A Study of Quantitative Genetics on Some Characters of the Meadowfoam Plant (*Limnanthes alba Benth.*)

A thesis presented in partial fulfilment of the requirements for the Master of Applied Science degree in Plant Science at Massey University

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March 1998

ABSTRACT

The meadowfoam plant is a moisture-loving native of the west coast of the North American continent near the borders of USA and Canada. It has recently stirred great interest in the chemical oil industry due to the potential of its seed oil to substitute for sperm whale oil. Due to the relative lack of published literature on this plant, an experiment was planned to study the quantitative genetics of some of its characters.

Thirty-six half-sib families were planted and the following characters were examined: plant height; diameter; uprightness; intensity of redness on branches and its distribution; leaf shape; period to first flower; seed set; mature seed retained; degree of seed shattering; and thousand-seed mass. Factor analysis was also performed on the flowering pattern of the plants.

Results indicated that all characters were heritable in the broad-sense, and all but two characters (diameter and degree of seed shatter) had significantly heritable narrow-sense heritabilities. The amount of genetic variability present in this species is also very high. Plant improvement methods based on selection are therefore recommended. Predictions on genetic advance show that the characters plant height, seed retention, leaf shape, and red intensity and distribution on branches showed greatest promise for rapid improvement.

ACKNOWLEDGEMENTS

I wish to express my gratitude to my supervisor, Dr. Ian L. Gordon, for both illuminating and expanding my mind through the duration of this work. His patient guidance has built up a strong foundation for me upon which academic confidence has been built. He has excelled in both roles as mentor and friend through my years at Massey.

The technical staff at Massey has also contributed greatly to lighten my load in this project. Many thanks to Mr. Mark Osborne for the initial field preparation work; and also to Mr. Robert Southward for his field advice, and his contribution of time and dedicated efforts in seed and data collection.

My friends have also contributed significantly in this project, in both physical effort and for providing camaraderie which has maintained my stress at 'healthy' levels. They are: *Chan* Weng Kong, *Chuah* Seong Kee, *Tan* Gaik Seong, *Tan* Seong Ho, *Teoh* Ee Ling (Ms.), and *Yang* Ming-Huang. In addition, *Yang* Ming-Huang gets special notice for being a very good friend at Massey for 5½ years.

This work would also not be possible without the emotional and financial support from my family, with whom weekly contact has lent me great support.

I also wish to thank the Singapore Government for the temporary suspension of my scholarship bond, and the facilitated provision of necessary documents to allow my continued sojourn in New Zealand.

The financial assistance provided by the Massey Masterate Scholarship and the Turners and Growers Research Grant is gratefully acknowledged.

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1.0 INTRODUCTION

1.1 Historical background to the meadowfoam plant

The meadowfoam plant (*Limnanthes alba*) has gained considerable interest due to the potential of myriad uses of its seed oil where industrial applications are concerned. It was first identified in the 1950s when the U. S. Department of Agriculture (USDA) conducted an extensive program to search for new crops amongst untested plants. The ideal new crop-plant candidate should fill a present or anticipated need and its usefulness should not compete with existing crops. This was done in part to alleviate the problems of massive surpluses of major food crops every year. Potential plant products and applications sought include cellulosic compounds for the pulp and paper industry, proteins for animal feed and industrial use, useful polysaccharides other than starch, natural toxins useful for pest and pathogen control, alkaloids, waxes, and unique vegetable oils (Earle *et al.*, 1959).

In the search for new plant products, oils receive special attention primarily because it has higher economic value per unit volume than proteins or fibres. It also has many applications in industry, the prime vehicle for value-addedness to a natural product. Industrial trends indicate increasing usage of oils as chemical intermediates in industry. It was of no surprise then, that greater interest was stirred when *Limnanthes* oil was named in 1971 as the most promising substitute for sperm whale oil (*Limnanthes* oil, together with *Crambe abyssinica* and *Simmondsia chinensis* (jojoba) oils were considered as possible substitutes for sperm whale oil (Hagemann and Rothfus, 1981)); after all sperm whale products were banned in 1969 when the Endangered Species Conservation Act was passed in the USA (Jolliff, *et al.*, 1981).

If successfully domesticated, demand for *Limnanthes* oil is expected to be strong, given that the US alone consumed 50 million pounds of sperm whale oil annually until 1972 for use in cosmetics, waxes, pharmaceuticals, lubricants, etc. Before attention was focused on its seed oil potential, the only cultivated species of *Limnanthes* was *L. douglasii* for its ornamental flowers (Purdy and Craig, 1987). The short life cycle and genetic variability suggests great potential for rapid crop improvement. The *Limnanthes* plants appeared to be efficient in the processing of raw matter and also produced a high ratio of seed to vegetative matter.

The seed oils are valuable because more than 95% of the fatty acids contain 20 or 22 C-chains which are mainly unsaturated at the 5C but sometimes at the 13C. This makes them suitable for a wide plethora of industrial uses such as waxes, lubricants, detergents, and plasticizers. Natural *Limnanthes* oil can be made into a liquid wax similar to jojoba oil, and when fully hydrogenated, a high quality solid wax about as hard as carnauba and candelilla waxes can be obtained. The oil content of *Limnanthes* seeds vary from 25-33% but fatty acid content of the C_{20}:1 type can be as high as 52-77% of the total seed oil (Higgins *et al.*, 1971).

1.2 Use of biometrics in plant improvement

Quantitative genetics deals with those traits which are expressed in a continuous spectrum rather than discrete classes. Most economic traits relating to yield fall within this definition. The manipulation of variation caused by genetic factors through breeding and selection forms the backbone of most plant breeding programs. The objective of plant breeding research is to enable better manipulation of these variations so that the desired qualities are realised.

2.0 MEADOWFOAM REVIEW

2.1 Limnanthes *systematics*

Limnanthes alba belongs to the family Limnanthaceae which is endemic to the west coast of the North American continent (Ornduff, 1971). Plant material was taken to England by David Douglas where the genus *Limnanthes* was first described in 1833 by Robert Brown. It is always found in very moist soil or even shallow water in valleys, foothills and mountains. Its name is derived from *limne* - marsh, and *anthos* - flower.

The family *Limnanthaceae* only has two genera: *Limnanthes* and the monotypic *Floerkea* (*F. proserpinacoides*). The differentiation into two genera is debatable (*Floerkea* and *Limnanthes*), even Brown remarked "examination proved these two plants to be so nearly akin that they might perhaps be included in the same genus." (Mason, 1952). The two genera are annual herbs, with the greatest species diversity in California. Mason describes 8 species and 11 varieties but two more species have since been added to this total.

F. proserpinacoides differs from *Limnanthes* in that it often occupies shaded and moist habitats and can be found widely from the Pacific to Atlantic Coasts. It is often overlooked because of its inconspicuous greenish flowers. The two genera are distinguished by flowers and cotyledons. *Floerkea* has hypogeous cotyledons and trimerous flowers, while *Limnanthes* has epigeous cotyledons and pentamerous flowers (except for *L. macounii* which has tetramerous flowers). Both have n = 5. The choice of the two characters for division into genera within the family seems to be arbitrary because many genera of angiosperms have different cotyledon positions within the same genus and this is sometimes under simple genetic control (e.g.: *Acer, Theobroma, Phaseolus*). The merosity of flower parts also vary within genera in other families (Ornduff and Crovello, 1968).

Limnanthes is naturally distributed on the Cascade-Sierra Nevada Range in northern California and southern Oregon, excluding *L. macounii* which is found on Vancouver Island in British Columbia (see Fig. 1, overleaf). The natural habitats of meadowfoam is along vernal streams, meadows, pools, or moist depressions of the valley grasslands in California and southern Oregon. It is observed to be tolerant in standing water provided several leaves can grow above the water surface. It is therefore suitable to be grown in poorly drained areas (Calhoun and Crane, 1978). The plant grows vegetatively during the cool climates and then matures rapidly during the warmer and drier climates before rapidly dying off (Higgins *et al.*, 1971).

In the Pacific coast of the USA, *Limnanthes* germinates in the late fall and completes its life cycle by April or June (Pierce and Jain, 1977). Mason (1959) reports that the warmer and longer days promote flowering. The soil pH requirements of *Limnanthes* are usually in the slightly acidic range of 5.5-6.7. *L. alba* is reported to grow on a soil pH of 6.2. The only known exception is *L gracilis gracilis* which grows in a soil pH of 7.2. All species of *Limnanthes* require wet to moist soils during germination and growing phases (Gentry and Miller, 1965).

The genus *Limnanthes* is again differentiated into two sections, depending on how the petals and sepals fold after flowering (Gentry and Miller, 1965). Those that fold outwards are grouped under section *Reflexae* and include the following species:

Section Reflexae

L. douglasii R. Br. (Robert Brown) var *douglasii*
L. douglasii var *sulphurea* C. T. Mason
L. douglasii var *nivea* C. T. Mason
L. douglasii var *rosea* (Benth) C. T. Mason
L. striata Jepson
L. bakeri J. T. Howell
L. macounii Trel.

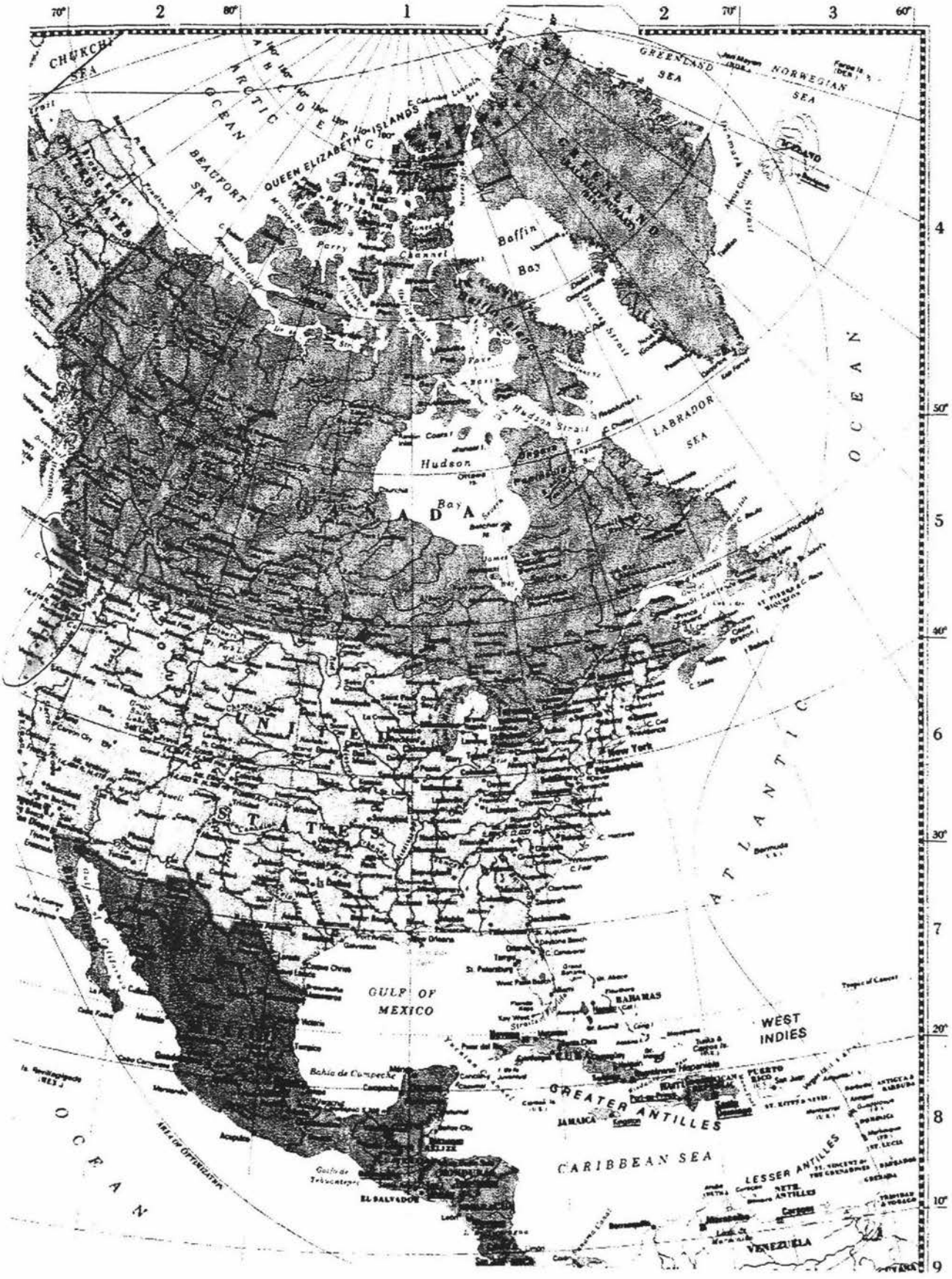


Fig. 1: Natural distribution of *Limnanthes sp.* [Map : Oxford University Press, 1996]
(marked in ink)

The other section has inward folding petals and sepals and are accordingly named section *Inflexae*. This section is considered more promising for domestication because it is regarded as being less prone to seed shattering. The species included in this section are:

Section *Inflexae*

L. gracilis Howell var *gracilis*
L. gracilis var *parishii* (Jepson) C. T. Mason
L. montana Jepson
L. alba Benth var *alba*
L. alba Benth var *versicolor* (Greene) C. T. Mason

L. floccosa Howell var *floccosa*
L. floccosa var *bellingeriana* (Peck) C. T. Mason
L. floccosa var *pumila* (Howell) C. T. Mason

Member species of the *Inflexae* section retain their seeds very well. Exceptional species highlighted were *L. alba alba* and *L. floccosa.* However, the entire flower head drops off during maturation of the latter species, therefore leaving *L. alba alba* as the species most promising as a potential crop where seed retention properties are concerned.

Members of the *Reflexae* group were less favoured because seeds from earlier flowers fall and are lost while the later flowers were still developing. It was also necessary to pull out the entire plants and let them dry on canvas sheets so that the seeds could be harvested. Gentry and Miller (1965) estimates that 10-20 % of seed production was lost or curtailed in this operation.

Member species between the two sections generally do not hybridise very well. The sole intersectional hybridisation known to be successful is that of *L. macounii x montana* (Ornduff, 1971).

Ecological survey of the genus showed a wide range of adaptability to different soil and weather conditions. This may be representative of the wide range of genetic diversity present in the genus. There also seems to be sufficient variability in growth form and seeding rates which should make selection for domestication promising. There is also some ability for interspecific crosses, this is important

because domestication usually involves some form of crossing within varieties or species within the genus (Miller *et al.*, 1964).

2.2 Habitat and life cycle

Seed germination occurs during the wet seasons of late autumn and winter. Flowering and seed production corresponds to dry conditions during spring. *Limnanthes alba* is suited to wet habitats exposed to full sun. It occurs mainly on the eastern margin of the Sacramento Valley of California and in the adjacent foothills of the Sierra Nevada from Merced County, north to Shasta County, along stream sides and pool edges, and in damp meadows. On the valley edge it is restricted almost entirely to the edges of vernal pools and ephemeral streams (Arroyo, 1973a).

As noted earlier by Gentry and Miller (1965), all species of meadowfoam require high levels of moisture for growth. The requirement by *Limnanthes alba* for wet habitats has been characterised by Pearson and Jolliff (1985). They discovered that, unlike many other crops, meadowfoam maintained an unusually high water potential which did not fall below -0.8 MPa. Furthermore, the stomata close even earlier before this level of water deficit was reached, at between -0.5 to -0.7 MPa. Their results suggest that stomata in meadowfoam leaves are sensitive to differences in leaf turgor between the guard cells and neighbouring cells, and stomata closure is independent of the overall leaf turgor. Hence stomata of meadowfoam plants are particularly sensitive to water stress, with very low tolerance to internal water deficits. Other than sensitive stomata, another factor which contributes to the low internal water deficit is the extensive fibrous root system. Pearson and Jolliff (1986a) has found that the roots were capable of extracting water at up to 150 cm of soil depth, although most of the water was obtained from the first 75 cm. While this may seem an over-stringent requirement for water, *L. alba* is considered to be less demanding of water amongst the genus (Gentry and Miller, 1965).

Preliminary studies by Brown and Jain (1979) have shown that *Limnanthes* is a long-day plant which requires 12-16 hrs of light for flowering. By manipulating light conditions, it is possible to grow three generations of plants in a year. Other information as measured during the study include:

- Days to flowering ~ 123.5 days (σ^2 10.7);
- No. of flowers per plant ~ 18.2 (σ^2 56.8);
- Seed set per flower ~ 2.5 (σ^2 0.83);
- Fecundity ~ 41.5 seeds per plant (σ^2 50.4).

The meadowfoam plants in their wild state are generally deemed unsuitable for commercial cultivation for a number of reasons. The following are some of the reasons as observed by Jolliff *et al.*(1981). Firstly, the plants are too short and have a severe prostrate growth habit which makes harvesting difficult. Protandry of the *L. alba* species results in heavy reliance of pollinator activity to effect fertilisation for seed yield, which can be unreliable. Plants in the genus exhibit seed shattering soon after maturity, representing a major loss of seed harvest. Finally, there is the tendency for seeds to enter secondary dormancy following a period of warmth (higher than 16°C). All of these qualities no doubt helped to contribute to its success in the natural habitat, but they serve only to frustrate the commercial grower.

2.3 Unique characteristics of Limnanthes *seed oil*

All the seed samples were relatively rich in oil (20-33%) and the amount of protein also ranged from 21-34%. This shows promise as both an oil crop and also as an animal meal (Miller *et al.*, 1964).

Earle *et al.* (1959) reported that meadowfoam seed oil was unusual because it had longer retention times in the gas chromatograph than common 18-carbon plant oils. Of the four oils, only erucic acid (a cis-13, 22-carbon monoene) was identified. One of the unknown fatty acids was identified by Bagby *et al.* (1961; cited in Calhoun *et al.*, 1981) as cis-5, cis-13 docosadienoic acid. It was not until 1969 that Smith *et al.* identified the other two unknown fatty acids as cis-5 eicosenoic acid; cis-5-docosenoic acid; and cis-13-docosenoic acid (Calhoun *et al.*, 1981).

While the fatty acid components of *Limnanthes* oil had been elucidated early on in its research, it was not until 1990 that Nikolova-Damyanova *et al.* managed to reveal the structure of the triacyglycerols, specifically that for *Limnanthes alba*. The resolving power of the high-performance liquid chromatography in reversed-phase mode revealed the 18 chemical species of triacylglycerols, with two other unknowns. Of these, three were more outstanding, and are presented in order of the fatty acid components: (Δ5-$C_{20:1}$)(Δ5-$C_{20:1}$)(Δ5-$C_{20:1}$) at 28.6%; (Δ5-$C_{20:1}$)(Δ5-$C_{20:1}$)(Δ 5,13-$C_{22:2}$) at 22.8%; (Δ5-$C_{20:1}$)(Δ5-$C_{20:1}$)(Δ13-$C_{22:1}$) at 15.0% (the Δ notation indicates the position of the double bond; while $C_{X:Y}$ refers to X number of carbon and Y number of double bonds). Altogether, these 3 triacylglycerols account for more than 66% of the seed oil content. Their results also showed that the top three fatty acids were cis-5-eicosenoic acid at 66.6%; cis-5,13-dodecasenoic acid at 16.0%; and cis-13-eicosenoic acid at 8.4%; accounting for 91% of the fatty acids in *Limnanthes* oil. The remaining fatty acids range from 16- to 22-carbon atom chains.

Fatty acid compositions show considerable variation in oil content. The study by Pierce and Jain (1977) showed that 9 out of 35 populations produced seeds where above 15% of the seed oil components were made up of the $C_{22:2}$ fatty acid; 4 populations had above 20% of the seed oil of the same fatty acid; while 8 populations had less than 10% of that fatty acid. The composition of the $C_{22:1}$ showed a similarly wide variation from 12.5% to 29.2% in seed oil composition.

The cis-5-eicosenoic acid isolated from *Limnanthes* oil is unique, no other fatty acid with a double bond in the carbon-5 position has been recorded in either plant or animal products as a constituent of a triglyceride (Smith *et al.*, 1960). The more usual position for an unsaturated bond in fatty acids would be at the $3n$ position, with n being a small integer. It can be argued that cis-5-eicosenoic acid conforms to this 'convention' (where n=5) by counting from the end opposite the carboxylic acid group, although this practice is counter to conventional numbering systems in organic chemistry nomenclature. However, a double bond at the carbon-5 position does exist in polyunsaturated fatty acids, such as the essential 5,8,11,14-eicosatetraenoic (arachidonic) acid. Other monounsaturated fatty acids which do not 'conform' to the $3n$ 'rule' also exist, such as 11-octadecenoic (vaccenic) acid.

The location of the cis-double bond which is unusually close to the acid group in the carbon chain (5C position) makes the molecule more versatile in transformation to other chemical derivatives. This is because the cis-double bond creates a greater kink in the carbon chain than a trans-double bond. At the same time, the position of the double bond near the carboxyl group (-COOH) also means that the straight portion of the carbon chain is shortened, and that the kinked portion of the carbon chain starts sooner. Both these factors means that the presence of a cis-5-carbon double bond results in a highly kinked molecule. In physical terms, the inter-molecular bonds are greatly weakened by the kinks, and the fatty acids stay in the liquid (oil) phase even at low temperatures and do not solidify into fats (Purdy and Craig, 1987). Another advantage of these unique positions of unsaturation is that they impart stability to the oil at high temperatures and make synthesis of chemical derivatives otherwise not possible or which had been more difficult to accomplish. It represents a renewable resource of hard wax, wax esters (as a sperm whale oil substitute), fatty alcohols and the long C chain fatty acids are useful for various industrial applications (Calhoun and Crane, 1978).

Limnanthes oil is also unusual among vegetable oils because it contains more than 95% fatty acids of 20- or 22-C chain while others more commonly possess 16- or 18-C chains such as cottonseed and soybean. The other common source of C_{20} or C_{22} fatty acids would be from fish oil such as herring oil. Even these sources only contain about 40% of these long-chain acids, as compared to 90% or more for *Limnanthes* oil. Another attraction of *Limnanthes* oil over fish oil is its relative lack of double bonds in its oils, which is an advantage for hydrogenation into wax products (Jolliff *et al.*, 1981). This led to the suggestion that the seed oil could be used as a high temperature lubricant, since larger molecules do not vapourise as easily as smaller molecules. The oil is also known to be extremely resistant to oxidation (Muuse *et al.*, 1992 cited in Jolliff *et al.*, 1993), not only because it has a long carbon chain with few double bonds, but also because the delta-5 position of its double bond is more stable than one in the middle of the fatty acid (Janick and Simon, 1990).

The unusual position of the unsaturated bond so close to the carboxyl acid group means that it is potentially very versatile in chemical transformations and should find wide-spread use in many industrial applications (Miller *et al.*, 1964). By comparison, the unsaturated bond or bonds in fatty acids from cotton or soybean occur at the 9th, 12th, and/or 15th carbon atom from the carboxyl end. Rapeseed and crambe oils are similar to *Limnanthes* because they contain up to 50% of erucic acid, which also has a 22-carbon chain but is unsaturated at the 13th C-atom. A comparison between rapeseed, crambe and meadowfoam oil is shown in table 1. The discovery that three of the four fatty acid chains were new prompted the interest in seed oil from this genus. (Gentry and Miller 1965). All the *Limnanthes* seed oils contain the same major constituent oils but in varying proportions. Other common fatty acids such as palmitic, stearic, oleic, linoleic, arachidic and myristic, occur in much smaller quantities (Pierce and Jain, 1977).

TABLE 1

Typical Compositions of Long Chain Fatty Acid Sources

	Meadowfoam	Rapeseed	Crambe
18:1	2	17	15
18:2	0.5	14	8
18:3	–	7	4
Other < C:20	1	3	3
20:0	0.5	0.5	1
20:1 (Δ5)	62.5	–	–
20:1 (Δ13)	–	9	4
22:1 (Δ5)	2.5	–	–
22:1 (Δ13)	12	48	59
22:2 (Δ5. Δ13)	18	–	–
Other > C:18	0.5	0.5	6
Total C:20 +	96.5	60	70

[Source: Purdy and Craig, 1987]

2.4 Novel oil extraction and content determination methods

Other than the conventional methods to extract vegetable oils used by industry, a novel way to maximise the yield of long chain fatty acids has been developed. This involves low temperature crystallisation of the *L. alba* acids in acetone (initial 0.05 g/ml) at -50°C which enriches eisosenoic acid in the precipitated fraction to 74%, while concentrating docosadienoic acid (70%) in the supernatant fraction (Chang and Rothfus, 1977).

Oil content determination is usually carried out by nuclear magnetic resonance (NMR) spectroscopy. A slight disadvantage to this method of oil determination is its destructive effect on the seeds, which can pose a problem to plant breeders with limited seeds that can be used for cultivar development. Patrick and Jolliff have recently (1997) demonstrated a non-destructive method for single-seed oil determination using near infrared transmission (NIT) spectroscopy. While the authors concede a slight drop in accuracy, the errors incurred using NIT is similar to NMR. NIT spectroscopy is advocated as a fast, efficient, and non-destructive way of determining seed oil content.

2.5 Industrial uses of Limnanthes *oil*

From the fatty acids with unsaturation at the carbon-5 position, numerous chemical intermediates have been derived: lactones; diepoxides; polymer mould-release agents; superior quality factises for rubber manufacture; dimer acids for polyamide synthesis; estolides for lubrication; and the chemical synthesis of hydroxy fatty acids. By using oxidative cleavage of the cis-5 double bonds, other acids such as pentadecanoic, glutaric, and suberic acids can be synthesised. Sulphurised meadowfoam oil has also been shown to be potentially useful for lubrication (Hayes and Kleiman, 1993). Erucic (cis-13-docosenoic) acid is currently used in its erucamide form as a slip agent during the manufacture of polyethylene sheets. Another use for it is as the sulphur polymer 'factice' in the rubber industry (Jolliff *et al.*, 1981). Its products can also be used as plasticisers, surfactants, and lubricants.

The other product left after fatty acids have been extracted would be glycerine. Glycerine is a common plant product obtained from numerous other oil sources as well. Some applications for it include paints, pharmaceutical products, baking aids, and also tobacco moisturiser (Anthony *et al.,* 1993).

The seed oil is deemed suitable as a suitable substitute for sperm whale oil due to the presence of its 20 and 22-C chain oils. Jojoba (*Simmondsia chinensis*) is another plant which is known to have 20 and 22-C straight chains in high concentrations. Using known processes of hydrogenation, the *Limnanthes* oil can be converted to a wax similar to jojoba. It has applications in plasticisizers, lubricants and detergents (Pierce and Jain, 1977). The long chain monoene and diene acids of *Limnanthes* oil are useful for synthesising diene and tetraene wax ester intermediates for prospective lubricant additives and PVC plasticisers (Chang and Rothfus, 1977).

Current commercial application of meadowfoam oil has been limited to high-value personal care products. The isopropyl esters of meadowfoam oil have a very low cloud point (-17°C) and low viscosity (10cp at 25°C), which is attractive to the cosmetic industry as it enables rapid adsorption into the skin (Purdy and Craig, 1987). As the price drops through improved agronomic qualities and utilisation experience, it is expected to have more widespread commercial uses that will reach the end-consumer (Patrick and Jolliff, 1997). Towards this aim, one of the objectives of the Oregon Meadowfoam Growers Association is to make the price of high quality meadowfoam oil drop to US$0.50 lb^{-1} in the near future (Purdy and Craig, 1987) .

Limnanthes seed oil may be converted to a jojoba oil-like substance by reactions already commercially practised (Miwa and Wolff, 1962). This means that all the potential uses of jojoba oil can be applicable to *Limnanthes* as well. Hydrogenation of these oils give it a good hardness and high melting points.

2.6 Oil content

The major fatty acid component was cis-5-eicosenoic acid (20-C) at 52-77%. The others were $C_{22:1}$ 8-29% $C_{22:2}$ 7-20%. Oil content from the seed ranged from 20-33% (Pierce and Jain, 1977). Fatty acids of longer carbon chains and higher levels of unsaturation are known to be an adaptive response to colder environments (Pierce and Jain, 1977). Perhaps that could account for its similarity to sperm whale oil, where one of its physiological roles would be to insulate against the cold in ocean depths. In light of this, perhaps more accessions could be sourced from colder climates as natural selection might produce populations with a naturally higher composition of $C_{22:2}$ fatty acids.

2.7 Potential use as animal feed

The meal remaining after oil extraction contains 21% crude protein (see table 2 for amino acid composition), 27% acid detergent fibre and 4.2% total glucosinolates (Throckmorton *et al.*, 1982). The glucosinolates were mainly (90%) meta-methoxyl benzyl glucosinolate, with most of the remainder being 2-hydroxy,-2-methyl propoyl glucosinolate (Purdy and Craig, 1987). The glucosinolates have been shown to have adverse effects in non-ruminant animals, such as heamolytic anaemia in cattle, as well as goitre in humans (Ellis, 1990). However, there was satisfactory performance of lambs fed on raw meadowfoam meal (Throckmorton *et al.*, 1982). Miller and Cheeke (1986) had also shown that raw meadowfoam meal may be used for beef cattle at up to 25% of their diets. Their results showed no overall difference in performance of beef cattle, although there was reduced average daily gain for the first four weeks. The authors attribute this to the presence of glucosinolates which affects palatability of the meal to cattle. However, there is evidence to show that meadowfoam meal may cause goitre in the offspring of goats (Jolliff *et al.*, 1981; White and Cheeke, 1983), so meadowfoam meal should be avoided during cattle pregnancy until further research isolates the cause of this malady. Perhaps one of the more obvious improvements that can be made to meadowfoam meal is the reduction of glucosinolate levels.

TABLE 2

Amino Acids in Meadowfoam Protein

	(g/16 g Nitrogen)		
Cysteine (half)	1.1	Lysine	5.1
Tyrosine	2.6	Arginine	7.4
Glycine	6.2	Methionine	1.3
Serine	3.9	Histidine	2.4
Alanine	4.0	Threonine	3.1
Aspartic acid	7.8	Leucine	6.4
Glutamic acid	16.4	Isoleucine	3.3
Proline	4.3	Valine	4.2
Ammonia	2.6	Phenylalanine	3.9

[Source: Purdy and Craig, 1987]

2.8 The choice of L. alba as a candidate for oil yield domestication

L. alba seems to be best suited for domestication and cultivation *en masse* because it is believed to possess superior seed retention properties in the genus, and due to its relative erect growth habit (Purdy and Craig, 1987). Preliminary studies in yield have resulted in around 2000 kg ha^{-1}, which is economically promising (Higgins *et al.*, 1971). *L. alba* also had high percentage viable pollen; of the 890 seeds counted only 22 were aborted (97.6% live seeds). This high reproductive success is important if seed yields were to be high.

L. alba is further classified into two subspecies: *L. alba alba* and *L alba versicolor*, which can be distinguished by several phenotypic traits. *Limnanthes alba versicolor* has white flowers that age pink, glabrous herbage and calyx; smooth to wrinkled nutlets; they are found in Sierra Nevada foothill area and in the mountains. *Limnanthes alba alba* has white flowers that does not age pink, pubescent herbage and calyx; tuberculate nutlets; and are mainly found in Great Central Valley of California. The two varieties are easily crossed and many viable seeds are obtained (Mason, 1952).

L. alba alba was reported to grow abundantly in fallow cultivated fields from Sacramento to Chico in California and appears to be best suited for light sandy soils and are also well-adapted to growing in valley slopes. As a result, *L alba* has less stringent water requirements. It is suggested that because this habitat was formed less than a hundred years ago, *L. alba alba* represents a fairly recent adaptive species in the genus. This rapid evolution is promising for domestication as it represents great potential for genetic improvement, being pre-adapted to soil conditions similar to cultivated lands. Gentry and Miller (1965) also indicated that the wild plants set seed profusely. Seed counts varied from 20-50 per plant in crowded conditions and up to 1000 per plant in diffuse conditions. While the maximum nutlet yield per flower is five, a reduction of the full complement frequently occurs as a result of inadequate pollination activity. This reduced number means that the resulting nutlets which develop tend to be of a larger size.

L. alba is predominantly an outbreeder and is slightly protandrous, with the delay between anthesis and stigma maturation ranging between one and three days. However, self-pollination is possible in *L. alba.* Emasculation does not hinder fertilisation or seed development. Foreign pollen was effective in fertilisation and subsequent seed formation. Therefore, *L. alba* is self-compatible and can also be easily cross-pollinated (Devine and Johnson, 1978).

2.9 Comparisons between L. alba *and* L. douglasii

It was earlier mentioned that *L. douglasii* was already a cultivated species used for ornamental purposes. However, as an oil crop, *L. alba* would be more promising. Comparing seed yields, *L. douglasii* produces large quantities (seed set efficiency 0.49-0.73) of small sized seeds, but *L. alba* produces fewer (seed set efficiency 0.40-0.61) and larger seeds. The seed weight of *L. alba* is higher than that for *L douglasii* (0.63-0.71 g 100^{-1}, mean 0.67g 100^{-1} for the former c.f. 0.39-0.52g 100^{-1}, mean 0.48g 100^{-1} for the latter) (Pierce and Jain, 1977). It would seem that seed size and seed numbers were compensating components of both species, and this was supported by Krebs and Jain (1985). They found that yield in *L. douglasii* populations was

correlated to seed set efficiency but not with seed weight. However, the reverse was true for *L. alba.*

For both species, *L. douglasii* and *L. alba*, there was a negative correlation between yield and days to full bloom, indicating that yield is greater with early flowering species. Yield was also positively correlated with floral asynchrony, so extended flowering time should be encouraged to maximise yield (Krebs and Jain, 1985). Based on these traits, *L. douglasii* would be superior since it has earlier flowering, and greater asynchrony of flowering (28 days of flowering c.f. *L. alba* with 21 days of flowering, on average), possibly aided by its more rapid development of a large leaf area index (Pierce and Jain, 1977).

Ultimately, the deciding factor was seed oil content, which swung the balance in favour of *L. alba*. The mean oil content of *L. alba,* at 26.9%, is higher than that for *L. douglasii* (22.05%) (and also higher than *L. floccosa* [21.7%], the other member of the *Inflexae* section with low seed shattering). This could be the result of a positive correlation between oil content and seed weight when comparing between the two species ($r = 0.62$; $P < 0.01$) (Pierce and Jain, 1977).

Another advantage of *L. alba* over *L. douglasii* was its shorter branch length. This may enable higher planting density to achieve greater yield per unit area (Pierce and Jain, 1977). The benefit of short branches was also supported by Krebs and Jain (1985) when they reported that shorter branch length (high first node to total branch length ratio) were associated with high yielding varieties of *L. douglasii.*

In addition, *L. alba* is preferred as the species for domestication due to its better seed retention, erect growth habit, and determinate growth. In light of the strengths of *L. douglasii*, if *L. alba* were to be the main species targeted for domestication, then more flowers per plant may be a good approach, coupled with early flowering and extended flowering period (Krebs and Jain, 1985).

2.10 Cultural notes on growing Limnanthes alba

The University of California, Davis, was one of the first institutions to do breeding work on *Limnanthes*. In 1976, a variety of *L. alba* called "Foamore", developed by Dr. Wheeler Calhoun (Brown *et al.*, 1979), was released for commercial production in Oregon. The "Foamore" cultivar was developed from an accession collected from northern California. It was after the release of this cultivar that more research was carried out to improve the understanding of yield components in meadowfoam.

2.10.1 Temperature

The best temperature for germination were around 16°C, or below. The optimum temperature for germination varied amongst species in the genus, from as low as 4°C for *Limnanthes alba* to 16°C *for L. douglasii.* The variation in optimum germination temperature within the genus may be due to the variety of natural habitats occupied by the species; from near sea level to as high as 5500 ft (more than 1800 m) above sea level, and from milder climates to severe summers and winters. In general, species with a broader range of optimum germination temperatures tend to be distributed over many habitats, while those limited to narrow climatic conditions also have more exacting requirements for germination (Toy and Willingham, 1966).

The rates of germination for *Limnanthes alba* at various temperatures are shown in table 3:

Table 3: Germination rates of *Limnanthes alba* at various temperatures

Temperature (°C)	Germination rate (%)
4	83
10	76
16	50
21	11.3
25	0
cooled from 25°C to 9°C	9

2.10.2 Secondary dormancy following warm temperature treatment

Following the discovery that *Limnanthes* seeds germinated poorly at high temperatures, Toy and Willingham (1967) also went on to investigate the extent of secondary dormancy induced in various species of *Limnanthes*. They subjected *Limnanthes* seeds to temperatures around 27°C for varying periods up to 14 days and tested their germination.

It was discovered that *Limnanthes alba versicolor*, and *Limnanthes striata* had very few seeds becoming dormant. *Limnanthes alba alba*, however, became increasingly dormant the longer the temperature was maintained at 27°C, with germination dropping from 77% without the warmth exposure to only 19% germination after 14 days of warmth exposure. A similar trend was also exhibited by *L. bakeri, L. douglasii nivea, L. floccosa floccosa, L. gracilis parishii,* and *L. montana*. For two subspecies, *L douglasii douglasii* and *L douglasii rosea*, the reaction to warmth was almost immediate, with germination dropping to 11% and 14% respectively after only 2 days' exposure to warmth. To prove that the seeds were not killed by the heat treatment and were indeed dormant, Toy and Willingham successfully revived most of the seeds after a period of dryness and then moisture at cool temperatures.

As *Limnanthes* plants are winter annuals, warmth-induced secondary dormancy serves as a protective mechanism in the event that heavy rains fall before winter. In their native habitats, seasonal rainfall usually occur in winter, and this mechanism helps to prevent them from germinating out-of-season when weather anomalies occur. However, it may be useful for the grower to obtain seeds which have this protective mechanism disabled. In this case, breeding with *L. alba versicolor* or *L. striata* may be desirable with this objective in mind.

2.10.3 Breaking of dormancy

Under natural conditions, cool and dark conditions are the most encouraging for germination of meadowfoam seeds (Toy and Willingham, 1966; Cheng *et al.*, 1997). To further encourage the breaking of dormancy, chemicals such as KNO_3 (Mmolawa, 1990, cited in Cheng *et al.*, 1997) and gibberellic acids GA_{4+7} (Hilhorst and Karssen, 1988) may be added to meadowfoam successfully (Cheng *et al.*, 1997). It was found that pre-chilling need not be recommended for optimum germination results; and that an alternating temperature regime such as 12 hours each of warmer and cooler temperatures may help to desensitise the seeds against becoming dormant at the higher temperature (Cheng *et al.*, 1997).

2.10.4 Flowering period and seed yield

Populations which were early-flowering gave the highest yield (Jain and Abuelgasim, 1981). This may possibly be due to the longer period of time which the plant can divert resources into seed production (average seed-fill period was found to be 30-31 days (Fiez *et al.*, 1991a). Krebs and Jain (1985) found that in addition to early-flowering, asynchrony in flowering, where flowering period is extended, is also a strong predictor to high yield. Early flowering (or the termination of vegetative growth) may be encouraged by having higher temperatures (maximum and minimum) in early spring, and also by having higher photon flux densities prior to flowering (Fiez *et al.*, 1991a).

2.10.5 Drainage and seed yield

Seed yields were not affected by soil water levels. Drainage did not increase the amount of seed produced (Calhoun and Crane, 1978). This comes as no surprise given the findings of Pearson and Jolliff (1985) about meadowfoam's low tolerance to internal water deficits (see pp 5-6 of this review).

2.10.6 Photosynthesis during maturity

It was found that the flowering period coincided with leaf senescence, with the leaf area index being at 0.1-0.2 at last bloom (Fiez *et al.*, 1991b). With leaf senescence starting shortly after anthesis, this means that assimilates being channelled into seeds were derived from a source other than the current photosynthates produced from leaves. A clue to where the other photo-assimilates may come from was offered by Seddigh *et al.* (1993) when he found that sepal photosynthesis differed from leaf photosynthesis by less than 3 μmol CO_2 $m^{-2}s^{-1}$, and the rate was even more than double that of leaves of early flowering plants. Having higher temperatures and stronger sunlight hours in early spring enables the plant to accumulate a store of assimilates that can be used for seed-fill and increase yield.

2.10.7 Pollination

The number of pollinator visits per receptive flower had a significant effect on seed set ($P = 0.011$), with flowers receiving 1, 6, and 11 honey bee (*Apis mellifera*) visits and setting 1.6, 2.3 and 3.3 seeds respectively. It was found that, on average, one bee visit deposited 15-22 pollen grains, and 6 bee visits deposited 43 pollen grains (Jahns and Jolliff, 1990). The effects of each additional bee visit does not seem to result in an additive increase in pollen grains deposited. The number of pollen grains deposited also had a significant effect on seed set ($P<0.01$). Under experimental conditions, 5 pollen grains were found to be sufficient to set 2.4 seeds on average, with 25 pollen grains producing 4.1 seeds on average (Jahns and Jolliff, 1990). This is clearly over-optimistic under field conditions and suggest that other factors, such as water stress, plant genotype, plant resource limitation, pollinator behaviour, and even timing may account for the lower than expected seed set.

To elaborate on the last point, a thesis by Jahns (1990, cited in Norberg *et al.*, 1993) noted that pollination 48 hrs after anthesis yielded three times as many seeds as at 24 or 72 hrs. It was also noted however, that heavy pollination and the subsequent heavier seed set slows down the formation of new flowers (Jain, 1979). This would be consistent with limited photosynthate resources of the plant, so heavy seed set in early

flowers may not necessarily improve yield, but rather allow a higher likelihood for the plant's potential seed set to be achieved sooner (if at all).

Still, there is a positive correlation found between pollinator visits and seed set in the field (Jahns and Jolliff, 1990; Norberg *et al.*, 1993), with Jolliff (1981) recommending four bee colonies per hectare. Cool, wet, and windy weather, the presence of other flowering plants and long distance from hive to meadowfoam will have detrimental effects on pollination (Jolliff, *et al.* 1981). There had also been a suggestion that instead of honey bees, another bee species, *Osmia lignaria propinqua* be used instead. This species is thought to be less adverse to inclement weather during foraging (Norberg *et al.*, 1993).

2.10.8 Fertiliser and seed yield

Calhoun and Crane (1978) reported that nitrogen application either decreased or had no effect on seed oil content of 'Foamore' meadowfoam. They also found that oil content decreased in meadowfoam on all three years that nitrogen was applied. However, its effects on seed oil composition was not reported. It was also discovered that ammonium sulphate produced a greater response in oil content than its corresponding nitrate at any given level. Adding ammonium sulphate at 100 kg/ha increased protein by as much as 29% while oil content decreased by 17%. For the nitrate, the increase in protein was 14% while the decrease for oil was 8%. The C-22 diene content was consistently increased in all years when nitrogen was applied. The sulphate had greater effect on its content than nitrate. At 100 kg/ha, ammonium sulphate increased C_{22} fatty acid content by 13% while the figure for extra nitrate was only 7%. Calhoun and Crane (1978) also reported that seed yield may also be reduced by nitrogen, so oil content was reduced in more than one way. It was concluded that while C_{22} diene content was increased, the gains were offset by the overall decrease in seed yield (Calhoun *et al.*, 1981).

In addition, nitrogen fertiliser delayed the onset of flowering (Johnson *et al.*, 1980; Pierce and Johnson, 1986). This delay may be brought about because nitrogen fertiliser increased vegetative growth (nitrogen fertilised plants were 8-10 cm taller at harvest (Calhoun and Crane, 1978)), which lowered light transmission levels. Since earliness of flowering is correlated to higher yield, this may be the reason why most reports indicated a lower yield. However, Pierce and Johnson (1986) found that the number of open flowers in one season was greater in nitrogen fertilised plots. Having more open flowers then led to more bee visits to the nitrogen fertilised plots than the control (Pierce and Johnson, 1986). The greater frequency of bee visits ought to have increased seed set efficiency, but this was not found to be so. Perhaps the bees could not get to the 'extra' flowers due to the extra branching which resulted in a denser canopy, or that seed set may be determined by other physiological or anatomical features.

Seed yield was also not found to be significantly different at fertiliser rates of between 50-100 kg N ha^{-1}. Therefore, the benefits of additional fertiliser, if any, quickly peters out at low rates of application. More importantly nitrogen fertilised plots produced seeds with lower seed oil content (Pierce and Johnson, 1986; Crane *et al.*, 1981). This can only be offset provided that seed numbers increase after nitrogen fertilisation. However, any increase in seed yield was not consistent, and cannot be relied on.

The development of the new cultivar 'Mermaid' (developed at Oregon State University) changed the scenario when it reportedly responded with increased yield when low rates of nitrogen fertiliser were applied (Jolliff, *et al.*, 1991). This cultivar is also superior to older ones with its better lodging resistance and higher yield. Pearson and Jolliff (1986) discovered that spring applications of nitrogen fertiliser improved seed yield by increased flower number. They found significant increases in seed yield with application rates of 50 and 100 kg N ha^{-1}. However, excessive rainfall during the flowering period may cause a lack of response to nitrogen fertiliser. In addition, there was no gain to be made when rates were increased to 100 kg N ha^{-1} compared to 50 kg N ha^{-1}. Seed set efficiency and seed weight were not affected. This finding was repeated by Jolliff *et al.* (1991) when they found that nitrogen fertiliser application of 50, 100, and 200 kg ha^{-1} increased flower numbers by 38, 72 and 92% respectively.

However, the application rate of 200 kg ha^{-1} was deemed to be unjustified for the marginal gain.

In addition, Fiez *et al.* (1991b) found no correlation between flower number and seed yield (n=24). Furthermore, at the 200 kg N ha^{-1} rate, dry matter production was only 6,530 kg ha^{-1} compared to more than 10,000 kg ha^{-1} for the plants receiving 50 kg N ha^{-1} (Pearson and Jolliff, 1986). High nitrogen fertiliser rates above 50 kg ha^{-1} may also induce lodging where conditions are conducive (Jolliff, and Seddigh, 1991), and also encourage invasion by gray mould fungus (*Botrytis sp.*) (McGahuey, 1986, cited in Jolliff *et al.,* 1993). Fertiliser applied during spring time was also found to be more useful than during autumn for flower number increase. Oil content of seeds was also found to increase during the 1982-3 growing season, but this result could not be consistently repeated, and had hitherto been unknown.

Jolliff and Seddigh (1991) concluded that increased dry matter production of 'Mermaid' meadowfoam was consistent following application of nitrogen fertiliser. This finding in itself represents a triumph of 'Mermaid' over the older 'Foamore', which did not produce consistent results with fertiliser application. However, the increase in dry matter production of 'Mermaid' varies from year to year. For example, Pearson and Jolliff's (1986) experiment found 'Mermaid' growing 40% taller and produced 50% more dry matter in the first year of fertiliser treatment than the second year. Hart and Young (1986, cited in Jolliff *et al.* 1993) reported that nitrogen levels in 'Mermaid' tissues were 1.5 - 1.7 g kg^{-1} in high yielding plants while 1.2 - 1.4 g kg^{-1} were found in lower yielding plants. While this may seem to encourage higher yields by extra nitrogen applications, they also found that concentrations above 1.8 g kg^{-1} would depress yield. It would be wise, therefore, to perhaps base nitrogen fertiliser applications on tissue nitrogen content test results.

It must be borne in mind that nitrogen fertiliser treatment encourages vegetative growth at the expense of reproductive development. In many cases nitrogen fertiliser delays the onset of floral initiation (Pearson and Jolliff, 1986; McGahuey, 1985 in Jolliff and Seddigh, 1991). This may shorten the flowering period, and if weather conditions are adverse for pollinator activity, this will confound the findings of fertiliser treatments to yield. Another way high nitrogen applications

may frustrate pollination attempts would be that the longer stems produced and higher stem branching may result in flowers opening below the canopy surface (Jolliff, *et al.*, 1993) and be by-passed by pollinators. Another reason *not* to fertilise came from Pearson and Jolliff (1986) who showed that oil yield is 81% higher when phytomass is low compared to the yield of the highest phytomass year.

In another case, 'Floral', a new cultivar derived from a cross between *Limnanthes floccosa grandiflora* and *Limnanthes alba alba*, the best yield results came from no nitrogen fertilisation, with soil supplied nitrogen estimated at 142-197 kg ha^{-1} (Hart and Young, 1986, cited in Jolliff *et al.* 1993). Perhaps soil should be tested for potential nitrogen supply before any nitrogen is applied, given that meadowfoam yield is depressed by high nitrogen levels.

From the above discussion, it does not seem advisable to apply nitrogen fertiliser to meadowfoam plantation. The plant seems to thrive in poorer soils and are penalised when planted in an over-rich environment. Response to nitrogen in soil also varies from cultivar to cultivar, and is sensitive to small changes of nitrogen concentration in its tissues.

2.10.9 Weed control

Acceptable weed control was also reported using propachlor (4.4-6.6 kg/ha) for pre-emergence weed control and diclofop (1.1-2.2 kg/ha) for selective weed control (Jolliff, *et al.* 1981; Jolliff, 1981). However, Waugh and Harrington (1994) reports that propachlor starts to reduce meadowfoam yield at rates above 2.2 kg ha^{-1}.

Waugh and Harrington (1994) had also done a fairly extensive study on the tolerance of meadowfoam to assorted herbicides under New Zealand conditions. Highlights of the report are listed below:

- Clopyralid should not be applied in excess of 0.3 kg ha^{-1}. At its higher rates, this controlled small-flowered buttercup (*Ranunculus parviflorus*) well.
- Haloxyfop at 0.25 kg ha^{-1} was tolerated, as well as in a mixture with 0.15 kg ha^{-1}. This mixture controlled annual poa (*Poa annua*), white clover (*Trifolium repens*) and groundsel (*Senecio vulgaris*). But its effects are generally considered narrow-

spectrum, as it failed to control scrambling speedwell (*Veronica persica*), spurrey (*Spergula arvensis*), toad rush (*Juncus bufonius*), chickweed (*Stellaria media*), and twin cress (*Coronopus didymus*). The addition of propachlor to the mixture improved its control over annual poa.

- Ethofumesate at 2.0 kg ha^{-1} was safely applied to control and prevent the re-establishment of annual poa and suppressed the growth of small-flowered buttercup. However, it failed to control scrambling speedwell, toad rush, groundsel, perennial ryegrass (*Lolium perenne*), broad-leaved dock (*Rumex obtusifolius*), hawksbeard (*Crepis capillaris*), and daisy (*Bellis perennis*).
- The following herbicides were not to be recommended: propyzamide; alachlor; chlorpropham; linuron; 2,4-DB; the fatty acid mixture of terbutryn, tribenuron, and triclopyr.

In conclusion, Waugh and Harrington recommend the use of ethofumesate, clopyralid and haloxyfop for reasonably weed-free meadowfoam production, but reminds growers to exercise sound cultural practices.

2.10.10 Harvesting and seed yield

Seed weight was significantly different at different harvest dates. Delaying the harvest is not recommended because it resulted in a continuous decrease in plant erectness and increased seed shattering. Harvesting one week before seed maturity gave the highest yield, possibly because seed shattering was reduced. At maturity or one week after maturity, seed losses of 20% and 37% were observed but the heaviest seeds and highest oil content were recorded at maturity. However, the higher seed retention of harvesting at one week before maturity did not impair the total seed yield by weight or oil content. Harvesting one week before maturity only caused a significant decrease in oil content for one year out of three (Johnson *et al.*, 1978). Another reason for early harvesting is that the 'green' seeds actually have a higher germination rate than mature 'brown' seeds (Cheng *et al.*, 1997). This was thought to be due to the oxidation of phenolic compounds which cause the brown coloration to add to the impermeability of the seed coat. Thus, harvesting one week before

maturity is recommended for optimal erectness and reduced seed loss due to shattering.

Paraquat (herbicide) was not effective as a harvesting aid because it encouraged seed shattering and a significant reduction in seed weight. Paraquat added resulted in reduction of seed oil content possibly due to chemically induced pre-mature ripening.

Seed losses are lowest when cut with a windrower timed during the period of more than 90% seed maturity. Windrowing should be done in the morning when there is enough dew to keep the flowers pliable to minimise seed shattering. Seed yields of up to 1,700 kg/ha has been attained but 2,750-3,300 kg/ha seems to be physiologically attainable (Jolliff, *et al.* 1981).

2.10.11 Phytomass and seed yield

In a bid to exploit the negative correlation between phytomass and oil yield, Norberg *et al.* (1993) tried to reduce phytomass by shading 'Mermaid' meadowfoam plants. It was found that shading increased seed yield by 35%, seed oil content by 8% and oil yield by 47%. Oil yield was also found to be correlated with seed number per unit area, but not with individual seed weight. Hence seed size is not indicative of oil content for this cultivar (This is contrary to the finding by Johnson *et al.* (1980) who found seed weight and oil content to be correlated at $r = 0.60$). Seed number per unit area was also found to be correlated to the number of open flowers per unit area but not with total flower buds nor seed set efficiency per flower. This may mean that it is better to improve oil yield by encouraging more flowers per unit area either by increased planting density or selection for more prolific flowering per plant, rather than high seed set efficiency. Shading may help to allow higher plant seeding by reducing the amount of vegetative growth, with more resources channelled into reproduction. Another effect of reduced vegetative growth by shading would be the reduction in spread of branches, thus allowing for higher planting density.

2.10.12 Seed set efficiency

A study by Fiez *et al.* (1991b) has found that seed weight and seed set efficiency per flower were important components for determining oil yield performance for 'Mermaid'. Higher seed weight were due to individual seed growth rates and were not found to be correlated to seed fill duration. Increased seed set per flower could be achieved by increased megagametophyte fertilisation, but this was also not found to be significantly correlated to bee visits, suggesting that there may be physiological factors affecting fertilisation success.

Another recent cultivar of meadowfoam is 'Floral', mentioned earlier. Agronomic traits and yield characteristics for this cultivar were determined by Jolliff *et al.* (1993). 'Floral' was found to be successfully grown at seeding rates which was twice that recommended for 'Mermaid'. Such high seeding rates enable quicker ground cover, forces the plants to be less prostrate, and hence easier to harvest (Jolliff *et al.*, 1981).

The higher density could be achieved due to the smaller plant size of 'Floral'. The smaller size also made the plants more resistant to seed shattering, which may have further boosted the yield figures. Seed yield was also found to be correlated to flower number (r^2=0.66, n=9, P=0.01). Although the number of flowers per plant decreased with higher seeding rates, this was compensated for by the sheer number of plants which made for an overall increase in flower number per unit area: for example, at seeding rates of 1.5 x 10^6 ha^{-1} there were 6346 flowers m^{-2}; while at 6.5 x 10^6 ha^{-1}, the flower numbers were at 9614 m^{-2} (Jolliff *et al.*, 1993).

The higher flower numbers per unit area could also serve to attract pollinators more effectively. To achieve more intense flowering per unit area, it was recommended that row spacing could be reduced to 6.6 cm from the more conventional spacing of 17.5 - 25.0 cm. The best yield results came from seeding rates of 6.5 x 10^6 ha^{-1} (about 35 kg ha^{-1}), no nitrogen fertilisation (soil supplied nitrogen estimated at 142-197 kg ha^{-1}), and pre-bloom irrigation (reiterating the need for high moisture requirements) (Jolliff *et al.*, 1993).

2.11 Future directions

A wish-list could be compiled to address the current shortcomings and agronomic inadequacies of meadowfoam. Some qualities of the ideal meadowfoam plant might be:

- erect and rapid vegetative growth especially in winter;
- late senescence of photosynthetic organs for greater accumulation of photosynthates;
- short flowering period (early-flowering) and a truncated vegetative phase for ease of harvesting and more resources channelled into seed;
- branches along the top half or two-thirds of the stem to allow for maximum photosynthesis and easy access to flowers for pollination;
- many flowers per plant to improve seed numbers and attract pollinators;
- self-fertilising ability to reduce dependence on pollinators;
- taller plant stature for easy harvesting;
- high seed set;
- large seed size;
- high oil content;
- non-seed shattering habits;
- if intended for animal feed, then glucosinolate levels should be reduced.

In the discussion of the advantages of meadowfoam seed oil, it was pointed out that the long carbon chains of its fatty acid enhanced its versatility in the production of chemical intermediates for industry. It might therefore be useful also to alter the fatty acid composition of its seed oils to include greater amounts of C_{22} fatty acids. However, from Pierce and Jain's (1977) work, the heritability values of oil content and fatty acid composition are thought to be low, based on a low between-family variance to within-family variance ratio. It might therefore be a better idea to source for populations with naturally high compositions of long-chain fatty acids. Since it had been shown that fatty acids of longer chains and higher unsaturation are adaptive to colder environments for chilling resistance (Lyons, 1973), a potentially promising source of germplasm with the tendency to produce long chain fatty acids may be in the colder environments of its natural habitat.

As for the development of a breeding program, the combined selection of best plants within best lines are recommended (Chozin, 1990), such as occurs in line selection and line breeding methods (Allard, 1960). This was because no notable trends were found in family/line performances and the values of heritability fluctuated wildly across generations (Chozin, 1990). However, this may have arisen due to sampling error from the small experiment.

2.11.1 Towards autogamous plants

Autogamy is considered fertility insurance in environments which do not favour the usual pollinators. It also has the advantage that only a single propagule is required to colonise new habitats. Levels of autogamy are based on degree of floral adaptation toward autogamy, greenhouse selfing ability, and the degree to which flowers are visited by bees in the field. Populations tending towards low autogamy are large flowered, possess nectar guides and protandry. More than 50% of their flowers are visited by bees in the field. Highly autogamous populations are small flowered, lack nectar guides and protandry. Less than 30% of their flowers are visited by bees in the field. It was found that all populations of *L. floccosa* are automatically self-pollinated in the greenhouse (Arroyo, 1973b).

Autogamy has become one of the objectives in meadowfoam breeding. This is because the protandry in *Limnanthes alba* makes outcrossing the major mechanism which effects fertilisation and seed set. This requirement is thought to hinder the realisation of potential yield, with heavy reliance on pollinator behaviour and its affecting variables, such as year to year weather conditions, timing of flowering, extent of vegetative growth through nitrogen fertilisation, etc. By having autogamous plants, the effects of these variables on yield would be minimised and the harvest could become more stable.

Another compelling reason for the development of autogamous plants is the heavy bee visits demand required for seed set. As noted earlier by Jahns and Jolliff (1990), 11 bee visits per flower in the field yielded only 3.3 seeds per flower. If 4,210 flowers m^{-2} open in one day, then 46,310 bee visits m^{-2} day^{-1} would be required for a yield of 3.3 seeds per flower (Norberg *et al.*, 1993)!

A study was undertaken by Jain (1978) to find the levels of outcrossing and inbreeding in natural *Limnanthes* alba populations. Outcrossing rates of *Limnanthes alba* were estimated by two genetic marker loci (est and got) varied between 43 - 97%. Heterozygosity was found to be between 12 and 27 %. Inbreeding depression was found to be significant in 4 of 7 populations monitored. In populations showing no inbreeding depression, autofertility increases with inbreeding, and seems to be under genetic control. This seems to be consistent with the hypothesis that inbreeders tend to arise in situations lacking pollinators.

It is therefore not surprising that crosses were attempted between *L. alba* and *L. floccosa*. The latter species was noted for low seed shattering and also belonged to the section *Inflexae*. The intention of the cross being to produce a plant with all the agronomic qualities of *L. alba* but with the autogamous property of *L. floccosa*.

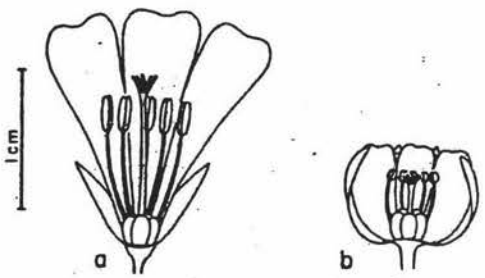


FIG. 2. Floral morphology of outcrossing *Limnanthes alba* (a) and autogamous *L. floccosa* (b).

Source: Arroyo, (1973a)

The outcrossed *L. alba* can be distinguished from an inbreeder such as *L. floccosa* in many ways. The outcrossed *L. alba* has larger petals and are fragrant, well-developed nectaries and nectar guides. It is self-compatible although very little seed is self-fertilised, since anther position is below the receptive stigma (see fig.2). Under insect-free conditions in the glasshouse, selfing ranges from 3-32% for *L. alba* as compared to 69-100% for *L. floccosa*. The former species also produces more pollen than the latter (467-933 thousand for *L. alba* versus 5-246 thousand for *L. floccosa*) There is also a degree of protandry which spaces anther dehiscence and stigma maturation from 2-3 days in *L. alba*. This contrasts with *L. floccosa* which have the anthers and stigma mature at the same time (Arroyo, 1973a).

In addition, *L. alba*, an outbreeder, shows little variation in flowering time. This is possibly because co-evolution with bee-timing means little leeway for timing variation if successful pollination were to be ensured. By comparison, *L. floccosa* has greater flowering time variation; inbreeders do not need tightly integrated timings with the emergence and flight periods of pollinators (Brown and Jain, 1979).

As a result of the different pollination strategies in the two species, allozyme variation at 11 loci in a large number of populations showed *L. alba* to be highly polymorphic in contrast to the virtual monomorphism within *L floccosa*. *L. alba* had 1.97 alleles per loci c.f. *L. floccosa* with only 1.02. *L. alba* also has 63% loci with polymorphism while *L. floccosa* has only 3 %. These results were based on 6 enzyme systems - esterase 2 loci phosphoglucomutase 2 loci, acid phosphatase (1 locus), leucine aminopeptidase (2 loci), glutamate oxaloacetate transaminase (2 loci) (Brown and Jain, 1979). Hopefully, the traits which enable self-pollination in *L. floccosa* would be transferred to *L. alba* while retaining the favourable agronomic qualities of the latter.

Attempts made by Jolliff *et al.* (1984) found that seeds were produced only when *L. floccosa* was used as the maternal parent. Initial attempts showed approximately 10% of the seeds produced germinated, but it was later determined that those were the result of self-fertilisation and were not hybrids. Successful hybrids were eventually achieved when *L. floccosa grandiflora* was used as the maternal parent. Seed yield from the F_1 plants were poor, at less than 1 g per plant. However, continued selection work up to F_4 progeny showed that some individuals managed to produced more than 10 g of seeds per plant. These results show the possibility of developing a self-fertilising *L. alba* plant type.

2.11.2 Male sterility in meadowfoam

Male sterile plants are potentially useful because they have often proven to be useful in experiments where crossing is involved. Kesseli and Jain (1984) have studied some *Limnanthes douglasii* populations which exhibited gynodioecy. They found that male sterile plants had aborted, whitish and small anthers with short stamens. These plants have a complete lack of pollen development beyond the pollen mother cell stage. Other plants had yellowish or reddish anthers of various sizes, but all had aborted pollen development at some stage after tetrad development. Some plants were incompletely male sterile and produced very low amounts of pollen grains.

In completely male sterile plants, the gynoecia resembled that of hermaphrodite plants, and were not reduced. However, petals, sepals and stamens were all significantly smaller ($P<0.001$). The flowers were also pure white, in contrast to the slight pinkish tinge typical of the species. The frequencies of male sterile plants in gynodioeic populations ranged from 0.04 to 0.24. Unfortunately, the authors (Kesseli and Jain, 1984) were unable to determine the genetic basis of male sterility in the paper, though some cytoplasmic and/or nuclear restorer factors are suspected.

Kesseli and Jain also investigated the gynodioeic populations for any difference in fitness between male steriles and hermaphrodite plants. It was found that male steriles had a significantly higher ($P<0.01$) number of flowers per plant. If this finding could be consistently reproduced, it might be worthwhile investigating the use of male sterile plants in meadowfoam plantations with a reduced proportion of hermaphrodite plants, since flower number per unit area is correlated to seed yield per unit area (Jolliff *et al.,* 1993). It was also found that male sterile plants were slightly larger (greater biomass; $P<0.05$). Yet the higher flower number is not due to a function relating to its size, since flower number per unit biomass was greater in male steriles than in hermaphrodites, indicative of greater reproductive allocation.

It might be argued that resource reallocation from the pollen mother cells might result in ovules of higher 'fitness' with less abortions. However, this difference in male sterile plants only conferred a slight fitness advantage over the hermaphrodites (fitness: 0.68-0.88, mean 0.81 relative to male steriles). This slight fitness advantage could easily become negligible for some populations, and indeed, quickly become a reproductive liability, since only half as many gametes would be produced by such plants. They might thence be eliminated, therefore accounting for the rarity of gynodioeic populations.

Another hypothesis for the presence of male sterile plants is that in those populations which possess them, selfing occurs more frequently and the male steriles are a mechanism to ensure outcrossing within the population as a defense against inbreeding depression (although inbreeding also allows an opportunity for rare beneficial recessive alleles to be expressed). This was found to be true in one of the populations. However, two of the gynodioeic populations (both *Limnanthes douglasii rosea*) studied by Kesseli and Jain displayed high degrees of polymorphic characters in the flowers. This was atypical for the subspecies as most populations were monomorphic for those characters. It was postulated that genetic exchange had occurred with *L. d. nivea* since some of these polymorphic forms resemble *L. d. nivea* flowers, and that the introduction of 'alien' nuclear and cytoplasmic alleles in the *L. d. rosea* populations resulted in incompatibility, hence the male steriles.

3.0 BIOMETRICAL REVIEW

3.1 Introduction to quantitative genetics

The performance of the individual can be measured by observing its *phenotype* (P). The phenotype can be thought of as the result of the individual's *genotype* (G) interacting with the given *environment* (E). This concept is denoted by the equation:

$$P = G + E$$

which was probably first partitioned in such manner by Fisher (1918). Since then, many others have adopted the terminology (e.g. Comstock and Robinson, 1948; Kempthorne, 1955; Moll and Stuber, 1974). When the genotype is grown in all possible environments, mean environmental deviation becomes zero, and the genotypic value is the same as the phenotypic value. Theoretically, there can be three possible genotypic values for a pair of alleles at a single locus, shown diagramatically below:

Genotype	A_1A_1	A_1A_2		A_2A_2
	--+------+---+----------+--			
Genotypic value	+a	d	0	-a

The above diagram shows the point of zero deviation from the mean to be mid-way between the two homozygote values, and A_1 is the allele that increases genotypic value. The genotypic value, d, of the heterozygote is dependent on the degree of dominance of A_1 over A_2. To measure the contribution of each locus to the population mean, the various genotypes must be weighted by the frequencies with which they occur in the population.

In any population, there will be the presence of some kind of family structure. Hence to gain a good understanding of population genetics, there must be some knowledge of the transmission of genetic values across generations. Parental genotypes do not offer this information, because genotypes cannot be transmitted, but rather, it is the alleles which are transmitted to create new genotype frequencies in the next generation. A measure must therefore be assigned to the genetic values which

offspring inherit from their parents. This is the *average effect of an allele* (α_i), and is defined as the absolute difference from substituting one allele for another. This average effect of an allele in a population is also dependent on the allele frequency within the population, so it is a property of the allele as well as of the gene.

While $\mathfrak{Go}_i$'s cannot be directly measured from the individual , one can infer their *breeding values* (A). If one individual is mated with several others, then the mean deviation of all its progeny from the population mean is a function of the genetic transmission value of the individual. Since only half the genes of progeny derive from the individual in question, this value must be doubled to obtain the breeding value.

The breeding value is thus one of the components of genotypic value. The remainder of the genotypic value is made up of the *dominance deviation* (D), so we can write:

$$G = A + D$$

Dominance deviation arises as a result of allelic interactions within a locus. It is the effect of the alleles which cannot be accounted for if the alleles are individually considered. Since both the average effect of an allele and the breeding value are dependent on allele frequency within the population, so too is the dominance deviation. Hence it is a property of both the gene and the population, not merely a measure of the extent of expression of the dominant allele.

In more complex cases, genotypic values are a result of not only a single locus but from the interaction of many loci. In these cases, the genotypic value partition must be extended to accommodate this *interaction deviation* (I) for *n* loci:

$$G = A_1 + D_1 + ... + A_n + D_n + I_{1...n}$$

Simplifying,

$$G = A + D + I$$

where A and D now represent the respective summed totals.

The transmission of genes governing quantitative traits is similar to those of qualitative traits. The difference being that usually many genes each with modest contributions to the trait are involved. Therefore, to study the collective effects of these genes, the use of statistical methods is necessary. From the earlier expression of P = G +E, we can partition the phenotypic variance as follows:

$$\sigma^2_P = \sigma^2_G + \sigma^2_E$$

and the genotypic variance may be further partitioned into:

$$\sigma^2_G = \sigma^2_A + \sigma^2_D + \sigma^2_I$$

where σ^2_A = variance of the breeding value, commonly referred to as the additive genetic variance (this is because in the absence of any dominance, alleles are said to act additively); σ^2_D = dominance variance; and σ^2_I is the epistatic or interaction variance.

The additive variance (σ^2_A) is the most important amongst the variance components because it describes the chief cause of resemblance between relatives. It is also one of the more readily obtained observations from the population. Additive variance is also important because it enables narrow-sense heritability to be found (discussed later).

If there are more than two alleles in the locus, then the additive variance arises from the α_i's of all the alleles, similarly the dominance variance will be the net result of all the dominance deviations.

When more than one locus is involved, the interaction variance (σ^2_I) becomes an additional component in genotypic variance. Firstly, one must ascertain how many loci are involved. For simplicity, the two-loci model will be considered. The type of inter-loci interaction must also be known, of which there are three types:

$$\sigma^2_I = \sigma^2_{AA} + \sigma^2_{AD} + \sigma^2_{DD}$$

where σ^2_{AA} = variance of breeding value interaction; σ^2_{DD} = variance of dominance deviation interaction; and σ^2_{AD} = variance of breeding value and dominance deviation interaction. The above may be further expanded to take into account more than two loci by the appropriate addition of more interactions between the other A and D values. As most quantitative traits are controlled by small step-wise values from many

loci, σ^2_I would be a frequent occurrence. Fortunately, when large numbers of loci are involved, the σ^2_I becomes small enough to be ignored (Falconer, 1989, pp 132).

3.2 Heritability

In order for plant selection during breeding programs to be successful, the character that is under study should be able to transmit its quality to its offspring. The extent to which the trait can be transmitted through the generations is known as its *heritability* (h^2). Not only is knowledge of heritability pre-requisite in a breeding program, it also allows some idea of expected gain through selection, and the construction of selection indices (e.g. Hazel, 1943). The definition of heritability is the ratio of genetic variability to total phenotypic variability, expressed as follows:

$$h^2 = \sigma^2_G / \sigma^2_P$$

The squared notation for heritability does not denote a compound variable, but reflects its make-up as variance components. Wright (1921) uses the symbol *h* to denote the coefficient of genetic determination, which is made up of the corresponding standard deviations.

One of the commonest ways of estimating heritability is to study the resemblance between relatives to determine how well the trait is genetically transmitted. Following this argument, the definition of heritability may be slightly altered to:

$$h^2_n = \sigma^2_A / \sigma^2_P$$

since σ^2_A is the component of genetic variability that is common among relatives. It makes sense to drop the non-additive genetic variances (σ^2_D, σ^2_I) because they are not retained across generations. The two definitions of heritability are differentiated by the term *broad-sense heritability* (h^2_b) for the former and *narrow-sense heritability* (h^2_n) for the latter.

For most cases, either type of heritability should be a reasonable estimate of the other, provided that the non-additive genetic variances (σ^2_D, σ^2_I) are not too great. Kempthorne (1955) noted that estimates of heritability are 'only mildly sensitive' to

non-additive effects of genes, having showed that coefficients of non-additive variance are only fractions.

Since heritability is dependent not only on the genotype but also on the environment, it is a property of the entire population living within a specific environment, and not that of the character. So if heritabilities are to be of use as a comparative statistic, then the two populations being compared should have similar genetic structures and live in similar environments.

3.3 Estimating heritability

The use of plant material where the familial relationship is known enables the estimation of the additive variance component which can lead to the narrow-sense heritability useful for plant breeding purposes. The prior discussion of partitioning of phenotypic variances simply into σ^2_G and σ^2_E observations is useful when dealing with wild populations or where familial relationships are unknown. The more usual way of estimating heritability is to use individuals which are related. There are three main methods of estimating heritability: (i) parent-offspring regression; (ii) as ratio of variance components from ANOVA; (iii) approximation of non-heritable variance from genetically uniform populations to estimate total genetic variance (Warner, 1952). The former two methods are more common than the last.

In parent-offspring regression narrow-sense heritability is regarded as the regression of the breeding value on the phenotypic value. So:

$$h^2_n = b_{AP}$$

where b is the regression coefficient. Expressed in this way, we may obtain a biometrical definition of heritability as *the slope of the linear regression line (if it exists) of the measurements of the character amongst offspring on the mean of the measurements of the character for their two parents* (Jacquard, 1983). For quantitative gene traits where there are many independent contributors, then the conditional expectation is for one variable to be the linear function of the other by the Central Limit Theorem (Jacquard, 1983).

Kempthorne (1953) suggests that the comparative resemblance between parent and offspring using regression is a better method, being less affected by environmental contributions than the comparison between contemporary relatives using intraclass correlations. However, Robertson (1959) found that the estimate of heritability obtained from a half-sib analysis is more accurate than that from a parent-offspring regression if the heritability is less than 0.25. To settle the disagreement, Hill and Nicholas (1974) have found substantial positive correlation between the results using both methods and have concluded that good experimental design provides robustness whichever method is used.

Since heritability has been defined as the ratio of two variance components, estimates of heritability must typically start with the estimation of the phenotypic variance components. This may be done by deducing genetic effects from data obtained by generation means (Anderson and Kempthorne, 1954; Hayman, 1958. Both works used inbred populations. Gardner and Eberhart, 1966, used random-mating populations). This method provides satisfactory precision without complex field designs. It also enables tests for non-allelic interactions; the presence of linkage; and also whether there are trigenic or higher gene interactions (van der Veen, 1959). It was pointed out that when there are epistatic interactions, the method of generation means fails to distinguish a unique value between additive and dominance variance (Hayman, 1958), but this limitation may be overcome when the epistatic variance is small (Hayman, 1960). Other criticism of the generation means method is that it can only be applied when gene frequencies are known, hence its use mainly with inbred parents; it also does not clearly show the relative importance of various gene interactions which it tests for (Sprague, 1966).

A more common method of partitioning phenotypic variability is through the use of variance components (Sprague, 1966). The analysis of variance (ANOVA) first described in detail by Fisher (1925, cited in Crump, 1946) has been widely used to test for the significance of treatment effects. However, because it can also be used to partition the relative contribution of variances from various sources, it is also a useful technique for estimating heritability, being a ratio of variance components. The techniques of using ANOVA to obtain variance components have also been well

discussed by other authors (Comstock and Robinson, 1948; Crump, 1946, 1951; Steel and Torrie, 1960) using various experimental models.

Heritability found using ANOVA may differ slightly in the composition of its variance components, and this reflects the peculiarities of the experimental model and practical constraints. Gordon (1978) lists three definitions for heritability estimated from ANOVA: (i) full-phenotype heritability = gene effects / total phenotypic variance; (ii) restricted phenotype heritability = gene effects / total phenotypic variance less all external non-genic effects; (iii) genetic influence = gene effects including all genic interactions / total phenotypic variance. The restricted phenotype heritability is usually adopted (e.g. Allard, 1960).

Another way of deriving narrow-sense heritability is to use sibling correlation. When familial structures are recorded, observations should be partitioned into family groups so as to exploit the knowledge of these relationships. This new partitioning will give rise to variances amongst family groups (σ^2_a), and within family groups (σ^2_w). The variance amongst family groups is common to all members of the family, and can also be referred to as the covariance of the family members. The proportion of variability accounted for between families as against the total variability will give a measure of how close the resemblance between families are. This is known as the intraclass correlation coefficient, expressed as *t*, below:

$$t = \sigma^2_a / (\sigma^2_w + \sigma^2_a)$$

The above relationship was first put forward by Kempthorne (1955) when he generalised that the correlation between relatives = covariance between relatives / total phenotypic variance.

Half-sibs differ from normal full siblings in that these individuals only share one parent in common. In this case, it is the female parent since it is laborious to have fully controlled pollination where the source of the male gametes are known. Since only one parent source is known, and assuming that its additive effect is α, then the group of half-sibs would have ½ α transmitted to them in common. The mean genotypic value that can be elucidated from the group of half-sibs is thus *half* the breeding value of the known parent. The covariance of the half-sib group can be

obtained by summing up all the sums of cross-products (products of the mean genotypic values squared and the expected frequencies) under conditions of random mating (table 4):

Table 4: Sum of cross products of genetic frequency and mean genotypic value

Common Parent Group	Frequency	Mean genotypic value	Sum of Cross Products
A_1A_1	p^2	$q\,\alpha$	$p^2(q\,\alpha)^2$
A_1A_2	$2pq$	$½(q-p)\,\alpha$	$½pq(q-p)^2\,\alpha^2$
A_2A_2	q^2	$-p\,\alpha$	$q^2(-p\,\alpha)^2$

The final result of $½(pq\alpha^2)$ which is ¼ the additive variance ($\sigma^2_A = 2pq\,\alpha^2$). A full treatment of this derivation can be found in Fisher (1918), and more recently in Falconer (1989).

The correlation amongst the half-sibs can then be expressed as the intraclass coefficient *t* as:

$$t = ¼\,(\sigma^2_A / \sigma^2_P)$$

assuming that: $\sigma^2_w + \sigma^2_a = \sigma^2_P$.

When *t* is expressed in this way, one notices that $t = ¼\,h^2_n$, and this offers one way of estimating narrow-sense heritability from the use of half-sib data.

3.4 The variance of heritability

The variance of the heritability is based on the variance of the primary estimator, which depends on the method chosen.

If heritability is estimated through parent-offspring regression, then by using the standard formula for the regression coefficient (b), we get:

$$\sigma^2_b = (N-2)^{-1}\,[(\sigma^2_Y / \sigma^2_X) - b^2]$$

where X is the independent variate; Y the dependent variate; and N the number of paired observations. Assuming that *b* is small enough that its square can be ignored, and N is large, we can simplify into:

$$\sigma^2_\beta \approx (N)^{-1}\,(\sigma^2_Y / \sigma^2_X)$$

substituting $\sigma^2_X = V_p / k$ where V_p is the phenotypic variance and k is the number of parents considered (i.e. 1 or 2); and $\sigma^2_Y = V_p [1+(n-1)t] / n$, where *n* is the number of individuals per family and *t* is the intra-class correlation between family members, we can re-express the approximate σ^2_b as:

$$\sigma^2_b = k[1+(n-1)t] / nN$$

where N is the total number of families (Falconer, 1989).

The above has assumed *n*, the number of individuals in each family analysed to be the same in all cases. In many real-life cases, this may not happen, either through design or accident. In such cases, the families will have to be weighted to give fair representation regardless of the progeny number. The intent of weighting is to assign weights inversely proportional to the variance of the regression so that all families would appear to uniformly have *n* offspring. A fuller discussion of estimating heritability by regression and the problem of unequal family size can be found in Kempthorne and Tandon (1953), and also some modification was made by Falconer (1963) so that each family is weighted to appear to have only one offspring.

In the case of sibling analysis using intra-class coefficients, the sampling variance of *t* is given by Fisher (1941, cited in Robertson, 1959; also in Falconer, 1989) to be:

$$\sigma^2_t = \frac{2[1+(n-1)t]^2(1-t)^2}{n(n-1)(N-1)}$$

where *n* is the number of individuals per family and *N* is the number of families. For the minimum value of σ^2_t, n should be $1/t$ (Anderson, 1959; Falconer, 1989). So for half-sib groups where $t = ¼ h^2_n$, *n* should be $4/h^2$. Since the value of h^2 is unknown, Falconer (1989) recommends that larger families should be planned because over-estimates are less damaging than under-estimates. As for the number of families, Robertson (1959) recommends 20-30 in a half-sib analysis when there is no previous evidence of heritability, family sizes for other cases are also discussed in his paper. This experiment should satisfy the requirements of minimising σ^2_t with 36 families of 14 individuals each replicated in 4 blocks.

Under favourable conditions, Falconer (1989) also simplifies $\sigma^2_t = 8t / nN$ (= $8t^2 / N$, in Robertson, 1959). To get the sampling variance of heritability for half-sibs, Falconer states that σ^2_t should be multiplied by 16. Therefore, by substituting $t = ¼ h^2_n$ for half-sibs, we can estimate :

$$\sigma^2_{h^2} = 16\,\sigma^2_t$$
$$= 32h^2 / nN.$$

The variance of heritability estimated from phenotypic variance components in randomised complete block (RCB) designs is not commonly stated. Osborne and Paterson (1952) may be considered pioneers in this field. In addition, Gordon (1979; *et al.*, 1972) also discusses in detail how standard errors and variances may be obtained from heritability estimated in RCB designs for annual and perennial species, and including environment effects such as sites and years.

To begin with obtaining heritability variance estimates from variance components, one needs the variance of the variance components first. This has been discussed by several authors, such as Crump (1946; 1951) and Satterthwaite (1946). In general (e.g. Gordon, 1972), this is expressed as:

$$V_{\sigma^2_t} = (n^2_t)^{-1} \sum_u \{2[E(MS_u)]^2 / f_u\}$$

where n is the appropriate divisor for the estimator σ^2_t ;

Ms_u is the u^{th} mean square of the σ^2_t ;

f_u is the degree of freedom of Ms_u

Some authors (e.g. Daniels, 1939, cited in Crump [1951]; and Gordon [1972]) suggest a correction of +2 for the degree of freedom as an aid to achieve the unbiased estimate.

Next, by denoting heritability as the ratio z between genotypic and phenotypic (restricted or full) effects, then the formula for the variance of the ratio z is:

$$\sigma^2_z = [\mu^2_y \sigma^2_x + \mu^2_x \sigma^2_y - 2\mu_x\mu_y cov(x,y)] / \mu^4_y$$

where σ^2 denotes variance; μ denotes expectation; x and y are the variance components corresponding to the numerator and denominator of the chosen definition of heritability.

Covariances have to be worked out for each individual term according to the components of x and y and involves the summation of variances and covariances of each component permutation.

4.0 Materials and Methods

4.1 The Experiment

4.1.1 Inference population

Thirty-six half-sib families were selected from the meadowfoam germplasm collection of Massey University. The families were derived from the University's open-pollinated composite of 'Moginie'. The 'Moginie' composite was made up of eight parental lines, six of which were from California (Davis) accessions; while the remaining two were mass selection bulks from the cultivars 'Foamore' and 'Mermaid' (Cheng, 1997). Eight of the families from the 'Moginie' composite were identified from prior principal component analysis to be, collectively, a fair representation of the entire species' genome. One family was selected from the original 'Moginie' composite, and another family was selected from a later generation of the same composite grown in 1995. The other twenty-six families were randomly chosen from the University's germplasm collection. The inclusion of the original 'Moginie' seeds and their most recent bulked descendants provide a reference to observe genetic drift.

4.1.2 Field design

The experiment was of the randomised complete block design with internal sampling. The size of the field was approximately 50 m by 30 m, and divided into four blocks. Each block was divided into 38 linear plots with 14 plant spaces. There was a distance of 0.75 m between each plant within the plot as well as between neighbouring plots. The plots on either side of the blocks were buffer blocks ('Moginie' 1995 accession used) and will not be analysed. Hence, only 36 plots in each block will be planted with experimental material, giving a total of 2,016 plants sown. The layout of the planting is in fig. 3. The packet numbers of each treatment refer to numerical nomenclature devised from previous work at the University. Every fifth plot in the field was labelled by a white peg with the appropriate number ahead of plant position 1. The first and last plots of each block were similarly numbered.

BLOCK 1

Plot:	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	0
Popn:	0	30	10	16	29	31	5	7	28	18	34	32	26	4	27	3	35	21	20	11	17	24	23	14	9	19	12	8	15	25	36	33	6	1	22	2	13	0

BLOCK 2

Plot:	0	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	0
Popn:	0	22	36	34	6	27	15	23	35	33	20	1	19	30	11	2	9	7	24	12	5	4	18	10	14	25	13	26	8	17	28	21	31	29	16	3	32	0

BLOCK 3

Plot:	0	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	0
Popn:	0	31	34	36	10	29	4	24	33	9	20	27	19	1	30	22	17	8	3	28	15	16	35	25	13	14	12	5	2	32	7	18	11	26	21	23	6	0

BLOCK 4

Plot:	0	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	0
Popn:	0	3	24	35	8	23	29	15	20	16	9	27	11	22	12	36	14	7	2	17	4	19	32	10	28	18	34	1	33	5	26	31	13	21	25	30	6	0

Key to treatments: 0 = Buffer Old = Original Moginie New = Latest Moginie Bulk (1995)

Popn	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Pkt	216	494	426	255	350	Old	***137***	***134***	424	391	***422***	387	38	283	164	465	73	445
Popn	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Pkt	***51***	107	43	***440***	356	New	450	***150***	210	86	179	***423***	228	***448***	346	162	184	178

Figure 3 : Layout of the experimental field

4.1.3 Cultural practices

4.1.3.1 Field preparation

The land was extensively cultivated by rotary hoe to clear it of all prior vegetation. Rills were marked across the length of the field at 0.75 m apart. Seeds were sown in autumn (11 May 1997) by hand with portable planting guides marked at 0.75 m intervals. At the same time backup seeds were sown in Jiffy pots, ready to replace any seeds in the field which failed to germinate. The backup seeds amounted to the equivalent of one complete block. No fertiliser was applied to this experiment because the literature suggested that this may have an adverse effect on seed yield.

An initial germination rate of approximately 85% was achieved in the field. Positions where seeds failed to germinate had replacement plants transplanted into them. The family made up from the original 'Moginie' accession was noted to have an extremely poor germination rate of 54% including the replacement plants. As such, only 38 of the plant positions for population 6 was filled, but it was present in all four blocks.

4.1.3.2 Irrigation

The plots were irrigated frequently from about one week after planting (19 May 1997); especially when the soil was dry, which happened frequently due to lack of rain and dry winds this season (due to a severe spell of the El Ninõ which resulted in unusually warm and dry weather). Each irrigation lasted for one hour, and was applied two to three times a week depending on weather conditions.

4.1.3.3 Pest control

There were also problems with animal pests, slugs and rabbits being the chief among them. Mesurol was applied twice by hand after the problem was first discovered. Slugs did not recur as a problem after the second application of Mesurol. Rabbit damage was successfully controlled by the erection of an 8kV electrified rabbit fence around the experimental blocks.

4.1.3.4 Weed control

There was also a problem with weeds, mainly twincress, grasses, rushes, groundsel, shepherd's purse, thistle, and some other weeds from the Asteraceae. The predominance of twincress and their resemblance to the young meadowfoam plants led to the decision to apply Nortron at 8 l ha^{-1} on 3 July 1997. This was not entirely successful and there was concern that further herbicide application may adversely affect the crop, given the already unfavourable weather conditions. Rotary hoeing was subsequently carried out on 17 Oct to remove all the inter-plot weeds. One month later, Versatil (2 l ha^{-1}) and Gallant (4 l ha^{-1}) was also applied by boom spray to kill thistles and rushes.

4.2 Characters Measured

4.2.1 Plant size and posture

(Characters 'Diameter', 'Height', 'Uprightness')

For each plant, the height and diameter were measured in centimetres at the stage when bud formation had just started. The ratio of plant height to diameter was also calculated from these and used as a guide to indicate the degree of uprightness of each plant. This ratio is of interest because more upright plants make harvesting easier.

4.2.2 Leaf shape

(Character 'Leaf shape')

Leaf shape was scored from 1 to 5, with 1 being the most slender (lanceolate) to 5 being the most rounded. The shape of meadowfoam leaves formed during each stage of growth change from being most rounded in the early juvenile stage to being very lanceolate in the mature stage. Once formed, the leaves do not change shape, so the shape of the leaves indicate at which stage they were formed. In order that leaf shape readings taken were comparable, a standardised period of the plant's development had to chosen. It was therefore decided that leaf shape readings were to be taken from the branches formed just below the first branches carrying flower buds. These were deemed to be leaves formed during the most 'mature' vegetative stage (see figure 4). Quantifying this character could help in cultivar identification.

1 2 3 4 5

Figure 4 : Leaf shape scores

4.2.3 Redness intensity and distribution on branches

(Characters 'Redness' and 'Distribution')

Some plants were observed to be flushed with redness to varying degrees. The intensity of redness on the branches of each plant was assigned, by visual inspection, a score of 1 to 5; one being a slight pinkish blush to five being dark-red (see figure 5). Half-scores exist for intermediate colours, and a zero is used to indicate the absence of redness. These were recorded from the plants just prior to their flowering.

The distribution of the of redness on branches were given meristic scores from 1 to 5; with 1 being 20% and 5 being 100%. A ½ score was sometimes awarded, so a score of 1½ means 30% of the branch was tinged with red.

Colour Score 0

Colour Score 3

Colour Score 1

Colour Score 4

Colour Score 2

Colour Score 5

Figure 5 : Colour Scores for redness

4.2.4 Date of first flowering

(Character '1st flower)

The date when the first flower buds are in full bloom is noted. This was used to calculate the number of days required from sowing to first full bloom. Such information may be used in the development of early- or late- flowering cultivar so that plantation activities may be staggered.

4.2.5 Pattern of flowering

(Characters 'Factor 1', 'Factor 2', 'Factor 3', and 'Factor 4')

At approximately one week intervals, the percentage of blooms in full flower was estimated by expressing them as decile intervals of 1 to 10, with each increment representing 10%. This was determined by comparing the estimated number of blooms in full flower with unopened and faded flowers. From these data, factors which described the pattern of flowering could be constructed. Data for the period of peak flowering character was collected over ten weeks (starting from 22 weeks after sowing) on the days listed below:

Week I - 21 Oct 97;	Week V - 21 Nov 97;	Week IX - 22 Dec 97;
Week II - 28 Oct 97;	Week VI - 28 Nov 97;	Week X - 30 Dec 97.
Week III - 6 Nov 97;	Week VII - 8 Dec 97;	
Week IV - 13 Nov 97;	Week VIII - 16 Dec 97;	

4.2.6 Number of fertilised ovules

(Character 'Seed set')

About three weeks into the flowering period, the number of fertilised ovules in five random flowers from each plant were observed and recorded. The reason for the relatively few flowers per plant observed was to conserve time so that harvesting could be completed with minimal seed shattering. Knowledge of the average quantity of seed set provides a reference when studying the propensity of the plants to shatter seeds.

4.2.7 Number of seeds retained at maturity per flower head

(Character 'Seeds retain')

Harvesting of seeds started in mid-December. This was done by hand for each plant, and as many flower heads as possible were collected. The seeds were then separated from the rest of the plant material and counted. The average number of ripe seeds from each flower head is calculated for each plant.

4.2.8 Degree of seed shattering

(Character 'Shatter')

By taking the difference between average number of fertilised ovules and average number of mature seeds for each plant, an estimate of the number of seeds shattered from each plant was obtained.

4.2.9 Average 1000-seed mass

(Character '1k seed-mass')

The total number of mature seeds collected from each plant was weighed and the average mass per 1000-seed for each plant was calculated. Seed mass is important because heavier seeds are more likely to yield more oil.

4.3 Data Analysis

4.3.1 Experimental Design and Statistical Model

In an experiment where it was expected that not all sources of variation were derived from treatment effects, steps must be taken to minimise the contribution of variance from other sources. In field experiments a likely source of additional variance is the position within the field: plants grown closer together experience conditions more alike than those grown further within the same field. Knowing this, steps must be taken to measure the extent of this variance so that it can later be excluded. With this mind, the randomised complete blocks with internal sampling design was adopted for this experiment (Steel and Torrie, 1960).

The statistical model is: $X_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ij} + \omega_k$

where X_{ijk} : the phenotype observed of the i^{th} treatment at the j^{th} block from the k^{th} plant

μ: mean

α_i: the i^{th} treatment effect (i = 1...f; f = no. of families)

β_j: the j^{th} block effect (j = 1...b; b = no. of blocks)

ε_{ij}: the interaction effect between the i^{th} treatment and the j^{th} block

ω_k: the within plot effect of the k^{th} plant (k = 1...p; p = no. of plants in plot)

The Infinite Random Universe Expectations of the Mean Squares [E(MS)] are given in table 5 for the above model:

Table 5: Expectations of Mean Squares

Source of variation	Degree of freedom	Mean Square	E(MS)
Blocks (b)	b-1	MS_β	$\sigma^2_\omega + p\sigma^2_\varepsilon + fp\sigma^2_\beta$
Treatments (f)	f-1	MS_α	$\sigma^2_\omega + p\sigma^2_\varepsilon + bp\sigma^2_\alpha$
Error	(b-1)(f-1)	MS_ε	$\sigma^2_\omega + p\sigma^2_\varepsilon$
Within plot (p)	bf(p-1)	MS_ω	σ^2_ω

4.3.2 Analysis of Variance

The main objective of the analysis of variance is to partition variance into various recognised sources. The mean squares from the E(MS) table would be used later for F-tests of significance; and the mean square equations used to derive the variance component for each partition of variation.

The SAS computer program was used in the calculation of means, mean squares, and appropriated F-tests. Significant groupings at the 5% level of significant differences were also determined.

The program AOVRCB (Gordon, *pers. comm.*, 1998) was used in the calculation variance components, heritabilities, and standard errors. The standard errors of variance components were developed by Crump (1946); and those of heritabilities were based on work by Osborne and Paterson (1952) and expanded by Gordon (1979; *et al.*, 1972).

4.3.3 Estimating heritability

Estimates of heritabilities were calculated using the program AOVRCB. Heritabilities would be presented at three levels: narrow-sense heritabilities; plant-level heritability; broad-sense heritabilities. Narrow-sense heritabilities is based on intra-class coefficient, *t*, which takes into account the half-sib family structure (Kempthorne, 1955). In the calculation of narrow-sense heritability, because 4 times the amongst-plot variance was used, the phenotypic variance needed to be adjusted with an additional 3 times the value of the amongst-plot variance in order to be comparable with the estimated genotypic variance. Plant-level heritability uses only the estimated genetic proportion of the within-plot variance (genetic coefficient, g_k) as the genotypic variance. Broad-sense heritability utilises the sum of genotypic variance at the plant- and population- levels, and includes non-additive genotypic variances as well (Allard, 1960; Falconer, 1989).

4.3.4 Estimating the genetic coefficient, g_k

The genetic coefficient (g_k) estimates how much of the variance expressed between plants was due to genetics. Unfortunately, there is no direct method of estimating genetic influence from phenotype observations. However, Smith (1938) had proposed a relationship between environmental variance of a larger plot expressed as sub-units (plants) of the plot. By estimating the environmental (error) variance at the plant level, the remainder of the variance could be attributed to genetic causes and g_k may be found.

Smith's equation allows the error (environmental) variance of a larger plot to be expressed in terms of sub-units as follows:

$$\sigma^2_n = \sigma^2_1 / n^b$$

where n : number of sub-units (plants) making up one experimental unit (plot)

σ^2_n :error variance of experimental unit

σ^2_1 : error variance of one sub-unit

b : coefficient of homoscedascity (extent of even randomisation)

The coefficient of homoscedascity for meadowfoam is not available in the literature, and was estimated by taking the average of published results in other seed crops: wheat; maize; sorghum; and soybean (Smith, 1938). For this experiment, the estimated value of *b* is 0.3925.

Re-arranging the equation for MS_ε, the error variance component can be found:

$$\sigma^2_\varepsilon = (MS_\varepsilon - \sigma^2_\omega) / p$$

and substituted as σ^2_n in the Harvey-Smith equation. The error variance at the plant-level could then be obtained with simple re-arrangement of the equation:

$$\sigma^2_1 = \sigma^2_n \cdot n^b$$

The genetic coefficient was then estimated as:

$$g_k = (\sigma^2_w - \sigma^2_e)/ \sigma^2_w$$

where σ^2_w : within-plot variance

σ^2_e : error variance at plant-level

The reliability of the estimate g_k so obtained hinges largely on the estimated coefficient of homoscedascity, *b*. The magnitude of *b* for any given crop is not constant and is dependent on the general growing conditions. For instance, Smith's paper (1938) quotes different values of *b* for the same crop depending on whether irrigation was applied. For some crops, the reported *b* was not constant but fell within a range of values.

In this experiment, the more conservative values for non-irrigated crops were used and where a range of values is quoted, the median value is adopted. This is despite the application of irrigation to this experiment. However, it should be noted that in this case, irrigation is used as a substitute for, and not to supplement, rain, due to the drought conditions brought about by the El Niño. It must also be borne in mind that the irrigation is more an attempt to keep the meadowfoam plant in moist conditions as regards its natural habitat, than towards any attempt to maximise yield.

The use of *b* from seed crops only is an attempt to bring the *b* estimate to a logical alignment with what might be expected for the meadowfoam plant if such information were available.

4.3.5 Analysing pattern of flowering

The character 'pattern of flowering' is a complex physiological behaviour that is dependent on many inter-related events, both environmental and genetic. In order that the minimum number of independent dimensions be found which can account for most of these variations observed, factor analysis by principal components was used (Cooley and Lohnes, 1971).

The raw data is transformed by standardised coefficients to create a composite score on an equalised scale. These new scores are known as factors. The prime property of the new net score over all attributes is that its variance represents a local maximum value. Since there are infinite sets of standardised coefficients, there will be many factors, and each factor will account for varying proportions of the variance in the data set.

A subsidiary feature of the composite score is orthogonality, so the variances accounted for by each factor does not overlap. A structure matrix can therefore be constructed which forms a hierarchy of 'local' maximum variance values. The proportion of variance accounted for by the first one or two factors therefore represent most of the variance while subsequent factors have diminishing accountability for the remaining variance. To achieve parsimony, a pre-determined cumulative threshold should be agreed upon so that less important factors may be neglected. For this experiment, it was set at 70%.

A method of applying the relevance of the factors to the input sources by awarding points as guidelines is presented here (Gordon, *pers. comm.,* 1997). Points are awarded to the standardised coefficients based on the percentage of the maximum coefficient value as indicated in table 6:

Table 6: Award of points to standardised coefficients of factor analysis

Percentage of maximum coefficient	Points
80 - 100 %	3
60 - 79 %	2
40 - 59%	1
20 - 39 %	½
0 - 19 %	0

Table 6 shows that the highest standardised coefficient receives 3 points. The *absolute* value of the next highest coefficient is compared against this coefficient to determine the number of points to be awarded.

A similar points system was also developed for the *absolute* values in the structure matrix, which shows the correlation between factors and the attributes they represent (table 7) :

Table 7: Award of points to values of structure matrix

Absolute value	Points
0.80 -0.99	3
0.60 - 0.79	2
0.40 - 0.59	1
0.20 - 0.39	½
0.00 - 0.19	0

In considering the award of points, only the *absolute* value is checked and the sign is ignored, this is because only the magnitude is important. Points derived from the two systems are then combined and checked against table 8 to determine the strength of emphasis of the factors against each of the inputs analysed.

Table 8 : Determining the strength of factor against input sources

std coeff \ struct	3	2	1	½	0
3	**STRONG**	**STRONG**	MEDIUM	*Suppressed*	*Suppressed*
2	**STRONG**	MEDIUM	MEDIUM	*weak*	*Suppressed*
1	*Enhanced*	*Enhanced*	*weak*	*weak*	*Suppressed*
½	pseudo	pseudo	*weak*	null	null
0	pseudo	pseudo	null	null	null

Enhanced and *suppressed* classifiers denote where discretion should be made as to the inclusion or exclusion of the relevance of the factors to the inputs (Gordon, *pers. comm., 1997).*

5.0 RESULTS AND DISCUSSION

5.1 Identifying the 'pattern of flowering' factors for further analysis

Further to the prior discussion on the need for compromise between parsimony and accountability of the factors, the cumulative variance accounted for by the factors obtained from the correlation matrix are presented in table 9. The table shows that 4 factors are sufficient to account for 73% of flowering behaviour, which exceeded the 70% threshold value decided upon for this experiment as discussed in the previous section.

The proportion of contribution by each factor was in descending order from factor 1 onwards. With a contribution of 32.72%, factor 1 was more than 1½ times more important than factor 2 which contributed only 19.65%. Factors 3 and 4 contributed about the same proportion, at 9.92% and 9.06% respectively. So factor 2 was about twice as important as each of factors 3 and 4.

The scores awarded to the structure matrix are presented in table 10; and those for the standardised score coefficients in table 11. Table 8 was used to interpret the scores and the results presented in table 12. Table 12 shows that factor 1 tends to explain late flowering peaks from weeks 7 to 10 inclusive. Factors 2 to 4 collectively emphasised peak flowering from weeks 1 through to 6: Factor 2 related to weeks 2,4,5; factor 3 to 1 and 6; and factor 4 to week 3 only. Hence, the factors may be named as follows:

Factor 1 ~ Factor for late flowering pattern

Factor 2 ~ Factor for mid flowering pattern

Factor 3 ~ Factor for bimodal flowering pattern (early and mid)

Factor 4 ~ Factor for 'week 3' flowering

Table 9 : **Proportion of variance accounted for by each factor**

Factor	*1*	*2*	*3*	*4*	*5*
Proportion	0.3272	0.1965	0.1065	0.0992	0.0906
Cumulative	0.3272	0.5327	0.6302	0.7295	0.8200

Factor	*6*	*7*	*8*	*9*	*10*
Proportion	0.0635	0.0562	0.0397	0.0147	0.0059
Cumulative	0.8835	0.9397	0.9794	0.9941	1.0000

Table 10 **Factor Pattern (Structure Matrix)**

Week	*Factor 1*	*pts*	*Factor 2*	*pts*	*Factor 3*	*pts*	*Factor 4*	*pts*
I	-0.04702	0	-0.28966	0.5	0.55868	1	-0.20988	0.5
II	-0.13404	0	-0.64995	2	0.33334	0.5	0.03780	0
III	-0.04023	0	-0.15211	0	0.06931	0	0.96756	3
IV	0.15536	0	0.77164	2	-0.17971	0	0.07186	0
V	0.26700	0.5	0.76462	2	0.27779	0.5	0.04677	0
VI	0.45743	1	0.33739	0.5	0.62613	2	0.03467	0
VII	0.86080	3	-0.03634	0	0.19573	0	0.03427	0
VIII	0.90904	3	-0.20192	0.5	-0.08464	0	-0.00094	0
IX	0.90632	3	-0.2458	0.5	-0.21274	0.5	-0.01383	0
X	0.74637	2	-0.19778	0.5	-0.21123	0.5	-0.02879	0

Table 11 **Standardised Score Coefficients**

Week	*Factor 1*	*pts*	*Factor 2*	*pts*	*Factor 3*	*pts*	*Factor 4*	*pts*
I	-0.01437	0	-0.14741	0	0.52464	2	-0.21149	0
II	-0.04096	0	-0.33076	2	0.31303	0.5	0.03809	0
III	-0.01230	0	-0.07741	0	0.06508	0	0.97497	3
IV	0.04748	0	0.39269	3	-0.16876	0	0.07241	0
V	0.08160	0	0.38912	2	0.26086	0.5	0.04713	0
VI	0.13979	0.5	0.1717	0.5	0.58797	3	0.03493	0
VII	0.26306	2	-0.01849	0	0.1838	0	0.03453	0
VIII	0.27780	3	-0.10276	0	-0.07948	0	-0.00095	0
IX	0.27697	2	-0.12509	0	-0.19977	0	-0.01394	0
X	0.22809	2	-0.10065	0	-0.19836	0	-0.02901	0

Table 12 **Interpretation of factors**

Week	Factor 1	Factor 2	Factor 3	Factor 4
I	null	null	MEDIUM	null
II	null	MEDIUM	null	null
III	null	null	null	**STRONG**
IV	null	**STRONG**	null	null
V	null	MEDIUM	null	null
VI	*weak*	null	**STRONG**	null
VII	**STRONG**	null	null	null
VIII	**STRONG**	null	null	null
IX	**STRONG**	null	null	null
X	**STRONG**	null	null	null

5.2 Analysis of Variance

5.2.1 Character means

The means and coefficients of variance (CV) of all the characters across the entire experiment are presented in table 13. The breakdown of each character mean by population is given in table 14 with the significance groupings and the least significant difference at the 5% level.

The data on seeds retained, seed mass, and degree of shattering were based on 26,674 flower heads and 54,863 seeds collected from 1,903 surviving plants. The mean number of flower heads collected per plant was about 14 and each plant yielded approximately 28 seeds which were collectible. While this appears less than that reviewed in the literature, it should be noted that this experiment is not geared towards maximum seed production.

The mean scores of fresh flowers present on each plant throughout the ten weeks is shown in table 15. It clearly shows that most flowers appeared in week 3, with flowering in weeks 4 and 2 coming in second- and third-most respectively.

5.2.2 Coefficients of variance

The coefficients of variance for all but one character are very high (table 13). The sole exception to this being the character for days to first flower, at CV = 2.66%. The mean breakdown by population shows that the difference between the quickest and slowest population to flower is a mere 10 days, or only a difference of 5 days about the mean. The analysis of variance sources will reveal whether this small variance is due more to uniform environmental conditions or exacting genetic behaviour. In any case, the low CV suggests little scope for the alteration of the plant's first flowering date.

Table 13: Means and coefficients of variance for all characters

Character	*Mean*	*C. V. (%)*
Diameter (cm)	13.958110	34.61679
Height (cm)	5.879960	27.08590
Uprightness	0.467403	34.67829
Leaf Shape	2.338076	26.45558
Redness	1.029160	97.46357
Distribution	2.128708	89.06277
1st Flower (days)	170.151100	2.66499
Seed set	3.552618	21.16054
Seeds retain	2.006274	46.96046
Shatter	1.561416	75.53654
1k seedmass (g)	5.831584	50.71562

Table 13a: Means and coefficients of variance for factors

Character	*Mean*	*C. V. (%)*
Factor 1	0.001632	9999.99
Factor 2	0.047715	4687.645
Factor 3	0.002596	9999.99
Factor 4	0.017891	7083.476

Table 14: Means by population

	Diameter (cm)		*Height (cm)*		*Uprightness*	
Population	*Mean*	*Sigf. Group*	*Mean*	*Sigf. Group*	*Mean*	*Sigf. Group*
1	13.8571	defghi	5.6339	hijklmno	0.43883	efghi
2	12.3214	hijk	5.3750	klmnop	0.52045	bc
3	14.0893	defgh	5.7143	ghijklmn	0.44595	efghi
4	12.9018	ghi	5.5893	hijklmno	0.48949	bcde
5	13.5545	efghi	5.9727	efghij	0.46651	cdefgh
6	11.0921	jk	5.0921	op	0.49139	bcde
7	16.6852	a	7.3519	ab	0.47266	cdefg
8	13.6518	defghi	6.5625	cde	0.53809	b
9	15.4000	abcd	5.9364	fghijk	0.43338	efghi
10	14.8571	bcdef	6.2679	defg	0.46586	cdefgh
11	16.0818	abc	6.9364	bc	0.47208	cdefg
12	14.8304	bcdef	6.6161	cd	0.48712	bcde
13	15.0545	abcdef	5.9000	fghijkl	0.44229	efghi
14	12.6339	ghij	5.1429	nop	0.48829	bcde
15	14.3125	cdefg	6.0446	defghi	0.44101	efghi
16	14.1071	defgh	5.9554	fghijk	0.47109	cdefg
17	13.7321	defghi	5.0893	op	0.40899	hij
18	15.3482	abcde	5.2321	mnop	0.36923	j
19	16.7563	a	6.0536	defghi	0.41320	ghij
20	16.3571	ab	7.5625	a	0.48935	bcde
21	14.9732	abcdef	5.7321	ghijklmn	0.41378	ghij
22	12.0714	ijk	4.4018	q	0.39959	ij
23	12.6964	ghij	6.9821	abc	0.62453	a
24	14.8571	bcdef	6.0536	defghi	0.45384	defghi
25	12.7321	ghij	4.8839	pq	0.45450	defghi
26	15.4196	abcd	6.4196	cdef	0.45477	defghi
27	12.8661	ghij	5.7679	ghijklm	0.47634	cdef
28	12.0714	ijk	5.8036	ghijklm	0.54371	b
29	10.7500	k	4.4464	q	0.47023	cdefg
30	12.9866	ghi	5.4732	ijklmnop	0.46158	cdefgh
31	13.8036	defghi	6.4554	cdef	0.51073	bcd
32	13.2500	fghi	5.4911	ijklmno	0.45316	defghi
33	14.0893	defgh	5.3304	lmnop	0.41546	ghij
34	13.6182	defghi	6.1727	defgh	0.51137	bcd
35	14.2857	cdefg	6.6250	cd	0.52183	bc
36	13.6429	defghi	5.4375	jklmno	0.4235	fghij
LSD (5%)	1.8058		0.5952		0.0606	

Table 14: Means by population (continued)

	Leaf Shape		*Redness*		*Distribution*	
Population	*Mean*	*Sigf. Group*	*Mean*	*Sigf. Group*	*Mean*	*Sigf. Group*
1	2.2246	h i j k l	0.8661	h i j k l m n	1.7143	h i j k l m
2	2.4821	b c d e f g	0.2768	r	0.8571	n
3	2.3750	f g h i j k	1.5714	c d e	3.7500	a
4	2.7818	a	1.3839	d e f	2.9107	b c d e
5	2.3000	g h i j k	1.9364	b c	3.0818	a b c d
6	2.4054	d e f g h i	0.8816	h i j k l m	2.2632	e f g h
7	2.3148	g h i j k	0.9259	g h i j k l	1.4074	j k l m n
8	2.3214	g h i j k	0.7679	i j k l m n o p	1.9732	g h i j k
9	2.6455	a b c	0.4182	p q r	1.0909	m n
10	2.3125	g h i j k	1.2411	e f g h	2.6696	d e f g
11	1.9545	m n	2.3704	a	3.5926	a b
12	2.3610	f g h i j k	0.8036	i j k l m n o	1.9821	g h i j k
13	1.8000	n	1.6727	b c d	2.7364	c d e f
14	2.3839	f g h i j	1.6607	b c d	2.6696	d e f g
15	2.1518	k l m	0.7321	j k l m n o p q	1.8304	h i j k l
16	2.3364	g h i j k	1.0714	f g h i j	1.6786	h i j k l m
17	2.2232	h i j k l	0.8839	h i j k l m	2.3036	e f g h
18	1.7589	n	0.4643	o p q r	1.1964	l m n
19	2.4464	b c d e f g h	0.6696	k l m n o p q	1.2946	k l m n
20	2.4196	c d e f g h i	0.8214	i j k l m n o	1.9107	h i j k
21	2.6607	a b	0.6429	k l m n o p q r	1.7232	h i j k l m
22	2.6161	a b c d e	1.0089	f g h i j k	2.7857	c d e f
23	2.3839	f g h i j	1.0179	f g h i j k	1.7589	h i j l k m
24	2.5909	a b c d e f	0.6875	k l m n o p q	2.1875	f g h
25	2.3482	g h i j k	0.5000	n o p q r	1.2768	k l m n
26	2.1964	i j k l	1.9464	b c	3.4107	a b c
27	2.4018	e f g h i	0.6161	l m n o p q r	1.3482	j k l m n
28	2.1607	j k l m	1.1071	f g h i j	2.8125	c d e f
29	2.6339	a b c d	0.3839	q r	1.4732	i j k l m n
30	2.3214	g h i j k	1.2768	e f g	2.1786	f g h i
31	2.4107	d e f g h i	1.9911	b	3.7054	a
32	2.4643	b c d e f g	0.7946	i j k l m n o	1.7143	h i j k l m
33	2.4455	b c d e f g h	0.5268	m n o p q r	0.9554	n
34	2.0556	l m	1.1000	f g h i j	2.0182	g h i j
35	2.3393	g h i j k	0.9091	g h i j k l	2.1182	f g h i
36	2.1518	k l m	1.1339	f g h i	2.3304	e f g h
LSD (5%)	0.2316		0.3751		0.7089	

Table 14: Means by population (continued)

	1st Flower (days)		*Seed set*		*Seed retained*	
Population	*Mean*	*Sigf. Group*	*Mean*	*Sigf. Group*	*Mean*	*Sigf. Group*
1	170.1964	i j k	3.2836	k l m	1.8406	g h i j k l
2	167.7321	m n o p	3.6945	d e f g h i	1.9879	d e f g h i j
3	166.2321	p q	3.0536	m n	1.7948	h i j k l
4	166.8393	o p q	3.6182	f g h i j	1.8143	g h i j k l
5	172.4727	c d e f	3.9091	b c d e	1.8709	f g h i j k
6	171.0286	f g h i j	3.4455	i j k	1.9265	f g h i j
7	176.9444	a	4.5283	a	2.2883	b c d e
8	175.3036	a b	4.4436	a	2.6891	a
9	167.4107	n o p q	3.1358	l m n	1.7581	i j k l
10	169.5000	j k l	3.7393	c d e f g h	2.0328	d e f g h i j
11	170.5185	h i j	3.8481	b c d e f	2.2084	b c d e f
12	171.3393	e f g h i	4.0545	b	1.9921	d e f g h i j
13	172.2182	d e f g	3.3385	j k l	2.1683	b c d e f g
14	170.6071	g h i j	3.6873	d e f g h i	2.0432	d e f g h i j
15	170.5000	h i j k	3.5963	f g h i j	1.9968	d e f g h i j
16	172.1071	d e f g h	3.9786	b c	2.3323	a b c d
17	167.3571	n o p q	3.2982	k l m	1.4951	l
18	166.0000	q	3.6370	e f g h i	1.9373	e f g h i j
19	168.2500	l m n o	3.0750	l m n	1.7441	j k l
20	171.5893	d e f g h i	3.4873	h i j k	2.0580	d e f g h i j
21	167.6786	m n o p q	2.9714	n	2.1392	b c d e f g h
22	168.8036	k l m n	3.2868	k l m	1.7880	h i j k l
23	175.9464	a	4.4618	a	2.4249	a b c
24	170.2545	i j k	3.4218	i j k	1.9490	e f g h i j
25	166.9643	o p q	2.9074	n	1.5339	k l
26	169.3393	j k l m	3.6464	e f g h i	2.1046	c d e f g h i j
27	171.5536	e f g h i	3.7786	b c d e f g	1.8989	f g h i j
28	167.1429	n o p q	2.8821	n	1.7945	h i j k l
29	168.8036	k l m n	3.4357	i j k	2.1075	c d e f g h i
30	168.4643	l m n o	2.9018	n	1.8101	g h i j k l
31	174.0727	b c	3.8036	b c d e f g	2.3438	a b c d
32	173.2679	c d	4.0500	b	2.4716	a b
33	168.1964	l m n o	2.8945	n	1.8053	h i j k l
34	172.8889	c d e	3.9396	b c d	2.1078	c d e f g h i
35	168.3214	l m n o	3.1286	l m n	2.0408	d e f g h i j
36	170.4286	h i j k	3.5393	g h i j k	1.9012	f g h i j
LSD (5%)	1.6989		0.2823		0.3606	

Table 14: Means by population (continued)

	Shatter		*1k Seedmass (g)*		*Factor 1*	
Population	*Mean*	*Sigf. Group*	*Mean*	*Sigf. Group*	*Mean*	*Sigf. Group*
1	1.4404	efghij	6.1106	bcdefghijk	-0.14160	defg
2	1.7103	bcdefgh	5.4815	efghijkl	0.00890	bcdefg
3	1.3299	ghijkl	5.9734	bcdefghijk	-0.23290	g
4	2.0911	ab	5.7536	cdefghijkl	0.10730	bcdef
5	2.0382	abc	5.0535	klm	0.18160	bcd
6	1.3735	fghijk	5.9329	bcdefghijk	0.25900	b
7	2.2425	a	4.4636	m	0.05150	bcdefg
8	1.7411	bcdefg	5.2520	hijkl	-0.02890	bcdefg
9	1.3419	ghijkl	5.5314	defghijkl	-0.13570	defg
10	1.7079	bcdefgh	5.5161	defghijkl	-0.07150	bcdefg
11	1.6273	cdefgh	5.5363	defghijkl	-0.14050	defg
12	2.0747	abc	5.8586	bcdefghijkl	-0.03150	bcdefg
13	1.1702	ijkl	5.3646	ghijkl	-0.05490	bcdefg
14	1.6307	cdefgh	5.1424	ijkl	-0.04600	bcdefg
15	1.5822	defghi	5.6501	defghijkl	-0.02920	bcdefg
16	1.7618	bcdefg	4.7714	lm	0.19670	bc
17	1.8294	abcde	6.5354	abcdef	0.92390	a
18	1.7014	bcdefgh	6.3295	abcdefgh	-0.20880	fg
19	1.2834	hijkl	5.7682	cdefghijkl	-0.08250	cdefg
20	1.4266	efghij	6.6199	abcd	-0.12500	cdefg
21	0.8928	l	6.5421	abcde	-0.21720	fg
22	1.5024	efghij	5.5831	defghijkl	0.01640	bcdefg
23	2.0280	abcd	5.0968	jklm	0.10040	bcdef
24	1.4728	efghij	6.2915	abcdefgh	0.86110	a
25	1.4436	efghij	6.9175	ab	0.15150	bcde
26	1.5287	efghij	7.2601	a	-0.12730	cdefg
27	1.8748	abcde	5.8814	bcdefghijkl	-0.1037	cdefg
28	1.1478	ijkl	6.2566	abcdefghi	-0.2163	fg
29	1.3258	ghijkl	6.4230	abcdefg	-0.1572	efg
30	1.0860	jkl	5.9449	bcdefghijk	-0.16	efg
31	1.5289	efghi	5.4217	efghijkl	0.0782	bcdefg
32	1.5747	defghi	5.4099	fghijkl	-0.0494	bcdefg
33	1.1150	jkl	6.2229	abcdefghij	-0.2079	fg
34	1.8189	abcdef	5.2956	ghijkl	-0.035	bcdefg
35	0.9512	kl	6.7952	abc	-0.0618	bcdefg
36	1.6468	bcdefgh	5.9186	bcdefghijk	-0.1079	cdefg
LSD (5%)	0.4533		1.1320		0.3319	

Table 14: Means by population (continued)

	Factor 2		*Factor 3*		*Factor 4*	
Population	*Mean*	*Sigf. Group*	*Mean*	*Sigf. Group*	*Mean*	*Sigf. Group*
1	-0.2116	g h i j k l m n	0.0153	e f g h i j k	0.1116	a b
2	-0.5503	j k l m n	0.0586	d e f g h i j	0.2201	a b
3	-0.8254	n	0.5969	a b c	-0.1428	a b
4	-0.3606	i j k l m n	0.7528	a	0.0422	a b
5	0.8854	a b c d	0.3701	b c d	-0.2543	b
6	-0.0291	f g h i j k l m n	0.0290	e f g h i j	0.0835	a b
7	1.1543	a b	-0.5581	o p	-0.1009	a b
8	0.7629	a b c d e f	-0.6807	p	-0.0951	a b
9	-0.3546	i j k l m n	0.2290	d e f	0.0436	a b
10	0.1756	c d e f g h i j k l	-0.0384	f g h i j k l	0.1044	a b
11	-0.0356	f g h i j k l m n	-0.3483	l m n o p	0.0801	a b
12	0.4053	b c d e f g h i	-0.2193	i j k l m n	-0.0329	a b
13	0.2636	b c d e f g h i j	-0.3148	k l m n o	-0.0476	a b
14	0.2228	c d e f g h i j k	0.0027	e f g h i j k	0.2180	a b
15	-0.2301	g h i j k l m n	-0.1090	g h i j k l m	0.0134	a b
16	0.9309	a b c	0.1485	d e f g h	0.0060	a b
17	-0.7057	m n	0.7336	a	-0.0199	a b
18	-0.5930	k l m n	0.3262	c d e	0.1748	a b
19	-0.2998	h i j k l m n	-0.0339	f g h i j k l	-0.2049	a b
20	0.0476	e f g h i j k l m	-0.4651	n o p	-0.0193	a b
21	-0.5549	j k l m n	-0.0448	f g h i j k l	0.0756	a b
22	-0.1637	g h i j k l m n	0.1930	d e f g	0.1906	a b
23	1.1777	a b	-0.3814	m n o p	0.0689	a b
24	0.1691	c d e f g h i j k l	0.6196	a b c	-0.0663	a b
25	-0.5537	j k l m n	0.6943	a b	-0.2080	a b
26	-0.0853	g h i j k l m n	-0.1432	h i j k l m n	0.2144	a b
27	0.0828	d e f g h i j k l m	-0.2270	i j k l m n o	-0.1379	a b
28	-0.6486	l m n	-0.0090	f g h i j k	-0.1706	a b
29	-0.4705	j k l m n	0.1484	d e f g h	-0.0822	a b
30	-0.5643	j k l m n	0.1059	d e f g h i	0.1324	a b
31	0.8564	a b c d e	-0.3506	l m n o p	0.0841	a b
32	0.5769	a b c d e f g	-0.5531	o p	0.1384	a b
33	-0.4967	j k l m n	-0.1392	f g h i j k l m n	0.0163	a b
34	0.5166	a b c d e f g h	-0.4700	n o p	-0.0245	a b
35	1.2676	a	0.3624	b c d	0.2492	a
36	0.0381	e f g h i j k l m	-0.2311	j k l m n o	0.0036	a b
LSD (5%)	0.8377		0.3334		0.4746	

Table 15 **Mean scores across all plants in the 10 weeks of flowering surveyed**

Week	Mean	Std Dev
I	0.11335	0.49772477
II	2.45139	1.78246933
III	5.87708	1.90076479
IV	4.11184	2.63689431
V	0.80101	1.57928851
VI	0.20655	0.79353323
VII	0.12292	0.59314254
VIII	0.02569	0.30261767
IX	0.00957	0.16316263
X	0.00202	0.06346807

Five other characters have CVs which are comparatively smaller, at less than 35%. Two of these relate to plant size, diameter and height, while uprightness is a secondary character being based on the ratio of the former two. Leaf shape and number of seed set are the remaining two characters with lower CVs.

Seed set is highly dependent on environmental factors since the plant is an outbreeder. The lower CV value for seed set may be due to the relative small size of the experimental field which ensures that all plants are accessible to the same group of pollinators. With a CV of 21.2%, seed set has the second lowest CV value among the characters surveyed.

The characters listed above with low CVs show that variation about the mean is not very great, this suggests the possibility that there might be reduced scope for the alteration of these characters in the plant. However, the plant's responsiveness to selection does not depend exclusively on pre-existing variance, as the equation for genetic advance will later show.

The large CV values for the other characters is to be expected since the plants are supposed to be representative of the spectrum of wild populations. One would expect low CV values for more characters in cultivated varieties where extensive genetic selection ensures that the plants responds similarly to a given environment.

The mean values of the factors from principal component analysis were all very low while variances were exceptionally high. This was to be expected since these factors were not real plant characters but mathematical constructs designed specifically to have a mean about zero (due to standardised scales) and large variances so as to be the best possible discriminator. For this reason, the means and CVs are presented separately in table 13a.

5.2.3 F-test results of the mean squares

The statistical model for ANOVA allows the partitioning of sources of variance into four levels: block; population; error (genotype-environment interaction); and within-plot. F-tests were not performed on the within-plot partition because it was at the lowest echelon of the model and thus had no valid denominator (table 16). The results for the 4 factors describing pattern of flowering are presented in a separate section because they were not true plant attributes.

Most characters had significant sources of variation from the partitions tested, except for six: diameter; degree of shatter; thousand-seed mass; flowering peak factors 1, 3, and 4. Of the six, degree of shatter; thousand-seed mass; and peak flowering factor 4 had non-significant contributions of variance from two partitions.

Peak flowering factor 3 (indicative of peak flowering in weeks 1 and 6 when flowering was surveyed) did not have significant variation from the 'block' source, suggesting that location within the experimental field was unimportant.

Plant diameter and peak flowering factor 1 (weeks 6-10) received no significant 'population' variance. These characters may have below-average genetic influence, since members within each population are half-sib families, which should tend to make populations different in phenotypic expression.

The characters 'shatter' and 'factor 4' did not have significant variance from both 'block' and 'population' partitions, so the error and within-plot partitions account for most of the variance. However, 'factor 4' also had a barely significant contribution from 'error' as a source of variance; it was significant only at the 10% level, with $p = 0.0573$. this means that 'within-plot' variance accounts for most of the variance of this character.

Table 16: **F-test significance of the mean squares**

Character	*Partition*	*df*	*F-test*	*Signif.*	*Probability*
Diameter	Block	3	3.67	*	0.0145
	Population	35	0.90	NS	0.6334
	Error	105	5.39	**!	0.0000
	Within-plot (all)	1847			
Height	Block	3	9.21	**!	0.0001
	Population	35	2.66	**!	0.0002
	Error	105	4.33	**!	0.0000
	Within-plot	1847			
Uprightness	Block	3	12.39	**!	0.0000
	Population	35	1.74	*	0.0169
	Error	105	2.81	**!	0.0000
	Within-plot	1847			
Leaf Shape	Block	3	3.01	*	0.0326
	Population	35	3.51	**!	0.0000
	Error	105	2.02	**!	0.0000
	Within-plot	1841			
Redness	Block	3	17.06	**!	0.0000
	Population	35	5.84	**!	0.0000
	Error	105	2.39	**!	0.0000
	Within-plot	1845			
Distribution	Block	3	8.10	**!	0.0002
	Population	35	6.14	**!	0.0000
	Error	1845			
	Within-plot				
1st flower	Block	3	2.30	(*)	0.0803
	Population	35	10.71	**!	0.0000
	Error	105	1.92	**!	0.0000
	Within-plot	1841			
Seed set	Block	3	4.77	**	0.0041
	Population	35	2.31	**!	0.0008
	Error	105	8.44	**!	0.0000
	Within-plot	1823			
Seed retained	Block	3	7.61	**!	0.0003
	Population	35	2.20	**!	0.0014
	Error	105	1.75	**!	0.0000
	Within-plot	1759			

Key to symbols' level of significance
(*) ~ 10%
* ~ 5%
** ~1%
**! ~ 0.1%

Table 16: **F-test significance of the mean squares (continued)**

Character	*Partition*	*df*	*F-test*	*Signif.*	*Probability*
Shatter	Block	3	1.95	NS	0.1243
	Population	35	1.07	NS	0.3861
	Error	105	3.70	**!	0.0000
	Within-plot	1743			
1k seed mass	Block	3	0.38	NS	0.7679
	Population	35	1.96	**	0.0050
	Error	105	1.18	NS	0.1091
	Within-plot	1759			
Factor 1	Block	3	2.96	*	0.0349
	Population	35	0.93	NS	0.5864
	Error	105	4.76	**!	0.0000
	Within-plot	1841			
Factor 2	Block	3	5.29	**	0.0023
	Population	35	2.56	**!	0.0002
	Error	105	1.53	**!	0.0009
	Within-plot	1841			
Factor 3	Block	3	0.62	NS	0.6048
	Population	35	3.20	**!	0.0000
	Error	105	3.17	**!	0.0000
	Within-plot	1841			
Factor 4	Block	3	1.57	NS	0.2008
	Population	35	0.49	NS	0.9915
	Error	105	1.24	(*)	0.0573
	Within-plot	1841			

Key to symbols' level of significance
(*) ~ 10%
* ~ 5%
** ~1%
**! ~ 0.1%

Thousand-seed mass had no significant variance at the 'block' and 'error' levels, so most of the variance stems from familial and within-plot differences. Both these sources of variation could potentially have high genetic influences, making this character appear to be largely unaffected by the environment of the experimental field at the time of the experiment.

A seventh character, '1st flower' had only a weakly significant (10%) source of variance at the population level ($p = 0.0803$). This also suggests that the position of the plants within the experimental field is not too important for the days required from sowing to flowering.

5.2.4 Variance components

The mean squares at each level (save the lowest) are made up by the sums of variance components at that level and appropriate lower levels as shown in the E(MS) table. To get a clearer picture, the variance components should be individually compared to appreciate the importance at that level.

The variance components are being presented numerically in table 17 with their standard errors, as well as pictorially in appendix 1 by histograms. In addition, figure 7 shows pie-charts depicting the variance component η^2 in percentages.

Table 17 shows that all non-significant mean square contribution to variance also have variance components which were nearly zero. For instance: 'Shatter' ~ 'block' 0.61% and 'population' 0.40%; '1k seed mass' ~ 'error' 1.31%. The 'block' partition for 'factor 4' was only a small positive number, 0.0023, accounting for just 0.14% of the variance, hence it was left out of the pie-chart. There were even negative components. These negative variance components, possible during the sampling of a low-value statistic, were only found in partitions which reported a non-significant contribution to variance: diameter ~ 'population'; 1k seed mass ~ 'block'; factor 1~ 'population'; factor 3 ~ 'block'; and factor 4 ~ 'population'. Negative estimates were rounded up to zero.

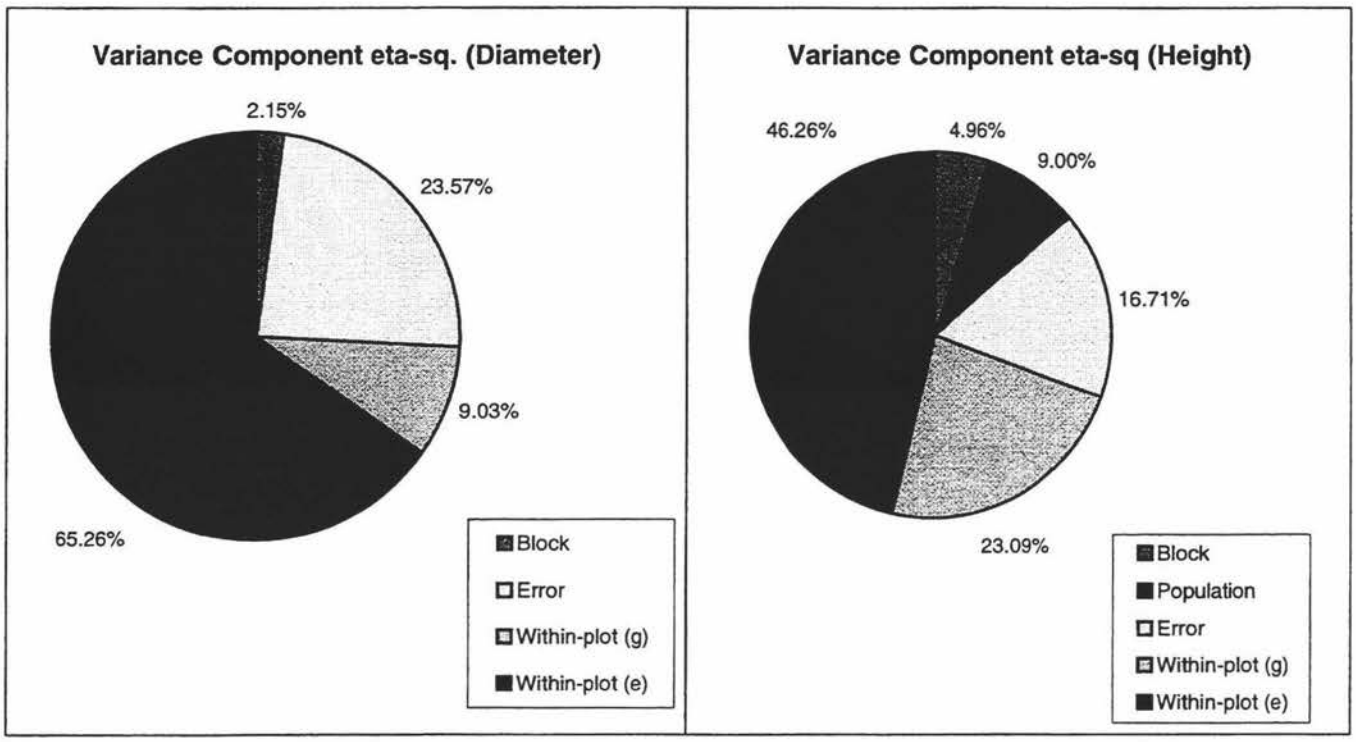
Table 17: **Variance Components**

	Variance Components	*s.e.*		*Variance Components*	*s.e.*
Diameter			**Height**		
Block	0.67484	0.758092	Block	0.18125	0.166035
Population	(-0.23285)	0.580104	Population	0.32910	0.129094
Error	7.40680	1.256509	Error	0.61105	0.109816
Within-plot (all)	23.34678	0.768261	Within-plot	2.53635	0.083462
Within-plot (g)	2.83802	0.093389	Within-plot (g)	0.84441	0.027786
Within-plot (e)	20.50876		Within-plot (e)	1.69194	
Uprightness			**Leaf Shape**		
Block	0.00169000	0.001498	Block	0.003140	0.003846
Population	0.00098000	0.000583	Population	0.035200	0.011930
Error	0.00343000	0.000738	Error	0.028400	0.007805
Within-plot	0.02627226	0.000865	Within-plot	0.382606	0.012611
Within-plot (g)	0.01677427	0.000552	Within-plot (g)	0.304289	0.010029
Within-plot (e)	0.00949799		Within-plot (e)	0.078317	
Redness			**Distribution**		
Block	0.0778400	0.067515	Block	0.078810	0.073429
Population	0.2110200	0.061164	Population	0.513620	0.147314
Error	0.1016000	0.024195	Error	0.139490	0.055827
Within-plot	1.0061223	0.033126	Within-plot	3.594385	0.118343
Within-plot (g)	0.7251863	0.023876	Within-plot (g)	3.208693	0.105644
Within-plot (e)	0.2809360		Within-plot (e)	0.385692	
1st flower			**Seed set**		
Block	0.10359	0.15006	Block	0.03653	0.037770
Population	6.96518	1.83915	Population	0.11441	0.049700
Error	1.37780	0.39906	Error	0.30781	0.048211
Within-plot	20.56181	0.67772	Within-plot	0.56513	0.018719
Within-plot (g)	16.76282	0.55250	Within-plot (g)	(-0.27306)	
Within-plot (e)	3.79899		Within-plot (e)	0.83820	

Table 17: **Variance Components (continued)**

	Variance Components	*s.e.*		*Variance Components*	*s.e.*
Seed retained			**Shatter**		
Block	0.02156	0.020270	Block	0.01040	0.017477
Population	0.03514	0.015942	Population	0.00681	0.028558
Error	0.05027	0.016365	Error	0.28707	0.054391
Within-plot	0.88766	0.029931	Within-plot	1.39123	0.047127
Within-plot (g)	0.75696	0.025525	Within-plot (g)	0.65357	0.022139
Within-plot (e)	0.13069		Within-plot (e)	0.73766	
1k seed mass					
Block	(-0.01335)	0.007431			
Population	0.18688	0.095214			
Error	0.11877	0.110027			
Within-plot	8.74695	0.294943			
Within-plot (g)	8.43815	0.284531			
Within-plot (e)	0.30880				
Factor 1			**Factor 2**		
Block	0.01477	0.018243	Block	0.06592	0.066416
Population	(-0.00482)	0.177270	Population	0.21591	0.086823
Error	0.21425	0.037478	Error	0.19065	0.077333
Within-plot	0.78515	0.025878	Within-plot	5.00291	0.164896
Within-plot (g)	0.19439	0.006407	Within-plot (g)	4.477225	0.147570
Within-plot (e)	0.59076		Within-plot (e)	0.525685	
Factor 3			**Factor 4**		
Block	(-0.0019)	0.002676	Block	0.00226	0.005138
Population	0.10029	0.035439	Population	(-0.0185)	0.006489
Error	0.12494	0.025246	Error	0.02738	0.020227
Within-plot	0.79226	0.026113	Within-plot	1.60609	0.052937
Within-plot (g)	0.44778	0.014759	Within-plot (g)	1.531365	0.050474
Within-plot (e)	0.34448		Within-plot (e)	0.074725	

Figure 6 : **Variance Component Eta-square Pie Charts**



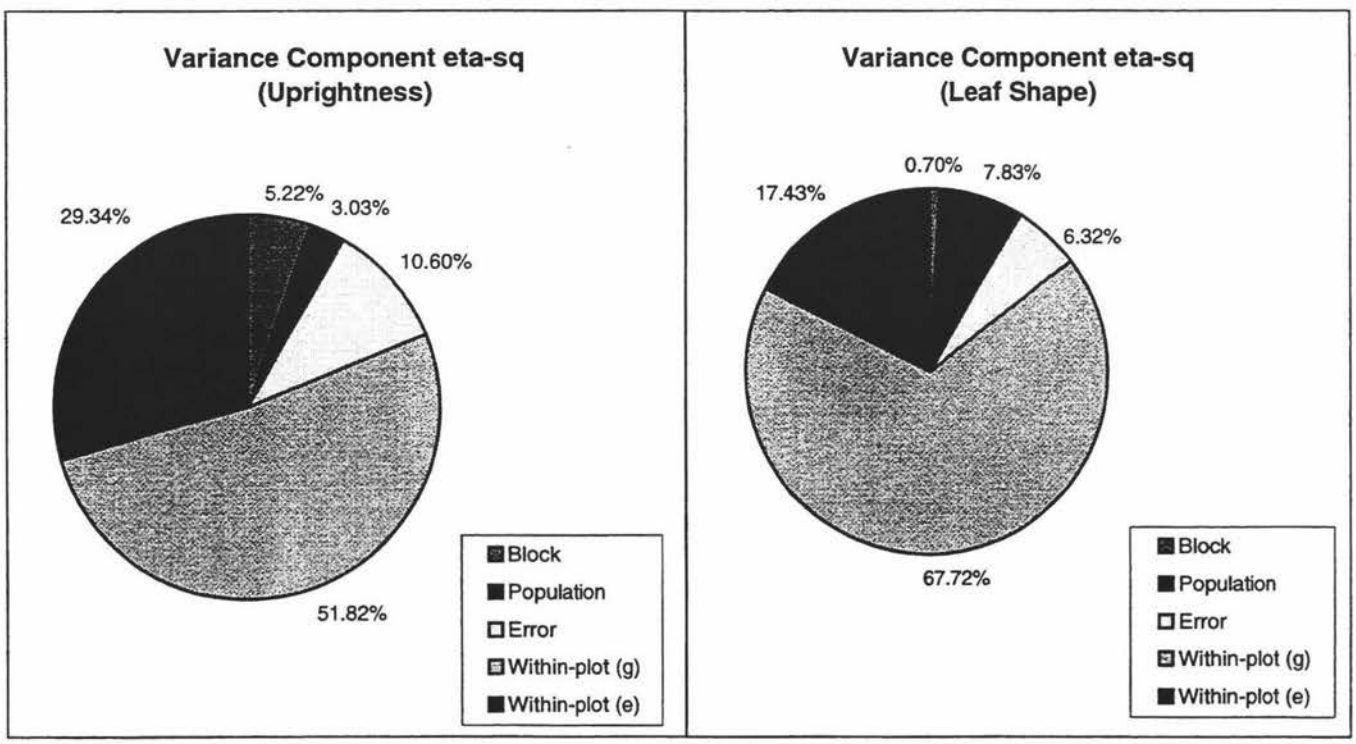
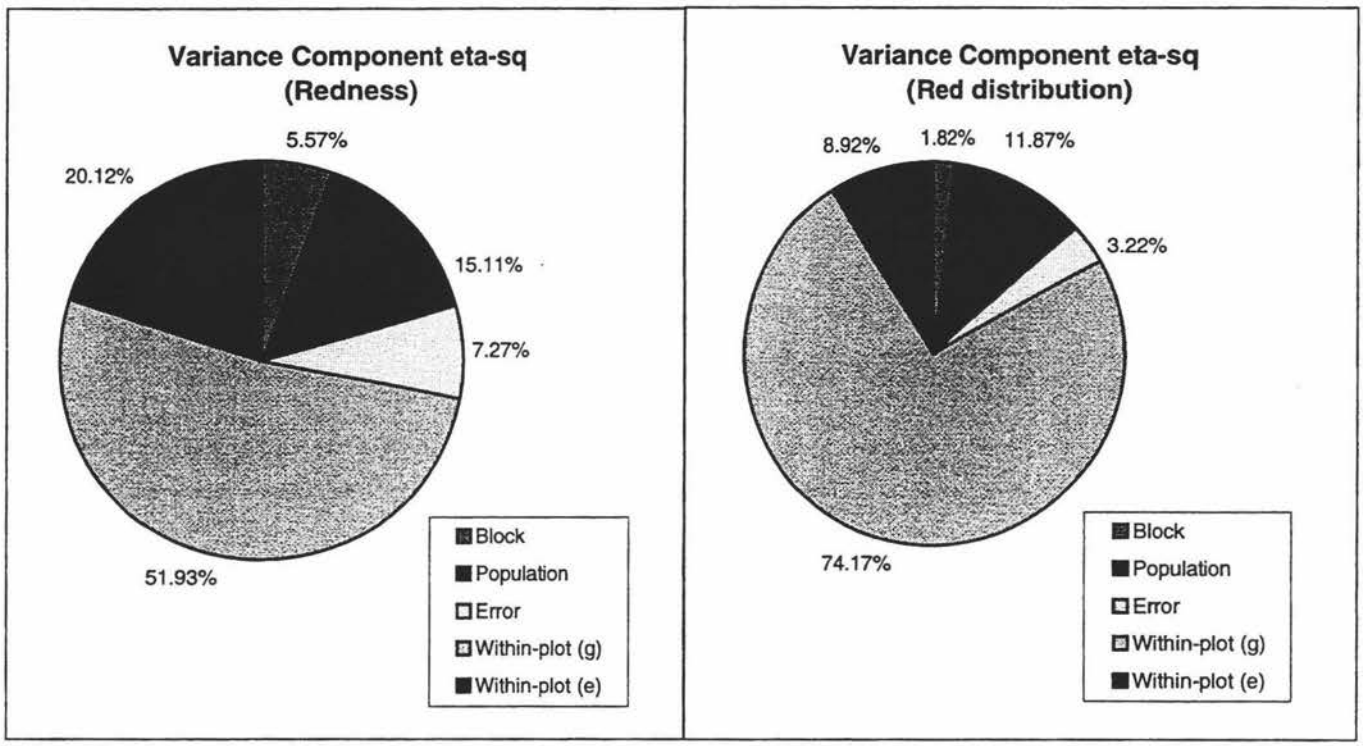


Figure 6 : **Variance Component Eta-square Pie Charts (continued)**



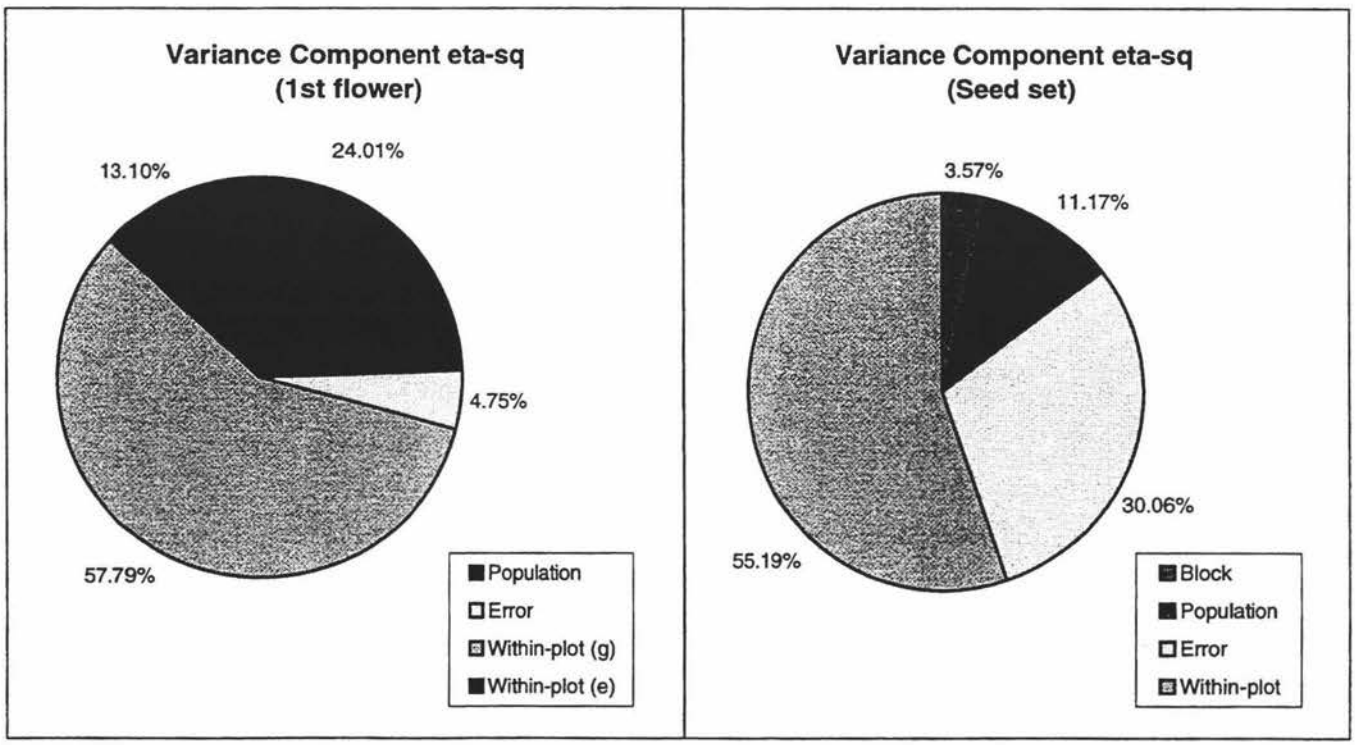


Figure 6 : **Variance Component Eta-square Pie Charts (continued)**

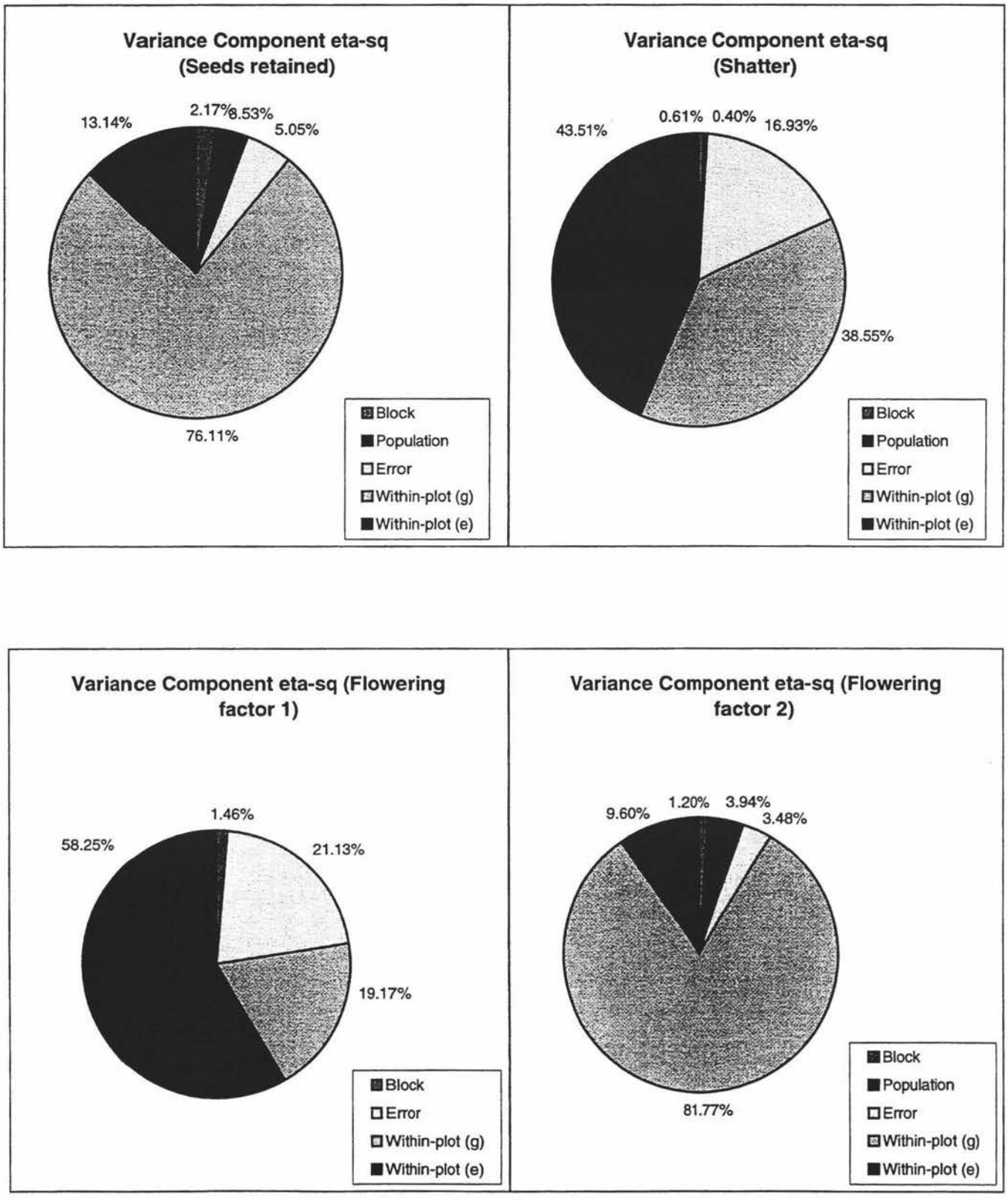
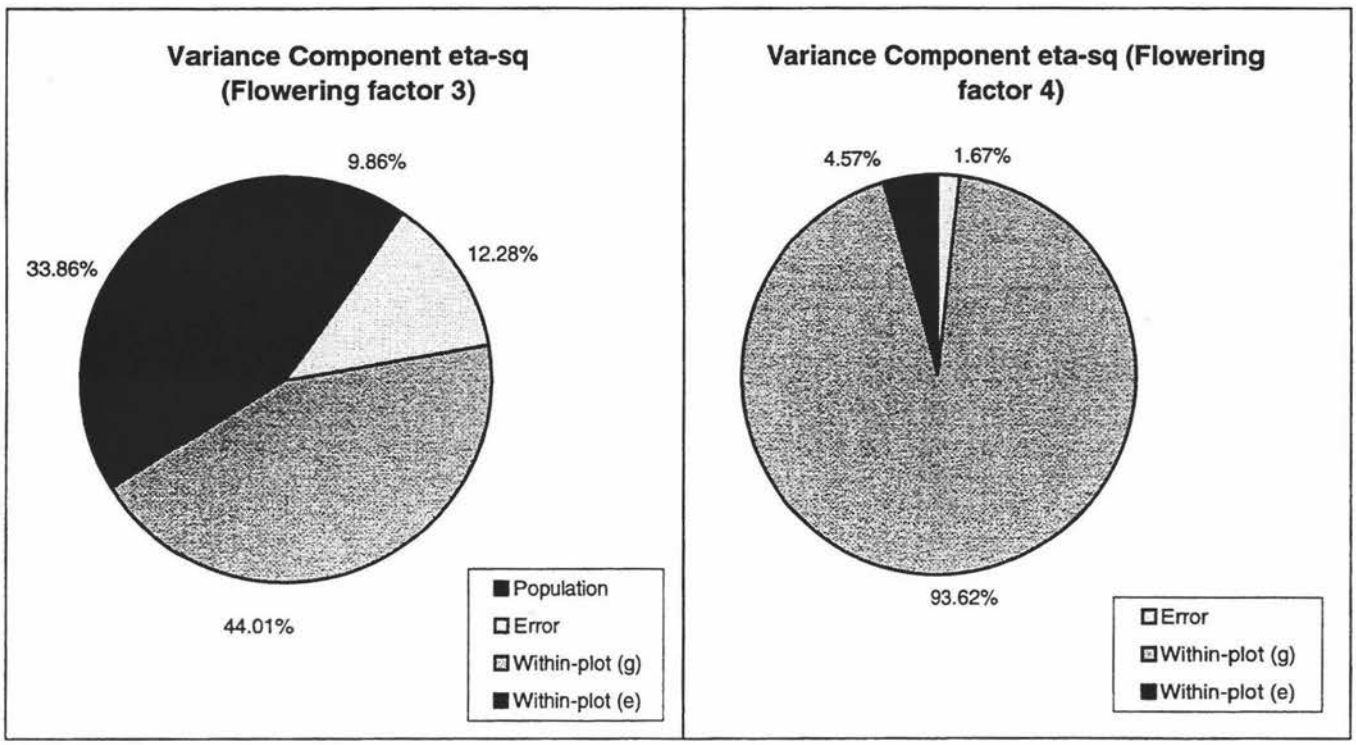
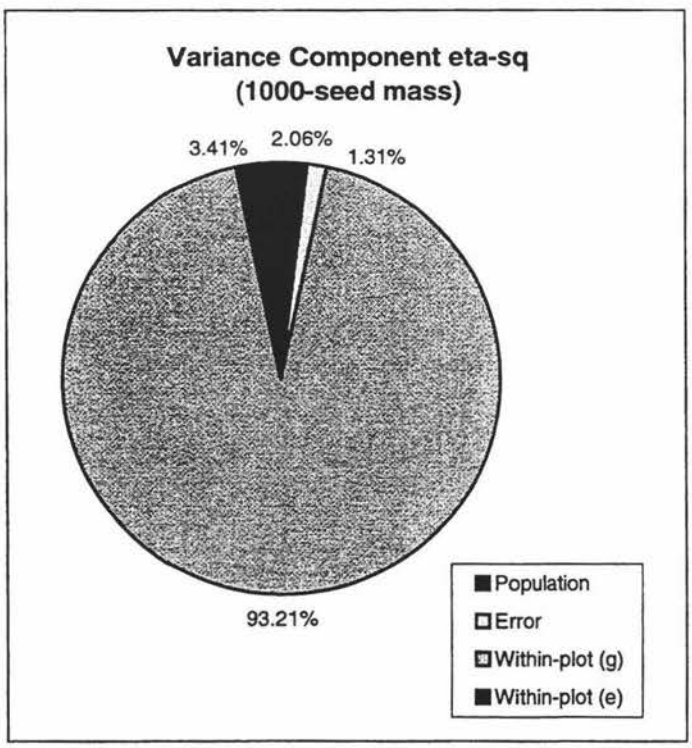


Figure 6 : **Variance Component Eta-square Pie Charts (continued)**





For all of the characters, the single largest variance component was the 'within-plot' partition. This is clearly shown by the data in table 17, and also illustrated by the histograms in appendix 1, where even though 'within-plot' is further partitioned into genetic or environmental causes, one or the other of the bars tower above the rest. This fact is also clearly demonstrated by the pie-charts in figure 7, where the relative sizes of the total within-plot variation always take up more than half the total, and frequently exceeds three-quarters. This is to be expected from plant families which have undergone little cultivation.

5.3 GENETIC INFLUENCE

5.3.1 Within-plot variance

Since the within-plot variance is the single largest source of variance, it would be of interest, therefore, to study this major source of variance further, to decipher the extent of genetics present. The genetic coefficients (g_k) derived allows estimates to be made in this regard. The other partition where genetics is likely to play a prominent role would be in 'population'. Together with the estimated genetic component in 'within-plot', this provides an idea of the role genetics play in character expression (table 18).

Five of the characters had g_k's which were less than 0.5: plant diameter (0.1216), height (0.3329), seed set (0), seed shatter (0.4698), and peak flowering factor 1 (mid-late peak) (0.2476). Seed set is noteworthy in having no genetic influence in the within-plot variance, testimony to the importance of external pollinators and the outbreeding nature of this plant. It is also of interest to note that two of the characters, plant diameter and factor 1, also have non-significant 'population' variance contribution (table 12). When 'population' η^2 and 'within-plot (genetic)' η^2 are added together, the above five characters are the only ones which have totals less than 50 %.

The character 'period to first flower' was earlier noted to have very low CV. The variance component η^2's for the within-plot genetic estimate and the 'population' contribution to variance were the top two sources of variance. This makes combined variance from genetic sources total 81.8%. It can be concluded that the low variance in this character was due to strict genetic control.

The two methods of examining genetic influence, g_k and η^2 genetic totals seem to produce rather consistent results in ranking the characters. This points to the relatedness between genetic influence at both the within-plot and population levels.

Table 18: Genetic coefficients and total proportion (%) of genetic influence in variance

	Genetic Coefficient	*rank**	*Genetic $\eta-2$ total (%)***	*rank**
Diameter	0.12155923	14	9.03	15
Height	0.33292211	12	32.09	12
Uprightness	0.63847841	9	54.85	9
Leaf Shape	0.79530601	7	75.55	7
Redness	0.72077353	8	67.04	8
Distribution	0.89269596	4	86.04	3
1st Flower	0.81524033	6	81.80	5
Seed set	-0.48318480	15	11.17	14
Seed retain	0.85276636	5	79.64	6
Shatter	0.46977808	11	38.95	11
1k seedmass	0.96469660	1	95.27	1
Factor 1	0.24757690	13	19.17	13
Factor 2	0.89492410	3	85.17	4
Factor 3	0.56519070	10	53.87	10
Factor 4	0.95347400	2	93.62	2

* rank 1 = highest
** Negative values are treated as 0

5.3.2 Heritabilities

In keeping with convention, the heritabilities based on restricted phenotypes, where 'block' variance is ignored in this case, will be presented and discussed (table 19). The heritabilities based on full phenotypes will be presented in appendix 3.

The table shows clearly that all the broad-sense heritability were significant, and only four characters had non-significant heritabilities. The four characters were: plant diameter (-0.031), degree of seed shattering (0.016), flowering peak factors 1 and 4 (-0.019 and -0.047 respectively). The negative values arose due to sampling error of a very small heritability, and will be considered as zero for our purposes. The non-significance of narrow-sense heritabilities in each of these characters were hinted at by the F-test of the 'population' mean square partition's contribution to variance. They were all also non-significant.

The other partition which may indicate genetic influence is the within-plot partition. While F-tests cannot be performed on the 'within-plot' partition, the observation from the variance components that 'within-plot' variation makes up the largest source of variance ensures that if the test had been possible, the 'within-plot' partition would most definitely be significant. In order for useful information to be obtained, the genetic component in this partition needs to be sieved out. As mentioned before, genetic coefficients are used for this purpose.

However, while high genetic coefficients may be indicative of great genetic influence in the within-plot partition, it does not necessarily imply a high heritability. For high heritability, there must be high genetic influence in the *phenotypic variance*, of which the within-plot partition makes up only a part. Low within-plot variance may still result in low heritability even if g_k's are high.

Table 19: Significance of heritabilities at various levels

Restricted Phenotypes

	Heritability (Narrow)	*s.e.*	*Signf.*	*Heritability (Broad)*	*s.e.*	*Signf.*	*Plant level Heritability*	*s.e.*	*Signf.*
Diameter	-0.031	0.07951	NS	0.085	0.01954	**!	0.093	0.00335	**!
Height	0.295	0.07816	**!	0.338	0.02281	**!	0.243	0.01083	**!
Uprightness	0.117	0.06176	(*)	0.579	0.0183	**!	0.547	0.01440	**!
Leaf Shape	0.255	0.06245	**!	0.761	0.01579	**!	0.682	0.02058	**!
Redness	0.432	0.05483	**!	0.710	0.01964	**!	0.550	0.02700	**!
Distribution	0.355	0.05858	**!	0.876	0.01273	**!	0.755	0.02763	**!
1st Flower	0.559	0.02804	**!	0.821	0.001648	**!	0.580	0.03789	**!
Seed set	0.344	0.09162	**!	0.116	0.04627	**!	n.a.	n.a.	n.a.
Seeds retain	0.130	0.05163	*	0.814	0.01495	**!	0.778	0.01623	**!
Shatter	0.016	0.06614	NS	0.392	0.01995	**!	0.388	0.01144	**!
1k seedmass	0.078	0.03676	*	0.953	0.01181	**!	0.932	0.01300	**!
Factor 1	-0.020	0.07337	NS	0.191	0.01929	**!	0.195	0.00649	**!
Factor 2	0.143	0.04921	**	0.868	0.01318	**!	0.828	0.01612	**!
Factor 3	0.304	0.07080	**!	0.539	0.02269	**!	0.440	0.01762	**!
Factor 4	-0.047	0.01703	NS	0.937	0.01204	**!	0.948	0.00940	**!

Key to symbols' level of significance	
(*)	~ 10%
*	~ 5%
**	~ 1%
**!	~ 0.1%

For the three characters 'diameter', 'shatter', and 'factor 1', low g_k's (about 2% of total variance or less) were coupled with non-significant population level variance, which strongly leads to low heritability.

Two other characters with low g_k's were 'height' and 'seed set', but these turned out significant narrow-sense heritabilities. One difference between these two characters and the three previously mentioned is the highly significant 'population' partition as a contributor to variance. Comparing the magnitude of this partition against the rest of the characters, it was noted that both 'height' and 'seed set' had fairly large variance contributions from 'population'. In fact, 'height' and 'seed set' ranked sixth and fourth in terms of magnitude of 'population' variance, with values 32.6% and 64.57% above the average 'population' contribution (6.79%) respectively.

The variance component approach to the calculation of heritability relies in no small way from having a large 'population' partition to contribute genetic influence. However, narrow-sense heritabilities also rely on knowledge of family structure. Narrow-sense heritability from half-sib families could be calculated from the intra-class coefficients (t) as noted in the review of literature (p. 42).

A component of t in the denominator is the within-plot variance (σ^2_w). A comparison of σ^2_w against the other characters reveal that 'height' and 'seed set' have the two lowest σ^2_w amongst all the characters surveyed (table 17). 'Seed set' had the smallest total 'within-plot' contribution to variance at just 55.19%, which was 31.24% below average (80.26%). 'Height' followed with the second lowest total 'within-plot' contribution to variance of 69.35% which was 13.6% below average.

The low σ^2_w values of these two characters helped to reduce the denominator of t and may have boosted the narrow-sense heritabilities which was 4 times t for half-sib families, and lead to the significant narrow-sense heritabilities.

The high 'population' contribution to variance and the low σ^2_w were probably two key factors which explained why the very low g_k's of 'height' and 'seed set' still produced a significant narrow-sense heritability result.

The character 'factor 4' (correlating to peak flowering during third week of observation), on the other hand, had the second highest g_k. The within-plot (genetic) variance also accounted for 93.62% of total phenotypic variance. By all indications, a high heritability ought to be expected. Indeed, the *broad-sense* heritability is exceptionally high at 0.947. The zero narrow-sense heritability can only be explained by assuming that the genetic variance comprises almost exclusively of dominance and epistatic variance.

There is also one character unique in having no plant-level heritability: seed set. The reason being due to the way this particular heritability is defined, being based on the estimated genetic proportion of the within-plot variance. Since the genetic coefficient for this character is (not unexpectedly) zero. This shows that the environment plays a large part in seed set, which is expected of a plant dependent on external pollinators.

5.3.3. Heritability variance components

Being defined as a ratio, heritability shows only the relative importance of the genotype to the phenotype expressed. While this allows for comparisons between different heritabilities, it does nothing to inform of the size of the variances by the genotypes or phenotypes. With regards to addressing this deficiency, table 20 displays the variance components of narrow-sense heritabilities and table 20a lists the components for broad-sense heritability.

The comparison of variance sizes between characters would be pointless since each is based on a different scale. To make them comparable, a common reference such as the mean of each variable would be needed. The differences between the variances would be examined instead.

The difference between full and restricted phenotypes is trivial, showing that the block effect is not very large. The phenotype for narrow-sense heritability is typically larger than that for broad, showing the effect of adjustment to accommodate the additional 'amongst-line' variance when using *t* to estimate narrow-sense heritability as discussed earlier. Anomalies where narrow-sense phenotypic variances was smaller were due to the negative estimate of additive variance (e.g. 'diameter).

The genotypic variance for broad-sense heritability can be expressed as the total of plant-level and population-level (between-families) variance. As such the former is usually greater than the latter except when population-level or plant-level genotypic variance is negative.

Similarly, additive variance, being a sub-set of genotypic variance is expected to be smaller in magnitude than the latter. In most cases, this was true but '1st flower', 'height', and 'seed set' lie contrary to this expectation. This disagreement could be put down to the different approaches used to arrive at the figures. For narrow-sense, the approach is more biometrical, using amongst and between family variances while broad-sense uses a variance components approach.

The standard errors are also an important statistic relating to the variances. This is because they measure the precision of the variance values. Reliable estimates of variances are required if the heritabilities are to be credible. The sizes of the standard errors of the variances therefore contribute to the significance of the heritability estimate. For example, both 'shatter' and 'diameter' have additive variance estimates where standard errors greatly overwhelm the estimates in magnitude. It was not surprising then that no significant narrow-sense heritability was found for them.

Table 20 : Narrow-sense heritability variance components

Character	Additive σ_2	s.e.	Phenotypic σ_2 (Restricted)	s.e.	Phenotypic σ_2 (Full)	s.e.
Diameter	-0.9314	2.32042	29.8222	0.52753	30.4970	0.92358
Height	1.3164	0.51638	4.4638	0.24624	4.6451	0.29699
Uprightness	0.0039	0.05933	0.0336	0.00121	0.0353	0.00192
Leaf Shape	0.1408	0.04772	0.5518	0.02542	0.5550	0.02571
Redness	0.8441	0.24466	1.9518	0.12381	2.0296	0.14102
Distribution	2.0545	0.58926	5.7884	0.30954	5.8672	0.31813
1st Flower	27.8607	7.35658	49.8003	3.71053	49.9039	3.71357
Seed set	0.4577	0.1988	1.3306	0.08865	1.3671	0.09636
Seed retain	0.1406	0.06377	1.0785	0.03898	1.1000	0.04393
Shatter	0.0273	0.11423	1.7056	0.04703	1.7160	0.05017
1k seedmass	0.7475	0.38086	9.6132	0.31461	9.5999	0.31470
Factor 1	-0.0193	0.07091	0.9801	0.02079	0.9949	0.02766
Factor 2	0.8636	0.34729	6.0572	0.21841	6.1231	0.22829
Factor 3	0.4012	0.14176	1.3184	0.07054	0.3165	0.07060
Factor 4	-0.074	0.02596	1.5595	0.04674	1.5617	0.04702

Table 20a : Broad-sense heritability variance components

Character	Genotypic σ_2 (Broad-sense)	s.e.	Genotypic σ_2 (plant level)	s.e.	Phenotypic σ_2 (Restricted)	s.e.	Phenotypic σ_2 (Full)	s.e.
Diameter	2.6052	0.58757	2.838	0.09339	30.5207	1.27762	31.1956	1.46334
Height	1.1735	0.13205	0.844	0.02779	3.4765	0.16933	3.6577	0.23609
Uprightness	0.0178	0.00080	0.017	0.00055	0.0307	0.00112	0.0324	0.00186
Leaf Shape	0.3395	0.01559	0.304	0.01003	0.4462	0.01758	0.4494	0.01793
Redness	0.9362	0.06566	0.725	0.02388	1.3187	0.07053	1.3966	0.09751
Distribution	3.7223	0.18128	3.209	0.10564	4.2475	0.18781	4.3263	0.20134
1st Flower	23.7280	1.92034	16.763	0.55250	28.9048	1.96366	29.0084	1.96772
Seed set	0.1144	0.04970	0.000	0.00000	0.9874	0.06271	1.0239	0.07254
Seed retain	0.7921	0.03009	0.757	0.02552	0.9731	0.03393	0.9946	0.03938
Shatter	0.6604	0.03613	0.654	0.02214	1.6851	0.06468	1.6955	0.06608
1k seedmass	8.6250	0.30004	8.438	0.28453	9.0526	0.29865	9.0392	0.29794
Factor 1	0.1896	0.01885	0.194	0.00641	0.9946	0.03989	1.0094	0.04319
Factor 2	4.6931	0.17122	4.477	0.14757	5.4095	0.18397	5.4754	0.19497
Factor 3	0.5481	0.03839	0.448	0.01476	1.0175	0.04647	1.0156	0.04626
Factor 4	1.5129	0.05089	1.531	0.05470	1.615	0.05148	1.6172	0.05157

5.4 Overall Discussion

5.4.1 Significance of heritability

All the characters surveyed had high genetic influences as indicated by the significant broad-sense heritabilities. Excluding factor analysis of flowering pattern (discussed in the next section), 9 out of 11 characters had highly significant narrow-sense heritabilities as well.

The non-significance of narrow-sense heritabilities means that additive genetic variance of those characters were zero or close to it. This implies that the characters (plant diameter and degree of seed shattering) are very poorly heritable across generations, and no selection will be effective on them. However, since no character had non-significant broad-sense heritabilities, we can surmise that for characters with non-significant narrow-sense heritabilities, all genetic variances were highly dominant and/or epistatic in nature. The effects of these intra- and inter- loci interactions would be most valuable in the cultivation of hybrid varieties.

5.4.2 Elucidation of flowering pattern through factor interpretaion

Table 19 showed that like most of the other characters, factors 2 and 3 had highly significant heritability in both the broad- and narrow- senses. The flowering pattern described by these 2 factors are thus very much under genetical control. While Factor 4 ('peak flowering at week 3') did not have a significant narrow-sense heritability, the very high broad-sense heritability suggests that inter-allelic and / or inter-loci interactions played a very large role in this factor. This means that while the effects described by 'factor 4' are not directly heritable, the high broad-sense heritability ensures that its characteristics will be maintained in the population even through the allelic recombination in the sexual cycles. It should not be surprising that week 3 flowering is accounted for by one factor exclusively, considering that table 15 suggests that most plants were blooming profusely at that week. Perhaps the most unusual result of the factors' heritabilities comes from 'factor 1', which had accounted for most of the variance, having a non-significant narrow-sense heritability. This

means that the most important factor accounting for flowering pattern behaviour in meadowfoam is environmental in nature.

To sum up the genetic control of flowering pattern at this point:

- Late flowering is a result of environmental circumstances;
- The strongest genetic factor (factor 2) emphasises a mid-period flowering pattern (weeks 4, 5);
- The next strongest genetic factor (factor 3) emphasises two flowering peak periods ~ early flowering (week 1) and mid-period flowering (week 6);
- The high broad-sense heritability of factor 3 ensures its continued presence in the population although it is not directly heritable.
- The genetic factors collectively account for flowering in weeks 1 to 6, while environmental influences contribute to flowering in weeks 7 to 10 of the survey period.

In order to check for general patterns of flowering and also to spot any individuals with unconventional flowering behaviour, scatter–plots of each plant's 'flowering pattern' scores against a pair of factors may be examined. Since factor 1 is due largely to environmental effects, the source of which had not been investigated, scatter-plots involving factor 1 will not be directly examined, but will be provided in the appendix (appendix 4). There are three possible scatter-plots involving pairs of the three factors, these are presented in figures 7a-c.

To interpret the plots, consider the following 'stereotypes':

- Early-floweringplant ~ this plant has positive scores in weeks 1 to 3 and negative scores in other weeks;
- Mid-period flowering plant ~ this plant has positive scores in weeks 4 to 6 and negative scores in other weeks;
- Late flowering plant ~ this plant has positive scores in weeks 7 to 10 and negative scores in other weeks;
- Evenly flowering plant ~ this plant has positive scores throughout the ten weeks.

The characteristics of the factors must also be borne in mind:

- Factor 2 ~ significant negative coefficient in week 2, significant positive coefficients in weeks 4 to 6.
- Factor 3 ~ significant positive coefficients in weeks 1 - 2 and 5 - 6.
- Factor 4 ~ large positive coefficient in week 3.

Considering the characteristics of the stereotype plants and the factors together:

For factor 2 and early-flowering plants ~ positive score * negative coefficient for week 2; negative scores * positive coefficients for weeks 4 to 6; net result is a *large negative composite score.*

Factor 2 and mid-flowering plants ~ negative score*negative coefficient for week 2; positive score*positive coefficients for weeks 4 to 6; net result is a *large positive composite score.*

Factor 2 and late-flowering plants ~ negative score*negative coefficient for week 2; negative score*positive coefficients for weeks 4 to 6; positive score*small negative coefficients for weeks 7 to 10; net *negative composite score.*

Factor 2 and evenly flowering plant ~ scores evenly positive throughout, so net score depends on size and sign of coefficients only; *net positive composite score.*

Factor 3 and early-flowering plants ~ positive score*positive coefficients for weeks 1 and 2; negative score*positive coefficients for weeks 5 and 6; *net slightly negative score due to size of coefficients.*

Factor 3 and mid-flowering plants ~ negative score*positive coefficients for weeks 1 and 2; positive score*positive coefficients for weeks 5 and 6; *net slightly positive score due to size of coefficients.*

Factor 3 and late-flowering plants ~ negative score*positive coefficients for weeks 1 and 2; negative score*positive coefficients for weeks 5 and 6; *net negative score.*

Factor 3 and evenly-flowering plants ~ *net positive score due to sign of coefficients.*

Factor 4 ~ due to large positive coefficient in week 3 and small coefficients in others, only early flowering plants, which may include week 3, get positive composite scores.

With the above points in mind, the scatter-plots can be more readily deciphered:

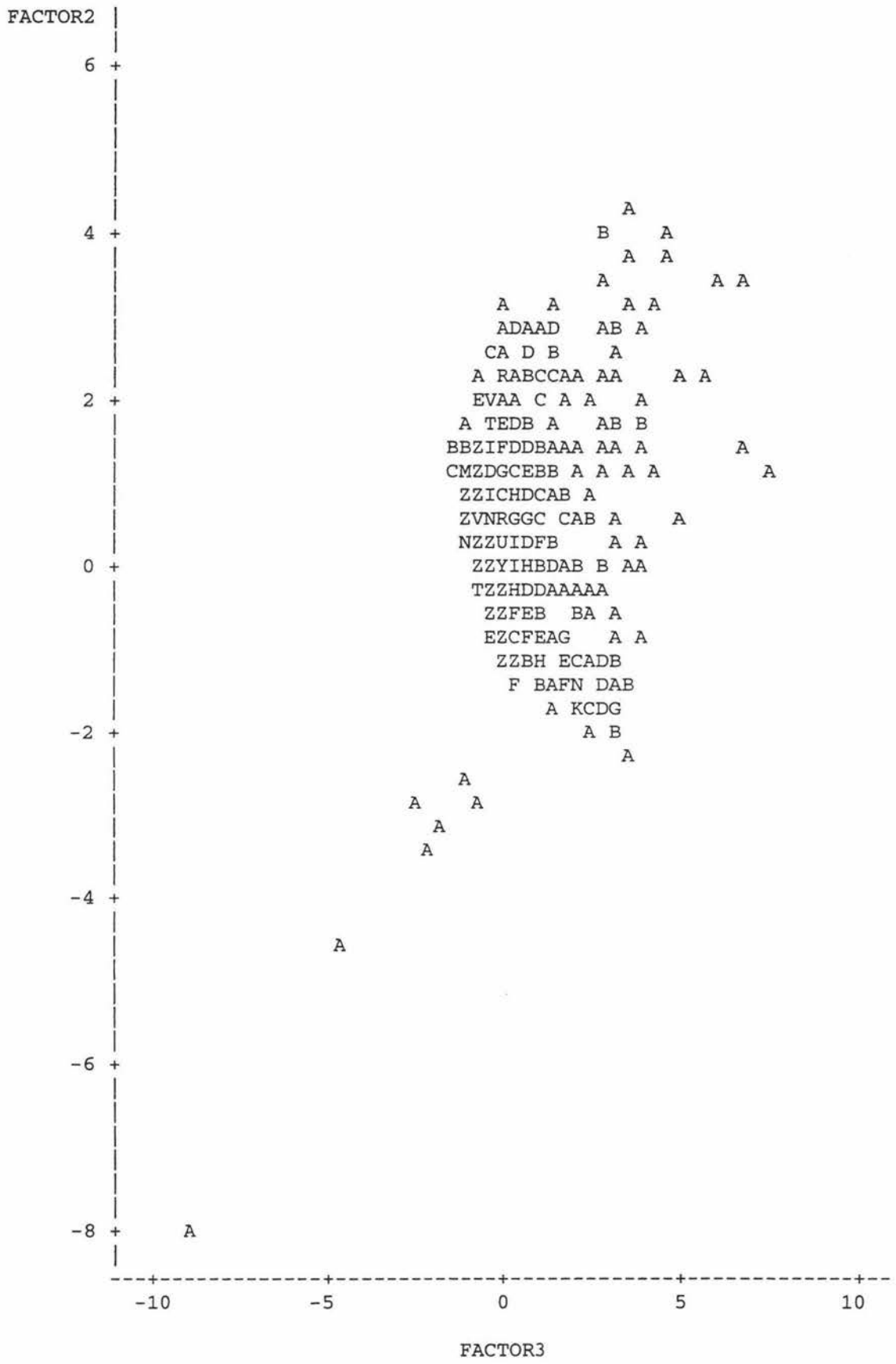
Factor2*Factor3 plot (Figure 7a) ~ mainly centred around the point (0,0), indicating many plants flower in early-mid period. There is also a group of more isolated plants in the lower left quadrant with low composite scores in both factors 2 and 3. These plants ought to represent the group which flowered later. The single plant with very high negatives in both factors may be an individual with two flowering periods, one very early and the other very late. There is also a less dense grouping in the upper right quadrant representing plants with high composite scores in factors 2 and 3. These may be plants with more even flowering distribution and perhaps a slight peak in the mid-period.

Factor2*Factor4 plot (Figure 7b) ~ many points occur in the positive half of factor 2, indicating that most plants flower in the mid-period. The spread across factor only indicates the extent of flowering which occurs in week 3. A handful of plants fall into the negative area of factor 2, these should be the same group of late flowering plants, spread out along the factor 4 axis according to the presence of flowersinweek 3. Not surprisingly, most of the late-flowering group did not have flowers in week 3, since they tend to be negative for factor 4. There is also a distinct linear band in the upper left quadrant. With highly negative factor 4 and positive factor 2, this group represents plants flowering strictly in the mid period after week 3 (high negative factor 4) and not in the 'late' weeks (positive factor 2).

Factor 3*Factor4 plot(Figure 7c) ~ This plot is rather similar to figure 7 b, since factor 3 also tends to favour the mid-flowering period plants. The group of late flowering plants appear in the lower half of the graph. There is also a linear band of early-(slightly negative factor 3) and mid-period(slightly positive factor 3) flowering plants which do not flower in week 3 (highly negative factor 4) near the (-3,0) portion of the graph.

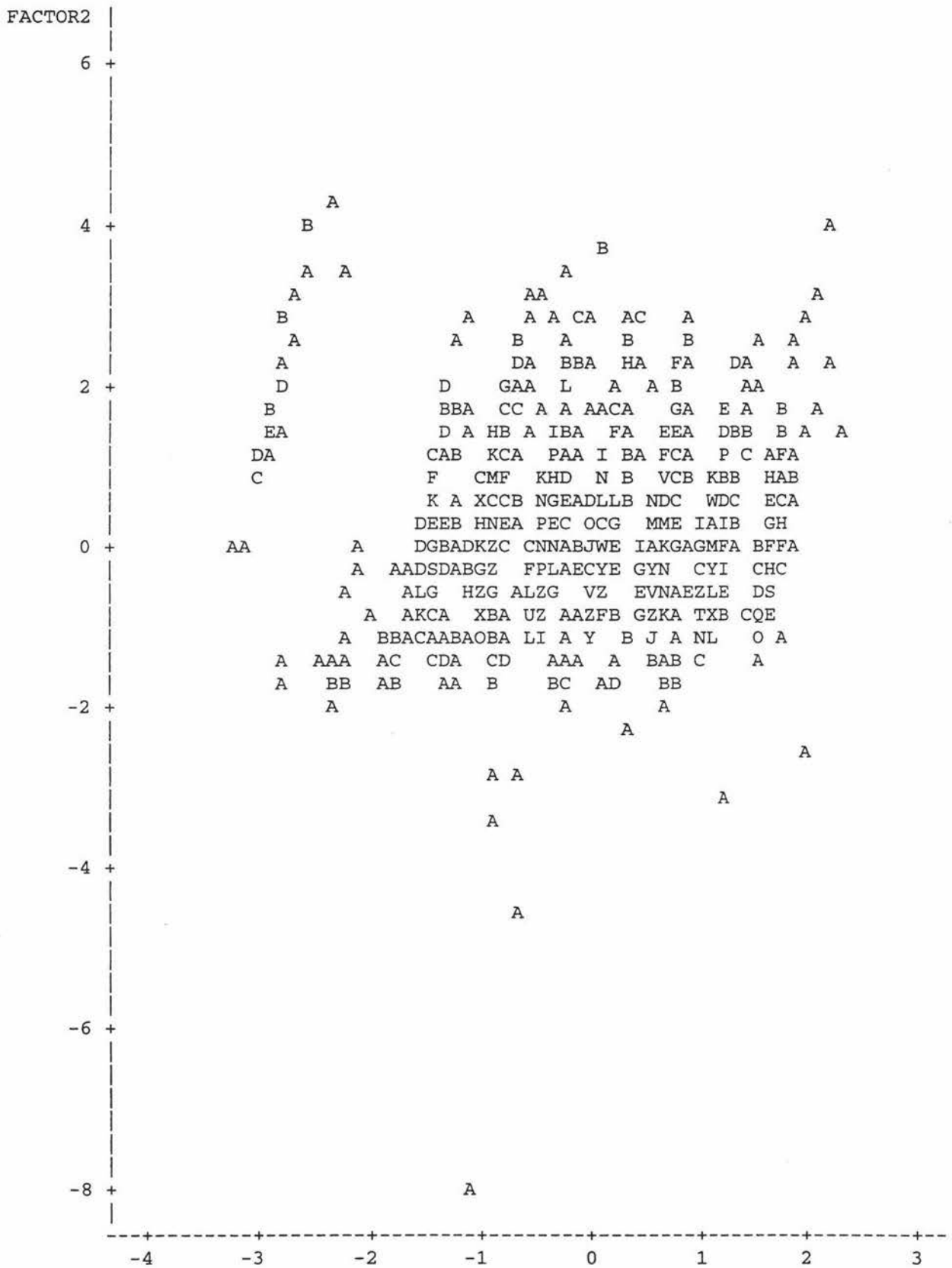
In conclusion, the scatter-plots reinforce the mid to slightly early flowering period of most plants (agreeing with table 15), and picks out a minority of plants which flowered late.

Figure 7a : Plot of FACTOR2*FACTOR3.
Legend: A = 1 obs, B = 2 obs, etc.



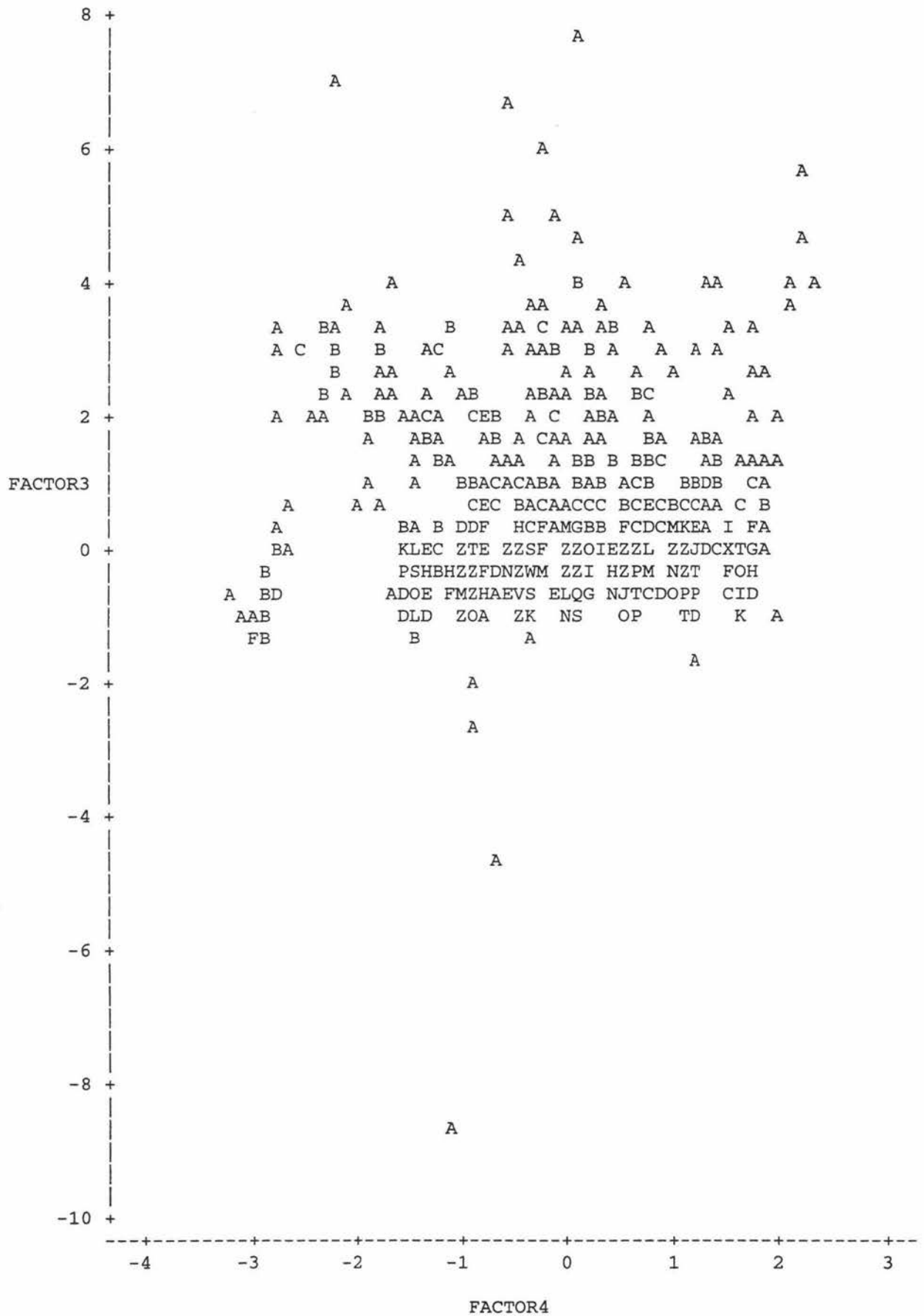
NOTE: 30 obs had missing values. 935 obs hidden.

Figure 7b : Plot of FACTOR2*FACTOR4.
Legend: A = 1 obs, B = 2 obs, etc.



NOTE: 30 obs had missing values. 79 obs hidden.

Figure 7c : Plot of FACTOR3*FACTOR4.
Legend: A = 1 obs, B = 2 obs, etc.



NOTE: 30 obs had missing values. 234 obs hidden.

5.4.3 The use of half-sibs in the estimation of narrow-sense heritability

It has often been noted that additive genetic variance is the chief cause of resemblance between relatives. Narrow-sense heritability uses additive variance as its genotype component in the calculation of heritability, so it measures the extent which a character is heritable. The use of half-sibs in estimating narrow-sense heritability is one of the best ways to do it because it enables the use of additive variance components without compounding it with non-additive variances.

When both parents are known, the covariance between parent and offspring is often compared to the 'mid-parent' value and assumes that both parents have the same variance. Under these circumstances, offspring-parent covariance is ½ the additive variance. Since half-sibs have only one known parent, no information exists for the other parent and the mid-parent value is unknown. The genotypic mean of the half-sib group is then assumed to be half that of the known parent's breeding value. The covariance between half-sibs is assumed to be half the additive variance of the known parent. Thus : $cov_{HS} = ¼ \sigma^2_A$.

If full sibs were used, the covariance would not be so simple. This is because when 2 individuals are mated together, genetic recombination produces 4 potentially different genotypes at each loci, and this assumes that all alleles are different. Therefore, each pair full-sib could have a ¼ chance that their genotypes are exactly the same at any particular locus. Not only would they then share the same additive variance, but also the same dominance variance. Should they also have the same genotype at one or more different loci, then even epistatic variance can be common between them. Their general covariances then become corrupted by non-additive variances and the use of amongst-plot variance in an experiment such as this would not cleanly produce an estimate of narrow-sense heritability. In fact, the covariance of full-sibs is written as: $cov_{FS} = ½ \sigma^2_A + ¼ \sigma^2_D$; and this ignores the possible interaction between the additive and non-additive variances too.

Having illustrated the differences between the two types of sibling, it is clear that the use of half-sibs in this experiment allows for a clean and confident method in the estimation of narrow-sense heritability which is of vital importance in many plant breeding programs.

5.4.4 Heritability in plant breeding decision-making

Both narrow- and broad- sense heritability denote genetic influence in phenotype character. This experiment has yielded results to indicate that many characters are significant with respect to both types of heritability and only a minority have significant broad-sense heritability only.

The key difference between the two types of heritability is the inclusion of non-additive genetic variance in the broad-sense heritability. Additive variance stems from the alleles present in parents, so they are directly heritable. Non-additive variance originates from the *combination* peculiar to the generation, which is non-heritable due to independent genetic assortment (except for some linked genes).

Choice of a breeding program should consider the key differences outlined. Where narrow-sense heritability is significant, continual selection for individuals or groups expressing the desired phenotype will lead to appreciable gains. Such methods include: Pure line selection; Pedigree breeding, pure line breeding, bulk population breeding; backcross breeding; simple recurrent selection. When narrow-sense heritability is non-significant but broad-sense heritability is significant, then one could still exploit the full genetic potential by encouraging new combinations which may result in hybrid vigour. These method include: hybrid cultivar development; synthetic cultivar development; and recurrent selection for good combining ability.

Since most characteristics have significant narrow-sense heritabilities, breeding methods involving selection should be encouraged. With high selection intensity, the improvements could be accelerated further.

5.4.5 Expected genetic advance

In order for a crop to be improved, it must possess certain pre-requisites. These being: relatively large genetic variability so that there is potential to 'create' a markedly different population; and the ability for the desired characters to be heritable. The rate at which the genetics of the base population approaches that of the desired selection will depend on the intensity of selection, and this is in part determined by resource constraints as well as strategic planning (Allard, 1960).

Genetic advance can therefore be expressed as the following:

$$\Delta G = i\, h^2_N\, \sigma_P$$

where ΔG : Genetic advance

i : Standardised selection differential (based on intensity)

h^2_N : Narrow-sense heritability

σ_P : Phenotypic standard deviation

Slight rearrangement of the above reveals an alternative form utilising additive variance:

$$\begin{aligned} \Delta G &= i\, h^2_N\, \sigma_P \\ &= i\, (\sigma^2_A / \sigma^2_P)\, \sigma_P \\ &= i\, (\sigma_A / \sigma_P)\, \sigma_A \\ &= i\, h_N\, \sigma_A \end{aligned}$$

This latter form is useful for estimating genetic advance of multivariate characters where the response of one is correlated with others. Since the correlation between characters was not studied here, the former equation will be used. Also, the use of the latter equation adds a further layer of assumption that the estimate additive variance used is precise; whereas the use of phenotypic variance avoids further assumption, being directly measured.

A sample genetic advance is presented in table 21, which assumes a selection intensity of 10% in an infinite base population (>500 plants). The narrow-sense heritability used is based on the restricted phenotype and the standardised selection differential under these conditions is 1.755 (Becker, 1967).

The ΔG's obtained for all the characters were all on different scales. In order to make them more comparable, they are re-expressed as percentages of the means found on table 13.

The factors 1 to 4, being mathematical constructs on a standardised scale, cannot be re-expressed in this way, since by definition, they are centred around zero. However, the magnitudes of the ΔG's will be of interest, bearing in mind that they may be applied in favour of, or against, what the factors represent. At around 0.6, the size of the ΔG's for factors 2 and 3 is relatively high, since most scores have magnitudes less than 5.

The top six characters in order of ranking all have ΔG's in excess of 10%. The 10% mark may be set arbitrarily as the threshold between 'high' and 'low' ΔG's in this case for convenience.

The characters for intensity of redness, distribution of redness on branches, seed set, height, leaf shape, and seeds retained, all have a 'high' expected response to genetic selection. The characters which were predicted to respond poorly are: diameter of plant, degree of seed shatter, period to first bloom, thousand-seed mass, and uprightness of plant.

Some of the ΔG results were not unexpected; such as those for red colour and distribution, since CVs and h^2_N were high. However, for most of the characters, there were conflicts between amount of variation and heritability. By applying this equation which integrates all components affecting genetic response, a clearer picture is presented to the plant breeder.

Table 21 : Genetic advance and its components

Character	*Heritability (narrow)*	σ (P)	Δ G	Δ G as % of *mean*	Rank
Diameter (cm)	0.000	5.4610	0.0000	0.0000	11
Height (cm)	0.295	2.1128	1.0938	18.6022	4
Uprightness	0.117	0.1833	0.0376	8.0444	7
Leaf Shape	0.255	0.7428	0.3324	14.2168	5
Redness	0.432	1.3971	1.0592	102.9189	1
Distribution	0.355	2.4059	1.4989	70.4136	2
1st Flower (days)	0.559	7.0569	6.9231	4.0688	9
Seed set	0.344	1.1539	0.6966	19.6081	3
Seeds retain	0.130	1.0385	0.2369	11.8080	6
Shatter	0.016	1.3060	0.0367	2.3504	10
1k seedmass (g)	0.078	3.1005	0.4244	7.2776	8
Factor 1	0.000	0.9900	0.0000	0.0000	n.a.
Factor 2	0.143	2.4611	0.6176	n.a.	n.a.
Factor 3	0.304	1.1482	0.6126	n.a.	n.a.
Factor 4	0.000	1.2488	0.0000	0.0000	n.a.

(i = 1.755)

5.4.6 Character consideration in future meadowfoam improvement programs

Plant height and ability of seed retention are two characters which come through strongly for agronomic interest. Since meadowfoam naturally have a ground-cover type of growth habit, taller plants confer advantages such as ease-of-harvest, easier inspection, and greater ability to out-compete weeds.

High seed yield by quantity hinges on two events: seed set and prevention of seed shatter. While seed set for outbreeding plants is highly dependent on the environment (mainly plenty of available pollinators and fine weather for their activity) and results show that little genetic improvement can be made in this regard, it is a limiting factor that may be easily overcome by the provision of extra bee hives. The other condition for high seed quantity is more difficult to overcome, for there is little in-field intervention that can be done to improve seed retention short of laboriously tying bags around each bloom, but only after pollination has occurred. The finding that seed retention ability can potentially receive a high genetic boost is most welcome to plant breeders.

The leaf is the plant's main photosynthetic organ, so being able to alter the leaves through selection is one way of increasing plant mass, which affects yield. The high ΔG for leaf shape selection is particularly useful because it may offer a visual method of increasing the plant's leaf-area index (LAI). This is especially important because meadowfoam leaves begin to senesce once flowering is initiated (Fiez *et al.*, 1991b). The typically slender, lanceolate leaves of mature meadowfoam plants further exacerbate the problem of reduced leaf area for photosynthesis. In fact, sepals begin to contribute significantly to photo-assimilates in mature plants (Seddigh *et al.*, 1993). Knowing that leaf-shape responds highly to selection opens the possibility of studying the relationship between shape and the leaf-area index (LAI) and ultimately to the possible correlation between the easily observed leaf shape and photosynthetic efficiency.

The genetically responsive quality of red stem colour and its distribution also means that cultivars can be selected for easily distinguishable shades of red, or the lack of it, as a possible cultivar identifier.

6.0 CONCLUSIONS

- There was high phenotypic variance in all characters surveyed except one: period to first flower.

- Maximum flowering in most plants occurred about 165-185 days after sowing (weeks II - IV).

- Plant size (height, diameter, and uprightness) and leaf shape were comparatively less variable about the mean.

- The high variance in most of the characters surveyed suggest that there is great potential for selection.

- The largest source of variance came from within-plot (intra-family variation).

- 9 out of 11 characters directly measured have significant heritabilities in both broad-sense and narrow-sense, indicative of high genetic influence in phenotype expression.

- Four characters (including 2 factors) have non-significant narrow-sense heritabilities: plant diameter ($h^2_N = 0$); degree of seed shattering ($h^2_N = 0.016$); flowering pattern factors 1 and 4 (both had $h^2_N = 0$). Non-significant narrow-sense heritabilities mean that no selection need be made on these characters, since these qualities would not be heritable across generations. In the case of peak flowering period, since factors 2 and 3 are heritable, then selection for mid-early flowering would still be possible.

- Since most characters have significant narrow-sense heritability, breeding methods involving selection of the best plants can be used to improve the crop.

- The four characters with non-significant narrow-sense heritability should still be considered for improvement in breeding methods utilising hybrid vigour and combining ability, such as hybrid cultivar development and recurrent selection emphasising combining ability.

- Selection for taller plants for ease-of harvest and good seed retention after ripening would yield the most genetic advance with respect to agronomic characters of immediate benefit.

- The genetically responsive qualities of redness intensity on the branches and colour distribution make them useful traits in cultivar development for identification purposes, and also as genetic markers in plant breeding work.

- Further studies on the correlation between leaf shape and photosynthetic efficiency may be worthwhile since the character 'leaf shape' is significantly heritable and holds good promise for genetic advance.

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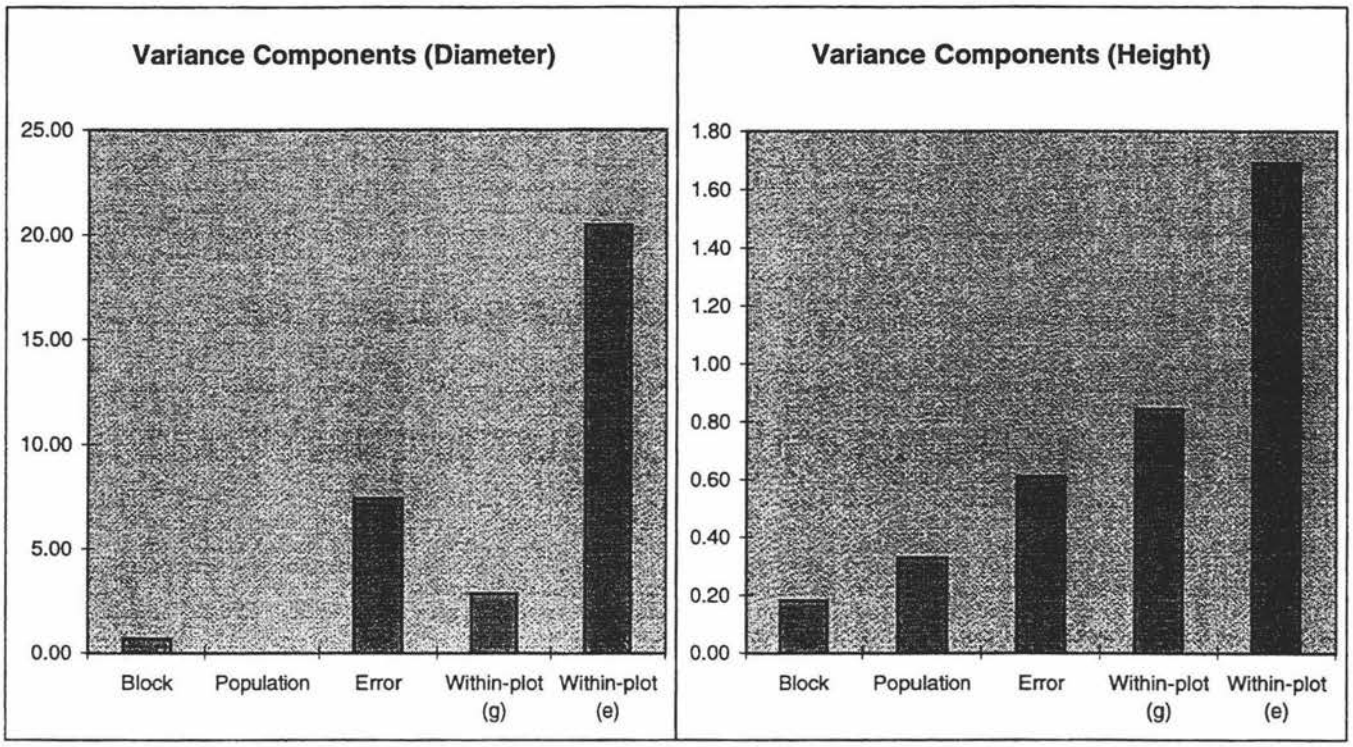
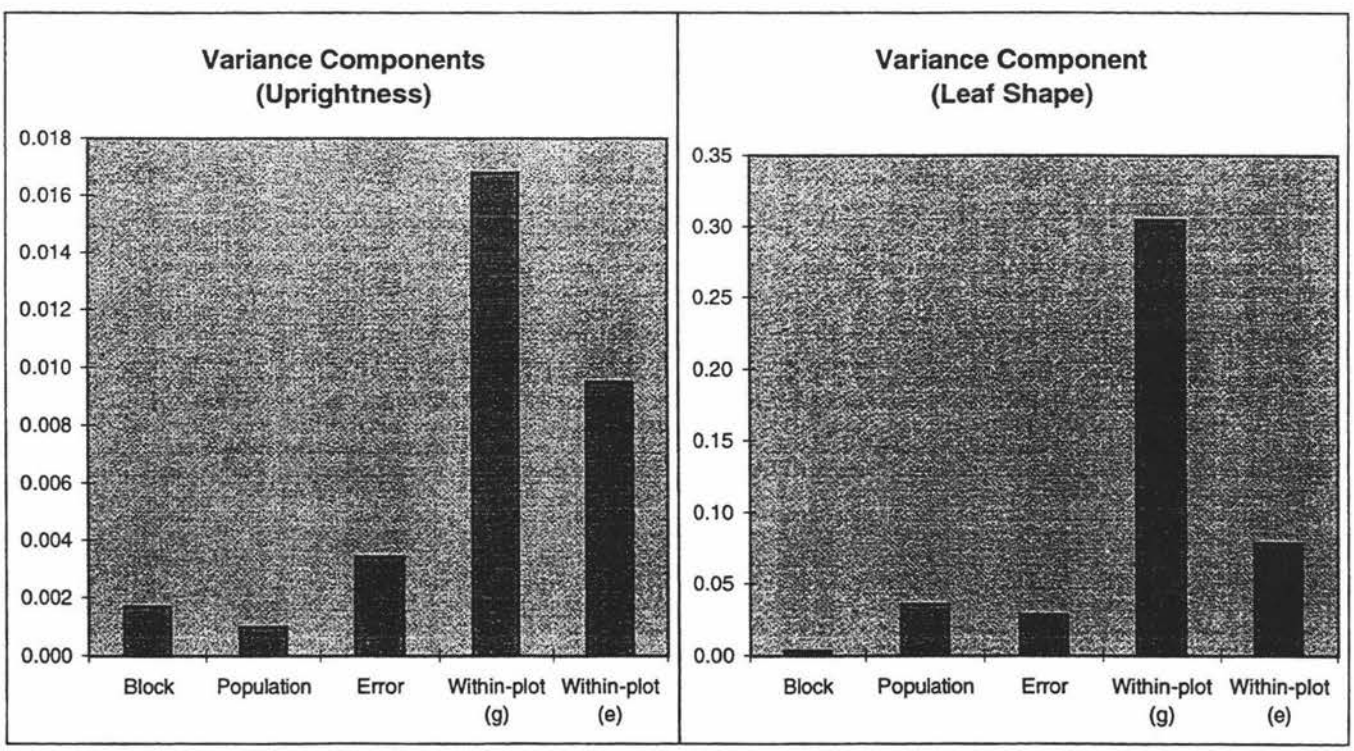
Appendix 1 : Mean Squares table

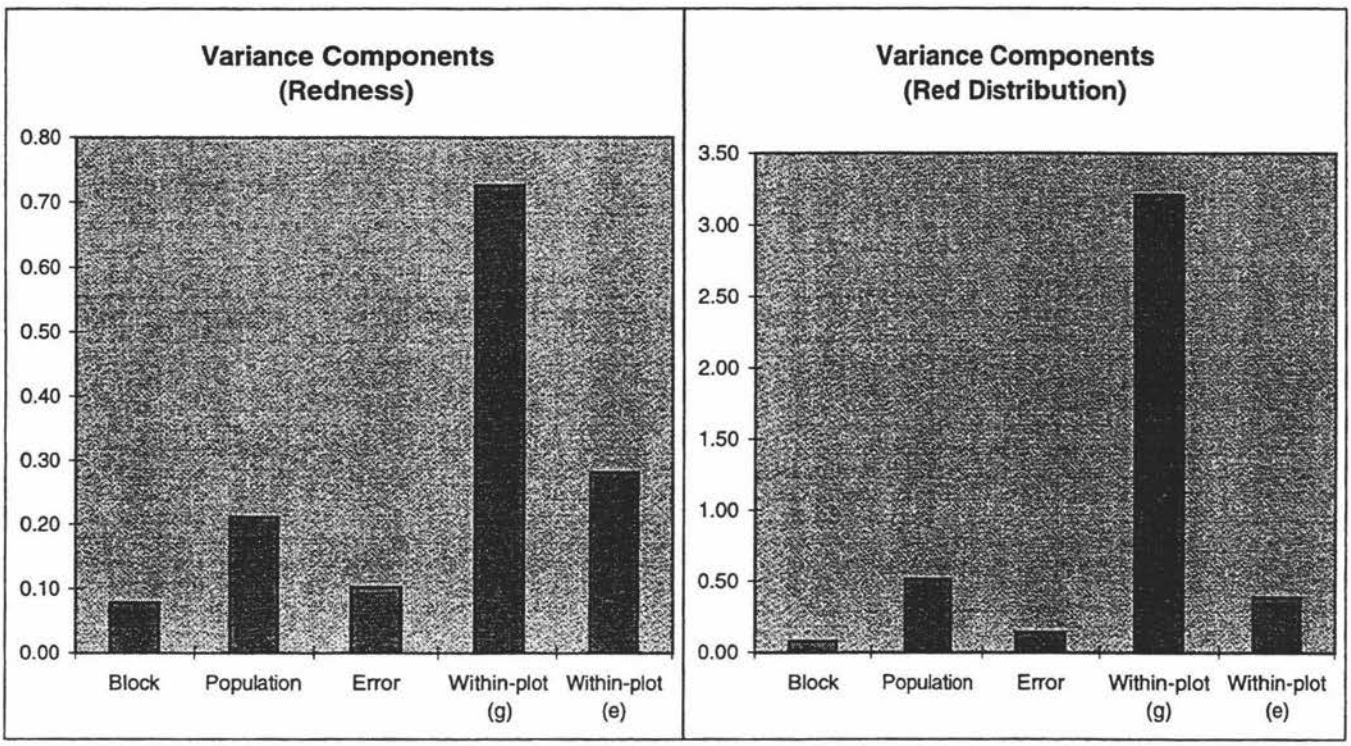
Character	*Partition*	*df*	*MS*	*Character*	*Partition*	*df*	*MS*
Diameter	Block	3	461.65659	Shatter	Block	3	10.06020
	Populations	35	112.87840		Populations	35	5.51022
	Error	105	125.75606		Error	105	5.15308
	Within-plot	1847	23.34678		Within-plot	1743	1.39123
Height	Block	3	101.20086	1k seed mass	Block	3	3.96302
	Populations	35	29.18608		Populations	35	20.19495
	Error	105	10.98497		Error	105	10.31651
	Within-plot	1847	2.53635		Within-plot	1759	8.74695
Uprightness	Block	3	0.91334				
	Populations	35	0.12794				
	Error	105	0.07370				
	Within-plot	1847	0.02627				
Leaf Shape	Block	3	2.33369	Factor 1	Block	3	11.06974
	Populations	35	2.71521		Populations	35	3.47291
	Error	105	0.77414		Error	105	3.73858
	Within-plot	1841	0.38261		Within-plot	1841	0.78515
Redness	Block	3	41.11471	Factor 2	Block	3	40.34563
	Populations	35	14.06816		Populations	35	19.53608
	Error	105	2.40951		Error	105	7.63101
	Within-plot	1845	1.00612		Within-plot	1841	5.00291
Distribution	Block	3	44.70894	Factor 3	Block	3	1.56962
	Populations	35	33.89870		Populations	35	8.04459
	Within-plot	1845	3.59438		Error	105	2.51446
					Within-plot	1841	0.79226
1st flower	Block	3	90.95899				
	Populations	35	432.60675	Factor 4	Block	3	3.10493
	Error	105	39.55434		Populations	35	0.96369
	Within-plot	1841	20.56181		Error	105	1.98357
					Within-plot	1841	1.60609
Seed set	Block	3	22.73325				
	Populations	35	11.02121				
	Error	105	4.76975				
	Within-plot	1823	0.56513				
Seed retained	Block	3	11.80809				
	Populations	35	3.40955				
	Error	105	1.55195				
	Within-plot	1759	0.88766				

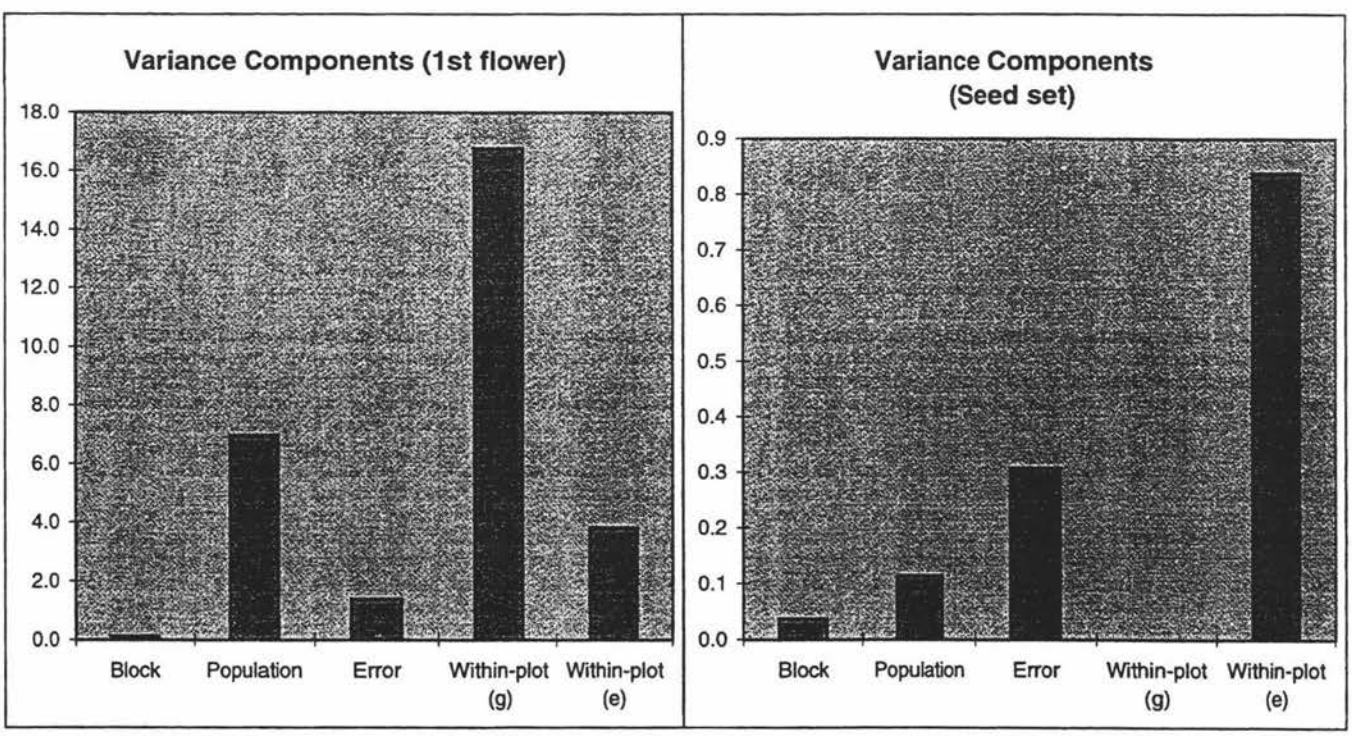
Appendix 2 : **Variance Component Histograms**



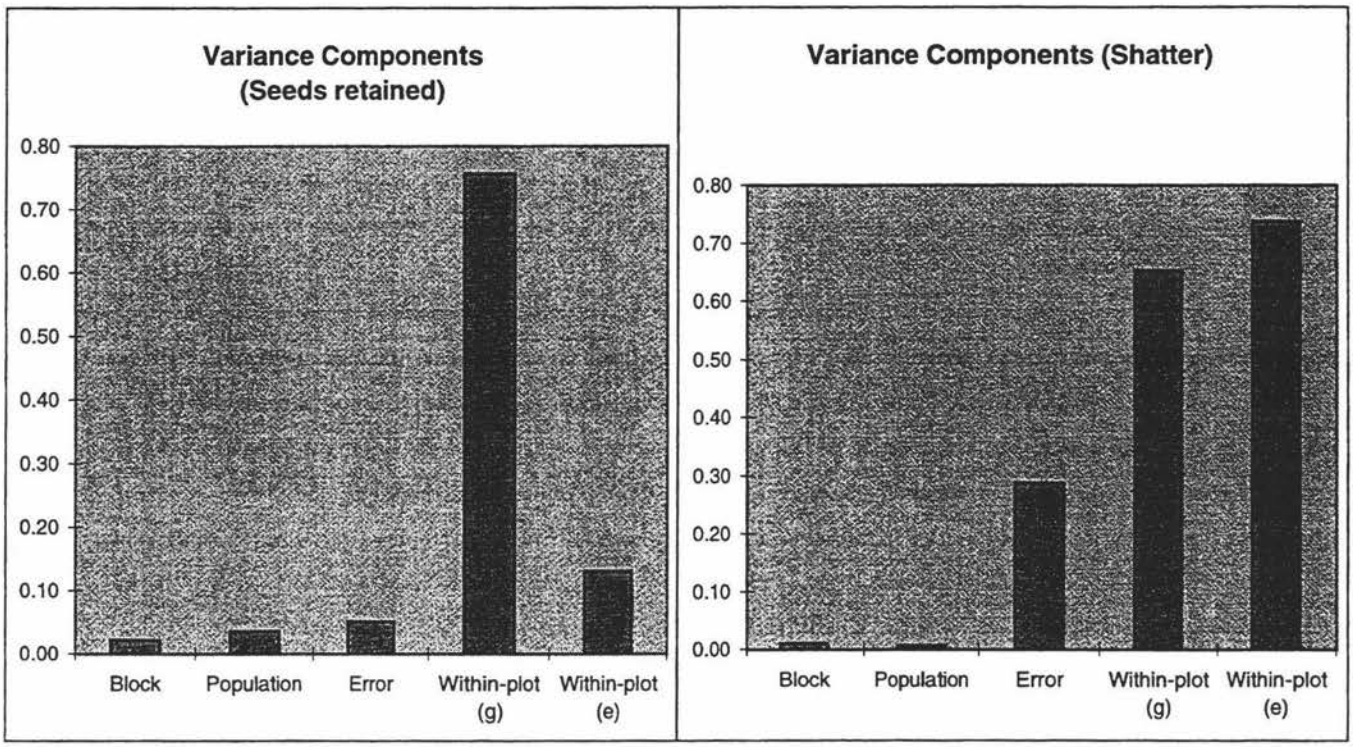


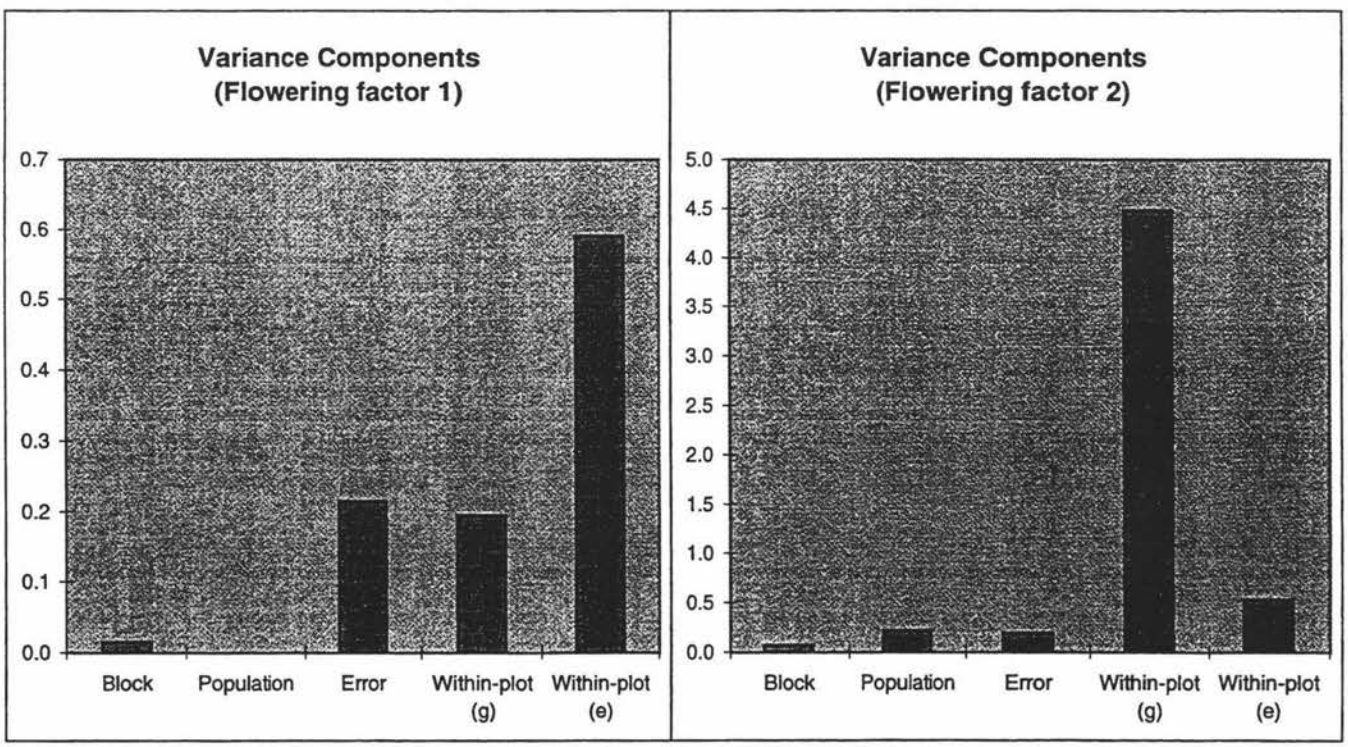
Appendix 2 : **Variance Component Histograms (continued)**



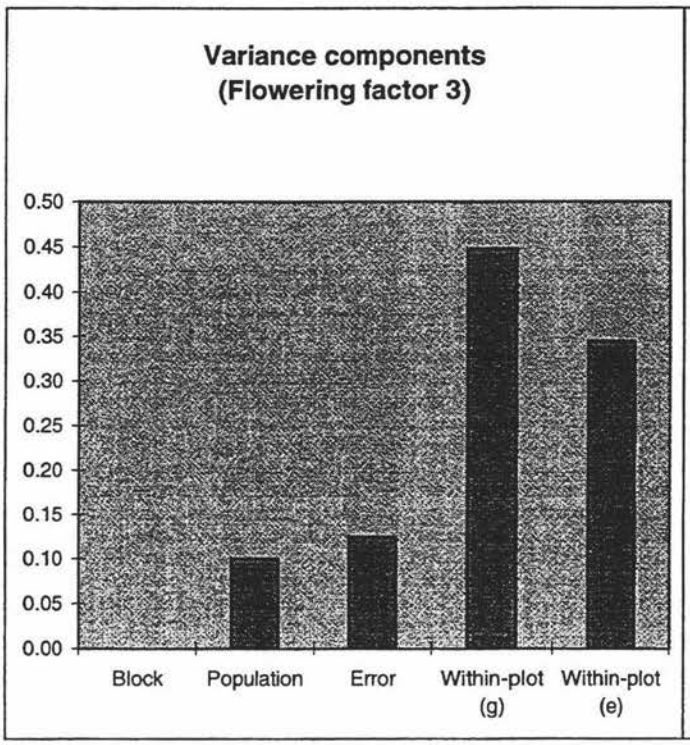


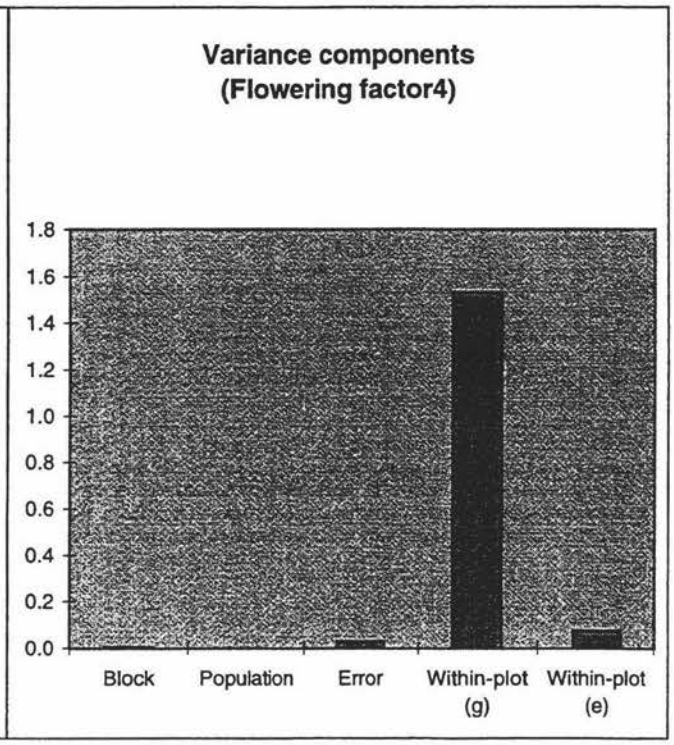
Appendix 2 : **Variance Component Histograms (continued)**

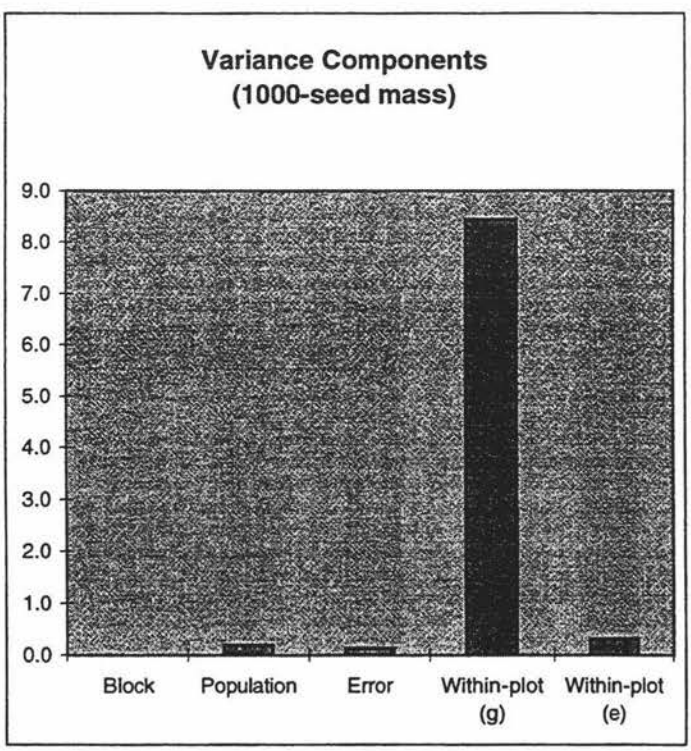




Appendix 2 : **Variance Component Histograms (continued)**







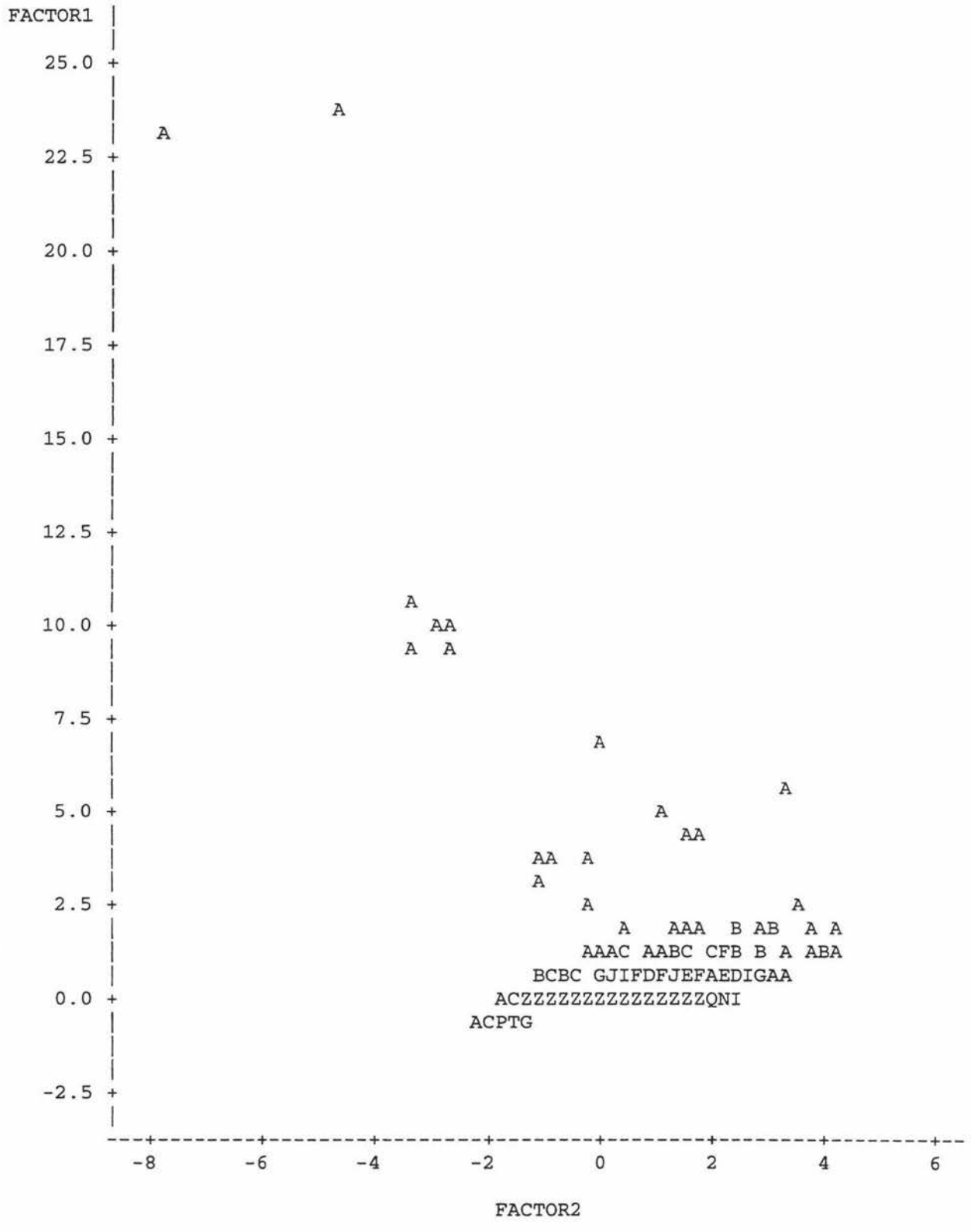
Appendix 3: Significance of heritability at various levels

Full Phenotypes

	Heritability (Narrow)	*s.e.*	*Signf.*	*Heritability (Broad)*	*s.e.*	*Signf.*	*Plant level Heritability*	*s.e.*	*Signf.*
Diameter	-0.031	0.07774	NS	0.084	0.01915	**!	0.091	0.00384	**!
Height	0.283	0.07739	**	0.321	0.03114	**!	0.231	0.01433	**!
Uprightness	0.111	0.05933	(*)	0.548	0.35056	**!	0.518	0.02727	**!
Leaf Shape	0.254	0.06226	**!	0.756	0.01668	**!	0.677	0.02101	**!
Redness	0.416	0.05825	**!	0.670	0.03771	**!	0.519	0.03487	**!
Distribution	0.350	0.08680	**!	0.860	0.01912	**!	0.742	0.02947	**!
1st Flower	0.558	0.02718	**!	0.818	0.01686	**!	0.578	0.03771	**!
Seed set	0.335	0.09140	**!	0.112	0.04489	*	n.a.	n.a.	n.a.
Seeds retain	0.128	0.05081	*	0.796	0.02160	**!	0.761	0.02191	**!
Shatter	0.016	0.06574	NS	0.389	0.01991	**!	0.385	0.01173	**!
1k seedmass	0.078	0.03680	*	0.954	0.01155	**!	0.934	0.01285	**!
Factor 1	-0.019	0.07227	NS	0.188	0.01914	**!	0.193	0.00708	**!
Factor 2	0.141	0.04878	**	0.857	0.0164	**!	0.818	0.01852	**!
Factor 3	0.305	0.07079	**!	0.540	0.0225	**!	0.441	0.01758	**!
Factor 4	-0.047	0.01702	NS	0.935	0.01208	**!	0.947	0.00957	**!

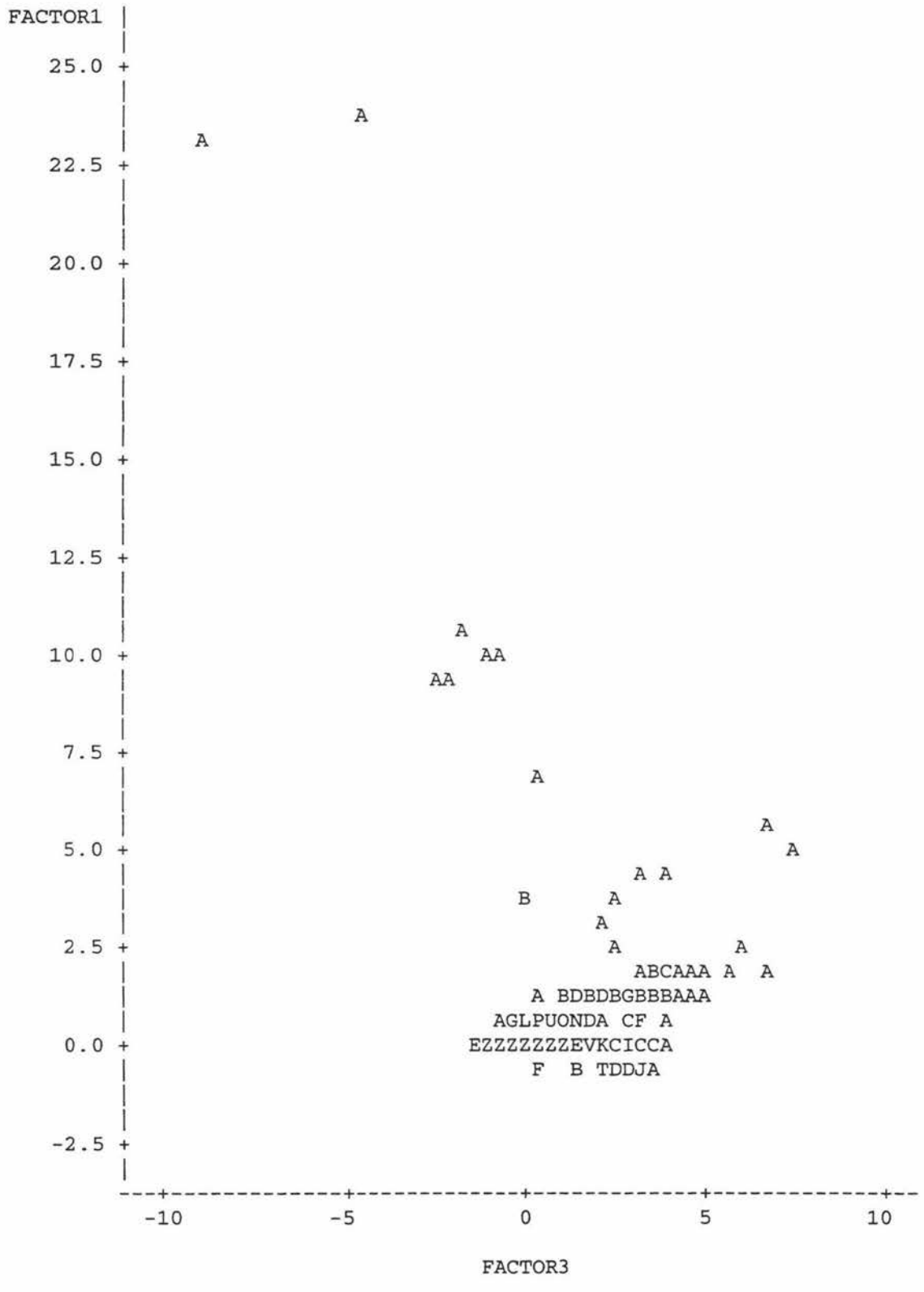
Key to symbols' level of significance	
(*)	~ 10%
*	~ 5%
**	~ 1%
**!	~ 0.1%

Appendix 4a: Plot of FACTOR1*FACTOR2.
Legend: A = 1 obs, B = 2 obs, etc.



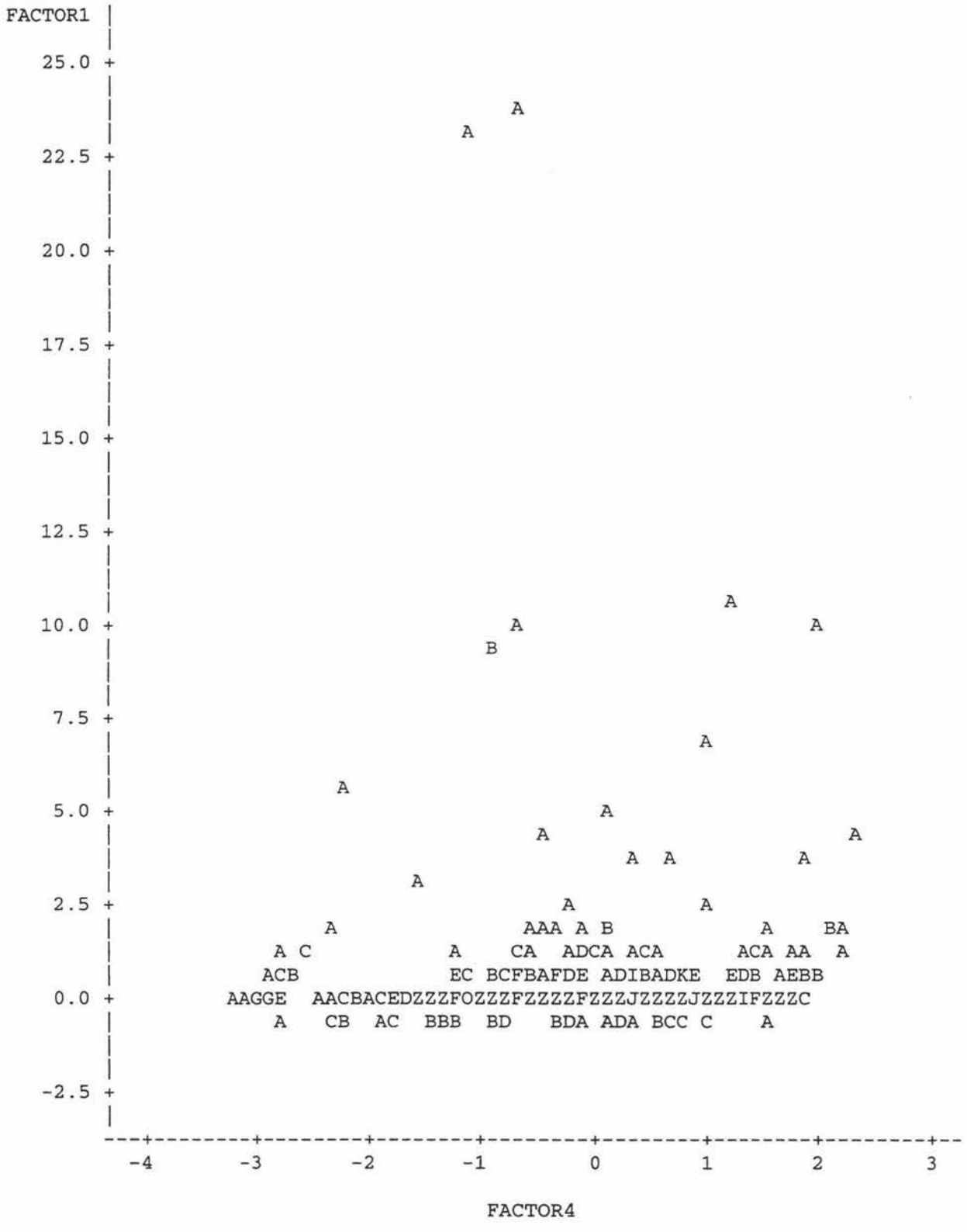
NOTE: 30 obs had missing values. 1343 obs hidden.

Appendix 4b: Plot of FACTOR1*FACTOR3.
Legend: A = 1 obs, B = 2 obs, etc.



NOTE: 30 obs had missing values. 1533 obs hidden.

Appendix 4c: Plot of FACTOR1*FACTOR4.
Legend: A = 1 obs, B = 2 obs, etc.



NOTE: 30 obs had missing values. 1067 obs hidden.