viability values presented as proportions transformed by arcsine.

Actual morph frequency values listed in Appendix 3.

Since even in greenhouse conditions self pollination yields only up to about 60% viability such high seed yields as those present in the natural populations suggest that at least moderate cross pollination may have been involved. However no conclusions can be drawn on the degree of cross pollination in the absence of progeny analysis, a point discussed later (Ch. 7).

Chapter Six

MECHANISMS OF SELECTION
2. DISCRIMINATIVE POLLINATION

1. Introduction
2. Experimental Theory
3. Experimental Method
4. Results
5. Conclusion

6.1 Introduction

The movements of bees foraging upon C. palustre at first appeared to be random. However during the summer of 1971 a series of experiments revealed that in fact the movements were very precise both with respect to the morph types visited and the direction of foraging. The present chapter is devoted to an account of the factors governing overall colour discrimination and the pattern of pollen flow between morph types. Directionality during foraging is treated later (Appendix One).

6.2 Experimental Theory

(a) Overall Discrimination

The ratio of morph types visited by a forager among a polymorphic population of plants may differ from the ratio of morph types present in the population as a consequence of several factors. If the morph types are distributed differently in the population then for a limited number of visits this effect will be apparent,

and will be of a magnitude dependent upon the degree of directionality during foraging (Appendix One). Differences in morph distribution will produce such effect over any number of visits if accompanied by non-randomness in the distribution of pollinators in the community, as could arise for instance if some plants were more frequented than others as a consequence of possessing more flowers or being in closer proximity to a bee nest.

If the foraging species can distinguish the different morph types then there may occur a tendency for the pollinator to restrict successive visits to plants of one morph type, irrespective of the actual morph type. Such a response would be of the type described by Tinbergen (1960) as a "searching image" response, instances of which have been described by De Ruiter (1952) and Tinbergen (1960) in the predation of insects by birds. No evidence for such response was obtained during the present work but the possibility of its involvement was implied by the widespread occurrence of flower constancy in bees (e.g. Darwin, 1876; Mather, 1947; Grant, 1949).

If the foraging species is not only capable of distinguishing the morph types but is attracted to different extents by the different types then there will occur an "intrinsic preference" for one or more of the types. The term "overall discrimination" may be used to describe the preferential pollination resulting from both search image response and intrinsic preference.

A search image response would be likely, by either of two ways, to produce preferential pollination of the most frequent morph type. In the first place as pointed out by Cain and Sheppard (1954) the extra effort involved in searching for successive morphs of a less frequent type might result in forages for this type being abandoned relatively more frequently. Secondly if two or more successive encounters with a given morph type would be necessary before a search image response would be evoked then the response would be evoked relatively more frequently for the more common morph type. Additionally it may be noted that intrinsic preference by promoting visits to morphs of a given type would increase the frequency with which search image responses would be elicited for that morph type. It is apparent therefore that overall discrimination is potentially the product of a search image response promoting visits to the more common morph type, and of an intrinsic preference response both in its action per se and in the promotion of a search image response for the preferred morph.

For a large number of flights a significant difference between the ratio of plants visited in a population and the ratio of plants present in the population will be a consequence only of either overall discrimination or a non-random distribution of foragers. In the experiments described below the distribution of foragers was assumed to be random since the plants appeared fairly uniform in size and flower production, and the distances travelled by

individual pollinators were of comparable magnitude to the areas of population studied. Accordingly the difference between the above ratios was taken as a measure of overall discrimination.

(b) Pollen Flow Analysis

Estimates of pollen flow between morph types were derived by recording the relative frequency of inter-morph moves of each type.

In addition values for the relative frequencies of inter-morph moves were calculated from the overall visits ratio on the assumption that these were solely determined by morph frequency and intrinsic preference. The difference between these sets of values were then interpreted as due to the effects of search image responses and heterogeneity in morph distribution. Comparison of these values for each of two populations together with quantification of the heterogeneity in morph distribution at either population by an independent technique enabled the influence of heterogeneity in morph distribution to be demonstrated and some indication to be gained of the probable importance of search image responses.

6.3 Experimental Method

Observations were conducted upon six populations. These comprised a very polymorphic seacliff population on the Gower Peninsula (Rhossili Down) and five mountain populations on the Brecon Beacons. The latter included a forest population (Beacons Forest) with a typical low frequency of white morphs (4.4%) relative to the height

of the population (1275 ft.). The remaining four populations were all highly polymorphic and were all located in fairly exposed pasture. They comprised two populations of the typical dimorphic purple and white type (Cantref and Forest Lodge), and two populations which contained high proportions of the intermediate and purplish white morphs (Mynydd Llangattwg and Mynydd Troed). The morph frequencies and principal pollinating species at each population are shown in figure 24.

At each population pollination during the daytime was overwhelmingly the work of bees. Night pollination was not considered during the present work, but this may have been of importance at least at Rhossili Down where moths were occasionally seen to visit the blossoms during the late evening. The possibility that significant colour discrimination may have been exercised during night pollination was implied by the evidence presented earlier (Introduction) that white flowers may be relatively more attractive at low light intensities.

At each population observations were conducted within a marked area enclosing several hundred plants and divided into rows each of about three metres width. The total number of plants of each morph type within each area was counted and the visits of bees to individual plants recorded. Observations were conducted separately on the different species present and in some cases on the different sexes present. In most cases a minimum of 500 plant visits were recorded for each species or sex considered. Individual bees were

Fig.24 Preferential pollination

Morph Ratio				Pollinating Species	Visits Ratio				Discrimination Probability			Species Frequency	
P	I	ppW	W		P	I	ppW	W	P	I	ppW	W	%
(a) Rhossili Down (SS 416896)													
208 (76.7)	0	5 (1.8)	58 (21.4)	A. mellifera	546 (71.4)	0	18 (2.4)	200 (26.2)	N.S.	-	N.S.	N.S.	ca.1
(b) Beacons Forest (SN 989180; popn.268)													
395 (95.6)	0	0	18 (4.4)	B. pratorum (female)	495 (96.3)	0	0	19 (3.7)	N.S.	-	-	N.S.	28.1
(c) Cantref (SN 992168; popn.269)													
521 (74.5)	0	0	178 (25.5)	B. agrorum	527 (74.1)	0	0	184 (25.9)	N.S.	-	-	N.S.	14.1
				B. lapponicus	480 (71.7)	0	0	189 (28.3)	N.S.	-	-	N.S.	1.3
				B. lucorum	809 (70.5)	0	0	338 (29.5)	(*)	-	-	*	79.6
				B. pratorum (female)	362 (58.9)	0	0	253 (41.1)	(***)	-	-	***	5.0

contd....

Fig.24 (contd)

Morph Ratio				Pollinating Species	Visits Ratio				Discrimination Probability				Species Frequency	
P	I	ppW	W		P	I	ppW	W	P	I	ppW	W		%
(d) Forest Lodge (SN 961247; popn.266)														
128 (36.0)	0	0	228 (64.0)	B. agrorum (male)	155 (37.3)	0	0	261 (62.7)	N.S.	-	-	N.S.		51.7
				B. agrorum (female)	162 (37.6)	0	0	269 (62.4)	N.S.	-	-	N.S.		23.0
				B. lapidarius (male)	170 (26.3)	0	0	476 (73.7)	(***)	-	-	***		8.2
				B. lapidarius (female)	80 (18.5)	0	0	353 (81.5)	(***)	-	-	***		1.1
				B. lapponicus	42 (11.6)	0	0	320 (88.4)	(***)	-	-	***		1.0
				B. lucorum	108 (17.5)	0	0	510 (82.5)	(***)	-	-	***		15.0
(e) Mynydd Llangattwg (SO 159166)														
163 (66.5)	1 (0.4)	80 (32.7)	1 (0.4)	B. agrorum	453 (67.5)	0	217 (32.3)	1 (0.1)	N.S.	-	N.S.	-		79.1
				B. lapidarius (male)	369 (63.6)	0	211 (36.4)	0	N.S.	-	N.S.	-		5.9
				B. lapidarius (female)	322 (75.4)	0	105 (24.6)	0	**	-	(**)	-		7.6
				B. lucorum	340 (53.5)	0	296 (46.5)	0	(***)	-	***	-		6.6

contd...

Fig.24 (contd)

Morph Ratio				Pollinating Species	Visits Ratio				Discrimination Probability			Species Frequency	
P	I	ppW	W		P	I	ppW	W	P	I	ppW	W	%
(f) Mynydd Troed (SO 171295)													
416 (61.3)	110 (16.2)	147 (21.7)	5 (0.7)	B. agrorum	379 (58.0)	122 (18.7)	149 (22.8)	3 (0.5)	N.S.	N.S.	N.S.	-	44.0
				B. lapidarius (male)	262 (65.2)	67 (16.7)	73 (18.2)	0	N.S.	N.S.	N.S.	-	29.0
				B. lapidarius (female)	213 (56.8)	75 (20.0)	87 (23.2)	0	N.S.	N.S.	N.S.	-	9.4
				B. lapponicus	324 (50.9)	114 (17.9)	198 (31.1)	0	(***)	N.S.	***	-	6.2
				B. lucorum	318 (56.5)	110 (19.5)	135 (24.0)	0	(*)	*	N.S.	-	11.3

Probability values calculated as described in text.

'*' = significant at 5% level; '**' = significant at 1% level; '***' = significant at 0.1% level.

Unbracketed probability values indicate morph types subject to preferential pollination and bracketed values indicate morph types discriminated against during pollination.

Bracketed values for morph ratio and visits ratio represent percentage values.

Species frequency values calculated from mean values on each day of observation.

followed for as long as possible in order to reduce human bias in the choice of plants.

At each population the great majority of the bees present belonged to a comparatively small number of species and accordingly observations were restricted to the common species in each case. At Beacons Forest due to limitation of time observations were restricted to only one of the common species present.

At half hourly or hourly intervals throughout the periods of observation the total number of bees of each type within the enclosed area was scored using the marked rows as an aid to scoring. At the time of each recording air temperature and humidity were measured using a whirling hygrometer and light intensity was recorded using an Eel portable photometer.

6.4 Results

(a) Overall Discrimination

The significance of the degree of discrimination displayed by each bee type was calculated separately for each morph type in each population. The tests were performed using Chi-squared to assess the difference between the observed and expected values for the ratio of the number of morphs of a particular type visited to the total number of alternative morphs present in each population.

The results are listed in figure 24. It was found that discriminative pollination was a frequent phenomenon and in most cases involved preferential pollination of the white morphs. Thus preferential pollination of the white morphs occurred in each of

the six cases in which species exhibited discriminative pollination in populations containing white morphs. In contrast discrimination was directed against the purple morphs in nine of the ten cases in which species exhibited discriminative pollination in populations containing purple morphs.

The net effect of such discriminative behaviour at the population level would be dependent upon the uniformity of the direction of preferential pollination, the relative frequency of the bees exhibiting such behaviour and their relative capacity for pollen transport. As mentioned the direction of discrimination quite consistently involved preferential pollination of the white morphs and discrimination against the purple morphs. Moreover many species were present in quite high frequency in populations in which they exhibited discrimination. The relative capacity of the species for pollen transport was less clear but since the smaller species, which may be presumed to have lower pollen carrying capacity, appeared relatively more active than the larger species it is possible that this may not be an important variable.

The degree of discrimination varied between species, between sexes and between the same sex in different populations. Whereas Bombus agrorum was uniformly non-discriminative in all populations each of the remaining species displayed discrimination. The morph visit ratios of the two sexes of B. agrorum were not significantly different when these sexes were scored separately at Forest Lodge, whereas the morph visit ratios of the two sexes of B. lapidarius

differed significantly in each of the three populations in which this species occurred ('p' < 0.01 in each case). The greatest difference between the sexes occurred in the two populations in which the degrees of discrimination were greatest (Mynydd Llangattwg and Forest Lodge) and in both cases the females were the most discriminative ('p' < 0.001 in each case).

Throughout the experimental period the highest intensity of foraging occurred on dry days that were calm and relatively warm. The proportion of different species foraging a population was not greatly affected by climatic fluctuation but in all the populations the proportion of male bees was considerably higher towards the end of the summer.

Variation between populations in the proportion of the sexual phases may in part explain the differences in the discriminative behaviour of species between populations. However other local influences appear to be operative since worker B. pratorum exhibited very different discriminative patterns at Cantref and Beacons Forest even though these populations were only half a mile apart. Discriminative behaviour may presumably be dependent upon such additional influences as light intensity and the frequency and density of morph types (Ch. 7).

(b) Pollen Flow Analysis

From the Cantref and the Forest Lodge observations values were obtained for the observed numbers of inter-morph flights of each type, viz. purple-purple, purple-white, white-purple and white-white.

For each bee species the observed frequency of each type was compared to that expected on a basis of morph frequency alone and to that calculated from the morph visits ratio on the assumption that the latter was determined solely by morph frequency and intrinsic preference. The comparisons were performed using Chi-squared to assess the difference between the observed and expected values for the ratio of the number of flights of a given type to the total number of alternative flights recorded. The results are listed in figure 25.

It was found that the observed frequency of flights between white morphs was significantly higher than that expected on a basis of morph frequency both for those species which exhibited preferential pollination of the white morphs and for certain of those which exhibited no overall discrimination. This effect represents a degree of non-random pollination which may contribute in part to the maintenance of the polymorphism (Ch. 7).

It was found that in the results obtained at Forest Lodge an extremely close correspondence existed between the observed visit sequences and those calculated from the morph visit ratios. Since it is likely that the effect of any search image responses would have been to increase the frequency of flights between like morphs to a level greater than that calculated from the morph visits ratio this result may be taken as implying that search image responses may have been of minimal importance.

However with the Cantref data the observed visit sequences

Fig. 25 Pollen flow analysis

FOREST LODGE

	DISCRIMINATIVE SPECIES								NON-DISCRIMINATIVE SPECIES		
	B. lucorum			B. lapidarius			B. lapponicus		B. agrorum		
(a) Deviations from values calculated from morph frequency											
BASE P	(***)	(***)	P	(***)	(***)	P	(***)	(***)	P	N.S.	N.S.
BASE W	(***)	***	W	(***)	***	W	(***)	***	W	N.S.	N.S.
	P	W		P	W		P	W		P	W
	RESPONSE										
(b) Deviations from values calculated from visits ratios											
BASE P	**	(*)	P	N.S.	N.S.	P	N.S.	N.S.	P	N.S.	N.S.
BASE W	N.S.	N.S.	W	N.S.	N.S.	W	N.S.	N.S.	W	N.S.	N.S.
	P	W		P	W		P	W		P	W
	RESPONSE										

CANTREF

	DISCRIMINATIVE SPECIES				NON-DISCRIMINATIVE SPECIES			
	B. lucorum		B. pratorum		B. lapponicus		B. agrorum	
(a) Deviations from values calculated from morph frequencies								
BASE P	N.S.	N.S.	P	(***)	***	P	N.S.	N.S.	P	*	(*)
BASE W	N.S.	***	W	***	***	W	N.S.	***	W	N.S.	*
	P	W		P	W		P	W		P	W
	RESPONSE										

contd....

Fig.25 (contd)

CANTREF (contd)

	DISCRIMINATIVE SPECIES				NON-DISCRIMINATIVE SPECIES					
	B. lucorum		B. pratorum		B. lapponicus			B. agrorum		
(b) Deviations from values calculated from visits ratios										
BASE P	*	(**)	P	(***)	N.S.	P	N.S.	(*)	P	*	(*)
BASE W	N.S.	N.S.	W	N.S.	***	W	(*)	***	W	(*)	*
	P	W		P	W		P	W		P	W
	RESPONSE										

In the tables are presented the significance values for the deviation of the observed numbers of inter-morph moves from those calculated from morph frequency ratios and morph visit ratios. The terms "base" and "response" refer respectively to the original plant and the subsequent plant visited on a given flight. Probability values calculated as described in text.

'*' = significant at 5% level; '**' = significant at 1% level; '***' = significant at 0.1% level.

Observed moves more frequent than calculated indicated by unbracketed probability values and those less frequent than calculated by bracketed values.

were clearly different from those calculated from the morph visit ratios. In a similar comparison it occurred that for the species showing no overall discrimination the frequencies of inter-morph flights expected on a basis of morph frequency alone differed significantly from the observed frequencies at Cantref but not at Forest Lodge. Both such effects are consistent with the conclusion derived earlier (Ch. 4) that the degrees of clumping of the morph types were significantly different at Cantref but not at Forest Lodge. As stated previously heterogeneity in morph distribution within a population may produce modification of visit sequences in addition to that produced by intrinsic preference and possibly a search image factor.

6.5 Conclusion

The morph types of C. palustre were found to be subject to discriminative pollination. In most cases this involved preferential pollination of the white morphs and discrimination against the purple morphs. Analysis of the patterns of pollen flow in two populations revealed that in each case pollen flow between the white morphs was more frequent than would have been expected from morph frequency alone. This effect may be attributed largely to intrinsic colour preferences and heterogeneity in the distribution of morph types.

Chapter Seven

DISCUSSION

The present work has come some way towards determining the ecological conditions correlated with the maintenance of the polymorphism. However the conditions associated with its restriction to seacliffs and mountains remain obscure, together with, as would be expected, the precise selective factors involved. It is possible that both may be associated with a reduction in insect pollination since this would be expected as a consequence of both the exposure of seacliffs and the exposure, mist, rain and low temperatures of mountains (Ch. 5).

However no accurate information was obtained on the levels of outbreeding in the natural populations. The plants raised in cultivation from seeds obtained from the population at Penmaen Marsh indicated that at least some outbreeding had occurred, while the high percentage viability of the seeds set in other populations on the Gower Peninsula suggested that the degree of outbreeding may have been moderate. However accurate assessment of degrees of outbreeding would obtain only from analysis of the progeny of natural populations in the light of more complete genetical studies and possibly the use of pigment analysis for the more precise identification of phenotype.

Pollination may be regarded as being limited in any situation in which pollen intermixture throughout a population is not complete.

The effect of such a situation on selection would be complex. Any morph type with a higher self compatibility would produce higher seed yields. However the greenhouse experiments indicated that at least the purple and white morph types did not differ in self compatibility. Secondly increased inbreeding would result in an increase in homozygosity which at the flower colour loci would produce an increase in the frequency of the white genotype. The degree of homozygosity so produced would augment a degree of homozygosity at the flower colour loci consequent upon discriminative pollination and certain forms of heterogeneity in morph distribution. The latter two effects would be operative at all levels of pollination, together with a degree of general inbreeding which Bateman (1947) has pointed out as being present even in large populations as a consequence of the limitations of pollination distance and seed dispersal.

However despite the production of some inbreeding with respect to flower colour it would be expected that the degree of general outbreeding of a morph type would be increased by preferential pollination in any situation of limited pollination. In addition it may be assumed on general grounds that the effect of outbreeding is to increase the fitness of a morph type. This was supported by the greenhouse experiments which although demonstrating that one generation of selfing did not lower the measured capacity for seed production nevertheless indicated that selfing was less efficient than cross pollination in the production of viable seed. It may

therefore be considered admissible that the localisation of the polymorphism on seacliffs and mountains is consistent with an increase in the relative fitness of the white morph type consequent upon preferential pollination in conditions of limited pollination.

It may be noted that outbreeding might be at an additional premium in situations in which total seed production is limited. The viability of the seeds set in the seacliff populations examined was high but it is possible that in some such populations seed production may be limited by an inhibitory effect of seaspray on pollen germination. Inhibition of pollen grain germination by seawater has been demonstrated by Eisikowitch (pers. comm.). In addition populations of the Gower Peninsula were heavily parasitised by the dipteran species Tephritis conura Lw. and Xyphosira miliaria Schr., the larvae of which inhabit the seed heads and consume the developing seeds. (I am indebted to Mr. E. Taylor of the Zoology Department at Oxford for identification of these species). Their potential effect was illustrated by a bulk collection of inflorescences taken from the Clyne Common population (popn. 57) during late July 1968. Of 55 capitula examined, 4 were found to be not infected, 11 to be slightly infected and the remaining 40 to have all ovules almost completely eaten away. It was not established whether the parasites were relatively more common among seacliff populations but it is clear that any localisation in their distribution might be of considerable ecological importance.

It is of importance also that the degree of discriminative

pollination in mountain and seacliff populations might be higher than that in lowland inland populations since both mountains and seacliffs are subject to relatively higher amounts of ultraviolet radiation (e.g. Geiger, 1950; Stair, 1952). Preliminary chromatographic work has indeed indicated that the floral pigments of each of the morph types include ultraviolet pigments. Future studies on pollination would demand a more detailed examination of the differences in floral pigmentation since whatever the relative importance of ultraviolet pigmentation it is possible that the discriminatory responses of bees might be more clearly defined in relation to pigment patterns than to the morph categories used in the present work.

It may be noted that an increase in the fitness of the white morph type relative to the purple morph type as a consequence of preferential pollination is capable of explaining the overall distribution pattern but is not necessarily evidence that the polymorphism is adaptive in the sense of Fisher (1930). In other words there is no evidence that polymorphic populations are on a basis of the above theory any more fit than monomorphic purple populations would be in the same situation. Cain and Sheppard (1954) have similarly noted that the correlation of selective forces with morph frequency in Cepaea nemoralis is not necessarily evidence that the polymorphisms concerned are adaptive. However in the present work it may be noted as possible that populations containing higher proportions of the white morphs may attract relatively higher numbers

of pollinators or encourage foraging for longer periods. If this were so then in conditions of limited pollination polymorphic populations would be more efficiently cross pollinated than monomorphic purple populations in the same situation, and accordingly the polymorphism could indeed be said to be adaptive. It is of relevance that among other members of the genus white flower colour may be a characteristic of the more alpine species (Introduction). It may be presumed that in these species, as is possibly the case in C. palustre, white flower colour is an adaptive characteristic in mountain conditions.

However some caution may be applied to the assumption that differences in morph fitness within populations are necessarily reflected at the populational level. Differences in the relative fitness of genotypes within and between populations have been demonstrated in Primula (Crosby, 1949), Mus (Lewontin, 1962), and Tribolium (Dawson, 1969). Of particular relevance is the work of Levin and Kerster (1970) on the pink and white flower colour polymorphism of Phlox pilosa. It was found that within polymorphic populations the white morphs produced the higher seed sets but that the mean seed production of each morph type was positively correlated with the frequency of the pink morphs. Moreover the authors considered that the differences in seed production both within and between populations were attributable to differences in the attractiveness of the morph types to insect pollinators.

Further influences are operative upon the polymorphism. On

seacliffs genetic drift may be of importance at least in the smaller populations. However some of the seacliff populations and most of the mountain populations which contained white morphs in high frequency were very large and remained fairly constant in size over at least two years of observation. Accordingly the role of genetic drift in these populations would appear likely to be minimal.

The selective effects operative upon seacliff populations are complex. While reduction in population size is accompanied by differential survival of the purple morphs, the smallest populations actually contain the lowest frequencies of these morphs. In addition while a limitation of pollination consequent upon exposure may explain the occurrence of the polymorphism on seacliffs, the most exposed populations in fact contain the lowest proportion of whites. However as noted above differences in the fitness of morph types within and between populations are not necessarily unusual and moreover as described in Chapter Three are in this particular case not necessarily inexplicable.

Among mountain populations both exposure and moisture have been demonstrated as being associated with selective effects. However the general interrelationship between climatic factors on mountains is such that the increase in white frequency with height may more accurately be correlated with the combined effects of increase in exposure, mist and rain and decrease in temperature. It is possible that the principal selective effect of these factors

is their combination to produce a limitation of pollination. As noted in Chapter Two the occasional presence of highly polymorphic populations on valley bases and the regular occurrence of predominantly purple populations in mountain forests are both suggestive of an individual effect of temperature, but neither case is conclusive evidence of the operation of temperature by an effect other than the limitation of pollination.

It is clear however that forest populations represent an unusual situation. As demonstrated by Beattie (1971) the activity of insect pollinators in forests is largely restricted to the limited regions of sunlight. It would appear probable that this represents a limitation of pollination which may be compensated only in part by any relative increase in pollinator activity consequent upon the calm conditions and relatively restricted temperature fluctuation of these habitats (Geiger, 1950). However despite this the white morphs were present at only low frequency. It is possible that at low light intensity the difference in attractiveness of the morph types may be reduced, for worker Bombus pratorum exhibited no preferential pollination in the forest population studied but a strong preferential pollination of white morphs in a moorland population only half a mile distant (Ch. 6). However evidence in other species suggests that white flowered plants may be more conspicuous in forest conditions (Introduction). It is possible that the morph frequencies may be principally determined by an effect of temperature or other factors in a manner other

than upon pollination; for instance these habitats were very moist and evidence suggests that the frequency of the white morphs may be lowest in moist conditions (Ch. 2).

The demonstration of intrapopulational selection in morph distribution within the relatively homogeneous habitat at Cantref demonstrates the probability that the maintenance of the polymorphism involves additional factors to those described above. Future work on the nature and intensity of the selective factors could profitably include experiments on the introduction of seed into new habitats or existing populations.

It is possible in particular that the purple morphs may possess a physiological advantage. Such might be responsible for the general preponderance of purple morphs in the majority of populations and possibly also the differential survival of these morphs in the conditions associated with a reduction in population size on seacliffs. It is certainly probable that a physiological heterozygous advantage of the purple morphs is involved in the maintenance of the polymorphism and that this may be responsible for the very common occurrence of white and intermediate morphs in low frequency in inland lowland populations. Heterozygous advantage has been proposed as an explanation for similar instances in Cepaea nemoralis (Cain and Sheppard, 1954) and Biston betularia (Kettlewell, 1958) in which polymorphisms have been maintained at low frequency in populations which on a basis of established selective trends would be expected to have become monomorphic.

However it may be noted that the actual degree of polymorphism of the predominantly purple populations may have been underestimated since as emphasised previously the purple morph category was not genetically uniform (Ch. 1). Assessment of the extent of such cryptic polymorphism must await more complete genetical studies and the identification of phenotype at the pigment level.

In this connection particular interest would be attached to the intermediate phenotype. The genetical evidence indicates that this category includes at least one homozygous and two heterozygous conditions. The evidence available on the distribution of this morph type in mountain populations suggests that at least certain genotypes of this category show a unimodal distribution at levels associated with an increase in frequency of the white genotype and a decrease in frequency of at least certain purple genotypes (figs. 11-15). This pattern of variation resembles the Hardy-Weinberg relationship between the heterozygote and the two homozygote frequencies for an allelic pair of genes, the frequency of the intermediate morphs resembling that of the heterozygote (Falconer, 1960). Such relationship might prove to be the case.

Appendix One

THE COMPONENTS OF DIRECTIONALITY IN BEE FORAGING

1. Introduction

2. Procedures

3. Results

4. Discussion

1. Introduction

The observations on pollination made at Rhossili Down and Cantref in 1971 included examination of the nature and interaction of the components of directionality in bee foraging. In this appendix is described how these components may be defined, separated and measured, and how the results obtained may relate to the breeding strategy of C. palustre.

Existing knowledge on bee flight directionality during foraging is largely the result of two papers. The first of these (Bateman, 1947) relates to the foraging of bumblebees in a radish plot. The directions of successive flights were recorded using two 180° categories. In this way a correlation was established between the direction of a flight and that of the preceding flight. However this directionality, termed in the present work "sequential directionality", was not very great.

Levin, Kerster and Niedzlek (1971) performed a more detailed experiment to study the directionality of bees and butterflies in the pollination of a Lythrum salicaria population. This experiment

involved a larger sample size and the use of more precise (45^{o}) direction categories. The values of sequential directionality so obtained were higher than those of Bateman and were quantified in terms of "unit directionality". This was defined as the increment of distance gained by pollen relative to the absolute flight distance. In addition it was established that in the butterflies studied there existed a tendency to turn to the right rather than the left. In the present work this force is termed the "turning force".

Two forces have thus been demonstrated as being involved in bee flight directionality; a sequential directionality and a turning force. In this work is described a third force, which is termed "overall directionality" and may be defined as the tendency of a foraging species to move in an overall direction during foraging.

2. Procedures

Observations were conducted on the Rhossili Down and Cantref populations of C. palustre described previously (Ch. 6). The observations at Rhossili Down were taken continuously from 14.00-20.30 hrs. on July 7 1971, and from 11.30-20.30 hrs. on July 8 1971. The observations at Cantref were taken from 9.30-21.00 hrs on August 6 1971, and 10.30-2100 hrs. on August 7 1971. Throughout the observations the weather was completely clear and sunny, with very little breeze. Observations were restricted to the principal pollinating species at each population, which for Rhossili Down

was Apis mellifera and for Cantref was Bombus lucorum.

In the Rhossili Down experiment the direction of each of 658 flights between inflorescences was recorded using two 180° categories, one comprising the 180° about east and the other the 180° about west. In the Cantref experiment the direction of each of 1671 flights was recorded, using eight 45° categories - north, north-east, etc.

3. Results

(a) Overall Directionality

Values were derived for the total number of flights in each direction category on each day of recording. Only the observations taken on the second day at Rhossili Down failed to demonstrate any overall directionality; on this day 190 flights were recorded as being in the 180° about east while 191 flights were recorded in the opposite direction. The results from the preceding day at Rhossili Down indicated a significant bias towards the east category, 163 flights being in this direction as opposed to 114 in the opposite direction (Chi-squared = 4.33, 'p' = 0.05-0.02). The results from Cantref indicated on the first day a progressive bias towards southerly flights, and on the following day a progressive bias in the opposite direction (figs. 26 & 27).

Fig.26 Overall directionality of Bombus lucorum flights at Cantref

	DIRECTION							
	N.	N.E.	E.	S.E.	S.	S.W.	W.	N.W.
August 6, 1971	33	48	54	64	70	51	39	30
August 7, 1971	219	215	213	130	94	118	108	185

Values indicate number of flights of each type.

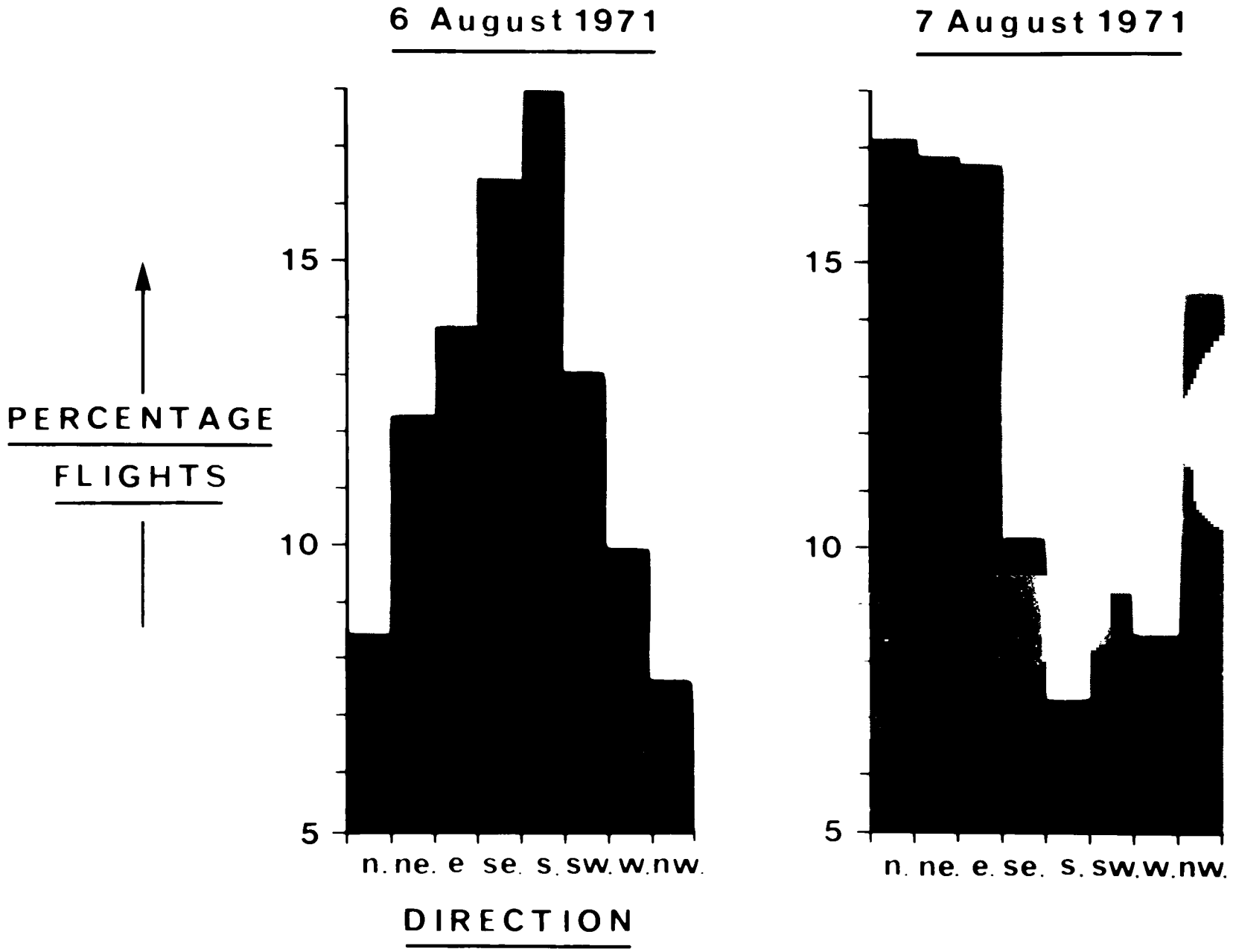


Fig. 27

Overall Directionality of Bombus lucorum Flights at Cantref

(b) Sequential Directionality

Rhossili Down

Figure 28 summarises, for the Rhossili Down population, the directional relationship between the first and the subsequent flight (the "base" and "response" flights) for each consecutive pair of flights recorded. It should be noted that, for the east base direction, the presence of a significantly larger number of response flights in the east than in the west response flight direction does not by itself constitute evidence of sequential directionality since the presence of the overall directionality favouring eastward flights would itself have produced at least some such effect.

The simplest evidence of sequential directionality derives from the response flights of the west base direction. Whereas for this base direction overall directionality alone would make the number of eastward responses greater than the number of westward responses, in the results obtained the reverse is the case and the significant deviation of the 95:145 ratio from 1:1 is sufficient to establish the existence of a strong sequential directionality (Chi-squared = 10.42, 'p' = 0.01-0.001). It is of course the case that whereas this significant deviation does indicate sequential directionality, the absence of a significant deviation could not be interpreted as indicating that sequential directionality was absent.

Fig.28 Directional relationships between Apis mellifera flights at Rhossili Down

		"RESPONSE" FLIGHT	
		East	West
"BASE" FLIGHT	East	179	95
	West	95	145

Values indicate number of flights of each type.

Fig.29 Directional relationships between Bombus lucorum flights at Cantref

"BASE" FLIGHT	"RESPONSE" FLIGHT							
	N.	N.E.	E.	S.E.	S.	S.W.	W.	N.W.
N.	59	44	32	10	14	14	19	43
N.E.	49	51	45	24	10	19	7	31
E.	34	42	54	48	23	12	8	17
S.E.	15	33	34	35	16	17	10	7
S.	15	10	23	22	33	26	14	13
S.W.	10	10	12	15	30	28	17	19
W.	13	14	13	7	17	18	31	17
N.W.	38	25	17	16	5	15	29	51

Values indicate number of flights of each type.

Cantref

As described previously, 1671 B. lucorum flights were observed and classified with respect to direction using an eight point scale. The results are listed in figure 29. Figure 30 shows the frequency distribution of response flights for each base flight direction. It was found that the distribution of response flight directions was such as to reach a maximum in almost each case at a value equal to that of the base flight direction. This indicates clearly that the bees exhibited a consistent sequential directionality.

The sequential directionality was quantified using the units of unit directionality proposed by Levin et al. (1971). In their method the number of flights in each direction category was multiplied by a vector representing the displacement of pollen resulting from movement in that direction. On the assumption that consecutive flights in the same direction resulted in a pollen displacement of 1 unit, the relative displacement corresponding to a 90^{o} deviation between consecutive flights was 0 units and that corresponding to a 45^{o} deviation was given by sine 45^{o}, i.e. 0.7071. The summed directionality value when divided by the total number of flights yielded the unit directionality.

This method however is appropriate only to a population showing no overall directionality. In the presence of an overall directionality towards a direction x, those response moves within the 180^{o} about x will have sequential directionality and overall

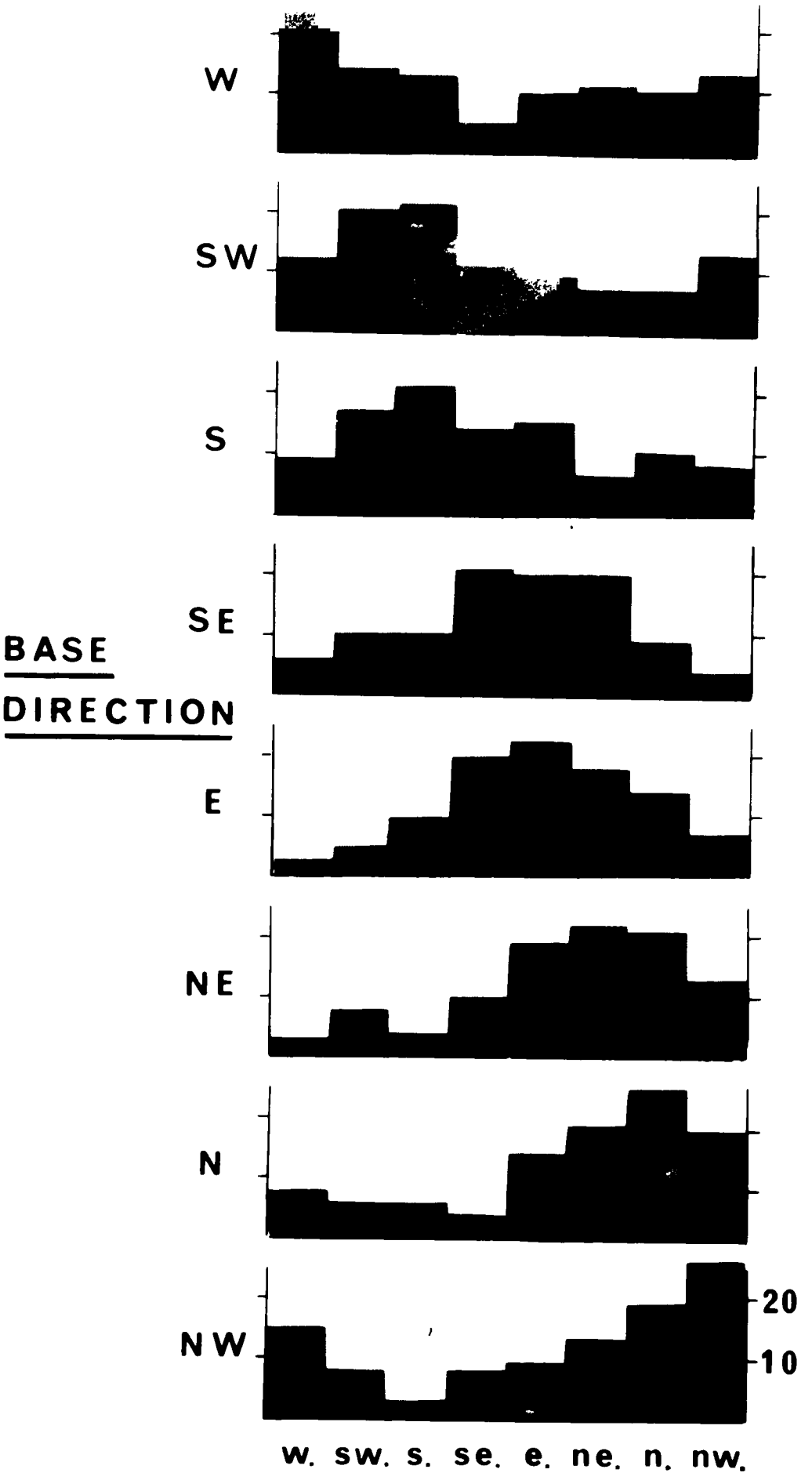


Fig. 30

Directionality in Successive Flights.-

Bombus lucorum, Cantref, August 6&7 1971.
Vertical axes represent percentage frequency distributions of response flights.

directionality acting in some unison. Consequently if overall directionality is ignored the degree of sequential directionality will be exaggerated. The reverse effect occurs in the opposite 180^{o} segment.

If the number of response moves in each direction category were equal overall directionality could be ignored and unit directionality calculated directly. However as a consequence of overall directionality the number of recorded responses increases with proximity to x and accordingly the amount of sequential directionality will be exaggerated. To overcome this the response moves were calculated as percentages of the total number of moves per response direction.

Using this method a value for the summed sequential directionality was obtained which when divided by the total number of flights (calculated as 100 per response direction) gave a unit directionality value of 0.31.

(c) Turning Forces

Using the Cantref data it was possible to calculate whether there existed a tendency for bees to turn more frequently to the left than to the right, or vice-versa. The method of Levin et al. (1971) involved a simple comparison of the numbers of right and left turns but again this was only applicable to a foraging population with no overall directionality. In the present case variation in the total numbers of flights observed for opposite directions with respect to x would have introduced apparent turning

forces consequent upon overall directionality. Accordingly the frequencies of right and left turns were again calculated as percentages per response direction.

The ratio obtained for the number of clockwise to anti-clockwise moves was 274.63:287.31. Since this ratio did not deviate significantly from 1:1 it was concluded that no turning force was apparent (Chi-squared = 0.286, 'p' = 0.80-0.50).

4. Discussion

The occurrence of an apparently frequent overall directionality in bee foraging is an interesting phenomenon which appears not to have been previously described. One might speculate that such a force, weak enough to allow deviations in the direction of foraging but sufficient nevertheless to exert an overall effect, could ensure a constant overall direction for a forage while eliminating the necessity for recording and integrating the directions of individual moves.

However the factors determining overall directionality remain obscure. A possible mechanism for its occurrence would be that bees might commence foraging in the region of population nearest the nest and tend to forage towards the opposite side of the population before returning to the nest. However the reversal of overall directionality over successive days at Cantref does not accord with such a view. It would appear more likely that overall directionality may be related to the influence of wind. Since

wind strength was very light throughout all the observations measurements were not taken of its direction but it nevertheless remains possible that its strength may have been sufficient to encourage flights in a given direction. It is certainly difficult to see how strong winds could fail to produce an overall directionality.

The demonstration of a sequential directionality in bee flight is in accord with the results of the previous workers (Bateman, 1947; Levin et al., 1971). The value of 0.31 for the unit sequential directionality of B. lucorum at Cantref was similar to the value of 0.39 obtained by Levin et al. (1971) for a mixed population of bees foraging upon Lythrum salicaria. The demonstration of the absence of a turning force in B. lucorum foraging accords with the result of the latter workers for bees.

The values of sequential directionality obtained during the present work were not sufficient to cause strongly directional feeding trends, a point emphasised by the previous workers. However both sequential directionality and overall directionality will be of importance in increasing the distance to which pollen is dispersed during foraging. The distance and direction of pollen transfer will be of importance in determining the pattern of pollen flow between morph types in any population in which the morph types show a heterogeneous distribution relative to each other (Ch. 6).

The effect of directionality in pollen transfer will be

dependent upon the degree to which pollen is carried over between plants by the foraging species. During population size fluctuations, such as those demonstrated on the Gower Peninsula (Ch. 3), the number of flowers per plant may be reduced during unfavourable years. Consequently increase would occur in the degree of pollen carry over and hence in the degree of gene flow between plants. Increase in the latter would also be promoted by an increase in pollinator flight distance consequent on any reduction in plant density (Levin and Kerster, 1969). The adaptive value of this is apparent if it is assumed that higher rates of gene flow are adaptive in unstable conditions (Levins, 1964).

Appendix Two

BREEDING RESULTS

Progeny ratios presented in the order

Purple : Intermediate : Purplish White : White

(1) Greenhouse Pollinations

(a) Self Pollinations

Clyne Common

Purple Morphs

Parent		Progeny Ratio		
7	13	5	0	0
10	2	0	0	2
12	1	0	0	0
38	12	0	0	0

Intermediate Morphs

3	0	1	0	2
5	1	0	0	0
8	7	9	0	6
11	55	40	0	39
14	12	4	0	12
15	1	1	0	1
17	3	24	0	24
25	0	1	0	2

White Morphs

1	0	0	0	10
2	0	0	0	21
9	0	0	0	30
19	0	0	0	37

(b) Cross Pollination

Clyne Common

Purple f. x White m.

Parents		Progeny Ratio		
18 x 6	7	0	0	2
27 x 28	1	0	0	0
33 x 31	12	0	0	5
33 x 37	2	0	0	0
34 x 28	1	0	0	0

White f. x Purple m.

6 x 18	0	0	0	8
19 x 4	1	0	0	14
19 x 7	9	0	14	1
28 x 30	18	0	0	3
28 x 34	0	0	0	10

Penmaen Marsh

Purple f. x White m.

Parents		Progeny Ratio		
a x b	11	0	0	0

White f. x Purple m.

c x d	6	0	0	22
e x f	18	0	0	12

(2) Natural Seed Sets

(a) Bishopston Valley

Purple Morphs

Parent		Progeny Ratio		
A	11	7	0	0

Intermediate Morphs				
A	1	63	0	0
C	1	39	2	0
E	1	45	13	4
N	0	22	3	2
White Morphs				
P	0	24	34	12

(b) Penmaen Marsh

Purple Morphs				
Parent		Progeny Ratio		
C	2	0	0	0
D	3	0	0	1
G	7	0	0	0
H	9	0	0	4
I	3	0	0	0
K	13	0	0	0
L	18	0	0	3
M	4	0	0	1
Intermediate Morphs				
A	2	1	0	0
White Morphs				
C	7	0	0	15
E	10	0	0	30
H	9	1	0	17

Appendix Three

MORPH FREQUENCIES OF THE GOWER POPULATIONS

Seasons 1-4 represent 1968-1971 respectively. Dashes represent seasons in which populations were unscored.

Altitude estimated to nearest 25ft. using 2.5" ordnance survey maps.

Popn. No.	Grid Reference	Altitude ft.	Season	Popn. Size	Morph Frequency P.	Int.	ppW.	W.
1	SS 527946	25	1	61	60	0	1	0
			2	27	27	0	0	0
			3	36	25	2	0	0
			4	71	50	1	0	0
2	SS 426935	25	1	176	160	0	0	16
			2	20	17	0	0	3
			3	19	10	0	0	0
			4	394	215	0	0	18
4	SS 415901	125	1	c.500	86	92	2	5
			2	c.600	137	149	17	2
			3	c.1000	119	92	6	4
			4	c.1250	282	49	2	0
5	SS 415897	125	1	120	106	0	0	14
			2	88	75	4	0	9
			3	433	240	4	0	56
			4	398	208	1	0	33
6	SS 416897	150	1	42	16	9	0	17
			2	46	24	1	1	20
			3	48	36	2	3	7
			4	203	98	3	0	44
7	SS 417888	175	1	65	65	0	0	0
			2	45	45	0	0	0
			3	58	38	0	0	0
			4	81	64	0	0	0

Popn. No.	Grid Reference	Altitude ft.	Season	Popn. Size	Morph Frequency P.	Int.	ppW.	W.
8	SS 418889	325	1	99	99	0	0	0
			2	94	94	0	0	0
			3	42	40	0	0	0
			4	486	339	0	0	0
9	SS 416890	125	1	105	105	0	0	0
			2	33	33	0	0	0
			3	235	105	1	0	0
			4	511	339	1	0	1
10	SS 418882	350	1	298	122	0	0	2
			2	254	246	6	1	1
			3	403	207	3	0	4
			4	c.1500	620	19	11	0
11	SS 414875	150	1	-	-	-	-	-
			2	-	-	-	-	-
			3	-	-	-	-	-
			4	369	116	0	0	0
12	SS 420873	200	1	320	55	37	2	8
			2	280	267	1	2	10
			3	53	12	0	0	1
			4	624	154	0	1	4
13	SS 434879	300	1	58	56	2	0	0
			2	17	16	1	0	0
			3	68	56	0	0	0
			4	669	516	4	0	0
14	SS 422872	175	1	210	105	0	0	1
			2	406	406	0	0	0
			3	203	96	0	0	0
			4	155	24	0	0	0
15	SS 424869	175	1	33	29	0	0	4
			2	69	66	0	0	3
			3	5	2	0	0	1
			4	25	7	0	0	0

Popn. No.	Grid Reference	Altitude ft.	Season	Popn. Size	Morph Frequency P.	Int.	ppW.	W.
16	SS 428866	200	1	127	123	0	0	4
			2	130	125	0	0	5
			3	15	11	0	0	0
			4	210	118	0	0	3
17	SS 446856	200	1	73	72	0	0	1
			2	51	51	0	0	0
			3	414	362	0	0	0
			4	257	54	0	0	0
18	SS 438860	175	1	188	188	0	0	0
			2	495	492	2	1	0
			3	151	121	0	0	0
			4	185	77	0	0	0
19	SS 448853	225	1	326	213	48	11	54
			2	118	91	7	1	19
			3	102	82	6	1	10
			4	87	48	0	0	1
20	SS 452853	200	1	213	200	0	0	13
			2	-	-	-	-	-
			3	-	-	-	-	-
			4	257	199	0	0	7
21	SS 454851	175	1	35	23	0	0	12
			2	146	69	1	0	76
			3	14	9	1	0	4
			4	44	28	2	0	8
23	SS 458849	125	1	163	131	0	0	32
			2	132	107	2	0	23
			3	68	49	0	0	17
			4	696	436	0	0	129
25	SS 460849	50	1	41	29	1	0	11
			2	56	43	1	0	12
			3	105	72	0	0	20
			4	124	59	0	0	14

Popn. No.	Grid Reference	Altitude ft.	Season	Popn. Size	Morph Frequency P.	Int.	ppW.	W.
26	SS 479859	200	1	15	13	0	0	2
			2	105	99	0	0	6
			3	7	5	0	0	1
			4	115	58	0	0	8
27	SS482857	200	1	287	200	2	0	85
			2	27	18	0	0	9
			3	38	26	1	0	8
			4	135	46	0	0	10
28	SS 484856	150	1	90	88	0	0	2
			2	4	4	0	0	0
			3	19	19	0	0	0
			4	273	177	0	0	16
29	SS 486857	200	1	49	42	0	0	7
			2	71	64	0	0	7
			3	45	33	0	0	2
			4	71	26	0	0	3
30	SS 488857	200	1	34	5	0	0	29
			2	7	4	0	0	3
			3	4	3	0	0	1
			4	17	3	0	0	10
31	SS 490856	175	1	25	10	0	0	15
			2	49	27	0	0	22
			3	174	88	0	0	78
			4	534	277	0	12	199
32	SS 497852	50	1	123	121	0	0	2
			2	93	90	1	0	2
			3	5	4	0	0	1
			4	31	14	0	3	0
33	SS 499853	175	1	80	78	1	0	1
			2	117	104	4	0	9
			3	7	6	0	0	1
			4	53	10	1	2	0

Popn. No.	Grid Reference	Altitude ft.	Season	Popn. Size	Morph Frequency P.	Int.	ppW.	W.
35	SS 509850	250	1	c.2000	400	4	4	1
			2	c.2000	314	1	0	3
			3	198	180	0	0	0
			4	1427	546	1	2	0
36	SS 480903	375	1	76	39	7	7	23
			2	123	74	22	10	17
			3	51	40	3	1	1
			4	102	30	3	3	5
37	SS 487900	450	1	201	180	6	2	13
			2	164	138	15	0	11
			3	394	110	5	1	31
			4	772	148	10	6	4
38	SS 503873	25	1	262	127	14	0	121
			2	47	21	3	0	23
			3	58	27	4	0	24
			4	13	6	1	3	1
39	SS 511881	50	1	74	72	0	0	2
			2	76	74	0	1	1
			3	103	87	0	0	2
			4	38	20	0	2	0
40	SS 525879	125	1	36	27	1	1	7
			2	4	4	0	0	0
			3	0	0	0	0	0
			4	3	3	0	0	0
41	SS 526879	150	1	100	53	8	8	31
			2	109	72	10	6	21
			3	114	60	12	13	21
			4	532	222	28	2	90
42	SS 528880	150	1	185	54	71	13	47
			2	49	17	17	3	12
			3	29	10	13	4	2
			4	82	31	7	0	7

Popn. No.	Grid Reference	Altitude ft.	Season	Popn. Size	Morph Frequency P.	Int.	ppW.	W.
43	SS 531877	125	1	220	42	2	0	30
			2	63	32	0	1	14
			3	582	323	10	15	128
			4	1360	469	15	2	136
45	SS 535883	25	1	212	106	4	6	96
			2	67	17	0	0	50
			3	448	204	9	17	203
			4	404	122	8	1	104
46	SS 537884	50	1	67	14	7	3	43
			2	47	19	0	1	27
			3	195	54	12	23	82
			4	103	19	6	0	20
47	SS 539884	125	1	180	58	44	30	48
			2	20	6	1	0	13
			3	12	2	2	3	3
			4	349	107	48	5	46
48	SS 565870	175	1	64	40	15	2	7
			2	159	140	17	0	2
			3	38	32	2	1	1
			4	50	13	2	0	0
49	SS 570865	275	1	52	29	7	1	15
			2	145	90	11	12	32
			3	87	57	12	0	12
			4	91	25	1	1	3
50	SS 567882	125	1	149	47	88	0	14
			2	2	1	1	0	0
			3	7	2	3	1	0
			4	49	8	19	1	4
51	SS 568880	75	1	145	4	133	6	2
			2	50	2	46	2	0
			3	154	6	130	0	2
			4	170	13	89	3	6

Popn. No.	Grid Reference	Altitude ft.	Season	Popn. Size	Morph Frequency P.	Int.	ppW.	W.
53	SS 579872	75	1	89	42	35	7	5
			2	14	7	4	2	1
			3	61	31	24	3	2
			4	36	11	5	0	1
54	SS 581873	150	1	186	150	34	1	1
			2	141	108	21	5	7
			3	163	112	30	8	3
			4	12	4	1	0	0
55	SS 587875	75	1	327	65	46	63	28
			2	0	0	0	0	0
			3	20	12	5	3	0
			4	104	23	16	3	2
57	SS 591895	275	1	95	35	23	16	21
			2	137	106	16	3	12
			3	148	55	5	1	17
			4	489	59	8	0	18
58	SS 595875	200	1	19	3	8	1	7
			2	18	10	2	0	6
			3	8	4	3	0	1
			4	23	6	3	1	2
59	SS 596873	50	1	50	31	8	5	6
			2	126	95	13	2	16
			3	217	159	27	10	10
			4	142	51	24	2	5
60	SS 573915	250	1	242	46	68	32	96
			2	116	30	22	8	56
			3	194	87	39	19	49
			4	554	123	14	14	37
61	SS 579924	300	1	101	41	43	6	10
			2	166	108	38	3	17
			3	162	57	20	5	7
			4	710	236	11	6	13

Popn. No.	Grid Reference	Altitude ft.	Season	Popn. Size	Morph Frequency P.	Int.	ppW.	W.
62	SN 613016	175	1	109	106	3	0	0
			2	22	22	0	0	0
			3	70	64	2	0	0
			4	30	23	0	0	0
71	SS 520885	350	1	85	84	0	0	1
			2	76	73	0	0	3
			3	173	140	0	0	3
			4	78	36	0	0	0
73	SS 416894	125	1	-	-	-	-	-
			2	86	44	2	0	40
			3	99	27	2	1	25
			4	97	25	1	0	12
74	SS 416893	125	1	-	-	-	-	-
			2	24	14	2	1	7
			3	2	1	0	0	0
			4	24	16	0	0	2
75	SS 492856	200	1	-	-	-	-	-
			2	16	13	0	0	3
			3	43	34	0	0	8
			4	20	7	0	0	0
76	SS 496854	100	1	-	-	-	-	-
			2	177	151	0	0	26
			3	21	16	0	0	2
			4	5	1	0	0	1
77	SS 526885	275	1	-	-	-	-	-
			2	235	126	4	2	103
			3	534	252	5	19	195
			4	480	88	1	1	65

Appendix Four

MORPH FREQUENCIES OF INLAND POPULATIONS AND PEMBROKESHIRE COASTAL POPULATIONS

Populations listed by counties arranged in alphabetical order.

Dates of scoring as follows:-

1968 : Populations 1-71

1969 : Populations 72-87

1970 : Populations 88-214 & 288-300

1971 : Populations 215-287

Moisture and exposure graded using a subjective 1-3 scale in each case, thus:

	Moisture	Exposure
1.	Moderately dry	Fairly sheltered
2.	Moderately damp	Moderately exposed
3.	Very damp	Very exposed

Altitude estimated to nearest 25 ft. using 2.5" ordnance survey maps.

Popn. No.	Grid Reference	Morph Frequency P.	I.	ppW.	W.	Size	Altitude ft.	Moisture	Exposure
	Berkshire								
292	SP 479073	100	0	0	0	900	400	-	-
293	SP 465073	159	0	0	0	159	450	-	-
	Breconshire								
88	SO 053136	100	0	0	0	1000	1100	1	2
89	SN 882542	109	0	0	0	130	875	2	3
90	SN 848278	100	0	0	0	1000	1075	2	2
91	SN 979239	136	0	0	0	148	900	3	2
92	SN 975233	106	0	0	0	109	1000	2	2
93	SN 893238	100	0	0	0	105	1000	2	3
94	SN 927155	100	0	0	0	140	1125	3	2
95	SN 891354	100	0	0	0	200	1250	3	2

Popn. No.	Grid Reference	Morph Frequency P.	I.	ppW.	W.	Popn. Size	Altitude ft.	Moisture	Exposure
96	SO 005132	100	0	0	0	500	875	2	2
97	SO 022286	100	0	0	0	300	475	3	2
98	SN 983288	100	1	0	0	200	525	2	2
100	SN 944099	98	2	0	0	121	900	3	2
101	SN 910367	98	2	0	0	1000	975	2	2
102	SO 006136	98	2	0	0	500	900	3	2
103	SO 002184	98	2	0	0	216	1475	3	3
104	SO 060173	86	2	0	0	92	975	1	3
105	SO 063170	386	0	0	1	389	875	2	3
106	SO 040245	516	0	0	2	518	800	1	2
107	SN 972225	101	0	0	1	115	1100	3	2
108	SO 056122	99	0	0	1	261	1200	3	2
109	SO 008284	99	0	0	1	300	500	2	2
111	SO 083173	148	1	0	2	179	775	2	2
112	SO 094183	111	5	0	2	119	625	2	2
113	SN 925298	98	0	0	2	1000	700	3	2
115	SO 055127	96	0	1	3	266	1125	1	2
116	SN 994434	96	1	0	3	200	1150	3	2
117	SN 893372	84	13	0	3	300	1300	3	2
118	SO 083169	85	0	0	3	95	1000	2	3
119	SN 945105	172	1	0	7	273	925	2	2
120	SN 887212	95	1	0	4	500	1125	2	2
121	SN 853216	94	2	0	4	450	1375	1	3
122	SO 117242	95	0	0	5	500	375	2	2
123	SN 853206	67	26	2	5	165	1225	2	2
124	SN 865246	91	2	0	7	1000	1175	2	2
126	SN 833275	81	11	0	8	170	1050	3	2
127	SN 860244	90	0	0	10	400	1350	1	2
128	SN 988178	90	0	0	10	175	1350	2	2
129	SN 983133	85	5	0	10	195	1200	3	3
130	SO 170295	99	26	2	15	900	1250	3	2
131	SN 980132	79	4	2	15	250	1225	3	2

Popn. No.	Grid Reference	Morph Frequency P.	I.	ppW.	W.	Popn. Size	Altitude ft.	Moisture	Exposure
132	SO 161169	83	0	0	17	500	1350	2	1
133	SN 992175	81	2	0	17	1500	1275	3	2
134	SN 888215	76	6	1	17	250	1150	3	2
135	SN 988189	82	0	0	18	300	1350	2	2
139	SN 889232	63	12	1	24	300	1125	3	2
140	SN 972210	96	5	0	33	208	1250	2	2
141	SN 987175	72	0	2	26	200	1425	3	2
142	SN 881206	73	0	0	27	131	1175	2	3
143	SN 992439	73	0	0	27	150	1125	2	2
144	SN 973216	71	2	0	27	300	1225	3	2
147	SN 852192	70	0	0	30	212	1100	2	2
148	SN 994166	64	1	1	34	1000	1175	3	2
149	SN 884210	64	0	0	36	300	1150	3	2
150	SN 986168	65	0	0	39	129	1475	3	3
153	SO 057116	60	0	0	40	300	1125	2	2
154	SN 967126	45	12	0	43	153	1150	3	2
155	SN 935125	46	0	0	54	400	1050	3	1
156	SO 160164	25	3	0	72	300	1525	3	1
157	SO 157168	17	0	0	83	250	1450	2	2
215	SO 185253	95	0	1	4	500	500	1	2
216	SO 186255	97	0	0	3	353	600	2	1
217	SO 182286	92	2	1	5	257	850	3	2
218	SN 951254	92	1	0	7	5000	1075	3	1
219	SN 962261	99	0	0	1	300	1075	1	3
220	SN 955257	49	1	0	50	149	1025	3	1
221	SN 909288	99	1	0	0	292	675	2	2
222	SN 872298	98	2	0	0	550	750	3	2
223	SN 837325	99	0	0	1	2500	500	1	2
228	SO 240152	87	0	3	10	500	275	2	1
229	SN 982246	100	0	0	0	1200	875	3	2
230	SN 949249	83	0	0	17	800	1050	3	1
231	SN 940237	97	0	0	3	3000	1000	3	1
232	SN 948262	97	0	0	3	750	1025	3	1

Popn. No.	Grid Reference	Morph Frequency P.	I.	ppW.	W.	Popn. Size	Altitude ft.	Moisture	Exposure
233	SN 946294	98	2	0	1	180	650	3	1
234	SN 948292	100	0	0	0	1000	700	3	1
236	SO 079259	98	1	0	1	1500	450	3	2
237	SO 122218	94	0	1	5	379	400	2	2
238	SO 085257	98	0	1	1	500	400	2	2
239	SO 178211	94	0	1	5	1000	400	3	2
240	SO 087316	99	0	0	1	1000	675	2	2
241	SO 116402	98	1	0	1	3000	500	3	1
242	SO 119398	99	0	1	0	308	375	2	3
243	SO 107421	90	2	0	8	1300	350	1	3
244	SO 061508	99	0	1	0	1010	400	3	2
245	SO 091280	100	0	0	0	1760	650	2	2
246	SO 111283	100	0	0	0	330	625	1	2
247	SO 117281	100	0	0	0	1500	525	3	2
248	SO 092296	100	0	0	0	1800	850	3	2
249	SO 133304	100	0	0	0	370	525	3	2
250	SO 139242	100	0	0	0	1500	525	3	2
251	SO 098333	100	0	0	0	600	500	2	3
252	SO 039254	98	0	0	2	550	850	3	1
253	SO 027360	100	0	0	0	500	725	2	2
254	SO 013385	100	0	0	0	1500	800	2	1
255	SN 981364	100	0	0	0	1150	800	2	2
256	SO 002328	100	0	0	0	500	775	2	3
257	SO 041259	98	0	0	2	1300	850	2	1
262	SO 126185	98	1	0	1	4000	550	3	2
263	SN 977315	100	0	0	0	800	650	2	2
264	SN 952336	97	3	0	0	1250	750	2	2
265	SN 936350	100	0	0	0	900	800	2	2
266	SN 961247	128	0	0	228	605	1075	3	2
267	SN 930293	100	0	0	0	1400	625	2	1
268	SN 989180	395	0	0	18	1200	1275	1	3
269	SN 992168	521	0	0	178	1700	1175	2	2
288	SO 013213	139	27	5	54	500	2550	3	3

Popn. No.	Grid Reference	Morph Frequency P.	I.	ppW.	W.	Popn. Size	Altitude ft.	Moisture	Exposure
289	SO 024204	492	161	12	181	500	2025	3	3
	Buckinghamshire								
291	SP 606147	100	0	0	0	2000	225	-	-
	Caernarvonshire								
159	SH 813455	100	0	0	0	300	1150	2	2
189	SH 764436	94	4	0	2	109	1550	3	3
	Carmarthenshire								
66	SN 736138	117	4	0	1	140	600	-	-
67	SN 728142	138	13	3	5	159	575	-	-
99	SN 738231	99	1	0	0	155	400	1	2
110	SN 719173	80	17	2	1	450	1300	3	2
125	SN 729182	50	43	0	7	200	1550	3	2
136	SN 793264	75	7	0	18	298	1100	3	2
224	SN 807344	100	0	0	0	550	350	1	2
225	SN 789349	99	0	1	0	132	275	1	3
226	SN 695293	100	0	0	0	566	150	3	2
227	SN 694285	99	1	0	0	1400	150	2	2
235	SN 878295	100	0	0	0	700	750	2	3
258	SN 824332	100	0	0	0	1300	450	2	3
259	SN 772352	100	0	0	0	750	250	3	2
260	SN 778338	98	0	0	2	1100	325	2	3
261	SN 815336	98	1	0	1	500	425	3	1
286	SN 253135	100	0	0	0	500	50	-	-
287	SN 524151	100	0	0	0	190	250	-	-
	Denbighshire								
158	SJ 005554	100	0	0	0	195	1475	2	2
161	SH 934448	94	0	0	0	300	1050	3	3
162	SH 964522	100	0	0	0	450	1075	3	2
163	SH 911480	100	0	0	0	900	1275	3	2
167	SH 961516	99	1	0	0	300	1150	2	3
168	SJ 009574	97	1	2	0	180	1350	3	3

Popn. No.	Grid Reference	Morph Frequency P.	I.	ppW.	W.	Popn. Size	Altitude ft.	Moisture	Exposure
169	SH 984574	98	1	1	0	300	1250	3	3
170	SH 929567	98	2	0	0	500	1275	3	2
171	SH 981569	98	2	0	0	220	1200	3	3
172	SH 950452	97	3	0	0	450	1125	3	2
173	SH 974561	97	3	0	0	204	1150	3	2
174	SH 917505	98	0	1	1	185	950	3	2
175	SH 997543	99	0	0	1	400	1400	3	2
176	SJ 007563	99	0	0	1	138	1525	3	3
177	SH 962495	98	1	0	1	400	1100	3	3
178	SH 995588	98	1	0	1	700	1250	3	2
182	SH 973610	95	4	0	1	130	1275	3	2
184	SH 951587	91	8	0	1	500	1500	3	2
187	SH 896542	96	2	0	2	400	1175	3	3
190	SH 934484	92	5	1	2	850	1225	3	2
191	SH 959593	54	7	37	2	120	1450	3	2
196	SH 921481	92	1	0	7	225	1275	3	2
197	SH 897485	89	3	1	7	1000	1275	3	2
201	SH 944583	69	22	1	8	1000	1475	3	2
205	SH 942578	83	7	0	10	1000	1450	3	2
	Glamorganshire								
114	SN 952066	95	3	0	2	300	625	2	3
	Merionethshire								
160	SH 840400	100	0	0	0	150	1075	3	3
164	SJ 008415	100	0	0	0	200	725	3	3
165	SH 981396	100	0	0	0	200	825	3	2
166	SJ 019379	100	0	0	0	364	500	2	3
179	SH 908450	98	1	0	1	200	1250	3	3
180	SJ 059419	98	1	0	1	200	450	2	3
181	SH 717424	96	3	0	1	300	950	3	2
183	SJ 014311	94	5	0	1	400	1550	3	2
186	SJ 015309	96	2	0	2	500	1525	3	2
188	SJ 005411	96	2	0	2	500	750	3	2

Popn. No.	Grid Reference	Morph Frequency P.	I.	ppW.	W.	Popn. Size	Altitude ft.	Moisture	Exposure
192	SH 805397	97	0	0	3	1000	1225	2	3
193	SH 732361	97	0	0	3	450	875	3	2
195	SJ 016307	88	6	0	6	501	1575	3	3
198	SH 790396	71	22	0	7	155	1500	3	3
199	SH 790395	50	43	0	7	124	1450	3	3
200	SH 949306	91	1	0	8	132	1000	3	2
202	SH 783397	87	4	0	9	200	1450	3	2
204	SH 906453	84	6	0	10	500	1325	3	2
206	SJ 009314	81	5	0	14	250	1525	2	3
207	SJ 009375	82	3	0	15	312	500	2	2
209	SH 875301	70	2	0	17	288	550	2	2
210	SH 754417	78	0	0	22	450	1425	3	3
211	SH 952300	70	7	1	22	156	1100	2	3
212	SH 798396	45	33	0	22	400	1500	3	3
214	SH 946267	46	0	0	40	157	1550	3	3
	Monmouthshire								
68	ST 392945	100	6	1	18	125	125	-	-
69	ST 413939	65	0	0	4	69	875	-	-
70	ST 409927	64	0	0	0	64	500	-	-
78	ST 437864	81	0	0	0	81	25	-	-
79	ST 438863	146	0	0	2	148	25	-	-
81	ST 443865	222	0	0	21	1200	25	-	-
82	ST 432932	42	0	0	3	45	500	-	-
83	ST 431935	100	1	0	5	700	525	-	-
84	SO 528012	125	1	0	4	130	150	-	-
85	SO 525022	141	0	0	4	145	350	-	-
86	SO 525024	112	0	0	5	125	400	-	-
151	SO 167131	59	2	0	39	300	1325	2	2
	Montgomeryshire								
185	SH 947267	97	1	0	2	143	1550	2	3
194	SH 936237	93	0	1	6	550	1450	3	2

Popn. No.	Grid Reference	Morph Frequency P.	I.	ppW.	W.	Popn. Size	Altitude ft.	Moisture	Exposure
203	SJ 048268	85	6	0	9	200	775	2	2
208	SJ 019301	77	6	0	17	350	1550	3	3
213	SJ 016305	69	8	0	23	240	1575	3	2
	Pembrokeshire								
270	SN 012409	51	0	0	0	60	275	-	-
271	SN 010399	48	107	22	0	224	100	-	-
272	SM 907408	99	0	0	0	114	75	-	-
273	SM 805285	100	0	0	0	550	300	-	-
274	SM 772263	98	0	2	0	175	200	-	-
275	SS 016974	100	0	0	0	187	100	-	-
276	SS 045977	107	0	0	0	107	200	-	-
277	SS 061972	100	0	0	0	206	150	-	-
279	SR 988948	87	0	0	13	1200	100	-	-
280	SR 993958	68	0	0	0	69	50	-	-
281	SM 887009	100	0	0	0	500	200	-	-
282	SM 025859	100	0	0	0	246	100	-	-
283	SM 855029	99	0	0	1	350	75	-	-
284	SM 877008	100	0	0	0	450	75	-	-
285	SN 122071	93	0	7	0	300	150	-	-
	West Riding of Yorkshire								
295	SD 884672	60	0	0	0	74	1225	-	-
296	SD 844624	134	7	0	0	299	1100	-	-
297	SD 877617	172	1	0	0	466	1050	-	-
298	SD 885616	117	27	9	59	261	1000	-	-
300	SD 881669	90	33	4	6	1500	1250	-	-

Appendix Five

BIBLIOGRAPHY

1. General List

2. References to white flowered types within the British Isles.

Titles of journals abbreviated, where possible, according to the World List of Scientific Periodicals, edition 4.

1. General List

Alston, J.L. & Bradshaw, A.D. (1966) Evolution in closely adjacent plant populations. 2. Agrostis stolonifera in maritime habitats. Heredity, Lond., 21, 649-664.

Anderson, E. (1933) Color variation in a Missouri colony of Hepatica acutiloba. Rhodora, 35, 66-67.

_____ (1936) Colour variation in eastern North American flowers as exemplified by Hepatica acutiloba. Rhodora, 38, 301-304.

_____ (1953) Introgressive Hybridisation. Biol. Rev., 28, 280-307.

Baker, H.G. (1961) The adaptations of flowering plants to nocturnal and crepuscular pollinators. Q. Rev. Biol., 36, 64-73.

Baldwin, J.T. (1970) White phase in flower development in Cypripedium acaule. Rhodora, 72, 142-143.

Barber, H.N. & Jackson, W.D. (1957) Natural selection in action in Eucalyptus. Nature, Lond., 179, 1267-1269.

Bateman, A.J. (1947) Contamination in seed crops. 3. Relation with isolation distance. Heredity, Lond., 1, 303-336.

Beattie, A.J. (1971) Itinerant pollinators in a forest. Madrono, 21, 120-124.

Beeks, R.M. (1962) Variation and hybridisation in southern California populations of Diplacus (Scrophulariaceae). Aliso, 5, 83-122.

Bonnier, G. (1894) Recherches expérimentales sur l'adaptation des plantes au climat alpin. Annls. Sci. nat. (Bot.), 20, 217-360.

Bradshaw, A.D. (1960) Population differentiation in Agrostis tenuis Sibth. 3. Populations in varied environments. New Phytol., 59, 92-103.

Bradshaw, A.D., McNeilly, T.S. & Gregory, R.P.G. (1965) Industrialization, evolution and the development of heavy metal tolerance in plants. Brit. Ecol. Soc. Symp., 5th. (1964), 327-343.

Brinkmann, W.A.R. (1971) What is a foehn? Weather, Lond., 26, 230-239.

Bruggemann, P.F. (1958) Insects and environments of the high arctic. Int. Congr. Ent., 10th. (1956), 1, 695-702.

Burkill, J.H. & Willis, J.C. (1894) Botanical notes from north Cardiganshire. J. Bot., Lond., 32, 4-10.

Butler, C.G. (1954) The World of the Honeybee. London.

Cain, A.J. & Sheppard, P.M. (1954) Natural selection in Cepaea. Genetics, Princeton, 39, 89-116.

Clapham, A.R., Tutin, T.G. & Warburg, E.F. (1962) Flora of the British Isles. Cambridge.

Cook, S.A. (1962) Genetic system, variation, and adaptation in Eschscholzia californica. Evolution, Lancaster, Pa., 16, 278-299.

Creed, E.R., Dowdeswell, W.H., Ford, E.B. & McWhirter, K.G. (1959) Evolutionary studies on Maniola jurtina: the English mainland 1956-57. Heredity, Lond., 13, 363-391.

Creed, E.R., Ford, E.B. & McWhirter, K.G. (1964) Evolutionary studies on Maniola jurtina: the Isles of Scilly, 1958-59. Heredity, Lond., 19, 471-488.

Crosby, J.L. (1949) Selection of an unfavorable gene complex. Evolution, Lancaster, Pa., 3, 212-230.

Czapik, R. (1958) Badania kariologiczne nad polskimi gatunkami rodzaju Cirsium Mill. em. Scop. (Karyological studies in species of Cirsium Mill. em. Scop. occurring in Poland). Acta Soc. Bot. Pol., 27, 483-489.

Daday, H. (1954) Gene frequencies in wild populations of Trifolium repens. 2. Distribution by altitude. Heredity, Lond., 8, 377-384.

Darwin, C. (1876) The Effects of Cross and Self-Fertilisation in the Vegetable Kingdom. London.

Dawson, P.S. (1969) A conflict between Darwinian fitness and population fitness in Tribolium "competition" experiments. Genetics, Princeton, 62, 413-419.

De Ruiter, L. (1952) Some aspects on the camouflage of stick caterpillars. Behaviour, 4, 222-232.

De Wildeman, E. & Durand, Th. (1899) Prodrome de la Flore Belge. Brussels.

De Wit, H.C.D. (1967) Plants of the World. London and New York.

Diver, C. (1940) The problem of closely related species living in the same area. The New Systematics (ed. Julian Huxley), Oxford.

Dobzhansky, T. & Pavlovsky, O. (1957) An experimental study of interaction between genetic drift and natural selection. Evolution, Lancaster, Pa., 11, 311-319.

Dowdeswell, W.H. & Ford, E.B. (1953) The influence of isolation on variability in the butterfly Maniola jurtina L. Symp. Soc. exp. Biol., 7, 254-273.

Dowdeswell, W.H., Ford, E.B. & McWhirter, K.G. (1960) Further studies on the evolution of Maniola jurtina in the Isles of Scilly. Heredity, Lond., 14, 333-364.

Dronamraju, K.R. (1960) Selective visits of butterflies to flowers; a possible factor in sympatric speciation. Nature, Lond., 186, 178.

Druce, G.C. (1911) The international phytogeographical excursion in the British Isles. New Phytol., 10, 306-328.

_____ (1930) The Flora of Northamptonshire. Arbroath.

_____ (1932) The Comital Flora of the British Isles. Arbroath.

Eisikowitch, D. & Galil, J. (1971) Effect of wind on the pollination of Pancratium maritimum L. (Amaryllidaceae) by hawkmoths (Lepidoptera : Sphingidae). J. Anim. Ecol., 40, 673-678.

Epling, C. & Dobzhansky, T. (1942) Genetics of natural populations. 6. Microgeographic races in Linanthus Parryae. Genetics, Princeton, 27, 317-332.

Epling, C., Lewis, H. & Ball, F.M. (1960) The breeding group and seed storage; a study in population dynamics. Evolution, Lancaster, Pa., 14, 238-255.

Falconer, D.S. (1960) Introduction to Quantitative Genetics. Edinburgh & London.

Fiori, A. (1969) Nuova Flora Analitica D'Italia. Bologna.

Fisher, R.A. (1930) The Genetical Theory of Natural Selection. Oxford.

Ford, E.B. (1940) Polymorphism and Taxonomy. The New Systematics (ed. Julian Huxley). Oxford.

_____ (1964) Ecological Genetics. London & New York.

Ford, H.D. & Ford, E.B. (1930) Fluctuation in numbers and its influence on variation in Melitaea aurinia. Trans. R. ent. Soc. Lond., 78, 345-351.

Free, J.B. (1955) The collection of food by bumblebees. Insectes soc., 2, 195-212.

FREE, J.B., & BUTLER, C.G. (1959) Bumblebees. London.

Gadella, T.W.J., & Kliphuis, E. (1963) Chromosome numbers of flowering plants in the Netherlands. Acta bot. neerl., 12, 195-230.

_____ (1966) Chromosome numbers of flowering plants in the Netherlands. 2. K. Akad. Wetenschap. Amsterdam Proc., Ser. C., 69, 541-556.

Geiger, R. (1950) The Climate near the Ground. Cambridge, Massachusetts.

Grant, V.E. (1949) Pollination systems as isolating mechanisms in the angiosperms. Evolution, Lancaster, Pa., 3, 82-97.

Gregor, J.W. (1930) Experiments on the genetics of wild populations. 1. Plantago maritima. J. Genet., 22, 15-25.

Gremli, A. (1886) Flore de la Suisse. Basle.

Grose, D. (1957) The Flora of Wiltshire. Devizes.

Hagerup, O (1950) Rain pollination. Biol. Meddr., 18, 1-19.

_____ (1951) Pollination in the Faroes, in spite of rain and poverty of insects. Biol. Meddr., 18, 1-48.

Hann, J. (1866) Zur Frage ueber den Ursprung des Foehns. Zeitschrift der Oesterreichischen Gesellschaft für Meteorologie, 1, 257-263.

_____ (1885) Einige Bemerkungen zur Entwicklungs-Geschichte der Ansichten über den Ursprung des Foehns. Met. Z., 2, 393-399.

Harding, J. (1970) Genetics of Lupinus. 2. The selective disadvantage of the pink flower colour mutant in Lupinus nanus. Evolution, Lancaster, Pa., 24, 120-27.

Harding, J. & Mankinen, C.B. (1971) Genetics of Lupinus. 3. Evidence for genetic differentiation and colonisation in Lupinus succulentus (Fabaceae). Madrono, 21, 222-235.

Heinrich, B. (1972) Temperature regulation in the bumblebee Bombus vagans; a field study. Science, 175, 185-187.

Hess, S.L. (1959) Introduction to Theoretical Meteorology. New York.

High Wycombe Terminal Forecast Facility (1961) Diurnal Variations of Ceiling and Visibility for selected Air Bases in England. High Wycombe.

Hocking, B. (1968) Insect-flower associations in the high arctic with special reference to nectar. Oikos, 19, 359-388.

Hopkins, B. (1954) A new method for determining the type of distribution of plant individuals. Ann. Bot. N.S., 18, 213-227.

Horwood, A.R. & Noel, C.W.F. (1933) The Flora of Leicestershire and Rutland. Oxford.

Hulkkonen, O. (1928) Zur biologie der südfinnischen Hummeln. Annls Univ. fenn. abo., Ser. A, 3, 1-81.

Hunter, R.F. & Grant, S.A. (1971) The effect of altitude on grass growth in East Scotland. J. appl. Ecol., 8, 1-19.

Huxley, A. (1967) Mountain Flowers. London.

Jain, S.K. & Bradshaw, A.D. (1966) Evolutionary divergence among adjacent plant populations. 1. The evidence and its theoretical analysis. Heredity, Lond., 21, 407-441.

Jain, S.K. & Joshi, B.C. (1962) Local differentiation in some natural populations of Justicia simplex. Genetics, Princeton, 47, 789-791.

Johnson, F.M., Kanapi, C.G., Richardson, R.H., Wheeler, M.R. & Stone, W.S. (1966) An analysis of polymorphism among isozyme loci in dark and light Drosophila ananassae strains from America and Western Samoa. Proc. natn. Acad. Sci. U.S.A., 56, 119-125.

Joshi, B.C. & Jain, S.K. (1964) Clinal variation in natural populations of Justicia simplex. Am. Nat., 98, 123-25.

Kettlewell, H.B.D. (1958) A survey of the frequencies of Biston betularia (L.) (Lep.) and its melanic forms in Great Britain. Heredity, Lond., 12, 51-72.

Klebs, G. (1906) Über Variationen der Blüten. Jb. wiss. Bot., 42, 155-320.

Kyhos, D.W. (1971) Evidence of different adaptations of flower colour variants of Encelia farinosa. Madrono, 21, 49-61.

Lamotte, M. (1951) Recherches sur la structure génétique des populations naturelles de Cepaea nemoralis (L.). Bull. biol. Fr. Belg. Suppl., 35, 1-239.

Levin, D.A. (1969) The challenge from a related species: a stimulus for saltational speciation. Am. Nat., 103, 316-322.

Levin, D.A. & Kerster, H.W. (1967) Natural selection for reproductive isolation in Phlox. Evolution, Lancaster, Pa., 21, 679-687.

———— (1969) The dependence of bee-mediated pollen and gene dispersal upon plant density. Evolution, Lancaster, Pa., 23, 560-571.

———— (1970) Phenotypic dimorphism and populational fitness in Phlox. Evolution, Lancaster, Pa., 24, 128-134.

Levin, D.A., Kerster, H.W. & Niedzlek, M. (1971) Pollinator flight directionality and its effect on pollen flow. Evolution, Lancaster, Pa., 25, 113-118.

Levins, R. (1964) The theory of fitness in a heterogeneous environment. 4. The adaptive significance of gene flow. Evolution, Lancaster, Pa., 18, 635-638.

Lewis, H. (1953) The mechanism of evolution in the genus Clarkia. Evolution, Lancaster, Pa., 7, 1-20.

———— (1962) Catastrophic selection as a factor in speciation. Evolution, Lancaster, Pa., 16, 257-271.

Lewontin, R.C. (1962) Interdeme selection controlling a polymorphism in the house mouse. Am. Nat., 96, 65-78.

Lewontin, R.C. & Hubby, J.L. (1966) A molecular approach to the study of genic heterozygosity in natural populations. 2. Amount of variation and degree of heterozygosity in natural populations of Drosophila pseudoobscura. Genetics, Princeton, 54, 595-609.

Lloyd, D.G. (1969) Petal color polymorphism in Leavenworthia (Cruciferae). Contr. Gray Herb. Harv., 198.

Lockwood, J.G. (1962) Occurrence of föhn winds in the British Isles. Met. Mag., 91, 57-65.

Lövkvist, B. (1962) A case of probable introgression in Cirsium. Bot. Notiser, 115, 385-386.

Lundegardh, H. (1924) Der Temperatur bei Kohlensäureassimilation und Atmung. Biochem. Z., 154, 193-234.

Mather, K. (1947) Species crosses in Antirrhinum. 1. Genetic isolation of the species majus, glutinosum and orontium. Heredity, Lond., 1, 175-186.

Mayr, E. (1963) Animal Species and Evolution. London & Cambridge, Massachusetts.

Meteorological Office (1952) Climatological Atlas of the British Isles. London.

Miyamoto, S. (1960) Observations on the behaviour of Bombus diversus Smith (Biological studies on Japanese bees, 13). Insectes soc., 7, 39-56.

Moore, R.J. & Frankton, C. (1962) Cytotaxonomic studies in the tribe Cynareae (Compositae). Can. J. Bot., 40, 281-299.

Narayanan, V. (1970) Diurnal variation of monsoon low cloud ceiling over Bombay Aerodrome (Santacruz). Indian J. Met. Geophys., 21, 128-130.

Pearsall, W.H. (1950) Mountains and Moorlands. London.

Pielou, E.C. (1959) The use of point to plant distances in the study of the pattern of plant populations. J. Ecol., 47, 607-613.

Poddubnaja (Arnoldi), V. (1931) Ein Versuch der Anwendung der embryologischen Methode bei der Lösung einiger systematischer Fragen. 1. Vergleichende embryologisch-zytologische Untersuchungen über die Gruppe Cynareae, Fam. Compositae. Beih. bot. Zbl., 48, 141-237.

Polunin, O. (1969) Flowers of Europe. Oxford.

Rau, P. (1924) Notes on captive colonies and homing of Bombus pennsylvanicus De Geer. Ann. ent. Soc. Am., 17, 368-381.

Rohweder, H. (1937) Versuch zur Erfassung der mengenmässigen Bedeckung des Darss und Zingst mit polyploiden Pflanzen. Ein Beitrag zur Bedeutung der Polyploidie bie der Eroberung neuer Lebensräume. Planta, 27, 501-549.

Rouy, G. (1905) Flore de France. Asnières & Paris.

Salter, J.H. (1928) The altitudinal range of flowering plants and ferns in Mid Wales. The North Western Naturalist, 1928.

Sampaio, G. (1946) Flora Portuguesa. Porto.

Schinz, H. & Keller, R. (1909) Flora der Schweiz. Zurich.

Sempala-Ntege, F.H.J. (1969) Some aspects of the physiology and ecology of Cirsium palustre (L.) Scop. and Cirsium vulgare (Savi) Ten. Ph.D. thesis, University of Lancaster.

Sheppard, P.M. (1952) Natural selection in two colonies of the polymorphic land snail Cepaea nemoralis. Heredity, Lond., 6, 233-238.

Stair, R. (1952) Ultraviolet radiant energy from the sun observed at 11,190 feet. J. Res. natn. Bur. Stand., 49, 227-234.

Streams, F.A. & Pimentel, D. (1961) Effect of immigration on the evolution of populations. Am. Nat., 95, 201-210.

Thoday, J.M. & Boam, T.B. (1959) Effects of disruptive selection. 2. Polymorphism and divergence without isolation. Heredity, Lond., 13, 205-218.

Thoday, J.M. & Gibson, J.B. (1962) Isolation by disruptive selection. Nature, Lond., 193, 1164-1166.

Tinbergen, L. (1960) The natural control of insects in pinewoods: 1. Factors influencing the intensity of predation by song birds. Archs. néerl. Zool., 13, 265-343.

Tischler, G. (1934) Die Bedeutungen der Polyploidie für die Verbreitung der Angiospermen erläutert an den Arten Schleswig-Holsteins, mit Ausblicken auf andere Florengebiete. Bot. Jb., 67, 1-36.

Waddington, C.H. (1957) The Strategy of the Genes. London.

Weatherby, C.A. (1925) Inconstancy in color-forms of Hepatics americana. Rhodora, 27, 131-132.

Weevers, Th. (1952) Flower colours and their frequency. Acta bot. neerl., 1, 81-92.

Weijer, J. (1952) The colour-differences in Epipactis helleborine (L.) Cr. Wats. & Coult., and the selection of the genetical varieties by environment. Genetica, 26, 1-32.

Willkomm, M. & Lange, J. (1870) Flora Hispanica. Stuttgart.

Wilson, G.F. (1929) Pollination of hardy fruits: insect visitors to fruit blossoms. Ann. appl. Biol., 16, 602-629.

Wilson, J. Warren (1957) Observations on the temperatures of arctic plants and their environment. J. Ecol., 45, 499-531.

Wright, S. (1931) Evolution in Mendelian populations. Genetics, Princeton, 16, 97-159.

_____ (1937) The distribution of gene frequencies in populations. Proc. natn. Acad. Sci. U.S.A., 23, 307-320.

_____ (1940) The statistical consequences of Mendelian heredity in relation to speciation. The New Systematics (ed. Julian Huxley), 161-183. Oxford.

_____ (1943) An analysis of local variability of flower color in Linanthus Parryae. Genetics, Princeton, 28, 139-156.

2. References to white flowered types within the British Isles

(a) Flora Records

White flowered specimens of the species are recorded in each of the following floras:

Amphlett, J. & Rea, C. (1909) The Botany of Worcestershire. Birmingham.

Bagnall, J.E. (1891) Flora of Warwickshire. Birmingham & London.

Buckman, J. (1844) Botany of the Environs of Cheltenham. Cheltenham.

Clapham, A.R. (ed.) (1969) Flora of Derbyshire. Derby.

Davey, J.G. (1953) Flora of Bedfordshire. Luton.

Dickinson, J. (1851) The Flora of Liverpool. Liverpool.

Druce, G.C. (1911) The international phytogeographical excursion in the British Isles. New Phytol., 10, 306-328.

_____ (1926) The Flora of Buckinghamshire. Arbroath.

_____ (1929) Flora of West Ross. Arbroath.

_____ (1930) The Flora of Northamptonshire. Arbroath.

Grose, D. (1957) The Flora of Wiltshire. Devizes.

Hennedy, R. (1874) Clydeside Flora. Glasgow.

Hodgson, W. (1898) Flora of Cumberland. Carlisle.

Horwood, A.R. & Noel, C.W.F. (1933) The Flora of Leicestershire and Rutland. London & Oxford.

Hyde, H.A. & Wade, A.E. (1934) Welsh Flowering Plants. Cardiff.

Johnson, G. (1829) A Flora of Berwick upon Tweed. Edinburgh & London.

Leicestershire Literary and Philosophical Society (1886) The Flora of Leicestershire. Edinburgh & London.

Leighton, W.A. (1841) A Flora of Shropshire. London.

Lester-Garland, L.V. (1903) Flora of the Island of Jersey. London.

Mackay, J.T. (1823) A Catalogue of the Plants found in Ireland, with Descriptions of some of the Rarer Sorts. Dublin.

Petch, C.P. & Swann, E.L. (1968) Flora of Norfolk. Norwich.

Purchas, W.H. & Ley, A. (1889) Flora of Herefordshire. Hereford.

Riddelsdell, H.J., Hedley, G.W. & Price, W.R. (1948) Flora of Gloucestershire. Arbroath.

Salter, J.H. (1935) The Flowering Plants and Ferns of Cardiganshire. Cardiff.

Scully, R.W. (1916) Flora of County Kerry. Dublin.

Storrie, J. (1886) The Flora of Cardiff. Cardiff.

Throw, A.H. (ed.) (1911) The Flora of Glamorgan. Cardiff.

Trail, J.W.H. (1923) Flora of Aberdeen. Aberdeen.

Vachell, E. (1927) Meeting of some of the Botanical Society of the British Isles in Glamorgan, 1927. Rep. botl. Soc. Exch. Club Br. Isl., 1927.

(b) Herbarium Specimens

The following is a list of the collectors and the sites of collection of the white flowered specimens present in the Fielding Druce Herbarium, Oxford.

Balfour, Sir I.B.	:	Dalwahouy Hill
Druce, G.C.	:	Berkhampstead, Herts; Corrie Ardran, Mid Perth; Cumberland; Ridge, Dorset; Surrey; Winter Corrie, Clova.
Haileybury, & I.S.C.	:	Middlesex
Loydell, A.	:	Middlesex
Palmer, C.E.	:	North Hants.
Roper, I.M.	:	Somerset
Stratton, F.	:	Isle of Wight
Walker, Miss	:	Boars Hill
Wright Mason, W.	:	Llanbedr, Merioneth