VARIATION IN *ASTER TRIPOLIUM* L., WITH PARTICULAR REFERENCE TO SOME BRITISH POPULATIONS.

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for the degree of Doctor of Philosophy

by

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" Species of the genus Aster are notoriously difficult to classify. Dr. Gray, the foremost student of the group, found them very puzzling. We find in his letters (Gray, '93)* many references like the following: " I am half dead with Aster. I got on very fairly until I got into the thick of the genus, among what I called Dumosi and Salicifolia. Here I work and work, but make no headway at all. I can't tell what are species and how to define any of them ..... I never was so boggled ..... If you hear of my breaking down utterly, and being sent to an asylum, you may lay it to Aster, which is a slow and fatal poison."."

Anderson, E. (1929) Ann. Mo. Bot. Gard. 16 p. 140.

* Gray, J. L. (1893). Letters of Asa Gray. pp. 696 - 697.

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ABSTRACT

(1) The following is a study of variation in *Aster tripolium* in relation to the ecology of the species and in view of its widespread occurence on British salt marshes. The main period of the investigation was between 1965 and 1968.

(2) Whilst recognisable as a taxonomically distinct species *A. tripolium* is highly variable at the phenotypic level, and at least 18 infraspecific taxa have been recognised in European populations by traditional methods. Most, or all, or these taxa are of doubtful status. It is unimportant whether varietal names are retained for extreme climatic types such as *arcticus* and *pannonicus*.

(3) In contrast to the extremely high level of phenotypic variation a sample of populations investigated were cytologically uniform. The diploid number 2n = 18 was counted in all plants from 14 British and 7 continental populations (including 3 varieties) and the karyotype of all 7 British and 5 continental populations studied was found to be highly uniform. The count is the first known count of British material. The karyotype has features which are thought to be evolutionarily primitive.

(4) *Aster tripolium* is both cross and self fertile. Three crosses between plants geographically separated by considerable distances have produced fertile fruit. There was no evidence of apomixis.

(5) A total of 56 populations were sampled, all but 15 of which were from the British Isles. Two sampling programmes were carried out, 39 populations being sampled as part of a programme designed to sample a small number of plants extensively within the species' range, and 17 populations being sampled as part of a programme designed to sample larger numbers of plants at distinct points along coastal gradients in 5 areas. Descriptions of these areas, 2 in Lincolnshire, 2 in Norfolk and 1 in Cheshire, are given. Populations from an inland site in Cheshire were also sampled.

(6) In order to make comparisons between populations from ecologically similar sites a subsample of 24 British salt marsh populations (17 'intensively' sampled and 7 from the 'extensive' sampling programme) was used to divide the salt marsh areas on which <u>Aster tripolium</u> occurs into three categories, high, mid, and low. Two methods were used to make this division. The first was based on a number of ecological factors related to tidal submergence studied at the 5 main coastal areas, and involved the recognition of a critical level separating two types of intertidal marsh. The second method used an association-analysis of the species recorded from each sampling area. There was a good measure of agreement between the results obtained by the two methods.

(7) Results obtained by comparative cultivation and by

growth of selected populations in a range of culture conditions suggest that the wide ecological amplitude of <u>Aster tripolium</u> in salt marshes is the result of adaptive divergence involving variables of fundamental biological importance such as life cycle, fruit size, germination requirements and growth in saline soils. Laboratory and field experiments were carried out to test further hypotheses relating to specific trends of differentiation.

(8) A north/south cline in year to first flowering in European populations was accompanied by similar variation in British salt marsh populations across the coastal zone. Populations from high marshes contained a high percentage of first-year flowering pauciennial types in contrast to those from mid and low marshes which were perennial and flowered for the first time in the second or third year of growth. First year flowering types flowered later and less synchronously than second or third year types. Growth under a spectrum of conditions indicated that high marsh pauciennials are able to respond to cultivation, behaving either as true annuals or, in certain conditions, overwintering or flowering more than once. The possible effects of variation in year of first flowering, number of flowering times and reproductive output was examined by a simple mathematical model.

(9) Populations sampled from points across the coastal zone in three areas showed a cline of variation in disc fruit size (accompanied in two areas by an inverse cline in fruit number

per head) - the high marsh populations having significantly smaller fruits. Fruits formed by ray florets were significantly smaller than those formed by disc florets. The wide variation in total fruit production per plant was largely a function of variation in the amount of axillary branching. The small within-population variation in fruit size under a range of conditions was interpreted as indicating that both fruit size and stability of fruit size were ecologically important characters. In particular the relationship between initial fruit size and seedling growth rate was examined and the hypothesis is advanced that the elimination of seedlings on unstable substrates by the mechanical effect of wave action may be an important factor selecting large fruited types on low marshes. This was supported by the variation in lateral root development and by studies of marked clumps of seedlings during the establishment phase in one low marsh area.

(10) Studies of germination in selected populations revealed variation from high to low marshes in the percentage of fruits requiring prechilling to break dormancy, the germination rate in dilutions of sea water, and the germination rate of large and small fruits. The ecological significance of this variation is discussed. Germination rates were slightly increased in all populations when the fruits were germinated in the dark.

(11) The growth of seedlings in nutrient culture solutions to which different amounts of sodium chloride had been added was studied. Within-population effects of increasing NaCl were

generally greater than between-population ones in short-term nutrient solution experiments. In particular growth depression was accompanied by reduced lateral root production. Survival and growth in soils given a range of simulated tidal treatments was relateable to the origins of the populations tested. Analyses of the ash of a low and a high marsh population grown in increasingly saline solutions suggested that the preferential absorption of potassium in high sodium environments may be an important feature of adaptation to growth in saline soils. Survival rates of high marsh populations in various conditions of cultivation in ordinary soils were consistently higher than those of mid marsh populations, which in turn were higher than those of low marsh populations. In one experiment larger numbers of low marsh than mid marsh plants were eliminated by competition with garden weeds.

(12) Variation in a number of morphological characters between high, mid, and low marsh populations was investigated by collateral cultivation. A number of these characters (branching woodiness, sub-basal leaf development, and number of heads per plant) were correlated with life cycle variation, whilst others (inflorescence height, height of perennating bud, basal leaf shape, rosette habit and leaf colour) revealed consistent patterns of variation from high to low marshes. Cultivation in different conditions suggests that these characters vary in their plasticity.

(13) The recent spread of the rayless form in south-east England is described and the results of controlled crossing experiments to determine the mode of inheritance of the ray floret character are discussed. The differential ecological distribution of full rayed, partly rayed and non rayed plants is described. The hypothesis is advanced that the spread of the rayless form may be explained by the selection of characters for which it is a pleiotropic marker enabling its enhanced survival in low marshes, and by the increase in such marshes created by the parallel spread of <u>Spartina anglica</u>. Adaptive divergence of the rayless form may be aided by the isolating factor of non-coincident flowering time, thus reducing gene flow between adjacent populations.

(14) Much of the variation in <u>Aster tripolium</u> from high to low salt marshes, and especially that between closely adjacent populations, was clinal, and discontinuities were blurred by numbers of intermediate types. The low incidence of clear-cut patterns of variation is thought to be due to a number of factors. These include (a) the graded, as opposed to sharply marked or stepped, variation in the environment, (b) the existence of different environmental gradients related to salinity, exposure and so on, (c) the strong possibility of gene flow between adjacent populations as a result of widespread outbreeding and effective fruit dispersal mechanisms, (d) the important role of plasticity in adaptation to fluctuating, heterogeneous environments

(e) the possibility that many of the characters being considered are under polygenic control, (f) the use in some experiments of seed, rather than mature plant, population samples, and (g) the stretched nature of the response of maritime plants to ordinary soils.

(15) Adaptation to maritime environments is believed to be a complex process involving adjustment to environmental factors at all stages of the plant's life cycle. In Aster tripolium such adaptation has involved a mixture of genetic and plastic responses which is likely to have included undetected physiological variation. The features which characterise the high marsh populations are commonly regarded as those exhibited by weedy aggressive species, a fact which is understandable in view of the increased competition with which such populations are faced.

The increasing effects of competition factors towards the upper zones and the increasing effects of factors related to tidal submergence towards the lower zones are thought to be the principal agents of disruptive selection, populations showing marked responses to them as the ecological limits of the species is approached. Although the intensity at which they do so may vary from one area to another these factors eventually become limiting, restricting the species to suitable maritime and paramaritime habitats.

GENERAL INTRODUCTION

The work reported in this thesis was generated by the observation that populations of Aster tripolium L. in this country are highly variable at a phenotypic level. It is concerned with an attempt to recognise, describe and quantify units of variation within the species and relate these to factors of ecological importance.

A number of aspects of variation has been examined including chromosome number, karyotype, floral and fruit characters, germination, seedling growth, morphological characters and life cycle. This range in the types of variation which were investigated presents certain difficulties for the organisation and presentation of this report. For this reason some discussion of the particular aspect of variation being considered is included in the text immediately following its description. A more general discussion of the total complex of variation within the species follows the final summary (Chapter 12).

The results of investigations are reported in four parts.

Part I. describes the background to the study, outlines the approach to the problem, contains an appraisal of the experimental methods used, and surveys and classifies the areas studied.

Part II is concerned with those aspects of variation relating to the reproductive biology of the plant and includes a chapter on fruit germination in selected populations.

Part III generally concerned with the dynamics of dispersal and establishment, describes a series of experiments which attempt to investigate the possible significance of, in particular, the variation in fruit characters described earlier (in Part II).

Part IV describes the variation in a number of morphological characters and in the ray floret character.

Part V summarises and discusses the overall variation pattern in relation to the ecology of the species.

Parts I - IV contain separate summaries of their contents.

PART I

INTRODUCTION, BACKGROUND AND METHODS

CHAPTER 1. INTRODUCTION

I Geographical distribution.

Aster tripolium L. occurs in suitable habitats throughout western and central Europe, its range extending southwards to the north coast of Africa (Tunis) and as far north as Varanger Fjord in northern Norway (c. 70 degrees N). Within this area it is most frequently found on the maritime marshes fringing the European sea coasts, including the Atlantic and North Sea coasts and extending from the Mediterranean to the east and west shores of the Baltic. It is absent from Iceland and the Faeroes.

Towards the eastern edge of its range as a maritime plant *A. tripolium* is locally common on the shores of inland lakes and saline areas of central Europe and Asia. It is recorded from several lakes in Lapland and from the Neusielder Sea, the Black Sea, the Caspian Sea, and as far east as Lake Baikal. As an inland 'salt-desert' plant it is found from the saline plains of north Germany and Poland and those of Hungary, Rumania, lower Austria, and northern Italy south-eastwards to the Taurus mountains and Persia and eastwards to the Atlas mountains and Siberia. It is also found around the inland sea and on the coast of Japan (Jinno 1956).

The species is absent from the New World. Its world distribution is indicated in Fig. 1.1 which is based on data extracted from various national Floras and from Clapham *et al*, (1942), Chapman (1960), Wendelberger (1950), Rikli (1943) and Adriani (1945).

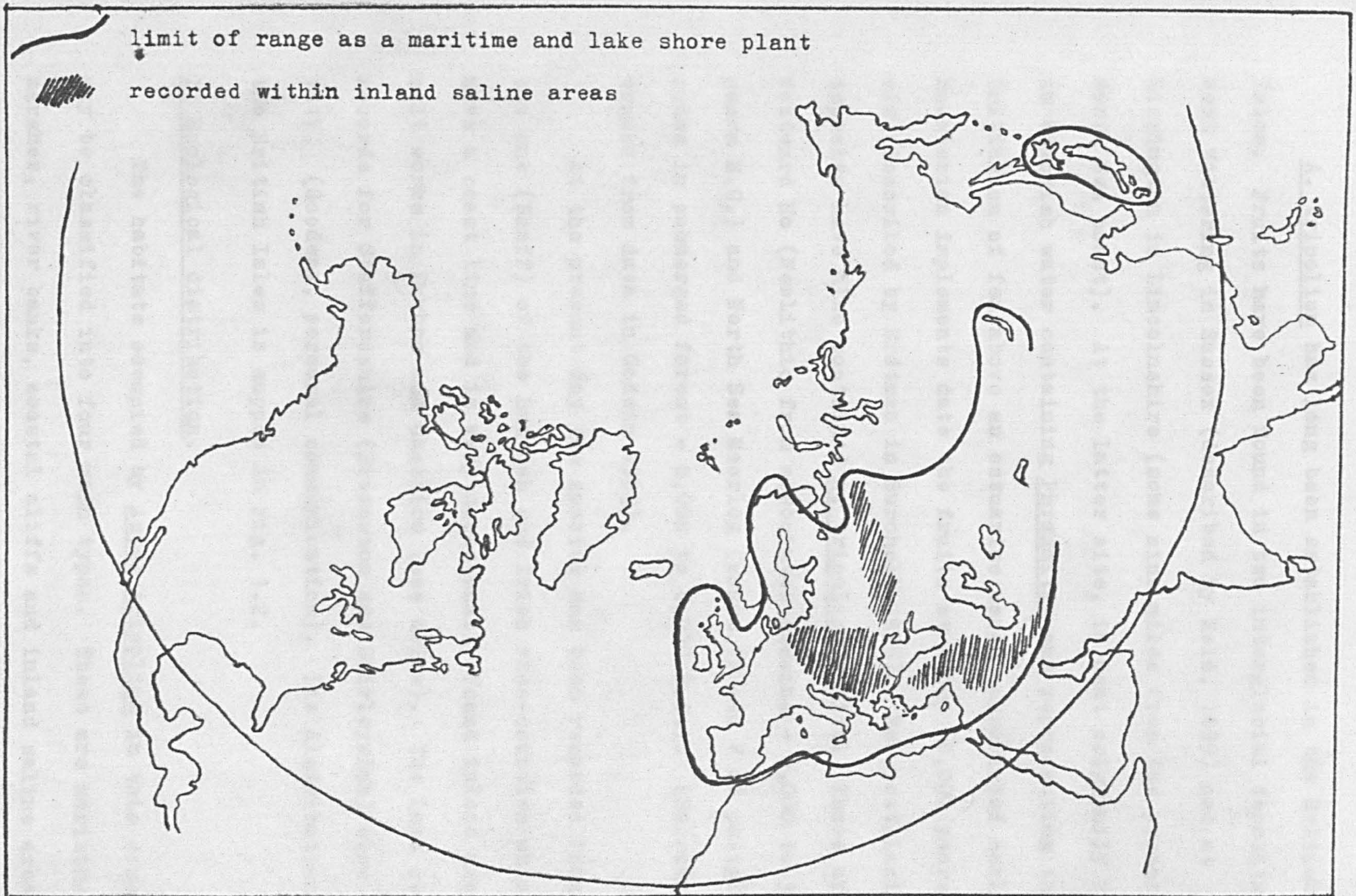

Fig. 1.1 World distribution of *Aster tripolium* L.

A. tripolium has long been established in the British Isles. Fruits have been found in two interglacial deposits, West Wittering in Sussex (described by Reid, 1899) and at Kirmington in Lincolnshire (some nine miles from the present North Sea coast). At the latter site, in peat originally formed in brackish water containing Phragmites and representing the initiation of fen above an estuarine marsh, associated early Mousterian implements date the fruits at about 60,000 years old (described by Erdtman in Burchell 1935). Two postglacial deposits have also contained A. tripolium fruits. These are at Westward Ho (Neolithic from associated remains - 5,000 to 3,000 years B.C.) and North Sea Moorlog (zones 1V and V of postglacial zones in submerged forest - 8,000 to 6,000 B.C.). (Sources traced from data in Godwin 1956).

At the present day the species has been recorded from all but one (Banff) of the British and Irish vice-counties which have a coast line and is very occasionally found inland around salt works in Durham and Cheshire (see below). The last certain records for Staffordshire (Braunston and Shirleywich) were in 1817. (Goodway, personal communication). Its distribution in the British Isles is mapped in Fig. 1.2.

II Ecological distribution.

The habitats occupied by Aster tripolium in this country may be classified into four main types. These are maritime salt marshes, river banks, coastal cliffs and inland saline areas. For the purpose of sampling, the salt marsh environments dealt

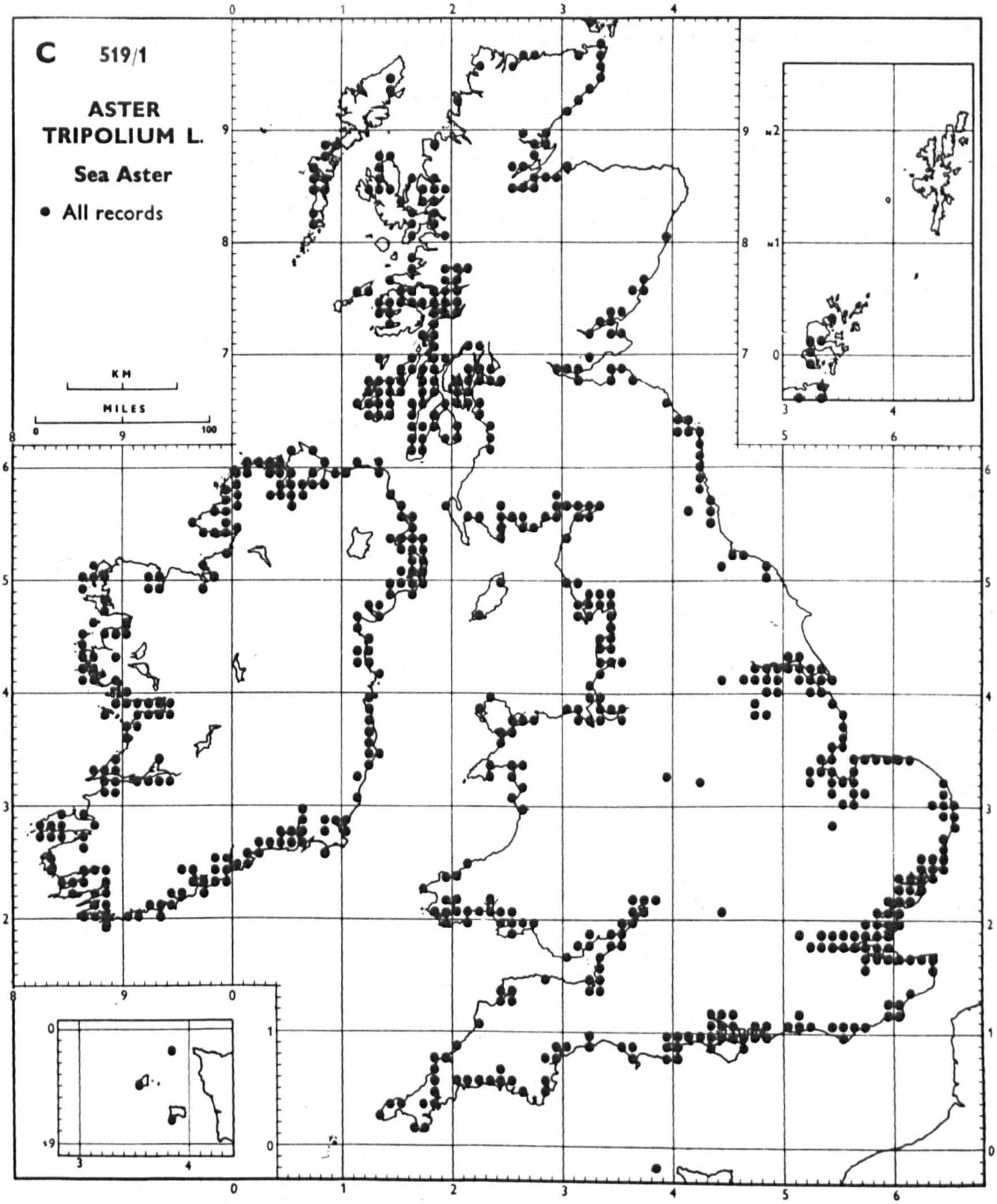

Fig. 1.2. Distribution of Aster tripolium in the British Isles (adapted, with permission, from Perring and Walters (1962)).

with in this study have been further subdivided into three main types, viz. high, mid, and low. The bases of this division, largely concerned with tidal relations and associated vegetation, are examined in Chapter 3. For the present the main habitat types may be listed as follows:-

(1) Low salt marsh
(2) Mid salt marsh } see following chapters.
(3) High salt marsh

(4) River banks. Many tidal rivers carry a fringe of halophytic vegetation along their banks for some distance inland and A. tripolium is often one of the last species to disappear from these linear colonies as one goes inland. Plants can be found on the banks of the Trent a few miles north of Gainsborough (personal observation - see also Howitt and Howitt 1963 p.107), on the Yorkshire Ouse beyond Goole (Lees 1888), and on the south Ouse well beyond Kings Lynn (personal observation). Its extension along river banks has led to its inclusion in the flora lists of Gloucestershire (along the Severn to vice-counties 53 and 54, Clapham et al 1942) and Cambridgeshire (on the Nene above Wisbech, Perring et al 1964). In the river bank habitat the plants are often luxuriant and may become very tall, as along the Tamar at Calstock (where I have found plants 5' in height), or along the Avon beyond Bristol (Bracher 1929). Lambert in B.E.C. Plant Notes (1945) reports a plant over 7' high and

flowering freely on a riverside embankment along the R. Yare at Surlingham, Norfolk.

(5) Coastal cliffs. A number of authors describe A. tripolium as a plant occasionally found on the tops and ledges of coastal cliffs, particularly in Pembrokeshire and Western Ireland (e.g. Goldsmith (1967), records it from cliffs on the island of Inishark, Co. Galway). Although unable to discover the species in this habitat during a limited search of the Cornish coast I am told that it occurs on cliffs at Durlston Head near Swanage (Ranwell in Perring, personal comunication).

(6) Inland areas. Inland salt marshes are nowhere extensive in England but some small areas around salt mines in Cheshire and County Durham support a more typically maritime flora including A. tripolium. Although absent from the salt spring overflows at Nantwich and Aldersey in Cheshire, described by Burke (1943), I have found the species on the banks of a salt 'flash' near Sandbach and near a salt works at Winsford (see Appendix IV). In former times it may have been more widespread in inland sites.

In addition to those habitats described above, the species may occasionally occur on thin mud over shingle, as at Cemlyn Bay, Anglesey and Dungeness (Scott 1960), in quayside walls (such as at Parkgate in Cheshire), or on waste land near docks. Many of these types of habitat are probably only very temporarily occupied by this species.

III The nature of the problem.

As can be seen from the above and from Fig. 1.2 Aster tripolium is largely confined to a narrow belt of maritime and paramaritime habitats extending around most of Britain. This restricted ecological range is especially interesting in the light of two particular facts. The first is that the species is not an obligate halophyte in the sense that, providing they are kept reasonably weed free, a large proportion of plants raised from seed flower and set fruit freely under conditions of cultivation in ordinary soils. The second fact is that the plant's distribution extends almost to the limits of this relatively narrow ecological zone. It has, for example, a large vertical range in salt marsh habitats. Unlike many salt marsh species A. tripolium is commonly found both in the lower zones on mobile mud associated with, for example, Salicornia species or Spartina anglica* , and in the upper zones with Juncus maritimus, Phragmites communis or Scirpus maritimus.

Salt marshes represent, in broad terms, an interface zone between two major environments; the marine and terrestrial. It

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* This name is used by Hubbard (1968) to refer to the more or less fertile amphidiploid (chromosome No. 2n = approx. 122) derived from the sterile primary hybrid S. X townsendii by doubling, It is, so far as is known, strictly a nomen nudum.

is to be expected that there are major differences in the habitat factors operating towards each end of this zone as the influence of the one environment lessens or increases in respect to the influence of the other. There are therefore good _a priori_ reasons for supposing that any species which successfully occupies the whole width of such a heterogeneous zone must have been subjected to a variety of selective forces. This variety could be expected to lead to multifarious adaptation. Previous ecological studies (see later) have suggested that salt marsh environments are frequently zoned. The stringency of the habitat factors related to the known (and predictable) variation in tidal factors which are largely responsible for this zonation should enable these zones to be recognised and thus the plants occupying them to be compared.

This project is thus addressed to two major questions:-

(1) What factors limit the spread of this species beyond a fairly narrow maritime belt? Why, on the one hand, is it not commonly found in nature in ordinary non-saline habitats fringing coastal marshes , and what, on the other hand, prevents its invasion of the very low zones of salt marshes occupied extensively by algal species and a few specialised seed plants?*

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* This is of course partly an artificial question. As opposed to its occurrence, the non-occurrence of a plant in a particular area may not be amenable to scientific explanation. However the pattern of variation in those plants which do extend beyond the normal limits of the belt is likely to be adaptive, and thus provide information on the reasons for the elimination of non-adapted plants in other areas.

(2) How does the species vary across the width of this maritime belt? How does this variation relate to the type of selection operating at different levels of a salt marsh and especially near either limit of the belt? In other words what aspects of the variation in this heterogeneous environment are likely to be of adaptive significance?

The bulk of this report is concerned with information relevant to an answer to question (2). The subdivision of the maritime belt into 'high', 'mid' and 'low' areas was made initially on the basis of tide-related factors and later on the basis of an association-analysis of the plant species. These are described in Chapter 3 (III).

CHAPTER 2. THE STUDY OF VARIATION.

The genus *Aster* is included in the section *Euaster* Gray of the tribe *Astereae*, one of thirteen traditional tribes of the *Compositae* (recognised by Cronquist (1955) as natural groups). A large temperate zone genus, it comprises about six hundred species in North America, Africa, Europe and Asia, and is a taxonomically critical group containing complexes of closely related heteroploid species. The complexity of variation within the genus in the abundant North American species is probably increased by localised introgression (Avers 1953).

By contrast the European *Aster tripolium*, originally described in Species Plantarum (Linne 1753), has traditionally been regarded as a good species*. *Tripolium vulgare* Nees (1818, 1832) is a post-Linnean synonym which grouped the species with four North American species (*A. paludosus*, *A. flexulosus*, *A. subulatus* and *A. pauciflorus*). The name *Eurybia maritima*, proposed by S.F. Gray (1821) recognises the many differences between this and other species of the genus *Aster* by placing it in a separate genus containing the one species.

* This not to imply that a well defined species is necessarily precluded from membership of a genus otherwise characterised by uncertain speciation. *Aster anomalus Englm.* is a North American member of the genus which has unmistakeable affinities with other intergrading species, yet is itself clear-cut and well defined (Anderson 1929). Good species may have close relatives which are 'bad' species.

'De Materia Medica', an account of the medicinal plants of Asia Minor compiled by Doiscorides about A.D. 60 suggests that the name 'tripolium' is derived from the fact that the plant was alleged to change colour three times a day (Melderis and Bangerter 1955). Grigson refers to the 'blue daisy of the saltings' as being 'already taken into Elizabethan gardens where, according to Gerard it 'waxed huge, great and ranke'' (Grigson 1955). This is probably a mistaken reference to species of Aster, collectively termed 'Michaelmas Daisies', introduced into cultivation from North America. Britton (1932) describes eighteen such naturalised and alien species occurring as garden escapes in Britain. Aster tripolium has variously been known as 'Sea Aster', 'Sea Starwort', 'Hogs beans', and 'Hoggibeans'.

I Variation (i) the classical account.

Despite both the ease with which traditional taxonomic methods separate A. tripolium from other species of the genus and the support for this provided by cytological evidence presented below (Ch. 4), the plant is extremely variable below the level of the average Linnaean species. The species is an excellent example of a taxon the discreteness of which can be established by orthodox methods on the basis of a nucleus of common characters; characters which may nevertheless vary greatly among themselves. Among all the plants examined in this study, whether in the field under experimental cultivation, or on herbarium sheets, there was not a single individual the specific identity of which was questionable; yet between-plant variation was considerable and extended to many customarily 'good' characters.

An indication of the nature and range of variation within the species is provided by examining the number of taxa which have been recognised at the infraspecific level. These taxa, usually designated 'varieties', have generally been delimited on the basis of morphological evidence alone. They thus represent the attempts of descriptive taxonomists to frame the lower taxonomic units in the face of variation occuring below the level of the species. Table 2.1 contains a list of the descriptions of infraspecific taxa thus far discovered in the European literature. Further general notes on the status of some of these are set out below. Suggestions for the retention of recognised taxa are not included and this question is returned to in the discussion (Ch. 12).

(1) var. pannonicus Jacq. This form is characterised by the upper cauline leaves, which are generally smaller and more linear-lanceolate than in the normal form. In addition the plant may become very bushy through the development of lateral branches in the axils of the stem leaves, has a well developed rosette of basal leaves, and generally has a more intense green colour. Hegi (1906) suggests that the heads of this form are larger than those of the type.

Var. pannonicus Jacq., elevated to a subspecies by some authors, is the characteristic form of the inland salt desert plants. It is recorded from central Germany and lower Austria

TABLE 2.1. INFRASPECIFIC TAXA DESCRIBED IN EUROPEAN POPULATIONS.

NAME	AUTHOR	SOURCE	DESCRIPTION	LOCALITY	STATUS
var. PANNONICUS	JACQUIN (1770)	JACQ. HORT. VIND. 1. p.3	INTENSE COLOUR, LINEAR-LANCEOLATE LEAVES, DEVP. IN AXIL OF STEM LEAVES	LOWER AUSTRIA, HUNGARY ITALY (PO VALLEY) RUMANIA	SEE TEXT.
var. DISCOIDEUS	REICHENBACH f.	?	CAPITULUM LACKING RAY FLORETS	BRITISH ISLES GERMANY, BELGIUM FRANCE, NETHERLANDS	SEE TEXT.
EURYBIA MARITIMA var. FLEXUOSA	S.F. GRAY (1818)	NAT. ARR. BRIT. PLANTS			
var. LONGICAULIS	(DUF.) ROUY	?	SLENDER STEM, LINEAR LEAVES OF AN UNUSUAL TYPE.	SPAIN, PORTUGAL BRITISH ISLES	SEE TEXT.
var. GLABER	BOLZON	?	FORM WITH GLABROUS INVOLUCRAL BRACTS.	BRITISH ISLES, ITALY	SEE TEXT.
var. DIFFUSUS ?	DE CANDOLLE (1836)	SPIRIBILLE ap HEGI (1918). PRODR. V.	BASAL BRANCHING	RUMANIA, SWEDEN	CONSTANT IN CULTIVATION (TURESS 1922
var. ARCTICUS	FRIES	?	DWARF FORM, BASAL BRANCHING	HEBRIDES, FINLAND NORWAY, SWEDEN.	SEE TEXT
var. SOLSTITIALIS	FOCKE	HEGI (1918)	EARLY-FLOWERING FORM	N. GERMANY.	NOT KNOWN
var. AUTUMNALIS	FOCKE.	HEGI (1918).	LATE-FLOWERING FORM	GERMANY, BALTIC COAST	NOT KNOWN
var. CRASSUS	V.J. CHAPMAN	?	SHORT, STOUT WITH LARGER LEAVES (CLIFF-TOP FORM)	BRITISH ISLES.	NOT KNOWN.
var. SALINUS	SCHRAD (D.C.)	FLORA ITALICA EXSICC.	LEAFY STEM, SERRATED LEAVES SALT-WATER FORM.	ITALY	NOT KNOWN.
var. GRACILIS	ROUY.	CHAPMAN (1960)	SLENDER, LAX FORM	?	NOT KNOWN
var. PYGMAEUS	BEGUINOT	FLORA ITALICA EXSICC.	BROAD-LEAVED, CHARACTERISTIC BRANCHING. SALT-WATER TYPE.	ITALY.	NOT KNOWN
f. DEPRESSUS	KIT (1863)	LINNAEA XXXII	DECUMBENT FORM	RUMANIA	NOT KNOWN.
f. TAUSCHERI	SOÓ (1924)	B.K. XXII	WHITE RAY FLORETS	NOT SPECIFIC, PROBABLY WIDESPREAD.	SINGLE GENE (DE VRIES 1901).
f. DEPASTUS	NYÁR	R.P.R. Flora	NOT GIVEN	RUMANIA	NOT KNOWN
ecad. MEDITTERANEUS	?	CHAPMAN (1960)	NOT GIVEN	MEDITTERANEAN REGION	NOT KNOWN
ecad MONSTROSA	?	CHAPMAN (1960)	EXTREME VEGETATIVE DEVELOPMENT	?	NOT KNOWN

(Hegi 1906), from the salt springs of the upper Po valley (Bertolani - Marchetti 1953), around the Nuesiedler See in Austro-Hungary (Wendelberger 1950), from central and northern Rumania (Tsopa 1939, Savulescu 1955), and is probably of Eastern Mediterranean affinities (Chapman 1960). Bequinot (in Flora Italica Exsiccata, ex herb LIVU) suggests that it is the form most frequently met with at some distance from the sea.

This form is constant in cultivation. Fruits obtained from Vienna germinated successfully and a sample of the plants is shown in Fig. 2.1. All seven plants grown flowered from fruit in a year and produced an average of 65.3 heads per plant. The mean number of ray florets, 40.33 for 35 heads counted, was significantly higher than that for the normal form (see Ch. 11), and the heads were generally large and showy.

(2) *var. longicaulis Duf*. The general description of this form is similar to that for *var. pannonicus*. Plants cultivated from fruit obtained from Portugal shared the linear leaf shape and profuse branching of the latter form, as well as its annual life habit and showy heads. By contrast they did not form basal rosettes of leaves (see Fig. 2.2). Reported only from the Iberian Peninsula on the continent, this form is also said to occur in Britain (Clapham *et al* 1942).

(3) *var. discoideus Rchb*. Chapter 12 is given over entirely to variation in the number of ray florets, the complete absence of which is said to be the only character diagnostic of this form.

Fig. 2.1. '<u>var. pannonicus</u>' from Austria.

(4) var. glaber Bolzon. The glabrous involucral bracts which characterise this form have not been found in any of the British material so far examined. The form is reported from northern Italy and in Britain from south Devon (Tozer in B.E.C. Report 1931 p. 654) and the Isle of Wight (Drabble and Long, op cit p.745).

(5) var. diffusus D.C. This form, described as dwarf form with a much-branched, generally prostrate habit, was collected and cultivated by Turesson and is described in his early classic paper (Turesson 1922). Although constant in cultivation, there was some variation between individuals in branching and in whether the main axis or the lateral branches were decumbent. Although much less tall than the normal form, there was some regression towards the type in height in the second year of growth (1920), plants reaching 10 - 16 cms. in height in contrast to their original 3 - 5 cms. (1919). The form may be widespread geographically, although Turesson suggests that (in Sweden?) it is confined ecologically to the drier parts of salt meadows. It is also recorded from central Rumania (Savulescu 1955).

(6) var. arcticus Fries. The description of this form contains all the characters required to fulfill the prescription given in the diagnosis of var. diffusus. It is recorded from a number of northerly localities including several of the Hebridean Islands (Heslop-Harrison 1948, 1951). A plant collected on North Uist and labelled as var. arcticus, which has been deposited in the University of Liverpool Herbarium, was less than

three inches high at flowering.

Both heritable and environmentally induced dwarfness has been observed among plants cultivated as part of this study. Many of these small prostrate plants exibit the morphological characteristics attributed to the forms diffusus and arcticus (see particularly Fig 10.16).

(7) var. solstitialis Focke and var. autumnalis Focke. Early and late flowering forms reported from Germany (Hegi 1906) have been given varietal status. Flowering time and its relation to life cycle is discussed in Ch. 4.

(8) var. crassus Chapman. This form, described by V.J. Chapman as a short stout plant with larger than usual leaves, is said to occur on cliffs in Pembroke, Cornwall and the Orkneys. A plant in the University of Liverpool Herbarium collected from Predannock Cliff on the Lizard peninsular fits the type description of var. crassus, although it is not labelled as such. Although he hoped to publish a separate Biological Flora account of the variety Professor Chapman was never actually able to see the plant in the field. (Chapman - personal correspondence 1966).

(9) Others. Information regarding the descriptions and status of other recognised variants is scanty. The white ray florets characterising the so-called var. tauscheri Soo were interestingly the subject of one of the very early experiments retesting Mendelian genetic ratios at the turn of the century. De Vries (1901 p.246) lists the white rayed form as an example

of a character controlled by a single recessive gene, the normal lilac rays comprising 63% of the F_2 generation. White rayed plants are commonly found in populations of A. tripolium and are particularly common on the salt marsh at Morston near Blakeney Point.

The range of variation in the morphological characters used to delimit other forms listed in Table 2.1 suggests that they are taxa of somewhat spurious significance. The literature is not without its nomenclatural difficulties generated by the precepts of orthodox classification. On specimens of the rayless form sent to the Botanical Exchange Club in 1915, for example, the comments were - 'Yes, the sub-var discoideus (Rchb),but this plant is also the var. glaber, Bolzon'.....

II Variation (ii) the present approach.

At the present time any claim to be adopting a 'genecological' approach to infraspecific variation must be accompanied by a detailed rationale. This is because of the variation in purpose of those claiming to be engaged in genecology and the divergence of interpretation and terminology to which this has led. Heslop-Harrison's recent review of the general field includes discussion of this divergence and contrasts the purposes of 'genecology' with those of 'biosystematics' and 'experimental taxonomy' (Heslop Harrison 1964). It would be inappropriate, therefore, to attempt a further evaluation here. There are, however, certain areas of dispute which should be considered at this stage in order that the terms employed in describing variation may not prejudice the interpretation of that variation. These are considered in three sections below.

(1) The first area of dispute centres around what might be called the 'ecotype v. ecocline' argument, an old sore which every student of infraspecific variation finds it necessary to reopen. Turesson originally defined the ecotype as 'the product arising as the result of the genotypical response of an (eco) species to a particular habitat' (Turesson 1922). This definition lacks the direct implication of discontinuous variation (and hence the possibility of a taxonomic aim) contained by his later use of latinised names such as _œcotypus arenarius_, _œcotypus littoralis_, or _œcotypus alpinus_ (Turesson 1925). The term 'ecocline' was derived from Huxley's neutral word 'cline', used to refer to a variational gradient of any sort within a population (Huxley 1938). The prefix 'eco-' denotes a correspondence of the gradient with an environmental factor. Its use implies that the pattern of variation is continuous. Thus 'ecotype' in the sense of Gregor (in his reviews of 1938 and 1944) is used to refer to an arbitrary reference point on a continuously varying cline.

Recent research has shown that the traditional conflict between the 'clinists', particularly Gregor and his co-workers, and those who have emphasised discontinuous variation, principally the Carnegie group, may not be a real one. A better understanding of genetic systems and the way in which selection may operate on these, coupled with more adequate sampling techniques, has shown that both continuous and discontinuous patterns of variation exist in nature, and that there are good reasons why they do. The factors which may be expected to effect the continuity of variation include both intrinsic mechanisms such as the breeding system, and external environmental factors. Such factors have been listed elsewhere (for example by Bradshaw (1962), Heslop-Harrison 1964), and their relevance to the pattern of variation in natural populations of Aster tripolium is discussed in the final chapter of this thesis. What is certain at this stage is that a prior committment to either a 'cline' or 'type' viewpoint is to anticipate fundamental facts not only about the evolution of a species, but also its biology.

(2) A second source of difficulty is presented by the vast and loaded terminology generated by the early disputes, and principally by what Langlet (1963) calls 'the attempt to force (the complex of local adaptations and ecological variability in various plants and animals) into one or other terminological system'.

Heywood (1959) presents the approach of a modern taxonomist to the current terminology and reviews the varied uses and abuses of the term 'ecotype'. If one's pronounced aim is not primarily taxonomic (and I do not see the present work as having this aim), the safest policy is to avoid completely the use of such equivocal terms. Alternative schemes, such as the 'deme' terminology proposed by Gilmour and Gregor (1939) and developed by Gilmour and Heslop-Harrison (1954), or the Danserian 'Comparium' system (Danser, 1929), are available for describing between-population variation in relatively neutral and unambiguous terms. The fact that neither of these is adopted here is due to the author's general suspicion that any formalised terminology one cares to use, whether it contains a flexible hierarchy or not, may be insufficiently protean to describe satisfactorily the complex of genetic and plastic modifications arising as a result of adaptation to environment.

Gregor and Watson (1961) point out that (at that time) 'the emphasis (in genecological work) is increasingly being transferred from attempts to delimit ecotypes to study of trends of ecotypic differentiation'. Harberd (1957) may have been the first to use the phrase 'genecological differentiation'. The full 'genecological differentiation' can imply that variation has been produced as a result of a genetic response to habitat (in Turesson's original sense of 1922), whereas the simple 'differentiation' may also cover cases of variation as a result of plastic response or of drift. All variation which shows a consistent pattern is differentiation; it lies with the investigator to demonstrate whether it is 'genecological'.

Rephrasing the present approach to variation in these terms the following general statement is permissible:- It is possible to distinguish three general levels of differentiation; (a) plastic, (b) genetic and adaptive, (c) genetic and non-adaptive. The traditional province of genecology has been to identify (b). Collateral cultivation has generally been used to isolate and eliminate (a) (but see under (3) below). Randomness with respect to habitat has been used to distinguish (c) from (b). A more interesting general approach might be to attempt to separate adaptive from non-adaptive variation. As well as differentiation due to (b) the adaptive class would also include that due to (a) (assuming for the time being that direct plastic modification of individuals is adaptive sui generis). Chapter 12 returns to this distinction between adaptive and non-adaptive variation during discussion of the variation described in the preceding chapters.

(3) A third major problem involves what might be called the 'vicious circle' situation. This concerns the nature of the evidence required to demonstrate adaptive variation. The traditional method of the garden trial contains the obvious danger of ending in a vicious circle if, in the first place, the pattern of variation of a morphological character leads one to conclude that it follows an ecological trend, and then, secondly, measurements of the same character are used as evidence to prove the pattern of the ecological variation. This point is made by Langlet (1963) who goes on to say 'moreover the mere absence of correlation between the measures of certain morphological properties in a number of local populations of Layia platyglossa and the latitudinal distribution of their original habitats has been misused for claiming that no ecological clines exist' (this is a reference to Clausen, 1951).

As Wilkins points out, following any controlled environment experiments 'there remains the final step of extrapolation to the wild, which is surely in most cases extremely speculative' (Wilkins 1960a). In order to make this final step techniques other than collateral cultivation are required. Any differences retained by plants from different habitats after cultivation are genetic, and, if consistent from population to population, and discounting the possibility of drift, provide strong circumstantial evidence that they are adaptive (since only natural selection is left to account for them). The danger lies in not being sure that such differences, and not ones closely related to them, for example by linkage, are the ones being selected. Two morphologically very different plants may share a common physiological property which is the real reason for their survival together in the same habitat, just as morphologically similar plants from different habitats may have been selected on the basis of their very different physiology.

These problems are especially acute where the nature of the selection is unknown or of low pressure. It is interesting that the most successful demonstrations of adaptive variation have concerned situations where the selection is particularly intense, as in the case of heavy metal tolerance in grasses (Bradshaw (1952) (1960), Wilkins 1960b) or industrial melanism in moths (Kettlewell 1955, 1956). In the present study an assessment of the nature of selection can only be made by reference to a complex of factors which may (or may not) be important in maritime environments. The final step, of attempting to show that a correlation between a particular variable and a prevailing habitat factor is the result of selection, must be extrapolatory. Some attempt to escape this position has been made in this investigation by a number of controlled culture and reciprocal transplant experiments (described in Part III). The time scale involved in studying adaptation in plants has dictated that much of the work presented here has been concerned with the first duty of genecological investigations, namely the detection of habitat-correlated morphological and physiological variation.

III Methods.

(a) Sampling

Different sampling procedures may yield different kinds of information; a fact evidenced by the 'ecotype v. ecocline' argument and the contrasting scales of the early genecologists and present day investigators. Although the principle of the garden trial remains the same, the selection of material for such trials will predetermine the nature and amount of the information which can be extracted from them. As the number of plants which can be grown is often restricted by the usual considerations of space it is worth considering in advance the basis on which material should be selected for trial.

The case for increasing the number of population samples at the expense of their individual size is presented by Harberd

(1961). In earlier papers both he (Harberd 1957, 1958) and Wilkins (1959) underline the inherent dangers of comparing populations using estimates of the relatedness of plants within large samples of the same population. In particular they are concerned with the nature of the statistic 'within-population variance', commonly used as part of the argument that, since individuals within a population vary less among themselves than they do in relation to those in a different population, then the differences between the two populations are adaptive, (on the basis that a large number of individuals have been independently selected in a particular area for characters which they share but were not shared by those presumed to have been eliminated). (The question of the possibility of drift is not discussed by these authors, but should clearly be considered).

Harberd is especially concerned by the decreased estimate of within-population variance which would result from measuring samples in which a single genotype is represented more than once (and possibly several times). The vegetative spread of many species, particularly perennial grasses, makes the chance repetition of genotypes in samples from local populations very likely. Harberd has recently produced evidence strongly suggestive of natural clonal spread on an extensive scale in <u>Holcus mollis</u>, one of possibly only four genotypes present in a large area of the Pentland Hills south of Edinburgh having colonised an area over half a mile across (Harberd 1967).

The fact that all the plants in a large population may be the direct descendents of a few founders in no way vitiates the concept of genetic response to environment as a principle of natural selection (selection and the founder principle are not mutually exclusive positions). Alternative sampling schemes are available which avoid the reliance on within-population variance as a measure of adaptedness. One of these is the collection of small samples from as many well-separated areas as possible, and the correlation of their means with particular environmental factors. Within the limits of the time and facilities available this has been attempted here and a list of areas sampled is given in Appendix I.

Coupled with this is the attempt to discover trends of differentiation in the species with respect to its wide ecological range on salt marshes. This exercise has necessitated the closer study of a limited number of well-defined areas, between which there may or may not be gene flow. It is believed that intensive sampling of plants from sites which form a clearly defined part of a salt marsh succession will yield data relevant to a discussion of the nature of adaptation to maritime environments (particularly in a short-lived species such as Aster tripolium). This is of course providing that any trends of differentiation are defined in terms of the comparison between populations at the equivalent stage of succession on different marshes. (In statistical parlance it can be said that one is stressing the use as error item of the variance between populations from ecologically similar habitats).

Therefore, in addition to collecting small samples from as many sites as possible, a small number of areas have been studied closely and populations on these sampled intensively. In dividing such areas into high, mid and low marshes external criteria (see Ch.3) have been used to define populations as reference points for genecological sampling.

Populations have been sampled as plants or fruit or both. The implications of this in terms of selection are considerable, and are returned to in discussion. For the present the history of plants described in the succeeding sections is given in each case where their variation is being considered.

(b) Treatments.

Collateral cultivation as a technique antedates the work of Turesson but remains, together with reciprocal and clone transplanting, the major way of distinguishing genetic from non-genetic variation. Its most important deficiency is that, in testing the response of different plants to a neutral environment, it may fail to detect important differences in the capacity of plants to respond to their own particular specialised environments. This may be especially true of growing maritime plants in ordinary soils. The capacity to respond, by direct plastic modification, to a range of environments (itself genetically determined) is surely a most important part of a plant's ability to survive. A single neutral environment allows for comparison of the observable genetic differences between plants at only one point on what may be a wide spectrum of response.

It seems also desirable to know not only which characters, or organs, are capable of modification by the imposition of different environments on identical genotypes, but also which populations show a wide range of such characters. In a fluctuating and relatively unstable environment such as the maritime one the flexibility of genotypes in adjusting to changing conditions may be of paramount importance in determining whether they survive to reproduce.

An attempt to assess the range of response of Aster tripolium populations to different environments has been made by growing plants from a limited number of populations under different cultivation conditions. An important source of error which must be acknowledged here is that the difficulty of obtaining clonal material has made impossible the use of identical genotypes in these tests. Fruits collected from the same population (at the same time) have been given such treatments. Thus it is only possible to gain an impression of the variability of a population in relation to treatment at the level of the population.

The conditions under which plants have been cultivated are as follows:- Plants collected in the wild were transfered to one of four treatments listed below. In the majority of cases where wild populations were sampled as fruit the fruit was generously sown in shallow plastic trays on a mixture of fine silt, sand, and peat (see below), and covered with a thin layer of sand.

The seedlings were pricked out after varying periods of time, and transferred to one (or in certain cases more than one) of the following treatments:-

(1) Cold house. Many of the plants introduced to the experimental garden at the beginning of this investigation were damaged by rabbit grazing. For this reason the majority of plants have been grown singly in 5" plastic pots on a bench about 3' high in an unheated greenhouse. The annual temperatures of the house fluctuated from 38 to 76° F, averaging about 48°F for a measured period from February to June. The spacing of the pots was adjusted to prevent overcrowding as the plants grew.

(2) Experimental garden. Latterly the protection afforded by wire cage (36' by 36' by 7' high), of the type normally used to protect fruit bushes from birds, has enabled plants to be grown in the open. These were planted in rows 2' apart and spaced at intervals of 18". The plot was situated in the south corner of the Botanic garden at Keele where poor drainage and the local slope of the land caused the soil to remain in a fairly waterlogged state. The pH of samples of air-dried soil ranged from 4.6 - 4.8.

(3) Open frame. Plants grown either singly in 5" pots or in wooden trays (15" by 9" by 4") were stood on a cinder base in open frames. These frames, on a concrete apron close to the greenhouses, were left uncovered apart from periods of heavy snow fall.

(4) Poor soil. Plants grown in the unheated greenhouse

or open frames were generally cultured in a soil made up of 7 parts fine silt (a fine-particled topsoil obtained from construction workings at nearby Audlem) to 3 parts coarse sedge peat to 2 parts coarse sand, (hereafter referred to as the 7:3:2 mix). A small smount of general purpose fertiliser was added at each mixing.

Some plants however were grown in a soil composed entirely of the Audlem silt. In contrast to the 7:3:2 mix, this 'poor soil', without added fertiliser, was never successfully colonised by local weeds, those pots in the open frames only occasionally supporting one or two very stunted Epilobium plants.

In addition to these general treatments certain plants were cultured under other conditions as part of particular short-term experiments. Details of these are given with the reports of the experiments.

The number of trials has been increased by concentrating where possible on seedling and young plant characters. This has allowed a greater turnover of material than the available space would have allowed were all the plants grown to maturity.

(c) Summary.

Natural populations of Aster tripolium have been sampled in two ways. (1) Small samples of either plants or fruits have been collected from widely separated areas, and (2) a few areas have been sampled intensively at what are thought to be ecologically distinct points across the maritime belt. This latter has involved the use of external criteria to distinguish three types of salt marsh, high, mid, and low, in the hope that a comparison of populations from the same type of marsh in different areas will reveal adaptive trends of differentiation

The populations have been compared under collateral cultivation in four main types of conditions. These have been used (1) to search for habitat-correlated variation, and (2) to gain an impression of the within-population variation in range of plastic response (at the general level of the breeding population). The pattern of differentiation revealed by (1) has been used to design further experiments in the hope of distinguishing adaptive from random variation.

CHAPTER 3.

THE AREAS STUDIED, AND THE CLASSIFICATION OF THE SAMPLED POPULATIONS

(Appendices I - VI form part of the information presented in this chapter).

I Those areas studied as part of the extensive sampling programme.

Samples of fruit or plants were obtained from a number of sites in the British Isles and Europe. These are indicated in Fig. 3.1. Appendix I gives a complete list of the material collected and in cultivation.

In addition to those obtained by post a number of samples were collected personally, and ecological notes on the areas from which these were taken are given as Appendix II.

No information is available on the methods used to collect those fruits obtained from the sites not sampled personally, and thus the relationship of any one fruit to the rest is not known. Those sampled directly in the field were collected as complete fruiting heads (1 head/plant) from plants at least 2 metres apart (this provides no guarantee that they are not from the same clone). For the purpose of collateral cultivation 1 fruit from each head collected was sown in the seedling trays.

II Those areas studied as part of the intensive sampling programme.

A small number of areas were specially chosen for detailed study and sampling. The basis of this choice was that the areas

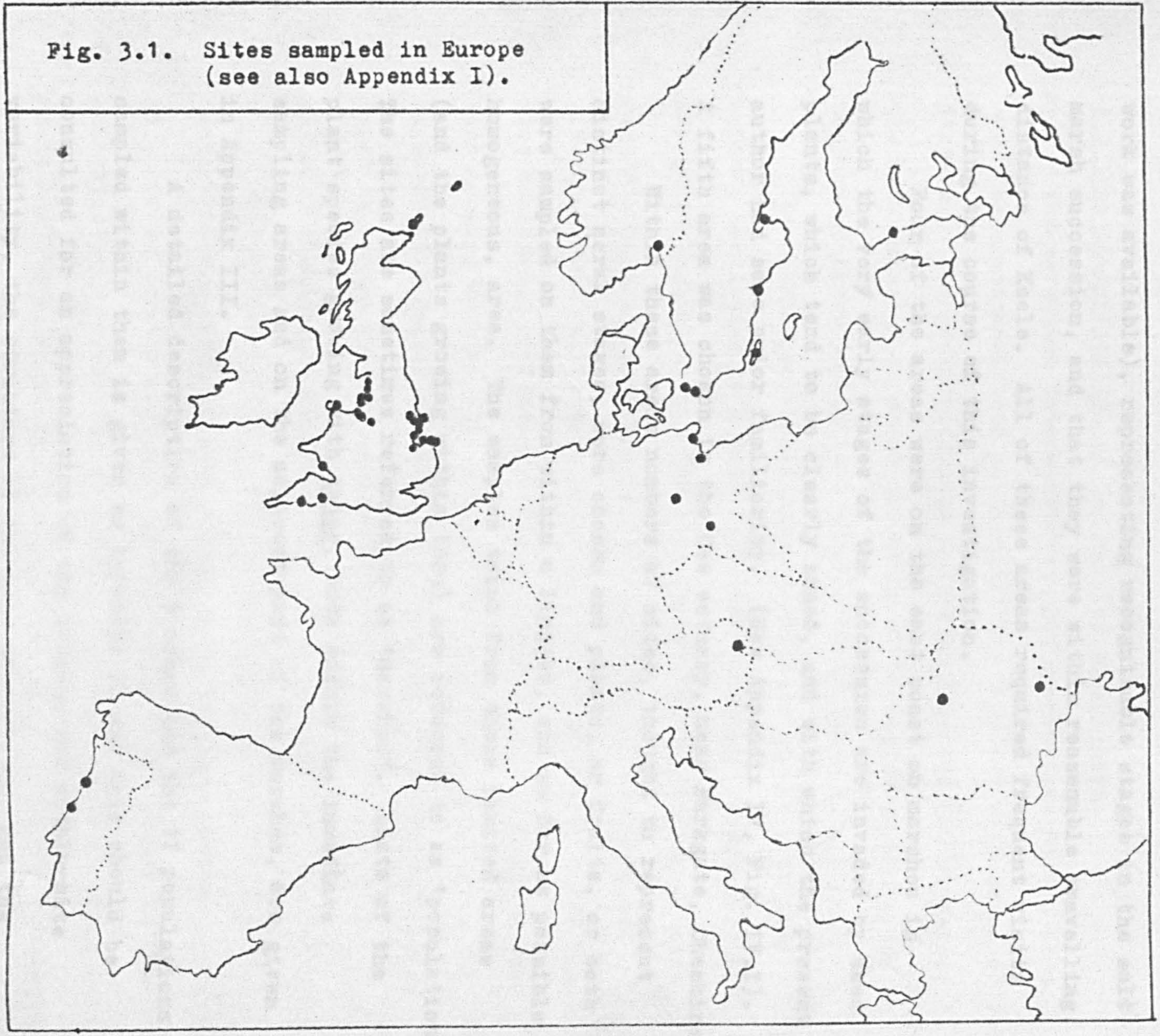

Fig. 3.1. Sites sampled in Europe
(see also Appendix I).

were well defined ecologically (in most cases a body of earlier work was available), representing recognisable stages in the salt marsh succession, and that they were within reasonable travelling distance of Keele. All of these areas required frequent visits during the course of this investigation.

Four of the areas were on the east coast on marshes in which the very early stages of the succession are invaded by seed plants, which tend to be clearly zoned, and with which the present author had some prior familiarity. (See Appendix IV, Fig. IV.I). A fifth area was chosen in the Dee estuary, near Parkgate, Cheshire.

Within these areas numbers of sites, thought to represent distinct seral stages, were chosen and plants, or fruits, or both were sampled on them from within a limited, and as far as possible homogeneous, area. The samples taken from these limited areas (and the plants growing within them) are referred to as 'populations'. The sites are sometimes referred to as 'marshes'. Lists of the plant species growing with Aster, both within the immediate sampling areas and on the adjacent part of the marshes, are given in Appendix III.

A detailed description of the 5 areas and the 17 populations sampled within them is given as Appendix IV and this should be consulted for an appreciation of the between-and within-site variability, the structure of the main populations, and the appearance of individuals in the field. The 17 populations sampled as part of this programme are listed in Table 3.1, nos. 1 - 17.

III The classification of the sampled populations.

The seventeen intensively sampled populations at the five maritime areas described in Appendix IV, together with seven of the personally sampled populations listed in Appendix II, represent the main material on which the study of variation in the species is based. These populations are listed in Table 3.1.

The classification of populations into groups with similar habitat conditions is a difficult, but necessary, prerequisite for any genecological work. The hope, expressed in Chapter 2, that populations could be compared from ecologically similar, but distant, areas relies on a satisfactory set of criteria for identifying ecological similarity. Two approaches to this problem have been adopted here:- (1) A classification based largely on the major habitat factor of tidal relations and (2) a classification based on common species association.

(a) Method (1). Tidal relations.

This method involves the use of a tidal curve and other factors in the physical environment to distinguish marshes differing in their salt-water regimes. The coincidence of changes in the characteristic vegetation at certain critical levels was regarded as an important factor in grouping marshes of ecological similarity together. The objective part of Method (1) has two elements; the recognition of a 'critical' level and the comparison of tidal

Table 3.1. The major maritime populations sampled.

No	Area	Grid ref.	population	code used in text.
1	Gibraltar Point Lincs.	TF 557578	Spartina marsh	G.Spa
2	"	TF 567588	Aster marsh	G.Ast
3	"	TF 559585	Limonium marsh	G.Lim
4	"	TF 569589	Agropyron marsh	G.Agr
5	Scolt Head Island Norfolk.	TF 802465	Cockle Bight	S.Cob
6	"	TF 800456	Beach Point (1965)	S.Bch'65
7	"	TF 800458	Beach Point (1967)	S.Bch
8	"	TF 805455	Plover marsh	S.Plo
9	"	TF 803466	Spiral marsh	S.Sol
10	Tetney Haven Lincs.	TA Northing 35 easting 04 to 06	The halosere	-
11	"	-	The old marsh	-
12	Morston, Norfolk	TG 004445	Morston marsh	-
13	Cley Norfolk	TG 047447	Cley	-
14	Dee Estuary Cheshire	within SJ 2777	Parkgate 1	P.1.
15	"	"	Parkgate 2	P.2.
16	"	"	Parkgate 3	P.3.
17	"	"	Parkgate 4	P.4.
18	Llys Dulas Anglesey	SH 479885	Anglesey A	-
19	"	"	Anglesey B	-
20	River Lune Lancs.	SD 455561	R. Lune	-
21	Hest Bank Lancs.	SD 467670	Hest Bank	-
22	River Wyre Lancs.	SD 368413	R. Wyre	-
23	Knott End Lancs.	SD 351487	Knott End	K.E.
24	Naze, R. Ribble Lancs.	SD 434272	Naze	-

relations above and below this level, and the identification of a separate group of marshes which are only rarely flooded by the tides. The subjective part of Method (1) consists of the fitting of marshes from areas for which no direct tide/elevation data are available into the framework of the objective categorisation.

Central to the method is the concept that at least one 'critical' level can be distinguished in intertidal areas. The critical level theory is derived mainly from the work of zoologists and is probably attributable to Coleman (1933). A broad statement of this hypothesis is that there are certain levels of the shore which are more important than others in limiting the vertical distribution of intertidal organisms. It has arisen from an attempt to explain the occurence of often sharp breaks in the distribution of these organisms (e.g. the 'barnacle'line').

The theory is based on the nature of tidal pattern, the regular alternation of neap and spring tides, and relies on the fact that the regular and predictable variations in both amplitude and daily duration of rise and fall affect, in a non-linear fashion, the periods of submergence and emersion at different levels. (The practice is adopted here of using the word 'emersion' to connote 'exposure to air' rather than 'exposure', used widely in ecology as an antonym of 'shelter').

The general subject of zonation, together with an example of how critical levels were calculated in the present investigation, is dealt with in some detail in Appendix VI. Of immediate relevance to this section is the fact that such calculations have generated curves of the type given in Fig. 3.2. In this figure, which is based on tidal data for Tetney Haven, Lincolnshire, the total number of tide-free days during the period February to October* is plotted against level (this can be level as based on tidal chart datum or on actual field datum - see Appendix VI). It can be seen that there is a particularly sharp increase in the amount of submergence from the 20' to the 21' tide level. Plants at these two levels, although separated by an elevation of only 1', experience markedly different tidal regimes.

That such a level may be 'critical' can be seen from Fig. 3.3 which gives the vertical range of some of the common species on the Tetney Halosere (an area which embraces a sharp transition zone see Appendix IV). Fig. 3.3 is based on a simple valence analysis, 10 quadrats being counted at random within each tidal level. It can be seen from this that the upper limit of two species, Salicornia and Enteromorpha, (and the approximate upper limit of two species, Spartina and Ulva), and the lower limit of three others (Halimione, Limonium, and Triglochin), correspond with the 20' tide level. It is of particular interest that Aster in this area exhibits a wide ecological amplitude (partly shared by

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* The period February to October has been used rather than the full yearly cycle. This is the period of active vegetative growth and is likely to be the most critical. For example Ranwell et al (1964) have shown that although the lower limit of Spartina anglica on south coast marshes is at a level where the plant is submerged for not more than 6 hours continuously, it may survive submergence outside the growing period of up to 9 hours.

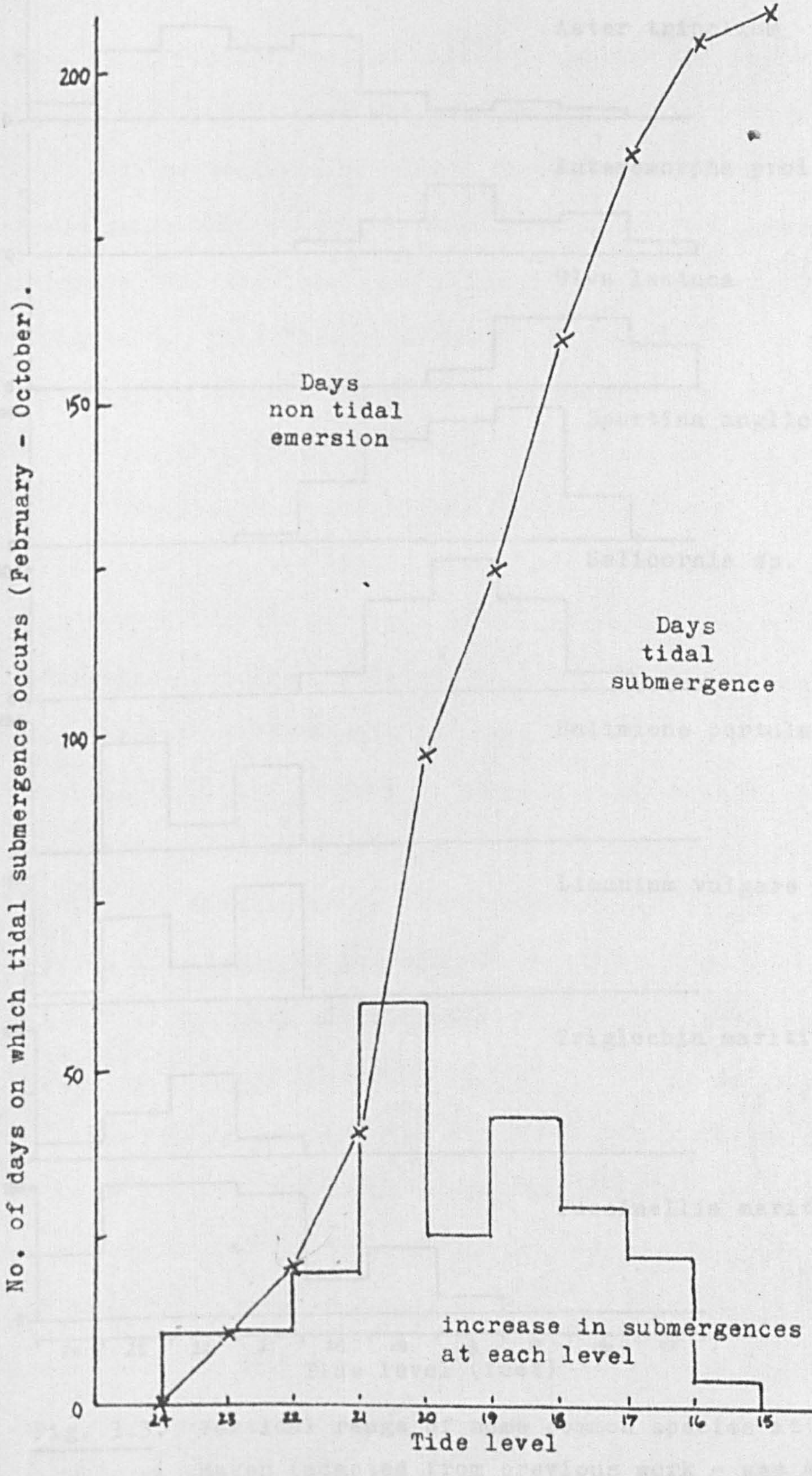
No. of days on which tidal submergence occurs (February - October).
200
150
100
50
0
24
23
22
21
20
19
18
17
16
15
Tide level
Days non tidal emersion
Days tidal submergence
increase in submergences at each level

Fig. 3.2.

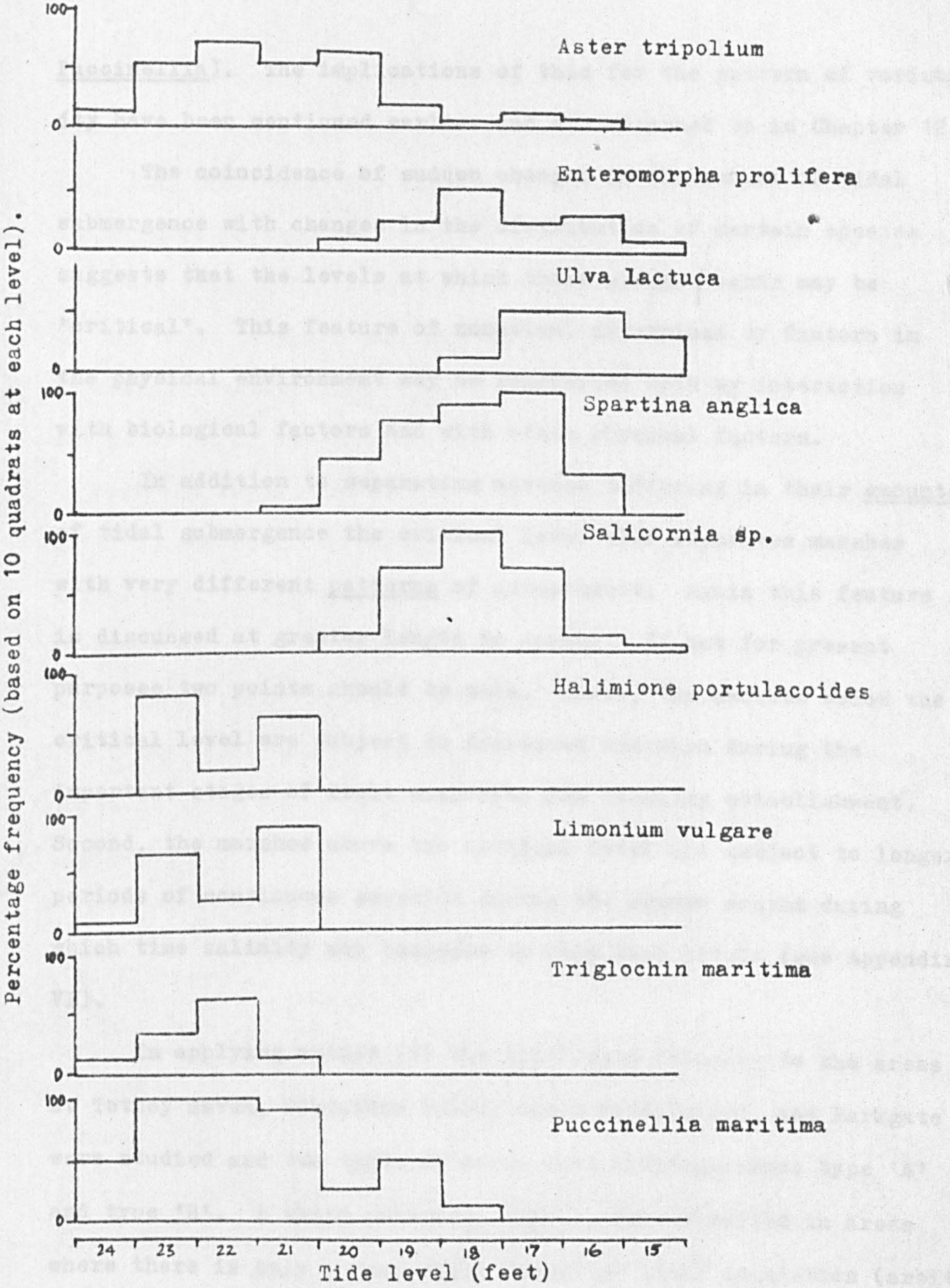

Fig. 3.3. Vertical range of some common species at Tetney Haven (adapted from previous work - see Appendix VI).

Puccinellia). The implications of this for the pattern of variability have been mentioned earlier and are returned to in Chapter 12.

The coincidence of sudden changes in the amount of tidal submergence with changes in the distribution of certain species suggests that the levels at which these changes occur may be 'critical'. This feature of zonation, determined by factors in the physical environment may be reinforced both by interaction with biological factors and with other physical factors.

In addition to separating marshes differing in their amounts of tidal submergence the critical level also separates marshes with very different patterns of submergence. Again this feature is discussed at greater length in Appendix VI but for present purposes two points should be made. First, the marshes below the critical level are subject to decreased emersion during the important stages of fruit dispersal and seedling establishment. Second, the marshes above the critical level are subject to longer periods of continuous emersion during the summer months during which time salinity may increase to very high levels (see Appendix VI).

In applying method (1) the tidal data relating to the areas at Tetney Haven, Gibraltar Point, Scolt Head Island, and Parkgate were studied and two types of marsh were distinguished; type 'A' and type 'B'. A third category, type C, was delimited in areas where there is only a very small amount of tidal inundation (arbitrarily defined as less than 10 tides/annum).

The division into type 'A' and type 'B' has relied on a tidal submergence curve for the area concerned (of the sort shown as Fig. 3.2) from which the theoretical critical level is determined, and a visit to the area to establish the extent of coverage of selected tides (of a height corresponding to the critical level). In the cases of Scolt Head and Parkgate previous work (by Chapman (1960a) and Marker; (1967) respectively) was available which verified the position of the critical level. This level varied in relation to mean sea level from area to area. At Tetney Haven and Gibraltar Point it was at about the 20' tide level (10' O.D. at Tetney Haven, actual O.D. at Gib. Pt. not known) and at Scolt Head Island it was at about 8' O.D. (at 8.10' O.D. according to Chapman when corrected from his arbitrary datum Island Zero Level).

The separation of marshes into the two types, A (below the critical level and B (above the critical level), gave the following division:-

	Type B	Type A
Gibraltar Point	Limonium marsh, Aster marsh.	Spartina marsh.
Scolt Head Island	Plover	Beach Point, Cockle bight Beach Point '65.
Tetney	Halosere, Old marsh.	
Parkgate	P2.	P3, P4.

The third category, type 'C', contained three marshes.

These were Agropyron marsh at Gibraltar Point (occasional seepage of seawater through a sluice, otherwise only flooded by storm surges e.g. 1953), Spiral marsh at Scolt Head Island (by overflowing of creek, <5 tides/annum) and P1 at Parkgate (by tides of 29' Liverpool C.D. or over, approximately 8 tides/annum).

The major feature of these types of salt marsh are listed in Table 3.2 which summarises the major differences in tidal relations described in Appendix VI and above. Also given are the positions of other populations sampled in this study. These have been obtained subjectively by extrapolation from the major vegetational features of areas for which no tidal data was available. In some cases the categorisation was easy on common sense grounds (e.g. Cley, to landward of sea wall with Phragmites dominant), in others it was more difficult (e.g. Morston, with a range of both typically 'A' and 'B' species).

(b) Method (2). Association-analysis.

More recently it has become possible to apply Method (2), association-analysis, to the classification of these areas*. The species lists taken from the immediate sampling area (i.e. the population lists) were used in association-analysis, as developed by Williams and Lambert (1959 and 1960), in which hierarchical divisions are used to separate the vegetation into groups which are considered to be ecologically meaningful. Williams and Lambert

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* My thanks are due to Dr. R. G. H. Bunce of the Nature Conservancy, Merlewood, who gave advice on this section of the study and who arranged for the data to be run at Southampton University.

TABLE 3.2. THE CLASSIFICATION OF SALTMARSHES BY METHOD (I). (See text and App. VI)

	TYPE 'A'	TYPE 'B'	TYPE 'C'
OCCURRENCE	RARE (ESPECIALLY ON W. and S COASTS)	COMMON (GENERAL SALT MARSH TYPE)	RARE DUE TO RECLAMATION
HEIGHT	BELOW CRITICAL LEVEL	ABOVE CRITICAL LEVEL	ALMOST REMOVED FROM TIDES.
TIDAL RELATIONS	COVERED BY BOTH HIGH NEAPS AND SPRINGS	REACHED BY ALL HIGH SPRINGS	RARELY REACHED BY SPRINGS
MAXIMUM PERIODS OF SUBMERGENCE	IN DECEMBER, JANUARY, APRIL, MAY JULY, OCTOBER	IN MARCH, APRIL, SEPTEMBER, OCTOBER	OCCASIONALLY SUBMERGED BY EQUINOCTIAL SPRINGS
NUMBER SUBMERG. FEB. to OCT.	+75	≥75	FEW ≥10.
MAXIMUM PERIODS OF EMERSION	SPRING, MORE RARELY AUTUMN	JUNE-AUGUST, DEC.-JANUARY.	ALMOST COMPLETE EMERSION
MINIMUM PERIOD OF CONTINUOUS EMERSION	C. 10 DAYS	C. 25-30 DAYS	UP TO 300+ DAYS
SALINITY	VARIABLE, RARELY ABOVE THAT OF SEA WATER.	VERY VARIABLE, OFTEN HIGH.	LOW, OFTEN FRESH WATER
DOMINANT VEGETATION	ALGAE, <u>SALICORNIA</u> sp. <u>SUAEDA MARITIMA</u> <u>SPARTINA ANGLICA</u>	VARIED. INCL. <u>PLANTAGO MARITIMA</u> <u>LIMONIUM VULGARE</u>, <u>ARMERIA MARITIMA</u> <u>HALIMIONE PORTULACOIDES</u> etc.	VARIED. INCL. <u>JUNCUS MARITIMUS</u> <u>SCIRPUS MARITIMUS</u>, <u>AGROPYRON PUNGENS</u>, <u>PHRAGMITES COMMUNIS</u>.
MARSHES STUDIED			
SCOLT HEAD	COCKLE BIGHT BEACH PT.	PLOVER MARSH	SPIRAL MARSH
GIBRALTAR POINT.	SPARTINA MARSH	ASTER MARSH LIMONIUM MARSH	AGROPYRON MARSH
PARKGATE	PARKGATE 4 PARKGATE 3	PARKGATE 2	PARKGATE 1.
OTHERS		KNOTT END ANGLESEY B R. WYRE HEST BANK	ANGLESEY A NAZE

calculated species correlations from all possible pairs and the properties of the resulting matrix were used to subdivide the stand populations into groups. The species with the highest coefficient of correlation (in this case $\sum\sqrt{\frac{\chi^2}{n}}$) was used at each stage to divide into groups. A diagram was used to represent the successive course of the division (which was terminated at a level of $\sum\sqrt{\frac{\chi^2}{n}}$ of $p = <.005$). Such a diagram for the 24 populations containing Aster is given as Fig. 3.4. The full matrix is given as Appendix III(b).

As can be seen from Fig. 3.4 the association-analysis extracted four groups of populations. The species used to divide the groups were, at successively decreasing levels of heterogeneity, Agrostis stolonifera, Festuca rubra and Plantago maritima. The groupings are given in Table 3.3 (column 2). An interpretation of these is included in (c) below.

(c) The categories 'high' 'mid' and 'low'.

The results of the two methods of classification can be compared with the help of Table 3.3.

It is apparent that the groups extracted by the analysis correspond closely with those recognised on the basis of Method (1). Thus Method (1), albeit partly subjective, seems a satisfactory one. Some obvious anomalies occur however. One of these is the status of the Spiral marsh population at Scolt Head, originally classified in terms of tidal relations as a 'C' marsh site. Its separation by the analysis from the other 'C' sites is due to the

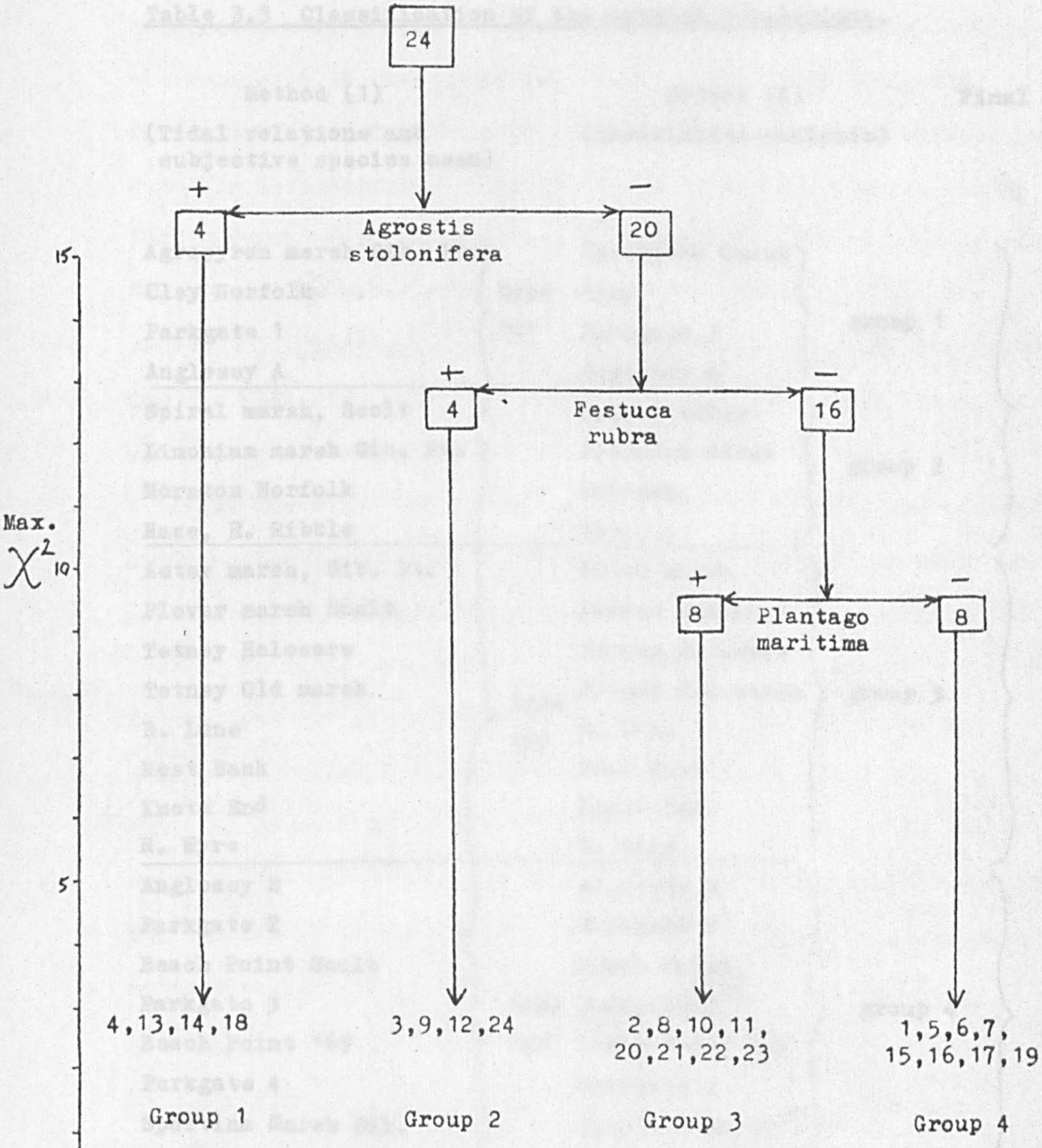

Fig. 3.4. Stand normal association-analysis of local marsh species data. (For full matrix see Appendix III).

Table 3.3 Classification of the sampled populations.

Method (1) (Tidal relations and subjective species assn)		Method (2) (association-analysis)		Final scheme
Agropyron marsh Gib. Pt.	type 'C'	Agropyron marsh	group 1	HIGH
Cley Norfolk		Cley		
Parkgate 1		Parkgate 1		
Anglesey A		Anglesey A		
Spiral marsh, Scolt		Spiral marsh	group 2	
Limonium marsh Gib. Pt.	type 'B'	Limonium marsh		
Morston Norfolk		Morston		
Naze, R. Ribble		Naze		
Aster marsh, Gib. Pt.		Aster marsh	group 3	MID
Plover marsh Scolt		Plover marsh		
Tetney Halosere		Tetney Halosere		
Tetney Old marsh		Tetney Old marsh		
R. Lune		R. Lune		
Hest Bank		Hest Bank		
Knott End		Knott End		
R. Wyre		R. Wyre		
Anglesey B		Anglesey B	group 4	LOW
Parkgate 2		Parkgate 2		
Beach Point Scolt	type 'A'	Beach Point		
Parkgate 3		Parkgate 3		
Beach Point '65		Beach Point '65		
Parkgate 4		Parkgate 4		
Spartina Marsh Gib.		Spartina marsh		
Cockle Bight Scolt		Cockle bight.		

lack of non-maritime species, a fact which could be related to the shingle-base on which it rests and its isolation by dunes from the remainder of the island and the mainland. Its inclusion in a group (Method (2) grp. 2) with the Limonium marsh at Gibraltar Point is interesting in view of the difficulty with which the latter marsh could be separated by Method (1) from the nearby 'C' marsh site the other side of Bulldog bank (Appendix IV). The presence of Artemisia maritima and Agropyron pungens at Morston and the estuarine position of Naze had also made these sites, of unknown tidal relations, difficult to classify by Method (1).

At the other end of the scheme only two marshes above the critical level, Anglesey B and Parkgate 2, have been moved by the association-analysis into a group with those below the critical level. No tidal data was available for Anglesey B and its inclusion in the type 'B' group had been subjective. The inclusion of Parkgate 2, clearly 'B' marsh in terms of tidal relations, in the analysis group 4 is interesting in view of the paucity of characteristic 'B' marsh species at this site. This may reflect one of the dangers of association-analysis of populations in areas such as salt marshes where a certain number of species, like Aster tripolium are characteristically ubiquitous in their habitat range. Cochlearia and Puccinellia, present at the P2 site, are both fairly wide-ranging species (see, for example, the range of the latter species at Tetney Haven in Fig. 3.3).

The results obtained by the two methods have been combined to arrive at a final system of classification. This recognises three groups of marshes, 'high', 'mid' and 'low'. The boundaries derived from Method (2), the association-analysis, since these have the benefit of complete objectivity, have been retained but, in order to broaden the categorisation, groups 1 and 2 have been merged together to form the category 'high' marsh. Thus the final scheme is as follows (see also Table 3.3):-

'High' marsh - populations Gib. Agr. to Naze (analysis grps. 1 and 2).
'Mid' marsh - populations Gib. Ast. to R. Wyre (analysis grp 3).
'Low' marsh - populations Anglesey B to S. Cob. (analysis grp. 4).

It is felt that, for the purposes of this investigation, a deliberately broad classification may be the most meaningful system for the study of variation in a wide-ranging species such as Aster. Ideally it should be possible to ordinate measured environmental variables with the variation in plant characters, and it may be that the multivariate techniques derived from phytosociology can be used in the future as a tool for habitat identification in genecological work. (A manuscript of a paper by Wilkins and Lewis (1969, in press)* has recently come to hand which proposes exactly this type of approach, employing in this case an ordination technique). However, in view of the known complexity of habitat factors and the fact that it has not been possible to account for this adequately, the broadness of the original

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* Now Wilkins and Lewis (1969).

scheme based on an apparently major habitat factor, the tides, has been retained in the final classification.

Thus no attempt has been made to identify 'zones' within the 'mid' category. This includes marshes of the 'general salt marsh' and salt pan type, which may vary considerably from area to area. Aster is a common member of the general salt marsh community at this level. It is interesting that, in the association-analysis this group is defined by the presence of Plantago maritima (and the absence of Agrostis and Festuca) a characteristic member of this community.

The low marsh group of populations (minus Agrostis, Festuca and Plantago) from the point of view of the analysis clearly form the 'dump' group. These marshes, where they exist (they may be absent on sandy substrates, particularly on the west coast). are commonly colonised by a wide range of algae and a small number of seed plants including Salicornia species, Suaeda maritima, Spartina anglica, and occasionally Puccinellia maritima. The 'Asteretum' zone recognised by Chapman as fringing the 'Salicornietum' on many East Anglian marshes represents the extension of Aster tripolium on to this low type marsh, and has no equivalent, according to him, in west and south coast types of succession (Chapman 1960 Chapter VI).

It is more difficult, on broad ecological criteria, to justify the amalgamation of the association-analysis groups 1 (plus Agrostis, minus Festuca and Plantago) and 2 (minus Agrostis, plus Festuca and Plantago) into a single 'high' marsh category. My own general observations and those of others (e.g. Ranwell (1968), Hannon and Bradshaw (1968)) confirm that Agrostis stolonifera is generally limited to the upper zones of salt marshes, and the importance which the analysis attaches to the discontinuity between stands containing or not containing Agrostis (the divisive species at the highest level of heterogeneity) may well reflect a highly significant ecological discontinuity. However, since their inclusion in the analysis in the first place depends on the presence of Aster within them, the stands can only be used in the analysis to reveal discontinuities within associations containing Aster and not within salt marsh associations as a whole. It seems fair, therefore, to interpret the groups from the point of view of the status of Aster within them. In groups 1 and 2 Aster is nearing the upper limit of its range and, in all the eight populations, is present with wide-ranging glycophytic grasses such as Agrostis stolonifera, Festuca rubra and Agropyron pungens (Puccinellia maritima, also present in other populations, is a specialised, restricted species). The environmental factors which normally prevent these species from colonising the general salt marsh community are no longer limiting, and it is suggested that their presence, together with the presence, in some stands, of brackish marsh species such as Phragmites communis and Scirpus maritimus, will greatly increase the general 'competitiveness' of the environment faced by Aster.

The broad categories of 'high', 'mid' and 'low' marsh have been delimited as a preliminary stage in the investigation. Where the tidal relations of individual marshes are known more exactly (for the seventeen intensively sampled populations) it is possible to rank populations within these three categories, and in the future it is hoped that component analysis can be used to extract possibly more subtle trends of differentiation within the species. For the present it is suggested that a preliminary analysis of the general patterns of differentiation is possible within the framework of the three major divisions, the habitats circumscribed by such divisions presenting the plants they contain with very different problems for survival.

SUMMARY OF PART I

(1) Aster tripolium L. is distributed throughout Western and central Europe and extends into Asia as a plant of inland saline areas. In the western part of its range, including Britain, it is largely confined to suitable habitats within a narrow maritime belt.

(2) Within this maritime belt the species is wide-ranging, being found at both extremes of the coastal zone along a transition from predominately marine to predominately terrestrial environments. In the light of the known variation in habitat factors at these extremes it was suggested that the ubiquity of the species in this respect must have been the product of adaptation by selection of suitable types in the respective environments. It was hoped that an investigation of (i) the elimination of plants at the extremes of its range, and (ii) the pattern of variation within the coastal zone, would provide information on the nature of that adaptation.

(3) Although a taxonomically discrete species A. tripolium is highly variable at the level of the phenotype and at least eighteen infraspecific taxa have been recognised in European populations using traditional methods. Some, or all, of these may be of spurious significance.

(4) The theoretical background of related studies was discussed and the approach to variation adopted in this project was outlined. This involved (i) the use of the traditional

garden trial to identify genetic variation, (ii) the use of a range of culture conditions to assess the range of plastic modification of certain characters, and (iii) further experiments to test the relevance of variation revealed by (i) to the prevailing habitat conditions.

(5) A range of European populations was sampled (i) on an extensive basis, small numbers of individuals being collected from geographically well separated areas, and (ii) on an intensive basis, (from British salt marsh populations only), larger numbers of individuals being collected from selected marshes thought to form a series from high to low at certain areas of the coastal zone. Populations were sampled as either fruits or plants or both.

(6) The coastal marshes from which populations were sampled as part of the intensive sampling programme were described in Appendix IV. These were four marshes at Gibraltar Point Lincolnshire, four marshes on Scolt Head Island Norfolk, two marshes at Tetney Haven, Lincolnshire, two marshes in the Blakeney area of N. Norfolk, and four marshes in the Dee estuary near Parkgate, Cheshire. Populations at two inland sites in Cheshire were described in Appendix V .

(7) The British salt marsh populations were divided into three main types, 'high', 'mid' and 'low'. Two methods of classification were used to make this division. These were based on (1) tidal relations and (2) species association (the background to method (1) was described in Appendix VI). It was suggested

that the division of marshes into three main groups has a valid ecological basis and should enable comparisons to be made between ecologically similar marshes in different areas.

PART II

REPRODUCTIVE BIOLOGY

CHAPTER 4.

CHROMOSOMES, THE BREEDING SYSTEM, LIFE CYCLE AND FLOWERING

I Chromosome number and karyotype.

The only reported chromosome number for Aster tripolium is 2n = 18. This number has been counted in material from a range of sources including Scandinavia (Love and Love, 1944), the Baltic coast both in Poland (Piotrowicz in Skalinska et al, 1959) and Germany (Wulff, 1937), Hungary (Polya, 1948), the Atlantic coast of Portugal (Rodriques, 1953), the northern Adreatic (Negodi 1941), and both coastal and inland areas of Japan (Tahara and Shimotomai, (1926), Jinno 1956). Other counts (listed in Darlington and Wylie (1955) and Love and Love 1961) all confirm this number. The count in Clapham et al (1962) is not of British material; no previous British counts have been recorded.

Material and methods. Somatic chromosomes were examined in root-tip squashes prepared either from primary radicles or from secondary roots of more mature plants growing in pots in the greenhouse. Excised roots were pretreated in a saturated solution of p-dichlorobenzene and were aerated throughout the 3-6 hour pretreatment period. A number of trials led to the best results being obtained by pretreating for 5 hours at the temperature of the laboratory or greenhouse in which the plants had previously been growing. The tips were then fixed (in a freshly made-up mixture of absolute ethyl alcohol, 10 parts: glacial acetic acid, 2 parts: chloroform, 2 parts: and formalin, 1 part), hydrolysed (in 1N hydrochloric acid at 60°C for 5 minutes), washed, and eventually stained in lactopropionic orcein. A range of British and foreign material was examined in this way and the counts are reported in Table 4.1.

The diploid number $2n = 18$ was found for a total of thirty seven plants from twenty-one populations (in some cases root-tips were examined from more than one plant per population). No supernumary chromosomes were found in the cells examined.

The chromosomes are large and, with pretreatment, relatively easy to separate. Photomicrographs and camera lucida drawings were made of several cells at or about mitotic metaphase, and these were used to analyse the karyotype in the species. It was possible to make such an analysis of a number of squashes, (see Table 4.1) despite the differential contraction rates of the constituent chromosomes.

An idiogram, based on the cells thus examined, is presented as Fig. 4.1. The complement consists of four pairs of metacentrics, the largest three of which (A-C) are difficult to distinguish one from another, four pairs with slightly submedian centromeres, two of which (E and F) are larger than D and one of which (H) is the smallest pair of the set, and a ninth, very distinctive pair (S), with submedian centromeres and with long arms which are clearly satellited. There are no apparent (gross) morphological differences between the karyotypes of any of the cells from the British and foreign material examined (which included the

Table 4.1. Chromosome counts

source	s/p	date determined	2n	karyotype analysis	no. of plants counted.
<u>British</u>					
Gibraltar Pt. Lincs	S	Nov. 1967	18	+	2
Gib. Pt. rayed plant	P	Oct. 1965	18	-	1
Gib. Pt. rayless plant	P	Oct. 1965	(18) ±1	-	1
Hest Bank, Lancs.	S	Oct. 1965	18	-	1
Knott End, Lancs.	S	Oct. 1965	18	-	1
Tetney Haven, Lincs.	P	Oct. 1965	18	-	1
"	S	Sept. 1966	18	+	2
Scolt Hd. Is. Norfolk	S	Sept. 1966	18	-	1
" Spiral marsh	S	Nov. 1967	18	+	4
" Cockle Bight	S	Nov. 1967	18	+	2
Llys Dulas Anglesey	P	Sept. 1966	18	+	1
Oyce, Orkneys	P	Sept. 1966	18	-	1
Winsford. Cheshire	S	Oct. 1967	18	+	3
Keele (genetic stock)	S	Oct. 1967	18	+	5
<u>Foreign</u>					
Wismar E. Germany	S	Sept. 1966	(18)±2	-	1
Helsinki Finland	S	Oct. 1967	18	+	2
Combra, Portugal*	P	Oct. 1967	18	+	2
Vienna Austria†	P	Oct. 1967	18	+	3
Halle Salle E.Germany	S	Nov. 1967	18	+	1
Mecklenburg E. Germany	S	Nov. 1967	18	-	1
Lund, Sweden.	S	Oct. 1967	18	+	1

S indicates radicle squash, P that tip came from mature plant.

* var. <u>longicaulis</u> † var. <u>pannonicus</u> ( ) = approximate count.

'varieties' pannonicus, discoideus and longicaulis). Some examples are given in Fig 4.2 - 4.5. In fact the karyotype is strikingly uniform from population to population. A camera lucida drawing of Japanese material (Huziwara 1959, p.191) is similar to the karyotype described above, although an earlier drawing (Shimotomai and Huziwara 1942) does not show a satellited pair.

In A. tripolium the satellited chromosome is of medium-size in relation to the rest of the haploid set, the antennae are of equal length and the trabants of equal size (see especially Fig. 4.3). The widespread existence of satellited chromosomes in the genera Aster, Gymnaster, Kalimeris and Heteropappus has been pointed out by Shimotomai and Huziwara (1942), who have used the similarity of antennae length in these chromosomes to recognise autopolyploids in the genus Gymnaster and the existence of trabants at unequal distances from the main arms to distinguish allopolyploids in the genus Aster. In contrast to the case of A. tripolium these chromosomes, designated L^{2E} - chromosomes, were usually the longest, or nearly the longest, in those species investigated by the two authors.

The basic chromosome number $X = 9$ is the most common in the Compositae as a whole, occuring in some genera of all thirteen tribes of the family , and is found particularly frequently in the Astereae (Darlington and Wylie 1955). Chromosomal evolution in the Compositae has been studied in several genera of the tribe Cichorieae (Stebbins 1958), notably the genus Crepis (Babcock 1947),

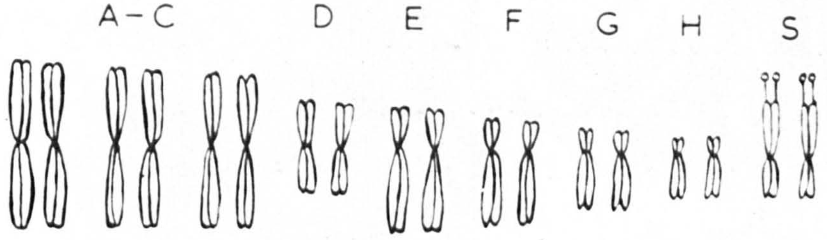


FIG. 4.1. IDIOGRAM, BASED ON ROOT TIP SQUASHES.

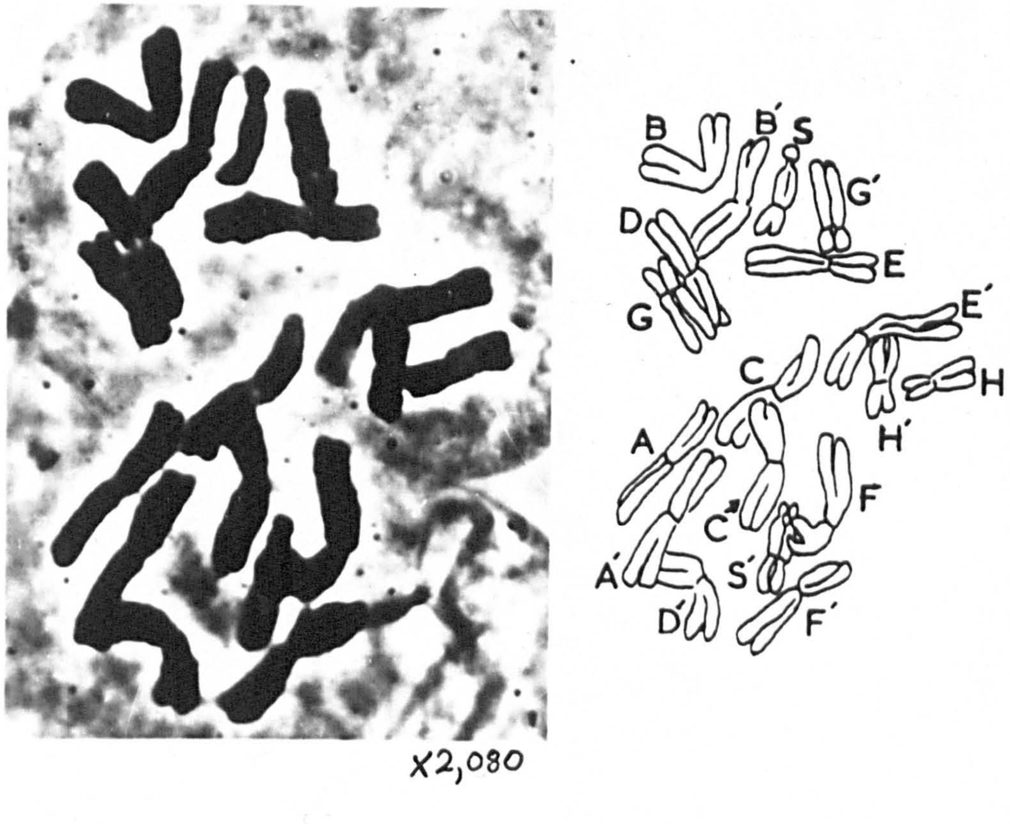


FIG. 4.2. SOMATIC CHROMOSOMES - var. pannonicus (VIENNA) 2n=18

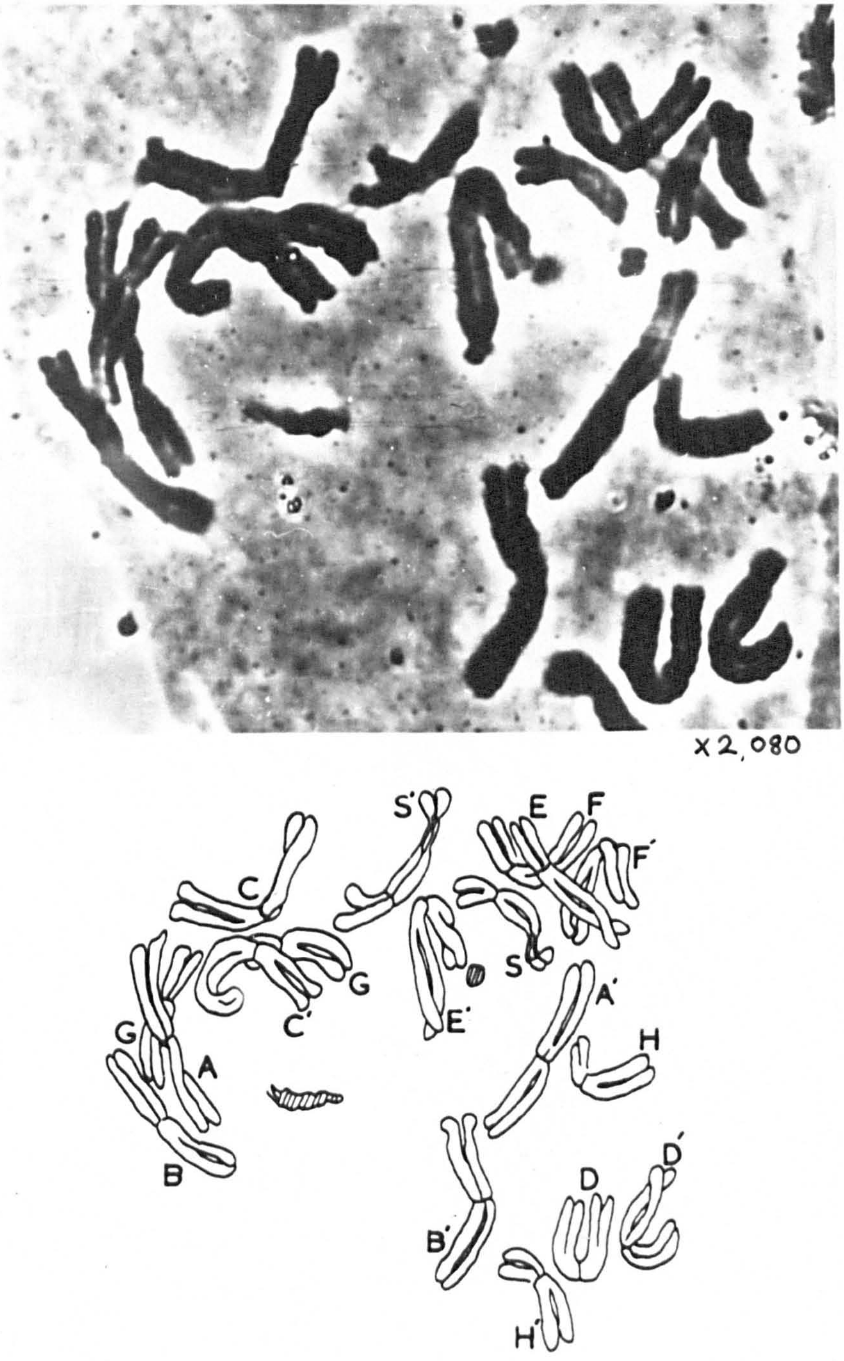


FIG. 4.3. SOMATIC CHROMOSOMES - material from WINSFORD (2n=18)

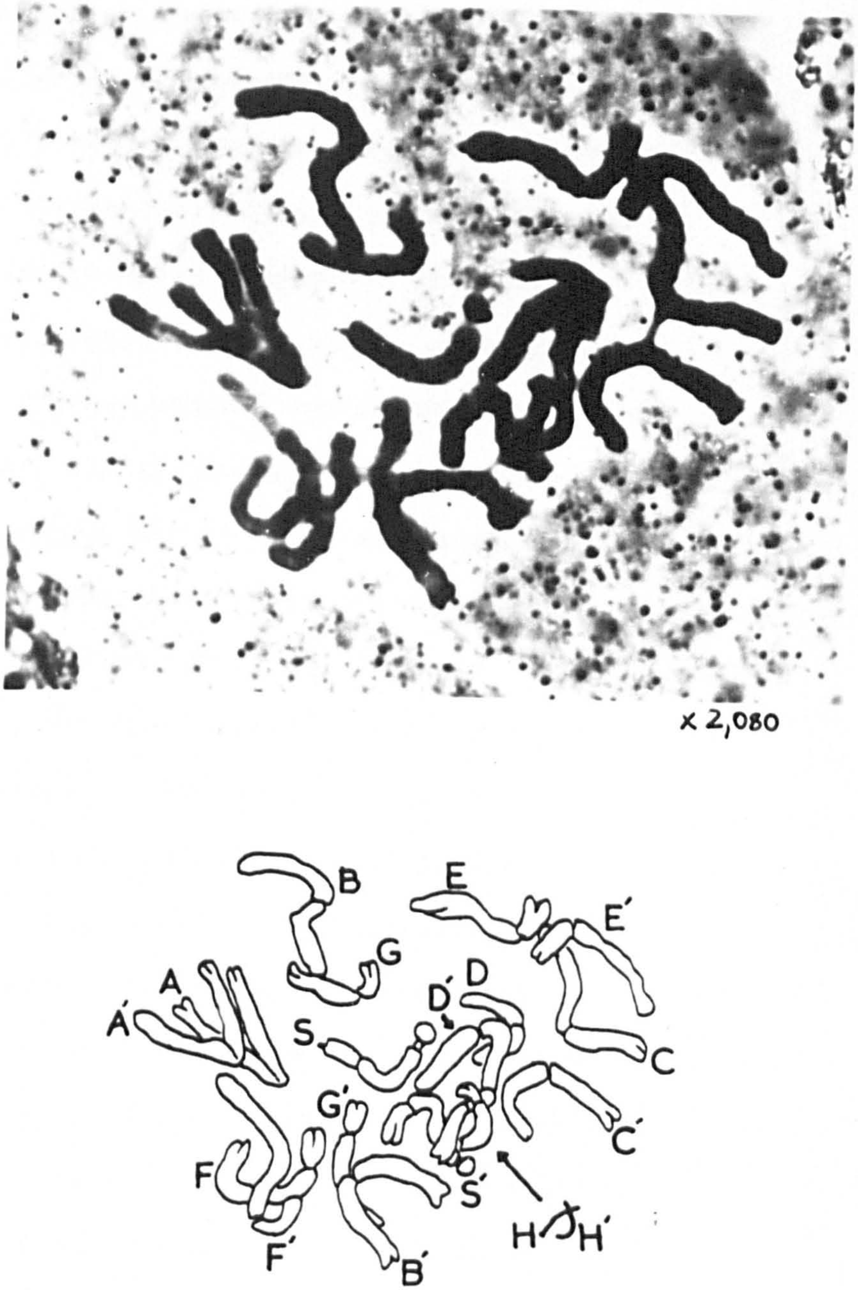


FIG. 4.5. SOMATIC CHROMOSOMES - var. <u>longicaulis</u> (PORTUGAL) 2n=18.

and within the subtribe Asterineae (Huziwara 1959). It is thought by these workers to have involved a reduction in the basic number and size of the chromosomes, as well as a trend from chromosomes with median centromeres to those with subterminal centromeres, and from symmetrical to asymmetrical karyotypes, (these are related and possibly widespread phenomena (Stebbins 1958, Davis and Heywood 1963, Ch.6) probably arising from a series of pericentric inversions, unequal reciprocal translocations, and eventual centromere, and adjoining heterochromatic segment, losses). Thus Aster tripolium may be regarded, on all counts, as karyotypically primitive. The chromosomes are exceptional in being the largest thus far found in the genus; they are largely metacentrics, or nearly so, and have a small overall size range from the largest to the smallest. In these respects the species is typical of the European and Asiatic representatives of the genus, all of which have X = 9, and which Huziwara (1959) is able to contrast with the more specialised North American species, where the basic numbers 5,8 and 9 are found.

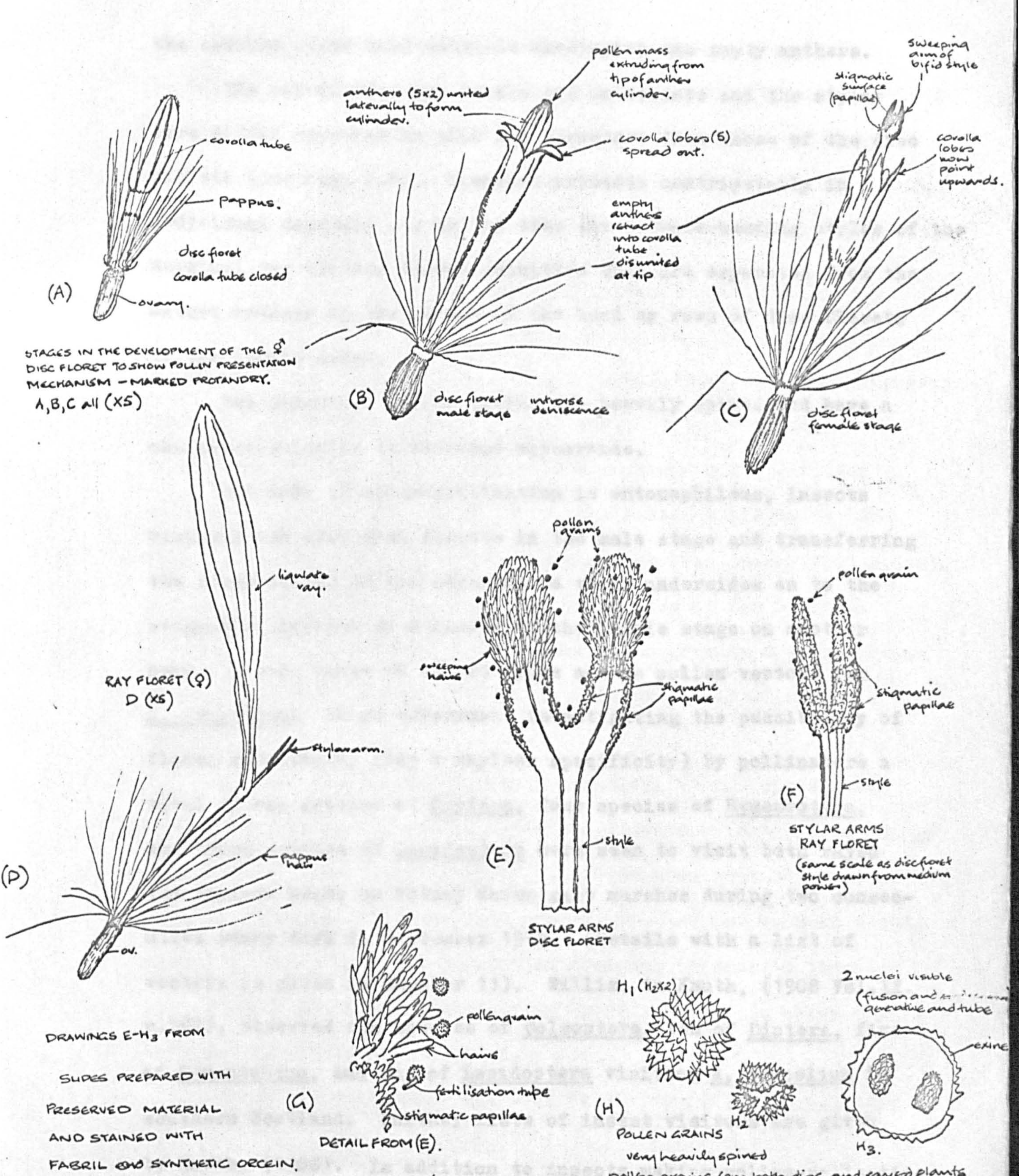
Aster tripolium appears to have a uniform, diploid karyotype throughout its Western and central European range. The above survey of scattered British and foreign material has failed to detect any tetraploids which may exist in natural populations of the species, although the existence of such tetraploids cannot be discounted. In addition it appears to have remained genetically isolated, there being no other species with which it has hybridised (or is likely to hybridise in the future). An examination of the karyotypes of potentially related species as drawn by Huziwara (1959) suggests that the species is not involved cytologically in the euploid series in the genus (2,3,4,6, and 8x taxa of the x = 9 basic number are known) or the few recognised aneuploids (largely trisomics). It is equally unlikely, on theoretical grounds as well as from cytological investigation, that, as a diploid monosomic, it formed the basis for further polyploidy in the group.

II Floral Biology.

The large showy heads vary greatly in diameter and in the number of florets they contain (see later) but, with the exception of the var. discoideus, they normally contain both ray and disc florets (the heterogamous condition). The central yellow disc florets are tubular, hermaphrodite and protandrous, so that the male and female stages of development are clearly recognisable in an individual floret. In the male stage anthers of the five epipetalous stamens project from the corolla tube, being united laterally to form a cylinder. Introrse dehiscence is followed by extrusion of pollen from the tip of the anther cylinder, a process partly effected by the elongation of the style, the upward sweeping arms of which bear apical hairs. The style continues to elongate until, in the female stage, the stylar arms eventually protude about two millimetres from the corolla tube, exposing the stigmatic papillae (see Fig. 4.6). At this stage

FIG. 4.6 ASTER TRIPOLIUM FLORAL CHARACTERS.

FLORET MORPHOLOGY.



STAGES IN THE DEVELOPMENT OF THE ⚥ DISC FLORET TO SHOW POLLEN PRESENTATION MECHANISM – MARKED PROTANDRY.
A, B, C all (X5)

RAY FLORET (♀)
D (X5)

DRAWINGS E-H_3 FROM SLIDES PREPARED WITH PRESERVED MATERIAL AND STAINED WITH FABRIL AND SYNTHETIC ORCEIN.

very heavily spined pollen grains from both disc and rayed plants appear microscopically identical under high power (X500).

H_1 and H_2 stained with FABRIL to show architecture of pollen grain surface. H_3 stained with synthetic orcein.

the corolla lobes bend outwards enveloping the empty anthers.

The ray florets are female and uniseriate and the stylar arms differ somewhat in size and structure from those of the disc florets (see Fig. 4.6). Anthesis proceeds centripetally in individual capitula and by the time the outward-bending styles of the marginal ray florets become receptive they are separated from the mature anthers in the centre of the head by rows of disc florets in the female stage.

The spherical pollen grains are heavily spined and have a characteristically three-lobed appearance.

The mode of cross-pollination is entomophilous, insects visiting one head with florets in the male stage and transferring the rough coated pollen adhering to their undersides on to the stigmatic papillae of a floret in the female stage on another head. A wide range of insect types act as pollen vectors for A. tripolium. In an experiment investigating the possibility of flower specificity (ray v rayless specificity) by pollinators a total of six species of Diptera, four species of Hymenoptera, and three species of Lepidoptera were seen to visit both rayed and rayless heads on Tetney Haven salt marshes during two consecutive sunny days in September 1963. (Details with a list of vectors is given in Chapter 11). Willis, in Knuth, (1908 Vol.II. p.582), observed one species of Coleoptera, ten of Diptera, five of Hymenoptera, and one of Lepidoptera visiting A. tripolium in southern Scotland. Further lists of insect visitors are given by Knuth, (1908). In addition to insects making pollen-collecting flights, pollen is probably transferred from floret to floret within the same head, and even from head to head, by small crawling insects such as aphids and small beetles, frequently found in the inflorescence.

III Breeding system.

The importance of the breeding system in determining the pattern of variation and consequent race formation in natural populations of higher plants is well known. This has been discussed by Baker (especially his reviews of 1951, 1953, 1959) whose development of its implications for divergence and speciation emphasises that an investigation of the breeding system is 'the very first requirement' in genecological studies (Baker 1953 p.118). Bradshaw (1962) has underlined the importance of the breeding system as an evolutionary factor tending to promote either discontinuity (in breeding) or continuity (out breeding) of infraspecific variation.

It is therefore desirable to establish at the outset the amount of natural crossing in wild populations. 'Natural crossing' is here used sensu Fryxell (1947 p.143) to mean that which occurs between individuals of the same population, and not to mean either naturally occuring inter-population crossing (discussed later) or naturally occuring interspecific hybridisation, to which the term has sometimes been applied.

Direct evidence from the wild is difficult to obtain. The use of a simple genetic marker or markers of known inheritance which has an easily classifiable phenotypic effect has been suggested as an accurate measure of natural crossing. Such a marker is not known in the case of Aster tripolium. The majority of cross- and self-pollinations from which plants have been raised to maturity have been undertaken to determine the mode of inheritance of the ray floret character (Ch. 11). This surely disqualifies them as suitable genetic markers; for the ray-floret character, affecting as it does the number of male-sterile units per plant, is likely to have an effect, of itself, on the amount of natural cross-pollination. Indeed the fact that the ray florets are more likely to be outcrossed whereas the hemaphrodite disc florets may be selfed, is a further complication of the pattern of natural crossing. It is therefore also difficult to compare the variation in characters known to be under genetic control in the products of controlled selfings and open pollination respectively.

Experiments on plants under cultivation have yielded information on the breeding system in the species, and from this indirect evidence of the amount of natural crossing can be obtained. This information, which is detailed below, suggests that A. tripolium is possibly generally outbreeding in the wild, with only occasional 'selfing'.

(1) Apomixis. A number of trials revealed no evidence for apomixis. As part of an initial programme of controlled pollinations (in September 1963) a number of controls were set

up to test the effectiveness of using both the ray and disc florets as female receptors, taking advantage of the marked protandry in the latter. These operations were carried out on plants in the wild at Tetney Haven and involved the bagging of heads prior to opening (with small transparent paper bags normally used to contain postage stamps), emasculation by removal of individual anther cylinders at the male stage, and the transfer of pollen from the previously-bagged male parent at the female stage. The results of this early series of cross-pollination are given in Table 4.2. It can be seen from this that, despite the difficulty and crudeness of the technique, the control failed to produce fruit in two cases and only five fruits (compared with a possible thirty) in two more.

This technique was used again to further test for evidence of apomixis in October 1966. Eight heads, five on a plant from Winsford and three on a plant from Portugal, all failed to produce a single viable fruit when emasculated in this way and bagged. All the fruits were thin and papery and failed to germinate when moistened on filter paper in a petri dish. Further tests for apomixis, using the ray florets only, failed to produce a single viable fruit (fruits were regularly produced in control crossings using this method).

(2) <u>Cross and self pollination</u>. The majority of cross pollination (25 out of a total of 37) were undertaken using only the female ray florets as seed parent. This more satisfactory

Table 4.2. Cross pollination programme, Sept., 1963.

Code No.	Descr*	Operation	Fertile fruits formed r.	 d.	Conclusion
T.12.1.A	R	Emasculated and	0	0	
T.12.2.B	D	bagged.	-	3	
T.12.3.C	R		0	0	Not apomictic
T.12.4.D	RD		lost		
T.12.5.D	RD		0	2	
T.12.6.D	RD		4	26	
T.12.7E	R		lost		Self-fertile
T.12.8.F	D	Bagged	lost		but see text.
T.12.9.F	D		-	22	
T.12.10.G	R		1	17	
N.14.1.H	D	Emasculated and	-	+	
N.14.2.H	D	pollen from	-	+	
N.14.3.H	D	Tetney D plants	lost		D x D cross
N.14.4.H	D	transferred	lost		fertile
N.14.5.H	D		-	+	
N.14.6.J	D	Emasculated and	lost		
N.14.7.J	D	pollen from	lost		D x R
N.14.8.J	D	Humberston	lost		cross fertile
N.14.9.J	D	R plants	lost		
N.14.13.K	D	transferred	lost		
N.14.10.K	D		-	+	
N.14.11.K	D		-	+	
N.14.12.K	D		-	+	
H.14.14.L	R	Emasculated and	lost		
H.15.1.L	R	pollen from	+	+	
H.15.2.L	R	Tetney D plants	+	+	
H.15.3.L	R	transferred	lost		R x D cross
H.15.4.M	R		+	+	fertile
H.15.5.M	R		+	removed	
H.15.6.M	R		lost		
H.15.8.M	R		lost		
H.15.9.N	R	Emasculated and	+	+	
H.15.10.N	R	pollen from	lost		R x R cross
H.16.1.P	R	Tetney R plants	+	+	fertile
H.16.4.P	R	transferred	+	+	
H.16.5.P	R		lost		

* R = fully rayed plants
D = discoid plants
RD = partially rayed plants
\+ fruits produced, number not counted.

method involved the removal of the disc florets of the plants to be used as seed parent prior to anthesis. These florets were replaced by loosely-packed cotton wool plugs and pollen from the male parent was later presented to the offered stigmas of the male-sterile ray florets either (1) by careful brushing with a camel hair brush, or (2) by rubbing the heads directly together. The heads were enclosed in water proof glassine bags throughout.

These later experiments (details of individual crosses are listed in Appendix VII) confirmed the results of the earlier method listed in Table 4.2. Viable fruits were readily obtained in both self - (but see below) and cross-pollinations.

The pattern of self-pollination in experimental material was rather curious. The stylar arms of the disc florets do not recurve, as in many Composites, but some pollen is generally trapped by the upward sweeping arms during growth through the pollen mass, making self pollination possible. Despite this a number (approximately one third) of bagged heads failed to produce good seed in cold house conditions. In contrast all the heads bagged in the wild, the open frame, and the experimental garden produced numbers of viable fruits. The reason for this discrepancy is not understood, but it may be due to a physiological 'upset' under greenhouse conditions. The fact that heads cross pollinated but otherwise similarly bagged produce viable fruit makes this phenomenon even more difficult to understand.

The meaning of the term 'self pollination', as applied to a Composite, may need some qualification. The experiments described above do not rule out the possibility that individual disc florets could be 'self-sterile', or that protandry necessitates that pollen from an adjoining less mature floret is needed to effect fertilisation (this latter is unlikely as it would mean an unusually early loss of the powers of germination of the pollen grains carried up on their own stigma). Thus selfing in the closely-packed Composite head may be acheived by crossing between florets of the same capitulum (strictly geitonogamy). This is unlikely to effect the pattern of variation as, even were the individual florets self-sterile geitonogamy will still be able to circumvent outbreeding and have exactly the same effect as inbreeding.

The fact that self fertilisation can take place in A. tripolium is not an obvious one. Despite their floral morphology, and the common occurence of reflexing styles, self incompatibility is widespread in the Compositae (Fryxell 1957). It was found in all twelve of the N. American species of Aster tested by East (1940) and in A. novae-angliae and A. multiflorous by Wetmore and Delisle (1939).

Cross pollination, using the ray florets as seed parents, readily produced viable fruit. In the majority of cases such controlled pollinations involved plants drawn from populations which were possibly able to exchange genes in the wild because of their nearness to one another. However the successful production of seed in the three following cases provides evidence that no internal barriers exist between some distant populations. The crosses between plants separated geographically by considerable distance were:- In 1966 plants from Cley in Norfolk were crossed with plants from the estuary of the R. Wyre in North Lancashire; in 1967 plants from Anglesey were crossed with plants from Gibraltar Point, and, more spectacularly, in the same year plants from Gibraltar Point were crossed with plants (3 separate plants) of the so-called var. <u>pannonicus</u> from Vienna. (Unfortunately F1 plants from the last cross have not yet flowered and their fertility has thus not been tested). F1 plants from the first two crosses produced viable seed on selfing. There was no evidence of a reduction of seed-setting, germination or F1 viability.

From the above it can be seen that both cross and self pollination are possible in the species. In this situation evidence as to actual amount of natural crossing which occurs in the wild has, in the absence a genetic 'marker', to be assessed cautiously from a study of the variation pattern. This pattern is described in later chapters but (1) the great range of variation in natural populations for characters known to be under genetic control, and (2) the blurring of discontinuities in such characters between adjacent populations, are circumstantial evidence that habitual outbreeding occurs. The morphology and protandrous development of the florets and their centripetal maturation (ensuring the separation of the marginal ray florets from pollen extruded from the central disc), together with the large numbers of suitable pollen vectors, even in the ecologically lower zones of salt marshes, may perhaps be regarded as further pieces of circumstantial evidence - at least the mechanisms tending to promote outbreeding are present.

IV Life cycle and phenology.

Aster tripolium is described in the literature as a short-lived perennial 'flowering in the second year from seed' (Clapham et al, 1942). Lovkvist (personal communication 1964) reports that he has rarely been successful 'in obtaining flowers the second year' in material cultivated at Lund (Southern Sweden).

As a result of comparative cultivation of both British and foreign material, and of field observation, information has been collected on (1) the time taken to flower from fruit, (2) the actual calender period of flowering and (3) the life cycle, in a number of populations. The range in variation of these three characters was considerable and both the extremes of first year, late flowering annuals and third or forth-year, early flowering perennials, were identified alongside many intermediates. The three characters are also intimately related both to one another and to growth habit. The information relating to life-cycle and flowering time is presented here, that relating to growth habit in Chapter 10, and that relating to their general correlation in Chapter 12.

(a) Foreign material. Plants grown from fruit received from a number of European botanic gardens have been cultivated at Keele under cold house conditions in normal daylight. The flowering behaviour of these is given in Table 4.3. The time of germination may not coincide in each case with the date sown (indicated in column 4) as variation in the period of delay between sowing and germination did occur, but was not recorded. However in those cases where the fruit was sown late in the year all germination had occured by September or October of the year sown and vegetative growth proceeded during the early winter. Flowering within a period of 12 months was counted as first year flowering.

The data from Table 4.3 are mapped in Fig. 4.7. There is an overall pattern of developmental periodicity in the European populations cultivated (a pattern discernible despite the small numbers of plants from certain localities). In general, plants from further north reach their flowering stage more slowly, consolidating vegetative development during the first, and perhaps second season of growth. This involves the development of a woody basal perennating system (see Chapter10). In contrast the more southerly types, and those from the inland saline areas of Central Europe, flower in one, or at the most two, seasons of growth. The localities are scattered and there are a number of important gaps. The general north/south cline may in fact contain two separate clines, an Atlantic maritime one and an inland continental one, (The central Austrian plants, although from

Table 4.3 Flowering from seed in plants of foreign origin (and in some British material of unknown ecology)

Culture no. (for Fig.4.7)	Source	date sown	no. plants	no. flowering in each year from seed #			yet to flower
				1st	2nd	3rd	
1	Helsinki, Finland	May 1966	3	0	0	-	2
2	Uppsala, Sweden	Feb. 1966	8	0	0	-	8
3	Oslo, Norway	June 1965	2	0	0	1	1
4	Askim(Vastergotland) Sweden	Feb. 1966	4	0	1	-	3
5	Oland Is. Sweden	Feb. 1966	38	0	0	-	38
6	Copenhagen Denmark	Aug. 1965*	2	1	0	-	1
7	Mecklenburg E.Germany	Aug. 1965*	10	6	3	-	0
8	Bad Durrenburg "	Aug. 1965*	10	10	0	0	0
9	Eiselben, "	June 1966	4	1	3	-	0
10	Halle Salle, "	Jan. 1965	7	5	1	-	0
11	Wein, Austria	Feb. 1967	7	7	-	-	0

Table 4.3 Continued:-

Culture no. (for Fig.4.7)	Source	date sown	no. plants	no. flowering in each year from seed #			yet to flower
				1st	2nd	3rd	
12	Cluj, Rumania	March 1965	9	8	1	-	0
13	Lasi oras, "	March 1966	1o	0	10	-	0
14	Porto, Portugal	March 1967	10	10	-	-	0
15	Ciombra, Portugal	Feb. 1966	12	12	-	-	0
	Ciombra, Portugal	March 1966	6	6	-	-	0
16	Fal estuary, Cornwall	Jan. 1966	5	0	1	-	4
17	Swansea, S.Wales	Feb. 1966	6	0	4	-	2

does not include reflowering in a subsequent year * autumn sowings (see text)

No.11 is described as var _pannonicus_ (Jacq), Nos.14 and 15 as var _longicaulis_ (Duf).

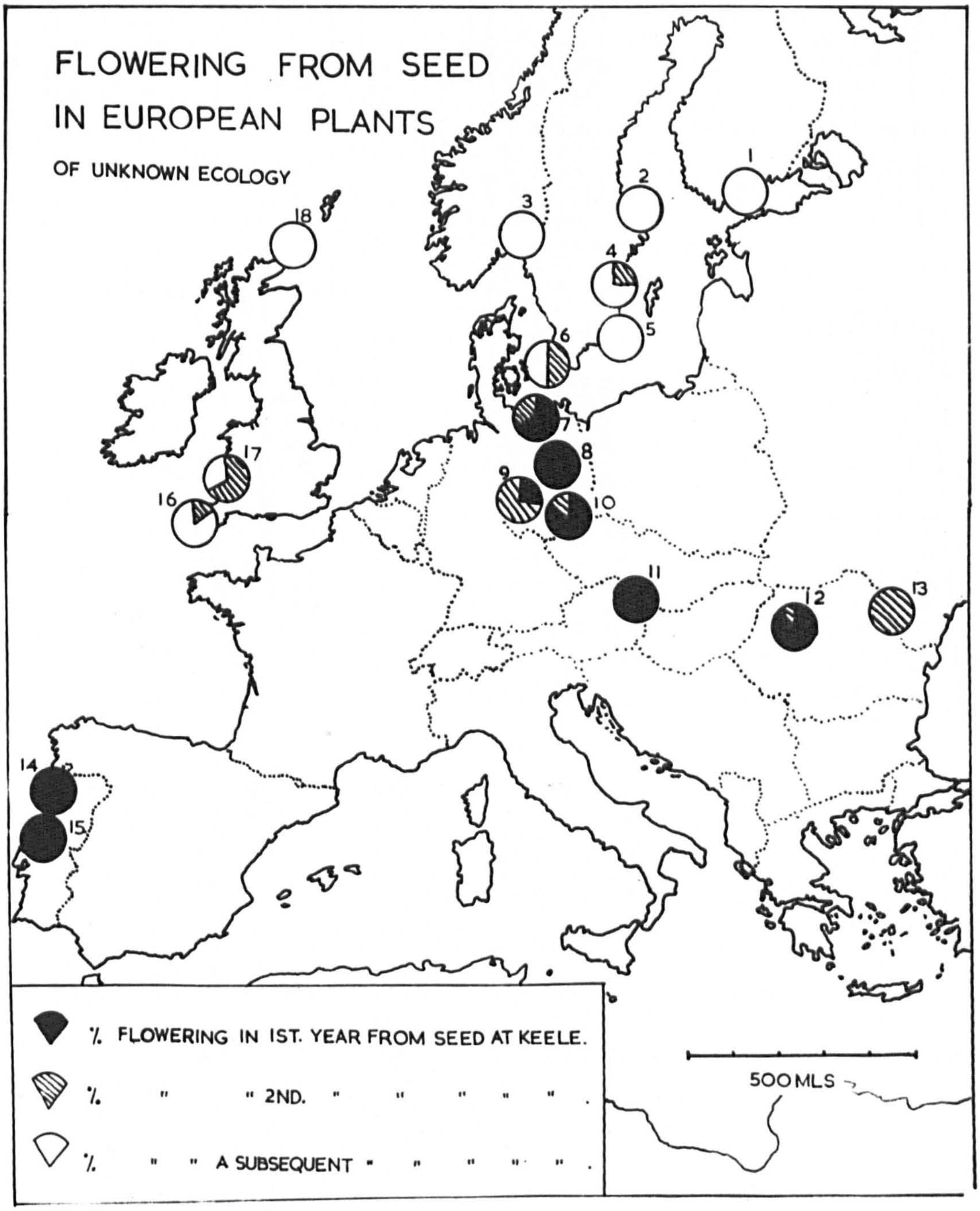


Fig. 4.7. (numbers refer to populations listed in Table 4.3).

further north and those from eastern Rumania, flowered earlier- i.e. all in their first year from fruit - as did north German inland plants in relation to those from southwest Britain).

The fruit received from Europe was locally - collected and of wild origin, and in most cases the exact details of the ecological conditions under which the seed parent was growing are not known. In consequence of this, the north/south and coastal/inland flowering cline may contain cases in which the sample is representative of a strictly local population only and thus a-typical for the region. That this may be possible is seen from the local variation, from population to population on the same marsh, revealed by cultivation of the intensively-sampled British salt marsh populations (described below). Thus the large-scale geographical variation of Fig. 4.7 is possibly superimposed on more local variation due to adaptation to local ecological conditions.

Information about the sites in N. Germany has been supplied by Dr. P. Gutte (Leipzig). The Mecklenburg site is on the north Baltic coast and estuarine, those at Bad Durrenburg and Eiselben on inland saline plains, and the Halle/Salle site is on a refuse pit from salt workings. Dr. Ranwell, who supplied the fruit, has given me details of the site in Cornwall which is in the mid-estuary of the R. Fal.

The problem of 'distortion' under the protected conditions of the greenhouse should be mentioned. A number of the plants from Portugal, Austria, Central Rumania and central Germany overwintered in the greenhouse following flowering and reflowered the subsequent year. In the wild these may be true 'annuals', dying after flowering, (but see below). The protected conditions of the greenhouse may also have enabled the autumn-sown populations to flower in the year after they were sown by allowing some vegetative growth during the early winter. The initiation of flowering stems *de novo* in autumn and late summer-germinants appears to be prevented by decreasing daylength (although mature plants already flowering may continue to flower into November and even December in the house). For this reason the early flowering of such plants in the year following germination (before August and within 12 months of sowing) has been classified as 'first year' flowering on the assumption that flowers might have been produced in the first season of growth but for the early interruption by winter conditions.

(b) British salt marsh material - Field observations.

The period of flowering in the wild on British salt marshes is from July to October, and in some upper zones may continue until the early frosts. Field observations indicate that, despite the fact they have been less recently flooded by the tide in a given year, plants in the upper salt marsh regions flower later than those lower down. Even in ecologically heterogenous or gently sloping marshes the evidence of earlier flowering as one approaches low water mark is apparent.

A quantitative measure of the relative flowering periods in Scolt Head populations was made on August 29th 1967 (1967 was a relatively late flowering year). Counts were made on Spiral (high), Beach Point and Cockle Bight (low) marshes in which plants were classified according to ten arbitrary categories of flowering grade (these categories are listed in Table 4.4). The results, based on about one hundred flowering stems per marsh, are graphed in Fig. 4.8.

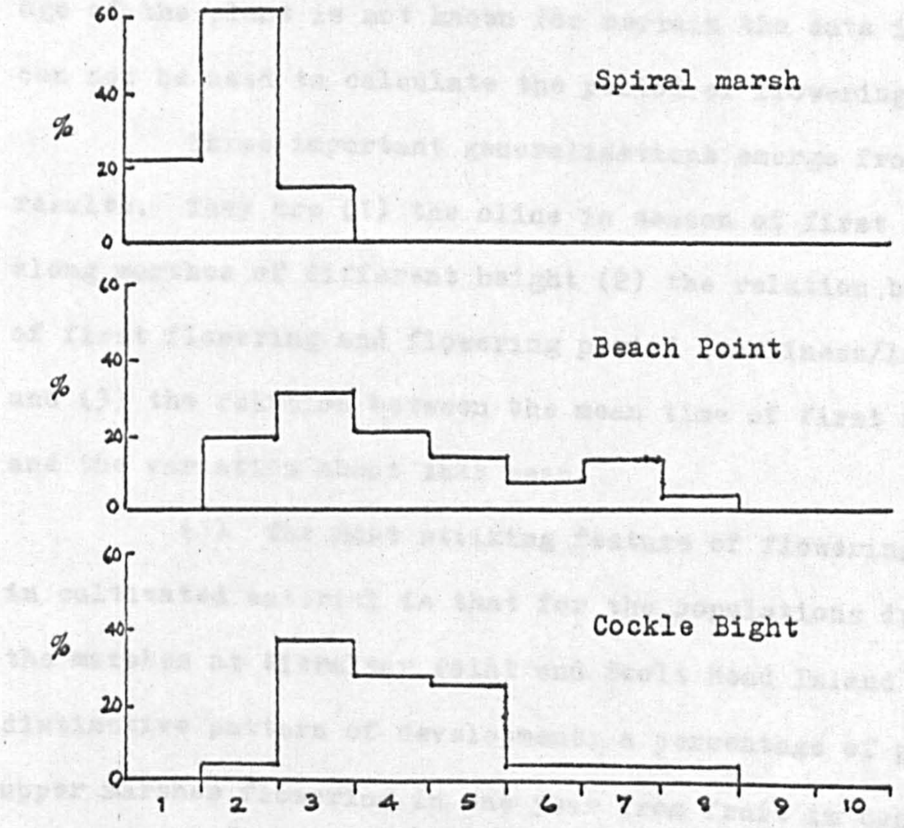
It can be seen that at a time when over 50% of the Cockle Bight plants and about 30% of the Beach Point plants are approaching the male stage and about to extrude pollen, 86% of the plants on Spiral marsh have not passed the bud stage. The remaining 14% of Spiral marsh plants are only beginning to reveal the immature disc florets at this stage. The time from the opening of the bud to the extrusion of the pollen mass is about 4 days, and to the opening of the bifid stigma a further 2 days. Thus the difference in flowering time may tend to isolate high and low marsh populations (see Ch.12). The greater variation for Beach Point plants to some extent reflects the ecological heterogeneity of that particular marsh.

(c) Cultivated material. Flowering was studied in cultivated material originally collected from the wild as either plants or fruit. Both the time taken to flower from fruit and the actual calender period of flowering were recorded.

The date of first flowering was arbitrarily defined as

Table 4.4.	Flowering grade
Class 1	flowering stem developing
2	many heads, none open
3	some heads about to open, some yellow showing
4	circa 5% of the heads showing yellow
5	circa 50% of the heads showing yellow
6	nearly all heads open, many ♂ stages
7	some heads in ♀ stage
8	many heads in ♀ stage, some red-brown in colour
9	nearly all heads red-brown, pappus hairs showing
10	mature seed easily removed from head.

Fig. 4.8. Flowering grades - Scolt Head Island 1967.



Flowering grade (August 29th 1967)

that on which the first ray ligules opened out at right angles to the pedicel. This was noted for each plant by frequent visits during the flowering period. Where periods of more than two days elapsed between visits, the date of first flowering was estimated from the current stage of flowering. The data '1st flowering index ' and 'mean date' were calculated. (See Tables 4.5 and 4.6). Flowering in a single plant normally continues in the greenhouse for a period of anything up to five weeks.

These data are summarised in Tables 4.5 and 4.6. (In the case of plants collected from the wild as plants it is not possible to say accurately whether any of these were from fruit which had germinated in the Spring prior to collection. As the age of the plant is not known for certain the data in Table 4.5 can not be used to calculate the period of flowering from fruit).

Three important generalisations emerge from these results. They are (1) the cline in season of first flowering along marshes of different height (2) the relation between season of first flowering and flowering period (earliness/lateness) and (3) the relation between the mean time of first flowering and the variation about that mean.

(1) The most striking feature of flowering behaviour in cultivated material is that for the populations drawn from the marshes at Gibraltar Point and Scolt Head Island there is a distinctive pattern of development; a percentage of plants from the upper marshes flowering in one year from fruit in contrast to

Table 4.5 Flowering in cultures collected from the wild as plants.

Source	type	code	no of plants to flower	date coll.	1st flowering from coll.		period of flowering	* fl. index	Var.	mean date*
					same yr	following yr				
Gibraltar Pt.Agr.	H	66/18	37	July 1966	24	13	Sept,Oct,Nov.	136±4.35	454.14	Oct 14th
" Lim.	H	66/19	23	"	16	7	Aug,Sept,Oct.	95.6±2.39	91.39	Sept 3rd
" Ast	M	66/20	30	"	17	13	Aug,Sept.	96.4±2.16	79.31	Sept 4th
" Spa.	L	66/21	20	"	16	4	Aug,Sept.	94.2±1.73	47.89	Sept 2nd
Scolt Head Cob	L	66/29	18	Aug. 1966	13	5	Aug,Sept.	98.1±2.71	95.49	Sept 8th
" Bch	L	66/27	15	"	12	3	Aug,Sept,Oct.	118.8±4.17	208.65	Sept26th
" Srl	H	66/28	19	"	14	5	Sept,Oct,Nov.	130.4±4.72	311.95	Oct 8th
" Plover marsh	M	66/30	3	"	3	-	Aug,Sept,Oct.	121.6±4.85	70.57	Sept29th
Anglesey A	H	66/22	12	July 1966	8	4	Aug,Sept.	85.5±3.25	84.47	Aug 24th
Anglesey B	L	66/22A	14	"	9	5	Aug,Sept.	81.0±2.39	51.41	Aug 20th
Morston Norfolk	H	65/44	3	Aug. 1965	0	3	Aug,Sept.	98.6±2.33	16.29	Sept 8th
Parkgate P1	H	66/32	5	Aug. 1966	5	-	Aug,Sept,Oct.	127.5±3.97	78.79	Oct 5th
Parkgate P4	L	66/33	5	Aug. 1966	2	-	Sept.	105.5±1.25	3.12	Sept 13th
Winsford A	-	66/23	7	July 1966	7	-	Aug,Sept.	88.4±2.07	29.99	Aug 27th
" B	-	66/24	2	"	2	-	Aug,Sept.	97.5±3.90	30.42	Sept 5th
" C	-	66/25	10	"	10	-	Aug,Sept.	86.1±4.17	173.86	Aug 25th
" D	-	66/26	5	"	5	-	Sept,Oct.	117.6±3.78	71.44	Sept 25th

* these values are comparable in all cases but Morston (collected in 1965), for their computation see foot of Table 4.6.

Table 4.6 Flowering in cultures collected from the wild as fruit

Source	code	type	no. of plants to flower	date sown	flowering from seed		flowering period	fl. index*	s^2	mean date**
					1st yr.	2nd yr.				
Gib. Pt. Agr	65/41A	H	14	Jan 1965	7	7	1st yr. Sept,Oct.	130.7±5.30	196.67	Oct 8th
							2nd yr. Apr,Sept	0.8±12.94	2177.31	June 1st
" Agr	67/13	H	13	Feb 1967	13 out of 20	-	n.r.	-	-	-
" Lim	67/14	H	2	Feb 1967	2 out of 16	-	Oct.	134.0±0.0	-	Oct 12th
" Ast	67/15	M	2	Feb 1967	2 out of 25	-	not recorded			
" Spa	67/16	L	0	Feb 1967	0 out of 10	-	" "			
Scolt Hd. Cob	67/10	L	0	Feb 1967	0 out of 10	-	" "			
" Bch	65/45A	L	6	Jan 1966	0	6	" " (competition expt)			
" Srl	67/12	H	5	Feb 1967	5 out of 23	-	Sept, Oct.	124.5±4.81	115.67	Oct 2nd
Cley Norfolk	65/46A	H	5	Jan 1965	3	2	Sept, Oct.	128.0±5.73	98.50	Oct 6th
R. Wyre Lancs	65/48A	M	7	"	0	7	June,July	16.1±4.38	134.31	June 16th
R. Lune Lancs.	65/50A	M	10	"	0	10	not recorded			
Knott End Lancs	65/49A	M	37	"	0	37	flowering in open frame			
Naze, Lancs	65/47A	H	21	"	0	21				

** N.B. 2 values relate to flowering during 1965, 2 to 1966 and 2 to 1967 and the relative dates are thus affected by seasonal differences.

* Flowering index in the population mean number of days after June 1st of first flowering in a particular year. When this value is written as an actual calender date this is referred to as "mean date".

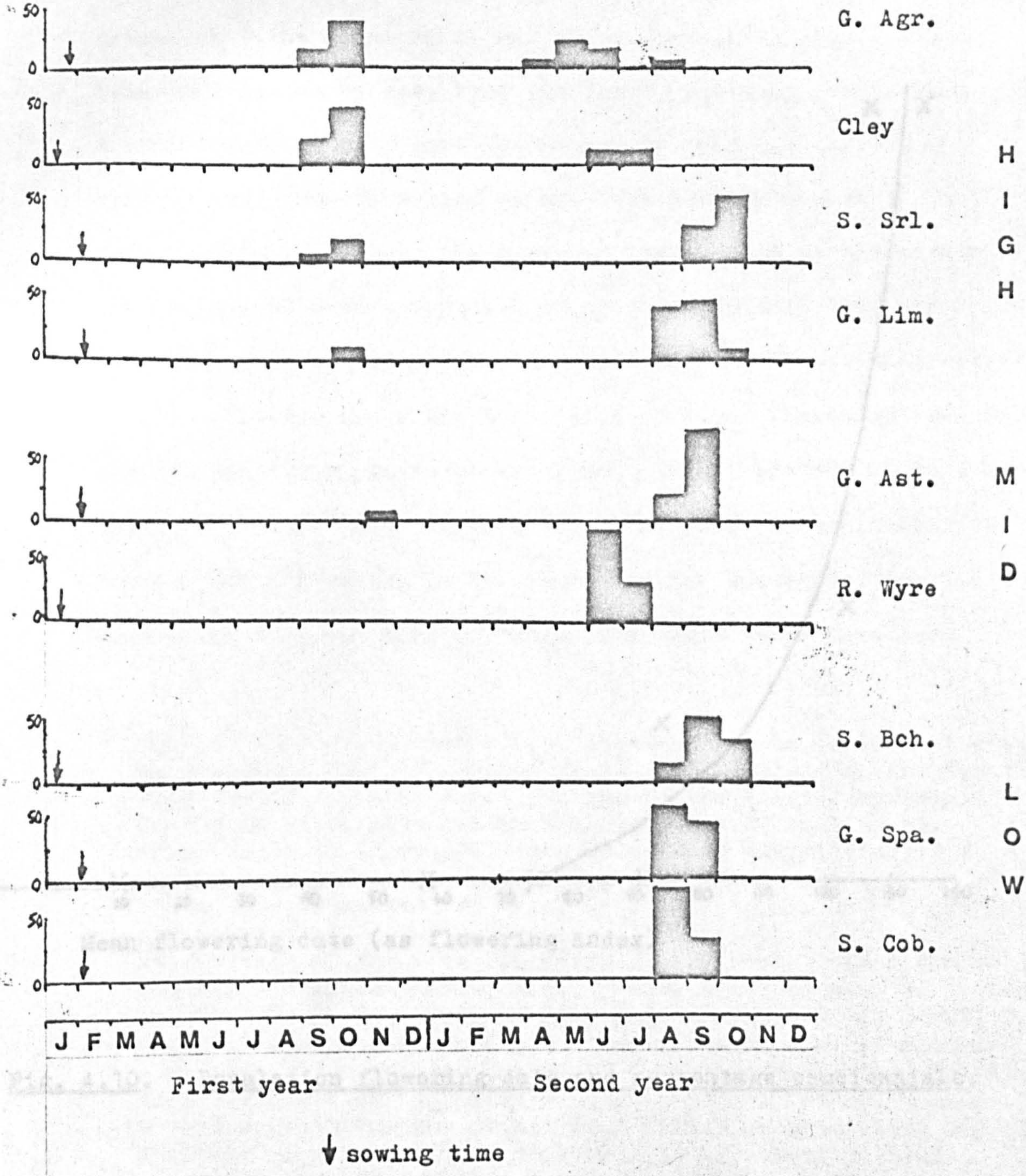
those from lower zones which largely flower in their second season (see Fig. 4.9). In those plants raised from fruit in which flowering data has been collected the fresh water population from Cley (high marsh) also contained plants which flowered in their first year, whereas none of the plants from four Lancashire marshes flowered until the second year. This feature can be seen in Fig. 4.9 (which does not include plants flowering again in a subsequent year).

This is further discussed below in relation to variation in life cycle and the data summarised in Table 4.7.

(2) The plotting of the percentage of plants in a population which flower in their first year against the average date of flowering (i.e. first flowering as expressed by the flowering index - see foot of Table 4.6) for that population reveals a further interesting feature. This plot is given as Fig. 4.10. The plants which flower in their first year from fruit have a higher flowering index than those which flower in a subsequent year. In other words first-year flowering plants on an average flower later. This may be anticipated on <u>a priori</u> physiological grounds, but is an interesting relation which can help to account for some of the variation in flowering time in populations of plants the ages of which are not known (see below).

(3) The dispersion about the mean first flowering date appears to vary in a way which suggests that populations with a high mean date (i.e. late flowerers) show more variation

Fig. 4.9. Flowering period in some salt marsh material based on cold house cultivation.



Reflowering plants are not included.

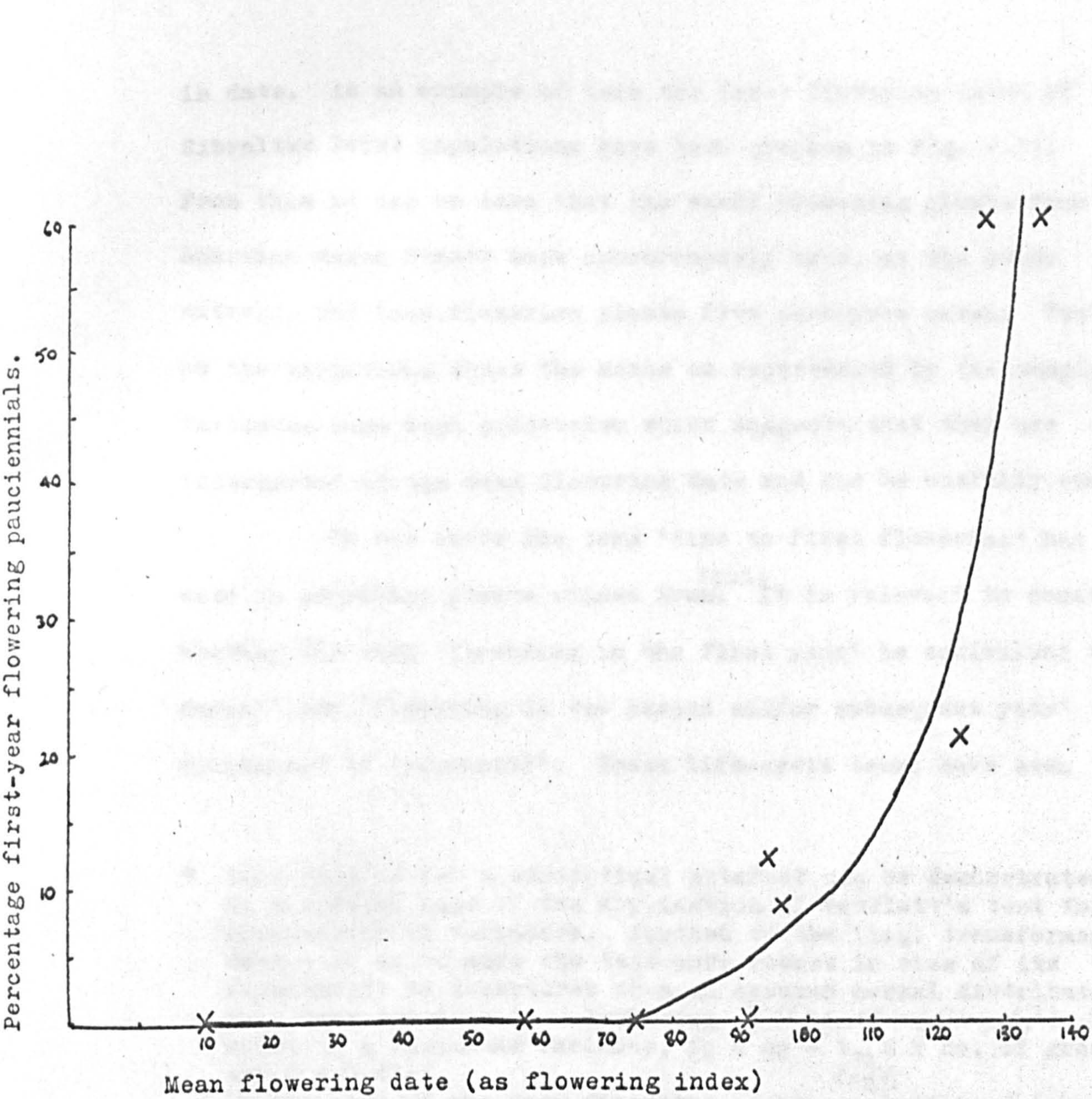


Fig. 4.10. Population flowering date and percentage pauciennials.

in date. As an example of this the first flowering dates of the Gibraltar Point populations have been graphed in Fig. 4.11. From this it can be seen that the early flowering plants from Spartina marsh flower more synchronously than, at the other extreme, the late flowering plants from Agropyron marsh. Tests on the variations about the means as represented by the sample variances have been undertaken which suggests that they are independent of the mean flowering date and can be usefully compared*.

In the above the term 'time to first flowering' has been used in comparing plants raised from fruit. It is relevant to consider whether the term 'flowering in the first year' is equivalent to 'annual', and 'flowering in the second and/or subsequent year' equivalent to 'perennial'. These life-cycle terms have been

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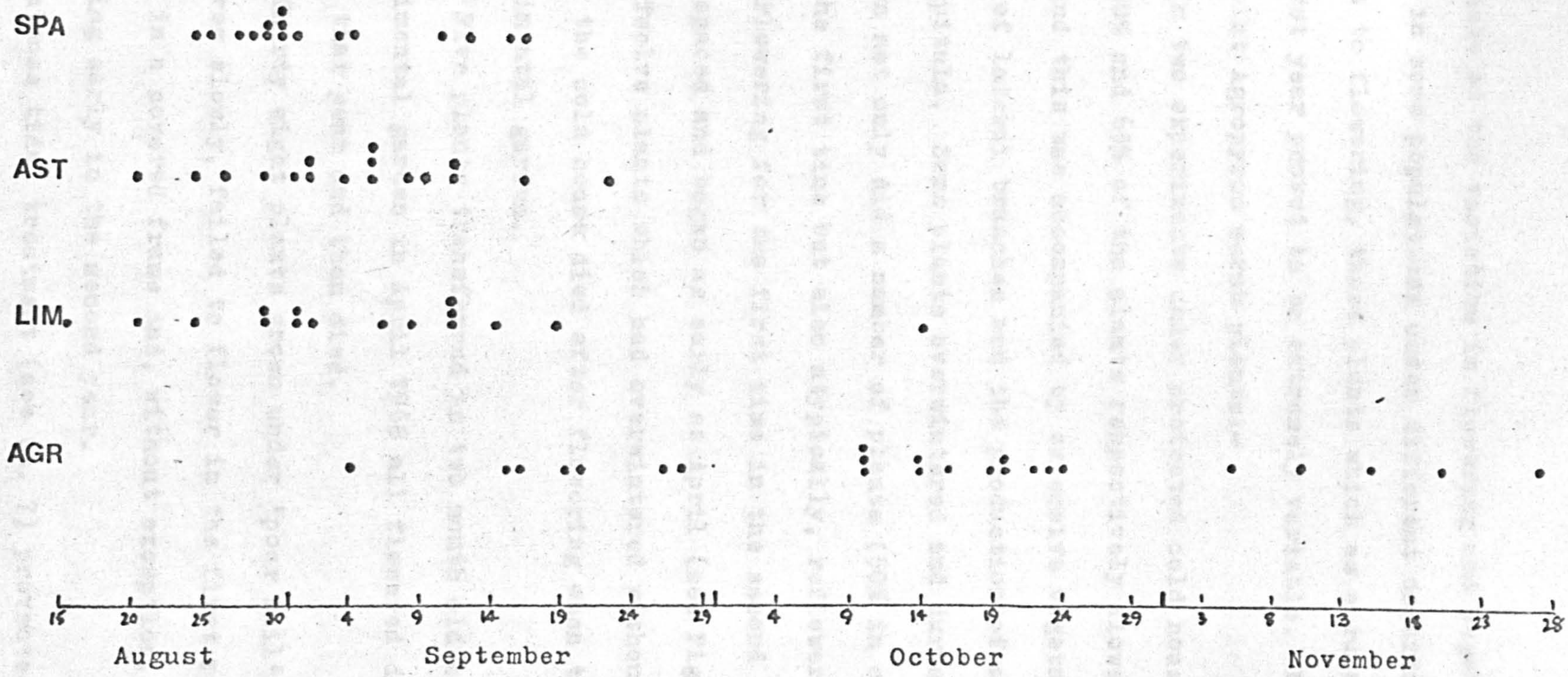
* That this is not a statistical artefact can be demonstrated by by a special case of the application of Bartlett's test for the homogeneity of variances. Applied to the (log) transformed data - so as to make the test more robust in view of its sensitivity to departures from an assumed normal distribution - this test consists in calculating $\frac{1}{C}(f \log_{10} S_i^2 - \sum f_i \log_{10} S_i^2)$ (B)
Where S_2 = estimated variance, $f_2 = n_2 - 1$, $f = \sum f_i$ k = no. of groups, and C = 0.4343
In the case of the four Gibraltar Point populations B (which is distributed approximately like χ^2 with K - 1 degrees of freedom) is equal to 36.21. This is significant at the 0.001 level, from which it can be concluded that the variances are not homogeneous. i.e. that the populations cannot be regarded as having equivalent true variances (σ^2). That sample variances are homogeneous (even though their true means might differ considerably) underlies all analyses of variance. In this case it may be disproved to point out that dispersions are significantly different.

Fig. 4.11. Earliest flowering dates, Gibraltar Point populations 1966.



avoided because of the variation in flowering and vegetative development in some populations under different conditions. With respect to flowering, those plants which as a rule flowered in their first year proved to be extremely variable. For example Gibraltar Point Agropyron marsh plants:-

(i) In two experiments under protected cold house conditions 50% and 65% of the plants respectively flowered late in the first year and this was accompanied by extensive vegetative development of lateral branches and the production of a large number of capitula. Some plants overwintered and during the second season not only did a number of plants (50% in one case) flower for the first time but also atypically, reflowering occurred in others. Flowering for the first time in the second year was irregularly spaced and began as early as April (see Fig. 4.9).

(ii) Twelve plants which had overwintered without flowering in the cold house died after flowering when transferred to the experimental garden.

(iii) Five plants transferred as two month old seedlings to the experimental garden in April 1968 all flowered during September of that year and then died.

(iv) Thirty eight plants grown under 'poor soil' conditions grew slowly, failed to flower in the first year, but overwintered in a covered frame and, without exception, died after flowering early in the second year.

(v) Intense tidal treatment (see Ch. 7) prevented all plants from developing a flowering stem in the first year. (But notice, in Ch. 7, those plants from mid and low marshes, normally subjected to tidal submergence in the wild, showed no propensity for first year flowering under tide-free cultivation).

In view of the plasticity shown by these, and other high marsh plants, their life cycle is best covered by the term 'pauciennial' introduced by Bocher *et al* (1955 p.29), to refer to all plants with a very limited duration of life and without any vegetative power of reproduction. They may be annual or biennial, and hapaxanthic (flowering only once) or not. This is in contrast to perennials in which there is obvious vegetative propagation (see below).

Table 4.7 has been drawn up with this distinction in mind and summarises the flowering behaviour under cold house conditions of those plants grown from fruit from marshes of different height.

The relationship between flowering behaviour in culture (as depicted in Table 4.7) and life-cycle in the wild needs to be considered. For example the plants from the Agropyron marsh at Gibraltar Point rarely overwinter in nature, thus behaving as true annuals. The remains of *Aster tripolium* on this marsh are difficult to find during the early spring and all the plants collected from this marsh in late spring or summer are clearly the products of that particular year's germination (this can be discerned by comparison with cultivated material of known age).

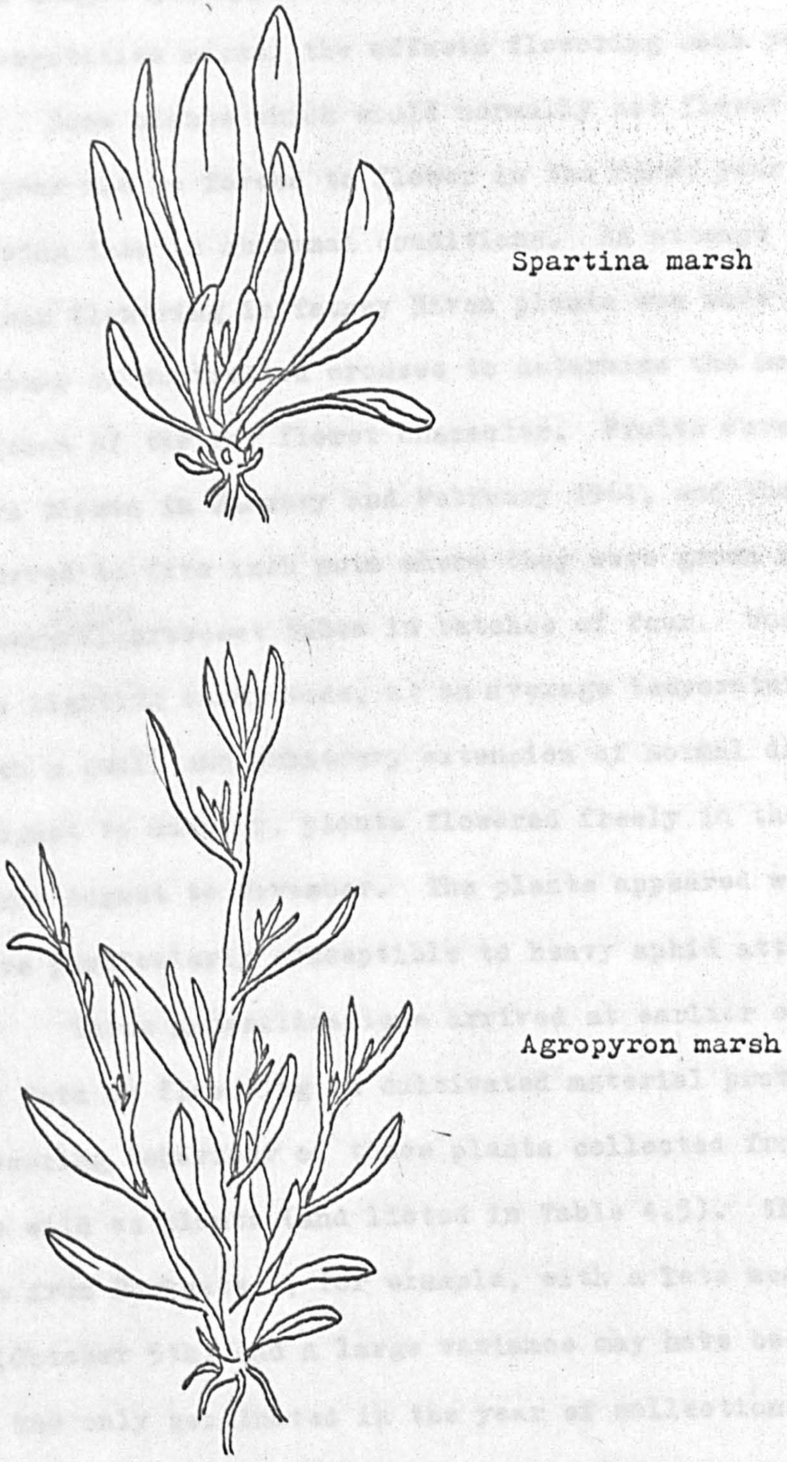
Table 4.7. Life cycle and flowering period in British salt marsh populations raised from seed under cold house conditions.

Ecological classification	Population	% first year flowering pauciennials	% perennials or second yr. pauciennials	month of main flowering
HIGH MARSH	Gib. Agropyron	50 - 60	40 - 50	Oct.
	Scolt Head Spiral marsh	21	79	Oct.
	Cley	60	40	Oct.
	Gib. Limonium	12	88	Sept.
	Naze	0	100	not recorded
MID MARSH	Gib. Aster	8	92	Sept.
	Tetney Haven	0	100	Sept.
	R. Lune	0	100	not recorded
	Knott End	0	100	not recorded
	R. Wyre	0	100	June
LOW MARSH	Scolt Head Beach Point	0	100	Sept.
	Gib. Spartina	0	100	Aug.
	Scolt Head Cockle Bight	0	100	Aug.

The large fluctuation of numbers of plants on this marsh from year to year is also indicative of an annual life cycle. The response of cultivated material in the experimental garden parallels the situation in the wild. Overwintering in the greenhouse and under poor soil regime is atypical and can be regarded as a part of the total spectrum of response in these biotypes to abnormal conditions (as can reflowering). The morphologically rather curious overwintering stages of Agropyron marsh plants carry their perennating buds several inches above the ground in a raised 'palm-tree' like rosette.

The ability of a particular plant to flower from fruit in a year is intimately related to what might be called its 'vegetative life-form', an aspect of its habit which is in most instances easier to recognise than to describe. Plants from the majority of salt marshes are characterised by an early period of vegetative growth culminating, at the end of the first season of growth, in a semi-rosette with the perennating buds held close to the soil surface in a typically hemicryptophyte condition. The degree of erectness of the overwintering rosette varies considerably as does the extent of basal thickening (see Ch.10, particularly Table 10.1, for data on growth habit variation); but whether a given plant is likely to overwinter is apparent quite early in its development. It is equally easy to recognise at an early stage those plants which are likely to flower in a single year from fruit. The latter show little lateral vegetative development or basal expansion, and the central leader soon elongates to form a flowering stem with few lateral branches. Fig. 4.12, drawings of representative 3 -month old plants from two Gibraltar Point populations, indicates the early contrast between first - and second-year flowering types. An examination of the non-tidal treatment plants in the 100 day tidal experiment described in Chapter 7 (Figs 7.1 and 7.2) underlines the ease with which the potentially annual types, in this case in the Agropyron and Spiral marsh populations, can be picked out. This is generally true of plants in the wild.

In contrast to the plasticity of pauciennial types, described above, flowering and reflowering in the low and mid marsh perennial types is much less variable or subject to changes in cultivation conditions. Plants collected as plants from Tetney Haven, for example have been retained for five years in culture, reflowering each year. Axillary buds on the stocky rhizome develop early in the growing season and form offsets which eventually become separated by the decay of the old rhizome. (It has been suggested (Clapham et al 1942) that natural propagation occurs by the detachment of the buds in the axils of radical leaves, although I have found no evidence to confirm this. Propagules from this source have been used artificially to obtain clonal material). Flowering in such perennial types is undisturbed by transfer to the experimental garden or to poor soil (although they may be affected in other ways - see later chapters) and in


Spartina marsh

Agropyron marsh

Fig. 4.12. Three-month old plants from two Gibraltar Point populations (based on photographs).

the wild single genotypes may spread over considerable areas by purely vegetative means, the offsets flowering each year.

Some plants which would normally not flower until their second year can be forced to flower in the first year from fruit by exposing them to abnormal conditions. An attempt to induce first year flowering in Tetney Haven plants was made as part of a programme of controlled crosses to determine the mode of inheritance of the ray floret character. Fruits were germinated in petri dishes in January and February 1964, and the seedlings transferred to five inch pots where they were grown under 4'40W MCF/U warm white fluorescent tubes in batches of four. Under these intense lighting conditions, at an average temperature of 55°F, and with a small and arbitrary extension of normal day-length from August to October, plants flowered freely in their first year from August to November. The plants appeared weak and spindly and were particularly susceptible to heavy aphid attack.

Three generalisations arrived at earlier on the basis of the data on flowering in cultivated material provide an indication of flowering behaviour of those plants collected from populations in the wild as plants (and listed in Table 4.5). The high marsh plants from Parkgate 1, for example, with a late mean flowering date (October 5th) and a large variance may have been plants which had only germinated in the year of collection, (their vegetative appearance also suggests this). On the other hand the plants from P4 (low marsh) flowered earlier and together (only 2 plants flowered from this population and the number is too small to draw a valid conclusion). The Anglesey A (high) population flowered on average slightly later and with a greater overall variation in first-flowering time than those from the ecologically less mature Anglesey B (low) site. The plants from N. Lancashire and Morston, ecologically similar marshes, showed early flowering and low variance, factors suggestive of a prior season of vegetative growth. It is worth noting that, in view of the powers of vegetative reproduction in such plants, synchronous flowering may be a product of, or reinforced by, genotypic uniformity over considerable areas.

The Winsford populations, too, show an interesting pattern of variation with respect to these characters. Plants from Winsford D, which have the vegetative appearance of pauciennials, flowered late and showed large variation in date. Plants from Winsford C reflect the heterogeneity of the habitat and could consist of a mixture of types, whereas plants from Winsford A are relatively early flowering, a fact consistent with their considerable vegetative growth.

Two East German populations showed a pattern of flowering consistent with that described for British material. The sample from the inland site at Bad Durrenburg (culture number 65/53) had a mean first flowering date at September 6th (98.101 ± 3.06)* in contrast to the estuarine Mecklenburg population (culture number 65/54) with a mean at August 15th (76.501 ± 2.87).

* Footnote next page.

Var. *pannonicus* from Vienna shows the wide variation in first flowering time of pauciennial types, although it is relatively early flowering in the greenhouse at Keele (fl. index = 103.85± 7.69, Sept. 11th) as is var. *longicaulis* from Portugal (mean date not recorded, but around late August of the first year).

Flowering time in British salt marsh material as observed in the field and described earlier exhibits a pattern of variation which can be explained in terms of the variation in life cycle of plants at different marsh heights. The contrast between pauciennial types, which in the case of Agropyron marsh and probably other high marshes are hapaxanthic and annual in nature, and perennial types, accounts for much of this. The mid and low marsh plants, having spent a prior season in a 'vegetative reinforcement stage' (Bocher *et al*, 1955), approach the flowering state earlier than those in the high marsh whose development, however rapid, is directly from fruit. The morphological appearance of the plants from these respective marshes in early summer is consistent with this observation. It would clearly be of value to observe the fate of individual seedlings in the wild on a number of marshes. (This has been done on the Spartina marsh at Gibraltar Point in relation to seedling elimination (see Chapter9),

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* As conventions in the literature appear to vary, it should be pointed out that the symbol '±' in the present thesis is followed, unless otherwise stated, by the standard error of the sample, defined as standard deviation divided by root n. ($\frac{s}{\sqrt{n}}$).

but the fate of the plants was not followed through to flowering). The fruit collected for comparative cultivation was, in all cases, the product of open, natural, pollination and thus, for example in high marsh areas, those plants which fail to flower in their first year may be eliminated in nature, whereas the protected cultivation conditions enabled them to survive without propogation until flowering occurred in the second year. Equally the small number of first year flowering types detected in the mid marsh population, Gib. Ast, may be eventually eliminated in the wild. This consideration, together with examination of the possible adaptive significance of the annual life habit in this species is returned to the Discussion.

CHAPTER 5. FRUIT VARIATION.

The 'seeds' of *Aster tripolium* are strictly monospermous fruits aggregated into heads. Such heads may be regarded, from the biological and evolutionary point of view, as single units or the equivalent of many seeded fruits. Zohary's discussion of the evolutionary trends in the fruiting head of the *Compositae* as a whole starts from essentially this viewpoint (Zohary 1950). Variation in the fruiting head of *A. tripolium*, studied mainly in British salt marsh populations, is described in this chapter under two main heads:- I, the polymorphism of ray and disc fruits, and II, variation in fruit production (including fruit size and number). III discusses the general implications of the results.

I Polymorphism.

The most obvious aspect of variation in the fruiting head is the contrast in size between the fruits produced by the ray and disc florets respectively. On any single head the ray fruits are generally about two thirds the size of the disc fruits. This is even true of the populations with extremely small disc fruits such as the Agropyron marsh population. (Size and weight are correlated factors - see below). With minor exceptions it is possible to pick out the fruits formed by ray and disc florets on individual heads even when the floral structures have decayed. (This was most difficult in the lower Parkgate populations P3 and P4).

Table 5.1 contains the summarised data of the mean weights of the two types of fruit in samples from six populations of rayed plants. Ten heads from each population (at random and sometimes more than one head from a single plant) were dissected, the ray and disc fruits counted and weighed separately and the mean weights of theray and disc fruits calculated for each head. (Significance was assessed by calculating Student's t for each sample pair, against the null hypothesis that the ray and disc fruit sample means were of equal weight). In every case the pairs of samples of ten heads were significantly different, the ray fruits being of significantly less weight.

Table 5.1.	Weights of ray and disc fruits. Sample size 10 heads/population.		
Population.	mean wt. ray fruits (mgms)	mean wt. disc fruits(mgms)	level of significance.
Gib. Pt. Agropyron	0.401±0.1	0.583±0.2	0.001
Scolt.Spiral Marsh	0.392±0.1	0.710±0.1	0.001
R. Lune Lancs.	1.296±0.6	1.904±0.8	0.001
Parkgate. P2	1.431±0.6	1.542±0.9	0.02
var. longicaulis. Portugal.	0.806±0.6	0.931±0.5	0.01
Cley, Norfolk	0.524±0.4	0.750±0.2	0.001

Apart from their overall size differences no other observable structual differences exist between the ray and disc florets. Pappus hair length, which varies from population to population (see below) does not vary in ray and disc fruits of the same head. Ray and disc fruits on the same head do not differ in their dormancy-breaking requirements although there is considerable variation in this respect from population to population (see Ch.6). The difference between the two types in germination rate is comparable to that which exists between small and large disc fruits (see experiments described in Chs. 6 and 8).

The production of two or more different types of seed by the same parent plant is a feature of higher plants which has received some attention in the past. Salisbury (1942) in describing a number of species with di- and tri-morphic fruits and seeds reserved the terms 'heteromorphism' or 'polymorphism' for such cases. More recently the interest in the phenomenon revived by the work of Harper and others on the autecology of 'weedy' species has, possibly through the emphasis of this work on the seed 'population', led to an extension of the term 'polymorphism' to cover cases in which the two morphs are produced by different plants (usually in the same population). Polymorphism in this wider sense is particularly common in, and seems to be largely restricted to, a group of the so-called 'weedy' families - the <u>Chenopodiaceae</u>, <u>Leguminosae</u>, <u>Cruciferae</u>, <u>Compositae</u> and <u>Gramineae</u> - a fact to which considerable significance has been attached by ecologists (Harper 1965).

The composite inflorescence, particularly in the heterogamous condition, lends itself ideally to dimorphism in the strict

Salisburyian sense, the variation commonly being maternal with the ray fruits differing from the disc fruits in size, structure and/or germination properties or requirements. The genus Dimorphotheca owes its name to this feature, the larger ray fruits germinating more rapidly than the smaller disc fruits in D. hybrida, and the larger disc fruits germinating more rapidly than the wrinkled marginal ray fruits in D. pluvialis (Becker on D. hybrida, Correns on D. pluvialis-cited by Salisbury 1942). Differences of a similar order between ray and disc fruits on the same head have been reported in Synedrella nodiflora (Purseglove in Harper 1965 p. 266) Galinosoga parviflora, Gutierrezia gymnospermoides, Hypochoeris glabra, Bidens cernua and B. triparta (cited by Salisbury 1942), Calendula species (Zohary cited by Harper 1965) and a large number of annual species of Crepis (Stebbins in Harper 1965 p. 267).

In addition to dimorphism of the ray and disc fruits the two types may themselves vary (in the same capitulum). Zohary (1950), for example, describes a gradual reduction of the Pappus from the centre towards the margin of the head in the closed form of Hedypnois cretica, and cites other examples of what he calls 'heterocarpy' in the Compositae. In Aster tripolium the variation in disc fruit size and structure both within and between heads on the same plant was characteristically small. Although no extensive measurements were made, repeated handling of the disc fruits for germination and other experiments suggested that the position of the head on the plant had no influence on the size of the disc fruits. By contrast the variation in disc fruit size between plants, particularly those from different populations, was considerable. This variation is described under II (b) below.

II Variation in fruit production.

Data have been collected on the reproductive capacity of plants, under cultivation and in the wild as measured by (a) the number of fruiting heads per plant, (b) the size and (c) the number of fruits per head.

(a) The number of heads per plant. Counts were made on samples of the populations under cultivation of the total number of fruiting heads borne by individual plants. These data are summarised in Table 5.2 and 5.3. Table 5.2 enables a comparison

Table 5.2	Number of heads/plant. Wild collected plants under cold house conditions.			
Source	Code	No. plants counted	mean no. heads.	range
Scolt Hd. Srl.	66/28	14	7. 9± 0.36	2- 18
" Bch.	66/27	10	48. 5± 11.27	6-184
" Cob.	66/29	13	42. 4± 13.56	10-196
Gib. Pt. Agr.	66/18	19	34. 1± 4.19	15- 88
" Lim.	66/19	16	72.56± 11.33	14-197
" Ast.	66/20	17	64.23± 7.15	10-124
" Spa.	66/21	14	81.35± 9.27	43-192
Anglesey A.	66/22	8	22.62± 7.07	5- 70
" B.	66/22A	8	17.25± 4.03	2-44
Winsford A.	66/23	7	15. 4± 4.04	4-32
" B.	66/24	2	12. 5± 3.91	7-18
" C.	66/25	9	100.4± 52.77	15-544
" D.	66/26	5	37. 2± 37.22	15- 88

to be made of the performance of those British populations collected from the wild as plants and grown under the standard cold house conditions. Table 5.3 summarises the performance of three populations under a range of environments. Additional data on plants grown from fruit and foreign material are included in the text.

Two immediate conclusions are evident. The first is that there is considerable variation in fruiting head production both within and between populations grown under similar conditions. The second is that, in those populations listed, this production is strongly influenced by the conditions under which the plants were grown.

The plants collected in the wild as plants prior to flowering and then transferred to the cold house produced, in the three populations counted, a similar number of heads to their counterparts in the wild. This suggests either that the cold house conditions have a similar effect on head production to natural ones, or that the number of heads produced by plants collected from the wild is to a large extent determined by the prior vegetative growth of these plants. Much of this growth occurred under markedly different conditions from those of the protected, warmer, greenhouse. However in the one case of plants collected from the wild as fruit in which head number was counted after growth in cold house conditions (Gib. Agr.) the number is similar

Table 5.3. Number of heads/plant under varying conditions.

		Gib. Agr.			Scolt Srl.			Bch. Pt.		
Collected	cultivation conditions	no plants counted	mean	range	no.	mean	range	no	mean	range
as plants	cold house	19	34.1 ± 4.19	15-88	14	7.9±0.36	2-18	10	48.5±11.27	6-189
as fruit	cold house (1st year)	6	36.26± 5.17	18.56						
"	cold house (2nd year)	5	231.8±22.47	187.312						
	wild	25	31.12± 7.72	8-79	10	5.3±2.24	2-26	10	56.6±12.13	20-100
as fruit	poor soil	20	11.5 ± 1.67	3-31						
as fruit	botanic garden	-	——	—	6	61.7±8.36	12-101			
as plants	open frame	-	——	—	-			10	14.0± 2.16	9-18

to that in the wild - at least in 1st year flowerers.

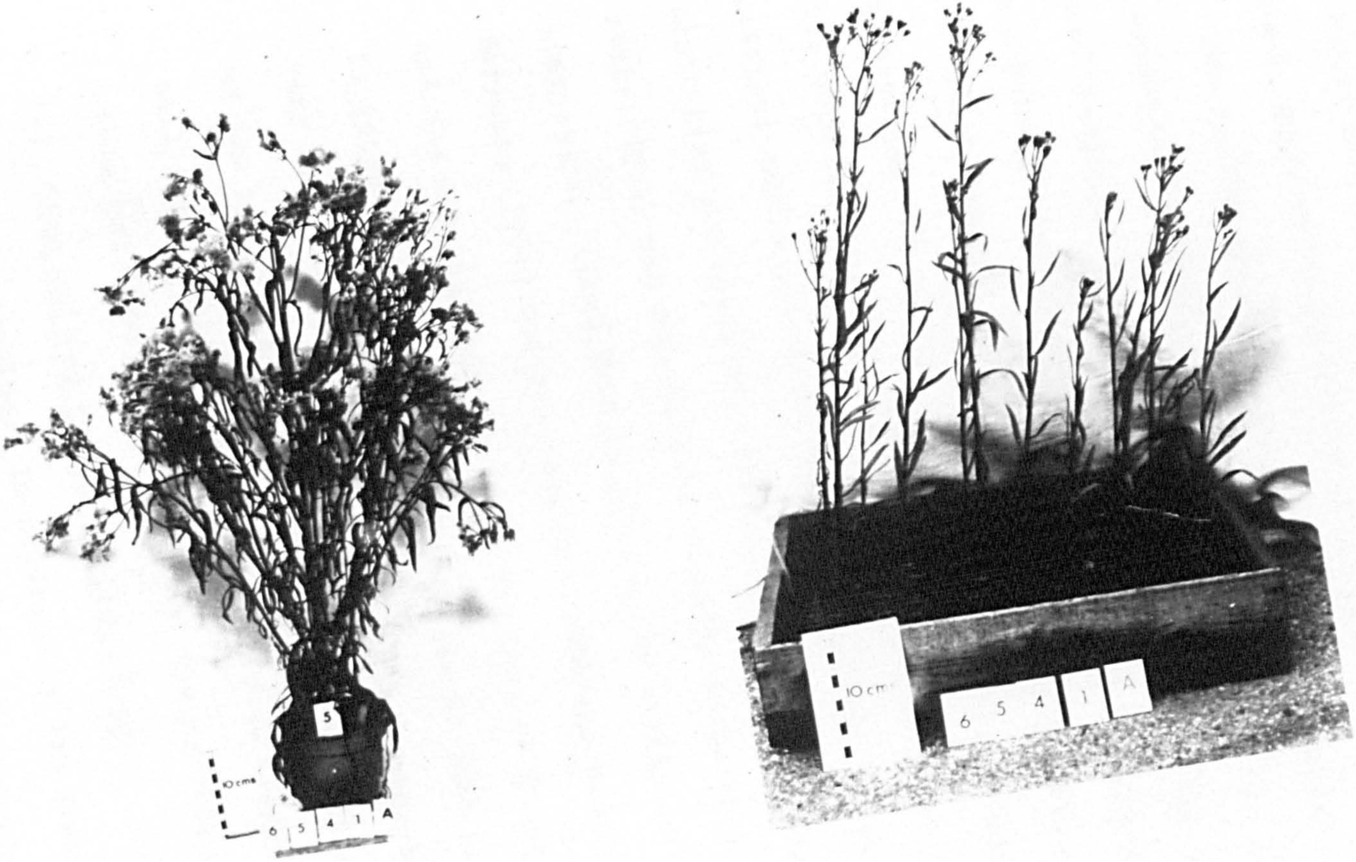
In general, populations at Gibraltar Point and Scolt Head containing pauciennial biotypes produce fewer heads per plant in comparable cultivation under greenhouse conditions than those containing perennials. (This with the exception of second-year flowering Agropyron marsh plants-see below). This is only true within geographical areas however (i.e. the Gibraltar Point series and the Scolt series). Plants from Cley (high marsh) had a mean head number of 76.66±13.48 with a range 44-97, and plants from the R. Wyre (mid marsh) collection yielded a mean of 99.83±6.64, range 82-128, heads/plant.

Of the two East German populations raised from fruit the plants from the inland site at Bad Durrenburg produced on average more heads than those from the estuarine site at Macklenburg (96.1±14.55, range 44-186, as opposed to 84.5±12.24, range 50-143). The var pannonicus from Vienna produced 65.3±4.49 heads/plant (range 43-139).

The effect of different treatments on plants from Gibraltar Point Agropyron Marsh reveals a wide range of plastic response in head production. Although clonal material was not available fruit from the same collection produced, with 'poor soil' treatment, plants with a significantly lower number of heads than those grown under cold house conditions (when compared with plants which flowered in their first year from fruit in the cold house t = 7.32 with 24 degrees of freedom, making p considerably less than 0.001). Those plants grown from fruit which first flowered in their second year bore very much larger number of heads (231.8±22.47). Plasticity of fruiting head number is not confined to pauciennial biotypes, as the decreased production of heads in open-frame grown Beach Pt. plants indicates. Nor is its direction obvious as the very much increased production of heads in Spiral marsh (high) plants grown in the botanic garden as opposed to the cold house shows. (These plants flowered the year after collection as plants, having been transplanted from pots to the experimental plot 3 weeks after collection.)

Fig 5.1 indicates the scale of this plastic response in Agropyron marsh (high) plants. The photographs were taken on the same day of (A) a representative plant of the second year flowering cold house material and (B) one of three trays of plants grown under 'poor soil' conditions.

As can be seen from Fig. 5.1 the parameter 'number of fruiting heads/plant' is a direct function of the amount of vegetative development as measured by the degree of lateral branching. The development of such branches in the axils of radical leaves produces the large number of heads in the ultimately cymose arrangement of the capitula. A reduction of the amount of branching causes a reduction in the number of heads. The variation with respect to such branching is discussed in Chapter 10. It seems that adverse environmental factors (which soil of low



A
GREENHOUSE

B
POOR SOIL

Fig. 5.1. (See text)

nutrient status coupled with infraspecific competition presumably is) produce a response in terms of a reduction in the amount of branching and thus in the number of fruiting heads.

The factors which may effect a growth character such as the amount of branching are numerous and complex, as are the plants' responses to them. Both the factors and the responses are little understood. Sufficient is known however from the work of agronomists and others to demand that caution be used in assessing the results of experiments in which factors such as soil moisture, density of sowing, spacing of pots, time of flowering, nutrient status and so on, have not been carefully isolated, controlled and measured. Only in such cases can one compare the scale, type, and direction of response in particularly plastic characters. (That this is possible is evidenced by work of Sorenson (1954) who was able to equate the nature of the reponse and the original habitat in populations of Capsella bursa-pastoris). In Aster tripolium a plastic response in head production with a very wide range of expressions has been demonstrated and some of the possible directions of this response indicated (to poor soil treatment). It is not possible to describe the between-population variation in response from the results obtained so far.

(b) Size and (c) number of fruit/head.

(i) Results. In contrast to the number of heads per plant the parameters 'mean fruit size' and 'number of fruits per head' show remarkably low plasticity.

Variation in the size (as determined by weight) and number of fruits per head has been examined in British salt marsh material. Three areas, Gibraltar Point, Scolt Head Island and Parkgate were sampled in the wild after flowering. Cultivated material from the populations at two of these, Gibraltar Point and Scolt Head, was also measured for the characters fruit size and number.

Flowering stems were collected randomly in the field (but some distance apart) at the various sites and a single head picked off at random from each stem. Heads were similarly taken at random from cultivated material. The heads were dissected and the total number of fruits, both ray and disc, were recorded, together with the number of these which appeared empty and flat (and thus unlikely to germinate - see later). A random sample of ten viable-looking disc fruits from each head was then weighed (on an Oertling 4 - figure balance accurate to 0.001 gms). Table 5.4 summarises the results of these measurements and the plot no. of fruits/head v. fruit weight is given in Fig.5.2 (Gibraltar Point populations) and Fig.5.3 (Scolt Head populations).

It can be seen from these that the character 'fruit weight' in particular has a within-population stability under the range of cultivation conditions tested. The number of fruits/head varies rather more*. In Scolt Head populations the variation in fruit number is greatest in the Beach Point sample and in Gibraltar

* Footnote next page.

Point populations it is greatest in Aster marsh populations (not counting poor soil conditions in Gib. Agr.). In five out of seven samples the effect of growth under cold house conditions was to increase slightly the number of fruits/head. The very low plasticity of fruit weight is discussed later.

The data relating to wild collected material are set out

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* This interpretation requires further explanation. The term 'stability' being essentially relative has to be applied subjectively. Fruit weight varies considerably within a given population (see Figs. 5.4, 5.5, and 5.6), despite the cline in mean value between populations in a given area. The fact that the mean values of the populations grown under different conditions do not, in any one case, overlap the mean values of another population (Figs. 5.2 and 5.3) has been taken as a measure of the 'stability' of the character fruit weight within a given population. This is open to argument as in one case the mean fruit weight of the population is increased by as much as 19% (Gib. Agr.) - the general increase varies from 1 - 10%. It would clearly be of considerable value to cultivate clonal material under a range of conditions and to measure 'stability' within populations along an axis of variation 'from 'unchanged' to 'increased by such-and-such an amount'.

Fruit number is less clearly canalised than fruit weight and, although the variation in mean value is nowhere greater than about 2%, this is sufficient to produce an overlap of the population means.

This dilemma underlines the major difficulty in using population means (rather than variation in ramets) as a measure of plasticity and it is hoped at a later date to present a more critical appraisal of fruit size stability using trials from selected pure lines. For the present it should be pointed out that the fact that dispersion has been ignored and that there are certain difficulties of interpretation, mean that the terms 'stability' or 'low plasticity' relate to the total pattern of variation in the character being thus described, and have a subjective element.

Table 5.4 Number of fruits/head and mean wt. of 10 disc fruits/head in wild and cultivated material.

Population	code	treatment	no heads in sample ‡	no fruit/ head	wt. 10 fruits/head (mgms.)
Gib. Agr.	-	wild	65	41.5±1.1	5.29±3.03
"	66/18	house	10	40.2±4.5	6.32±7.8
"	66/41A*	poor soil	10	38.8±7.8	5.17±9.1
"	"	botanic gdn	4	43.5±3.1	5.80±3.0
Gib. Lim.	-	wild	103	33.8±0.9	8.89±3.82
"	66/19	house	11	38.3±2.0	9.01±2.2
Gib. Ast.	-	wild	80	26.1±0.9	11.49±6.00
"	66/20	house	9	29.9±1.9	11.03±10.3
Gib. Spa.	-	wild	74	26,0±0.6	14.07±5.09
"	66/21	house	10	26.5±2.9	15.75±6.4
Scolt Srl.	-	wild	50	23.9±0.9	6.43±3.17
"	66/28	house	10	27.4±2.3	7.02±5.2
"	66/28	frame	6	25.2±1.4	6.67±6.8
Scolt Bch.	-	wild	50	20.5±0.6	13.79±7.49
"	66/27	house	10	24.8±1.5	14.92±3.7
"	66/45A*	Botanic gdn	4	27.4±0.5	13.95±2.8
Scolt Cob.	-	wild	50	22.7±0.7	15.62±4.20
"	66/29	house	10	20.5±7.6	16.81±6.2

* grown from fruit

‡ 1 head from each of n plants.

in Table 5.5. Histograms based on the raw data of fruit weight in the three areas are given as Fig. 5.4, 5.5, and 5.6. Figs. 5.7, 5.8, and 5.9 are photographs of representative samples of 10 fruits (not in each case from a single head)from each of the eleven populations, the total sample weights of which are close to the mean value (weight of 10 disc fruits/head) for each population. The photographs are of Gibraltar Point (5.7), Scolt Head (5.8) and Parkgate (5.9) populations respectively.

At all three sites there is a clear clinal variation in mean (disc) fruit weight, plants from the high marsh populations producing smaller fruits than those from the lower marshes. Further, in the 3 areas high marsh populations (Agr. P1. Srl. Lim.) have smaller fruits than mid marsh (Ast.) which are smaller than low marsh (P2, Bch., P3, P4, Spa., Cob.). If the populations from the two east coast areas are ranked in order of ecological maturity (as determined by height, tidal relations and associated vegetation - see Table 3.2) there is an exact correspondence with their mean fruit weight (Viz - in order high to low marsh Gib. Agr. (5.29 mgms), Scolt Srl. (6.43 mgms), Gib. Lim. (8.89 mgms), Gib. Ast. (11.49 mgms), Scolt Bch. (13.79 mgms), Gib. Spa. (14.07 mgms) and Scolt Cob. (15.62 mgms). The Parkgate marsh is more difficult to compare ecologically with the other two, (and does not contain the ray-less form (Chapter 11)).

The sample dispersion of fruit weight, as for some other characters, reflects the ecological heterogeneity of the marsh

Table 5.5 Data Summary. Fruit Weight and Number (wild collected material).

Population	no. heads examined	number fruits/head			level of p *	weight ten disc fruits/ head.			level of p. *
		$\bar{x}$	S^2	st.e.		$\bar{x}$(mgs.)	S^2	st.e.	
<u>Gib. Pt.</u>									
Agropyron marsh	65	41.5	73.92	1.1	0.001	5.29	60.02	3.03	0.001
Limonium "	103	33.8	86.56	0.9		8.89	150.49	3.82	
Aster "	80	26.1	64.19	0.9		11.49	288.19	6.00	
Spartina "	74	26.0	25.89	0.6		14.07	192.10	5.09	
<u>Scolt Head</u>									
Spiral marsh	50	23.9	44.93	0.9	0.1	6.43	50.42	3.17	0.001
Beach Point	50	20.5	15.16	0.6		13.79	281.01	7.49	
Upper Cockle Bight	50	22.7	21.76	0.7		15.62	88.45	4.20	
<u>Parkgate</u> P1	25	36.4	39.71	1.3	0.2	9.34	115.57	6.79	0.001
P2	25	38.8	33.54	1.2		17.56	189.73	8.71	
P3	25	37.9	53.11	1.5		20.83	203.18	9.01	
P4	25	42.1	34.11	1.2		24.44	274.91	10.48	

* Based on an analysis of variance (in the case of Gibraltar Point populations on a sub-sample of 50 heads/population).

Each level of p is calculated on difference between populations in one area.

Fig. 5.2 Mean disc fruit weight and number - Gibraltar Point populations.

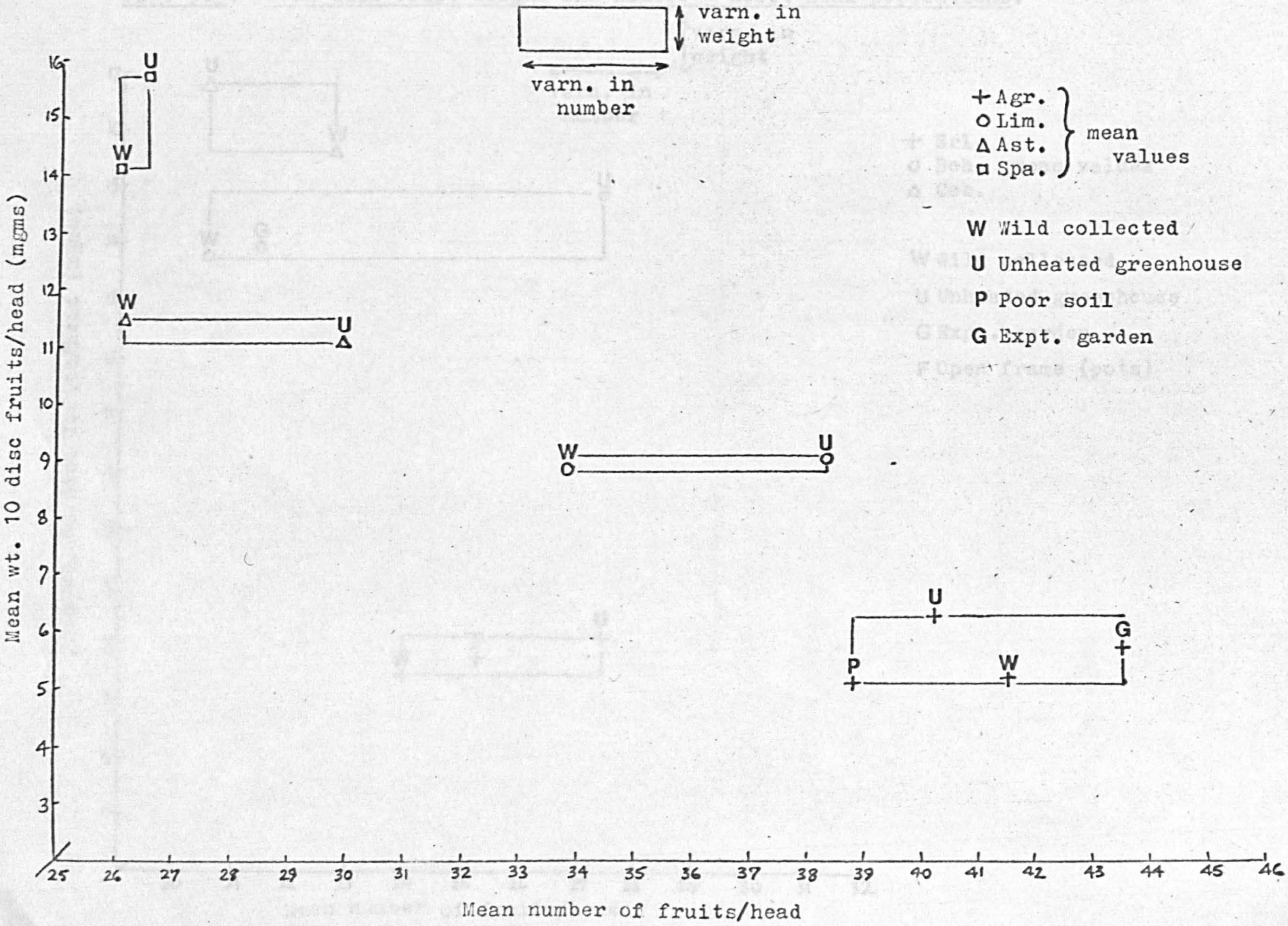


Fig. 5.3. Mean disc fruit weight and number - Scolt Head populations.

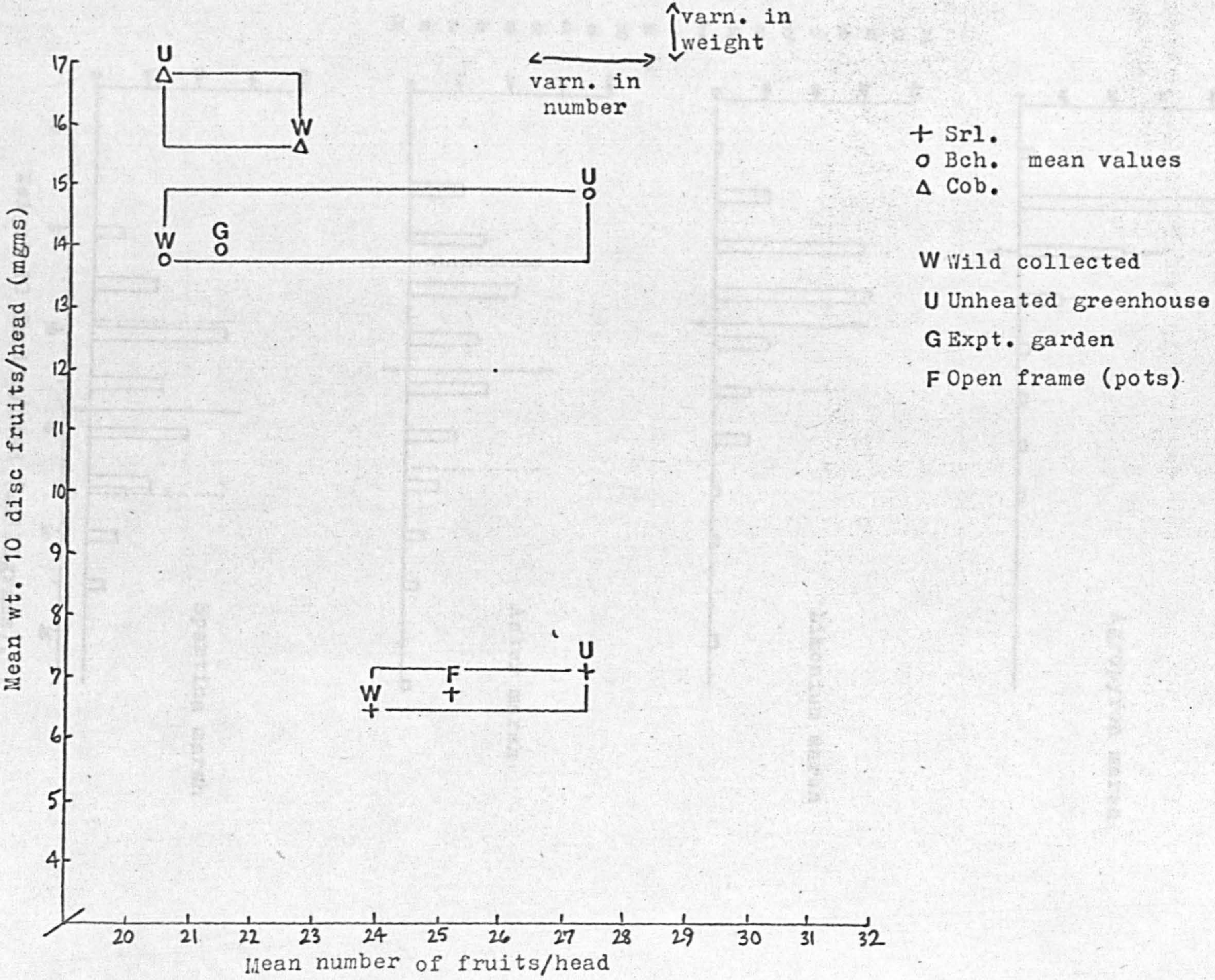


Fig. 5.4. Weights of disc fruits - Gibraltar Point populations.

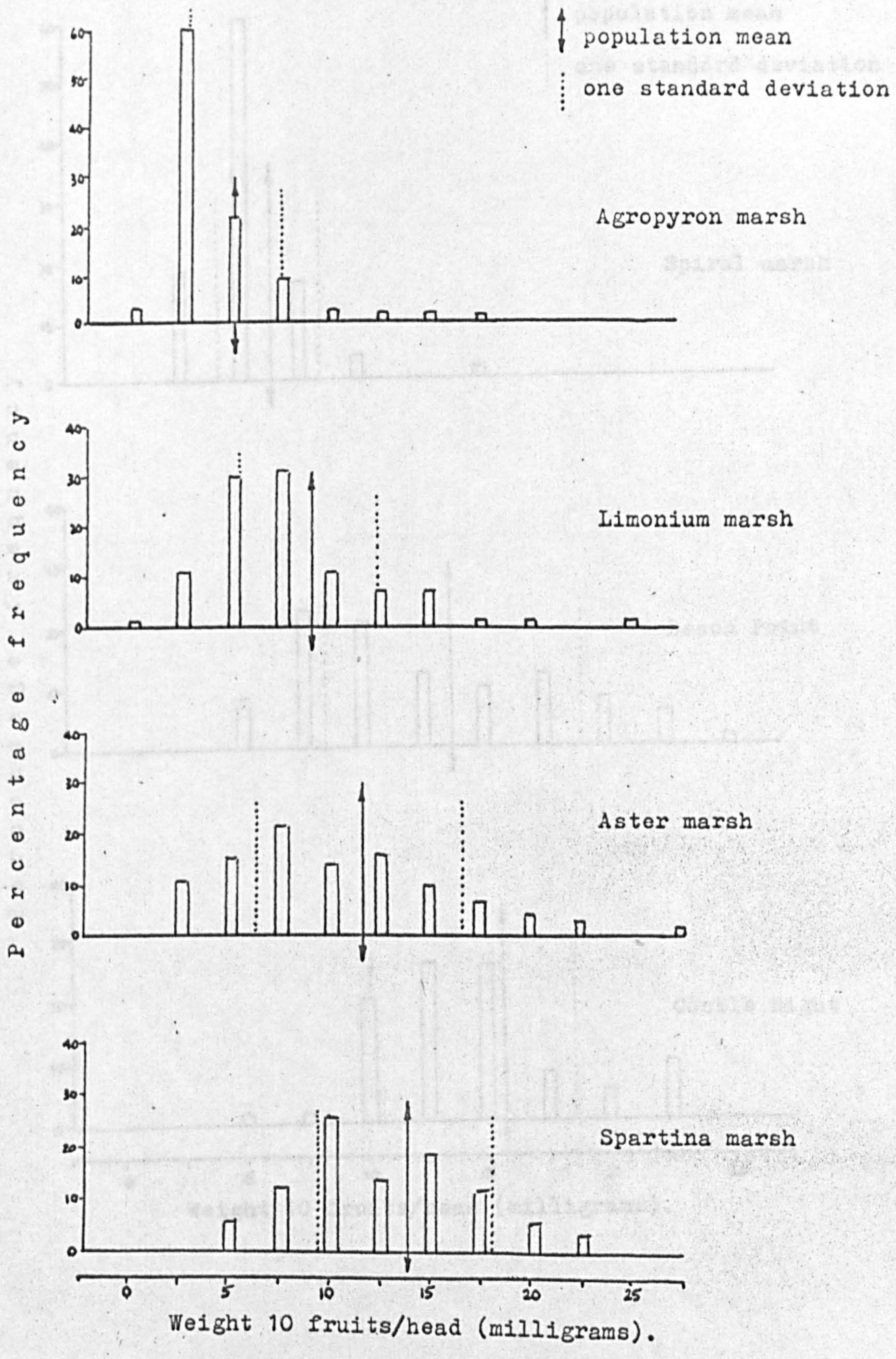


Fig. 5.5. Weights of disc fruits - Scolt Head populations.

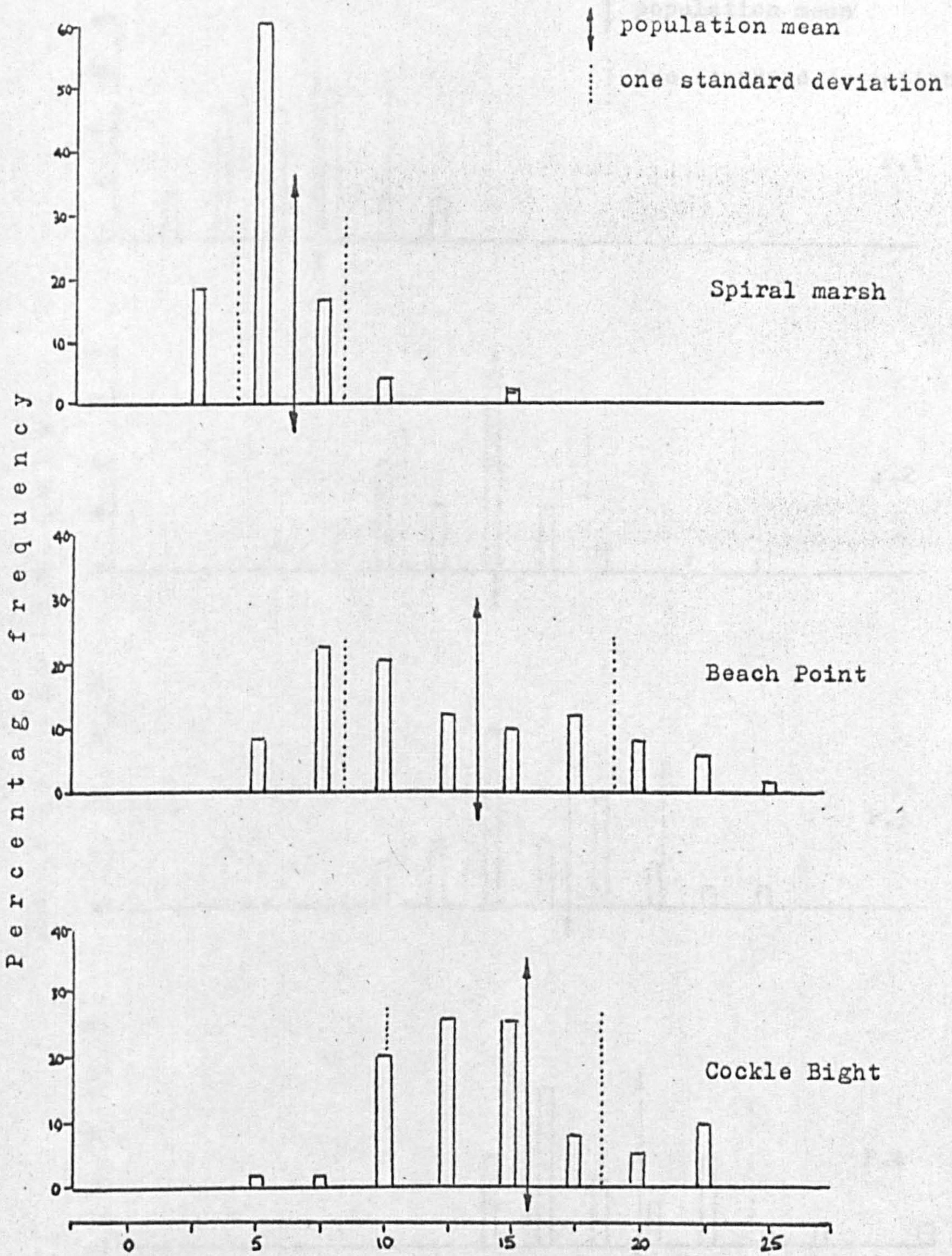
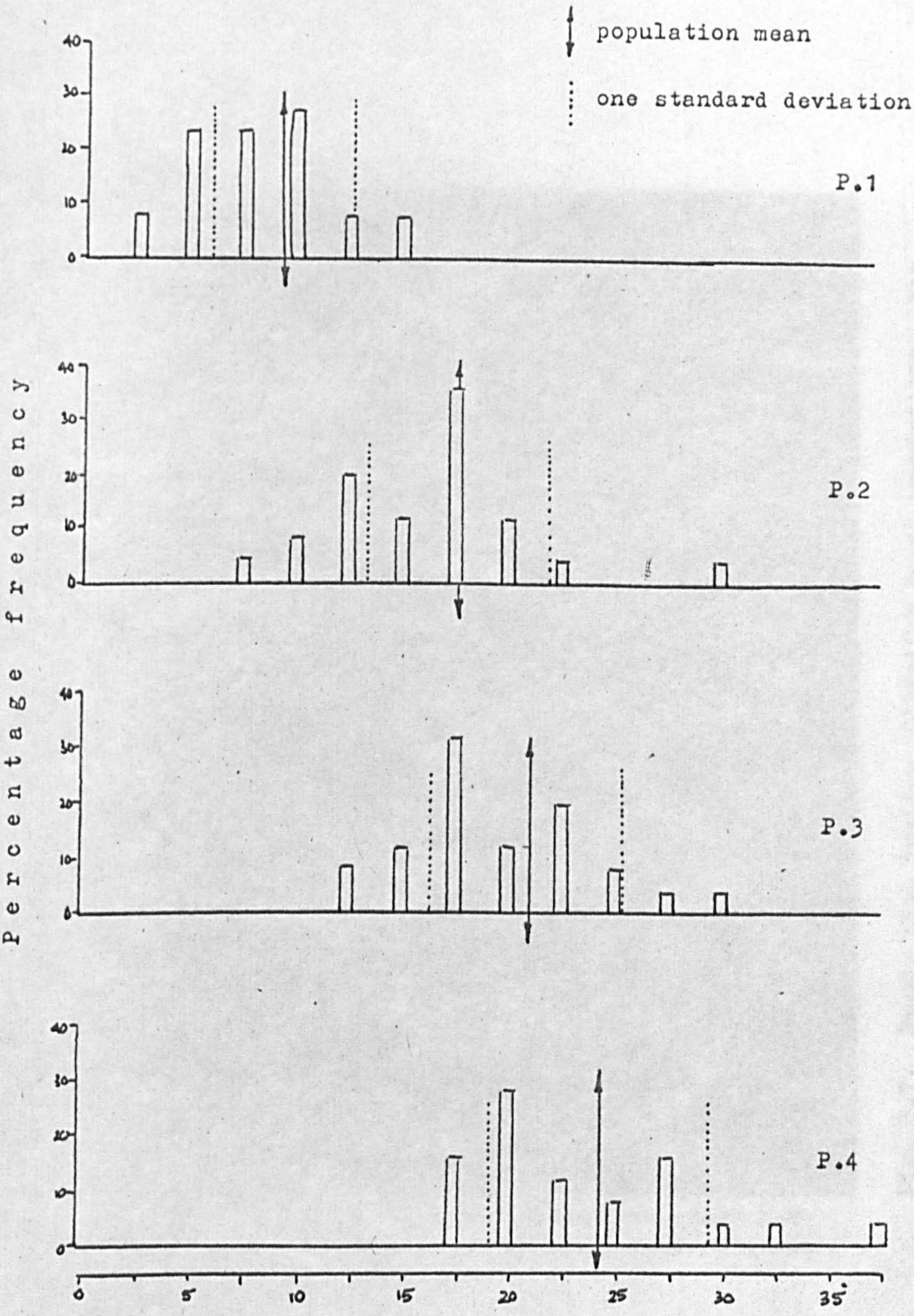


Fig. 5.6. Weights of disc fruits - Parkgate populations.



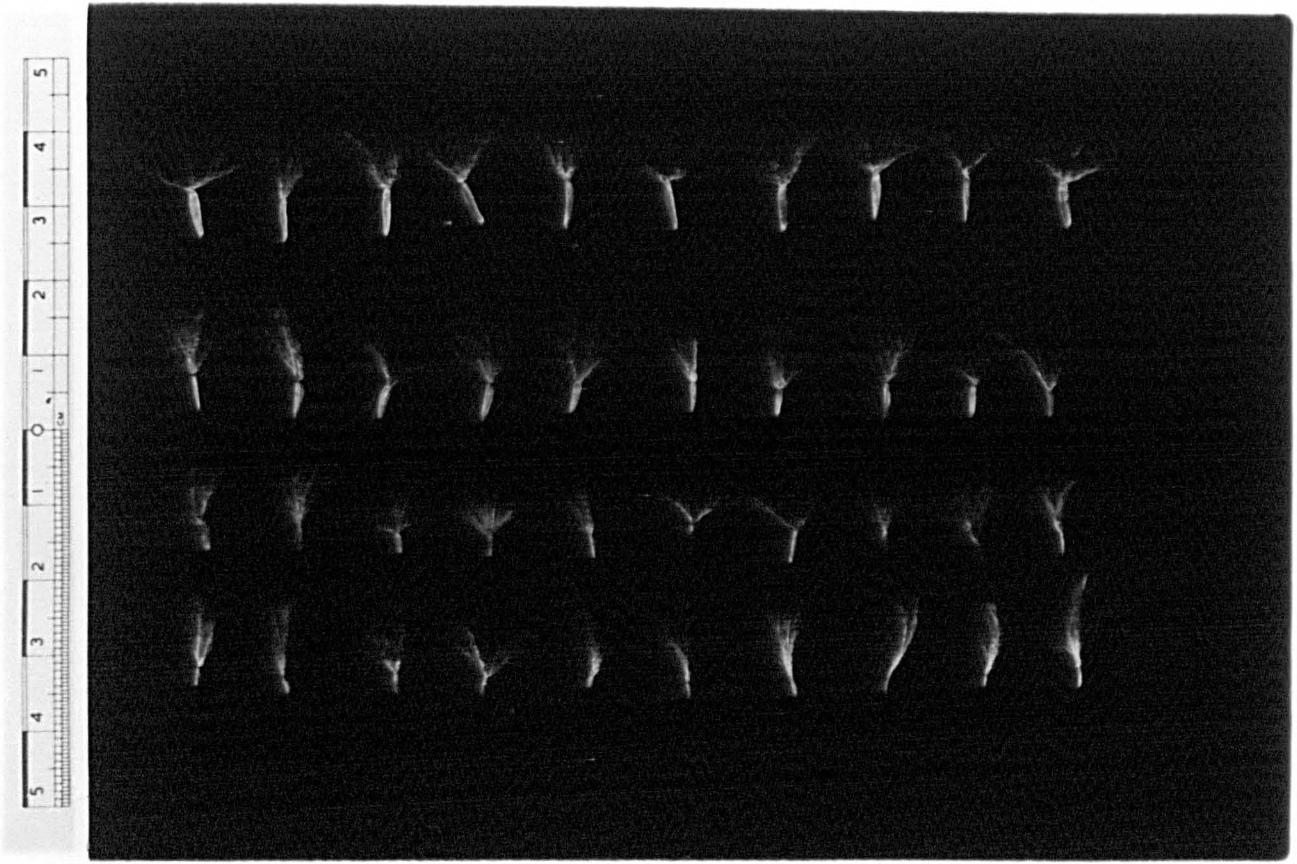


Fig. 5.7. Sample disc fruits. Gibraltar Point populations. Top row: Spa., 2nd row: Ast., 3rd row: Lim., Bottom row: Agr.

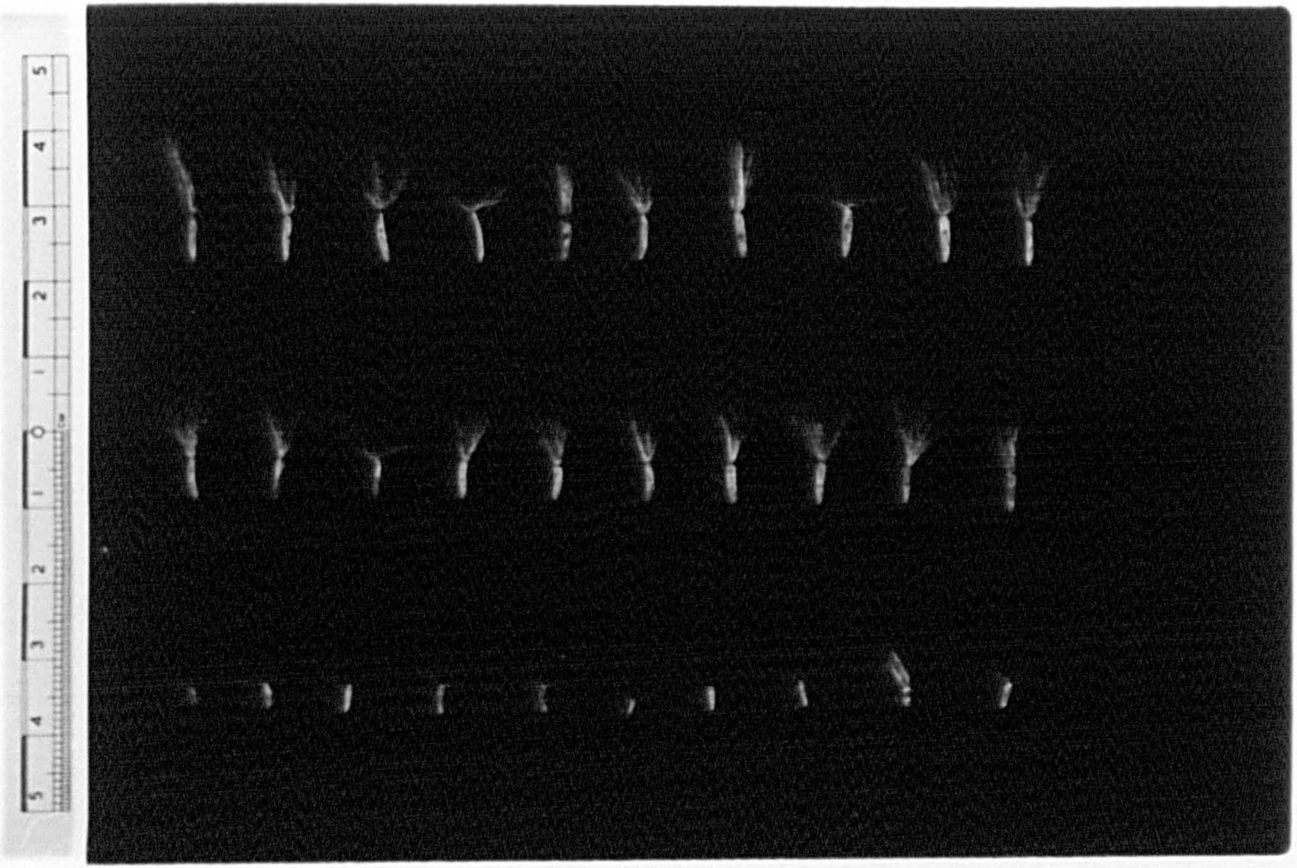


Fig. 5.8. Sample disc fruit. Scolt Head populations. Top row: Cob., Middle row: Bch., Bottom row: Srl.

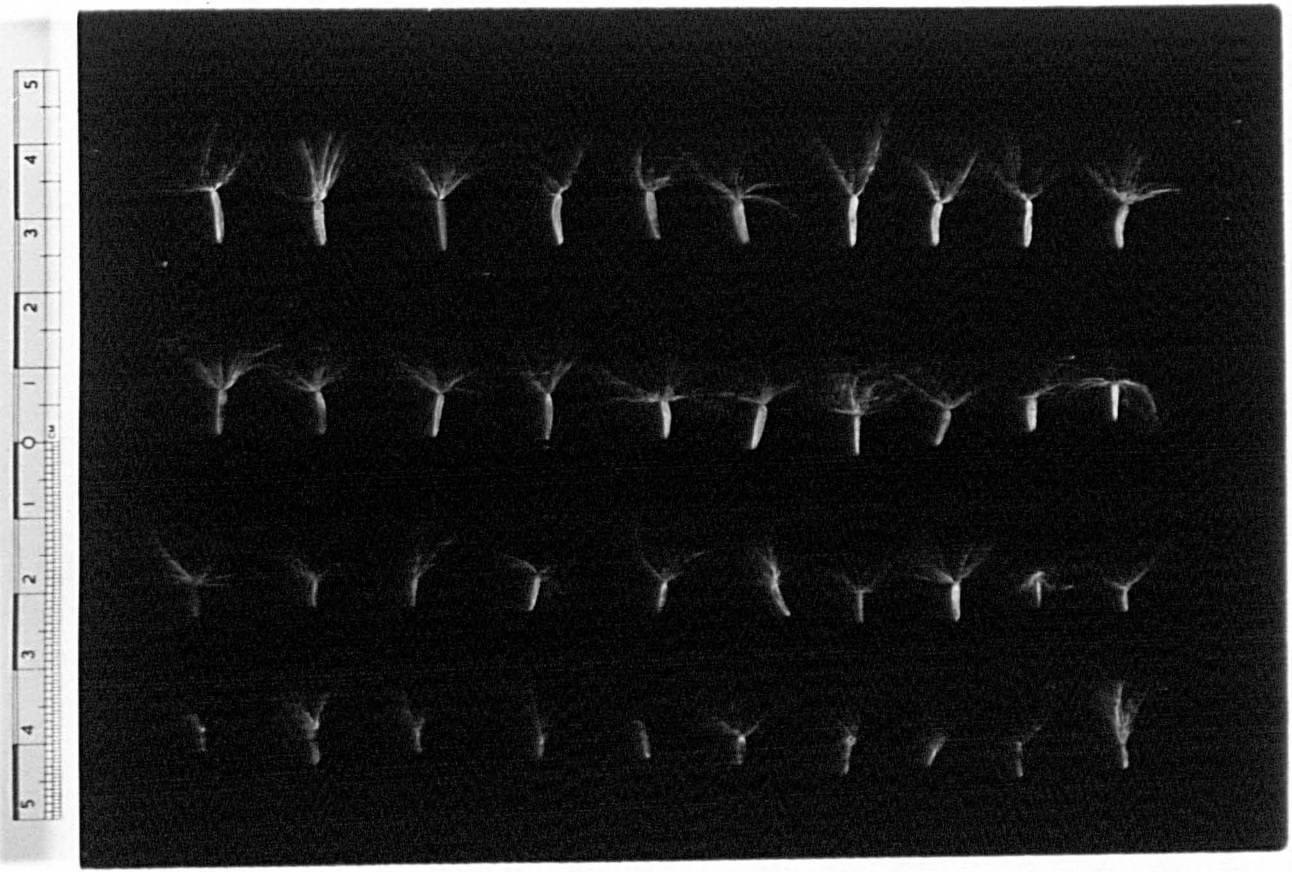


Fig. 5.9. Sample disc fruits. Parkgate populations. Top row P4, 2nd row: P3, 3rd row: P2, Bottom row: P1.

from which it was collected. This is illustrated by the large sample variance of the Scolt Beach Point and Gibraltar Point Aster samples, and the small variance of the Spiral Marsh sample. The negative skew of the Agropyron marsh histogram is explained by the fact that only viable-looking fruits were selected for weighing and thus a lower limit to weight may have been fixed artificially (there is also likely to be such a limit naturally).

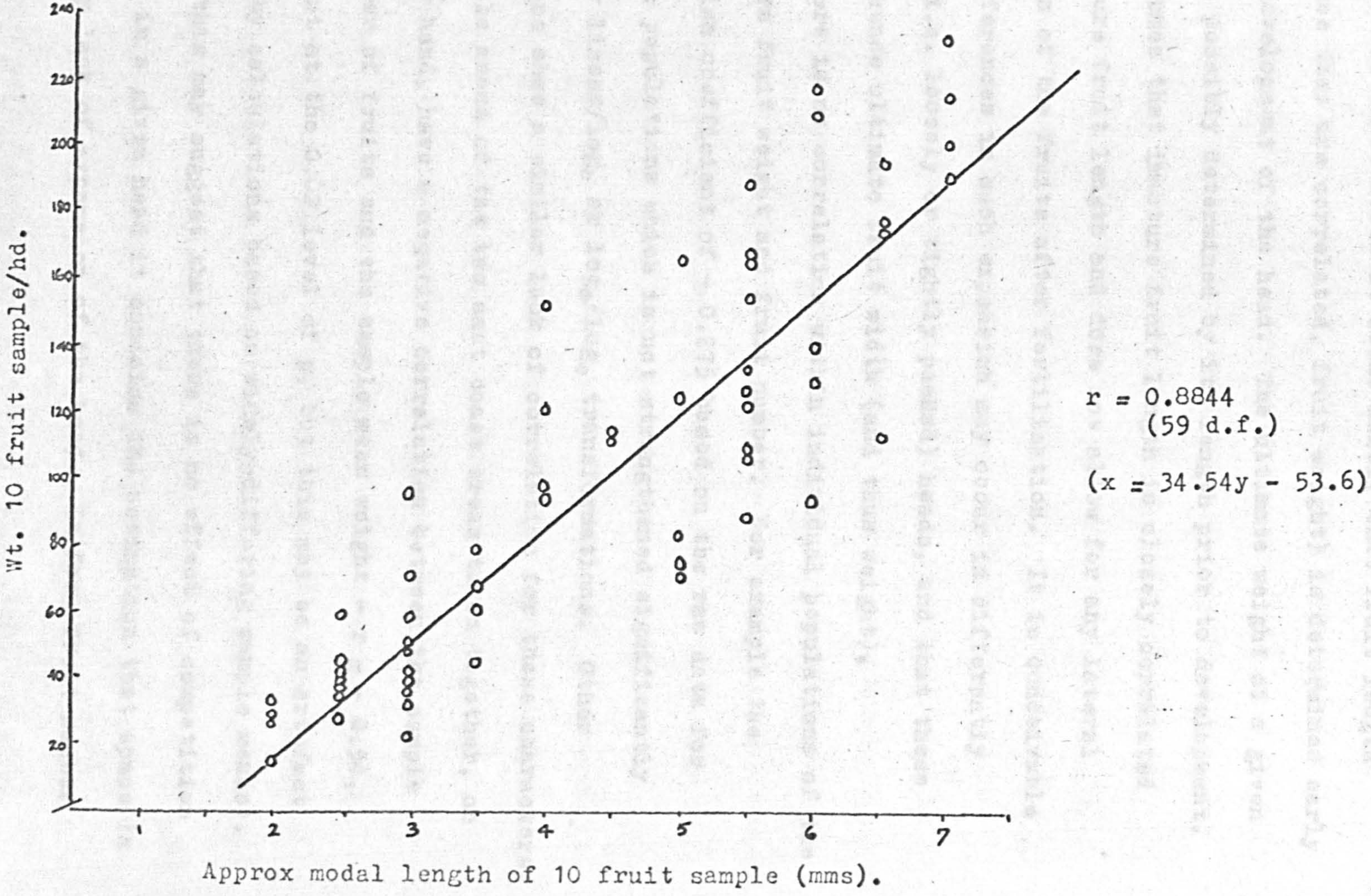
The cline in fruit weight is paralleled by an inverse cline in the number of fruits/head at Gibraltar Point and Scolt Head. At Parkgate however the contrast between the production of many small fruits and few large ones is not evident. No such trend is apparent in these populations.

(ii) Factors affecting fruit size. Apart from genetic control of both actual fruit size and stability of fruit size (see III (b) below) other factors may affect the size of fruits.

One of these is the spacing of the fruits in the head. During preliminary investigations the individual lengths of fruits were measured as well as their weights. It became evident that length and weight were closely correlated and the time-consuming measurement of length was abandoned. Before this was done, however, sufficient data for Fig. 5.10 was obtained. Fig. 5.10 graphs the correlation between fruit weight and length for 600 fruits from Gibraltar Point plants (single points representing the mean of a group of 10 fruits from one head).

Distinct variation in the length of the immature fruit from population to population can be observed in the head prior to

Fig. 5.10. Correlation between seed length and weight. (Sixty plants from Gibraltar Point).



r = 0.8844
(59 d.f.)

($x = 34.54y - 53.6$)

anthesis. In other words it seems likely that fruit length (and since they are correlated, fruit weight) is determined early in the development of the head. The ultimate weight of a given fruit is possibly determined by its length prior to development. This assumes that immature fruit length is closely correlated with mature fruit length and does not allow for any lateral expansion of the fruits after fertilisation. It is conceivable that differences in such expansion may occur in differently spaced (i.e. loosely or tightly packed) heads, and that these may influence ultimate fruit width (and thus weight).

There is no correlation within individual populations of the parameters fruit weight and fruit number. For example the correlation coefficient of - 0.275 based on the raw data for Gib. Agr. populations which is not strengthened significantly by either linear/$\log_e$ or $\log_e$/$\log_e$ transformations. Other populations show a similar lack of correlation for these characters. (The sample means of the two east coast areas taken together, on the other hand, have a negative correlation between the sample mean number of fruits and the sample mean weight - $r = - 0.94$, significant at the 0.02 level of p, but this may be an artefact produced by calculations based on widely-differing sample means). Although this may suggest that there is no effect of competition for space in a given head it contains the assumption that space is limited by lack of expansion of the head. In fact fruit number is likely to vary with head diameter, which was considerably variable, but was not measured. On the other hand ultimate head diameter, fruit number, fruit length, and fruit size may all be laid down early in the development of individual capitula and thus not be affected by either spacing or the presence of thin inviable fruits (see below).

The development of floret initials was not studied, but the work of Khan (1967) on the processes of fruit development in the sunflower indicates that, in this species, fruit size is influenced by, in cultivated varieties, what happens during the initiation and completion of floret initials, or in wild varieties, the stages right from the beginning of floret primordia development.

(iii) Other variation. Two further aspects of variation should be mentioned. The first, variation in the numbers of inviable fruits, will affect reproductive capacity; the second, variation in pappus hair length, may affect fruit dispersal.

Most of the fruiting heads examined contained varying numbers of thin, papery fruits which appeared inviable. Their emptiness was demonstrated by the complete failure of a sample of these fruits to germinate on moistened filter paper. The numbers of such fruits was recorded for wild-collected populations and the data are produced in Table 5.6.

On the two east coast marshes plants from the lowest marshes contain the smallest proportion of inviable fruits. This is not so for the Parkgate populations. The effect of this variation

Table 5.6 % 'inviable' fruits.

Gibraltar Point marshes				Scolt Head marshes				Parkgate marsh			
Popul	no hds counted	$\bar{X}$ inviable no.fruit/ head	% inviable	popul	no hds counted	$\bar{X}$ inviables/ heads	% inviable	popul	$\bar{X}$ inviable fruits per head	no hds counted	% inviable
Agr.	65	12.60	30.33%	Sol	50	9.62	40.11%	P1	9.52	25	26.12%
Lim.	103	10.83	32.05%	Bch.	50	4.00	19.49%	P2	7.72	25	19.91%
Ast.	80	7.92	30.36%	Cob.	50	2.78	12.23%	P3	5.64	25	14.87%
Spa.	74	4.06	15.58%					P4	8.86	25	21.05%

on reproductive capacity is discussed under III below.

It has been pointed out to me by Dr. D. H. Dalby (personal discussion) that considerable variation in the length of pappus hairs of A. tripolium fruits is found in nature. This variation was noticed in a number of the populations examined, but, apart from the very short pappus of almost all the Spiral marsh plants (see Fig. 5.8), no major between-population variation was revealed by a cursory examination. It was decided not to make detailed measurements of this character.

III General considerations.

(a) Reproductive capacity.

The data presented in II above can be used to assess the respective outputs of the different populations. Reproductive output, in terms of fruit weight and number, can be considered in two ways; the potential output and the actual output.

To calculate the former the variation in the number of heads per plant and that in the proportion of inviable fruits may be left aside for the present. Considering first the two east coast areas together, the output of a single plant with an equal number of heads (in this case 10) is as follows (Table 5.7a).

Although the output in terms of total fruit weight/plant appears generally higher in low marsh plants the number of units (individual fruits) over which this is spread is lower in these plants. Thus the lack of high individual fruit weight is offset

Table 5.7a. Potential output of 1 plant with 10 heads from each population.

Output	Computation	Gibraltar Point				Scolt Head.			Units
		Agr.	Lim.	Ast.	Spa.	Srl.	Bch.	Cob.	
mean total number of fruits per plant	$\bar{X}$ no. fruit per head x 10.	415	338	261	260	239	205	227	fruits.
mean total fruit weight per plant.	$\bar{X}$ fruit wt. x $\bar{X}$ no. of fruits per hd.x 10	219	300	299	367	154	282	352	mgms (approx)

by the production of large numbers of fruits. This contrast between low numbers of large fruits and high numbers of small fruits is one which is returned to in Discussion. However if one goes on to consider the actual variation in production by taking into account both the variation in number of heads/plant (under cold house conditions) and the variation in the proportion of inviable fruits/ head, the output of a single plant under cold house conditions, would be as follows:-

Table 5.7b Theoretical actual output of a single plant. E. coast marshes.

Output	Computation	Gibraltar Point				Scolt Head			Units
		Agr.	Lim.	Ast.	Spa.	Sol.	Bch.	Cob.	
Mean total no. viable fruits/plant	$\bar{X}$ no. viable fruits/hd. x no. heads/ plant.	984	1,677	1,167	1,792	113	706	867	fruits
Mean total fruit weight/plant	$\bar{X}$ no. viable fruit x $\bar{X}$ fruit wt. x $\bar{X}$ no. heads.	522	1,493	1,342	2,527	73	975	1,352	mgms (approx)

From this it can be seen that the inverse relationship between fruit size and number breaks down completely. In a single harvest an individual plant from the highest marsh at Gibraltar Point produces about a half the total number of fruits produced by a plant on the lowest marsh. The corresponding ratio at Scolt Head is less than a seventh. In terms of fruit weight the ratio highest to lowest marsh is about one fifth at Gibraltar Point and one eighteenth at Scolt Head.

The Parkgate populations are characterised by a marked variation in fruit size unaccompanied by an inverse variation in number of fruits. No data is available on the actual output but the potential output of a single plant with ten heads is:-

Table 5.7c	Potential output of a single plant (with 10 heads) Parkgate population.				
Output	Computation	P1	P2	P3	P4
Mean total no. of fruits/plant	$\bar{X}$ total fr/head x 10	364	388	379	421
Mean total fruit wt/plant	$\bar{X}$ fruit wt. x no fruits/head x 10	340	681	790	1029

General observations in the field indicate that there are fewer heads/plant in the upper marsh populations (P1) at Parkgate. This would affect the actual production in the same way as it does in the east coast populations for which data are available.

(b) <u>The stability of fruit size</u>.

The most striking aspects of fruit variation are (1) the clinal variation in fruit size of populations along the three marsh areas studied and (2) the low plasticity of fruit size in all the populations examined. It is suggested below that these may be related phenomena.

The work of agronomists, referred to earlier, on the effects of density on infraspecific variation has indicated that certain characters such as plant size, leaf number, stem length, and hairiness are plastic whereas others, particularly floral characters, are not. A great deal of evidence supports this view. Stebbins (1950) relates the differences in plasticity of such characters to the period of time over which they develop (and thus the amount of time during which they may be subject to environmental influences). Harper's observation that species with indeterminate growth generally respond to density by varying the number of parts, whereas those with determinate growth tend to respond by varying the size of parts, is really an extension of this argument (Harper 1961).

Seed size is a character which is extremely stable in species with indeterminate growth. Numerous examples exist of species in which this character changes little in response to density. These include <u>Linum usitatissimum</u> (Khan 1963), <u>Papaver</u> species (Harper 1963), <u>Vicia faba</u> (Hodgson and Blackman, 1956) and varieties of spring wheat (Quinlan and Sagar, 1965). Other examples are cited by Bradshaw (1965) and Donald (1963).

On the other hand examples of species with determinate growth in which seed size is plastic are not easy to find. The data of Clements et al (1929), later partly supported by Khan (1967), on fruit size in Helianthus annuus provides perhaps the major piece of evidence to support the view of Harper (1961) expressed above. Later work on this species by Khan, however, reveals that whereas the cultivated varieties are plastic in this respect the wild varieties are not, but show a high degree of canalisation (Khan 1967).

It is therefore relevant to ask why Aster tripolium, a species with determinate growth and thus one in which fruit size is 'potentially plastic' (in terms of Harper's view), has such a low plasticity for this character. The answer, it is felt, lies in the pattern of clinal variation. By this it is meant that for the environment to have selected large and small fruits on low and high marshes in three separate regions then fruit size must be of evolutionary importance. It must be of adaptive significance.

The fact that fruit size generally is of selective importance has been suggested by a number of authors. It is implied, for example by Salisbury's observation that smaller seeded plants are generally found in 'open' habitats and larger seeded ones in 'closed' habitats (Salisbury 1942) (but see Chapter 12).

It is important to distinguish between the selection of genes controlling fruit size and those controlling canalisation of fruit size. Large (or small) fruit size could be obtained in Aster tripolium, as in cultivated sunflowers, by selection of the genes directly responsible for this property without a parallel selection for genes favouring fruit size stability. (The mounting evidence that canalisation is separately controlled by genetic factors and can be acted on by selective factors in the same way as a 'normal' character is presented by Bradshaw (1965)). Selection for fruit size, however, appears to have been accompanied in Aster, by selection for stability of fruit size. Possible reasons for this are advanced later in this thesis (Chapter 12). For the present it serves to underline the importance with which fruit size variation in the species must be regarded and is the reason why a number of experiments described in the following chapters (particularly Chs. 6,8and 9) are concerned with this particular aspect of variation.

CHAPTER 6. GERMINATION.

This chapter describes and discusses a series of germination tests on selected populations. It seemed reasonable to concentrate on those aspects of germination which are likely to be relevant to the plant's natural habitat. Most of the trials were carried out by testing germination on (or between) layers of moistened filter paper in disposable plastic petri dishes. Unless otherwise stated the filter paper was moistened with distilled water (4 ml./dish). The trials which took place in the light were placed inside a seed germination chamber of a standard type, (Copenhagen bath), those which took place in the dark inside an incubator. Others were carried out on nylon platforms in polystyrene beakers of the sort described in Chapter 8 (Fig. 8.1A). As only the major effects were sought the number of replicates was reduced to a minimum in those trials which were replicated.

I The effect of light.

Preliminary trials were undertaken in which the fruits were germinated at lab. temperature (18°C ± 2°) in continuous light. These revealed that germination was generally high in low marsh populations but low in high marsh populations.

200 wild collected fruits from each population at Gibraltar Point were tested and the germination rate recorded. A fruit was deemed to have germinated on the date of the initial emergence of the primary root (although some roots did not continue to

elongate after initial emergence). The results of this experiment (expt. 5 Dec. 1967) are recorded in Table 6.1 and the data are plotted in Fig. 6.1.

Table 6.1	Germination at room temperature in continuous light - Gibraltar Point populations.												
	% of fruits germinated on day after soaking												
Populations	3	4	5	6	8	9	10	11	12	13	14	15	16
G. Spa.	15	39	50	62	63	66	69	73	73	75	78	78	80
G. Ast.	7	13	14	15	17	17	21	21	21	22	22	23	26
G. Lim.	1	1	1	1	1	3	3	3	3	4	4	4	4
G. Agr.	0	0	0	0	0	1	1	2	3	4	4	5	6

The fruits were removed on the 16th day and classified according to whether they (i) had germinated and showed good cotyledon and primary root growth (ii) had germinated but subsequently aborted (iii) were infected by fungi or (iv) had not germinated. The final counts were (in %s) -

	class (i)	class (ii)	class (iii)	class (iv)
G. Spa.	80	11	2	7
G. Ast.	26	20	8	48
G. Lim.	4	1	4	91
G. Agr.	6	1	10	83

A parallel experiment was carried out in an unlit incubator. The results are given in Table 6.2 and the data plotted in Fig. 6.2.

Fig. 6.1. Germination at room temperature in continuous light (Gibraltar Point populations).

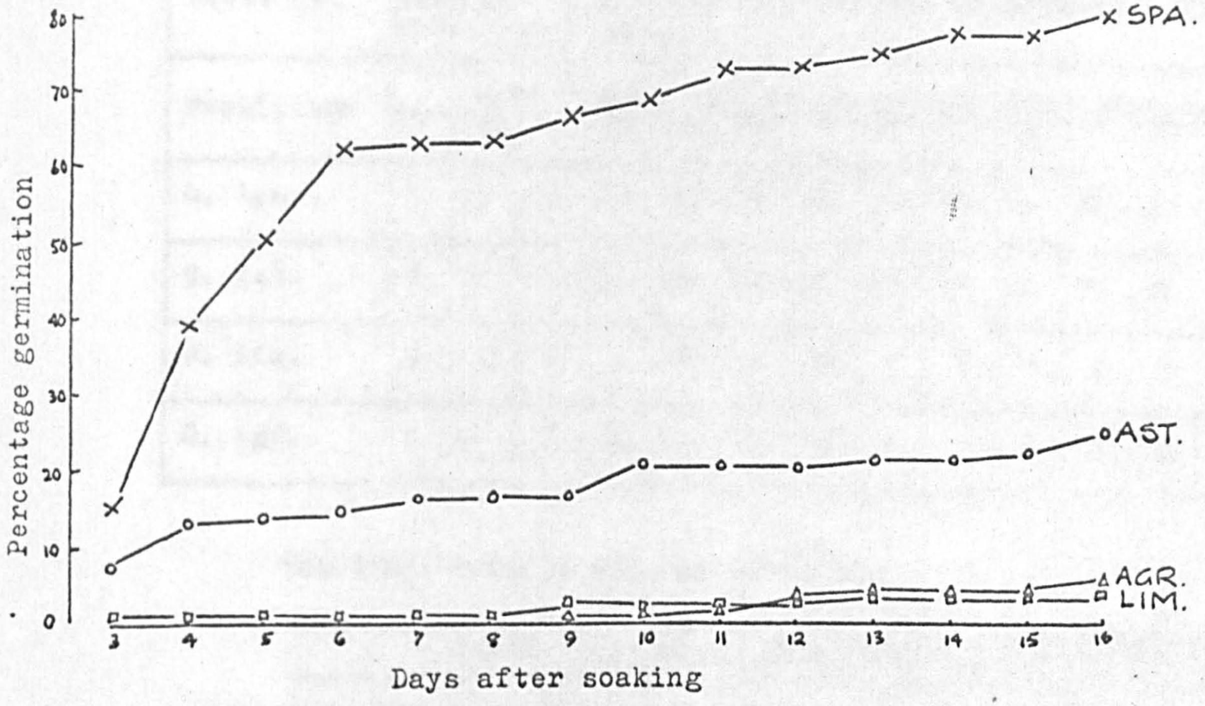


Fig. 6.2. Germination at room temperature in darkness (Gibraltar Point populations).

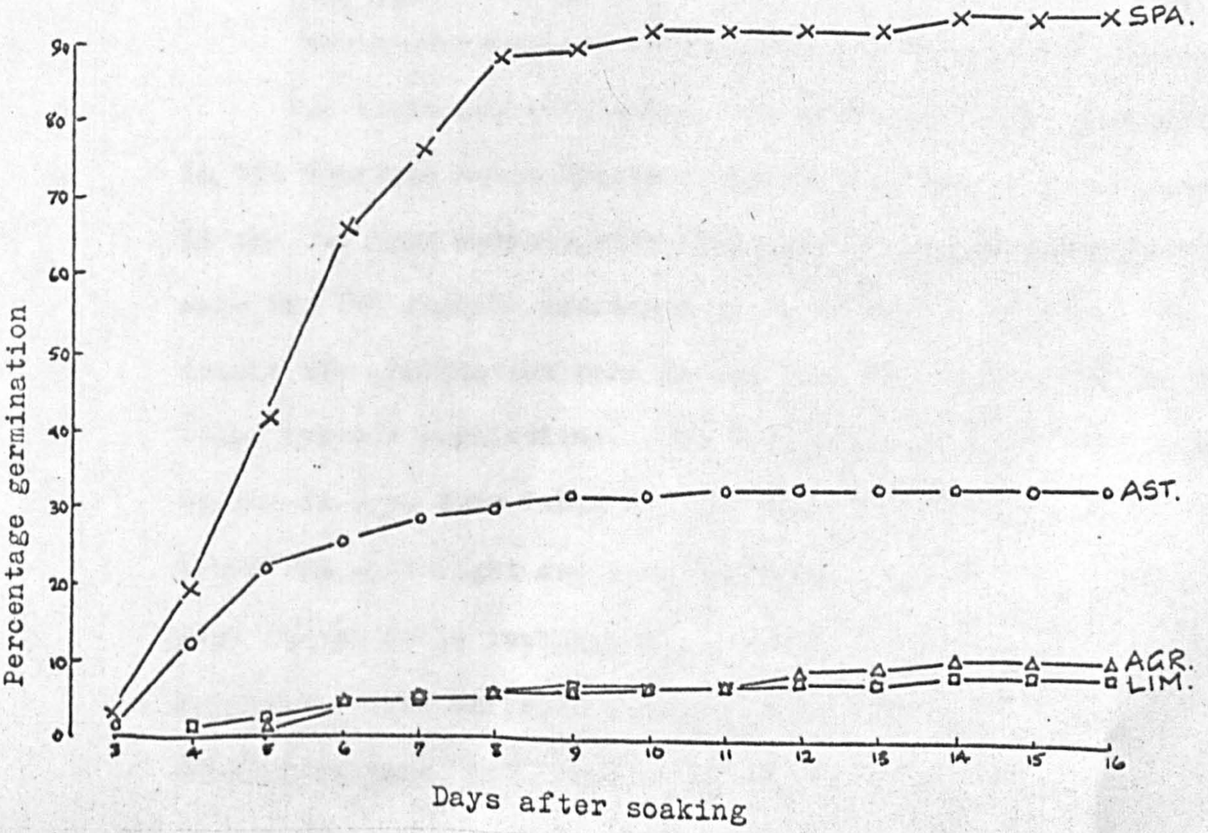


Table 6.2 Germination at room temperature in darkness - Gib. Point population.

Population	% of fruits germinated on day after soaking.													
	3	4	5	6	7	8	9	10	11	12	13	14	15	16
G. Spa.	3	19	41	66	76	88	89	91	91	91	92	93	93	93
G. Ast.	1	12	22	25	28	29	31	31	32	32	33	34	35	35
G. Lim.	0	1	2	4	5	5	6	6	7	7	7	8	8	8
G. Agr.	0	0	1	4	5	5	5	6	7	8	9	10	10	10

The final results were as follows:-

	class (i)	class (ii)	class (iii)	class (iv)
G. Spa.	93	0	1	6
G. Ast.	35	4	8	53
G. Lim.	8	2	0	90
G. Agr.	10	0	5	85

Two facts are noticeable, the high percentage germination in the Spartina marsh fruits compared with the very low percentage in the two high marshes, with Aster marsh, intermediate in performance, and the overall increased germination in the dark. In all trials the germination rate in the dark was higher than in the light for all populations. The difference in this rate varied as can be seen from Table 6.3 in which all the germination trials involving both light and dark conditions in distilled water have been listed (this includes those to which pretreatments have been applied). This difference remained constant under a number of treatments (e.g. dilutions of sea water, prechilling - see below).

Table 6.3 Comparison of light and dark germination in a number of populations (in distilled water) (with varying pretreatment-not indicated).

Population	light (%germin.)	Dark (%germin.)	increase in dark (%)
Beach Point	75.6	78.8	3.2
Knott End	60.0	68.5	8.5
Parkgate P1	13.9	22.2	8.3
Parkgate P4	32.1	34.7	2.6
Gib. Spa.	80.0	93.0	13.0
Gib. Ast.	26.0	35.0	9.0
Gib. Lim.	4.0	8.0	4.0
Gib. Agr. (1)	6.0	10.0	4.0
" (2)	43.8	51.0	7.2

II Dormancy and the effect of prechilling.

The failure of the apparently viable high marsh fruits to germinate in the preliminary trials suggested that they might be dormant. Many of the fruits from these populations had imbibed water following the initial soaking and, although swelling considerably, had not germinated.

The effect of a prior period of cold treatment was tested. The fruits which had failed to germinate in the preliminary trial described earlier, together with a number of petri dishes of fruits which had been soaked at the same time and from which the (uncounted) germinated fruits had been removed, were given one of three treatments. Twenty days after their initial soaking they were either (1) placed in a refrigerated room (2 - 5° C) for

24 hours, (2) placed in the same room for 72 hours, or (3) transferred to a refrigerator (-3°C) for 24 hours. A number of the dishes of each population were not treated and remained as a control. Following these treatments the samples were divided and half of them placed in the illuminated germination chamber at 20°C. The other half were placed in an unlit incubator at the same temperature. The results (after 15 days) given in Table 6.4 are expressed as percentages. The actual numbers in each treatment varied but averaged about 50 fruits in each subsample.

Table 6.4	Effect of various cold treatments on germination in Gibraltar Point populations (Expt. 1 Jan. 1968).						
Pretreatment	24 hrs.2-5°C		72hrs.2-5°C		24hrs. -°C		none
Germination cond. 15°C.	light	dark	light	dark	light	dark	continuous light
Gib. Spa	78	92	91	94	0	0	7
Gib. Ast.	70	68	80	93	0	0	0
Gib. Lim.	25	50	31	69	0	0	0
Gib. Agr.	31	7	100	100	0	0	1

The figures in Table 6.4 relate to the germination performance of those fruits which had not germinated 20 days after the initial soaking and are thus %s of the residue of fruits. Germination of these was considerably increased by prechilling in all populations. In the case of Agropyron marsh and, to a lesser extent, Limonium marsh, fruits,the increase in the period of prechilling from 24 to 72 hours considerably increased the number of germinants. No fruits germinated after 24 hours freezing at - 3°C.

A further experiment (Expt. 2) to test the effects of prechilling was undertaken. Fruits from the Gibraltar Point populations were soaked for 24 hours in the dark and transferred to one of three treatments: (1) prechilling (2-5°C) for 30 hours (2) prechilling for 100 hrs and (3) no prechilling. The unchilled fruits were soaked two days after the others and the fruits were all placed in the germination chamber at 15°C. The percentage germination after 15 days is indicated in Table 6.5.

Table 6.5	Effect of cold treatment on germination (in light) (Expt. 2).		
Pretreatment	30 hrs. 2-5°C.	100 hrs. 2-5°C	none
G. Spa.	86.3	40.5	86.6
G. Ast.	22.6	12.5	27.5
G. Lim.	11.1	6.5	3.5
G. Agr.	22.7	15.5	2.5

The results from this experiment are somewhat at variance with those of the earlier one. Although the control is similar to that described earlier (Table 6.1) germination is lower in all cases of pretreated fruits. Despite this the performance of G. Lim. and G. Agr. fruits was increased by prechilling whereas G. Ast. and G. Spa. fruits are apparently affected adversely by prolonged cold treatment.

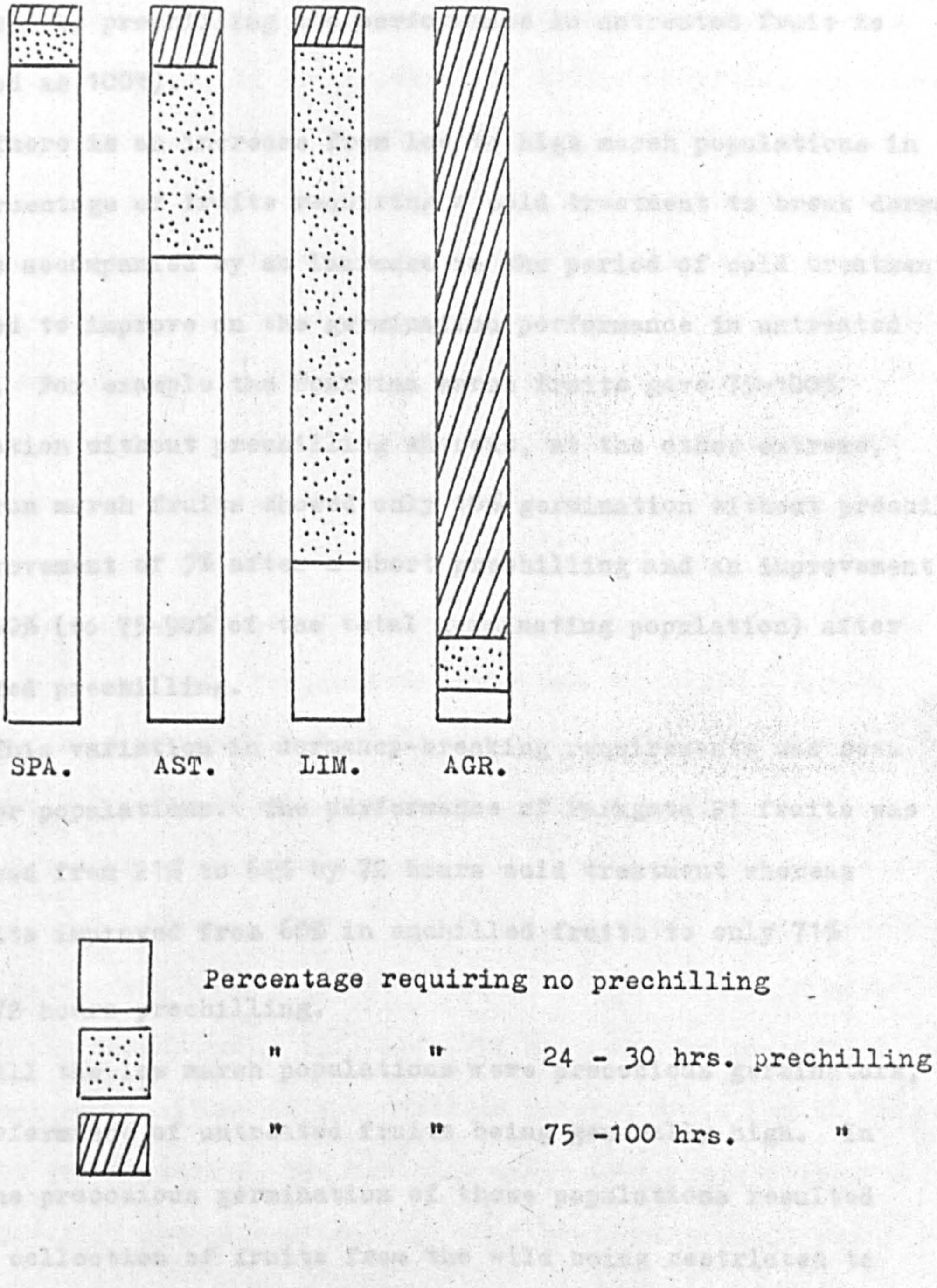
The disparities between the two experiments in the germination of the pretreated fruits may be due to the differences in the period of soaking prior to cold treatment (20 days v 24 hrs.), differences in the periods of prechilling (24 v 30, 75 v 100 hrs.), the differences in the temperature to which the fruits were returned (20°C v 15°C) or to changes in the viability of the fruits (the experiments were carried out 6 weeks apart). All these are likely to be critical factors and more work is required, using more replicates, to examine the effects of prechilling more closely.

Table 6.6 Prechilling requirement of Gibraltar Point populations

	Expt.	Approx % of germinants not requiring prechilling.	Approx. % of germinants requiring short prechilling (24-30 hrs.).	Approx. % of germinants requiring longer prechilling (75-100 hrs.).
Gib. Spa.	1	85	12	3
"	2	100	0	0
Gib. Ast.	1	30	55	15
"	2	100	0	0
G. Lim.	1	5	85	10
"	2	40	60	0
G. Agr.	1	10	15	75
"	2	10	0	90

However certain general trends common to both experiments, are evident. The results of the two experiments are listed in Table 6.6 and plotted in Fig. 6.3. (These do not account for apparently inviable fruits and regard, for example, unless the

Fig. 6.3. Prechilling requirement of fruits from Gibraltar Point populations (mean value of two experiments).



actual performance is improved by prolonged treatment, those requiring short pretreatment as the % of the total population requiring prechilling of any sort. If the performance was not increased by prechilling the performance in untreated fruit is regarded as 100%).

There is an increase from low to high marsh populations in the percentage of fruits requiring a cold treatnent to break dormancy. This is accompanied by an increase in the period of cold treatment required to improve on the germination performance in untreated fruits. For example the Spartina marsh fruits gave 75-100% germination without prechilling whereas, at the other extreme, Agropyron marsh fruits showed only 10% germination without prechilling, an improvement of 5% after a short prechilling and an improvement of 60-80% (to 75-90% of the total germinating population) after prolonged prechilling.

This variation in dormancy-breaking requirements was seen in other populations. The performance of Parkgate P1 fruits was increased from 21% to 64% by 72 hours cold treatment whereas P4 fruits improved from 68% in unchilled fruits to only 71% after 72 hours prechilling.

All the low marsh populations were precocious germinators, the performance of untreated fruits being generally high. In fact the precocious germination of these populations resulted in the collection of fruits from the wild being restricted to dry days. Large numbers were lost from the Cockle Bight, two

two Blakeney Point, and Gibraltar Point Spartina marsh populations because of germination of fruit in the heads enclosed in polythene bags during transport to Keele.

The dormancy of the high marsh fruits is apparently innate. Numbers of fruits failed to germinate in both prechilling experiments, especially the second (14% G. Spa., 90% G. Lim., 70% G. Ast., and 80% G. Agr.). These undoubtedly contained a great proportion of viable fruits, the innate dormancy of which was not broken by the particular conditions of the experiment. It is likely that a more prolonged period of prechilling is required although a number of factors may be responsible for innate dormancy. These include the immaturity of the embryo, the impermeability of the testa to water or to gases, special requirements for light or temperature, the presence of germination inhibitors and so on. Such factors have been reviewed by Mayer and Poljakoff - Mayber (1963) and Barton (1965). None of them (apart from the prechilling requirement) have been investigated in <u>Aster tripolium</u>.

Innate dormancy is particularly characteristic of the seeds of annual weeds (Harper 1959). The innate dormancy of the majority of high marsh fruits in <u>Aster</u> is especially interesting in view of this, and their annual life cycle in nature. The possible ecological implications of the relation between variation in life cycle and germination in low and high marsh fruits are presented in Chapter 12. The general ecological significance of dormancy and germination is discussed by Harper (1959) and Wareing (1965).

Enforced (as opposed to innate) dormancy in Aster tripolium can occur by soaking the fruits in dilutions of sea water (see below).

III The effect of salt concentration.

Germination was tested in a range of dilutions of Artificial Sea Water. The fruits were placed on filter paper saturated with the appropriate solution (4 ml. per dish) in a petri dish. In a preliminary experiment wild-collected fruits from Beach Pt., Scolt (low marsh), Knott End, (mid marsh) and Gib. Agropyron (high marsh) were tested, a total of 1,050 fruits/population being used. The samples were subdivided and half of the dishes covered with metal foil. The samples were subdivided and half of the dishes covered with metal foil. The results are given in Table 6.7, and plotted in Fig. 6.4a.

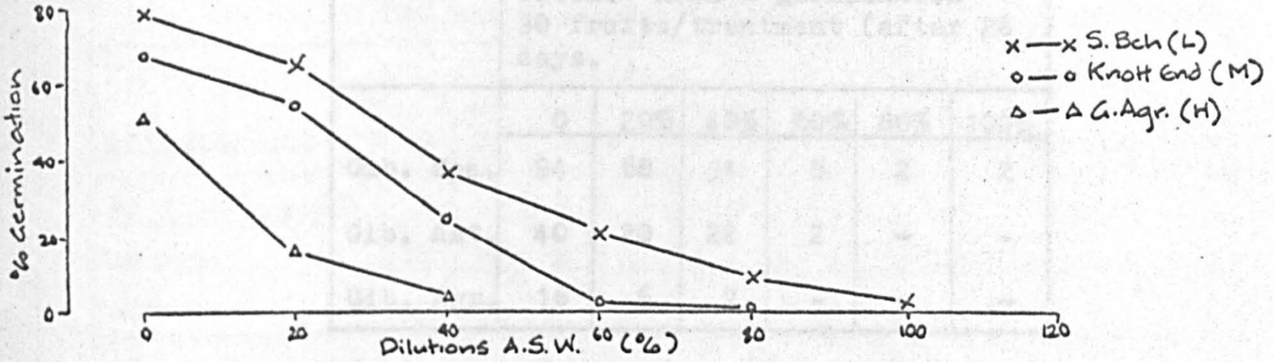
Table 6.7	Germination in Artificial Sea Water. Mean % germination 150 fruits per trial (after 28 days)							
Dilutions of A.S.W.		0	20%	40%	60%	80%	100%	120%
Scolt	light	75.6	59.5	36.0	13.0	3.6	0	0
Beach Point	dark	78.8	65.5	36.5	20.4	9.0	2.0	0
Knott End	light	60.0	44.5	6.6	2.5	0	0	0
	dark	68.5	55.0	24.4	2.5	0.5	0	0
Gib. Agropyron	light	43.8	23.0	3.3	0	0	0	n.t.
	dark	51.0	16.6	5.0	0	0	0	n.t.

n.t. = not tested.

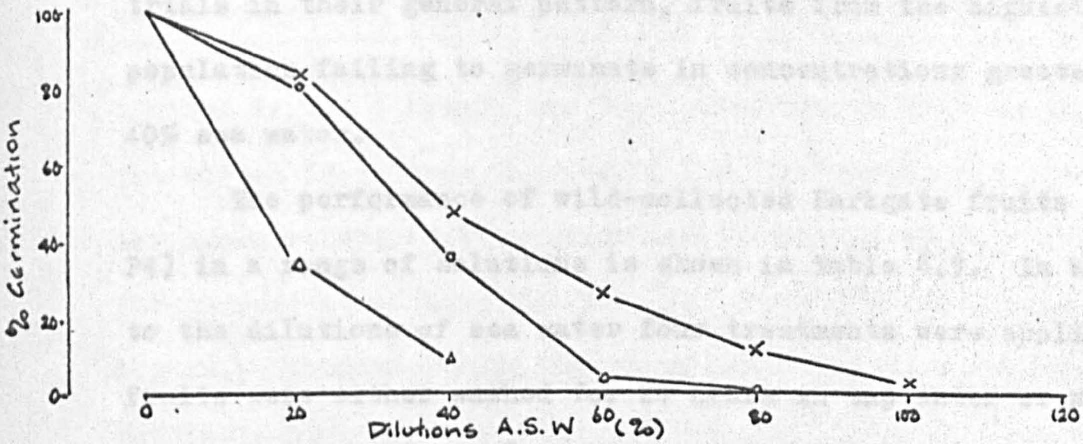
These results are striking in that they suggest that percentage germination in increasing concentrations of artificial sea water is dependent on the origin of the fruits. This trial was carried out before those in which prechilling was employed to break dormancy, and thus there are between-population differences in the distilled water germination performance which makes the effect of seawater on the different populations difficult to compare. (It is interesting that the performance of the Gib. Agr., fruits without prechilling is much higher here than in the later trials, a fact which may be partly explained by the longer period of testing - 4 weeks - or the fluctuating temperatures of the laboratory during the present trial). However, if the germination in distilled water is regarded as the maximum germination ability of the sample (i.e. 100%) the performances in 20%, 40% and 60% sea water represent for Beach Point a decrease of only 13%, 50% and 57% respectively; whereas for Gib. Agropyron the decrease is 58%, 92% and 100% respectively (see Fig. 6.4b). This suggests that sea water has a differential effect, depressing germination less in low marsh populations than in high marsh ones.

In a further series of trials fruits from three Gibraltar Point marshes were tested. 50 fruits per treatment were moistened with the appropriate solution in a petri dish kept in the dark at 20^{O}C. The results are shown in Table 6.8 and plotted, on a percentage reduction basis, in Fig. 6.4c.

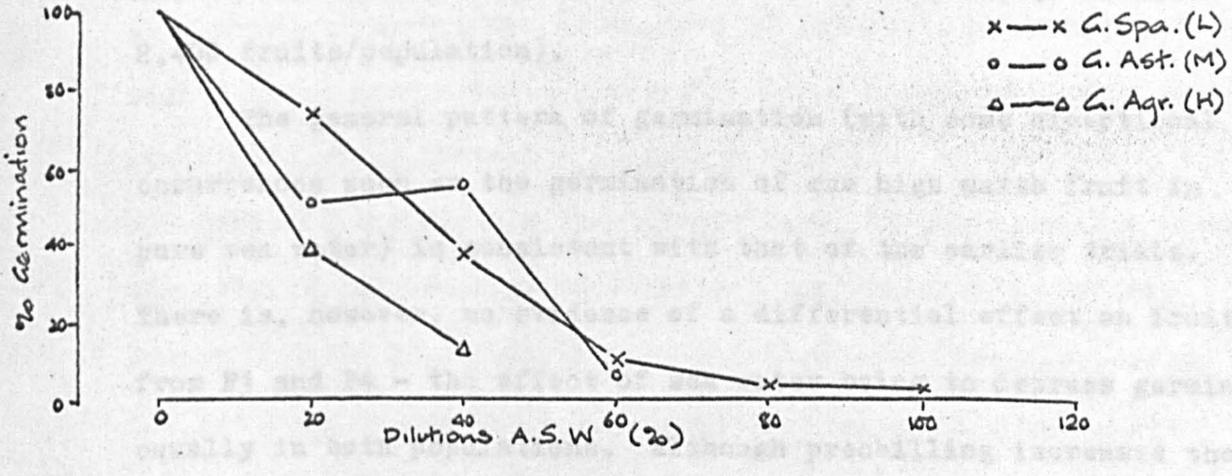
Fig. 6.4. Germination in dilutions of Artificial Sea Water.



(a) Actual % germination in 3 populations (from high, mid and low marshes).



(b) % germination with distilled water performance as 100% (pops.above).



(c) % germination in 3 Gibraltar Point populations (distilled water performance as 100%).

Table 6.8 Germination in Artificial Sea Water. Mean % germination 50 fruits/treatment (after 28 days.

	0	20%	40%	60%	80%	100%
Gib. Spa.	94	68	34	8	2	2
Gib. Ast.	40	20	22	2	-	-
Gib. Agr.	16	6	2	-	-	-

These results agree closely with those of the earlier trials in their general pattern, fruits from the highest marsh population failing to germinate in concentrations greater than 40% sea water.

The performance of wild-collected Parkgate fruits (P1 and P4) in a range of solutions is shown in Table 6.9. In addition to the dilutions of sea water four treatments were applied: the fruits were either washed for 24 hours in tap water or not, and either prechilled at 5°C for 24 hours or not. All germination was tested at 25°C. 100 fruits per cell were used, (a total of 2,400 fruits/population).

The general pattern of germination (with some exceptional occurrences such as the germination of one high marsh fruit in pure sea water) is consistent with that of the earlier trials. There is, however, no evidence of a differential effect on fruit from P1 and P4 - the effect of sea water being to depress germination equally in both populations. Although prechilling increased the average germination, from 12.0% in untreated fruits to 15.9% in

Table 6.9	Germination in Artificial Sea Water. The effect of washing and prechilling on high and low marsh											
	prechilled						not prechilled					
Dilution ASW	0	20	40	60	80	100	0	20	40	60	80	100
P4 (low marsh)												
washed	64	36	4	0	4	0	49	28	19	2	2	0
unwashed	65	41	13	7	0	1	43	34	8	7	1	0
P1 (high marsh)												
washed	36	21	3	0	1	0	33	10	0	2	0	1
unwashed	27	17	12	0	0	0	24	13	14	0	0	0

treated fruits (largely due to an increase in high marsh germinants), the effect of washing was negligible, increasing the average performance from 13.6% in unwashed to only 14.4% in washed fruits. The purpose of washing had been to remove, by dissolving them, any salts from the testa which may have prevented or delayed germination.

In addition to decreasing the numbers of germinants saline solutions may also delay their time of germination as compared with that in pure water. Although a large number of fruits swell soon after immersion in saline solutions in most cases the emergence of the radicle does not follow. If these swollen fruits are removed and transferred to dishes containing fresh water, germination is often very quick to follow this transfer. As it has not been tested there is no evidence on whether the overall performance of any population in fresh water is improved by an initial soaking in sea water, as found for a number of populations of Limonium vulgare by Boorman (1966 and 1968).

The results obtained above confirm the findings of Poma (1922), Montfort and Brandup (1927), Chapman (1942) and some others for A. tripolium in demonstrating that germination is highest in fresh water and decreases with addition of salt. The findings of Schratz (1936) and Stocker (1928) that the origin of the parent plant (the salinity of its habitat) affects germination are also underlined. (This phenomenon was also discovered in Limonium species by Boorman (1966)).

IV The effect of fruit size.

Fruits from Beach Point populations on Scolt Head Island (one of the very few populations containing both large and small-fruited plants) were used to test the effect on germination rate of initial fruit size. One head each from ten large-fruited and ten small-fruited plants was sown, a sample of 10 fruits from each head being weighed. A total of 125 large, and 140 small fruits was tested. The mean weights of the ten ten-fruit samples were 22.12±1.8 mgs. and 8.12±1.0 mgs. respectively. The fruits were germinated in petri dishes in distilled water in laboratory conditions at 18°C, and the number of germinated fruits scored daily for 14 days. These numbers, expressed as %s of the total large- or small-fruit population, are given in Table 6.10.

The results, graphed in Fig. 6.5, indicate that a greater

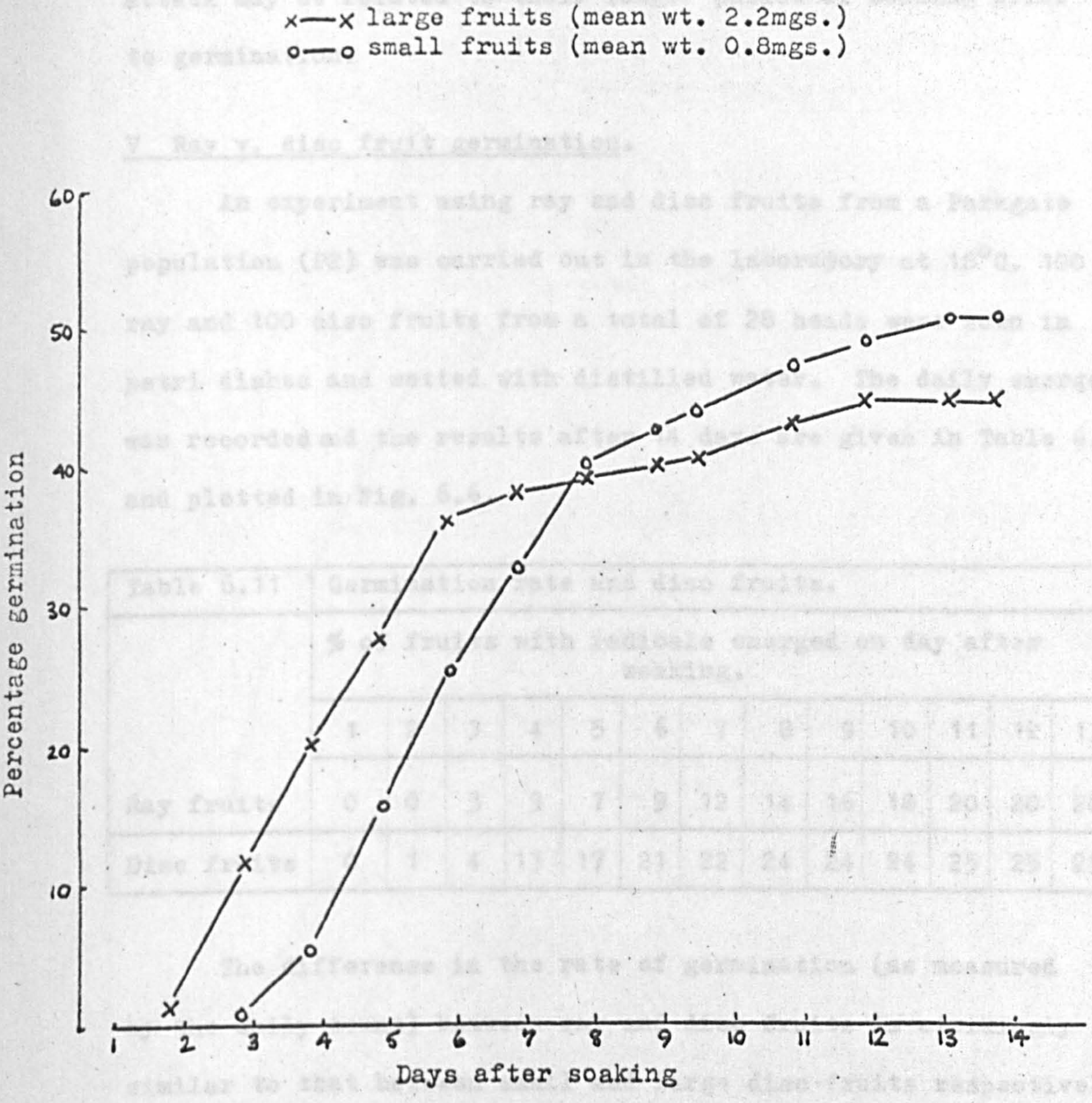
Table 6.10	Germination rate in large and small fruits						
	% of fruits with radicals emerged on day after soaking.						
Day	1	2	3	4	5	6	7
Large fruits ($\bar{X}$ wt.2.2mgs)	0	1.6	11.2	20.0	27.2	36.0	37.6
Small fruits ($\bar{x}$ wt.0.8mgs)	0	0	0.7	5.0	15.7	25.0	32.8
Day	8	9	10	11	12	13	14
Large fruits	39.2	40.0	40.8	43.2	44.8	44.8	44.8
Small fruits	40.0	42.8	43.6	47.1	49.3	50.7	50.7

proportion of large fruits than small fruits germinate rapidly, so that, for example, after 4 days 20% of the total large-fruit sample had germinated in contrast to only 5% of the small-fruit sample. Unfortunately trials to further test this difference in rate were impossible due to a shortage of suitable fruits.

After 14 days most of the seedlings were transferred to pots as part of an experiment described in Chapter 8. However further germination occurred in the remaining fruits and the final figures after 28 days were :- (classes (i) - (iv) as for earlier trials).

	class (i)	class (ii)	class (iii)	class (iv)
Large fruits	65.6	0.8	7.2	26.4
Small fruits	56.4	2.1	28.6	12.8

Fig. 6.5. Germination rate - large and small fruits. (S. Bch.)



The apparently greater susceptibility of small fruits to fungal attack may be related to their longer period of soaking prior to germination.

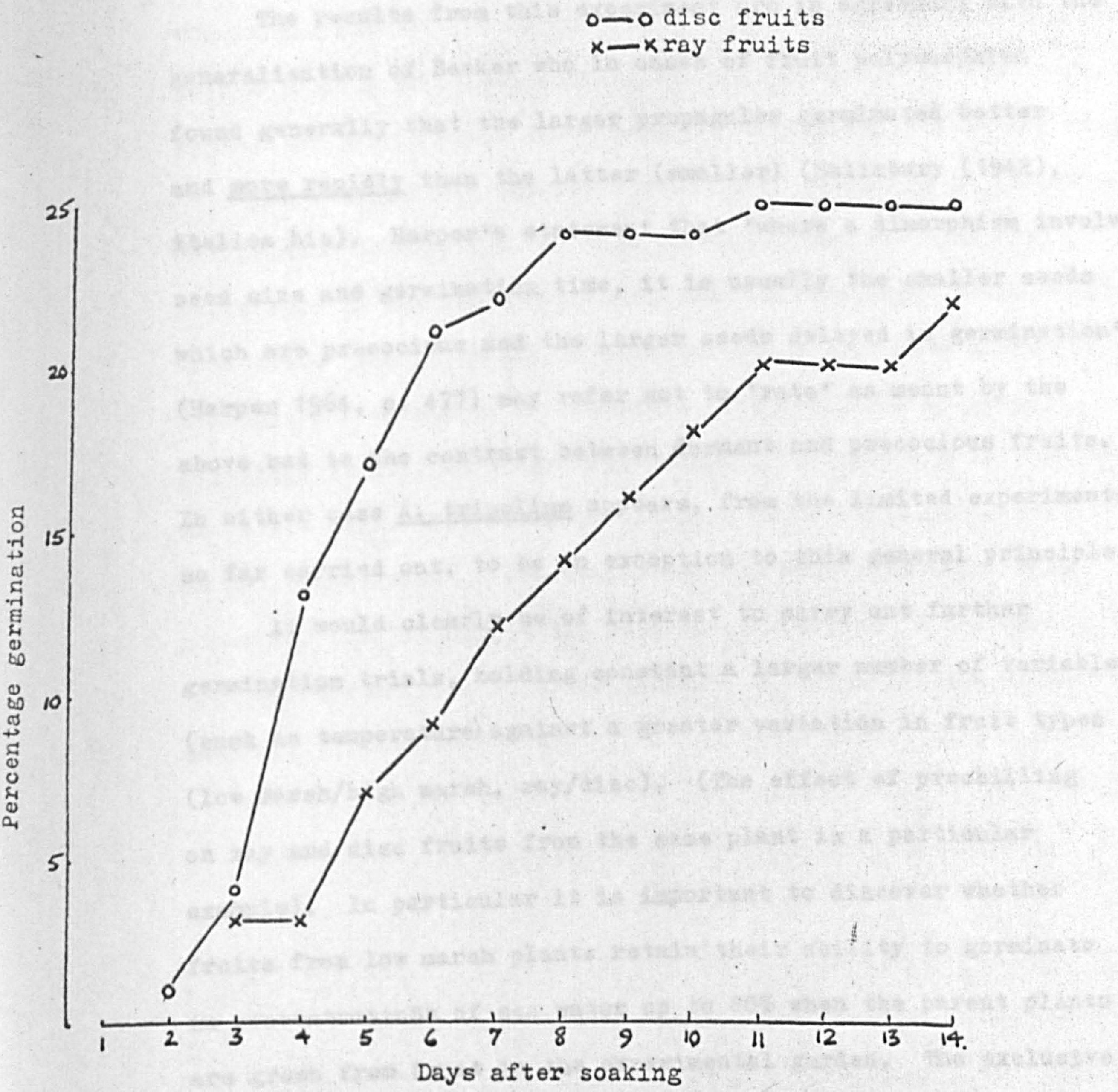
V Ray v. disc fruit germination.

An experiment using ray and disc fruits from a Parkgate population (P2) was carried out in the laboratory at 18°C. 100 ray and 100 disc fruits from a total of 28 heads were sown in petri dishes and wetted with distilled water. The daily emergence was recorded and the results after 14 days are given in Table 6.11 and plotted in Fig. 6.6.

Table 6.11	Germination rate and disc fruits.													
	% of fruits with radicals emerged on day after soaking.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Ray fruits	0	0	3	3	7	9	12	14	16	18	20	20	20	22
Disc fruits	0	1	4	13	17	21	22	24	24	24	25	25	25	25

The difference in the rate of germination (as measured by the daily count) between ray and disc fruits is strikingly similar to that between small and large disc fruits respectively (see IV above) - the smaller ray fruit sample germinating at a slower rate than that of the larger disc fruits. (The overall low germination may have been due to the accidental selection of immature fruits as in order to be sure of having ray or disc

Fig. 6.6. Germination rate in ray and disc fruits (P2 population).



fruits, only fruits to which the withered perianth was still attached were used for this trial).

The results from this experiment are in agreement with the generalisation of Becker who in cases of fruit polymorphism found generally that the larger propagules germinated better and *more rapidly* than the latter (smaller) (Salisbury (1942), italics his). Harper's statement that 'where a dimorphism involves seed size and germination time, it is usually the smaller seeds which are precocious and the larger seeds delayed in germination' (Harper 1964, p. 477) may refer not to 'rate' as meant by the above but to the contrast between dormant and precocious fruits. In either case *A. tripolium* appears, from the limited experiments so far carried out, to be an exception to this general principle.

It would clearly be of interest to carry out further germination trials, holding constant a larger number of variables (such as temperature) against a greater variation in fruit types (low marsh/high marsh, ray/disc). (The effect of prechilling on ray and disc fruits from the same plant is a particular example). In particular it is important to discover whether fruits from low marsh plants retain their ability to germinate in concentrations of sea water up to 80% when the parent plants are grown from fruit in the experimental garden. The exclusive use of wild-collected fruits has meant that any genetic component of variation in germination requirements cannot be detected, and features such as the ability to germinate in saline solutions may be entirely determined by environmental influences during the development of the parent plant. Collections of fruits have been made from crossings carried out in the course of this investigation and it is hoped that these may be tested at a future date.

However certain important aspects of variation in germination performance between low and high marsh populations have been isolated. In particular the variation in dormancy-breaking requirements and that in response to saline media will have important ecologic consequences. For example, the inability of Aster tripolium fruits, whatever their origin, to germinate in undiluted sea water means that their germination in the wild may depend on the concurrence of low neap tides and high rainfall. This aspect of the biology of the species is examined in the next section, which deals with the dynamics of dispersal and establishment.

SUMMARY OF PART II

(1) The diploid number $2n = 18$ was counted in all plants from 14 British and 7 European populations (including the varieties *longicaulis*, *pannonicus* and *discoideus*). This confirmed all previous counts and is the first known count of British material.

(2) The karyotype was found to be strikingly uniform in all plants examined (from 7 British and 5 European populations, and including 3 varieties). An idiogram was constructed. Four chromosome pairs with median centromeres and five pairs with submedian centromeres, one pair of which have a distinctly satellited arm, were identified and labelled individually in all the cells examined. The karyotype in the species has a number of features thought to be evolutionarily primitive.

(3) *Aster tripolium* is both cross-and self-fertile. Viable fruits were readily obtained using two different methods of controlled cross pollination. Selfing also occurred in enclosed bags but there was a reduction in the amount of good seed set in these bags under greenhouse conditions. None of the plants tested were apomictic.

(4) Cross-fertility in the species can extend over considerable distances. In the two cases of crosses between an east and west coast British population F1 plants were produced which produced viable fruits on selfing. A cross between a plant from Lincolnshire and one from Austria has produced viable fruits and F1 plants (the fertility of which has not been measured).

(5) The amount of natural crossing is difficult to estimate. A number of mechanisms tending to promote outbreeding exist but their effectiveness is not known. They include structural mechanisms, protandry, and the presence of a large number of suitable pollen vectors.

(6) There was a cline from north to south in the percentage of plants in European populations which flowered during the first, second or a subsequent year from germination. The northernmost populations contained no first-or second-year flowering types, the southernmost populations contained all first-year flowering types, whilst those from central Europe may have contained a mixture of all three types.

(7) A similar variation in time to first flowering existed across the width of the maritime belt, a percentage of all high marsh populations flowering from fruit in one year as opposed to those from lower zones all of which flowered in the second or subsequent year (apart from a small number of first year flowerers in the Gib. Aster marsh population).

(8) The term 'paucienniaI' has been used to distinguish those plants which have no means of vegetative propagation. A high marsh population consisting largely of pauciennial types (Gib. Agr.) was extremely plastic with respect to flowering behaviour. Under a range of treatments plants flowered during their first year, reflowered the following year and then died (greenhouse), failed to flower the first year (tidal treatment, poor soil treatment),

or flowered for the first time during the second year (poor soil treatment). Although fruits from the same population were used (and not clonal material) the variation was consistent within treatments and may be regarded as plasticity of the character 'time taken to first flowering'. Mid and low marsh populations were less plastic in this respect although first year flowering in normally second year flowering types was induced under extreme conditions.

(9) Populations of plants which flowered during the first year from fruit flowered, on average, later and less synchronously than those consisting of 2nd or 3rd year flowering types. These characters (late flowering and uneven flowering) could be used to estimate the age at first flowering of plants which have been collected from the wild as plants. In some cases the appearance of wild-collected plants in the field was a guide to the likelihood of their flowering during the year of collection. (It had to be established by other means whether they were seedlings or had developed from lateral buds of a mature plant). The variation in flowering time of first-and second-year flowering types may explain the variation in flowering time of plants (of unknown age) observed as one proceeds from high to low marshes in a given area of the coastal zone.

(10) The fruits of <u>Aster tripolium</u> are dimorphic, the ray florets producing smaller fruits than those produced by the disc florets. Apart from a difference in germination rate the difference

in size is the only character separating ray and disc fruits.

(11) There was a cline in the population mean size of the disc fruits in populations along a gradient from high to low marshes in three separate areas (Gibraltar Point, Scolt Head and Parkgate). This cline (measured in terms of fruit weight, which is correlated with fruit length) remained consistent for the east coast populations from both areas when these were ranked according to the classification proposed in Part I. In these two areas the cline in disc fruit weight was accompanied by an inverse cline in disc fruit number. Fruit weight and number were not correlated within individual populations, although the population means for these parameters were significantly correlated. At Parkgate there was no significant variation in fruit number.

(12) When grown under a range of conditions the population mean disc fruit size did not differ significantly for a given population. This was regarded as an indication of the stability of fruit size. The mean number of fruits/head also varied little in the same population grown under a range of conditions. The stability of fruit size in particular was, in view of the clinal variation in three separate areas, felt to be of ecological and adaptive significance.

(13) The number of fruiting heads per plant varied considerably in response to different treatments. This variation was related to the production of axillary branches. Poor soil and competitive conditions significantly reduced the amount of lateral branching

and thus head production.

(14) The reproductive output of plants was largely dependent on head production. With equal head production high marsh populations on the east coast areas produced many small fruits in contrast to low marsh populations which produced fewer, larger fruits. When the actual variation in head production under standard cold house conditions (and the different proportions of inviable fruits produced) was taken into account however, high marsh populations produced, at each flowering, fewer viable fruits than low marsh populations.

(15) The percentage germination in all fruit samples tested under both conditions was increased when the fruits were germinated in the dark (as opposed to the light). This increase varied from population to population but was independent of pretreatment conditions.

(16) Using Gibraltar Point populations there was an increase from low marsh to high marsh populations in the percentage of wild collected fruits requiring a cold treatment to break dormancy. This was accompanied by an increase in the time of pretreatment required to improve the germination level. All other low marsh fruits tested were precocious germinators and one other high marsh population tested (P1) showed innate dormancy.

(17) The variation in dormancy-breaking requirement varied from population to population but not from small to large fruits within the same population. Two groups of fruit (small and large) from Beach Point differed in their germination rate, the smaller

fruits germinating more slowly as indicated by the number of fruits to have germinated by a given day after soaking. This variation was of the same order as that between ray (small) and disc (large) fruits from the same population.

(18) The germination of wild-collected fruits in dilutions of artificial sea water suggested that their performance was related to the origin of the parent plants. Thus low marsh fruits were better able to germinate in high concentrations of sea water than were high marsh fruits, although all types showed maximum germination in distilled water. This confirmed previous work. The effect of sea water was to reduce the amount of germination and to delay the time of germination. Washing the fruits in one experiment did not significantly increase the overall germination rate.

PART III

DISPERSAL AND ESTABLISHMENT

CHAPTER 7. THE EFFECT OF TIDAL TREATMENT

I The tides as a selective agency.

The effects of periodic tidal submergence on higher plants may be considered under three heads. These are the effects due to (1) actual submergence by sea water, (2) the saline nature of sea water, and (3) the movement of sea water.

(1) When plants are submerged photosynthesis may be reduced or cease due to diminished carbon dioxide supply, a reduction in the amount of incident light, or an alteration in its spectral composition. Transpiration will be affected by stomatal closure, the root environment may be altered, and the oxygen supply diminished so that normal respiration is affected.

(2) High concentrations of salts in the soil have a profound effect on many aspects of growth, including ion uptake, basal metabolism, respiration, and seed germination.

(3) The incoming tide may exert a purely mechanical effect on the plant, which may be particularly important in the establishment of seedlings, and may help to disperse seeds.

Controlled experiments on (1) have not been included in this study - there is, in fact, little experimental evidence for the sort of response to submergence mentioned above (and by Chapman 1964), many of which are probably based on purely theoretical considerations.

Some of the effects of (2) and (3) have been tested experimentally and their relevance to the selection of suitable biotypes in the low and mid marsh environments is dealt with in the following chapters. As an introduction to this work an experiment attempting to measure the gross effects of simulated tidal treatments is described below.

II The effect of experimental 'tide' treatment.

An experiment was set up to test the effect of simulated tidal treatments on the germination and growth of selected populations. Wild-collected fruits from four Gibraltar Point and three Scolt Head populations were sown in small plastic trays on a 7:3:2 mixture of Audlem soil, peat and sand. Fifty fruits from a single population, each from a different plant (as far as could be determined in the field) were sown in each tray. Four trays of each of the seven populations were prepared. The fruits were covered with a thin sprinkling of sand to prevent their being washed away during the experimental treatment. The trays, which were prepared on February 28th (1967) and separated into four groups, were each subjected to a different salt-water regime for a period of 100 days, during which time they remained in an unheated greenhouse (average temperature 48°F, range 38-76°F).

The four levels of treatment were based on calculated equivalents of the 24', 22', 20.5' and 19' levels with respect to tide heights at Immingham. (Using Fig. VI.3). Thus plants receiving treatment 1(≡ 24' tide level) remained free of tidal submergence for the whole period, whilst those receiving treatments 2, 3 and 4 were subjected to a 'tide' on each day during the 100 days when it would be expected that they would be covered by a tide in nature (not allowing for variation from the predicted heights). Treatment 4 is equivalent to that 1' below the 'critical' level, treatment 3 to that 6" above it. Each 'tide' consisted of a gentle watering, with a small plastic watering can fitted with a long narrow rose, of a constant amount of sea water (800mls./ treatment). The sea water was stored in an aerated carboy in the greenhouse. Exceptions to the natural regimes were (1) the trays were given only one 'tide' per tidal day, and this constantly in the early morning, and (2) that, irrespective of the tidal programme, they received a daily watering of 800 mls./7 trays of rainwater in the early evening. The extra technical difficulties involved in attempting to give 'tides' at the exact hour of their natural counterparts, and of randomly varying the amount of rain waterings and their coincidence with the tides, were felt to be greater than could be justified by the possibly greater accuracy of the result. (These variables may be extremely important however. Experiments on germination (Ch. 6) and seedling growth (Ch. 8) suggest that the diluting effect of heavy spring showers, particularly when coincident with the beginning of a number of days of continuous emersion is possibly important for seedling establishment).

The number of plants in each tray was counted at regular intervals, and the surviving plants were harvested on the 101st day

Photographs of the plants prior to harvesting are given in Fig. 7.1 (Gibraltar Point populations) and Fig. 7.2 (Scolt Head populations).

(a) Survival.

The number of plants surviving after 100 days was not in all cases equal to the number of fruits which germinated, due to the death of some seedlings. Seedlings which died did so mainly at the cotyledon stage and mainly in treatments 3 and 4. The percentage of plants (of the 50 fruits sown) surviving after 100 days in each tray is given in Table 7.1.

Table 7.1 Tidal treatment. % survival.

	Population						
	Gibraltar Point				Scolt Head		
Treatment	Spa	Ast	Lim	Agr	Cob	Bch	Srl
1	6	30	32	40	8	20	52
2	26	12	22	34	16	36	48
3	14	16	28	26	20	42	38
4	24	20	20	20	10	6	6

There is a between-population trend in the % survival of plants under treatment 1, as can be seen from Fig. 7.3 in which the populations are ranked according to marsh type from low to high. Despite the consistent pattern, in relation to different treatments, of within-population variation in the two highest (Agr. and Srl) and the two lowest (Cob. and Spa.) marshes seen

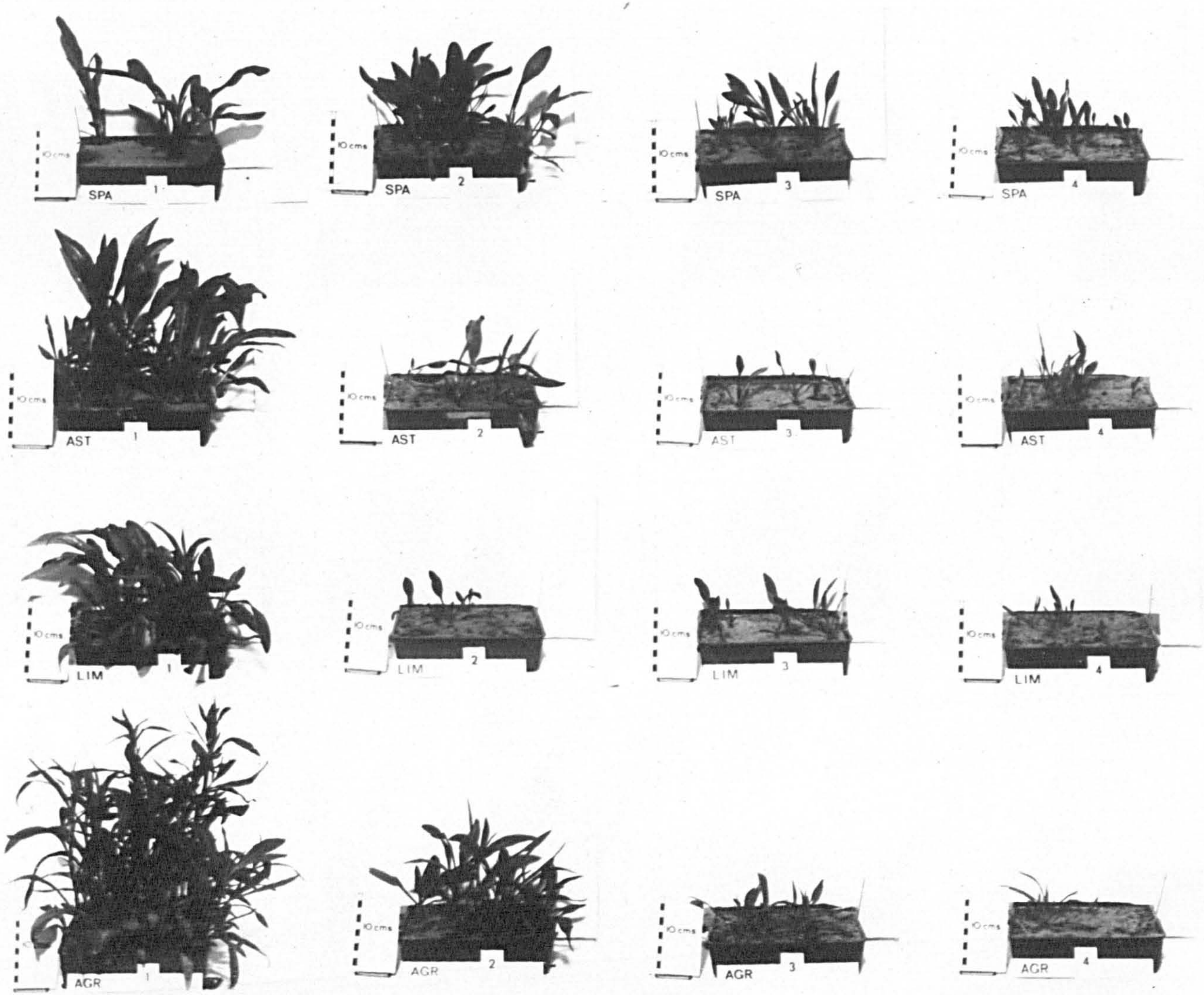


Fig 7.1 Tidal treatment experiment - Gib. Pt. popns. prior to harvesting.

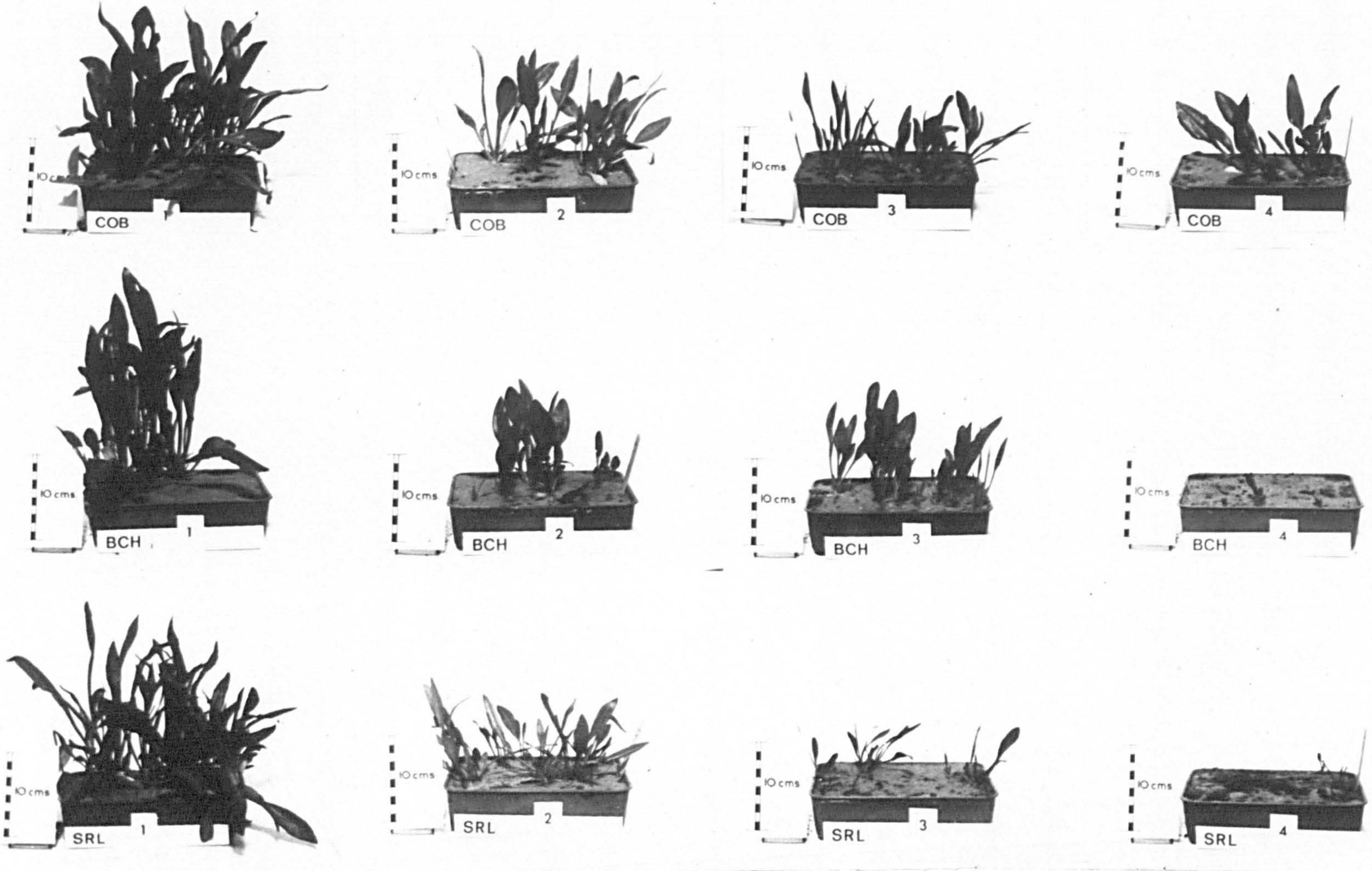


Fig 7.2 Tidal treatment experiment - Scolt Head popns. prior to harvesting.

in Table 7.1, there is, when the actual number of survivors is considered, no reverse trend in treatment 4 (Fig. 7.3). Thus, although the relative number of high marsh types surviving under full tidal treatment is lower than under completely, or relatively, tide-free conditions, the actual numbers are similar to those for low marsh types.

The relatively low germination and survival of low marsh plants under tide-free treatment is not consistent with their high germination rate in non saline water under Petri plate conditions. Petri plate experiments were run concurrently with this experiment and continued to give high germination of Spa. and Cob. fruits. The conditions in periodically-watered soils may be very different to those obtaining in petri experiments on germination (described in Ch. 6).

The pattern of germination in relation to the periods of tidal watering is particularly interesting. Figs. 7.4 and 7.5 are graphs* of the actual totals of plant numbers as counted at intervals during the course of the experiment. Two observations can be made. First it can be seen that the fruits of low marsh populations reach an early peak of germination to which they do not add, whereas there is a more gradual increase in the number of germinating fruits in high marsh populations. This is consistent

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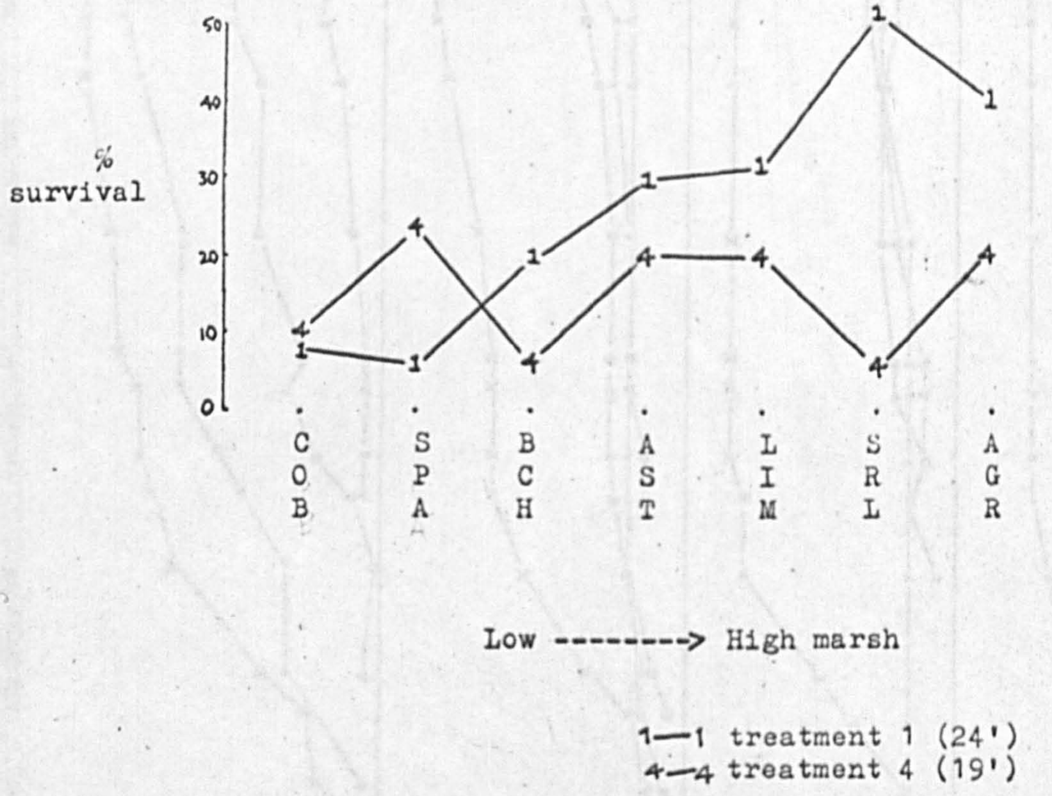
* These are not true 'graphs' in that it is not strictly permissible to join the points by straight lines. The actual number of plants present at a given time between two observations cannot be extrapolated from the 'graph' because the disappearance of seedlings was discontinuous and unpredictable.

Fig 7.3 Tidal treatment experiment. Percentage survival and marsh type (Treatments 1 and 4 only).



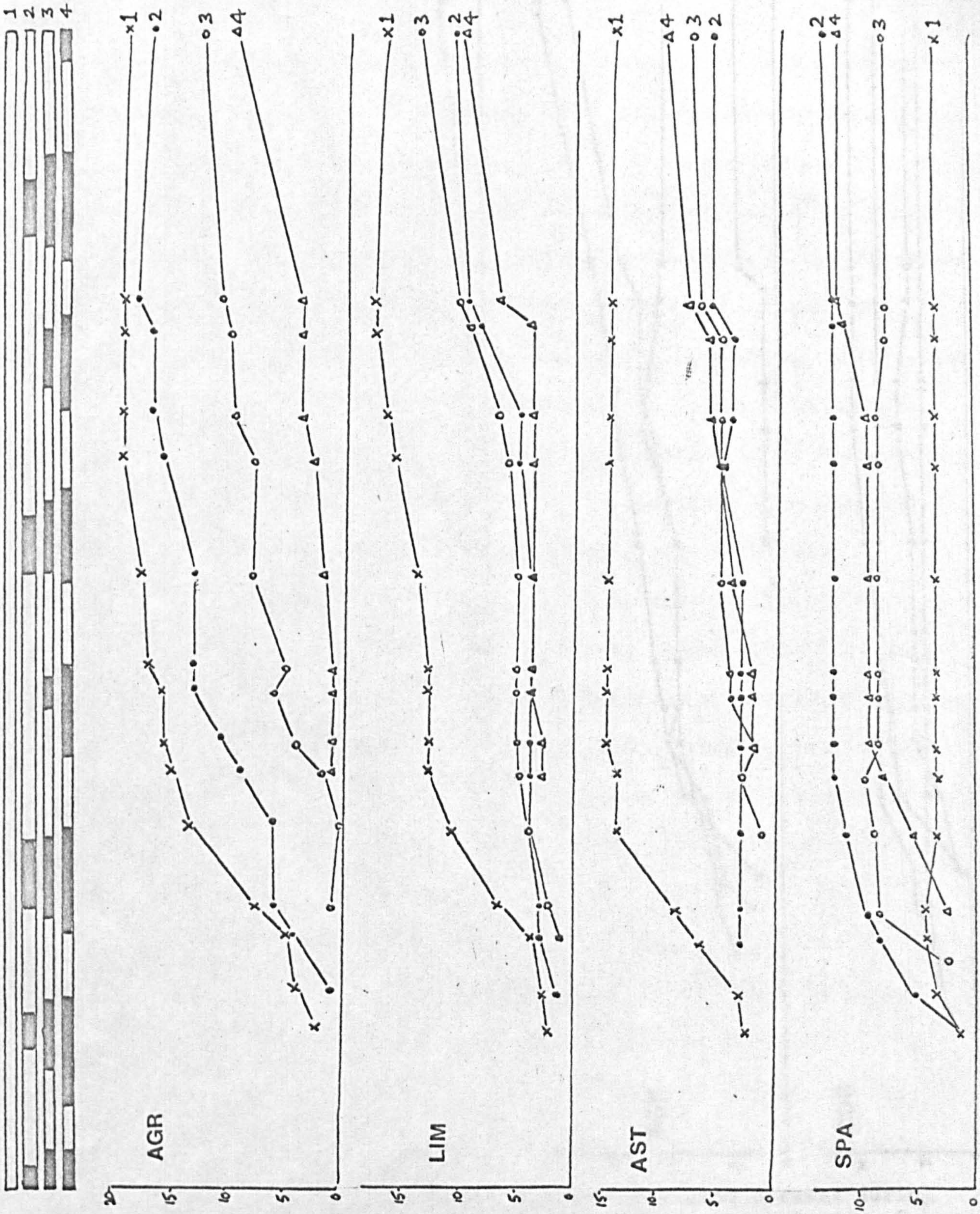


Fig 7.4 Tidal treatment experiment. Numbers of plants present during 100-day period, Gib.Pt. Popns (Treatments 1 ×—×, 2 •—•, 3 ∘—∘, 4 △—△)(Tide days ▬, non-tide days ▭ , see text).

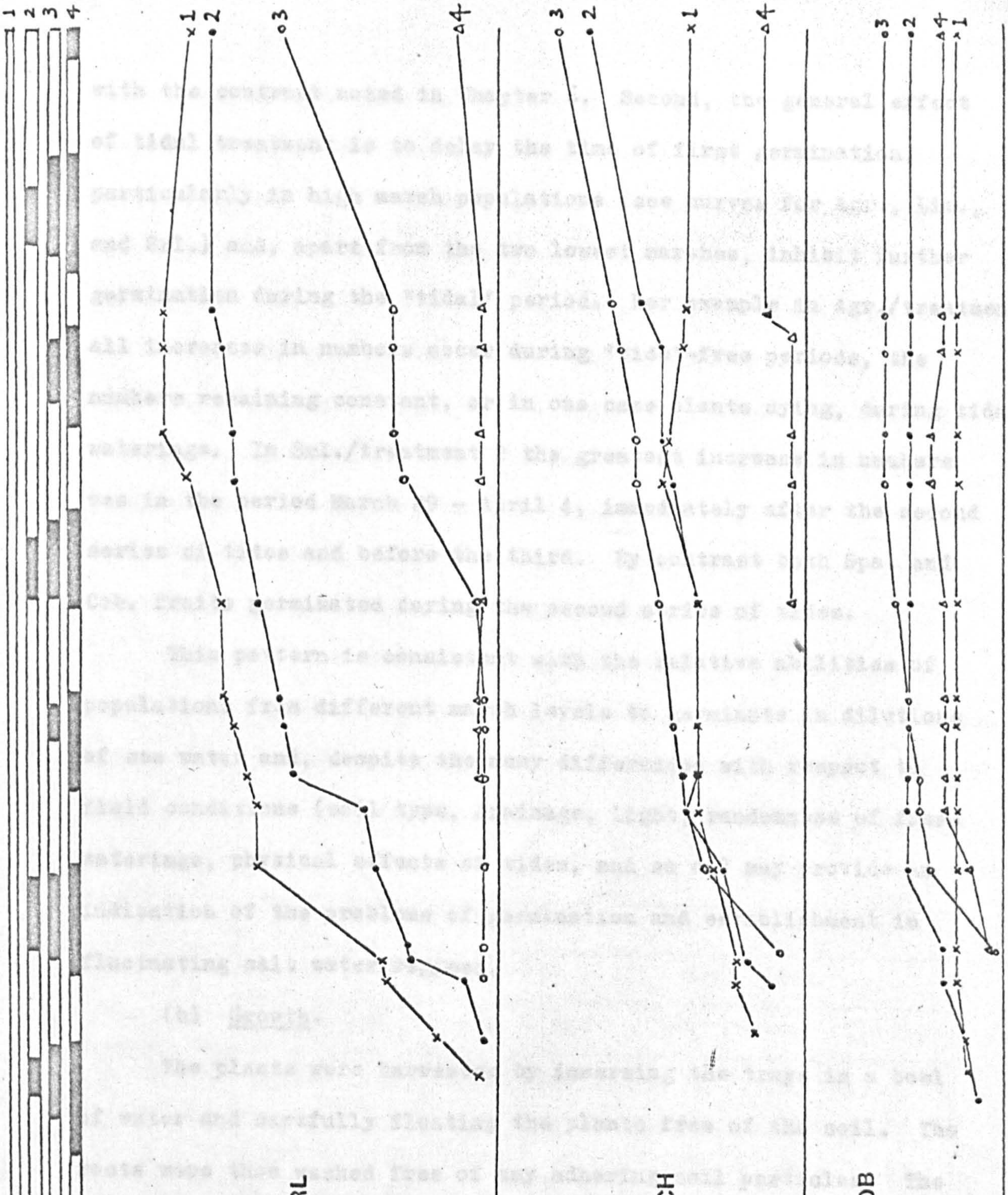


Fig 7.5 Tidal treatment experiment. Numbers of plants present during 100-day period Scolt Head popns (Treatments 1 ×—×, 2 •—•, 3 o—o, 4 Δ—Δ) (Tide days ▬, non-tide days ▭, see text).

with the contrast noted in Chapter 6. Second, the general effect of tidal treatment is to delay the time of first germination, particularly in high marsh populations (see curves for Agr., Lim., and Srl.) and, apart from the two lowest marshes, inhibit further germination during the 'tidal' period. For example in Agr./treatment 3 all increases in numbers occur during 'tide'-free periods, the numbers remaining constant, or in one case plants dying, during tidal waterings. In Srl./treatment 2 the greatest increase in numbers was in the period March 29 - April 4, immediately after the second series of tides and before the third. By contrast both Spa. and Cob. fruits germinated during the second series of tides.

This pattern is consistent with the relative abilities of populations from different marsh levels to germinate in dilutions of sea water and, despite the many differences with respect to field conditions (soil type, drainage, light, randomness of fresh waterings, physical effects of tides, and so on) may provide an indication of the problems of germination and establishment in fluctuating salt water regimes.

(b) <u>Growth</u>.

The plants were harvested by immersing the trays in a bowl of water and carefully floating the plants free of the soil. The roots were then washed free of any adhering soil particles. The number of leaves on each plant was noted and roots and tops were oven dried separately in folded paper towels for 36 hours at 95°C. Although at the time leaf number, the dry weight of roots and the dry weight of tops were calculated individually for all plants beyond the cotyledon stage, it is proposed that only total 'biomass' (the total dry weight of all plants/tray) be considered. The total biomass can not be compared statistically as a design deficiency has resulted in no true replication. However an account of the data is given below and it will be seen from this, and especially from a study of the photographs in Figs. 7.1 and 7.2, that there is good agreement between the types of response by populations from similar marsh levels. Further, there is evidence of a trend between treatments and, in the absence of an objective test these provide evidence of the reliability of the results.

The total biomass data are given in Table 7.2 (based on dry weights of individual roots and tops weighed on a top-loading balance correct to the nearest 0.005 gm.). There is an interesting within-population variation in response to tidal treatment. Populations from the two highest marshes (Agr. and Srl.) gave lower yields as the amount of tidal treatment increased and the other high marsh population (Lim.) gave comparatively poor yields in tidal

Table 7.2	Total biomass; tidal treatment expt. (wts. in gms.).						
	Gibraltar Point				Scolt Head		
Treatment	Spa	Ast	Lim	Agr	Cob	Bch	Srl
1	8.67	115.43	92.48	444.40	31.78	73.80	245.63
2	70.01	7.77	4.40	71.40	32.92	36.68	64.79
3	11.52	4.92	13.16	14.24	20.20	55.60	13.68
4	14.64	6.30	2.35	4.28	10.43	0.19	0.02

in tidal as opposed to tide free conditions. At the other extreme the two lowest marsh populations (Spa. and Cob.) showed an increase in yield under tidal treatment 2 and, although yield was depressed in treatments 3 and 4 was relatively less reduced than in the other populations. The Bch. population was severely depressed by treatment 4 (corresponding to that for a height 1' below the calculated 'critical' level - equivalent to 20' C.D.) but was relatively less depressed in treatments 2 and 3 than the Ast. population.

Between-population comparisons should not be drawn, because of the variation in relative growth rates (not measured) and the differences in the age structure of the different populations. These differences can be seen in Fig. 7.1 and 7.2. The trays of the pauciennial types, the Agr., Srl., and to a smaller degree the Lim., populations, contain both plants about to flower and newly-germinated seedlings; those of lower marsh populations are generally more uniform in age. Tidal treatment may have had an effect on growth similar to that on germination, plants growing more slowly during periods of tidal watering (there were several seedlings in treatments 3 and 4 which appeared to remain in the cotyledon stage for long periods of time).

Rather than compare total yields it may be more relevant to ask which populations produced, in respective treatments, individuals likely to survive to overwinter and/or produce fruit. A number of Srl., Agr., and, a smaller number of Lim., individuals would clearly have flowered in treatment 1 had this not been harvested (in early June). It is difficult to estimate how many individuals were likely to overwinter but it would appear that some plants from all populations may survive in treatment 1. On the other hand, in treatment 4, the chances of some Spa. and Cob. plants having developed sufficient reserves to overwinter are, judging from their appearance at harvesting, possibly greater than those of other populations (in this treatment).

CHAPTER 8. SEEDLING GROWTH.

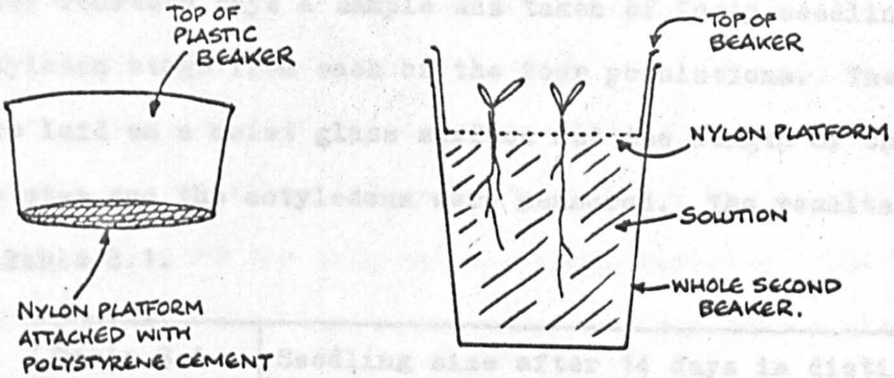
Recent work in causal plant ecology has indicated that the development of the seedling may be of paramount importance in the distribution and ecology of a species. A striking example is provided by the work of Davison on the 'calcicole' problem in parts of Derbyshire and where the vegetation exactly reflects the mosaic of acid and calcareous soils (Davison 1964). He shows that the germination of acid-soil and calcareous-soil species is largely unaffected by soil type and that the failure and death of some acid-soil species on calcareous soils occurs, through a number of largely physical factors, at the young seedling stage.

This, and other, examples have prompted a number of experiments investigating some of the factors affecting the growth and elimination of Aster tripolium seedlings. These experiments are reported in this and the following chapter. The experimental methods varied and are described for each experiment. Both the reports and discussion of the experiments dealt with in this chapter are grouped under two headings I, the effect of initial fruit size on seedling growth and II, the effect of salt concentration on seedling growth.

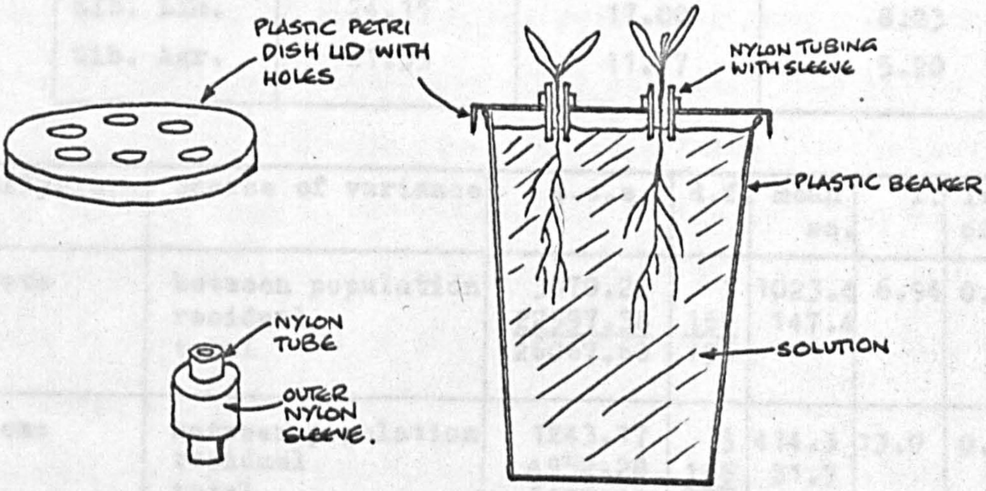
I The effect of initial fruit size.

Samples of wild collected fruits from the four Gibraltar Point populations were germinated on nylon platforms over distilled water in the 'apparatus' depicted in Fig. 8.1.(A). The fruit was

Fig 8.1



A. Apparatus for germination and early seedling growth.



B. Apparatus for seedling growth experiments.

prechilled in moist paper towels inside polythene bags in a refrigerated room for 48 hours. Sixteen beakers were set up in the germinator and approximately fifty fruits 'sown' in each beaker. After fourteen days a sample was taken of forty seedlings in the cotyledon stage from each of the four populations. The seedlings were laid on a moist glass surface and the length of the radicle, the stem and the cotyledons were measured. The results are given in Table 8.1.

Table 8.1	Seedling size after 14 days in distilled water - Gibraltar Point populations. Data (mean of 40 individuals/population).		
	mean length root (mms.)	mean length stem (mms.)	mean length cotyledons.
Gib. Spa	32.35	19.35	10.92
Gib. Ast.	29.47	18.36	9.05
Gib. Lim.	24.15	17.80	8.23
Gib. Agr.	21.33	11.77	5.20

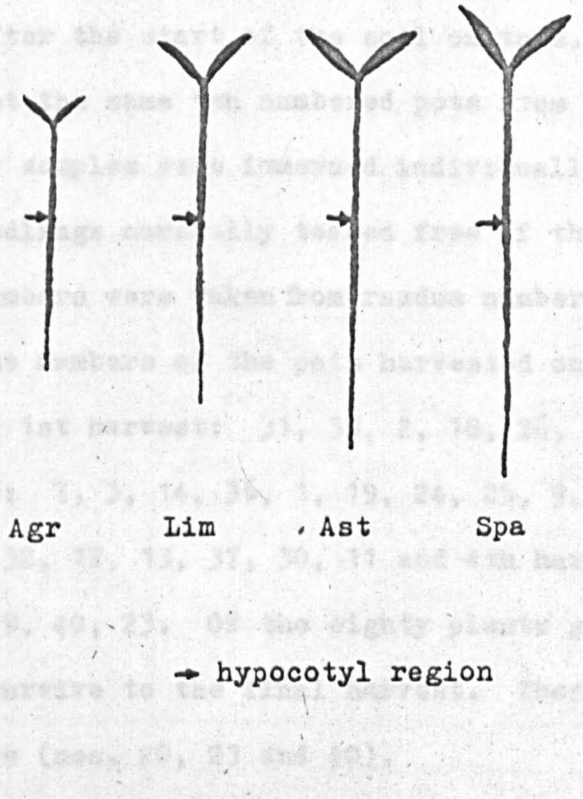
Analysis:-	Source of variance	s.o.s.	d.f.	mean sq.	f.	level of p.
roots	between population	3070.22	3	1023.4	6.94	0.001
	residual	22997.38	156	147.4		
	total	26067.60	159			
stems	between population	1243.17	3	414.3	13.0	0.001
	residual	4952.28	156	31.7		
	total	6195.45	159			
cotyledons	between population	682.34	3	227.4	50.9	0.001
	residual	696.06	156	4.4		
	total	1378.40				

and scale drawings of 'mean plants' after 14 days are given in Fig. 8.2.

There is a close correspondence between seedling size and the known cline in fruit weight for these populations (see Ch. 5). The original sample of fruit was random and the weights were not recorded, but the differences in seedling size suggest that seedling growth, at least without added nutrients and for a short period, largely reflected the original embryonic reserves. (Differences in germination time, which also occurred, may have tended to reinforce the differences in seedling size as no account was taken of the age of a seedling, except that the cotyledons must have opened out).

To eliminate the effect of fruit size, further experiments were carried out using both fruits of different sizes from the same marsh, and fruits of approximately equal size from ecologically different marshes. A subsample of 40 seedlings each of the 100 large and 100 small fruits from the Scolt Beach Point population which had been used in the germination trials (see Chapter 6) was transferred from germination beakers after 14 days to 3 inch pots filled with Audlem soil to which a small quantity of Fisons G.H.5 fertiliser had been added, (1 seedling/pot). Each pot was marked with the pot number (1-40), the fruit size (large or small) and the number of the source plant. To aid early recovery from transplanting the randomly ordered pots were stood in large plastic trays filled with water to the soil level. The water was allowed

Fig 8.2 'Mean' plants after 18 days in distilled water.
(Wild collected fruits)



to evaporate away until the trays contained about a ½inch of water and this level was then kept constant by watering (no top watering was carried out). These conditions were considered to be optimum for root development. The entire experiment took place in an unheated greenhouse.

A random sample of the plants was harvested at 3-weekly intervals, (from Feb. 13th to May 13th 1967), the fourth and final harvest being 14 weeks after the initial soaking of the fruit and 12 weeks after the start of the soil culture. On the occasion of each harvest the same ten numbered pots from both the large and small fruit samples were immersed individually in a bowl of water and the seedlings carefully teased free of the soil and washed (the pot numbers were taken from random number tables in Fisher and Yates). The numbers of the pots harvested on each occasion were as follows: 1st harvest: 31, 39, 2, 18, 26, 10, 16, 28, 35, 15,. 2nd harvest: 7, 3, 14, 36, 1, 19, 24, 25, 9, 27. 3rd harvest: 33, 5, 21, 34, 32, 12, 13, 37, 30, 11 and 4th harvest: 38, 6, 22, 20 8, 4, 17, 29, 40, 23. Of the eighty plants grown only three plants failed to survive to the final harvest. These were all from small fruits (nos. 20, 23 and 40).

For the purpose of analysis the plants are dealt with as two groups; those developing from small fruits and those developing from large fruits, with mean weights per 10 fruit sample of 8.12± 1.0mgs. and 22.12± 1.81 mgs. respectively. As both the original samples of 100 fruits were comprised of ten subsamples of ten disc fruits from one head, and as the measure of fruit weight was based on the mean weight of each of these samples of ten fruits, the weights of individual fruits are not known. For this reason direct correlations of the various growth parameters with actual fruit weight, a technique employed for example by Thomas (1966) for within-population variation in Lolium perenne, are not possible. The comparison made here between the two extreme grades of very large and very small thus assumes that a constant relationship between fruit size and growth exists for fruits of any size within a population.

Before they were transferred to 3 inch pots the cotyledonous seedlings were measured after 14 days growth in distilled water. The results are presented in Table 8.2.

Table 8.2	Seedling size after 14 days in distilled water - Beach Point plants. Data (mean of 40 replicates) measured in mms.		
	mean root length	mean stem length	mean cotyledon length
large fruits (22.12 mgs/10 fruit)	33.95	15.40	10.13
small fruit (8.12 mgs/10 fruit)	5.85	7.15	4.55

Analysis:-	source of variance	s.o.s.	d.f	mean sq.	F.	level of p.
roots	between population residual total	15792.20 5035.00 20827.20	1 78 79	15792.20 64.55	244.65	0.001
stems	between population residual total	1361.25 760.70 2121.95	1 78 79	1361.25 9.75	139.61	0.001

Analysis:- (continued)	source of variance	s.o.s.	d.f.	mean sq.	F.	level of p.
cotyledons	between population residual total	621.61 186.28 807.89	1 78 79	621.61 2.38	261.18	0.001

The differences between the two groups in root, stem and cotyledon length after 14 days are highly significant.

At the first harvest, taken 5 weeks after the original soaking and 3 weeks after transplanting to soil culture, a number of measures of growth were made. These were the length of the main root, the number of lateral roots, the length of each lateral root (giving total root length), root dry weight, the length of the stem, the length of the cotyledons, the number of true leaves plus the area of each leaf (obtained by tracing the leaf outline onto graph paper) and the dry weight of the tops (oven dried at 95°C for 36 hours). The data obtained is summarised in Table 8.3 (overleaf).

At this stage the differences in mean values for all the measures of growth between large and small fruited plants were all highly significant.

The measurement of all the growth parameters obtained at the first harvest proved impossible at harvests 2, 3 and 4 because of the size of the plants. On these occasions root dry weight, tops dry weight, and leaf number were measured. The results are summarised in Table 8.4.

The plants from large and small fruits are significantly different in terms of dry weight up to the third harvest, despite

Table 8.3	First harvest (after 3 weeks soil culture). Beach Point plants.	
Mean values / plant	plants from large fruit	plants from small fruit
Roots		
length main root (mms)	56.9± 6.19	17.9 ± 5.16
number lateral roots	19.1± 1.60	7.3 ± 1.18
total root length (mms)	283.7±24.98	82.2 ±16.31
dry wt. roots (mgs)	1.64± 0.13	0.72± 0.09
tops		
length stem (mms)	17.4 ± 1.14	8.6 ± 0.68
length cotyledon (mms)	17.3 ± 0.69	11.1 ± 0.62
number of true leaves	3.6 ± 0.16	2.6 ± 0.26
leaf area/plant (mms^2)	190.3 ±19.89	47.0 ±16.99
dry wt. tops (mgs)	8.29± 0.76	2.52± 0.40
TOTAL		
total dry wt. (mgs)	9.93± 0.84	3.24± 0.49

All significantly different at 0.01 level of p or below.

an increase in variance accompanying the increase in mean weight. Although at the first harvest the plants from large fruit had significantly more true leaves (see Table 8,3), (the variance ratio $F = 10.41$ where $n_1 = 1$, $n_2 = 18$ and p between 0.01 and 0.001), this difference soon disappears (see Fig. 8.4). The small-fruit plants are in every sense smaller-sized versions of the large-fruit ones up to the thrd harvest; leaf number, and presumably rate of leaf appearance remaining more or less similar in both groups

Table 8.4. Second, third and fourth harvests. Beach Point plants

Harvest	Values - plants from large fruit			Values - plants from small fruit			level of p.
		mean ± s.e.	variance	mean ± s.e.	variance	t.	
2nd harvest	dry wt. roots	17.62 ± 3.29	108.59	6.19 ± 1.99	39.89	2.96	.01-.001
(after 6 weeks soil culture)	dry wt. tops	92.82 ± 16.98	2881.89	36.27 ± 10.07	1013.03	2.87	.01-.001
	total dry wt.	110.44 ± 20.07	4016.31	42.46 ± 12.05	1451.01	2.91	.01-.001
	leaf number	8.6 ± 0.96	9.28	9.0 ± 1.18	14.00	-	n.s.
3rd harvest	dry wt. roots	137.41 ± 37.76	14241.48	64.73 ± 13.84	1914.83	1.81	.1-.05
(after 9 weeks soil culture)	dry wt. tops	417.24 ± 93.41	87134.06	266.61 ± 52.31	27331.16	1.41	n.s.
	total dry wt.	554.65 ± 129.08	166396.07	331.34 ± 64.77	41903.61	1.55	n.s.
	leaf number	19.1 ± 2.09	43.68	18.0 ± 2.04	41.6	-	n.s.
4th harvest	dry wt. roots	515.67 ± 161.81	261471.13	531.20 ± 125.42	110477.90	0.07	n.s.
(after 12 weeks soil culture)	dry wt. tops	760.61 ± 164.75	271065.29	744.60 ± 146.99	151735.80	0.01	n.s.
	total dry wt.	1276.28 ± 318.98	1016055.40	1275.80 ± 266.68	499435.11	0.001	n.s.
	leaf number	19.9 ± 2.24	50.48	19.4 ± 1.61	25.80	-	-

Dry weights in mgms.

n.s. = not significant

in all but the first harvest.

The difference in initial fruit size might have continued to exert an influence on seedling size beyond the 12 week duration of the experiment under less competitive conditions. The plants harvested finally were clearly suffering to various extents from the effects of pot binding and the large variance in the 3rd and final harvests may partly be a product of the arrival at different times at the critical stage where root overcrowding had a deleterious effect on growth. One would expect the effect of fruit size to decrease with time, although Thomas (1966) has shown that in <u>Lolium perenne</u> the long-term cumulative effect of seed weight is still considerable (interestingly the only character which appears to be unaffected by seed weight in <u>Lolium perenne</u> is rate of leaf appearance). Salisbury (1929) cites the work of Eitingen on Oaks, in which the differences of height and leaf area between trees grown from large and small acorns respectively were persistent over a period of at least 8 years.

Whatever the rate at which the effect of initial fruit size tends to become negligible it can be seen from the above experiment that fruit size exerts its greatest influence during the first 8 and possibly 10, weeks (see Figs. 8.3 (graph log. dry wt./harvest) and 8.6 (photographs of harvested plants)). As measured by the value of Students' t the null hypothesis that the harvested samples have equivalent mean total dry weights can be safely rejected up to a point after the 3rd harvest - see Fig. 8.5 (since the

Fig 8.3 Fruit size experiment. Dry weights large and small fruits at each harvest.

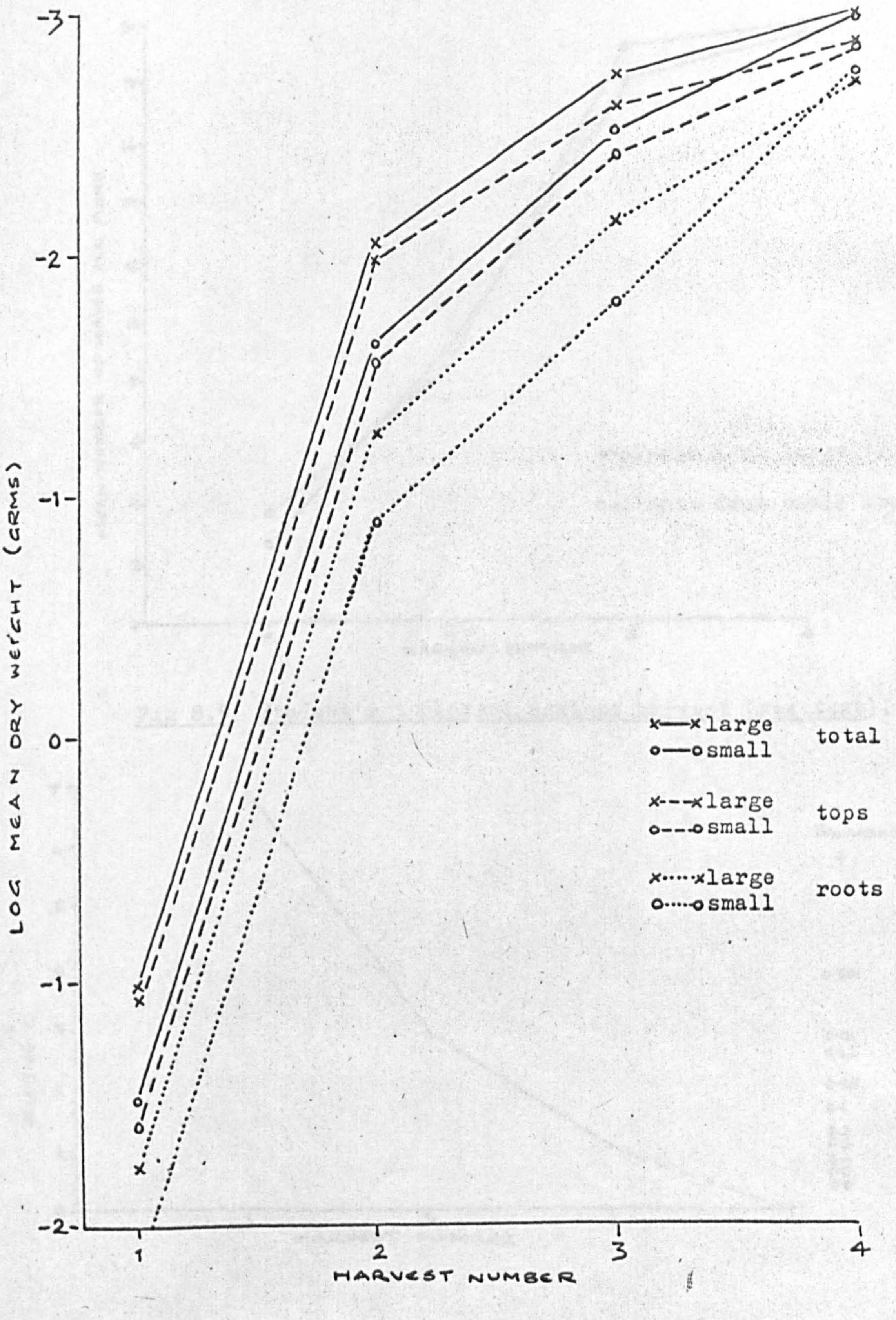


Fig 8.4 Number of leaves per plant in plants from large and small fruits.

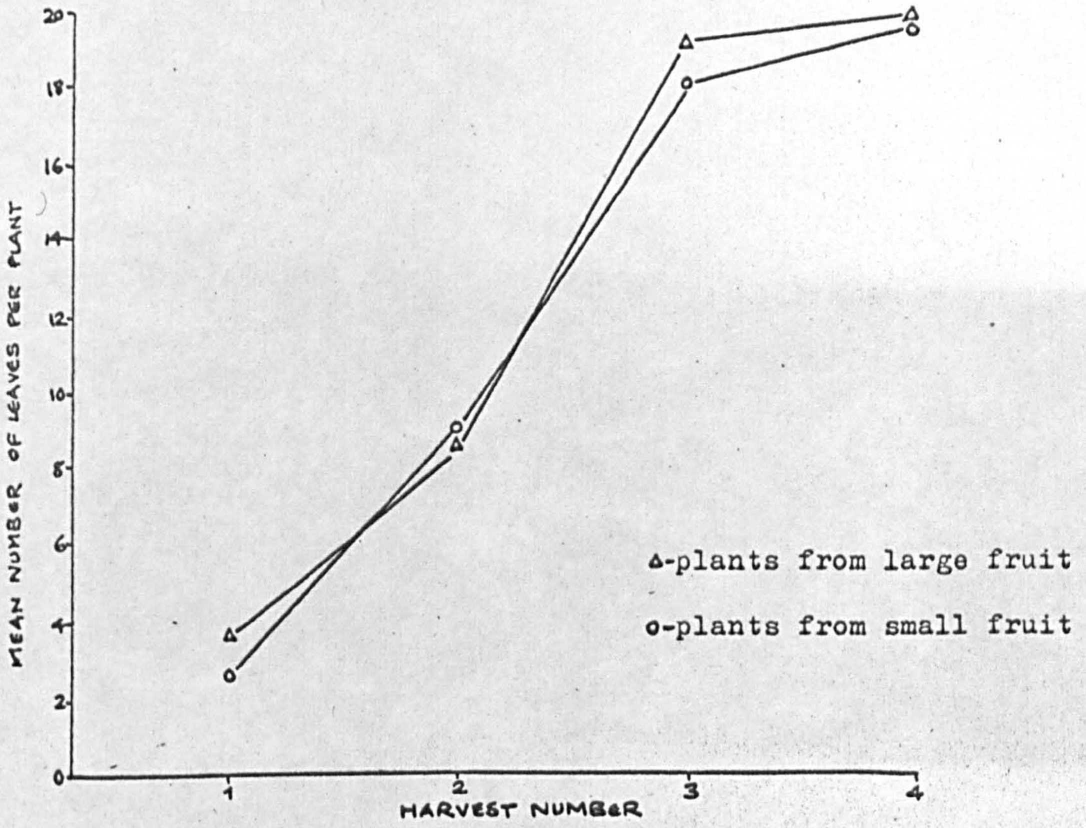
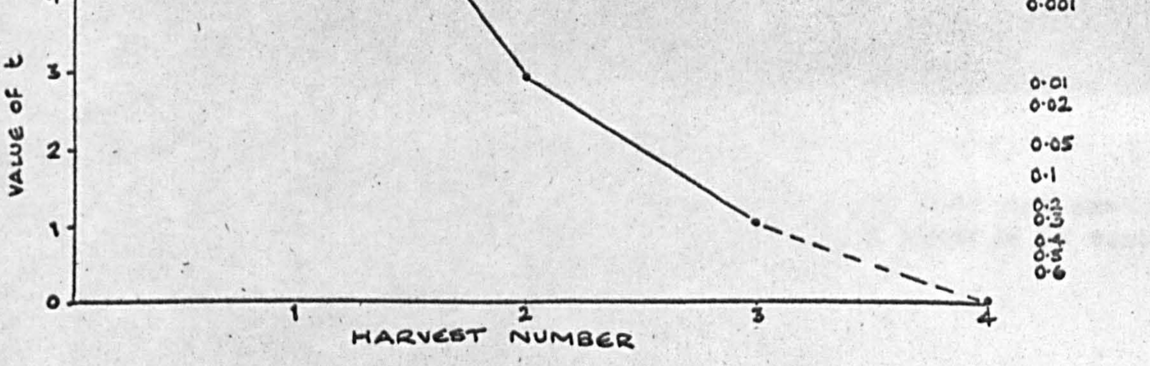


Fig 8.5 Student's t plotted against harvest (see text).



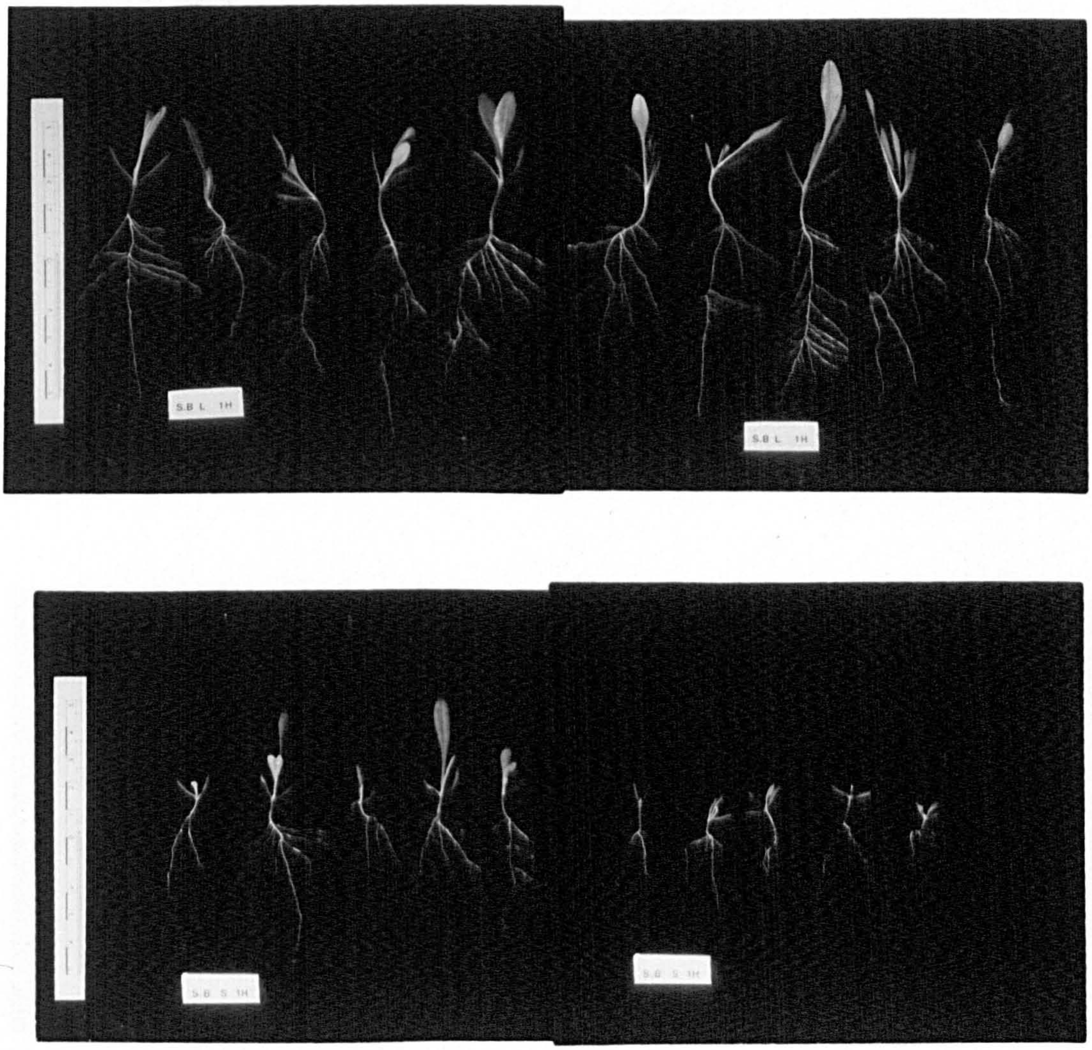


Fig 8.6 (a) Plants from large fruits (top row) and small fruits (bottom row) at first harvest (3 weeks growth in soil).

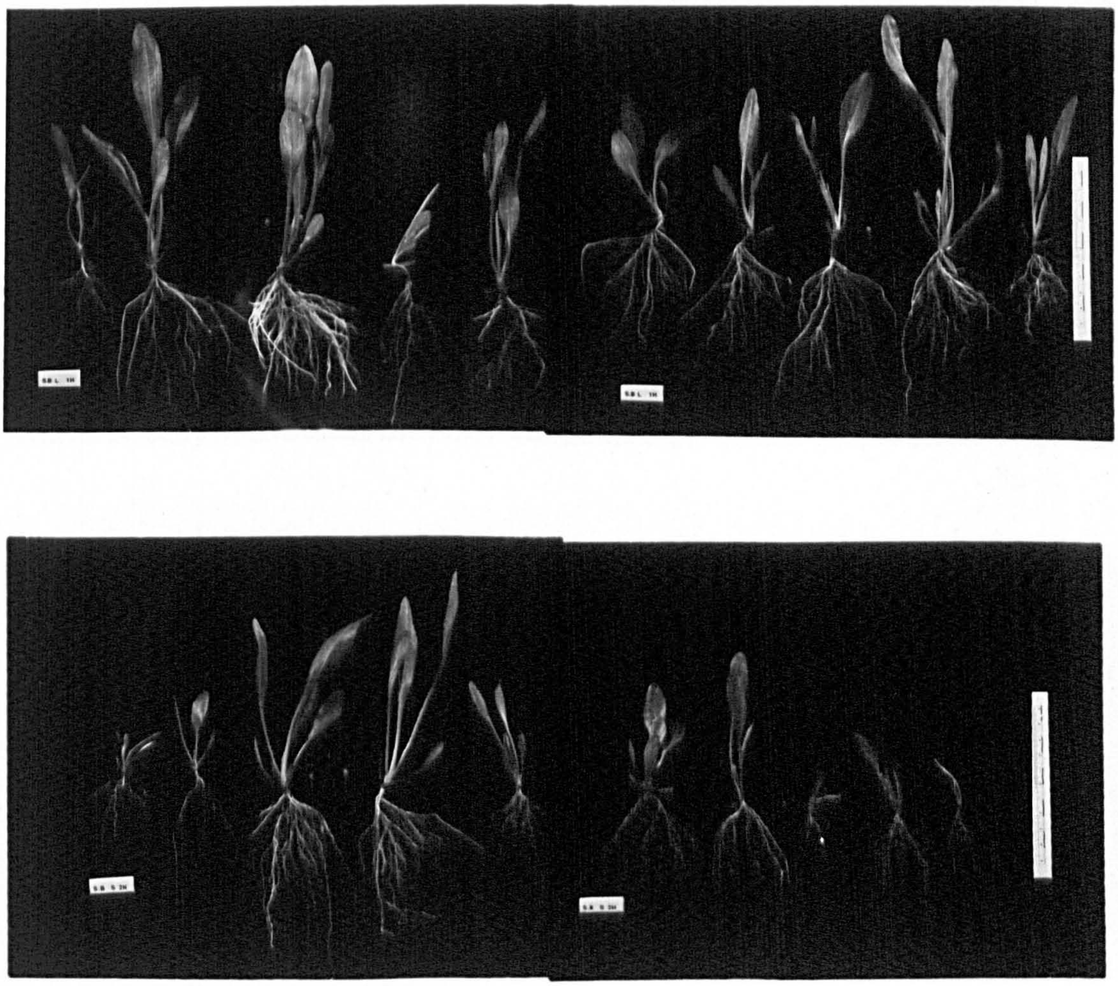


Fig 8.6 (b) Plants from large fruits (top row) and small fruits (bottom row) at second harvest (6 weeks growth in soil).

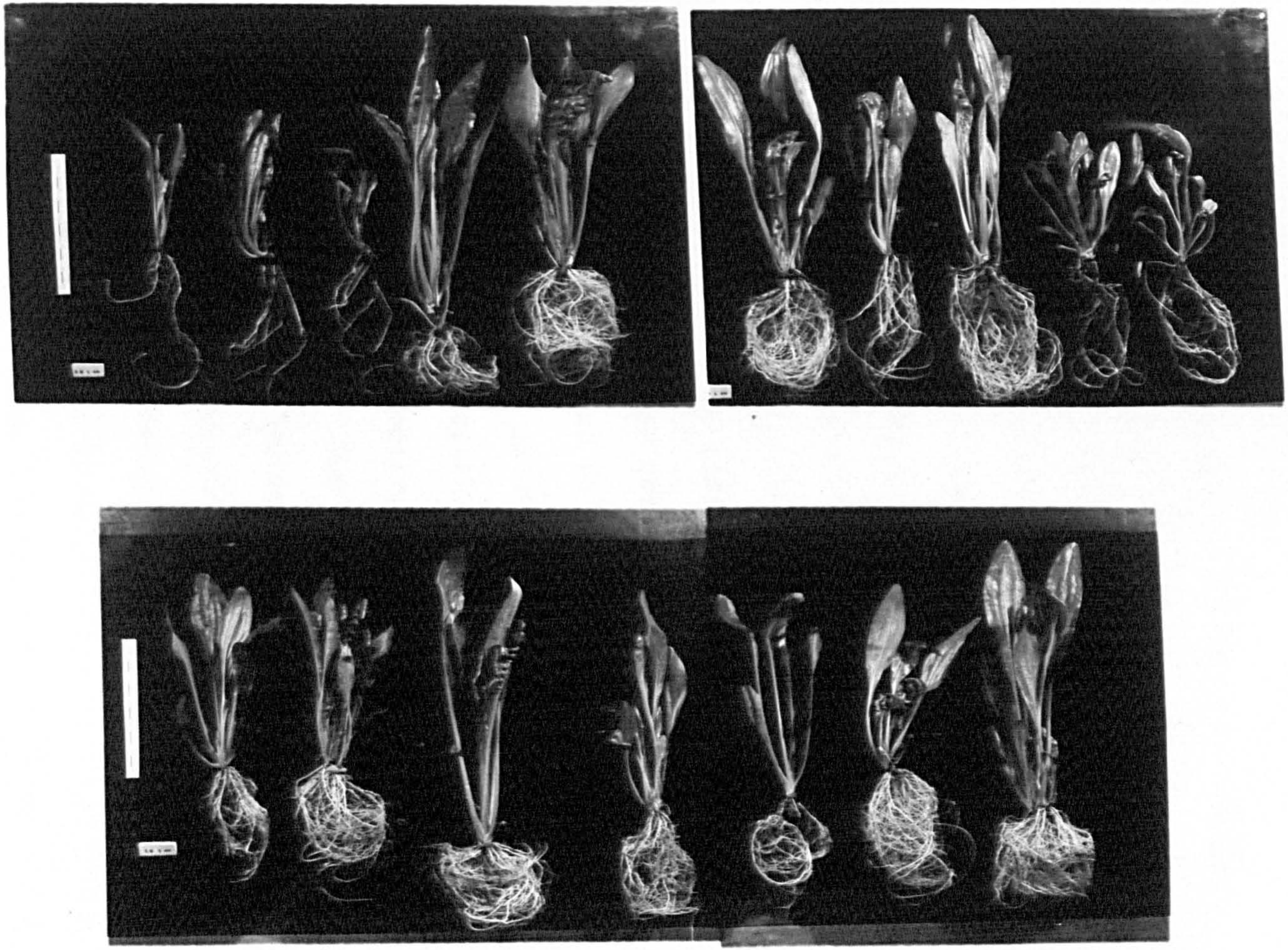


Fig 8.6 (d) Plants from large fruits (top row) and small fruits (bottom row) at final harvest (12 weeks growth in soil).

values of t are comparing dry weight at separate, and statistically independent, sample times, and the degrees of freedom are with the exception of the final harvest, constant, they can be validly compared).

Thus plants from large fruits will be at an advantage in any environment selecting large vigorous seedlings. Such an environment is provided by the highly competitive stage of sward establishment (the relevence of this to Aster marshes is discussed in Ch. 12). Black (1958 and 1960), using pure stands of Trifolium subterraneum, has demonstrated that the high initial vigour conferred by large seed does not decrease in mixed stands of small and large seeded plants, of this species, under competition, but, on the contrary, leads to the death of the smaller seedlings.

Whatever the critera of vigour measured studies using within-population variance may lead to the assumption that all or any aspects of seedling size at a given point are related to initial fruit size. This ignores the possibility that the size of the seedling may also depend on comparative growth rates, which may vary independently of fruit size. For example two populations of Teucrium scorodonia studied by Hutchinson showed ecotypic differentiation in respect of cotyledon size, seedlings from shaded habitats having large cotyledons compared with those from unshaded habitats; The difference in cotyledon size, however, was not dependent upon initial seed size differences (Hutchinson 1967). A between-population study of Lolium perenne by Cooper

(1964) has indicated that different cultivars (from Aberystwyth and Algeria) have very different patterns and rates of seedling development, depending on the environment. Such examples suggest that between population differences in seedlings developing from fruit of approximately equal size may provide a pointer to the nature of selected types, if not selection, in the respective environments.

Samples of fruit of plants from Knott End, (65/49A) a typical 'mid' marsh environment with salt pans and a close sward of vegetation on 2 - 3ft. of fine silt above sand, and from the lower particularly mobile, sandy soil of Beach Point (65/45A) were carefully chosen to include fruits of approximately equal weight (Knott End 11.12 ± 1.4 mgs/10 fruits, Beach Point 11.25 ± 1.1 mgs/10 fruits). Of the 100 fruits of each sample sown in beakers of the type depicted in Fig. 8.1.A (10 fruits/beaker) 53 Knott End seedlings and 58 Beach Point seedlings were harvested (on March 3rd 1967) after 20 days in distilled water. The details of the various measurements of seedling growth in the two populations are given in Table 8.5.

Comparing these data with those in Tables 8.1 and 8.2 it can be seen that (1) cotyledon and stem length are significantly different as in earlier trials (and therefore the differences are not solely due to differences in fruit size) and (2) that, although the difference in root length is no longer significant there is a significant difference in the number of lateral roots. This last

Table 8.5. Seedling growth after 20 days glass-distilled water. Knott End and Beach Point plants of approximately equal initial fruit size.

per plant mean values		length of main root	length of avr. lateral root	no. of laterals	length of stem	length of cotyledons *	no. of other leaves	length of true leaves	total plant length	
Knott End ($\bar{x}$=11.12±1.4mgs./10fr)		35.17	7.72	0.34	10.22	8.65	= $\frac{24}{53}$	4.54	67.36	n=53
Beach Point ($\bar{x}$=11.52±1.1mgs/10fr)		30.59	5.79	1.17	14.79	10.70	= $\frac{14}{58}$	4.64	74.71	n=58
Analysis	t	1.15		4.68	6.59	4.01	1.37	0.16	1.45	
	p	.2 - .3		>0.001	>0.001	>0.001	.1 - .2	n.s.	.2 - .1	

All measurements of length in mms.

*1 cotyledon/plant only measured.

feature, the contrast between a long main root with few laterals and a shorter but highly branched rooting system, was particularly striking. In Beach Point seedlings 18.17% of the total root length was due to laterals compared with only 6.94% for Knott End plants. Rooting pattern is further examined in experiments described in the next section on salt tolerance. It is interesting that, if total plant length is used as a measure of seedling size, the difference between the two populations is only significant at the 20% level. The size of individual parts varies independently of total size from population to population.

Fruits from a wild-collected sample of Knott End and Beach Point plants sown in trays in a 7:3:2 Audlem soil:peat:sand soil are shown in Fig. 8.7 after 4 weeks (a) and 8 weeks (b) growth. The sample mean fruit weights were 12.89 ± 3.72 mgs/10 fruits/head and 13.79 ± 7.49 mgs/10 fruits / head respectively. No measurement of seedling vigour was taken as the seedlings were pricked out into 6 inch pots or transferred to the experimental garden as part of a different experiment, but the overall picture of more vigorous development of shoots by the Beach Point population is clearly seen after 8 weeks growth under these conditions.

The Beach Point and Knott End habitats differ with respect to associated vegetation (low Asteretum/mature Limonietem), locality (E. Coast /W. Coast), tidal relations and type of marsh (low/mid) substrate (sandy mobile/firm mud), and the ray floret character (from rayless/rayed communities). It may be that

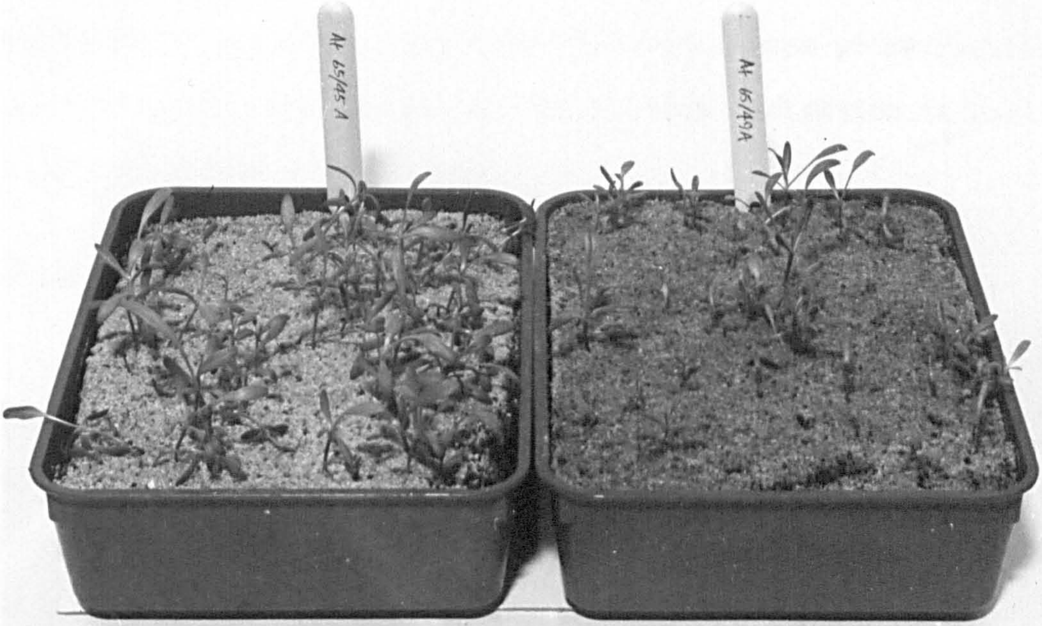


Fig 8.7 Beach Point (4ft) and Knott End (1ft) populations grown in trays
(a) above, after 4 weeks; (b) below, after 8 weeks.

superimposed on the correlation between fruit size and seedling vigour found in populations from the same series of marshes, there are differences due to the effects of local selection in such widely separated populations.

II The effect of salt concentration.

The literature relating to the physiology of salt tolerance is particularly diverse. Experimental work has tended to concentrate on the effects on plant growth due to the unusually high osmotic pressure of saline media or on the effects due to the superabundance of certain ions, notably Na^+ and Cl^-, in such media. In theory it should be possible to distinguish between osmotic and specific ion effects by the addition of isosmotic solutions of various salts to a base nutrient medium. In such a case any deviation in growth response for one salt in comparison with another will indicate the presence of an ion effect in addition to the normal osmotic one. This technique has been employed, for example, by Gauch and Wadleigh (1944) using Phaseolus vulgaris, and by several other workers. More usually, however, one aspect of salt tolerance is concentrated on at the expense of the other, although both must almost always operate simultaneously in nature. It is symptomatic of the present diversity of approach that in two recent papers on salt tolerance in the mangrove species Avicennia marina Scholander (1968) concentrates on the mechanism of separating fresh water from the sea by an ultrafiltration process involving an active maintenance of high internal solute pressure, whereas Rains and Epstein (1967) study the preferential absorption of potassium by leaf tissue in the presence of high concentrations of sodium chloride.

In addition to the separation of approach to these two mutually dependent aspects of the problems of growth in high external salt concentrations, the material studied has tended to fall into one of two groups; either adapted plants (halophytes) or glycophytes (usually crop and forage plants). The evidence gained from studies of the toxic effects of certain salts on crop plants may or may not be relevant to the study of how salt tolerant species are able to survive in habitats unsuitable for normal plant growth. Bernstein and Hayward (1958~~a and 1958b~~), Hayward and Bernstein (1958), Hayward (1956) and Chapman (1966) review the general problem of the physiology of salt tolerance as related to both glycophytes and halophytes, and Uphof (1941) reviews the earlier literature on the physiology of halophytes in particular.

The diversity of approach and material has led to the postulation of a wide range of physiological mechanisms enabling plants to survive in saline soils, all of which may be important. For this reason it was decided in the preliminary nutrient culture experiments described below to measure, without regard to an evaluation of the underlying causal mechanisms, the gross effects on a number of growth parameters of increasing NaCl concentrations.

In addition to gross effects some information relating to the absorption and transport of the major monovalent cations Na^+ and K^+ has been obtained by photometric analysis of the ash of plants grown in nutrient culture. The significance of this information is discussed.

(a) Gross effects.

A series of experiments in which seedlings were grown in a culture solution was undertaken. In the first experiment fruits from Knott End and Beach Point, Scolt Head Island were pregerminated in distilled water and, after being measured, the seedlings were transferred to numbered tubes (wrapped in metal foil and corked with a strip of foam rubber) containing the appropriate solution. A basic nutrient solution (a modified Hoagland's medium) was used which contained Calcium nitrate (5mls. Molar soln./litre), Potassium nitrate (5ml./1), Magnesium sulphate (2ml./1), and Potassium dihydrogen orthophosphate (1ml./1), plus a trace element solution (0.7 gms. Ferrous sulphate, 0.015 gms. Copper sulphate, 0.7 gms. Zinc sulphate, 0.01 gms. Manganese sulphate, and 0.01 gms. Sodium molybdate made up to 100ml.) of which 1 ml./1 was added. The sulphate radicals of the trace element salts were used to exclude chlorides (normally used) and the pH was adjusted to 6.5 using 2 mls. of 40% Potassium hydroxide (tested with pH meter).

These basic constituents were added in the appropriate amount to a litre of distilled water or a 1%, 2%, 3% or 5% NaCl solution. (The NaCl concentration of the medium was increased in terms of

% solutions in accordance with the precedent of Montfort and Brandup (1928), Eijk (1938), Baumeister and Schmidt (1962) and others in similar experiments). Root growth was measured every 3 days and the solutions were changed twice weekly. The culture tubes were kept in a laboratory at room temperature (16 - 20°C) throughout the experiment.

The seedlings in solutions in which the base nutrient had been added to a 2% or more NaCl solution showed no increase in root length. After a period of 15 days all seedlings given this treatment had withered. Not allowing for the base medium, the concentration of 2% NaCl is osmotically equivalent to a salinity of 21‰ or two thirds that of standard sea water (calculated from depression of freezing point data). Older plants under better conditions of root aeration undoubtedly survive higher salinities than this, although their growth may be depressed. Root growth in the seedlings cultured in Base + Distilled water, and Base + 1% NaCl was compared after a total of 4 weeks growth. The results (means of six seedlings/treatment) are summarised in Table 8.6 and photographs of the plants are given in Fig. 8.8.

Two effects of NaCl may be noted. (1) In Beach Point seedlings the mean length of the main root is increased in base + 1% NaCl and the laterals are slightly decreased, whereas in Knott End seedlings both the main root and the lateral root length are greatly decreased in NaCl. (2) A marked effect of increased NaCl concentration appears to be the inhibition of lateral root development

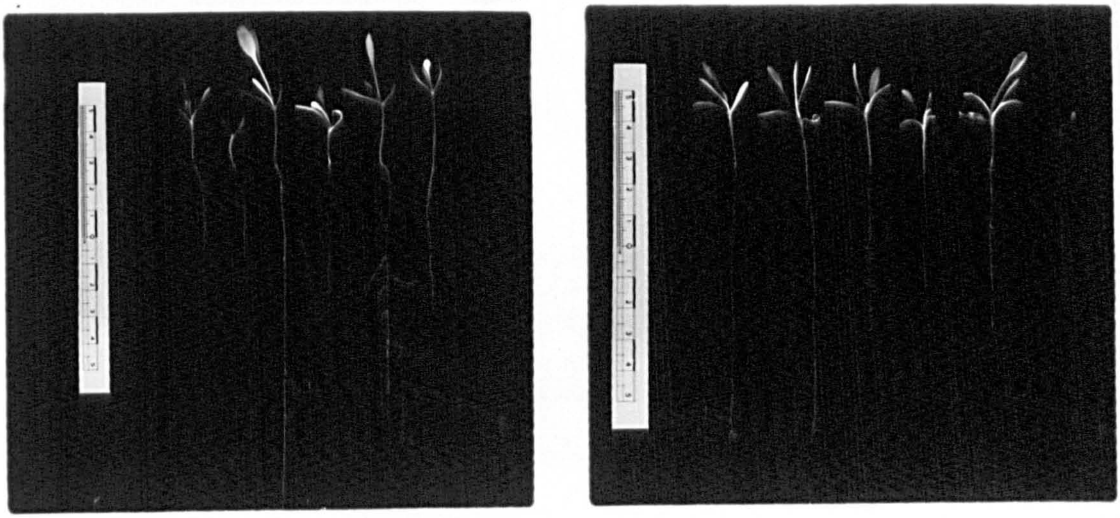
Table 8.6.	Root growth in Nutrient + Distilled water and Nutrient + 1% NaCl after 28 days (mean of 6 replicated/treatment). (mms.)							
		MAIN ROOT			LATERAL ROOTS			
To base nutrient soln.	Source	$\bar{x}$ initial length	$\bar{x}$ final length	mean increase	$\bar{x}$ initial length	$\bar{x}$ total length (inc. initial)	increase length	mean no	$\bar{x}$ total increment
Distilled water	Scolt Bch.	33.5±5.05	50.8±8.87	17.3±5.17	5.3	43.2±5.70	37.8	10.8±0.42	55.2±8.62
	Knott End	40.5±6.08	61.5±4.03	21.0±7.18	1.0	28.5±10.91	27.5	4.7±1.30	48.5±13.33
1% NaCl	Scolt Bch.	39.2±5.05	67.5±10.76	28.3±8.42	1.3	26.2±12.23	24.8	3.8±1.13	53.2±20.06
	Knott End	56.7±4.76	65.8±6.48	9.2±2.82	0.2	6.8±2.60	6.6	3.2±1.21	15.8±4.46

		%final root length due to laterals	% increment due to laterals
Distilled water	Beach Pt.	45.92	68.58
	Knott	31.66	56.70
1% NaCl	Beach Pt.	27.93	46.70
	Knott	9.40	42.10

Distilled

+ 1% NaCl

Fig 8.8 Seedlings after 4 weeks growth in nutrient medium. Top row: Beach Point seedlings from base nutrient plus distilled water (left) and base nutrient plus 1% NaCl solution (right). Bottom row: Knott End seedlings (arranged as above).



and growth. Both populations show a significant reduction in the number of lateral roots produced in base + 1% NaCl solution. The between-population difference in rooting pattern, expressed as lateral root number, and % final root length due to laterals, in Table 8.6, can be seen in Fig. 8.8.

This contrast in lateral root development, noted earlier in Table 8.5, was retained in populations cultured in base medium + 0.5% NaCl harvested after 35 days. After this period of time Beach Point plants produced a mean of 16 laterals/plant whereas Knott End plants developed only 7.25 laterals/plant (based on 12 seedlings in each population). Photographs of the plants, in which the contrast in rooting pattern can be seen, are given in Fig. 8.9.

A third experiment was undertaken in which material originally collected from Gibraltar Point was cultured under improved conditions of aeration. Fruits were collected from plants grown in the experimental garden (collected from the wild as plants) and were pregerminated in petri dishes. The seedlings were transferred to the 'apparatus' drawn in Fig. 8.1(B) (5 seedlings/beaker). The base nutrient medium used earlier was made up in concentrations of 0 (distilled water), 0.5%, 1.0% and 1.5% Sodium chloride solutions. The solutions were aerated throughout the experiment and changed in the beakers every three days. The beakers were placed inside a chamber constructed of angle iron and covered with polythene sheeting. Ten seedlings per population per treatment were used and the beakers (2 per treatment/population) were randomised within

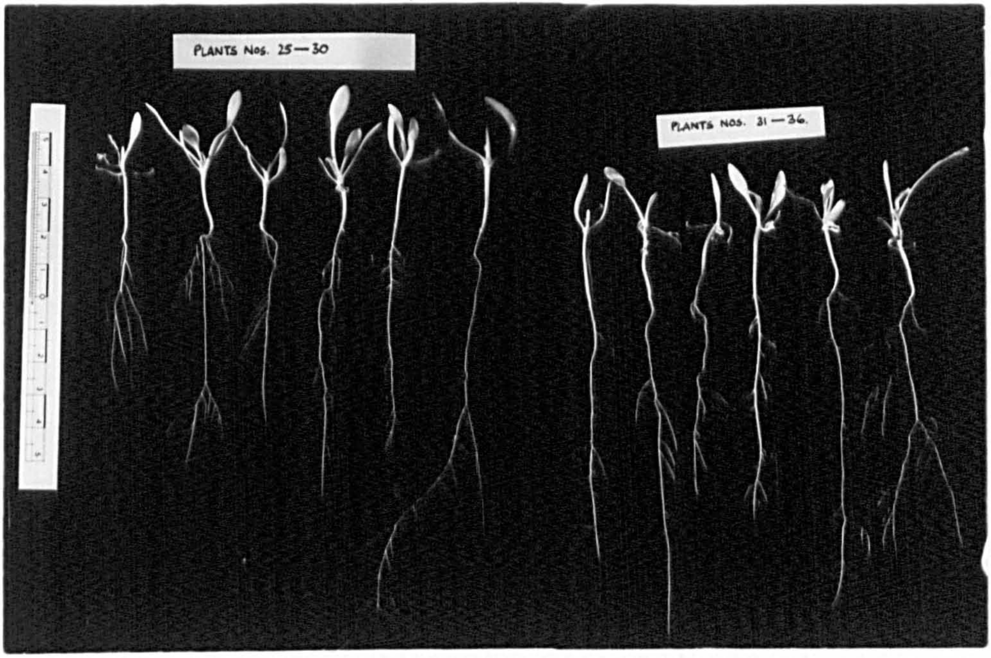
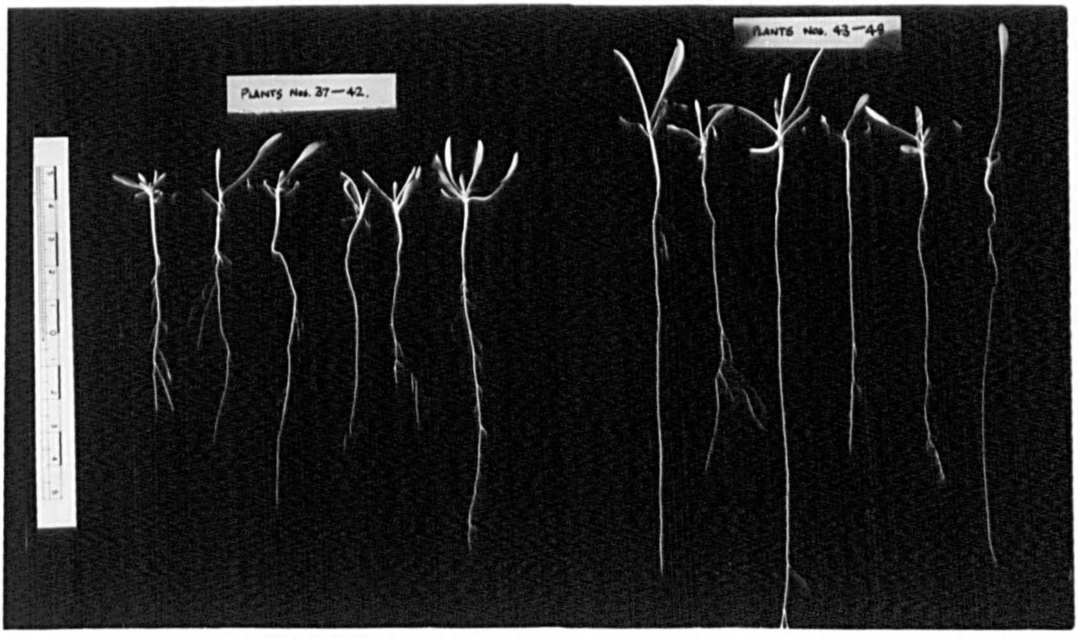


Fig 8.9 Seedlings after 5 weeks growth in nutrient medium plus 0.5% NaCl solution. Above: Beach Point. Below: Knott End.



the chamber. Large beakers of distilled water containing paper 'wicks' were placed at each end of the chambers.

The seedlings were harvested after 4 weeks growth, and the results are presented in Table 8.7. A number of measurements of both root and top growth were taken. Subsamples of 5 plants/ population/treatment were separated into tops and roots, wrapped in weighed metal foil and oven dried at 90°C for 40 hours. An analysis of the dry weights is given in Table 8.8.

Two conclusions are apparent. The first is that there is no obvious pattern of growth depression between treatments as indicated by most of the yield parameters. The significant difference in Spa. (low marsh) tops shows no general pattern on inspection and is due largely to poor growth at 1.5% NaCl. Although growth was generally depressed in all populations at 1.5% NaCl the overall between-treatment differences are largely insignificant (on the other hand, because of initial fruit size differences, the between population variation, in all but that of roots at 1.5% remains significant). The second conclusion is that the yield as measured by dry weights may bear no relation to the actual general appearance of the seedlings. For example although the Spa. plants in Base medium gave high values for the various measures of growth they looked yellow and developed necrotic spots on the leaves during the final week of growth.

The pattern of lateral root development was similar to that observed in earlier experiments; a general and significant reduction

Table 8.7 Nutrient culture experiment - Gibraltar Point populations. Data summary (mean values only - 10 plants/treatments) except dry wts. (subsamples 5 plants/treatment).

	Population Medium	SPARTINA MARSH				ASTER MARSH				LIMONIUM MARSH				AGROPYRON MARSH				Units
		Base	0.5%	1.0%	1.5%	Base	0.5%	1.0%	1.5%	Base	0.5%	1.0%	1.5%	Base	0.5%	1.0%	1.5%	
	Length main root	60.2	79.5	75.5	53.9	64.6	77.3	98.1	49.4	55.4	96.4	65.3	42.9	61.1	73.3	84.9	50.2	mms
R	Length lateral	42.7	35.0	34.1	26.1	27.0	37.4	14.8	8.9	25.4	8.7	10.0	21.4	31.4	12.4	30.7	23.5	mms
O	Total length	102.9	114.5	109.6	79.0	91.6	114.7	112.9	58.3	80.0	105.1	75.3	64.3	92.5	85.7	115.6	73.7	mms
O	Number laterals	7.7	2.6	2.3	1.3	5.6	3.7	1.6	0.4	4.8	1.6	2.4	0.8	4.5	2.1	2.6	1.1	-
T	Root increase	73.6	87.8	81.4	48.4	62.2	85.6	74.0	35.5	56.1	77.2	55.0	41.6	69.0	65.6	93.1	54.5	mms*
S	% increase	330.2	417.4	321.2	150.6	216.1	327.2	258.9	160.0	227.7	310.6	281.9	197.8	283.8	382.7	440.1	288.0	-//
	Dry weight	4.38	3.88	4.04	2.70	2.95	2.92	2.90	1.67	2.95	3.30	1.82	1.60	1.66	2.27	3.40	2.20	mgs
T	Number leaves	3.5	3.7	3.0	2.9	3.8	3.1	3.2	2.0	3.5	3.6	3.3	2.9	3.2	2.9	2.6	2.7	-
O P	Leaf area	38.6	45.6	44.4	22.9	28.4	34.1	34.0	16.8	26.4	33.6	20.3	11.9	23.9	20.1	42.0	18.8	Sq. mms
S	Dry weights	13.01	11.64	14.24	10.52	7.92	9.55	9.95	6.83	7.00	7.55	6.12	4.57	4.76	4.75	7.17	5.07	mgs
	Total dry weight	17.42	15.52	18.28	13.22	10.87	12.47	12.85	8.50	9.95	10.85	7.94	6.17	6.42	7.02	10.57	7.27	mgs
	Appearance	Chlorotic yellow spots	Pale green	healthy	Pale green	Pale green	healthy	healthy	Pale green	healthy	healthy	healthy	Pale green	healthy	healthy	Pale yellow	Chlorotic	-

* Final root length - original root length

// $\frac{\text{Final - original}}{\text{original}}$ X 100

Table 8.8		Nutrient culture experiment. Gibraltar Point popns. Analysis dry wt. data (5 plants/treatment)				
Wts. in mgms.		TOPS				
Population treatment		Spa	Ast.	Lim	Agr	Analysis of variance between popn.level of p.
Base	$\bar{x}$	13.02	7.92	7.00	4.76	0.001
	s.e.	0.35	1.46	0.54	1.02	
	S^2	0.62	8.68	1.20	4.17	
+ 0.5% NaCl	$\bar{x}$	11.64	9.55	7.55	4.75	0.001
	s.e.	1.30	0.56	1.52	0.65	
	S^2	8.60	1.25	9.25	1.77	
+ 1.0% NaCl	$\bar{x}$	14.24	9.95	6.12	7.17	0.001
	s.e.	1.99	1.78	1.25	0.67	
	S^2	20.05	12.70	6.32	1.82	
+ 1.5% NaCl	$\bar{x}$	10.52	6.83	4.57	5.07	0.01
	s.e.	1.63	1.63	0.76	0.69	
	S^2	13.50	10.65	2.30	1.93	
Analysis of variance between treatment level of p.		0.01	n.s.	n.s.	n.s.	

continued ....

Table 8.8 continued ....

Population treatment		ROOTS				
		Spa	Ast	Lim	Agr	Analysis of variance between popn.level of p.
Base	$\bar{x}$	4.38	2.95	2.95	1.66	
	s.e.	0.29	0.27	0.27	0.39	0.01
	s^2	0.42	1.60	0.30	0.62	
+ 0.5% NaCl	$\bar{x}$	3.88	2.92	3.30	2.27	
	s.e.	0.40	0.38	0.51	0.37	0.05
	s^2	0.82	0.52	1.50	0.55	
+ 1.0% NaCl	$\bar{x}$	4.04	2.90	1.82	3.40	
	s.e.	0.22	0.05	0.26	0.40	0.01
	s^2	0.25	0.12	0.26	0.65	
+ 1.5% NaCl	$\bar{x}$	2.70	1.67	1.60	2.20	
	s.e.	0.29	0.31	0.30	0.27	n.s.
	s^2	0.42	0.40	0.38	0.30	
Analysis of variance between treatment of level of p.		0.05	n.s.	0.05	n.s.	

in the mean number of lateral roots/plant in increasing salt concentrations. (The between-treatment differences in lateral root number giving F ratios of 14.3 ($p = 0.001$) for Spa., 4.9 ($p = 0.01$) for Agr., 16.2 ($p = 0.001$) for Lim., and 18.8 ($p = 0.001$) for Ast.). The mean lateral root numbers are plotted in Fig. 8.10. It is interesting that, in the base medium, the development of lateral roots decreases from low to high marsh populations. This corresponds with the early difference between Knott End (a mid marsh) and Beach Point (a low marsh) for this character.

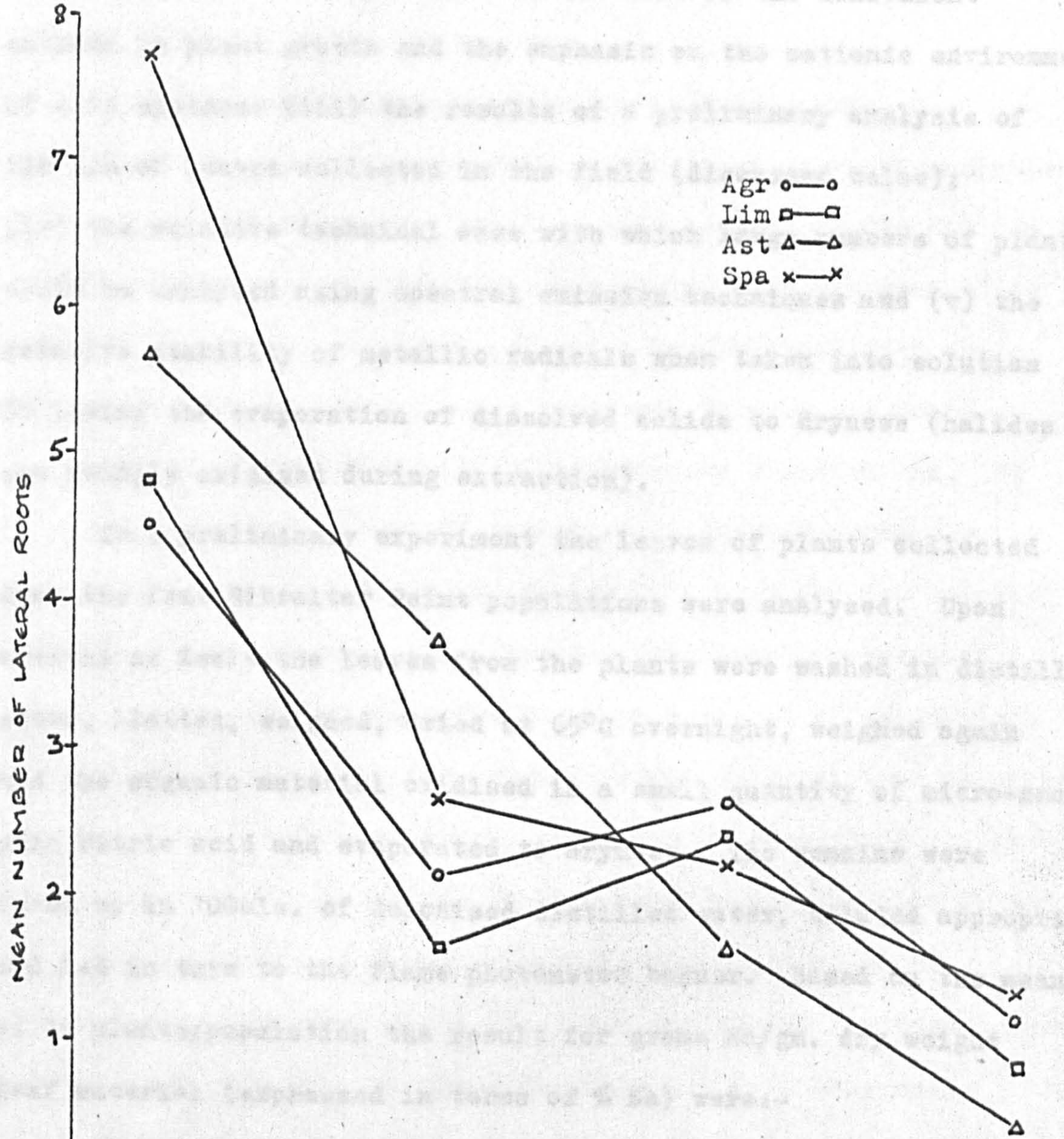
As, in general, the seedlings appear to survive under nutrient culture conditions up to 1.5% NaCl it would be of interest to extend these experiments to a period of time over which between-population differences in growth response may be detectable.

(b) Na^+ and K^+ absorption.

In addition to the comparison of gross yield parameters described in the above section some information has been gained on the effect of increasing salt concentration on the absorption and transport of the cations Na^+ and K^+.

The decision to concentrate on this particular aspect of growth in saline media was based on a number of factors. These were (i) the accumulating body of evidence, reviewed by Epstein and Jefferies (1964), suggesting that selective ion transport in plants has a genetic basis, and its concomitant possibility that selection may have occured for genotypes excelling in a specific detectable ability enabling them to survive in high sodium environ-

Fig 8.10 Number of lateral roots in plants grown in nutrient media.



ments (ii) the evidence from work on other halophytes, some of it cited later, of the importance of the role of the monovalent cations in plant growth and the emphasis on the cationic environment of cell systems; (iii) the results of a preliminary analysis of the ash of leaves collected in the field (discussed below); (iv) the relative technical ease with which large numbers of plants could be analysed using spectral emission techniques and (v) the relative stability of metallic radicals when taken into solution following the evaporation of dissolved solids to dryness (halides are readily oxidised during extraction).

In a preliminary experiment the leaves of plants collected from the four Gibraltar Point populations were analysed. Upon arrival at Keele the leaves from the plants were washed in distilled water, blotted, weighed, dried at 65°C overnight, weighed again and the organic material oxidised in a small quantity of micro-analytic Nitric acid and evaporated to dryness. The remains were taken up in 100mls. of deionised distilled water, diluted appropriately and led in turn to the flame photometer burner. Based on the mean of 20 plants/population the result for grams Na/gm. dry weight leaf material (expressed in terms of % Na) were:-

Agropyron marsh	44.9	mgms./gm. dry wt.	± 4.1
Limonium marsh	57.4	"	± 6.1
Aster marsh	46.2	"	± 3.5
Spartina marsh	85.6	"	± 2.7

(Overall differences significant at 0.001 level of p.).

The clear differences in the concentrations of Na$^+$ in the leaves of plants from the four populations suggest that differences in Na$^+$ metabolism may exist.

The samples of weighed plants from the Agropyron and Spartina marsh populations which had been cultured in the experiment described in (a) above were analysed with respect to the Na$^+$ and K$^+$ content of their roots and tops. The method used was similar to that for the leaves of plants collected in the field with the exceptions that (1) the material dissilved in HNo$_3$ was taken up in 50 ml. of deionised distilled water before dilution and, (2) in the photometry, Na$^+$ and K$^+$ content was estimated using the central part of the scale by reference to a high and low standard solution, sprayed before and after the test sample respectively (instead of a calibration curve based on a number of standards). The amounts of Na$^+$ and K$^+$ in the original dried material were calculated and from this the concentration, expressed as mgms. Na (or K)/gm. ash was obtained. In all, 72 samples were tested for both Na and K - 5 plants/treatment (tops and roots) for Spartina marsh plants and 4 plants/treatment (tops and roots) for Agropyron marsh plants. The data is summarised in Table 8.9 (see overleaf).

In comparing the values set out in Table 8.9 it should be borne in mind that the major contrast is between a high and low marsh population cultured in media ranging from 0% NaCl in which Na is present only as a trace element (0.009 ppm. from the Na molybdate) to one which there is a superabundance of exchangeable

Table 8.9 Na and K content after 48 days growth of roots and tops of two Gibraltar Point populations grown in increasing NaCl concentrations. (expressed in mgms./g. dry wt. plant material).

Conc of NaCl to base nutrient.		0		0.5%		1.0%		1.5%	
		Na	K	Na	K	Na	K	Na	K
Agropyron marsh (mean of 4 plants/ treatment).	tops	26.1	98.2	275.1	77.5	329.2	46.9	447.3	48.6
	roots	37.1	64.9	183.6	49.7	237.5	40.5	252.1	41.9
Spartina marsh (mean of 5 plants/ treatment).	tops	23.4	98.5	70.6	100.9	94.2	63.8	126.9	44.2
	roots	61.3	78.2	55.5	68.3	98.0	64.0	136.4	72.5

(The between treatment differences for both ions and both populations are all significant at the 0.001 level of p).

Na ions (5,897 ppm. in 1.5% NaCl), and in which the K levels are constant (at about 790 ppm.). It is not possible to relate the Na or K content of the plants to that of the solutions in which they were grown, as these were frequently replenished and any ions already accumulated from earlier solutions of the same concentration may have been retained by the plant.

The summarised data contained in Table 8.9 is set out in Figs 8.11a and b. In general it can be seen that:-

(1) With increasing external Na$^+$ concentrations, Agropyron marsh plants accumulate higher Na$^+$ concentrations than Spartina marsh plants in both tops and roots

and (2) K$^+$ concentrations in the tops of both Agr. and Spa.

plants are lowered by increasing external Na^+ concentrations. K^+ concentrations in the roots are less effected by high external Na^+ concentrations.

To examine this relationship more closely the individual per plant data relating to tops have been plotted in Fig. 8.12. In both populations there is a negative correlation between internal Na^+ content of tops and internal K^+ content of tops. (For Spa. $r = - 0.723$, for Agr., $r = - 0.722$, both significant at the 0.001 level of p). (The assumption of a linear correlation is purely arbitrary). However the difference in slope of the two regression lines in Fig. 8.12 suggests that the balance between Na and K ions in the tops differs in the two populations. Although Spa. plants are (in high external Na^+ environments) unable to accumulate more K^+ in the tops than Agr., plants they appear to be able to restrict the accumulation of Na ions to low levels. This difference in performance may be of relevance to plant growth - especially in the light of what is known about the roles of Na and K in plant growth.

The concentration of K^+ in plants generally exceeds that of any other cation. Evidence which underlines its role as an indispensible macronutrient for normal growth is reviewed by Evans and Sorger (1966) who lists the effects of growth in K^+ deficient soils, some of the symptoms of which, such as chlorosis and the development of necrotic spots on the leaves, are well known.

On the other hand some controversy surrounds the question

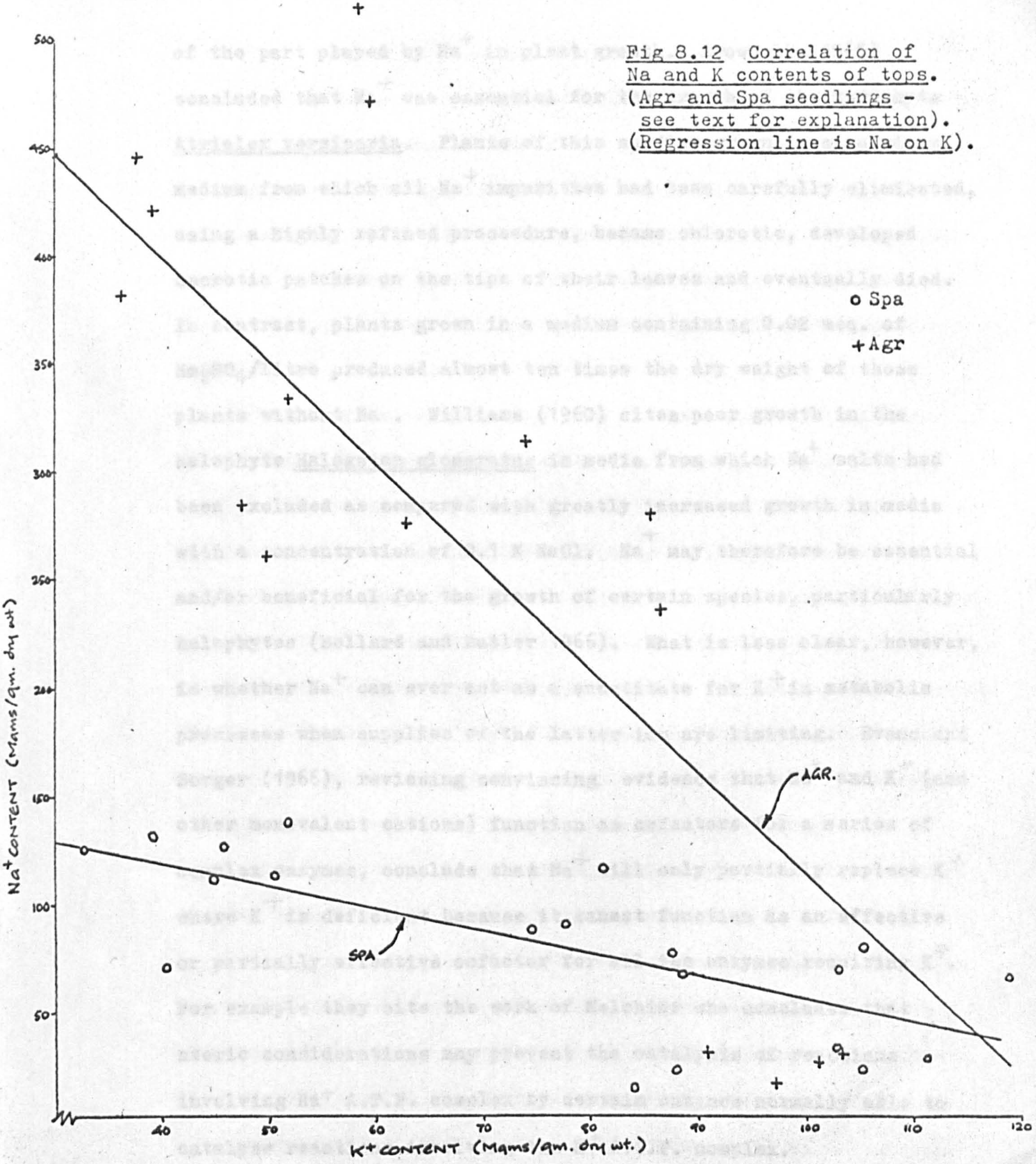


Fig 8.12 Correlation of Na and K contents of tops. (Agr and Spa seedlings - see text for explanation). (Regression line is Na on K).

of the part played by Na^+ in plant growth. Brownell (1965) concluded that Na^+ was essential for the growth of the xerophyte Atriplex versicaria. Plants of this species grown in a nutrient medium from which all Na^+ impurities had been carefully eliminated, using a highly refined proceedure, became chlorotic, developed necrotic patches on the tips of their leaves and eventually died. In contrast, plants grown in a medium containing 0.02 meq. of Na_2SO_4/litre produced almost ten times the dry weight of those plants without Na . Williams (1960) cites poor growth in the halophyte Halogeton glomeratus in media from which Na^+ salts had been excluded as compared with greatly increased growth in media with a concentration of 0.1 N NaCl. Na^+ may therefore be essential and/or beneficial for the growth of certain species, particularly halophytes (Bollard and Butler 1966). What is less clear, however, is whether Na^+ can ever act as a substitute for K^+ in metabolic processes when supplies of the latter ion are limiting. Evans and Sorger (1966), reviewing convincing evidence that Na^+ and K^+ (and other monovalent cations) function as cofactors for a series of complex enzymes, conclude that Na^+ will only partially replace K^+ where K^+ is deficient because it cannot function as an effective or partially effective cofactor for all the enzymes requiring K^+. For example they cite the work of Melchior who concludes that steric considerations may prevent the catalysis of reactions involving Na^+ A.T.P. complex by certain enzymes normally able to catalyse reactions involving the K^+ A.T.P. complex.

Apart from its possible role as an essential element in some species, and as a partial substitute for K under certain conditions, a third aspect of the effect of Na on plant growth can be distinguished, viz its affect in excessive quantities. The toxic effect of excess Na on plants is well known, particularly from studies on crop and forage species. Increasing levels of exchangeable Na in the soil may result in decreased absorption of other elements, particularly K and Ca .

The implications of this work on the respective concentrations of K$^+$ and Na$^+$ in the nutrient culture experiment are as follows. In the base nutrient made up in distilled water the plants face a minimal supply of Na, but have adequate K . In the other 3 treatments the supplies of Na , increase to an excess and although K concentrations in the ambient medium remain the same, concentrations in the cell sap may be lowered due to the decreased absorption of K in favour of the more plentiful Na (this will depend on the nature of the absorptive mechanism - see below). Assuming that K is essential for healthy growth in reasonably large quantities, and that Na cannot entirely replace it, any decrease in its concentration in the cells may result in K deficiency symptoms. This could explain the chlorosis observed in Agropyron marsh plants in 1.0 and 1.5% NaCl solutions, although as the % K content of the dry matter is generally higher than that at which K deficiency occurs in other species (Evans and Sorger, 1966, p.51) it could equally well be a result of toxic levels of Na . The chlorosis observed in Spartina marsh plants in the base medium may be explained in terms of Na deficiency in a Na requiring type. As Agropyron marsh plants with equally low concentrations of Na in the tops showed no chlorosis in the base medium they may not require Na in any appreciable quantity. The consistency with which Spartina marsh and other low marsh plants, tended to become yellow when grown in the experimental garden may be explained if such plants ~~are~~ require high Na levels ~~requiring~~ (independent tests of the soil in the garden give an average reading of 0.17 meg. Na/100gms air dried soil).

Reference to Table 8.9 and Fig. 8.12 reveals that Spartina marsh plants accumulate K in the tops without the accompanying high accumulation of Na observed in Agropyron marsh plants. Current interpretations of the mechanism of salt uptake in plants favour the active and independent absorption of different ions, although the assumption that only one type of ion, cation or anion has to be absorbed actively has been challenged in a recent review by Brouwer (1965). Bange and Van Vliet (1961), working on maize postulate a K^+-specific translocation carrier with a high affinity for K^+ which transports this ion from the root vacuoles containing both Na^+ and K^+, previously absorbed by a non specific cation carrier, to the leaves. Heimann (1966) concludes that sodium intruding a plant through the roots is mainly retained by them, and a barrier of a still unexplained kind prevents its transfer to the trunk or stem, and from there into the petioles and leaves. On the other hand potassium ions move freely to the foliage. Lower concentrations of Na^+ and Ca^{++} in the shoots than the roots is reported by Greenway and Rogers (1963) in Agropyron elongatum and Hordeum vulgare, two apparently salt tolerant varieties of Western Australia. Huffaker and Wallace (1959a, 1959b), working with corn, avocado, soybean, citrus and raddish, present data which show that these species accumulate considerable amounts of Na in the roots and that they translocate Na varying distances through the root and stem, depending on species, Na level and K level. One would expect any mechanism which allows the filtering of K^+ ions into the tops whilst excluding high levels of toxic Na ions to be selected in highly sodic environments. Fig. 8.12 may well indicate the presence of such a mechanism in Spartina marsh plants. That such a mechanism is an aspect of halophytic competence in coping with high salt levels is strongly suggested by the work of Rains and Epstein (1967) on Avicennia referred to earlier. The preferential absorption of Potassium by leaf tissue at both low (1m M) and high (10m M) concentrations of Potassium was little affected by Sodium chloride concentrations up to 200mM. Black's study of ion uptake in the non-halophyte Atriplex versicaria (Black 1960) also points to a K^+ specific carrier although higher concentrations of NaCl in the leaves than in the roots, possibly due to other alkali cation carriers suitable for Na^+ absorption, were not (unlike Agropyron marsh plants in 1.5% NaCl solution) accompanied by symptoms of toxicity.

On the other hand Heimann (1966 p. 206 - 7) suggests that salt-sensitive plants react to high concentrations of Na^+ by an increased absorption of K$^+$ to restore cationic balance in the plant, and only 'salt-tolerant' plants such as beet or barley actually depress K$^+$ levels in the plant with increasing external Na$^+$ concentrations.

As interpreted above the results of Na$^+$ and K$^+$ analysis of Spartina and Agropyron marsh seedlings correspond more closely with visual observations of the degree of chlorosis in the leaves than with measurements of the various growth parameters (apart from lateral root number). The concentrations of Na$^+$ and K$^+$ in the tops of seedlings after 4 weeks growth may indicate in some cases the beginnings of nutritional disorders, the affect on growth of which would be felt after a longer period of culture. Although the maintainance of high internal solute pressures are desirable in halophytic species an inability to descriminate between Na$^+$ and K under conditions of high external Na$^+$ may, in plants normally facing low Na concentrations in nature, result in uncritical osmoregulatory uptake of Na$^+$ in concentrations which eventually prove toxic or depress the uptake of nutritionally essential K$^+$.

III Summary

Summarising the experimental work on seedling growth in this chapter it may be concluded that:-

(i) There is a correlation between seedling size and initial fruit size which may persist over a number of weeks. In an experiment comparing the growth of seedlings from two sets of fruit, one consisting of fruit almost three times the weight of the other, initial fruit size continued to have a significant effect on seedling size up to a period of 11 weeks after germination (but not after this period).

(ii) Seedlings from widely separated populations exhibit variation in lateral root development irrespective of fruit size.

(iii) In general depression of seedling growth occurs in solutions containing increasing concentrations of Sodium chloride up to about 2%, at which wilting and death occur although optimal growth may occur in media to which a solution of 0.5 or 1% NaCl have been added.

(iv) The most marked effect of increasing NaCl concentrations is the inhibition of the development of lateral roots.

(v) After 4 weeks growth the pattern of leaf chlorosis in seedlings bears no relation to estimates of yield using dry weight, leaf area, and root length, on which the effects of initial fruit size are still evident.

(vi) These preliminary experiments have failed to detect a major between-population morphological difference in growth response to increasing salt concentration. Within the range of concentrations tested the within-population optima for various yield parameters show no relation to the origin of the parent plant. Agropyron marsh seedlings, for example, which are found in a relatively salt free soil in nature, show optimum growth, as indicated by dry weight, in a medium made up in 1% NaCl.

(vii) Observations from only a single experiment, but one producing highly consistent results suggest that the contrast in gradient of two regression lines inversely correlating the Na^+ and K^+ content of the tops of seedlings may reflect differences in the ability of low and high marsh populations to cope with high external Na^+ environments. It is suggested, on theoretical grounds, that one aspect of adaption to such environments may be the capacity preferentially to absorb K^+ from media containing excess Na^+. Further to this the possibly greater plasticity of adapted plants in replacing K^+ by Na^+ in certain reactions may have led, through selection, to a preference for the latter ion in a greater number of metabolites (to an extent that growth is affected by limited supplies of Na^+ even when K^+ is plentiful).

(viii) The contrast between estimates of growth based on various yield parameters on the one hand, and on lateral root development, leaf chlorosis, and Na^+ and K^+ absorption on the other, suggests that initial growth of seedlings, as measured by the former group of parameters, may give no indication of their relative chances of survival to fruiting.

CHAPTER 9. SEEDLING ELIMINATION.

In genecological studies it is often possible to detect variation in a character which is correlated with the environment. Beacause this process takes some time the much more difficult exercise of examining the relation between this variation and the environment experimentally, and attempting to prove that this variation is adaptive, may not be approached in short-term studies. This study is no exception. The early detection of a fruit-size cline in Aster tripolium, however, has permitted the completion of three field experiments which may give some indication of the possible adaptive significance of variation in fruit size, germination requirements, and seedling vigour. These experiments, reported in this chapter, relate to (1) the effect of mechanical wave action on seedling elimination, (2) the fate of fruit introduced to selected sites, and (3) the fate of transplants introduced to selected sites. In addition to these, an experiment measuring the effects of competition with glycophytes under experimental garden conditions is reported, together with information relating to the survival of plants in ordinary soils.

Discussion of the results contains the premise that in theory fruits from plants in any part of a salt marsh may be dispersed to other parts, so that at an appropriate time viable fruit from high marsh plants is present on low marshes and vice versa. This premise is examined at the outset.

I Fruit dispersal.

Their possession of a well developed pappus suggests that the fruits of A. tripolium are mainly wind dispersed. Air borne fruits are not an uncommon sight, particularly on high marshes, in the late autumn. However seedlings are commonly found in closely aggregated clumps, particularly on many low marshes, which suggests that the fruits from a single capitulum may tend to adhere to one another (the pappi are commonly entangled), or the entire capitulum may fall to the ground.

A further dispersal agent may be the tides, especially the high equinoctial springs. Dalby (1963) suggests that tides commonly disperse the seeds of Salicornia pusilla, the fruiting heads of which can remain afloat for up to three months. Praeger (1913) reports that fruits of Aster tripolium remained afloat for up to five days in contrast to, for example, Plantago maritima, Cochlearia officinalis and Salicornia radicans, the seeds of which all sank in less than a minute. Unshaken beakers of sea water have contained a large number of floating fruits after a period of seven days in the laboratory at Keele, although regular shaking tended to sink all the fruits in less than 4 days. Both Guppy (1906) and Ridley (1930) report the germination of Aster fruits under water (sea water) and subsequent floating of the seedlings. They suggest that seedlings 'carried up into less saline waters by a rising tide might establish themselves up river' (Ridley p. 190).

I have no evidence for or against this possibility although unanchored seedlings can often be found on open mud in spring. (They may have been uprooted by the tides - see below). Guppy further suggests that Snow-Buntings, which are alleged to eat the fruit, may help to disperse the fruits which adhere to their feathers by the pappus. Seed eating birds are unlikely to evacuate the fruits undamaged.

Wind dispersal is likely to be most effective on the high marshes where the fruits are less heavy and, with the notable exception of Spiral marsh plants, the pappi large. In low marsh plants the infrequency of complete drying out of the fruiting heads may add to the difficulties of dispersing heavy fruits. Here tidal dispersal may be instrumental in transporting fruits up onto the higher marshes. By contrast fruits from high marsh plants, being near the tidal limit, and because of the nature of the flow of water off the marsh, may tend to be deposited by the ebbing tide in the neighbourhood of the parent plant. The chances of dispersal away from a particular marsh will be affected by the local physiography. For example on Scolt Head Island fruits produced on Spiral marsh can be dispersed to the Cockle Bight via the main drainage creek, but unless they are also wind dispersed (and then over a 6' dune ridge) the direction of tidal flow precludes their dispersal to Beach Point.

Although it is generally easy to describe the ways in which fruits could be dispersed it is diffecult to discover how effective these mechanisms are in the field. Webb's treatment of the subject of dispersal underlines this fact particularly well (Webb 1966). All that can be said is that mechanisms promoting wind, and to a lesser extent, tide dispersal of Aster tripolium exist, and, if they are reasonably efficient, the viable fruit population on a given marsh area probably contains a proportion of fruits from plants from a different marsh area. These fruits will find themselves in ecological conditions often vastly different from those experienced by at least their maternal, and probably both, their parents.

II The mechanical effect of wave action on seedling elimination.

The tides on low marsh areas tend to ebb and flow as a continuous sheet of water, as opposed to on mid and high marsh where they rise and fall along an intricate series of creeks. It was suggested in Chapter 7 that the tides may exert a purely mechanical effect on seedlings on the open mobile mud of low marsh flats. This suggestion was partly based on the apposite study of Wiehe (1935) who showed that the mortality of seedlings of Salicornia europea was a direct result of the tidal water washing them out from their original site. Based on results obtained from a study of five permanent transects running at right angles to the tide line on the open Dovey estuary, Wiehe's results also serve to emphasise the contrast between tidal conditions operating above and below the critical level separating mid and low marshes. A study of Wiehe's Fig. 3. p.328 reveals that percentage mortality of seedlings is high in the 'neap tide' zone and comparatively low in the 'spring tide' zone and that there is a sharp transition between the zones (as opposed to a gradual increase in percentage mortality corresponding to the increase in periods between successive tidal inundations, which might, theoretically, be expected.). This provides strong experimental evidence to support the earlier suggestion (see Ch. 3 and Appendix VI) that the number of days of continuous non-tide emersion during the critical high spring cycle of the vernal equinox may be important in the establishment of a plant from seed.

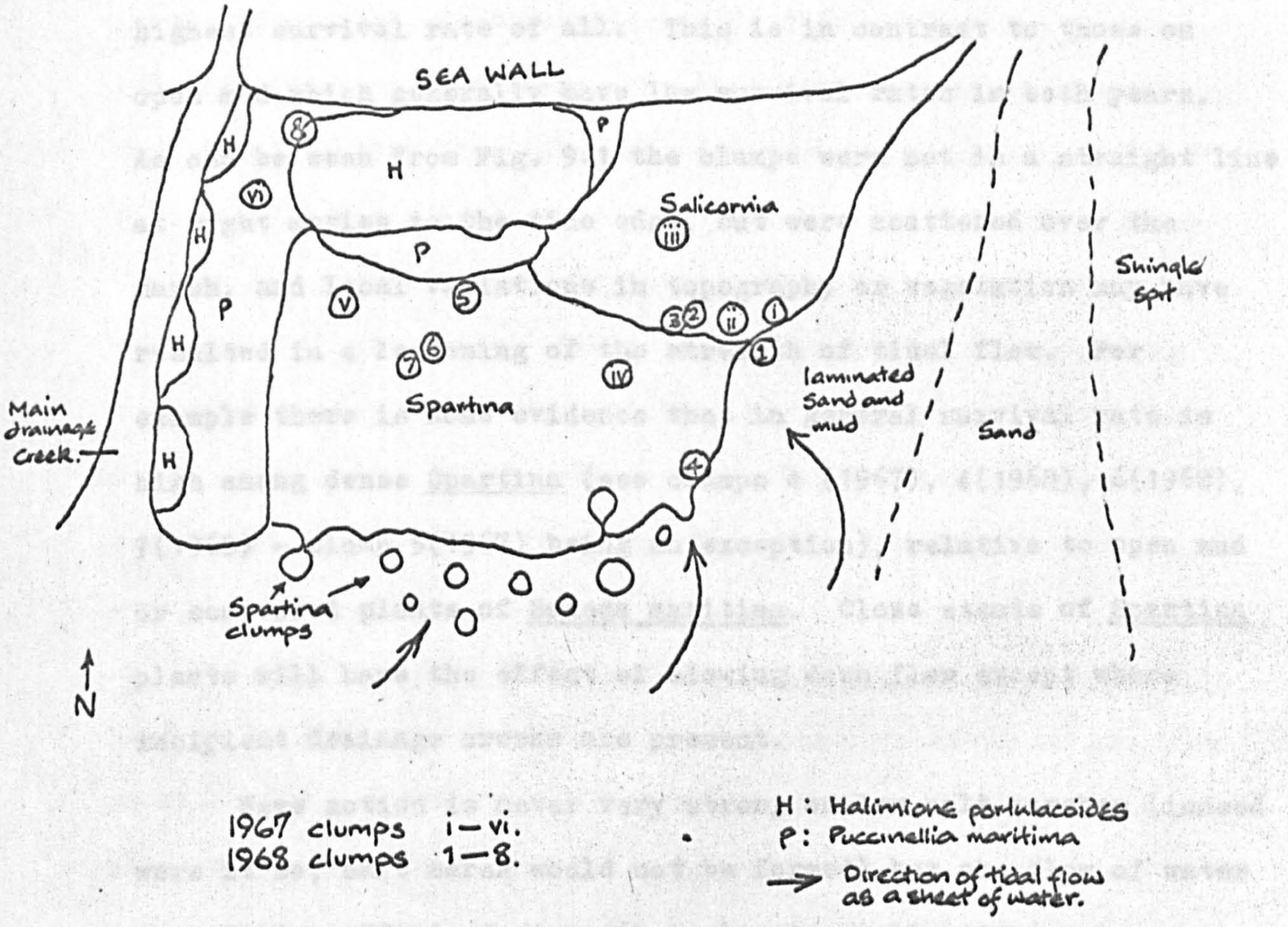
A field experiment attempting to repeat Wiehe's work, but on Aster tripolium seedlings, was carried out during 1967 and 1968. An important exception to Wiehe's programme was the fact that, instead of using line transects, selected groups of seedlings were marked in the Spring by a stake. This was necessary because the seedlings, unlike those of Salicornia were not dispersed throughout the marsh in high densities, but tended to occur in quantity only in isolated clumps. (See Fig. VI. 3 b). Six such clumps were marked in 1967 and eight were marked in 1968. The approximate positions of these clumps are marked on Fig. 9.1. The seedlings were generally all at the cotyledon stage and any seedlings too small to identify as Aster were carefully removed. The number of seedlings counted in each clump at the date of marking and the number counted during early summer (at 5 and 6 leaf stages) are given in Table 9.1. In this table the clumps are arranged in order according to the approximate sequence in which they would be covered by an incoming tide (but see below) and the composition of surrounding vegetation is given for each clump.

Table 9.1. Survival of naturally set seedlings - Gibraltar Point *Spartina* marsh.

1967 clump	no of seedlings March 25th	no of seedlings July 15th	assoc vegetation	% survival
1	6	1	open mud *Salicornia*	16.6
2	11	0	open mud *Salicornia*	0
3	21	2	scattered *Suaeda* *Spartina*	9.5
4	8	3	dense *Spartina*	37.5
5	5	0	dense *Spartina*	0
6	6	6	patches of *Puccinellia*	100
TOTAL	57	12		
1968	no of seedlings April 7th	no of seedlings June 2nd		
1	14	0	open mud *Salicornia*	0
3	12	2	dense *Suaeda*	16.6
4	7	4	dense *Spartina*	57.1
5	7	1	dense *Spartina*	14.3
6	17	8	*Spartina* several adult *Aster* plants	47.0
7	9	3	dense *Spartina*	33.3
8	5	4	open space near *Halimione*	80.0
TOTAL	71	22		

(N.B. the position of clump 2 1968 was not discovered in June).

Fig 9.1 Sketch map of Spartina marsh, Gibraltar Point showing approximate sites of marked seedling clumps.



The number of both clumps and seedlings are perhaps rather low, but the survival of seedlings does not show the consistent pattern in relation to tidal flooding shown by Wiehe for Salicornia. Despite this there are certain trends worthy of note. The two clumps in 1967 and 1968, furthest from the tide edge have the highest survival rate of all. This is in contrast to those on open mud which generally have low survival rates in both years. As can be seen from Fig. 9.1 the clumps were not in a straight line at right angles to the tide edge, but were scattered over the marsh, and local variations in topography or vegetation may have resulted in a lessening of the strength of tidal flow. For example there is some evidence that in general survival rate is high among dense Spartina (see clumps 4 (1967), 4(1968), 6(1968), 7(1968) - clump 5(1967) being an exception), relative to open mud or scattered plants of Suaeda maritima. Close stands of Spartina plants will have the effect of slowing down flow except where incipient drainage creeks are present.

Wave action is never very strong on low salt marshes (indeed were it so, salt marsh would not be formed) but the flow of water may attain sufficient strength in local swirling movements (particularly under a depth of water where wind-created wave action is strong) to disturb the mobile mud of the lowest zones and uproot poorly anchored plants.

The differences in overall survival rates in the two years may be related to the timing of the 1st and 2nd counts. In 1967, where 21% of all seedlings survived to the second count, the first count (on March 25th) was made just prior to the highest spring tides of the year and the 2nd count was made during the midsummer neaps (see Fig. VI3).

In 1968, on the other hand, where 31% of all seedlings survived, the 1st count was made about 6 days after the previous 19' tide and seedlings counted on this occasion may have developed from fruit in that time (thus avoiding the first high springs of that year). The second counts in 1968, on June 2nd, was earlier than in the previous year and further thinning may have occured subsequent to the count. There is no evidence to eliminate the possibility that further germination may have occured after the first count in any of the clumps. Seedlings which had germinated subsequent to the first count may have been included in the second count.

It appears, from Wiehe's study, that Salicornia seedlings need to be left uncovered for two or three days after germination to allow for a sufficiently firm root anchorage. Aster fruits, however, which cannot germinate in undiluted sea water (see Ch. 6) will require longer and the actual time needed will depend on the coincidence of diluting spring showers with breaks in the pattern of tidal coverage. (This could partly explain the relative success of the two species in colonising the lower zones of salt marshes). It may be that the fruits of high marsh plants dispersed to lower zones are prevented from establishing themselves there because of their inability to germinate in highly saline environments. Should the salinity be lowered by rainfall the less rapid germination and the smaller seedling size associated with small fruits (see Ch. 8) may lead to the elimination of the small-fruited high marsh plants under such tidal conditions. Certainly one would expect, on mobile mud under constant innundation, selection to favour rapidly germinating vigorous seedlings with well branched rooting systems which develop in a relatively short time-features characteristic of the large-fruited low marsh genotypes.

III The fate of fruits introduced to selected sites.

Wild collected fruits were introduced to three marshes at the east end of Scolt Head Island on Sept. 16th 1967. Three batches of fruit were used:- (1) from Gibraltar Point Agropyron marsh (coll. 1967), (2) from Gibraltar Point Spartina marsh (coll. 1967) and (3) from the Cockle Bight on Scolt Head (coll. 1967). One hundred fruits from each of these areas were introduced to each of the three marshes on Scolt Head. These were (a) Spiral marsh, (b) the middle portion of Beach Point to the north of the sand blow-off and (c) the <u>Aster</u> zone of the Upper Cockle Bight. Thus fruits from one high (Gib. Agr.) and two low (Gib. Spa, Scolt Cob.) marshes were introduced to a high and two different low marshes.

The fruits were held in position by a circle of fine nylon mesh glued to the upper half of a plastic beakers. The beakers and mesh (in fact the inverted top part of the seedling culture apparatus in Fig. 8.1A) were held by an anodised plant label stake sunk into the soil and bent over at the top. The source of the fruit in each cup was labelled by scratching a code letter on the top of the stake. The position of the three seed cups on each marsh was mapped.

The seed cups were collected the following spring on April 14th 1968. Each of the sites was photographed before collection and the photographs are given as Fig. 9.2. On returning to the laboratory the entire core of soil in each cup was taken out and inverted before being photographed - see Fig. 9.3. The soil in each cup was then carefully sieved and the seedlings it contained were classified into three groups:- (i) Those with green cotyledons or emergent radicles which if not confined by the nylon mesh may have shown healthy growth, (II) aborted seedlings or those still contained by the testa and (iii) decayed seedlings which appeared to have germinated at some time well before the date of collection. Ungerminated fruits were also common in a number of cups but these were not counted. The number of seedlings in each category recovered from each cup are given in Table 9.2.

The number of seedlings present are expressed as a percentage of the number of fruits initially sown and therefore combine the effects of the environment on germination and mortality over the period September to April. Of particular interest are the numbers of potentially healthy seedlings. These have germinated, and are developing, in the early part of the growing season. Selection in the form of tidal washings or physiological extremes may eliminate them but if they survive these there is then a chance

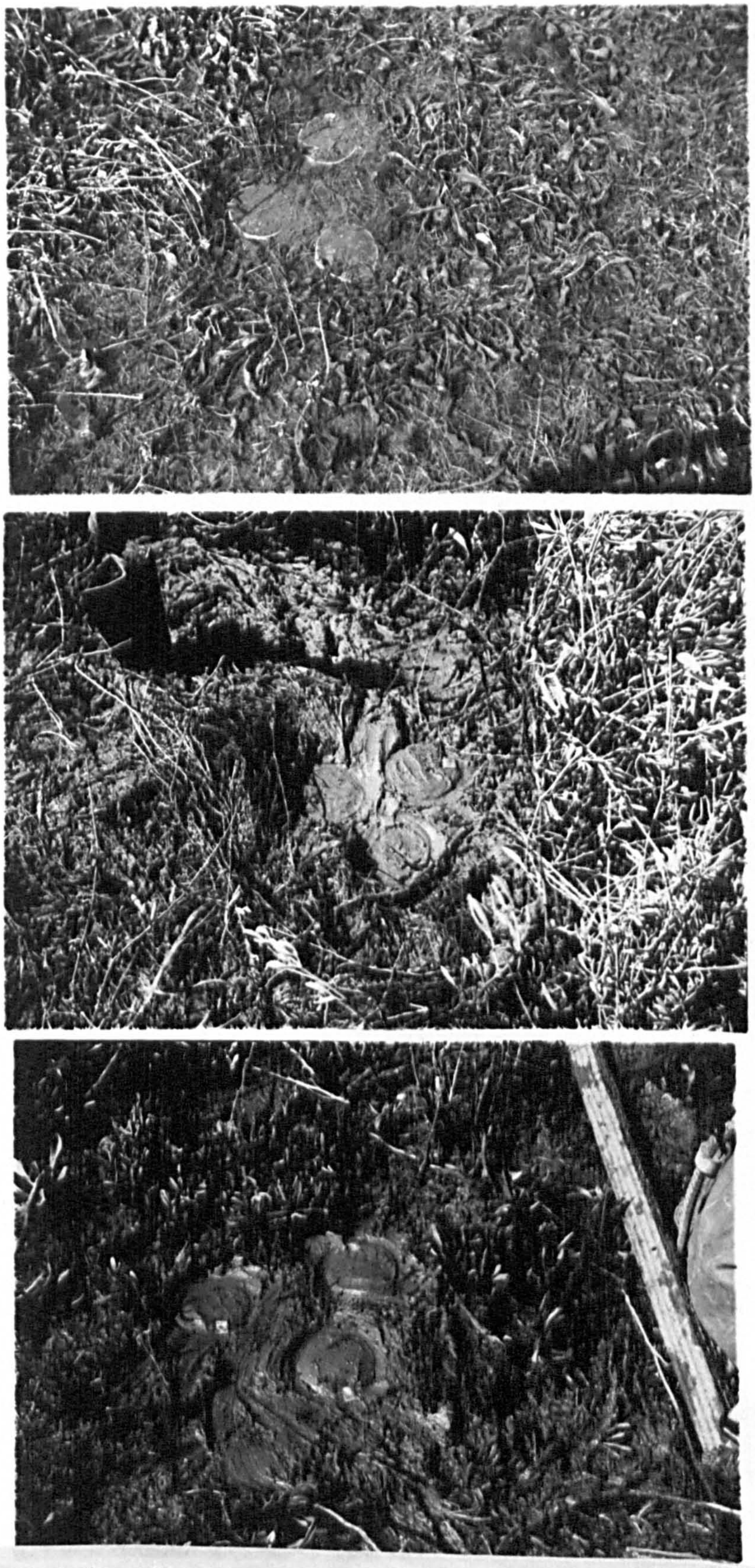


Fig 9.2 Seed cups in situ on Spiral marsh (top), Beach Point (middle) and Cockle Bight (bottom) on Scolt Head-April 1968.

Fig 9.3 Seed cups with contents as recovered from Spiral marsh (top), Beach Point (middle) and Cockle Bight (bottom) (S:Spartina marsh, A:Agropyron marsh, C:Cockle Bight).

Table 9.2.	Fruit introduced to Scolt Head Island on Sept. 16th. 1967. Seedlings recovered April 14th, 1968.								
Site / Source	Spiral marsh near drainage creek in Limonium sward			Beach Point near Halimione along creek edge			Cockle Bight in Aster with Suaeda maritima		
	(i)	(ii)	(iii)	(i)	(ii)	(iii)	(i)	(ii)	(iii)
Agropyron Gib. Pt.	4	2	0	1	0	0	0	0	0
Spartina Gib. Pt.	5	0	1	63	19	9	19	4	2
Cockle Bight Scolt Head	10	0	1	24	0	1	27	4	1

Categories (i), (ii), (iii) - see text.

Total number of fruits to show germination

Site / Source	Spiral marsh	Beach Point	Cockle Bight
Agropyron	6	1	0
Spartina	6	91	25
Cockle Bight	11	25	32

that they may eventually produce fruits. It is of course not possible to anticipate how selection may work against them later, but up to the point of collection in April they have survived the environmental conditions affecting their germination and early growth. This much is a minimum requirement of all successful plants.

Fruits from Gibraltar Point Agropyron marsh had a low survival rate overall but those introduced to Spiral marsh, which had only been reached by the unusually high springs of the previous week, gave some evidence of potential establishment. (A tide of 27' or more will partly flood the Spiral marsh. According to the warden Mr. R. Chestney this had happened during the week prior to collection). No Agropyron marsh fruits had germinated on the Cockle Bight. Spartina marsh fruits had a very high germination rate (63%) on Beach Point, a marsh which interestingly corresponds well in other respects with the Spartina marsh at Gibraltar Point (see for example associated vegetation list, tide level, and weight of <u>Aster</u> fruits). Cockle Bight fruits had their highest germination rate on the Cockle Bight itself but fruits also survived on Beach Point and Spiral marsh.

Mature plants were present in large numbers at each of the three sites although they may not have been established from seed. Only on Beach Point and the Cockle Bight were seedlings of <u>A. tripolium</u> found outside the seed cups at the time of collection, their numbers being much higher on Beach Point. A search for naturally set seedlings on Spiral marsh was unrewarded.

An impression of the respective rates of accretion at the three sites can be gained from the photographs in Fig. 9.2. The Spiral marsh site was relatively undisturbed whereas the other two sites were retraced with some difficulty as the cups were covered with a considerable layer of mud which had to be scraped from the surface of the mesh before they could be harvested. Whether, when not enclosed, fruits are buried, or whether they settle back on to the surface of the mud after the tide has receeded is not known. Milton (1939) reports small numbers of buried fruits of A. tripolium on a salt marsh considering their abundance at the surface.

IV The fate of transplants introduced to selected sites.

The difficulty of obtaining large amounts of clonal material in a short period has prevented any extensive transplant experiments up to the present time, although it is hoped to report at a later date on the progress of both transplanted ramets and sib seedlings introduced to Gibraltar Point in 1968. However the results of a limited and somewhat unsuccessful transplant experiment are detailed below.

The original material was collected from site A at Winsford in July 1966. A single plant was separated into three clusters which were planted out in 5" pots. These were further broken up and after a period of growth in wooden trays were planted in the experimental garden on June 2nd 1967. The resultant 18 ramets were introduced to three sites (6 plants/site) on the marsh at Parkgate on July 10th 1967. Photographs of the sites are given in Fig. 9.4. The six plants at each site were planted in a circle, each plant equidistant from a central marker stake, and watered with tap water brought from Keele. The details of the sites were as follows:-

1. On marsh P3 amid *Suaeda maritima*, *Salicornia sp*., *Puccinellia maritima* and *Spartina anglica* were present close by.
2. On marsh P2 in a sward of *Puccinellia maritima*. Many *Aster* plants surrounding the sward.
3. On marsh P1 among *Scirpus maritimus* with *Triglochin maritima*, *Festuca rubra*, *Phragmites communis* and *Cochlearia officinalis* were present close by.

The transplants, which may be termed 'phytometers' after Cavers and Harper (1967a, were revisited on Oct. 2nd 1967. Only one plant had survived at Site 1, no live plants were present at site 2 and only two plants were present at site 3 (one of which had flowered and was 60cms. tall). The plant at site 1 had only 2 green leaves compared with the average of about 6 leaves when transplanted. The number of survivors are too low to base any conclusion on, and the intention of measuring various yield parameters was abandoned.

The low survival rate may have been due to a long spell of

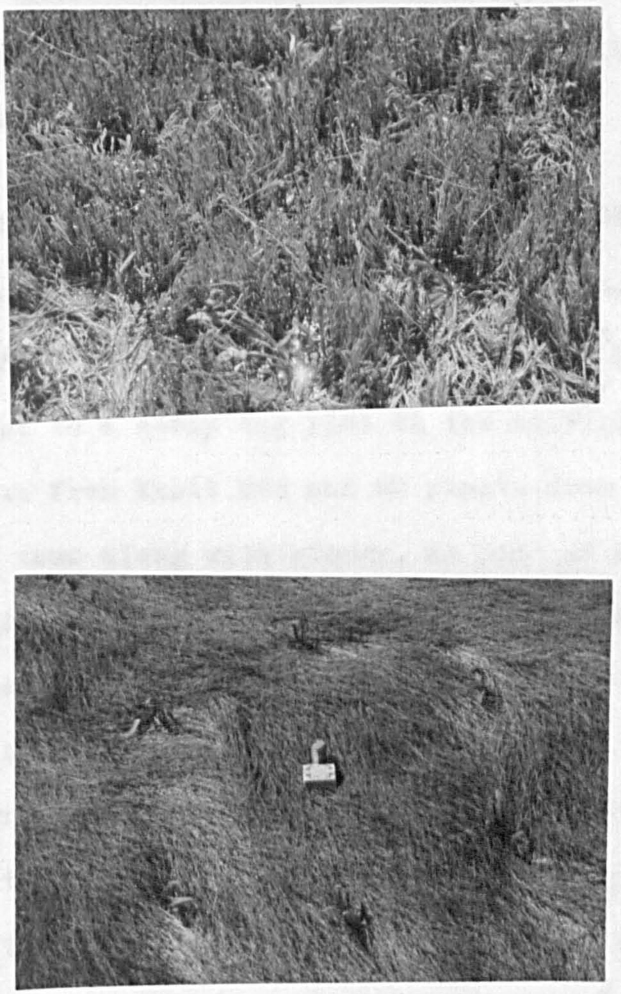


Fig 9.4 Transplant sites at Parkgate. Top:P3, middle:P2, bottom:P1.

hot dry weather which immediately followed the date of planting or to the inability of the plants to adjust to maritime conditions after a long period of growth in ordinary soil.

V Competition with glycophytes and growth in ordinary soils.

In June 1966 5-month old plants grown from fruit collected in 1965 from Knott End and from Beach Point, Scolt Head Island, were transplanted to a newly dug plot in the experimental garden. Eighty six plants from Knott End and 80 plants from Beach Point were planted in rows along with others, as part of a programme of collateral cultivation. Photographs of a random sample of the plants after they were picked out from trays before transfer to the garden are given in Fig. 9.5.

Early signs of predation (apparently by rabbits and grey squirrels) led to covering the rows of plants with 'cloches' of wire netting. This made weeding almost impossible and it was decided to abandon the plants to their fate, awaiting the building of a permanent protective wire cage. The plot became choked with weeds and remained like this until August 18th when the plants were lifted and the site cleared to erect the wire cage. (See Fig. 9.6).

Of the 80 original Beach Point plants only 11 (13.7%) had survived less than 3 months competition with the local weed population in contrast to 41 (47.7%) of the original 86 Knott End plants. The interference 'stress' due to the weeds was very high.

Fig 9.5 5-month old plants from Beach Point (above) and Knott End (below) prior to transplanting to the experimental garden.

Fig. 9.7 shows a Knott End plant photographed among the plants which had previously covered it completely. The species were:-

Stellaria media a.
Ranunculus repens c.
Rumex acetosa c.
Polygon persicaria c.
Dactylis glomerata o.
Potentilla anserina o.
Holcus lanatus o.
Plantago major o.
Plantago lanceolata o.
Senecio vulgaris r.
Ranunculus acris r.
Bellis perennis r.

The ability of mid marsh population from Knott End to survive competition in greater numbers than the low marsh Beach Point population is typical of a general pattern of growth and survival in ordinary soils. Even when assiduously weeded and planted in single pots a greater proportion of the low marsh plants from several marshes died during cultivation. Fig. 9.8 shows the history of the Knott End and Beach Point populations from the original pricking out of 87 Knott End and 108 Beach Point seedlings on April 22nd 1966, to the following March.

Table 9.3 contains information, extracted from a catalogue of the stocks under cultivation, relating to the survival of plants grown in ordinary soils. No attempt has been made to make a detailed comparison of the performance of individual populations as certain details such as times and amounts of weeding, number of plants and length of treatment, are not known and 'survival' time varied (as indicated in the table). Where treatment and period are comparable, however, the survival rates bear out the general

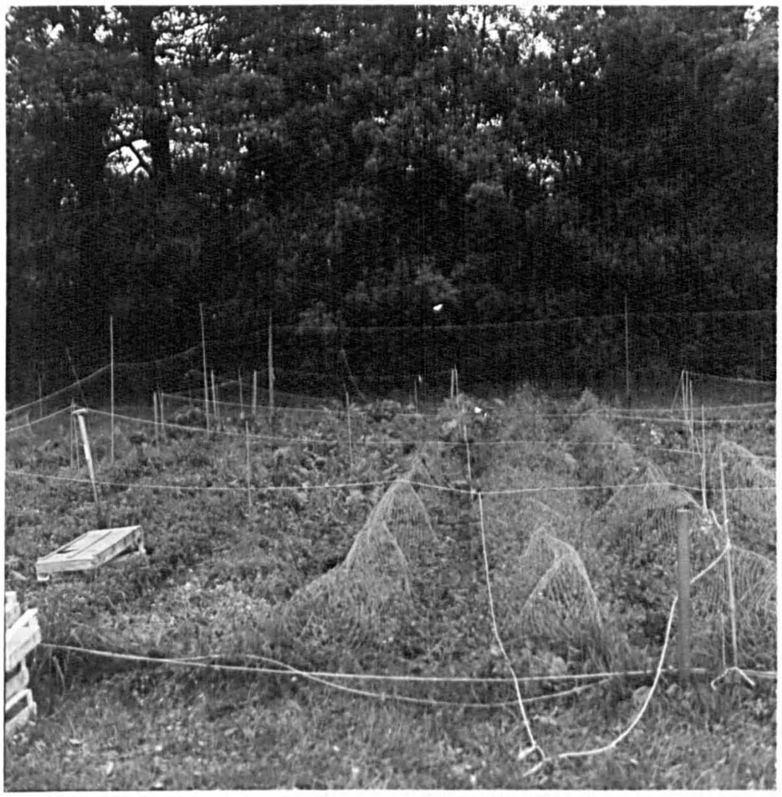


Fig 9.6 'Competition' plot in Botanic Garden at Keele.

Fig 9.7 Knott End plant among local weed population (see text).

Fig. 9.8 Survival of Knott End and Beach Point plants during various stages of cultivation.

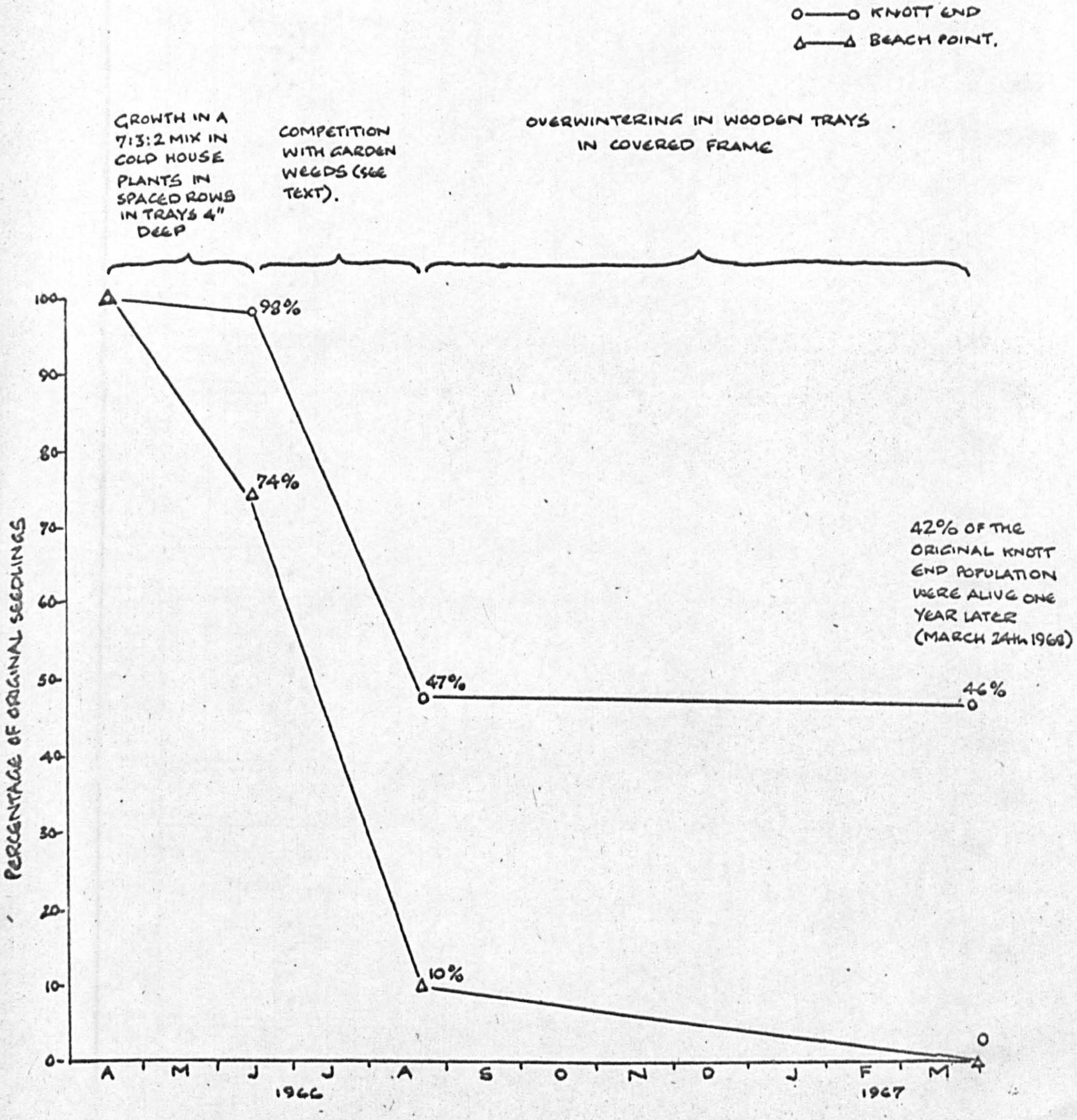


TABLE 9.3 Survival of plants in ordinary soils (data extracted from general stock catalogue)

Code	Popn.	from wild-collected plant (P) or fruit (S)	No. of seedlings pricked out or plants potted	no. surviving	treatment	Period of growth (months)	% survival
MARITIME POPULATIONS							
65/41A	Gib.Agropyron	S	108	94	H/trays	6	87.0
	"	P	54	46	F/Trays*	9	85.2
66/18	"	P	37	33	H/pots	(9)	89.2
65/46A	Cley	S	6	6	H/Trays	4	100
		P	6	6	G	(12)	100
66/32	Parkgate Pl.	P	5	5	H/pots	6	100
		P	16	14	G	6	87.5
66/22	Anglesey A	P	16	14	H/pots	(9)	87.5
66/28	Spiral marsh	P	15	15	H/pots	(9)	100
		P	12	9	F/pots	8	75.0
66/19	Gib.Limonium	P	23	20	H/pots	(9)	86.9
65/47A	Naze	S	54	22	H/trays	4	40.7
Total rate HIGH marsh pops. all treatments, all periods			352	284			83%
66/20	Gib. Aster	P	30	16	H/pots	(9)	53.3
66/30	Plover mrsh.	P	10	9	H/pots	(9)	90.0
66/2	Tetneyold	S	30	19	H/pots	(20)	63.3
65/50A	R.Lune	S	12	12	G	(12)	100
65/48A	R.Wyre	S	25	12	G	9	48.0
Total rate MID marsh all treatments, all periods			107	68			63.5%
66/22A	Anglesey B	P	14	5	H/pots	(9)	35.7
66/27	Beach Point	P	28	14	H/pots	9	50.0
66/33	Parkgate P4	P	5	2	H/pots	6	40.0
66/21	Gib.Spartina	P	20	7	H/pots	(9)	35.0
66/29	Cockle Bight	S	15	4	F/trays	(9)	26.7
	"	P	9	3	H/pots	(9)	33.3
Total rate LOW marsh all treatments, all periods			91	35			38.4%
INLAND POPULATIONS							
66/23	Winsford A	P	12	12	H/pots	5	100
	"	P	11	10	G	(9)	90.9
66/24	" B	P	4	3	H/pots	5	75.0
66/25	" C	P	14	12	H/pots	5	85.7
66/26	" D	P	5	3	H/pots	5	60.0
Total rate WINSFORD all treatments, all periods			46	40			86.9%

H = Cold house
G = Garden
F = openframe

( ) indicates overwintering
* poor soil

trend. (For example Gib. Agropyron marsh 66/18 - 89% survived, Gib. Limonium marsh 66/19 - 87% survived, Gib. Aster marsh 66/20 53% survived and Gib. Spartina marsh 66/21 - 35% survived). One can thus expect to lose in culture on average 15% of the plants grown from a high marsh population, 30% from a mid marsh population and 60% from a low marsh population. These sorts of figures confirm the general impression gained of survival rate amongst those population from maritime areas for which no accurate record of initial numbers was kept.

Apart from the occasional case of serious damage by slugs in cultivation the reasons for the failure of plants is presumably physiological and unknown. The general symptom often preceeding death was chlorosis of the leaves which may be related to a possible requirement for sodium in higher quantities than that found in ordinary soils (see Chapter 8). It is not possible to say that plants from a given population generally grew 'well' or 'better' in ordinary soils. Several plants from populations with a high death rate flourished vegetatively and fruited freely. Only the proportion of plants lost from a population reflected the origin of that population. In terms of both soil types and the amount of interference from other plants the conditions of experimental cultivation most closely resemble those obtaining in the relatively tide free upper zones of maritime salt marshes. The differences in competitive ability and survival rate (between plants from different marsh levels) under such conditions thus reflect differences of the kind which may lead to the elimination of less well adapted types in the upper zones.

Chapter 12 contains further discussion of the adaptive significance of fruit size, germination requirements, seedling vigour, and ability to grow and compete with glycophytes in ordinary soils.

VI Summary.

The work on seedling elimination reported in this chapter may be summarised as follows.

(i) It was assumed that the dispersal mechanisms of *Aster* fruits are efficient, the main agents being wind and tidal water, and that, in theory, fruits from low marsh populations are dispersed to high marshes and *vice versa*. The nature of tidal flow makes the former a more likely contingency where tide dispersal operates and the latter more likely where wind dispersal operates.

(ii) The pattern of elimination in marked clumps (probably fruits from the same capitulum) partly supports the suggestion that the mechanical action of the tides may be an important means by which seedlings are eliminated. The different survival rates in sheltered and open sites respectively and the different rates in two years with different amounts of tidal washings are evidence of this type of selection. It is suggested that this would lead to the elimination of high marsh seedlings in the lower marshes as they require lower salinities for germination and gave a slower rate of root growth than low marsh types. Those low marsh types which can germinate at relatively higher salinities and have rapid root growth would be favoured under these conditions.

(iii) The germination of wild collected fruits from 2 low and 1 high marsh populations when introduced to 2 low and 1 high marsh at Scolt Head Island was relatable to their origins. The fruits from the high marsh population (Gib. Agr.) showed very poor germination ($> 1\%$) on the two low marshes whereas some germination occured of low marsh fruits (Gib. Spa. and S. Cob.) introduced to the high marsh. The fruits of all populations showed optimum germination in the marsh most closely resembling that of their origin. The experiment was conducted to correspond with what was regarded as the natural period for germination and seedling establishment (Sept. - April).

(iv) The different survival rates of plants from low, mid and high marshes when grown in garden soils shows a pattern which can be related to the closeness of these conditions to those from which they were collected. Thus high marsh plants, found in the wild in relatively tide-free conditions, survive well in culture, whereas a high percentage of low marsh plants die. In particular a mid and a low marsh population showed different survival rate after a period of competition with indigenous weeds in the botanic garden.

SUMMARY OF PART III

(1) The selective nature of the tides was discussed and experiments relevant to the possible selective effects of (i) the physical movement and (ii) the high NaCl concentrations, of sea water were described.

(2) In an experiment partly simulating tidal treatment the pattern of germination and growth in populationsfrom two series of high to low marshes was examined. Fruits from high marsh populations showed decreased germination with increased tidal treatment and were unable to germinate during periods of simulated tidal coverage. By contrast fruit from low marsh populations were able to germinate and grow (although less well) under conditions corresponding to intense tidal treatment. The general pattern of growth as measured by total biomass in all populations was relatable to their origin.

(3) Seedling size during the early stages of growth was directly correlated with initial fruit size. This was felt to be an important factor in the elimination of seedlings by the physical movement of tides in the lower zones. A field experiment, in which the change in number of marked clumps of naturally set seedlings was recorded after the equinoctial spring tides in two consequtive years, suggested that the elimination of seedlings occurs during this period. The difference in elimination rate in exposed and sheltered positions and after differing periods of tidal action suggests that mechanical elimination may be important.

(4) Seedlings from different populations have a different pattern of lateral root development irrespective of fruit size. In two separate experiments the lowest marsh populations showed increased development of lateral roots compared with populations from higher marshes (Beach Point/Knott End: Gib. populations).

(5) When grown in the various conditions of cultivation which were used in this project a proportion of the plants from low marsh populations consistently failed to survive. This proportion was less for mid marsh populations and much less for high marsh populations. No attempt was made to measure the different effects of soil type, spacing or competition on the survival of plants from different marsh types, but the general pattern of survival in ordinary soils described above was apparently similar under a range of conditions. In one experiment the competitive superiority of a mid marsh population as compared with a low marsh population in competition with garden weeds was demonstrated.

(6) Seedling growth in most populations is increasingly depressed by the increased addition of NaCl to a base nutrient medium. Death occurs above concentrations of about 2% NaCl. The most marked effect of increased NaCl concentration is a reduction in the number of lateral roots. In short-term experiments the appearance of seedlings (i,e. the amount of leaf chlorosis) bore no relation to various yield parameters and there were no obvious between-population differences in yield which could be related

to the origin of the parent populations.

(7) The literature relating to the physiology of salt tolerance was reviewed. This reveals a diversity of approach to the problem. One experiment revealed the contrasting abilities of seedlings from a high (Gib. Agr.) and a low (Gib. Spa.) population to absorb K ions from solutions containing increasing concentrations of Na ions. This was thought to be important and the possible implications were discussed. Na and K content of the tops of seedlings was related to the amount of leaf chlorosis but not to estimates of seedling size.

(8) The possible means of dispersal of Aster fruits was discussed. Both wind and water may commonly act as dispersal agents.

(9) Selected fruits were introduced to three marshes on Scolt Head Island. Their pattern of germination was examined and fruits from each of three populations showed optimum germination in the marsh which most closely resembled (ecologically) that from which they were collected. The results of an experiment using transplanted clonal material were unsatisfactory due to low survival rates in the field.