PROBLEMS IN THE IDENTIFICATION AND UTILISATION OF INTERSPECIFIC HYBRIDS OF *POA* IN A PLANT BREEDING PROGRAMME

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SUMMARY

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Twenty-four Poa ampla X P. pratensis hybrids were obtained following high temperature treatment of P. ampla prior to anthesis, and 49 hybrids following short day treatment of P. ampla; parental biotypes of both species were highly apomictic. The amphimictic species P. iberica and P. longifolia were both crossed with P. pratensis; four F1 hybrids were obtained from each series of crosses. Somatic chromosome numbers of F_1 hybrids indicated that probably 60 per cent were derived from two reduced gametes, 34 per cent from one unreduced and one reduced gamete, a further six per cent could not be classified. Most F_1 hybrids were fertile but 12.3 per cent were sterile or nearly so. Principal co-ordinate analysis and canonical analysis of discriminance on data from spaced plant field trials were used to confirm identification of hybrids and maternal types.

Data from two spaced plant field trials with eleven hybrid progenies and six control parental biotypes were used to examine the possibility of distinguishing between predominantly apomictic and predominantly sexual families. Discrimination between different types of progeny was possible from examination of both the relative magnitude of determinants from within family dispersion matrices and the clustering of individual points on scatter diagrams from principal component, principal co-ordinate or canonical analyses. Similar techniques also enabled separation of apomictic and sexual families on seedling characters. Aberrants could be distinguished from maternal individuals using multivariate methods; precision was improved where data from

vegetative clones were used. Univariate analyses were helpful in confirming results. The need for a more objective procedure is discussed. Three to six variates gave adequate discrimination, characters were chosen for high repeatability, ease of measurement and low correlations.

Two trials were conducted, the first on P. ampla and P. pratensis biotypes, the second on eleven hybrid progenies to examine the influence of light regimes from just before ear emergence until anthesis on the resulting seedling progenies. Both photoperiod and total irradiance affected seedling characters and variability but the direction and magnitude of response differed between biotypes of P. pratensis and between hybrids. Results from P. ampla indicated that a higher proportion of sexual seed matured after ten hour than after twenty hour photoperiod treatments. Different pollen parents used in pair crosses between P. ampla and P. pratensis biotypes gave progenies differing in seedling characters and in homogeneity of dispersions from the same maternal parent.

The need firstly for improved techniques for assessment of apomixis, and secondly for more information on the control of apomixis and the developmental physiology of reproduction in facultative apomicts is discussed, with the broad objective of increasing the scope of a breeding programme.

.... and he shall separate them one from another, as a shepherd divideth his sheep from the goats: And he shall set the sheep on his right hand but the goats on the left.

Matthew 25: 32 and 33.

GENERAL OBJECTIVES

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The work reported here was all carried out at the Scottish Plant Breeding Station within the broad context of a breeding programme based on the facultative apomict Poa pratensis. The ultimate objective is to produce a grass for use in upland hill sheep systems where there is a requirement for high quality pasture production during spring and late autumn. A programme of introgressive hybridisation was started in 1968 by Dr. Patricia J. Watson with the aim of introducing into P. pratensis the characteristic vigorous early and late season growth exhibited by the three foreign species P. ampla, P. iberica and P. longifolia.

The overall objective of this project was to fully exploit the flexibility inherent in the breeding system; two main areas were investigated. First, the possibility of maximising recombination in the sexual phase which follows hybridisation by manipulation of the environment to promote sexual rather than apomictic seed production. Second, effective screening for elite, highly apomictic individuals each of which will produce uniform maternal progenies and may therefore form the basis of a new cultivar.

The particular problems examined were:-

1. Production of F_1 interspecific hybrids. This necessitated altering the balance between apomictic and sexual reproduction in favour of the latter.

2. Separation of F_1 hybrids from maternal individuals. Somatic chromosome counts, visual assessment and multivariate statistical methods were all used.

3. Discrimination between highly apomictic and predominantly sexual hybrids from progeny tests; and recognition of individual aberrants (sexual or cytological) within partially apomictic families. Visual assessment on mature plants revealed a "grey area" where phenotypically variable apomicts could not be distinguished from plants morphologically similar but differing in genotype. Cytological methods were time consuming and not sufficiently reliable for this purpose. Methods of evaluation based on easily measured and agronomically useful characters were examined using univariate and multivariate statistical techniques.

4. The possibility of adapting methods used on mature plants for screening seedling progenies. If successful, this would enable earlier separation of predominantly sexual plants (for further crossing) from predominantly apomictic plants (for agronomic evaluation).

5. The influence of different light regimes imposed during inflorescence development on apomictic seed production in P. ampla, P. pratensis and on interspecific hybrids. Seedling progenies were examined.

6. The effect of different pollent parents on seed production and seedling development from highly apomictic biotypes of P. ampla and P. pratensis.

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CHAPTER 1

GENERAL INTRODUCTION

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GENERAL INTRODUCTION

A breeding programme was started at the Scottish Plant Breeding Station in 1965 with the objective of producing a grass for use in improved pastures on marginal and hill land (Ann. Rep. Scott. Soc. Res. Plant Breed., 1966).

Systems of hill sheep production developed by the Hill Farming Research Organisation depend on both increased pasture productivity and on increased efficiency in its utilisation (Eadie, 1970, 1972). The two critical periods in nutrition of the hill ewe are prior to parturition and during lactation, and prior to mating (Gunn, 1967 and Russel, 1967). High productivity is therefore required in an improved hill pasture from April to August and from October to mid-December.

Earlier work at the Scottish Plant Breeding Station on the improvement of hill pasture had shown that indigenous Poa pratensis (sensu lato) was responsive both to an increase in level of fertiliser nitrogen and to intensive grazing pressure (Gregor, Watson and Connell, 1950 and Gregor and Watson, 1953). It is a grass widely used in Europe and the U.S.A. and there are many commercial cultivars available from countries in these areas.

The Poa pratensis species complex has been variously divided and sub-divided into species, sub-species, forms and varieties, but most current classifications recognise the following five main ecological groups as either species or sub-species:-

(a) P. pratensis L. sensu stricto

(b) P. angustifolia (L.) Lindb. fil.

(c) P. irrigata (Lindm.) Lindb. fil. incl. P. subcaerulea Sm.

(d) P. alpigena (Fr.) Hiitonen

(e) P. arctica R. Br.

In the British Isles only (a), (b) and (c) occur naturally. Where a distinction between groups is necessary the classification of Hubbard (1968) will be used; otherwise all biotypes within the species complex will be referred to as simply P. pratensis.

In 1965-7 a collection of commercial cultivars of P. pratensis (s. lato) from the U.S.A., Europe and Russia was examined by Dr. Patricia Watson (Ann. Rep. Scott. Soc. Res. Plant Breed., 1967). At the same time a collection of named wild populations of P. pratensis (s. lato) from Europe and England, and a collection of sixty ecotypes from different habitats all over Scotland were also in trials at Pentlandfield. Most of the cultivars were morphologically like P. pratensis (s. stricto) although a few were like P. angustifolia or like P. subcaerulea. The wild Scottish biotypes all appeared to be P. subcaerulea; a few of these wild populations gave dry matter yields as high as the highest yielding cultivars under spaced plant trial conditions (Ann. Rep. Scott. Soc. Res. Plant Breed., 1968). On the basis of these results it seemed sensible to attempt to develop lines of indigenous Scottish P. pratensis which would be more productive early and late in the season for use in hill sheep systems.

Several foreign species of Poa had also been included in the above trials. Three of these were of particular interest since they were summer dormant, grew actively in autumn and spring, and

spring, and remained green throughout the winter; they were all vigorous and erect bunch grasses. P. ampla Merr. is from the north-western U.S.A. where it grows on prairies and open grassy hillsides between 900 m and 300 m (Keck, 1965). P. iberica Fisch et Mey and P. longifolia Trin. are both endemic in mountainous areas around the Black Sea where they grow in alpine meadows; they are considered to be morphologically identical although tetraploid (2n = 28) and hexaploid (2n = 42) respectively (Almgård, 1966).

Work at the Carnegie Institute has shown that interspecific hybrids can be obtained between many, some rather distantly related, species of Poa (Clausen, Keck and Hiesey, 1944, 1945 and 1946). The prevalence of polyploidy in the genus and the marked tolerance to aneuploidy facilitate the survival of hybrids, and the widespread occurrence of apomictic seed production also helps to maintain chromosomally unbalanced genotypes (Clausen, 1961).

Gustaffson's (1946) terminology for apomixis will be used. He referred to sexually reproducing organisms as being amphimictic, and to those with the process of fertilization wholly or partially lost as being apomictic. Apomicts may reproduce by seeds (agamospermy) or by vegetative reproduction (e.g. bulbils, runners). In agamospermous plants the gametophyte stage may be by-passed by the development of the embryo directly from a nucellar or integumental outgrowth; this is adventitious embryony. Alternatively the gametophyte is present and may develop either by diplospory, when it arises from the megaspore mother cell, or by apospory when it arises from a somatic cell in the nucellus or chalaza.

Müntzing (1933) first showed that P. pratensis was agamospermous. Subsequently Åkerberg (1939, 1942), Tinney (1940) and others have demonstrated that development of the gametophyte is by apospory and that fertilization of the central nucleus is required for endosperm formation and full development of the embryo (pseudogamy). Aposporous development starts when a cell in the nucellus becomes differentiated at about the time of the first meiotic division of the macrospore mother cell. Meiosis may proceed normally leading to the formation of a typical eight nucleate embryo sac. More often the macrospores degenerate during or after the second meiotic division. The differentiated somatic cell in the nucellus becomes enlarged and divides mictotically to form an eight nucleate embryo sac structurally indistinguishable from a "normal" one except that the chromosome complement is not reduced. The egg cell may divide to form a multicellular proembryo before anthesis. Polyembryony may occur due to the development of both aposporous and sexual embryo sacs; two or more aposporous embryo sacs occasionally arise in one ovule. Pollen formation is usually regular (Åkerberg, 1942).

Müntzing (1940), Åkerberg (1942) and Nygren (1951) all distinguish two steps in the formation of apomictic seed in P. pratensis; first the production of aposporous initials and second the parthenogenetic development of the egg cell. This suggests that seed may be produced by four main pathways; an egg cell may have a reduced or an unreduced chromosome complement, and may develop parthenogenetically or require fertilization.

Any plants which deviate morphologically from the norm in

a predominantly apomictic family are generally identified simply as aberrants unless their precise origin is known. Åkerberg (1942) observed from a study of twelve biotypes representative of all species in the P. pratensis complex except P. arctica, that the progenies of aberrants were usually variable indicating a mainly sexual seed production regardless of their mode of origin.

The proportion of aberrants produced by any one biotype may be influenced by the pollen parent (Åkerberg, 1939) or by the environment (Nygren, 1951 and Clausen, Hiesey and Nobs, 1954). Grazi, Umaerus and Åkerberg (1961) discuss the different stages at which competition may occur between apomictic and aberrant individuals, these range from the timing of the appearance of embryo sac initials to differences in genotype-environment interactions of mature plants. Clausen et al.(1956 and 1958) and Watson and Clausen (1961) reported very widely differing responses of families to differences in the environment which were observed during transplant experiments. They also observed that strains which appeared phenotypically uniform in one environment and were therefore thought to be highly apomictic, were found to be very variable at other transplant stations showing that seed production had in fact been mainly sexual.

Müntzing (1940), Åkerberg (1942), Clausen et al.(1946), Almgård (1966) and van Dijk (1974) all found that F_1 hybrids of Poa from interspecific and intraspecific crosses produced mostly sexual seed even when both parents were highly apomictic. A wide range of segregants was generally obtained in the second generation showing a variable degree of recombination

between the parental characters. In later generations there was a gradual shift towards apomixis. Müntzing (1940) postulated that apomixis in Poa is due to a rather delicate genetic balance of special constellations of genes and chromosomes which can be upset in various ways, and particularly by a quantitative change in the chromosomes. In the cross P. longifolia X P. pratensis Almgård (1966) found functional apomixis to be completely recessive although the presence of aposporous embryo sacs showed a dominant pattern of inheritance.

Evidence from polyhaploid lines of P. pratensis which closely resembled P. trivialis (Kiellander, 1942 and Åkerberg and Bingefors, 1953), and from the wide range of morphological and physiological variability in the species complex (Clausen et al, 1951), suggests that P. pratensis is itself of hybrid origin with a high degree of autopolyploidy. It falls into Harlan and de Wet's (1975) category of class I polyploids "in which chromosome increase occurs in the first generation through union of gametes one or both of which are unreduced". Clausen (1961) concludes from the range of Poa material examined by the Carnegie Institute team that many species in the genus and P. pratensis in particular have been able to absorb genomes from other species due to the occurrence of occasional sexual aberrants in a predominantly apomictic and polyploid breeding system.

The three main phases in a breeding programme based on a facultative apomict such as P. pratensis are: firstly to shift the balance in favour of sexual reproduction to enable initial intra- or interspecific hybridisation; secondly to maintain sexual reproduction in progenies to allow further cycles of

crossing and recombination; and thirdly to test useful biotypes and select highly apomictic lines for multiplication.

Since apomictic seed production in Poa is known to be influenced by environmental factors (loc. cit.), more precise information on the quantitative effects of some of these factors on the biotypes under test would be of great value. A prerequisite to such quantitative measures is a satisfactory technique for distinguishing apomictic from aberrant individuals. Moreover such a technique is essential for screening progenies in the breeding programme.

The investigations reported here concern the production of interspecific hybrids of Poa and the methods used in distinguishing hybrid from maternal offspring. Field data from hybrid progenies are then used to explore possible statistical techniques for separating apomictic from segregating progenies and individual aberrants within otherwise apomictic progenies. The application of similar techniques to seedling data is investigated and the influence of different light regimes on the balance between aposporous and sexual seed production is examined on parent biotypes and on some hybrid progenies.

The general objective of this aspect of the work is to develop techniques which will enable rapid assessment of breeding behaviour of selected plants leading to separation of the predominantly sexual plants, which can subsequently be treated as a "normal" outbreeding population, from the predominantly apomictic plants which can be tested as potential new cultivars.

CHAPTER 2

STATISTICAL METHODS

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All variates were tested for normality of distribution using a program which gave a measure of skewness and kurtosis (Sokal and Rohlf, 1969). Transformations were carried out where necessary to minimise skewness and kurtosis. Homogeneity of variances was tested using Bartlett's method (Snedecor and Cochran, 1967) and homogeneity of dispersion matrices using the method given by Seal (1964). Canonical analysis of discriminance was carried out using a computer program based on the algorithm given by Seal (loc. cit.) and developed at the Scottish Plant Breeding Station. The Euclidean distance between every pair of groups was computed as the square root of the sum of squared distances of the canonical variates. Distances were then scaled by dividing each by the maximum distance and multiplying by 1000. The position of the centroid was defined by the means of the canonical variates. Standard computer programs were used for principal component (Hope, 1968) and principal co-ordinate (Blackith and Reyment, 1971) analyses, and for analyses of variance.

The convention is adopted of probabilities being signified throughout as *, 0.05 to 0.01; **, 0.01 to 0.001, and ***, < 0.001.

CHAPTER 3

PRODUCTION OF INTERSPECIFIC HYBRIDS

(TRIALS A and B)

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PRODUCTION OF INTERSPECIFIC HYBRIDS (TRIALS A AND B)

INTRODUCTION

The range of variability observed in seasonal growth patterns of 60 Scottish ecotypes of P. pratensis (Ann. Rep. Scott. Soc. Res. Plant Breed., 1968) was clearly too limited to form the basis of a breeding programme. A programme of interspecific hybridisation of P. pratensis with P. ampla, P. iberica and P. longifolia (Fig.1) was therefore proposed. The objective was to obtain a hybrid with the morphological characters of P. pratensis but with the characteristic vigorous early and late seasonal growth which all three foreign species exhibited.

P. ampla is, like P. pratensis, a facultative apomict; Nygren (1951) showed that embryo sacs are produced by apospory and that egg cells ~~do~~ could not divide in matured sacs and ~~cannot~~ therefore be fertilized. There are no known incompatability barriers between the two species; Clausen et al. (1944, 1945) first produced hybrids between them using P. pratensis as the pollen parent. In the Pentlandfield environment both species are highly apomictic and early attempts to cross them failed to reveal any hybrids (Ann. Rep. Scott. Soc. Res. Plant Breed., 1969).

A means of shifting the equilibrium between apomictic and sexual seed formation was required since when this balance is upset an increase in sexual aberrants usually results (Müntzing, 1940). Julén (1954, 1958) and Grazi et al. (1961) used X-ray irradiation to "break" the apomixis in P. pratensis. The

FIGURE 1

Poa iberica

Poa longifolia

Poa ampla

Poa pratensis

Mature plants of four species used as parents
in production of interspecific hybrids

simplest method suggested is to change the environment before and during flowering (Nygren, 1953). Grazi et al.(loc. cit.) observed a reduction in the number of aposporous embryo sac initials in a plant of cv. Fylking which flowered in early spring in a greenhouse compared with others which flowered later in the season. A response similar to that observed in Calamagrostis spp. (Nygren, 1946).

One biotype of P. ampla was known to produce more aberrants at Pullman (latitude 46° N) than at Pentlandfield (56° N), and more again at Stanford, California (38° N) where there was considerable disturbance in its flowering behaviour (Watson and Clausen, 1961). It seemed possible that the proportion of aberrants in P. ampla would be increased if plants were moved before flowering to an environment more like that at Stanford.

P. iberica and P. longifolia are both amphimictic but interspecific hybrids with other species are not easily obtained (Nygren and Åkerberg, 1957). Clausen et al.(1945) produced a few hybrids with P. longifolia but they were very weak and not useful agronomically. Almgård (1960, 1966) found that P. longifolia hybridised with P. Chaixii Vill. and with P. hybrida Gaud. relatively easily; he also obtained nine hybrids with P. pratensis but only three of these F_1 hybrids were fertile. F_1 hybrids between P. longifolia and P. pratensis were reported by van Dijk (1974) to be mostly sexual and fertile.

Screening progenies from crosses for possible hybrids can be a problem where large numbers are involved. The possibility of using canonical analysis of discriminance and principal co-ordinate analysis to help in distinguishing maternal from hybrid individuals is investigated.

MATERIALS AND METHODS

Production of 1968 hybrids P. ampla X P. pratensis

Two biotypes of P. ampla and ten of P. pratensis (Table 1) were transferred just before anthesis from the open field to a heated greenhouse with a day temperature of c. 30°C and a night temperature of c. 16°C. Individual plants of the two species were pair-crossed but seed was harvested only from the P. ampla parent.

The seed was germinated on filter paper and pricked on into individual pots. Multiple seedlings resulting from polyembryony were separated before they were pricked out. All multiple seedlings and those seedlings which showed any evidence of deviation in habit from P. ampla, together with one plant of each of the parental biotypes, were vegetatively cloned into twelve ramets. Ten ramets of each clone were planted out in 1969 in a fully randomised spaced plant trial, Trial A. The remaining two ramets were kept for cytological examination.

Production of 1968 hybrids P. iberica X P. pratensis and P. longifolia X P. pratensis

Pair-crosses of two genotypes of P. iberica and two of P. longifolia with five biotypes of P. pratensis (Table 2) were made in an unheated greenhouse. Seed was harvested from all the plants.

All viable seedlings from P. iberica and P. longifolia, the few seedlings from P. pratensis which appeared possibly to be hybrids, and the parent plants were vegetatively cloned in the same way as the P. ampla material and included in Trial A.

TABLE 1 - Sources of parental material used in production of 1968 P. ampla X P. pratensis interspecific hybrids

Species	Index No.	Source
P. ampla	42	Sherman CIW 4172. Supplied by Plant Materials Centre, Soil Conservation Service, Pullman, U.S.A.
"	45	P.846 CIW 4178. Supplied by Plant Materials Centre, Soil Conservation Service, Pullman, U.S.A.
P. pratensis	109	Maritime community, Skåne, S. Sweden. Supplied by Dr. A. Müntzing.
"	119	Mountains, S. Hordaland, Norway. Supplied by Dr. A. Müntzing.
"	136	Rough hill pasture, 150 m, Roxburgh, Scotland.
"	159	Roadside, 30 m, Kirkcudbright, Scotland.
"	167	Moorland near stream, 300 m, Banff, Scotland.
"	168-1	Near stream by road, 280 m, Aberdeen, Scotland.
"	168-3	" " " " " " "
"	172	Hill pasture, 230 m, Perth, Scotland.
"	183	Maritime grassland, 0 m, Argyll, Scotland.
"	189	" " 0 m, " "

TABLE 2 - Source of parental material used in production of 1968 P. iberica X P. pratensis and P. longifolia X P. pratensis interspecific hybrids

Species	Index No.	Source
P. iberica	59-8	Supplied by Hortus Botanicus, Stavropolensis, U.S.S.R.
"	59-11	Supplied by Hortus Botanicus, Stavropolensis, U.S.S.R.
P. longifolia	107-2	Supplied by Dr. G. Almgård, Dept. of Genetics and Plant Breeding, Uppsala, Sweden.
"	107-4	Supplied by Dr. G. Almgård, Dept. of Genetics and Plant Breeding, Uppsala, Sweden.
P. pratensis	14	Roadside, Cévennes district. Supplied by Laboratoire de Recherches sur les Plantes Forragères, Rouen, France.
"	142	Moorland, 270 m, Angus, Scotland.
"	145-2	Roadside, 210 m, Perth, Scotland.
"	160	Roadside, 150 m, Dumfries, Scotland.
"	190	Moorland by stream, 120 m, Argyll, Scotland.

Production of 1969 P. ampla X P. pratensis hybrids

The number of possible hybrids between P. ampla and P. pratensis obtained in 1968 was lower than expected, so in 1969 environmental conditions more nearly like those at Stanford, California were simulated with a short photoperiod as well as increased temperatures.

A simple dark chamber made with heavy black polythene and fitted with fluorescent strip lights controlled by time switches was erected in a heated greenhouse. The length of photoperiod was calculated from tables in the Nautical Almanac 1969 to be equivalent to that at Stanford on the same date (Table 4); it was adjusted at intervals during the period of treatment.

Plants from two biotypes of P. ampla were lifted from the field where they had overwintered and moved into the dark chamber at a date calculated to give them 40 days of short day, high temperature treatment prior to the expected time of anthesis. Five Scottish biotypes of P. pratensis (Table 3) were brought into the greenhouse when P. ampla plants were near anthesis. The two species were mass pollinated.

All seed from the (non-rhizomatous) P. ampla parents was sown as soon as it was mature. Possible hybrid seedlings were selected by their prostrate or rhizomatous habit. The selected seedlings and their parents were vegetatively cloned into twelve parts, ten ramets of each were planted out in 1970 in a fully randomised spaced plant trial: Trial B. The other two ramets were kept for cytological examination.

TABLE 3 - Sources of parental material used in production of 1969 P. ampla X P. pratensis interspecific hybrids

Species	Index No.	Source
P. ampla	42	See Table 1
"	57	Supplied by Washington Agricultural Experimental Station, Pullman, Washington, U.S.A.
P. pratensis	137	Moorland, 150 m, Roxburgh, Scotland.
"	145-1	Roadside, 210 m, Perth, Scotland.
"	148	Rough pasture, 180 m, Perth, Scotland.
"	179	Roadside, 140 m, Sutherland, Scotland.
"	191	Lochside, 120 m, Perth, Scotland.

TABLE 4 - Daylength and mean monthly temperatures Edinburgh and Stanford for six months January-June

		1st Jan.	1st Feb.	1st Mar.	1st Apr.	1st May	1st June
Edinburgh	sunrise	8.25	7.55	6.52	5.35	4.20	3.25
55° 57' N	sunset	15.35	16.35	17.35	18.40	19.40	20.40
	difference	7.10	8.40	10.43	13.05	15.20	17.15
	temp. av. max.	6°C	7°C	8°C	11°C	14°C	17°C
	" av. min.	1°C	1°C	2°C	3°C	7°C	8°C
Stanford	sunrise	7.10	7.05	6.35	5.45	5.05	4.40
37° 26' N	sunset	16.50	17.20	18.05	18.25	18.55	19.20
	difference	9.40	10.15	11.30	12.40	13.50	14.40
	temp. av. max.	11°C	16°C	17°C	20°C	23°C	25°C
	" av. min.	3°C	4°C	6°C	7°C	9°C	10°C

Trial A field records

The following variates were recorded on all ramets:-

EE (half weeks), ear emergence - date on which third panicle appeared above ligule of flag leaf.

PH (cm), plant height - measured from ground to top of second tallest inflorescence.

PD (cm), plant diameter - greatest spread measured on 17.9.1970

FLL (mm), flag leaf length - lamina length measured on representative flowering tiller.

FLB (mm), flag leaf breadth - measured half way along lamina.

No. Pan, number of panicles

SG (categories 1 to 3), spring growth - category 1, most leaves dead; category 3, most leaves green; recorded 22.2.1971.

Trial B field records

The following variates were recorded on all ramets:-

EE, ear emergence - as for Trial A

PH, plant height - " " " "

PD, plant diameter - " " " "

LL (mm), leaf length - length of lamina on last mature leaf on representative vegetative tiller.

LB (mm), leaf breadth - measured half way along lamina of above leaf.

SG1 (categories 1 to 10), spring growth - category 1, < 10 per cent of leaves green; category 10 > 90 per cent of leaves green; recorded on 18.2.1972.

SG2 (categories 1 to 10), as SG1 - recorded 15.3.1972.

SG3 (" " " "), " " - " 20.4.1972.

Hab (categories 1 to 5), habit - category 1, erect and non-rhizomatous; category 5, prostrate with long rhizomes.

AuH (categories 1 to 3), auricle hairs - category 1, no hairs; category 3, very hairy.

LfH (categories 1 to 3), leaf hairs - as for AuH.

Pan L (cm), panicle length - length of rachis from lowest branch in inflorescence to top, measured on representative flowering tiller.

Sp L (mm), spikelet length - measured on spikelet from centre of panicle.

Sp B (mm), spikelet breadth - maximum breadth of above spikelet.

Sd/Sp, number of seeds in spikelet - mean from 3 spikelets.

Sd H (categories 1 to 3), seed hairs - category 1, no hairs on lemma; category 3, very hairy lemma.

Statistical analyses, Trials A and B

Analyses were carried out separately on data from 1968 *P. ampla* X *P. pratensis* possible hybrids and parents (Trial A), 1968 *P. iberica* X *P. pratensis* and *P. longifolia* X *P. pratensis* possible hybrids and parents (Trial A), and on 1969 *P. ampla* X *P. pratensis* possible hybrids and parents (Trial B).

Cytology

Somatic chromosome counts were made on root tips or on leaf preparations. Root tips were prepared using $2\frac{1}{2}$ hours pre-treatment in a saturated solution of α bromonaphthalene in 0.05 per cent saponin followed by overnight fixation in glacial acetic acid and hydrolysis for 14 minutes in N. hydrochloric acid held at 60°C. They were stained for 1 to 2 hours in Feulgen, macerated in a 3 per cent pectinase solution for c. half an hour and mounted in fast green in glycerol or in 2 per cent acetocarmine. Leaf bud tissues were prepared by stripping off all the outer leaf sheaths on an actively growing vegetative tiller, any roots at the base of the tiller were cut off. Only 8 to 10 minutes was needed for hydrolysis, otherwise the procedure was the same as that used for root tips.

Counts were taken from at least three different cells; where there was a wide discrepancy in the counts more cells were examined.

Pollen stainabilities (Carroll, 1975) were assessed using five or six anthers from different spikelets for each preparation. They were squashed in Gram's iodine and 200 pollen grains examined. Darkly stained grains of regular shape were assessed as viable pollen. All plants which gave very low stainability

counts were checked. At least two preparations were made and counted for each hybrid and from the parental material. This method gives an estimate from the appearance of the pollen of its viability. Actual viability was not tested. Plants showing abnormal anther development were noted.

A rough assessment of the relative proportions of good (hard) seed was obtained from greenhouse crosses in 1970 and 1971.

RESULTS

1968 hybrids P. ampla X P. pratensis

Where polyembryony occurred the first seedling to develop was almost invariably maternal in appearance. Many of those which germinated later were less vigorous initially but appeared to be hybrids.

After twelve months in the field some of the 47 possible hybrid clones were seen to be of maternal type and were indistinguishable by eye from the P. ampla clone. The remaining plants varied in habit but all showed some character of P. pratensis (Fig.2).

Clone means for the seven variates are shown in Table 5 together with somatic chromosome numbers, pollen stainabilities and seed production. Clone 224 was omitted from all analyses since eight of the ten original ramets died during 1969. Field trial data from the maternal clones are shown in Appendix Table 1. Correlation coefficients were calculated from clone means between all seven variates measured in Trial A (Table 6).

FIGURE 2

P. ampla, *P. pratensis* and three *P. ampla* X *P. pratensis* F_1 hybrids showing range in habit

TABLE 5 - Notes

1. Chromosome number, accurate to $\pm$ 5. Clones 199, 226-2 and 227-2 see also text.
2. Pollen stainability.
3. Anther development; G, all anthers normal; N, a few anthers not fully developed; P, most anthers with poor or abnormal development; S, sterile.
4. Seed production; assessed from two ramets, controlled crosses in greenhouse, 1970; G, 1000 good seeds; N, 100-1000 good seeds; P, 1-1000 good seeds; S, no good seeds.
5. Number of ramets surviving in field trial.
6. Square root transformation.
7. Data squared X 10^{-3}.
8. Log_{10} transformation.
9. Square root transformation.
10. " " "
11. " " "

TABLE 5 - Trial A - 1968 hybrids P. ampla X P. pratensis, cytological and field trial data (clone means) for parents (P) and hybrids (H)

	Clone No.	Chro.[1] No.	PS[2] A[3] S[4] %	R[5]	EE[6]	PH[7] (cm)	PD[8] (cm)	FLL[9] (mm)	FLB[10] (mm)	No Pan[11]	SG
♀ P	42	56	42 M -	10	1.00	4.41	0.92	9.61	1.72	3.44	2.20
♂ P	189	100	71 G	10	2.21	2.62	1.70	6.95	2.23	4.45	1.60
H	197-2	105 3n	66 M G	9	1.99	5.20	1.32	8.43	2.05	4.27	1.78
♂ P	183	89	67 G	10	2.19	1.93	1.38	6.39	2.23	3.92	1.00
H	198-2	110 3n	31 P M	9	1.61	2.84	1.24	6.81	1.96	3.66	1.33
H	199	77 2n	41 G M	10	1.89	1.94	0.84	6.00	1.78	4.02	1.00
♂ P	172	81	73 G	10	1.89	2.20	1.52	6.03	2.09	5.68	1.20
H	200-2	65 2n	70 G G	10	1.89	1.48	1.04	5.55	1.69	2.86	1.30
♂ P	168-1	72	28 P	10	2.02	1.48	1.61	5.61	2.14	4.94	1.00
H	201	90 3n	63 M G	10	1.79	2.32	1.27	6.14	1.97	4.65	1.20
♂ P	119	81	95 G	10	2.21	3.72	1.66	6.00	2.36	4.63	1.60
H	204	102 3n	82 G P	10	2.02	3.93	0.86	7.89	1.69	3.42	1.80
♂ P	109	71	79 M	10	1.94	1.68	1.64	5.57	2.26	4.12	1.00
H	208	- -	- - S	9	2.37	0.15	0.95	6.87	1.41	1.88	1.00
♂ P	136	95	- -	-	-	-	-	-	-	-	-
H	212	64 2n	- S P	10	2.86	0.65	1.21	6.68	2.07	2.13	1.30
H	213	80 2n	- - G	9	2.20	3.14	1.14	7.71	1.98	3.32	1.22
H	214-2	95 3n	78 G G	10	1.86	4.61	1.35	8.22	1.95	5.27	2.10
H	215-2	102 3n	87 G G	10	1.87	4.00	1.35	7.43	2.24	4.82	2.50
H	216	75 2n	- - P	9	2.28	2.81	0.94	5.54	1.78	2.72	1.00
H	219	67 2n	17 P P	9	2.45	1.92	1.33	5.52	1.82	5.36	1.00
♂ P	168-3	86	60 M	6	2.02	1.00	0.98	5.14	1.93	3.80	1.17
H	220	70 2n	84 G G	10	2.14	3.92	1.19	7.08	1.83	5.83	1.00
H	221	72 2n	93 G M	10	2.14	2.83	1.24	5.88	1.75	7.90	1.00
H	222	85 3n	88 G G	10	2.19	2.96	1.20	4.96	1.65	2.39	1.10
H	223-2	109 3n	37 G G	10	1.75	4.09	1.39	5.71	1.66	4.88	1.00
H	224	- -	- - -	2	2.35	0.90	0.60	6.08	1.58	1.23	1.00
♂ P	159	90	91 G	10	1.99	2.07	1.68	6.52	2.30	5.95	1.30
H	226-2	113 3n	78 G G	10	1.73	3.61	1.28	7.65	1.97	4.91	1.80
H	227-2	107 3n	76 M G	10	1.63	2.86	1.05	7.36	1.94	5.19	1.10
H	228-2	111 3n	87 P M	10	1.92	2.77	1.08	8.15	2.02	4.98	1.40
H	229-2	77 2n	72 G G	10	1.65	3.10	1.09	8.36	2.01	5.13	1.70
♂ P	167	98	79 G	10	2.09	1.84	1.59	6.13	2.09	4.70	1.00
H	231-2	100 3n	48 G G	10	1.78	1.21	1.11	6.89	2.14	4.66	1.10
H	232-2	90 3n	84 M G	10	1.64	1.64	1.09	7.31	2.05	3.54	1.30

TABLE 6 - Trial A. Correlation coefficients (r) calculated from clone means (a) top right of table, 1968 hybrids P. ampla X P. pratensis and parents; and (b) bottom left of table, 1968 hybrids P. iberica X P. pratensis and P. longifolia X P. pratensis and parents

(b) \ (a)	EE	PH	PD	FLL	FLB	No Pan	SG
EE		-0.860	0.543	-0.893	-0.016	-0.194	-0.871
PH	-0.364		-0.439	0.904	0.045	0.241	0.913
PD	0.207	-0.622		-0.566	0.557	0.266	-0.479
FLL	-0.296	0.939	-0.588		0.065	0.123	0.941
FLB	-0.170	0.903	-0.615	0.912		0.265	0.127
No Pan	-0.048	-0.747	0.823	-0.674	-0.755		0.111
SG	-0.356	0.547	-0.220	0.678	0.434	-0.321	

(a) $r > \pm 0.428$ ***, $r > \pm 0.341$ **, $r > \pm 0.263$ *

(b) $r > \pm 0.693$ ***, $r > \pm 0.575$ **, $r > \pm 0.456$ *

Data from the 56 clones were tested for homogeneity of within-clone dispersions. Since one clone had fewer than seven surviving ramets, and the dispersion matrix for seven variates would therefore have been singular, it was not possible to test for homogeneity from all seven variates together. The first three and the last four variates were tested separately and gave chi-square values of 817.46 *** for 330 df and 1637.27 *** for 550 df respectively indicating that dispersions were heterogeneous.

Canonical analysis is thought to be sufficiently robust to withstand small discrepancies between dispersion matrices

(Hope, 1968); so analyses were carried out on all seven variates and on the first three variates (EE, PH and PD). The first canonical variate accounted for 79.03 per cent and 84.52 per cent of the total variation respectively in the two analyses. When clone means were plotted on the first and second canonical variates there was in each case a clear separation along the first axis of all clones similar morphologically to P. ampla from the remaining clones (figs. 3 and 4).

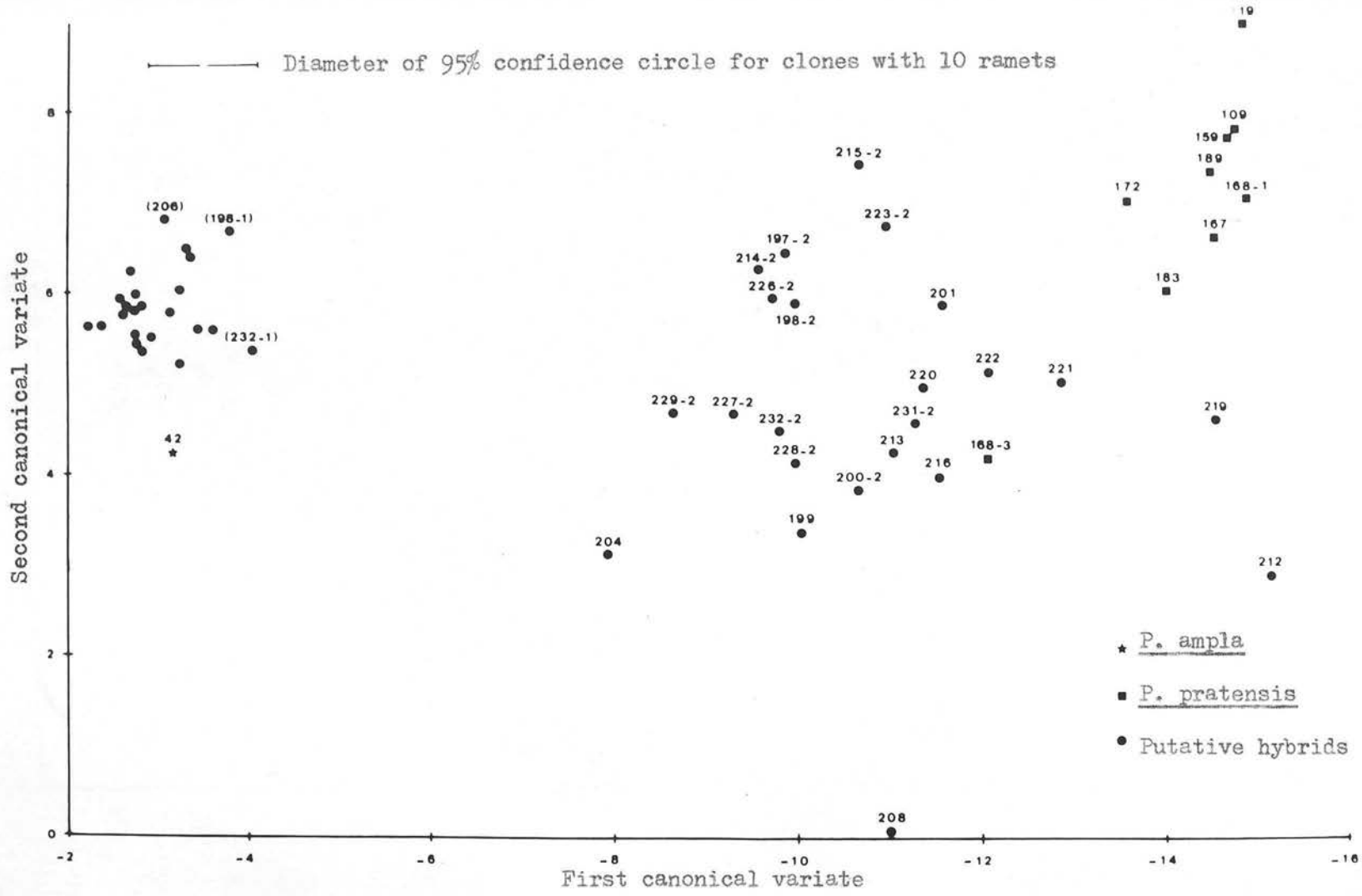
Principal co-ordinate analyses were also carried out on all seven and on the first three variates using clone means since this method also gives a scatter diagram but has no requirement for homogeneity of within group dispersions, (figs. 5 and 6). A comparison of diagrams from the two analyses showed substantially similar grouping of parental biotypes, maternal offspring and hybrids, although some of the individual relationships differed on the second axis.

Somatic chromosome numbers and pollen stainabilities were only counted on those clones which could be seen clearly to be of hybrid origin. Accurate chromosome counts were not possible, although a greater precision was possible in some plants than in others. In particular three clones gave widely discrepant results when checked, as follows: clone 199, c. 90 and c. 77 (fig.11); clone 226-2, c. 82 and c. 113; and clone 227-2, c. 80 and c. 107. There was no reason to believe any mistake was made in sampling or counting although some of the check counts were made on the original material after periods of up to five years. Morphological chimaeras have appeared in a few hybrids but there were no visible differences in the clones which gave the discrepant

results. Chromosome numbers were determined for some of the maternal types. Most gave 2n = 48 or 56, one was lower (2n = 42) and a few were higher (upto 2n = 68).

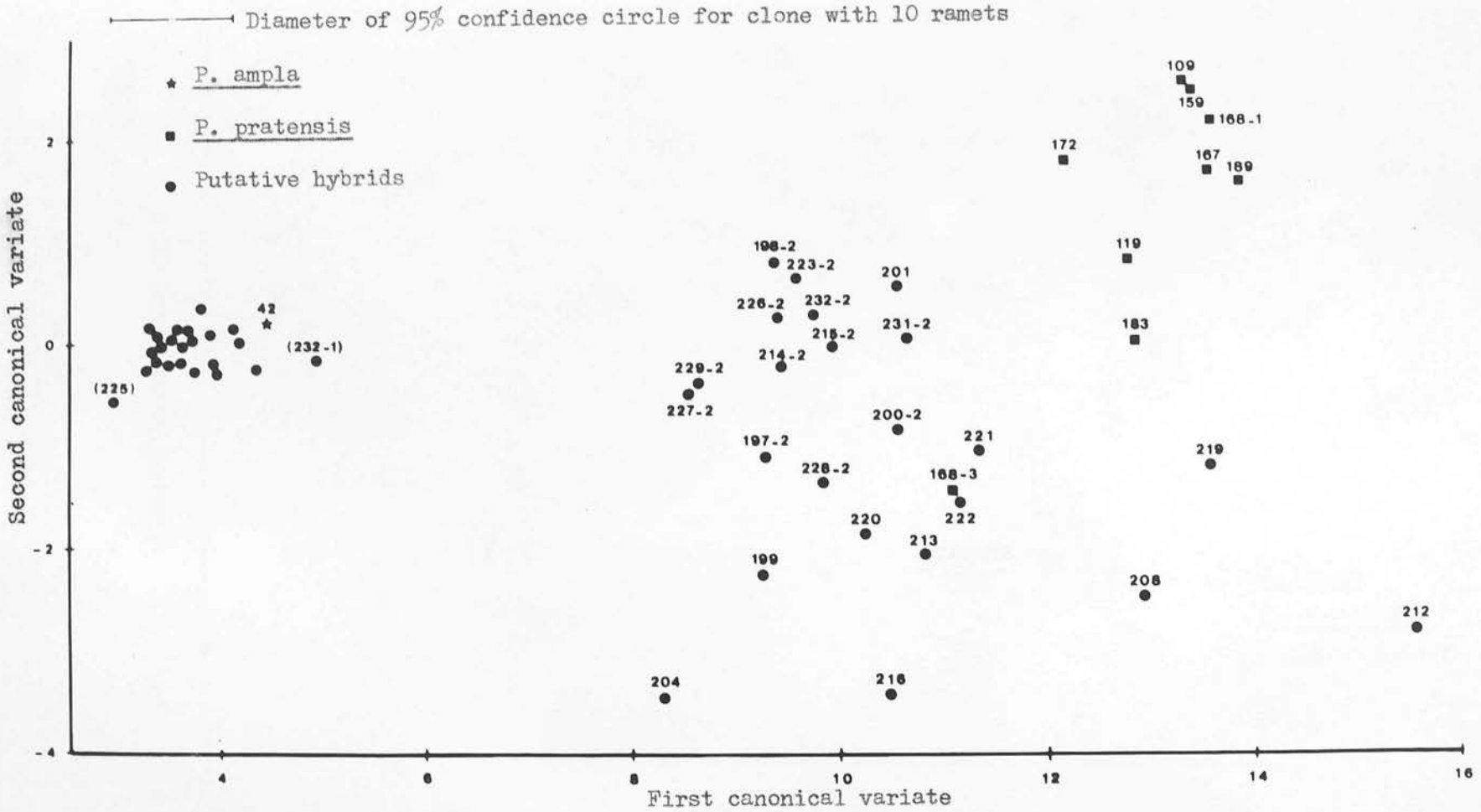
Some hybrids had a chromosome number close to the expected value assuming fertilization of either a reduced or an unreduced egg cell by a reduced male gamete. Others did not show any clear relationship to the expected values. There was no obvious clustering of "diploid" and "triploid" hybrids on the first, second or third canonical variates or principal co-ordinates.

Anther development, pollen stainability and seed production were good or moderately good on most hybrids. No assessment of fertility was possible on 208, 216 or 224 which were all very weak plants; 198-2, 219 and 228-2 had very poorly developed anthers and only yielded a small amount of viable seed; 212 was almost entirely sterile.



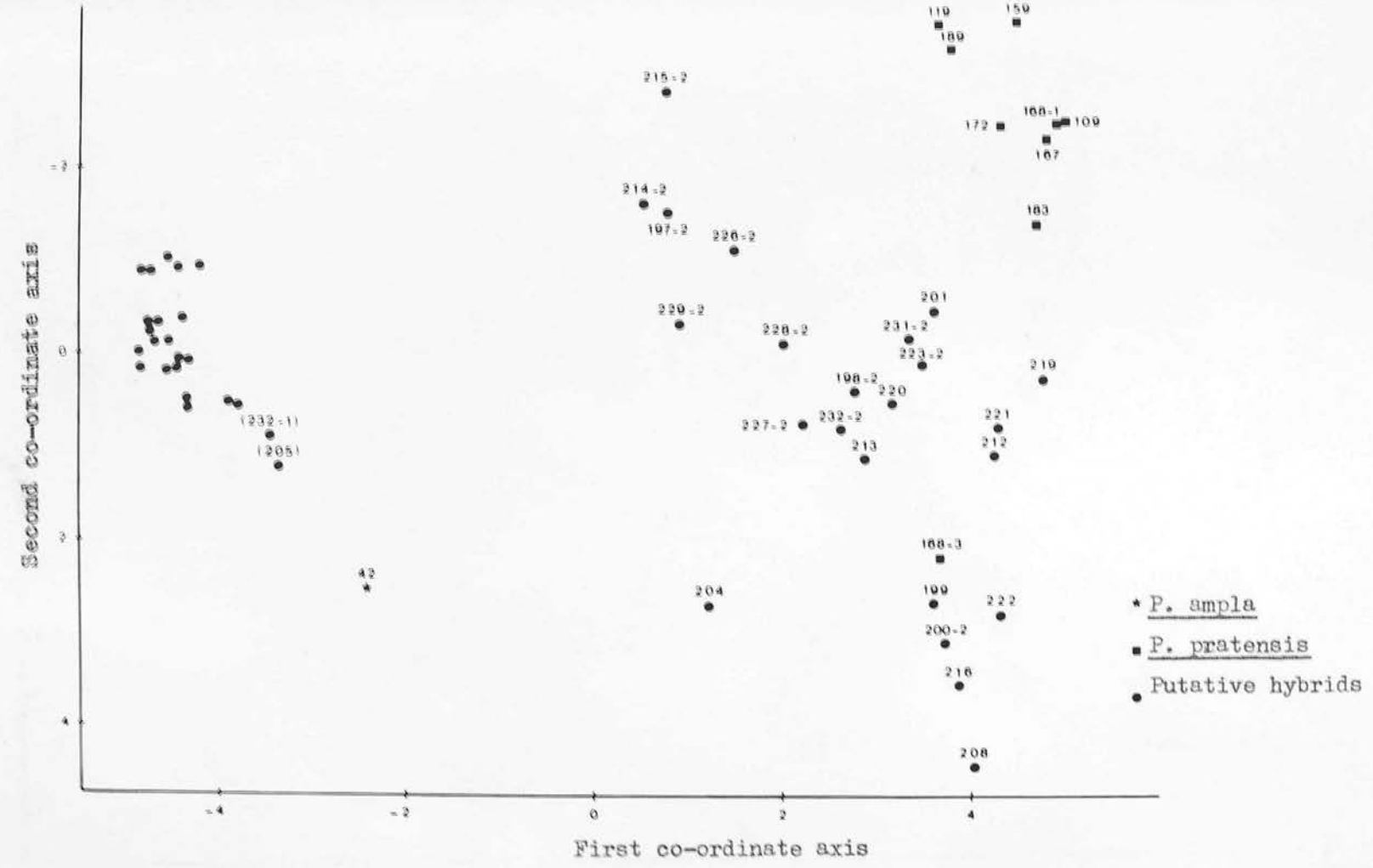
Trial A 1968 P. ampla X P. pratensis putative hybrids and parents. Canonical analysis on seven variates

FIGURE 3



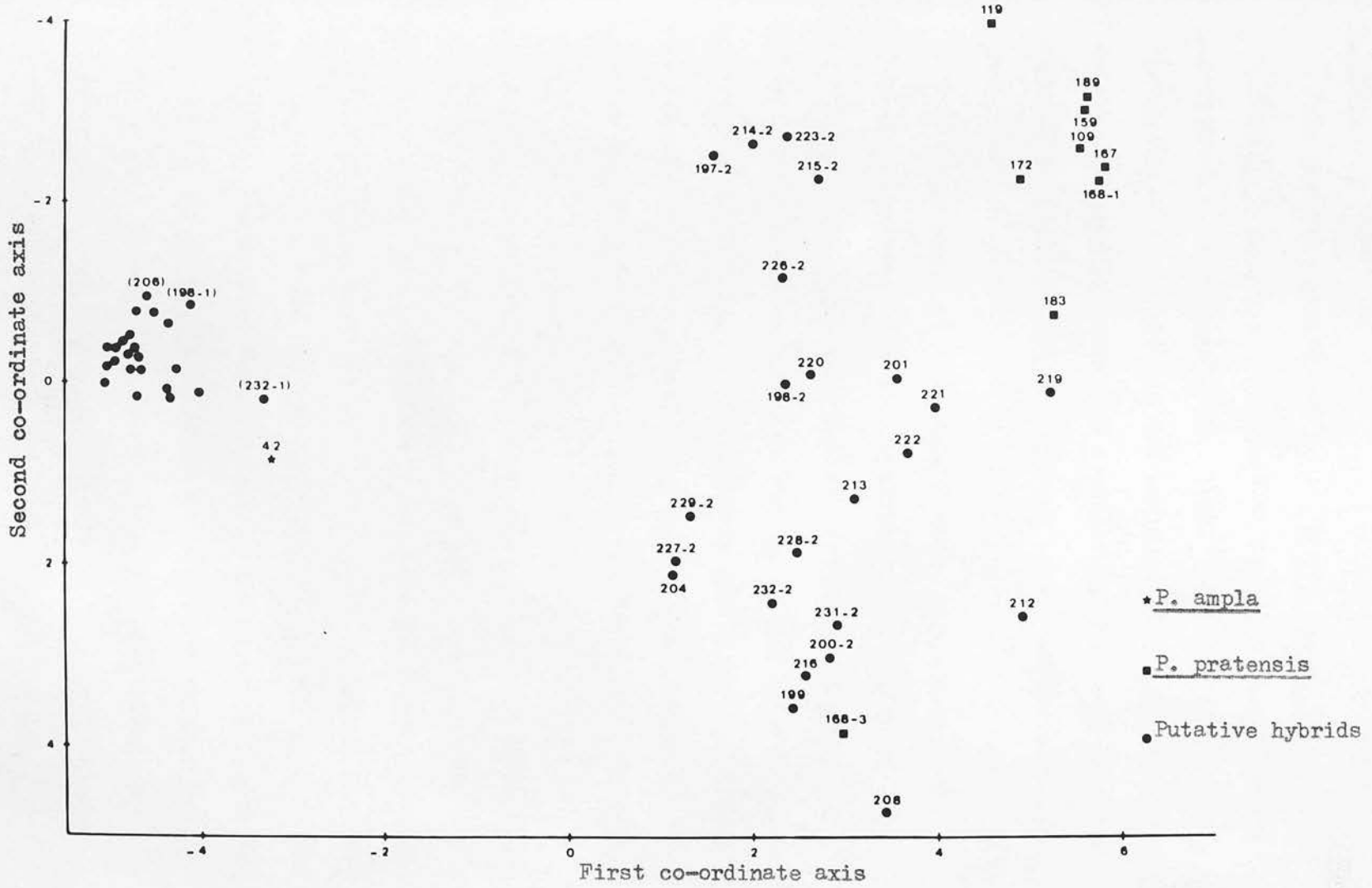
Trial A 1968 P. ampla X P. pratensis putative hybrids and parents. Canonical analysis on three variates

FIGURE 4



Trial A 1968 P. ampla X P. pratensis putative hybrids and parents. Principal co-ordinate analysis on seven variates

FIGURE 5



Trial A 1968 P. ampla X P. pratensis putative hybrids and parents. Principal co-ordinate analysis on three variates

FIGURE 6

1968 hybrids P. iberica X P. pratensis and P. longifolia X P. pratensis

Five possible hybrids of P. pratensis and P. iberica and five with P. longifolia were detected by their morphological characteristics at the seedling stage. Plant 237-2 was very dwarf and soon died; it was not included in the field trial. Only three ramets of clone 239 survived in the field so it was omitted from analyses but it appeared almost definitely to be a hybrid.

The clone means of transformed data, chromosome numbers, pollen stainabilities and seed production are all shown in Table 7 and correlation coefficients are shown in Table 6. Within-clone dispersion matrices for EE, PH and PD were again found to be heterogeneous (chi square = 242.23 *** for 108 df). Data from the nineteen clones were included in canonical analyses on all seven variates and on the first three variates only.

A high proportion of the total variability was accounted for by the first canonical variate in both analyses: 83.79 per cent from seven variates and 87.79 per cent from three variates. The plots of clone means on first and second canonical variates were very similar from the two analyses (figs.7 and 8). The two parental types were separated along the first axis with the exception of P. pratensis 14 which occurs about mid-way between the extremes; it was a wild French biotype identified as P. pratensis (s. str.) whereas the other P. pratensis biotypes represented were Scottish and more like P. subcaerulea Sm. The proximity of P. iberica and P. longifolia confirms other

reports of their close relationship (Almgård, 1960).

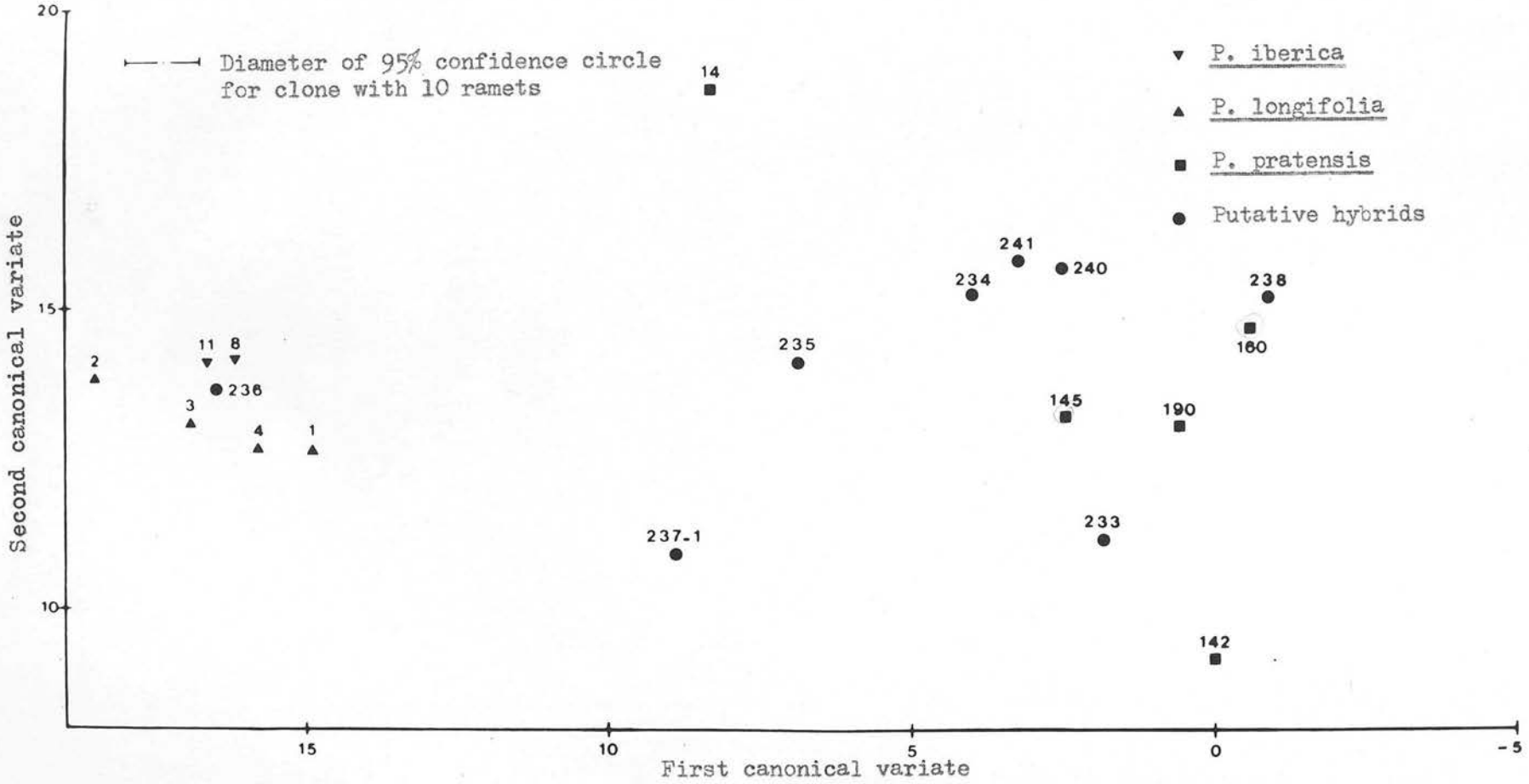
Clone 236 was morphologically very like its P. iberica parent (figs.7 and 8); the chromosome number (2n = 28) indicated that it was probably a result of self-pollination. Both 233 and 237-1 gave low chromosome counts, 2n = 48 and 45 respectively but were evidently hybrids. It seemed possible that 238 was a maternal type from P. pratensis 145 but since neither this plant nor its progeny were sufficiently like 145 to confirm the possibility it was classed as probably hybrid.

TABLE 7 - Trial A - 1968 hybrids P. iberica X P. pratensis and P. longifolia X P. pratensis; cytological and field trial data (clone means) from parents (P), hybrids (H), maternal offspring (M) and non-parents (NP)

	Clone No.	Chrq. No.[1]	PS[2] A[3] S[4] %	R[5]	EE SQRT	PH	PD SQRT	FLL Log_{10}	FLB Log_{10}	NoPan Log_{10}	SG
♀P	59-4	28	70 G	-	1.37+	116.2+	2.99+	2.29+	0.92+	0.64+	2.25+
♂P	190	81	84 G	10	1.54	41.6	5.30	1.45	0.64	1.44	1.00
H	233	48 2n	59 M M	9	1.72	37.3	4.02	1.47	0.70	1.06	1.11
♀P	59-8	28	72 G	10	1.08	109.7	2.86	2.31	0.92	0.61	2.40
♂P	145	80	38 G	10	1.75	42.9	4.56	1.63	0.71	1.32	1.80
H	234	68 2n	82 G M	10	1.75	45.4	4.89	1.83	0.67	1.26	2.80
H	235	73 2n	54 G P	7	2.44	63.0	4.57	1.99	0.77	0.65	2.00
♀P	59-8	28	72 G	10	1.08	109.7	2.86	2.31	0.92	0.61	2.40
♂P	14	73	70 P	10	1.54	95.5	6.57	2.06	0.86	1.37	2.30
M	236	28	46 G P	10	1.67	113.7	2.71	2.18	0.83	0.53	2.70
♀P	107-2	42	94 G	7	1.65	135.3	3.04	2.22	0.98	0.22	2.00
♂P	142	91	91 G	7	1.89	20.7	3.38	1.15	0.58	0.78	1.29
H	237-1	45 2n	- - -	6	2.58	74.0	2.91	2.02	0.96	0.56	1.00
♀P	145-2	80	38 G	10	1.75	42.9	4.56	1.63	0.71	1.32	1.80
♂P	59-8	28	72 G	10	1.08	109.7	2.86	2.31	0.92	0.61	2.40
H	238	82,95 2n	87 G M	10	1.95	32.6	6.25	1.58	0.76	1.24	1.70
♀P	160	86	79 G	10	1.97	42.1	6.54	1.44	0.57	1.38	1.10
♂P	107-4	42	94 G	4	2.12	112.0	2.78	2.16	0.94	0.08	2.00
H	239	- -	- - -	3	2.24	44.7	2.30	1.83	0.76	0.01	1.00
H	240	- -	- - P	8	2.09	55.4	6.24	1.61	0.73	0.96	2.00
H	241	102 3n	77 G M	8	1.93	56.6	6.20	1.65	0.73	0.83	2.00
NP	59-11	(P. iberica)		10	1.66	122.7	3.13	2.27	0.93	0.66	2.10
NP	107-1	(P.longifolia)		7	1.83	103.6	2.75	2.09	0.89	0.15	2.14
NP	107-3	(P.longifolia)		9	1.61	121.6	2.63	2.20	0.98	0.39	2.00

+ Mean value from 59-8 and 59-11; 59-4 not included in field trial.

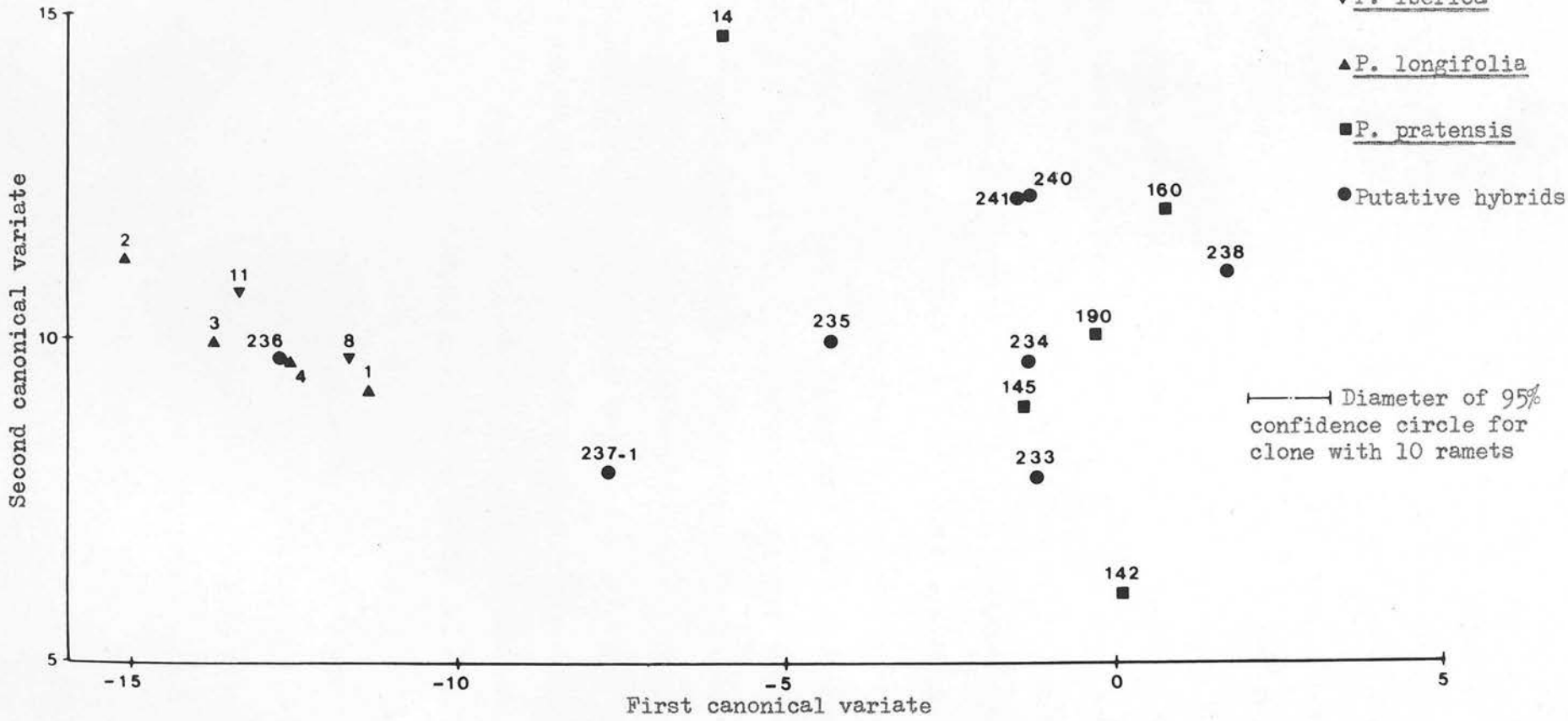
1. See notes for Table 5
2. " " " " "
3. " " " " "
4. " " " " "
5. " " " " "



Trial A 1968 P. iberica X P. pratensis and P. longifolia X P. pratensis putative hybrids and parents

Canonical analysis on seven variates

FIGURE 7



Trial A 1968 P. iberica X P. pratensis and P. longifolia X P. pratensis putative hybrids and parents

Canonical analysis on three variates

FIGURE 8

1969 hybrids P. ampla X P. pratensis

In Trial B the 83 plants selected at the seedling stage as possible hybrids showed a range of variation from plants indistinguishable morphologically from P. pratensis to those which appeared identical to P. ampla. All ramets of clone 247 died in thefield and only one ramet of clone 254 survived.

The variates spring growth (3), ligule hairs and leaf hairs all had very skewed distributions even after transformation and so they were omitted from analyses. Correlation coefficients between the remaining thirteen variates are shown in Table 8.

Parent and hybrid clone means for thirteen variates, chromosome numbers, pollen stainabilities and seed production are all shown in Table 9; clone means from maternal offspring are in Appendix Table 2. The first three variates were tested for homogeneity of within clone dispersions; as with data from Trial A the test indicated that dispersion matrices were heterogeneous ($p < 0.001$). Canonical analyses were carried out on the first three variates (EE, PH and PD), and on all thirteen variates; 74.7 per cent and 68.8 per cent of the total variation was accounted for by the first canonical variate in the two analyses respectively. Positions of clone means on the first and second canonical variates were plotted; scatter diagrams from the two analyses both distinguished P. ampla and the maternal (non-hybrid) plants from all others. The relative positions of clones were very similar but the P. ampla types were even more clearly separated from other clones when thirteen variates were included in the analysis (figs.9 and 10).

Forty-nine clones were identified as hybrids. Since the parental plants were mass pollinated the range of possible chromosome numbers expected in "diploid" and "triploid" hybrids was calculated from the extremes in the range of parental counts assuming that multiples of seven chromosomes were inherited. This gave a diploid range of 62 to 83 and a triploid range of 91 to 112. Four clones (245, 267, 297 and 303) had chromosome numbers of c. 56, but with the possible exception of 267, they were clearly hybrid in character rather than being non-hybrid aberrants of P. ampla. There were twenty-seven clones in the "diploid" range, eight clones with counts between 83 and 91, nine clones in the "triploid" range and two with higher counts than expected for triploids. The latter two clones, 287 and 289, had counts of c. 120 and c. 130 respectively; they were probably tetraploid and derived from unreduced gametes.

There was no clear relationship between morphological characters and chromosome number. Diploid hybrids had a greater tendency to male or female sterility; approximately 65 per cent of diploids had poor seed production or pollen development compared to c. 30 per cent in all other categories of hybrid.

TABLE 8 - Trial B - 1969 hybrids P. ampla X P. pratensis and parents, correlation coefficients (r) calculated from 89 clone means

	EE	PH	PD	LL	LB	SG1	SG2	Habit	Pan L	Sp L	Sp B	Sd/Sp	Sd H
EE													
PH	-0.577												
PD	0.216	0.152											
LL	-0.501	0.814	0.140										
LB	0.022	0.334	0.689	0.437									
SG 1	-0.123	0.457	0.705	0.534	0.728								
SG 2	-0.581	0.767	0.305	0.851	0.487	0.644							
Habit	0.287	-0.106	0.920	-0.344	0.538	0.529	-0.035						
Pan L	-0.600	0.784	-0.253	0.665	0.012	0.062	0.548	-0.565					
Sp L	-0.424	0.561	-0.296	0.751	0.091	0.076	0.505	-0.570	0.658				
Sp B	0.215	0.334	0.695	0.367	0.614	0.569	0.338	0.577	-0.047	0.093			
Sd/Sp	-0.137	0.527	0.223	0.786	0.452	0.456	0.618	-0.102	0.333	0.772	0.489		
Sd H	0.255	-0.038	0.820	-0.163	0.371	0.387	-0.054	0.898	-0.403	-0.450	0.542	0.012	

$r > \pm 0.357^{***}$, $r > \pm 0.238^{**}$, $r > \pm 0.217^{*}$

TABLE 9 - Trial B 1969 P. ampla X P. pratensis hybrids and parents; cytological and field data

Clone No.	Chro.[1] No.	PS[2] A[3] S[4] %	No Ram[5]	EE Log_{10}	PH (mm) $SQx10^{-5}$	PD (mm) Log_{10}	LL (mm) $SQRTx10^{-1}$	LB (mm) SQRT	SG1 $SQx10^{-1}$	SG2 $SQx10^{-1}$	Hab Log_{10}	Pan L (mm) $x10^{-2}$	Sp L (mm) SQRT	Sp B (mm) SQRT	Sd/Sp SQRT	Sd H SQRT
P 42-6	56	42 G	10	0.45	6.77	2.05	1.90	1.69	0.45	6.10	0	1.74	3.06	1.41	2.11	1.17
P 42-10	56	52 G	10	0.44	7.26	2.05	2.11	1.80	0.85	6.25	0	1.75	3.08	1.41	2.34	1.20
P 57-6	63	53 G	8	0.41	7.02	2.01	1.99	1.71	0.71	6.21	0	1.70	3.02	1.31	2.23	1.10
P 137	102	74 G	6	0.60	1.56	2.43	1.20	1.94	0.82	2.20	0.58	0.70	2.30	0.60	2.01	1.73
P 145	80	38 G	10	0.74	7.24	2.74	1.46	1.70	0.85	2.65	0.70	0.91	2.17	1.60	2.02	1.73
P 148	82	93 G	8	0.83	3.26	2.65	1.29	2.07	0.90	2.41	0.69	0.76	2.17	1.66	2.02	1.64
P 179	83	85 G	8	0.78	2.78	2.40	1.13	2.01	0.90	2.05	0.57	0.62	2.24	1.57	1.85	1.69
P 191	83	86 G	9	0.81	2.61	2.56	1.29	2.11	0.90	2.12	0.61	0.76	2.07	1.52	1.85	1.57
H 242	70 2n	8 P P	10	0.92	3.54	2.15	1.25	1.63	0.37	1.96	0.30	0.96	2.32	1.33	1.80	1.39
H 243	70 2n	14 P M	10	0.75	4.04	2.14	1.26	1.58	0.55	2.36	0.30	1.09	2.36	1.38	1.77	1.39
H 244	70 2n	60 G P	10	0.97	2.81	2.14	1.21	1.74	0.45	0.69	0.39	0.98	2.65	1.46	2.20	1.32
H 245	56(2n)	37 G M	10	0.77	5.07	2.35	1.48	1.74	0.40	3.62	0.39	1.31	2.36	1.37	1.92	1.40
H 249	62 2n	- - -	10	0.78	2.00	2.19	1.04	1.51	0.37	1.13	0.35	0.98	1.94	1.14	1.48	1.19
H 253	70 2n	- - M	10	0.75	3.79	2.03	1.12	1.65	0.40	2.10	0.39	1.16	2.19	1.25	1.60	1.22
H 254	73 2n	93 G -	1	0.79	6.40	2.70	1.51	1.73	0.90	1.60	0.70	1.22	2.24	1.87	2.24	1.73
H 256	74 2n	72 G M	8	0.73	1.20	2.04	0.96	1.49	0.35	0.76	0.39	0.63	2.31	1.37	1.76	1.33
H 257	95 3n	58 M G	10	0.59	6.79	2.33	1.62	2.06	0.90	5.82	0.41	1.45	2.76	1.41	1.90	1.43
H 261-2	92 3n	51 M G	10	0.71	3.38	2.35	1.27	1.71	0.45	2.41	0.44	1.13	2.67	1.39	1.96	1.63
H 262	77 2n	35 P G	10	0.59	3.11	2.40	1.37	1.74	0.40	4.38	0.53	0.98	2.50	1.41	1.99	1.52
H 266	68 2n	- - S	5	0.99	0.16	1.88	0.90	1.40	0.10	0.16	0.30	0.44	2.05	1.13	1.40	1.29
H 267	57(2n)	- - -	9	0.46	5.76	1.90	1.88	1.58	0.42	4.21	0	1.58	2.81	1.35	1.97	1.25
H 270	79 2n	77 G G	10	0.78	2.87	2.46	1.26	1.81	0.65	3.18	0.55	1.01	2.42	1.43	1.88	1.56
H 271	70 2n	63 G P	7	0.77	1.16	2.03	0.93	1.64	0.14	0.90	0.43	0.62	2.13	1.21	1.68	1.55
H 273	72,98	40 G M	10	0.79	2.68	2.65	1.21	1.91	0.90	3.51	0.62	0.63	2.21	1.65	1.90	1.60
H 277	102 3n	57 G -	9	0.69	2.26	2.03	0.90	1.45	0.37	0.58	0.34	0.97	2.32	1.28	1.56	1.43

continued.....

TABLE 9 - Trial B 1969 P. ampla X P. pratensis hybrids and parents; cytological and field data (cont'd.)

Clone No.	Chro.[1] No.	PS[2] A[3] S[4] %	No Ram[5]	EE Log_{10}	PH (mm) $SQx10^{-5}$	PD (mm) Log_{10}	LL (mm) $SQRTx10^{-1}$	LB (mm) SQRT	SG1 $SQx10^{-1}$	SG2 $SQx10^{-1}$	Hab Log_{10}	Pan L (mm) $x10^{-2}$	Sp L (mm) SQRT	Sp B (mm) SQRT	Sd/Sp SQRT	Sd H SQRT
H 278	104 3n	32 M M	8	0.57	4.84	2.00	1.56	1.64	0.59	3.40	0.19	1.40	2.62	1.26	1.91	1.39
H 279	100 3n	85 G M	9	0.63	4.37	2.26	1.11	1.75	0.62	1.57	0.46	1.20	2.30	1.39	1.68	1.47
H 281-1	84(2n)	62 G G	10	0.78	3.84	2.61	1.33	1.95	0.85	3.05	0.68	0.86	2.21	1.61	1.78	1.63
H 281-2	87(2n)	62 G G	10	0.78	4.59	2.63	1.47	1.88	0.90	3.18	0.66	0.98	2.20	1.60	1.83	1.70
H 282	82 2n	89 G G	10	0.77	2.23	2.48	1.18	2.09	0.55	1.64	0.58	0.76	2.17	1.56	1.89	1.70
H 284	78 2n	88 G M	7	0.78	1.27	2.11	0.91	1.65	0.23	0.37	0.38	0.64	2.10	1.24	1.55	1.28
H 286	85(2n)	0 S G	9	0.78	2.33	2.13	1.01	1.61	0.40	0.68	0.36	0.96	2.16	1.23	1.52	1.17
H 287	120 3n	81 G G	8	0.78	4.94	2.65	1.39	1.90	0.90	2.05	0.70	1.04	2.21	1.58	1.77	1.69
H 288	73 2n	28 G M	8	0.81	1.87	1.91	1.08	1.69	0.29	0.95	0.30	0.93	2.20	1.36	1.73	1.47
H 289	130(3n)	0 S M	9	0.70	1.70	2.23	1.10	2.11	0.46	1.02	0.46	1.05	2.10	1.14	1.43	1.17
H 290-2	81 2n	— — G	7	0.72	1.15	2.18	0.97	1.53	0.19	0.71	0.43	0.90	2.11	1.30	1.55	1.49
H 291-2	84(2n)	52 M M	4	0.78	2.57	2.04	0.88	1.54	0.33	0.58	0.43	1.15	2.10	1.10	1.39	1.31
H 292	75 2n	28 G G	10	0.77	6.26	2.40	1.39	1.89	0.90	3.95	0.48	1.02	2.44	1.33	1.96	1.44
H 293	65 2n	72 G P	3	0.49	4.13	1.67	1.72	1.76	0.40	0.10	0.30	1.21	2.76	1.28	1.63	1.00
H 294	70 2n	25 M P	3	0.92	0.96	2.33	1.05	1.94	0.40	0.90	0.52	0.59	2.29	1.41	1.72	1.24
H 297	56(2n)	48 G G	10	0.63	7.14	2.26	1.52	2.10	0.45	4.38	0.37	1.38	2.38	1.47	1.84	1.32
H 298	67 2n	85 G S	6	0.96	2.97	2.12	1.46	1.82	0.30	0.33	0.30	0.81	2.91	1.41	2.09	1.28
H 299	70 2n	59 G P	10	0.98	2.32	2.31	1.23	1.83	0.40	1.15	0.47	1.03	2.83	1.58	2.25	1.53
H 302	72 2n	37 P M	10	0.78	4.15	2.27	1.29	1.86	0.45	2.84	0.51	1.22	2.50	1.39	1.89	1.28
H 303	55 2n	88 G —	8	0.98	1.80	2.17	1.26	1.66	0.43	0.63	0.39	0.92	2.54	1.49	2.19	1.47
H 304-2	98 3n	57 M M	10	0.63	4.75	2.16	1.24	1.59	0.22	1.19	0.41	1.31	2.36	1.33	1.47	1.56
H 305	88(2n)	58 G M	10	0.71	6.73	2.23	1.29	1.78	0.34	1.80	0.32	1.81	2.38	1.35	1.69	1.63
H 306	82 2n	53 G G	9	0.78	4.15	2.55	1.39	1.92	0.90	2.93	0.60	0.96	2.29	1.52	1.87	1.58
H 307	90(3n)	60 G G	10	0.78	4.08	2.58	1.49	1.94	0.90	3.16	0.66	0.90	2.14	1.46	1.78	1.70
H 308	81 2n	78 G G	9	0.76	2.00	1.99	1.04	1.42	0.39	1.51	0.32	0.90	2.02	1.16	1.47	1.43

continued.....

TABLE 9 - Trial B 1969 P. ampla X P. pratensis hybrids and parents; cytological and field data (cont'd.)

Clone No.	Chro.[1] No.	PS[2]A[3]S[4] %	No. Ram[5]	EE Log_{10}	PH (mm) $SQx10^{-5}$	PD (mm) Log_{10}	LL (mm) $SQRTx10^{-1}$	LB (mm) SQRT	SG1 $SQx10^{-1}$	SG2 $SQx10^{-1}$	Hab Log_{10}	Pan L (mm) $x10^{-2}$	Sp L (mm) SQRT	Sp B (mm) SQRT	Sd/Sp SQRT	Sd H SQRT
H 309	74 2n	- - M	7	0.82	1.23	1.90	0.90	1.60	0.27	0.19	0.33	0.82	2.23	1.23	1.40	1.34
H 310	70 2n	37 M M	10	0.81	1.39	2.40	1.05	1.61	0.40	0.55	0.50	0.94	2.30	1.31	1.68	1.49
H 311	70 2n	- - P	10	0.80	1.89	2.32	1.36	1.81	0.85	3.64	0.42	0.75	2.26	1.31	1.62	1.20
H 312	70 2n	67 G G	7	0.83	2.03	2.11	0.97	1.58	0.40	0.69	0.40	0.73	2.10	1.18	1.60	1.49
H 314-2	107 3n	68 M M	10	0.76	2.72	2.11	1.06	1.53	0.45	0.83	0.42	1.14	2.25	1.32	1.48	1.49
H 316	90(3n)	51 P S	10	0.87	3.93	2.35	1.60	2.02	0.90	3.99	0.42	1.23	2.36	1.41	1.94	1.46
H 318	92 3n	- P M	10	0.78	3.59	2.39	1.48	1.85	0.70	3.51	0.37	1.28	2.46	1.53	1.86	1.34

Notes

1. Chromosome number, accurate to ± 5.

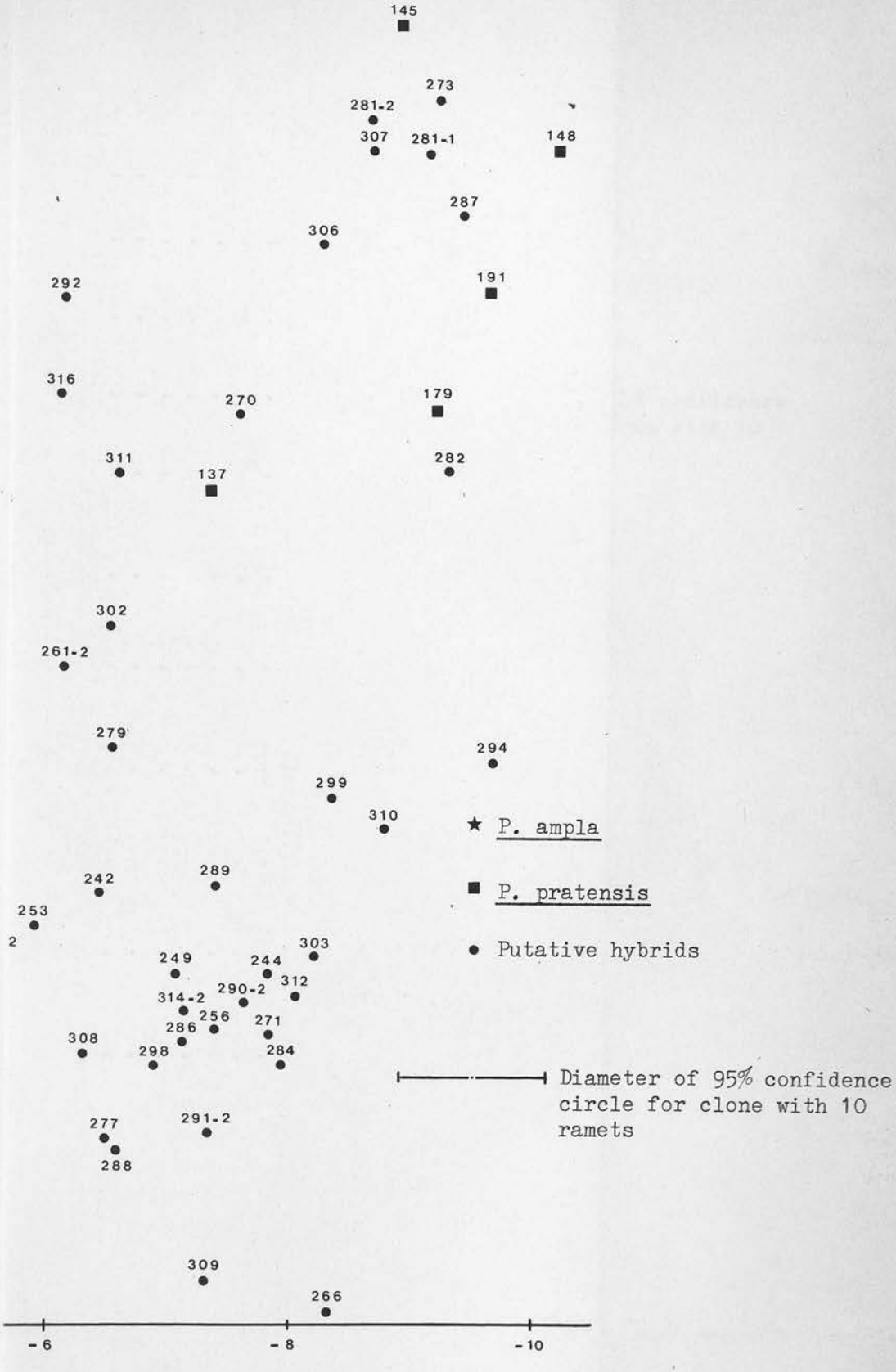
2. Pollen stainability.

3. Anther development; G, all anthers normal; M, a few anthers not fully developed; P, most anthers with poor or abnormal development; S, sterile.

4. Seed production; assessed from two ramets, controlled crosses in greenhouse, 1971; G,>1,000 good seeds; M, 100-1000 good seeds; P, 1-100 good seeds; S, no good seeds.

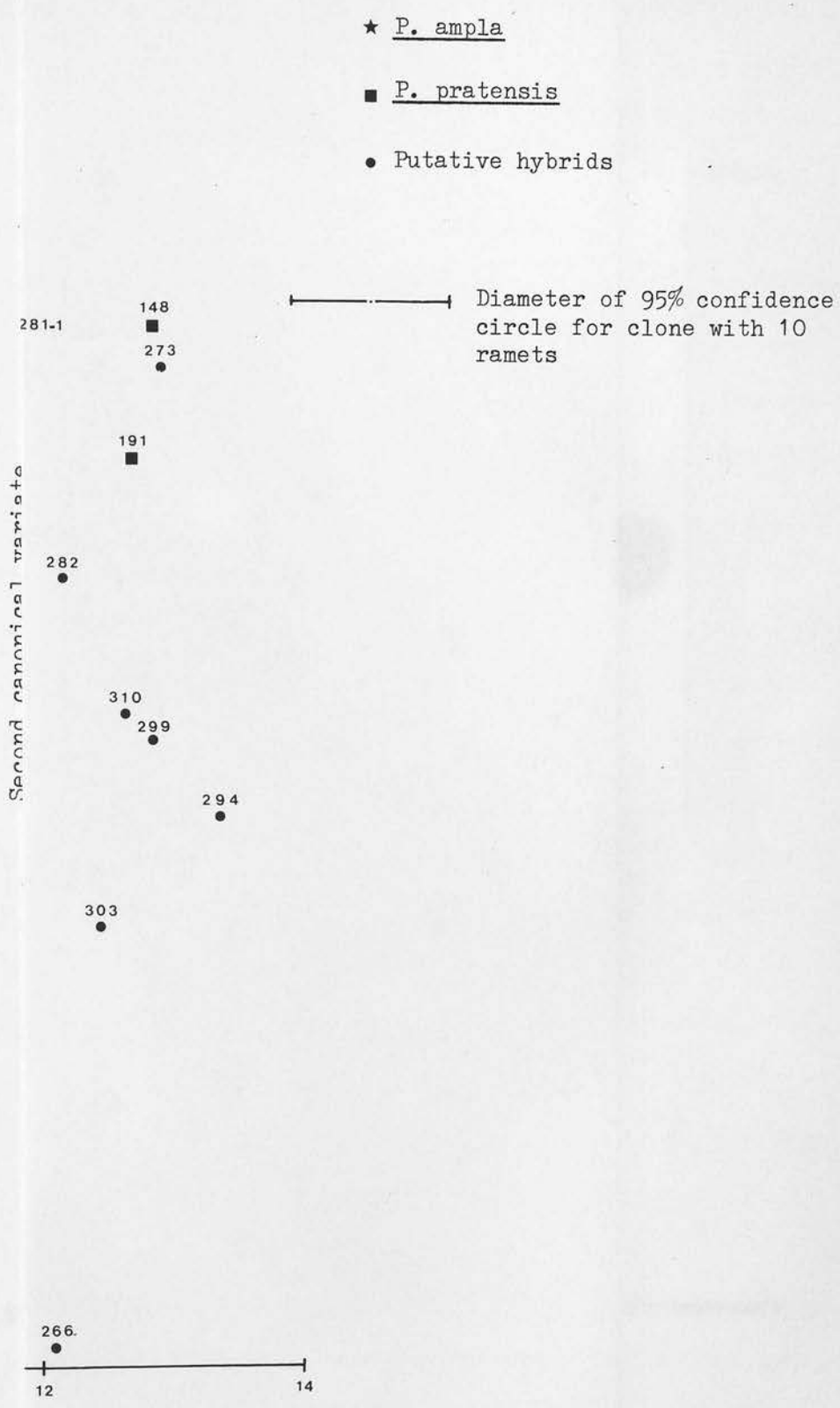
5. Number of ramets surviving in field trial.

FIGURE 9



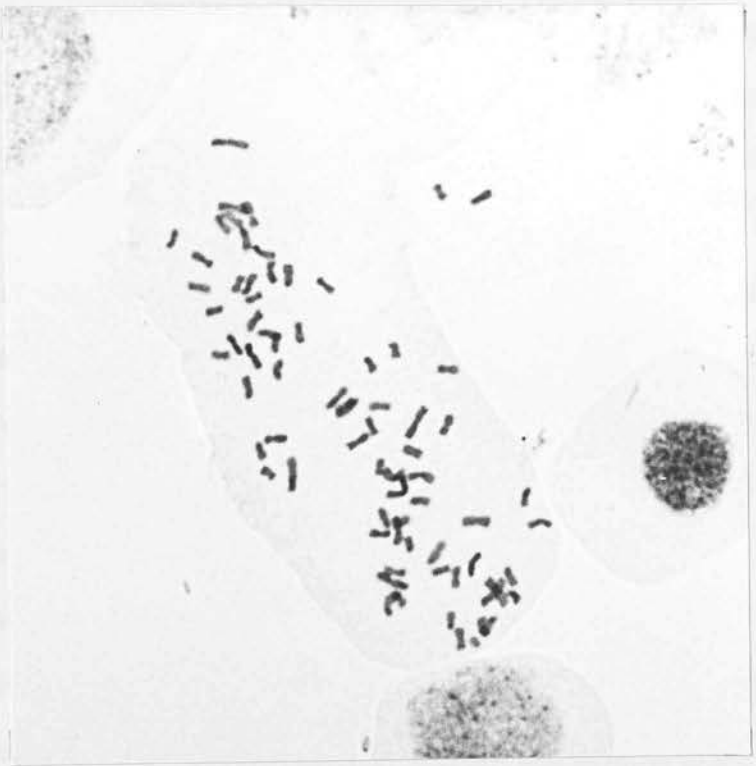
is on thirteen variates

FIGURE 10



ysis on three variates

FIGURE 11



Root tip squash of hybrid 199 showing metaphase chromosomes ($2n = 77 \pm 5$), X 1450

DISCUSSION

There has been no reported comprehensive cytological examination of Scottish ecotypes of P. subcaerulea which formed the basis of the P. pratensis (s. lato) parental material in crosses. Åkerberg (1942) examined seven Swedish biotypes, 2n = 84 to 95, of which two were sexual and the other five highly apomictic. Chromosome numbers from 2n = 82 to 147 were reported on Icelandic P. irrigata by Löve (1952). He examined 700 biotypes and only twenty per cent of these were aneuploid, 2n = 112 and 119 occurred most frequently; seed formation was aposporous and pseudogamous and no sexual embryo sacs were seen. Barling (1962) obtained chromosome counts from 2n = 54 to 109 in P. subcaerulea biotypes from England and Wales and, although meiosis was generally regular, observed univalents and an uneven segregation of chromosomes in some pollen mother cells. The chromosome numbers determined for Scottish biotypes used as parental material in this series of hybridisations ranged from 2n = 72 to 102. Meiosis and embryology were not studied at all but the morphological uniformity observed in earlier field trials had indicated a high level of apomixis occurred in these lines.

In P. ampla irregular meiotic divisions were reported by Grun (1950, 1952) and the occurrence of a mean of five or six univalents per cell at first meiotic metaphase (1955). Almgård (1960) observed that meiosis was very irregular in P. longifolia with usually eight to ten univalents present.

The irregular segregation of chromosomes at meiosis and the frequent occurrence of univalents in three of the species used in hybridisations must account for much of the variation in chromo-

some numbers of the hybrids from expected levels.

Disconcertingly big discrepancies in somatic chromosome numbers were observed in some hybrids, the greatest being 2n = 82 and 113. Previous reports of chromosome counts ranging from 60 to more than 100 in the same plant of a Scottish biotype of P. subcaerulea (Ann. Rep. Scott. Soc. Res. Plant Breed., 1964); and of a mean deviation within plants of P. pratensis L. of 4.7 and a maximum deviation of 9 (Speckman and van Dijk, 1972), both concluded that aneusomaty is a regular phenomenon in these species. It would appear likely that the differences in somatic chromosome number seen within the interspecific hybrids were also due to aneusomaty. However, the somatic chromosome numbers were a valuable aid in confirming identifications of the interspecific hybrids and in assessing their probable origin from reduced or unreduced gametes, although the results from these interspecific hybrids tend to support van Dijk's (1974) observation that the chromosome number of P. longifolia X P. pratensis hybrids could not immediately be derived from the parents.

In 1969 there were more diploid than triploid P. ampla hybrids, indicating that egg cells were more often fertilized from sexual than from aposporous embryo sacs. As P. ampla has previously been reported to produce very few aberrants under normal conditions at Pentlandfield, this result may reflect an actual increase in the number of sexual embryo sacs maturing under the imposed environment. A detailed study of the embryology would be needed to establish the stage in development at which the change in proportion of the two types of embryo sac occurred due to the altered environment.

The use of a short photoperiod and increased temperatures prior to anthesis was more successful than the use of increased temperature alone in increasing the proportion of fertilized egg cells in P. ampla. It confirms the earlier report by Watson and Clausen (1961) that the proportion of aberrants may be influenced by the total environment. It also indicates that daylength has a direct influence on the balance between sexual and asexual reproduction in P. ampla similar to that reported by Knox and Heslop-Harrison (1963) for the facultative apomict Dichanthium aristatum. They showed that plants kept in an eight hour photoperiod after floral induction had between 68 and 79 per cent aposporous embryo sacs whereas plants given a sixteen hour photoperiod only developed 27 to 46 per cent aposporous sacs. Knox (1967) further demonstrated a marked association between daylength during development of the inflorescence and the degree of apomixis at six different latitudes.

The F_1 hybrids showed a range in morphological variation between the parental types although rather more P. pratensis-like characters were evident (figs.3-7). This may have been due to the initial screening at the seedling stage for hybrids. Most seedlings were selected as hybrids by their early tendency to a rhizomatous or prostrate habit; these were both very distinctive characters and could not have been inherited from the maternal parents which were all very erect and non-rhizomatous. The method of selection probably led to some hybrids without these characteristics being overlooked. But since all seedlings which were even possible hybrids were selected, and the "possible" hybrids actually included some maternal types in both Trials A

and B, it seems unlikely that many hybrids were missed at the seedling stage.

There was no clear relationship between chromosome number and morphology in the hybrids and there were no consistent differences between diploids and triploids, although there was a tendency for diploids to be less fertile.

Scatter diagrams from canonical analysis and from principal co-ordinate analysis both enabled a very clear separation of maternal types from hybrids which confirmed visual records. In addition the multivariate analyses clarified the position of some clones which would have been difficult to place as maternal or hybrid by eye alone or from somatic chromosome numbers, although conversely, chromosome counts may enable classification of some clones in the "grey area" on diagrams. The three characters ear emergence, plant height and plant diameter distinguished the main morphological types almost as well as seven variates (for 1968 hybrids) and thirteen variates (for 1969 hybrids). There were only minor differences evident in the relationships between clones when more characters were used.

CHAPTER 4

HYBRID PROGENIES

(TRIALS C and D)

CHAPTER 4

HYBRID PROGENIES (TRIALS C AND D)

INTRODUCTION

Progenies from 1968 and 1969 F_1 hybrids were planted out in field trials in 1971 and 1972 respectively. These trials provided data (1) for assessment of the proportion of viable seed produced apomictically by the maternal plant; (2) for individual phenotype selections which, if highly apomictic, could be multiplied immediately for further testing, and (3) for selection of maternal plants. Only the results relating to (1) are reported here.

There are several possible methods for detecting the relative proportions of aposporous and sexual seed produced by a facultative apomict. Embryological studies in P. pratensis have been used to investigate the sequence of development of the two types of embryo sac and to assess the predominant breeding system within a biotype (Müntzing, 1940, Åkerberg, 1942). Nygren (1951) showed that they could also be used to predict breeding behaviour. His observed numbers of aposporous and sexual embryo sacs related fairly closely to assessments of morphological uniformity of progenies in the field, but in 28.1 per cent of ovules both types of embryo sac developed so that either or both might give a viable embryo. Knox (1967) and Knox and Heslop-Harrison (1963) used the incidence of aposporous and sexual sacs in Dichanthium aristatum as a quantitative measure of the degree of apomixis due to changes in the environment before and during flowering. However, when large numbers of plants need to be screened as in a breeding programme, it becomes a major task to measure the relative frequency of the two types of embryo sac for each individual.

Somatic chromosome counts on progeny and maternal plants should also give a good indication of the predominant breeding system. Plants developing parthenogenetically from aposporous initials would be expected to have the same chromosome number as the maternal parent and aberrants to have chromosome numbers differing according to their origin. Some segregating progenies might also be expected to have counts in the same range as the maternal parent, and since experience with the F_1 hybrids has shown that exact counts are very difficult to obtain and that they cannot always be predicted, it seems unlikely that chromosome counts could be used as a reliable method of screening in this material.

The use of a marker gene would be invaluable but at present none is known in this material; Marshall and Brown (1974) have suggested models for estimation of the level of apomixis in various breeding systems. These involve progeny testing known genotypes taken at random from populations polymorphic for specific marker loci. P. pratensis does not lend itself easily to genetic studies being both a facultative apomict and highly polyploid so the behaviour of a marker gene could be difficult to predict. Nittler and Kenny (1972, 1975) have been able to distinguish between some cultivars of P. pratensis grown in sand culture with solutions lacking various essential elements. The omission of calcium and of nitrogen both resulted in marked differences in response between some of the cultivars but at least one-third of the tested cultivars gave an intermediate or mixed response.

Almgård (1969) examined a partially apomictic family of P. pratensis using an isozyme technique. Eight out of 55 seedlings

were identified morphologically and cytologically as being produced sexually but another three aberrant plants were identified by deviating oxidase and peroxidase patterns compared to the maternal plant.

The most generally used method for determining the proportion of apomicts is from morphological field characters observed in progeny tests (Tinney and Aamodt, 1940, Funk and Han, 1967). Morphologically uniform progenies are assumed to represent highly apomictic maternal parents although further progeny tests have not always confirmed these assumptions (Nielsen, 1945; Smith and Nielsen, 1945). The possibility of a predominantly sexual maternal parent with many dominant genes giving a phenotypically uniform progeny of maternal type should be taken into consideration in these tests. Almgård (1966) used a combination of morphological and cytological methods to investigate the balance between sexual and apomictic seed formation and the inheritance of apospory in four P. longifolia X P. pratensis hybrids. He found that the presence of aposporous embryo sacs was not a reliable indication of the proportion of functioning apomictic seed set, but that the most useful measure of apomictic seed production in his material was the number of multiple seeds formed.

In the current breeding programme c. 100 plants need to be screened in a year for apomictic seed production. Since progeny rows are planted out for initial assessment of field characters under spaced plant conditions, it seems sensible to investigate the reliability of these characters in the separations of segregating from predominantly apomictic families, and in distinguishing apomicts from aberrant individuals. Some possible methods are

investigated using field characters in two trials using 1969 hybrid progenies, the six parental biotypes are included as highly apomictic standards.

MATERIALS AND METHODS

Progenies from 1968 F_1 hybrids

1968 F_1 hybrids were pair-crossed, selfed and open pollinated in the greenhouse. In 1971, seed from the successful crosses and from the parental biotypes was germinated on filter paper, pricked out into seed boxes and planted out in the field in two replications of 20 spaced plants per progeny. There were 67 families derived from 18 *P. ampla* X *P. pratensis* hybrids, nine families from four *P. iberica* X *P. pratensis* hybrids, and two from *P. longifolia* X *P. pratensis* hybrids.

Progenies were recorded for selection purposes and no detail of results or analyses will be presented here. A visual assessment of morphological uniformity within each family was used as a measure of apomixis.

Progenies from 1969 F_1 hybrids

1969 F_1 hybrids were pair-crossed, selfed and open pollinated in 1971 in the greenhouse. After thrashing, good seed from each pollination was selected (filled and hard seeds were assumed to be potentially viable) and counted. There were 71 pollinations from 38 maternal F1 hybrids which yielded more than 60 viable seeds; all were included in this series of trials. One hundred good seeds were counted from field open pollinated seed lots of the five parental biotypes of *P. pratensis* and one of *P. ampla*

Seed was germinated on filter paper in germination trays and

pricked out into John Innes 2 potting compost in batches of ten seedlings as they germinated. No detailed records were taken at the seedling stage but there was considerable variation evident in vigour and habit both between and within different families.

Trial C - eleven of the more vigorous seedling hybrid progenies from different maternal parents which showed a range of within family variation were selected for further study. Ten plants from each of these families and ten plants from each of the six parental lines were vegetatively cloned into five ramets. They were planted out in the field at 60 cm square spacing in five blocks. The 170 clones were fully randomised within each block. The sources of the 17 populations are listed in Table 10.

Trial D - seed from one of the 71 hybrid progenies did not germinate so was omitted from further trial. Forty plants from each of the remaining 70 hybrid progenies and from each of the six parental lines were planted out in the field at 60 cm square spacing. Rows of ten plants per family were randomised in four blocks.

Field records, Trials C and D

The two trials were adjacent in the field and, as far as possible, they were recorded at the same time. The following records were taken:

Code	Record	Trial C	Trial D
EE	Ear emergence (half weeks), 3.5.73 to 13.6.73	-	✓
PH	Plant height (mm), as for Trial A	✓	✓
PD 1	Plant diameter (mm), 25.6.73 (mean of greatest spread and that at right angles to it)	✓	-

Code	Record	Trial C	Trial D
PD 2	Plant diameter (mm), 14.11.73 (mean of greatest spread and that at right angles to it	✓	✓
LL	Leaf length (mm), as for Trial B	✓	✓
LB	Leaf breadth (mm), " " " "	✓	✓
No Pan	Number of panicles	✓	-
SG 1	Spring growth 21.1.74, 10 categories as for Trial B	✓	)
SG 2	Spring growth 14.3.74, 10 categories as for Trial B	✓	) total score used
SG 3	Spring growth 3.4.74, 10 categories as for Trial B	✓	)
SG 4	Spring growth 16.4.74, 10 categories as for Trial B	✓	)
FW	Fresh weight (g per plant) 4.9.73, each plant cut to c. 60 mm	✓	✓
Pan L	Panicle length (mm), as for Trial B	✓	✓ +
Pan B	Panicle breadth (mm), length of longest branch in bottom whorl of inflorescence	✓	✓ +
Sp L	Spikelet length (mm), as for Trial B	✓	✓ +
Sp B	Spikelet breadth, (mm), as for Trial B	✓	✓ +
Sd/Sp	Number of seeds per spikelet, as for Trial B	✓	✓ +
Sd H	Seed hairs	✓	✓ +
Br A	Branch angle (categories 1-5), angle formed between main axis and lowest branch of inflorescence; $1 < 36^{\circ}$ from vertical to $5 > 144^{\circ}$ from vertical	✓	✓ +
RC	Rachis colour (categories 1-5); 1 green to 5 purple	✓	✓ +
Rust	14.8.73 (10 categories); $1 < 10\%$ leaves infected to $10 > 90\%$ leaves infected	✓	✓ +
Mil	Mildew 14.8.73 (10 categories); as for rust	✓	✓ +
Apo	Apomixis 11.7.73, morphological uniformity	✓	✓ +

+ Recorded on only two blocks in Trial D

TABLE 10 - Trial C Source of 17 populations in trial

Family code	Source of seed
1	1969 hybrid 257 X 145
2	" " 261-2 X 148
3	" " 273 open pollinated
4	" " 281-1 X 314-2
5	" " 281-2 X 270
6	" " 287 X 172
7	" " 292 open pollinated
8	" " 297 X 159
9	" " 306 X 289
10	" " 307 X 191
11	" " 318 open pollinated
12	P. pratensis 137 open pollinated (field)
13	" 145 " " "
14	" 148 " " "
15	" 179 " " "
16	" 191 " " "
17	P. ampla 42 " " "

RESULTS

Progenies from 1968 F_1 hybrids

Nearly all progenies appeared to be segregating and showed a wide range in size and morphology. A few individual plants approached the very rhizomatous habit of *P. pratensis* and some offspring from 235 looked very much like *P. iberica*. There were none which closely resembled *P. ampla* although the characteristic erect habit and stiff, glaucous leaves were evident in many of the families. The two progenies from 238 were similar in habit to *P. pratensis* although less vigorous; they were less variable than other hybrid families but rather more variable than *P. pratensis*.

It was apparent from this trial that all of the F_1 hybrids represented, except possibly 238, were predominantly amphimictic.

Progenies from 1969 F_1 hybrids

Trial C - Clones with only one ramet surviving or with missing scores where no flowers developed were omitted from analyses. Families 2, 8, 11 and 17 each had either one or two clones which were weak and did not survive in the field. Five variates (SG 4, Br A, RC, rust and mildew) were omitted from all analyses since no suitable transformation was found to reduce the high degree of skewness and kurtosis present. Correlation coefficients calculated from ramets and from clone means are shown in Table 11. As the two scores for plant diameter and the three for spring growth were highly correlated only PD 2 and SG 2 were used in subsequent analyses.

The univariate test for homogeneity of variances showed the 17 within family variances to be heterogeneous ($p < 0.001$) for

TABLE 11 - Trial C Correlation coefficients (r) between 16 characters calculated from 164 clone means (genotypic correlations - G) and from 703 ramets (phenotypic correlations - P)

P \ G	PH	PD(1)	PD(2)	LL	LB	SG(1)	SG(2)	SG(3)	FW	No Pan	Pan L	Pan B	Sp L	Sp B	Sd/Sp	SdH
PH	-	0.041	0.070	0.749	0.150	0.059	0.227	0.124	0.080	0.157	0.717	0.614	0.327	-0.246	0.469	-0.206
PD(1)	0.130	-	0.974	0.263	0.023	0.348	0.444	0.732	0.772	0.622	-0.426	-0.057	-0.756	0.601	-0.432	0.703
PD(2)	0.111	0.797	-	0.336	0.026	0.410	0.514	0.778	0.684	0.687	-0.332	0.043	-0.771	0.558	-0.473	0.788
LL	0.506	0.243	0.251	-	0.362	0.143	0.255	0.333	0.168	0.416	0.568	0.656	0.008	-0.071	0.153	0.151
LB	0.081	0.045	0.056	0.184	-	0.009	-0.153	0.042	0.342	-0.023	0.093	0.481	0.024	0.118	0.001	0.308
SG(1)	0.080	0.221	0.301	0.113	0.062	-	0.882	0.781	0.600	0.345	-0.169	-0.068	-0.184	0.175	-0.059	0.211
SG(2)	0.187	0.290	0.379	0.166	-0.040	0.738	-	0.851	0.511	0.380	-0.073	-0.081	-0.236	0.132	-0.046	0.199
SG(3)	0.143	0.494	0.592	0.208	0.042	0.667	0.750	-	0.737	0.701	-0.314	-0.070	-0.616	0.374	-0.346	0.576
FW	0.186	0.594	0.585	0.252	0.173	0.455	0.379	0.558	-	0.431	-0.447	-0.077	-0.502	0.416	-0.257	0.454
No Pan	0.247	0.476	0.503	0.296	-0.060	0.249	0.253	0.447	0.392	-	-0.160	0.225	-0.690	0.106	-0.585	0.602
Pan L	0.598	-0.235	-0.217	0.344	0.039	-0.051	0.023	-0.147	-0.248	-0.040	-	0.782	0.653	-0.322	0.583	-0.394
Pan B	0.510	0.029	0.064	0.359	0.185	-0.051	-0.047	-0.026	-0.040	0.143	0.647	-	0.205	-0.061	0.215	0.107
Sp L	0.235	-0.503	-0.569	0.005	0.015	-0.152	-0.178	-0.430	-0.343	-0.459	0.491	0.176	-	-0.332	0.848	-0.774
Sp B	-0.128	0.237	0.272	-0.035	0.103	0.082	0.052	0.173	0.145	-0.047	-0.130	0.029	-0.046	-	-0.056	0.618
Sd/Sp	0.257	-0.206	-0.246	0.050	0.026	-0.074	-0.070	-0.167	-0.149	-0.331	0.336	0.149	0.693	0.180	-	-0.614
SdH	-0.103	0.378	0.462	0.107	0.116	0.151	0.126	0.347	0.249	0.313	-0.223	0.071	-0.441	0.294	-0.250	-

$r_G > \pm 0.250^{***}, > \pm 0.198^{**}, > \pm 0.152^{*}$; $r_P > \pm 0.123^{***}, > \pm 0.096^{**}, > \pm 0.073^{*}$

all characters except panicle number and seed hairs. Dispersion matrices from the five P. pratensis families based on plant height, plant diameter and spring growth were heterogeneous χ^2 64.993 *** for 24 df. The test was repeated omitting one probably aberrant clone from four of the families and gave χ^2 20.612 (ns) for 24 df, indicating that, in the absence of aberrants, within family dispersions of these apomictic families were homogeneous. Determinants from dispersion matrices based on the three characters used above, and on six characters (plant height, plant diameter, spring growth, leaf length and breadth and number of panicles) are shown in Table 12.

Analysis of variance and canonical analysis are known to be "robust" statistical techniques and were not expected to be seriously affected by the heterogeneity evidently present. However, some caution in interpretation of the results is necessary.

Analyses of variance and intraclass correlations on thirteen variates are shown in Table 13. All variates except spikelet breadth showed significant differences between clones within families and between families ($p < 0.01$). The intraclass correlation (Snedecor and Cochran, 1967) is the ratio of total genetic to total phenotypic variation:

$$\frac{\sigma^2_f + \sigma^2_{cl/f}}{\sigma^2_f + \sigma^2_{cl/f} + \sigma^2_{r/cl}}$$

The components of variance for families, σ^2_f, clones within families, $\sigma^2_{cl/f}$, and for ramets within clones, $\sigma^2_{r/cl}$, are calculated using the coefficients of the components in the expected mean squares shown in Table 13. The intraclass correlation is equivalent to repeatability as defined by Killick

TABLE 12 - Trial C Determinants from family variance-co-variance matrices calculated (a) from six variates (PH, PD, SG, LL, LB, No Pan) and (b) from three variates (PH, PD, SG)

Family	Determinant	
	(a) $\times 10^{2}$	(b) $\times 10^{-4}$
1	2776.1	220.4
2	193.7	62.7
3	117.4	70.5
4	1.0	6.3
5	3.6	2.2
6	466.3	45.5
7	0.7	2.5
8	9505.4	422.8
9	13.1	3.8
10	214.7	142.3
11	757540.0	5730.4
12	4.2	7.1
13	125.3	184.8
14	13.0	4.1
15	1.6	16.9
16	7.9	39.1
17	2588.0	1236.8

TABLE 13 - Trial C Analyses of variance and intraclass correlations

Source	df	PH(mm) MSx10^{-3}	PD2(mm) MSx10^{-3}	LL(mm) SQRT MS	LB(mm) MS	SG2 SQUARE MS	FW(g) SQRT MS	No Pan MS
Between families	16	245.84***	491.25***	52.604***	5.7590***	1617.7***	268.08***	209.81***
Between clones	147	23.77***	10.42***	7.918***	1.0572***	134.7***	9.90***	12.48***
Between ramets	539	4.04	4.53	3.167	0.5598	40.9	5.26	6.55
Intraclass correlation		0.7120 ±0.0742	0.7419 ±0.0692	0.4090 ±0.0897	0.2912 ±0.0785	0.5859 ±0.0884	0.5828 ±0.0887	0.4851 ±0.0919

Source	df	Pan L(mm) SQRT MS	Pan B(mm) MS	Sp L(mm) Log_{10} MSx10^2	Sp B(mm) Log_{10} MSx10^2	Sd/Sp SQRT MSx10^2	Sd H MS
Between families	16	34.293***	3011.4***	31.635***	10.555***	77.494***	12.384***
Between clones	147	1.249***	164.5***	0.745***	0.762	6.590***	0.528***
Between ramets	539	0.494	56.8	0.331	0.640	3.517	0.374
Intraclass correlation		0.6642 ±0.0810	0.6238 ±0.0853	0.7188 ±0.0731	0.2935 ±0.0788	0.4091 ±0.0897	0.4636 ±0.0917

Coefficients of MS: component (1) 41.27; component (2) 4.38: 4.27; component (3) 1.0 1.0 1.0

(1972). Its standard error estimated for full-sib families is:

$$\sigma_t = \sqrt{\frac{2\left[1 + (n-1)t\right]^2 (1-t)^2}{n(n-1)(N-1)}}$$

where t is intraclass correlation, n is number of observations (harmonic mean for number of ramets per family = 39.445) and N is number of families. Standard errors may be slightly underestimated since there were both full-sib and half-sib families in the trial.

All 164 clones were included in a canonical analysis on the six field characters which were also measured on all individuals in Trial D. All six latent roots were significant ($p < 0.001$) indicating that the clone means could be separated on the associated canonical variates in the six dimensions (Hope, 1968). The first and second canonical variates together accounted for 69.22 per cent of the total variation. A second canonical analysis on the three characters plant height, plant diameter and spring growth gave three significant latent roots ($p < 0.001$), the first and second together accounted for 84.54 per cent of the variance. The positions of clone means were plotted on the first two canonical variates from both analyses (figs.12 and 13, in pocket).

Clone means and analysis of variance tables for each family are shown in Appendix Tables 3-19 (pages 185 to 201). Variates showing significant differences between clones ($p < 0.05$) are shown family by family with clone indices ordered from least to greatest. The Q-method (Snedecor and Cochran, 1967) was used to test differences between clone means. The advantage of this method is that the probability of erroneous claims of significance is much smaller than in the use of an L.S.D. ($p \geq 0.95$).

Snedecor and Cochran also note that fewer real differences will be detected. In eight out of a total of 70 tests using the Q-method no differences were detected between the two extreme clone means where the variance ratio had indicated that differences existed. ($p < 0.05$). An L.S.D. was calculated as a check to test these differences ($p < 0.01$).

Results from analyses of variance and canonical analyses are presented family by family and clones are classified as (1) apomicts, (2) aberrants or (3) segregants to indicate their probable origin from (1) an aposporous embryo sac by parthenogenesis, (2) a cytological variant of (1) or (3) normal sexual reproduction; but strictly none of these classes are verified for the plants discussed.

Family 1

PH	2	1	3	9	4	7	5	6	10	8	
LB	4	6	7	1	5	3	9	10	2	8	n.s.Q, $L.S.D._{0.01}$
FW	9	1	8	4	2	3	6	7	5	10	
PD	8	9	3	7	5	10	6	2	4	1	
SG	8	9	7	4	5	6	2	10	3	1	
Pan L	2	3	9	4	8	6	5	1	7	10	

Canonical analyses, distance from centroid:

Clone No.	1	2	3	4	5	6	7	8	9	10
6 variates:	2.98	2.82	3.12	3.30	3.49	3.60	3.36	7.23	4.69	4.52
3 variates:	2.52	2.01	2.85	2.65	3.06	3.12	3.33	6.74	3.94	4.09

Clones 3, 4, 5, 6 and 7 form a loose cluster (possibly apomicts); clones 2 and 9 a little separated, clones 1, 8 and 10 rather distant; probably some segregants.

Family 2

PH	4	3	6	5	8	2	7	1
LL	4	8	2	5	6	3	7	1
SG	4	8	2	6	1	5	3	7
Pan L	4	8	3	1	5	2	7	6
Pan B	4	8	3	7	2	1	6	5
Sd/Sp	8	1	7	3	6	2	5	4

Canonical analyses, distance from centroid:

Clone No.	1	2	3	4	5	6	7	8
6 variates:	2.67	3.14	4.51	6.59	3.60	4.38	2.73	3.83
3 variates:	2.41	3.03	4.23	6.16	3.20	4.14	2.41	3.51

Clones 1, 2, 7 and 3, 5, 6 and 8 form two very loose groups, possibly some apomicts, with clone 4 (smaller) distant from all others. General wide scatter indicates probably also some segregants.

Family 3

PH	9	1	10	5	7	3	6	2	4	8
PD	9	7	1	10	2	5	3	6	4	8
Pan B	9	5	10	3	6	7	1	2	4	8
Sp L	9	4	7	10	6	5	2	3	8	1

Canonical analyses, distance from centroid:

Clone No.	1	2	3	4	5	6	7	8	9	10
6 variates:	3.84	3.46	3.23	3.24	2.58	2.73	2.44	3.56	3.89	3.25
3 variates:	3.77	3.05	2.99	3.13	2.41	2.42	2.30	3.36	3.53	2.94

Clone 9 (smaller and possibly aberrant) separated from all other clones which form a cluster, probably apomictic but some variation in size.

Family 4

PH	5	10	1	9	4	2	7	8	3	6	
SG	5	3	4	8	2	10	6	7	1	9	n.s.Q, L.S.D.$_{0.01}$
Pan L	5	1	9	10	4	7	6	2	3	8	
Pan B	5	10	2	4	3	1	7	9	6	8	

Canonical analyses, distance from centroid:

Clone No.	1	2	3	4	5	6	7	8	9	10
6 variates:	1.39	1.60	1.38	1.87	2.08	2.04	2.03	1.60	1.84	0.92
3 variates:	1.18	0.67	1.22	1.21	1.70	1.84	1.30	1.13	1.70	0.81

All clones in dense group, probably apomicts, except clone 5 which is smaller and probably aberrant.

Family 5

No differences between clones in analyses of variance.

Canonical analyses, distance from centroid:

Clone No.	1	2	3	4	5	6	7	8	9	10
6 variates:	2.05	3.10	2.57	1.24	1.59	2.35	1.72	2.67	1.77	1.99
3 variates:	1.68	1.09	1.53	0.76	1.30	1.78	0.96	2.23	1.04	1.59

All clones tightly clustered, probably apomictic.

Family 6

PH	8	6	3	4	1	7	9	10	5	2	
No Pan	8	4	9	6	10	1	3	7	2	5	
LL	8	6	3	9	7	10	5	1	4	2	
LB	3	1	9	6	8	5	2	4	7	10	n.s.Q, L.S.D.$_{0.01}$
FW	8	6	4	3	10	5	7	9	1	2	
SG	6	8	5	2	7	10	9	1	3	4	
Sd H	6	8	2	7	9	1	5	3	4	10	

Canonical analyses, distance from centroid:

Clone No.	1	2	3	4	5	6	7	8	9	10
6 variates:	3.03	3.18	2.37	2.56	2.70	2.65	2.50	2.76	2.36	2.17
3 variates:	2.78	2.35	1.97	1.41	2.59	2.58	2.29	2.68	1.56	1.63

Clones 6 and 8 smaller and possibly aberrants, separated from other eight clones which form dense cluster and are probably apomicts.

Family 7

SG 3 5 2 8 9 1 4 6 7 10 ns.Q, L.S.D.$_{0.01}$

Pan L 3 1 8 2 9 7 4 5 10 6

Canonical analyses, distance from centroid:

Clone No.	1	2	3	4	5	6	7	8	9	10
6 variates:	3.33	3.50	3.20	2.87	3.09	2.88	3.28	2.21	2.88	2.26
3 variates:	3.15	3.27	3.11	2.76	3.05	2.87	3.20	2.15	2.67	2.12

All clones form a group and are probably apomicts but clone 3 (small) and 10 (vigorous) peripheral and possibly aberrant.

Family 8

PH 6 7 9 8 1 2 3 4 5

LL 7 8 9 6 1 2 5 3 4

SG 6 7 8 9 2 4 5 3 1 n.s.Q, L.S.D.$_{0.01}$

Pan L 6 7 8 9 1 3 4 2 5

Pan B 7 8 6 9 4 2 5 1 3 n.s.Q, L.S.D.$_{0.01}$

Sd/Sp 7 8 6 1 2 3 5 4 9

Canonical analyses, distance from centroid:

Clone No.	1	2	3	4	5	6	7	8	9
6 variates:	1.87	5.40	4.94	5.91	5.70	5.99	5.67	4.88	5.65
3 variates:	1.57	4.91	4.00	4.98	4.95	5.93	5.37	4.75	5.03

Clones 2, 3, 4 and 5 form a loose cluster of possible apomicts separated from a "tail" of clones 8, 9, 7 with clone 6 most distant (smallest). Clone 1 separate from all other clones. General scatter indicates presence of some segregants.

Family 9

No Pan	6	8	3	4	5	2	10	7	9	1
LL	10	8	3	7	5	4	2	6	9	1
LB	10	5	9	2	4	1	7	6	3	8
FW	8	3	6	4	10	7	2	1	5	9
SpL	2	10	7	1	4	5	9	6	3	8

Canonical analyses, distance from centroid:

Clone No.	1	2	3	4	5	6	7	8	9	10
6 variates:	2.72	1.56	1.77	0.90	1.88	1.55	2.20	2.70	2.17	3.32
3 variates:	1.36	0.79	1.22	0.56	1.47	1.00	0.74	2.02	0.90	1.17

Clone 8 small and slightly separated, possibly aberrant; all other clones form a group, probably apomicts but some variation in size evident.

Family 10

PD 10 2 5 3 7 9 6 1 8 4

Canonical analyses, distance from centroid:

Clone No.	1	2	3	4	5	6	7	8	9	10
6 variates:	1.87	2.42	1.97	3.00	1.33	1.52	1.35	2.35	1.57	1.25
3 variates:	1.35	0.49	1.40	2.45	0.54	1.31	1.10	2.00	0.98	0.52

All clones form a dense cluster, probably apomicts.

Family 11

PH 3 7 9 2 5 6 8 4 1

No Pan 9 4 3 8 6 2 1 5 7

LL 3 7 8 2 9 6 1 5 4

LB 3 2 7 6 5 8 9 4 1

FW 3 7 2 8 9 5 4 6 1

PD 3 2 9 4 6 7 8 5 1

SG 3 2 6 7 5 4 9 1 8

Pan L 3 6 7 5 9 2 1 8 4

Pan B 7 6 3 2 9 5 1 8 4

SpL 3 1 7 5 6 9 4 2 8

SpB 3 7 8 1 5 2 9 6 4

Sd/Sp 3 7 6 5 1 9 2 8 4

SdH 3 6 8 5 2 4 9 7 1 n.s.Q, L.S.D.$_{0.05}$

Canonical analyses, distance from centroid:

Clone No.	1	2	3	4	5	6	7	8	9
6 variates:	3.74	4.51	6.81	4.74	2.44	4.13	4.99	4.79	3.86
3 variates:	2.61	4.30	6.04	3.44	2.32	3.84	3.81	4.28	3.65

All clones scattered, no clustering indicating wide segregation.

Family 12

PH 7 2 3 10 5 9 6 8 4 1

No Pan 7 4 9 5 3 10 8 6 2 1

Pan L 7 3 10 2 6 5 4 9 8 1

SpL 2 4 3 10 5 7 9 6 1 8

Sd/Sp 4 2 3 9 5 6 7 1 10 8 n.s.Q, L.S.D.$_{0.01}$

Canonical analyses, distance from centroid:

Clone No.	1	2	3	4	5	6	7	8	9	10
6 variates:	1.45	2.64	2.64	1.67	1.79	1.87	4.38	2.08	1.61	1.91
3 variates:	0.92	1.88	2.57	1.54	1.67	1.34	4.24	1.95	1.48	1.70

Clone 7 distant from all others, probable aberrant. All other clones form group, probably apomicts.

Family 13

PH	9	1	8	2	7	10	5	6	3	4
PD	9	6	7	3	1	10	8	2	4	5
SG	9	1	7	4	6	3	10	2	5	8
Pan L	9	10	4	1	6	3	2	8	5	7

Canonical analyses, distance from centroid:

Clone No.	1	2	3	4	5	6	7	8	9	10
6 variates:	3.37	4.08	3.64	4.85	4.59	3.75	3.28	3.77	3.14	3.79
3 variates:	2.35	3.47	3.23	4.02	3.84	2.66	2.59	3.36	3.00	3.14

Clone 9 (small) distant from all other clones, probable apomict. Other clones form group with clones 1 and 6 peripheral; probably apomictic.

Family 14

There were no differences between clones in analyses of variance.

Canonical analyses, distance from centroid:

Clone No.	1	2	3	4	5	6	7	8	9	10
6 variates:	2.23	1.66	2.51	1.41	1.88	1.36	2.30	1.40	2.27	2.21
3 variates:	2.03	1.33	1.75	1.10	1.56	1.08	1.58	0.72	2.15	1.95

All clones in tight cluster, probably all apomicts.

Family 15

PH 7 1 5 2 3 9 4 6 8 10

Canonical analyses, distance from centroid:

Clone No.	1	2	3	4	5	6	7	8	9	10
6 variates:	2.19	2.14	1.29	1.38	1.64	1.75	3.65	1.78	2.19	1.59
3 variates:	1.75	1.45	0.87	0.88	1.48	1.35	3.41	1.49	2.03	1.18

Clone 7 distant, probably aberrant. Other clones form a group of probable apomicts with clones 8 and 10 peripheral.

Family 16

PH 7 9 5 10 3 1 8 6 4 2

No Pan 7 10 5 9 3 2 8 6 1 4

PD 7 10 3 5 8 9 5 1 4 2

Canonical analyses, distance from centroid:

Clone No.	1	2	3	4	5	6	7	8	9	10
6 variates:	1.95	1.96	1.43	2.42	2.41	1.12	4.25	1.34	2.07	1.39
3 variates:	1.69	1.83	1.11	1.83	2.18	0.79	4.09	1.10	1.94	1.32

Clone 7 small and separated from other clones, probable aberrant. Other clones form group of probable apomicts.

Family 17

PH	2	6	3	1	4	5	8	7
SpL	2	3	4	7	5	6	8	1
Sd/Sp	2	3	4	5	6	8	1	7

Canonical analyses, distance from centroid:

Clone No.	1	2	3	4	5	6	7	8
6 variates:	5.43	2.62	5.30	5.29	6.13	3.64	6.06	6.62
3 variates:	4.46	1.95	4.82	4.57	5.50	2.91	5.63	6.20

Clones 5, 7, 8 are most vigorous, forming loose group with clones 2 and 6 small and most distant. Scatter of clones indicates some segregants probably present.

Trial D - Only data from the seventeen families included in Trial C were analysed. The visual estimate of relative morphological uniformity (fig.14) on each row of ten plants showed that hybrid families 3, 4, 5, 6, 9 and 10 were all comparable in uniformity with the parental biotypes. There was some variation in size evident in these six progenies and in several of them at least one plant which looked sufficiently different (usually also much smaller in size) to be classed as aberrant. Families 2 and 11 showed a wide range in variation and all plants from these families appeared to be the result of a normal sexual reproductive cycle. Families 1, 7 and 8 had varying proportions of plants which were morphologically similar in appearance indicating that

FIGURE 14

On left, three highly apomictic morphologically uniform families and on right, three morphologically variable families derived from predominantly sexual maternal parents

the maternal parent had yielded a mixture of apomictic and amphimictic seed.

Only the seven characters scored on plants in all four blocks were used in the following analyses since it was thought to be more important to include the maximum number of individuals than the maximum number of variates and the results from Trial C had indicated that large differences between individuals could be detected without information from the more detailed panicle records.

Correlation coefficients between the seven variates calculated from 618 individuals are shown in Table 14.

Table 14 - Trial D Correlation coefficients between seven characters calculated from 618 individuals

	EE	PH	PD	LL	LB	SG	FW
EE							
PH	-0.328						
PD	0.182	-0.009					
LL	-0.155	0.517	0.137				
LB	0.089	-0.042	-0.009	0.150			
SG	-0.277	0.434	0.136	0.394	-0.027		
FW	0.015	0.234	0.372	0.281	0.181	0.524	

$r > \pm 0.131$ ***, $r > \pm 0.103$**, $r > \pm 0.079$*

Family means and variances are shown in Tables 15 and 16 respectively. Within family variances were heterogeneous for all seven characters. Determinants from within family variance-co-variance matrices on the seven variates are also shown in Table 16.

Principal co-ordinate and principal component analyses were carried out on families 1, 11 and 14 separately using all seven

TABLE 15 - Trial D Family means for seven variates

FAMILY	NO. INDIV.	EE nil	PH (mm) nil	PD (mm) nil	LL (mm) SQRT	LB (mm) SQRT	SG SQUARE	FW (g) SQRT
1	36	7.83	624	183	17.34	2.181	644	10.08
2	37	14.89	442	233	15.06	1.901	616	8.64
3	36	13.97	465	436	16.06	2.086	935	15.36
4	39	13.41	571	369	17.82	1.916	798	11.92
5	38	13.68	571	417	17.79	1.995	882	11.75
6	38	13.37	621	504	18.69	2.236	709	11.56
7	38	11.45	571	209	15.49	2.029	813	9.98
8	25	8.96	636	154	16.24	2.142	448	6.80
9	38	13.74	563	422	17.72	2.022	859	11.22
10	38	13.90	566	441	18.14	2.000	837	12.01
11	30	14.93	516	184	15.15	1.919	540	7.94
12	37	2.03	478	387	15.77	1.985	729	12.12
13	36	8.72	721	538	18.23	1.833	897	9.82
14	39	14.49	530	411	16.52	2.128	793	14.06
15	39	12.31	496	400	15.03	2.037	841	13.58
16	39	15.10	495	437	17.16	2.087	878	13.28
17	35	1.86	747	152	19.85	1.991	1239	12.59

TABLE 16 - Trial D Family variances, determinants of dispersion matrices from seven variates, and tests for homogeneity of variances and dispersions

FAMILY	EE	PH (mm) $\times 10^{-3}$	PD (mm) $\times 10^{-2}$	LL (mm)	LB (mm) $\times 10^{-2}$	SG $\times 10^{-3}$	FW (g)	Det $\times 10^{-3}$
1	25.00	9.32	31.39	4.981	4.980	105.38	15.37	13161
2	37.88	14.23	78.78	5.253	3.559	107.14	13.95	62245
3	5.74	5.35	65.40	2.099	3.917	17.40	13.41	722
4	5.41	1.66	18.17	3.131	2.214	9.08	3.10	9
5	7.09	2.56	31.44	4.537	2.066	11.83	5.60	54
6	7.91	5.04	57.22	3.820	2.794	15.44	7.46	378
7	18.42	14.37	30.44	5.714	4.413	40.88	7.70	998
8	47.96	24.22	52.67	5.186	5.322	71.15	7.29	17014
9	5.17	1.26	38.55	2.625	1.586	9.86	4.99	15
10	3.61	1.53	30.72	6.772	2.709	8.29	3.55	22
11	16.48	14.98	40.18	6.580	5.193	84.08	15.22	22146
12	9.69	3.38	50.98	3.965	3.564	29.76	4.10	1038
13	5.29	3.17	50.94	3.911	2.802	12.92	4.36	443
14	3.47	5.75	29.64	1.528	3.599	13.57	9.08	110
15	7.01	10.45	80.63	3.039	2.897	29.21	18.95	1342
16	4.67	5.98	76.10	3.524	3.006	27.55	10.79	451
17	5.42	5.76	11.68	4.980	0.952	177.76	10.13	852
χ^2 (16df)	229.28 ***	192.14 ***	113.41 ***	87.50 ***	48.20 ***	408.61 ***	81.54 ***	1570.4+ ***

+ χ^2 with 448 df.

variates and the three variates ear emergence, plant height and plant diameter. The seventeen family means were included in each analysis so that the variation between individuals within each family could be compared with the variation between families. Individuals were plotted on the 1st and 2nd principal co-ordinates and on the 1st and 2nd principal components (Appendix figs.1 to 12, pages 202 to 213).

The relationships between individuals in a family and between family means were broadly similar from the two analyses using either seven or three variates. This result indicated that either form of analysis could be used on this data to give a cluster diagram.

A comparison of families 11 (probably segregating) and 14 (probably apomictic) shows less variation between individuals in 14 relative to the family means than in 11. But since 11 was probably the most variable family being investigated it seemed unlikely that a similar comparison would help in the description of a less variable family.

All 618 individuals were included in principal component analyses on seven and on three variates. Positions of individuals in each family were plotted separately on the 1st and 2nd principal components; diagrams from the analysis on seven variates are shown in figs.15 to 23. Plots from the two analyses were similar but some small differences were noticeable and have been taken into account in the following comments.

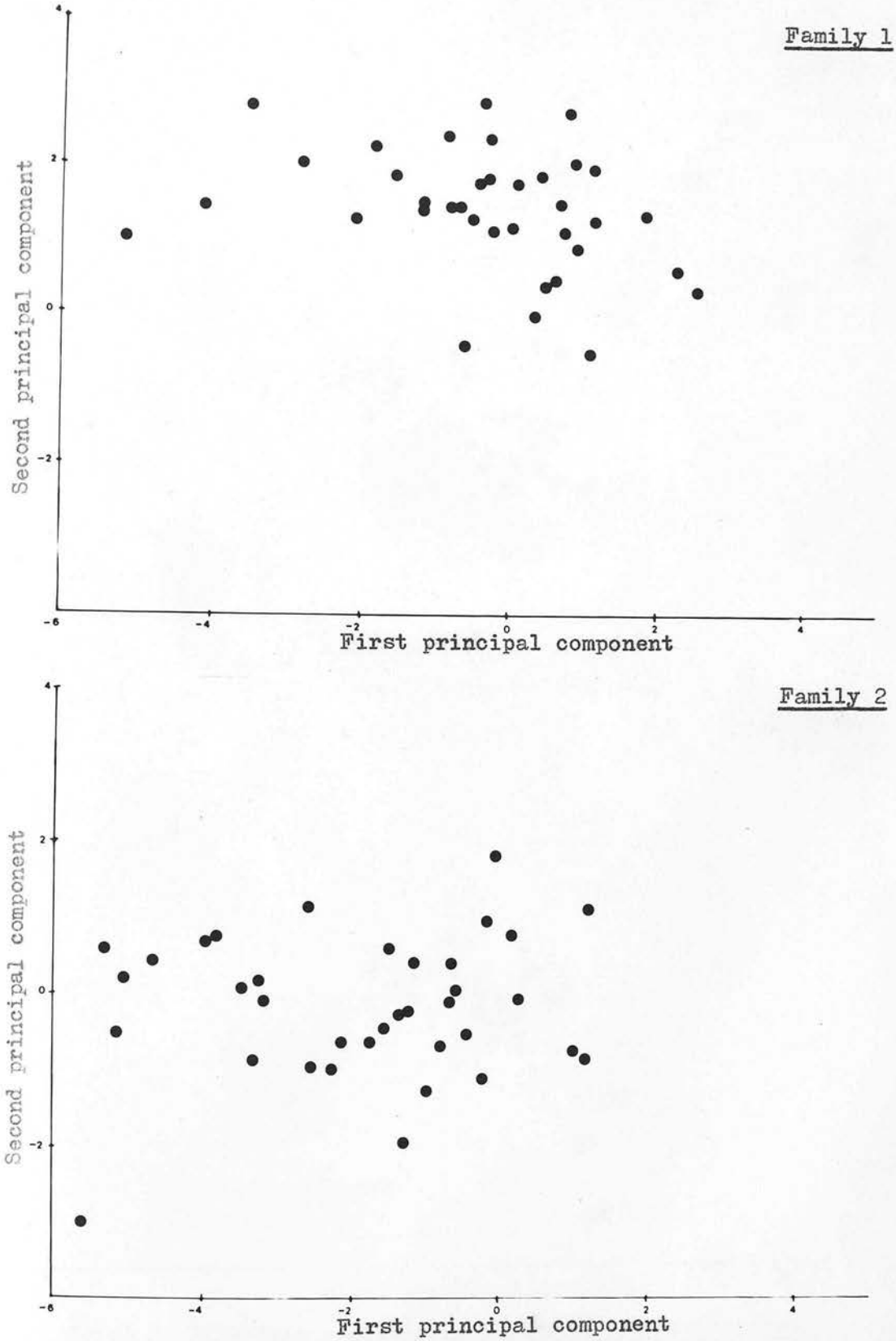
The number of surviving plants (Table 15) in each progeny gives an indication of the occurrence of aberrants. Approximately one plant per family was lost during cultivations; other

missing plants were probably weak aberrants which died in the course of the trial. A summary of the results from Trials C and D (Table 17) shows the approximate percentage of apomicts in each of the seventeen families. Each percentage is probably accurate within the range ± 10 per cent.

TABLE 17 - Trials C and D Number of probably apomicts in each of seventeen families

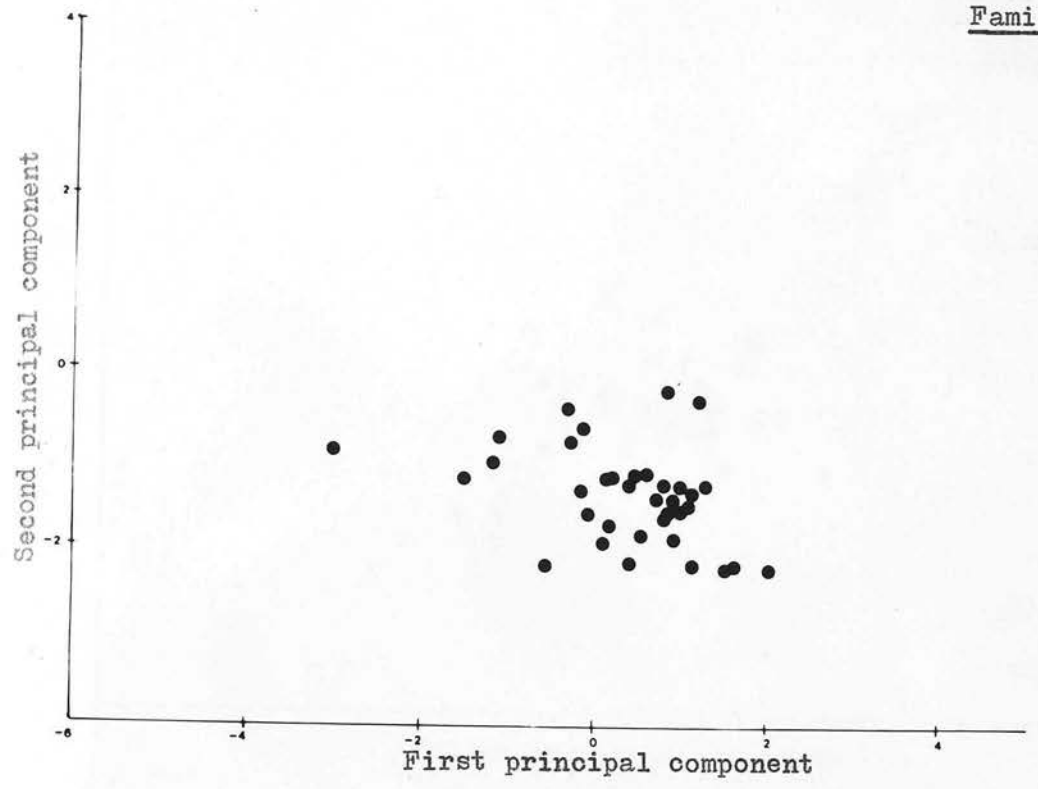
Family	Trial C	Trial D	Total	Percentage
1	5	10	15	30.6
2	0	0	0	0
3	9	26	35	71.4
4	9	39	48	98.0
5	10	36	46	93.9
6	8	37	45	91.8
7	8	29	37	75.5
8	4	11	15	30.6
9	9	37	46	93.9
10	10	36	46	93.9
11	0	0	0	0
12	9	36	45	91.8
13	9	36	45	91.8
14	10	38	48	98.0
15	9	30	39	79.6
16	9	34	43	87.8
17	3	28	31	63.3

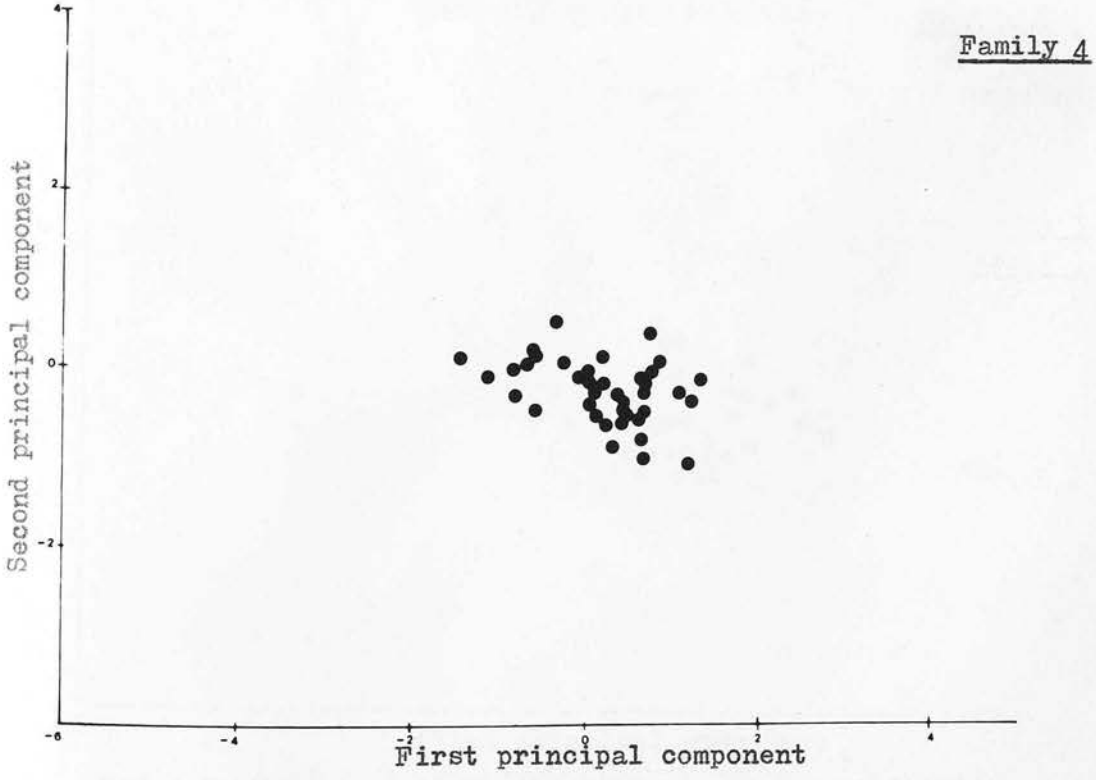
FIGURE 15



Trial D Principal component analysis on 7 variates over all 618 individuals in 17 families

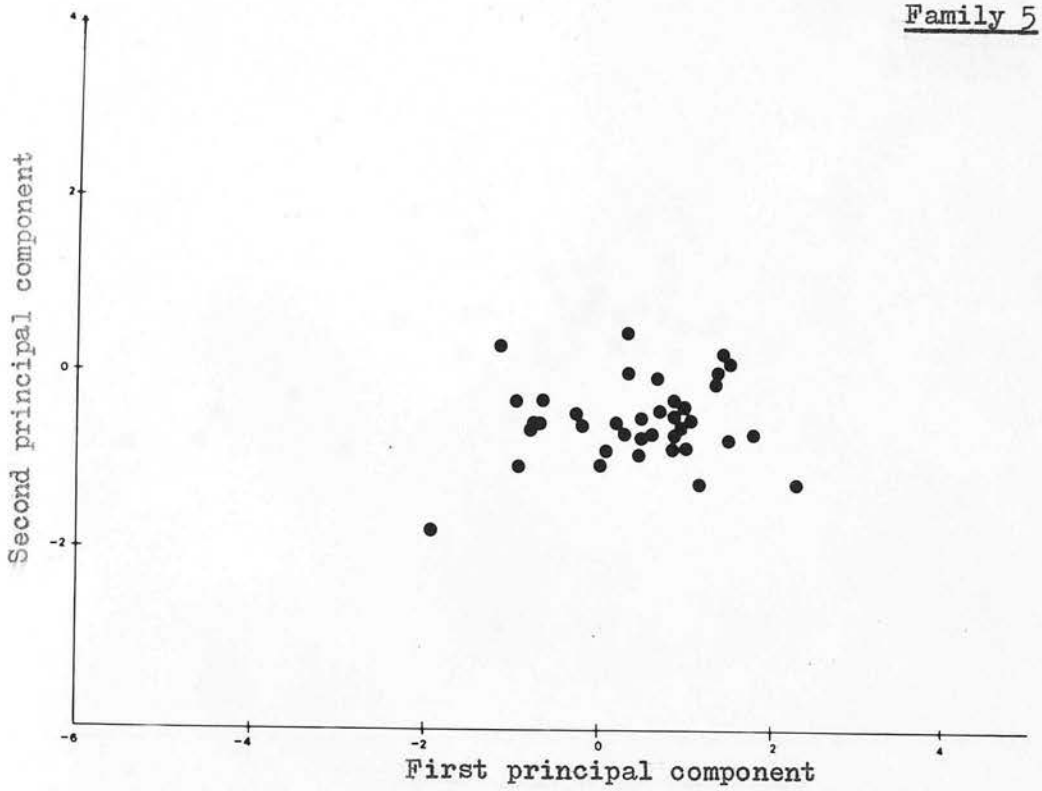
FIGURE 16

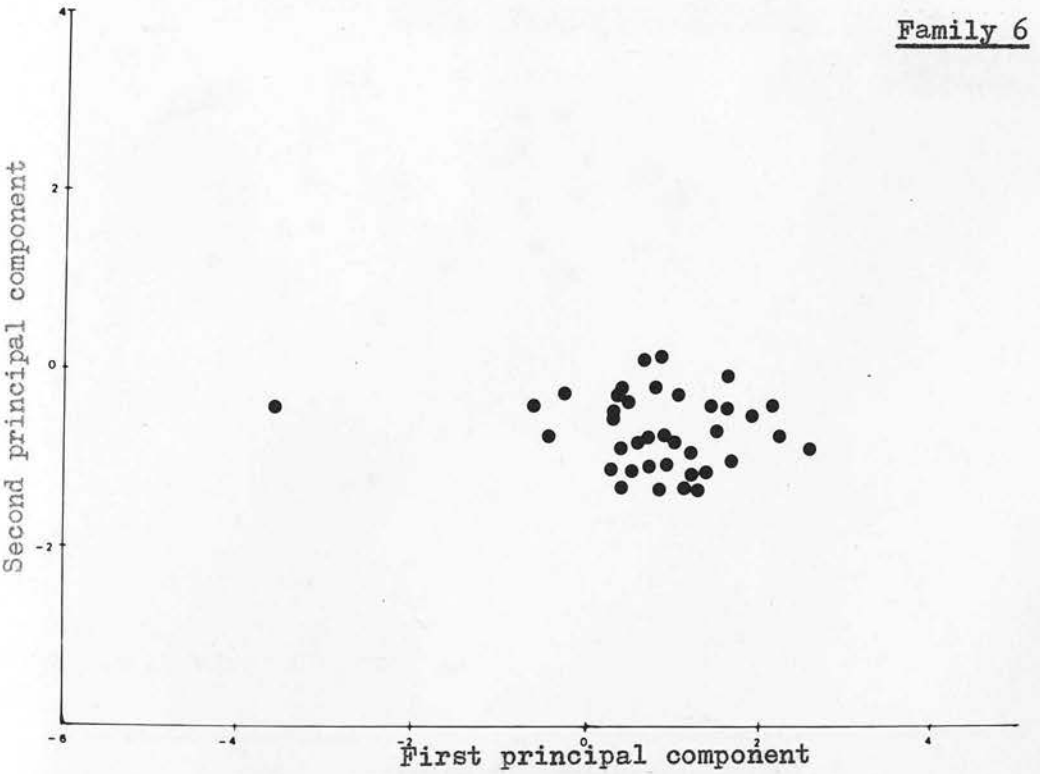




Trial D Principal component analysis on 7 variates over all 618 individuals in 17 families

FIGURE 17

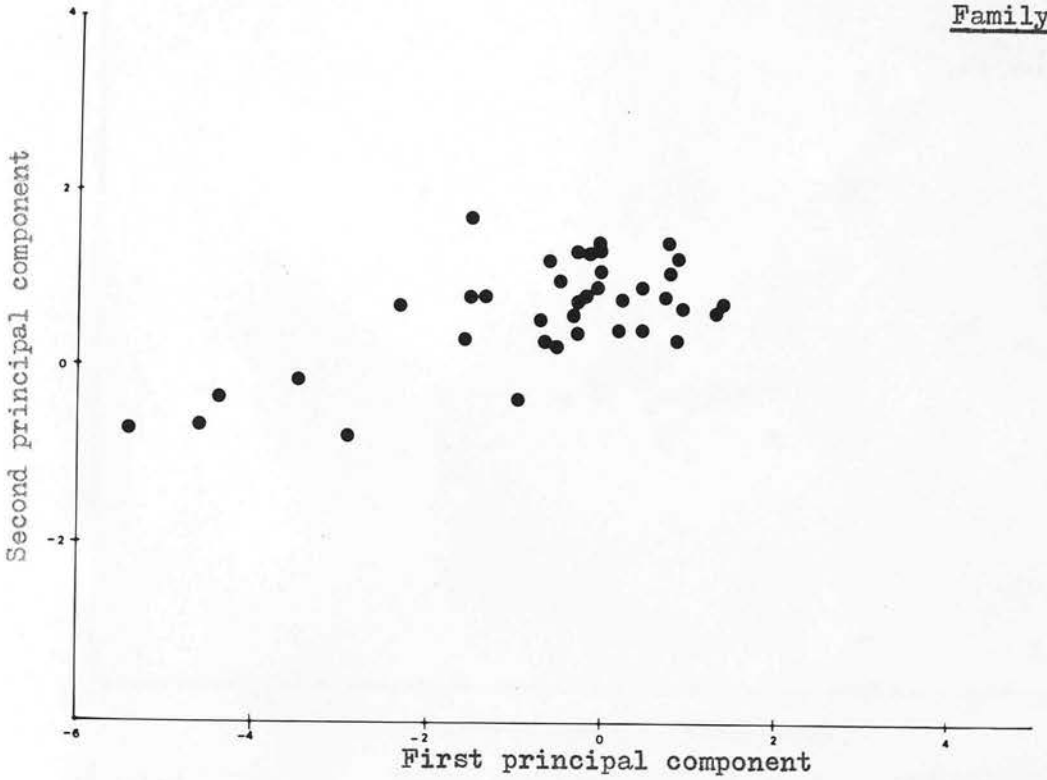




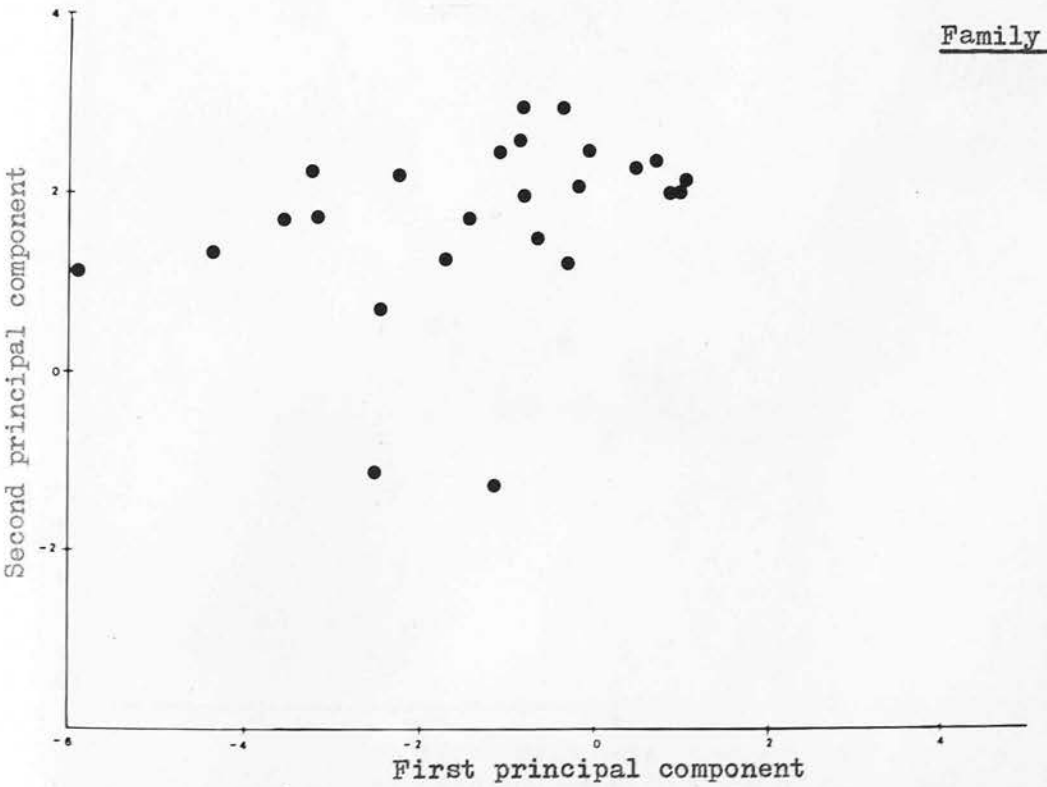
Trial D Principal component analysis on 7 variates over all 618 individuals in 17 families

FIGURE 18

Family 7

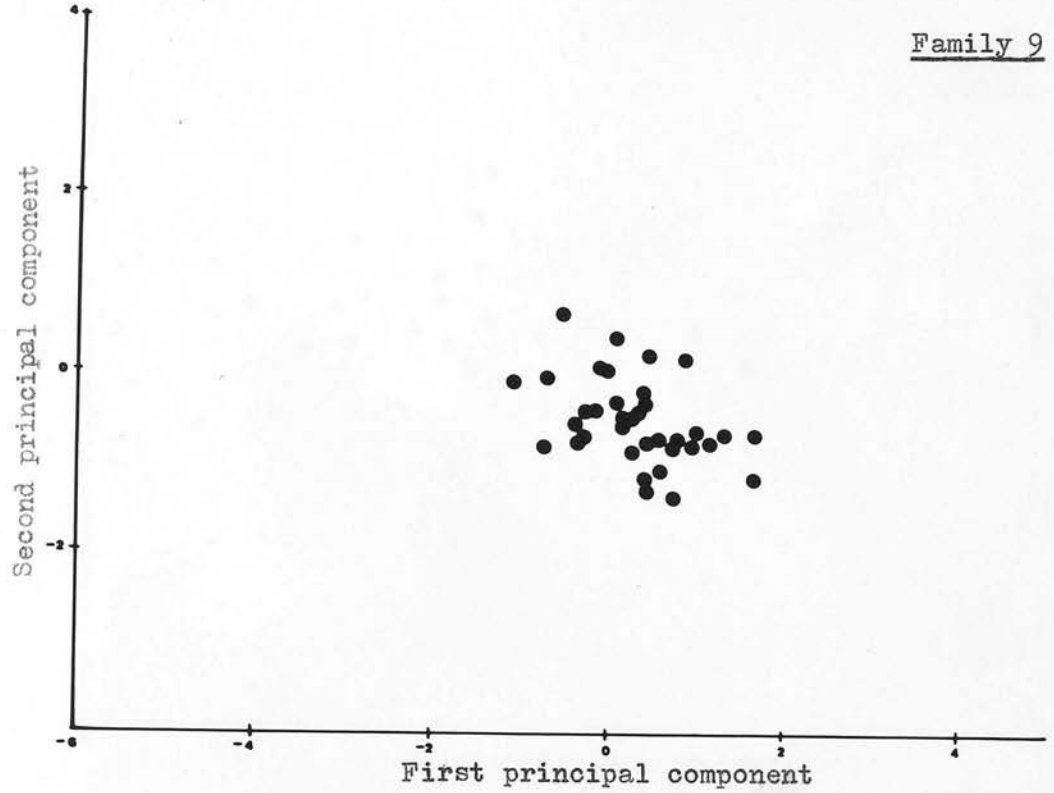


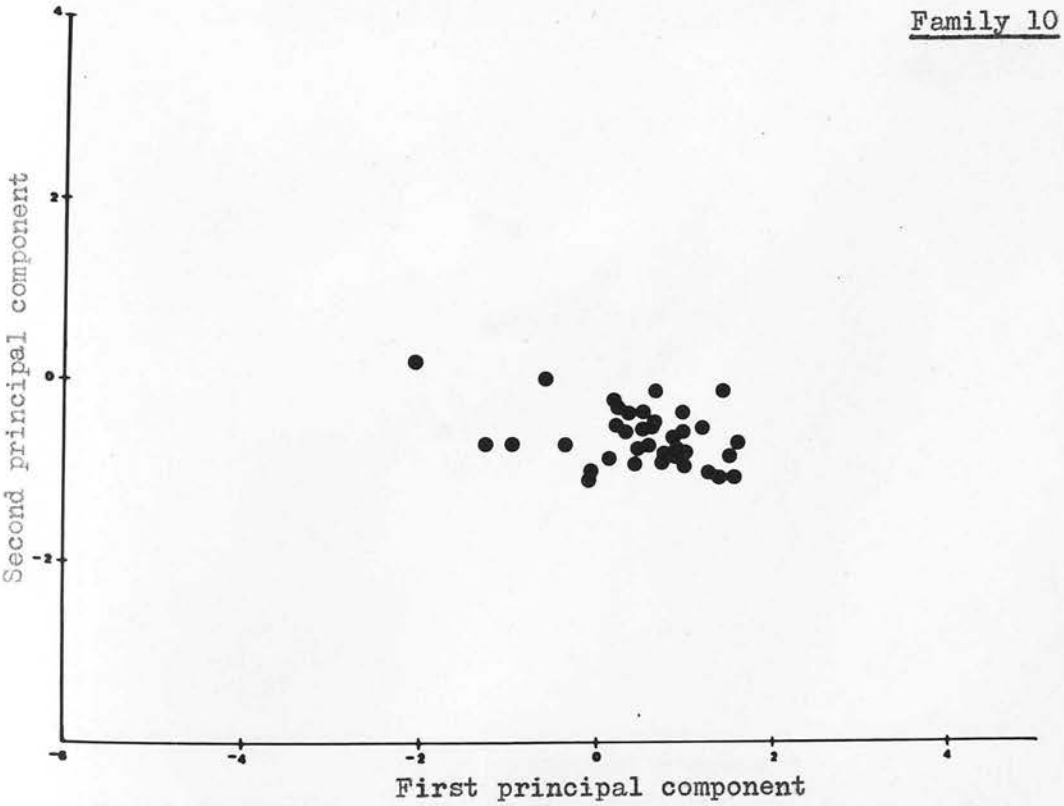
Family 8



Trial D Principal component analysis on 7 variates over all 618 individuals in 17 families

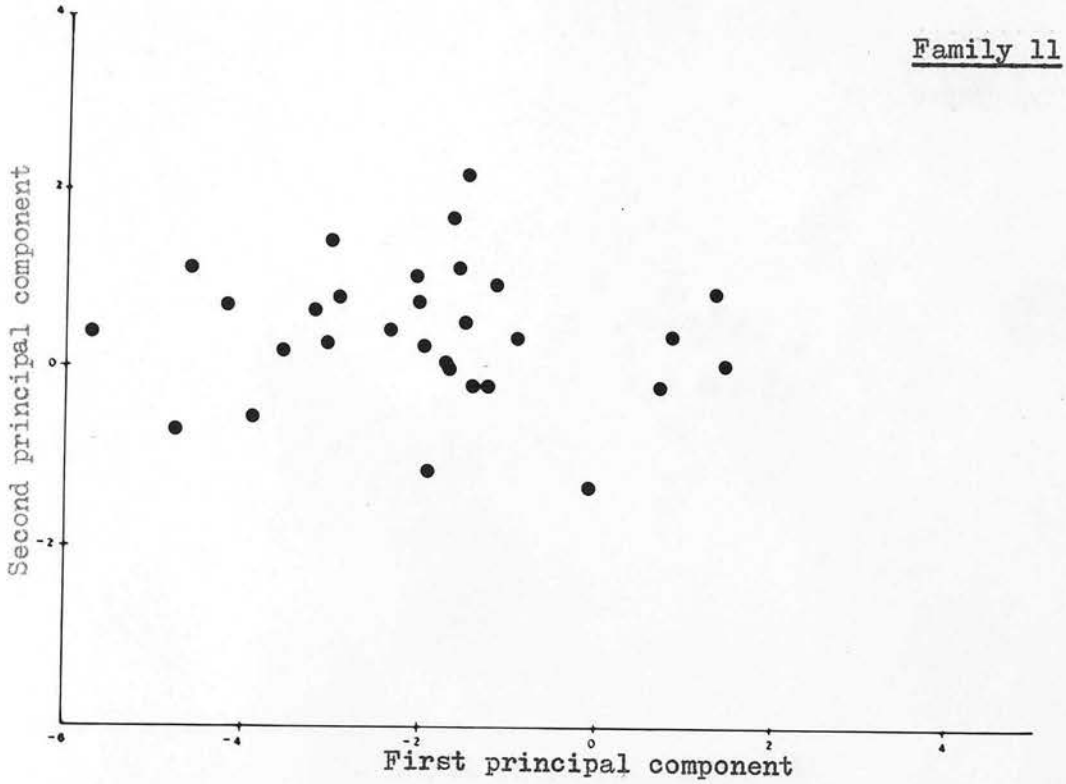
FIGURE 19

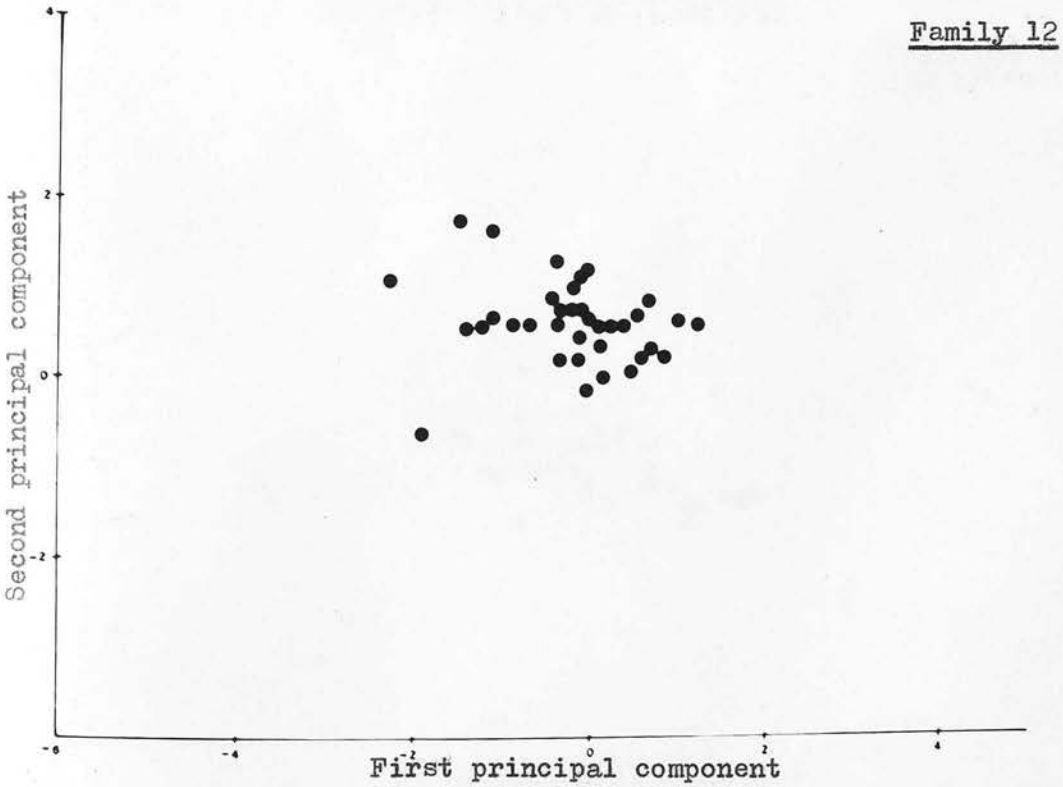




Trial D Principal component analysis on 7 variates over all 618 individuals in 17 families

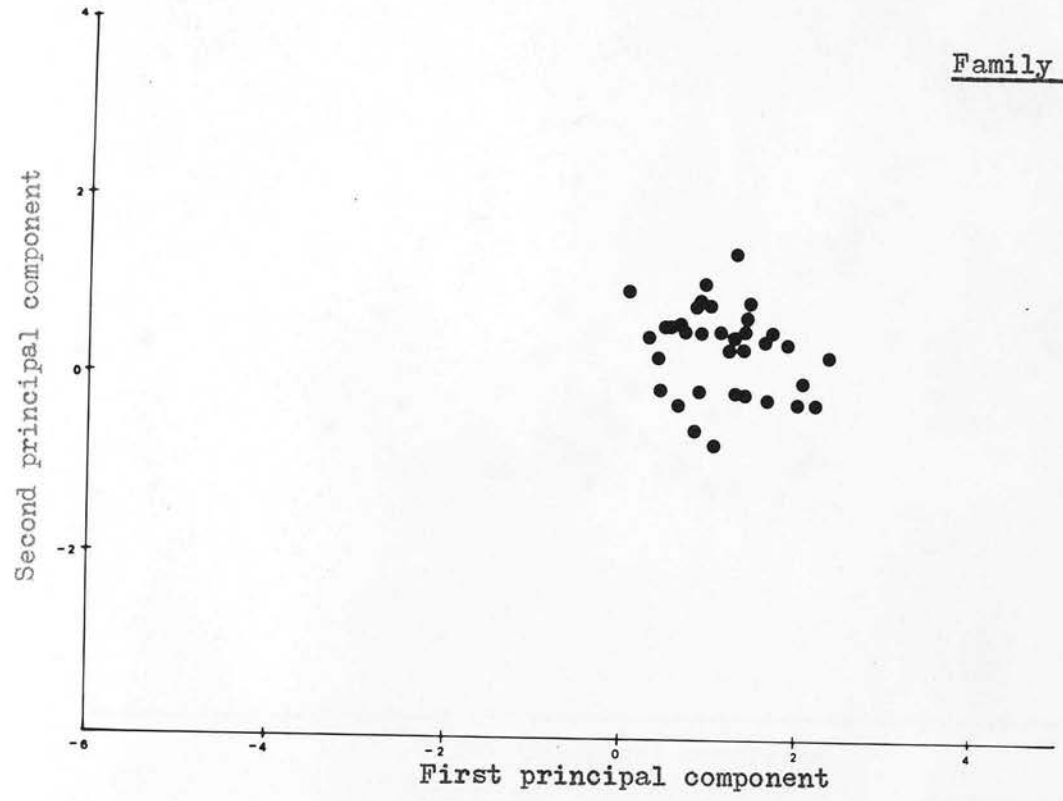
FIGURE 20

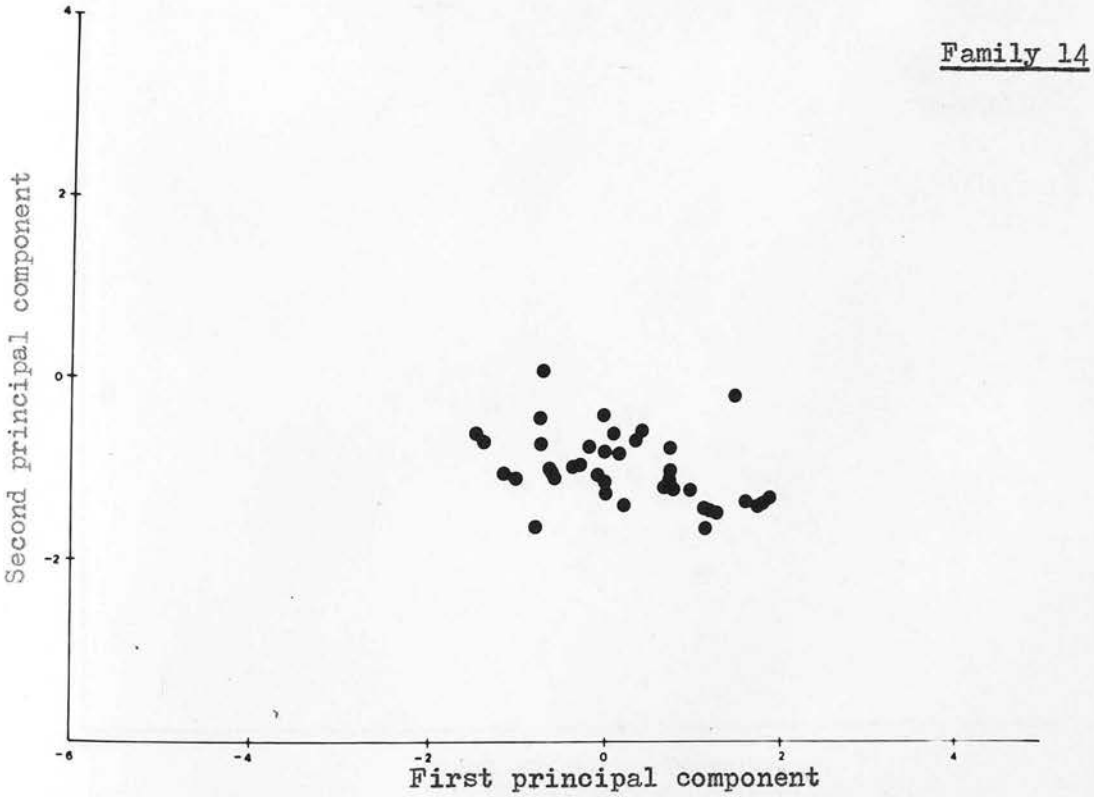




Trial D Principal component analysis on 7 variates over all 618 individuals in 17 families

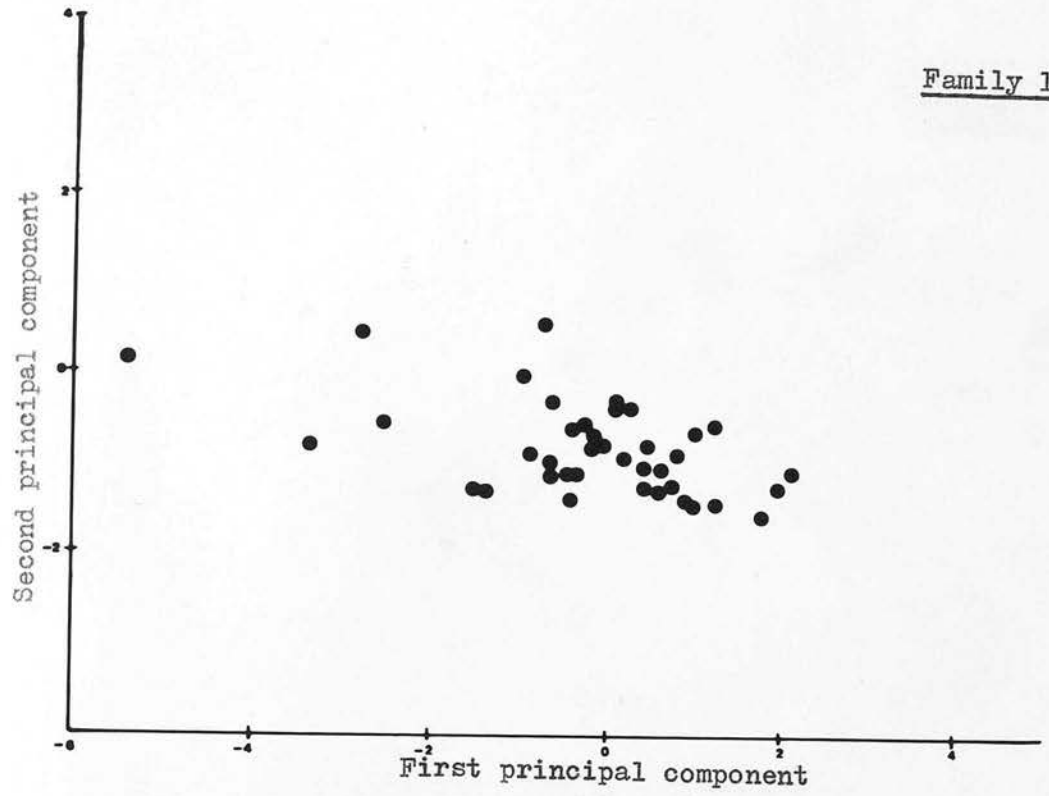
FIGURE 21

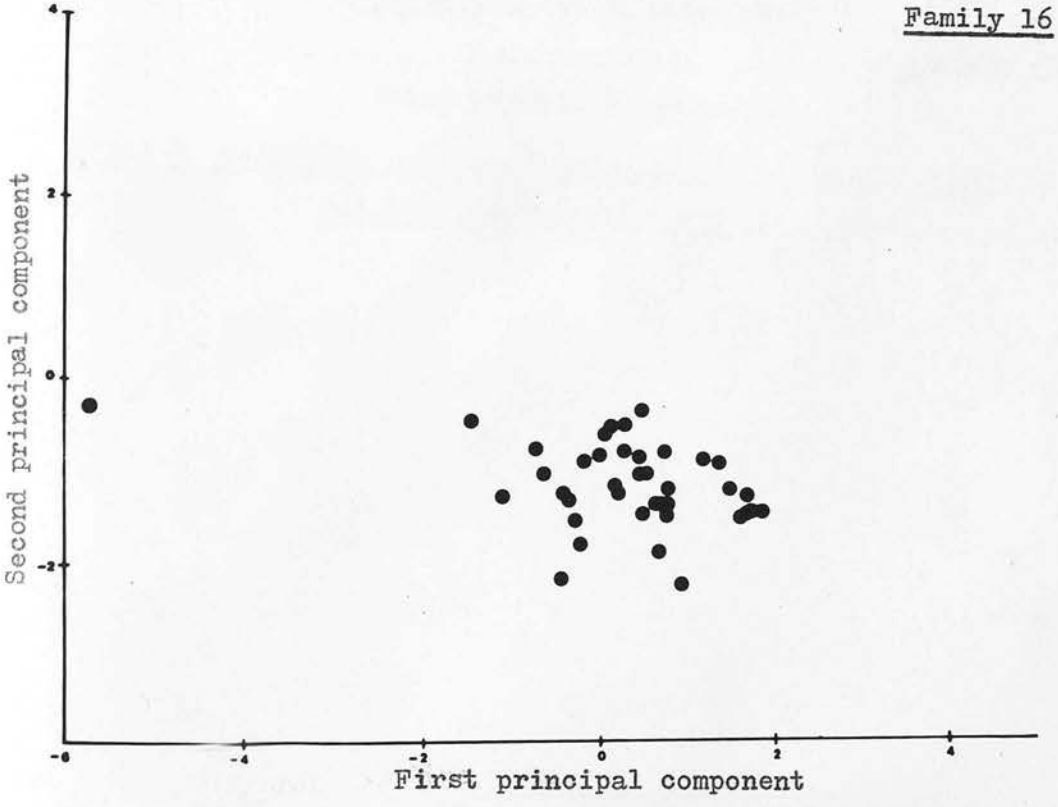




Trial D Principal component analysis on 7 variates over all 618 individuals in 17 families

FIGURE 22

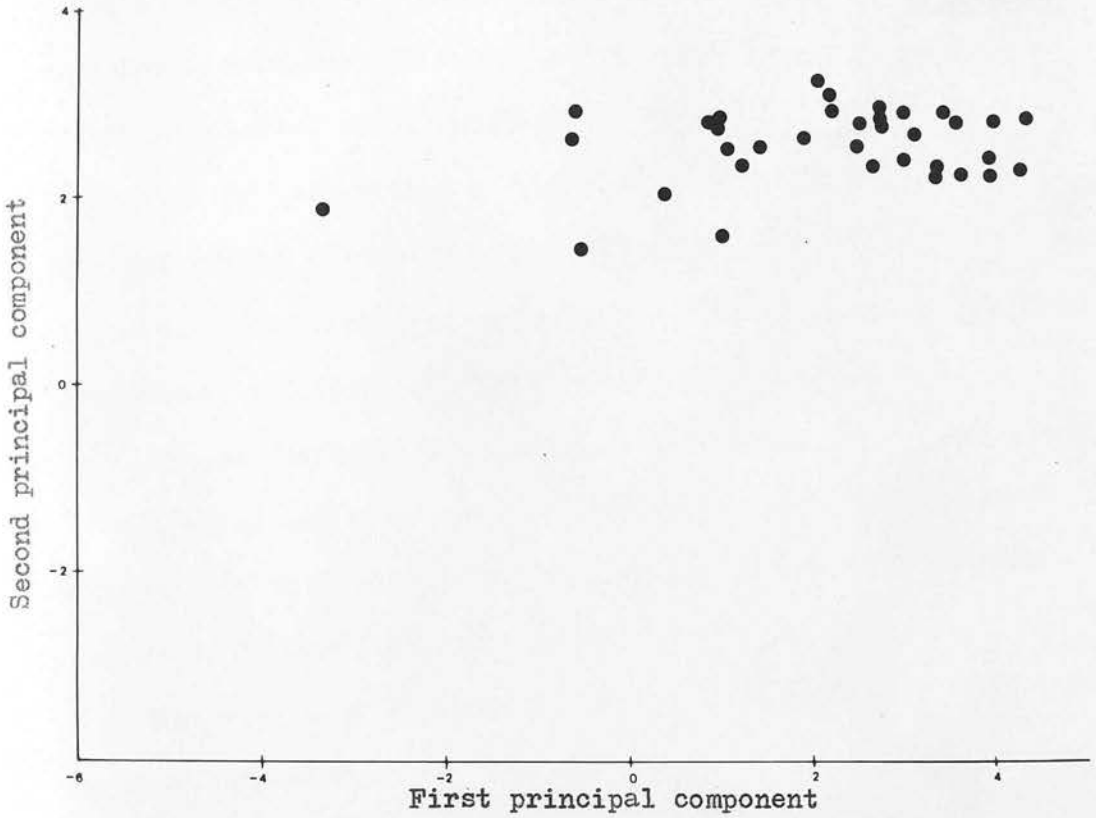




Trial D Principal component analysis on 7 variates over all 618 individuals in 17 families

FIGURE 23

Family 17



Trial D Principal component analysis on 7 variates over all 618 individuals in 17 families

DISCUSSION

The main objective of Trials C and D reported here was to find a method for distinguishing seed clones produced from apomictic maternal parents from the progenies of amphimictic maternal plants, and if possible to detect the proportion of each type of seed within a family of "mixed" origin. Since any successful method must be applicable to the screening of large numbers of progenies in the context of the plant breeding programme, an essentially simple method is required.

Eye estimates of morphological uniformity on progeny rows gave a quick and easy rough guide to the separation of the extremes of variability, but tended to be subjective and the intermediate families were difficult to classify.

The choice of characters for use in analyses was influenced by ease and accuracy of measurement and by their possible use as criteria for selection. Highly correlated characters were omitted and those with high intra-class correlations were included as far as possible. All variates were tested for normality; this was necessary since tests for homogeneity of variances ~~was~~ are very sensitive to departures from normality (Snedecor and Cochran, 1967). Variates which were not normally distributed either before or after $\log_{10}$, square root or square transformation were omitted. The four characters which satisfied the above requirements and were found generally to have the best discriminatory power were ear emergence, plant height, plant diameter and spring growth. The effective separation of individuals obtained when using these variates was possibly due

to their tendency to maximise character differences of the two parental species.

Observations on progenies of 1968 hybrids as spaced plants indicated that probably all the viable seed from 1968 F_1 hybrids was produced sexually. Clausen (1961) reported that the majority of F_1 interspecific hybrids of Poa between apomictic species were amphimictic and suggested that hybridisation upsets the delicately balanced apomictic system by combining two genomes that are adjusted individually but not mutually. Almgård (1966) and van Dijk (1974) also reported F_1 interspecific hybrids with P. pratensis to produce sexual seed. The 1969 F_1 hybrids were therefore expected to produce predominantly sexual seed and hence, segregating progenies.

It is apparent from the results of Trials C and D that the parental biotypes of P. pratensis produced differing proportions of apomictic seed (80 per cent to 98 per cent) and that P. ampla in these trials was very variable with probably only 63 per cent of viable seed produced apomictically. Also only two of the eleven hybrid progenies appeared to be derived from maternal plants with entirely sexual seed production, and five hybrid families were as uniform as the highly apomictic P. pratensis lines.

Progenies from 37 of the F_1 1969 hybrids were included in Trial D; rough estimates indicated that c. 16 per cent of the hybrids were highly apomictic, c. 33 per cent produced a mixture of apomictic and sexual seed, and c. 51 per cent were entirely amphimictic. The rather high proportion of apomictic progenies present in Trial C was probably due in part to the selection of

eleven of the more vigorous seedling progenies for study. In this material both seedling and mature plants from apomictic hybrids are generally more vigorous than those from non-apomicts. Clausen et al. (1954) reported a similar difference in vigour.

A straightforward comparison of the magnitude of family variances and of determinants from variance-co-variance matrices distinguished highly apomictic from predominantly segregating progenies, but the occurrence of even a low frequency of aberrants in a highly apomictic family inflated the variances and it was not possible to identify such families without additional information. The relative size of determinants was found to be helpful in the interpretation of plots from multivariate analyses but the test for homogeneity of dispersions was found to be too sensitive to be very useful with these data. The test carried out on the five P. pratensis families in Trial C showed that in the absence of aberrants the variance-co-variance matrices were homogeneous. This suggests the possibility of devising a method for testing progenies against a known apomict, the most distant individuals being successively deleted until the two dispersions become homogeneous.

It becomes clear, then, that before a progeny can be judged to have developed from apomictic or sexually produced seed, the relationships of all individuals in the progeny must be examined, and the within family dispersion must be compared with that of a known apomictic family. This becomes essentially a problem in numerical taxonomy and the three techniques used for clustering data all gave diagrams which could be used to distinguish aberrant individuals within families. It was, however, necessary

to compare the positions of individuals on more than two axes of variation, or from two different analyses to confirm relationships between them. The distance from the centroid calculated in the canonical analyses on Trial C data was of value in confirming a clone as aberrant, but distance measures between individuals were not used.

The differences in dispersion between apomictic, partially apomictic and segregating progenies were clearly illustrated when individuals from all seventeen families were included in the same analysis then plotted family by family on the same scale (Trial D). Segregating progenies showed all individuals separated and widely scattered while the apomicts formed tight clusters. The variation in plant size which was seen in the field even in predominantly apomictic families also showed in the scatter along the second axis (figs.15 to 23) which was found to represent mainly size variation. There were some differences in degree of variability between families classified as apomictic as well as those due to size. There must inevitably be a "grey area" where the possibility mentioned earlier of a maternal parent with many dominant genes would give a progeny of relatively uniform, maternal-like hybrids which could not be distinguished from an apomictic progeny. Aneuploid aberrants would also be expected to occur relatively frequently in this material and some might be only marginally different in phenotype from apomicts in the same family; it is probable that they were a source of variation within seed clones.

Diagrams from partially apomictic families were the most difficult to interpret particularly where there was only a small

CHAPTER 5

THE INFLUENCE OF LIGHT REGIMES ON APOMICTIC SEED PRODUCTION IN P. AMPLA AND P. PRATENSIS

(TRIALS E and F)

CHAPTER 5

THE INFLUENCE OF LIGHT REGIMES ON APOMICTIC SEED PRODUCTION IN P. AMPLA AND P. PRATENSIS

INTRODUCTION

The 1969 P. ampla X P. pratensis hybrids were obtained, after exposure to a reduced daylength regime, from a biotype of P. ampla known to be highly apomictic in the Edinburgh environment. There was also earlier evidence of a higher proportion of sexual seed developing in the shorter daylengths at Pullman and at Stanford than at Pentlandfield (Watson and Clausen, 1961). However, there is no indication that this is a response to photoperiodic stimulus rather than to differences in total irradiance nor whether other environmental factors were involved.

There is little information on the influence of daylength on seed production in P. pratensis although Han (1970) reported that photoperiod did not affect the proportion of aberrant seeds produced.

The length of the photoperiod is known to affect inflorescence development in fully induced P. pratensis cv. Merion (Lindsey and Peterson, 1962). An eight hour photoperiod prevented heading but a half hour light break during the sixteen hours dark promoted flowering, as did continuous light. The rate of inflorescence development was also influenced by increasing length of photoperiod up to sixteen hours, a similar response to that observed for Lolium temulentum (Cooper, 1956).

Total irradiance affects the supply of photosynthate to the developing inflorescence and may influence the total number of florets developing in a panicle (Ryle, 1965, 1967), as well as

the final seed weight as in Lolium perenne and L. temulentum (Bean, 1973). Where more than one embryo sac initial occurred within an ovule of P. pratensis, Grazi et al (1961) postulated that competition for nutrients might influence the number of functional embryos maturing. It seems possible that in facultative apomicts such as P. ampla and P. pratensis environmental factors could play a large part in determining the relative numbers of aposporous and of sexual embryo sac initials produced and maturing. Preliminary investigations are reported here on the influence of different light regimes on type of seed production in one biotype of P. ampla and five Scottish biotypes of P. pratensis.

In Trial F the biotypes were used as recurrent parents and intercrossed within each of five light regimes. The experiment was analysed as a series of North Carolina I type designs. Variability of seedling progenies was assessed as it might be expected to reflect any alteration in the proportion of aberrant seeds which matured as a result of different light regimes during inflorescence development.

Trial E - Influence of two light regimes on seed production in P. ampla and P. pratensis

One biotype of P. ampla (42) and three biotypes of P. pratensis (145, 159 and 190) were vegetatively cloned into six ramets each and kept outside throughout the winter. Three ramets from each biotype were randomised inside a simple dark chamber constructed in an unheated greenhouse, and subjected to an eight hour photoperiod; the other three ramets from each biotype were randomised and subjected to a 16 hour photoperiod on an adjacent bench. Daylight was supplemented by warm-white fluorescent strip lighting to give a minimum illuminance of 7000 lux; photoperiod treatments started on 20th March 1973.

The biotypes were allowed to mass pollinate. Seed was harvested, thrashed and counted from individual panicles. Pollen samples were taken for stainability tests. A summary of data from the harvested panicles is shown in Table 18.

TABLE 18 - Trial E Summary of data from harvested panicles and pollen stainabilities

	P. ampla 42		P. pratensis 145		P. pratensis 159		P. pratensis 190	
	8 hr	16 hr	8 hr	16 hr	8 hr	16 hr	8 hr	16 hr
Number of panicles	8	16	37	23	1	1	3	4
Florets per panicle	267	440	748	519	210	605	84	190
Total seed set	28	895	13	1078	50	218	7	522
Hard seed (%)	1.31	12.72	0.05	9.03	23.81	36.03	2.79	68.78
Pollen stainability (%)	28.5	61.0	0.0	71.0	95.0	79.0	80.5	89.0

The very low seed production from all clones except P. pratensis 159 after short day treatment meant that a comparison of progenies derived from ramets in the two light regimes was not possible. The trial was therefore abandoned at this stage.

Where possible two panicles had been sampled from each ramet for embryological studies on the morning that the first florets were at anthesis. The objective was firstly to investigate the possibility of using ovule squashes to assess the proportion of multicellular proembryos and the frequency of polyembryony at, or just prior to anthesis; and secondly to examine the possibility of using such counts to supplement information obtained from progeny tests in the assessment of the proportion of aposporous and sexual seed produced.

In a preliminary examination of ovule squashes in acetocarmine it was possible to identify some multicellular proembryos. But it became evident that a more detailed study of the embryology was required than time or the material would permit. Since no comparison with progeny performance was possible due to the low seed production, this aspect of the trial was also discontinued.

Trial F - The influence of five light regimes on seed production and on subsequent seedling development in P. ampla and P. pratensis

MATERIALS AND METHODS

Ten ramets of each of one P. ampla (42) and five P. pratensis (145, 159, 172, 189 and 190) biotypes were over-wintered in pots outside, and brought into a greenhouse on 2nd April 1974.

Dark cabinets were constructed with heavy light-proof curtains which were opened daily for 6 to 8 hours. Warm-white fluorescent strip lights provided supplementary illuminance. Three basic light regimes were used:

A, 10 hour photoperiod with minimum illuminance 8000 lux plus 14 hours dark;

B, 20 hour photoperiod, 10 hours as A plus 10 hours with maximum illuminance 50 lux provided by a tungsten filament light plus 4 hours dark;

C, 20 hour photoperiod with minimum illuminance 8000 lux plus 4 hours dark.

Two further light regimes used were:

AC, ramets transferred at 50 per cent ear emergence from A to C;

CA, ramets transferred at 50 per cent ear emergence from C to A.

Initially there were four ramets from each clone in A and C and two ramets in B. Ramets under all light regimes were in one greenhouse; temperatures were controlled manually by means of vents and blinds to give a "normal" range of 10° to 25°C. The

minimum temperature recorded was 5°C and the maximum 33°C. The ear emergence date for each panicle was noted. Biotypes were pair crossed or selfed immediately before first anthesis using pollen-proof pergamine bags.

Seed production from P. pratensis 172 and 189 was low so these two biotypes were omitted from progeny tests. One hundred and eighty seeds from each of the five light regimes were germinated from the other four biotypes, sixty seeds from each of three crosses within a light regime. Seedlings were pricked out as they germinated into John Innes 2 potting compost in individual three inch pots. Plots of eight seedlings from each cross were randomised in four blocks on sandbenches; they were grown under a minimum photoperiod of 16 hours with supplementary fluorescent lighting (minimum illuminance 8000 lux).

The following records were taken:

1000 seed weight (mg) - calculated from weight of 60 seeds from each cross;

Mult. - number of multiple seedlings in sample of 32 pricked out from each cross;

Ab - number of seedlings in each sample of 32 which appeared to differ in habit from others in progeny;

Germ. - number of seeds in sample of 60 which had germinated after eight days.

All seedlings were scored for:-

GD - germination date, scored as number of days from sowing to emergence of coleoptile;

2LD - second leaf date, number of days from sowing till second leaf mature;

4LD - fourth leaf date, number of days from sowing till fourth leaf mature. 4LD-2LD used in analyses, number of days between second and fourth leaf maturity dates.

2LL (mm) - length of second leaf lamina

2LB (mm) - breadth of " " "

4LL (mm) - length of fourth " "

4LB (mm) - breadth of " " "

No T(4L) - number of tillers at fourth leaf maturity date

No T(H) - " " " " harvest. T/D, number of tillers produced per day between sowing and harvest date used in analyses.

FW (mg) - fresh weight per seedling, cut to 5 mm above soil c.9 weeks after germination. FW/D - fresh weight increment per day used in analyses.

Colour (categories 1 to 5) - leaf sheath colour; category 1 green, category 5 purple.

Lig (categories 1 to 5) - ligule length; category 1 less than 1 mm, category 5 more than 4 mm .

Au H (categories 1 to 5) - auricle hairs; category 1 no hairs visible, category 5 very hairy.

LA (categories 1 to 5) - leaf angle of third leaf recorded on fourth leaf maturity date; category 1 lamina 0 - 36° from vertical, category 5 lamina 144-180° from vertical.

TA (categories 1 to 9) - tiller angle recorded at harvest; category 1 erect, category 9 prostrate and rhizomatous.

Multiple seedlings were separated as soon as was possible without causing any damage, and the first seedling to appear was recorded.

RESULTS

The median ear emergence date was delayed on all P.pratensis biotypes in light regime A compared with B or C (Table 19).

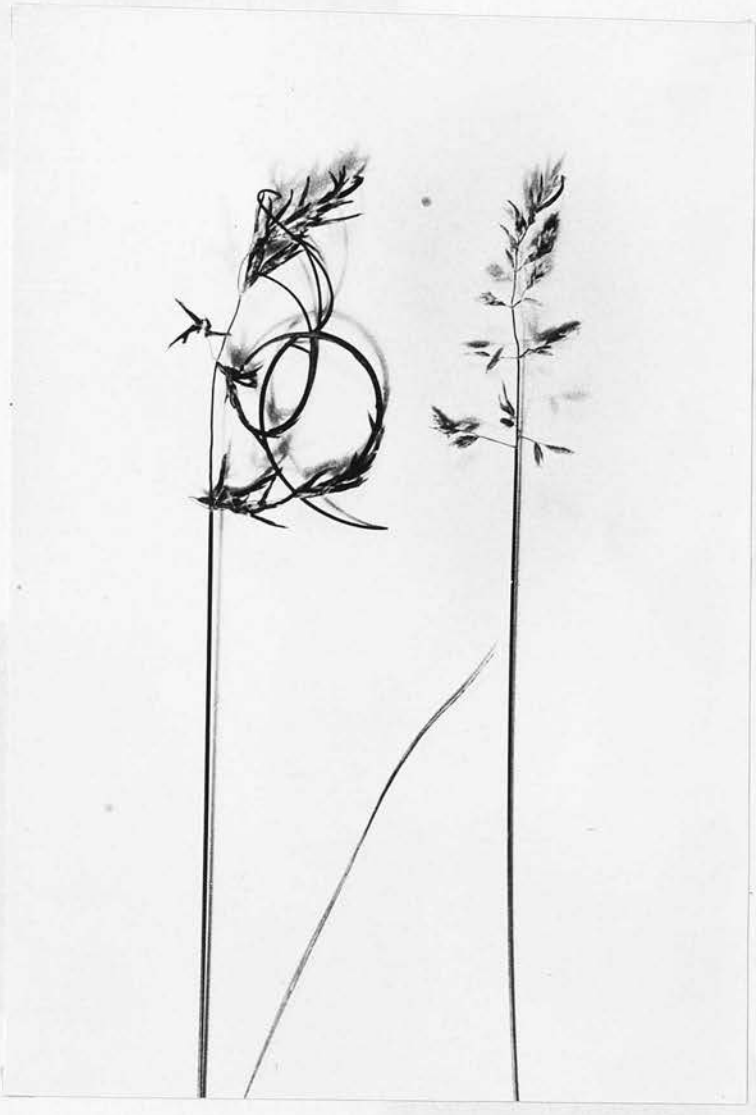
TABLE 19 - Trial F Number of days after start of treatment to median ear emergence and total number of panicles for each biotype

Biotype	A		B		C	
	EE	No pan	EE	No Pan	EE	No pan
42	17	26	15	20	16	31
145	43	35	25	19	24	34
159	41	34	29	23	29	34
172	41	15	33	3	31	18
189	65	13	38	12	33	10
190	36	10	25	2	22	28

As mentioned above, very few good (hard) seeds were produced from P. pratensis 172 in A (11 seeds), or from P. pratensis 189 in A (13 seeds) or in AC (8 seeds). These two biotypes were omitted from the progeny test to avoid missing items in the experiment. Most florets in P. pratensis 189 on panicles which developed in A and AC, but not on panicles from CA, formed vegetative proliferations (fig.24). There was no visible evidence of vegetative proliferation in any other biotype.

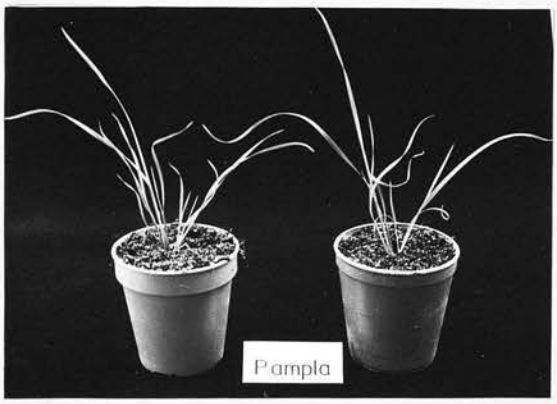
A few seedlings died during the experiment and a total of 1902 seedlings were included in analyses rather than the 1920 which had been pricked out. Seedlings from P. ampla and the three biotypes of P. pratensis are shown at fourth leaf in fig.25. Character correlations between all the quantitative variates are shown in Table 20 as calculated (a) from all 1902 individuals and (b) from sixty sample means (four biotypes X

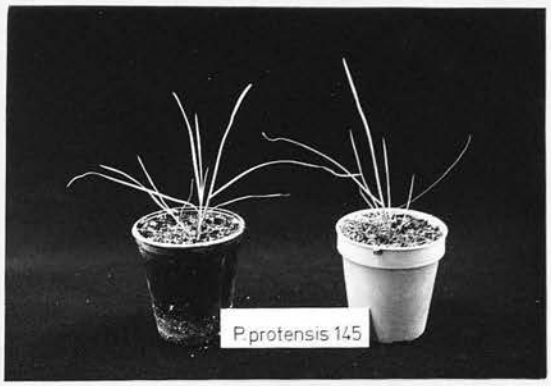
FIGURE 24

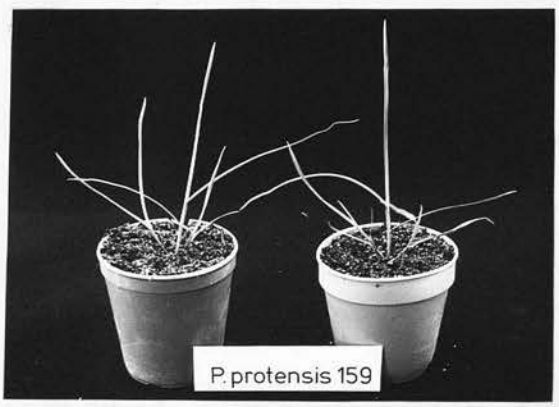


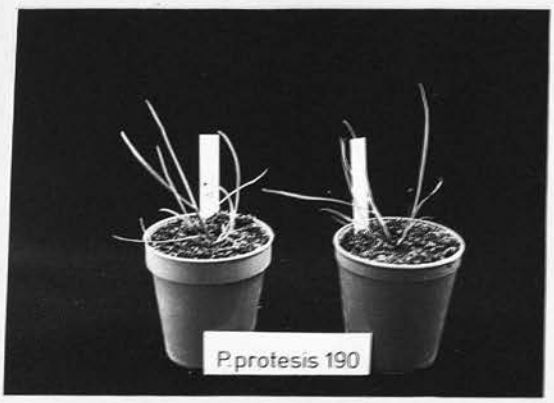
Trial F. Light regime A (ten hour photoperiod at minimum illuminance of 8000 lux). Two panicles from P. pratensis 189 which developed vegetative proliferations.

FIGURE 25









Trial F. Seedlings from four biotypes at fourth leaf.

TABLE 20 - Trial F Correlation coefficients between quantitative variates calculated from (a) 1902 individuals and (b) 60 sample means

(b) \ (a)	1000 Sd Wt	Mult	Ab	Germ	2LD	2LL	2LB	4LL	4LB	No T	T/D	FW/D
1000 Sd Wt	-	-	-	-	-	-	-	-	-	-	-	-
Mult	-0.386		-	-	-	-	-	-	-	-	-	-
Ab	-0.231	-0.065		-	-	-	-	-	-	-	-	-
Germ	0.352	-0.059	0.238		-	-	-	-	-	-	-	-
2LD	-0.536	0.207	0.199	-0.376		-0.424	-0.503	-0.299	-0.465	-0.476	-0.428	-0.617
2LL	0.001	-0.167	0.098	0.016	-0.290		0.317	0.604	0.656	0.395	0.349	0.504
2LB	0.746	-0.234	-0.404	0.068	-0.665	-0.034		0.453	0.484	0.538	0.308	0.459
4LL	0.254	-0.078	-0.418	-0.040	-0.521	0.400	0.618		0.500	0.383	0.254	0.437
4LB	0.196	0.225	0.120	0.439	-0.461	0.539	0.241	0.292		0.301	0.256	0.664
No T	0.548	-0.485	-0.323	-0.228	-0.483	0.067	0.662	0.323	-0.204		0.551	0.472
T/D	-0.141	-0.204	-0.218	-0.573	-0.287	0.252	0.229	0.371	-0.173	0.513		0.602
FW/D	0.485	-0.088	-0.115	0.500	-0.756	0.386	0.522	0.338	0.796	0.220	0.025	

(a) $r > \pm 0.250$ *, $r > \pm 0.325$ **, $r > \pm 0.408$ ***

(b) $r > \pm 0.045$ *, $r > \pm 0.059$ **, $r > \pm 0.075$ ***

three crosses within each of five light regimes).

Seed weight showed a negative correlation with the number of multiple seedlings and with second leaf date ($p < 0.01$); the latter indicated that seedlings from heavier seed reached second leaf maturity earlier. It was also positively correlated with early germination, second leaf breadth, number of tillers and fresh weight increment per day ($p < 0.01$).

The number of multiple seedlings and of probable aberrants were not correlated over all, but in P. pratensis 145 there was a negative correlation ($p < 0.05$). The number of aberrants did however show an overall negative correlation with both second leaf breadth and fourth leaf length ($p < 0.01$) which supported the general observation that the aberrant individuals tend to be smaller. In P. ampla there were more probable aberrants recorded from the samples with lower 1000 seed weights ($p < 0.01$).

The negative correlation ($r = -0.376^{**}$) between germination after eight days and second leaf maturity date is rather misleading due to the different methods of scoring; in fact samples with earlier germination also reached second leaf maturity earlier. In P. pratensis 190 there was a negative correlation between 1000 seed weight and both early germination and second leaf date ($p < 0.05$), indicating that in this biotype fewer of the heavier seed lots had germinated on day eight but that they subsequently germinated and reached second leaf maturity earlier.

Analyses of variance are shown in Tables 21 and 22 and biotype means for crosses within light regimes in Appendix Tables 20 to 28. All characters showed differences between the four biotypes ($p < 0.01$) and eight variates showed differences

between the light regimes (Table 23).

TABLE 21 - Trial F Analyses of variance based on sixty sample means (four biotypes, three crosses within five light regimes)

Source	df	1000 Sd Wt (mg) $MSx10^{-2}$	Mult $Log_{10}(data+1)$ MS	AB Log_{10} MS	Germ (D8) $Log_{10}(data+1)$ MS
Populations	3	2252.43***	0.75382***	0.19574**	3.28404***
Light regimes	4	102.57*	0.07437	0.13324**	0.71202***
P X L	12	114.54**	0.11556*	0.05149	0.14104**
Residual	40	31.70	0.05272	0.03379	0.05236

There was a clear distinction between seedlings from A and those from C, with the exception of second leaf length. Those from light regime A had lighter seed, more aberrants, earlier germination and second leaf dates but narrower second leaves and fewer tillers. The number of aberrants, colour, and to some extent also early germination, showed similar close relationships from A and AC and from C and CA indicating that the light regime before ear emergence influenced these characters. The intermediate position of B suggested that it was not a strictly photoperiodic response. Seed weight, second leaf breadth and number of tillers were all greater from the two light treatments with 20 hours illuminance at the higher light intensity after ear emergence. Crosses within light regimes for each biotype showed differences ($p < 0.05$) for eleven of the fourteen characters measured on all the seedlings. They were non-orthogonal so that direct comparisons would be meaningless. However these results do indicate that there were some differences

TABLE 22 - Trial F Analyses of variance from data recorded on individual seedlings

Source	df	2LD Log_{10} MS X 10^2	4LD-2LD Log_{10} MS X 10^2	2LL (mm) MS	2LB (mmX10)SQRT MS	4LL (mm) MS x 10^{-3}	4LB (mm) MS	No T(4L) SQRT MS X 10^2
Populations (P)	3	30.762***	14.155***	2198.2***	32.450***	36.360***	27.746***	664.14***
Light regimes (L)	4	4.213**	0.798	800.3**	1.447***	0.840	0.259	30.13***
Replicates (R)	3	3.134***	4.409***	2336.9***	0.532***	4.864***	0.190	14.35*
P X L	12	3.420***	0.760	1329.3***	0.659**	1.892	1.051***	37.93***
P X R	9	1.117***	0.765***	129.9	0.129	1.640***	0.139	9.08*
L X R	12	0.534**	0.495**	146.2	0.080	0.292	0.142	7.46
P X L X R	36	0.537***	0.506***	208.9	0.202***	1.038***	0.231**	5.41
Crosses(C)/L X P	40	1.790***	0.349	779.9***	0.394***	1.546*	0.511***	17.30***
R X C/L X P	120	0.644***	0.496***	327.7**	0.121**	0.900***	0.181**	7.30**
Residual	1680	0.209	0.206	179.7	0.073	0.365	0.128	4.39

continued.....

TABLE 22 - Trial F Analyses of variance from data recorded on individual seedlings (continued)

Source	df	T/D SQRT MS X 10^2	FW/D SQRT MS	Colour MS	Lig (data + 1)Log_{10} MS X 10^2	Au H MS	LA Data squared MS	TA MS
Populations	3	54.326***	31.345***	1120.314***	910.230***	745.019***	5099.9***	1602.48***
Light regimes	4	1.661	0.410	0.996**	0.131	0.643	10.2	0.79
Replicates	3	4.239***	14.477***	1.077**	0.842**	0.607	67.8***	63.72***
P X L	12	3.837***	2.433***	0.815*	0.220	1.766**	21.0***	3.26
P X R	9	1.710***	1.121***	0.309	0.297	0.945*	13.7*	5.37***
L X R	12	1.940***	0.723***	0.383	0.188	0.736*	12.7*	1.54
P X L X R	36	0.938**	0.416*	0.398*	0.209	0.598*	9.0	2.08**
Crosses/ L X P	40	1.895*	1.242**	0.349	0.240	1.016**	15.2*	2.55*
R X C/L X P	120	1.180***	0.696***	0.380**	0.208	0.527*	9.9**	1.55**
Residual	1680	0.459	0.258	0.273	0.192	0.406	6.5	1.09

in seedling characters due to the different pollen parents.

TABLE 23 - Trial F Light regime means for eight characters with significant main effect in analysis of variance

Variate (transformed)	A	B	C	AC	CA	SE
1000 Sd Wt	407.75	388.58	461.58	442.33	411.08	±16.25
Ab	0.8532	0.7079	0.6352	0.8572	0.6633	± 0.0531
Germ (8D)	1.2487	0.8765	0.5659	0.9087	0.8341	± 0.0660
2LD	1.4695	1.4967	1.4917	1.4824	1.4815	± 0.0037
2LL	45.465	41.782	44.580	42.941	44.180	± 0.6840
2LB	0.3019	0.3070	0.3166	0.3124	0.3036	± 0.0014
No T (4L)	1.8362	1.8560	1.9081	1.8657	1.8448	± 0.0107
Colour	3.1674	3.2359	3.3024	3.2129	3.2630	± 0.0267

Variates with significant P X Linteractions ($p < 0.05$) are listed for each biotype in turn. Light regime codes are shown ordered by size; the means which do not differ ($p > 0.05$) are linked.

P. ampla 42:

Mult	A	CA	B	AC	C	Least
Germ (8D)	A	CA	AC	C	B	Least
2LD	A	AC	CA	B	C	Latest
2LB	A	CA	C	AC	B	Broadest
No T (4L)	A	CA	C	AC	B	Most
T/D	A	AC	CA	B	C	Most

A differed from B and C in early germination and in second leaf breadth which suggested that these two characters were influenced by length of photoperiod. Results from A and CA were similar for all six characters, and from B and C for all characters except tiller number. The latter results also indicated that some response to photoperiodic treatments occurred particularly in the post ear-emergence period.

P. pratensis 145:

1000 Sd Wt	A	AC	C	B	CA	Least
Mult	A	AC	CA	B	C	Most
Germ (8D)	A	AC	B	C	CA	Least early
2LD	A	CA	AC	C	B	Latest
2LL	A	C	CA	AC	B	Shortest
2LB	A	AC	C	CA	B	Narrowest
4LB	A	CA	AC	C	B	Narrowest
No T (4L)	CA	A	AC	C	B	Least
T/D	AC	CA	A	C	B	Least
FW/D	CA	A	AC	C	B	Least
LA	CA	B	A	C	AC	Greatest

Mean values from A and B differed for all characters except the number of multiple seedlings and leaf angle. B and C gave similar results except for second leaf length, tiller number and number of tillers per day. Seedlings from A and AC were similar except for second leaf length and fourth leaf breadth, but there was no clear relationship between CA and either A or C. These results suggest that most of the characters were influenced by differences in photoperiod rather than by differences in total irradiance, and that maternal plants were more receptive in the pre-ear emergence period.

P. pratensis 159:

1000 Sd Wt	A	B	AC	C	CA	Greatest
Mult	A	C	B	AC	CA	Least
Germ (8D)	A	B	CA	AC	C	Least early
2LD	A	CA	C	AC	B	Latest
2LL	B	A	AC	C	CA	Longest
2LB	A	CA	B	AC	C	Broadest
4LB	AC	B	A	C	CA	Broadest
Colour	B	A	CA	AC	C	Most purple
Au H	B	AC	A	C	CA	Most hairy

Results were similar from light regimes A and B except for 1000 seed weight and second leaf maturity date; this suggests that there was a similar overall response to ten hours illuminance at the higher light intensity. None of the other results were consistent with light treatments and there was no evidence of a response to the different photoperiodic treatments.

P. pratensis 190:

1000 Sd Wt	B CA AC A C	Greatest
Mult	A AC CA B C	Most
Germ (8D)	A B AC CA C	Least early
2LD	CA A AC B C	Earliest
2LL	A C B AC CA	Shortest
2LB	A CA B AC C	Broadest
4LB	CA B AC A C	Broadest
No T	A CA AC B C	Most
T/D	A CA AC B C	Most
FW/D	A CA AC B C	Most
Colour	A AC B CA C	Most purple

Au H	AC CA A B C	Most hairy
LA	A AC B CA C	Greatest

The effect of light regimes on seedling characters was not consistent in P. pratensis 190, although A and AC gave similar results for all characters except second leaf length, indicating that the short day treatment had a relatively greater influence before than after ear emergence on the characters measured. Differences in total irradiance on the maternal plants led to differences in early germination, second leaf breadth and leaf angle. There was no obvious pattern of response in 1000 seed weight, second leaf length, fourth leaf breadth or auricle hairs. The remaining six characters all appeared to be influenced predominantly by the length of photoperiod.

Seven quantitative characters (2LD, 2LL, 2LB, 4LL, 4LB, No T (4L) and FW/D) were included in a canonical analysis on twenty groups derived from the four biotypes X five light regimes. The first three canonical variates accounted for 56.90 per cent, 29.40 per cent and 6.96 per cent of the variability respectively. The twenty group means were plotted on first and second (fig.26) and first and third (fig.27) canonical variates. Ninety-five per cent confidence limits (Seal, 1964) are shown.

In P. ampla 42, the positions of group means B, C and AC were all similar but A and CA both differed fromthis main grouping and from each other. The largest distance was 285, between A and B.

A and CA were similar in P. pratensis 145 but AC differed

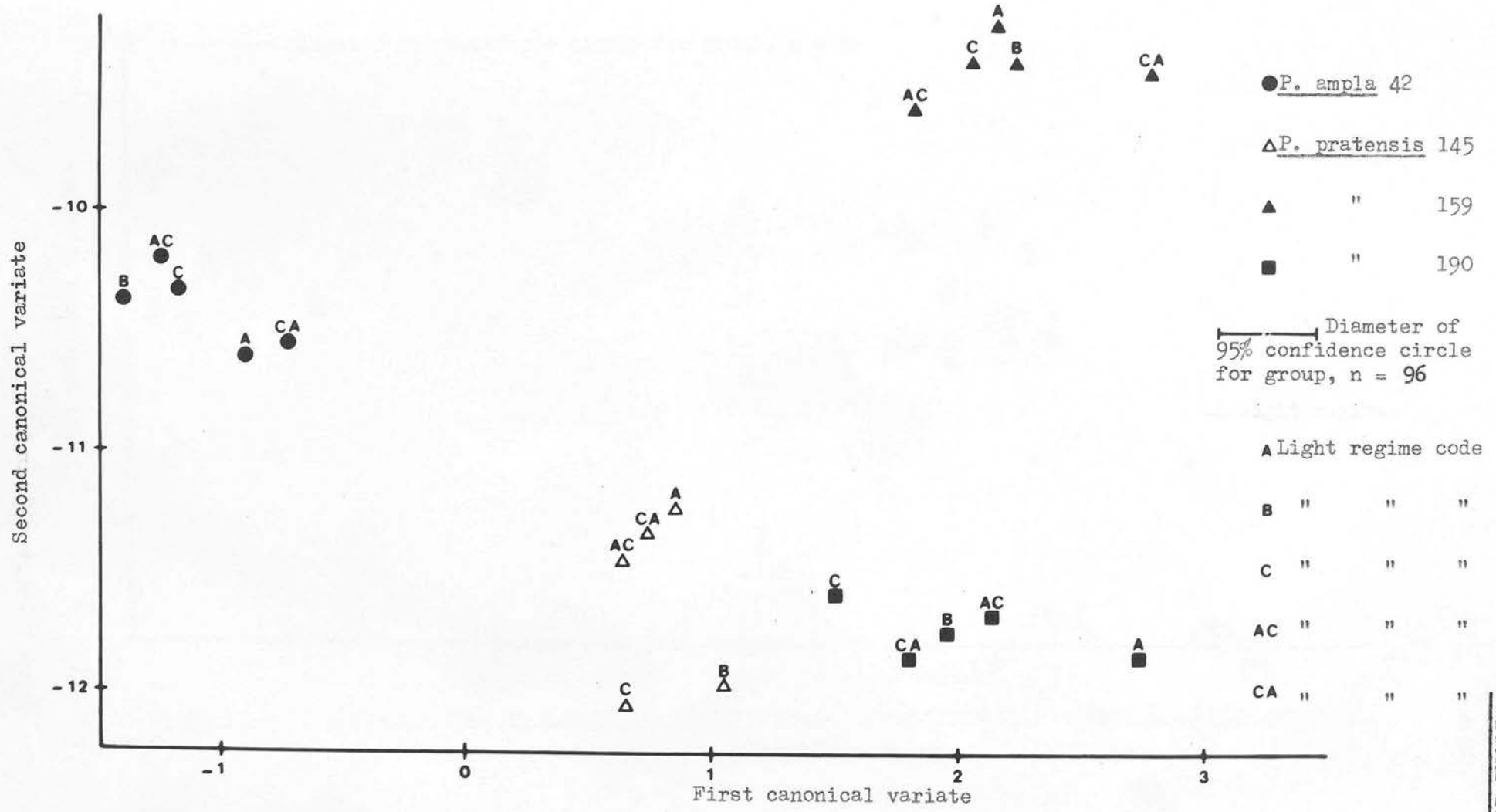
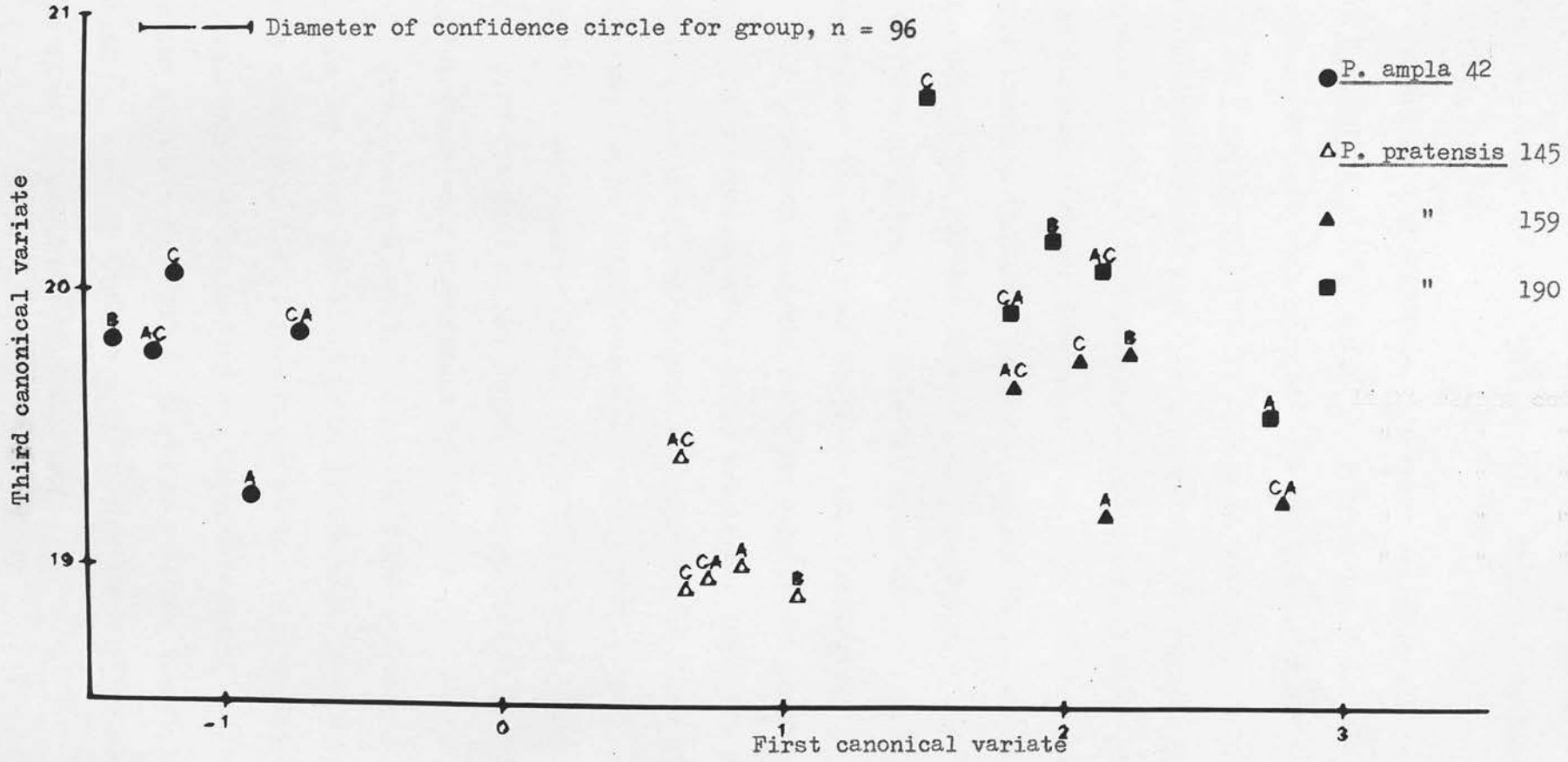


FIGURE 26

Trial F Canonical analysis on 7 seedling characters, 20 group means (4 biotypes X 5 light regimes) plotted on first and second canonical variates



Trial F Canonical analysis on 7 seedling characters, 20 group means (4 biotypes X 5 light regimes) plotted on first and third canonical variates

FIGURE 27

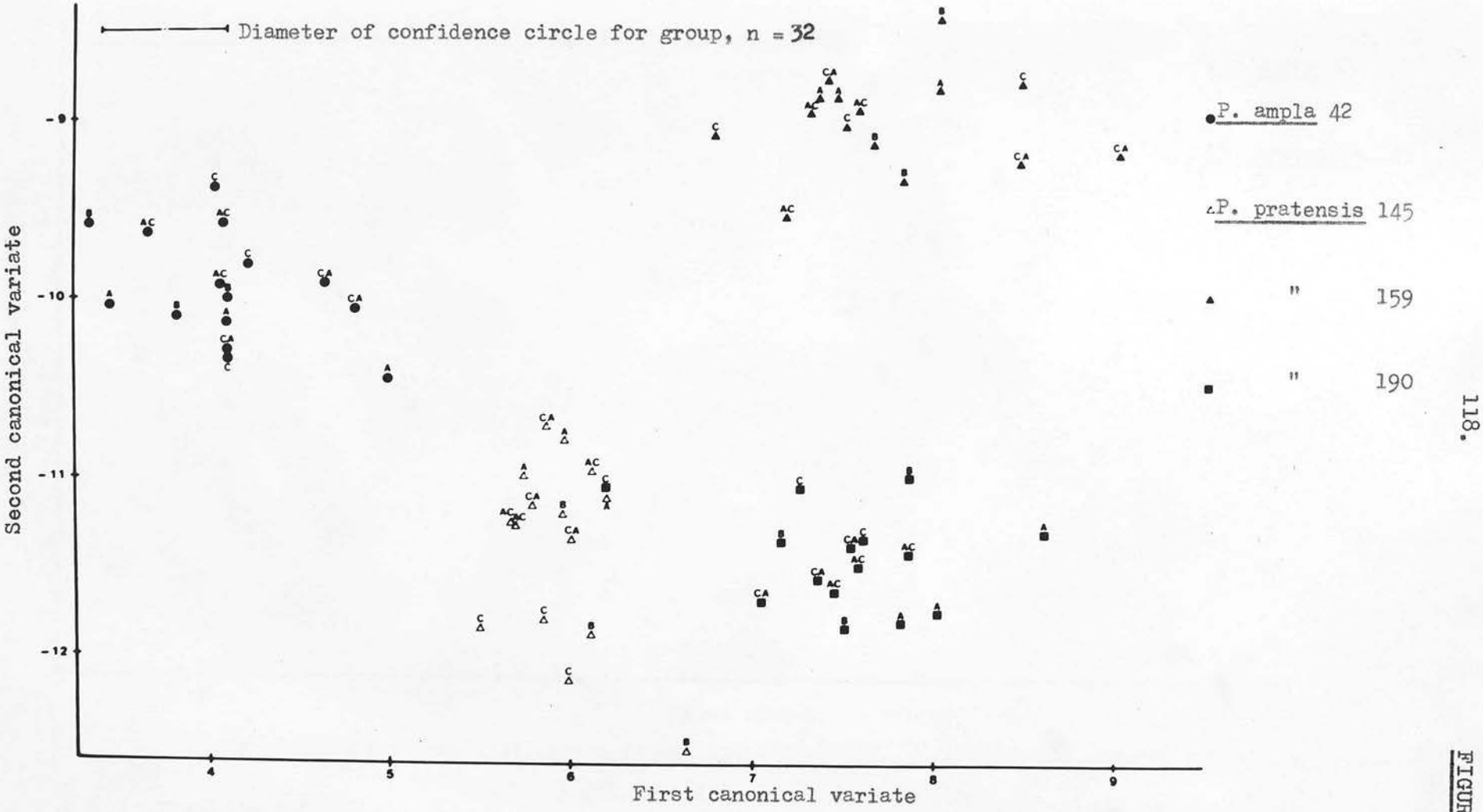
from all other group means. B and C were adjacent but separated from all other groups; the greatest distance was between AC and C (296).

A and CA differed from each other and from all other groups in P. pratensis 159; B and AC differed but C overlapped them both. The greatest distance was 272 between A and CA.

In P. pratensis 190, B, AC and CA were all similar and lay in an intermediate position between the two extreme groups A and C which differed from each other and from all other groups with the largest distance between them of 396. This grouping suggests that the main influence of light regimes on these seven characters was due to the difference in total irradiance. The relative positions of group means from the other three biotypes appeared to reflect the different photoperiodic treatments.

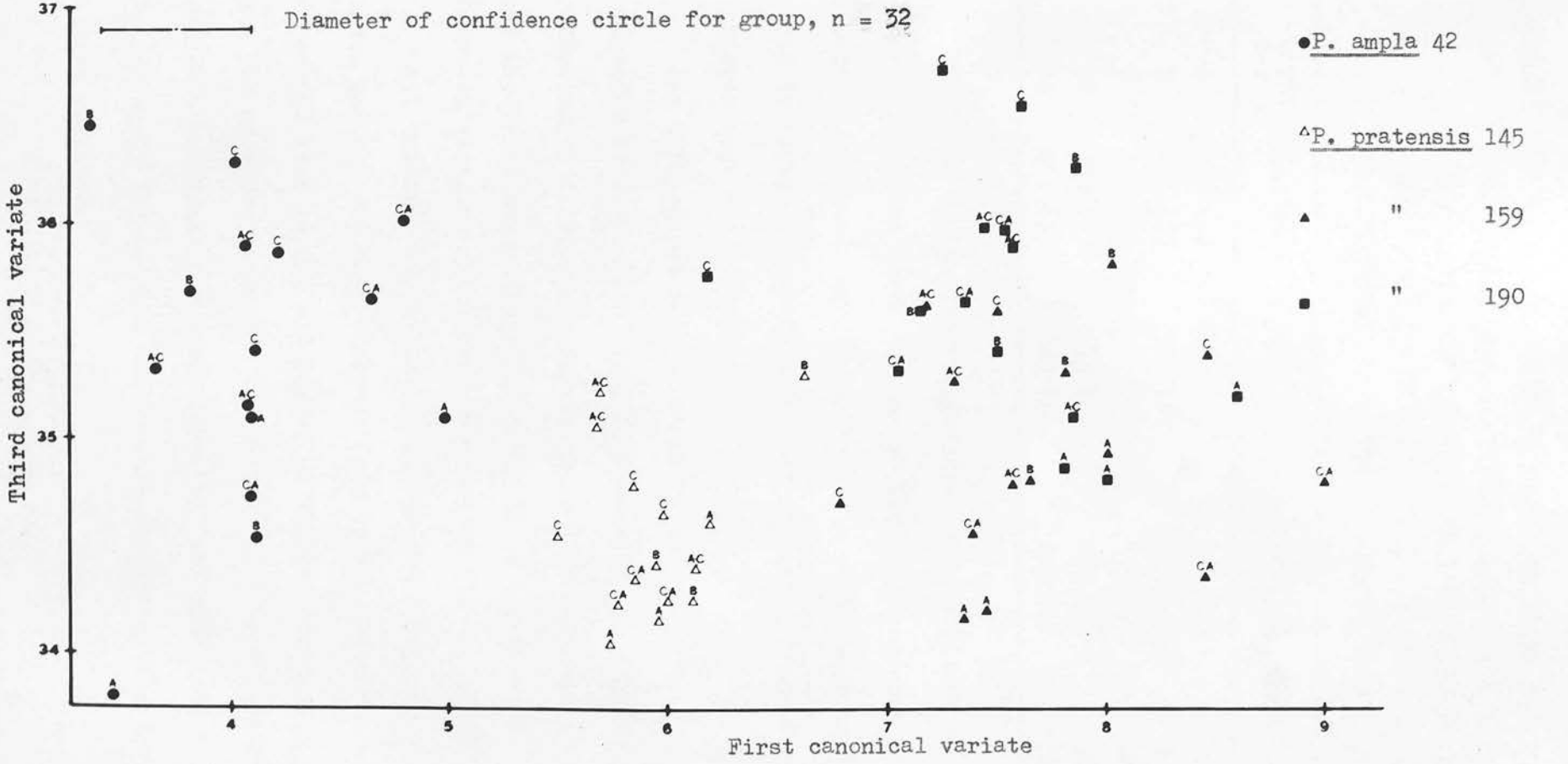
A canonical analysis was also carried out on the sixty group means from crosses within light treatments using the same seven variates as above, to examine any differences which might be attributable to pollen parents. The first, second and third canonical variates accounted for 50.39 per cent, 25.04 per cent and 10.20 per cent of the total variation respectively; all the latent roots were significant ($p < 0.001$).

Group means were plotted on the first and second (fig.28), and on the first and third (fig.29) canonical variates; 95 per cent confidence limits were calculated. The greatest distance between pairs of means from the three crosses within each light regime is shown in Table 24 together with the number of pairs of means (0, 1, 2 or 3) which were outside the confidence limits on the first three canonical variates.



Trial F Canonical analysis on 7 seedling characters, 60 group means plotted on first and second canonical variates

FIGURE 28



Trial F Canonical analysis on 7 seedling characters, 60 group means plotted on first and third canonical variates

FIGURE 29

TABLE 24 - Trial F Canonical analysis on sixty groups; maximum distance between means from three crosses within each light regime for each biotype, and (in parentheses) number of pairs of means which differ in position on 1st, 2nd and 3rd canonical variates

Light regime	P. ampla 42	P. pratensis 145	P. pratensis 159	P. pratensis 190
A	386 (3)	199 (1)	234 (2)	250 (2)
B	431 (3)	579 (2)	226 (2)	209 (2)
C	273 (1)	272 (0)	373 (3)	295 (2)
AC	270 (2)	173 (2)	212 (1)	301 (2)
CA	297 (2)	149 (0)	429 (3)	198 (1)

Only two of the twenty groups of means did not show differences between the three crosses on the first, second or third canonical variates, and it was possible that these would also be found to differ if positions on the remaining four canonical variates were also examined.

Any differences in proportion of aberrant seedlings in progenies from the five light regimes might be expected to be reflected by differences in variance. An analysis of variance was therefore carried out on within block variances from the fourteen variates which were recorded on all seedlings (Table 25).

All variates except sheath colour and ligule showed differences between the four biotypes ($p < 0.01$). With the exception of second leaf length and number of tillers per day, P. ampla 42 had the greatest variances for quantitative variates, but the smallest variances for auricle hairs, leaf angle and tiller angle. P. pratensis 159 had the smallest variances for all nine quantitative variates.

TABLE 25 - Trial F Analyses of variance on within-block variances

Source	df	2LD MS X 10^8	4LD-2LD MS X 10^8	2LL MS X 10^{-2}	2LB MS X 10^4	4LL MS X 10^{-3}	4LB MS X 10^4	No T MS X 10^5
Populations	3	10081.3***	2049.82***	1201.92***	373.216***	1566.80***	489.481**	3387.87***
Light regimes	4	597.4	296.96	384.91**	6.681	233.36	123.844	75.21
Replicates	3	541.9	107.40	126.51	41.471	86.99	281.099*	199.43
P X L	12	682.6**	525.90	321.60***	26.168	137.36**	251.481**	261.78*
P X R	9	387.1	342.85	175.37*	15.396	97.50	68.434	164.45
L X R	12	441.9	331.28	133.97	22.254	102.40*	72.498	100.36
P X L X R	36	372.7	296.98	91.24	23.216	79.55	88.248	101.61
Crosses/L X P	40	409.7*	259.15	121.98	24.375	57.92	61.419	161.75
R X C/L X P	120	265.3	344.72	87.02	21.105	54.71	87.332	120.64

continued.....

TABLE 25 - Trial F Analyses of variance on within-block variances (continued)

Source	df	T/D MS X 10^6	FW/D MS X 10^4	Colour MS X 10^8	Ligule MS X 10^8	Au H MS X 10^8	LA MS	TA MS
Populations	3	250.032***	2916.18***	3354.17	3286.89	69402.0***	1139.80***	20.1799***
Light regimes	4	34.042*	195.70	6322.69**	3597.73	1348.0	56.51	0.4684
Replicates	3	29.996	354.37	1864.03	4245.69	2028.4	201.90***	0.9665*
P X L	12	29.065**	491.57	3172.25	2636.81	2854.8	56.34*	0.3904
P X R	9	20.115	269.91	2140.88	2478.10	2722.0	46.17	0.3451
L X R	12	13.146	352.93	1250.98	1984.04	1733.6	39.62	0.1080
P X L X R	36	10.032	220.60	1688.66	1909.66	1506.9	29.90	0.3713
Crosses/L X P	40	12.467	241.66	1212.88	2171.59	2060.7	20.98	0.3652
R X C/L X P	120	11.776	290.23	1762.22	2353.65	2391.7	29.00	0.3402

Second leaf length, tillers per day and colour all showed a significant main effect for light regimes ($p < 0.05$). Variances were similar from B and C, and from B and CA for all three variates. AC and A had the greatest variances except for tiller production where A was smaller than AC.

Significant biotype X light regime interactions ($p < 0.05$) are presented for each biotype in turn with the light regime codes ordered by mean size of the variance. Those not differing in size ($p > 0.05$) are marked.

P. ampla 42:

2LD	C	AC	B	A	CA	Greatest
2LL	C	B	AC	CA	A	Greatest
4LL	C	B	AC	CA	A	Greatest
No T (4L)	AC	B	A	C	CA	Greatest
T/D	B	CA	AC	A	C	Greatest

Variances from B, C and AC were all similar, except for tiller production per day, indicating that the 20 hour photoperiodic treatment resulted in smaller variances and that this response to photoperiod occurred after ear emergence.

P. pratensis 145:

2LD	A	B	CA	AC	C	Greatest
2LL	B	CA	A	C	AC	Greatest

4LL	CA B C A AC	Greatest
4LB	A CA B C AC	Greatest
No T (4L)	CA A C B AC	Greatest
T/D	CA A C B AC	Greatest
LA	B C AC CA A	Greatest

Variances from light regime B were similar to those from A for all variates except leaf angle, and similar to those from C for all variates except second leaf date and second leaf length. The treatments A and CA resulted in small variances for the six quantitative variates although for leaf angle they gave the greatest variances. AC either had the greatest variance, or did not differ from the greatest variance, in all characters. There did not appear to be any overall effect on variances due to the different light regimes although treatments with only 10 hours of higher intensity illuminance throughout, or after ear emergence generally gave smaller variances. The change at ear emergence from light regime A to C and vice versa did seem to accentuate differences in variance for most characters.

P. pratensis 159:

LA	AC A CA C B	Greatest

Variances for leaf angle were smaller following the shorter photoperiod treatment, especially when this occurred prior to ear emergence. There were no differences for any other characters.

P. pratensis 190:

2LD	B	A	AC	C	CA	Greatest
2LL	C	B	A	AC	CA	Greatest
4LL	C	B	CA	AC	A	Greatest
4LB	B	C	CA	AC	A	Greatest
LA	B	C	AC	CA	A	Greatest

Light regimes B and C resulted in the smallest variances and were similar except for second leaf length. Variances from A and AC were similar, except for leaf angle; the short photoperiod tended to give greater variances for these characters than the long photoperiod, even when it occurred only before or after ear emergence.

Determinants for each biotype - light regime combination were calculated for variance-co-variance matrices using the seven characters used in canonical analyses. Heterogeneity of dispersions was tested for light regimes within each biotype (Table 26).

TABLE 26 - Trial F Determinants ($x10^{-17}$) of variance-co-variance matrices for progenies of four biotypes from five light regimes (calculated from seven quantitative characters)

Biotype	A	B	C	AC	CA	df	χ^2
P. ampla 42	981.15	454.42	114.56	459.43	1070.10	112	202.70***
P. pratensis 145	8.60	58.52	40.02	64.77	2.49	112	283.60***
" 159	1.57	13.30	6.92	8.31	35.15	112	244.43***
" 190	217.81	14.63	24.30	133.45	57.77	112	328.62***

Determinants were also calculated for crosses within light regimes and the heterogeneity of each set of three dispersions was tested (Table 27) to examine differences due to pollen parents.

Principal component analyses were carried out for each biotype on the fourteen seedling characters from the light regimes with the greatest and smallest determinants (Table 26). The frequency of occurrence of individuals in the extreme ten positions on each component was calculated. Individuals which occurred three or more times at the extremes were counted as possible aberrants; these figures expressed as percentages are shown in Table 28 in comparison with the group determinants and the eye estimates of probably aberrant seedlings scored immediately before plants were cut.

TABLE 27 - Trial F Determinants ($x10^{-17}$) of dispersion matrices from seven quantitative characters, and tests for homogeneity of dispersions for crosses within light regimes

Biotype	Light regime	Cross 1	Cross 2	Cross 3	df	Chi-square	
P. ampla 42	A	302.290	664.400	170.740	56	68.87	ns
	B	39.567	254.650	52.546	56	105.17	***
	C	29.642	10.093	107.580	56	95.71	***
	AC	462.960	431.960	19.665	56	72.30	ns
	CA	1221.900	321.790	162.260	56	72.38	ns
P.pratensis 145	A	12.991	0.924	0.523	56	108.33	***
	B	2.937	2.687	26.053	56	114.62	***
	C	35.118	28.439	3.499	56	62.66	ns
	AC	38.565	15.108	19.283	56	73.13	ns
	CA	0.122	2.851	0.294	56	125.45	***
P.pratensis 159	A	1.089	0.124	0.105	56	122.55	***
	B	4.359	3.552	5.824	56	73.86	ns
	C	0.900	1.352	2.186	56	83.97	**
	AC	1.287	12.452	0.440	56	108.71	***
	CA	11.149	4.948	4.203	56	85.24	**
P.pratensis 190	A	25.404	49.969	341.480	56	76.67	*
	B	1.402	16.020	1.602	56	105.27	***
	C	1.222	11.666	6.962	56	105.90	***
	AC	9.762	65.486	50.429	56	89.90	**
	CA	23.808	47.033	17.987	56	50.13	ns

TABLE 28 - Trial F Percentages of possibly aberrant seedlings estimated by eye and from extremities of principal component analyses, and determinants from dispersion matrices

Biotype	Light regime	Eye estimates of possible aberrants (per cent)	Frequency counts from principal components (per cent)	Determinant of dispersion matrix ($x10^{-17}$)
42	C	14.1	14.1	114.6
	CA	14.6	25.0	1070.1
145	AC	20.9	29.7	64.8
	CA	15.6	17.7	2.5
159	A	21.9	8.3	1.6
	CA	17.7	32.3	35.2
190	A	42.7	33.3	217.8
	B	47.9	8.3	14.6

DISCUSSION

Analyses of variance and canonic analyses both showed that the characters measured on seedling progenies in Trial F clearly distinguished all four biotypes from each other. Variances, and determinants of dispersion matrices derived from seven quantitative characters, were also distinctive and were greatest for P. ampla 42 and smallest from P. pratensis 159. The smallest determinant (Table 26) for P. ampla from light regime C (114.5) was larger than the greatest for either P. pratensis 145 (58.5 for light regime B) or P. pratensis 159 (35.2 for light regime CA).

Each biotype showed a different response to the five light regimes for most of the seedling characters and it is clear that, by definition, biotype-environment interactions occurred. The canonic analysis on seven variates for twenty groups gave further evidence of interaction. The rather consistent differences in size of variances and determinants characteristic of each biotype indicated that there may be differences in stability (Mather, 1953; and Freeman, 1973).

P. ampla 42: analyses of variance and the canonical analysis showed similar results on progenies from the three light regimes B, C and AC. Variances were also generally similar and smaller than those from either A or CA. The two latter groups had greater determinants although in general seedlings from these treatments were less vigorous. It appeared that the exposure of this biotype to a 10 hour photoperiod either throughout the period of treatment, or after ear emergence, influenced ovule and seed development with the result that seedling progenies were less vigorous and more variable with more multiple seedlings than

those from a 20 hour photoperiod.

P. pratensis 145: progenies from A and CA were similar in seedling characters and more vigorous than those from other light regimes. They also had smaller variances and determinants. Progenies from B and C were similar, but those from AC had the greatest variance for most characters. In the canonic analysis it differed from all other groups. The differences appeared to be due to the photoperiod treatments with the 10 hour photoperiod resulting in more vigorous and less variable seedlings than 20 hours. The two light regimes AC and CA gave the most and least extreme variability respectively, indicating that a different response to daylength occurred before and after ear emergence. However, there was no difference in the number of multiple seedlings from these two treatments, most occurred in C.

P. pratensis 159: there was no evidence that any differences in seedling characters occurred as a result of photoperiod treatments during ovule and seed development. The smallest determinants were from light regimes A and AC; the change from C to A at ear emergence caused the greatest variability, a result reflected in the canonic analysis where A and CA were more distant from each other than from any other progeny of this biotype.

P. pratensis 190: the results from this biotype suggest that there was an overall effect of short (10 hour) photoperiods tending to lead to less vigorous seedling progenies showing greater variability than those from long (20 hour) photoperiods. Several of the seedling characters (e.g. early germination, second leaf breadth and leaf angle) also appeared to be

influencedby the total irradiance. Progenies from AC were generally similar to those from A so probably the light regime in the period before ear emergence was more critical than after ear emergence. It seemed likely from these results and from the canonical analysis that the response to long or short photoperiod was modified by the total irradiance received in any 24 hour period.

The differences which occurred between families from each biotype after the different light treatments could not be identified clearly as being due to differences in the proportion of aposporous and sexual embryo sacs which matured. This emphasised the need for a satisfactory method of assessing the relative proportions of the two types of seed produced by a facultative apomict. The difficulty of scoring aberrant seedlings by eye was exemplified by the comparison with frequency counts from a principal components analysis of seedlings occurring at the extremities of the components, (Table 28). Quantitative characters scored on individual seedlings gave a clearer indication of development relative to the rest of that family than a single score of phenotypic uniformity among plots of eight full-sib seedlings.

Evidently the choice of characters is important and information on the heritability of seedling characters would assist in the selection of those most suitable. Differences in time of germination and the occurrence of multiple seedlings in Trial F both decreased the uniformity at the start of the experiment and necessitated a compromise from the ideal situation in which no selection of young seedlings would be practised.

The establishment of a time scale for the different stages

in reproductive development of the biotypes examined is clearly a prerequisite to any attempt to relate the biotype-environment interaction reported here to its physiological basis. P. pratensis has a requirement for both a short photoperiod and low temperatures for floral induction (Peterson and Loomis, 1949). Lindsey and Peterson (1962) showed that maximum induction of the cultivar Merion did not occur until late February at Davis, California; and that there is a long day requirement for floral development. They showed an increasing inhibition of floral development on fully induced plants with an increase in duration of the dark period from eight to sixteen hours.

All the clones used in Trials E and F should have been fully induced since they were kept in natural conditions outside until 20th March and 1st April respectively. The 10 hour photoperiod in Trial F delayed the median ear emergence date in all P. pratensis biotypes although not in P. ampla. Since it appeared that some of the biotypes tested showed a different response to daylength before and after ear emergence, it would be helpful to be able to relate the stages in ovule and embryo sac development to the period of treatment.

Tinney (1940) observed that at the time of ear emergence, the ovule in the basal florets of the spikelets consisted of outer and inner integuments and a nucellus with a well differentiated macrospore mother cell. The aposporous initial was occasionally visible in the nucellus at this early stage, but generally was not apparent until meiosis was complete. Grazi et al. (1961) made a similar observation that there was considerable variation in the time of appearance of aposporous initials

from prophase until tetrad stage in the pollen, or even later; they also noted that the duration of the period when developing legitimate and aposporous embryo sacs occurred in the same ovule varied between clones. On the basis of these observations it would appear that the light regime would be more likely to influence development of sexual embryo sacs before ear emergence, and of aposporous embryo sacs after ear emergence.

Both Nygren (1951) and Grazi et al.(1961) suggested that competition occurs between developing embryo sacs within an ovule; Nygren stated that the sac in which the nuclei are the first to divide is usually successful but that aposporous sacs located close to vessels carrying nutrients may develop in preference to more advanced sexual sacs. Supply of photosynthate would be influenced by the light regime and, if competition for nutrients does occur, could affect the developing embryo sacs. However, since no details are known of the physiological mechanisms by which the light regime influences these stages of reproductive development, any attempt to explain the results must be purely speculative.

In Trial E the percentage seed set was severely reduced in all four biotypes after an eight hour daylength compared to a sixteen hour daylength. A ten hour photoperiod (Trial F) reduced seed set in P. pratensis 172 and 189 compared to a 20 hour photoperiod. Vegetative proliferations were produced on inflorescences of the latter biotype following exposure to the ten hour photoperiod before ear emergence; a response similar to that reported for S 48 timothy plants by Langer and Ryle (1958).

Differences occurred between half-sib families from all four

biotypes where different pollen parents had been used indicating that some seed developed from sexual reproduction, and that the variation was probably due to differences in phenotype of hybrids from the different pollen parents.

It appeared from the results of Trial F that daylength did not have the relatively straightforward effect on apomictic seed production in the biotypes tested which has been reported for other grasses (Knox, 1967; Knox and Heslop-Harrison, 1963). The genotype-environment interactions which occurred between the P. pratensis biotypes indicated a complex relationship which might be clarified by a detailed study of the reproductive development and with the use of regression techniques (Breese, 1969; and Hill, 1976). The increased variability in P. ampla progenies from maternal plants which had been exposed to short daylength treatments was in agreement with earlier results reported (Chapter 3) where more hybrid seedlings were identified following short day treatment.

CHAPTER 6

THE INFLUENCE OF LIGHT REGIMES ON SEED PRODUCTION AND RESULTING SEEDLING PROGENIES FROM INTERSPECIFIC HYBRIDS OF POA. (TRIAL G)

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INTRODUCTION

The P. pratensis cultivars currently available in the U.K. are reported to be slow to establish on reseeded marginal and hill land (Newbould, 1973). Results from a trial using nine wild P. pratensis biotypes each sown with wild white clover at Pentlandfield supported this observation. The sown grass made a negligible contribution to the sward in the first two seasons although by the third season ground cover varied from 47 per cent up to 83 per cent (Ann. Rep. Scott. Soc. Res. Plant Breed., 1974). It therefore appeared that some assessment of seedling vigour in the hybrid progenies would be desirable in the breeding programme.

As already reported, differences were observed between and within seedling progenies from the 1969 P. ampla X P. pratensis hybrids. An examination of the nature of this variability in seedling families is clearly required before a screening technique can be developed. There are advantages both in time and space involved in assessment of seedling progenies rather than mature plants in the field. The possibility of extending to seedlings the techniques already used on data from mature plants to distinguish highly apomictic from segregating families seemed worth investigation.

The ability to predict the proportion of apomictic seed produced by interspecific hybrids under different environmental conditions would be useful. Seed production from any possible new cultivar could be affected when grown in regions with an

environment different from that in which initial selection and assessment was made.

The following trial is an exploratory investigation into the effect of different light regimes on seed production from eleven hybrid progenies, and into possible methods of assessment of the variability in seedling families.

MATERIALS AND METHODS

One plant was taken from each of eleven families which showed high mean fresh weight yields and high spring growth scores in the 1968 hybrid progenies field trial. The source of each family is shown in Table 29.

TABLE 29 - Origin of eleven vegetative clones used in Trial G

Clone Code number	Maternal parent (Index number)	Cross (Index number of pollen parent)
1	200-2	X 197-2 (half sib)
2	213	open pollinated
3	214-2	X 155 (*P. pratensis*)
4	227-2	X 238 (1968 hybrid)
5	231-2	X 240 (" ")
6	233	self pollinated
7	234	X 190 (*P. pratensis*)
8	235	X 107-2 (*P. longifolia*)
9	238	X 227-2 (1968 hybrid)
10	241	X 107-2 (*P. longifolia*)
11	240	X 231-2 (1968 hybrid)

Plants with ear emergence dates in the same seven day period were chosen and lifted from the field in October 1973. Each was cloned into forty groups of tillers of equal size which were over-wintered in pots outside. All 11 X 40 ramets were brought into an unheated greenhouse at the end of March 1974. On 2nd April

ten ramets of each clone were completely randomised in two blocks within each of the following four light regimes:-

A - as for Trial F

B - " " " F

C - " " " F

D - control, normal greenhouse conditions, no supplementary lighting.

All ramets were allowed to open pollinate and were moved onto a bench without supplementary lighting when they had finished flowering. Florets were sampled for pollen stainability. Seed was harvested from each ramet separately and fifty good (filled and hard) seeds were counted out. The five lots of fifty seeds from each clone within a block (hereafter called samples) were bulked, giving a total of eighty-eight seed lots, two samples from each of eleven clones for four light regimes.

The following records were taken on all ramets:-

EE - ear emergence (half-weeks from 16th April); at least three panicles showing above ligule of flag leaves.

Anth - anthesis (half-weeks from 16th April); first florets observed to be at anthesis.

No Pan - number of panicles

PH - plant height (mm); measured from base to top of second longest flowering tiller

FLL - flag leaf length (mm); length of lamina on above tiller

FLB - flag leaf breadth (mm); breadth of above leaf measured half way along lamina

Pan L - panicle length (mm); length of main axis from lowest branch to top, on representative flowering tiller

Pan B - panicle breadth (mm); length of longest branch in bottom whorl of above tiller

FL/Sp - number of florets per spikelet; mean of three spikelets from centre of above panicle

Sd/R - seed per ramet (mg); total weight of unsorted seed

The following records were obtained from the eighty-eight samples:-

1000 Sd Wt - 1000 seed weight (mg); assessed from bulked 250 seed lots

% Germ - percentage of seeds germinated from samples of 40 seeds

% Mult - percentage of multiple seedlings from samples of 20 seeds

Assessment of seedling progenies

Twenty seeds from each of the eighty-eight bulked seed lots were sown individually in John Innes 2 potting compost in 7.5 cm pots to avoid selection during pricking out. A further twenty seeds from each seed lot were germinated on filter paper in Petri dishes; the seedlings were used to fill gaps where seed did not germinate in the pots. Multiple seedlings were separated and recorded individually where possible. Five seedlings from each of the eighty-eight seed lots were completely randomised in four blocks. Supplementary lighting gave a minimum illuminance of 8000 lux for 16 hours daily.

The following records were taken on all seedlings:-

GD - germination date; as for Trial F

2LD - second leaf maturity date; as for Trial F

4LD - fourth " " " " " " "

2LL - length of second leaf lamina (mm)

2LB - breadth " " " " (mm)

4LL - length of fourth " " (mm)

No T - number of tillers more than 20 mm long 12 weeks after sowing

Size - seedling size 12 weeks after sowing; 9 categories, 1 very dwarf to 9 very vigorous

Colour - colour of leaf sheath, as for Trial F

Lig - ligule length, as for Trial F

Au H - auricle hairs, " " " "

Sh H - hairs on leaf sheath; 5 categories as for Au H

LA - leaf angle, as for Trial F

TA - tiller angle, as for Trial F

Final records were taken twelve weeks after sowing. All seedlings in one replication were kept so that the mature plants could be scored (in 1976) and the relative discriminatory power of data recorded on seedlings and mature plants could be compared. Seedlings in the other three blocks were cut 5 mm above soil level and total aerial shoot fresh weights were recorded individually. The date of appearance and initial orientation of first and second tillers were also scored but these tillers did not develop in all seedlings. In order to avoid incomplete data sets records on tillers and seedling fresh weights were omitted from all analyses.

RESULTS

The first ear emergence was recorded on 16th April (category 1) on ramets in B and C, and the first ramets reached anthesis on 10th May (category 8) in light regime C. The dates were fourteen and thirty-eight days respectively after commencing the treatments. The four light regimes evidently influenced the rate of elongation of flowering stems (figs.30 and 31).

Pollen stainabilities are shown in Table 30.

TABLE 30 - Trial G Percentage pollen stainabilities for eleven clones in four light regimes (mean of two samples)

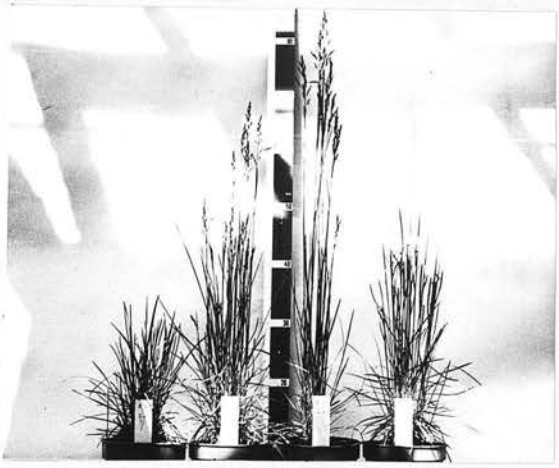
Clone	Light regime			
	A	B	C	D
1	45.8	63.0	15.7	38.0
2	78.0	63.0	52.0	45.5
3	67.0	68.5	33.3	88.5
4	75.0	79.3	85.3	76.5
5	73.5	74.0	65.0	75.5
6	59.8	78.5	66.0	63.8
7	75.5	87.3	89.0	88.5
8	78.0	78.3	86.0	83.0
9	88.0	89.3	83.0	82.0
10	71.5	86.0	94.8	87.5
11	85.0	87.0	81.8	78.0

The light regimes did not appear to affect pollen viability as measured by this method, except possibly treatment C where clones 1 and 3 had fewer regular and darkly stained pollen grains than samples from the other treatments.

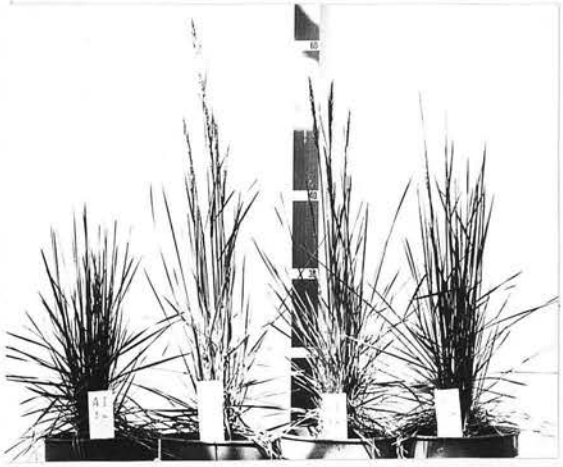
In seedling progenies from clones 4 and 8 the minimum number of five seedlings per plot was not recorded due to insufficient

FIGURE 30

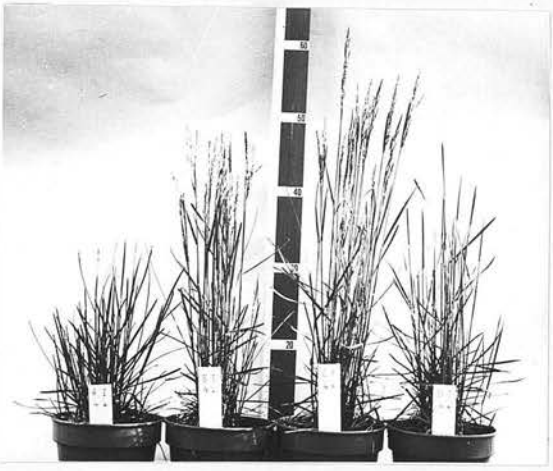
Clone 1



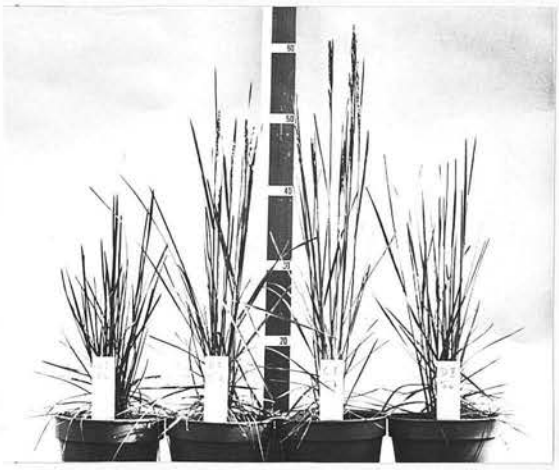
Clone 2



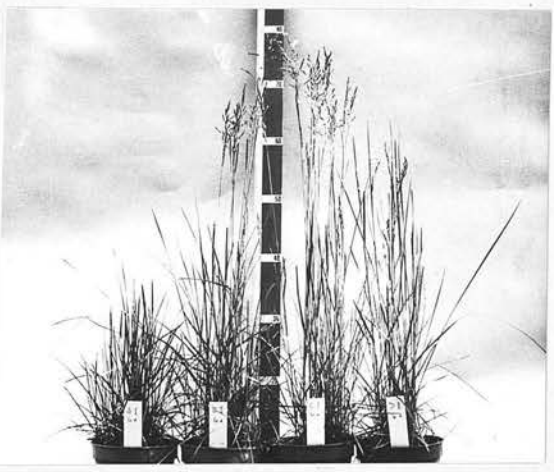
Clone 3



Clone 4



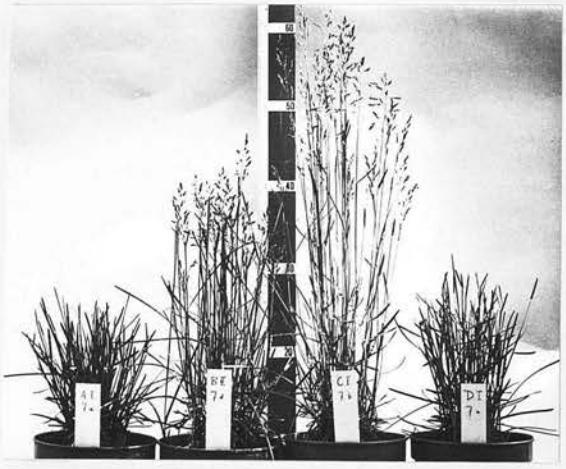
Clone 5



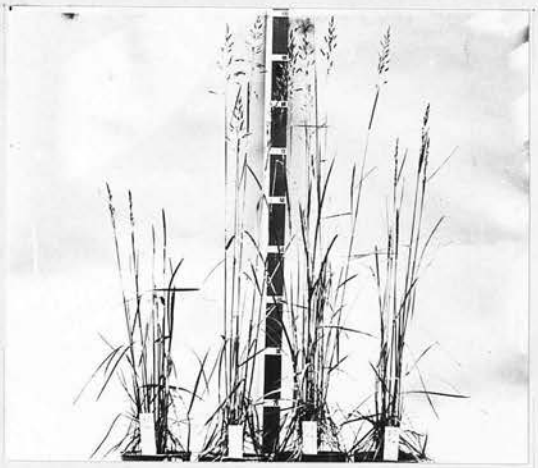
Clone 6

Trial G. Clones 1 to 6 showing different stages of development in the four light regimes (from left: A, B, C and D) twenty-seven days after start of treatment.

FIGURE 31

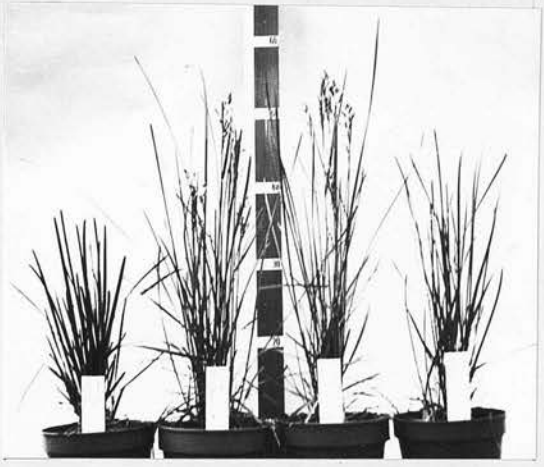


Clone 7



Clone 8

Clone 9



Clone 10

Clone 11

Trial G. Clones 7 to 11 showing different stages of development in the four light regimes (from left: A, B, C and D) twenty-seven days after start of treatment.

viable seed being produced. Germination was very slow and rather spasmodic in families 2 and 6, resulting in low numbers of seedlings; the most marked effect was in family 2 from light regime C where only six out of a total of eighty seeds germinated. The required number of seeds germinated in the other seven families although some died or did not develop to the necessary stage for recording and were omitted from analyses. A total of 1522 seedlings were recorded, and when summed over samples within light regimes, 168 from the expected total of 176 plots (4 replications X 4 light regimes X 11 familes) were represented. The eight plots where no seedlings were recorded were from families 2, 4 and 6.

After testing variates for normality and performing the necessary transformations, correlation coefficients were calculated from forty-four means between all twenty-seven variates from maternal clones and seedling progenies (Appendix Tables 29 and 30). Correlation coefficients between fifteen of the variates calculated from forty-four means are also shown in Table 31, together with correlation coefficients from seedling data calculated from all 1522 seedlings. The high positive correlation between germination date, second leaf date and fourth leaf date ($r \geqslant 0.840^{***}$) indicated that the omission of germination date and fourth leaf date from analyses would not lead to any serious loss of information.

Preliminary analyses on data from families 1 and 3 were carried out with three factors: replications, light regimes and samples within light regimes. The only variate which showed significant differences between samples within light regimes was

TABLE 31 - Trial G Correlation coefficients calculated from (a) 44 group means, data from maternal clones and seedlings (top right of table), and (b) data from 1522 seedlings (bottom left of table)

(b) (a)	EE	Anth	PH	FL/Sp	Sd/R	Sd Wt	% Germ	% Mult	GD	2LD	2LL	2LB	No T	Size	TA	
GD		0.872	-0.429	-0.129	-0.273	-0.003	0.188	0.167	-0.249	-0.258	-0.252	0.192	0.118	0.098	0.104	EE
2LD	0.913		-0.071	-0.279	-0.164	0.239	0.071	0.123	-0.297	-0.332	-0.263	0.092	0.136	-0.005	-0.148	Anth
4LD	0.840	0.934		-0.188	0.537	0.234	-0.495	0.053	0.233	0.157	0.171	-0.252	0.252	-0.352	-0.358	PH
2LL	0.100	0.092	0.036		-0.203	-0.038	-0.078	0.127	0.089	0.113	-0.165	-0.075	-0.046	-0.096	-0.052	FL/Sp
2LB	-0.221	-0.241	-0.241	0.486		-0.319	-0.244	0.245	0.439	0.346	0.252	-0.367	0.481	-0.234	0.103	Sd/R
4LL	-0.127	-0.120	-0.131	0.731	0.527		0.261	-0.160	-0.479	-0.492	-0.352	0.484	-0.208	-0.039	-0.622	Sd Wt
No T	-0.303	-0.375	-0.442	0.220	0.309	0.205		-0.219	-0.553	-0.543	0.042	0.588	-0.056	0.394	-0.093	% Germ
Size	-0.369	-0.419	-0.469	0.474	0.541	0.575	0.525		0.485	0.425	-0.175	-0.420	0.071	-0.640	-0.036	% Mult
Colour	0.039	0.050	0.058	0.282	0.011	0.263	-0.190	0.213		0.977	0.338	-0.464	-0.108	-0.360	0.513	GD
Lig	0.028	0.021	0.036	0.049	0.180	0.070	0.175	0.054	-0.451		0.311	-0.438	-0.228	-0.322	0.541	2LD
Au H	0.012	0.043	0.027	0.338	0.260	0.407	-0.115	0.374	0.528	-0.225		0.047	0.102	0.393	0.439	2LL
Sh H	-0.032	-0.034	-0.043	0.225	0.243	0.350	0.002	0.275	0.206	-0.022	0.583		0.023	0.560	-0.020	2LB
LA	-0.137	-0.165	-0.173	0.205	-0.031	0.205	0.136	0.103	0.014	0.063	-0.068	-0.072		0.094	-0.085	No T
TA	0.100	0.078	0.008	0.365	0.202	0.336	0.090	0.401	0.280	-0.108	0.418	0.188	-0.060		0.383	Size
																TA

	GD	2LD	4LD	2LL	2LB	4LL	No T	Size	Colour	Lig	Au H	Sh H	LA	TA	-

(a) $r \geq \pm 0.298$*, $r \geq \pm 0.385$**, $r \geq \pm 0.481$***, and (b) $r \geq \pm 0.050$*, $r \geq \pm 0.066$**, $r \geq \pm 0.084$***

second leaf date in family 3 ($p < 0.05$). In family 1, fourth leaf length and sheath colour both showed differences in the replications X samples within light regimes interaction ($p < 0.05$). These results indicated that there were some differences in seedling development from the two samples within the light treatments but most variates gave similar results. In all following analyses data from the two samples are bulked to give a maximum of ten seedlings per replication from each of the forty-four family X light regime combinations.

Analyses of variance on data from the clones are shown in Table 32. Data from seedling progenies were initially analysed using 128 plot means derived from families 1, 3, 5, 7, 8, 9, 10 and 11 which did not have missing plot values; results are shown in Table 33.

All variates, whether recorded on parental clones or on seedling progenies, showed differences between the eleven populations ($p < 0.01$) except for the mean number of florets per spikelet. Nine variates recorded on the clones and three variates recorded on seedlings showed overall differences due to the light regimes (Table 34). Only ear emergence and anthesis showed a clear response to photoperiod although another five variates showed differences between means from light regime A and those from B and C. Total seed yield per ramet was the only variate which showed a clear response to total daily irradiance although four other variates also had means from A and B which differed from C. Most variates appeared to be influenced by both photoperiod and the level of illuminance. Ramets from A, which received the short photoperiod and the lower level of daily

TABLE 32 - Trial G Analyses of variance on data from eleven hybrid clones after exposure to four light regimes during flowering

Source	df	EE (half weeks) SQRT MS	Anth (half weeks) MS	No Pan SQRT MS	PH (mm x 10) SQRT MS	FLL (mm) Log_{10} MS x 10^2	FLB(mm x 10) SQRT MS	Pan L(mm) SQRT MS
Clones (CL)	10	0.7161***	7.776***	7.5463***	9.398***	26.567***	2.2180***	11.839***
Light regimes (L)	3	6.7159***	84.856***	0.5559	23.005***	0.693	0.0564	3.946***
CL X L	30	0.0328***	0.419***	0.0827	0.235***	0.808***	0.0698	0.265
Samples (S)/L	4	0.0249**	0.393**	0.2351*	0.291***	0.315	0.0758	0.093
CL X S/L	40	0.0071	0.087	0.1047	0.039	0.368	0.0507	0.161*
Residual	339	0.0053	0.103	0.0933	0.040	0.334	0.0490	0.112

Source	df	Pan B (mm) SQRT MS	FL/Sp Log_{10} MS X 10^2	Sd/R (mg) SQRT MS	1000 Sd Wt (mg) Squared x 10^{-4} MS	% Germ Arcsin MS	% Mult Arcsin MS
Clones	10	2.1494***	0.4043	289.62***	1792.6***	969.28***	894.39***
Light regimes	3	5.1613***	9.5092*	422.45**	391.4***	356.08***	102.92
CL X L	30	0.2524	0.6558***	7.98**	56.6**	95.74*	74.87
Samples/L	4	0.2061	0.9409**	20.78***	9.2	112.63	50.58
CL X S/L	40	0.1819*	0.2810	4.61	20.0	50.83	60.04
Residual	339	0.1132	0.2293	3.78	———	———	———

TABLE 33 - Trial G Analyses of variance on data from eight hybrid progenies grown from seed harvested from clones exposed to four light regimes during flowering. (Three progenies omitted due to very incomplete data sets.)

Source	df	2LD Log_{10} MS x 10^3	2LL (mm) MS	2LB (mm) MS x 10^3	4LL (mm) Squared MS x 10^{-6}	No T SQRT MS x 10^2	Size MS x 10
Blocks (B)	3	17.881***	36.27	18.303	25.214	42.646***	83.536***
Light regimes (L)	3	27.329***	183.09**	23.496*	18.083	13.160	8.210
Progenies (P)	7	50.922***	1334.07***	99.929***	279.526***	79.028***	34.326**
B X L	9	0.931	67.35	10.262	44.916*	6.546**	9.503**
B X P	21	1.297	47.12	7.488	42.515**	2.540	7.276**
L X P	21	2.238***	82.16*	15.438**	49.908	4.074	6.918**
B X L X P	63	1.042	58.52	7.391	31.939**	4.182**	3.156
Residual	1081	0.921	45.14	7.484	20.877	2.353	3.348

continued.....

TABLE 33 - Trial G Analyses of variance on data from eight hybrid progenies grown from seed harvested from clones exposed to four light regimes during flowering. (Three progenies omitted due to very incomplete data sets.) (Continued)

Source	df	Colour MS x 10	Lig SQRT MS x 10^2	Au H MS x 10	Sh H SQRT MS x 10^2	LA SQRT MS x 10^2	TA MS x 10
Blocks (B)	3	12.244***	0.637	2.466	1.094	0.963	7.215***
Light regimes (L)	3	0.679	1.055	2.542	0.604	0.672	2.482
Progenies (P)	7	225.080***	102.602***	162.617***	23.784***	31.441***	93.913***
B X L	9	2.077**	1.264	1.415	1.780*	1.215	2.484**
B X P	21	1.420*	1.045	1.977**	1.591**	0.447	2.367***
L X P	21	0.966	1.774	2.837***	1.475*	0.713	2.669*
B X L X P	63	1.234*	1.181**	1.080	0.950	0.682	1.431*
Residual	1081	0.853	0.789	1.034	0.826	0.811	0.997

TABLE 34 - Trial G Light regime means for variates showing differences over all clones or seedling progenies

Variate		Light regime				Light regime codes ordered by size and non-significant differences marked ($p > 0.05$)			
		A	B	C	D				
EE	SQRT	3.204	2.132	2.065	2.829	A	D	B	C
Anth		13.578	10.134	10.164	13.523	A	D	C	B
PH (cm)	SQRT	7.190	8.452	9.220	9.462	A	B	C	D
Pan L (mm)	SQRT	9.279	9.791	9.839	10.315	A	B	C	D
Pan B (mm)	SQRT	5.392	5.625	5.622	6.490	A	C	B	D
FL/Sp (x 10)	Log_{10}	1.592	1.556	1.622	1.470	C	A	B	D
Sd/R (mg)	SQRT	18.266	20.791	25.041	28.115	A	B	C	D
1000 Sd Wt(mg) Squared x 10^{-4}		33.285	36.111	43.004	39.590	A	B	D	C
% Germ	Arcsin	60.326	55.322	50.922	53.136	A	B	D	C
2LD+	Log_{10}	1.603	1.632	1.673	1.647	A	B	D	C
2LL+ (mm)		57.760	59.414	60.843	63.405	A	B	C	D
2LB+ (mm)		0.9704	0.9992	1.0126	1.0352	A	B	C	D

+ Means from eight seedling progenies; progenies 2, 4 and 6 omitted.

irradiance, always showed extreme values compared with light regimes C or D. They had later ear emergence and anthesis, smaller panicles and seed but a higher total percentage of seed germinated; seedlings reached second leaf maturity earlier but second leaves were smaller.

Eight variates recorded on the eleven parental clones showed clone X light regime interactions ($p < 0.05$, Table 32) and seven variates recorded on seedlings showed family X light regime interactions ($p < 0.05$, Table 33) when data from eight families were analysed.

One way analyses of variance were carried out on seedling data from families 2, 4, 6 and 8 separately. The remaining families were also analysed separately using data from individual seedlings. The number of seedlings, and variate means for each clone and seedling family from each light regime are shown in Appendix Tables 31 to 44.

A canonical analysis was performed on forty-four groups (eleven families X four light regimes) using the twelve variates recorded on seedling progenies. The first, second and third canonical variates accounted for 53.87, 16.30 and 11.34 per cent of the total variation respectively. The positions of the forty-four means were plotted on the first three canonical variates (figs.32 and 33). Ninety-five per cent confidence limits were calculated for all groups; those for the control group, D in each progeny are shown in figs.32 and 33. The distance between each pair of family means from light regime D is shown in Table 35.

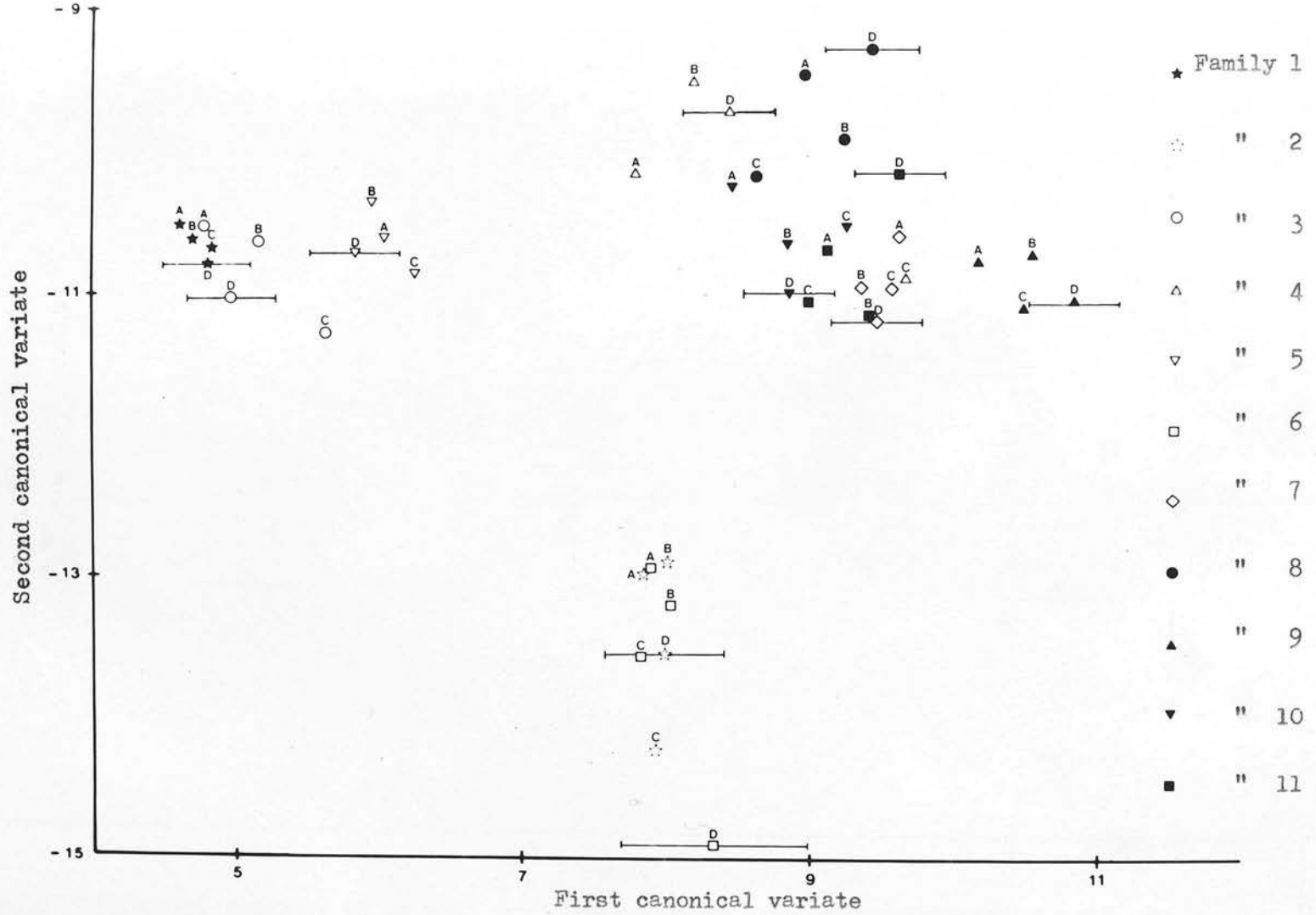
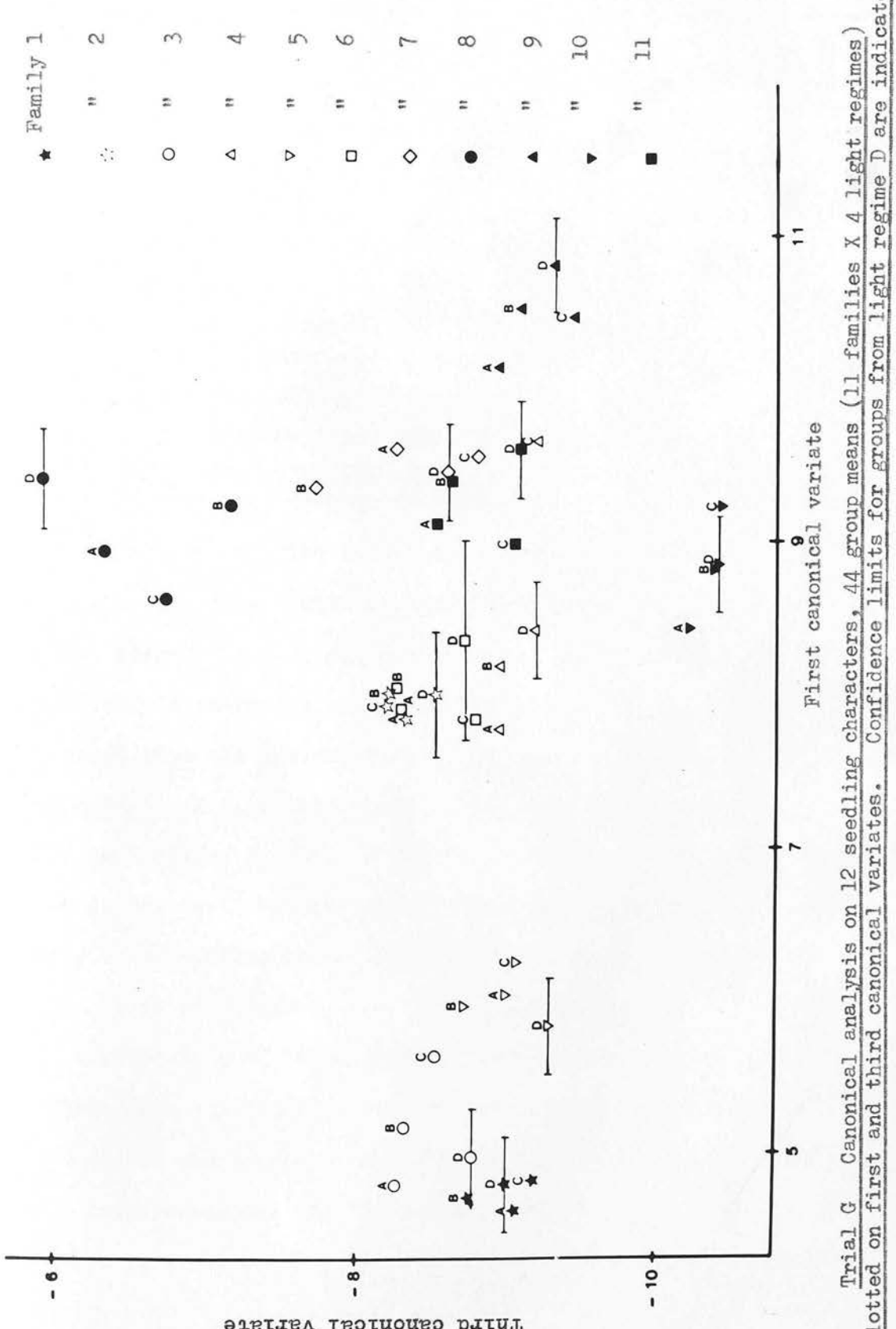


FIGURE 32

Trial G Canonical analysis on 12 seedling characters, 44 group means (11 families X 4 light regimes) plotted on first and second canonical variates. Confidence limits for groups from light regime D are indicated.

FIGURE 33



Trial G Canonical analysis on 12 seedling characters, 44 group means (11 families X 4 light regimes) plotted on first and third canonical variates. Confidence limits for groups from light regime D are indicated.

TABLE 35 - Trial G Canonical analysis; distance between each pair of family means from light regime D (control)

Family	1	2	3	4	5	6	7	8	9	10
1										
2	660									
3	249	622								
4	645	618	629							
5	311	559	352	463						
6	850	358	800	871	821					
7	740	446	729	332	587	711				
8	892	805	879	659	817	1000	608			
9	905	610	883	508	789	736	384	682		
10	706	509	695	362	517	759	346	762	441	
11	762	623	759	376	608	819	358	643	342	452

Figure 32 shows the families separated into three clusters which were consistent with general observations of the morphological characteristics of both maternal clones and their progenies. A central cluster was formed by families 7, 9, 10 and 11, which were all derived from P. iberica X P. pratensis or P. longifolia X P. pratensis hybrids. Family 4 linked this group to families 1, 3 and 5 which all showed many P. ampla characteristics. Familes 2 and 6 were like P. pratensis in habit; family 8 was similar morphologically to P. iberica but did not show a close relationship with any other family.

A comparison of light regime means for each progeny was possible from the distance between each pair of means (Table 36) and from the confidence limits calculated for group means on the first three canonical variates. The latter method showed that families 1, 2, 5, 6 and 7 did not differ between light regimes.

In progeny 3, A and D differed from C; in progeny 4, A differed from D, and A, B and D all differed from C; in progeny 8, C differed from D; in progeny 10, A differed from C; and in progenies 9 and 11, A differed from D.

TABLE 36 - Trial G Canonical analysis; distance between each pair of light regime means for eleven families

Family	A-B	A-C	A-D	B-C	B-D	C-D
1	126	144	124	148	110	102
2	187	336	170	375	199	230
3	124	309	160	216	125	266
4	245	667	223	645	193	615
5	198	191	234	171	142	149
6	143	190	496	156	443	373
7	173	242	199	217	246	195
8	253	252	229	208	336	338
9	138	205	192	170	103	158
10	224	341	247	174	146	171
11	186	216	231	153	204	233

The eleven family variances (Appendix Table 45) were tested for homogeneity using Bartlett's test. All twelve variates recorded on seedlings gave heterogeneous variances ($p < 0.001$). The four light regime variances for all variates within each family were also tested for homogeneity; results are shown in Table 37.

Determinants of the family variance-co-variance matrices were calculated from (a) the six quantitative seedling characters (2LD, 2LL, 2LB, 4LL, No T and Size); (b) the six qualitative seedling characters (colour, ligule, Au H, Sh H, LA and TA); and (c) from all twelve variates together; they are shown in Table 38.

TABLE 37 - Trial G Tests for homogeneity of light regime variances within families. Probability levels for tests showing significant differences (heterogeneity) between variances are indicated with asterisks

Family	2LD	2LL	2LB	4LL	No T	Size
1	ns	ns	ns	**	ns	ns
2	ns	*	ns	ns	ns	ns
3	*	ns	ns	*	*	ns
4	ns	ns	ns	ns	ns	*
5	ns	ns	ns	ns	ns	ns
6	***	ns	*	ns	ns	ns
7	ns	ns	ns	ns	ns	ns
8	**	ns	ns	ns	ns	ns
9	ns	ns	ns	ns	ns	ns
10	ns	ns	ns	ns	ns	ns
11	*	ns	ns	ns	ns	ns

Family	Colour	Lig	Au H	Sh H	LA	TA
1	ns	ns	ns	*	ns	ns
2	ns	ns	***	*	ns	ns
3	ns	*	***	***	ns	***
4	ns	ns	ns	ns	ns	ns
5	ns	ns	ns	ns	ns	ns
6	***	ns	***	***	ns	ns
7	***	ns	ns	ns	ns	ns
8	***	ns	ns	ns	ns	ns
9	ns	ns	ns	ns	ns	ns
10	ns	ns	ns	ns	ns	ns
11	ns	ns	ns	***	ns	ns

$p > 0.05$, ns; $p < 0.05$, *; $p < 0.01$, **; $p < 0.001$, ***

TABLE 38 - Trial G Determinants of dispersion matrices based on (a) six quantitative, (b) six qualitative and (c) all twelve seedling variates; and tests for homogeneity

Family	Determinants		
	(a) $x10^{-8}$	(b) $x10^{-7}$	(c) $x10^{-2}$
1	30.12	60.91	8.25
2	10.25	28.41	1.07
3	156.02	6.09	3.95
4	105.05	4533.70	952.03
5	504.23	1245.80	2741.00
6	20.36	46.38	3.18
7	330.37	6412.10	4520.40
8	528.51	2575.40	3821.40
9	22.99	146.82	9.25
10	182.66	959.93	665.79
11	89.87	845.72	209.21
χ^2 (210 df)	910.90 ***	1656.70 ***	-

Between family dispersions were highly heterogeneous for both (a) and (b) and were consequently assumed to be heterogeneous also for (c); there was a greater range of dispersions from the qualitative than from the quantitative characters. The families fell into three rough groups based on the relative size of determinants as calculated from (a), (b) and (c). Families 1, 2, 3, 6 and 9 had small determinants; families 4, 10 and 11 had determinants of intermediate size although qualitative variates gave the second greatest determinant for family 4; and families 5, 7 and 8 had the largest determinants.

Homogeneity of dispersions between light regimes was tested for each family from (a) six quantitative variates and (b) six

qualitative variates. The determinants and chi-squares from the tests are shown in Table 39. In families 5, 9 and 10 dispersions were homogeneous between light regimes for both quantitative and qualitative characters. Families 2, 3 and 6 had heterogeneous dispersions for both (a) and (b), and families 4, 7 and 11 were heterogeneous for (a) but not for (b). However, although family 4 dispersions were homogeneous for (b) from light regimes A, B and D (as shown), they might be assumed to be heterogeneous with C included since this determinant would by definition be zero. The relative size of determinants from different light regimes was not consistent between families.

Data from the six quantitative variates from all seedlings in the control light treatment, D, were included in a principal component analysis. The first, second and third principal components took up 49.0, 21.0 and 12.0 per cent of the total variance. The positions of individuals were plotted on the first and second, and on the first and third principal components family by family (Appendix figs. 12 to 23).

Rough estimates of morphological uniformity of seedlings within a progeny were made twelve weeks after sowing; these gave an indication of the overall proportion of apomictically produced seed (Table 40). Field open pollinated seed was also harvested from the eleven clones used in Trial G and ten of the resulting progenies were included in a spaced plant field trial. An assessment of phenotypic uniformity was made on the mature plants by three independent observers and the percentage of putative apomicts calculated from the mean of the three records. These figures are included in Table 40 together with a summary of the results from Trial G.

TABLE 39 - Trial G Determinants of dispersion matrices for light regimes within families, and tests for homogeneity of dispersions

(a) six quantitative variates (determinants $\times 10^{-10}$)

Family	Light regime A	B	C	D	χ^2(63 df)
1	705.00	1099.50	2332.90	5398.40	71.77
2	1268.70	403.31	6.27	253.11	88.10*
3	1153.10	6571.30	13622.00	15512.00	101.24**
4	2236.90	5016.30	0.19	6431.90	94.82**
5	29999.00	60012.00	18611.00	28599.00	56.97
6	1159.50	824.02	658.77	0.02	115.38***
7	7886.10	13166.00	12930.00	23349.00	85.49*
8	1488.80	24498.00	51717.00	30468.00	79.32
9	1085.40	885.25	2592.00	536.94	81.71
10	11016.00	7031.70	9297.00	5169.70	80.63
11	2584.30	3701.10	4611.90	3315.30	99.26**

(b) six qualitative variates (determinants $\times 10^{-9}$)

Family	Light regime A	B	C	D	χ^2(63 df)
1	1004.80	1167.80	6936.00	8369.80	102.47**
2	1394.10	397.52	2.45	616.40	193.59***
3	1.91	1061.10	428.13	4.38	386.16***
4	169080.00	96816.00	+	146710.00	45.49++
5	106610.00	73753.00	128490.00	42340.00	58.91
6	5109.20	1617.30	854.75	0.004	152.47***
7	558720.00	362030.00	210920.00	515330.00	64.21
8	12747.00	525470.00	33875.00	33898.00	87.93*
9	15012.00	3101.70	19500.00	4935.30	70.37
10	37139.00	70522.00	78800.00	61086.00	59.63
11	45018.00	22093.00	60788.00	87739.00	72.60

\+ omitted due to occurrence of invariate data for sheathcolour

++ 42 df

TABLE 40 - Trial G Summary of results from seedling progenies

Family	Differences between light regimes				Family dispersions	Percentage of apomicts (eye estimates)	
	Anova No. of variates significant	Canonics	Variances No. of variates heterogeneous	Dispersions			
				Quant. Qual.	Size	Seedlings	Mature plants*
1	2 variates	Similar	2 variates	Hom Het	Small	75	27
2	4 "	"	3 "	Het "	"	90	91
3	2 "	Different	7 "	" "	"	25	18
4	5 "	"	1 "	" (")	Intermediate	50	46
5	1 "	Similar	0 "	Hom Hom	Large	25	-
6	3 "	"	5 "	Het Het	Small	90	89
7	7 "	"	1 "	" Hom	Large	25	24
8	1 "	Different	2 "	Hom Het	"	10	21
9	4 "	"	0 "	" Hom	Small	90	82
10	6 "	"	0 "	" "	Intermediate	75	64
11	8 "	"	2 "	Het "	"	50	40

* Progenies derived from open pollination in the field.

DISCUSSION

Visual examination of the seedling progenies showed that there were differences between them which made it possible to assign many of the individual seedlings to the correct family. These differences were substantiated and clarified by analyses of variance and canonical analysis of characters measured on individual seedlings. Differences in the qualitative characters leaf sheath colour, ligule length, hairiness and leaf and tiller angle were easier to distinguish by eye than differences in quantitative characters. But since such differences could be due to major gene differences they were not expected to be a reliable guide to the proportion of apomictic seed produced as measured by the variability of progenies.

Differences in quantitative characters were also masked to some extent by differences in the stage of development of seedlings which were due largely to the wide range in germination dates. Families 2 and 6 had particularly late and poor germination, subsequent germination tests in a range of temperature and light regimes gave similar results to those reported in Trial G. The relatively frequent occurrence of multiple seedlings, which were usually smaller than singles, also tended to increase variability in quantitative characters. It is obviously desirable to avoid any form of selection of germinating seedlings since earliness of germination could be associated with the occurrence of aberrants. But some modification of the method used in this trial is necessary to overcome these difficulties.

Determinants from family dispersion matrices showed that a range in variation occurred between the seedling progenies. The scatter of individual points on plots from the principal component analysis did not show such a clear distinction between those progenies thought to be derived from predominantly apomictic seed and those from sexually produced seed as had been obtained with data from mature plants (Trials C and D); this may have been due to errors in visual assessment or to the less effective discriminating properties of the recorded seedling characters. The scatter diagrams appeared to relate more closely to eye estimates of uniformity than to the size of determinants from dispersion matrices. In particular, family 3 showed a relatively wide scatter and the seedlings were observed to be phenotypically variable although determinants were relatively small.

Estimates of morphological uniformity from mature plants were included as a check on the rough estimates obtained from seedlings. Since seed was obtained from the field rather than a greenhouse environment some differences might be expected, but the overall agreement with the assessment of seedling progenies was surprisingly good. Family 1 only gave a noticeably different result; the relatively high level of uniformity noted in the seedlings has subsequently been confirmed on plants which were kept for further observation. So this may reflect a true difference in variability between the two seed lots.

Families 5, 9 and 10 which showed no differences in variability following the different light treatments were sexual, apomictic and partially apomictic respectively. All the other

families, which also represented the three probable categories of seed production, showed some evidence of heterogeneity of dispersions. There was no consistent trend in size of dispersions with the light regimes which indicated that there was no overall response pattern in these hybrid progenies to light treatments.

There was also evidence of biotype-light regime interactions from analyses of variance on data from clones and seedling progenies and from the canonical analysis. Data from the parental clones showed that the period to ear emergence and to anthesis was influenced by photoperiod. Panicle size, number of mature florets and seed size were all influenced by light treatments. Seed size particularly showed a response to increased illuminance. It appeared that response of seedling progenies to light regimes was due to the combined effects of photoperiod and total daily irradiance. It was not possible from the data recorded in this trial to distinguish in seedling progenies between the effect of differences in seed development and of possible changes in the proportion of apomictic seed produced due to the light treatments. It should be possible to discriminate between phenotypic and genotypic variability in seedling progenies but more detailed information is needed on the influence of the environment on seed production and on the heritability of seedling characters. However, it was clear that there were differences both in means and in variability of seedling progenies after parental clones had been subjected to four different light regimes.

CHAPTER 7

GENERAL DISCUSSION

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Clausen (1961) observed that high polyploidy and predominantly apomictic seed formation have enabled P. pratensis to evolve into one of the most highly buffered and tolerant species complexes in the Northern hemisphere. These characteristics also offer great potential in any breeding programme but at the same time cause problems in practice.

Many breeding programmes based on Poa spp involve an initial inter- or intra-specific hybridisation (Nygren and Åkerberg, 1957; Funk and Han, 1967, van Dijk, 1974). Most F_1 hybrids, even between two highly apomictic biotypes, produce sexual seed. Hybrid progenies show different degrees of recombination between the parental biotypes and of the variability previously fixed in each of the parents by apomixis (Clausen, loc. cit.). Later hybrid generations tend to return to the apomictic state (Nygren, 1953), and in the second stage of the breeding programme, highly apomictic biotypes with the required characteristics can be selected.

Heslop-Harrison (1961) was doubtful if this type of hybridisation involving whole genomes initially would allow the high level of recombination required (between and within genomes) to give a population with a smooth quantitative expression of characters equivalent to that of a panmictic population. However, recombination occurs between and within the genomes in some of the Poa hybrids and Clausen, Hiesey and Nobs (1954) observed that the range of variability within a single F_4 progeny

from a P. ampla X P. pratensis hybrid was indicative of the extent of potential variability of the parental strains.

The scope of a breeding programme as outlined above could be extended by maintenance of the sexual phase, if necessary by manipulation of the environment, with the objective of allowing a free flow of variation and making recurrent selection possible. This approach emphasises the necessity for a reliable technique for assessment of apomictic seed production.

The work reported in earlier chapters was all closely related to development of the Poa breeding programme at Pentlandfield and was on three main topics. Firstly, the production and recognition of F_1 interspecific hybrids. Secondly, the possible use of statistical techniques to distinguish predominantly sexual from predominantly apomictic progenies, and for assessment of the proportion of aberrants within a partially apomictic progeny; and thirdly, the influence of light regimes prior to anthesis on seed production.

Differences in variability between and within families may be due to several factors. The primary difference is between progenies derived from apomictic and those from amphimictic maternal parents. Both may show biotype-environmental interactions which affect assessment; this was demonstrated by Watson and Clausen (1961) with sexual progenies some of which appeared phenotypically uniform in one environment and very variable in another. Data from Trials C, D and F indicated that there were differences also in variability between highly apomictic families. These results suggest that assessment from progeny tests should if possible be made in more than one environment (Nygren, 1953), or by growing

second generation progenies (Myers, 1943).

There are several other possible sources of variation within an apomictic, or partially apomictic family. Differences in degree of aneuploidy would be expected in some seed clones and might lead to differences in phenotype. Major gene mutations might occur. Aberrants may also be produced by several different pathways as listed by Åkerberg (1939) and Grazi et al.(1961), and in varying proportions with apomictic seed; they may be conspicuously different from apomicts or almost indistinguishable.

In the early stages of a plant breeding programme the important distinction is between the two broad categories of sexual and apomictic seed production; the latter problem of variation within a highly apomictic line is more likely to arise with possible new cultivars and has been encountered in some existing cultivars of P. pratensis (Duich and Musser, 1959).

Heterogeneity of intra (vegetative) clone variances and dispersions was observed in Trials A, B and C; this might have been overcome by increased replication but there are several factors similar to those listed above which may have increased heterogeneity. There may have been differences between the biotypes in their stability in the environment used. Possibly in some clones there were differences in plasmatype of vegetative ramets (Breese, Hayward and Thomas, 1965). Or maybe a more stringent test for normality of distribution was needed; data were transformed to minimise skewness and kurtosis but tests for homogeneity of variances and dispersions are noted to be very sensitive to departures from normality.

The difference in range of morphological variation between predominantly sexual and predominantly apomictic families can be recorded visually on mature plants (Trial D) but is much less obvious at the seedling stage (Trial G). The relative magnitude of determinants from family dispersion matrices is intuitively the nearest statistic equivalent to an overall visual assessment. One disadvantage in the use of determinants without complementary scatter diagrams is that dispersion in a uniform family may be increased disproportionately with the occurrence of one, or a few, very divergent aberrants. A comparison of intra family variability from scatter diagrams was possible when data from all families were included in one analysis, then individuals in each family were plotted separately. Segregating progenies generally gave a wide but evenly distributed scatter of points whereas partially apomictic progenies showed a cluster with aberrants as outliers. The inclusion of data from one or more highly apomictic families as controls is essential for a reliable assessment particularly if, as with seedlings, a visual record of uniformity is difficult to obtain.

The three forms of multivariate analysis (principal component, principal co-ordinate and canonical analysis) which were used all gave comparable results from scatter diagrams, particularly when relationships between individuals or vegetative clones were considered over more than two axes of variation. This conformity between results despite differences in the method of analysis supports their validity.

Where biotype-environment interactions exist and the relative dispersion of families or the similarity between indivi-

duals is being tested, the precision would be greatly increased if there was a measure of environmental variation. The confidence limits which can be defined for a group mean in canonical analysis (Seal, 1964) are based on the number of individuals in the group. They are not a measure of intra group dispersion. It might be possible to derive a measure of environmental variation if vegetatively cloned control plants were included with progeny rows.

The choice of characters is obviously an important factor in increasing the efficiency of these methods. The minimum number of characters with high repeatability, quick to record and of agricultural interest which together give maximum discrimination is required. More information on these criteria is needed, particularly from seedlings, to increase the precision of assessment. Some of this information is not easy to obtain due to the breeding system which makes biometrical analysis of characters difficult.

The need for greater precision in estimates of the expression of apomixis logically leads to the central problem: the genetic and physiological control of apomixis.

Powers (1945) proposed a general genetical model for the control of apomixis with three pairs of genes controlling meiosis, fertilization of the egg and parthenogenetic development of the embryo respectively. But as Stebbins (1950) points out, the situation in many apomicts may be more complex than this. However, Bashaw, Hovin and Holt (1970) found the inheritance of apomixis in Cenchrus ciliaris and the hybrid C. ciliaris X C. setigerus to be relatively simple being controlled by two genes and epistasis.

Müntzing (1940) first suggested that apomixis in P. pratensis was controlled by a constellation of recessive genes. This

suggestion was supported by Åkerberg (1942) who also emphasised the independence of apospory and parthenocarpic development, and by Nygren (1953). Almgård (1966) also concluded that apospory was governed by a delicate polygenic system which was independent of the system controlling pseudogamy.

The mathematical models proposed by Marshall and Brown (1974) for estimation of the level of apomixis using marker genes are at present of theoretical interest only in this material. But possibly treatment of apomictic seed production as a super character (Mather and Jinks, 1971) equivalent in some respects to seed yield in cereals and grasses might help to clarify some aspects of its expression. A study of some of the component sub-characters such as spikelet and floret numbers within a panicle, production of aposporous initials, degeneration of legitimate cells during or after meiosis, parthenogenetic development to form a multicellular proembryo before anthesis, and development of the embryo (or pro-embryo) and endosperm following fertilization of the central nucleus would help in understanding the functioning of the system as a whole.

Most authors who have studied the embryology of P. pratensis accept that competition may occur between sexual and aposporous embryo sacs and at later stages of development (Nygren, 1951; Grazi et al, 1961). Competition is probably affected either directly or indirectly by environmental factors. The preliminary results from Trials F and G indicated that the light regime prior to anthesis may influence the proportion of sexual or aposporous seed maturing. More detailed studies on the influence of environmental factors on reproductive development might substan-

tiate the evidence for competition and indicate the critical stages during which competition may occur.

Evidence for the influence of the pollen parent on development of apomictic seed in Poa has previously been based on rather small samples which makes interpretation of results more difficult. Grazi et al.(1961) reported that predominantly apomictic plants gave variable numbers of maternal offspring when crossed with different pollen parents which, in turn, gave different results in other crosses; but assessment was based on only twelve plants per progeny and did not permit any firm conclusions. Clausen, Hiesey and Nobs (1962) showed that in one apomictic P. ampla biotype seventeen unreduced and five reduced egg cells developed after pollination by a P. pratensis biotype, but when pollinated by a P. pratensis X P. alpigena hybrid only six reduced egg cells developed. They conclude that fertilization does not occur at random among the apomictic Poas. Results from Trial F where different pollen parents were used in the initial pair crosses support these earlier results, but the nature of the variation due to different pollen parents could not be identified from this trial.

Investigations which have been reported here were carried out on P. pratensis biotypes, P. ampla and the hybrids P. ampla X P. pratensis, P. iberica X P. pratensis and P. longifolia X P. pratensis which are all in the Poa breeding programme at the Scottish Plant Breeding Station. A relatively wide range of biotypes was examined so that results and any techniques developed from the preliminary work should be applicable to material in the breeding programme.

It is clearly essential to develop a reliable technique for assessment of apomixis before any other aspects of its expression and control can be investigated. The application of biometrical methods, and especially the use of multivariate analyses, should enable screening of large numbers of individuals and progenies; but in more detailed studies the complementary cytological and embryological methods are still required.

The scope of the breeding programme is currently restricted by the lack of information on the genetic control of apomixis, the developmental physiology of reproductive development, and the influence on both of environmental factors. The long term objective should be to obtain increased control of the reproductive system in facultative apomicts so that firstly, the flow of genetic variability is maximised in the sexual phase and secondly, highly apomictic seed production is reinstated in selected biotypes.

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I declare that this thesis is my own work with the exception of trials reported in Chapter 3 where the author made a significant contribution.

APPENDIX

CHAPTER 3

PRODUCTION OF INTERSPECIFIC HYBRIDS

(TRIALS A and B)

APPENDIX TABLES 1 and 2

APPENDIX TABLE 1

Trial A - 1968 P. ampla X P. pratensis putative hybrids, field trial data from maternal-type clones

Clone No.	No. Ram.	EE	PH (cm)	PD (cm)	FLL (mm)	FLB (mm)	No Pan	SG
		SQRT	SQx10^{-3}	Log$_{10}$	SQRT	SQRT	SQRT	
197-1	10	1.07	5.67	0.97	10.67	1.97	4.37	3.00
198-1	9	1.11	6.24	1.09	10.34	2.11	4.21	2.78
225	10	1.00	6.48	0.94	10.90	1.94	5.22	2.80
200-1	9	1.11	6.40	1.06	10.53	2.08	3.81	2.89
202	7	1.00	5.80	0.95	10.35	1.92	5.85	2.71
205	10	1.00	5.46	0.98	9.75	1.78	4.72	2.30
206	10	1.00	6.80	1.08	10.59	2.04	4.92	2.70
207	10	1.00	5.80	0.99	10.21	1.94	4.87	2.60
209	10	1.07	6.15	1.01	10.20	1.97	4.40	2.70
210	10	1.04	6.17	1.00	10.62	1.86	4.59	2.50
211-1	10	1.00	6.26	1.01	11.20	2.07	5.03	2.90
211-2	10	1.00	6.34	1.01	10.82	1.97	4.77	2.50
214-1	10	1.12	5.48	0.99	10.34	2.04	3.49	2.50
215-1	9	1.00	6.11	1.07	10.52	2.05	4.92	2.67
217	10	1.00	5.89	0.99	10.62	1.95	4.08	2.50
218	10	1.04	5.56	1.01	10.19	1.95	4.11	2.40
226-1	9	1.00	6.21	0.99	10.93	2.02	4.62	2.67
228-1	10	1.00	5.99	1.02	10.62	1.97	4.54	2.80
229-1	10	1.00	6.30	0.98	10.77	1.92	4.71	2.80
230-1	10	1.00	6.60	1.06	11.07	2.02	5.05	2.80
230-2	10	1.00	6.19	1.02	11.05	2.02	4.38	2.70
231-1	10	1.00	6.15	1.03	10.73	1.99	4.40	2.80
232-1	10	1.17	5.11	1.01	9.63	1.89	4.04	2.70

Trial B 1969 P. ampla X P. pratensis putative hybrids, field data from maternal-type clones

Clone No.	No. Ram.	EE Log_{10}	PH (mm) $SQx10^{-5}$	PD (mm) Log_{10}	LL (mm) $SQRTx10^{-1}$	LB (mm) SQRT	SG1 $SQx10^{-1}$	SG2 $SQx10^{-1}$	Hab Log_{10}	Pan L (mm) $x10^{-2}$	Sp L (mm) SQRT	Sp B (mm) SQRT	Sd/Sp SQRT	Sd H SQRT
246	10	0.53	6.43	2.01	1.97	1.79	0.67	5.92	0	1.74	3.14	1.40	2.32	1.08
248	10	0.42	7.10	2.04	2.03	1.84	0.65	6.25	0	1.71	3.32	1.43	2.49	1.08
250	10	0.49	7.60	2.05	2.01	1.81	0.45	6.40	0	1.85	3.01	1.41	2.23	1.26
251	10	0.41	7.83	2.11	2.01	1.78	0.55	6.40	0	1.86	3.04	1.37	2.21	1.24
252	10	0.44	7.74	2.05	2.06	1.84	0.65	6.40	0	1.87	3.06	1.23	2.08	1.41
255	10	0.46	6.94	2.04	2.02	1.84	0.50	6.40	0	1.68	3.09	1.41	2.36	1.21
258	10	0.42	7.36	2.07	2.07	1.79	0.85	6.40	0	1.81	3.10	1.34	2.21	1.08
259	10	0.47	7.08	2.03	1.94	1.81	0.60	6.10	0	1.74	3.03	1.41	2.23	1.15
260	10	0.50	6.98	2.07	1.99	1.78	0.60	6.25	0	1.77	3.17	1.41	2.29	1.17
263	10	0.44	7.36	2.03	2.07	1.76	0.70	6.40	0	1.78	3.16	1.37	2.30	1.20
264	10	0.48	7.52	2.03	1.97	1.77	0.55	6.25	0.05	1.76	3.10	1.40	2.23	1.19
265	10	0.48	7.05	2.09	1.96	1.77	0.65	6.25	0	1.68	3.10	1.37	2.26	1.07
268	10	0.50	7.24	2.04	1.96	1.78	0.45	6.25	0.05	1.75	3.22	1.41	2.32	1.17
269	10	0.53	7.00	2.04	1.98	1.79	0.52	6.25	0	1.74	3.17	1.34	2.29	1.08
272	10	0.42	7.75	2.09	2.09	1.77	0.55	6.10	0	1.74	3.02	1.29	2.10	1.08
274	10	0.46	7.18	2.07	2.07	1.74	0.60	6.57	0	1.63	3.20	1.40	2.41	1.12
275	10	0.45	6.55	2.05	1.90	1.73	0.60	6.01	0.06	1.60	2.93	1.37	2.21	1.20

continued.....

APPENDIX TABLE 2 (continued)

Trial B 1969 P. ampla X P. pratensis putative hybrids, field data from maternal-type clones

Clone No.	No. Ram.	EE Log_{10}	PH (mm) $SQx10^{-5}$	PD (mm) Log_{10}	LL (mm) $SQRTx10^{-1}$	LB (mm) SQRT	SG1 $SQx10^{-1}$	SG2 $SQx10^{-1}$	Hab Log_{10}	Pan L (mm) $x10^{-2}$	Sp L (mm) SQRT	Sp B (mm) SQRT	Sd/Sp SQRT	Sd H SQRT
276	9	0.45	6.27	2.01	1.96	1.73	0.46	6.07	0	1.55	2.98	1.39	2.18	1.26
280	10	0.37	7.04	2.09	1.94	1.76	0.75	6.40	0	1.77	2.94	1.41	2.30	1.27
283	10	0.47	7.16	2.01	1.96	1.78	0.60	6.25	0	1.77	2.95	1.34	2.18	1.27
285	10	0.47	6.89	2.00	1.99	1.82	0.65	6.40	0	1.77	3.06	1.33	2.25	1.12
295	10	0.39	7.03	2.01	1.93	1.68	0.67	6.40	0	1.73	2.92	1.29	2.22	1.00
296	10	0.48	7.16	2.02	1.97	1.77	0.75	6.40	0	1.79	3.01	1.40	2.32	1.04
300	10	0.44	7.87	2.06	2.04	1.73	0.75	6.40	0	1.79	3.03	1.25	2.13	1.17
301	10	0.45	7.10	2.00	1.97	1.69	0.55	6.25	0	1.76	2.98	1.37	2.16	1.17
304-1	10	0.39	7.25	2.10	2.03	1.74	0.85	6.40	0	1.80	3.03	1.38	2.27	1.16
313	10	0.46	7.15	2.02	2.05	1.76	0.65	6.25	0	1.77	2.97	1.33	2.13	1.17
315	10	0.47	6.83	2.03	1.94	1.75	0.55	6.25	0	1.75	3.00	1.35	2.25	1.16
317	10	0.37	7.50	2.08	1.93	1.82	0.65	6.10	0	1.75	2.97	1.41	2.27	1.08
319	10	0.46	7.32	2.05	2.02	1.78	0.85	6.25	0	1.79	3.01	1.35	2.21	1.28
320	10	0.51	6.96	2.05	2.00	1.76	0.55	6.40	0	1.68	3.04	1.35	2.22	1.23
321	10	0.49	7.12	2.03	2.00	1.81	0.65	6.40	0	1.73	3.08	1.41	2.25	1.17
321a	10	0.46	7.41	2.08	1.99	1.78	0.70	6.40	0	1.69	2.94	1.38	2.20	1.12

CHAPTER 4

HYBRID PROGENIES (TRIALS C and D)

APPENDIX TABLES 3 to 19

and

APPENDIX FIGURES 1 to 12

Trial C - Family 1 - clone means and analyses of variance

Clone No	No. Ramets	PH (mm)	PD (mm)	LL (mm)	LB (mm)	SG	FW (g)	No Pan	Pan L (mm)	Pan B (mm)	Sp L (mm)	Sp B (mm)	Sd/Sp	Sd H
				SQRT		SQUARE	SQRT	SQRT	SQRT		Log_{10}	Log_{10}	SQRT	
1	3	573.3	243.3	15.93	4.000	36.67	8.35	4.97	11.63	59.00	0.985	0.477	2.229	1.67
2	3	553.3	200.0	17.70	4.667	22.67	9.08	3.07	10.28	45.00	0.971	0.418	2.570	2.00
3	5	606.0	158.0	16.99	4.400	27.60	9.23	5.42	10.88	52.60	1.040	0.371	2.435	1.60
4	5	632.0	200.0	17.89	2.800	14.80	8.88	4.96	11.22	62.60	1.023	0.371	2.446	1.60
5	3	660.0	176.7	19.03	4.000	16.33	10.04	4.88	11.53	60.67	1.053	0.301	2.509	1.00
6	5	678.0	184.0	16.89	3.000	16.80	9.49	5.59	11.45	61.60	1.025	0.371	2.514	1.80
7	5	652.0	168.0	17.45	3.400	13.00	9.61	7.55	11.87	65.00	1.008	0.407	2.342	2.20
8	3	840.0	116.7	18.24	4.667	2.00	8.59	4.79	11.42	61.67	1.028	0.418	2.378	1.33
9	5	626.0	146.0	19.46	4.400	7.00	7.72	2.63	10.96	54.20	1.024	0.371	2.322	2.20
10	5	772.0	182.0	19.06	4.400	25.80	13.09	5.93	12.67	66.40	1.025	0.371	2.450	1.40
Mean		659.8	176.2	17.89	3.905	18.05	9.48	5.08	11.42	59.31	1.020	0.385	2.419	1.71

Analysis of variance. Coefficient of between clone component of expected mean square = 4.17

Source	df	MS $\times 10^{-2}$	MS $\times 10^{-2}$	MS	MS	MS	MS	MS	MS	MS $\times 10^{-3}$	MS $\times 10^{-3}$	MS $\times 10^{-2}$	MS
Between clones	9	269.78 **	39.68 **	5.023	2.076	382.26 **	10.063 *	8.717	1.731 **	165.12	1.938	6.694	3.762	0.582
Ramets within clones	32	70.22	9.03	2.896	0.654	96.55	4.156	4.593	0.532	104.15	3.462	8.270	5.967	0.292

Trial C - Family 2 - clone means and analyses of variance

Clone No.	No. Ramets	PH (mm)	PD (mm)	LL (mm)	LB (mm)	SG	FW (g)	No Pan	Pan L (mm)	Pan B (mm)	Sp L (mm)	Sp B (mm)	Sd/Sp	Sd H
				SQRT		SQUARE	SQRT	SQRT	SQRT		Log_{10}	Log_{10}	SQRT	
1	2	520.0	190.0	18.88	4.000	17.00	6.98	5.51	9.22	43.00	0.874	0.477	2.000	2.00
2	5	512.0	170.0	16.13	3.800	11.80	7.24	7.30	9.53	42.80	0.803	0.407	2.094	2.00
3	4	340.0	187.5	17.63	3.250	22.75	6.95	6.60	9.11	34.00	0.795	0.301	2.000	2.75
4	2	245.0	145.0	11.94	2.500	5.00	6.05	2.08	7.87	22.00	0.841	0.301	2.343	2.00
5	4	405.0	212.5	16.37	3.250	20.50	6.33	8.40	9.43	45.00	0.812	0.389	2.110	2.00
6	5	364.0	170.0	17.12	3.200	13.60	6.87	6.65	9.75	44.40	0.845	0.407	2.047	2.60
7	4	512.5	195.0	17.93	3.250	27.75	9.28	8.96	9.71	41.50	0.826	0.345	2.000	2.50
8	4	432.5	185.0	15.21	3.750	10.50	4.90	7.75	8.22	24.75	0.826	0.301	1.866	2.00
Mean		422.3	183.0	16.55	3.400	16.57	6.88	7.06	9.21	38.23	0.824	0.366	2.043	2.27

Analysis of variance. Coefficient of between clone component of expected mean square = 3.70

Source	df	MS $x10^{-2}$	MS $x10^{-2}$	MS	MS	MS	MS	MS	MS	MS	MS $x10^{3}$	MS $x10^{3}$	MS $x10^{2}$	MS
Between clones	7	286.34 ***	12.61	10.797 **	0.5857	183.98 **	6.018	11.412	1.533 **	270.23 **	1.999	12.480	5.070 *	0.4167
Ramets within clones	22	30.23	18.64	2.534	0.5045	36.52	5.098	7.412	0.324	56.90	2.343	5.850	1.803	0.3159

Trial C - Family 3 - clone means and analyses of variance

Clone No.	No. Ramets	PH (mm)	PD (mm)	LL (mm)	LB (mm)	SG	FW (g)	No Pan	Pan L (mm)	Pan B (mm)	Sp L (mm)	Sp B (mm)	Sd/Sp	Sd H
		nil	nil	SQRT	nil	SQUARE	SQRT	SQRT	SQRT	nil	Log_{10}	Log_{10}	SQRT	nil
1	5	430.0	382.0	16.18	4.000	38.60	14.67	6.45	8.58	42.60	0.843	0.467	2.189	2.40
2	5	528.0	384.0	16.05	3.400	41.20	16.61	6.15	8.68	42.80	0.792	0.336	2.094	2.00
3	5	516.0	418.0	16.20	3.600	38.60	18.07	7.76	8.72	36.40	0.805	0.442	2.047	2.40
4	5	536.0	480.0	17.18	3.800	36.40	15.79	9.13	9.04	42.80	0.762	0.407	1.946	2.20
5	5	484.0	404.0	16.57	4.400	29.40	16.75	7.62	8.53	35.60	0.778	0.407	1.994	2.00
6	5	526.0	420.0	16.49	3.400	34.20	14.59	8.92	8.42	40.20	0.776	0.407	2.041	2.00
7	5	504.0	374.0	16.61	4.200	33.80	16.26	7.86	8.66	40.60	0.762	0.407	1.994	2.40
8	2	540.0	530.0	18.02	5.000	30.50	19.22	8.47	8.83	46.00	0.812	0.477	2.000	3.50
9	3	373.3	300.0	14.80	3.333	25.67	12.90	2.66	7.88	26.67	0.725	0.360	1.911	2.00
10	5	478.0	382.0	17.34	3.200	36.40	12.29	9.44	8.74	36.20	0.776	0.442	2.047	2.20
Mean		493.6	404.0	16.52	3.778	35.13	15.61	7.589	8.63	39.07	0.784	0.413	2.033	2.24

Analysis of variance. Coefficient of between clone component of expected mean square = 4.47

Source	df	MS $x10^{-2}$	MS $x10^{-2}$	MS	MS	MS	MS	MS	MS	MS	MS $x10^{3}$	MS $x10^{3}$	MS $x10^{2}$	MS
Between clones	9	103.12 **	118.53 *	2.352	1.101	89.96	17.412	14.403	0.3325	101.57 *	4.160 *	7.763	2.698	0.5123
Ramets within clones	35	31.04	46.34	1.516	1.196	55.70	11.457	6.855	0.2804	37.91	1.562	8.288	2.102	0.2771

Trial C – Family 4 – clone means and analyses of variance

Clone No.	No. Ramets	PH (mm)	PD (mm)	LL (mm)	LB (mm)	SG	FW (g)	No Pan	Pan L (mm)	Pan B (mm)	Sp L (mm)	Sp B (mm)	Sd/Sp	Sd H
				SQRT		SQUARE	SQRT	SQRT	SQRT		Log_{10}	Log_{10}	SQRT	
1	5	604.0	366.0	18.29	3.800	31.60	11.64	10.53	9.77	58.80	0.747	0.336	1.930	3.40
2	5	618.0	368.0	18.91	3.800	27.20	11.19	12.33	10.21	57.20	0.747	0.371	1.994	2.80
3	5	624.0	416.0	19.30	4.000	25.00	11.80	10.49	10.32	58.20	0.776	0.407	2.084	2.80
4	5	612.0	416.0	19.69	3.800	25.00	11.85	12.13	10.13	57.20	0.744	0.301	1.940	2.80
5	5	488.0	298.0	17.64	3.600	21.40	7.91	9.33	8.64	41.00	0.715	0.301	1.877	2.60
6	3	636.7	453.3	19.10	4.000	28.67	13.06	11.85	10.19	63.33	0.699	0.418	1.911	3.67
7	5	620.0	410.0	20.31	4.000	29.40	11.89	12.26	10.19	60.20	0.792	0.442	2.094	3.00
8	5	620.0	410.0	19.16	4.000	25.00	11.19	11.78	10.56	68.20	0.747	0.407	2.047	3.20
9	5	608.0	428.0	18.09	4.400	31.60	11.64	10.12	9.95	60.40	0.760	0.371	2.047	2.40
10	4	587.5	375.0	18.26	3.750	27.75	10.95	9.42	10.11	53.00	0.714	0.345	1.866	3.00
Mean		600.6	391.9	18.88	3.915	27.19	11.24	11.02	10.00	57.62	0.746	0.368	1.984	2.94

Analysis of variance. Coefficient of between clone component of expected mean square = 4.69

Source	df	MS $x10^{-2}$	MS $x10^{-2}$	MS	MS	MS	MS	MS	MS	MS	MS $x10^{3}$	MS $x10^{3}$	MS $x10^{2}$	MS
Between clones	9	85.53 **	87.07	3.215	0.2344	51.81 *	8.093	6.496	1.367 **	245.16 ***	3.475	11.340	3.387	0.5935
Ramets within clones	37	23.92	49.88	4.371	0.4743	21.65	3.902	8.653	0.420	40.34	2.586	6.551	4.454	0.3640

Trial C - Family 5 - clone means and analyses of variance

Clone No.	No. Ramets	PH (mm)	PD (mm)	LL (mm)	LB (mm)	SG	FW (g)	No Pan	Pan L (mm)	Pan B (mm)	Sp L (mm)	Sp B (mm)	Sd/Sp	Sd H
				SQRT		SQUARE	SQRT	SQRT	SQRT		Log_{10}	Log_{10}	SQRT	
1	5	630.0	440.0	20.21	4.200	29.40	11.58	11.56	9.94	62.20	0.727	0.336	1.877	2.80
2	5	610.0	408.0	23.11	4.200	25.00	12.35	11.82	9.97	55.20	0.715	0.371	2.047	2.60
3	4	617.5	422.5	19.15	3.750	30.50	12.60	14.14	10.33	64.00	0.739	0.389	1.925	3.25
4	5	626.0	380.0	19.85	4.400	25.00	12.17	9.46	10.43	61.20	0.762	0.442	2.094	2.60
5	5	614.0	422.0	18.60	4.000	25.00	11.23	11.39	10.06	59.00	0.747	0.407	1.946	3.00
6	5	624.0	454.0	19.92	4.200	25.00	12.17	12.78	10.19	56.80	0.744	0.371	2.088	3.00
7	5	634.0	350.0	18.59	4.000	29.40	11.05	12.43	10.70	62.00	0.715	0.301	1.946	2.80
8	5	634.0	484.0	19.23	4.000	25.00	11.60	13.31	10.51	61.60	0.776	0.371	1.946	3.00
9	4	597.5	397.5	18.13	4.000	27.75	12.35	12.12	10.20	59.75	0.739	0.301	1.992	3.00
10	4	660.0	422.5	19.23	3.750	22.75	12.73	12.58	10.36	59.75	0.719	0.345	1.933	3.25
Mean		624.7	418.3	19.65	4.064	26.45	11.95	12.11	10.27	60.09	0.739	0.365	1.981	2.92

Analysis of variance. Coefficient of between clone component of expected mean square = 4.70

Source	df	MS $x10^{-2}$	MS $x10^{-2}$	MS	MS	MS	MS	MS	MS	MS	MS $x10^{3}$	MS $x10^{3}$	MS $x10^{2}$	MS
Between clones	9	12.02	70.49	9.435	0.1898	29.64	1.556	7.429	0.294	33.31	2.006	9.307	2.649	0.240
Ramets within clones	37	8.20	33.79	4.481	0.4622	15.21	3.142	7.110	0.257	37.35	2.494	6.831	3.342	0.365

Trial C - Family 6 - clone means and analyses of variance

Clone No.	No. Ramets	PH (mm)	PD (mm)	LL (mm)	LB (mm)	SG	FW (g)	No Pan	Pan L (mm)	Pan B (mm)	Sp L (mm)	Sp B (mm)	Sd/Sp	Sd H
				SQRT		SQUARE	SQRT	SQRT	SQRT		Log_{10}	Log_{10}	SQRT	
1	5	672.0	516.0	20.37	4.000	25.00	11.80	8.77	10.75	64.80	0.762	0.442	2.047	3.20
2	5	728.0	426.0	22.56	4.600	23.20	12.55	10.03	11.48	66.20	0.805	0.442	2.047	3.00
3	5	664.0	458.0	17.48	3.400	25.00	10.41	9.02	11.05	72.40	0.776	0.442	2.047	3.80
4	5	666.0	406.0	20.44	4.600	25.00	9.71	5.91	11.23	67.80	0.832	0.477	2.189	3.80
5	4	697.5	485.0	20.11	4.500	22.75	11.09	10.47	11.60	72.75	0.828	0.433	2.177	3.50
6	5	518.0	426.0	16.86	4.200	14.60	8.57	7.41	10.40	50.60	0.832	0.407	2.227	2.20
7	5	672.0	476.0	19.53	4.800	23.20	11.51	9.98	10.13	69.40	0.776	0.477	2.047	3.00
8	4	442.5	317.5	16.79	4.250	15.25	7.32	4.90	10.54	61.25	0.843	0.477	2.230	2.50
9	5	674.0	410.0	17.87	4.000	23.60	11.68	6.10	10.42	62.80	0.803	0.477	2.142	3.00
10	5	686.0	398.0	19.56	4.800	23.20	10.92	7.91	11.42	66.80	0.805	0.442	1.994	3.80
Mean		645.0	433.1	19.19	4.313	22.21	10.61	8.07	10.90	65.42	0.805	0.451	2.111	3.19

Analysis of variance. Coefficient of between clone component of expected mean square = 4.80

Source	df	MS $x10^{-2}$	MS $x10^{-2}$	MS	MS	MS	MS	MS	MS	MS	MS $x10^{3}$	MS $x10^{3}$	MS $x10^{2}$	MS
Between clones	9	348.94 ***	137.66	16.641 **	0.9514 *	69.51 **	11.717 *	16.979 ***	1.303	198.06	3.638	2.857	3.634	1.479 **
Ramets within clones	38	30.93	87.56	4.896	0.4145	20.80	4.980	3.948	0.848	103.66	3.301	4.203	2.006	0.368

Trial C - Family 7 - clone means and analyses of variance

Clone No.	No. Ramets	PH (mm)	PD (mm)	LL (mm) SQRT	LB (mm)	SG SQUARE	FW (g) SQRT	No Pan SQRT	Pan L (mm) SQRT	Pan B (mm)	Sp L (mm) Log_{10}	Sp B (mm) Log_{10}	Sd/Sp SQRT	Sd H
1	5	718.0	202.0	19.65	4.400	23.20	10.97	9.31	10.00	53.80	0.911	0.301	2.227	1.80
2	3	720.0	203.3	17.49	3.000	19.00	10.63	8.22	10.36	53.00	0.882	0.301	2.307	2.00
3	4	682.5	190.0	17.59	4.250	16.50	8.84	7.98	9.57	47.50	0.916	0.345	2.230	1.75
4	5	698.0	214.0	19.32	4.200	25.00	10.43	8.71	10.54	52.40	0.871	0.301	2.322	2.00
5	5	728.0	236.0	19.15	4.000	18.60	11.72	9.86	10.70	52.60	0.943	0.301	2.407	1.60
6	5	714.0	222.0	18.72	3.800	25.00	10.67	8.87	10.79	54.00	0.902	0.301	2.232	2.00
7	5	734.0	218.0	17.59	4.000	27.20	10.03	9.26	10.44	53.00	0.822	0.336	2.135	1.80
8	5	678.0	248.0	18.22	4.200	21.40	11.23	8.52	10.07	47.60	0.929	0.301	2.322	2.00
9	5	698.0	224.0	19.99	4.200	21.40	10.55	9.51	10.38	51.40	0.942	0.301	2.314	2.00
10	4	700.0	280.0	19.69	4.000	27.75	11.66	10.83	10.71	53.50	0.852	0.301	1.913	2.25
Mean		707.2	224.1	18.80	4.044	22.67	10.69	9.13	10.36	51.89	0.898	0.309	2.245	1.91

Analysis of variance. Coefficient of between clone component of error mean square = 4.59

Source	df	MS $x10^{-2}$	MS $x10^{-2}$	MS	MS	MS	MS	MS	MS	MS	MS $x10^{-3}$	MS $x10^{-3}$	MS $x10^{-2}$	MS
Between clones	9	16.46	27.97	3.983	0.5292	61.46 *	3.016	2.893	0.643 *	26.10	7.663	1.251	8.112	0.150
Ramets within clones	36	24.03	28.54	2.589	0.4764	26.03	4.014	3.309	0.227	30.16	7.017	1.335	3.865	0.175

Trial C - Family 8 - clone means and analyses of variance

Clone No.	No. Ramets	PH (mm)	PD (mm)	LL (mm)	LB (mm)	SG	FW (g)	No Pan	Pan L (mm)	Pan B (mm)	Sp L (mm)	Sp B (mm)	Sd/Sp	Sd H
				SQRT		SQUARE	SQRT	SQRT	SQRT		Log_{10}	Log_{10}	SQRT	
1	2	575.0	235.0	17.27	4.500	20.00	9.01	7.67	10.67	69.00	0.801	0.301	2.118	2.50
2	4	732.5	137.5	18.75	4.250	7.00	6.96	3.99	11.62	66.75	0.914	0.301	2.230	2.00
3	4	740.0	170.0	20.00	5.250	16.50	8.00	4.65	11.58	69.25	0.897	0.301	2.230	1.75
4	4	772.5	147.5	20.10	4.500	10.50	6.89	3.05	11.61	66.00	0.966	0.301	2.337	2.00
5	4	790.0	145.0	18.85	5.250	15.25	7.62	5.04	11.71	67.25	0.909	0.301	2.284	1.75
6	2	350.0	50.0	16.28	3.500	1.00	3.35	1.21	9.49	53.50	0.874	0.301	2.000	1.50
7	2	410.0	70.0	13.37	3.500	1.00	2.70	1.00	9.77	46.00	0.841	0.301	1.866	2.00
8	4	485.0	107.5	14.67	3.250	1.00	4.61	5.32	10.11	52.00	0.872	0.226	1.933	1.75
9	2	475.0	80.0	14.90	5.000	1.00	4.32	0.71	10.39	60.50	0.903	0.301	2.450	1.00
Mean		632.1	132.1	17.61	4.393	8.821	6.25	3.91	10.97	62.25	0.895	0.290	2.176	1.82

Analysis of variance. Coefficient of between clone component of expected mean square = 3.07

Source	df	MS $x10^{-2}$	MS $x10^{-2}$	MS	MS	MS	MS	MS	MS	MS	MS $x10^{-3}$	MS $x10^{-3}$	MS $x10^{-2}$	MS
Between clones	8	832.15 **	72.12	18.520 **	1.897	160.92 *	12.343	12.339	2.264 **	204.00 *	6.116	2.427	10.260 *	0.357
Ramets within clones	19	219.66	40.30	3.406	1.237	56.04	8.792	6.357	0.427	78.28	5.255	3.576	3.206	0.171

Trial C - Family 9 - clone means and analyses of variance

Clone No.	No. Ramets	PH (mm)	PD (mm)	LL (mm)	LB (mm)	SG	FW (g)	No Pan	Pan L (mm)	Pan B (mm)	Sp L (mm)	Sp B (mm)	Sd/Sp	Sd H
				SQRT		SQUARE	SQRT	SQRT	SQRT		Log_{10}	Log_{10}		
1	5	622.0	424.0	21.68	4.200	27.20	11.94	13.20	10.33	58.00	0.744	0.336	2.000	3.40
2	5	594.0	380.0	18.37	4.000	27.20	11.54	11.83	10.09	50.60	0.715	0.371	1.934	2.60
3	5	520.0	332.0	17.13	4.800	25.40	9.20	7.74	9.23	54.20	0.828	0.371	2.094	3.40
4	5	564.0	348.0	18.32	4.000	24.00	9.68	9.58	9.88	50.40	0.747	0.371	1.946	3.20
5	5	622.0	432.0	17.82	3.600	27.20	12.05	11.62	10.64	57.40	0.746	0.301	2.088	3.00
6	3	533.3	330.0	18.81	4.667	22.00	9.64	6.94	9.86	55.33	0.801	0.418	2.079	3.00
7	4	585.0	367.5	17.47	4.250	27.75	10.68	12.86	10.07	55.75	0.739	0.345	1.992	3.00
8	2	470.0	280.0	16.58	5.000	25.00	7.21	7.58	9.51	53.50	0.841	0.389	2.000	3.50
9	5	618.0	366.0	20.32	3.800	29.40	12.78	13.01	10.25	61.80	0.760	0.442	2.077	2.40
10	4	560.0	322.5	14.10	3.000	30.50	10.46	12.05	9.62	51.75	0.719	0.389	1.925	2.25
Mean		577.2	365.6	18.23	4.070	26.77	10.79	10.94	9.99	55.00	0.758	0.371	2.014	2.95

Analysis of variance. Coefficient of between clone component of expected mean square = 4.27

Source	df	MS $x10^{-2}$	MS $x10^{-2}$	MS	MS	MS	MS	MS	MS	MS	MS $x10^{3}$	MS $x10^{3}$	MS $x10^{2}$	MS
Between clones	9	85.80	81.37	18.374 **	1.308 **	24.35	9.566 *	22.638 *	0.795	62.24	6.995 *	7.451	2.026	0.784
Ramets within clones	33	41.04	73.22	4.370	0.334	38.62	4.119	9.591	0.465	74.12	2.829	7.628	4.238	0.571

Trial C - Family 10 - clone means and analyses of variance

Clone No.	No. Ramets	PH (mm)	PD (mm)	LL (mm)	LB (mm)	SG	FW (g)	No Pan	Pan L (mm)	Pan B (mm)	Sp L (mm)	Sp B (mm)	Sd/Sp	Sd H
				SQRT		SQUARE	SQRT	SQRT	SQRT		Log_{10}	Log_{10}	SQRT	
1	5	616.0	424.0	18.56	4.200	27.20	11.54	12.16	10.29	61.20	0.789	0.371	2.094	2.60
2	4	565.0	322.5	19.25	4.500	25.00	9.49	12.66	10.15	60.25	0.719	0.345	2.000	3.50
3	3	613.3	386.7	17.98	3.000	32.33	12.49	10.05	10.15	61.00	0.725	0.301	1.821	2.67
4	4	650.0	497.5	19.40	3.500	27.75	12.57	13.98	10.47	60.00	0.739	0.345	2.000	2.75
5	4	605.0	370.0	20.01	4.500	25.50	12.30	8.31	10.35	59.25	0.739	0.301	1.992	2.75
6	5	594.0	418.0	19.05	4.000	27.20	12.52	10.62	9.88	54.60	0.744	0.407	1.946	3.90
7	5	614.0	392.0	19.18	4.000	29.40	12.24	10.78	10.27	60.20	0.760	0.336	1.994	2.80
8	5	624.0	452.0	20.26	3.600	32.00	11.99	11.13	10.28	60.60	0.747	0.407	2.041	2.40
9	5	626.0	394.0	18.59	3.400	27.20	12.24	11.63	10.25	58.40	0.744	0.336	2.088	2.80
10	5	566.0	312.0	18.09	3.600	23.00	9.25	10.39	9.81	54.80	0.811	0.301	1.983	2.40
Mean		607.1	397.3	19.05	3.844	27.56	11.64	11.19	10.18	58.89	0.754	0.348	2.004	2.76

Analysis of variance. Coefficient of between clone component of expected mean square = 4.49

Source	df	MS $x10^{-2}$	MS $x10^{-2}$	MS	MS	MS	MS	MS	MS	MS	MS $x10^{-3}$	MS $x10^{-3}$	MS $x10^{-2}$	MS
Between clones	9	30.82	136.78 *	2.439	0.946	37.01	6.963	9.801	0.196	28.11	3.681	7.236	2.258	0.438
Ramets within clones	35	32.40	59.82	2.200	0.497	51.49	6.302	11.001	0.319	85.81	3.721	5.936	3.275	0.411

Trial C - Family 11 - clone means and analyses of variance

Clone No.	No. Ramets	PH (mm)	PD (mm)	LL (mm)	LB (mm)	SG	FW (g)	No Pan	Pan L (mm)	Pan B (mm)	Sp L (mm)	Sp B (mm)	Sd/Sp	Sd H
				SQRT		SQUARE	SQRT	SQRT	SQRT		Log_{10}	Log_{10}	SQRT	
1	5	750.0	328.0	17.63	5.600	29.40	12.55	7.36	11.23	59.20	0.818	0.336	2.142	2.40
2	4	497.5	127.5	16.03	2.750	3.25	4.61	7.09	11.16	45.25	1.041	0.345	2.544	1.75
3	3	320.0	66.7	10.36	2.667	1.00	3.29	3.62	9.20	41.33	0.693	0.301	1.626	1.33
4	4	662.5	137.5	20.15	5.250	21.50	9.22	2.79	12.83	84.75	0.977	0.508	2.691	2.00
5	5	592.0	208.0	17.80	3.200	15.00	8.80	7.59	10.67	58.40	0.857	0.336	2.088	1.40
6	3	640.0	140.0	17.42	3.000	9.67	9.60	5.11	9.39	39.33	0.859	0.360	2.061	1.33
7	4	402.5	150.0	12.32	2.750	14.75	3.74	8.67	10.42	34.75	0.828	0.301	1.866	2.25
8	3	646.7	183.3	15.84	4.000	44.67	5.34	4.56	12.59	77.00	1.090	0.301	2.646	1.33
9	4	427.5	130.0	16.37	4.000	22.75	6.87	1.90	10.68	50.25	0.924	0.345	2.280	2.00
Mean		556.9	172.3	16.21	3.771	18.20	7.406	5.61	10.96	54.89	0.897	0.350	2.219	1.80

Analysis of variance. Coefficient of between clone component of expected mean square = 3.87

Source	df	MS $x10^{-2}$	MS $x10^{-2}$	MS	MS	MS	MS	MS	MS	MS	MS $x10^{3}$	MS $x10^{3}$	MS $x10^{2}$	MS
Between clones	8	783.64 ***	233.22 ***	31.646 ***	5.157 ***	619.23 ***	39.950 ***	23.015 ***	5.133 ***	1068.15 ***	51.150 ***	15.840 *	45.260 ***	0.713
Ramets within clones	26	37.56	23.48	1.722	0.497	44.38	2.920	4.757	0.394	44.55	4.715	4.943	3.915	0.304

Trial C - Family 12 - clone means and analyses of variance

Clone No.	No. Ramets	PH (mm)	PD (mm)	LL (mm)	LB (mm)	SG	FW (g)	No Pan	Pan L (mm)	Pan B (mm)	Sp L (mm)	Sp B (mm)	Sd/Sp	Sd H
				SQRT		SQUARE	SQRT	SQRT	SQRT		Log_{10}	Log_{10}	SQRT	
1	4	545.0	355.0	17.37	3.750	25.00	10.42	10.25	10.29	46.25	0.809	0.508	2.177	3.25
2	4	477.5	312.5	16.25	3.250	20.50	8.92	10.18	9.13	45.75	0.675	0.477	1.933	2.75
3	5	504.0	454.0	17.35	3.800	23.20	10.41	8.76	9.01	45.20	0.760	0.442	2.041	2.80
4	5	522.0	380.0	16.69	4.000	21.40	10.11	7.82	9.27	42.40	0.731	0.442	1.893	3.00
5	5	510.0	382.0	17.13	3.600	23.60	11.35	8.12	9.23	47.40	0.762	0.502	2.094	3.60
6	5	512.0	340.0	16.99	4.400	23.20	10.20	9.36	9.19	42.80	0.805	0.477	2.142	3.40
7	4	347.5	375.0	16.84	3.250	17.00	8.31	4.03	8.56	32.25	0.790	0.464	2.171	2.75
8	5	514.0	410.0	17.85	3.800	21.80	11.40	9.32	9.43	42.60	0.830	0.477	2.189	3.40
9	4	510.0	357.5	16.79	4.000	22.75	10.95	8.03	9.40	46.00	0.795	0.508	2.059	3.00
10	5	504.0	358.0	17.52	4.200	19.60	10.72	8.88	9.04	47.00	0.760	0.442	2.184	2.80
Mean		496.7	374.3	17.10	3.826	21.85	10.33	8.50	9.25	43.87	0.772	0.473	2.089	3.09

Analysis of variance. Coefficient of between clone component of expected mean square = 4.59

Source	df	MS $x10^{-2}$	MS $x10^{-2}$	MS	MS	MS	MS	MS	MS	MS	MS $x10^{-3}$	MS $x10^{-3}$	MS $x10^{-2}$	MS
Between clones	9	119.82 ***	70.78	0.976	0.618	22.69	4.217	12.828	0.787 **	82.20	8.827 **	3.254	5.250 *	0.467
Ramets within clones	36	23.99	52.23	3.125	0.585	33.33	3.370	4.131	0.206	42.10	2.867	4.343	2.391	0.596

Trial C - Family 13 - clone means and analysis of variance

Clone No.	No. Ramets	PH (mm)	PD (mm)	LL (mm)	LB (mm)	SG	FW (g)	No Pan	Pan L (mm)	Pan B (mm)	Sp L (mm)	Sp B (mm)	Sd/Sp	Sd H
				SQRT		SQUARE	SQRT	SQRT	SQRT		Log_{10}	Log_{10}	SQRT	
1	5	656.0	486.0	19.17	2.800	29.40	9.60	7.18	10.45	47.80	0.770	0.407	2.239	2.40
2	5	736.0	518.0	19.21	3.000	36.00	10.88	8.96	10.63	51.60	0.792	0.371	2.322	3.00
3	4	760.0	482.5	19.73	3.000	33.25	11.60	10.80	10.61	50.25	0.778	0.345	2.230	2.00
4	5	806.0	528.0	19.19	2.800	31.60	10.51	8.82	10.38	44.40	0.778	0.407	2.189	2.60
5	5	752.0	544.0	18.76	2.600	36.00	12.25	9.94	10.85	52.40	0.787	0.396	2.221	2.40
6	5	752.0	364.0	19.16	2.800	32.00	10.33	6.76	10.48	40.20	0.818	0.336	2.322	2.20
7	5	736.0	450.0	19.94	3.000	29.40	10.33	8.78	10.85	58.00	0.812	0.467	2.395	2.20
8	4	720.0	517.5	20.02	3.500	36.00	10.83	8.73	10.74	44.00	0.758	0.389	2.171	2.50
9	5	414.0	274.0	17.08	3.600	16.00	8.85	7.31	8.72	38.00	0.805	0.477	2.279	1.80
10	4	745.0	490.0	19.31	3.000	33.25	10.18	8.80	10.36	44.75	0.739	0.433	2.333	2.00
Mean		705.5	463.4	19.12	3.000	31.11	10.513	8.55	10.40	47.19	0.785	0.404	2.272	2.32

Analysis of variance. Coefficient of between clone component of expected mean square = 4.70

Source	df	MS $x10^{-2}$	MS $x10^{-2}$	MS	MS	MS	MS	MS	MS	MS	MS $x10^{3}$	MS $x10^{3}$	MS $x10^{2}$	MS
Between clones	9	597.22 ***	351.63 ***	3.304	0.467	171.93 ***	4.398	7.216	1.892 **	180.73	2.727	10.370	2.403	0.579
Ramets within clones	37	46.77	52.00	4.388	0.319	28.90	5.834	5.832	0.478	89.21	3.189	9.087	6.086	0.351

Trial C - Family 14 - clone means and analyses of variance

Clone No.	No. Ramets	PH (mm)	PD (mm)	LL (mm)	LB (mm)	SG	FW (g)	No Pan	Pan L (mm)	Pan B (mm)	Sp L (mm)	Sp B (mm)	Sd/Sp	Sd H
				SQRT		SQUARE	SQRT	SQRT	SQRT		Log_{10}	Log_{10}	SQRT	
1	5	556.0	378.0	17.94	4.600	13.20	14.29	8.07	9.31	51.40	0.803	0.407	2.142	2.60
2	4	590.0	407.5	17.40	4.500	20.50	17.40	7.34	9.84	51.75	0.828	0.477	2.236	3.25
3	4	600.0	425.0	17.79	5.250	18.25	17.01	7.26	9.51	55.25	0.795	0.389	2.118	2.75
4	4	617.5	350.0	17.99	4.500	18.25	16.98	7.57	9.52	57.75	0.778	0.433	2.236	3.25
5	4	555.0	365.0	19.14	4.500	16.00	15.82	8.05	9.24	49.75	0.775	0.477	2.177	2.75
6	5	548.0	350.0	17.71	4.400	19.60	13.55	6.38	8.93	48.80	0.776	0.407	2.279	2.80
7	5	588.0	420.0	17.54	5.000	19.60	15.30	10.16	9.31	49.60	0.792	0.407	2.232	2.80
8	4	620.0	372.5	16.88	4.250	22.75	16.70	10.28	9.24	49.00	0.778	0.477	2.059	2.75
9	3	616.7	460.0	17.62	4.000	19.00	14.44	8.15	9.38	54.00	0.774	0.418	2.079	3.00
10	4	592.5	425.0	17.94	4.000	16.50	15.69	6.45	8.89	47.25	0.778	0.389	2.177	2.25
Mean		586.0	392.9	17.79	4.524	18.29	15.65	7.99	9.31	51.29	0.788	0.427	2.179	2.81

Analysis of variance. Coefficient of between clone component of expected mean square = 4.19

Source	df	MS $x10^{-2}$	MS $x10^{-2}$	MS	MS	MS	MS	MS	MS	MS	MS $x10^{-3}$	MS $x10^{-3}$	MS $x10^{-2}$	MS
Between clones	9	31.13	54.14	1.335	0.620	31.24	7.507	7.962	0.329	43.74	1.230	5.357	2.214	0.353
Ramets within clones	32	49.31	44.10	1.552	0.591	21.86	7.326	4.861	0.221	20.84	1.716	6.800	1.321	0.416

Trial C - Family 15 - clone means and analyses of variance

Clone No.	No. Ramets	PH (mm)	PD (mm)	LL (mm)	LB (mm)	SG	FW (g)	No Pan	Pan L (mm)	Pan B (mm)	Sp L (mm)	Sp B (mm)	Sd/Sp	Sd H
				SQRT		SQUARE	SQRT	SQRT	SQRT		Log_{10}	Log_{10}	SQRT	
1	4	505.0	365.0	15.61	4.250	28.25	12.11	6.62	8.11	38.00	0.755	0.389	2.051	3.50
2	4	535.0	307.5	16.06	3.000	30.50	12.21	8.51	8.64	37.00	0.739	0.433	2.118	3.25
3	5	552.0	342.0	16.46	3.600	27.20	13.09	8.39	8.21	36.60	0.715	0.336	2.094	2.60
4	5	560.0	372.0	16.43	4.000	25.40	14.15	9.08	8.48	41.00	0.744	0.371	2.142	2.60
5	5	524.0	348.0	16.60	3.800	29.40	13.38	7.42	8.68	42.60	0.805	0.477	2.236	3.20
6	5	576.0	368.0	16.44	3.600	32.00	13.51	8.59	8.59	39.00	0.760	0.336	2.131	3.00
7	5	436.0	440.0	16.58	4.600	29.40	11.95	4.54	8.21	32.60	0.820	0.477	2.084	3.40
8	5	596.0	344.0	16.66	4.000	33.80	14.17	9.05	8.96	43.60	0.731	0.407	2.142	3.00
9	4	555.0	360.0	16.96	3.750	36.00	12.55	9.15	8.37	38.50	0.758	0.433	2.236	3.00
10	3	610.0	286.7	16.63	3.667	29.33	13.29	9.64	8.55	41.33	0.725	0.360	1.911	2.33
Mean		542.9	356.9	16.45	3.844	30.07	13.08	8.03	8.49	39.00	0.757	0.403	2.122	3.00

Analysis of variance. Coefficient of between clone component of expected mean square = 4.49

Source	df	MS $x10^{-2}$	MS $x10^{-2}$	MS	MS	MS	MS	MS	MS	MS	MS $x10^{-3}$	MS $x10^{-3}$	MS $x10^{-2}$	MS
Between clones	9	111.63 **	70.54 *	0.554	0.816	44.35	3.043	10.798	0.320	51.28	5.444	13.130	3.187	0.576
Ramets within clones	35	36.76	45.91	2.071	0.416	51.59	2.429	6.064	0.393	29.44	3.875	6.350	3.062	0.423

Trial C - Family 16 - clone means and analyses of variance

Clone No.	No. Ramets	PH (mm)	PD (mm)	LL (mm)	LB (mm)	SG	FW (g)	No Pan	Pan L (mm)	Pan B (mm)	Sp L (mm)	Sp B (mm)	Sd/Sp	Sd H
				SQRT		SQUARE	SQRT	SQRT	SQRT		Log_{10}	Log_{10}	SQRT	
1	4	565.0	432.5	18.38	4.500	22.75	15.65	10.07	9.29	49.00	0.699	0.301	1.867	2.75
2	5	590.0	452.0	19.35	4.400	23.20	13.89	9.17	9.38	47.60	0.680	0.311	1.829	2.40
3	5	560.0	372.0	18.94	4.400	20.00	14.13	8.92	9.19	48.40	0.711	0.336	1.930	2.60
4	5	578.0	448.0	18.72	4.400	23.20	14.08	12.17	9.27	48.60	0.676	0.371	1.987	2.80
5	4	505.0	417.5	18.52	4.750	27.75	13.44	7.55	9.32	46.25	0.699	0.389	1.933	3.00
6	4	577.5	380.0	18.96	4.250	25.00	14.07	9.71	9.31	44.75	0.739	0.389	1.925	2.50
7	4	350.0	275.0	16.69	4.500	30.50	9.36	2.62	8.73	40.00	0.758	0.389	2.059	3.25
8	4	567.5	392.5	19.16	4.000	22.75	13.94	9.51	8.97	46.00	0.695	0.345	1.866	3.00
9	5	504.0	396.0	18.47	4.000	21.40	12.93	8.82	9.23	46.00	0.715	0.301	1.839	2.40
10	5	530.0	316.0	17.45	4.200	18.20	11.97	7.13	9.15	44.00	0.715	0.371	1.893	2.80
Mean		534.9	389.1	18.48	4.333	23.22	13.35	8.641	9.19	46.16	0.708	0.349	1.911	2.73

Analysis of variance. Coefficient of between clone component of expected mean square = 4.49

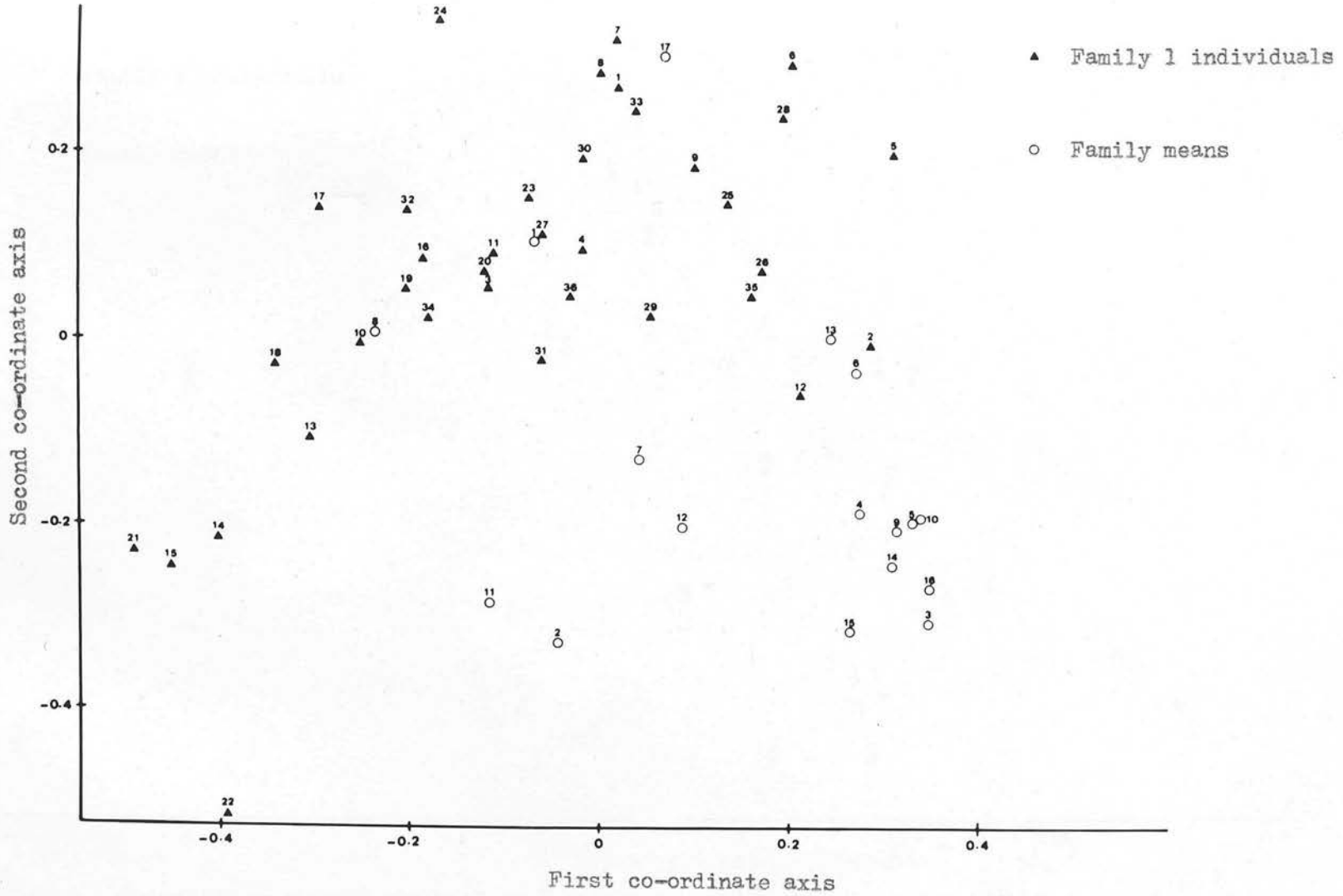
Source	df	MS $x10^{-2}$	MS $x10^{-2}$	MS	MS	MS	MS	MS	MS	MS	MS $x10^{3}$	MS $x10^{3}$	MS $x10^{2}$	MS
Between clones	9	208.86 ***	143.07 **	2.888	0.233	55.88	11.782	26.830 *	0.158	31.20	2.768	5.892	2.203	0.344
Ramets within clones	35	21.19	38.97	2.580	0.740	30.48	6.939	10.590	0.183	28.95	2.506	10.500	4.247	0.506

Trial C - Family 17 - clone means and analyses of variance

Clone No.	No. Ramets	PH (mm)	PD (mm)	LL (mm)	LB (mm)	SG	FW (g)	No Pan	Pan L (mm)	Pan B (mm)	Sp L (mm)	Sp B (mm)	Sd/Sp	Sd H
				SQRT		SQUARE	SQRT	SQRT	SQRT		Log_{10}	Log_{10}	SQRT	
1	2	680.0	85.0	21.15	4.000	16.00	5.40	2.95	12.26	54.50	1.114	0.151	2.450	1.00
2	2	565.0	220.0	17.69	2.500	17.00	8.47	8.23	10.18	40.00	0.801	0.301	1.732	2.00
3	2	645.0	75.0	19.30	4.000	37.00	6.27	3.35	11.77	36.00	0.977	0.301	2.225	1.00
4	3	680.0	103.3	18.35	3.667	36.00	7.25	2.48	12.18	46.00	0.999	0.301	2.229	1.00
5	3	743.3	126.7	21.39	3.333	44.67	8.64	5.57	13.08	50.00	1.040	0.201	2.373	1.00
6	3	570.0	140.0	16.69	3.333	23.33	4.89	2.16	10.80	42.00	1.040	0.301	2.378	1.33
7	4	825.0	132.5	19.66	3.750	35.75	10.07	5.78	10.70	47.50	1.038	0.301	2.491	1.50
8	3	780.0	130.0	20.43	4.000	49.00	11.29	5.61	12.90	57.33	1.076	0.301	2.444	1.00
Mean		700.0	126.8	19.34	3.591	33.73	8.04	4.53	11.73	47.14	1.018	0.274	2.320	1.23

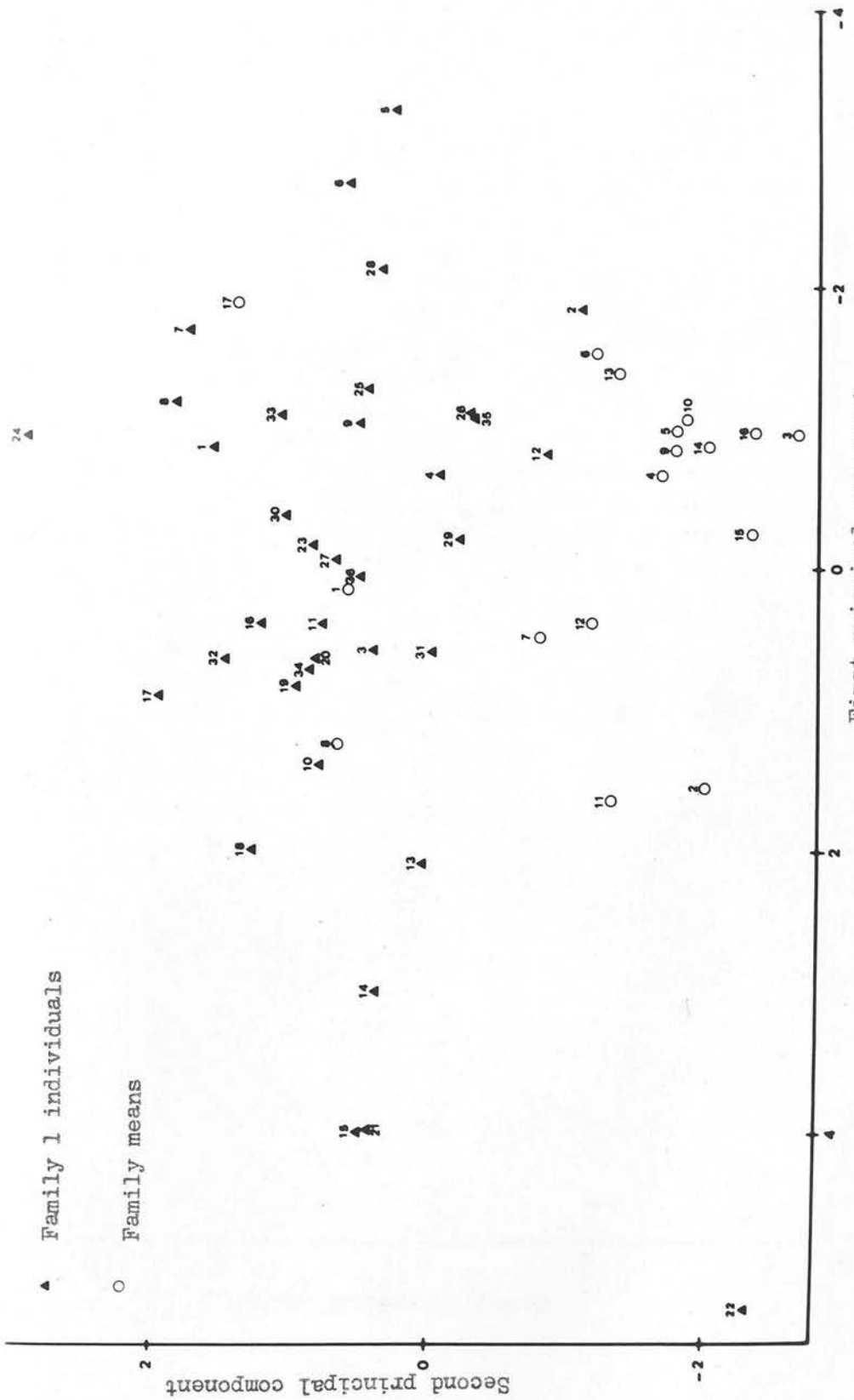
Analysis of variance. Coefficient of between clone component of expected mean square = 2.73

Source	df	MS $x10^{-2}$	MS $x10^{-2}$	MS	MS	MS	MS	MS	MS	MS	MS $x10^{3}$	MS $x10^{3}$	MS $x10^{2}$	MS
Between clones	7	260.76 **	40.81	7.485	0.581	374.90	14.476	11.090	3.198	125.49	18.800 **	8.433	13.570 *	0.314
Ramets within clones	14	50.48	41.36	5.766	0.232	140.29	6.657	5.457	4.971	83.01	3.815	7.550	4.362	0.262

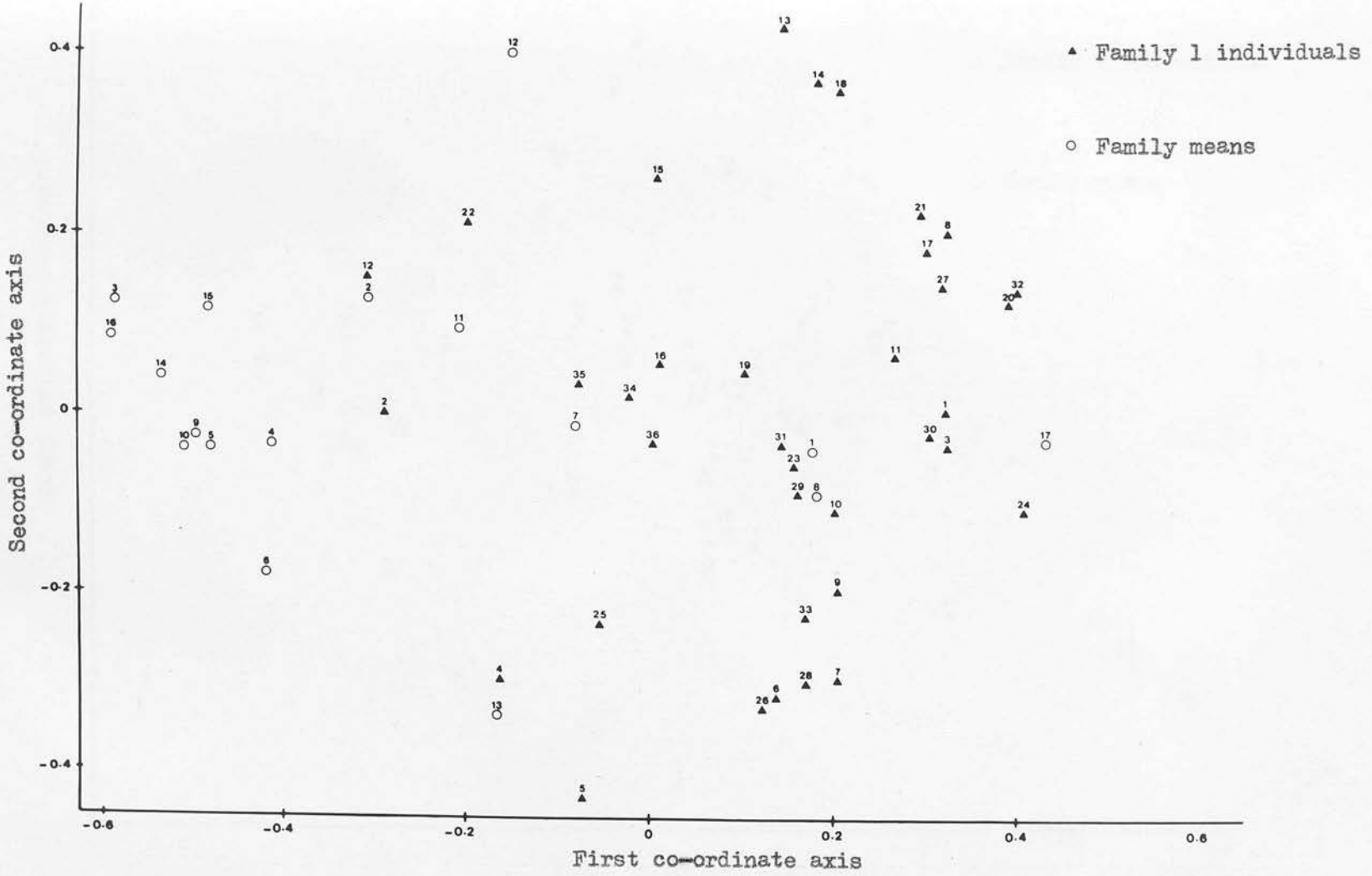


Trial D Principal co-ordinate analysis on 7 variates, family 1 individuals and 17 family means

APPENDIX FIGURE 2

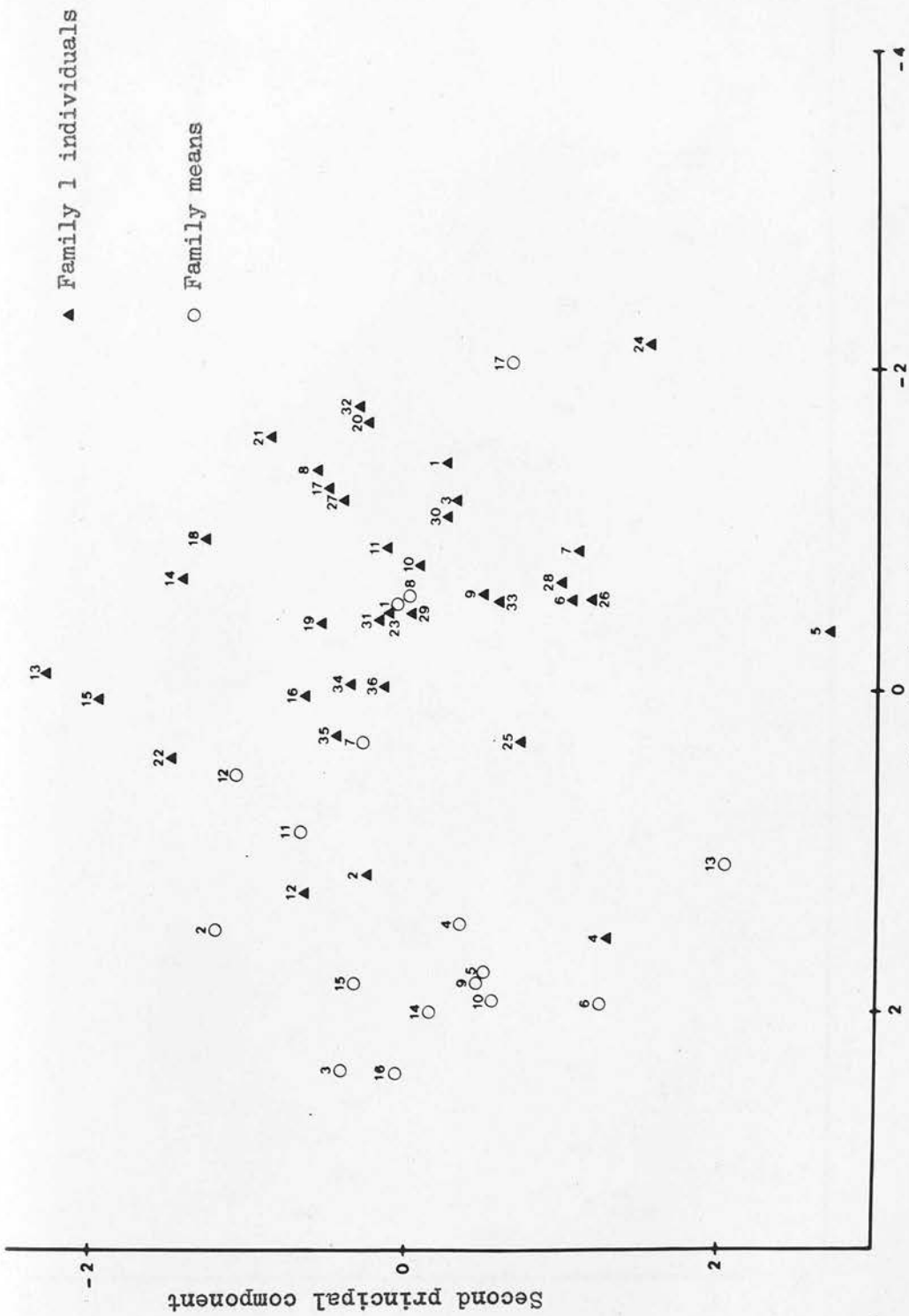


Trial D Principal component analysis on 7 variates, family 1 individuals and 17 family means

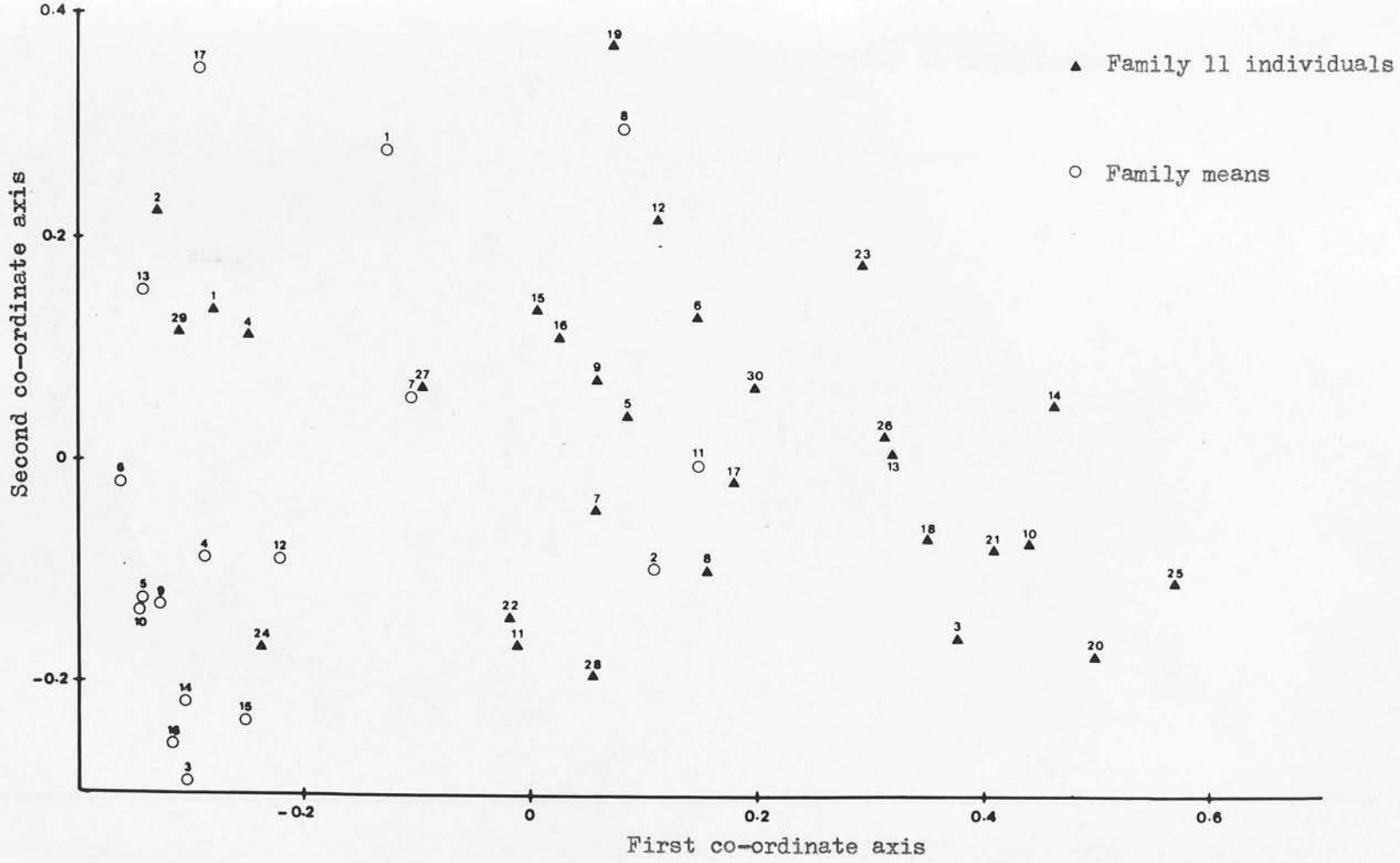


Trial D Principal co-ordinate analysis on 3 variates, family 1 individuals and 17 family means

APPENDIX FIGURE 4

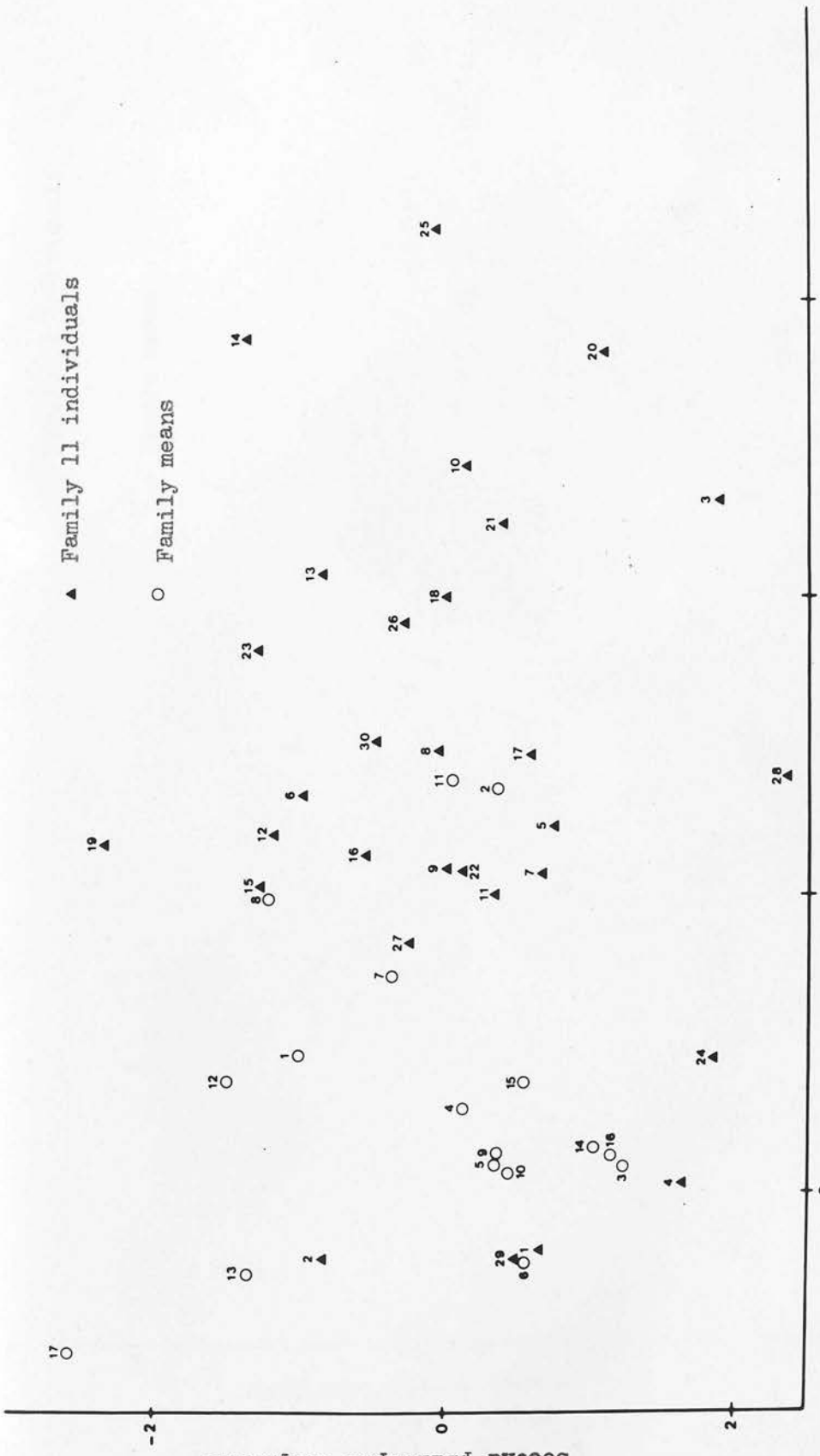


Trial D Principal component analysis on 3 variates, family 1 individuals and 17 family means

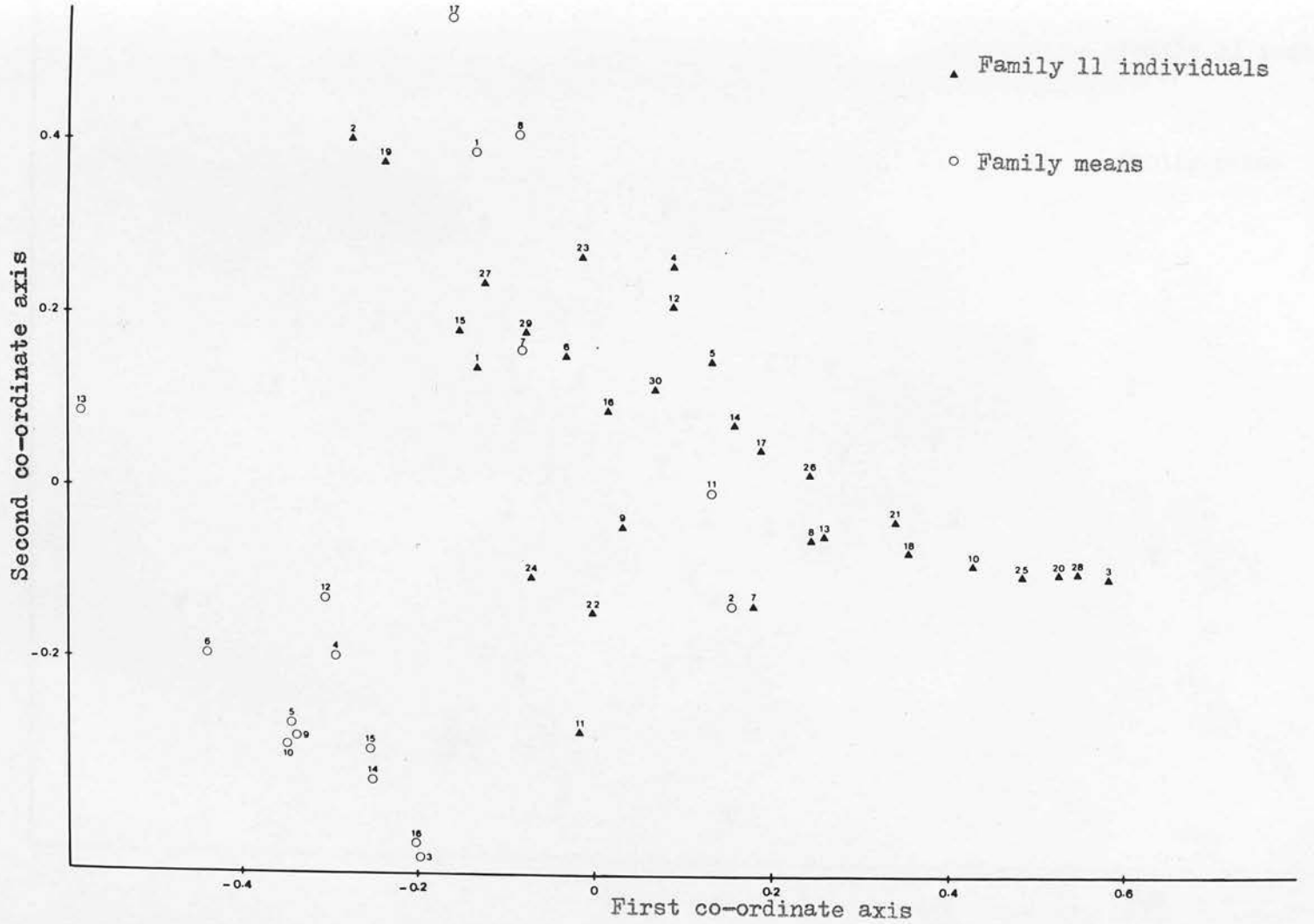


Trial D Principal co-ordinate analysis on 7 variates, family 11 individuals and 17 family means

APPENDIX FIGURE 6

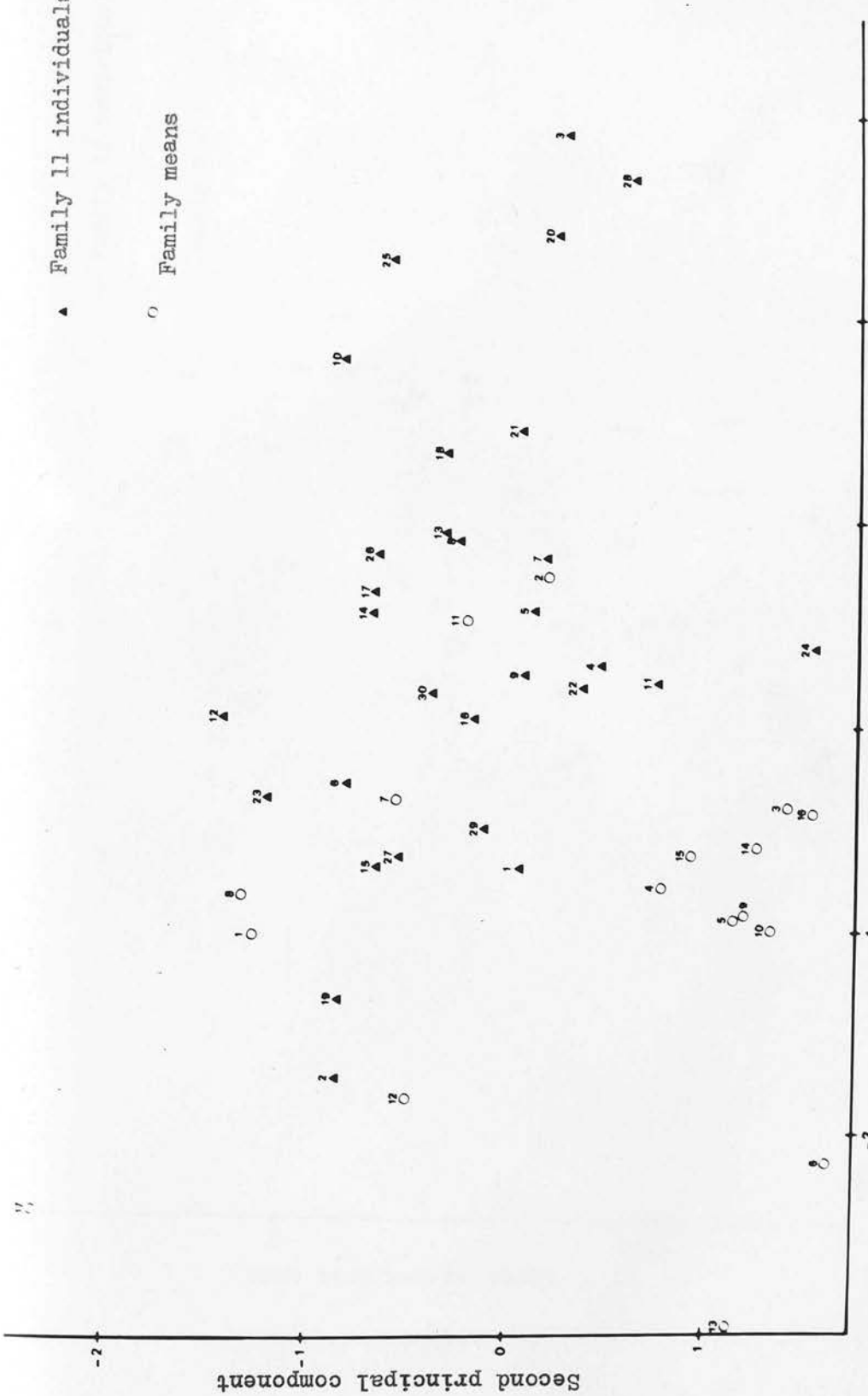


Trial D Principal component analysis on 7 variates, family 11 individuals and 17 family means

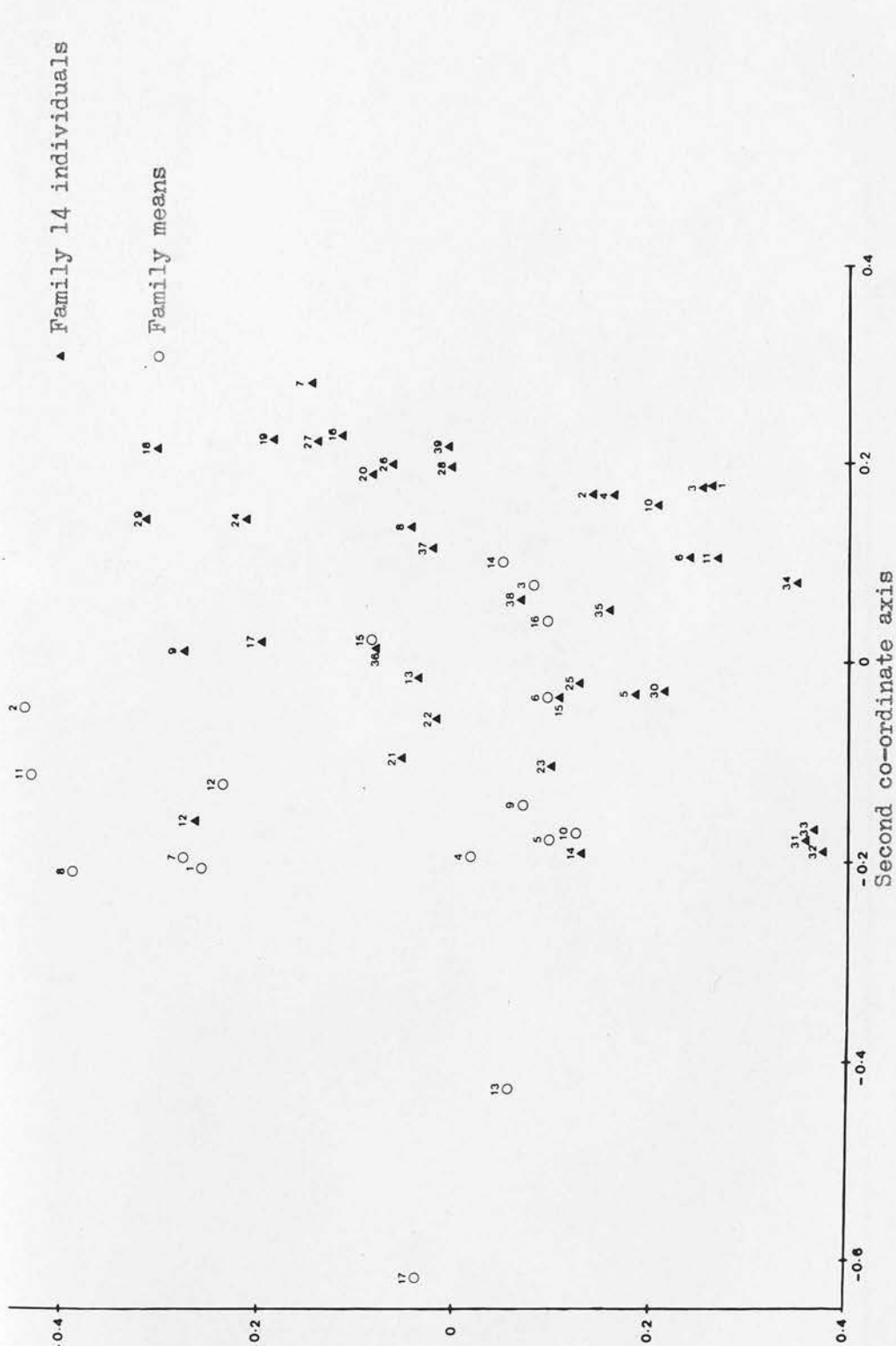


Trial D Principal co-ordinate analysis on 3 variates, family 11 individuals and 17 family means

APPENDIX FIGURE 8

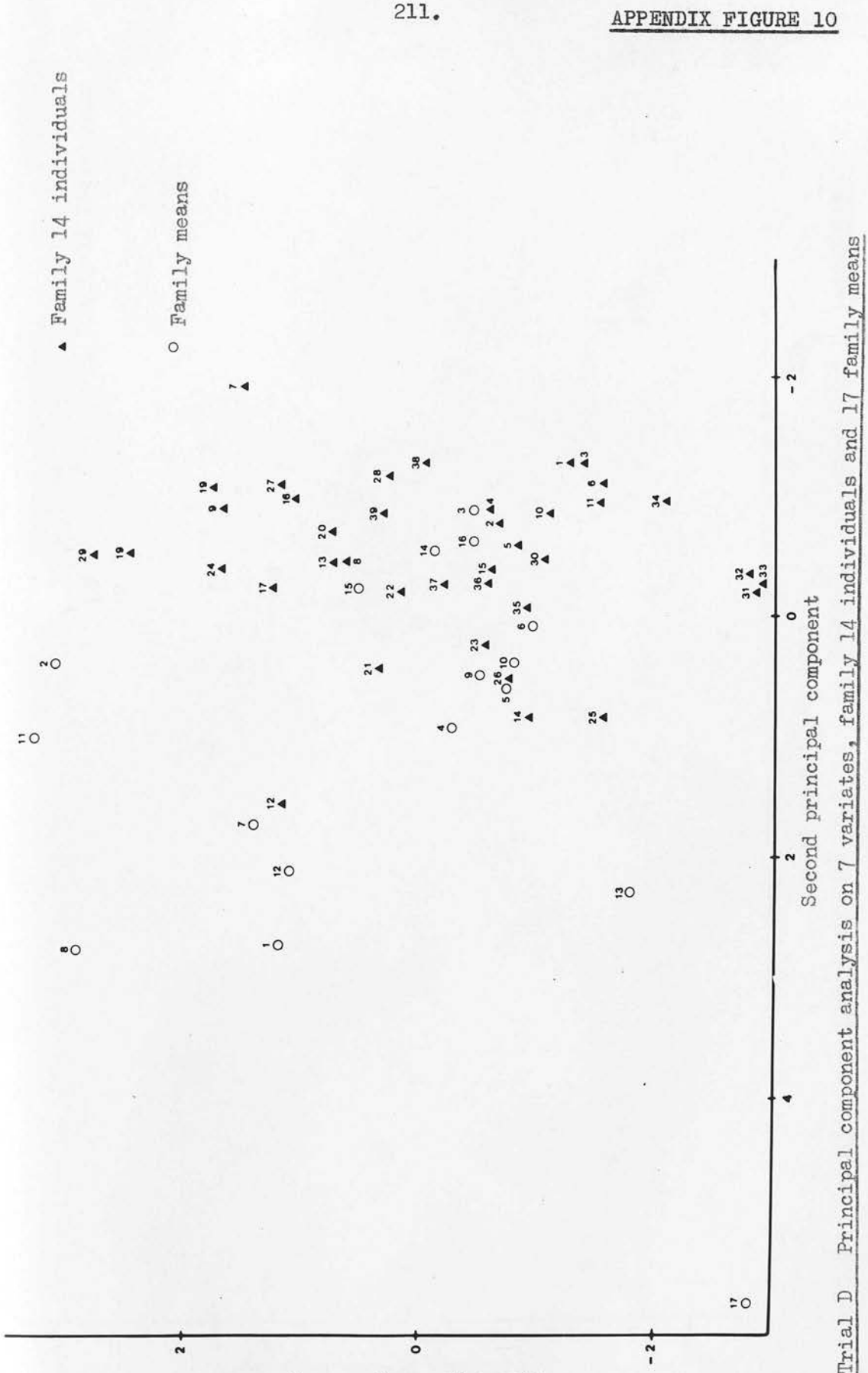


Trial D Principal component analysis on 3 variates, family 11 individuals and 17 family means



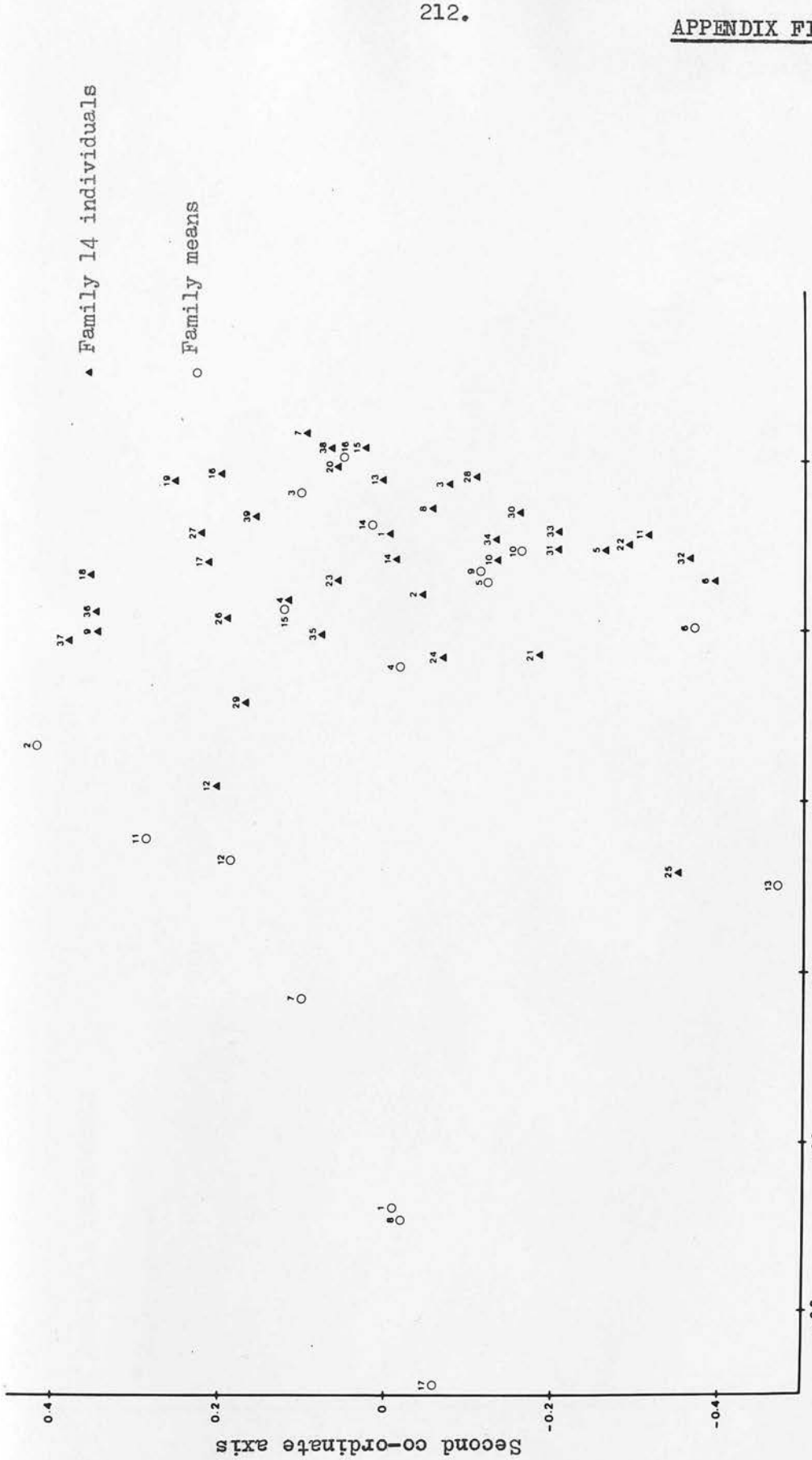
Trial D Principal co-ordinate analysis on 7 variates, family 14 individuals and 17 family means

APPENDIX FIGURE 10



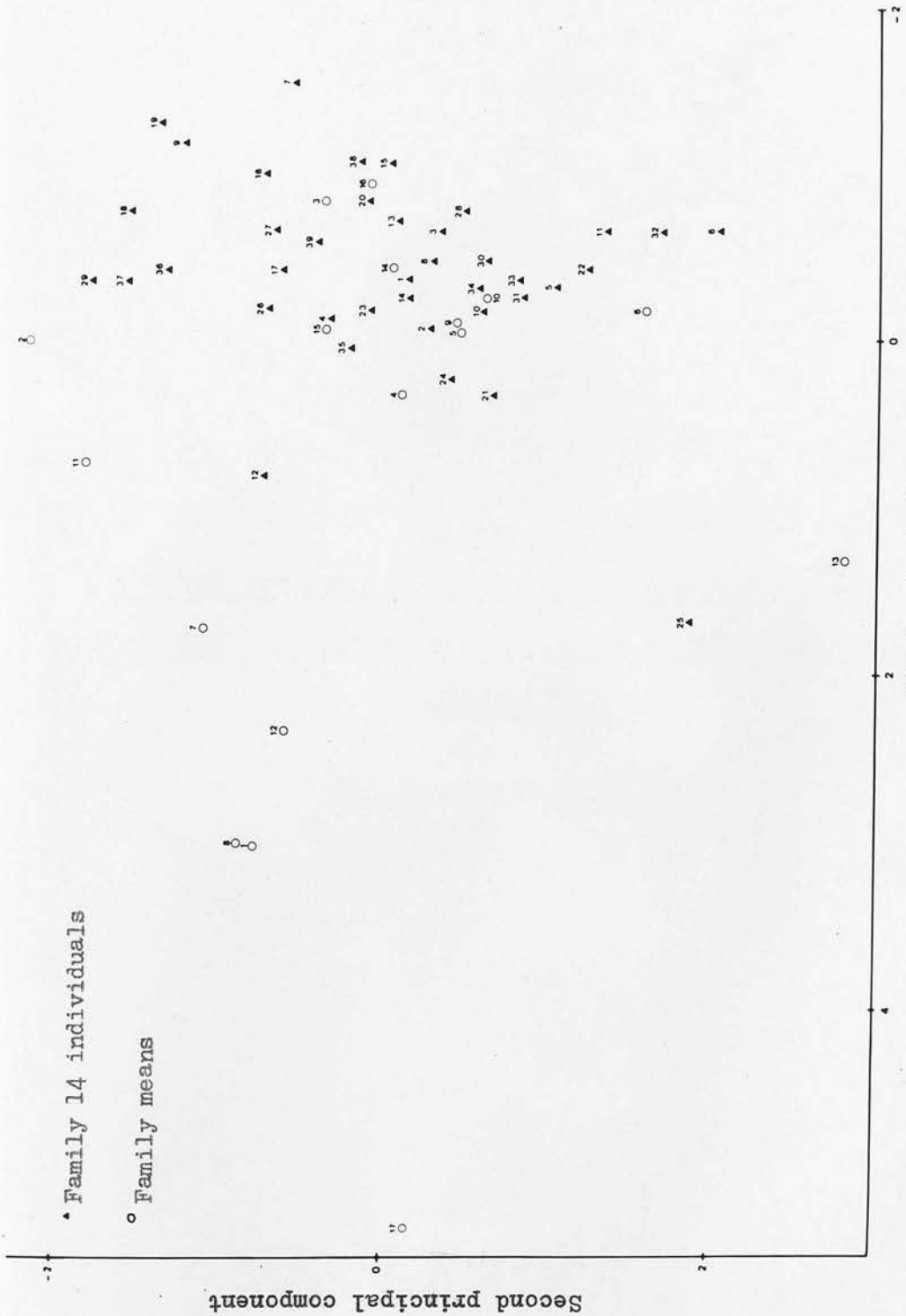
Trial D Principal component analysis on 7 variates, family 14 individuals and 17 family means

APPENDIX FIGURE 11



Trial D Principal co-ordinate analysis on 3 variates, family 14 individuals and 17 family means

APPENDIX FIGURE 12



Trial D Principal component analysis on 3 variates, family 14 individuals and 17 family means

CHAPTER 5

THE INFLUENCE OF LIGHT REGIMES ON APOMICTIC SEED PRODUCTION IN P. AMPLA AND P. PRATENSIS

(TRIALS E and F)

APPENDIX TABLES 20 to 28

APPENDIX TABLE 20

Trial F - Light regime means for three crosses within each biotype

Biotype	Cross	Light regime				
		A	B	C	AC	CA
1000 Seed weight (mg)						
P. ampla 42	1	648	473	592	670	573
	2	655	642	670	582	538
	3	502	593	525	545	613
P. pratensis 145	1	393	258	265	387	206
	2	372	232	307	331	190
	3	343	252	365	302	210
P. pratensis 159	1	290	372	412	535	529
	2	255	340	556	458	490
	3	290	418	470	375	485
P. pratensis 190	1	459	373	444	403	372
	2	393	377	553	368	410
	3	293	333	380	352	317
Number of multiple seedlings (Log_{10} (data + 1))						
P. ampla 42	1	0.301	0	0	0	0
	2	0.301	0.301	0	0	0.301
	3	0.602	0.301	0	0.301	0.301
P. pratensis 145	1	0.602	0.301	0.778	0	0.477
	2	0	1.114	0.699	0.477	0.477
	3	0	0.301	0.699	0.699	0.699
P. pratensis 159	1	1.041	0.602	0.699	0.778	0.699
	2	0.778	0.778	0.954	0.954	0.301
	3	0.845	0.845	0.845	0.477	0
P. pratensis 190	1	0	0.602	0.477	0	0.301
	2	0.301	0.301	0.477	0.301	0
	3	0	0.602	0.602	0.477	0.602

APPENDIX TABLE 21

Trial F - Light regime means for three crosses within each biotype

Biotype	Cross	Light regime				
		A	B	C	AC	CA
Number of aberrant seedlings (Log_{10})						
P. ampla 42	1	0.602	0.699	0.477	0.477	0.602
	2	0.477	0.301	0.477	0.845	0.845
	3	0.903	0.477	0.954	0.477	0.477
P. pratensis 145	1	0.845	0.778	0.477	0.903	0.477
	2	0.699	0.477	0.477	1.000	0.845
	3	0.845	0.845	0.602	0.699	0.699
P. pratensis 159	1	0.778	0.954	0.477	0.954	0.778
	2	0.699	0.903	0.699	0.778	0.903
	3	1.000	0.778	0.903	0.602	0.477
P. pratensis 190	1	1.204	0.477	0.477	1.230	0.778
	2	1.146	0.903	1.000	1.176	0.602
	3	1.041	0.903	0.602	1.146	0.477
Number of germinated seeds - day 8 (Log_{10} (data + 1))						
P. ampla 42	1	1.431	0.699	0.903	1.114	1.204
	2	1.380	1.230	0.845	0.903	0.845
	3	1.602	0.477	1.079	0.954	1.362
P. pratensis 145	1	0.301	0	0.301	0.477	0
	2	0.845	0	0	0.301	0
	3	0.301	0.301	0	0.477	0
P. pratensis 159	1	1.602	1.362	0.602	0.845	1.114
	2	1.568	1.519	0.903	1.204	1.544
	3	1.613	1.462	1.380	1.342	1.519
P. pratensis 190	1	1.531	1.146	0	0.778	1.041
	2	1.431	1.146	0	1.255	0.778
	3	1.380	1.176	0.778	1.255	0.602

APPENDIX TABLE 22

Trial F - Light regime means for three crosses within each biotype

Biotype	Cross	Light regime				
		A	B	C	AC	CA
Second leaf date (Log_{10})						
P. ampla 42	1	1.413	1.480	1.467	1.439	1.457
	2	1.445	1.427	1.445	1.475	1.477
	3	1.465	1.474	1.480	1.443	1.442
P. pratensis 145	1	1.485	1.469	1.563	1.485	1.460
	2	1.463	1.620	1.535	1.493	1.494
	3	1.461	1.541	1.502	1.482	1.469
P. pratensis 159	1	1.445	1.486	1.483	1.500	1.467
	2	1.462	1.484	1.473	1.471	1.477
	3	1.455	1.472	1.462	1.466	1.463
P. pratensis 190	1	1.505	1.485	1.487	1.497	1.514
	2	1.504	1.516	1.497	1.518	1.522
	3	1.531	1.505	1.503	1.517	1.536
Fourth leaf date - second leaf date (Log_{10})						
P. ampla 42	1	1.144	1.175	1.137	1.153	1.139
	2	1.124	1.159	1.142	1.160	1.134
	3	1.119	1.154	1.142	1.145	1.145
P. pratensis 145	1	1.130	1.132	1.118	1.150	1.147
	2	1.147	1.111	1.137	1.145	1.135
	3	1.139	1.133	1.148	1.143	1.141
P. pratensis 159	1	1.146	1.173	1.173	1.177	1.152
	2	1.165	1.169	1.177	1.174	1.153
	3	1.152	1.153	1.142	1.163	1.140
P. pratensis 190	1	1.171	1.177	1.180	1.177	1.184
	2	1.191	1.178	1.165	1.193	1.172
	3	1.171	1.161	1.171	1.161	1.186

APPENDIX TABLE 23

Trial F - Light regime means for three crosses within each biotype

Biotype	Cross	Light regime				
		A	B	C	AC	CA
Second leaf length (mm)						
P. ampla 42	1	51.25	39.84	39.16	40.78	38.47
	2	39.81	45.72	38.28	41.13	40.50
	3	35.26	38.45	39.25	42.94	39.44
P. pratensis 145	1	42.66	47.00	39.19	46.41	46.88
	2	54.48	31.34	43.63	40.15	45.25
	3	50.81	41.56	55.31	42.47	43.38
P. pratensis 159	1	42.25	42.03	43.97	39.59	49.88
	2	47.25	39.81	48.75	44.19	54.00
	3	39.28	41.81	45.53	47.59	56.31
P. pratensis 190	1	54.25	48.22	49.03	52.10	44.69
	2	48.09	44.25	47.34	38.34	40.25
	3	40.03	41.53	45.50	39.84	31.13
Second leaf breadth (mm x 10, SQRT)						
P. ampla 42	1	3.462	3.495	3.463	3.488	3.347
	2	3.333	3.381	3.584	3.553	3.394
	3	3.135	3.722	3.360	3.513	3.284
P. pratensis 145	1	2.941	2.968	2.859	3.085	2.959
	2	3.090	2.736	2.966	3.006	2.916
	3	3.070	2.856	3.105	2.938	3.000
P. pratensis 159	1	3.033	3.036	3.190	3.121	3.257
	2	3.103	3.023	3.306	3.199	3.035
	3	2.981	3.198	3.052	3.127	2.955
P. pratensis 190	1	2.783	2.867	3.154	3.030	2.828
	2	2.666	2.741	3.065	2.655	2.795
	3	2.628	2.830	2.907	2.772	2.660

APPENDIX TABLE 24

Trial F - Light regime means for three crosses within each biotype

Biotype	Cross	Light regime				
		A	B	C	AC	CA
Fourth leaf length (mm)						
P. ampla 42	1	116.06	107.35	101.94	108.06	92.00
	2	97.69	111.75	94.25	101.59	99.94
	3	89.58	101.32	104.18	101.97	98.84
P. pratensis 145	1	90.78	97.41	97.68	94.94	101.37
	2	110.16	89.25	103.34	88.15	104.72
	3	104.00	98.56	111.97	93.25	99.69
P. pratensis 159	1	98.97	91.72	93.47	90.50	106.41
	2	110.56	97.32	106.84	95.56	109.19
	3	89.16	90.28	94.06	104.91	100.84
P. pratensis 190	1	89.75	91.34	96.06	87.50	84.78
	2	85.47	82.78	77.25	75.87	81.66
	3	79.44	78.12	77.84	77.22	74.03
Fourth leaf breadth (mm)						
P. ampla 42	1	2.403	2.345	2.369	2.338	2.306
	2	2.231	2.500	2.459	2.463	2.453
	3	2.213	2.426	2.236	2.450	2.244
P. pratensis 145	1	2.284	2.334	2.058	2.344	2.334
	2	2.468	1.931	2.238	2.193	2.294
	3	2.497	2.131	2.413	2.266	2.341
P. pratensis 159	1	2.716	2.681	2.753	2.575	2.969
	2	2.844	2.694	2.884	2.778	2.981
	3	2.738	2.906	2.925	2.819	2.878
P. pratensis 190	1	2.641	2.419	2.563	2.633	2.250
	2	2.381	2.275	2.538	2.194	2.297
	3	2.200	2.403	2.459	2.297	2.053

APPENDIX TABLE 25

Trial F - Light regime means for three crosses within each biotype

Biotype	Cross	Light regime				
		A	B	C	AC	CA
Number of tillers at fourth leaf (SQRT)						
P. ampla 42	1	2.019	2.040	2.020	2.055	1.944
	2	1.975	2.120	2.080	2.046	2.044
	3	1.925	2.086	1.975	1.983	1.990
P. pratensis 145	1	1.828	1.912	1.721	1.947	1.950
	2	1.921	1.605	1.859	1.889	1.876
	3	1.911	1.714	1.987	1.795	1.873
P. pratensis 159	1	1.719	1.711	1.730	1.721	1.746
	2	1.740	1.697	1.798	1.753	1.717
	3	1.721	1.746	1.781	1.749	1.650
P. pratensis 190	1	1.808	1.972	2.052	1.949	1.801
	2	1.791	1.816	1.967	1.665	1.832
	3	1.676	1.855	1.956	1.837	1.716
Tillers per day (SQRT)						
P. ampla 42	1	0.504	0.530	0.543	0.537	0.536
	2	0.514	0.529	0.553	0.514	0.540
	3	0.494	0.529	0.519	0.517	0.504
P. pratensis 145	1	0.549	0.585	0.510	0.594	0.599
	2	0.608	0.465	0.565	0.588	0.563
	3	0.586	0.491	0.602	0.583	0.597
P. pratensis 159	1	0.494	0.487	0.502	0.483	0.479
	2	0.505	0.485	0.471	0.496	0.478
	3	0.498	0.511	0.525	0.503	0.502
P. pratensis 190	1	0.501	0.519	0.513	0.520	0.480
	2	0.478	0.488	0.514	0.462	0.502
	3	0.452	0.507	0.515	0.485	0.475

APPENDIX TABLE 26

Trial F - Light regime means for three crosses within each biotype

Biotype	Cross	Light regime				
		A	B	C	AC	CA
Fresh weight production (mg per day (SQRT))						
P. ampla 42	1	2.082	2.054	2.183	2.321	2.044
	2	2.034	2.146	2.212	1.975	2.132
	3	1.783	2.081	2.015	2.044	1.903
P. pratensis 145	1	1.649	1.816	1.236	1.775	1.898
	2	1.993	0.935	1.545	1.689	1.784
	3	1.894	1.300	1.802	1.690	1.865
P. pratensis 159	1	2.167	2.144	2.229	2.013	2.147
	2	2.303	2.076	2.056	2.200	2.299
	3	2.214	2.340	2.446	2.381	2.627
P. pratensis 190	1	1.963	2.179	2.005	2.004	1.706
	2	1.733	1.739	2.024	1.565	1.886
	3	1.417	2.067	2.048	1.756	1.530
Colour of leaf sheath						
P. ampla 42	1	1.313	1.355	1.188	1.438	1.219
	2	1.219	1.344	1.156	1.406	1.313
	3	1.226	1.355	1.250	1.219	1.219
P. pratensis 145	1	3.625	3.938	3.968	3.875	3.875
	2	3.871	3.938	3.906	3.741	4.031
	3	4.032	3.844	3.969	3.844	3.969
P. pratensis 159	1	2.750	2.750	3.000	3.094	2.938
	2	2.875	2.807	3.031	2.625	2.781
	3	2.938	2.750	3.219	3.063	3.000
P. pratensis 190	1	4.719	5.000	5.000	4.867	5.000
	2	4.719	4.938	4.938	4.719	5.000
	3	4.719	4.813	5.000	4.688	4.813

APPENDIX TABLE 27

Trial F - Light regime means for three crosses within each biotype

Biotype	Cross	Light regime				
		A	B	C	AC	CA
Ligule (Log_{10} (data + 1))						
P. ampla 42	1	0.761	0.775	0.778	0.778	0.773
	2	0.776	0.763	0.778	0.747	0.778
	3	0.747	0.778	0.761	0.778	0.765
P. pratensis 145	1	0.483	0.486	0.481	0.485	0.477
	2	0.481	0.481	0.481	0.482	0.481
	3	0.489	0.481	0.481	0.481	0.475
P. pratensis 159	1	0.600	0.597	0.604	0.574	0.584
	2	0.605	0.590	0.598	0.596	0.611
	3	0.590	0.571	0.590	0.597	0.589
P. pratensis 190	1	0.470	0.477	0.477	0.469	0.464
	2	0.468	0.477	0.477	0.470	0.477
	3	0.468	0.485	0.481	0.470	0.472
Auricle hairs						
P. ampla 42	1	1.000	1.000	1.000	1.031	1.000
	2	1.000	1.000	1.000	1.000	1.000
	3	1.000	1.000	1.000	1.000	1.000
P. pratensis 145	1	1.125	1.063	1.161	1.500	1.188
	2	1.258	1.031	1.094	1.185	1.344
	3	1.097	1.250	1.063	1.281	1.188
P. pratensis 159	1	3.594	3.469	3.656	3.406	3.688
	2	3.906	3.548	4.031	3.563	4.313
	3	3.469	3.594	3.688	3.969	4.063
P. pratensis 190	1	2.094	2.594	2.531	2.233	1.813
	2	2.250	2.063	2.125	1.719	2.156
	3	1.969	2.000	2.063	1.844	1.969

APPENDIX TABLE 28

Trial F - Light regime means for three crosses within each biotype

Biotype	Cross	Light regime				
		A	B	C	AC	CA
Leaf angle (data squared)						
P. ampla 42	1	1.094	1.000	1.000	1.188	1.000
	2	1.094	1.000	1.094	1.188	1.000
	3	1.194	1.000	1.214	1.250	1.094
P. pratensis 145	1	7.625	6.969	7.613	7.594	5.625
	2	6.581	8.063	7.594	7.148	7.188
	3	7.194	6.094	6.656	8.188	6.344
P. pratensis 159	1	8.969	9.969	8.250	7.813	8.625
	2	8.250	8.226	7.500	9.250	7.625
	3	7.344	7.781	8.313	6.813	7.438
P. pratensis 190	1	6.656	7.656	7.438	8.300	6.875
	2	6.813	6.656	8.781	6.438	8.406
	3	6.344	7.563	8.656	6.406	8.500
Tiller angle						
P. ampla 42	1	2.469	2.710	2.688	2.688	2.594
	2	2.563	2.500	2.625	2.625	2.625
	3	2.645	2.871	2.393	2.688	2.531
P. pratensis 145	1	6.281	6.375	5.645	6.188	6.688
	2	6.258	5.063	5.906	6.148	6.344
	3	6.258	6.031	6.219	6.156	5.781
P. pratensis 159	1	6.750	6.625	6.594	6.781	6.031
	2	7.125	6.516	5.813	6.781	6.469
	3	6.469	6.719	6.844	6.813	6.813
P. pratensis 190	1	5.719	6.250	6.281	5.667	5.969
	2	6.313	6.063	5.938	5.938	5.813
	3	5.844	6.094	6.250	5.500	6.000

CHAPTER 6

THE INFLUENCE OF LIGHT REGIMES ON SEED PRODUCTION AND RESULTING SEEDLING PROGENIES FROM INTERSPECIFIC HYBRIDS OF POA. (TRIAL G)

APPENDIX TABLES 29 to 45

and

APPENDIX FIGURES 13 to 23

Trial G - Correlation coefficients calculated (a) from 44 clone X light regime means (top right) and (b) from 44 seedling progeny X light regime means (bottom left)

(b) \ (a)	EE	Anth	No Pan	PH	FLL	FLB	Pan L	Pan B	FL/Sp	Sd/R	Sd Wt	% Germ	% Mult	
GD		0.872	-0.196	-0.429	-0.001	-0.238	-0.073	-0.012	-0.129	-0.273	-0.003	0.188	0.167	EE
2LD	0.977		-0.366	-0.071	0.315	0.021	0.277	0.304	-0.279	-0.164	0.239	0.071	0.123	Anth
4LD	0.936	0.978		-0.072	-0.613	-0.500	-0.446	-0.380	-0.069	0.531	-0.696	-0.150	-0.090	No Pan
2LL	0.338	0.311	0.249		0.495	0.186	0.666	0.514	-0.188	0.537	0.234	-0.495	0.053	PH
2LB	-0.464	-0.438	-0.386	0.047		0.642	0.696	0.419	0.019	-0.238	0.418	-0.254	-0.038	FLL
4LL	-0.034	-0.005	-0.007	0.785	0.355		0.331	0.451	-0.056	-0.453	0.437	0.053	-0.381	FLB
No T	-0.108	-0.228	-0.345	0.102	0.023	-0.108		0.519	-0.140	0.097	0.607	-0.070	-0.072	Pan L
Size	-0.360	-0.322	-0.338	0.393	0.560	0.561	0.094		-0.378	0.072	0.396	-0.143	-0.003	Pan B
Colour	0.133	0.202	0.202	0.499	-0.119	0.432	-0.486	0.403		-0.203	-0.038	-0.078	0.127	FL/Sp
Lig	-0.177	-0.195	-0.179	-0.379	0.359	-0.287	0.396	-0.236	-0.860		-0.319	-0.244	0.245	Sd/R
Au H	-0.035	0.085	0.164	0.377	0.351	0.580	-0.594	0.571	0.748	-0.464		0.261	-0.160	Sd Wt
Sh H	-0.005	0.115	0.207	0.098	0.187	0.417	-0.617	0.280	0.414	-0.144	0.702		-0.219	% Germ
LA	-0.426	-0.502	-0.550	0.076	-0.123	0.123	0.213	-0.073	0.031	-0.175	-0.206	-0.395		% Mult
TA	0.513	0.541	0.521	0.439	-0.020	0.300	-0.085	0.383	0.419	-0.410	0.447	0.158	-0.463	
(b) \ (a)	GD	2LD	4LD	2LL	2LB	4LL	No T	Size	Colour	Lig	Au H	Sh H	LA	TA

$r > \pm 0.298$ *, $r > \pm 0.385$ **, $r > \pm 0.481$ ***

Trial G - Correlation coefficients between data from parental clones and seedling progenies, calculated from 44 population X light regime means

	GD	2LD	4LD	2LL	2LB	4LL	No T	Size	Colour	Lig	Am H	Sh H	LA	TA
EE	-0.249	-0.258	-0.219	-0.252	0.192	-0.071	0.118	0.098	-0.345	0.331	-0.056	-0.006	-0.160	0.104
Anth	-0.297	-0.332	-0.315	-0.263	0.092	-0.100	0.136	-0.005	-0.350	0.305	-0.182	-0.097	0.102	-0.148
No Pan	0.248	0.272	0.234	0.176	-0.367	-0.028	0.109	0.008	0.217	-0.232	0.004	-0.073	-0.094	0.373
PH	0.233	0.157	0.067	0.171	-0.252	-0.024	0.252	-0.352	-0.149	0.089	-0.465	-0.269	0.340	-0.358
FLL	-0.205	-0.262	-0.298	-0.101	-0.100	-0.080	0.138	-0.230	-0.310	0.214	-0.394	-0.164	0.342	-0.530
FLB	-0.247	-0.223	-0.170	0.136	0.010	0.285	-0.389	0.094	0.333	-0.306	0.312	0.343	0.293	-0.301
Pan L	-0.363	-0.419	-0.458	-0.252	0.061	-0.194	0.253	-0.245	-0.399	0.440	-0.463	-0.162	0.421	-0.768
Pan B	0.076	0.085	0.091	-0.008	-0.004	0.053	-0.222	-0.143	0.055	0.065	0.097	0.227	0.103	-0.169
FL/Sp	0.089	0.113	0.130	-0.165	-0.075	-0.211	-0.046	-0.096	-0.115	0.062	-0.145	0.035	-0.311	-0.052
Sd/R	0.439	0.346	0.241	0.252	-0.367	-0.132	0.481	-0.234	-0.054	-0.027	-0.381	-0.444	0.181	0.103
Sd Wt	-0.479	-0.492	-0.436	-0.352	0.484	-0.042	-0.208	-0.039	-0.198	0.251	-0.025	0.100	0.297	-0.622
% Germ	-0.553	-0.543	-0.473	0.042	0.588	0.156	-0.056	0.394	0.144	0.043	0.398	0.068	0.097	-0.093
% Mult	0.485	0.425	0.397	-0.175	-0.420	-0.345	0.071	-0.640	-0.308	0.184	-0.378	-0.256	-0.049	-0.036

$r \geq \pm 0.298$ *, $r \geq \pm 0.385$ **, $r \geq \pm 0.481$ ***

APPENDIX TABLE 31

Trial G - Clone X light regime means

Ear emergence (square root)

Clone	Light regime				
	A	B	C	D	Mean
1	3.680	2.595	2.585	3.000	2.965
2	3.220	2.020	1.785	2.735	2.440
3	3.145	2.210	2.210	2.755	2.580
4	3.445	2.185	2.115	3.000	2.686
5	3.420	2.360	2.340	3.095	2.804
6	3.075	1.990	1.890	2.680	2.409
7	3.290	2.060	1.785	2.930	2.516
8	2.555	1.500	1.395	2.335	1.946
9	2.585	1.885	1.725	2.605	2.200
10	3.385	2.340	2.290	3.015	2.758
11	3.445	2.305	2.590	2.965	2.826

Anthesis

Clone	Light regime				
	A	B	C	D	Mean
1	15.290	11.000	11.300	13.875	12.866
2	14.000	10.300	9.300	13.700	11.825
3	13.700	10.575	11.000	13.700	12.244
4	13.700	9.600	10.000	13.600	11.725
5	14.900	11.600	11.400	15.300	13.300
6	13.400	9.300	9.300	13.100	11.275
7	12.100	9.000	9.000	12.900	10.750
8	13.365	10.300	10.200	13.800	11.916
9	10.500	9.000	8.300	11.300	9.775
10	14.000	10.400	10.500	13.575	12.119
11	14.400	10.400	11.500	13.900	12.550

APPENDIX TABLE 32

Trial G - Clone X light regime means

Number of panicles (square root)

Clone	Light regime				
	A	B	C	D	Mean
1	2.075	2.745	2.535	3.060	2.604
2	3.705	3.565	3.850	3.825	3.736
3	3.170	3.090	3.205	3.165	3.158
4	3.520	3.735	4.060	4.305	3.905
5	2.500	2.230	2.695	2.620	2.511
6	3.705	3.950	3.880	3.725	3.815
7	4.235	4.495	4.710	4.245	4.421
8	2.005	1.895	2.275	2.180	2.089
9	4.130	4.080	4.730	4.550	4.373
10	1.760	1.745	1.965	1.765	1.809
11	1.865	1.615	2.010	2.480	1.993

Plant height (cm, square root)

Clone	Light regime				
	A	B	C	D	Mean
1	7.130	9.230	9.630	10.095	9.021
2	8.535	9.770	10.250	10.170	9.681
3	7.460	8.720	9.270	9.675	8.781
4	6.665	7.810	8.365	8.960	7.950
5	6.140	7.930	9.450	9.630	8.288
6	8.760	9.865	10.515	10.670	9.953
7	6.400	7.250	8.735	8.600	7.746
8	8.835	10.260	11.300	11.030	10.356
9	6.260	6.320	7.085	7.720	6.846
10	6.590	8.030	8.820	9.055	8.124
11	6.315	7.790	8.005	8.480	7.648

APPENDIX TABLE 33

Trial G - Clone X light regime means

Flag leaf length (mm, Log_{10})

Clone	Light regime				
	A	B	C	D	Mean
1	1.927	1.771	1.710	1.863	1.818
2	1.683	1.781	1.734	1.743	1.735
3	1.681	1.619	1.591	1.604	1.623
4	1.737	1.580	1.564	1.737	1.654
5	1.766	1.862	1.818	1.722	1.792
6	1.702	1.761	1.735	1.832	1.757
7	1.266	1.438	1.357	1.276	1.334
8	2.056	1.984	1.934	2.015	1.997
9	1.431	1.434	1.435	1.414	1.428
10	1.740	1.692	1.730	1.681	1.711
11	1.675	1.668	1.624	1.515	1.620

Flag leaf breadth (mm $x10^{-1}$, square root)

Clone	Light regime				
	A	B	C	D	Mean
1	5.355	5.115	5.025	5.325	5.205
2	4.985	5.315	5.095	4.765	5.040
3	4.925	4.735	4.740	4.645	4.761
4	5.330	5.235	5.200	5.740	5.376
5	5.715	5.950	5.860	5.635	5.790
6	4.975	5.415	5.240	5.420	5.263
7	4.950	5.315	4.935	4.675	4.969
8	6.845	6.500	6.700	6.765	6.703
9	5.445	5.420	5.440	5.315	5.405
10	5.775	5.730	5.860	5.680	5.761
11	5.330	5.525	5.320	4.945	5.280

APPENDIX TABLE 34

Trial G - Clone X light regime means

Panicle length (mm, square root)

Clone	Light regime				
	A	B	C	D	Mean
1	11.240	10.810	11.150	12.190	11.348
2	8.595	9.990	8.800	9.880	9.316
3	10.555	10.740	11.025	11.620	10.985
4	8.790	9.320	10.080	10.365	9.639
5	9.525	9.550	9.980	10.815	9.968
6	9.225	10.135	9.445	10.310	9.779
7	7.855	9.095	8.775	9.435	8.790
8	11.370	11.705	12.100	12.140	11.829
9	7.120	7.620	8.140	7.525	7.601
10	9.490	9.400	9.685	10.055	9.658
11	8.300	9.335	9.045	9.125	8.951

Panicle breadth (mm, square root)

Clone	Light regime				
	A	B	C	D	Mean
1	4.175	5.220	5.560	6.300	5.314
2	5.265	5.960	4.960	5.970	5.539
3	5.470	5.395	5.560	6.795	5.805
4	5.140	5.530	6.050	6.915	5.909
5	5.500	5.480	5.775	7.105	5.965
6	5.485	6.085	5.545	6.490	5.901
7	4.570	5.055	5.190	5.415	5.058
8	6.495	6.095	6.485	7.200	6.569
9	5.025	4.820	5.295	5.530	5.168
10	6.680	6.495	6.005	7.560	6.685
11	5.510	5.745	5.420	6.115	5.698

APPENDIX TABLE 35

Trial G - Clone X light regime means

Mean number of florets per spikelet (x10, Log_{10})

Clone	Light regime				
	A	B	C	D	Mean
1	1.565	1.571	1.664	1.491	1.573
2	1.547	1.547	1.559	1.498	1.537
3	1.636	1.495	1.632	1.470	1.558
4	1.591	1.599	1.707	1.301	1.549
5	1.606	1.582	1.680	1.468	1.583
6	1.664	1.608	1.584	1.575	1.607
7	1.552	1.564	1.568	1.473	1.539
8	1.639	1.560	1.559	1.383	1.535
9	1.553	1.567	1.571	1.521	1.553
10	1.593	1.565	1.657	1.486	1.575
11	1.564	1.458	1.667	1.508	1.549

Total weight of seed per ramet (mg, square root)

Clone	Light regime				
	A	B	C	D	Mean
1	12.500	21.705	22.335	28.575	21.279
2	24.400	28.745	29.940	33.245	29.083
3	27.050	29.545	32.540	34.785	30.980
4	12.230	11.110	17.730	21.205	15.569
5	11.595	13.275	19.190	20.645	16.176
6	26.605	31.490	31.690	37.435	31.805
7	19.500	24.945	31.635	32.850	27.233
8	15.670	16.680	23.335	26.140	20.456
9	22.360	19.760	27.350	29.885	24.839
10	15.190	14.930	19.340	17.970	16.858
11	13.830	16.515	20.370	26.530	19.311

APPENDIX TABLE 36

Trial G - Clone X light regime means

1000 seed weight (mg, data squared $x10^{-4}$)

Clone	Light regime				
	A	B	C	D	Mean
1	40.045	45.795	45.225	48.945	45.003
2	21.230	16.825	19.870	18.575	19.125
3	36.060	49.280	51.845	49.465	46.663
4	37.120	35.950	36.775	34.345	36.048
5	43.800	52.685	63.150	58.375	54.503
6	17.770	15.365	20.530	16.580	17.561
7	23.280	25.805	41.800	32.695	30.895
8	58.060	45.105	65.725	65.785	58.669
9	17.420	14.215	19.905	16.730	17.068
10	40.145	43.045	48.540	41.995	43.431
11	31.200	53.150	59.675	52.000	49.006

Percentage germination (arcsin)

Clone	Light regime				
	A	B	C	D	Mean
1	63.470	60.900	64.170	67.210	63.938
2	49.360	30.500	28.970	31.245	35.019
3	74.315	60.105	55.250	60.250	62.480
4	54.480	46.090	40.890	53.735	48.799
5	45.110	49.360	50.035	50.035	48.635
6	53.735	43.565	34.495	19.565	37.840
7	56.010	67.210	59.925	60.835	60.995
8	74.990	51.605	50.250	57.090	58.484
9	67.495	67.890	72.140	68.250	68.944
10	61.225	62.555	46.445	55.375	56.400
11	63.400	68.765	57.575	60.900	62.660

APPENDIX TABLE 37

Trial G - Clone X light regime means

Percentage of multiple seedlings (arcsin)

Clone	Light regime				
	A	B	C	D	Mean
1	6.460	6.460	5.985	0.000	4.726
2	36.055	27.965	24.390	28.835	29.311
3	36.810	15.035	26.180	28.185	26.553
4	6.460	0.000	0.000	0.000	1.615
5	17.850	11.100	15.300	6.125	12.596
6	33.870	21.615	10.045	13.280	19.703
7	15.100	18.915	17.715	10.565	15.574
8	0.000	0.000	0.000	6.655	1.664
9	0.000	0.000	0.000	0.000	0.000
10	10.350	29.180	28.720	27.355	23.901
11	20.605	22.650	14.550	6.125	15.983

APPENDIX TABLE 38

Trial G - Number of seedlings recorded and analysed

Family	Light regime				Total
	A	B	C	D	
1	40	40	40	40	160
2	40	21	18	23	102
3	40	40	40	40	160
4	39	17	6	39	101
5	30	39	40	39	148
6	40	37	24	9	110
7	39	40	38	40	157
8	14	39	19	36	108
9	39	40	40	40	159
10	40	40	38	40	158
11	39	40	40	40	159
TOTAL	400	393	343	386	1522

APPENDIX TABLE 39

Trial G - Family X light regime means

Second leaf maturity date (Log_{10})

Family	Light regime				
	A	B	C	D	Mean
1	1.574	1.559	1.594	1.562	1.572
2	1.713	1.754	1.751	1.751	1.737
3	1.559	1.590	1.664	1.611	1.606
4	1.582	1.626	1.699	1.619	1.611
5	1.630	1.611	1.647	1.641	1.632
6	1.699	1.716	1.759	1.766	1.723
7	1.613	1.638	1.706	1.676	1.659
8	1.555	1.582	1.575	1.576	1.572
9	1.641	1.671	1.719	1.705	1.684
10	1.653	1.739	1.787	1.772	1.737
11	1.597	1.668	1.691	1.634	1.647

Second leaf length (mm)

Family	Light regime				
	A	B	C	D	Mean
1	55.00	62.67	61.87	62.20	60.44
2	61.20	58.67	80.61	63.57	64.64
3	45.67	47.70	52.82	48.17	48.59
4	60.51	57.88	42.17	55.80	57.16
5	38.36	47.78	48.70	50.49	46.33
6	69.90	65.03	65.54	63.11	66.75
7	64.31	52.65	58.65	72.82	62.11
8	70.44	67.37	66.33	71.14	68.82
9	68.32	70.22	78.00	72.75	72.32
10	68.92	66.87	61.20	65.47	65.62
11	51.06	60.05	59.17	64.20	58.62

APPENDIX TABLE 40

Trial G - Family X light regime means

Second leaf breadth (mm)

Family	Light regime				
	A	B	C	D	Mean
1	1.070	1.185	1.175	1.160	1.148
2	0.758	0.724	0.794	0.778	0.762
3	0.938	0.975	0.908	0.988	0.952
4	1.095	1.082	0.850	0.951	1.023
5	0.832	1.033	0.945	1.039	0.962
6	0.815	0.797	0.846	0.711	0.807
7	0.913	0.810	0.971	0.993	0.922
8	0.919	0.906	0.957	0.928	0.928
9	1.013	0.988	1.045	0.990	1.009
10	1.098	1.060	1.050	0.980	1.047
11	0.981	1.038	1.050	1.205	1.068

Fourth leaf length (mm, data squared x 10^{-4})

Family	Light regime				
	A	B	C	D	Mean
1	2.169	2.720	2.854	2.713	2.614
2	2.147	2.111	3.225	2.505	2.411
3	1.735	1.796	2.188	1.587	1.826
4	3.000	3.159	1.324	2.753	2.832
5	1.724	2.328	2.277	2.404	2.183
6	2.752	2.154	2.271	2.375	2.415
7	3.180	2.522	2.446	3.206	2.838
8	2.921	3.131	2.339	3.312	2.926
9	2.865	2.858	3.201	2.753	2.919
10	3.621	3.003	2.673	2.800	3.024
11	2.287	2.308	2.528	2.975	2.524

APPENDIX TABLE 41

Trial G - Family X light regime means

Number of tillers (square root)

Family	Light regime				
	A	B	C	D	Mean
1	2.249	2.474	2.156	2.306	2.297
2	1.905	1.879	2.257	2.107	2.007
3	2.306	2.222	1.997	2.368	2.223
4	1.962	1.685	1.539	1.682	1.782
5	1.596	1.874	1.644	1.800	1.728
6	2.013	1.933	2.076	2.486	2.039
7	1.913	1.757	1.740	1.862	1.818
8	1.815	1.909	1.810	1.752	1.821
9	1.996	1.912	1.887	1.917	1.928
10	1.890	1.676	1.585	1.636	1.696
11	1.999	1.753	1.791	1.848	1.848

Size

Family	Light regime				
	A	B	C	D	Mean
1	5.450	6.300	5.725	5.925	5.850
2	4.575	4.762	5.056	4.870	4.765
3	4.775	4.850	4.775	5.050	4.863
4	6.385	5.294	5.667	5.359	5.762
5	4.969	5.275	4.525	5.003	4.943
6	5.200	4.541	4.875	5.333	4.918
7	6.086	5.150	5.438	5.475	5.537
8	5.406	6.225	5.277	5.597	5.626
9	6.064	6.450	6.175	6.250	6.235
10	6.500	4.950	4.888	5.225	5.391
11	5.853	5.350	5.575	6.500	5.819

APPENDIX TABLE 42

Trial G - Family X light regime means

Colour

Family	Light regime				
	A	B	C	D	Mean
1	1.525	1.725	1.725	1.750	1.681
2	3.025	2.714	3.056	3.044	2.971
3	1.775	1.975	1.875	1.900	1.881
4	3.051	3.412	4.667	3.513	3.386
5	1.865	2.131	1.850	1.892	1.934
6	3.050	3.135	2.833	3.000	3.027
7	4.164	4.075	4.069	4.200	4.127
8	4.531	4.283	4.384	4.637	4.459
9	4.317	4.350	4.500	4.350	4.379
10	3.200	3.450	3.725	3.250	3.406
11	3.772	3.925	3.425	3.950	3.768

Ligule (square root)

Family	Light regime				
	A	B	C	D	Mean
1	2.024	2.003	2.026	2.000	2.013
2	1.589	1.543	1.626	1.599	1.588
3	1.952	1.928	1.918	2.025	1.956
4	1.682	1.661	1.533	1.734	1.690
5	1.782	1.774	1.696	1.867	1.780
6	1.616	1.545	1.611	1.520	1.583
7	1.412	1.322	1.459	1.527	1.430
8	1.558	1.431	1.330	1.319	1.410
9	1.415	1.444	1.549	1.441	1.462
10	1.764	1.771	1.790	1.804	1.782
11	1.377	1.402	1.496	1.445	1.430

Trial G - Family X light regime means

Auricle hairs

Family	Light regime				
	A	B	C	D	Mean
1	1.200	1.150	1.200	1.150	1.175
2	1.100	1.191	1.000	1.130	1.108
3	1.050	1.075	1.025	1.000	1.038
4	2.667	3.000	2.500	3.282	2.951
5	1.510	1.542	1.575	1.478	1.526
6	1.275	1.135	1.083	1.000	1.164
7	3.278	2.425	2.781	2.900	2.846
8	2.969	2.956	1.893	2.665	2.620
9	3.358	3.600	3.650	3.775	3.596
10	3.700	3.600	3.488	3.325	3.528
11	2.581	2.325	2.475	3.125	2.626

Sheath hairs (square root)

Family	Light regime				
	A	B	C	D	Mean
1	1.060	1.068	1.144	1.095	1.092
2	1.031	1.020	1.069	1.072	1.045
3	1.000	1.010	1.021	1.031	1.016
4	1.207	1.224	1.373	1.298	1.255
5	1.242	1.248	1.223	1.134	1.212
6	1.021	1.101	1.086	1.092	1.068
7	1.380	1.236	1.275	1.289	1.295
8	1.242	1.306	1.105	1.171	1.206
9	1.111	1.124	1.269	1.161	1.166
10	1.487	1.416	1.329	1.335	1.392
11	1.082	1.052	1.083	1.120	1.084

APPENDIX TABLE 44

Trial G - Family X light regime means

Leaf angle (square root)

Family	Light regime				
	A	B	C	D	Mean
1	1.580	1.669	1.589	1.610	1.612
2	1.623	1.731	1.703	1.631	1.661
3	1.818	1.774	1.828	1.762	1.796
4	1.771	1.709	1.276	1.748	1.722
5	1.606	1.680	1.564	1.636	1.622
6	1.665	1.691	1.613	1.626	1.659
7	1.698	1.685	1.608	1.676	1.667
8	1.937	1.866	1.905	2.046	1.939
9	1.556	1.605	1.581	1.600	1.586
10	1.453	1.463	1.504	1.493	1.478
11	1.646	1.668	1.682	1.692	1.672

Tiller angle

Family	Light regime				
	A	B	C	D	Mean
1	2.175	2.325	2.425	2.675	2.400
2	3.175	2.810	3.500	3.261	3.177
3	2.100	2.100	2.200	2.300	2.175
4	3.821	2.824	3.000	3.513	3.485
5	2.448	2.311	2.500	2.636	2.474
6	3.400	3.514	3.417	4.222	3.509
7	3.547	2.850	3.094	3.475	3.242
8	2.219	2.556	1.821	1.488	2.021
9	3.914	4.000	3.975	4.250	4.035
10	3.700	3.225	3.044	3.325	3.323
11	3.786	3.750	3.600	4.050	3.797

APPENDIX TABLE 45

Trial G Family variances (x 10^3) from data recorded on seedlings and tests for homogeneity of variances

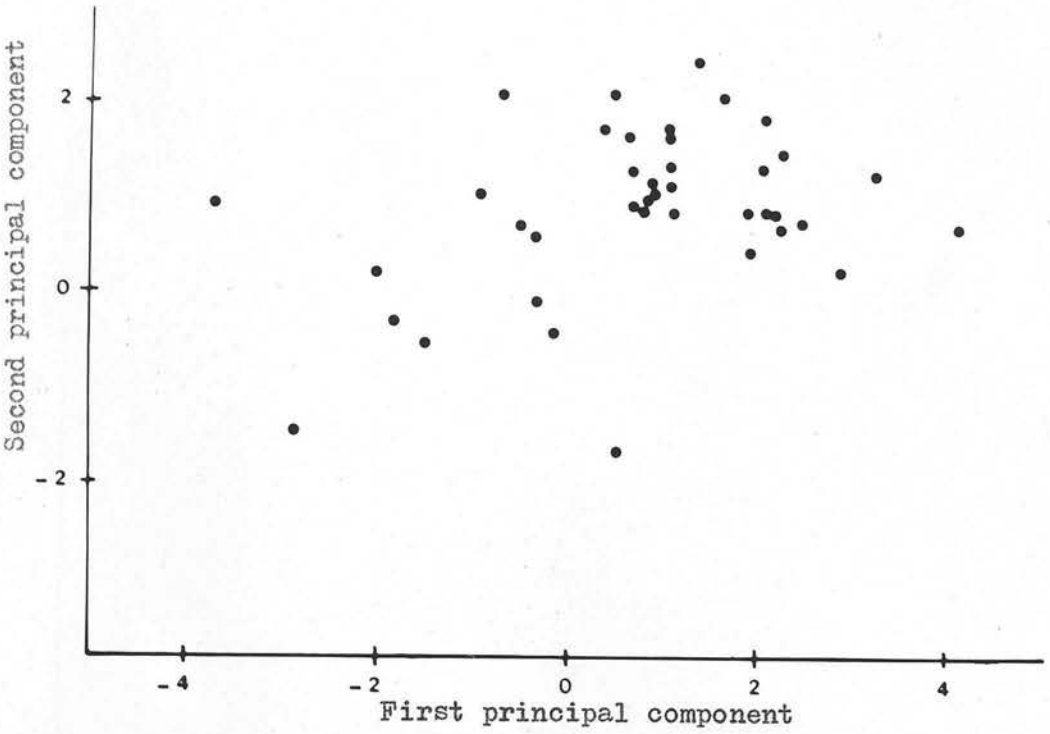
Family	2LD+	2LL	2LB	4LL	No T	Size
1	63	22	51	1944	234	2710
2	45	37	15	677	267	1746
3	101	37	73	1264	354	3088
4	77	43	101	2549	212	4083
5	128	51	91	2240	261	4026
6	52	30	17	913	265	1310
7	81	56	78	2585	230	3725
8	69	62	78	2751	310	4548
9	68	26	36	1189	101	2053
10	131	38	52	1645	136	3148
11	82	30	65	1430	141	2492
χ^{2}++	77.46	73.59	185.53	113.94	101.53	81.98

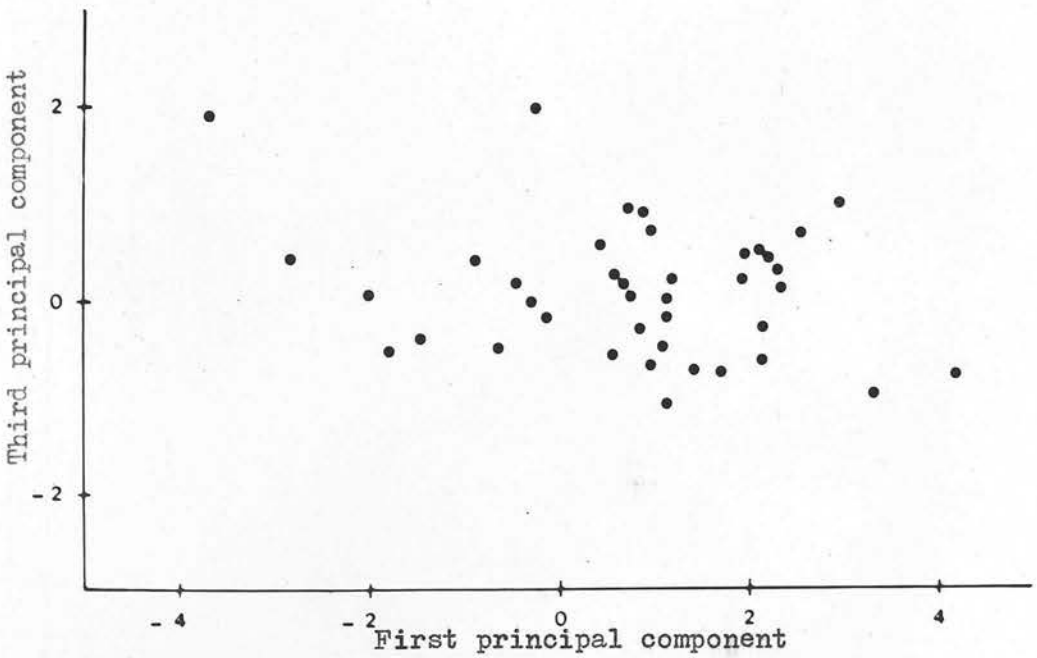
Family	Colour	Lig	Au H	Sh H	LA	TA
1	646	60	309	46	83	506
2	801	46	137	17	46	1216
3	898	96	49	6	70	472
4	1059	66	1428	100	84	1592
5	785	108	768	105	65	1000
6	596	40	285	24	38	1060
7	997	77	1609	129	78	1582
8	602	80	1832	111	53	1373
9	490	26	774	68	54	638
10	841	43	1015	89	74	946
11	939	62	1121	38	53	1052
χ^{2}++	39.49	117.43	606.14	462.62	39.74	119.28

+ variances x 10^4

++ χ^2 with 10 df > 29.59, $p < 0.001$

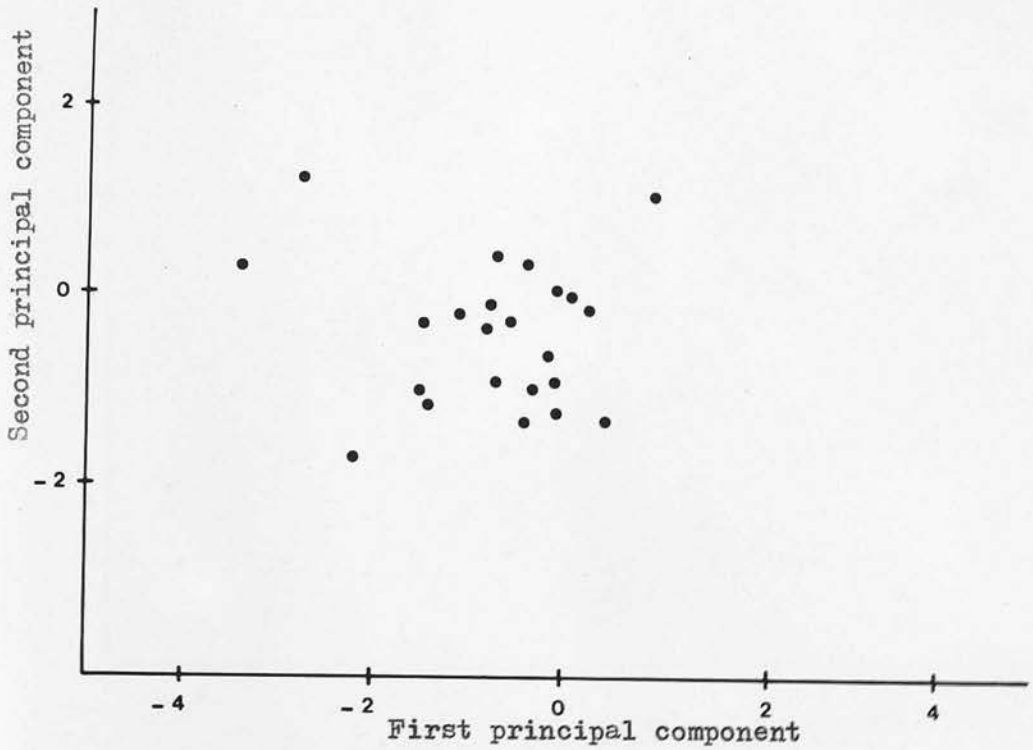
APPENDIX FIGURE 13

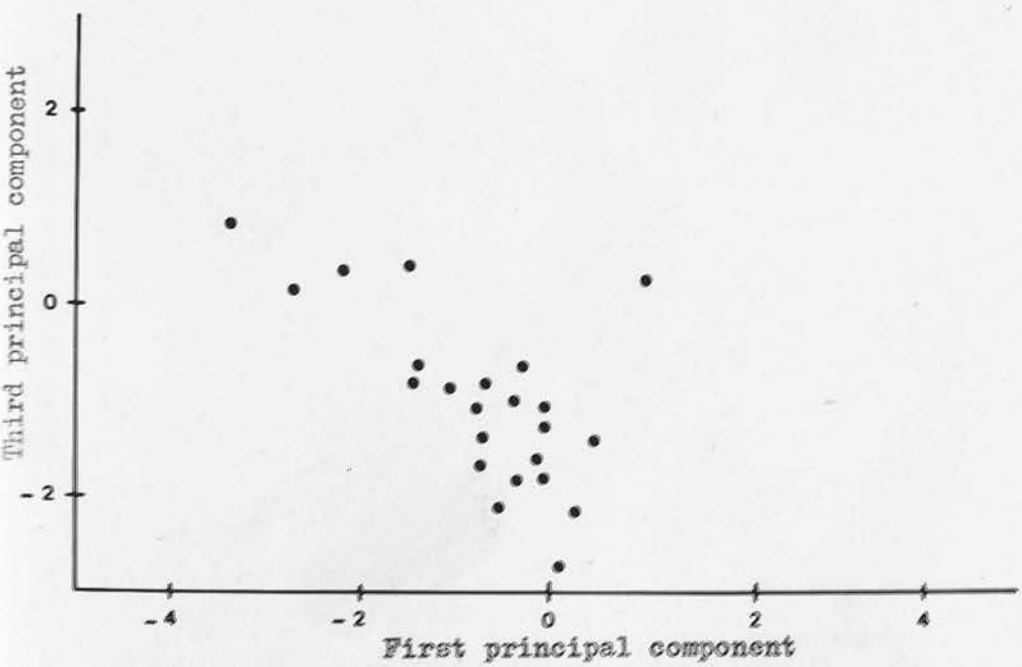




Trial G Principal component analysis on 6 variates, all seedlings from light regime D (control): family 1

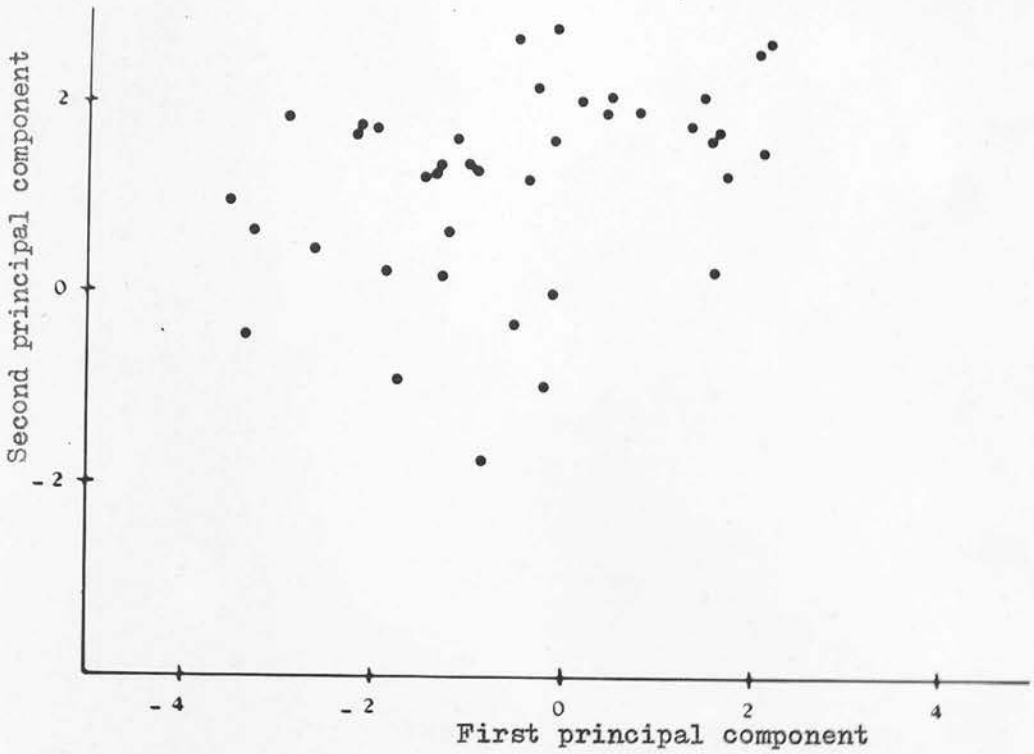
APPENDIX FIGURE 14

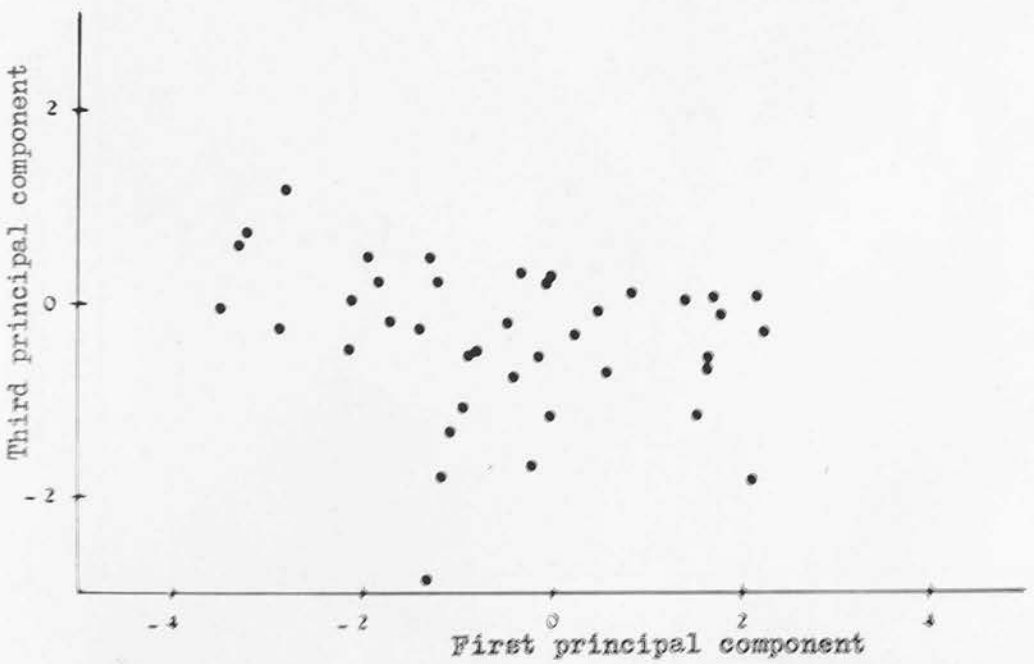




Trial G Principal component analysis on 6 variates, seedlings from light regime D (control): family 2.

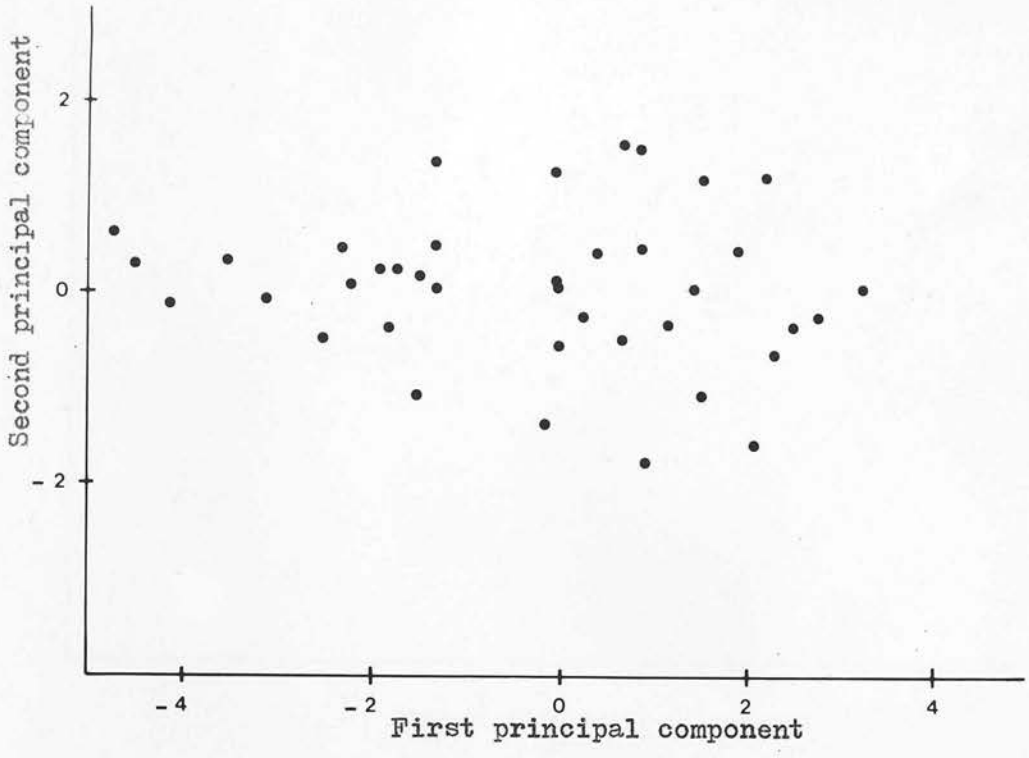
APPENDIX FIGURE 15

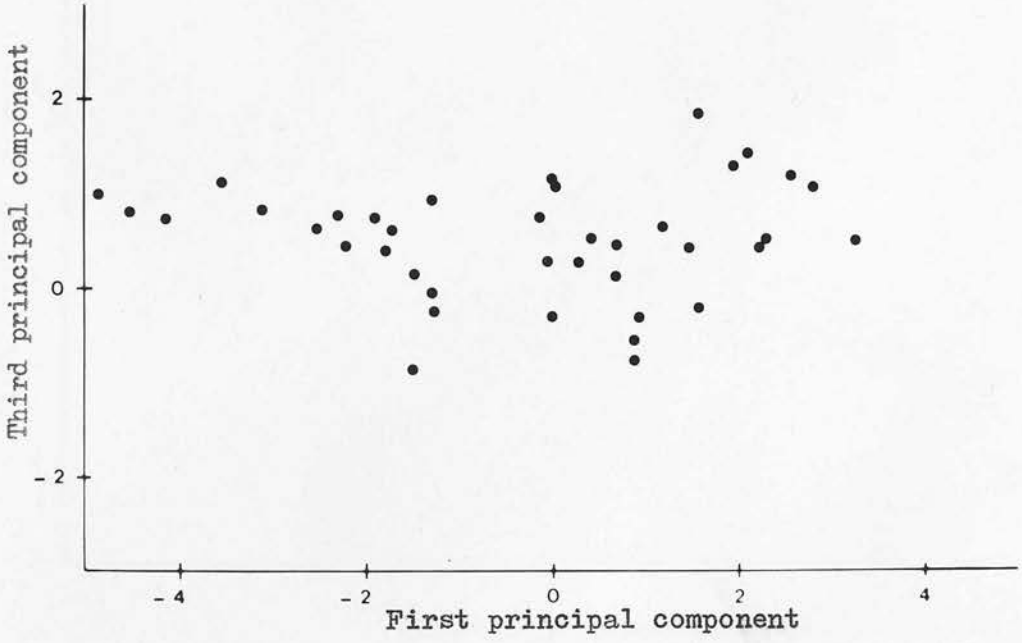




Trial G Principal component analysis on 6 variates, seedlings from light regime D (control): family 3.

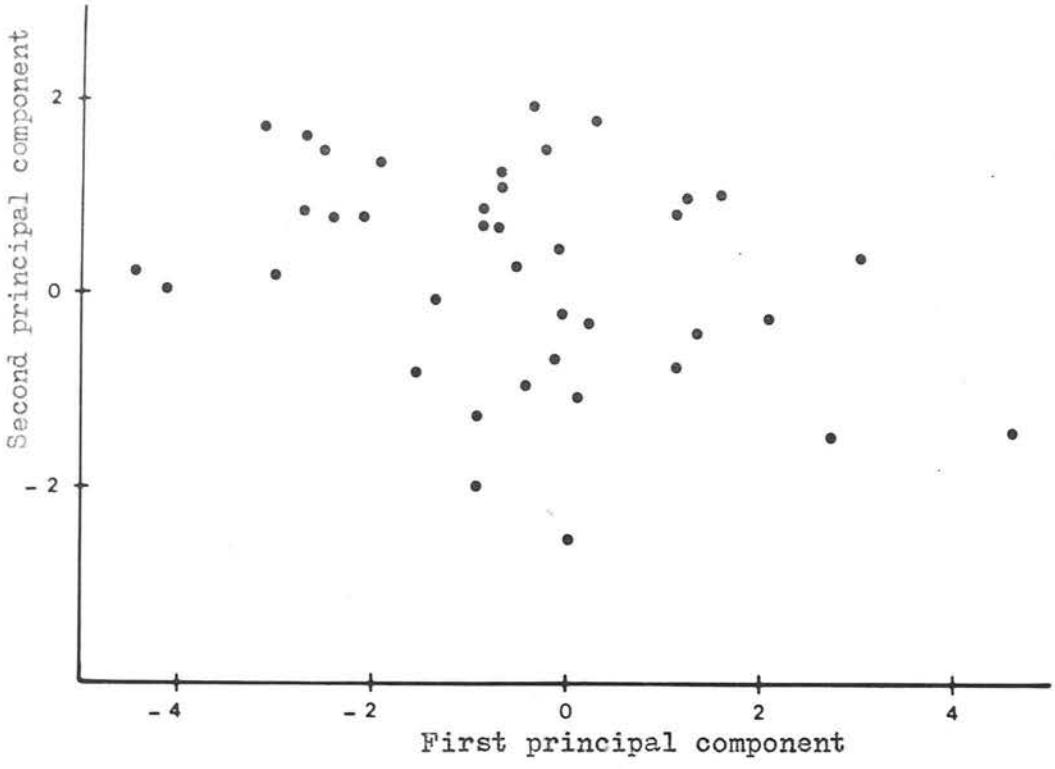
APPENDIX FIGURE 16

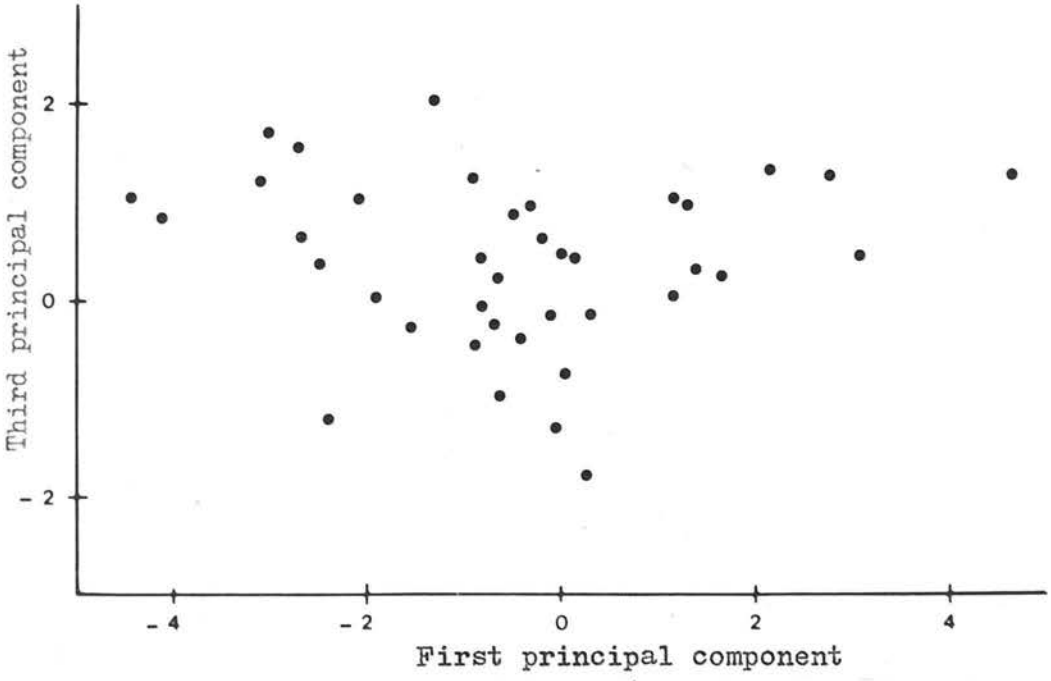




Trial G Principal component analysis on 6 variates, all seedlings from light regime D (control): family 4

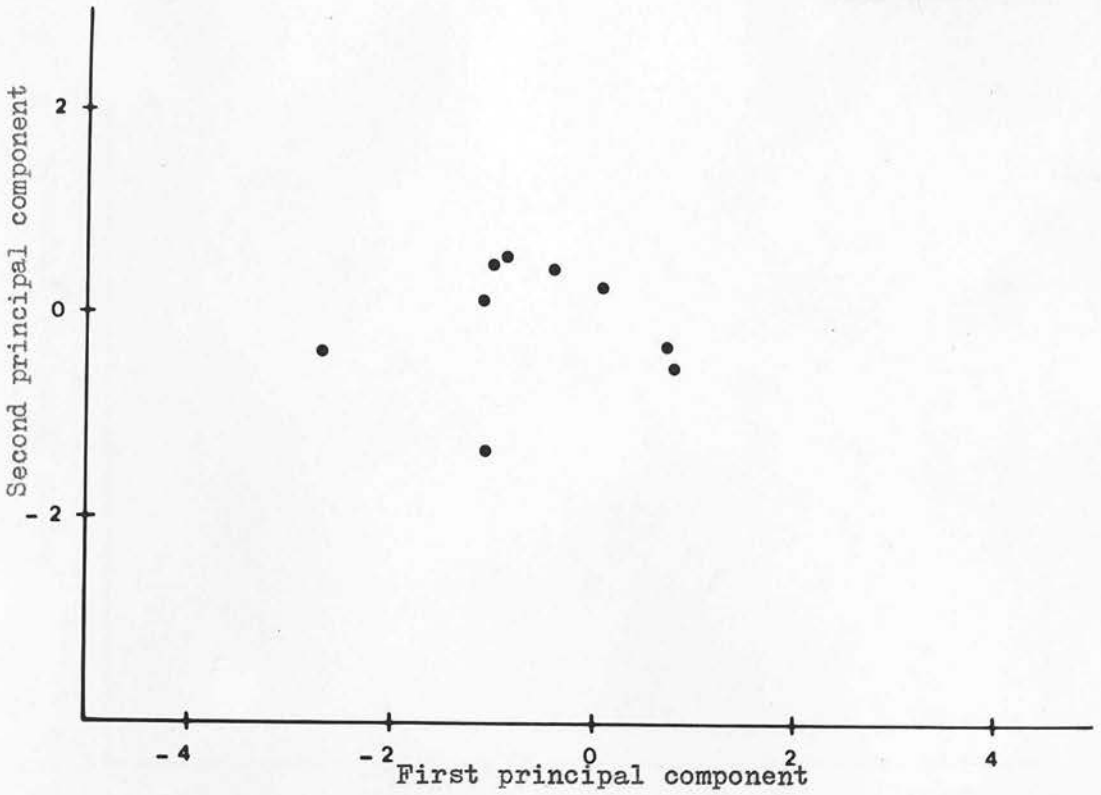
APPENDIX FIGURE 17

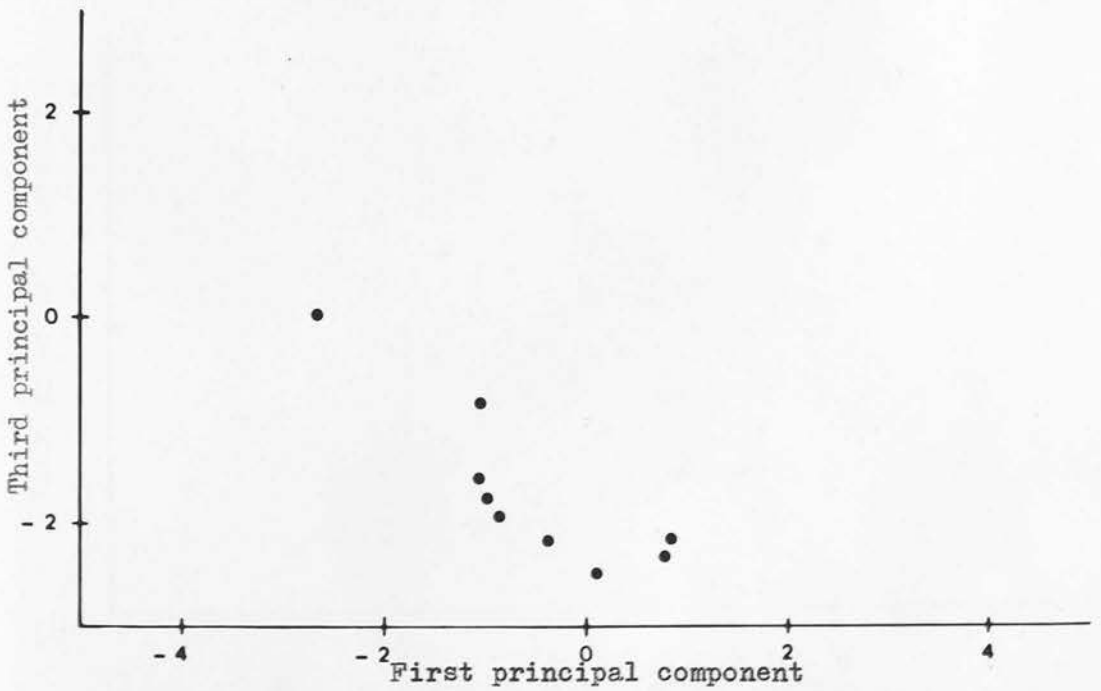




Trial G Principal component analysis on 6 variates, all seedlings from light regime D (control): family 5

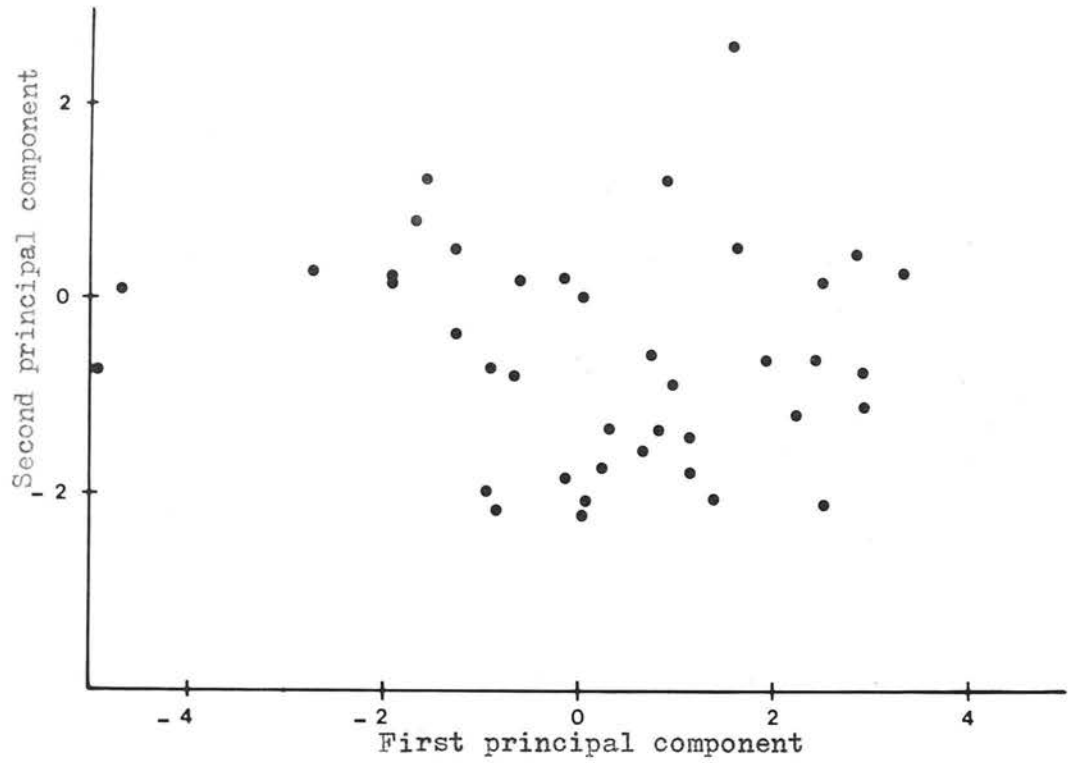
APPENDIX FIGURE 18

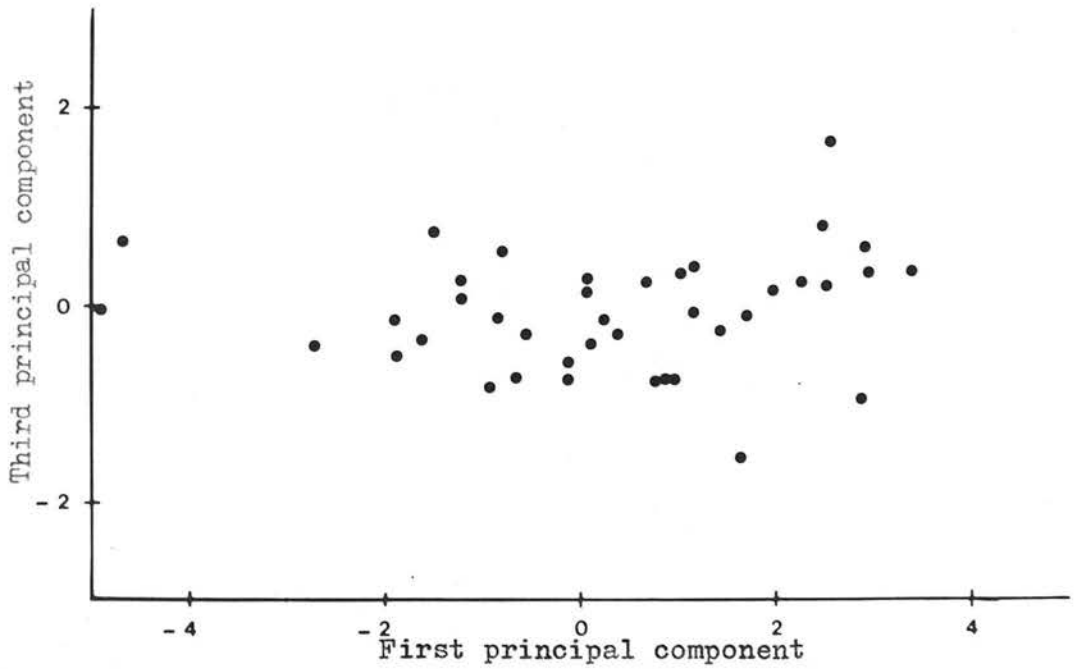




Trial G Principal component analysis on 6 variates, all seedlings from light regime D (control): family 6

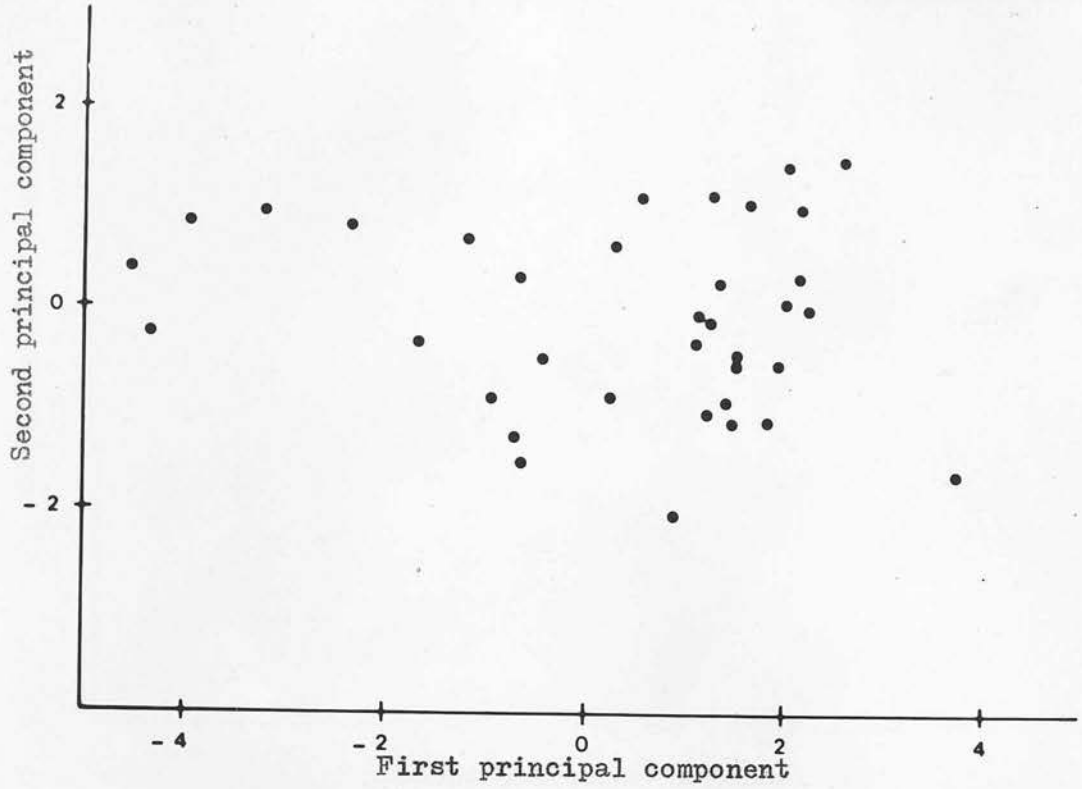
APPENDIX FIGURE 19

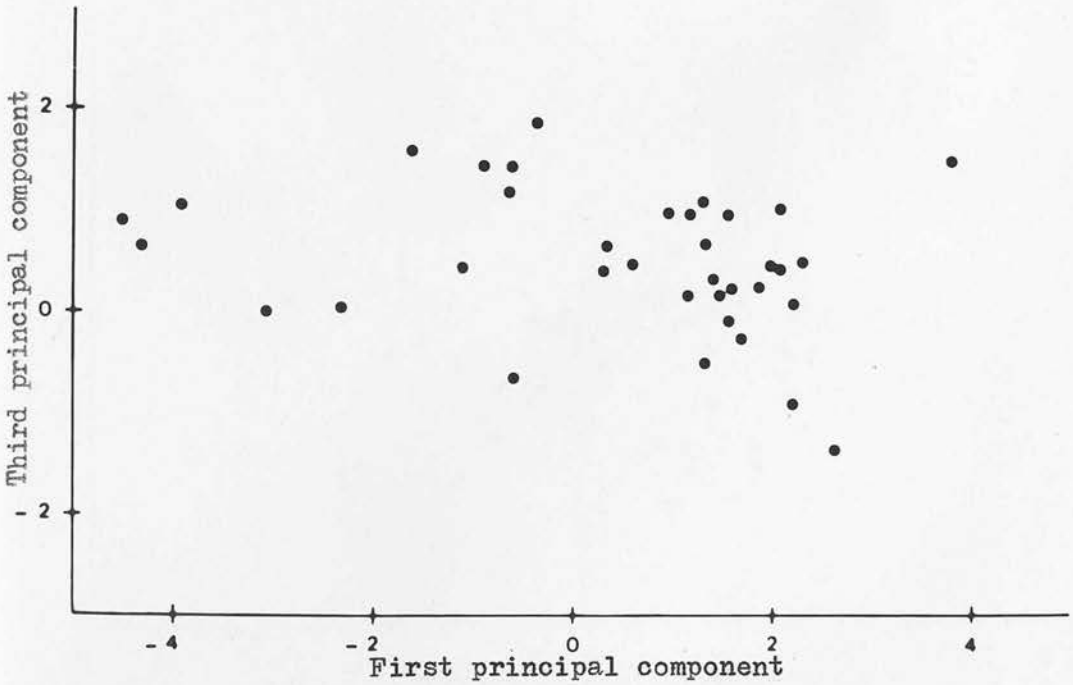




Trial G Principal component analysis on 6 variates, all seedlings from light regime D (control): family 7

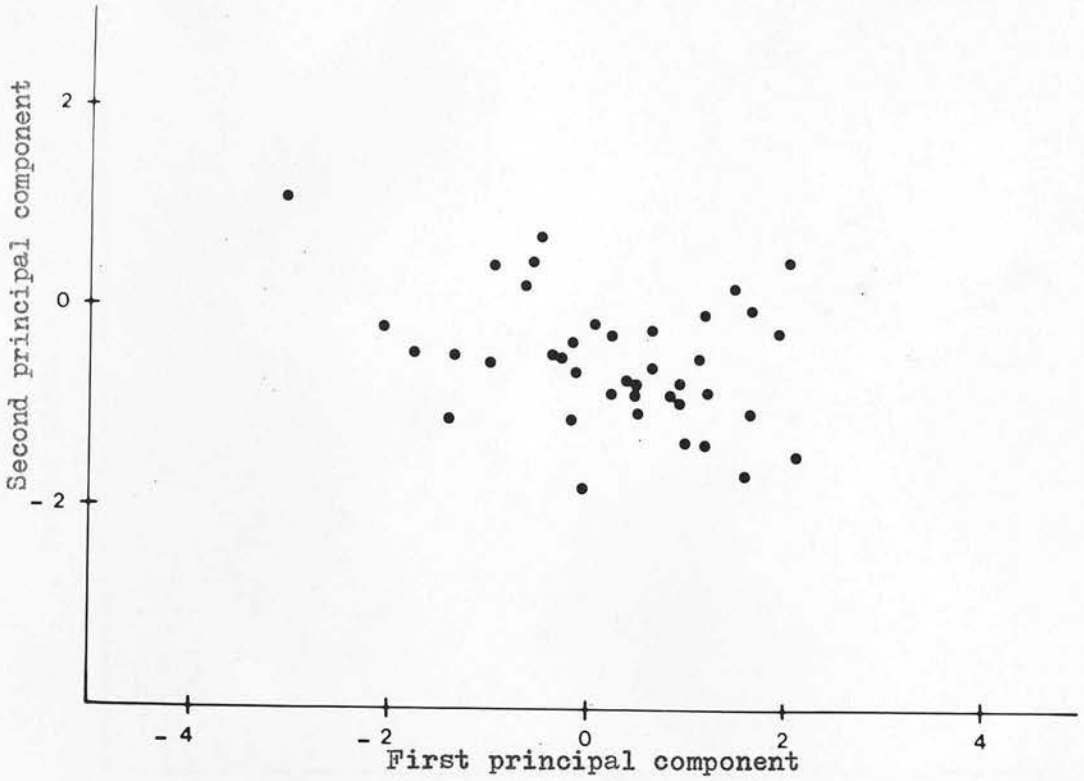
APPENDIX FIGURE 20

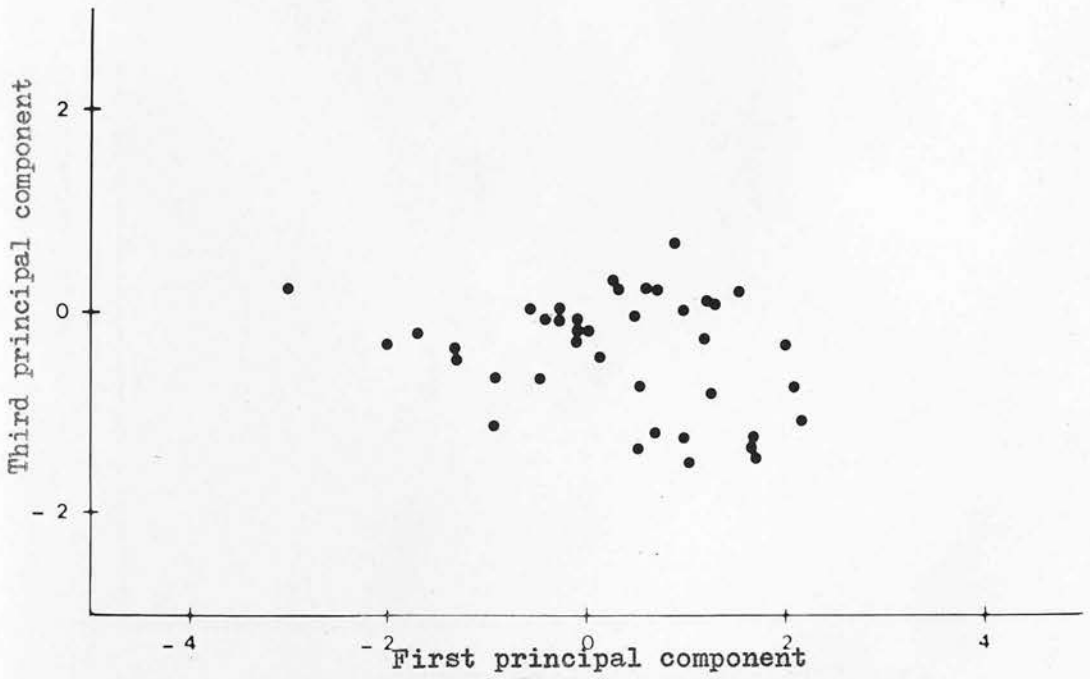




Trial G Principal component analysis on 6 variates, all seedlings from light regime D (control): family 8

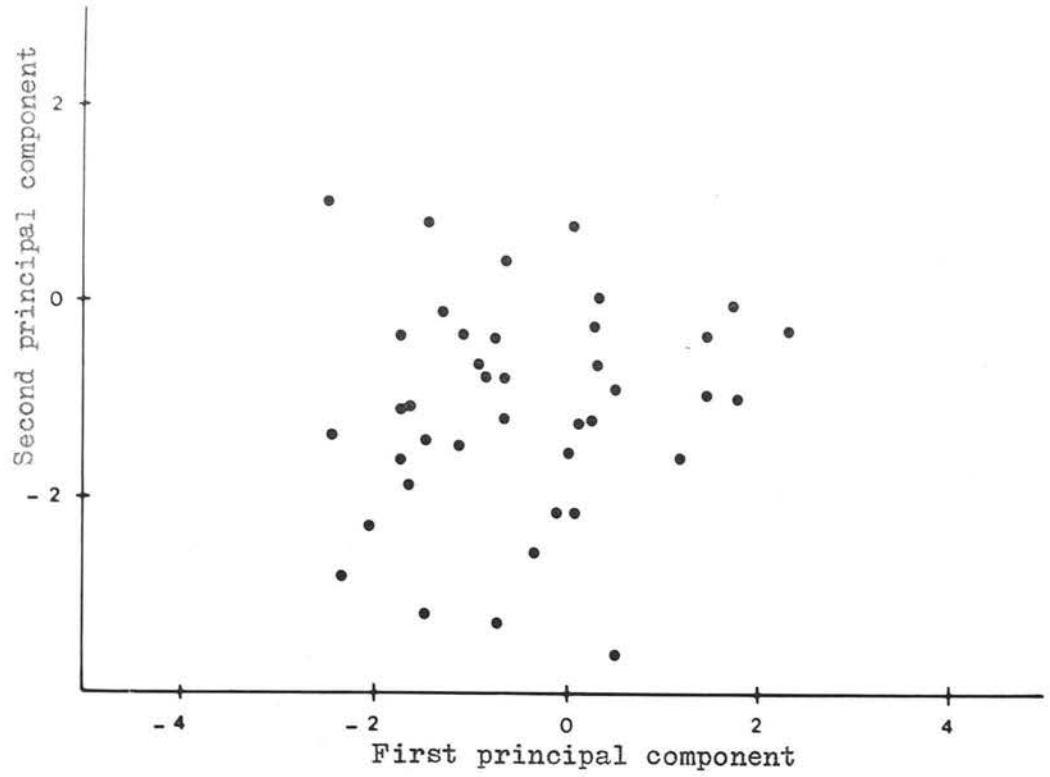
APPENDIX FIGURE 21

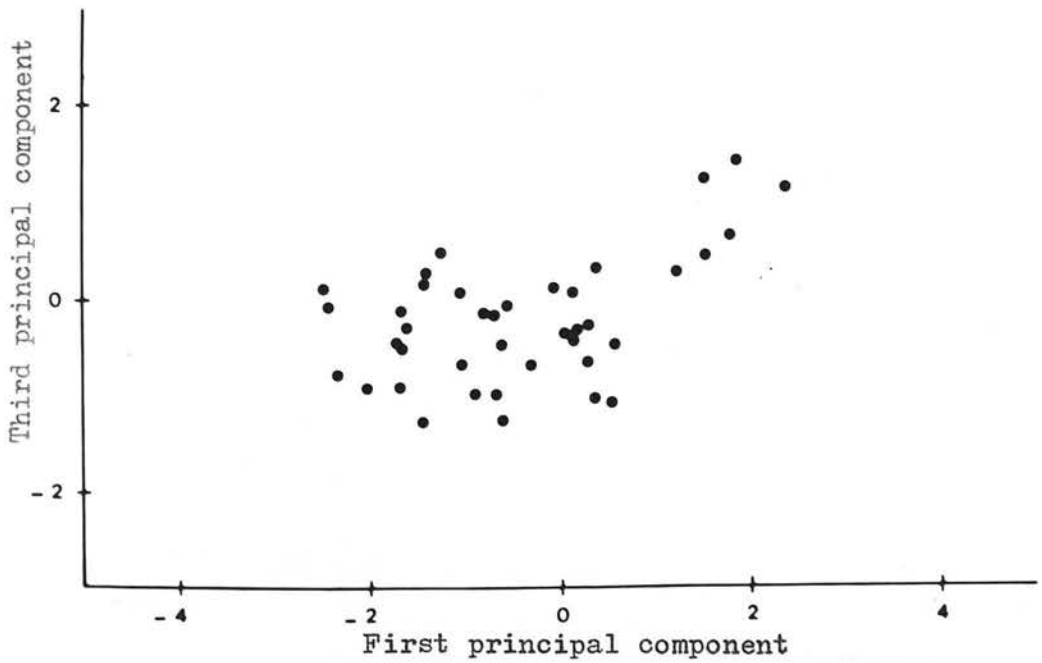




Trial G Principal component analysis on 6 variates, all seedlings from light regime D (control): family 9

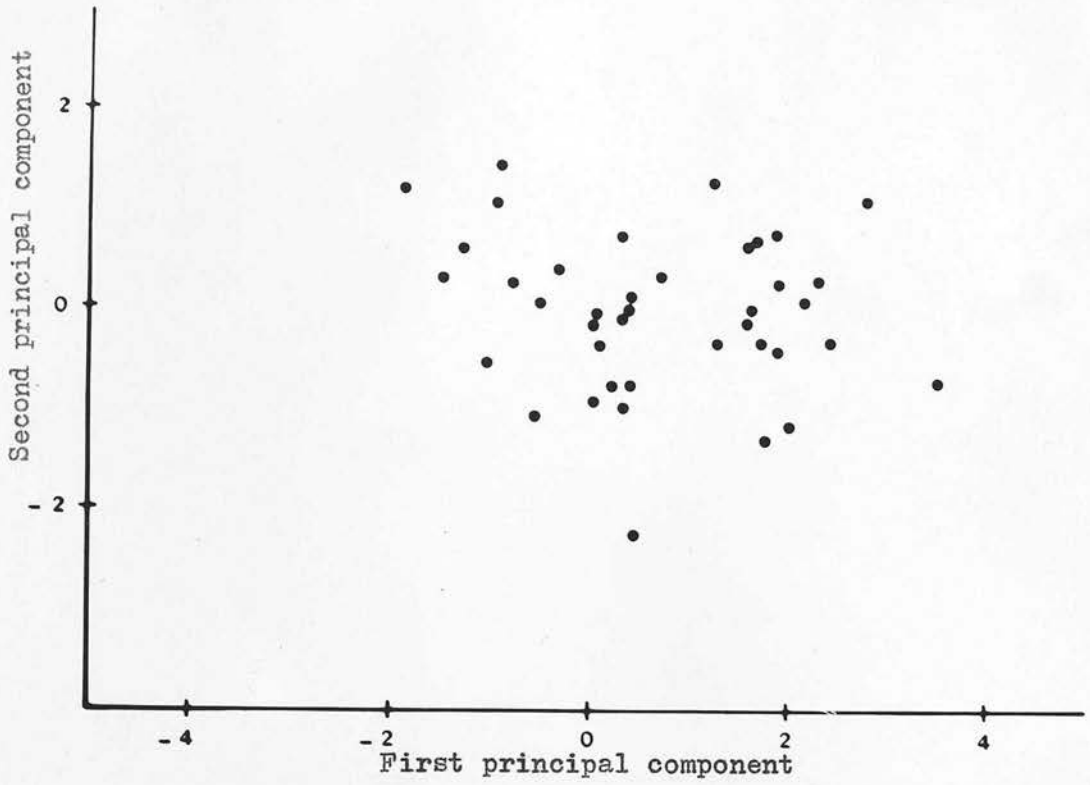
APPENDIX FIGURE 22

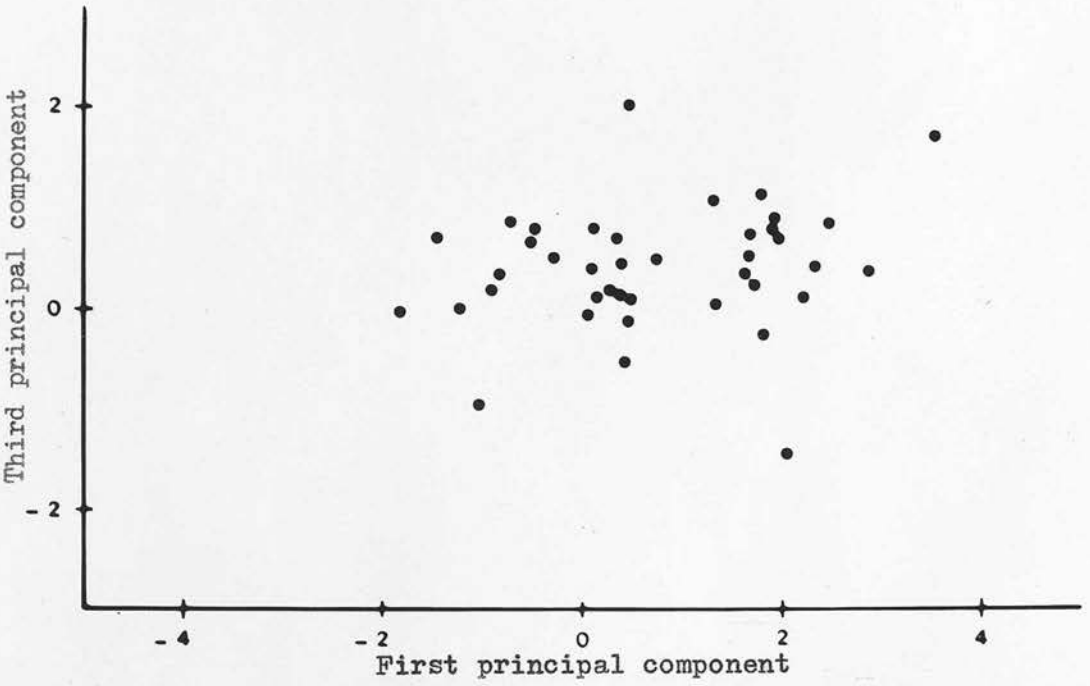




Trial G Principal component analysis on 6 variates, all seedlings from light regime D (control): family 10

APPENDIX FIGURE 23

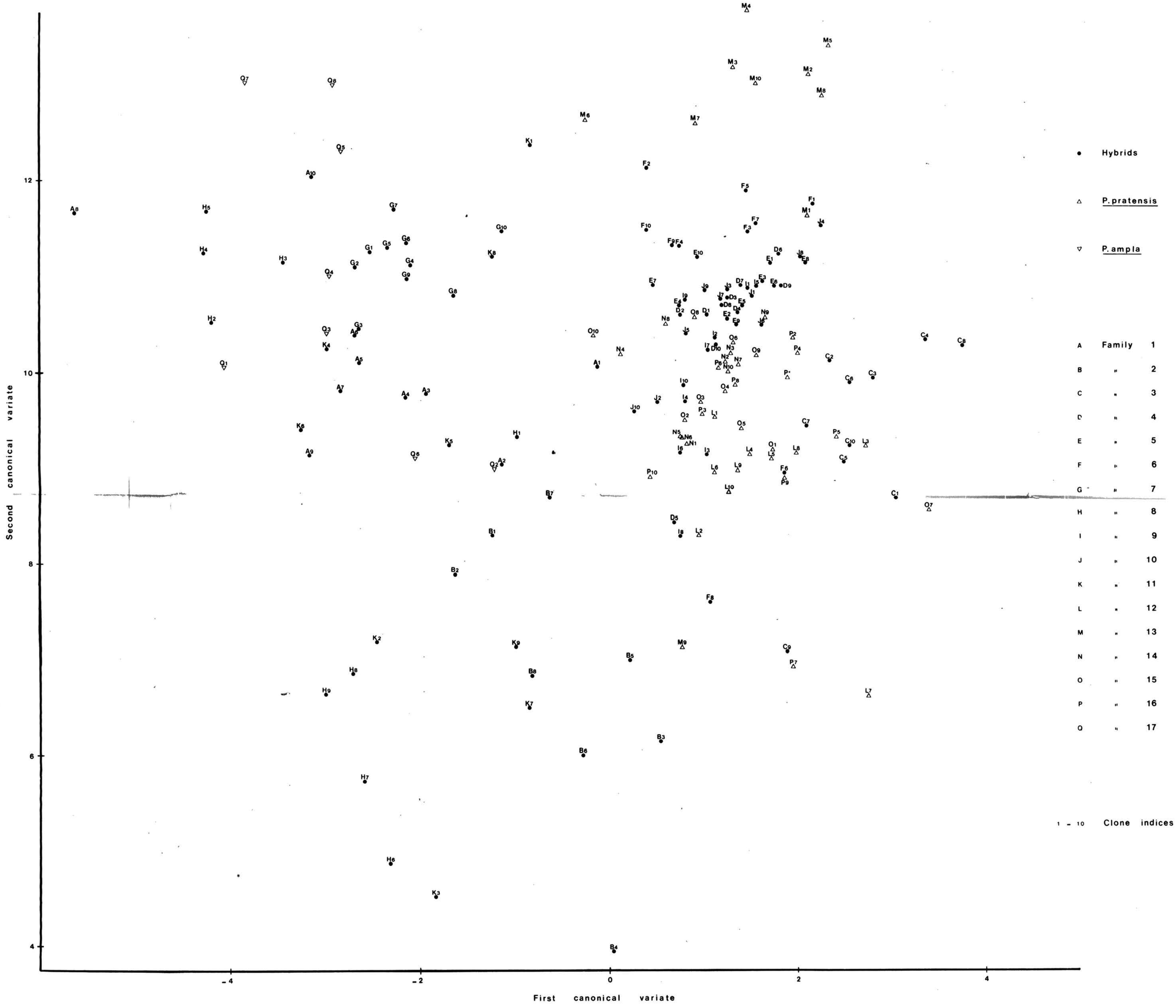




Trial G Principal component analysis on 6 variates, all seedlings from light regime D (control): family 11

TRIAL C CANONICAL ANALYSIS ON 164 CLONES FROM 17 FAMILIES (3 VARIATES)

FIGURE 13



TRIAL C CANONICAL ANALYSIS ON 164 CLONES FROM 17 FAMILIES (6 VARIATES) FIGURE 12