Briggs & Walters. 1997. Plant Variation and Evolution (3rd ed.). Cambridge University Press

Chapter 8 pp. 167-190

8

Infraspecific variation and the ecotype concept

We saw in Chapter 7 how different breeding systems can be expected to produce different patterns of variation. If we are to understand the variation patterns actually found in nature and the processes which give rise to these patterns, we must discover how the potential variation in seeds relates to the variation of reproductively mature plants. Historically, the first advances in this field were made by means of comparisons between plants belonging to the same species but from different populations. Taxonomists, biometricians and, later, geneticists became interested in genetic variation in the wild and many of their studies converged at one point, namely the controversy over the 'reality' of the infraspecific groups, which could be distinguished in nature, whether they were the subspecies or varieties of the taxonomist or the 'local races' of the biometrician. A new look at this old question was provided by the famous research of Turesson published in the early 1920s.

Turesson's pioneer studies and other experiments

At the time of Turesson's experiments, the question of the reality of 'local races' was combined with another controversial issue, namely how much of the observed variation in natural populations was the result of the direct modification of plants subjected to severe environmental stresses? By the end of the nineteenth century, many botanists reasoned that distinctive infraspecific variants were merely 'habitat modifications'. Turesson, however, pointed out that in all previous cases known to him, only a partial test of the 'habitat modification' hypothesis had been carried out. For example, he considered the studies of *Lathyrus japonicus* undertaken by Schmidt (1899). Baltic populations of this plant have dorsiventral leaves, whilst on the North Sea coast of Denmark the plant has isolateral leaves. Schmidt

showed, by experiment, that watering the Baltic variant with sodium chloride solutions induced a leaf structure typical of Danish plants. Given that the North Sea has a higher percentage content of salt than Baltic waters, Schmidt deduced that the leaf structure of the plants on the North Sea coast of Denmark was merely a habitat modification.

The logic of this type of deduction did not satisfy Turesson. His approach to the problem was to grow samples of several variants of a species in a standard garden, to see if 'distinctiveness' was retained or lost. He collected living plants (and in certain cases seeds) of many common species from a variety of natural habitats in southern Sweden and grew them in experimental gardens first at Malmö (1916–18) and subsequently at the Institute of Genetics at Åkarp. In this way he studied, for example, shade variants, dwarf lowland plants from coastal habitats and succulent variants, in most cases growing these plants alongside collections of the same species collected from ordinary inland habitats (Turesson, 1922*a*, *b*).

In some cases the distinctness of the variants was lost in cultivation in an inland garden, but usually the distinctive plants originating from extreme habitats retained their characteristics in cultivation even in the absence of shading, salting, etc. These observations were clearly at odds with the notion that extreme variants were nothing more than habitat modifications and the persistence of distinct variants under standard conditions suggested to Turesson that the variation had a genetic basis.

Many of Turesson's early experiments were carried out on the Composite *Hieracium umbellatum*. This plant is common in southern Sweden where its principal habitats – woodland, sandy fields, dunes and cliff tops – may all be found. In each of these habitats a distinctive plant was discovered in the field. By careful sampling and cultivation, Turesson found that, with few exceptions (for example, certain prostrate plants from sandy fields) distinctive variants retained their characteristics in cultivation. The results of these experiments were consistent with those obtained in studies of other species and again Turesson considered that patterns of residual difference had a genetic basis.

H. umbellatum is a common plant in southern Sweden, and Turesson was able to collect many samples from each habitat type. A close study of his extensive collections after a number of years of cultivation suggested to him the possibility that habitat-correlated patterns of genetic variation were present, that is to say, in a particular habitat of *H. umbellatum* a certain race of characteristic morphology was invariably present. In the appropriate habitat, there was to be found a dune race, a woodland race, etc. Turesson called these local races 'ecotypes' and described five, as follows (note that in these descriptions he considered anatomical and physiological traits (e.g. flowering times) as well as morphological features):

1. *An ecotype from shifting dunes.*
   Narrow leaves and slender, less erect, sometimes more or less prostrate stems. Marked power of shoot regeneration in autumn. Leaves tough and thick with three to four layers of palisade cells. Fruiting in early September.
2. *An ecotype from sandy fields and stationary dunes.*
   As 1, but power of shoot regeneration in autumn weak or lacking. Extremely prostrate in growth habit.
3. *An ecotype from western sea cliffs.*
   Broad leaves and more or less prostrate stems. Growth form contracted and bushy. Cells of leaves more or less distended. Fruiting late September to early October.
4. *An ecotype from eastern sea cliffs.*
   As 3, but plants tall and almost as erect as in 5.
5. *An ecotype from open woodland.*
   Stout, erect plants with lanceolate leaves of intermediate width. Leaves thinner with two or, at most, three palisade layers. Fruiting in September.

Turesson notes that additional ecotypes might be discovered in future studies.

H. umbellatum is a member of a genus famed for its apomictic reproduction. In considering Turesson's results it seems essential, therefore, to take into account the breeding behaviour of the plant. In a partial examination of the breeding system of his material, Turesson performed castration experiments, removing the upper half of unopened flower-heads with a razor. No fruits developed. This evidence supports the view that reproduction is sexual and not obligately apomictic. Plants of *H. umbellatum* proved in fact to be self-incompatible, and artificial crosses between plants of the dune ecotype and between plants of the cliff ecotype produced progenies in which the ecotypic characteristics of each were perpetuated, confirming the genetic basis of the discovered differences. Lövkvist (1962) has re-sampled at many of Turesson's *H. umbellatum* sites, and found broadly similar patterns of variation in cultivation trials. He also re-examined the breeding system of southern Swedish material of *H. umbellatum* and found no evidence of apomixis. However, apomixis *has* been reported in this species (e.g. Bergman, 1935, 1941, and references cited therein) and may influence patterns of variation elsewhere.

Considering the origin of ecotypes, Turesson made two important

deductions. He concluded, first, that the finding of widespread habitat-correlated genetic variation does not support the view that the variation patterns are largely governed by chance; rather the evidence suggests that natural selection operates in natural populations, well-adapted genotypes being selected in each habitat. This idea is expressed many times in Turesson's writings, for example, he says (1925): 'Ecotypes ... do not originate through sporadic variation preserved by chance isolation; they are, on the contrary, to be considered as products arising through the sorting and controlling effect of habitat factors upon the heterogeneous species-population'. Turesson further concluded that a close study of the variation within and between ecotypes of *H. umbellatum* revealed patterns of leaf morphology which suggested a 'local' origin for coastal ecotypes from the widespread inland populations. It was possible that an appropriate ecotype could be produced many times, that is to say polytopically, and it was not necessary to postulate the invasion of Sweden by fully formed standard ecotypes after the last glaciation.

In a series of long papers published from 1922 onwards, Turesson eventually described ecotypes in more than 50 common European species. His first papers were about the plants of southern Sweden, but later (1925, 1930) he experimented with material collected from distant localities in all parts of Europe and also showed physiological differences between some of his stocks (1927*a*, *b*). Analysis of the behaviour of his extensive collections in cultivation enabled him eventually to distinguish two kinds of ecotypes, namely edaphic and climatic ecotypes, where the most important environmental effects were soil type (as in the case of *H. umbellatum* in southern Sweden) and the climatic influences, respectively.

As early as the beginning of the eighteenth century, there was a considerable amount of observational evidence that common species did not flower at the same time in different localities. For example, Linnaeus (1737) noted the different flowering times of Marsh Marigold (*Caltha palustris*) (March in the Netherlands, April to May in different parts of Sweden, June in Lapland). Quetelet (1846), having studied the dates of first flowering of Lilac (*Syringa vulgaris*) in different parts of Europe, came to the conclusion that there was a retardation of 34 days for each advance of 10° northwards in latitude. He also compared flowering at different altitudes above sea-level, and discovered a retardation of 5 days for every 100 m increase in elevation. The important environmental factor controlling flowering was thought to be temperature. Turesson, studying the behaviour in cultivation of a large number of spring-flowering species, clearly demonstrated the importance of persisting genetic differences between

plants originating from different climatic regions. Southern plants of such species flowered earlier in Turesson's experimental garden than plants of the same species collected from northern latitudes. He suggested that this group of plants is adapted to flower in the period immediately preceding the leafing of trees, a phenomenon which occurs earlier in the year in southern latitudes than in northern Europe.

In the botanical literature of the nineteenth century, there are scattered reports that alpine plants flower earlier than lowland ones when both are cultivated in lowland gardens. Turesson's extensive experiments with species such as *Campanula rotundifolia* (Table 8.1) and *Geum rivale* enabled him to demonstrate that alpine ecotypes were smaller and retained their early flowering habit in cultivation. He also carried out research upon summer-flowering plants, showing that northern ecotypes were early flowering and of moderate height, while southern plants were late-flowering and tall. Western Europe was characterised by late-flowering plants of low growth; from eastern Europe, on the other hand, came taller early-flowering ecotypes.

Turesson's contribution to our understanding of the patterns of variation within species is of very great importance; he demonstrated clearly the widespread occurrence of infraspecific habitat-correlated genetic variation. Adaptation to the environment was sometimes by plastic responses, but more frequently it had a genetic basis. Such studies were grouped together under the name of 'genecology' and the work was the model for many studies by other botanists. The work of Stapledon (1928) is of special interest. Using the common pasture grass *Dactylis glomerata*, he studied the influence of hay cutting and animal grazing, and described a third class of ecotype, namely the 'biotic ecotype'. His work is summarised in Table 8.2.

Scandinavian botanists have made many notable contributions to genecology and it is appropriate at this point to give an example of the important experiments of Bøcher. He used the Turessonian technique of cultivation in a standard garden to examine the variation and flowering behaviour of collections of many European plants, and carried the analysis of variation into an important new area, namely the study of the timing of flowering in relation to the life history of the plant. For example, he discovered in cultivation experiments with *Prunella vulgaris* (1949) that there were two main growth types in Europe, namely plants with a short vegetative phase, flowering in their first year, and plants with a longer vegetative phase, flowering in their second year. This latter group was further subdivided into plants which were short-lived and perennial types. The distribution of the two main types – first- and second-year flowerers –

Table 8.1. *Geographic variation in* Campanula rotundifolia

(a) Results of transplant experiments from Turesson (1925) (Means of five measurements given)

		Field no.	Transplanted from	Length of stems (mm)	Width of middle-stem-leaves (mm)	Number of flowers on stems	Length of corolla (mm)	Width of corolla in the middle (mm)	Width of corolla at mouth (mm)	Length of corolla lobes (mm)	Length of calyx lobes (mm)	Power of regeneration of basal rosette-leaves	Year of collection	No. of plants
Norway		99	Vitemölla	547.75	2.18	23.25	18.63	16.50	22.45	7.33	6.33	none-weak	1920	8
and		206	Åhus	650.54	2.16	27.49	19.93	16.45	22.82	7.65	7.29	none-weak	1922	13
Sweden		270	Ulriksdal	334.30	1.86	20.33	17.13	14.99	20.2	7.02	5.63	none-weak	1921	14
	M	298	Åre	308.43	2.97	11.5	22.12	21.0	25.91	9.54	5.88	mostly strong	1921	17
		349	Bergen	378.67	2.73	9.19	20.56	20.53	25.67	8.41	6.36	weak-strong	1922	7
		240	Trondhjem	336	2.03	15.97	21.0	18.34	25.06	8.39	5.80	weak-strong	1922	14
Central	M	19–25	Abisko (seeds)	250.10	1.99	13.97	24.47	20.48	27.68	9.32	7.90	strong	1921	seeds
Europe		770	Freiburg	278.56	2.12	19.86	20.44	18.89	24.54	8.5	6.56	none-weak	1923	16
	M	796	Feldberg	224.66	4.29	6.88	23.45	21.82	25.32	8.76	7.89	strong	1923	14

(b) Progeny trial, from Turesson (1930)

Field no.		Source	No. of plants	Height (cm)			Earliness of flowering[a]		
				Mean	σ	m±	Mean	σ	m±
770		Freiburg	20	68.9	5.89	1.32	1.60	0.35	0.29
796	M	Feldberg	20	29.5	2.41	0.54	5.00	0.00	0.00
270		Ulriksdal	20	47.1	5.47	1.22	2.80	0.44	0.10
298	M	Åre	20	33.4	3.75	0.84	5.00	0.00	0.00

[a]note that a large mean corresponds to earlier flowering
M = montane localities

Table 8.2. *Biotic ecotypes in* Dactylis glomerata. *Stapledon (1928) discovered that grassland use determined the type of* Dactylis *present in a particular area*

		Per cent growth type				Per cent over 100 cm	Per cent flowering behaviour			
		Hay	'Cup'	Tussock	Pasture		Early 1	2	3	Late 4
Commercial hay stocks	A	59	36	2	3	78	40	50	9	1
	B	66	31	1	2	78	61	32	6	1
Old pastures		15	23	6	56	15	11	35	38	16
Hedgerows and thickets		26	35	25	14	31	17	35	34	14

Hay types with their taller early-flowering plants were distinct from the shorter, later-flowering plants characteristic of grazed pasture. Pasture types had many more tillers than hay types and a smaller percentage of tillers produced inflorescences. Plants from hedgerows had a wide range of variants. Even though this experiment did not reveal a discontinuous pattern of variation, Stapledon was content to interpret his results in terms of 'biotic ecotypes'. (See Warwick & Briggs, 1978*a*, *b*, for a partial review of recent work on 'hay' and 'pasture' ecotypes.)

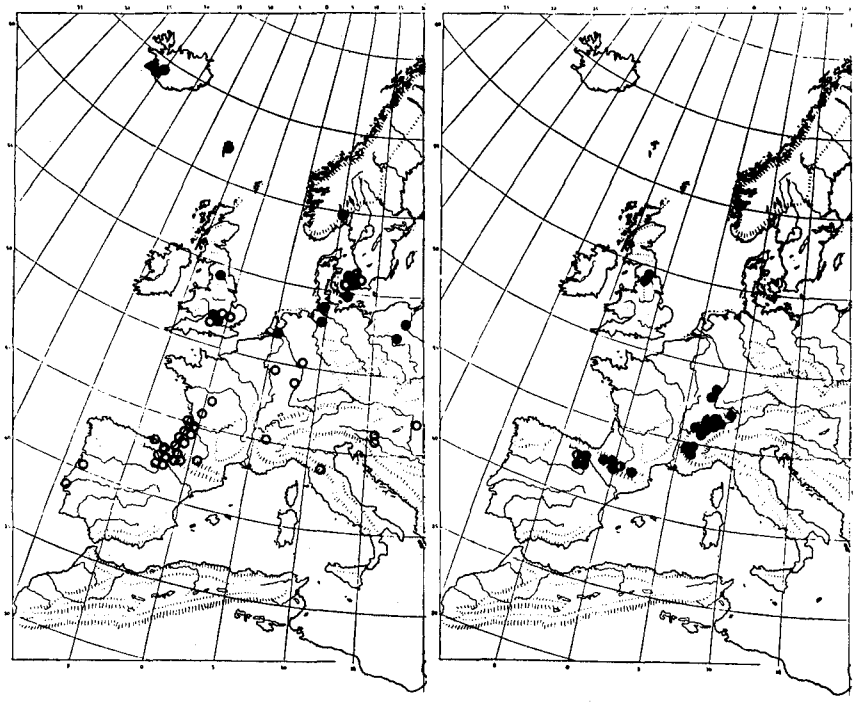

Fig. 8.1. Distribution in Europe of first-year flowering and second-year flowering types of *Prunella vulgaris*: the first (with short rosette stage) indicated by open rings, the second (with long-lasting rosette stage) by filled circles. All 75 samples were sown and cultivated simultaneously during 1950–51. On the map on the left are 51 lowland samples. On the map on the right are 24 samples from montane stations. The tendency towards second-year flowering in the northerly direction and from the lowland to the highland areas is evident. (From Bøcher, 1963.)

proved most interesting (Fig. 8.1); for example, in Mediterranean regions subject to summer drought, only short-lived annual plants were found, whilst in areas with different climatic conditions biennial or perennial types were characteristic. Such patterns are likely to be the result of natural selection: only those plants whose life history 'fits' the growing season of a particular area will survive in the long-term.

Experiments by American botanists

Some of the most famous experiments on ecotypes were carried out by Clausen, Keck & Hiesey (1940) on different species of plants collected on a

200-mile transect across Central California, from a 'Mediterranean' climate in the west to an 'alpine' climate in the east. Turesson's method of studying ecotypes was to grow all his collections in a lowland garden. Such a method has the limitation that it may not allow certain traits to be revealed (e.g. tolerance or sensitivity to frost or drought). In an attempt to overcome this difficulty, Clausen and his co-workers carried out experiments with many gardens, and finally used three: at Stanford (30 m above sea-level), Mather (1400 m) and Timberline (3050 m). To illustrate the very different conditions in the gardens, Fig. 8.2 gives climatic details for sites near Stanford and Timberline. Of special importance are the extremes of temperature and the differences in the length of the growing season. In each garden, plants were grown spaced out in weed-free plots protected from grazing. The experimental plantings consisted, in the main, of clonally propagated stocks, each individual being grown and divided, and a ramet of each planted in each garden. Thus the growth and performance of each individual from samples collected from a range of different sites could be studied in a 'Mediterranean', an intermediate and an 'alpine' garden. Climatic ecotypes were studied in many species, particular attention being paid to *Potentilla glandulosa*, a species found from the coastal hills near the west coast of California to high altitudes in the Sierra Nevada. Their experiments made it possible to test the behaviour of diverse stocks in very different standard gardens. For example, they discovered that most lowland stocks died in the harsh climate of the alpine garden, and at the Stanford garden plants originating from high altitude remained winter-dormant under conditions which stimulated growth of lowland samples. Clausen and his associates (1940) decided that there were four distinct climatic ecotypes in *P. glandulosa*, corresponding to the following taxa: subsp. *typica* (lowland); subsp. *reflexa* and subsp. *hanseni* (intermediate altitudes); and subsp. *nevadensis* (alpine) (Table 8.3). Clausen & Hiesey (1958) suggested that each subspecies was in fact made up of two or more ecotypes. Their hypothesis that ecotypic variants of *P. glandulosa* differed genetically received support from a comprehensive series of crossing experiments.

Other American botanists made studies of ecotypes using the transplant stations at Stanford, Mather and Timberline. Lawrence (1945), for example, studied ecotypes of the grass *Deschampsia cespitosa*, discovering differences in survival in different stations (Fig. 8.3). Of special interest were his studies of reproduction in the different transplants; although all individuals survived at Timberline, only the stocks native to that area were able to produce seeds in the short growing season. Such a finding, which is of crucial importance in understanding the genecology of the species, could

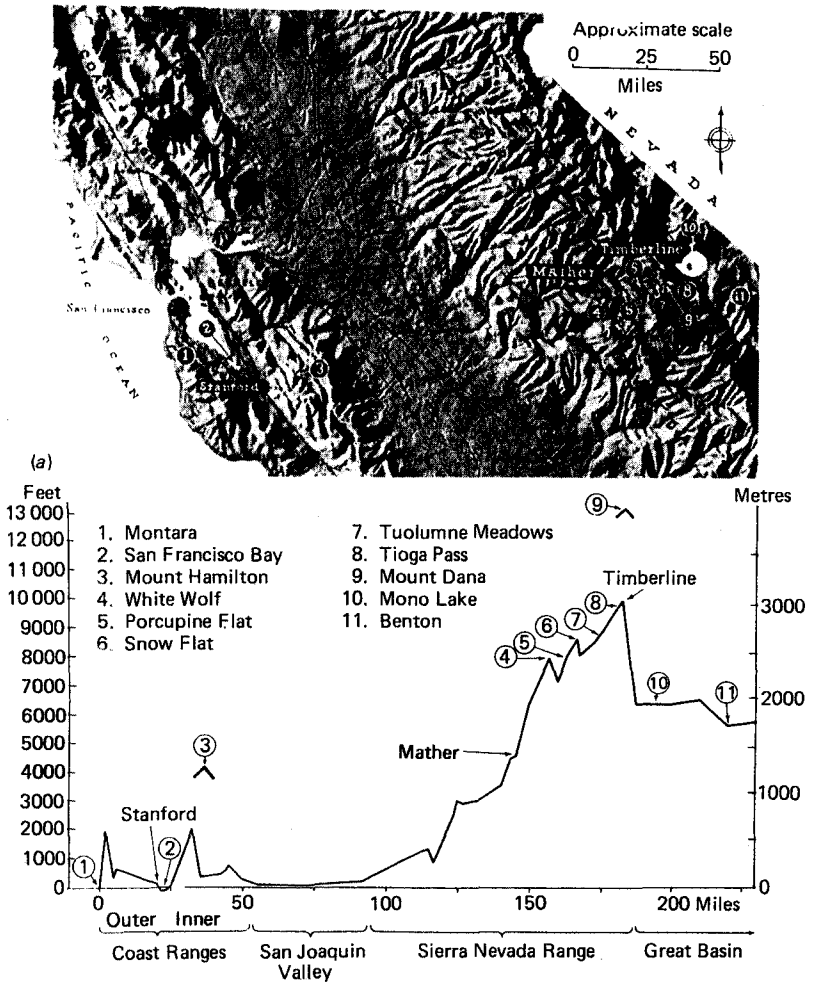

Fig. 8.2. Map and climatic details for Stanford and Timberline sites in Central California, used for the famous transplant experiments of Clausen, Keck & Hiesey. (*a*) Diagrammatic transect showing heights above sea-level. (*b*) Graphs showing annual variation in temperature and precipitation (US Weather Bureau data for 1925–35 inclusive) near Stanford and Timberline. In the lowland site with 'Mediterranean-type' climate (Stanford), active growth is possible throughout the year, whereas at Timberline (*c.* 3000 m) the active growth period is restricted to July and August. At Stanford, average annual precipitation was 31.7 cm; there was no snowfall except for traces in 1931 and 1932. At Timberline, average annual precipitation was 74.1 cm. Maximum temperature = average of the highest monthly temperatures. Mean temperature = average of the mean monthly temperatures obtained from daily readings. Precipitation = average monthly precipitation. Minimum temperature = average of the lowest monthly temperatures. (From Clausen, Keck & Hiesey, 1940.)

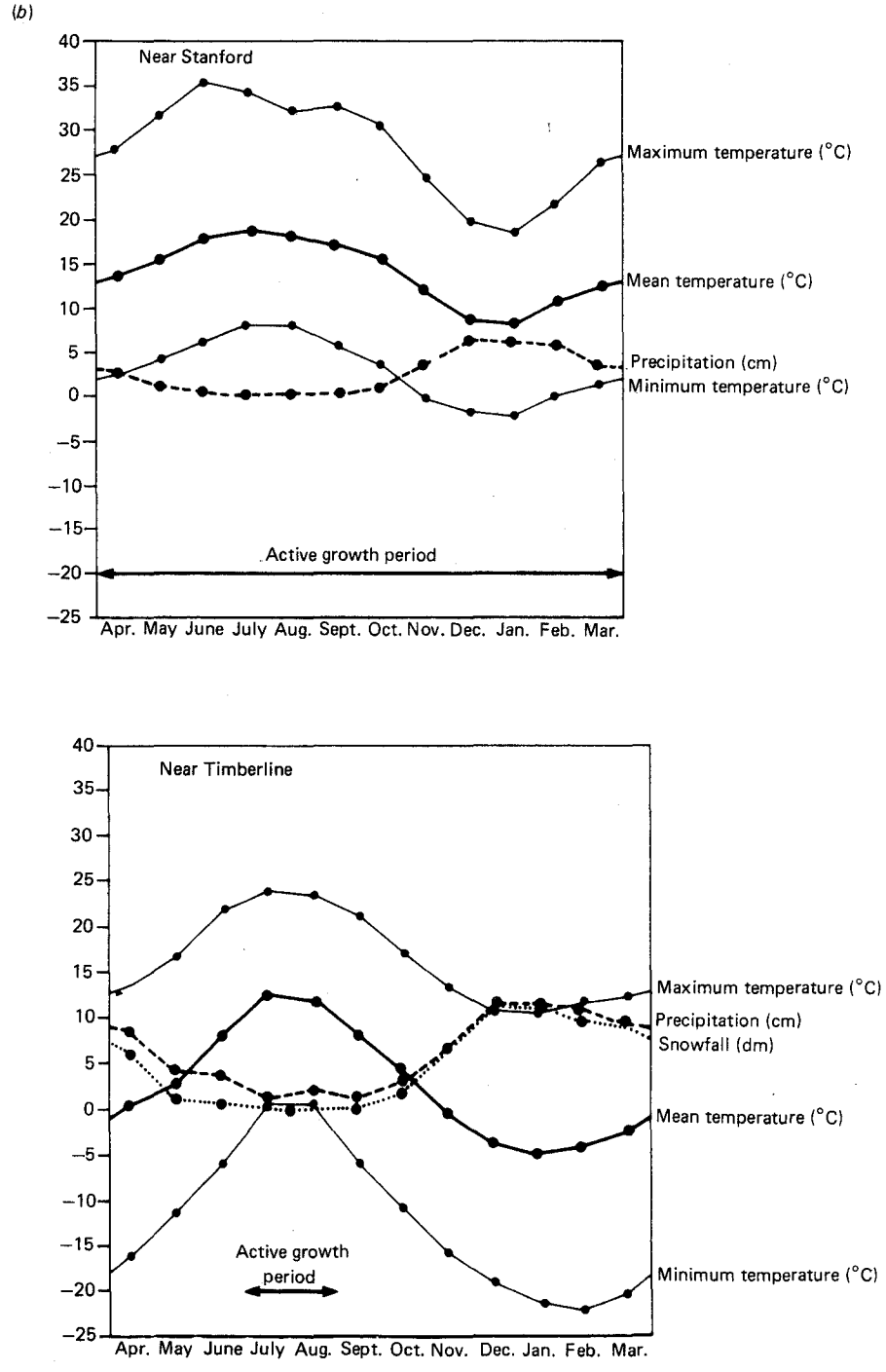

Table 8.3. *A summary of the characteristics of the ecotypic subspecies of* Potentilla glandulosa *along the Central Californian transect (Data from Clausen & Hiesey, 1958, as summarised by Heslop-Harrison, 1964)*

	typica	*reflexa*	*hanseni*	*nevadensis*
Distribution	Coast ranges and lower Sierra Nevada	Low and middle altitudes of Sierra Nevada	Meadows, midaltitudes of Sierra Nevada	High altitudes of Sierra Nevada
Habitat	Soft chaparral and open woods	Dryish, open timbered slopes	Moist meadows	Moist, sunny slopes
Climatic tolerances as experimentally determined	Coastal to middle altitudes	Coastal to middle altitudes	Middle and high altitudes (poor survival near coast)	Middle and high altitudes (poor survival near coast)
Seasonal periodicity at Stanford (alt. 30 m)	Winter- and summer-active	Winter-active or -dormant; summer-active	Winter-dormant, summer-active	Winter-dormant, summer-active
Internal variation	Wide, probably several 'ecotypes'	Wide, probably several 'ecotypes'	Wide, at least two 'ecotypes'	Moderate, at least two 'ecotypes'
Self-compatibility	Self-fertile	Self-fertile	Undetermined	Self-sterile

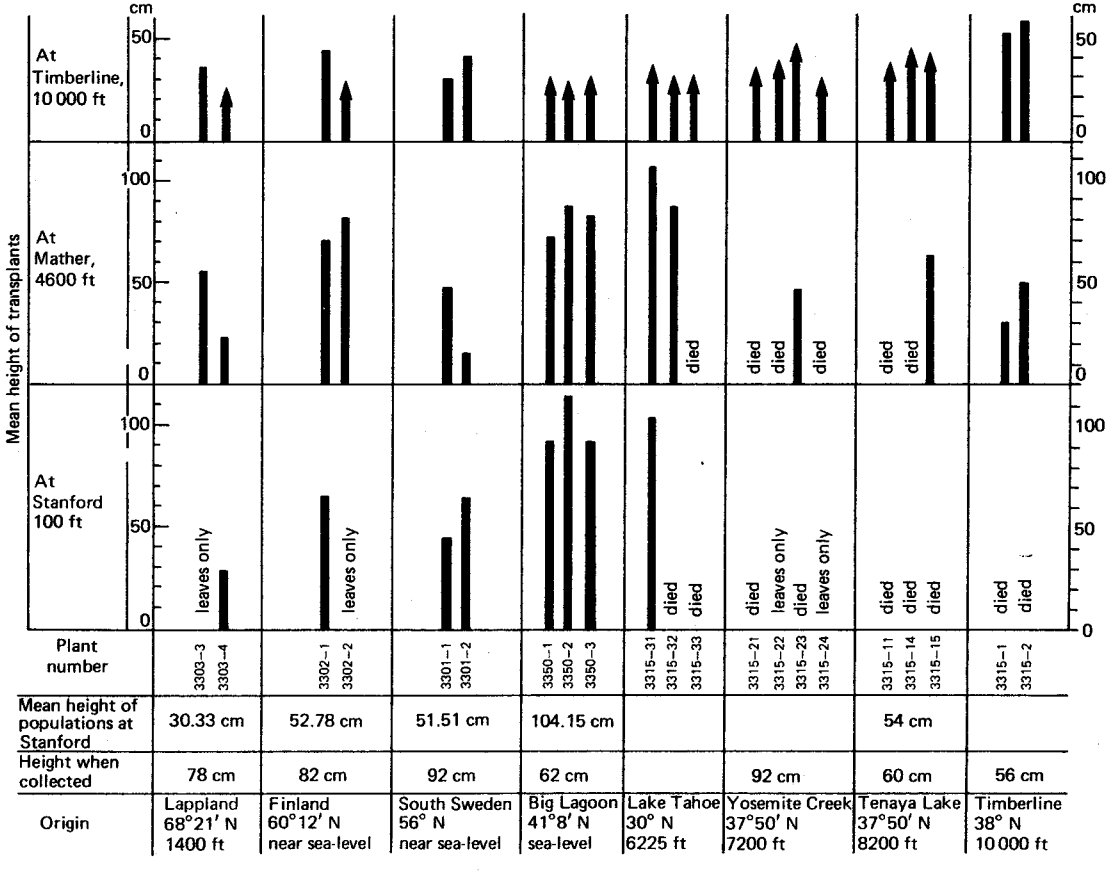

Fig. 8.3. Mean heights of plants of *Deschampsia cespitosa* (*D. caespitosa*) of diverse origin grown at the transplant stations Stanford, Mather and Timberline, in Central California. (The arrowheads on certain columns at Timberline signify that these individuals did not reach maturity in any year.) (From Lawrence, 1945.)

not have been revealed in a lowland garden. A further point of general interest is revealed by their results with plants of *D. cespitosa* from Finland (latitude 60° N) and South Sweden (latitude 56° N). When these plants were grown at low altitudes at Stanford (38° N), many of them became viviparous, a character not expressed in their native habitats. Growth in a garden with very different climatic characteristics may provoke an unusual response from plants.

Experiments with several gardens separated by great distances are expensive to maintain, and botanists have devised ways of investigating ecotypes by varying the conditions in a single garden or laboratory. Turesson's experiments were carried out in a lowland garden on fertile soil and in describing edaphic ecotypes he inferred the importance of soil differences in the wild. A more direct approach to the study of patterns of variation in relation to edaphic factors was made by Kruckeberg (1951, 1954). In one experiment, fruits of *Achillea borealis* were collected from serpentine and non-serpentine sites in California. (Serpentine is a rock type which gives rise to soil with high levels of magnesium and low levels of calcium.) Two tons each, of a serpentine and a fertile soil, were collected and transported to the University of California Botanical Gardens, and stocks were grown from seed in soil bins, or pots, of the two soil types. Stocks raised from seed of plants native to serpentine soils grew well on the serpentine test soil, but, in contrast, plants from other soil types (shales, basalt, etc.) generally (though not always) grew badly or died (Fig. 8.4). Kruckeberg's results on *A. borealis* and other species are consistent with the idea that a common species found on different soil types may be made up of a number of edaphic ecotypes.

A second example of the way in which diverse stocks may be presented with different environments in one garden or laboratory is provided by the use of glasshouses, growth chambers, etc., in which daylength, temperature and other factors may be varied. Samples may be tested in a variety of artificially controlled environments, in which, for instance, the responses of different stocks may be monitored under different daylengths. In the first experiments studying the effect of different daylengths, plants were grown on movable trucks. After a period of natural daylight plants were moved into light-proof structures where they could be either in total darkness or given supplementary light from artificial sources. A good example of this type of experiment is provided by Larsen (1947), who studied *Andropogon scoparius*, a widespread and important forage grass in North America. Plants were collected from 12 localities from 28°15′N in Texas to 47°10′N in North Dakota. The grasses were given constant daylengths of 13, 14 and 15

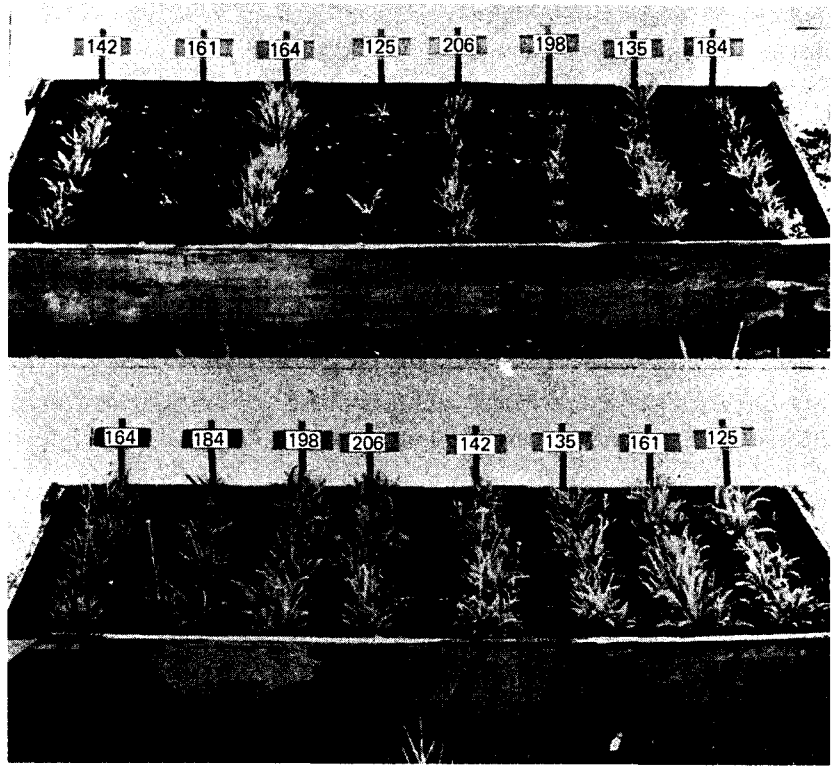

Fig. 8.4. Experiments with *Achillea borealis*, grown on serpentine soil (above) and non-serpentine soil (below). All eight samples grew well on the fertile, non-serpentine soil, whilst three of the four samples from the non-serpentine soil (161, 125, 198) grew badly on serpentine soil. The fourth sample from non-serpentine soil (206) grew unexpectedly well on serpentine soil, however. (From Kruckeberg, 1951.)

hours of light. None of the 12 samples flowered at 13 hours. Plants from the southern USA required a 14-hour photoperiod for floral induction, but a photoperiod of 15 hours was necessary for flowering in many northern plants. Figure 8.5 illustrates the relation between latitude and daylength at different times of year. *A. scoparius* plants growing in the southern USA naturally come into flower after receiving a photoperiod of 14 hours. Northern plants, with longer summer days, need a 15-hour day to come into flower.

As more sophisticated equipment became available, growth chambers were constructed in which many environmental factors (e.g. temperature, daylength) could be controlled. Adjacent chambers could be used to subject

plants to different conditions. A splendid example of such studies is provided by the experiments of Mooney & Billings (1961) who studied *Oxyria digyna* collected from sites between 38° N and 76° N in North America. Other botanists have continued to be fascinated by the different photoperiodic responses of plants from different geographic areas. The work of McMillan (1970, 1971) on *Xanthium strumarium* provides an impressive example. Physiological studies are advancing our understanding of ecotypes, and the reviews of Heslop-Harrison (1964), Hiesey & Milner (1965) and Bannister (1976) may be consulted for details of early studies. In recent years, the field of physiological ecology has expanded greatly. Specialist reviews give details of research in different fields: e.g. frost survival (Sakai & Larcher, 1987); photosynthesis (Evans, Caemmerer & Adams, 1988); multiple stresses (Mooney, Winner & Pell, 1991); resource

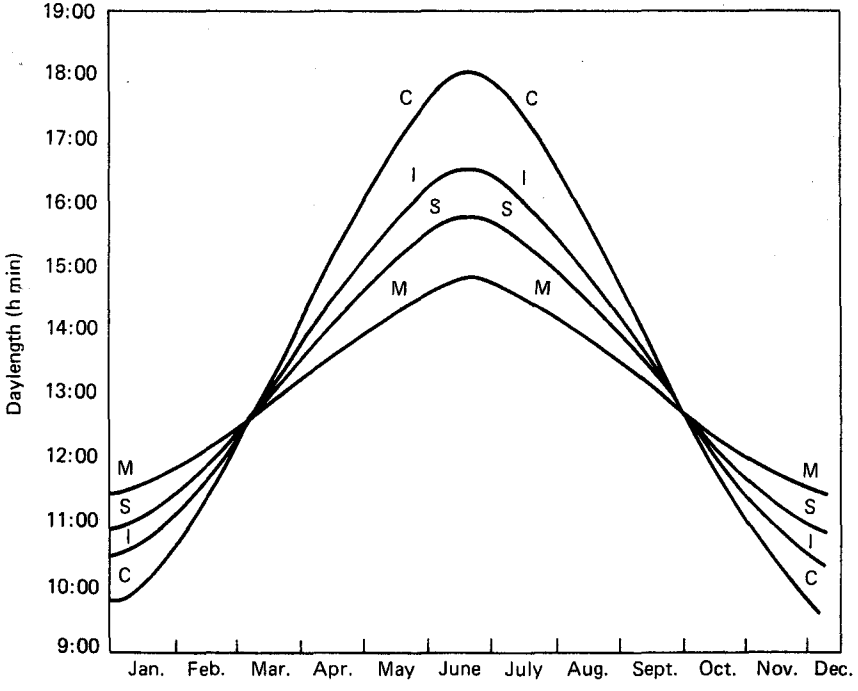

Fig. 8.5. Relation between latitude and daylength at different times of the year. Daylength includes twilight of that intensity receivable when the sun is 6° or less below the horizon, thus adding about 1 hour to the daylength between sunrise and sunset. M = Miami, FL, latitude *c.* 26°N; S = San Francisco, CA, *c.* 37°N; I = Ithaca, NY, *c.* 42°N; C = southern Canada, 50°N. (From Curtis & Clark, 1950.)

use (Townsend & Calow, 1981); and physiological ecology of woody plants (Kozlowski, Kramer & Pallardy, 1991).

The widespread occurrence of ecotypes

As a result of experiments in which plants have been grown in gardens or under controlled conditions, ecotypes have been described in hundreds of species. There is evidence that ecotypes occur not only in outbreeding species but also in species apparently predominantly inbreeding. There are also numerous studies of facultatively apomictic plants in which ecotypic patterns have been described, for example the grass *Poa pratensis* (Smith, Nielsen & Ahlgren, 1946) and *Potentilla gracilis* (Clausen, Keck & Hiesey, 1940).

Of special interest is the finding of genetic heterogeneity in plants which are apparently obligately apomictic. Turesson (1943) discovered, within collections of European *Alchemilla glabra, A. monticola* (*A. pastoralis*) and *A. filicaulis*, that plants from Lapland and montane areas were earlier flowering in cultivation than lowland stocks. The patterns of variation appeared to be ecotypic, but Turesson called the variants 'agamotypes' in recognition of the breeding system of *Alchemilla*. Bradshaw (1963*a*, *b*, 1964) and Walters (1970, 1986*a*) have described dwarf variants of an ecotypic nature in *Alchemilla*, the origin of which is plausibly due to selection in response to grazing by sheep (Fig. 8.6).

Clines

In the experiments outlined above, the researchers were content to describe their material in terms of distinct local races, often using the term 'ecotype'. However, the ecotype concept was not without its critics. Langlet (1934), for example, pointed out that the most important habitat factors, such as temperature and rainfall, commonly varied in a continuous fashion, and thus one would expect graded variation in many widespread species rather than discontinuous variation.

Support for this view was provided by Gregor (1930, 1938) who made an intensive study of *Plantago maritima* in northern Britain. Representative seed collections were made and plants were grown in an experimental garden of the Scottish Society for Research in Plant Breeding. Table 8.4 gives results obtained by Gregor (1946) in similar studies. In this case all three sample zones are from the Forth estuary in eastern Scotland. If collections of *P. maritima* taken from different sites along a gradient from

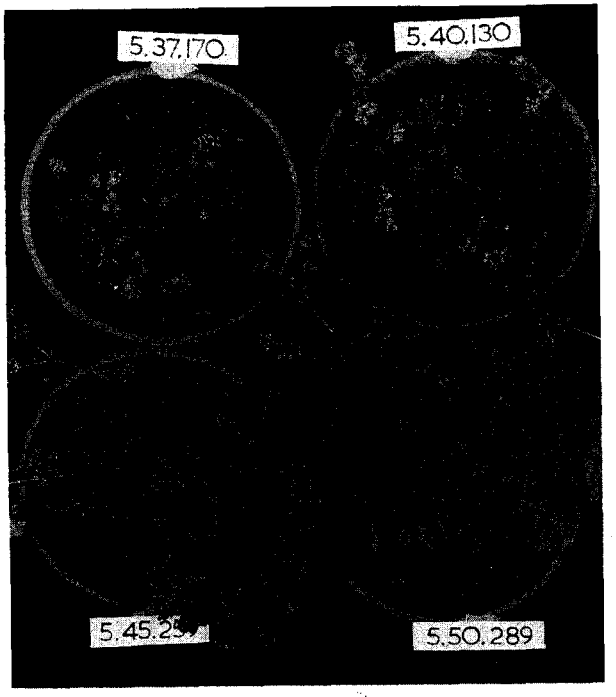

Fig. 8.6. Dwarf variants of *Alchemilla* from the North Pennine Hills, England. Four transplants from grazed mountain localities, grown under standard conditions for nine months at Durham University experimental grounds. Top row: two separate transplants of *A. minima* from Ingleborough. Bottom row: *A. filicaulis*; left, transplant from Mickle Fell; right, transplant from Moor House National Nature Reserve. *A. minima* retains its very dwarf habit in cultivation, in contrast to *A. filicaulis*. (From Bradshaw, 1964.)

high to low salt concentration are compared, a progressive increase in scape height is found. In a similar fashion there are increases in: scape volume and thickness; leaf length, breadth and spread; and seed length. Figure 8.7 illustrates the different growth-habit types found in *P. maritima*. As Table 8.4 shows, it is only in the upper marsh that erect plants predominate.

In 1938, Huxley, after surveying the literature, coined the useful term 'cline' for character variations in relation to environmental gradients. Thus, a graded pattern associated with ecological gradients is referred to as an ecocline (a good example of this is Gregor's *P. maritima* result). If the pattern is correlated with geographical factors, the term topocline can be employed. Clinal variation has been described in a large number of species and a small selection of examples is given in Table 8.5 and Fig. 8.8.

Table 8.4. *Results of soil analyses (air-dried samples) and cultivation experiments with* Plantago maritima *(Gregor, 1946)*

Habitat	Mean scape length (cm)	Habit grades (Percentage of sample in each grade) 1	2	3	4	5
Waterlogged mud zone (salt concentration 2.5%)	23.0 ± 0.58	74.5	21.6	3.9	—	—
Intermediate habitats with intermediate salt concentrations	38.6 ± 0.57	10.8	20.6	66.7	2.0	—
Fertile coastal meadow above high tide mark (salt concentration 0.25%)	48.9 ± 0.54	—	2.0	61.6	35.4	1.0

How far are intraspecific patterns of variation explicable in terms of ecotypes and clines? Experiments, for example, by Bradshaw (1959*a*, *b*, *c*, 1960) on the grass *Agrostis capillaris* (*A. tenuis*), have shown that much more complex patterns may be found in nature. Careful collections of living specimens of this grass were made mostly from localities in Wales. The stocks were grown, and then cloned material was planted into a number of experimental plots in North and Mid-Wales, with an altitudinal range from sea-level to about 800 m. A wide range of different responses was demonstrated by these experiments. Not only were plants different morphologically but there were also physiological differences. For example, certain plants grew well on soils containing lead and other heavy metal residues; others, indistinguishable from them morphologically, died on this type of soil (we shall return to this interesting phenomenon of tolerance of heavy metal ions in Chapter 9). At this point it is important to note that Bradshaw could not delimit ecotypes in *A. capillaris*. This was not because extreme variants were not found in extreme habitats. On the contrary, many very distinctive plants were discovered: for instance, dense cushion plants from the exposed Atlantic cliffs at West Dale, South Wales. The problem was that, even though habitat-correlated variation could be demonstrated, the fact that all kinds of intermediate plants were discovered made it utterly impossible to decide where one ecotype ended and another began.

Does the concept of clines help in this situation? Bradshaw studied his material closely with this idea in mind. In many areas, even though clines

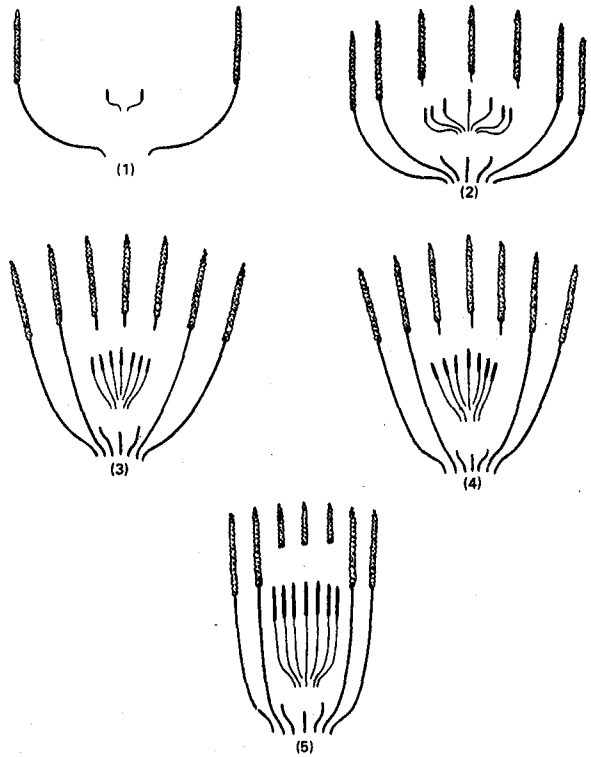

Fig. 8.7. Variation in *Plantago maritima*. For purposes of classification, Gregor divided his material into five grades, illustrated diagrammatically here. There was, however, no sharp line of demarcation between one grade and the next. (From Gregor, 1930, 1938.)

might be described, he decided that the environmental gradients and the associated variation were too complex.

What then, determines the patterns of intraspecific variation found in the wild? How can one reconcile the distinct ecotypes of Turesson and Clausen with the complex variation found by Bradshaw and many other researchers?

Factors influencing the variation pattern

Of first importance is the type of sampling technique used. Turesson and many other botanists collected widely spaced samples, whereas Gregor and Bradshaw carried out intensive sampling in small areas. Widely spaced samples taken from extreme habitats may exhibit a pattern of distinct

Table 8.5. *Some examples of clinal variation*

Species	Variation	Reference
Allium schoenoprasum	Longitudinal cline in chromosome banding pattern in eastern North America	Tardif & Morisset (1991)
Anthoxanthum odoratum	Clines for various characters at mine/pasture boundary	Antonovics & Bradshaw (1970)
Asclepias tuberosa	Clines for flower colour and leaf-shape in North America	Woodson (1964)
Blandfordia grandiflora	Morphological & reproductive characters in Australia	Ramsey, Cairns & Vaughton (1994)
Dactylis glomerata complex	Clinal variation in European populations in glutamate oxaloacetate transaminase gene frequencies (GOT I Locus)	Lumaret (1984)
Eschscholzia californica	Clines in California for various features	Cook (1962)
Eucalyptus spp.	Graded patterns of leaf glaucousness with extreme 'waxy' types in exposed habitats	Thomas & Barber (1974)
Geranium robertianum	Clines for hairiness	Baker (1954)
Geranium sanguineum	Decrease in leaf-lobe breadth west to east in Europe	Bøcher & Lewis (1962)
Holcus lanatus	First-year flowering in Southeast Europe. Second-year flowering in northern Europe	Bøcher & Larsen (1958)
Juniperus virginiana	Clines in terpenoid content northeast Texas to Washington DC	Flake, von Rudloff & Turner (1969)
Lotus corniculatus	Flower colour variation in North England. Dark-keeled variant rare in West, increasing in frequency eastwards.	Crawford & Jones (1986)
Pinus strobus	Decrease in leaf length and number of stomata, increase in number of resin ducts, with increasing latitude in North America	Mergen (1963)
Silene latifolia	Clinal variation in seed morphology	Prentice (1986)
Viola riviniana	Clines in plant size	Valentine (1941)

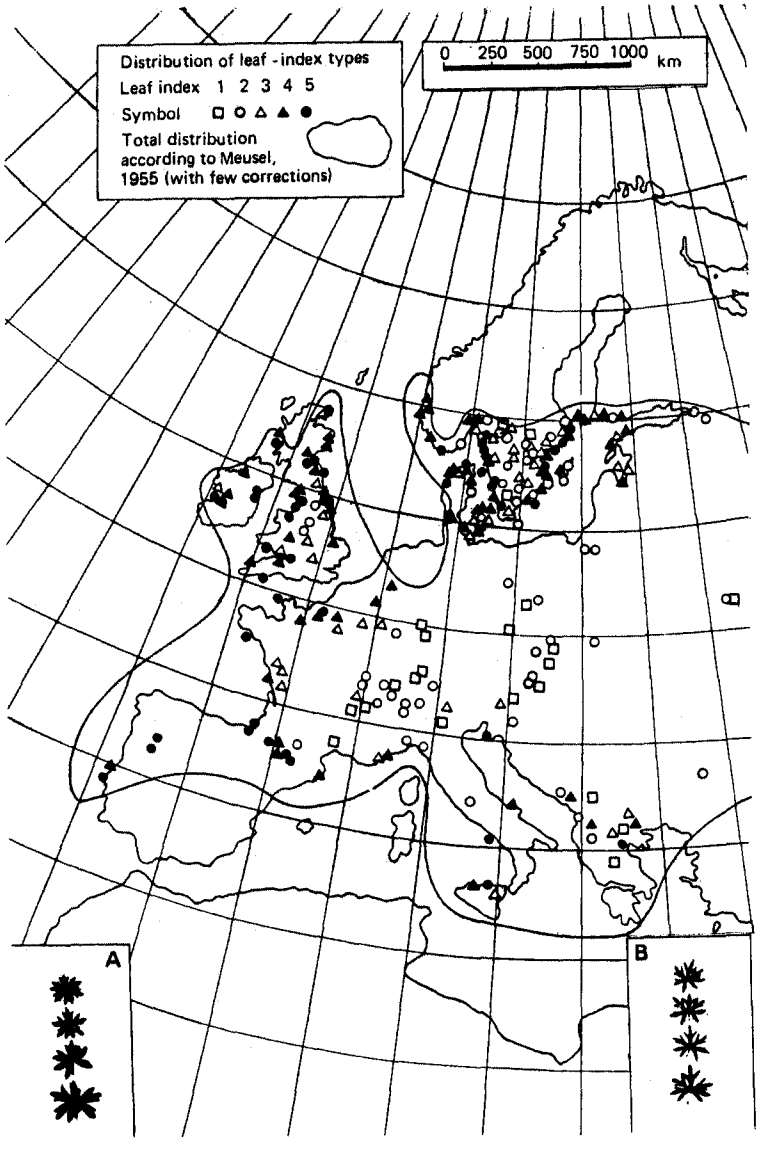

Fig. 8.8. Clinal variation in *Geranium sanguineum* (Bøcher & Lewis, 1962). At first sight there seems to be a more or less simple topocline for leaf-lobe width across Europe, plants from North and West Europe usually having broad-lobed leaves (leaf index 4 and 5), as in inset A. On the other hand, material from continental Europe often has narrow leaf-lobes (leaf index 1 and 2), as in inset B. The distribution map of leaf index values for herbarium material suggests, however, that the variation is more complex. It seems likely, in view of the occurrence of broad-lobed plants on the east coast of Sweden and in the Mediterranean area, that this leaf type is associated with coastal climatic conditions. Narrow-lobed plants, found in dry limestones of inland Britain and Sweden, seem to be found wherever continental climatic conditions occur.

ecotypes. In contrast, samples taken from along smooth, regular gradients of soil or altitude may well give a pattern of clinal variation in the experimental garden. If, however, sampling is carried out in small areas, the plants being collected at random rather than along particular gradients, then experiment might reveal very complex patterns. Thus, in a very real sense, the mode of sampling largely determines the patterns 'discovered' in cultivation experiments.

Another aspect of sampling is important. An experimenter can choose either to collect a representative seed sample or to dig up mature plants. If both types of sampling are carried out on a single population, different patterns of variation might well be found. This is because mature plants have survived the rigours of natural selection. Seed collections, on the other hand, give an estimate of potential rather than actual variation. If several adjacent populations in different environments are examined, in a case where pollen can be transported from one population to another, sampling of mature individuals might well reveal a pattern of more or less distinct ecotypes. On the other hand, because of gene flow between populations, seed samples will seem to reveal a more complex pattern in the same case.

Ecological, historical and geographical factors also influence the patterns discovered in experiments. If a species is found as small, non-contiguous populations, or if it has populations inhabiting two or more very different types of habitat, then the pattern of variation in the wild is more likely to be that of distinct ecotypes. In contrast, common species, which throughout their geographical range are more or less continuously distributed over many habitats, will in all probability exhibit complex patterns of continuous variation. Also, the mode of pollination is important. Small populations of insect-pollinated species often exhibit ecotypic discontinuities, but these are less likely to occur in widespread wind-pollinated species.

Since Turesson's time there has clearly been a change of outlook. Ecotypes are now regarded as nothing more than prominent reference points in an array of less distinct ecotypic populations (Gregor, 1944). Some experimenters have been reluctant to designate ecotypes; they have instead carefully recorded the patterns of 'ecotypic differentiation' found in particular experiments (see, for example, Quinn, 1978). However, despite the difficulties of defining the word 'ecotype', on-line searches of the Institute for Scientific Information database of recent scientific publications (via BIDS; Bath Information and Data Services, England) reveal that it is still being used for local and regional variants. Also, some of the regional, biochemical and developmental variants of *Arabidopsis thaliana*

are referred to as 'ecotypes' in British, European and US stock lists and in publications.

With hindsight one can see in Turesson's own results the possibility that, in common species, variation patterns were more complex than the ecotype concept implied. For instance, where sandy fields and dunes were found as adjacent habitats, a considerable number of intermediate *Hieracium umbellatum* plants were found linking the two ecotypes. Similarly, in *Leontodon autumnalis*, Turesson (1922*b*) found a complex situation where meadows and pastureland ran down to the sea.

The refining of genecological experiments

Early cultivation experiments were often very crude; a few plants were dug up in the wild and planted in a garden. As we have just seen, the pattern of sampling will to a very large extent determine the outcome of an experiment. Furthermore, while a simple garden technique may serve to study major differences between population samples, the study of fine scale variation has resulted in the devising of improved cultivation and other experiments. Thus, as genecology has developed, the methods of sampling and cultivation have been refined to enable statistical analysis of finer and finer differences between samples of plants.

Sampling populations

Much time and effort may be spent in growing and measuring plants and analysing results, but very little attention may have been given to sampling strategies; indeed, the word 'strategy' may be entirely inappropriate for samples of seed snatched at brief roadside stops on car journeys or obtained from Botanic Garden seed lists.

If statistical analysis is to be performed on the results, then ideally a random sample of plants must be collected. Ward (1974) has described a simple way in which two people may collect such a sample. Having decided on the area to be sampled, the recorders count the number of individuals in the area (or subsection of an area if the plant population is very large). A decision is then made on the size of the sample, say 25 plants out of 250. Using a table of random numbers (as found, for instance, in Fisher & Yates, 1963) or numbers 'drawn out of a hat', 25 numbers within the range 1–250 are 'selected' and placed in ascending order: say 5, 8, 14, 27, etc. On traversing the sample area again, one person calls out the number of each individual, 1–250, while the second person labels the individuals to be

sampled, the 5th, 8th, etc., as determined by the random numbers. This random sample is then used for experimental investigation. Other methods of random sampling are discussed by Yates (1960), Cochran (1963), Greig-Smith (1964) and Green (1979). There are some theoretical and practical difficulties to be faced in undertaking such a sampling procedure, which will now be considered.

If the experimenter is studying apparent hybridisation, a random sample might not include all the 'interesting' plants of an area. A deliberate sampling of the plants of the area might be more appropriate in such circumstances. Should the study involve the investigation of variation across a vegetational discontinuity, e.g. woodland to grassland, it might be more informative to collect plants from a transect (sampling at, say, metre intervals) across the ecotone rather than collect a random sample. All will depend on the hypothesis being tested. There are many habitats where the collection of random samples is very difficult (e.g. tropical rain forests, aquatic and wetland habitats, cliffs). However, where the collection of such samples is a practical possibility it should be seriously considered.

Since populations often contain individuals at all stages of growth and development from seeds and seedlings to adult plants, a truly random sample should perhaps contain individuals in several different age classes. In practice, a subset of the population is often sampled. The following might usefully be distinguished:

1. 'Individuals' present as ungerminated seed in the soil ('seed bank').
2. Seedlings, a transitory stage in many habitats, but more important in some plant communities. For instance, in tropical rain forests many tree species growing in deep shade have a long seedling stage; only if disturbance in the canopy causes greater illumination of the ground flora do the seedling trees develop into adults.
3. Immature individuals.
4. Mature individuals.
5. Seeds attached to 4.
6. Diseased and damaged plants. Sometimes, as in the case of the 'choke' disease of grasses caused by fungus, the plants are vegetatively vigorous but the fungal infestation suppresses the formation of inflorescences (Bradshaw, 1959*c*).

Subsets 4 and 5 are most commonly sampled by experimenters. Different subsets may reveal quite different spectra of variation; we shall see examples in Chapter 9 when we consider attempts to study the effects of natural selection by comparing the variation of different subsets in cultivation.

One of the biggest difficulties in sampling populations concerns the definition of an individual. In open vegetation it is usually possible to define individuals in annual plants and to see patches of individual perennial plants. In closed swards, however, the problem is more difficult. Sometimes the presence of 'marker' genes (e.g. leaf marks in *Trifolium repens*: Davies, 1963) might reveal the extent of particular individuals; but such markers are rare. Theoretically it might be possible to trace root systems in an attempt to establish the extent of individuals, but the practical difficulties are enormous. Furthermore, in some plants, e.g. certain forest trees, root-grafts occur which unite the root systems of several different individuals (Graham & Bormann, 1966; Böhm, 1979). Recent studies of patterns of allozymes in Strangler Figs (*Ficus* species), which form a woody sheath around many tropical trees, provide evidence that apparent individuals are in reality genetic mosaics, caused by root fusions of a number of plants (Thomson *et al.*, 1991).

The problem of defining the individual is further complicated by clone formation, in which the vegetative continuity of an individual breaks down, producing a clonal patch of several individuals of identical genotype (Harper, 1978). Evidence for clonal populations is usually circumstantial, but direct evidence is available in the case of certain self-incompatible species which are very variable morphologically. Variability has been studied in garden trials of population samples, and the material classified into different individuals on the basis of morphology, phenology, susceptibility to pests and diseases, etc. The behaviour of different plants in crossing experiments is then studied. Crosses between dissimilar-looking plants may yield a 'full seed-set', from which we may infer that the plants have different *S* alleles and are different genotypically. Conversely, crosses between plants which are morphologically indistinguishable may yield little (or no) seed, and can be thought to share the same *S* alleles and to be of the same genotype or 'isoclonal'. This method was used to study variation in populations of the grass *Festuca rubra* (see Table 8.6). Some caution is necessary in interpreting experiments of this type, as the method depends upon a thorough knowledge of the type of incompatibility mechanism involved – a requirement almost never satisfied with wild species.

Recently, investigations using molecular methods have greatly increased our understanding of populations of clonally propagating species. For instance, in a study of a population of Bracken (*Pteridium aquilinum*) in Virginia using 6 polymorphic isozyme loci, as many as 45 genotypes were detected in the study area (Parks & Werth, 1993). Moreover, some of these clones were very extensive. In an investigation of a population of the same

Table 8.6. *Some examples of studies of clones (Lines of evidence: F. = field observations; C. = cultivation trials; H. = hybridisations; I. = electrophoretic studies of isozymes; M. = DNA fingerprinting)*

C.	*Anemone nemorosa* (von Bothmer *et al.*, 1971): large number of clonal patches, of limited size, in Swedish habitats.
C.F.I.	*Betula glandulosa* (Hermanutz, Innes & Weis, 1989): clones mapped on Baffin Island, at northern limit of species.
F.I.	*Decondon verticillatus* (Eckert & Barrett, 1993): a survey of this tristylous species reveals that at the northern margin of its range in eastern North America, populations may consist of only one of the three style variants and reproduction is exclusively by clonal propagation.
C.H.	*Festuca rubra* (Harberd, 1961): evidence of many genetically different individuals in a study of an area of South Scotland. One particular variant occurred at points *c.* 220 m apart. If this area was achieved by radial growth then the clone must be *c.* 400–1000 years old. However, perhaps the present distribution has been achieved by dispersal of fragments by animals or other causes, or as a consequence of vivipary, which has been recorded in this species (Smith, 1965). Widespread clones also found in *Festuca ovina* (Harberd, 1962).
F.I.	*Larrea tridentata* (Sternberg, 1976; Vasek, 1980): extensive clonal patches, visible on aerial photographs, in the Mojave Desert, California. By radiocarbon dating oldest clone may be 11 700 years old. Isozyme studies reveal that parts of apparent clones are indeed isoclonal.
C.H.	*Lysimachia nummularia* (Dahlgren, 1922; Bittrich & Kadereit, 1988): self-sterile clones found in many parts of North and Central Europe; presumably sexual reproduction only takes place in populations where individuals with different *S*-alleles occur together.
F.M.	*Phragmites australis* (Neuhaus *et al.*, 1993): large and small clones 'mapped' in Berlin and Northeast Germany using DNA fingerprinting (*a.* by digestion using restriction enzymes *Alu*I or *Dra*I, with the oligonucleotide $[GATA]_4$ used as a probe in hybridisation; or *b.* by RAPD reactions followed by separation of the amplification products on agarose gels and staining with ethidium bromide).
F.M.	*Populus tremuloides* (Rogstad, Nybom & Schaal, 1991): clones of various sizes mapped using DNA fingerprinting; Fig. 8.9 (digestion with restriction enzymes *Dra*I, *Hae*III or *Hinf*I and hybridization with the M13 probe).
F.I.	*Solidago altissima* (Maddox *et al.*,1989): using isozyme markers, clones were mapped in sites at different stages of old field succession near Ithaca, New York.
F.	*Ulmus* spp. (Rackham, 1975): by studying in British woodlands patterns of morphological variation together with incidence of fungal diseases and timing of coming into leaf and leaf fall, evidence of very extensive clonal patches was discovered.

species in North Wales, an extensive triploid clone was detected and this was mapped using isozyme markers (Sheffield *et al.*, 1993). On the basis of various lines of evidence it would seem that extensive clones, probably of great age in some instances, occur in some habitats (see Table 8.6 and Fig. 8.9).

Why is knowledge of the extent of individual genotypes important in sampling? Suppose we collect two population samples, A and B. Fortuitously, sample A could consist of 25 pieces of a widespread clone, whilst sample B could consist of material of 25 genetically different individuals. A comparison of the two 'populations' in a cultivation trial is likely to show that they are different, but interpreting this difference as a real population difference could be misleading. Perhaps population A *is* largely composed of the clonally propagated individuals of one genotype, while B is variable; on the other hand, populations A and B might both be variable, and the multiple sampling of one clone in population A might be merely the

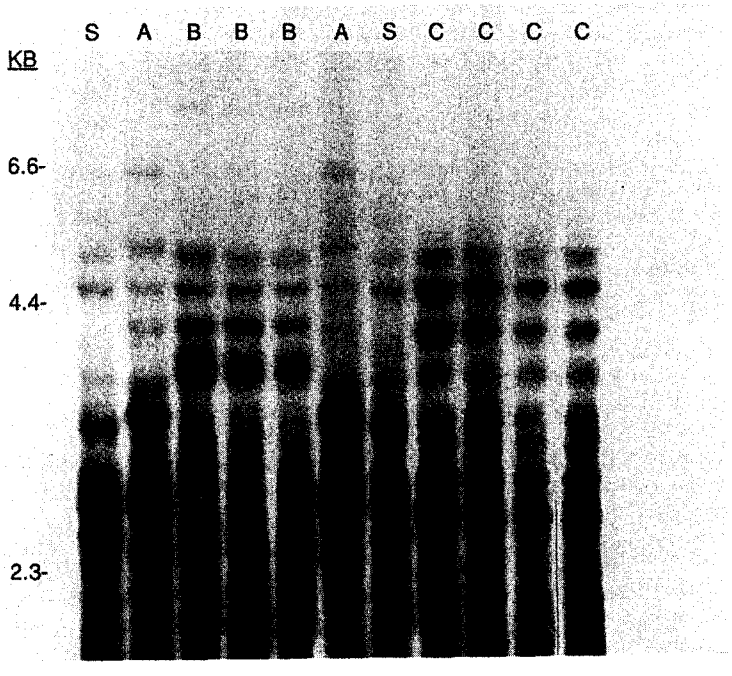

Fig. 8.9. The use of DNA fingerprinting to examine genetic diversity and clonal growth in *Populus tremuloides*. (From Rogstad, Nybom & Schaal, 1991). Initially, on morphological grounds, it was postulated that material from a site in Colorado was of two clones. DNA fingerprinting revealed that three clonal genotypes (A, B, C) occurred in the area. S = standards.

consequence of poor sampling technique. Harberd and others, who have made a special study of the problem (Harberd, 1957, 1958; Wilkins, 1959, 1960; Ward, 1974), recommend that spaced samples be collected from populations. From all the evidence available the probable maximum extent of clonal patches is estimated. Sampling at points separated by distances greater than this estimated clonal patch size is then carried out. Widely spaced samples are to be recommended to counteract another problem which arises in studying plant populations. Fruits and seeds are often shed very close to the plant which produced them and 'family groups of close relatives', perhaps involving several generations, may be found (see, for example, Linhart *et al.*, 1981). Distorted comparisons can arise if a sample containing a group of closely related plants is matched against a set whose members are totally unrelated.

It must be noted, however, that wide spacing of samples is somewhat at odds with the present trend of studying small systems in detail. Such studies as those of Smith (1965, 1972), and Harper and associates (Harper, 1983) reveal enormous variation within sites. There seems to be no easy solution to the problems raised by clonal populations; the experimentalist must make the best judgement possible in each situation in relation to the hypothesis under consideration.

Another question to be resolved before sampling is undertaken concerns the number of sites and samples within sites. Suppose we study a single site with two different soil types, A and B. Patterns of variation may be revealed in samples drawn from the two subsites A and B, and at the end of an experiment some differences related to soil type may be found in plants originating from the two subsites. The experimenter must then decide whether the differences are ecotypic or whether they owe their origin to random variation. With one A/B comparison it is difficult to rule out random events (Wilkins, 1959). A more penetrating study of the patterns of variation might be made by studying several areas, where subsites of type A and B are juxtaposed. Furthermore, in collecting from the wild, a bulk seed sample may be made to represent each of the subsites A and B, or the seeds from a random collection of mature individuals may be separately collected and packeted at each subsite. Family lines may then be grown, patterns of variation within lines offering some insights into the breeding system of the plants under study. This type of sampling – a hierarchical or nested pattern – has much to recommend it, allowing not only a number of A/B comparisons to be made, but also providing some information on variation within subsites. For instance, the plants under study might be obligate apomicts; while the progenies of different 'seed parents' might differ, there

might be little or no variation within progenies. In this circumstance, the cultivation of plants from bulked seed samples would fail to reveal an important strand in the variation pattern.

Cultivation experiments

A study of variation usually requires cultivation of plants. This is true not only of field collections brought into a common environment to investigate the nature of variation patterns, but also of many sophisticated genetic and physiological studies. In many cases, the experimenter wishes to grow material from diverse sources under the same conditions. Thus, if population samples are collected in the wild and if there are interesting phenotypic differences between populations, a Turessonian cultivation experiment might be carried out, to see if differences between populations persist in cultivation.

At first sight a requirement to grow material 'under the same conditions' appears to present little difficulty. A moment's reflection, however, is sufficient to remind the reader of the variation in soil fertility, drainage, pests and diseases within even the most uniform experimental plot in the garden or field. The notion that glasshouses provide a uniform environment is quickly dispelled by studying investigations of yields of vegetable crops on benches in different parts of experimental glasshouses (see, for example, the little-known experiments of Lawrence, 1950).

In designing genecological experiments, the botanist has had much to learn from the agricultural scientist. Farmers wish to grow high-yielding varieties of crop plants and, since the middle of the last century, research workers have struggled to perfect experiments designed to study yield. In this short book we cannot provide a complete review of this interesting subject and will confine our attentions to a few important general issues. Notable advances in the design of field experiments came with the work of Fisher, who studied the famous long-term Broadbalk Wheat experiment at Rothamsted Research Station in South England (Fig. 8.10). A book on the life of Fisher (Box, 1978) provides a useful historical review of field experimentation and explores in detail Fisher's many contributions to the subject.

The basic ideas behind the design of cultivation trials are as follows:

1. Experiments must be designed with sufficient replications of the varieties, populations, treatments, etc. Thus, in a simple experiment on yield in, say, Spring Wheat, several plots of each variety must be grown.

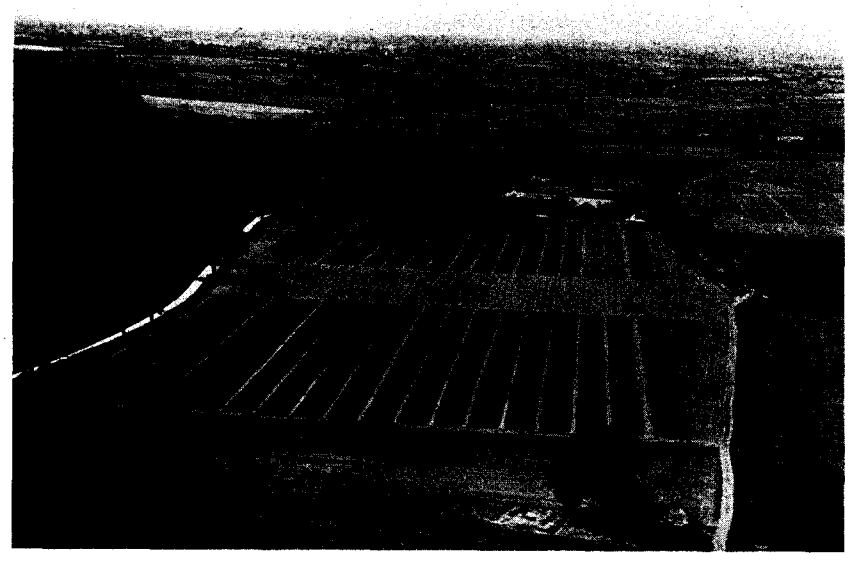

Fig. 8.10. Layout of the famous Broadbalk field experiment at Rothamsted, Herts, England, studied by R. A. Fisher. Experimental crops of Winter Wheat have been grown continuously in these plots since 1843. Photograph taken in 1954. © Rothamsted Experimental Station.

2. Soil fertility and other edaphic factors often vary across garden plots and fields, but it is commonly found that adjacent sites have similar fertility, etc. Thus, Fisher (1935) recommended that the ground available be divided into uniform blocks (not necessarily square). Each block should contain a full complement of the material under study. Within blocks the small plots of each variety should be *randomly* arranged. In early experiments in agriculture and forestry it was hoped that, by careful husbandry, varieties could be given the same conditions. But a critical approach to experimentation suggests that this is a forlorn hope; it is impossible to ignore the variability induced by environmental factors. With a proper layout of experiments, differences between blocks can be *measured* to give an estimate of the random element of variation introduced into the experiment.
3. Another important factor in the design of field experiments is the effect of position. If plants are growing in blocks, those in the centre of the block will be surrounded by neighbouring individuals; in contrast, plants on the margins of blocks are likely to be adjacent to bare soil and subject to very different amounts of root and shoot competition. Thus, it is

recommended that 'guard rows' of similar plants be planted around the blocks, to provide uniform conditions for the experimental material. Guard rows, usually of the same species as the plants under study, are discarded at final harvesting of the experiment.

It is clear that these ideas can with profit be incorporated into the design of genecological experiments, and indeed advanced field trial techniques were employed in the famous genecological experiments of Gregor and his associates in studies of variation in *Plantago maritima*, to which we have already referred (Gregor, 1930, 1939; Gregor, Davey & Lang, 1936; Gregor & Lang, 1950).

In a simple genecological experiment each individual, say of plants A, B, C and D, may be clonally propagated, the experimental garden may be divided into small blocks and a ramet of each individual of A, B, C and D planted in a weed-free plot surrounded by guard rows of the same species. The position of each ramet within blocks is determined by random numbers.

The fundamental ideas influencing the layout of simple field trials may also be incorporated into the design of more complex genecological experiments such as population trials, family lines and experiments involving populations given various treatments. Several excellent books with fully worked examples of various designs are now available for the biologist. Especially suitable for beginners are: Salmon & Hanson (1964); Bishop (1971); Parker (1973); and Clarke (1980). More advanced treatment will be found in: Campbell (1974); Ridgman (1975); Snedecor & Cochran (1980); Sokal & Rohlf (1981); Yates (1981); Stuart (1984); and Mead (1988).

Studies of agricultural crops have resulted in other important insights into the design of field experiments. At first sight it would seem reasonable to suppose that repeated experiments with the same varieties (or genotypes) would 'give the same results'. In practice there are considerable differences from year to year in the results of experiments estimating yield in cultivated stocks. The principal causes of variability are differences in weather, and changes in the incidence and severity of various pests and diseases (which are themselves probably correlated with past or present weather conditions). An experiment by Nelson (1967) emphasises the importance of year-by-year differences in a genecological experiment. He studied variation in *Prunella vulgaris* collected from many sites in the USA, by growing material at Berkeley, California. Usually there is no winter frost in this area, but, exceptionally, a very cold period occurred from 20–24 January 1962, providing him with a unique experiment, which revealed that some of his plants were frost-sensitive.

In designing genecological experiments other factors must be taken into account:

1. In experiments begun with samples of seeds, Roach & Wulff (1987) point out that there may be maternal effects, i.e. there may be a 'contribution of the maternal parent to the phenotype of its offspring beyond the equal chromosomal contribution expected from each parent'. Such contributions may be: (a) cytoplasmic; (b) flow from the greater contribution of maternal genes ($2n$) than male genes (n) to the ($3n$) endosperm; or (c) result from the fact that maternal tissues contribute to developing fruits and seeds. Thus, maternal effects are often manifest in differences in seed size and mineral composition. Roach & Wulff (1987) review the various techniques available to measure maternal effects in cultivation trials. Evidence for paternal effects – via 'male' cytoplasm – is also reviewed in the same paper. Maternal and paternal effects are examples of what are sometimes called 'carry-over' effects.
2. 'Carry-over' effects are also possible in experiments begun with vegetative material, such as clone transplants. Thus, the length of an experiment may be crucial if the investigation involves material dug up from the wild and transplanted into a garden for, as Turesson (1961) discovered, an extended period of adjustment may be necessary before plants may be said to have outgrown the effects of their original habitats. Indeed, it may be difficult to convince a sceptic that a complete adjustment is ever made, especially in the case of woody plants. Experiments with herbaceous plants have also been revealing. For instance, from a 43-year-old pasture in Canada, Evans & Turkington (1988) grew samples of *Trifolium repens* collected from patches dominated by different species of grasses. At the end of a field trial, lasting for 4 months, significant differences were detected between the samples for a number of characters. Then, a second trial was begun with the same samples, using material produced by vegetative propagation. It is of very great interest that there were no significant differences between the samples after 27 months. This experiment is a clear indication of the importance of 'carry-over' effects in relation to the duration of experiments. A second example makes some further important points. 'Carry-over' effects could arise from the use of unequal-sized pieces of material used to begin clone experiments. In a study of many facets of garden trials, Davies & Snaydon (1989) examined the effect of tiller size – small versus large – in the grass *Anthoxanthum odoratum* on a number of measures of performance in a garden trial. They discovered no evidence

for a major problem with 'carry-over' effects in this case, as there were no differences in survival, height or date of flowering. However, large tillers produced slightly larger plants.

3. The pretreatment of seeds and seedlings prior to the experiment is very important. There will be differences in the speed of development of plants between those sown as seed and those set out in the field as young plants. The timing of the experiment in relation to such seasonal factors as cold periods may also be crucial. Thus, some plants will not flower unless subjected to cold treatments, and spring and autumn sowing will yield different results.
4. The treatment of plants during the experiment has a profound effect upon their growth and performance. The experimenter must decide whether to water plants in dry weather, apply fertilisers, etc.
5. The incidence of pests and diseases causes considerable problems. In particular, experiments in glasshouses often turn into a struggle to control various insect and fungal pests, and the liberal use of pesticides may be the only means of 'preserving' the experiment. It is important to realise that 'spot-treatments' of badly infected individual plants may seriously affect the randomised design of the experiment. In the design of garden and field trials, on the other hand, the decision is often taken to allow non-catastrophic invasions of pests and diseases to take their toll on the experimental material. In this way it may be possible to see if any individuals or populations are resistant to fungal or insect attack. Studies of the effects of non-fatal pests and diseases may add a further dimension to our knowledge of population variation.
6. Agricultural experiments are often designed to be left until the final harvest when estimates of yield are made on fruiting material, and in other cases the experiment is so constructed as to permit regular intermediate harvests at selected periods between sowing and final harvest. Such experiments may be poor models for experiments in the ecological genetics of plants, in which a great deal of information may be gathered by 'non-destructive scoring' of the plants over weeks or months. For instance, given adequate spacing between plants, plant height at different times could be measured, and the timing of flowering and fruiting could be studied. Also, samples of leaves could be removed for study, provided that all the material in the experiment is treated alike. Thus, a good deal of quantitative information might be obtained by repeated scoring of an experiment. Sometimes it is unnecessary to make measurements; the stages of development or incidence of damage by pests may be recorded by classifying the material into a small number of 'character states'.

The designed experiment

So far we have discussed a number of important factors in the design of genecological experiments. For both the experimenter and the botanist who wishes to interpret the scientific literature, it is crucial to take proper account of the problems, and possibilities of sampling and cultivation. These are elements in a larger canvas, however. Many authors have stressed that genecologists should aim at a *designed experiment* in which hypothesis, sampling, cultivation, analysis and interpretation all take their proper places.

The generation of germinal ideas is a mysterious process. Armed with a knowledge of the literature, provoked by the observations and comments of others, the botanist notices something of interest in the patterns of variation. From this initial interest an idea emerges for an experiment. The process by which ideas occur to experimenters is not to be seen as a mechanical process, but as a creative act much as is required for practice of the arts. Next, the experimenter formulates a hypothesis leading to an experimental investigation, the results of which are used to consider whether the hypothesis is confirmed or rejected. As part of the investigation the results may be subjected to statistical tests.

The best way to appreciate the different elements in the designed experiment is to study an example. We have chosen to present the results of a simple study on *Plantago major* (Warwick & Briggs, 1979). Our account should be seen as a simplified introduction to a central concern of science, namely how to devise, execute and interpret experiments. We hope that biologists reading our account will be encouraged to study the many excellent introductory books (which we have noted above) on the design and statistical analysis of experiments.

An experiment to study the variation in Plantago major *growing on droves (grassy tracks) at Wicken Fen Nature Reserve, Cambridgeshire, England*

Many thousands of visitors visit the famous Wicken Fen Nature Reserve each year and the droves (grassy tracks) which cross the Fen are subject to severe trampling pressure. *P. major* occurs in the heavily trampled areas (as a small, prostrate plant) and also in the adjacent grassy sward (in which it is a larger, erect plant).

Ecotypic differentiation has been reported in *P. major* (Turesson, 1925; Groot & Boschuizen, 1970; Mølgaard, 1976) and, as we shall see in Chapter 9 there is evidence from a number of genecological studies which suggests

that differentiation might occur over short distances, despite gene flow. Therefore, the possibility exists that dwarf, prostrate variants might be selected on the pathway, while taller plants would be at a premium in the adjacent grassy sward. Thus, we could formulate the hypothesis that samples taken from the wild might retain their distinctness in cultivation. As the differences involved are those of size, our hypothesis is not very precise in its present form. We cannot make any definite prediction as to the degree of difference to be retained; indeed as we are dealing with quantitative differences it is not at first sight clear how one can make a prediction as to the degree of difference which 'needs' to be retained in order to accept the hypothesis. So far our hypothesis is too vague. However, a precise hypothesis is possible in this case, namely that on cultivation we expect *no* difference between groups of plants after cultivation. Such a hypothesis is known as a 'null hypothesis'. The concept of the null hypothesis is widely used in biology and such a hypothesis, that zero difference is expected between two sample groups, should always be formulated as part of a designed experiment, for a precise initial hypothesis is likely to lead to a well-designed investigation.

Unbiased samples, 10 from the trampled area and 10 from adjacent grassy swards, were collected in the autumn of 1974. *P. major* is not a clonally propagating species (although it may be cloned in gardens: Marsden-Jones & Turrill, 1945), but spaced samples were taken at least 10 m apart. Plant material was potted up in John Innes No. 1 compost and the pots, which were randomly arranged, were plunged to the rims in the sand of an outdoor plunge bed. Spacing between pots was very generous and guard rows were not necessary.

In order to allow us to examine the null hypothesis, a statistical test is necessary to enable us to compare the two groups of samples. The test should allow us to compare the variation between and within groups. Clearly variation between groups (from trampled path versus adjacent grassy sward) is only likely to be significant if it can be shown to be significantly greater than variation within groups. We shall use for our test the analysis of variance technique, which works by estimating the significance of variation between groups by comparing it with variation within groups. The variation in some measurable trait of 20 plants of *P. major* is, by this test, partitioned in such a way as to enable us to see the variation due to subsites at Wicken, while at the same time giving us an estimate of the variation within groups.

The steps in the analysis of variance are a simple extension of those used in Chapter 3. To recapitulate, we showed that:

$$\text{variance } (s^2) = \frac{\sum(x - \bar{x})^2}{n - 1}$$

The sum of the squares of the deviations from the mean could be calculated by subtraction of each value from the mean, squaring the difference and summing the resulting squared deviations. Alternatively, we suggested that, if a calculating machine is available, the sum of (deviations from mean)^2 (sum of squares) could more readily be calculated by employing the formula:

$$\text{sum of squares} = \sum x^2 - \frac{\left(\sum x\right)^2}{n}$$

Where $\frac{\left(\sum x\right)^2}{n}$ is known as the Correction Factor or Term, *C*.

We may now examine (Table 8.7) the steps in the calculation of simple analysis of variance on the *P. major* experiment. The *null hypothesis* is that there is no difference in leaf length between plants grown from trampled drove and from grassy sward.

If this null hypothesis is to be confirmed then there should be little or no difference between the variances between and within groups. To estimate the relative size of these two variances we calculate the variance ratio (the *F* value – in honour of R.A. Fisher who developed analysis of variance). If, however, there is a real difference between groups, we would expect variation between groups to exceed that of the variance within groups. Tables of probabilities appropriate to different values of *F* are available. In the case of the *P. major* experiment, it is clear that a good deal of the variation is *within* groups and that the difference between groups is small. The mean values are very similar. Indeed, leaf length of plants grown from the small plants of the trampled area slightly exceeds that for the samples from the tall sward. The null hypothesis, that there is no statistically significant difference between the two groups of plants, is supported by our results. On the strength of present evidence, we have no reason to suppose that ecotypic differentiation has occurred in the trampled and tall sward subsites.

The *P. major* investigation was part of a more extensive study of this species in various grasslands (Warwick & Briggs, 1979, 1980*b*). Table 8.8 sets out another comparison. Small phenotypes were found not only in trampled areas (as on the droves at Wicken), but also in closely mown lawns. Samples of plants from the Botanic Garden lawn in Cambridge and

Table 8.7. Plantago major: *length of longest leaf (cm) in plants after* c. 10 *months cultivation in the Botanic Garden, Cambridge*

Wicken Fen: trampled areas on droves	Wicken Fen: grassy swards adjacent to droves
30.5	33.3
33.4	28.0
25.5	21.9
34.2	26.0
27.4	24.0
26.5	28.4
31.5	32.2
29.3	27.0
24.8	26.3
28.0	26.0
Mean 29.110	27.310
Total 291.100	273.100

Grand total = 564.200

$$\text{Correction factor} = \frac{564.200^2}{20} = 15916.082$$

$$\text{Sum of squares (total)} = (30.5^2 + 33.4^2 + 25.5^2 \ldots 26.0^2) - C$$
$$= 16133.080 - 15916.082 = 216.998$$

Having calculated the total sum of squares we now calculate the variations between and within groups.

Between groups is estimated by

$$\frac{291.100^2}{10} + \frac{273.100^2}{10} - C = 15932.282 - 15916.082 = 16.200$$

Within groups is estimated by subtracting 16.200 from the total sum of squares.

Within groups sum of squares = 216.998 − 16.200 = 200.798

Subdivision of the sum of squares into its two parts has been accomplished and the degrees of freedom (19 in all: one less than the number of observations) may now be determined for each component. Between groups: 2 groups, therefore 1 degree of freedom. Within groups: 10 observations per group, each loses 1 degree of freedom, total 18.

The analysis of variance may now be set out in a table showing the sources of variation, the divisions of degrees of freedom and sum of squares. Mean squares (variances) are now calculated. The between-groups mean square gives the variance of the two groups about the grand mean, while the within-groups variance gives the variance of individual values about the two sample means.

Source of variation	Degrees of freedom	Sum of squares	Mean square (variance)	Variance ratio (F)	Probability
Between groups	1	16.200	16.200	1.452	> 0.05
Within groups	18	200.798	11.155		
Total	19	216.998			

Table 8.8. Plantago major: *the effect of* c. *10 months cultivation on samples of small phenotype from Wicken droves and Botanic Garden lawns; length of longest leaf (cm)*

Wicken: trampled areas on droves	Botanic Garden lawns
30.5	11.8
33.4	20.7
25.5	8.9
34.2	22.6
27.4	24.0
26.5	14.1
31.5	13.1
29.3	16.0
24.8	12.5
28.0	12.0
Mean 29.110	15.570
Total 291.100	155.700

Grand total = 446.800

$$\text{Correction factor} = \frac{446.800^2}{20} = 9981.512$$

$$\text{Sum of squares (total)} = 30.5^2 + 33.4^2 + 25.5^2 \ldots 12.0^2 - C$$
$$= 11228.860 - 9981.512 = 1247.348$$

$$\text{Between groups sum of squares} = \frac{291.100^2}{10} + \frac{155.700^2}{10} - C$$
$$= 916.658$$

Within groups sum of squares = 1247.348 − 916.658 = 330.690

Source of variation	Degrees of freedom	Sum of squares	Mean square (variance)	Variance ratio (F)	Probability
Between groups	1	916.658	916.658	49.895	< 0.001
Within groups	18	330.690	18.372		
Total	19	1247.348			

from Wicken droves (trampled areas) were compared. The variation between groups in this case is statistically significantly greater than the variation within groups. Therefore, the null hypothesis, namely that samples do not differ in leaf length, receives no support from the experiment. There would appear to be a real difference in leaf length between the two samples. In Warwick & Briggs (1979, 1980*b*) details are given of the

highly distinctive plants of *P. major* discovered in the lawns of Cambridge colleges and gardens.

Our examples of analysis of variance are of a very simple kind, with division of the variation into two parts. Much more elaborate experiments may be devised and the 'overall variation' discovered in experiments may be divided into many parts estimating, where appropriate, the variation due to blocks, population differences, family lines within populations, interacting factors, random events, etc. By looking at the relative magnitude of different segments of the variation, very considerable insights into population variation may be obtained.

Analysis of variance is a most elegant technique, which must, however, be used with care. It should only be employed in analyses where the results are 'normally distributed' and in which the variances of the contributing population samples, treatment values, etc., are equal or approximately so. Various tests have been devised to study the 'properties' of arrays of figures to see if they are appropriate for analysis of variance (for details of Bartlett's test see Salmon & Hanson, 1964; Sokal & Rohlf, 1981). Sometimes it is possible to 'transform' the results to produce equality of variances. For instance, the unsatisfactory raw data may be converted to square roots or to logarithms. If the results cannot be satisfactorily transformed, then other statistical tests – so called non-parametric tests – may be applied (Sokal & Rohlf, 1981). Such tests do not make any assumptions that the figures from the experiment are normally distributed or have equal variances. Non-parametric tests should be more widely used in biology, for the results of many experiments and observations show enormous departures from normality.

The interpretation of experiments

Whatever the results of particular experiments, there are usually grounds for a cautious interpretation of genecological studies.

However many plants are grown, or studied in experiments, the size of samples that can conveniently be grown is often minute relative to the size of wild or semi-natural populations. For instance, according to the estimates of Barling (1955), populations of *Ranunculus bulbosus* may reach 257 000 per acre in the English Cotswolds, and continuous populations in adjacent fields of pasture were estimated to contain 14 000 000 plants. Such figures are by no means exceptional.

Many experiments are carried out in conditions remote from those in nature. For example, studies of metal tolerance in plants involve measurements of root growth in very simple culture solutions (see Chapter 9). In the wild, plants grow in soils where conditions are quite different. The attempt to simplify situations in order to study individual factors is clearly justified, but the investigator must not make too facile an extrapolation from simple laboratory tests to the natural situation.

As in the case of metal tolerance, many experimentalists isolate individual factors of presumed importance and make special studies of the tolerances of population samples. A fascination with the study of critical or limiting factors should not blind the student of evolution to the fact that the concept of a factor is an abstraction. Often particular factors are chosen for study, largely because the means to control or vary them in precise ways are available in laboratories. It is often forgotten that plants respond to their environments as a functioning whole. Realising this difficulty, a number of botanists are becoming interested in experimental studies of the adaptive significance of variation in plants by carrying out experiments in the field. The garden trial with its weed-free, spaced plants is not entirely satisfactory as a means of studying 'adaptation', for the competitive interaction between plants is absent. Thus, there has been a revival of interest in the reciprocal clone-transplant experiment, in which cloned material of diverse origin is transplanted into swards subject to different treatments. By close mapping and labelling of plants, the survival and growth of transplants may be studied (see Chapter 9). Care in the layout and recording of such experiments may overcome the difficulties which, as we saw in Chapter 6, cast doubt on the historic studies of Bonnier and Clements. By studying the way plants behave in such experiments, the experimentalist may have a very direct insight into the responses of plants to 'whole' environments. There is obviously a place for both tolerance tests and reciprocal transplant investigations in the repertoire of techniques available to the genecologist.

A final problem facing the experimentalist is that of deciding the causes of the underlying patterns of variation under study. Even after long and complex experiments, it is not possible to conclude with certainty that residual variation in, say, a garden trial, is 'genetic' in origin; breeding experiments are necessary to see if characteristics are transmitted by seed. As we shall see in the next chapter, which reviews recent studies in genecology, the advent of molecular methods has transformed the study of population variation.