

**LEGUMES IN ANTIQUITY: A MICROMORPHOLOGICAL  
INVESTIGATION OF SEEDS OF THE VICIEAE.**

by

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FRONTISPIECE

Lathyrus blepharicarpus (x3).

In Syria, the seeds of this wild member of the Viciaeae are "much liked by people of the country" (Post, 1986, p. 293).

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ABSTRACT.

Early evidence for the importance of legumes in nutrition is limited by difficulties in the identification and interpretation of pulse remains from archaeological contexts.

The main aims of this work are to seek in a detailed survey of legume seed micromorphology, criteria that can be used i) for the identification of species and, ii) as evidence of cultivation. Assessment of the role of SEM in archaeobotany is a supplementary aim.

Archaeological evidence of pulses in the Old World is first reviewed, followed by the rationale for the selection of members of the tribe Viciae as the species researched.

The second part of the thesis is a background study of the Viciae and its four major genera Vicia, Lathyrus, Lens and Pisum. Brief accounts of their taxonomy, seed morphology and geographical distribution are followed by an examination of traditional Old World systems of pulse agronomy.

Chapter three concerns the material and methods of

research. The results are described qualitatively in chapter four, and following multivariate analysis in chapters five and six. The seventh chapter describes some miscellaneous procedures.

The eighth chapter is a discussion of the results. Identifications down to species level using seed micromorphology are restricted to certain taxa, contrary to some published reports; but allocation of specimens to a higher taxonomic rank, using an array of criteria, is usually more realistic. Most useful characters are found in features in the hilar region. Clear evidence of cultivation has only been observed in the genus Pisum. Using SEM, similar data may be recorded equally in fresh seeds and fragments of charred archaeological specimens.

It is concluded that seeds of the Viciae usually do not exhibit sufficient morphological differences for species identification. Evidence for cultivation may rather be sought in biochemical changes that facilitate control of the germination in seeds of cultigens. Future research into pulse biochemistry and agronomy is anticipated.

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TITLE. LEGUMES IN ANTIQUITY: A  
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## 1. INTRODUCTION.

The role of legumes in agriculture has been an increasingly important focus of international research over the past years, during which time the world has seen major crises in food supply in many areas of the Third World. The amount of work on legume crops reflects the interest in improving human nutrition by raising the levels of pulse production (Ker, 1979, p.26; Sanderson & Roy, 1979, p.42). Agricultural research, particularly in the arid regions of North Africa and Southeast Asia (for example Hawtin & Chancellor, 1979; Saxena & Varma, 1985) has been accompanied by international meetings to discuss a number of topics related to legumes, including (i) the wider agronomic aspects of the pulses and forage and fodder crops in temperate regions (Summerfield & Bunting, 1980, a; Summerfield & Roberts, 1985b; Summerfield, 1988); (ii) the taxonomy, genetics, biochemistry and distribution of related wild legume species worldwide (Polhill & Raven, 1981; Stirton, 1987; Ladizinsky, 1989; Zohary, 1989b); and (iii) ethnographic studies on the food-preparing strategies of traditional societies (Aykroyd & Doughty, 1982). Many of these data on legumes are being incorporated in international databases (for example Allkin et al., 1986).

More recently a debate has arisen concerning the agricultural origins of the cereals and pulses: it has been asked (i) whether both types of food plant were

taken into cultivation simultaneously, as wild-type crops, using similar techniques (Zohary,1989a); or (ii) whether the legumes were semi-domesticated separately prior to cultivation, and were added to the crop repertoire already modified by man's selection during gathering from the wild (Ladizinsky,1987,b). This debate stimulated the hypothesis that legumes might have preceded the cereals in early farming (Kislev & Bar-Yosef,1988). Several avenues of research have been followed in order to try to clarify the position of legumes as crops in early cultivation: (i) fieldwork amongst the modern cultivars and their wild relatives (Zohary,1973/4;1977;1983;1986;1989b); (ii) investigations on the genetics and biochemistry of modern plants; (iii) plant breeding studies, to determine the wild progenitors and closest relatives of the crop species (for example, Ladizinsky,1985,1989; Ladizinsky et al.,1983; Zohary & Plitmann,1979) and (iv) from examinations of the archaeological record (Hopf,1986; Kislev,1985;1989). Many issues remain unresolved.

### 1.1. Legumes in the archaeological record.

In the Old World systems of seed agriculture, the importance of grain legumes, or pulses, is well-recognised. Together with the cereals, they play complementary roles in supplying the essential amino acids in the diet (Aykroyd & Doughty,1982,p.82). This association has been shown to be of some antiquity and is

known from the earliest archaeological records (Zohary & Hopf, 1973; 1988, p.83). Legumes, however, are usually found in lesser amounts than the cereals in archaeological assemblages. This has resulted in a similar disparity in the literature relating to archaeobotanical remains (Zohary & Hopf, 1973).

It has been suggested that this bias in the archaeological record is due to the circumstances of deposition (Dennell, 1972, p.151; 1978, p.18; Renfrew, 1973, p.104); for example, cereals, more commonly than legumes, may be parched prior to storage, which can lead to accidental charring and the preservation of some of the grains. This view, however, has been questioned by van Zeist et al. (1984) in the light of some early evidence from the Levant, where certain archaeological sites have been found with a predominance of legumes amongst the plant remains.

The accounts of the earliest remains of pulses in the Old World have recently been reviewed by authors such as Hopf (1986). She has outlined the types of remains found, discussed the contexts of their recovery, and the conditions for their preservation, and described their different physical states.

By far the majority of the earliest remains of legumes in the Old World have been preserved by charring. The accidental burning of seeds during food preparation and

the deliberate burning of leguminous plant material account for some of the circumstances of preservation by charring; both refuse matter and fuel might be included; the fuel may consist of fresh plants or of dung, derived from leguminous plant fodder. Major charring episodes, such as conflagrations involving whole buildings or villages, may result in the preservation of entire storage areas; in such contexts, large volumes of seeds may be recovered, sometimes in their original containers. The seeds may represent a store of pure or mixed crops, and they may be associated with the seeds of some of the weeds that contaminated the crop in the field. The analyses of such assemblages can contribute to the creation of models of ancient farming.

#### 1.1.1. Recovery of the legume seeds.

The charred seed remains may be recovered by hand picking, by the dry-sieving of fill, and by flotation, a technique adopted in the late 1960's, and which is usually the most effective method.

On many of the early excavations, flotation was not practised; from any such deposits the recovery of the plant remains may be far from complete, as at the Late Bronze Age site of Deir 'Alla in Jordan (van Zeist & Heeres, 1973).

The techniques of recovery, the contexts chosen for the

excavation and the selection of the sites themselves have influenced the archaeobotanical record, and consequently affected the interpretations of plant remains that have been made (Hopf,1986).

#### 1.1.2. Origins of plant food production.

Many archaeological sites in the Old World dating from the Mesolithic have yielded the remains of food plants, including legumes, and these have provided the basis for theories on the beginnings of cultivation. Various early theories concerning the origin of food production in southwest Asia have been discussed in a survey by Wright (1971), and criticized by Hole (included in Wright,1971) for lacking climatic considerations. Flannery (1969) and Redman (1977) are amongst those who have attempted an environmental reconstruction for this region during the period when cultivation is believed to have been developing. More recent reviews for this area include those of Harris (1977) and Moore (1985;1989). Summaries of the plant remains from early sites in the Near East and Europe include those of Helbaek (1960), Hubbard (1980) and Renfrew (1973). In addition, a number of reviews have recently been published on the evidence for the earliest cultivation in the Old World, encompassing the origin and development of the cultigens, and the spread of agriculture (Hopf,1986; Kislev,1984; Kislev & Bar-Yosef,1988; Ladizinsky,1989; Zohary,1973/4;1986;1989b; Zohary & Hopf,1988).

As a consequence of the ready availability of this current information, a detailed review of the archaeobotanical literature is not included in this thesis. However, a few examples are cited in the following paragraphs to illustrate the evidence for the use of legumes prior to the onset of cultivation, and their importance from the first stages of cultivation.

#### 1.1.2.1. Evidence for the gathering of wild legumes prior to cultivation.

Evidence has been found of the gathering of legume seeds prior to the initiation of cultivation. At Abu Hureyra in Syria, for example, the Epipalaeolithic levels contain the seeds of many wild plant species, including the grains of grasses, the seeds of small-seeded legumes, such as species of Trifolium, and edible wild legumes such as lentils and some vetches (Hillman et al., 1989). Similarly at Mureybit, the seeds of lentils and vetches have been recovered in association with grains of wild cereals, and the legume Astragalus, (van Zeist, 1970; van Zeist & Bakker-Heeres, 1984). The publications of Costantini (1989) and Vaquer et al. (1986), exemplify analyses of plant assemblages from Mediterranean Mesolithic sites in which wild legumes are well represented.

#### 1.1.2.2. The beginnings of cultivation.

The first definite evidence of cultivation in the Old World can be observed in charred plant material from sites in the Near East in levels from the Pre-Pottery Neolithic period, dating from 8000 to 7000 BC (Zohary, 1986; Zohary & Hopf, 1988, p.42). Predominating amongst these plant remains are the caryopses and chaff fragments of the cereals emmer, einkorn and barley. These bear morphological signs of selection under cultivation. Commonly associated with these remains are the seeds of a number of large-seeded legume species, usually in lesser concentrations. The species most frequently represented are *Lens culinaris*, *Pisum sativum*, *Vicia ervilia* and *Cicer arietinum*; less commonly found are *Vicia faba* and *Lathyrus sativus* (Hopf, 1986; Zohary & Hopf, 1988, p.83).

In the Near East, the sites of Jarmo in Iraqi Kurdistan (Helbaek, 1959; 1960; Watson, 1983), Jericho in the Lower Jordan Valley (Hopf, 1983) and Çayonu in eastern Turkey (van Zeist, 1972; 1977), are examples which have yielded similar plant assemblages from this period, which have included large-seeded legumes together with larger amounts of both wild and cultivated wheats and barleys.

#### 1.1.2.3. The Neolithic; established cultivation.

By the ceramic Neolithic period, there is evidence that cultivation was well-established, both in the Near East,



and in the Eastern Mediterranean, and pulses commonly occur together with the cereals. The plant remains, for example, from Erbaba in Central Anatolia (van Zeist & Buitenhuis, 1983), and from Nea Nikomedeia in Greece (van Zeist & Bottema, 1971), include high proportions of the seeds of the large-seeded legume crop species, mainly pea, bitter vetch, lentil and chickpea.

Storage contexts have yielded large deposits of the seeds of single species. These have provided evidence for the large-scale production of pulses: an early example has been found at the site of Yiftah-el in Israel, dating from about 7000 BC; here, in a silo, an enormous hoard of 1,400,000 lentils and over 2000 horse beans has been recovered, both found as pure deposits (Garfinkel et al., 1987; 1988; Kislev, 1985).

#### 1.1.2.4. The spread of cultivation.

Although some authors still favour theories of the indigenous development of agriculture outside Western Asia (Barker, 1985, p.250; Dennell, 1983, pp.163-165), the conventional view of agricultural origins holds that cultivation was established in the Near East and quickly expanded to Europe and Central Asia (Zohary & Hopf, 1988, pp.208,209). By the sixth millenium, it had spread to Greece (Hopf, 1962; Kroll, 1981; Renfrew, 1979; van Zeist & Bottema, 1971), the Danubian basin (Renfrew, 1974; 1979), Egypt (Wenke & Casini, 1989; Zohary &

Hopf, 1988, p.189), Caucasia (Lisitsina, 1984) and the Indian subcontinent (Costantini, 1981; Costantini & Costantini-Biasini, 1985); although pulses have not been recovered yet from the earliest agrarian sites in the latter two regions, the major cultivated species einkorn, emmer, barley, flax, lentil, chickpea, bitter vetch and pea commonly occur in association and are believed to have been introduced together as "founder crops" (Zohary, 1989b, p.358).

## 1.2. Identification and interpretation of charred ancient legumes.

Difficulties inherent in the identification and interpretation of legume seeds are described below. These problems are compounded in ancient seed remains.

### 1.2.1. Identification of ancient legume seeds.

The seeds of most grain legumes can be identified to genus by their gross morphology. Ancient charred legume seeds are commonly identified on the basis of their shape and size, and the form of the hilum, when matched with modern comparatives. However, some legume taxa, particularly those of wild status, have seeds with very similar morphologies; seeds of the pea/vetch/lentil type have been described as "enigmatic" (van Zeist & Bakker-Heeres, 1985, p.286), and it may be impossible to identify even modern seeds beyond tribal level.

Archaeological legume seeds are usually altered by charring and the depositional process. Commonly they are lacking most of their surface features, and thus pose even greater problems in diagnosis. Some of the identifications in the literature are insecure; and much of the ancient pulse material remains without firm diagnosis to the level of species or even genus.

#### 1.2.2. Ecological evidence used to aid identification of legume seeds.

The ecological requirements of some taxa have been used as evidence to aid the diagnoses of species; current environmental data has been employed to create models of past conditions in particular areas. Using such a model, the ancient seeds of a wild lentil, for example, have been identified to species level on the basis of a knowledge of the preferred habitat of modern populations, and the ecological constraints believed to have been pertaining during the relevant period in antiquity (Hansen, in press).

#### 1.2.3. Evidence of cultivation in ancient legumes.

The cultivated or wild status of legume seeds is similarly difficult to determine. This problem has been discussed by Hopf (1986) amongst many other archaeobotanists. The characters that develop in the cultigens following selection under cultivation, are

described below (2.4.2). They include reduced dehiscence, reduced seed dormancy and seed gigantism. The following paragraph briefly describes the evidence conventionally used to interpret the status of charred ancient pulses.

#### 1.2.3.1. Morphological evidence for reduced dehiscence in remains of ancient legumes.

Helbaek (1970) has described the changes in cereal chaff that provide evidence of the reduced dehiscence and thus of the cultivated status of cereals; this can be observed in ancient charred material, and can be used as evidence for the beginnings of cultivation (Zohary & Hopf, 1988, p.42). The pods of cultivated legumes carry the equivalent evidence of cultivation. However no ancient charred pod fragments of the large-seeded legumes of the Old World have yet been recovered, and thus this form of evidence is lacking for the pulses (Ladizinsky, 1979; Zohary & Hopf, 1973).

#### 1.2.3.2. An increase in seed size.

The charred seeds of legumes, such as lentils and unidentified species of vetch or vetchling, that are present together with the early cultigens of wheat and barley, are commonly small and within the size range of wild species. Seed gigantism is a development following selection during cultivation that is believed to occur at a late stage (Hopf, 1986). However, on some early sites

which span pre- and post-agrarian periods, a small increase in the size of the legume seeds can be observed at a time when cereal cultivation has been deduced. An example comes from Franchthi Cave in the Argolid of Greece. From levels dating from 11000 BC small lentils and a few vetch seeds have been recovered with the grains of wild cereals. At levels dating from 6000 BC, cultivated emmer and barley occur. At this stage the associated lentils are slightly larger. This size change is abrupt; this has prompted the interpretation that the seeds were introduced with cultivation from an external source (Hansen, in press).

#### 1.2.3.3. Evidence for reduced dormancy.

A relatively thin testa and a smooth testa surface, are two additional characters that have been cited by Hopf (1986) as evidence of cultivation in the seeds of some legumes. These characters have been associated with an increased rate of imbibition and thus with a reduction in seed dormancy. These concomitants of cultivation, described below (2.4.2.2.), can be seen, for example, in the charred remains of archaeological peas. Helbaek (1970) used the texture of the testa to identify wild ancient peas at Hacilar, and cultivated peas at Çatal Hüyük, sites on the Konya Plain, Turkey; and, similarly, van Zeist (1972) used the testa texture to identify the peas at Çayonu. All pulses, however, do not exhibit these characters.

1.2.3.4. Circumstantial evidence for legume cultivation.

The interpretation of legume cultivation on early archaeological sites has largely been based upon various types of circumstantial evidence. These can be demonstrated by the following examples.

(i) The association of seeds of large-seeded legume species with the grains of known cereal cultigens in the plant remains from early sites has been employed as evidence for legume cultivation. It is the basis for the widespread assumption that cereals and legumes were taken into cultivation together from earliest times (Zohary, 1986; Zohary & Hopf, 1988, p. 83).

(ii) Large deposits of the seeds of one taxon may be interpreted as a sample of a harvest. In one example, Kislev (1989), referring to the seeds from the genus Lathyrus, has suggested that part of a crop might be represented when the number of ancient seeds in one deposit exceeded one hundred. Obviously the particular numbers would be based on the estimation that it would be unlikely that such an amount of seeds would have been gathered from the wild, an assumption which is often impossible to validate.

(iii) A third line of evidence comes from seed assemblages that may accompany putative cultigens. Certain weedy species are commonly associated with

particular crops, but do not regularly occur with the closely-related wild species under natural conditions. The presence of seeds from the weed species could indicate that the major component of the seed deposit was cultivated. This ecological reasoning has been used by Garfinkel et al. (1988), with reference to the presence, at the site of Yiftah-el in Israel, of the seventh millenium BC, of seeds of Galium tricornutum amongst the lentils; the latter thus were identified as the cultigen, Lens culinaris.

In summary, it can be seen that, excepting the genus Pisum, no morphological characteristics have been found in legume seeds that can be used as unequivocal evidence of their cultivation at the very earliest stages (Zohary & Hopf, 1988, pp.92,110). The circumstantial evidence is highly speculative. There is an apparent need for alternative criteria that can be used to establish more accurately the status of the pulses on early agrarian sites.

### 1.3. Selection of the taxa for research into alternative criteria.

From the archaeological record, it can be seen that the seeds of large-seeded legumes, that are commonly found in association with ostensibly-cultivated cereal grains,

from early Neolithic occupations, are restricted to relatively few taxa. Just six species embrace the main pulses cultivated in the Old World, and they are found throughout all periods since the earliest pre-agrarian periods: they are lentil, pea, broad bean, bitter vetch, grass pea and chickpea (Hopf,1986; Ladizinsky,1989; Zohary,1989b; Zohary & Hopf,1988,pp.83,109). Their use is also documented from historical times (see 2.5.4.,below). All are still important food resources, with the exception of bitter vetch, which was once a human food, but is now usually reserved for use as animal feed or fodder.

It was decided to focus the research upon the seeds of a single tribe, the Viciaeae, in order to be able to include a number of the wild taxa in the study. It is believed that a broader understanding of a narrow range of plants might be more useful than a more superficial study of leguminous plants drawn from a wider taxonomic group. The vetch tribe, or Viciaeae, includes the vetches, lentils, vetchlings and peas; it thus incorporates all the important cultivated legumes in the Old World, with the exception of chickpea. This tribe, therefore, has been selected to provide the seed material examined for this thesis. The taxa examined include all the major cultigens in the Viciaeae, their wild progenitors, or closest wild relatives, and representatives from many different taxonomic sections of the genera selected. Replicate populations of many of the taxa have been included to



allow studies of components of variation within species.

1.4. Application of the scanning electron microscope (SEM) in the study of fresh seeds.

Over the past ten to fifteen years, the increasing availability and widening range of applications of the electron microscope in the natural sciences has resulted in a number of studies of seed micromorphology and anatomy. More than twenty years ago observations of seed surfaces were being made by SEM; at that time it was anticipated that this would become a routine technique for the study of seed micromorphology (Heywood, 1969). Some of the early publications have been reviewed by Brisson & Petersen (1976), and enthusiastic reports of applications of SEM have prompted a number of investigations. These have focussed particularly on the micromorphology of the surface of the seedcoat, or testa. The work undertaken by Lersten (1981), and Lersten & Gunn (1982), led to expectations that newly-observed testa characters would facilitate the identification of legume seeds. Further work in the early 1980's, including some preliminary studies on charred archaeological pulse remains, also gave promising results (Stocks, 1980).

As has been stated above (1.3), characters of the gross morphology do not separate seeds of many legumes; however, it is possible that micromorphological and anatomical characters are sufficiently different in the

seeds of some closely-related legume species to be useful for their identification and interpretation.

#### 1.4.1. SEM of charred archaeological seeds.

SEM is a technique that has proved to be useful when applied to charred material. The charred remains of ancient wood has been examined, and have illustrated that the fine anatomical features are commonly preserved in charcoal, and can be recorded by SEM, using the same techniques as are used for fresh plant material (Prior & Alvin,1983). Charred archaeological seeds and grains have also been scanned; the early observations showed that ancient seeds can retain much of their micromorphology (Conolly,1976). Following this earliest work, SEM has been applied to fresh and charred archaeological seeds in the identification of the ancient cereal grains by matching their micromorphology (Körber-Grohne,1981; Körber-Grohne & Piening,1980; Colledge,1988). Similar work has been published for the seeds of other species, such as Chenopodium (Smith,1988), and a few pulses (Kislev,1986; Stocks,1980).

The following section consists of a elaboration of the aims of this thesis; these will be achieved by employing the technique of SEM.



1.5. Aims of the project.

1.5.1. The first aim: to seek identificatory criteria in seeds of the Viciaeae.

In the light of the need to increase our understanding of the role of the legumes as major dietary components since earliest times, and in view of the difficulties in the identification and interpretation of legume seeds, the first major aim has been to seek new characters in the micromorphology and anatomy of seeds of the cultigens in the Viciaeae and their wild relatives, that can be used to identify the seeds (i) to the level of genus; and (ii) to the level of species. These characters will be sought primarily in fresh modern seeds

1.5.1.1. Methods employed to achieve first aim.

(i) An investigation of the micromorphology of the surface of the testa surface is the initial undertaking. To record structural variations, observations are made (a) on different areas of the seed; (b) of two or more specimens of any one taxon; (c) on seeds of replicate populations of certain taxa. The taxa include all the grain crop species in the Viciaeae used for human food, some species that probably were once part of the human diet, their wild progenitors where known, and some wild species that provide a range of seed morphology and micromorphology, and represent many different taxonomic

sections in the tribe.

(ii) To supplement the data, more extensive surveys of the testa are undertaken, including the structures associated with the hilum, and the layers of the testa. The primary selection of the characters to be recorded is based upon the observations in published accounts of other workers, and also upon features which are thought likely to be taxonomically stable (Bell, 1969, p.71).

#### 1.5.2. The second aim: evidence of cultigens.

The need for criteria with which to establish the cultivated or wild status of legume seeds has dictated the second and equally important aim: namely, to find characters in the morphology and anatomy of seeds of members of the Viciaeae, that can be used as evidence of cultivation, or otherwise.

##### 1.5.2.1. Methods employed to achieve second aim.

(i) Observations concentrate on those testa features that are likely to show changes following selection under cultivation; the selection of features used in this exercise is governed by (a) the published accounts of testa features showing such changes; (b) other factors, including biochemical and physiological differences in the cultigens that might be correlated with changes in the micromorphology.

### 1.5.3. Statistical methods.

In order to standardise the observations, and to reduce subjective error, it is attempted to record the data numerically. If the dataset is of sufficient size for valid statistical analysis, multivariate analyses are employed.

### 1.5.4. Relevance of data from fresh seeds to charred archaeological seed remains.

Because of the altered state of ancient plant material, it is necessary to demonstrate the relevance to archaeological seeds of the observations made of fresh modern material. This is achieved in two ways: (i) the effects of charring are investigated. Modern seeds are charred; the charring regime is determined experimentally during the project. Differences in the seed micromorphology following charring are investigated. (ii) ancient charred legume seeds are studied and compared with both fresh and charred modern seeds. With all types of seed material, similar observations are made, employing similar standardised techniques.

### 1.5.5. Evaluation of SEM applied to fresh and ancient seed micromorphology: a supplementary aim.

SEM is a technique that has been successfully applied to both fresh modern plant material, and to ancient charred

plant material (see 1.5.1. above). In this project, observations of the micromorphology are made of seeds in both states and it is necessary to compare the data recorded on both types of material. It is believed that the opportunity to employ a single technique throughout the project will facilitate the standardisation of observations and eliminate some potential sources of error. The evaluation of SEM, when applied to the observation of testa micromorphology of fresh modern, charred modern and charred archaeological seeds is a supplementary aim.

#### 1.6. The structure of the thesis.

In this first chapter the basis for the research in the thesis has been described, and the selection of the tribe Viciaeae has been discussed. The second chapter briefly considers the taxonomy, morphology and anatomy of the seeds of members of this tribe as conventionally described, and the distribution and ecology of various species. This is followed by an account of the cultivated species. The chapter is completed by a more detailed description of some traditional agronomical techniques applied in the cultivation of pulses.

This background has provided the foundation from which the main research has been conducted, and which is next described. The seed materials are listed, and methods of microscopy are set out with explanations for their

selection; some associated techniques that have been employed are described. This third chapter is followed by the results of a survey of the micromorphology and anatomy of fresh seeds, which are assessed qualitatively and expressed as an atlas for each taxon in the study (chapter four). The atlas is illustrated by a series of plates, contained in a second volume. A summary of these data is made. The results of the SEM of charred modern seeds and of charred archaeological seed remains are described. The following chapters, five and six, outline the methods of numerical analysis that have been applied to the microscopical data, with the results. A short seventh chapter describes some miscellaneous procedures and observations that have contributed to the thesis.

Finally, the eighth and ninth chapters are those of the discussion, followed by the conclusions. Some potentially useful directions for future research are suggested.

## 2. THE VICIEAE.

In this chapter the taxonomy, the seed morphology and anatomy and the distribution of the Vicieae are described. The development of the cultigens is discussed, and notes on the main cultigens are included. These topics provide a general background to the tribe.

### 2.1. Taxonomy.

#### 2.1.1. Introduction.

The taxonomy of the Vicieae forms the subject of this section; the tribe is set in its classificatory context, and its genera are listed.

Legume taxonomy has been extensively studied in recent years, most notably at the Royal Botanic Gardens Kew, where research has resulted in a number of detailed publications (Polhill & Raven, 1981; Stirton, 1987). The treatment of this complex subject here is necessarily highly abbreviated, but attempts have been made to provide a sufficient background for the general understanding of the place of the Vicieae within the plant kingdom, and to indicate the range of attributes of morphology, anatomy, biochemistry and genetics that has contributed to the classification.

Beginning with the legume family, and the papilionoid



subfamily, general traits are described within the tribe Viciaeae, followed by a brief description of the morphological characters conventionally used to separate the genera.

### 2.1.2. The family Leguminosae.

The Leguminosae (Fabaceae) is the third largest family of flowering plants, and includes some 650 genera. They are of worldwide distribution, being found in all continents, and they occupy a great diversity of ecological niches, from those in the most extreme conditions of cold and aridity, to those of temperate environments and tropical rainforests, at altitudes ranging from sea level to high mountain plateaux (Adams & Pipoly, 1980).

This wide-ranging distribution of the legumes reflects a great versatility of morphology and physiological behaviour, which has complicated the taxonomy. Consequently aspects of morphology, anatomy, cytology and biochemistry have all been drawn upon to set subfamilial and tribal boundaries (Polhill et al., 1981).

The family is subdivided into three subfamilies: the Caesalpinioideae, Mimosoideae, and Papilionoideae (Faboideae) (Polhill et al., 1981). The latter are characterised by the papilionoid flower, a hilar valve in the seed, and a distinctive phytochemistry (Polhill, 1981).

### 2.1.3. The subfamily Papilionoideae.

The Papilionoideae are comprised of approximately 440 genera, which include trees and lianes in the tropics, and shrubs and herbs in temperate climates (Polhill, 1981). In his system published in 1865, Bentham (cited in Polhill, 1981) separated some temperate groups on the basis of leaf morphology, distinguishing the Viciae by their typically paripinnate leaves and tendrils. Cytological research by Senn (1938) and others has clarified many of these early taxonomic separations. For example, the Viciae have a karyotype of  $x=7$ , probably derived from an ancestral  $x=8$ , showing a relationship with the similarly temperate tribe Trifolieae with  $x=8$ , and a clear separation from tribes with tropical affinities, such as the Phaseolae, with  $x=11$  (Goldblatt, 1981).

Tribal boundaries have been clarified further by chemotaxonomic research, exemplified by the work of Ingham (1981) on phytoalexins, the secondary metabolites that accumulate in the plant in response to pathogenic invasion. It has been shown that the phytoalexins produced in the Viciae are compounds that are either very rare in or absent from other papilionoid tribes. Similarly, the chemistry of the anthocyanin pigments separates the Viciae from related tribes (Harborne, 1971).

Thus early classification systems have been reinforced or reorganised following the coordination of information from many lines of approach, which have been described by Heywood (1984). As stated by Polhill et al. (1981,p.24), "up to now the basic classification of the family has remained remarkably stable", yet it is appreciated that new techniques and further field research are extending the possibilities for a more detailed understanding of this complex plant group.

#### 2.1.4. The tribe Viciaeae.

All members of this tribe are temperate herbs ranging from annuals and biennials to perennials in life cycle. Germination is exclusively hypogeal, and this characteristic and the tendrillous leaf have been considered to represent residual traits of an ancestral forest flora (Polhill & van der Maesen,1985). These combined characters are distinctive to the Viciaeae, and have been regarded as of overriding significance in taxonomic considerations within the Papilionoideae. However it should be noted that etendrillous species do occur in some genera in the tribe (Kupicha,1977).

A detailed survey of the systematics of the Viciaeae has been published by Kupicha (1977), who considers that this tribe of five genera forms a small distinct group having no obvious connections with other tribes (Kupicha,1981). She admits that "generic limits in the Viciaeae are still

a matter of debate" (Kupicha,1977). In 1964 Abrus was reclassified and placed in its own tribe (Hutchinson,1964), and as recently as 1977 Cicer similarly was allotted tribal status (Kupicha,1977). Currently Vicia, Lathyrus, Lens, Pisum and Vavilovia are accepted as the five member genera in the Viciaeae.

These genera are closely related, and many of their species have had varied generic classifications. Vicia, Lathyrus and Lens are particularly close. The form of the style is of particular importance in separating the genera. The main morphological taxonomic characters of the genera are given below. They are listed in their conventional taxonomic sequence.

Vicia. Kupicha (1976) has described the intrageneric structure of Vicia and discussed the variable traits within the genus. The main distinguishing features are the angled stem, which is never winged; a calyx with teeth of unequal length and with an oblique mouth; a staminal tube with oblique apex; and a hairy style, usually dorsally or laterally compressed. The genus includes about 140 species.

Lens. This genus has been described as a "Vicia with some Lathyrus characters" by Cubero (1981,p.18), who has listed in detail the main taxonomic characters by which it may be distinguished. These are exemplified by the deeply divided calyx, in which the teeth are at least

twice as long as the tube. The dorsally-compressed style is fringed with hairs on the upper side (Davis & Plitmann, 1970). There are five species that have been reclassified by Ladizinsky (1986), in a system used throughout this project, but which has not as yet achieved universal acceptance.

Lathyrus. This genus has received detailed description also by Kupicha (1983). The stem is winged or unwinged; the calyx as in Vicia may have unequal teeth and an oblique mouth; however the staminal tube typically is truncate at the apex, and only rarely oblique; the style is dorsally compressed, and adaxially hairy. About 150 species are recognised.

Pisum. This genus shows a close relationship with the genus Lathyrus. The smooth or terete stems of this genus differentiate it from most Lathyrus species, as do the toothed stipules, broad calyx teeth and a style with retroflexed margins and hairy distal region on the upper surface. There are two species, both of annual habit (Davis, 1970, pp. 370-372).

Vavilovia. Previously considered to be a species of Pisum, the single species of Vavilovia may be distinguished by its perennial habit, small stipules and slender calyx teeth, although in stylar morphology it is similar to Pisum (Davis, 1970, p. 372). As seeds from this genus were not available during the research period of

the project, it has not been included.

Throughout this work, the classifications of Vicia and Lathyrus as set out by Kupicha (1976,1983), those of Pisum by Davis (1970,p.370-373) and Lens by Ladizinsky (1986) are used. The specific nomenclatures are those used in the Viciaeae Database at Southampton University (Allkin et al.,1986), for all species except those of Lens (Ladizinsky,1986).

## 2.2. Seed morphology and anatomy in the Viciaeae, and their use in identification.

### 2.2.1. Introduction.

Bartlott (1981;1984) has examined the structure and microstructure of seeds. While he agrees that, to most taxonomists, data relating to seeds are of "great significance for the classification of angiosperm taxa", he considers that such data have had little influence in shaping classifications. This is certainly borne out in the Viciaeae, where seed characters have seldom been employed to delimit the tribe or assess its relationship with other tribes (Lersten & Gunn, 1982,p.1). However, as is mentioned above, the presence of hilar valves in seeds is considered important at a higher taxonomic level, in the subdivision of the subfamily Papilionoideae from the Caesalpinioideae and the Mimosoideae within the Leguminosae. Corner (1951,p.117) has stressed the

fundamental importance of seed structure in the classification of the subfamily Papilionoideae, "perhaps even to generic rank".

A comprehensive survey of the historical documentation dealing with the morphology and anatomy of legume seeds has been published by Lersten & Gunn (1982, pp.1-10), who have also reviewed the more recent literature. The morphology and anatomy of the seeds of legumes have been described by Corner (1951) and Rolston (1978), amongst others. Histological observations have been made by such workers as Winton & Winton (1935, pp.314-327), Moeller (1905), Vaughan (1970, p.129-139) and Gassner (1973). More recently, surveys have been made of the seed coat by scanning electron microscope, notably by Bridges & Bragg (1983), LaSota et al. (1979), Lersten (1979;1981), Lersten & Gunn (1982) and Saint-Martin (1978). Such studies have formed the basis for this new research.

The morphology and anatomy of the seeds of the Viciaeae are described below, and the features stressed are those which conventionally have been used to separate the species.

2.2.2. External morphology and anatomy of the seed and the testa surface in the Viciaeae.

The external gross morphology of the seeds of the Viciaeae has been described by Lersten & Gunn (1982, pp.2,3). The

seeds are spherical or compressed-oblong with modifications to this shape caused by pressure from adjacent seeds in the pod, or from the walls of the pod itself. Kupicha (1977) has recorded a light ridge on the seed surface that marks the position of the radicle.

The surface of the seedcoat, or testa, varies in texture. It is usually smooth, ranging from shiny to matt, or even velvet y; it may also be wrinkled, or roughened by ridges or tubercles visible to the naked eye (Red'kina & Khoroshailov, 1974).

The hilum is the zone of attachment of the funicle to the seed; it is characterised in the subfamily, as mentioned above (section 2.1.2.), by having a valvar structure of two lips and a central slit. The micropyle is a small pore at one end of the hilum, lying towards and above the radicle. At the other end of the hilum, lying at varying positions from the edge of the hilar rim, to opposite the hilum on its trajectory, is the lens, a conical projection of the testa (Corner, 1951). The hilar rim is reduced relative to that of the seeds of related tribes and is usually smooth; in some species, for instance in Vicia grandiflora, funicular remnants persist on the edges of the hilum to varying extents (Gunn, 1981; Lersten & Gunn, 1982, p.24).

The testa colour, a seed character often with identificatory significance (Bartlott, 1984), has



conventionally received much attention in the Viciaeae, particularly in Vicia (Nozzolillo et al., 1989; Red'kina & Khoroshailov, 1974; Voronchikhin, 1981) and Lens (Barulina, 1928; 1930). The testa may be monochrome white, yellow, orange, red, brown, green, purple or black; it may be spotted, mottled or streaked with combinations of these colours; it may be patterned with dark areas surrounding the hilum or on the lens. The pigments include a number of different compounds such as chlorophyll, anthocyanin and tannin (Nozzolillo et al., 1989). The hilum itself may be dark or light in colour. The genetics of testa pigmentation have been investigated in Vicia faba; three types of phenolic compounds, flavones, flavonols and anthocyanins are responsible for the range of seed colours in this species, and their production is controlled by seven alleles; probably more alleles control the proportions of the different pigments; black testas are thought to be due to the presence of the compound phlorbaphene (Nozzolillo et al., 1989), and the pigment responsible for black hila is melanin (Higgins et al., 1981). The genetics of mottling of the testa similarly has been studied, for example in lentil (Wilson & Hudson, 1978, 1979).

A small group of morphological characters of the testa surface has been used by Gunn (1970) to construct a key to separate the seeds of 100 species of Vicia. They are the length of the hilum as a percentage of the seed circumference, the distance between the lens and the edge

of the hilum, the position of the micropyle, and the shape of the hilum. The hilum has been classified according to its shape and length as circumlinear (with a length-breadth ratio greater than 10, occupying more than 70% of the circumference), linear (with length-breadth ratio of 5 to 7, occupying less than 50% of the circumference), oblong (with a length-breadth ratio of less than 5, also occupying less than 50% of the circumference), wedge-shaped (with convergent margins, but otherwise like the oblong hila), or oval (occupying less than 20% of the circumference). Other workers such as Kupicha (1976), Red'kina & Khoroshailov (1974), Voronchikhin (1981) and Zertova (1962) have also found a number of these criteria taxonomically useful in Vicia.

In Lathyrus by contrast, seed characters have played a more minor role in classification. Kupicha (1983) considers them to be of slight taxonomic value. Seed shape and size, and seedcoat texture all show a range of form similar to those seen in species of Vicia. Hilum length, and the distance between lens and hilum, are more uniform throughout this genus than in Vicia.

Characters of the seeds of Lens have been described in some detail by Barulina (1928;1930). She has used seed diameter and thickness, seed weight, testa colour and patterning, and testa surface texture, as well as testa thickness to separate not only some different lentil species, but also many subspecies and varieties of Lens

culinaris. However all five species recognised by Ladzinsky (1986) have not been separated using seed characters.

### 2.2.3. Gross morphology of the embryo in the Viciaeae.

At maturity, the seeds of members of the Viciaeae are exalbuminous, containing virtually no endospermic tissue, (Kupicha, 1981). The embryo lies directly beneath the testa.

The pair of cotyledons, swollen and modified as storage organs, are responsible for the basic shape and size of the seed. The tapered radicle is curved and lies closely against the surface of the cotyledons. The plumule is borne on the embryonic axis approximately at right angles to the radicle, and is embedded between the cotyledons. Gunn (1981) considers these characters to be of significance for the separation of the Papilionaceae from the other leguminous subfamilies.

### 2.2.4. Internal morphology and anatomy of the testa in the Viciaeae.

The testa consists of two cell layers beneath a uniformly thin cuticle (Corner, 1951): a single outer layer of Malpighian or palisade cells, narrow, columnar and elongated towards the external surface; and a single inner layer of hourglass cells, large, and bobbin-shaped

with intercellular air-spaces. Beneath these is a very attenuated layer of crushed and disorganised parenchyma, which is all that remains in the mature seed of the inner integument and nucellar tissues of the ovule. The term "remnant layer" (Lersten & Gunn, 1982, p.5) is used throughout this work in reference to these crushed tissues.

The palisade cells have outer periclinal walls that exhibit a characteristic patterning on the seed surface; this fine relief, termed "secondary sculpture" by Barthlott (1984), is termed "primary patterning" throughout this thesis. In the Viciaeae this has been described as papillose (Lersten & Gunn, 1982, p.5), a single papilla arising from each cell, but Saint-Martin (1978) has referred to a reticulate topography in Lathyrus aphaca, and Roti-Michelozzi & Serrato-Valenti (1986) have described variations from the papillose testa in four species of Vicia. Biochemical investigations have shown that the papillae in Pisum are formed from suberised caps on the cell walls (Spurny, 1964). A larger-scale surface topography is sometimes present on the testa, which presents as a rugosity visible to the naked eye; described as "mounds" by Lersten & Gunn (1982, p.5), and formed by local elongations of groups of palisade cells, this topography is termed "secondary patterning", or "mounds", throughout this thesis. Within the palisade cell layer is the "light line", a zone of diffraction that lies in each cell, and is visible under

light microscopy. Once thought to represent the boundary between two cell layers, it is now said to be an optical effect due to differences in the cell lumen (Corner, 1951). It is not a feature considered to be of taxonomic significance at tribal level (Lersten & Gunn, 1982, p.4).

The vascular tissue of the testa consists of an extension of the ovule vascular bundle, running from the edge of the hilum, within a parenchymatous layer, around the periphery of the seed beneath the hourglass cells. This normally simple unbranched vascular strand (Kupicha, 1977) appears to vary slightly, occasionally bifurcating, for example sometimes in Vicia faba (Kuhn, 1927), or branching, as in Pisum sativum (Hardham, 1976).

#### 2.2.5. Hilar anatomy in the Viciaeae.

The hilum is comprised of a pair of hilar lips separated by a slit (see paragraphs 2.1.2., 2.2.4., above). Here the palisade is covered by a second layer, the counter-palisade, which derives from the funicle (Corner, 1951). The hourglass cells are absent. Beneath the hilar slit lies a rod of tracheid-like elements known as tracheoids, running from the edge of the micropyle to the ovule vascular bundle, with which it appears to merge. The tracheid bar, elliptical to round in cross-section, is embedded in stellate aerenchyma (Lersten, 1982; Lersten & Gunn, 1982, p.6).

The remarkably uniform nature of the tracheid bar within the Papilionoideae has been noted. Amongst the Viciaeae, Lathyrus shows the greatest range in shape of this structure. The tracheoids are arranged approximately perpendicular to the hilum, and are pitted. The pits have been recorded as regularly arranged and surprisingly large and regular in size and shape, with virtually a complete loss of primary cell wall, and typically with plain borders, or, at most, only a few small warts (Lersten, 1982; Lersten & Gunn, 1982, p.6).

#### 2.2.6. Cotyledon morphology and anatomy in the Viciaeae.

The surface of the cotyledons at the junction with the radicle has been classified in the Papilionoideae as plain or embellished, depending upon the extent of overlap of cotyledon tissue over the radicle. In the Viciaeae, the radicle lies closely against an unembellished cotyledon surface (Gunn, 1981).

The fine structure of the cotyledons in legumes has received comparatively little attention from anatomists, except for the cotyledons of some pulses that have attracted interest as food resources (Patel et al., 1979). Following a study of cotyledon morphology, a system of classification has been formulated by Smith (1981). Four types have been separated on leaf-like characters, and further subdivided into two groups on the distribution of the vascular strands. The Viciaeae have been classed

together as having hypogeal germination with fleshy storage cotyledons, showing no differentiation into palisade and mesophyll tissues, and with a vascular system confined to one plane.

The large storage cells of the cotyledons have enlarged nuclei. Although members of the Viciaeae characteristically are diploid (Kupicha,1981), these cotyledon cells have been shown to be polyploid. Such endopolyploidy is thought to be present throughout the tribe Viciaeae and is correlated with the presence of starch grain. While cotyledon anatomy and histology seem to have a place in the classification above tribal level, and even within certain tribes, within the tribe Viciaeae micromorphological and anatomical characters of the cotyledon appear to be uniform (Smith,1981).

### 2.3. Geographical distribution and ecology of the Viciaeae. Some characteristic habits of the tribe.

Different views have been held regarding the original distribution of the tribe Viciaeae. A number of authors have considered that the tribe has a distribution centre in the Mediterranean region, as cited by Simola (1986). However, current opinion holds that the Viciaeae belong to a group of tribes spreading originally from the Sino-Indian region further east, into the temperate

regions mainly in the northern hemisphere (Polhill & van der Maesen, 1985).

The legumes are adapted to a wide variety of environments. It has been concluded that this property is due to the matching of a number of adaptive characteristics of certain taxa with the environmental requirements, rather than the result of any single factor (Adams & Pipoly, 1980). The Viciae demonstrate this characteristic. In any one area, many ecological niches may be exploited by members of this tribe. For example, tendrillous species retain the ability of their forest-living forbears to compete for sunlight within a crowded habitat by climbing, using associated plant species as support; however, both Lathyrus and Vicia include a group of species, the oroboid types, which are mainly etendrillous, of erect form, with densely racemose inflorescences, and which inhabit more open woodlands, in predominantly north-eastern temperate regions (Fedevov, 1939; Kupicha, 1981).

Major factors influencing distribution are daylength and temperature. Overall plant size, branching pattern, initiation of flowering, pod set and seed maturation are largely governed by these factors. Flowers are mainly but not exclusively self-fertile. Growth is typically indeterminate, and the earliest flowering nodes set the most pods (Summerfield & Wien, 1980). The indeterminate and associated free-branching habit tends to be



advantageous under drought conditions, when water is diverted from old leaves to the apical meristems; although as a consequence flowers and pods may be shed, more fruits may be initiated from newly generated nodes (Elston & Bunting, 1980). An extended period of fruiting is the result.

#### 2.4. Cultivated members of the Viciaeae.

##### 2.4.1. Introduction.

Legumes are exploited by man for a number of different end products, including human food, animal feed and fodder, and green manure for soil enrichment.

Different parts of the plant may be utilised, such as vegetative organs, both in the green and dry states, and the seeds, also both when green and immature, and when ripe. This project centres upon the dry mature seeds, or pulses, predominantly used in human diet; but, since a flexibility of utilisation is a feature of legume production, a broader view of the exploitation of members of the Viciaeae will be taken in this section.

##### 2.4.2. Development of the cultigens: changes in fruits and seeds of legumes associated with selection under cultivation.

Evolutionary trends in the legume family show that the

Vicieae are a relatively advanced tribe, being for example herbaceous with a tendency for autogamy (Polhill,1981). Legume cultigens tend to exhibit a number of traits that are also associated with the natural evolution in the family, such as gigantism, and an erect habit with compact growth (Smartt,1978). Selection by man for optimal yield has favoured plants with large seeds, a property that has been found to be associated with a reduction in seed number, and this trait may also be accompanied by a generalised gigantism of vegetative parts. Seed gigantism in the pulses appears to be acquired over a prolonged time period (Ladizinsky,1979a), a phenomenon that is certainly demonstrated by the archaeological evidence: the remains of legumes from the earliest agricultural sites show that the seeds are within the size range of their wild ancestors, and seed size seems to have been increasing in cultivated species into historical times (Hopf,1986). Thus this trait, which might perhaps be one of the most obvious criteria to use as evidence of cultivation, is not observed in legume seeds which may have derived from cultivated populations in early agrarian contexts.

Other characters of more direct agronomic significance have been selected by practices of cultivation. These favour the increased control by man over dissemination and germination: in taxa developed as grain legume crops, both dehiscence of the pods and seed dormancy are either reduced or lost.

It has been postulated that in cultigens the reduction of seed dispersal mechanisms and an increased germination rate are changes from the wild states that arise early during the initial stages of cultivation. This is borne out by recent experimental work on cereals (Hillman & Davies, 1990, in press). Thus evidence of these changes in archaeological specimens might pinpoint the earliest stages in the process of agricultural development.

Changes to the fruits and seeds of legumes following cultivation are described below.

#### 2.4.2.1. Reduced dehiscence.

Dissemination in the Viciaeae is effected by dehiscence of the pod. This tends to be explosive, and the ripe seeds of most wild species may be dispersed for up to several metres. This is an obvious disadvantage to those gathering the seed, and has led to the selection under cultivation of populations with reduced dehiscence.

Dehiscence is a function of the anatomical structure of the pod wall. Sixteen types of pod wall anatomy have been described in various legumes. The type recorded in the wild species of Vicia, Lens, Lathyrus and Pisum that have been investigated has thick-walled, elongated epidermal cells beneath which is a single layer of fibres, diagonally orientated in the opposite direction to the epidermal cells (Fahn & Zohary, 1955). The result

of a reduction in this lignified fibrous layer is a loss of dehiscence, which is under genetic control. For example in lentil and pea, indehiscence is controlled by a single recessive Mendelian factor (Ladizinsky, 1979a, 1979b). In Vicia ervilia it is controlled by two major genes and probably also some minor ones (Ladizinsky & van Oss, 1984).

Unfortunately, this change in pod anatomy associated with cultivation has rarely been observed in archaeological plant remains. It is more common that, in legumes, the remains of seeds rather than fruits are recovered; this has been referred to both by Helbaek (1969) in consideration of the plant remains from the early neolithic site of Ali Kosh, and also by Hopf (1983) in relation to the site of Jericho. In exceptional circumstances of preservation, legume pods occasionally have been found; for example, in the cave site of Coxcatlan in Meso-America, the pods of Phaseolus vulgaris, preserved by desiccation, have provided the evidence for their cultivation: they have reduced fibrous parchment layers, indicating a decrease in dehiscence (Kaplan, 1965). Similar evidence in the remains of species of the Viciaeae has not been found (Zohary & Hopf, 1973).

#### 2.4.2.2. Loss of seed dormancy.

The seeds of wild legumes typically have a high level of

dormancy, and single populations may germinate over a wide period of several weeks or even months. Under cultivation, populations have been selected that germinate more readily, having low levels of dormancy.

The physiological change in the seed, that results in an increase in the rate of germination, is associated with changes in the properties of the testa. Legumes are characteristically hard-seeded. The testas of most wild species are relatively impermeable to water and the seeds are dormant, while those of the cultigens are more permeable and less dormant. The genetics of seed dormancy has been studied in a number of legume taxa. In lentil, dormancy is controlled by a single gene (Ladizinsky, 1985); in Vicia sativa two genes are involved (Donnelly, 1970). Clearly, while different genetic mechanisms operate in different taxa, it appears that few loci are implicated, and it has been postulated that loss of dormancy in a population may arise comparatively abruptly under conditions of positive selection (Ladizinsky, 1987, b).

Investigations have been carried out to determine which tissues might be responsible for conferring the impermeable properties upon the testas of dormant legumes seeds. Although early work suggested that the cuticle was water-repellent, it has now been demonstrated that this layer is permeable (Baciu-Michlaus, 1970). The main features of the testa that confer the hard-seeded

properties have been summarised by Rolston (1978) and Werker (1980/81), who have stated that the palisade cells play some part, but that other tissues may be involved. The thickened external periclinal walls of palisade cells have suberin encrustations in Pisum (Spurny, 1964). Yet there is no evidence of suberin in the testas of many other hard-seeded legumes. Ballard (1973) considers that while suberisation may be an important barrier against the penetration by water, other factors situated more deeply within the palisade layer are also of significance. Some work on legume species from other related tribes may also be of relevance to members of the Viciaeae. Experiments on Coronilla varia have shown that an impermeable layer exists towards or beneath the base of the palisade cells (McKee et al., 1977). Using histochemical techniques, an impermeable callose layer was found beneath the testa in the remnant layer of Trifolium repens (Bhalla & Slattery, 1984). This biochemical evidence of hard-seededness has not as yet been correlated with any anatomical feature that could provide visible evidence of seed dormancy.

It is generally held that the seeds of cultigens have a thinner testa than the seeds of their wild relatives (Hopf, 1986), as has been found indeed in Pisum (Werker et al., 1979). Also cultigens are said to have testas that are superficially smooth at low magnifications (Hopf, 1986). This is certainly the case in Pisum sativum, where seeds of the wild subspecies, P. sativum elatius,

have rough granular testas, readily distinguished from the smoother-coated seeds of the subspecies P. sativum sativum. These characters are usually observable in ancient seed remains, and they have been employed to identify charred archaeological specimens (Helbaek, 1970; Zohary & Hopf, 1973). However, firm anatomical evidence of soft- or hard-seededness is usually lacking.

Some research into the chemical nature of seed dormancy has been undertaken; this has shown some correlation between dormancy and the pigmentation of the testa. Werker et al. (1979) have investigated the biochemistry of the testa in Pisum, and found that during maturation of the seeds of wild taxa, there is oxidation of some phenolic compounds to form quinones within either the palisade or the hourglass cells; this produces a continuous impermeable layer. This is associated with a darkening of the testa colour. In cultigens, phenols are reduced in concentration or absent; this property is correlated with a light-coloured testa in the mature seed (Marbach & Mayer, 1975). No attempts have been made as yet to determine the biochemistry of archaeological legume seed remains.

The altered biochemistry associated with selection for reduced seed dormancy, and the light-coloured testa may also be reflected in a decrease in some of the unpalatable or anti-nutritious compounds that are a feature of many legume seeds (Appendix 1).

#### 2.4.2.3. Reduction in toxin levels, and light-coloured testas.

A characteristic of legumes is their potential toxicity, both to man and to other fauna. Secondary metabolites with toxic properties may accumulate in the various organs at different concentrations. The mature seeds of many taxa contain compounds which may be poisonous or anti-metabolic in action, and their presence may be indicated by the testa colour. With some exceptions, the cultigens in the Viciaeae are not reputed to be dangerous or unpalatable to eat, but there is documentary evidence that the seeds of some earlier cultivated forms were inedible without some prior detoxification treatment (see Appendix 1).

Tannins, for example, which are water-soluble phenolic compounds, can confer a bitter astringency upon seeds; this decreases their palatability (Haslam, 1979). Tannins are known to possess undesirable anti-nutritional properties both for man (Aykroyd & Doughty, 1982, p.42), and for other animals (Harborne, 1877, p.135), and tend to be concentrated within the testa (Werker et al., 1979). They are the compounds associated with hard-seededness. High concentrations of tannins have been correlated with a dark testa colour in Pisum (see section 2.4.2.2.). Work on the genetics of pigmentation in some legumes has been carried out by Vaillancourt et al. (1986), who have recorded that in Lens culinaris and Vicia faba, the



absence of tannin in the testa is controlled by a single recessive gene. Where no tannin is found, the flower, stems and testa are lacking in the anthocyanin pigments; the presence of tannin is correlated with pigmentation of these organs. Ladizinsky (1979a) has reported however that in lentil, while the colours of the testa and epicotyl are each controlled by a single Mendelian factor, flower colour is controlled by at least three genes. So, in some taxa at least, while testa colour seems to be correlated with the concentration of tannins in the testa, the colour of other organs may not be directly related. This situation is similar to that seen in the tribe Phaseolae: in Phaseolus vulgaris, for example, many genes seem to control the testa pigmentation, but the leucoanthocyanins that pigment the flower are governed by one gene (Feenstra, 1960). Concentration levels of tannins appear to be reduced in populations with low seed-dormancy which have been selected under cultivation; and light-coloured testas in the species of the Viciae that have been investigated do seem to be correlated with low tannin levels.

Lathyrus sativus has been the subject of much research relating to the content in the seeds of the neurotoxin \*ODAP. Attempts have been made to breed toxin-free strains of the cultivar. Associated with these attempts have been searches for a correlation between flower colour or seed colour and the toxicity of the seeds, which could facilitate selection of such strains. Some claims have

\* beta N-oxalyl amino Lalpha,beta,diamino proprionic acid

been made that varieties with light cream-coloured seeds have low concentrations of toxin (Dahiya,1976). However other workers have not found any clear correlation between flower colour, testa colour and toxin levels (Quader et al.,1986). The research into readily identifiable breeding strains of toxin-free Lathyrus sativus continues.

It is not known whether farmers in antiquity selected for low seed toxicity as such, or whether certain toxic compounds by association with hardseededness suffered negative discrimination. Although a feature of the development of the grain legume cultigens is selection for low levels of certain biochemical compounds, including some toxins (Smartt & Hymowitz,1985), there is evidence that this cannot always be assumed (Bressani & Elias,1980,a). Further, the point has also been made that many seed crops are consumed when in a green, immature state, at which stage the concentrations of certain phenolic compounds in the seeds may be lower than when the seeds are ripe (Bate-Smith, in discussion following Feenstra,1960).

The properties that are associated with testas of different colours give different selective advantages. Dark seeds may be advantageous to the plant; the higher concentrations of tannins that have been associated with dark testas are known to impart protection against animal predation, and also to affect the storage properties of

the seed (Marbach & Mayer, 1974). Both of these factors can be of advantage to the farmer.

There are records of a disadvantage to legumes that is associated with a light-coloured testa. It has been found that in the two pulses, Phaseolus vulgaris and Cicer arietinum, in seeds with white testas there is a higher rate of water uptake; this has been associated with a looser adherence of the white testa to the cotyledons than is found in brown- and black-coated seeds. This condition facilitates the passage of water through the seed; however, it can cause damage to the embryo during imbibition, which results in a decreased germination rate in the crop (Matthews et al., 1988). This condition is found in pulse crops of other tribes and may well also occur in members of the Viciaeae. It might explain in part the reason for the lack of selection for a light testa in many of the landraces. The degree of testa adherence has not been examined in this project; the process of charring tends to loosen or detach the testa from the cotyledon surfaces, and thus in charred ancient seeds this character is unlikely to be observable.

#### 2.4.2.4. Range of variation in seed characters of cultigens in the Viciaeae.

It has been observed that, in the Viciaeae, the cultigens generally display a wider range of variation in such seed characteristics as size, shape and colour than the wild

taxa (Ladizinsky, 1989). The pulses that are recovered in archaeological assemblages are usually low in numbers; their morphology may be changed due to charring, and the seed colour cannot be observed; it is thus impossible to draw firm conclusions about such a range of variation in antiquity.

#### 2.4.2.5. Summary of the visible evidence of cultivation in the morphology and anatomy of ancient seeds.

An increase in seed size is not observed in the early stages of cultivation (see section 1.2.3.2.). A reduction in hardseededness may be reflected by the single morphological character of a relatively thin testa, which is sometimes accompanied by a smoother texture of the testa surface. Evidence of the change in biochemistry that underlies the reduction in seed dormancy is not directly visible; in fresh seeds a light testa colour may be evidence of a particular biochemistry, however, this criterion obviously cannot be observed in charred seeds. Zohary (1986) is amongst those who acknowledge the lack of criteria for the recognition of the cultivated state in seeds of legumes, and particularly in the remains of charred seeds from archaeological contexts.

2.4.3. Species of the Viciaeae that have been cultivated as pulses.

Today the number of species of the Viciaeae grown as pulses is restricted to perhaps four or five in common use. In the past, other species were cultivated for human food; some of these are more familiar in present times as animal feed and fodder crops. Taxa known to have been cultivated are shown in Table 1; this list is unlikely to be comprehensive.

The following paragraphs contain brief descriptions of the major pulse crops in the Viciaeae, with mentions of some of the less common contributors to human diet in this plant group. They are all annuals and usually grown in temperate, cool season environments. Some of the local names for the cultigens are given. They are examined following the conventional taxonomic sequence of genera, and, within each genus, in the order of economic importance of species and subspecies, as shown in Table 2.

Table 1. Species in the Viciaeae that have been Cultivated.

(Sources: Aykroyd & Doughty, 1982; Davis, 1970; Duke, 1981; Ladizinsky, 1987; Summerfield & Roberts, 1985; Thulin, 1983; Townsend & Guest, 1974; Zhukovsky, 1924)

Taxon	Use/Place (where known)
<u>Vicia articulata</u>	pulse. Turkey
<u>V. benghalensis</u>	fodder. India
<u>V. cracca</u>	fodder. China, Japan
<u>V. ervilia</u>	pulse. N. East
<u>V. faba</u>	pulse. Widely
<u>V. graminea</u>	forage. S. America
<u>V. hirsuta</u>	fodder. India
<u>V. johannis</u>	pulse. Turkey
<u>V. michauxii</u>	forage. Portugal
<u>V. narbonensis</u>	pulse, forage
<u>V. pannonica</u>	pulse. Turkey
<u>V. sativa ssp. nigra</u>	green manure
<u>V. sativa ssp. sativa</u>	pulse, fodder. Europe
<u>V. tenuifolia</u>	forage. Iraq
<u>V. unijuga</u>	forage. Siberia, Japan
<u>V. villosa</u>	green manure. USA, Ethiopia
<u>Lens culinaris</u>	pulse. Widely
<u>L. nigricans</u>	pulse? France
<u>Lathyrus annuus</u>	pulse. Egypt
<u>L. aphaca</u>	fodder. India
<u>L. cicera</u>	pulse. N. Africa
<u>L. clymenum</u>	pulse. Greece
<u>L. gorgoni</u>	fodder
<u>L. hirsutus</u>	forage. USA
<u>L. latifolius</u>	horticulture. Turkey, Europe.
<u>L. ochrus</u>	pulse. Greece
<u>L. odoratus</u>	horticulture. Widely
<u>L. pratensis</u>	forage
<u>L. rotundifolius</u>	horticulture. Widely
<u>L. sativus</u>	pulse. India, N. Africa
<u>L. sylvestris</u>	forage
<u>L. tingitanus</u>	N. Africa
<u>L. tuberosus</u>	tubers. W. Asia
<u>Pisum sativum</u>	
<u>ssp. elatius</u>	fodder
<u>P. sativum</u>	
<u>ssp. sativum var. arvense</u>	pulse. Widely
<u>P. sativum</u>	
<u>ssp. sativum var. sativum</u>	pulse. Widely

Table 2. List of Cultivated Taxa in the Viciaeae Described in the Text.

Vicia faba  
V. ervilia  
V. sativa sativa  
V. benghalensis  
V. monantha  
V. narbonensis  
V. pannonica  
V. villosa  
Lens culinaris  
L. nigricans  
Lathyrus sativus  
L. cicera  
L. blepharicarpus  
L. gorgoni  
L. clymenum  
L. ochrus  
Pisum sativum sativum

2.4.3.1. Vicia.

Vicia faba - the broad bean, ful (Egypt and Lebanon), pi-ouro (Coptic), bakla (India and Turkey).

This species is represented by four taxa that have been classified variously. However the current taxonomic opinion groups them as follows (Cubero, 1974): Vicia faba subspecies faba, varieties major, equina and minor. They are distinguished mainly by seed size. These taxa have a single mainstem, and are largely allogamous. They produce seed in a variety of colours. V. faba subspecies paucijuga, is a small dark-seeded and branching taxon, which is autogamous (Cubero & Suso, 1981).

The species in all cases is robust and erect. It may be determinate or indeterminate, and as stated above, both self- and cross-pollinating types are found. Several

features, such as the large size and the woolly lining to the pods, are unique to this species in the Viciaeae (Bond et al., 1985; Lawes, 1980).

Vicia faba is only known as a cultigen. Genetically with  $2n=12$ , the DNA content is unusually high. Attempts at hybridisation with the closest wild relatives in section Faba, all of which have the chromosome number  $2n=14$ , of the Vicia narbonensis group, and V. bithynica, have proved unsuccessful (Cubero, 1982; Cubero & Suso, 1981; Pickersgill et al., 1985; Smartt, 1981, 1984). Investigations by electrophoresis of the testa proteins in seeds of members of section Faba have shown that all varieties of Vicia faba differ markedly from the wild species, and that they themselves have such similar seed protein profiles that they probably are monophyletic in origin (Ladizinsky, 1975). Recent analyses of the flavonoids have shown distinct differences between Vicia faba and related members of section Faba (Perrino et al., 1989). The wild progenitor is not known; the current view is that either it is extinct, or that it is an undiscovered self-pollinating species which occupies a restricted habitat probably in the Middle East (Ladizinsky, 1975; Zohary, 1977). The smallest- and darkest-seeded forms of the V. faba minor and V. faba paucijuga subspecies found today in Afghanistan and the Indian subcontinent are most similar to the other Vicia species, and they are believed to be closest to the ancestral species (Ladizinsky, 1975; Lawes et al., 1983).



Today Vicia faba is grown widely throughout all continents, the highest production of seed currently being in China. The immature seeds are commonly consumed in Europe. Dry seeds may be hulled and ground into flour. The smaller-seeded varieties are used as animal feed, and were fed particularly to horses in the early part of this century. The green tops of the plants are occasionally used as human food. Straw by-products are valued as animal fodder, and for constructional applications, such as brick-making in Egypt. The empty pods are used as compost (Bond et al., 1985; Hebblethwaite et al., 1983; Nassib et al., 1988). The earliest archaeological remains of this species have been identified from the seventh millennium site of Yiftah'el in Israel (Kislev, 1985). This recently-acquired Near Eastern evidence of the faba bean has changed the previously-held view that the distribution of V.faba was predominantly in the Mediterranean region and southern Europe during the initial stages of its cultivation (Schultze-Motel, 1972). The earliest examples have all been identified as the minor variety, and are thought to have had the dark-coloured testa that has been found to be dominant over the lighter forms and more easterly in distribution (Lawes et al., 1983).

Vicia ervilia - Bitter Vetch, French Lentil (Russia), karaburcak (Turkey).

This species is etendrillous, with characteristic

torulose pods (having constrictions between the seeds) and angular seeds. It exists today in both wild and cultivated forms. These readily hybridise. The chromosome number is  $2n=14$  (Ladizinsky & van Oss, 1984).

The crop today is most commonly grown for animal feed. This restricted use is usually considered to be due to the presence of a bitter alkaloid in the seeds; these, when consumed in quantity without prior treatment, can be toxic to man and to some farm animals (see Appendix 1).

Vicia ervilia is now not widely grown; it has been cultivated mainly in Afghanistan, Russia, Spain, Greece, Cyprus, Turkey and other Near Eastern countries such as Jordan and Syria; it has sometimes exceeded the lentil in importance as a pulse crop, having the advantage of tolerance of a higher latitude and altitude than lentil. The greatest concentration of different forms of the species occurs in the eastern Mediterranean region. The wild populations may be found in Quercus scrub and on screes, at field edges and roadsides (Davis, 1970), and at high elevations up to 2000 metres (Ladizinsky & van Oss, 1984).

There is a narrow range of variation in the size of the seeds of this species, a property that has been explained by Barulina (1930) because of it having been mainly cultivated as a fodder crop, rather than for its grains. However, in the past it has been a human food. It has

been found in pure storage deposits at a number of archaeological sites, such as Çayonu in the Near East (van Zeist, 1972). There is early documentation of its use and detoxification, for instance in the Hebrew text, the Mishnah (Shabbath 1.1-2.3). It has been suggested that the bitter quality of the seeds, while thought to be a deterrent to the taste buds of Western Europeans today, may have been acceptable or even desirable to other societies (Kislev, pers.com). Certainly this phenomenon has been recorded in certain communities in Latin America with some forms of Phaseolus vulgaris (Bressani & Elias, 1980, a).

V. sativa - common vetch, tare, akta and akvi (India).

The Vicia sativa aggregate is a complex group of annuals with a variable genetic complement of  $2n=10, 12, \text{ or } 14$ , 12 being the most usual number (Zohary & Plitmann, 1979).

The common vetch, V. sativa<sup>sup.</sup> nigra, is a wild taxon, that is today also cultivated as a green manure and fodder crop. The self-pollinating cultigen, V. sativa<sup>sup.</sup> sativa, is grown as a pulse for animal feed, particularly for poultry and pigeons in Turkey and southern Europe (Jones, 1983; Nesbitt, pers.com.), and previously for horses in India (McCombie Young, 1927). It tends to be associated with primary grain crops, such as lentil, where forms may be selected with lentil-shaped seeds, known as V. sativa<sup>sup.</sup> sativa<sup>var.</sup> lentisperma, an often-cited

example of crop mimicry, due to a recessive mutation. In commercially-available supplies of lentils obtained from northern Europe, particularly those from Poland and West Germany, it has been estimated that commonly up to 1% of the grains are those of the mimicking vetches (Barrett,1983; Barulina,1930; Kuhn, in press; Hanelt,1986; Rowlands,1959). This phenomenon has also been observed in northwest India and Afghanistan (Vavilov,1951,p.88).

It has been recorded in North America that the pods and seeds of vetch (presumably Vicia sativa), gathered from the wild, are "irresistible", when cooked and eaten (Morton,1963).

The seeds of this vetch contain a neurotoxin (beta cyano-L-alanine), which was originally, but is no longer, thought to be implicated in the aetiology of lathyrism (Roy et al.,1986).

Vicia benghalensis (purple vetch), V.monantha (bard vetch), V.narbonensis (Narbonne vetch), V.annonica (Hungarian vetch), V.villosa (hairy vetch).

These are examples of vetches which are today still cultivated in Iraq, Turkey and other parts of Europe and central Asia. They are now grown mainly in the USA, as animal feed, fodder crops, hay and silage, and green manure, but were once more widely used

(Duke, 1981, pp. 271-286; Townsend & Guest, 1974). V. monantha is mentioned by Barulina (1930) as a type of "lentil" crop once important in Spain and Portugal. Vicia narbonensis is still commonly found in fallow fields in Palestine (Hopf, 1983).

#### 2.4.3.2. Lens

Lens culinaris - lentil, mercimek (Turkey), 'adas (Lebanon), masur (India), phacos (Greece).

The lentil is cultivated primarily as human food, and is divided on the basis of seed size into one of two groups: microsperma with a maximum seed diameter of less than six millimetres, and macrosperma with a seed diameter of more than six millimetres (Cubero, 1981). All the lentil species are small branching annuals; the cultigen, although usually larger in form than its wild relatives, is still low and tends to sprawl. The wild progenitor is L. orientalis (Ladizinsky, 1979; Zohary, 1972); it grows on mountainsides, in screes, among oak-forests and in fallow fields in Europe, North Africa and Asia (Davis, 1970, p. 327; Townsend & Guest, 1974, p. 548). Populations from Israel have a genetic constitution that is particularly close to that of the cultigen (Zohary, 1983). Small-seeded forms with dark-coloured testas are found in the Indian subcontinent, Afghanistan and Ethiopia and the southern Soviet states, where relatively undeveloped landraces are still cultivated.

The important role of the lentil as a staple food is well-documented (for example, Abu-Shakra & Tannous, 1981; Aykroyd & Doughty, 1982, p. 92; Cubero, 1981; Duke, 1981, pp. 110-113). Classical sources refer to the famous Egyptian lentils of Alexandria and Pelusium (in North Sinai) (Darby et al., 1977, p. 688); today the areas most associated with their consumption are the Middle East, Russia, Pakistan, India and Ethiopia, though at present the greatest exporters are the USA, Canada and Turkey (Blain, 1988). The nutritional value of lentils is well-recognised (Abu-Shakra & Tannous, 1981; Kuzali et al., 1966), and the straw by-products of the crop are highly valued as animal feed (Nygaard & Hawtin, 1981). The archaeological record provides ample evidence of the antiquity of its cultivation (see section 1.1.2. above).

Instances have been recorded in northwest India and Afghanistan of mimicry by lentils of Vicia sativa, similar to the better-known converse situation described above (Vavilov, 1951, p. 88).

L. nigricans- black-podded lentil.

This wild species is typically small-seeded, but occasional populations with larger seeds are found in southeast Europe. It has been suggested on the basis of cytogenetic evidence that these are segetal forms developed from populations which were cultivated in antiquity, and have since reacquired dehiscence. More typical wild

populations are found in Western Asia (Ladizinsky et al., 1983; Ladizinsky & van Oss, 1984).

References can be found to the French and one-flowered lentils in some publications. These respectively are Vicia ervilia and V.monantha (Barulina, 1930) (see paragraphs under 2.4.5.1 above).

#### 2.4.3.3. Lathyrus

Lathyrus sativus- chickling pea, blue chickling, grass pea, mutters (previously in England), jilbein (Turkey), julban (Syria), hurtuman (Iraq), khesari, chapta and matra (India), teora (Bengal), mattar (Punjab), jarosse, Gesse cultivee and dent-de-brebis (France), Riga pea (Russia).

This cultigen, with the chromosome<sup>n°</sup>  $2n=14$ , is closely related to a number of wild species of section Lathyrus. The closest wild relative is usually considered to be L.cicera, though this species is as yet unverified as the progenitor (Jackson & Yunus, 1984). It is grown today largely in the Indian subcontinent and mainly for use as an animal feed (Duke 1981, pp.107-110). In times of drought it is a useful food for man, regardless of its toxic properties (see Appendix 1). Until historic times the seeds have been an important human food around the Mediterranean region, in southern Europe and North Africa, as well as in mid-Europe and the Middle East

(Stockman,1917). The green pods have also been used for human food (Townsend & Guest,1974,p.556). The straw and husk by-products are used for animal feed (Bharati,1986) and this species is commonly grown as a fodder crop in Europe and the Middle East, where it can also be found as a weed in cereal fields (Davis,1970,p.360). Today it is most commonly grown in the Indian subcontinent, and is the highest in proportion of the grain legumes consumed in Bangladesh (Kaul et al.,1986).

Lathyrus sativus is said to be a cultigen of secondary importance, and it has not been highly-developed as a crop species; however, its seed dispersal systems are reduced, so that mature pods tend to remain closed, unlike those of the wild related species of Lathyrus (Ladizinsky,1979a). Smartt (1981) considers that the cultigen is still at an evolutionary stage with no apparent discontinuity in morphology from the wild varieties of the species. Archaeological remains have often been difficult to identify with certainty to the level of species, but there are claims of its early use as human food, for example, from the Mesolithic period around the Mediterranean, from France to the Near East (Marinval,1986).

L.cicera - dwarf chickling, jarosse and Gesse chiche (France), cicerchia (Italy).

Today this species is only cultivated as a fodder crop,



and is usually found as a wild species. However, there is documentation that its seeds have formed part of the human diet, particularly in parts of North Africa, such as Algeria, and in southern Europe, for example, in France (Stockman,1917). Wild populations today grow in Pinus and Quercus forests, and on rocky slopes as well as at fieldsides in southern Europe and the Middle East (Davis,1970). Like its close relative, L.sativus, it contains the lathyrogen ODAP, responsible for the neuropathological condition lathyrism (Bell,1971; Roy et al.,1986). Archaeological remains of this species have been associated with those of L.sativus (Marinval,1986).

Lathyrus blepharicarpus - ul-julaybineh (Syria) and L.gorgoni -hurtuman haiya (Iraq).

The seeds from other close relatives of Lathyrus sativus, in the same taxonomic section Lathyrus, have also probably formed part of the human diet in the past. For instance, it has been recorded that the seeds of the wild species L.blepharicarpus are gathered in Syria as a casual food, and "are much liked by the people of the country" (Post,1896,p.293). L.gorgoni is documented as a spring animal fodder in Iraq (Townsend & Guest,1974,p.559).

Lathyrus clymenum -Spanish vetch.

This scrambling annual species is glabrous with a broadly

winged stem and large pillow-shaped seeds. It is rarely grown today; for example it can still be found on the Greek island of Santorini (Sarpaki & Jones, in press). In the past it was used as a pulse crop in Algeria, the Levant, and Spain (Davis, 1970, p. 365; Townsend & Guest, 1974, p. 554). Wild populations, mainly found around the Mediterranean regions and in Turkey, tend to grow near water and by fields (Davis, 1970, p. 365). The seeds contain the toxin ODAP, that causes lathyrism (Bell, 1971; Roy et al., 1986).

Lathyrus ochrus - yellow pea, louvana (Cyprus).

This similarly scrambling and glabrous species normally needs the support of other plants. Although not now considered to be of any great economic importance, this species is still cultivated for its dry seeds in Cyprus (Photiades & Alexandrou, 1979) and on the Greek island of Patmos on a small scale (Townsend & Guest, 1974, p. 554). In the wild it is found chiefly in grassy places, such as at the edges of woods in southeast Europe and Turkey (Davis, 1970, p. 366).

#### 2.4.3.4. Pisum

Pisum sativum sativum - pea, kalon, kulai or mattur (India), bezelye (Turkey), bizilla (Lebanon), buzilla (Iraq), bishla (Syria), polka (Kurd).

The wild progenitor is P.sativum elatius pumilio, previously known as P.humile, populations from Turkey and Syria being most closely related to the cultigen. The chromosome number is  $2n=14$  (Ben-Ze'ev & Zohary, 1973; Zohary, 1983).

Early cultigens grown as a pulse for human food were dark-seeded varieties similar to the types of field pea produced today as animal feed. Their seeds needed treatment prior to the final cooking process to remove the bitter-tasting tannins from the testa (Drummond & Wilbraham, 1939).

Today this crop is grown for human consumption as a pulse, for its immature green seeds, and for the whole immature fruit. It is a cool season species, performing best between 10 and 30C and between the latitudes of the Tropic of Cancer and 50N. It has a comparatively short growing season of 80 to 100 days. Today nearly 80% of the total grain is produced in Russia and China (Davies et al., 1985; Blain, 1988); but some varieties can tolerate hotter conditions such as are found in India (Pate, 1977). The wild subspecies is most commonly found in Turkey

(Davis, 1970, pp. 371, 372).

Archaeological evidence from many of the earliest Neolithic sites in the Near East, such as Çayonu (van Zeist, 1972), and more prolifically from later sites, such as Çatal Hüyük and Hacilar (Helbaek, 1970), testifies to the importance of pea from these times. However there is variable evidence for its cultivation. For example, in Turkey at approximately the same period, the sixth millennium BC, the smooth-coated seeds of the cultigen predominated at Çayonu and Çatal Hüyük, but at Hacilar the charred seed remains have mainly included the rough-coated wild pea.

#### 2.4.4. Gathering of wild legumes.

The brief accounts of the main species in the Viciaeae that are part of the human diet today, or in the recent past, have included some species that are regarded as wild; records have revealed that the opportunistic gathering and consuming of the seeds of large-seeded wild legumes, particularly in the green, immature state, is a common practice (see 2.4.3.1., 2.4.3.3. above). Field "snacking" is the bonus for field workers in all societies today, as exemplified by the relished consumption in Turkey of seeds of the wild purple pea, Pisum sativum elatius, described by Helbaek (1970, p. 227).

There are advantages in the seeds in an immature state

over those that are fully ripe, which include palatability, and often relatively low toxicity (see Appendix 1), digestibility when uncooked, and the availability on plants of seeds within the non-dehisced pod.

The evidence for similar practices in antiquity is lacking, but it is suggested that such "casually harvested" immature wild legumes, although probably invisible in the archaeological record, made an important contribution to the diet of early societies, both before and after the development of agriculture.

The following section comprises descriptions of some of the processes traditionally and currently employed in the cultivation of legumes; the documentary evidence for some of the past practices is examined; finally, the attitude of farmers towards their legume crops is considered (Butler, in press b).

While the relevance of this agronomic section for archaeobotanists concerns the construction of models of crop processing for the interpretation of charred ancient pulses, its importance in this thesis mainly relates to an understanding of the pressures resulting from selection under cultivation, that may be responsible for some of the properties of the seeds of the cultigens in the Viciaeae.

## 2.5. Cultivation of species of the Viciaeae.

### 2.5.1. Applications of ethnographic data.

In the late 1960's, Grimes (1969) expounded the need to combine biological, ethnographical and archaeological work to increase the data for the further understanding of the earliest agriculture. Since that time, a number of studies of traditional agriculture have been undertaken (for example Charles, 1985; Dennell, 1976; Hillman, 1984a, 1984b, 1985; Hubbard, 1976; Jones, 1983, 1984), which have formed the basis for the construction of models which have been used in the interpretation of archaeological plant remains. These models have largely been concerned with seed agriculture. They have been concentrated upon cereal production. The processes associated with pulse cultivation have seldom been incorporated. Consequently consideration will be given below to some of the technologies recorded for grain legume production in the Old World, which do not always parallel methods of cereal processing.

An examination of some of the processes that are involved in the growing of legume crops by traditional methods, can also further the understanding of the development of the cultigens (Smith, 1986). It is this aspect that has the greatest relevance to this thesis, in which evidence for cultivation is being sought in seeds. Its importance has governed the inclusion of this relatively

detailed account of agronomic methods, mainly culled from sources that may not be easily accessed by archaeobotanists.

#### 2.5.2. Introduction to legume agronomy in the Old World.

Traditional agriculture, as it is observed surviving in the Near East and the more remote areas of eastern Europe, is recorded commonly in terms of the lack of mechanisation, and the use of hand-implements and animal power. Particular assemblages of grain and straw and weed by-products result from the various harvesting stages; analyses of archaeological crop residues depend upon a knowledge of such procedures. This conventional treatment has governed the approach in the subsequent section on monocropping, which lists various stages sequentially as they are practised in pulse production. Tillage procedures are first described, followed by the techniques of plant manipulation from sowing to harvesting and grain-cleaning.

A brief summary of alternative agronomic strategies found in the temperate Old World is given. These perhaps are more relevant to a consideration of the earliest methods of cultivation. An attempt is then made to understand the role of the pulses in farming, from the point of view of the farmer, in order to assess the potential position of some of these alternatives in the earliest stages of

cultivation.

### 2.5.3. Monocropping of pulses.

The importance of grain legumes in the human diet today is reflected in the estimation that over 70% of pulse production worldwide is used in the form of dry seed for human consumption, rising to 90% in Southeast Asia (Malik et al., 1988). The highest yields are mainly products of monocropping systems. The different pulse crop species are often cultivated using similar practices, since they broadly share the same environments (Buddenhagen & Richards, 1988). Water requirements can affect the choice of crop, since Lathyrus sativus and lentil tolerate dry soils, and broad bean and pea are cultivated under wetter conditions (Summerfield & Roberts, 1985a).

#### 2.5.3.1. Preparation of the seed-bed.

When the seed-bed is prepared the remains of the crop and the weeds of the previous season are removed and the soil surface is broken-up ready for planting. These activities are governed by the nature and scheduling of the previous crop, the availability of labour and equipment, the soil-type and the climate.

Commonly, cultivation of a legume crop will follow cereals or fallow in rotation. After a cereal harvest, the stubble is usually grazed. Subsequently, the



remaining weeds and stubble are ploughed under. Similarly after a fallow season, the land is ploughed. Differences are found in the extent of weed cover that tends to result from cultivation and fallow; more weeds tend to grow after a cereal harvest, since the weed species will have shed their seed while the crop was standing prior to harvest and grazing, and thus actually be encouraged to proliferate by the practice (Basler, 1979).

The timing of the ploughing is dependant upon the availability of soil moisture, perhaps the main constraint upon legume growing (Papendick et al., 1988). In areas of autumn rainfall, the soil may be tilled shortly after the first rain. This tends to stimulate the germination of weeds, which are destroyed during tilling, before the seedlings become established, or they may be removed by hand before they set seed. In West and Southern Turkey, for example, the ground for lentils is prepared by one or two ploughings and weeding in the late summer, between August and September. The importance of weeding is greater in relation to legume crops than to most others: characteristically legume species do not compete well, particularly at the seedling stage. Even for such cultivars as Vicia faba, which is robust and erect in habit, yield loss has been estimated at over 50% where weeding has been neglected (Kukula et al., 1985), while the lentil harvest can be reduced by as much as 75% (Muehlbauer et al., 1985). In those regions where there

is little or no autumn rainfall, the priority is to conserve soil moisture. Seed-bed preparation consists of up to three shallow ploughings, or merely raking, to remove the weed cover and break up the soil crust. Evaporation may be reduced by levelling the furrows to decrease the surface area; planking, the flattening of furrows by a piece of wood, is commonly employed in India (Lal, 1985).

In regions such as Southern Iraq, where there is likelihood of soil erosion, this is minimised by coarse ploughing to leave large clods in the fine silty soils (Charles, 1988). In certain areas, it is the practice to reduce all tilling and so retain the stubble of the previous harvest, to prevent excess erosion from either water or wind (Harris & Pala, 1987). This conservation tillage is a practice that is currently being applied to many regions in the developing world (Papendick et al., 1988).

Stubble may be left standing in the fields over the winter prior to a spring sowing season; it may be burnt off as the initial tilling procedure. This is the practice in Central Anatolia and the Iraqi Plateau. Stubble-burning may reduce disease, but has in fact been found to have little effect on weeds or subsequent seed yield of the crop (Harris & Pala, 1987).

### 2.5.3.2. Sowing.

The sowing time is the result of a compromise between the provision of the maximum growing period for the crop, and the possible effects of a number of variables. These include the availability of water, the likelihood of frost, the presence of weed competitors, parasites and predators, as well as temperature fluctuations and day-length. Thus where conditions allow, autumn and winter are the preferred seasons for planting in the Old World, but different crop species are influenced differently by the various factors, and separate regimes may be used in the same areas for each species.

Typically, for lentils in the Eastern Mediterranean region, such as Greece, and coastal Turkey, Southern Turkey and India, the sowing time is in late October and early November (Jones, 1983; Sakar et al., 1988; Lal, 1985). In Syria and Jordan, the sowing period may be somewhat later, from December to January (El-Matt, 1988; Antoun & Quol, 1979; Saxena, 1979). Soil moisture is the main limiting factor in these areas where winters are relatively mild. Experimental plantings of lentil have yielded up to 100% better yields in the autumn than in the spring (Sakar et al., 1988). However in the Central Plateau of Turkey, Iran and Iraq, temperatures fall rapidly in the autumn, and frost damage curtails the establishment of early plant growth; therefore, lentils are not planted until early spring (Harris, 1979).

Broad bean is often spring sown. In central Asia, the

relatively high water requirements of this species demand the rainfed conditions usually present in the spring; winter sowings have to be supported by irrigation. In eastern Europe, and western Asia, broad bean grown as a pulse is not a major crop today (Bond et al., 1985). Egypt is known to be an important centre for broad bean growing, but irrigation is then almost invariably practised.

Peas are particularly sensitive to high temperature and drought stress. They are normally grown in the cool season, when soil moisture is sufficient or autumn rainfall has arrived (Davies et al., 1985).

The method of sowing varies with the crop species and is dependant upon the size of the seed (Charles, 1985). Small-seeded species, the lentils, vetches, Lathyrus species and smaller varieties of pea, are broadcast. In West Asia and most of the Indian subcontinent, broadcasting is followed by light ploughing; however this is known to produce irregular stands due to the unevenness of the soil cover. Broadcasting over shallow furrows or on ridges, followed by levelling, gives a more uniform result.

Sowing may be followed by light ploughing or harrowing to cover the seed, but often, as is usual in Bangladesh, seeds may be sown without subsequent burying, in spite of the risk of heavy loss to birds (Malik et al., 1988). The

usual planting depth varies from ten centimetres in Syria (El-Matt,1979) to four or five centimetres in cooler areas in the Near East (Saxena,1979).

Hand placement, where one or more seeds are placed by hand into prepared holes, on ridges or along furrows, is the more usual method where seeds are large, as in the bigger pea varieties, and broad bean (Diekmann & Papazian,1985; Mayouf,1979). For lentil too, drilled sowings are occasionally carried out in northern Syria and Jordan, and give heavier yields than broadcast sowings (Saxena,1981; Snobar et al,1988).

Fertilisers in the form of manure, or dung cakes, may be incorporated into the ground with the seeds, but they are not part of the soil treatment traditionally used in legume cultivation (Lahoud et al.,1979; Eser,1979). Where soil moisture is very limited, as is the case in parts of India, seeds may be soaked before planting, or they may be planted at a depth where the soil is still damp; this requires plantings from ten to twenty-five centimetres in depth (Papendick et al.,1988; Smithson et al.,1985). Traditionally, most warm temperate pulse crops are rainfed (Papendick et al.,1988); irrigation is not a usual practice for legumes (Nassib et al.,1988), although some dry regions, such as the Central Anatolian Plateau, do have a long tradition of irrigation (Hillman, pers.comm).

The sowing rate giving the optimal plant density and best yield varies with the location and the species and variety of cultivar. Figures quoted by Snobar et al. (1988) refer mainly to modern practices used to obtain the maximum yields, rather than to traditional sowings. However these workers reported that farmers in Jordan plant from 50 to 200 kilograms of seeds per hectare in dryland areas; the higher weights are found in Syria (El-Matt,1979) and Ethiopia (Telaye,1979). Of interest is the conclusion that where the population density of the plants is increased above a certain level, the plants tend to be taller with fewer branches and fewer pods, and therefore provide a lower seed yield (Murinda & Saxena,1985). However, a lower number of side branches has been found to increase the production of fruiting nodes, and hence seed yield, in determinate varieties of Vicia faba, thus a high planting density may be sometimes recommended (Pilbeam et al.,1990).

Broad bean, hand placed into drills and ridges, is commonly planted at the rate of one to two seeds, fifteen to twenty centimetres apart in two rows per ridge, in Egypt for example, and at a slightly lower density in Cyprus (Snobar et al.,1988).

#### 2.5.3.3. Harvesting.

Hand harvesting of grain legumes is still practised in much of West Asia and in certain areas of Southern

Europe. Some cultivars have a growth habit that is branched and sprawling, and often cannot be satisfactorily cut by machine, though specialised equipment has been designed to pull plants up from the soil, or cut plants below the soil surface (Diekmann & Papazian, 1985). The description given by Jones (1983; 1984), of pulse-harvesting techniques on the Cycladic island of Amorgos, is similar to those recorded in Turkey and Syria by Hillman (1984a), and observed on Crete and Cyprus and in southeastern Turkey as part of this project.

The legume plants are uprooted by hand or blunt sickle, or are cut at low level by sickle or scythe. Weeds are left standing where possible. The cut crop is piled into small heaps regularly distributed across the field, weighed down by stones in windy regions, and left to dry. The drying period varies with the location and prevailing climatic conditions, and may last from a few days (Jones, 1983) to a few weeks (Lal, 1985) or several months. Typically at this stage, the crop retains most or all of the straw, and includes some weeds; these are often climbing species, often wild legumes, that grow in intimate association with the crop.

The time of harvest varies with the climate, and growing period of the cultigen. In southern Turkey, for instance, May is the typical harvest month for lentils. The stage at which the crop is cut is finely judged.

Shattering of the pods can result in heavy losses of yield when the plants have reached complete maturity (Lal,1985). A compromise must be reached between the ripeness of the majority of the pods and their seeds, and yet some greenness of most of the plants, when the moisture content is still about 30% (Haddad et al,1988), and in lentil, when around 60% of the pods are golden brown (Erskine,1985), or the leaves are yellow (Lal,1985). In spite of such careful timing, up to a quarter of the yield may be lost through pod shatter. Even in the cooler climates of Britain, and in the developed cultigen Vicia faba, pod shatter can be a problem: it was documented a century ago, that in Norfolk beans were normally harvested on cloudy days to reduce the loss in yield from seed shedding (Rider Haggard,1899,p.329).

Records have been made of the work involved in hand-pulling pulses. In Syria, for lentil harvesting, between 12 and 20 man-days of work per hectare are usual (Khayrallah,1981). In Jordan, 10 days' work has been noted as sufficient (Haddad et al.,1988). Today the expense of this labour is one of the biggest incentives towards mechanisation (Hawtin & Potts,1988; Sakar et al.,1988).

#### 2.5.3.4. Threshing.

Threshing normally takes place near or immediately



adjacent to the village on areas of relatively level ground trodden to a hard floor. However, where fields are remote, smaller local floors may be constructed. In Greece these are usually less than six metres in diameter (Cheetham,1982). Today they sometimes have concrete surfaces.

Bundles of the dried crop are carried to the floors using animal transport where available. This is by ox, donkey or mule. Movement of the dry material generally takes place at periods of the day when the strongest sun and highest temperatures are least likely, in order to reduce pod shatter and seed loss. The straw and pods are broken up by animal hooves, drag rollers or threshing sledges. It has been noted that the traction animals usually eat the seeds as they work (Cheetham,1982). Where the harvest is small, or during adverse weather conditions when processing has to take place under cover, sticks or flails may be employed (Hillman,1981). In Spain, small bundles of lentils may be hung on walls and beaten with sticks to release the grain (Chocarro, pers.comm.).

#### 2.5.3.5. Winnowing.

Winnowing is carried out by means of a variety of equipment, ranging from hands, where the harvest is very small, to forks, shovels, fans or baskets (Cheetham,1982; Jones,1983; Hillman,1981;1984a). The time taken depends upon the size of the harvest and the wind strength.

Jones (1983) surprisingly recorded that still days were favoured for winnowing because of the greater degree of control that could be exercised over the deposition of the fractions. Usually however, a steady gust-free breeze is considered optimal, as described by Hillman (1984b) in connection with cereals.

Care is normally taken to retrieve all residues; the value of legume straw and pod by-products for animal feed is very high (Amirshahi, 1979). Lentil straw is considered to be of particular value, and is fed to the most prized animals (Erskine, 1985; Khayrallah, 1981). In Syria and Jordan, and other regions where the dry seasons limit the availability of other fodder, lentil residues may actually have more commercial value than the seeds themselves (Muelhbauer et al., 1985).

#### 2.5.3.6. Sieving and cleaning.

Crop sieving processes for pulses have been described in detail by Hillman (1981, 1984a). Large and heavy fractions, the straw nodes and pod fragments, are separated by coarse sieves of mesh sizes that may vary with the crop. For example, one coarse sieve for lentils recorded in southern Turkey had a mesh size ranging from 4 to 5 millimetres. The small weed seeds and other impurities that have separated with the crop may be removed by fine sieving. Hand sorting is a final stage usually carried out immediately prior to processing the

grain for food, the cleanings commonly being used as poultry feed (Jones, 1983; 1984).

#### 2.5.3.7. Note on implements.

It has been observed that implements connected with legume cultivation by traditional methods are usually the same as those used for cereals, that equipment specialized for processing pulses is not normally used. Ploughs, drilling tools, sickles, scythes, threshing sledges, winnowing forks and sieves, the animal transport and traction, are common to both types of crop. This usually means with the availability of a single repertoire of implements, that when there is a common harvest season for more than one crop, the pulses are relegated, and priority is given to any of the cereal species, which carry more commercial value.

The construction of threshing sledges has been described by Cheetham (1982) and Fox & Pearlman (1987).

There are a few exceptional references to specialised scythes with short blades for reaping peas and beans in nineteenth century Denmark (Begtrup, 1806, and Niemann, 1807, as cited by Steensberg, 1943, pp. 239, 240).

#### 2.5.3.8. Labour.

The type of labour similarly is common to seed crops:

animals are usually driven by men, the operation of large or specialized equipment for sowing, threshing and winnowing is male work. Females traditionally do the processing that may be carried out by hand, such as the hand harvesting, weeding and grain-cleaning, together with the use of the least complicated implements such as sieves and sickles. Thus the techniques of seed agriculture used to be divided approximately evenly between the sexes. However with the introduction of progressively more mechanisation, it has been noted that now men are beginning to undertake an increased proportion of the agricultural work (Rassam & Tully, 1986). At peak periods in harvest time whole families commonly work together.

Described above is the system of monocropping pulses, together with the crop processing techniques that have been compared by Hillman (1981, 1984a, 1985) with those used for free-threshing cereals. However other systems have been developed and are practised today in a number of developing countries, and some of those considered below may have had a particular relevance in antiquity.

#### 2.5.4. Multiple cropping.

Certain environments and soils will support more than one cropping per unit of land per season. Where water

supplies are sufficient, and the growing period prior to harvest is short, yield can be more than doubled in this way. Legumes, having soil-enriching properties, are particularly suitable as components of these systems (Tiwari,1979). In Ethiopia, lentils are often planted as a second crop immediately after the wheat or barley harvest; Lathyrus sativus similarly may follow a summer chickpea crop (Westphal,1973). In India, following the main summer harvest of maize, sorghum or jute, the land may be used for lentil cultivation supported by the moisture retained in the soils from the earlier monsoon rains. In the north east of the Indian subcontinent sometimes a third crop may be planted, although it is usual to apply some irrigation at this stage (Postan,1843, as cited in Fentress,1982,p.366; Saxena,1981).

These practices represent a variation of the standard monocropping. This intensification strategy has been considered by Thomas (1983) in his work on agriculture in the 3rd millennium BC in Northwest Pakistan; similarly in relation to the Indus valley, Fentress (1982) suggests that she has found evidence for multiple cropping at the site of Mohenjo-Daro, involving pulses and other crops under irrigation.

#### 2.5.5. Mixed cropping.

Mixed cropping systems, where more than one species are

grown together in the same plot, are well-known today throughout Asia, North Africa and Europe. Often they are associated with tree crops, tubers or the production of annual species as animal fodder. Equally, grain crops for human consumption are grown as components of mixed systems, and in these legumes play a major role. Such systems are not uncommon and are well-documented in the agricultural literature. However, comparatively little consideration seems to have been given to these alternatives to monocropping by those concerned either in the debates on the origins of seed agriculture or in development of the grain cultivars, or in the interpretation of archaeological material.

#### 2.5.5.1. Relay planting.

The Indian subcontinent, in particular, displays a number of alternatives to monoculture involving pulses. Relay planting, known as utera, may involve the broadcasting of legume seeds into a standing crop of rice in dry paddy. Sowing takes place ten days to three weeks prior to the rice harvest. The legume crop then utilises the residual soil moisture from the monsoons, while the rate of evaporation <sup>decreases</sup> because of the rice cover. This system is common in India and Nepal where lentil and Lathyrus sativus are deemed to be particularly suitable for growing in this way. In Northern India, distinctions are made between species most suitable for planting; when the rice crop is near to harvest, chickpea is planted, and

when the paddy crop is to be left standing for longer, pea and Lathyrus sativus are favoured (Bharati,1986; Bhattarai et al.,1988; Saxena,1981; Tiwari,1979).

#### 2.5.5.2. Intersowing.

Somewhat similar is the practice of intersowing cereals with legumes in Western Asia. In Lower Iraq, for example, broad beans and barley are sown together and harvested separately. The barley may be harvested by careful cutting with a sickle at the green stage, up to three times successively before the beans have ripened. The beans are then harvested by hand picking of individual pods (Guest,1930, as cited in Townsend & Guest,1974,p.543).

In Ethiopia, broad beans and peas are commonly grown in association, and harvesting is timed to the stage when most of the leaves have dropped and the upper pods are yellow. This takes place when most of the pods are ripe and yet not all of them have dried; the beans and peas are treated separately. The crop is cut with sickles, and left to dry in the fields in stacks which may be covered with grass as long as from four to six weeks. the crop is then threshed by trampling or flailed with sticks, and winnowed (Telaye,1979; Westphal,1974).

#### 2.5.5.3. Field edge sowing.

A common practice is the sowing of pulses on field edges, surrounding standing cereal crops. This is exemplified in Northern India where lentil may be grown around the wheat or, more rarely, the barley fields in high densities. The cereal is usually harvested in the period from March to May, depending upon the altitude, but the lentil may ripen and be harvested as early as February. When the ripening period coincides for both crops, harvesting and subsequent grain processing may be carried out together for the mixed crop, or bijra, which is then treated as a single product for storage and in food preparation (Atkinson, 1980, pp.684,685,694).

#### 2.5.5.4. Legumes as protection against soil erosion.

In India grain legumes are commonly planted singly or in lines between spaced rows of other crops to prevent or reduce soil erosion and improve soil structure; this follows by virtue of their relatively deep root systems and spreading growth habits (Tiwari,1979).

#### 2.5.5.5. Broadcast mixed sowing.

A more complex system of mixed cropping of pulses involves the broadcast sowing of a number of different species in associations that resemble fodder cropping. Such mixed sowing has been practised widely in the Indian



subcontinent. The individual species in these complex mixtures grow and ripen at different rates and times, and require harvesting by hand picking as each matures. This entails the covering of the same plot a number of times, which inevitably results in some loss of yield by treading. The grains are cleaned into separate populations for storage and use (Bharati, pers. comm.).

As examples of this system, in India, chickpea may be planted with wheat, barley, linseed or mustard; broad bean may be intersown with castor, groundnut, or cotton (Tiwari, 1979). In Nepal, mixtures of chickpea, lentil, pea, broad bean and Lathyrus sativus are sown with rice, maize, linseed or mustard in a variety of combinations (Bhattarai et al., 1988). Chickpea and lentil are commonly intercropped with mustard in Bangladesh (Papendick et al., 1988). It is also reported that broad bean, pea and Lathyrus sativus are grown mixed with barley in Afghanistan (Wassimi, 1979).

Mixed cropping involving legumes is commonly found in many situations; it may be practised with taxa from tribes other than Viciaeae in different geographical regions; sometimes members of the Viciaeae are cultivated in regions normally considered to be outside temperate zones. In the early nineteenth century in Botswana all kinds of grain and pulse, including broad bean, were sown "promiscuously", reaped together, and the seeds stored indiscriminately in earth granaries. These yields were

subsequently used without selection usually in the form of flour for bread making, or as a gruel (Barrow, as cited in Tannahill, 1975, p. 279). Today in northern Nigeria, for example, cowpea, a member of the tribe Phaseoleae, and sorghum are intersown; this association appears to be long-standing: the relations between the developmental cycles in these two crops are so close as to be held as evidence of co-evolution (Bunting, 1990). Also in Africa, at high altitudes in Burundi, pea is intercropped with maize or Phaseolus vulgaris (Nijimbere et al., 1988). As another example, Lupinus mutabilis, a member of the tribe Genisteae, is planted with Solanum tuberosum in the high Andes in Bolivia (Altieri & Merrick, 1987).

These examples of mixed cultivation systems involving pulses illustrate a traditional attitude towards seed agriculture that appears to be worldwide.

#### 2.5.5.6. Advantages of mixed cropping.

The extent of success of mixed cropping of legumes with other species relates to the different growth requirements that each species withdraws from the soil, and the degree of competition. Generally legumes compete poorly with species that have large leaf canopies. Yet species such as mustard can act as a supporting medium

for plants that climb or sprawl, and thus reduce the likelihood of lodging (Saxena,1981). Certain species in association, make particularly effective use of the soil moisture, as they have different rooting depths (Bhattarai et al.,1988; Tiwari,1979).

#### 2.5.5.7. Seeding ratios.

The seeding ratios between species vary: but equal weights of cereal and legume seed mixtures have been recorded (Mirchandani & Misra,1975).

#### 2.5.5.8. Labour.

The labour input required in mixed cropping is minimal for most stages except for harvesting itself, which is highly labour intensive. Traditionally, it appears that hand-harvesting is predominantly women's work (Rassam & Tully,1986). Thus this method of cultivation would seem to be benefitted where surplus labour is available and there is a large female work force.

#### 2.5.6. Consideration of the cropping systems.

The range of cropping systems represents responses to different environmental conditions, and differing attitudes of the farmers towards their various crops.

As mentioned above, monocropping of pulses is usual in

regions with autumn or winter rains. Where the winters are mild, autumn or winter sowings are practised. This is the case in the eastern Mediterranean, and at the lower altitudes in Turkey and Syria. Where winters are more severe, and carry risk of damage to crops from frost and snow, spring sown monocropping is normal; this can be seen on the higher ground in central Turkey, Iraq and Iran.

In temperate regions, mixed cropping for grain harvest seems more usual where crops are supported on soil moisture retained from rainfall in the summer, as in Afghanistan, the north of the Indian subcontinent, southern Iraq and north Africa.

Today, however, mixed cropping is readily observed across all these regions in the temperate Old World, when cultivation is intended for animal feed. Thus Jones (1983) describes on the Cycladic island of Amorgos, how Vicia sativa and Lathyrus sativus may be grown separately for feed grain, and together as a mixed crop for hay. Broad beans and cereals were commonly grown together as a fodder crop widely across Europe up to the early nineteenth century (Bond et al., 1985). At that time there are records in Norfolk of rye and vetches being sown together for sheep fodder (Rider Haggard, 1899, p.369). In north-central Turkey, Vicia sativa is sown as a green fodder with autumn-sown Triticum durum (Hillman, pers. comm.). Ladizinsky (1989)

and Ladizinsky et al. (1983) have recorded the growing of barley and lentil together on terraces in Alpine France and Italy in historical times. Apparently, this was a very ancient practice, but it was discontinued during this century.

The difference that is most important between mixed cropping for human food and for feed, lies in the harvesting and processing, rather than the cultivation. Pulses, as has been described above (2.5.3.3.), are gathered, pulled or cut as individual species when ripe, or nearly so. Crops for feed are commonly pulled or cut as hay in the green to dry stage, including all the species together in one operation. The hay is left to dry in the fields, in a similar fashion to pulses under monocropping, before being transported in bundles to the villages, or to the markets for sale.

The possible role of producing animal feed in the development of early agriculture has been examined by Bohrer (1972), and she remarks that Lathyrus sativus, for example, when grown primarily as fodder, can become a famine food for man in times of nutritional stress by the addition of threshing to separate the grains from the straw residues. However Bohrer does not discuss the growing systems for pulses.

Direct evidence from most easily-observed current non-mechanised practices of legume cultivation, such as those

outlined above (2.5.3.-2.5.5.), may give the impression that mixed cropping represents no more than a regional variation in cropping strategy. Monocropping, that seems to underlie most archaeological interpretations of seed cultivation in the Old World, certainly is the system most commonly observed today as "traditional agriculture" for legumes and cereals alike, in the Near East, where, in the past, archaeological investigations of early seed cultivation have centred.

The extent to which mixed cropping systems may have played a major role in the development of early seed agriculture in the Old World seems to have received relatively little attention. In the case of pulses, at least, it would appear to merit more serious consideration.

Apart from modern ethnography, a further source of evidence for traditional agricultural practices is provided by historical documents, which is examined in the following section.

2.5.7. Documented evidence for legume cultivation in the ancient Old World.

Records of early agricultural and food processing practices involving pulses in Europe, the Near East and

Egypt can be found in a number of sources, in the form of both textual and pictorial evidence. This information about the role of legumes in the ancient Old World adds a valuable extra dimension to the ethnographic observations that can be applied to archaeological interpretations. Cited below are some examples from various regions and periods, which illustrate the available types of this evidence.

#### 2.5.7.1. Textual evidence for methods of pulse cultivation.

Texts in Aramaic and Sumerian scripts from ancient Mesopotamia, describing the cultivation of emmer and barley, also refer to "small" or minor crops; these include lentil and vetch. From the Old Babylonian period, two tablets from Shemshara mention fields of pulses. Commonly the translations of the cuneiform are uncertain, as has been discussed by Stol (1985); however, their interpretations reveal that lentils, broad beans and chickpeas were winter crops, sown in September and harvested in May in Babylonia, and that broad beans were also grown in Palestine (Dalman, 1932, p.271; El-Samarraie, 1972, p.68). Translations of Akkadian texts refer to pea or possibly a species of Lathyrus, which was sown by broadcasting. The cultivation of other pulses with small seeds has been inferred from various Akkadian texts, but whether the references mean species of Vicia or Lathyrus has not been resolved (Stol, 1985). In the

cuneiform texts on the British Museum tablets of Ur III Girsu, reference is made to plots where are grown legumes and other plants, possibly including mustard, but it is not known whether this describes mixed cropping or crop rotation; also there are records of very small amounts of lentils being sown with the barley in temple plots, and "big pulses" being sown in a mixture and harvested with cereals (Maekawa,1985).

Biblical references to the cultivation of legumes are few, one of which mentions a plot of lentils, which was treated with some respect during conflict (Bible, Samuel II,23:11). It seems that in this instance, monocropping was the agricultural method employed.

Evidence of pulse cultivation in Asiatic Thrace, at Calpe Haven, is given by Xenophon, who described the local soil conditions as good and loamy, suitable for cereals and pulses of all sorts (cited in Bedigian & Harlan,1986).

Amongst the classical sources, the detailed records of Theophrastus (Book 8), writing in the fourth century BC on the Greek island of Lesbos, stand out as perhaps the most comprehensive accounts of early legume cultivation. The sowing season of broad bean and "okhros" (L.sativus) was early, at the "setting of the Pleiades"; lentil, Vicia sativa, and pea were late sown, in early spring; Vicia ervilia and chickpea may be sown at either season (p.143). The germination period varied from four to five



days for all species except for broad bean, which could take up to twenty days (p.147). It was stated that on the island of Melos, pulses did not grow as abundantly as did the cereals (p.157). This seems to imply that at Lesbos, pulses were more important. The most prevalent weeds were recorded, the "axe weed" (probably a species of Lathyrus) infesting Vicia sativa and lentil crops, and Galium being particularly associated with lentil (p.193). The best species for storing were listed as chickpea, lupin and vetch (p.211). The cooking and nutritional qualities of pulses are described; it was noted that white-seeded varieties were sweeter than those with coloured seeds, that bitter vetch was more digestible when sown in the spring and that the character of the soil could affect the cooking properties of broad bean and other legumes (p.197).

The later records of Columella (Book II) describe field crop husbandry in southern Spain during the first century AD, and include interesting details of some of the agricultural practices. Some of these are described below.

(i) Broad bean, lentil and pea were included amongst the legume crops for human food (p.139). Pea was said to prefer loose soil and warm wet conditions (p.157). Broad bean thrived in heavy soil; it was usually sown in the late autumn, but occasionally sown in the early spring. Beans were harvested early in the morning, before dawn;

the pods were dried on the threshing floor, and beaten with sticks to release their seeds. If these were cooled and then stored immediately, no weevil damage would result (pp.159-165). Lentils needed a drier soil, and could be sown either early or late. After threshing, flotation in water would separate a light fraction containing seeds that were discarded. After drying in the sun, the heavier seeds were sometimes mixed with ashes, before being sealed in the stores with gypsum (pp.165-167). For long-term storage, it was recommended that the seeds should be re-threshed to remove weevils (p.219). Commonly the legumes were threshed before the other crops, to smooth the floor for the cereal grains, which were presumably considered a more delicate product (p.215).

(ii) Unusually, fodder crops have been described, amongst which were vetch (presumably Vicia sativa) (p.175), chickling vetch (Lathyrus sativus or L.cicera) and bitter vetch (p.179). Fodder crops were usually grown under dry conditions on broken ground in either the autumn or winter. Chickling vetch was sown in rich soil in January or February, or sometimes in November (p.169). It was considered that bitter vetch was less harmful to cattle if sown before March (p.179). An interesting reference is made to barley, vetch and other legumes being fed green to animals. In some regions, oxen were fed the crushed seeds of chickling vetch after soaking and mixing with chaff; this legume crop was said to look like a small

chickpea, but the seeds were blacker in colour. It is not clear whether Lathyrus sativus or L.cicera is the species here, but it is stated that the processed seeds were not unpleasant for humans (p.181). The straw from broad beans was also used for hay (p.183).

#### 2.5.7.2. Historical references to the use of pulses.

There are references to pulses as food in the Bible. David was brought parched corn with beans, lentils and a parched pulse (Bible, Samuel II, 17:28). There is also the well-known account of the experimental feeding of youths with pulses at the court of Nebuchanezzar (Bible, Daniel, 1:11-14). An interesting reference is made to the use of a mixture of the grains of wheat, barley, beans, lentils, millet and spelt to make bread (Bible, Ezekiel, 4:9-12).

Evidence can be found of the use of pulses as conveniently portable forms of food. On a fragment of papyrus from el-Hibeh in Middle Egypt, dating from the 21st. Dynasty in the eleventh century BC, the writings of Wen-Amon record a journey to Phoenicia. He was sent provisions, which included twenty sacks of lentils, with one sack for his own use (Pritchard, 1958).

The value attributed to pulses in the past may be deduced from records of Rameses III, which report the offering of 11998 jars of shelled beans to the Nile god (Darby et

al.,1977,p.684). Lentils commanded the same price as wheat in 113 BC, as recorded in another papyrus (SP.II 398,516A, cited by Darby et al.,1977,p.688).

Records relating to the Roman army in AD 199, state that by far the most common vegetables supplied by the villages of Egypt for the military diet were beans and lentils (Davies,1971).

The well-known taboo attached to the broad bean has been described by several classical authors. Pythagorus, who reputedly was a victim of favism, and who is said to have died rather than enter a bean field (Arie,1959), attributed the taboo to the belief that beans were made of the same matter as man and therefore were proscribed as food; Diodorus took a different view and said that priests were denied beans in their diet as a form of self-denial; Pliny ascribed the avoidance of faba beans to their insomniac properties (Darby et al.,1977,p.683). Herodotus (II:37), writing around 450 BC, commented that "none of the Egyptian priests would eat beans (Vicia faba) or even look upon them as they are an unclean kind of pulse".

The detoxification of pulses is described in the Hebrew text, the Mishnah (Shabbath I,5,translated by Danby,1933,p.101); here reference is made to the soaking of Vicia ervilia seeds in water prior to their preparation for food.

The early use of pulses in medicine is documented in the Syriac book of medicines (Budge,1913). Most of the remedies seem to involve legume seeds in the form of flour, and often these are recommended for application as poultices, rather than for internal consumption; the seeds of vetches, including bitter vetch, beans and chickpeas, as well as Melilotus and lupins were used as plasters. Bitter vetch appears to have played a large part in these cures. Infusions of lentil seem to have been popular for many complaints, from streaming eyes and diarrhoea, to heart and liver disease.

#### 2.5.7.3. Pictorial evidence of pulses in the ancient Old World.

The decoration on pottery can provide another source of information on the importance of certain legumes. A vase excavated on the Greek island of Thera at Akrotiri is decorated with motifs of vetches (Doumas,1983). These might represent Lathyrus clymenum; archaeological evidence for it has been found on Thera, ancient Santorini (Sarpaki & Jones, in press).

Egyptian wall paintings provide more evidence of the role of the pulses in antiquity; an example may be found in the tomb of Rameses III, which depicts the preparation of a dish of lentils (Darby et al., 1977.p.687).

#### 2.5.7.4. Conclusions drawn from the documented evidence.

Most of the documentary evidence underlines the relevance of the ethnographic records made from present-day traditional forms of agriculture. The documentation however tends to be late in date relative to the onset of cultivation; it is the product of highly sophisticated societies, and thus refers to agronomic practices and food-processing activities during phases of intensive food production. References unsurprisingly seem to relate mainly to the records made for official bodies, such as the court or the army, or to individuals of an elevated social standing; they pertain mainly to field agriculture rather than to small garden plots (Halstead, 1987), to monocropping rather than to mixed cropping and the gathering of wild plants for food, unless animal fodder is the end product. Evidence for mixed cultivation remains elusive.

#### 2.5.8. Farming attitudes.

An examination of the attitude of the farmer provides some insight into the rationale that underlies the operation of the prevailing agricultural practices. This may help to throw some light on past systems of cultivation.

Legumes are seldom grown for maximum productivity. In spite of their important role in nutrition, the aspect of

pulses as crops that is most greatly exploited by the farmer seems to be their characteristic tolerance of relatively adverse growing conditions. Many agricultural systems reflect a priority of attention given to and demanded by cereals, with a minimum of expenditure of input into pulses. Thus, traditionally, legumes are grown on marginal soils, often with limited availability of water, with little or no tillage, and the addition of no manure. The growing season may be relegated to non-optimal periods when the ground has been released from competing croppings, and when the labour and equipment can be diverted from other tasks (Papendick et al., 1988).

In traditional agriculture as practised today, this attitude of the farmer is difficult to reconcile with the constantly-reiterated statements about the significant position of legumes in the repertoire of food plants (Agostini & Khan, 1988; Brady, 1988; Malik et al., 1988; Marsi, 1979). However, if such an attitude is itself part of a long tradition in agriculture, it might be a reflection of an adaptation in farming most in sympathy with the natural requirements of the species, and a utilisation of those species in the most versatile way.

Characteristically most members of the Viciaeae are tendrillous, and have a sprawling or climbing habit, as described above, often being supported on associated vegetation. They disseminate explosively, and thus tend to establish diffuse populations. It can be seen

therefore, that mixed cropping systems can closely reproduce conditions obtaining in the wild. The nodulating root systems, typical of legumes, support growth in habitats where some other species find insufficient nutrient. Hypogeal germination allows for the development of seedlings under cool conditions with some degree of frost protection. Some species, notably Lathyrus sativus and lentil, have a marked tolerance of drought. Generally, the members of the Viciae compete poorly, and grow best under conditions that act against strong growth of those plants that might otherwise dominate the habitat. The cultivation of pulses on marginal ground may be well tolerated by those taxa, the conditions discriminating against other species, and favouring the pulses.

Under optimal climatic conditions, and on fertile soils, the harvest from legume crops may not represent very good yields compared to those from cereals (Sanderson & Roy, 1979, p.39). This may be explained in part because commonly pulses reflect an intense adaptation to local environmental conditions, which conventionally have been those of marginal land. Often these crops have an extended period of maturation. Also, the indeterminate flowering habit of legumes produces successions of seed, and thus a non-synchronous harvest, a property which is unaffected by the environment (Swaminathan, 1975; Swaminathan & Jain, 1975). Against the results of poor conditions however, these cultigens can represent an



insurance. In times of famine, in particular, they can provide a fail-safe source of grain. Together with the value of the seeds for human food, the straw and pod by-product as animal feed is, as has been described above, of equal or greater worth. In times of food glut, the green plants can be ploughed-in and add to the nutrient status of the soil. The extensive root systems may be exploited for reducing soil erosion (Tiwari,1979). Thus pulse crops can be used by the farmer with great versatility under a number of different circumstances, often with a minimum of expense in terms of labour or land treatment.

The benefits of agrosystems employing multiple cropping methods are recognised today by those concerned with the current world food situation. Such agronomic strategies are said to promote a diversity of diet and income resources, a stability of production, the minimisation of risk, a reduced incidence of insect and disease, an efficient use of labour, a relative intensification of production using limited resources, and a maximisation of returns under low levels of technology (Altieri & Merrick,1987).

2.5.9. Possible effects of cropping methods on the development of the cultigens.

The antiquity of the mixed cropping strategy is unknown, but it is of interest that most areas that apply such

systems for pulses use landraces, that have been defined as "traditional cultivars, not subject to scientific selection; often a population or a mixture of related genotypes" (Polhill & van der Maesen, 1985, p.32). These small-seeded varieties can respond successfully to a range of environmental conditions in their particular geographical region, and usually show the least modification from the wild progenitors (Erskine, 1985; Malik et al., 1988; Telaye, 1979; Wassimi, 1979). The landraces are often associated with populations of closely-related wild and weedy forms (Altieri & Merrick, 1987), and, despite the characteristic autogamy of the Viciaeae, outbreeding can occur, which maintains the genetic variability of the cultigens.

The landraces retain much of the ability to adapt; this reflects the important characteristic of the legume taxa of an ability to exploit a wide range of environments (Adams & Pipoly, 1980) (see 2.5.5.above). Genetic diversity and phenotypic plasticity in the wild species result in a great flexibility of response to the requirements of different habitats.

Within populations of even the most highly-developed legume cultigens exist individuals that exhibit a range of variation in morphology and anatomy, physiology and biochemistry. The retention of a variation in shape, size and colour of the seeds, the amount of pod dehiscence, and the extent of seed dormancy may reflect the fact that

the farmer did not apply the selection pressures that would result in a more homogeneous crop.

2.5.10. Systems practised during earliest cultivation of legumes.

The possibility that the mixed cropping of legumes was widely practised in antiquity is of great importance to archaeobotanists. In the absence of direct evidence, it has been the convention amongst those working on seed agriculture in the Old World to use models of monocropping for both cereals and pulses. The ethnographic evidence presented above suggests that early legume cultivation should be differently viewed.

2.6. The basis for the experimental work of the project.

The background to the tribe Viciaeae given in this chapter 2 has provided the basis from which the experimental work for this project has been conducted. The selection of taxa from which to take the seeds as the subjects of the microscopic survey, and the methods employed, have largely depended upon the taxonomic relationships within the tribe, and the published data from previous morphological and anatomical investigations.

The following sections (chapters 3,4,5,6,7) concern the experimental research carried out as the major component of the project.

### 3. MATERIALS AND MICROSCOPIC METHODS.

#### 3.1. Introduction.

The difficulties of identifying the remains of ancient seeds of members of the Viciaeae have been described above (paragraph 1.2.1.): even fresh seeds are frequently impossible to separate to the level of species or even to the level of genus by the conventional means; this is by matching, using comparative reference material employing characters of the gross morphology observed with the unaided eye and with optical microscopy at low magnifications.

The primary requirement of this project has been to find new characters in legume seeds that can be used for the identification of fresh material. Since gross morphology does not appear to supply such characters, it was decided to examine the micromorphology and anatomy of legume seeds. The relatively high magnifications required for such investigations, together with the good resolution and depth of field, can be achieved by scanning electron microscopy (SEM), a technique that is becoming more readily available and that has been applied successfully to the study of seed micromorphology and anatomy (Brisson & Peterson, 1976). Thus SEM was chosen as the major technique for this project, which centres upon a survey of the micromorphology and anatomy of Viciaeae seeds.

In order to establish the direction of research, a pilot study using SEM was undertaken on seeds of representative species of a number of different taxonomic groups in the single genus Lathyrus, which in the Viciaeae is considered to show the greatest variation in seed form (Lersten & Gunn, 1982, p.6). The results of this initial restricted research project were sufficiently encouraging to stimulate further investigations along similar lines (Butler, 1986).

The material selected for study falls into three groups: fresh modern seeds, charred modern seeds and charred archaeological material, as explained below.

On the basis of the results of the preliminary work, the project was designed to examine by SEM fresh seeds of members of all four major genera in the Viciaeae, particularly the cultivated taxa and their closest wild relatives. However throughout the project it has also been necessary to employ light microscopy and use a variety of other techniques that complement and lead towards a better understanding of the features observed by SEM.

The vast majority of archaeological seed remains have been charred in antiquity, and the physical and chemical changes that have taken place in the charred seeds compound the difficulties in identification; so throughout and in parallel with the main survey of fresh

seeds, observations have also been made on charred seeds. Although it is a standard practice in archaeobotany to char seeds for comparative purposes, there is surprisingly little documentation of the subject. Thus it has been necessary to undertake the experimental charring of fresh material, primarily to establish a standardised regime for the production of charred material to use comparatively for the identification of archaeological seed remains, and also to observe in detail the morphological and anatomical changes due to heat.

The conditions of deposition and the subsequent recovery of the charred seeds from archaeological sites result in varying amounts of degradation of the material. Some features tend to be reduced in definition or lost. Thus it has been important to establish the relevance of the observations recorded in modern material to the archaeological remains: "one must work from the fossil" (Körber-Grohne, pers comm.). Therefore throughout the survey charred archaeological specimens have been examined by the same techniques as the modern specimens.

The approach to the project outlined above is described in detail in the following sections; tables of the material employed are presented, and the methods given.

### 3.2. Materials.

#### 3.2.1. The pilot study.

From the genus Lathyrus, single populations of nine species, two populations of three species, and three populations of one species were selected. The seeds were from both annual and perennial species, having a range of testa textures and colours, representing wild taxa, species or subspecies sometimes cultivated for animal grain feed or grown as fodder crops, and grain cultigens (see Table 3).

Table 3. Material Used in Pilot Study.

No.	Species	Section	Life Period		Testa Texture		Testa Colour		Use
			annual(a)/	perennial(p)	rough(r)/	smooth(s)	light/	dark(1-5)	
1	<u>Lathyrus annuus</u>	Lathyrus	a	r	3				wild/fodder
2	<u>L.aphaca</u>	Aphaca	a	s	3				wild
3	<u>L.cicera</u>	Lathyrus	a	s	3				wild/fodder
4	<u>L.clymenum</u>	Clymenum	a	s	3/4				wild/feed
5	<u>L.hirsutus</u>	Lathyrus	a	r	3				wild/fodder
6	<u>L.inconspicuus</u>	Linearicarpus	a	s	2				wild
7	<u>L.laxifolius</u>	Pratensis	p	s	4				wild
8	<u>L.niger</u>	Orobus	p	s	3/4				wild
9	<u>L.nissolia</u>	Nissolia	a	r	2				wild
10	<u>L.ochrus</u>	Clymenum	a	s	3				wild/feed
11	<u>L.pratensis</u>	Pratensis	p	s	5				wild
12	<u>L.sativus</u>	Lathyrus	a	s	1/2				cultigen
13	<u>L.sylvestris</u>	Lathyrus	p	s	4				wild

#### 3.2.2. The main project.

The main project was designed to examine seeds of all four major genera in the Viciaeae. Material from the fifth genus, Vavilovia, represented by a single wild species, and outside the main scope of this study, was unavailable during the

research period.

As with the pilot study, the seeds were taken from species from a wide range of types, wild and cultivated, annual and perennial. All taxa used are Old World in distribution, specimens being supplied from collections made in South West Asian countries including Israel, Jordan, Syria and Turkey, and the Indian subcontinent, as well as Europe with Britain, and North Africa. Many seeds are the progeny grown on in this country of foreign reference seed collections. Most specimens are samples from vouchered accessions. Some seeds of highly-developed modern cultigens were commercially obtained. All the seeds have had their identifications carefully checked. Several populations of some taxa have been studied in order to record phenotypic variations in morphology and anatomy. The seeds are listed with population codings in Tables 4 and 5 at the end of the chapter.

The taxa selected for this study include all the major cultivated pulse crop species in the Viciaeae, their progenitors where known, or most of what are believed to be their closest wild relatives, the weedy species in the Viciaeae which are commonly associated with these crops, species that are now cultivated as animal feed and fodder, and wild species. All the lentil taxa and most of the pea taxa have been studied. The species that have been omitted from the project are thus wild and mainly those of Vicia and Lathyrus. The wild species that have been included have been



selected on the basis of their ready availability and Old World distributions, their close relationship with major cultigens, their membership of a range of taxonomic sections, and their different morphologies. Those taxa that have been represented by a number of populations have been selected largely because of their ready availability and range of gross morphologies.

No records are available of the number of seeds per pod, pods per plant or individual plants that have contributed to the seed specimens employed in this project. The components of variation that have been recorded throughout this survey thus reflect the general variability within a population.

### 3.2.3. Storage conditions for reference collections of seeds.

The seeds were stored either at room temperatures in paper envelopes, or stoppered glass containers; or under cool refrigeration in stoppered glass containers.

### 3.2.4. The archaeological material.

To establish the relevance of the data from fresh material to the archaeological remains of legume seeds, investigation of the latter formed an important part of the project. Charred pulses were supplied from sampled storage and burial contexts that had been excavated on seven archaeological

sites; they have formed the archaeobotanical study material (Table 6). The legume seeds, with a single exception, were supplied with identifications to species level, or to cf. species, that had been made using conventional criteria of the gross morphology, and from circumstantial evidence. Reference has been made to these ancient seeds by these identities; and on that basis, the specimens were selected as representatives of the main pulse crops. The seed of one taxon had been identified only to tribal level, as a seed from a member of the Viciaeae. The validity of the identifications is investigated in the following chapter 4.

### 3.3. Methods.

#### 3.3.1. Light microscopy.

##### 3.3.1.1. Reflected light microscopy.

The morphology of modern seeds was examined with the naked eye, and at low magnifications with a x10 hand lens, and using a Wild M8 zoom stereoscopic microscope, providing magnifications up to x50.

##### (i) Examination of seed external morphology.

Observations were made of the seed surface of five specimens from each population. The colour and texture of the testa were recorded. Using an eyepiece graticule, measurements were made of the length and the maximum width of the hilum,

and the distance between the centre of the lens and the nearest edge of the hilum. Using a drawing tube attachment to the microscope, drawings were made of the outline of each specimen at the maximum circumference of the seed, including the whole length of the hilum. Calculations of the hilum length expressed as a percentage of the circumference were made on these scale drawings, using a digitiser, with a program written at the Department of Pure and Applied Biology, Imperial College London.

(ii) Examination of embryo morphology.

Initially, observations were made on two specimens from each population. The seeds were soaked in water for thirty minutes. The softened testa was removed. One cotyledon was broken from the embryo, exposing the plumule, and the angle between the plumule and the radicle was measured. Following the observation that characteristically this angle is an approximate right angle, and that no consistent departure has been recorded from this, this procedure was discontinued. Similarly the length of the radicle and plumule were initially recorded. However, in stored mature seeds it was noted that frequently there is evidence of the start of germination, with a change in dimensions of the embryonic components. Since archaeological legume seed material is often in this state, it was decided that to rely upon radical or plumule length as potentially diagnostic characters might prove misleading. Thus these observations were also discontinued.

3.3.1.2. Transmitted light microscopy.

The anatomy of the tracheid bar beneath the hilum, and of certain features of the testa were examined in seeds of some species of Lathyrus. Serial sections made transversely across the hilum were stained. The species were selected as representatives with different gross morphologies (seed shape and size, and hilum length) and being both wild and cultivated taxa.

(i) Preparation of thin sections.

Transverse sections of the hilum.

Whole seeds of Lathyrus pratensis, L.sativus, L.cicera and L.clymenum were soaked in water for twenty four hours. The testa was removed and a rectangular portion including the entire hilum was excised. This was mounted on a Frigistor freezing stage in a dilute solution of gum acacia (approximately 2%), and using a Reichart sledge microtome, the blade having been set at an angle of 17, sections at right angles transversely across the hilum were cut at a thickness of 30um.

Longitudinal sections through the testa.

Similarly thin sections across the testa were cut at a thickness of 20um. in L.pratensis.

(ii) Histological staining techniques.

The thin sections were stained by methods developed and practised in the Jodrell Laboratory, Royal Botanic Gardens, Kew (Alvin, pers. comm.).

Safranin and alcian blue.

To wet sections were added one drop of safranin, at a concentration of 1% in 30% alcohol, and two drops of a 4% aqueous solution of alcian blue. After five minutes staining, immersion in 50%, 70% and absolute alcohol in ascending concentrations removed excess stain and dehydrated the sections. Preparations were made on slides in the mountant "Euparal", and subsequently the slides required drying for six to eight weeks to become permanent. The tracheid bar and parenchyma stain blue, and the malpighian cells stains red, while the hourglass cells remain unstained.

Phloroglucinol and hydrochloric acid.

Sections were placed in phloroglucinol solution for five minutes, acidified with a drop of concentrated hydrochloric acid and mounted in dilute glycerol, making temporary preparations. The tracheid bar stains pink. Staining by this method is transitory, and thus photographic records were made of slides prepared by this technique.

#### Sudan IV.

Sections were placed in Sudan IV solution for five minutes and then cleared in 70% alcohol and mounted in glycerol making temporary preparations. The cuticle stains red.

#### (iii) Anatomical observations.

Observations were made of the stained sections at magnifications of between x50 and x400 using a Gillet & Siebert trans-illuminating microscope. A permanent record of the impermanently-stained sections was obtained by photography with a microscope-mounted Riechart KAM ES camera system using Ektachrome daylight film (100 ASA) with a Wrattan 80B filter.

#### 3.3.2. Scanning electron microscopy (SEM) of fresh seeds.

##### 3.3.2.1. Examination of the external micromorphology of the testa.

The seeds generally were mounted for scanning without prior treatment. Some workers have recommended dipping in absolute ethanol to prepare the surface (Lersten & Gunn, 1982; Roti-Michelozzi & Serrato-Valenti, 1986), but because of the need to preserve details of the micromorphology with the minimum of possibility of creating artifacts, and the risk of distortion following dehydration

of the seed, this was not practised. However the following treatments were carried out on some specimens to clarify the nature of certain features, and to facilitate some observations.

(i) Preparation of testa surface.

Ultrasound.

In some populations the seeds are covered in a variety of debris adherent to the testa surface, obscuring the micromorphological characters. This debris has been removed by immersion in distilled water containing a wetting agent (a drop of detergent), and placing in an ultrasonic bath for thirty seconds. After rinsing in water the seeds are air-dried. This procedure does no apparent damage to the seeds; it removes fungal hyphae and most surface contamination, including the seed dressing that had covered one commercially-obtained sample of Vicia faba, though in this case an extended treatment period of sixty seconds has been required.

Enzymic digestion.

In some populations the seeds have a surface deposit that resists the above treatment and appears to be organic in nature; it has been suggested that this derives from the pericarp of the pod, or that it is related to the activities of a pathogen, prior to dehiscence (Butler, in press,a).

After the method of Lester & Durrands (1984), a brief immersion in a dilute solution of a proteolytic enzyme (biological detergent) removes the surface contamination that obliterated topographic detail on the seeds, which, following washing in water and air-drying, show no apparent resultant damage or artifact.

#### Treatment with wax solvents.

In two species the seed surface was seen under SEM to be covered with crystalline plate-like or rod-like deposits. To investigate the nature of these deposits, whole seeds of Lathyrus ochrus and L.inconspicuus were dipped in the organic solvents chloroform and ether, mixed in proportions of one-to-one by volume (Baker, 1982). Subsequent scanning showed that this treatment dissolved the deposits, on the basis of which they were identified as cuticular waxes (Alvin, pers. comm.).

#### (ii) Mounting the specimen for SEM.

The whole seeds were mounted on Cambridge aluminium stubs using a water-based adhesive, Durafix or UHU. These glues retain a slight plasticity when dry, which accommodates the differential stresses which may result when the living hygroscopic legume seeds take up atmospheric water after mounting and prior to scanning. Hard-setting glues, such as araldite, or conducting cements, such as carbon or silver dag, which are commonly recommended as mountants, are



brittle when dry and have proved less satisfactory (Butler, 1988). Trails of silver dag (Acheson Electrodag 915) were applied from the seed surface to the edge of the stub, across the adhesive to maximise the electrical contact, and minimise the likelihood of charging (Brisson & Peterson, 1976).

The seeds were mounted to show the length of the hilum in three-quarter view, with the testa adjacent to the hilum and at the midseed region readily visible. Where the hilum is too long to be visible along its whole length at the lowest magnification that can be achieved, the micropyle end has been exposed. Where the seeds are exceptionally large, as in Vicia faba and cannot fit on a stub, the seed has been bisected in a plane parallel with the hilum, and the two portions have been mounted separately.

#### 3.3.2.2. Examination of testa and hilar anatomy.

(i) Preparation and mounting of pressure-fractured specimens.

To expose the tissues of the hilum and associated structures, and to reveal the structure of the testa layers, seeds were bisected transversely across the hilum by pressure fracture, using a heavy blade. Usually only one of the two resultant faces proved suitable for scanning. The half seeds were mounted with the fractured surface parallel with the surface of the stub and uppermost, using the same

procedures as described for whole seeds.

(ii) Preparation and mounting of thin sections.

Thin sections of the testa and the tracheid bar were cut as described above (3.3.1.b.(i)). Wet sections were floated on to Cambridge stubs covered in double-sided adhesive tape. The sections were air-dried.

3.3.2.3. Coating the specimen.

The specimens were coated in gold with a water-cooled Polaron E5000 sputter coater to a depth of 30 to 40nm, applied at 9kV over an extended period of 12 minutes in order to achieve a small grain size to the coating.

3.3.2.4. Storage of SEM stubs.

The mounted specimens on stubs when coated in gold were fixed in blocks of polystyrene and stored in plastic boxes with snap lids over silica gel at room temperature. It is possible to rescan specimens stored in this way, provided that they are recoated in gold before each scanning. Two or even three sputter-coatings with gold to the same thickness do not appear to affect the observations made under the range of magnifications employed. All stubs were allowed to equilibrate with the environmental conditions of the cool, dry, basement SEM laboratory for at least forty minutes prior to coating and scanning. This precaution was practised

in order to minimise the possible effects of wide variations in temperature and relative humidity on such hygroscopic specimens as dry, fresh legume seeds.

#### 3.3.2.5. Scanning.

Scanning was carried out mainly on a PSEM500, at voltages of 12kV and 25 kV, and also on a JEOL35, at 12 kV, at magnifications between x12 and x10,000.

##### (i) Photography.

Electron-micrographs were taken on 35mm.PAN F film (50 ASA), developed in perceptol, or on 120mm.HP5 film (400 ASA), developed in ID11 developer, and printed on Kodak bromide glossy photographic paper, grades 2-4, of normal to extra-hard quality.

#### 3.3.2.6. Recording the characters.

Early in the survey it was found that some electronic aberration occurred at low magnifications in the PSEM500. Following tests on the instrument, it was established that at magnifications greater than x50 the distortion was insignificant. However to reduce to a minimum the possible effects of aberration, photographs were taken where possible from the same angles, and at standardised positions and magnifications. Micromorphological and anatomical features were recorded by qualitative descriptions of these electron

micrographs, and by direct measurement from the micrographs.

It is impossible to record by SEM the complete data from single seed specimens of each population; differently-mounted specimens necessarily have been used for observations of surface and internal seed features. However, for the final dataset, measurements made on single specimens have provided the data from external seed features, and separate single specimens for all internal testa characters.

### 3.3.3. Investigation of charred archaeological remains.

Central to this project is the establishment of the relevance of the characters recorded in fresh legume seeds to the charred remains of archaeological seed specimens. There are two requirements for this.

(i) Primarily, an examination of archaeological charred seed remains can determine the extent of deterioration and change in the micromorphology and anatomy of such material, by comparison with the modern fresh seeds. Thus throughout the study of fresh seeds, regular reference was made to archaeological specimens.

(ii) Secondly, an investigation into the effects of experimental heating on modern fresh seeds can produce a record of the changes in seed micromorphology and anatomy due to charring. Such a procedure may be used also to establish a standardised regime for the production of

charred specimens of modern seeds to be employed as comparatives for the identification of archaeological remains, which are routinely employed in archaeobotany.

#### 3.3.3.1. SEM of archaeological seed remains.

Specimens were carefully selected from samples of well-preserved archaeobotanical charred remains. Where possible whole seeds were used, which were judged by their appearance to the naked eye and at low magnifications, to have retained a high proportion of the original features of the gross morphology. Thus the seed shape and size could be observed and the hilum was retained together with at least some of the testa adjacent to the hilum and at midseed. Similar seeds also were bisected across the hilum, where there was sufficient material. In most archaeobotanical samples, however, it is rare to find many seeds still bearing the hilum, and because of the salient characters that hila carry, it is desirable to preserve these intact. Fortunately it was found that frequently amongst the charred seed remains the detached hila surrounded by a rim of testa have been preserved. These fragments of tissue usually bear most of the relevant micromorphological and anatomical features. The hila can be bisected transversely by pressure-fracture and mounted to show the same structures in the hilum region as are scanned in the bisected specimens of whole seeds.

All these specimens were mounted for SEM, coated and scanned as described for fresh seeds (paragraph 3.3.2.b)(ii)). Small

fragments of hilum and testa, which were very light, could alternatively be mounted with double-sided adhesive tape, which allows greater control in the positioning of the specimen than do the water-based glues.

### 3.3.3.2. Examination of charred fresh seeds.

#### (i) Introduction.

In view of the necessity of considering the effect of charring upon seeds when identifying charred ancient seed remains using modern comparatives, there is surprisingly little literature on the subject. Hopf (1955;1975) examined grain size in wheat and barley heated to 220°C from four to six hours; and she also experimented on barley with temperatures ranging between 50 and 250°C for ten hours. Bowman (1966) charred wheat and barley over a temperature range from 150 to 500°C for up to 16 hours. He found that charring between 250 and 300°C for one to 16 hours could produce grains with distortions similar to those seen in charred ancient cereals. Wilson (1984) is one of the few people who have recorded the effects of charring pulses; she charred lentils and peas in both wet and dry states, in soil or "solo", using temperatures of 250 to 550°C for 16 to 30 hours; the numbers of seeds that survived under these different regimes was recorded, rather than their morphological state. Cope & Chaloner (1985) and Prior & Alvin (1983) have examined the effects of different temperatures on the histology and chemistry of wood tissues.

Charles & Colledge (pers. comm.) have undertaken charring experiments on cereals, and produced comparatives for detailed morphological and micromorphological study of charred ancient cereal remains using a temperature of 235C for 16 hours. This work has formed the basis for the following experiments to find a suitable regime to produce comparatives for the remains of charred ancient pulses.

(ii) Charring experiments.

Experimental charring was carried out in a Gallenkamp Hotspot furnace, with a temperature range up to 1000C, using a number of regimes. The performance of the muffle furnace dictated the lowest temperature employed. The highest temperature applied was 400°C, under which conditions the seeds char within one hour, and tend to fragment.

A number of procedures were used to investigate the effect of different heating conditions. Some samples were initially brought to constant weight by heating for 24 hours at 100°C. These were stored in a desiccator prior to charring. The effects of a gradual increase to maximum temperature, and a gradual cooling to room temperature after charring, were recorded. Reduction in the availability of oxygen during charring was achieved either by covering in foil the tubes containing the seeds, or by burying the seeds in sand during charring. The different experimental charring conditions are listed in Table 7.

Table 7. Experimental Charring Conditions.

Temperature °C	Heating Period (hours)	Pre-treatment at 100°C for 24 hours	Slow Heating	Slow Cooling	Availability of oxygen
235	16	x	0	0	in open tubes
235	16	x	x	0	in open tubes
235	16	x	0	x	in open tubes
235	17	x	x	x	under sand
400	1	x	0	x	under sand
320	4	x	0	0	under sand
300	2	x	0	0	foil-wrapped
300	2	0	0	0	in open tubes

The results were recorded by naked eye and also by SEM. Refinements to the charring methods, such as the gradual increase to temperature, and the prior drying of seeds to constant weight, have had little effect upon the end-result, as observed by the techniques used in this project, when applied with the conditions described.

The regime selected as a standardised procedure for subsequent use in the preparation of comparatives was heating at 300°C for two hours in open tubes. When charred under these conditions, modern legume seeds compare well, both in gross morphology and in micromorphology and anatomy, with well-preserved charred archaeological legume seeds.

(iii) SEM of charred comparatives.

The charred pulses were mounted for SEM either as whole seeds or bisected; they were coated and scanned following the same procedures that were used for fresh seeds.



3.3.3.3. Electron spin resonance (ESR) of charred seeds, both modern and ancient, to determine thermal histories.

ESR has been carried out on some ancient charred pulses. The results do not appear to be plausible. They are discussed at this stage to explain why the technique has not been pursued further.

Following the pioneer work of Hillman et al. (1985) on the application of ESR to determine the thermal histories ancient cereal grains, ESR of pulses has been undertaken for a similar purpose by Dr. Keith Sales and his team at the London University ESR Unit, in the Chemistry Department, Queen Mary College London.

(i) Calibration curves from charred modern seeds.

Fresh seeds of both Lens culinaris (4 populations) and Pisum sativum sativum (3 populations) were charred at temperatures ranging between 100°C and 400°C, at intervals of 50°C. ESR was carried out on these charred seeds and the manganese and organic carbon signals were analysed, and displayed graphically to provide calibration curves for each species.

(ii) ESR analysis of ancient pulses.

Similar analyses were carried out on ancient charred peas from Catal Huyuk, and ancient charred lentils from Mycenae. Thermal profiles of the ancient seeds were derived by

calibration of the data with the curves produced from the modern material.

(iii) Results of ESR of ancient pulses.

The maximum temperature of previous heating (MTPH) in antiquity was calculated to be approximately  $210^{\circ}\text{C}$  for the peas, and  $430^{\circ}\text{C}$  for the lentils. The appearance of these ancient seeds, both to the naked eye, and under SEM, appears similar to that of modern seeds charred at a temperature of about  $300^{\circ}\text{C}$ ; although the time period for the heating episodes in antiquity cannot be ascertained, and thus there is uncertainty about their effects,  $210^{\circ}\text{C}$  seems to be too low a temperature to have produced the changes observed in the ancient peas;  $430^{\circ}\text{C}$  conversely appears too high a temperature for the ancient lentils not to have effected greater change. It is suggested therefore that these calculations of thermal histories of ancient pulses using ESR are discrepant.

Some published results of ESR support this suggestion: charred emmer grains were recovered from the granaries at Mycenae, within the same context that contained the lentils; it can be assumed that both types of grain were charred during the same episode of conflagration in antiquity, and thus had been subjected to very similar charring conditions. The emmer has been analysed by ESR in the same laboratory, and the MTPH of  $250^{\circ}\text{C}$  has been calculated (Hillman et al., 1985), which is much lower than than the temperature of  $430^{\circ}\text{C}$  calculated for the lentils.

It is concluded that further work on the ESR of charred ancient seeds is required before this technique can be confidently applied to calculate the conditions of charring in antiquity.

The results of the research using the methods described above are described in the following chapter 4.

Table 4. Fresh Seed Material

(Numbers for each taxon given here are consistent throughout all tables, and are the same as the Operational Taxonomic Units (OTU's) 1-106 used in the multivariate analyses, and the dataset, Table 15.)

No.	Species	Population
1	<u>Vicia bithynica</u> (L.)L.	VIC 855/77
2	<u>V. cracca</u> L.	GCH/84
3	<u>V. ervilia</u> (L.)Willd.	GCH Jordan 85
4	" "	GCH 3761
5	" "	(R)
6	" "	770141P78
7	" "	790144P78
8	" "	790141P78
9	" "	770144P78
10	<u>V. faba</u> L. subsp. <u>faba</u> var. <u>minor</u> Beck	H/53 PBI
11	" "	EAB85 Banner
12	" "	van Zeist
13	<u>V. faba</u> L. subsp. <u>paucijuga</u> (Alef.) Murat	GCH
14	<u>V. lutea</u> L.	780361P80
15	<u>V. melanops</u> Sibth. & Smith	(R)
16	<u>V. narbonensis</u> L.	38Israel
17	<u>V. noeana</u> Reuter ex Boiss.	GCH/CMD 129
18	<u>V. orobus</u> DC	780276P83
19	<u>V. peregrina</u> L.	(R) 40
20	" "	808037P80
21	" "	VIC315/79
22	<u>V. sativa</u> L. subsp. <u>nigra</u> (L.) Ehr.	EABBlot

23	"	"	EABButser
24	<u>V.sativa</u> L.	subsp. <u>sativa</u>	Rifa-at
25	"	"	alba (R)
26	"	"	EABCrete
27	<u>V.sativa</u> L.	subsp. <u>sativa</u> var. <u>lentisperma</u>	lent.
28	<u>V.sepium</u> L.		EABBlet
29	"		780487P80
30	"		EAB17
31	<u>V.sylvatica</u> L.		780254P80
32	<u>V.tetrasperma</u> (L.)	Schreber	GCH/84
33	"	"	EAB11
34	<u>V.villosa</u> Roth	subsp. <u>varia</u> (Host)	Corbiere 800026P81
35	"	"	780369P81
36	<u>Lathyrus annuus</u> L.		790196P83
37	<u>L.aphaca</u> L.		780007P80
38	"		835006
39	<u>L.cicera</u> L.		790523P80
40	"		K395/G
41	"		(R)
42	<u>L.clymenum</u> L.		790539P83
43	"		790524P80
44	<u>L.heterophyllus</u> L.		(R)
45	<u>L.hirsutus</u> L.		790332P80
46	<u>L.inconspicuus</u> L.		790204
47	<u>L.laxiflorus</u> (Desf.)	Kuntze	780347P81
48	<u>L.niger</u> (L.)	Bernh.	780051P80
49	"	"	780052P82
50	<u>L.nissolia</u> L.		790498P80
51	"		780061P78

52	"	780061P80
53	<u>L.ochrus</u> (L.) DC	780349P80
54	"	K319/4
55	<u>L.pratensis</u> L.	Muswell Hill
56	"	770131P82
57	"	780566P80
58	"	N.Ireland
59	<u>L.sativus</u> L.	GCHGrassPea
60	"	Gelveri
61	"	127BB
62	"	Rifa-at
63	<u>L.sylvestris</u> L.	780019P79
64	"	780019P80
65	"	780567P80
66	"	EAB19
67	<u>L.tuberosus</u> L.	780393P80
68	<u>Pisum sativum</u> L.subsp. <u>elatus</u> (MBieb.)Asch&Graebner	RMN786
69	"	RMN806
70	"	RMN821
71	<u>Pisum sativum</u> L.subsp. <u>sativum</u> var. <u>arvense</u> (L.)Poiret	B1545
72	"	Maple Pea
73	"	IndianHFS
74	"	PartridgePea
75	<u>Pisum sativum</u> L.subsp. <u>sativum</u> var. <u>sativum</u>	Whiteseeded
76	"	soupmix
77	"	jomardii
78	<u>Lens culinaris</u> Medik.	UK
79	"	greenCardiff
80	"	Canada

81	"	"	WIR1957
82	"	"	WIR1386
83	"	"	WIR2387
84	"	"	WIR2110
85	<u>Lens ervoides</u>	(Brign.) Grande	770118P80
86	"	"	L28
87	"	"	L32
88	"	"	L90
89	"	"	WIR2221
90	<u>Lens nigricans</u>	(M.Bieb.) Webb & Bert	780247P80
91	"	"	L59
92	"	"	L62
93	"	"	L65
94	"	"	L68
95	"	"	WIR2365
96	<u>Lens odemensis</u>	Ladizinsky	L36
97	"	"	L37
98	"	"	L38
99	"	"	L112
100	"	"	L170
101	<u>Lens orientalis</u>	(Boiss.) Sch	770119P80
102	"	"	L22
103	"	"	L26
104	"	"	L40
105	"	"	L130
106	"	"	WIR2290

Table 5. Fresh Seed Material Used for SEM only

(Species numbers are the same as those in the dataset, Table .)

No. Species	Population
107 <u>Lathyrus blepharicarpus</u> Boiss.	867490
108 " "	868173
109 <u>L.cicera</u> L.	867117
110 <u>L.gorgoni</u> Parl.	867476
111 <u>L.pseudocicera</u> Pampan.	868023
112 " "	867563

Table 6. Charred Archaeological Seeds Used for SEM only

No. Species	Period	Site
113 <u>Vicia ervilia</u>	Bronze Age	Assiros, Greece
114 <u>Pisum sativum elatius</u>	Bronze Age	Çatal Hüyük, Turkey
115 <u>Pisum sativum sativum arvense</u>	Bronze Age	Çatal Hüyük, Turkey
116 <u>Lens cf. culinaris</u>	Iron Age	Gordion, Turkey
117 Viciaeae sp.	Iron Age	Gordion, Turkey
118 <u>Pisum sativum sativum arvense</u>	Bronze Age	Manerba, Italy
119 <u>Vicia faba</u>	Bronze Age	Pella, Jordan
120 <u>Lathyrus cf. sativus</u>	Bronze Age	Servia, Greece
121 <u>Pisum sativum sativum arvense</u>	Bronze Age	Tell Brak, Iraq



## 4. RESULTS.

### 4.1. Introduction.

This study comprises a survey of the morphology and micromorphology of fresh seeds of members of the tribe Viciaeae. As described above (section 3.2.1.), the main survey was preceded by a pilot study of seeds from species of Lathyrus, listed in Table 3. The results of this preliminary work have been incorporated in the main survey.

The primary data consist of observations made at magnifications up to x10,000 as described above, and recorded as a series of electron micrographs. The plates (volume 2) are representative examples of these observations, and have been selected to illustrate the characters recorded in the dataset as well as to show some of the basic anatomy that has been reported by other workers. Initially the interpretations of these observations have been purely subjective, and are the basis of qualitative descriptive records of the taxa investigated. These are presented as an atlas, with reference to the plates and comprise the first part of this chapter (4.2).

The second part consists of a summary of the range of features that has been observed (4.3).

The third part describes the changes in the morphology and micromorphology of seeds after charring (4.4); this is followed by observations on charred archaeological material (4.5).

4.2. The morphology, micromorphology and anatomy of the seeds of the Viciaeae: the qualitative results of SEM.

4.2.1. An atlas of morphological, micromorphological and anatomical characters of seeds of the Viciaeae, with notes on the range of variation.

4.2.1.1. Introduction.

The morphological, micromorphological and anatomical characters of the fresh seeds observed under SEM, and recorded on micrographs, are described below for each taxon, with a note of the number of populations of each that have been studied. Variations between the characters of different populations of the same species are recorded.

The characters described are by no means an exhaustive list. An attempt has been made to confine this study to features that are particularly diagnostic for the identification of taxa, and that are likely to be observable in charred tissue. The criteria selected therefore have largely been those that (i) are considered to be potentially useful in legume classification

(Gunn,1970,1981; Prakash,1987; Stirton,pers.comm), (ii) have been recorded in recent publications on testa morphology and anatomy in legumes (Lersten,1979; Lersten,1981; Lersten,1982; Lersten & Gunn,1982), (iii) have been investigated in relation to legume physiology (Marbach & Mayer,1975; Werker et al.,1979) in fresh material, and (iv) that are also known to be observable in charred archaeological specimens (Hopf,1986). Thus emphasis has been laid on the external testa topography, the hilum and its associated features, particularly the tracheid bar, and the dimensions of the cell layers in the testa.

For each taxon in this study descriptions have been made of (i) the gross morphology, which includes the shape of the seed in outline and in transection, the shapes of the hilum and micropyle, and the position of the lens relative to the hilum. These are followed by (ii), general descriptions of the testa topography at standardised positions on the seed; these positions are (a) immediately adjacent to the hilum, and (b) approximately at the seed "equator", lateral to the hilum; this latter position is termed "midseed" throughout the thesis. (iii) The internal micromorphology of the seed is then described, primarily in the hilar region, including the profile of the hilum in transection, the shape of the tracheid bar in section, and some details of tracheoid anatomy. (iv) Finally the dimensions in longitudinal surface of the three testa

layers are considered.

Where populations with particularly dark or light-coloured testas have been studied, attention is drawn to characters that might be expected to be correlated with such testa colours, as potential indicators of particular physiological properties.

Certain features of more general botanical interest have also been recorded and are described.

The hilum gross morphology has been classified into five categories based on length relative to the seed circumference, and shape, in accordance with the criteria of Gunn (1970).

Topographical descriptions of the testa surface have been rendered as simply as possible, rather than using specialist terminology, as employed by Lersten (1981), which seems open to some confusion by subjective interpretation. Surface patterns produced by the topography of individual palisade cell walls are termed "primary patterning". Rugosities of the testa surface, which are visible to the naked eye, or under low magnifications, and caused by zonal elongations of groups of palisade cells, are termed "secondary patterning", or "mounds", after Lersten (1981).

The "distribution" of the testa pits refers to the

evenness of their density, as even or uneven; the "arrangement" of pits refers to the patterns of their distribution, which may be regular, either opposite or alternate, or irregular, using the terminology of Stirton (pers.comm).

The term "aetiology" is used when reference is made to the assignment of a cause for the manifestation of a feature.

It must be emphasised that the data recorded here apply to the seed specimens taken from the particular populations studied and are not necessarily representative of the total range of variation that can occur in each taxon. It is appreciated that a wider range of variation may occur in certain tissues and features than has been observed in this project, and this must be borne in mind when comparing these results with any others.

The genera below are listed in the conventional taxonomic sequence (Davis, 1970; Townsend & Guest, 1974). Species within genera have been listed alphabetically, for greater ease of reference (as in the plates, volume 2).

Throughout the following descriptions, the numbers of illustrative plates and figures contained in volume 2 are cited. Table 8 lists the species that are illustrated and their plate numbers (which is repeated as Table 20 in

Volume 2 with the legends, for ease of reference).

Table 8. List of Species Illustrated in the Plates.

Plate Number		Facing Page Number
1.	<u>Vicia bithynica</u>	489
2.	" "	490
3.	<u>V. cracca cracca</u>	491
4.	" "	492
5.	<u>V. ervilia</u>	493
6.	" "	494
7.	" "	495
8.	<u>V. faba</u>	496
9.	" "	497
10.	" "	498
11.	<u>V. lutea</u>	499
12.	" "	500
13.	<u>V. melanops</u>	501
14.	" "	502
15.	<u>V. narbonensis</u>	503
16.	" "	504
17.	<u>V. noeana</u>	505
18.	" "	506
19.	<u>V. orobus</u>	507
20.	" "	508
21.	<u>V. peregrina</u>	509
22.	" "	510
23.	" "	511
24.	<u>V. sativa nigra</u>	512
25.	" "	513
26.	<u>V. sativa sativa</u>	514
27.	" "	515
28.	" "	516
29.	<u>V. <sup>sp.</sup>sativa <sup>syn.</sup>sativa <sup>var.</sup>lentisperma</u>	517
30.	<u>V. sepium</u>	518
31.	" "	519
32.	<u>V. sylvatica</u>	520
33.	" "	521
34.	<u>V. tetrasperma</u>	522
35.	" "	523
36.	<u>V. villosa varia</u>	524
37.	" "	525
38.	<u>Lens culinaris</u>	526
39.	" "	527
40.	" "	528
41.	<u>L. ervoides</u>	529
42.	" "	530
43.	<u>L. nigricans</u>	531
44.	" "	532
45.	<u>L. odemensis</u>	533
46.	" "	534
47.	<u>L. orientalis</u>	535
48.	" "	536

49.	<u>Lathyrus annuus</u>	537
50.	" "	538
51.	<u>L. aphaca</u>	539
52.	" "	540
53.	<u>L. blepharicarpus</u>	541
54.	<u>L. cicera</u>	542
55.	" "	543
56.	<u>L. clymenum</u>	544
57.	" "	545
58.	" "	546
59.	<u>L. gorgoni</u>	547
60.	<u>L. heterophyllus</u>	548
61.	" "	549
62.	<u>L. hirsutus</u>	550
63.	" "	551
64.	<u>L. inconspicuus</u>	552
65.	" "	553
66.	<u>L. laxiflorus</u>	554
67.	<u>L. niger niger</u>	555
68.	" "	556
69.	<u>L. nissolia</u>	557
70.	" "	558
71.	<u>L. ochrus</u>	559
72.	" "	560
73.	<u>L. pratensis</u>	561
74.	" "	562
75.	<u>L. pseudocicera</u>	563
76.	<u>L. sativus</u>	564
77.	" "	565
78.	<u>L. sylvestris</u>	566
79.	" "	567
80.	<u>L. tuberosus</u>	568
81.	" "	569
82.	<u>Pisum<sup>sp.</sup> sativum<sup>subsp.</sup> elatius</u>	570
83.	" " " "	571
84.	<u>Pisum<sup>sp.</sup> sativum<sup>subsp.</sup> sativum<sup>var.</sup> arvense</u>	572
85.	" " " "	573
86.	<u>P.<sup>sp.</sup> sativum<sup>subsp.</sup> sativum<sup>var.</sup> sativum</u>	574
87.	" " " "	575
88.	<u>Vicia ervilia</u> , archaeological	576
89.	<u>Pisum<sup>sp.</sup> sativum<sup>subsp.</sup> elatius</u> "	577
90.	" " " "	578
91.	<u>P.<sup>sp.</sup> sativum<sup>subsp.</sup> sativum<sup>var.</sup> arvense</u> "	579
92.	<u>Lens cf. culinaris</u> and Viciaeae species "	580
93.	" " " "	581
94.	<u>Pisum<sup>sp.</sup> sativum<sup>subsp.</sup> elatius</u> "	582
95.	<u>Vicia faba</u> "	583
96.	<u>Lathyrus<sup>sp.</sup> sativus</u> "	584
97.	<u>Pisum<sup>sp.</sup> sativum<sup>subsp.</sup> sativum<sup>var.</sup> arvense</u> "	585



4.2.1.2. Descriptive results of the microscopic survey.

Vicia.

Vicia bithynica. (Plates 1,2. Number of populations studied: 1)

i. Gross seed morphology. The seeds are subrectangular in shape, with a smooth testa and prominent lens (Plate 1, Fig.1). The hilum is oval-shaped (Plate 1, Fig.2), and the micropyle is small and oval (Plate 1, Fig.3).

ii. Micromorphology of the testa surface. The testa surface is papillose; each acute papilla occupies the entirety of the external surface of each palisade cell. Fine even ridges radiate from the apex of each papilla with some confluence between the ridges of adjacent papillae. Some regions of the testa have areas where compression has obscured the outline of individual papillae (Plate 1, Fig.4). The papillae on palisade cells adjacent to the hilum (Plate 1, Fig.5,6) are slightly more acute than those on cells lateral to the hilum (Plate 1, Fig.7,8).

iii. Micromorphology of the internal hilar region. The lips of the hilum do not protrude and in transverse plane have an even, level surface (Plate 2, Fig.1). The tracheid bar is seven tracheoids wide (Plate 2, Fig.2). Tracheoid pits are evenly distributed in a regular manner (Plate

2, Fig. 3), having both alternate and opposite arrangements. The pits vary from oval to subrectangular in shape, as do the pit apertures. The pit borders are plain, warty or slightly vestured (Plate 2, Fig. 4-6).

iv. Micromorphology of the internal testa layers. The palisade cells are of uniform length over the seed (Plate 2, Fig. 6-8). Hourglass cells are short and barely discernible in longitudinal plane lateral to the hilum, at midseed and at the opposite pole to the hilum. The remnant layer is thick.

Vicia cracca. (Plates 3, 4. Number of populations studied: 2)

i. Gross seed morphology. The seeds are oval, both in outline and in section, with a smooth testa (Plate 3, Fig. 1; Plate 4, Fig. 1). The hilum is oblong, and the micropyle is oval (Plate 3, Fig. 1, 2).

ii. Micromorphology of the testa surface. The testa surface is papillose. Variation in detailed testa topography is present in specimens from a single population. The papillae may be moderately acute and ridged, occupying the entire external surface of the palisade cells, and with some ridges being confluent between adjacent papillae (Plate 3, Fig. 3, 4). Alternatively, the papillae may be low and confined to the central area of the external surface of each palisade

cell. Here, ridging outlines the cell walls, varying in prominence, and tending to be most prominent in positions lateral to the hilum (Plate 3, Fig. 5-7). Some specimens have areas of testa with a range of cell sizes and topographies (Plate 3, Fig. 8). The palisade cells may be overlaid by a deposit (Plate 3, Fig. 9).

iii. Micromorphology of the internal hilar region. The lips of the hilum are slightly protruding (Plate 4, Fig. 2). Three tracheoids occupy the width of the tracheid bar (Plate 4, Fig. 3). The tracheoid pits are unevenly distributed in an irregular manner (Plate 4, Fig. 4). They are oval or round in shape with slit-shaped or oval apertures. The pit borders are plain, warty and vestured (Plate 4, Fig. 5, 6).

iv. Micromorphology of the internal testa layers. The palisade cells are even in height over the seed. Hourglass cells, although long adjacent to the hilum, are very short over most of the seed. The remnant layer is thin (Plate 4, Fig. 7-9).

Vicia ervilia. (Plates 5, 6, 7. Number of populations studied: 7).

i. Gross seed morphology. The seeds are angular to rounded and triangular-isodiametric in shape (Plate 5, Fig. 1; Plate 6, Fig. 1), with a smooth testa. The hilum is oval to wedge-shaped, and the micropyle is oval to

round (Plate 5, Fig,2,3; Plate 7, Fig 4).

ii. Micromorphology of the testa surface. The testa surface is papillose. Acute papillae occupy most of the external surface of the palisade cells, with prominent ridges running from the apex of each papilla and interlocking with those from adjacent cells (Plate 5, Fig.4-7). There is variation in thickness of the ridges, which tend to be narrower on cells nearer the hilum (Plate 5, Fig.5,7). The papillae on cells in the same region may vary in height and definition in seeds from different populations (Plate 5, Fig.6,8).

iii. Micromorphology of the internal hilar region. The lips of the hilum do not protrude (Plate 6, Fig.2). The tracheid bar is embedded in deep parenchymatous tissue (Plate 6, Fig.2). The tracheid bar is 4 to 6 tracheoids wide (Plate 6, Fig.3). The tracheoid pits are usually evenly distributed in an irregular manner, and may be slit, oval, round or subrectangular in shape, with a similar variety in shape of pit aperture. The pit borders may be plain, warty or vestured, and may show remnants of the primary cell wall (Plate 6, Fig.4,5).

iv. Micromorphology of the internal testa layers. The palisade cells are moderate in height. The hourglass cells show relatively small increases in length adjacent to the hilum (Plate 6, Fig.4,5).

Vicia faba. (Plates 8,9,10.) Vicia faba ssp.faba var. minor. (Number of populations studied: 3) Vicia faba ssp.paucijuga. (Number of populations studied: 1).

i. Gross seed morphology. The seeds are subrectangular in outline, with a smooth testa. The hilum is wedge-shaped (Plate 8, Fig.1) and, uniquely in the Viciaeae, situated along the shorter edge of the seed. The micropyle is slit-shaped to triangular (Plate 8, Fig.2).

ii. Micromorphology of the testa surface. The testa surface is smooth, with the external surfaces of the palisade cells slightly domed and often slightly undulating (Plate 8, Fig.3). Each such domed cell wall may have a reticulate surface, formed from ridges which are either indistinct and ill-defined (Plate 8, Fig.6), or more prominent (Plate 8, Fig.8) in both subspecies studied. In some seed specimens of the subspecies, V.faba paucijuga, testa pits have been recorded.

iii. Micromorphology of the internal hilar region. The hilum is slightly sunken beneath the testa surface and the hilar lips do not protrude (Plate 9, Fig,1). The tracheid bar is large and may occupy virtually the full depth between the hilar slit and the cotyledon surface (Plate 9, Fig.2; Plate 10, Fig,1). The tracheoids are evenly and regularly pitted in a mainly alternate arrangement (Plate 9, Fig.3). The pits are slit, oval, round and subrectangular in shape, with similar shapes of

pit aperture. The pit borders are plain, warty and vestured (Plate 9, Fig. 4, 5; Plate 10, Fig. 3, 5).

iv. Micromorphology of the internal testa layers. The palisade layer is of moderate depth (Plate 10, Fig. 6, 8) in both subspecies. The hourglass cells are long adjacent to the hilum, and are also relatively long lateral to the hilum at midseed and lateral to the hilum; the remnant layer is also thick (Plate 9, Fig. 6-8; Plate 10, Fig. 7). The pits recorded on the surface of the testa have not been observed in section.

Vicia lutea. (Plates 11, 12. Number of populations studied: 1)

i. Gross seed morphology. The seeds are obovate in outline, with a slightly rugose to smooth testa, The hilum is oblong, and the micropyle is oval in shape (Plate 11, Fig. 1, 2).

ii. Micromorphology of the testa surface. The testa surface is papillose and variably undulating, with reduced secondary patterning (Plate 11, Fig. 3-5; Plate 12, Fig. 5). The papillae are low and ridged with some intercalation of ridges and confluence of ridges between adjacent papillae (Plate 11, Fig. 7, 8). There is some distortion of papillae and variation in papillar height relative to the position upon the undulations (Plate 11, Fig. 6).

iii. Micromorphology of the internal hilar region. The hilar lips do not protrude (Plate 12, Fig.1). The tracheid bar is six tracheoids wide (Plate 12, Fig.2). The tracheoids are evenly pitted, but the pits are irregularly arranged (Plate 12, Fig.3,4). The pits are oval to round in shape, as are the pit apertures. The pit borders are plain or slightly warty.

iv. Micromorphology of the internal layers. The palisade cell layer is of moderate and slightly variable thickness over the seed surface (Plate 12, Fig.6,7,8). The hourglass cells are short adjacent to the hilum (Plate 12, Fig.6) and scarcely discernible lateral and opposite to the hilum (Plate 12, Fig.7,8). The remnant layer is easily visible at all positions on the testa.

Vicia melanops. (Plates 13,14. Number of populations studied: 1).

i. Gross seed morphology. The seed is oval to subrectangular in shape, with a smooth testa (Plate 13, Fig.1). The hilum is wedge-shaped, and the micropyle is slit-shaped to oval (Plate 13, Fig.3). The lens is prominent (Plate 13, Fig.1).

ii. Micromorphology of the testa surface. The testa surface is papillose, the papillae being more acute adjacent than lateral to the hilum (Plate 13, Fig.5,7). Rarely, the papillar ridges are confluent between

adjacent papillae.

iii. Micromorphology of the internal hilar region. The hilar lips do not protrude. The tracheid bar occupies most of the area between the hilar slit and the cotyledons (Plate 14, Fig. 1). The tracheid bar is three tracheoids wide (Plate 14, Fig. 2). The tracheoid pits are evenly distributed and regularly arranged in an alternate manner. The pits vary from slit-shaped, oval and round to subrectangular in shape, as do the pit apertures. The pit borders are predominantly warty, but may also be plain or vestured (Plate 14, Fig. 3, 4).

iv. Micromorphology of the internal testa layers. Moderately thick palisade cells are of constant depth over the seed. The hourglass cells are long adjacent to the hilum (Plate 14, Fig. 5), and readily discernible lateral to the hilum at the midseed position (Plate 14, Fig. 6).

Vicia narbonensis. (Plates 15, 16. Number of populations studied: 1).

i. Gross seed morphology. The seed is subrectangular in shape, with a smooth testa. The hilum is oval and the micropyle is slit-shaped. The lens is large and prominent (Plate 15, Fig. 1-3).

ii. Micromorphology of the testa surface. The testa



surface is papillose with blunt papillae occupying most of the palisade cell exterior wall. The ridges along the sides of the papillae intercalate with those of adjacent cells. The micromorphology is remarkably uniform; differences have not been observed between characters on different areas of the testa on the same seed, or between different specimens (Plate 15, Fig. 4-6).

iii. Micromorphology of the cotyledon surface. On either side of the embryonic axis are two kidney-shaped patches of highly enlarged cells, which represent the petioles of the cotyledons. These are visible in all seeds of the Viciaeae, and have been observed fortuitously under SEM during this study. They have not been routinely recorded in this survey, but one example is illustrated here for general botanical interest (Plate 15, Fig. 7, 8).

iv. Micromorphology of the internal hilar region. The hilum is flat in transection, without protruding lips. The tracheid bar is rounded and occupies most of the area between the hilar slit and the cotyledon surface (Plate 16, Fig. 1, 2). The tracheid bar has five tracheoids in its width. The tracheoid pits are evenly distributed in a regular alternate arrangement, and they may be slit, oval, round or subrectangular in shape, as are their apertures. While the pit borders are mainly warty, some are plain or vestured (Plate 16, Fig. 3-6). Remnants of the primary cell wall may be retained (Plate 16, Fig. 5).

v. Micromorphology of the internal testa layers. The testa is moderately thick. The palisade cells are of regular depth over the seed. The hourglass cells are moderately long adjacent to the hilum (Plate 16, Fig. 7, 8).

Vicia noeana. (Plates 17, 18. Number of populations studied: 1)

i. Gross seed morphology. The seeds are angular in outline and round in section (Plate 17, Fig. 1; Plate 18, Fig. 1), with a smooth testa and wedge-shaped hilum (Plate 17, Fig. 1, 2). The micropyle is oval (Plate 17, Fig. 3).

ii. Micromorphology of the testa surface. The testa is papillose, with blunt papillae, both adjacent to the hilum and at midseed (Plate 17, Fig. 4-8). Bundles of ridges longitudinally running down some papilla intercalate with those from adjacent cells (Plate 17, Fig. 8).

iii. Micromorphology of the internal hilar region. The hilar lips do not protrude (Plate 18, Fig. 2). The tracheid bar is relatively rounded, with three tracheoids in the width (Plate 18, Fig. 3). The tracheoid pits are evenly distributed and regularly, alternately arranged (Plate 18, Fig. 4). They may be slit, oval, round or subrectangular in shape, as are the pit apertures. Although the pits largely plain-bordered, a few warts may

be seen (Plate 18, Fig.5,6).

iv.Micromorphology of the internal testa layers. The palisade cells are uniformly of moderate length over the seed. The hourglass cells are very long adjacent to the hilum and readily discernible at midseed and opposite the hilum (Plate 18, Fig.7-9). The remnant layer is thick.

Vicia orobus. (Plates 19,20. Number of populations studied: 1)

i.Gross seed morphology. The seed is oval in outline and round in transection (Plate 19, Fig.1), with a smooth testa. The hilum is linear and the micropyle is oval in shape.

ii.Micromorphology of the testa surface. The testa surface is papillose, with rounded papillae covered by fine ridges. Some adjacent papillae are connected by low ridges to form irregular chains (Plate 19, Fig.2,3). The palisade cells may have ridges outlining the cell walls (Plate 19, Fig.4-7).

iii.Micromorphology of the internal hilar region. The lips of the hilum do not protrude (Plate 20, Fig.1). The tracheid bar is narrow in transection, but comprises five narrow tracheoids in its width (Plate 20, Fig.2). The tracheoid pits are evenly distributed and regularly, alternately arranged. The pits may be slit-shaped, oval,

round or subrectangular in shape, with similarly shaped apertures. The pit borders are elaborately vestured, but some plain and warty borders have been recorded (Plate 20, Fig. 3, 4).

iv. Micromorphology of the internal testa layers. The palisade cells are of uniform length over the seed. The hourglass cells are moderately long adjacent to the hilum, and easily discernible at midseed and opposite the hilum (Plate 20, Fig. 5-8). The remnant layer is moderately thick.

Vicia peregrina. (Plates 21-23. Number of populations studied: 3).

i. Gross seed morphology. The seed is oval in outline and round in transection (Plate 21, Fig. 1; Plate 22, Fig. 1). The testa is smooth. The hilum is wedge-shaped (Plate 21, Fig. 2). The micropyle is slit-shaped (Plate 21, Fig. 3). The lens is not prominent (Plate 21, Fig. 1).

ii. Micromorphology of the testa surface. The testa surface is papillose, the papillae being slightly broader on cells adjacent to the hilum. The ridges running longitudinally down the sides of each papilla may interconnect with those of adjacent papillae (Plate 21, Fig. 4, 5). A deposit overlies the papillae mainly in areas lateral to the hilum (Plate 21, Fig. 7, 8), and also, to a lesser extent, adjacent to the hilum in some

populations (Plate 21, Fig. 6). In some specimens of one population, testa pits have been recorded (Plate 21, Fig. 9).

iii. Micromorphology of the internal hilar region. The lips of the hilum may project very slightly (Plate 22, Fig. 2), or be level with the testa surface (Plate 23, Fig. 2). The tracheid bar is round in transection, and comprises four to seven tracheoids in its width. The tracheoid pits are usually evenly distributed in a regular and alternate arrangement (Plate 22, Fig. 4, 5; Plate 23, Fig. 5), but sometimes are unevenly distributed. Pits are slit, oval, round or subrectangular in shape, as are the pit apertures. The pit borders are plain, warty or vestured (Plate 22, Fig. 4-6; Plate 23, Fig. 5, 6). The primary pit walls may be retained (Plate 23, Fig. 4).

iv. Micromorphology of the internal testa layers. The palisade layer is of even thickness. Hourglass cells are long adjacent to the hilum (Plate 22, Fig. 7; Plate 23, Fig. 7), and are short, though discernible lateral to and opposite the hilum (Plate 22, Fig. 8, 9; Plate 23, Fig. 8, 9). The testa may vary in depth between populations (Plate 22, Fig. 7-9; Plate 23, Fig. 7-9).

Vicia sativa ssp. nigra. (Plates 24, 25. Number of populations studied: 2).

i. Gross seed morphology. The seed shape is oval in

outline and round in transection (Plate 24, Fig.1; Plate 25, Fig.1). The testa is smooth. The hilum is wedge-shaped (Plate 24, Fig.2,4). The micropyle is slit-shaped or round (Plate 24, Fig.3). The lens is not prominent.

ii. Micromorphology of the testa surface. The testa surface is papillose. Some ridges running longitudinally down the papillae intercalate with those of adjacent cells (Plate 24, Fig.5-8). The papillae lateral to the hilum may be broader than those adjacent to it (Plate 24, Fig.7,5).

iii. Micromorphology of the internal hilar region. The hilar lips protrude (Plate 25, Fig.2). The tracheid bar is of moderate width comprising three tracheoids (Plate 25, Fig.2). The tracheoid pits are unevenly distributed in an irregular arrangement (Plate 25, Fig.3-5), and are slit-shaped, oval or round in shape, with apertures generally oval or round. The pits are plain-bordered, warty or vested, and may bear remnants of the primary cell wall (Plate 25, Fig.4).

iv. Micromorphology of the internal testa layers. The palisade cells are uniform in length. The hourglass cells are moderately long adjacent to the hilum (Plate 25, Fig.6), and short elsewhere over the seed (Plate 25, Fig.7,8). The remnant layer is thin, and barely discernible.

Vicia sativa ssp. sativa. (Plates 26, 27, 28. Number of populations studied: 3)

i. Gross seed morphology. The seed is subrectangular in outline (Plate 26, Fig. 1), and rounded in transection (Plate 27, Fig. 1). The testa is smooth. The hilum is wedge-shaped and the micropyle is slit-shaped (Plate 26, Fig. 2, 3). The lens is not prominent (Plate 26, Fig. 1).

ii. Micromorphology of the testa surface. The testa surface is papillose with acute papillae (Plate 26, Fig. 4-6), similar to that in the wild subspecies V. sativa nigra (Plate 24, Fig. 5-8). Some populations have a deposit overlying the papillae, particularly at midseed (Plate 26, Fig. 7, 8).

iii. Micromorphology of the internal hilar region. The lips of the hilum protrude. The tracheid bar is four to five tracheoids wide (Plate 27, Fig. 2). The tracheoid pits vary between populations, and may be evenly, regularly pitted (Plate 27, Fig. 3), or irregularly pitted. The pits may be slit-shaped (Plate 27, Fig. 5), oval (Plate 27, Fig. 6), round or subrectangular, and have apertures with the same range of shapes, which may vary in adjacent tracheoids (Plate 27, Fig. 7). The pit borders may be plain, warty or vestured in all specimens, whether with a white testa (Plate 27, Fig. 8), light brown testa (Plate 27, Fig. 4-6), or black testa (Plate 27, Fig. 7).

iv. Micromorphology of the internal testa layers. The palisade cells are of uniform length over the seed, with moderately long hourglass cells adjacent to the hilum, and often barely discernible hourglass cells in other regions (Plate 28, Fig.1,2). Specimens from a white-seeded population (Plate 28, Fig.4), and a black-seeded population (Plate 28, Fig.5,7), both have thinner testas than the seed examined from the brown mottled population (Plate 28, Fig.1-3).

Vicia sativa ssp. sativa var. lentisperma. (Plate 29. Number of populations studied: 1).

i. Gross seed morphology. The seed is round in outline (Plate 29, Fig.1), and compressed in transection (Plate 29, Fig.4), similar to that of Lens, but having less sharp edges. The surface is smooth, and the hilum is wedge-shaped with a slit-shaped micropyle.

ii. Micromorphology of the testa surface. The testa has papillae that may be reduced to domes. Those on cells adjacent to the hilum are very low with a fine reticulum surrounding the papillar bases (Plate 29, Fig.2). The papillae lateral to the hilum at midseed are blunt with a coarser reticulum (Plate 29, Fig.3). The testa patterning may appear very similar to that of Vicia faba, and is totally unlike that of any Lens species.

iii. Micromorphology of the internal hilar region. The



hilar lips protrude (Plate 29, Fig. 4, 5). The tracheid bar is narrow, three tracheoids in width (Plate 29, Fig. 5). The tracheoid pits are unevenly and irregularly distributed, and vary in shape and aperture shape as seen in the more common variety of the subspecies. The pit borders are mainly plain (Plate 29, Fig. 6), but a few warts and vestures have been observed.

iv. Micromorphology of the internal testa layers. The palisade cells are of moderate depth. The hourglass cells are moderately long adjacent to the hilum, and readily discernible lateral and opposite to the hilum. The remnant layer is relatively thick (Plate 29, Fig. 7-9).

Vicia sepium. (Plates 30, 31. Number of populations studied: 3).

i. Gross seed morphology. The seed shape is oval, both in outline and transection (Plate 30, Fig. 1; Plate 31, Fig. 1). The testa is smooth. The hilum is usually circumlinear, though some shorter linear hila occur. The micropyle is oval to triangular (Plate 30, Fig. 2).

ii. Micromorphology of the testa surface. The testa is papillose. The papillae typically are covered at the apex with a reticulum of ridges (Plate 30, Fig. 3). The palisade cell walls are outlined by ridges that vary in thickness in seeds of different populations (Plate 30, Fig. 4, 6). Some regions of the testa show less distinct features

(Plate 30, Fig. 5), or lack a reticulum (Plate 30, Fig. 6). Such variations between single specimens from the same population, are illustrated in Plate 30, Fig. 3-8.

iii. Micromorphology of the internal hilar region. The hilar lips may protrude (Plate 31, Fig. 2), or may be flat. The tracheid bar is of moderate width, although typically being only three tracheoids wide. The tracheoid pits are unevenly distributed and of irregular arrangement (Plate 31, Fig. 4, 5). The pits and their associated apertures may be slit-shaped, oval, round or subrectangular. The pit borders are usually ornamented with warts or vestures, and remnants of the primary cell wall may be present.

iv. Micromorphology of the internal testa layers. The palisade layer is moderately thick. The hourglass cells are long adjacent to the hilum (Plate 31, Fig. 6) and short over the remainder of the seed (Plate 31, Fig. 7, 8). The remnant layer is thick.

Vicia sylvatica. (Plates 32, 33. Number of populations studied: 1).

i. Gross seed morphology. The seed is round in outline and transection (Plate 32, Fig. 1; Plate 33, Fig. 1), with a smooth testa. The hilum is linear, and the micropyle is oval (Plate 32, Fig. 2).

ii. Micromorphology of the testa surface. The testa

surface is papillose, the papillae are covered in coarse longitudinal ridges, which form a twisted "knot" on the apex of each papilla, more clearly expressed on cells at midseed, lateral to the hilum (Plate 32, Fig. 5-7), than on cells adjacent to the hilum (Plate 32, Fig. 3, 4, 8). Some specimens show a slight ridging outlining the cell walls (Plate 32, Fig. 6, 8).

iii. Micromorphology of the internal hilar region. The hilar lips do not protrude (Plate 33, Fig. 2). The tracheid bar is narrow (Plate 33, Fig. 3) and three tracheoids wide. The tracheoid pits are evenly distributed, but irregularly arranged, and vary in shape, as do their apertures, from slit-shaped to oval, round and subrectangular. The pit borders may be plain, or ornamented with warts and vestures (Plate 33, Fig. 4, 5).

iv. Micromorphology of the internal testa layers. The palisade cells are uniformly of moderate length. The hourglass cells are only moderately long adjacent to the hilum, and shorter but visible over the remainder of the seed. The remnant layer is thick (Plate 33, Fig. 6-8).

Vicia tetrasperma. (Plates 34, 35. Number of populations studied: 2)

i. Gross seed morphology. The seed is oval in outline, and round in transection (Plate 34, Fig. 1; Plate 35, Fig. 1). The testa is smooth. The hilum is wedge-

shaped, and the micropyle is oval to round in shape (Plate 34, Fig. 2, 3).

ii. Micromorphology of the testa surface. The testa is papillose; the papillae are linked in chains due to a linear arrangement of papillae of similar length adjacent to shorter papillae (Plate 34, Fig. 5, 7). These uneven papillae are ridged, some of the ridges being confluent between adjacent cells (Plate 34, Fig. 6, 8). A deposit overlies the testa papillae in some specimens (Plate 34, Fig. 4).

iii. Micromorphology of the internal hilar region. The lips of the hilum protrude (Plate 35, Fig. 2). The tracheid bar is narrow and comprises three or four tracheoids in its width (Plate 35, Fig. 3). The tracheoid pits are very uneven in distribution and irregularly arranged (Plate 35, Fig. 4). The pits are mainly oval with oval or round pit apertures. The pits may be plain-bordered, warty (Plate 35, Fig. 5), or vestured.

iv. Micromorphology of the internal testa layers. The palisade cells are slightly longer adjacent to the hilum than over the rest of the seed. The hourglass cells are of moderate length adjacent to the hilum, and scarcely discernible elsewhere. The remnant layer is thin (Plate 35, Fig. 6-8).

Vicia villosa ssp. varia. (Plates 36, 37. Number of

populations studied: 2).

i. Gross seed morphology. The seed is oval in profile and round in transection (Plate 36, Fig.1; Plate 37, Fig.1). The testa is smooth. The hilum is oval to wedge-shaped and the micropyle is oval (Plate 36, Fig.1-3).

ii. Micromorphology of the testa surface. The testa surface is papillose. Ridges commonly outline the cell walls lateral to the hilum (Plate 36, Fig.5), but, more rarely, may be absent (Plate 36, Fig.8). Broad ridges along the papillae intercalate between adjacent papillae.

iii. Micromorphology of the internal hilar region. The hilar lips do not protrude (Plate 37, Fig.2). The tracheid bar is rounded and has six to seven tracheoids in its width (Plate 37, Fig.2). The tracheoids are evenly or unevenly pitted in an irregular (Plate 37, Fig.3), or regular arrangement (Plate 37, Fig.4-6). The pit shape varies from slit, oval and round to subrectangular, as does that of the pit apertures. The pit borders may be plain (Plate 37, Fig.6), warty (Plate 37, Fig.5), or vestured (Plate 37, Fig.4).

iv. Micromorphology of the internal testa layers. The palisade cells are slightly longer adjacent to the hilum. The hourglass cells are long adjacent to the hilum, and readily visible across the rest of the seed. The remnant layer is thick (Plate 37, Fig.7-9).

Lens

Lens culinaris (Plates 38,39,40. Number of populations studied: 7)

i. Gross seed morphology. The seed is circular in outline and lenticular in transection (Plate 38, Fig.1,2). The seeds from microsperma populations (Plate 39, Fig.1), which are of smaller diameter, are broader relative to the diameter than those of the macrosperma populations (Plate 39, Fig.2). The testa is smooth. The hilum is wedge-shaped (Plate 38, Fig.3). The micropyle is oval.

ii. Micromorphology of the testa surface. The surface topography of the testa is highly reduced; the domed palisade cells may be dimpled adjacent to the hilum, or at the edges of the seed (Plate 38, Fig.4). Adjacent to the hilum they are low and irregular in plan (Plate 38, Fig.5,7), at midseed they may form papillae, which are more uniform in outline, and are usually embedded in a deposit (Plate 38, Fig.6,8), which can be removed by enzymic digestion (Plate 40, Fig.1,2).

iii. Micromorphology of the internal hilar region. The hilum protrudes in a characteristically conical form in transection (Plate 40, Fig.1,3). The tracheid bar is narrow in transection, and is three tracheoids wide (Plate 39, Fig.4). The tracheoid pits are unevenly distributed and irregularly arranged. The pits are most

commonly oval or round in shape, but slit-shaped and subrectangular pits can also be observed. The pit borders are nearly always plain (Plate 39, Fig. 5), but warts and vestures have been recorded in some populations (Plate 39, Fig. 6).

iv. Micromorphology of the internal testa layers. The testa is thin. The palisade cells are relatively and evenly short. The hourglass cells are long adjacent to the hilum (Plate 39, Fig. 7), and barely discernible over the remainder of the seed (Plate 39, Fig. 8, 9). The remnant layer is thin.

Lens ervoides. (Plates 41, 42. Number of populations studied: 5)

i. Gross seed morphology. The seed is round in outline, and lenticular in transection (Plate 41, Fig. 1; Plate 42, Fig. 1). The testa is smooth. The hilum is wedge-shaped, and consistently longer than in the other four species of Lens. The micropyle is a slit (Plate 41, Fig. 2).

ii. Micromorphology of the testa surface. The testa is papillose to domed. The papillae are highly reduced and irregular in outline adjacent to the hilum (Plate 41, Fig. 3, 5), and more regular in outline at midseed (Plate 41, Fig. 4, 6).

iii. Micromorphology of the internal hilar region. The lips of the hilum protrude (Plate 42, Fig. 2). The tracheid bar is narrow, and three to four tracheoids in width (Plate 42, Fig. 3). The tracheoid pits are unevenly distributed in an irregular arrangement. Pits are largely oval or round in shape, although slit-shaped and subrectangular pits are present in some populations. Remnants of the pit membrane may be found. The pit borders may be plain or warty. Vestures have not been observed (Plate 42, Fig. 4).

iv. Micromorphology of the internal testa layers. The testa is generally thin. The palisade cells are relatively short. The hourglass cells are long adjacent to the hilum and very short elsewhere (Plate 42, Fig. 5-7). Opposite the hilum, narrow strands have been observed, which stand erect on the surface of the cotyledon beneath the testa in specimens of some populations (Plate 42, Fig. 7, 8). Their significance is not understood.

Lens nigricans. (Plates 43, 44. Number of populations studied: 6)

i. Gross seed morphology. The seed is circular in outline and lenticular in transection (Plate 43, Fig. 1; Plate 44, Fig. 1). The testa is smooth. The hilum is wedge-shaped, and micropyle is triangular (Plate 43, Fig. 2, 3).

ii. Micromorphology of the testa surface. The testa



surface has reduced papillae. The papillae adjacent to the hilum are irregular in outline and may be dimpled (Plate 43, Fig. 3, 5). The papillae are more regular in outline at midseed, and may be covered by a deposit moulded around them (Plate 43, Fig. 6). The blunted tip and straight sides of the characteristic papillar form are shown in Plate 43, Fig. 7, 10.

iii. Micromorphology of the internal hilar region. The lips of the hilum may protrude markedly, slightly (Plate 44, Fig. 2), or be flat. The tracheid bar is narrow, but may be from three to as many as six tracheoids in width (Plate 44, Fig. 3). The pits are most commonly evenly distributed in a regular alternate arrangement, though some specimens in some populations have uneven pitting. Slit-shaped pits are rare, and most are oval or round, as are the pit apertures. The pit borders are usually warty or vestured, unlike those of the specimens from other species of Lens observed in this study (Plate 44, Fig. 4-7).

iv. Micromorphology of the internal testa layers. The testa is thin. The palisade cells are relatively short. The hourglass cells are long adjacent to the hilum (Plate 44, Fig. 2, 8), and short elsewhere (Plate 44, Fig. 9, 10). The remnant layer is of moderate thickness.

Lens odemensis. (Plates 45, 46. Number of populations studied: 5).

i. Gross seed morphology. The seed is round in outline and lenticular in transection (Plate 45, Fig. 1; Plate 46, Fig. 1). The testa is smooth, but appears velvety to the naked eye in fresh specimens. The hilum is wedge-shaped (Plate 45, Fig. 2), and micropyle is variously oval, round or triangular.

ii. Micromorphology of the testa surface. The testa is papillose. The papillae adjacent to the hilum are oval in outline with ridges round the base (Plate 45, Fig. 3, 5). At midseed, in all specimens of all populations examined, the testa has very highly acute, non-ridged papillae, which are most clearly observed when scanned obliquely (Plate 45, Fig. 4, 6).

iii. Micromorphology of the internal hilar region. The hilum has protruding lips, and a conical profile (Plate 46, Fig. 2). The tracheid bar is narrow, and usually four tracheoids wide (Plate 46, Fig. 2, 5). The tracheoid pits are most commonly unevenly distributed and irregularly arranged (Plate 46, Fig. 3), although one population has been observed with even pitting. The pits vary in shape from slit-like, oval and round, to subrectangular, as do the apertures. The pit borders are most commonly plain (Plate 46, Fig. 4), but some warts and very occasional vestures have been recorded.

iv. Micromorphology of the internal testa layers. The testa is relatively thin. The palisade cells are

uniformly short. The hourglass cells are long adjacent to the hilum (Plate 46, Fig.7), and readily discernible on the rest of the seed (Plate 46, Fig.8). The remnant layer is thin.

Lens orientalis. (Plate 47,48. Number of populations studied: 6).

i. Gross seed morphology. The seed is round in outline and lenticular in transection (Plate 47, Fig.1; Plate 48, Fig.1). The testa is smooth. The hilum is wedge-shaped and the micropyle is round or triangular (Plate 47, Fig.2).

ii. Micromorphology of the testa surface. The testa is papillose. The papillae are reduced, and irregular in outline adjacent to the hilum, with ridges around the base (Plate 47, Fig.3). A deposit between papillae may obscure the topography at midseed (Plate 47, Fig.4). One population exhibited the very acute papillae very similar to those characteristic of Lens odemensis, although in a reduced form (Plate 47, Fig.7).

iii. Micromorphology of the internal hilar region. The hilar lips protrude (Plate 48, Fig.2). The tracheid bar is narrow, three to four tracheoids wide. The pits are unevenly distributed and irregularly arranged (Plate 48, Fig.3). The pits are commonly oval or round, though in some populations slit-shaped and subrectangular pits have

been observed. The pit apertures are similarly shaped. The pits are usually plain-bordered (Plate 48, Fig. 4). No vestures have been recorded, but some warts are usually present in all populations.

iv. Micromorphology of the testa layers. The testa is thin. The palisade cells are uniformly short. The hourglass cells are of moderate length adjacent to the hilum, and short over the rest of the seed. The remnant layer is thin (Plate 48, Fig. 5-8).

#### Lathyrus.

##### Lathyrus annuus.

(Plates 49, 50. Number of populations studied: 1).

i. Gross seed morphology. The seed is round in profile and transection (Plate 49, Fig. 1). The testa is markedly rugose. The hilum is wedge-shaped and the micropyle is oval (Plate 49, Fig. 1). The lens is readily visible, its surface lacking the rugosity of the rest of the testa (Plate 49, Fig. 1, 4).

ii. Micromorphology of the testa surface. The rugose testa is papillose, the rugosity formed from groups of elongated palisade cells making conical projections forming mounds and ridges (Plate 49, Fig. 3, 4, 5). The individual papillae are very low and covered with a fine reticulum of ridges (Plate 49, Fig. 6, 7).

iii. Micromorphology of the internal hilar region. The hilar lips are flat, but the hilum as a whole is slightly sunken (Plate 50, Fig.1). The tracheid bar is moderately wide, comprising three to four tracheoids in its width (Plate 50, Fig.3). The tracheoid pits are unevenly distributed and irregularly arranged (Plate 50, Fig.4). Their shape varies from slit-shaped or oval, to round, as do the pit apertures. Only plain-bordered pits have been observed in this species, a unique finding in this survey (Plate 50, Fig.4), however it is stressed that but a single population has been observed.

iv. Micromorphology of the internal testa layers. The thickness of the palisade layer varies across the seed, forming rugosities as described above. The hourglass cells are relatively short throughout. The remnant layer is thin (Plate 50, Fig.5).

Lathyrus aphaca. (Plates 51, 52. Number of populations studied: 2)

i. Gross seed morphology. The seed is subrectangular to oval in outline, and oval in transection (Plate 51, Fig.1,2; Plate 52, Fig.1). The testa is smooth. The hilum and micropyle are both oval (Plate 51, Fig.1,3). The lens is not prominent.

ii. Micromorphology of the testa surface. The palisade cells are not papillose, but are coarsely reticulate,

often with ridging outlining the cell walls (Plate 51, Fig.5-8). Some specimens have domed palisade cells immediately adjacent to the hilum, covered with ridges (Plate 51, Fig.4). The specimen from the black-seeded population displays a variation in topography, having a finer reticulum (Plate 51, Fig,8). Treatment with chloroform and ether clarifies the patterning, although there are no overt indications of the presence of cuticular wax (Plate 51, Fig.6).

iii. Micromorphology of the internal hilar region. The hilum is flat, with no protrusion of hilar lips. The tracheid bar is moderate in width, and three to four tracheoids wide (Plate 52, Fig.2). The tracheoid pits are evenly distributed but generally irregular in arrangement (Plate 52, Fig.3,4), and vary in shape from slit-shaped, or oval to round, as do the apertures. The pit borders may be plain, or vested, but are mainly warty (Plate 52, Fig.4).

iv. Micromorphology of the internal testa layers. The palisade cells are of moderate depth. The hourglass cells are only moderately long adjacent to the hilum, and short across the rest of the seed. The remnant layer is thick. Specimens from a black-seeded population have large rhombic crystals in the remnant layer, only visible adjacent to the hilum (Plate 52, Fig.5-7). The same specimens also have testas slightly thicker than those of the brown-seeded population (Plate 52, Fig.10,7).

Lathyrus blepharicarpus. (Plate 53. Number of populations studied: 2)

i. Gross seed morphology. The seeds are angular, chisel-shaped to rounded, and oval to subrectangular in transection with the hilum on the wider edge (Plate 53, Fig. 1). The testa is very slightly rough. The hilum is wedge-shaped. The micropyle is a slit. The lens is prominent (Plate 53, Fig. 1, 2).

ii. Micromorphology of the testa surface. The testa has very slight secondary patterning forming barely discernible mounds; it is papillose, fine ridges surrounding the apex of each papilla, and sometimes intercalating with those from adjacent papillae near the hilum (Plate 53, Fig. 3). Faint ridging may be observed outlining the cell walls lateral to the hilum in some specimens (Plate 53, Fig. 4).

iii. Micromorphology of the internal hilar region. The hilum is flat and sunken below the testa surface (Plate 53, Fig. 5). The tracheid bar is moderately wide with ten tracheoids in the width. The pits are evenly distributed in a regular alternate arrangement (Plate 53, Fig. 6). Their shape varies from slit-shaped, oval and round to subrectangular, with similar pit apertures. The pit borders are plain (Plate 53, Fig. 6) or warty. Some remnants of the primary cell wall are retained.

iv. Micromorphology of the internal testa layers. The palisade layer is moderately thick and even over the seed. The hourglass cells are moderately long adjacent to the hilum and readily visible lateral to the hilum at midseed and opposite the hilum. The remnant layer is moderately thick. (Plate 53, Fig. 7-9).

Lathyrus cicera. (Plates 54, 55, 56. Number of populations studied: 4).

i. Gross seed morphology. The seed is angular, and chisel-shaped, and subtriangular in transection (Plate 54, Fig. 1; Plate 55, Fig. 1), with the hilum at one end of the narrow rectangular face. The testa is smooth. The hilum is oval to wedge-shaped, and the micropyle is a slit (Plate 54, Fig. 2). The lens is prominent.

ii. Micromorphology of the testa surface. The testa is papillose, with fine ridges covering the sides of each papilla, that may intercalate with those of adjacent papillae (Plate 54, Fig. 3, 5). The papillae lateral to the hilum (Plate 54, Fig. 5) are less acute than those adjacent to the hilum (Plate 54, Fig. 3), and show a distinctive ridging around the base. Some specimens in one population have pits in the testa surface (Plate 54, Fig. 9).

iii. Micromorphology of the internal hilar region. The flat hilum is slightly sunken (Plate 55, Fig. 2). The



tracheid bar is oval in transection and six to nine tracheoids wide (Plate 55, Fig. 3). The pits are generally evenly distributed and regularly arranged, ranging from slit, oval and round to subrectangular in shape, as do the pit apertures. The pit borders may be plain, warty or vestured (Plate 55, Fig. 4).

iv. Micromorphology of the internal testa layers. The palisade cells are of uniform thickness over the seed. Hourglass cells are readily visible over the remainder of the seed. The remnant layer is relatively thick (Plate 55, Fig. 6-8). A section through the testa surface pits shows the pits penetrate to the hourglass cells (Plate 55, Fig. 5).

Lathyrus clymenum. (Plates 57, 58. Number of populations studied: 2).

i. Gross seed morphology. The seed is subrectangular to oval in outline, and oval in transection (Plate 57, Fig. 1; Plate 58, Fig. 2). The testa is smooth. The hilum is wedge-shaped and the micropyle is a slit (Plate 57, Fig. 1, 2).

ii. Micromorphology of the testa surface. The testa is papillose, with ridges down the papillae intercalating between papillae, and sometimes outlining the cell walls. These ridges may be coarse (Plate 57, Fig. 3, 4) or fine (Plate 57, Fig. 5, 6) in different populations.

iii. Micromorphology of the internal hilar region. The hilar lips are slightly protruding and the hilum may be slightly sunken (Plate 58, Fig. 3, 10). The tracheid bar is moderately wide, comprising six tracheoids in its width (Plate 58, Fig. 3, 10). The tracheoid pits are evenly distributed in a regular alternate arrangement and may vary in shape between slit, oval and round to subrectangular with similarly shaped apertures. The pit borders may be plain, warty or vestured (Plate 58, Fig. 4, 5).

iv. Micromorphology of the internal testa layers. The palisade layer is moderately thick. Hourglass cells are long adjacent to the hilum, and readily discernible around the remainder of the seed. The remnant layer is discernible but not thick (Plate 58, Fig. 4, 7, 8).

Lathyrus gorgoni. (Plate 59. Number of populations studied: 1).

i. Gross seed morphology. The seed is subrectangular in outline (Plate 59, Fig. 1), and triangular to rounded in transection. The testa is smooth. The hilum lies across the corner of the rectangular edge, and is oval to wedge-shaped. The micropyle is a slit.

ii. Micromorphology of the testa surface. The testa is papillose. The papillae are ridged, ridges between adjacent papillae intercalating, particularly in areas

adjacent to the hilum (Plate 59, Fig.3). Lateral to the hilum a heavy deposit may be present, overlying the papillae (Plate 59, Fig.4). However different areas in the same specimen may be free from this. Ridges may outline the palisade cell walls (Plate 59, Fig.5).

iii. Micromorphology of the internal hilar region. The hilar lips are flat, but the hilum is markedly sunken in profile (Plate 59, Fig.2). The tracheid bar is rounded, comprising at least ten tracheoids in its width. The tracheoid pits are evenly distributed in a regular alternate arrangement, and these may be slit-shaped, oval, round or subrectangular with similarly shaped apertures. The pit borders are plain, warty and vestured, with different ornamentation occurring in immediately adjacent tracheoids (Plate 59, Fig.6-8).

iv. Micromorphology of the internal testa layers. The depth of the palisade cells is usually slightly thicker adjacent to the hilum, than opposite it. The hourglass cells are only moderately long adjacent to the hilum, and scarcely visible around the remainder of the seed. The remnant layer is thick (Plate 59, Fig.9-11).

Lathyrus heterophyllus. (Plates 60,61. Number of populations studied: 1).

i. Gross seed morphology. The seed is oval in outline, and round in transection (Plate 60, Fig.1; Plate

61, Fig. 1). The testa is rugose and ridged (Plate 60, Fig. 2). The hilum is linear, and the micropyle is round (Plate 60, Fig. 2, 3).

ii. Micromorphology of the testa surface. The testa surface is papillose. Groups of elongated palisade cells form marked ridges (Plate 60, Fig. 4). Individual lines of adjacent papillae are elongated in chains between the ridges (Plate 60, Fig. 5). The papillae are ridged with much intercalation of ridges between adjacent papillae, both near the hilum, and at midseed (Plate 60, Fig. 5, 8).

iii. Micromorphology of the internal hilar region. The hilar lips protrude slightly, and the hilum is slightly sunken (Plate 61, Fig. 2). The tracheid bar is of moderate width, comprising three tracheoids in the width (Plate 61, Fig. 3). The tracheoid pits are evenly distributed in a regular alternate arrangement. The pits vary in shape from slit-like, which is particularly common (Plate 61, Fig. 4), to oval, round or subrectangular (Plate 61, Fig. 5). The pit borders may be plain, warty or vestured.

iv. Micromorphology of the internal testa layers. The length of the palisade cells varies over the seed surface (Plate 61, Fig. 6-8). The hourglass cells are relatively long over the whole seed, but not markedly so adjacent to the hilum. The remnant layer is thin.

Lathyrus hirsutus. (Plates 62,63. Number of populations studied: 1).

i. Gross seed morphology. The seed is round both in outline and in transection (Plate 62, Fig.1; Plate 63, Fig.1). The testa is highly rugose. The hilum is oval and the micropyle is a slit to oval in shape (Plate 62, Fig.3).

ii. Micromorphology of the testa surface. The rough texture of the testa is a result of the elongation of large groups of palisade cells to form mounds and ridges (Plate 62, Fig.4,6). The testa is papillose, adjacent papillae are elongated in lines to form chains linked by coarse ridges between shorter papillae (Plate 62, Fig.7).

iii. Micromorphology of the internal hilar region. The hilum is flat and not sunken. The tracheid bar is rounded and comprises six tracheoids in its width (Plate 63, Fig.2). The tracheoid pits are evenly distributed in a regular alternate arrangement. Their shape may be slit-like (Plate 63, Fig.5), oval and round (Plate 63, Fig.4,6), and subrectangular (Plate 63, Fig.3). The pit apertures are similarly shaped. The pit borders may be plain, warty or vestured. The warts are often very small. Some primary pit membranes may be retained (Plate 63, Fig.6).

iv. Micromorphology of the testa layers. The palisade layer is uneven in depth over the surface of the seed

(Plate 63, Fig. 7). The hourglass calls are short. The remnant layer is thin (Plate 63, Fig. 7).

Lathyrus inconspicuus. (Plates 64, 65. Number of populations studied: 1).

i. Gross seed morphology. The seed is rounded to angular in outline and oval in transection (Plate 64, Fig. 1; Plate 65, Fig. 1). The testa is smooth and waxy-looking. The hilum is oval and the micropyle is a slit (Plate 64, Fig. 2).

ii. Micromorphology of the testa surface. The testa surface is not papillose, nor of the common reticulate form. The palisade cells walls are outlined by thick angular ridges, and covered by a deposit of fine short rods, particularly prevalent near to the hilum (Plate 64, Fig. 3-6). Treatment of the seeds by immersion for one minute in a 1:1 mixture of chloroform and ether, a wax solvent, removes these rods (Plate 64, Fig. 7), which have therefore been interpreted as cuticular wax.

iii. Micromorphology of the internal hilar region. The hilar lips are slightly protruding, and the hilum is sunken (Plate 65, Fig. 2). The tracheid bar is rounded and four to five tracheoids wide. The tracheoid pits are unevenly distributed in an irregular arrangement. The pit shape varies from oval to round. The pit apertures are slit-shaped, oval or round. Pit borders are plain or

warty. No vestures have been observed (Plate 65, Fig. 3, 4).

iv. Micromorphology of the internal testa layers. The palisade cells are even in length. The hourglass cells are only moderate in length adjacent to the hilum, and short over the rest of the seed (Plate 65, Fig. 5, 6). The remnant layer is thin.

Lathyrus laxiflorus. (Plate 66. Number of populations studied: 1).

i. Gross seed morphology. The seed is oval to subrectangular in outline and oval in transection. The testa is smooth (Plate 66, Fig. 1, 4).

ii. Micromorphology of the testa surface. The testa is not papillose. The external walls of the palisade cells are finely reticulate with ridges radiating in a stellate fashion, the cells being flatter at midseed. Those adjacent to the hilum may be slightly domed and dimpled (Plate 66, Fig. 2, 3).

iii. Micromorphology of the internal hilar region. The hilar lips protrude (Plate 66, Fig. 5). The tracheid bar is relatively narrow, with three tracheids in the width (Plate 66, Fig. 5). The tracheoid pits are unevenly distributed in an irregular arrangement and may be oval or slit-shaped. The pit apertures are slit, oval or round in shape. The pit borders are plain or warty. No vestures

have been observed. Remnants of the primary cell wall may be retained (Plate 66, Fig. 6).

iv. Micromorphology of the internal testa layers. The palisade cells are uniform in length and are relatively short. The hourglass cells are long adjacent to the hilum (Plate 66, Fig. 7), but are not easily discernible over the rest of the seed (Plate 66, Fig. 8, 9). The remnant layer is thin.

Lathyrus niger ssp. niger. (Plates 67, 68. Number of populations studied: 2).

i. Gross seed morphology. The seeds are oval in outline and round in transection (Plate 67, Fig. 1: Plate 68, Fig. 1). The hilum is wedge-shaped to oblong, the micropyle is narrowly oval (Plate 67, Fig. 1, 2). The testa is smooth.

ii. Micromorphology of the testa surface. The testa is typically non-papillose with a fine stellate reticulum on the exterior walls of the palisade cells (Plate 67, Fig. 4). In areas adjacent to the hilum these cell walls are slightly domed and dimpled (Plate 67, Fig. 3). In some specimens of one population, pits have been recorded in the testa surface.

iii. Micromorphology of the internal hilar region. The hilar lips protrude and the hilum is slightly sunken



(Plate 68, Fig. 2). The tracheid bar is narrow, three or four tracheoids wide. The pits are of uneven distribution mainly irregularly arranged. The pits are slit-shaped, oval or round, as are the pit apertures. The pit borders are plain, warty or vestured (Plate 68, Fig. 3, 4).

iv. Micromorphology of the internal testa layers. The palisade cells are even in length over the seed. The hourglass cells adjacent to the hilum are long (Plate 68, Fig. 5), but barely discernible at midseed or opposite the hilum (Plate 68, Fig. 6, 7). The remnant layer is thin.

Lathyrus nissolia. (Plates 69, 70. Number of populations studied: 3).

i. Gross seed morphology. The seeds are drum-shaped to sub-cubic in shape, and angular to round in transection (Plate 69, Fig. 1: Plate 70, Fig. 1). The testa is rough. The hilum is oval and the micropyle is a slit (Plate 69, Fig. 2, 3).

ii. Micromorphology of the testa surface. The roughness of the testa is caused by localised groups of elongated palisade cells forming mounds, separated by areas of flat-walled cells covered by a fine reticulum of ridges (Plate 69, Fig. 1, 4-7). The seeds from different populations show minor differences in the fineness of the reticulum (Plate 69, Fig. 5, 7).

iii. Micromorphology of the internal hilar region. The hilum is flat, but may be slightly sunken (Plate 70, Fig. 2). The tracheid bar is rounded in transection and comprises four to five tracheoids in the width (Plate 70, Fig. 3). The tracheoid pits are unevenly distributed in an irregular arrangement. The pits are slit-shaped, oval, round or subrectangular, and their pit apertures are of a similar range in shape. The pit borders may be plain, warty or vestured. The primary cell walls may be retained (Plate 70, Fig. 4, 5).

iv. Micromorphology of the internal testa layers. The palisade cells vary in length over the seed, forming mounds as described above. The hourglass cells are of moderate length adjacent to the hilum, and rarely discernible over the rest of the seed. The remnant layer is relatively thick (Plate 70, Fig. 6-8).

Lathyrus ochrus. (Plates 71, 72. Number of populations studied: 2).

i. Gross seed morphology. The seed is round in shape (Plate 71, Fig. 1; Plate 72, Fig. 2), and the testa has a waxy texture. The hilum is wedge-shaped to oblong, and the micropyle is a slit (Plate 71, Fig. 1, 2). The lens is prominent.

ii. Micromorphology of the testa surface. The testa is papillose. However the papillae are largely obscured by

the presence of a heavy deposit of angular platy crystals, most well-defined adjacent to the hilum (Plate 71, Fig. 3, 4), but also present at midseed (Plate 71, Fig. 5, 6) and all other testa areas. These crystals disappear following treatment of seeds with 1:1 mixtures of chloroform and ether (Plate 71, Fig. 7), a wax solvent, but incomplete treatment may leave a stringy partial deposit upon the papillae (Plate 71, Fig. 8). The crystals, because of both their form and their solubility in the wax solvent, have been interpreted as cuticular wax.

iii. Micromorphology of the internal hilar region. The hilar lips protrude markedly (Plate 72, Fig. 2). The tracheid bar is moderate in width, and three tracheoids wide. The tracheoid pits are evenly distributed, but unevenly arranged, an unusual combination of characters in this study, and are slit, oval or round, with similar apertures. The pit borders are plain, warty or vested (Plate 72, Fig. 3-7).

iv. Micromorphology of the internal testa layers. The palisade cells are even in length over the seed. The hourglass cells are moderately long adjacent to the hilum, and short over the remainder of the seed. The remnant layer is relatively thick (Plate 72, Fig. 7-9).

Lathyrus pratensis. (Plates 73, 74. Number of populations studied: 4).

i. Gross seed morphology. The seeds are oval in outline and in transection (Plate 73, Fig. 1). The testa is smooth. The hilum is wedge-shaped, and the micropyle is a slit or oval (Plate 73, Fig. 2, 3).

ii. Micromorphology of the testa surface. The testa is not papillose, but the external cell walls of the palisade cells are reticulate and flat, particularly at midseed (Plate 73, Fig. 6, 7), different populations showing slight variations in the thickness of the reticulate ridges. Adjacent to the hilum the palisade cells may be slightly domed and dimpled with a stellate reticulum (Plate 73, Fig. 5). In some specimens of one population, pits have been recorded in the testa surface.

iii. Micromorphology of the internal hilar region. The hilar lips may protrude very slightly (Plate 74, Fig. 2), or be flat. The hilum may be slightly sunken. The tracheid bar is relatively narrow, and four to five tracheoids wide. The tracheoid pits are of uneven distribution (Plate 74, Fig. 3), and mainly irregularly arranged, although small areas in some tracheoids do have alternate pitting. The pits may be slit, oval, round or subrectangular in shape, as are the pit apertures. The pit borders are plain, warty or vestured and some primary cell walls remnants may be retained (Plate 74, Fig. 3-5).

iv. Micromorphology of the internal testa layers. The palisade cells are of even length over the seed. The

hourglass cells are moderately long adjacent to the hilum (Plate 74, Fig. 1, 9) and short though visible over the remainder of the seed (Plate 74, Fig. 10, 11). Sections through the testa pits in one population demonstrate that they penetrate to the hourglass cell layer (Plate 74, Fig. 9).

Lathyrus pseudocicera. (Plate 75. Number of populations studied: 2).

i. Gross seed morphology. The seeds are angular and chisel-shaped, and triangular in transection (Plate 75, Fig. 1). The testa is very slightly rough. The hilum is oval to wedge-shaped, and the micropyle is a slit. (Plate 75, Fig. 2). The lens is prominent.

ii. Micromorphology of the testa surface. Groups of palisade cells are elongated to form very low mounds. The external cell walls are papillose, coarsely ridged adjacent to the hilum (Plate 75, Fig. 3), and more finely ridged at midseed (Plate 75, Fig. 4), with some intercalation of ridges between papillae.

iii. Micromorphology of the internal hilar region. The hilum is usually flat, but sometimes may be sunken (Plate 75, Fig. 2, 5). The tracheid bar is rounded and nine to ten tracheoids wide (Plate 75, Fig. 5). Tracheoid pits are evenly distributed in a regular alternate arrangement and vary from slit, oval and round to subrectangular in

shape, with similarly shaped apertures (Plate 75, Fig. 6). The pit borders are plain, warty (Plate 75, Fig. 6) or slightly vestured. Remnants of the primary cell wall may be retained.

iv. Micromorphology of the internal testa layers. The palisade cells are slightly uneven in length, resulting in the slightly roughened testa. The hourglass cells are relatively short adjacent to the hilum and short over the rest of the seed. The remnant layer is moderately thick (Plate 75, Fig. 7-9).

Lathyrus sativus. (Plates 76, 77. Number of populations studied: 4).

i. Gross seed morphology. The seed is angular and chisel-shaped (Plate 76, Fig. 1) and triangular in transection. Specimens from the smaller-seeded populations tend to be rounder, and those from large-seeded populations are more angular. The testa is smooth. The hilum is oval to wedge-shaped, and situated at one end of the narrow, rectangular face. The micropyle is a slit. The lens is prominent (Plate 76, Fig. 2).

ii. Micromorphology of the testa surface. The testa is papillose, the most distinct topography being seen usually on areas adjacent to the hilum (Plate 76, Fig. 3, 4, 6, 7). The acuteness of the papillae varies with the region of the testa in a single specimen (Plate

76, Fig. 3, 4) and with different specimens from different populations (Plate 76, Fig. 3, 6, 7). Ridges along the papillae may intercalate with those from adjacent papillae (Plate 65, Fig. 4). The papillae may be well-defined at midseed (Plate 76, Fig. 5), or, particularly on specimens from large-seeded populations, they can be virtually obscured (Plate 76, Fig. 8), and they may be low and diffusely arranged, or acute and more densely distributed. In some specimens from one population with very large seeds, pits were recorded in the testa surface.

iii. Micromorphology of the internal hilar region. The hilum is flat or with slightly protruding lips (Plate 77, Fig. 1), and sunken. The tracheid bar is round, and from seven to ten tracheoids wide (Plate 77, Fig. 2). The tracheoid pits are evenly distributed and regularly, alternately arranged. They may be slit-like and long, oval, round or subrectangular, with similar apertures, and can vary in immediately adjacent tracheoids (Plate 77, Fig. 3). The pit borders are plain, warty or vestured (Plate 77, Fig. 4, 5).

iv. Micromorphology of the internal testa layers. The palisade cells are even in length over the seed surface. The hourglass cells adjacent to the hilum are long (Plate 77, Fig. 6), but hard to distinguish over the remainder of the seed. The remnant layer is of moderate thickness (Plate 77, Fig. 6-8).

Lathyrus sylvestris. (Plates 78,79. Number of populations studied: 4).

i. Gross seed morphology. The seed is obovate in outline and round in transection (Plate 78, Fig.1; Plate 79, Fig.1). The hilum is linear and the micropyle is round (Plate 78, Fig.2). The testa is slightly rugose (Plate 78, Fig.1).

ii. Micromorphology of the testa surface. The testa is papillose (Plate 78, Fig.3). The localised elongations of the palisade cells result in undulating ridges and low mounds on the testa (Plate 78, Fig.6). Individual papillae may be longer on adjacent papillae and connected by ridges to form chains, more discernible adjacent to the hilum (Plate 78, Fig.4) than at midseed (Plate 78, Fig.5). Most commonly there is well-developed ridging over the papillae that forms a reticulum (Plate 78, Fig.6,7), and typically is wrinkled transversely along the ridges (Plate 78, Fig.7).

iii. Micromorphology of the internal hilar region. The hilum is flat (Plate 79, Fig.2). The tracheid bar is rounded and four tracheoids wide (Plate 79, Fig.3). The pits are usually unevenly distributed and irregularly arranged. They are slit, oval, round or subrectangular in shape with similarly-shaped apertures. The pit borders are usually plain (Plate 79, Fig.4,5), but may be warty or vestured.



iv. Micromorphology of the internal testa layers. The palisade cells are of moderate length. The hourglass cells are long adjacent to the hilum, and short but readily discernible over the rest of the seed. The remnant layer is relatively thick (Plate 79, Fig. 6-8).

Lathyrus tuberosus. (Plate 80. Number of populations studied: 1).

i. Gross seed morphology. The seed shape is obovate in outline and rounded in transection. The testa is slightly rugose; the hilum is wedge-shaped. The micropyle is a slit (Plate 80, Fig. 1, 2).

ii. Micromorphology of the testa surface. The testa is papillose. Groups of palisade cells are elongated to form low mounds and ridges. The ridges on the papillae may intercalate in large groups with those of adjacent papillae, linking the papillae in chains (Plate 80, Fig. 3-6). The papillae chains tend to be distorted on areas over mounds (Plate 80, Fig. 4, 6). The distribution of papillae is denser at midseed than near the hilum (Plate 80, Fig. 3, 5).

iii. Micromorphology of the internal hilar region. The hilum is flat. The tracheid bar is rounded and six tracheoids wide. The pits are of even distribution and irregularly arranged (Plate 80, Fig. 7). The pits are slit-like, oval and round as are the pit apertures. The

pit borders are plain or warty. Vestures have not been recorded. The primary cell wall may be imperforate in some tracheoids, or retained as remnants.

iv. Micromorphology of the internal testa layers. The palisade cells are of uneven length over the seed. The hourglass cells are relatively short adjacent to the hilum, and barely discernible over the rest of the seed. The remnant layer is thick (Plate 80, Fig. 9).

Pisum.

Pisum sativum ssp. elatius. (Plates 81, 82, 83. Number of populations studied: 3)

i. Gross seed morphology. The seed is round in shape, with a rough testa (Plate 81, Fig. 1; Plate 82, Fig. 1). The hilum is oval, and the micropyle is a slit (Plate 81, Fig. 2). The lens is not prominent.

ii. Micromorphology of the testa surface. The rugosity of the seed is caused by localised groups of palisade cells elongated to form conical mounds (Plate 81, Fig. 3). These mounds may be distributed at varying densities, with the bases occupying almost the whole surface, as illustrated, or more widely spaced and interspersed with flat areas. The testa is papillose, papillae between mounds having clearly-defined ridges intercalating between adjacent papillae (Plate 81, Fig. 5, 6), and those upon mounds being

somewhat distorted (Plate 81, Fig. 8). The papillae adjacent to the hilum show a reduction in ridging and appear oval in outline (Plate 81, Fig. 4).

iii. Micromorphology of the internal hilar region. The hilum has rounded and protruding lips (Plate 82, Fig. 2). The tracheid bar is rounded in transection with seven to nine tracheoids in the width. The tracheoid pits are unevenly distributed and yet usually regularly arranged. The pits and pit apertures are slit-shaped (Plate 82, Fig. 5), oval and round (Plate 82, Fig. 4, 6). The pit borders are most commonly plain (Plate 82, Fig. 4), with some warts (Plate 82, Fig. 6). No vestures have been recorded.

iv. Micromorphology of the internal testa layers. The palisade cells vary in length as described above. The hourglass cells are only moderately long, or short, near the hilum (Plate 82, Fig. 7, 8). The remnant layer is thin.

Pisum sativum ssp. sativum var. arvense. (Plates 84, 85.  
Number of populations studied: 4)

i. Gross seed morphology. The seed is subspherical in shape (Plate 84, Fig. 1; Plate 85, Fig. 1). The testa is smooth (Plate 84, Fig. 1). The hilum is wedge-shaped and the micropyle is a slit (Plate 84, Fig. 2).

ii. Micromorphology of the testa surface. Although the

testa may appear to be smooth at low magnifications, very low mounds are discernible under higher magnifications (Plate 84, Fig. 3, 4). They are formed from slight elongations of palisade cells in localised groups. The testa is papillose, each low papilla being covered in coarse ridges, producing a stellate reticulum (Plate 84, Fig. 5), most marked at midseed, where the papillae are flatter (Plate 84, Fig. 6, 8, 9).

iii. Micromorphology of the internal hilar region. The hilar lips may protrude very slightly (Plate 85, Fig. 2). The tracheid bar is rounded in transection and five to nine tracheoids in width. The pits are evenly distributed and arranged alternately (Plate 85, Fig. 3). The pits may be slit-shaped, oval (Plate 85, Fig. 4), or round, occasionally subrectangular, with similarly shaped apertures. The pit borders are usually plain, but warts have been recorded (Plate 85, Fig. 4). Vestures have not been observed.

iv. Micromorphology of the internal testa layers. The palisade cells are mainly of even length and short. The hourglass cells are relatively short, even adjacent to the hilum. The remnant layer is thin (Plate 85, Fig. 7) or moderately thick.

Pisum sativum ssp. sativum var. sativum. (Plates 86, 87.

Number of populations studied: 3).

i. Gross seed morphology. The seed is subspherical (Plate 86, Fig. 1), or drum-shaped. The testa is smooth. The hilum is oval and the micropyle is a slit (Plate 86, Fig. 2).

ii. Micromorphology of the testa surface. The testa is barely papillose, papillae at midseed and adjacent to the hilum being flattened, similar to those seen at midseed in P. sativum sativum arvense, described above (Plate 86, Fig. 3-5). In P. sativum sativum sativum form jomardii the papillae are more acute (Plate 86, Fig. 8). All papillae are covered by ridges radiating from the apices in a stellate fashion and covering the entire testa surface.

iii. Micromorphology of the internal hilar region. The hilar region in transection is broad and the hilar lips protrude (Plate 87, Fig. 1). The tracheid bar is rounded and five to six tracheoids wide. The tracheoid pits may be even or uneven in distribution, and regular or irregular in arrangement. Their shape is most commonly slit-like, but oval and round pits occur. In one population a few subrectangular pits have been recorded. The pit apertures are similarly shaped. The pit borders are most frequently warty, with few plain borders (Plate 87, Fig. 4-6).

iv. Micromorphology of the internal testa layers. The palisade cells are uniformly short. Hourglass cells are relatively short adjacent to the hilum, and barely

visible in the remainder of the seed. The remnant layer may be thick (Plate 87, Fig.7-9) or moderately thin.

#### 4.3. General description of testa micromorphology in seeds of the Viciaeae.

Much of the testa micromorphology observed in this study has been recorded in the previous research of other workers. However, some of these new results do not agree with the earlier work. Consequently, it has been considered useful to include amongst the data described here, some of those features that have already been reported, as well as the new findings. A comparison of the new data with that of previously published observations is made below (section 8.1).

##### 4.3.1. The testa surface.

The testa of seeds of members of the Viciaeae exhibits two types of feature that project above the primary patterning of the surface: the lens and the secondary patterning. Both of these features arise from the palisade layer alone. The anatomy of the lens is illustrated in the longitudinal surface view of a seed of Lathyrus sativus (Plate 77, Fig.2). The conical projection of the lens can be seen to be the result of a group of palisade cells that are elongated. This is similar to the anatomy of secondary patterning or "mounds", which is due to groups of elongated palisade cells, visible in the

testa of Lathyrus annuus (Plate 50, Fig.5), which is described in following paragraphs.

The most characteristic form of the testa surface of the seeds of the taxa in this study is papillose. Each papilla is an outgrowth from an individual palisade cell, shown in Vicia lutea (Plate 12, Fig.5).

The area of cell wall occupied by the papillar base varies with the species. Most commonly virtually the entire cell wall is covered by a papilla, as seen in Vicia cracca (Plate 3, Fig.3), and V.lutea (Plate 11, Fig.7). Sometimes the papillar base is narrower, as in V.melanops (Plate 13, Fig.7) and V.peregrina (Plate 21, Fig.6); or, even more markedly, the papillae are separated by a wider margin, as in V.ervilia (Plate 5, Fig.7).

In all taxa except in the genus Lens (Plate 45, Fig.4,6), the papillae are ridged, the longitudinal ridges usually intercalating between those of adjacent papillae, and covering the testa between papillae. The extent of ridging varies between species, and between populations of the same species, exemplified by Vicia ervilia (Plate 5, Fig.5,7). In certain taxa, the form of the ridges is distinctive: for example, in Vicia sylvatica (Plate 32, Fig.5,6), the ridges are twisted to varying extents into "knots" at the apex of each papilla; in V.sepium (Plate 30, Fig.3), and Lathyrus sylvestris (Plate

78, Fig. 7) the ridges form a reticulum at the apex, and in the latter they are transversely wrinkled.

Ridges may outline the palisade cell walls. However these features seem usually to be inconsistent within species, or between different areas of a single specimen. The ridges vary in thickness and definition, as exemplified on the seeds of Vicia cracca (Plate 3, Fig. 3, 4).

In taxa such as Vicia narbonensis (Plate 15, Fig. 4), the papillae are sometimes shouldered, and the angle of taper of the papillae changes along their length. The apices of the papillae may be curved or even flattened, as exemplified in some seed specimens of Vicia bithynica (Plate 1, Fig. 7, 8). The manifestation of both these features appears to be unstable. They may be intrinsic to the testa, or perhaps they are induced prior to dehiscence in some specimens, by pressure upon the seed by the pod or by adjacent seeds. They may equally be artifactual, produced under the vacuum of the sputter-coater, or of the SEM. It has not been possible during this study to clarify the aetiology of these characters.

The topographic patterning of the testa varies over the surface of the seed. The external cell walls of the palisade cells vary in shape and size with their position on the seed, producing a variable distribution of the surface features. Immediately adjacent to the hilum the shape of the palisade cells is usually slightly



elongated, appearing in surface view as approximately rectangular; at the midseed region, these cells have sides of approximately equal length. The height of the papillae also may vary with the position on the seed. In Pisum sativum sativum arvense (Plate 84, Fig. 5, 6, 8, 9) for example, the papillae tend to be flatter at the midseed region. Usually the form of the patterning seen on positions of the seed adjacent to the hilum is more clearly discernible than that at midseed.

The widest range of papillar height may be observed in Lens; the seeds of Lens odemensis (Plate 45, Fig. 4, 6), as observed in this survey, consistently have the most acutely papillose testa; they may be contrasted with the very flattened palisade cells of the other Lens species (Plates 38, 41, 43, 47), although one population of L. orientalis (Plate 47, Fig. 7) has also been recorded as acutely papillose. As further examples of the reduced palisade cell topography, all populations of Vicia faba (Plate 8, Fig. 6, 8) have flat cell surfaces with very little pattern detail.

Papillae on an individual seed may be heteroge~~ous~~<sup>ous</sup> in width and height, even when there is no secondary patterning: rows of slightly larger papillae, amongst those of a standard size, may be arranged to form chains, as seen in Vicia orobus (Plate 19, Fig. 2), V. tetrasperma (Plate 34, Fig. 5, 7), Lathyrus heterophyllus (Plate 60, Fig. 5) and L. hirsutus (Plate 62, Fig. 4, 6). In the

latter two species, these palisade cell chains can be discerned to be distinct from the secondary patterning over which they occur.

Other topographies exist, particularly in the genus Lathyrus, as exemplified by (i) the reticulate testa of L.aphaca (Plate 51, Fig.5-8), and (ii) the thick ridges outlining the cell walls in L.inconspicuus (Plate 64, Fig.7). The form of this type of patterning varies within species, as seen in L.pratensis (Plate 73, Fig.5-7), both in the shape of the reticular patterning, and in the thickness of the ridges that define it. Because of the complex and variable nature of these other topographies, and because of the intended application of the results of this survey to charred seeds with altered testa surfaces, it has not been attempted to classify them individually, and they have been recorded collectively as "non-papillose", or "reticulate". In some taxa with reticulate testas, the palisade cells adjacent to the hilum may be very slightly domed and have a dimpled centre. This condition has been recorded in Lathyrus laxiflorus (Plate 66 Fig.2,3), L.niger (Plate 67, Fig.3), and L.pratensis (Plate 73, Fig.5). In some populations of L.niger these domed and dimpled cells have also been observed at midseed (Plate 67, Fig.8).

Secondary patterning has been observed mainly in members of the two genera Lathyrus and Pisum, but also to a very minor extent in Vicia. It is created by groups of long

palisade cells, termed "mounds", after Lersten (1981). Their morphologies have proved to be unique to the taxon in all the examples examined in this study. These features may occur together with papillose or non-papillose primary surface topography. Thus Lathyrus nissolia (Plate 69, Fig. 5-7) has mounds overlaid by a reticulate surface; Pisum sativum elatius (Plate 81, Fig. 3) has mounds overlaid by a papillose surface; Lathyrus annuus (Plate 49, Fig. 1) has mounds coalescing to form large ridges with a papillose surface; L. hirsutus (Plate 62, Fig. 4, 6) has mounds with a linked-chain papillose surface (see the seventh paragraph in this section). The seeds of some taxa have a reduced form of secondary patterning that is not visible to the naked eye, yet can be observed by SEM. Vicia lutea (Plate 11, Fig. 3), Lathyrus blepharicarpus (Plate 53, Fig. 3) and Pisum sativum sativum arvense (Plate 84, Fig. 3, 4) fall into this category.

Another feature on the testa surface is cuticular wax. This is discussed in detail below (section 8.1.6.1.); it has been observed in the populations of just two species, Lathyrus inconspicuus (Plate 64) and L. ochrus (Plate 71), and has been consistently present on all specimens of these species examined, although it has varied in extent, even on individual seed specimens.

A deposit that may obscure the surface patterning has been observed on the surface of the seeds of many taxa,

exemplified by Vicia cracca (Plate 3, Fig. 9), V. peregrina (Plate 21, Fig. 7, 8) and Lens culinaris (Plate 38, Fig. 6, 8). It commonly occurs in the greatest density at midseed, lateral to the hilum. It can be discontinuous in distribution, as in Lens culinaris (Plate 38, Fig. 6), or exist as virtually solid sheets of material that have the appearance of having been laid down as a liquid which has solidified between the papillae (seen in Vicia peregrina, Plate 31, Fig. 8). The heavy deposits have been removed from certain specimens by digestion with proteolytic enzymes; they thus appear to have been proteinaceous. The deposits have the appearance of having been superimposed upon the seed from an external source; it has not been possible within the confines of this project to investigate their aetiology (8.1.6.2. below). Testa surfaces with a light deposit have not been treated with enzymes, since it was considered preferable to retain the testa surface in its natural state where possible, to minimise the possibility of creating artifacts; it is uncertain whether all the deposits have the same composition and aetiology.

4.3.1.1. Seed identification from surface micromorphology: a key.

The key (Figure 1) that has been constructed below employs 20 surface characters to separate the 44 taxa observed by SEM in this project. The characters selected survive charring, and are potentially observable in

ancient seeds. The applications of this key are limited, due to the facts that (i) many species are not included and (ii) it is likely that the range of micromorphological variation is greater than has been observed in such a restricted survey. The key clearly demonstrates the restricted separations that have been achieved using testa topography. It can be used, however, to facilitate the identification of certain wild genera, and separate the single cultigen Vicia faba from some of the most closely-related wild species.

Figure 1. Key to Viciaeae Using Characters of Testa Micromorphology.

(Some taxa are represented in more than a single category.)

1a. seed visibly rugose	2
b. seed not visibly rugose	6
2a. secondary patterning as "mounds"	4
b. "mounds" coalesced into "ridges"	3
3a. secondary patterning covers entire seed surface	<u>L.hirsutus</u>
b. secondary patterning interspersed by flat areas on the seed	<u>L.heterophyllus</u>
4a. primary patterning reticulate	<u>L.nissolia</u>
b. primary patterning papillose	5
5a. secondary patterning covers entire seed surface	<u>L.annuus</u>
b. secondary patterning interspersed by flat areas on the seed	<u>P.sativum elatius</u>
6a. secondary patterning visible by SEM	7
b. no secondary patterning	10
7a. papillae reticulate at apex	<u>L.sylvestris</u>
b. papillae not reticulate at apex	8
8a. papillae linked in chains	<u>L.tuberosus</u>
b. papillae free	9
9a. marked confluence of ridges from adjacent papillae	<u>P.sativum sativum arvense</u>
b. more discrete papillae	( <u>V.lutea</u> <u>L.blepharicarpus</u> )
10a. non-papillose primary patterning	11
b. papillose primary patterning	13
11a. reticulate patterning	12
b. non-reticulate patterning	<u>L.inconspicuus</u>
12a. reticulum stellate, radiating from centre of cell	( <u>L.laxiflorus</u> <u>L.niger</u> <u>L.pratensis</u> )
b. reticulum not stellate	<u>L.aphaca</u>
13a. papillose primary patterning	
b. reduced primary patterning	14

- 14a. central domes, or highly reduced papillae, surrounded by ridges (Lens culinaris  
(L. ervoides  
(L. nigricans  
(L. orientalis
- b. each dome covering entire cell surface, with superimposed fine reticulum (V. faba  
(V. faba paucijuga  
(V. sativa sativa lentisperma
- 15a. very acute papillae without ridges Lodemensis  
b. ridged papillae 16
- 16a. ridges twisted at apex of papilla V. sylvatica  
b. ridges not twisted 16
- 17a. papillae reticulate at apex V. sepium  
b. papillae not reticulate at apex 18
- 18a. ridges confluent between adjacent papillae (V. orobus  
(V. tetrasperma  
(L. tuberosus  
19
- b. ridges not confluent
- 19a. cell walls ridged (V. cracca  
(V. villosa  
(L. clymenum  
20
- b. cell walls not ridged
- 20a. papillae flat and widely dispersed (L. sativus  
(P. sativum sativum sativum  
(V. bithynica  
(V. ervilia  
(V. melanops  
(V. narbonensis  
(V. noeana  
(V. peregrina  
(V. sativa nigra  
(V. sativa sativa  
(V. sativa sativa lentisperma  
(L. cicera  
(L. gorgoni  
(L. ochrus  
(L. pseudocicera  
(L. sativus

In the following sections the micromorphology of internal features of the testa are described.

#### 4.3.2. The micromorphology of the hilar region.

The hilum in tranverse view can be seen to consist of the double palisade layer, forming the hilar lips, with the slit between, and the tracheid bar embedded in aerenchyma. The profile of the hilum in this view may be variously protruding or sunken. Lens species (Plates 39,42,44,46,48) have seeds with pointed profiles; Vicia bithynica (Plate 2) has seeds which exemplify the flat profile; Lathyrus sativus seeds (Plate 77, Fig.1) typify those with sunken hila. The aerenchyma beneath the hilum varies in depth along the length of the hilum, thus it is important always to bisect the seeds close to the centre position of the hilum, in order to achieve some standardisation of the observations. The shape of the aerenchyma as seen in transverse view appears to vary with the species, and is related to the hilar profile; a broad shield-shaped area of aerenchyma characterises seeds of the shape of Lathyrus sativus (Plate 77, Fig.2); a narrower aerenchyma zone is found in Vicia faba (Plate 9, Fig.2; Plate 10, Fig.1), where the embedded tracheid bar occupies the full depth from the hilum to the cotyledons.

#### 4.3.3. The micromorphology of the tracheid bar.

The tracheid bar in transverse view varies from round to



narrowly oval in shape, and has from three (in Lens species, for example, Plate 39, Fig.4; Plate 41, Fig.3) to ten tracheoids (in Lathyrus sativus, Plate 77, Fig.2)) in its width. The number of tracheoids in the width of the tracheid bar seems to be a function of the total width of the bar. The length of the tracheoids is difficult to estimate or measure; these elements are arranged approximately perpendicular to the hilum slit but may be slightly oblique in orientation. Consequently the surface of the bisected seed usually does not show the complete length of any tracheoid, but parts of a number of tracheoids broken along their length.

The depth of the bar relative to the depth of aerenchyma varies along the length of the tracheid bar, as has been shown in the serial sections cut along the length of the tracheid bar in Lathyrus cicera, L.clymenum and L.pratensis, and varies in the seeds of different taxa when measured at the standard position midway along the hilum that has been selected for bisection of the seeds.

4.3.4. Tracheoid anatomy: the distribution, micromorphology and anatomy of tracheoid pits.

The anatomy of the tracheoids appears to vary between species. The distribution of the pits is commonly regular and is even and alternate in arrangement. Frequently, however, areas of the tracheoid wall can be seen where the pits are unevenly scattered, as in

Lathyrus pratensis (Plate 74, Fig.3).

Tracheoid pits may vary from slit-like to oval, round or rectangular in shape, and similarly in the shapes of the pit apertures. Most commonly a mixture of pit morphologies is observed within a single seed specimen, as seen in Lathyrus pratensis (Plate 74, Fig.4,5).

The pit borders may be variously ornamented, being plain-bordered, as in Lathyrus annuus (Plate 50, Fig.4), warty, as in Lens nigricans (Plate 44, Fig.4), or vestured, as in Vicia cracca (Plate 4, Fig.6). The ornamentation may vary in different populations of the same species, and even within the same seed specimen as in Vicia faba (Plate 9, Fig.4,5; Plate 10, Fig.3,5). Plain-bordered pits with occasional warts seem to be the most common. Elaborate vestures are rare, being most developed in Vicia orobus (Plate 20, Fig.3,4) amongst the species observed in this project. Observations of white, brown-mottled and black seeds of Vicia sativa sativa have shown that testa colour does not appear to be correlated with any particular pit anatomy (Plate 27, Fig.4-8).

The primary cell membrane occasionally has been recorded in the tracheoid pits, as in Vicia narbonensis (Plate 16, Fig.5).

Observations made by SEM of the tracheid bar in thin sections of the testa in Lathyrus cicera, L.clymenum and

L.pratensis have demonstrated the consistent nature of tracheoid micromorphology. The form of the pits does not appear to vary with the position of the tracheoids: pits that connect with the parenchyma surrounding the tracheid bar display the same range of variation that is found in pits of tracheoids that intercommunicate at the centre of the tracheid bar: similarly, the micromorphology of the tracheoids does not appear to vary with the position along the length of the tracheid bar.

#### 4.3.5. The internal micromorphology and anatomy of the testa.

The cells of the testa layers have been illustrated in longitudinal section at high magnifications to show their anatomy; palisade cells are illustrated in Vicia lutea (Plate 12, Fig.5); an hourglass cell is shown in Vicia orobus (Plate 20, Fig.8). The papillose testa surface can be seen to be a product of an outgrowth of each palisade cell wall. The characteristic shape and fluted appearance of the hourglass cells is visible.

The testa depth has been a particular focus of the data collection; measurements of cell layers may be readily collected from fresh seeds, and are also potentially measurable in archaeological specimens. Features within the cell layers have been excluded from the recorded observations, since they are frequently not observable both in certain seed specimens and particularly in

charred tissues.

To minimise any variations in the thickness of the testa that might accompany changes in the levels of hydration of the dry, fresh seeds, which are stored under various conditions of temperature and humidity (see 3.2.3.), stubs of seed specimens were allowed to equilibrate with the environmental conditions of the SEM laboratory prior to making observations by SEM (see 3.3.2.d).

The testa may vary in depth in different populations of the same taxon, as in Vicia peregrina (Plate 22, Fig.7; Plate 23, Fig.7-9). In Vicia sativa sativa observations made on seeds with testas of three different colours from three populations, show that the white and black testas are both consistently thinner than the brown mottled testas. Here, testa thickness does not correlate with the depth of colour of the seed. The mottled character has not been investigated sufficiently for any conclusions to be drawn from its presence.

The hourglass cells vary in depth over the seed. The palisade cells tend not to vary in length with their position on the seed. Variations in the total testa depth therefore reflect mainly the variations in hourglass cell length. The species Pisum sativum shows the most marked intraspecific variation in characters relating to testa thickness: seeds of P.sativum sativum consistently have thinner testas than those of P.sativum

elatus.

The thickest testas are those with secondary patterning, and this feature is found only in wild taxa. The thinnest testas are those in the genus Lens of all four wild species and the cultigen.

#### 4.3.6. Vascular tissue in the testa.

Occasionally the xylem elements of vascular tissue can be observed lying beneath the testa at the opposite side of the seed to the hilum, as illustrated in Lens nigricans (Plate 44, Fig.10), and Lathyrus annuus (Plate 49, Fig.6). These represent the single vascular strand that runs around the periphery of the seed (Kupicha, 1977). The vascular strand is not commonly observable in seeds that have been bisected in this project; and it has not contributed to the observations recorded in the dataset.

#### 4.3.7. Connections between the testa and the cotyledons.

Beneath the testa, on the cotyledon surface situated opposite the hilum, very narrow strands of tissue have been recorded. They appear to emerge perpendicular to the cotyledon surface and to connect with the testa. These have been observed only in Lens species, and are illustrated here in Lens ervoides (Plate 42, Fig.7,8). Their significance is unknown.

#### 4.3.8. Evidence of possible seed pathology in the Viciaeae.

Certain features have been recorded throughout this survey that are of irregular occurrence, and that have appeared to be atypical of the micromorphology of the taxon concerned. It has not been possible within the confines of this project fully to investigate these phenomena. The possibility that a physiological disorder or a pathological condition underlies some of the observations is discussed in greater detail in appendix 2.

##### 4.3.8.1. Fungal infections.

Some fresh seeds from reference collections are covered with a slight "bloom". These specimens are revealed under SEM to be covered by fungal hyphae. Specimens may also bear very few hyphae and appear normal to the naked eye. The fungus can easily be removed by the ultrasonic treatment described above (section 3.3.2.a)(i)). The seed topography appears not to be affected by such topical infections, which appear to have invaded the seeds after harvest and during storage.

##### 4.3.8.2. Cavities between the cotyledons.

Some seeds of species in all four genera in the survey have been hollow, with cavities between the cotyledons.

These have been recorded in Vicia peregrina, Lens nigricans, L.orientalis, Lathyrus hirsutus, and Pisum sativum elatius. This hollowness of the seeds does not appear consistently in any taxon, and has been interpreted as the manifestation of the physiological disorder "hollow heart" (Heydecker & Kohistani,1969), which is discussed in appendix 2 below.

#### 4.3.8.3. Testa pits.

Pits have been observed in the testa surfaces of seeds of six taxa: Vicia faba paucijuga, V.peregrina (Plate 21, Fig.9), Lathyrus cicera (Plate 54, Fig.9), L.niger, L.pratensis, and L.sativus. They are of irregular occurrence; they are not present on all seeds in a population; and they are of irregular distribution and density, being mainly concentrated in the region around the hilum. Thin sections through the pitted testa of Lathyrus pratensis pit have suggested that the pits do not penetrate the full thickness of the testa; the surface of the cotyledons beneath the pits has not been studied by SEM.

The possibility that these features have a pathological aetiology is discussed below (appendix 2).

#### 4.3.8.4. Invasion of seeds by insects.

Rarely during the survey, upon bisection, seed specimens

have been observed in which the cotyledons were largely eaten away, the seeds consisting of little more than the testa with a thin lining of cotyledonous tissue, with, within the cavity, the deceased larva of an insect. Although the testa and hilum surface have been of normal appearance, the tracheid bar consists of tracheoids that are flattened, with imperforate, slit-like pits. An example is illustrated by a charred seed specimen of Pisum sativum elatius (Plate 83, Fig.6). Externally the seeds appear to the naked eye to be normal, with no sign of invasion by an insect. Insect predation of legume seeds is discussed in appendix 2.

#### 4.4. Changes to the morphology and anatomy following charring.

The results of charring have been observed mainly after two hours heating at 300°C (see section 3.3.3.b) above). This regime was selected to reproduce the physical state of charred archaeobotanical seeds, as judged by the naked eye, and also to reproduce approximately the state as observed under SEM of the altered micromorphology of charred ancient seeds. It is impossible to estimate to what extent the size of ancient seeds had been altered by charring in antiquity, since the original seed size is unknown, and therefore the effects of charring on seed size have not been considered when selecting the routine charring regime. The probability is acknowledged that under different charring conditions other artefacts of



charring can occur in seeds than those that are described below.

4.4.1. Effects of charring on seed shape and size.

Charring under the regimes employed in this study seems to have little effect upon the size and shape of seeds of the Viciaeae regardless of the original gross morphology. This is illustrated in the Plates listed in Table 9.

Table 9. List of Plates Illustrating both Fresh and Charred Seeds of the Same Species, Representing a Variety of Seed Shapes.

Species	Seed Shape	Plate(P) and Figure(F)	
		Charred	Fresh
<u>Lathyrus annuus</u>	spherical	P49 F2	P49 F1
<u>Lathyrus hirsutus</u>	spherical	P62 F2	P62 F1
<u>Vicia ervilia</u>	angular	P8 F1	P6 F1
<u>Lathyrus cicera</u>	angular	P56 F1	P54 F1
<u>Vicia sativa nigra</u>	obovate	P24 F2	P24 F1
<u>Vicia villosa varia</u>	obovate	P36 F2	P36 F1
<u>Lathyrus aphaca</u>	obovate	P51 F2	P51 F1
<u>Vicia melanops</u>	subrect- angular	P13 F2	P13 F1

Comparisons between fresh and charred seeds of the same populations of each species illustrate the typical lack of marked dimensional change that is observed following charring at 300°C for two hours. When higher temperatures are employed, however, the seed may become distorted or fragmented.

An example of distortion following charring is illustrated in Plate 47, Fig.8, showing a seed of Lens orientalis which has become inflated from the normal

lenticular shape (seen in Plate 47, Fig.1) following charring at 400°C for one hour. The gaping cotyledons enclose a cavity, and there is a hole leading to it. While it may be possible to interpret this solely as the result of charring at high temperature, it is believed that it is more likely to be due to a predisposing factor in the seed itself, such as "hollow heart", a condition described in appendix 2.

4.4.1.1. Note: observations made following accidental charring above 400°C: some additional data.

During one charring episode, undertaken subsequent to the main research period of the project, a fault in the thermostat of the muffle furnace resulted in an accidental increase in temperature above 400°C (the exact temperature could not be ascertained in the circumstances). Seeds of Pisum sativum sativum, and Lens culinaris were removed from the furnace fully charred after only 35 minutes. There appeared to the naked eye to be changes in the dimensions of both species after the charring, and so measurements were made on fifty specimens each, of both fresh and charred seeds of the two species, listed in Table 10, below. The dimensions selected for measurement were the same for seeds of both species: the maximum diameter, and the seed thickness.

Table 10. Dimensions of Samples of 50 Fresh Seeds, Uncharred and after Charring above 400°C.

Seed species	Physical State	
	Fresh	Charred
<u>Pisum sativum</u>	mm.	
Maximum diameter	10.3	9.2
Minimum diameter	7.0	6.0
Mean diameter	7.8	7.8
Maximum thickness	6.9	7.2
Minimum thickness	4.9	5.0
Mean thickness	5.9	5.9
<u>Lens culinaris</u>		
Maximum diameter	6.2	6.7
Minimum diameter	3.8	4.1
Mean diameter	4.9	5.1
Maximum thickness	3.5	4.0
Minimum thickness	2.1	2.8
Mean thickness	2.7	3.3

The seeds of Pisum, from a wrinkled-seeded population, became more spherical and smooth in contour following charring; but were unchanged in dimensions. The seeds of Lens exhibited an appreciable increase in size; no concavities developed on the inner surface of the cotyledons to account for the slight increase in thickness. The testa on the seeds of both species was retained on most specimens, although on the lentils it was split around the periphery of the seed. The increased size of the cotyledons in the lentils appears largely to have been accommodated within a testa that has increased in surface area. It has not been possible within the period of the project to investigate by SEM the changes in anatomy that have contributed to the size increase that resulted from this accidental episode. It has only been possible to record the changes in gross morphology.

#### 4.4.2. The charred testa.

Characteristically the testa cracks on charring as a result of the differential stresses within the testa layers, presumably due to the release of water from the seed and some expansion of the embryo, as illustrated in Vicia ervilia (Plate 7, Fig.1). The thickness of the testa seems to be a factor in determining the type of cracking. Thus the surface, particularly of thin testas, may become crazed with numerous fine cracks, as shown in Vicia sativa nigra (Plate 24, Fig.4), Lathyrus aphaca (Plate 52, Fig.1) and Lens orientalis (Plate 47, Fig.8). By contrast, thick testas appear to adhere more strongly to the cotyledon surfaces, and deep cracks tend to penetrate to the surface of the embryo (Vicia ervilia, Plate 7, Fig.2; V.melanops, Plate 13, Fig.2,4), or even into the tissues within (Lathyrus annuus, Plate 49, Fig.2; L.hirsutus, Plate 62, Fig.2).

Certain areas on the testa often show marked tendencies to crack, exemplified by the lens (Vicia villosa, Plate 36, Fig.2; Lathyrus aphaca, Plate 52, Fig.2). The hilum, too, represents a region of marked differential stress, such that cracks may appear on it (Vicia melanops, Plate 13, Fig.4), or around it (Vicia ervilia, Plate 7, Fig.3; V.villosa, Plate 36, Fig.2; Lathyrus pratensis, Plate 73, Fig.4). The testa may be lost around the hilum (Lens culinaris, Plate 40, Fig.3); or, conversely, the hilum may detach and the testa preferentially be retained. Usually

a halo of testa remains attached around the hilum, and is demarcated by the cushion of parenchyma surrounding the tracheid bar beneath the hilum. Thus a detached hilum usually consists of an area covered by palisade and counter palisade layers, beneath which is the the tracheid bar embedded in parenchyma, as illustrated in Lathyrus annuus (Plate 50, Fig. 8).

A thin testa may exhibit a high degree of plasticity when heated, and patches may curl away from the cotyledon surface (Lens culinaris, Plate 40, Fig. 4). When such testa fragments become detached, they have a misleading appearance by being concave on their external surface, and thus are "inside out"; extra care must be taken in establishing the correct orientation of each such fragment before its examination and interpretation are attempted.

The testa itself may fracture along or between its cell layers. The hourglass cells represent a plane of relative weakness, and commonly shear across their narrowest width, leaving areas of broken cell bases over the seed surface. These often have the misleading appearance of large angular papillae (Lathyrus cicera, Plate 55, Fig. 8, 12; L. pratensis, Plate 73, Fig. 8, 12; Lens culinaris, Plate 40, Fig. 7;). It is common for a single charred seed to exhibit areas where each of the different layers is exposed, thus showing the intact external testa surface, broken hourglass cell bases, parenchymatous

remnant cell layers, and the surface of the cotyledon itself. Each of these layers may be relatively thin, and it may be surprisingly difficult to establish the identity of a particular charred surface without recourse to SEM (Lens culinaris, Plate 40, Fig. 6, 7).

Commonly the seed loses the entire testa, often together with the radicle, and is preserved as the charred cotyledons and embryonic axis. The testa may be shed in fragments of various sizes, which can be examined in isolation by SEM.

#### 4.4.3. Micromorphology of the charred testa surface.

The surface of the testa usually shows some changes in topographical patterning following charring.

The secondary patterning, exemplified in Lathyrus annuus, is typically little changed after charring, although fine cracks may appear through the mounds (Plate 49, Fig. 8). Major cracks through the testa and cotyledons appear to absorb the greater part of the heat-induced stresses in such seeds where the testa is thick. Often observable effects of charring on this secondary patterning are negligible or absent (Lathyrus hirsutus, Plate 62, Fig. 8; L. nissolia, Plate 69, Fig. 8; Pisum sativum elatius, Plate 83, Fig. 7, 8).

On testas with papillose topographies, charring usually

does not change the density or height of the papillae. The bases of papillae may contract, resulting in a flattened area between papillae which have themselves become narrower and thus more acute. The ridges along the papillae become reduced in number, and some of those that intercalate between cells appear to fuse. Ridging outlining the palisade cell walls tends to become reduced.

A rarely-observed phenomenon is the fracture on charring of the apices of the papillae (Vicia ervilia: Plate 7, Fig.6).

In summary, the results of charring, at temperatures of 300C or under, typically produce minimal changes to micromorphology of the papillae themselves, perhaps no more than a slight shrinkage of the bases, giving more acute edges to topographical features. This may be seen particularly in papillae of palisade cells lateral to the hilum. However the very acute papillae in Lens odemensis seem very little altered after charring. Most changes seem to affect the ridging around and between papillae. Plates illustrating comparisons between the testa surfaces of fresh and charred seeds are listed in Table 11.

Table 11. List of Plates Comparing Fresh and Charred Papillose Testa Surfaces.

Species	Plate(P) and Figure(F) Numbers.	
	Charred	Fresh
<u>Vicia melanops</u>	P13 F6,8	P13 F5,7
<u>V.sativa nigra</u>	P24 F6,9	P24 F5,7
<u>V.villosa varia</u>	P36 F6,7	P36 F4,5
<u>Lens culinaris</u>	P40 F5,6	P38 F6,8
<u>L.ervoides</u>	P41 F7,8	P41 F3-6
<u>L.nigricans</u>	P43 F8-10	P43 F5-7
<u>L.odemensis</u>	P45 F7,8	P45 F4,5
<u>Lathyrus sativus</u>	P76 F9,10	P76 F3-7

The non-papillose testas also show little alteration to the basic patterning after charring. Reticulate patterns on the palisade cells, exemplified by L.aphaca (Plate 51, Fig.7), and L.nissolia, between mounds, (Plate 69, Fig.8) tend to become sharper as the ridges become more acute. Conversely, on some specimens reticulations may become less well-defined, as seen here in Lathyrus pratensis (Plate 73, Fig. 9-11). However these changes due to charring can be overshadowed by the potential variability between populations of the same species in this type of topography, described above (section 4.3.1.). The domed and dimpled patterning that can sometimes be found particularly near the hilum on non-papillose testas becomes more exaggerated after charring, as seen in seeds of Lathyrus niger (Plate 67, Fig.5-8).

In the two species that have cuticular wax on the seeds, the effect of charring in the populations studied in this project appears to be variable. In Lathyrus ochrus the



wax crystals seem to melt to form stringy deposits on the papillae (Plate 71, Fig. 8). In L. inconspicuus the wax rods largely disappear on charring (Plate 64, Fig. 7).

The charred testa has a tendency to fracture across the hourglass cells, as described above. Such broken hourglass cell bases are illustrated in Lathyrus cicera (Plate 55, Fig. 5), L. pratensis (Plate 73, Fig. 8), and Lens culinaris (Plate 40, Fig. 7). These fractured cells may appear very similar to the papillae on palisade cells which have narrowed and become angular following charring, as seen in Lens culinaris (Plate 40, Fig. 6, 7). However there is a major difference in scale of approximately one order of magnitude between the size of the hourglass cells and the palisade cells in this species: generally the former are visible by optical microscopy. The scale must be borne in mind when observing the surfaces of charred legume seeds to prevent the formation of erroneous conclusions.

Where charring has occurred at the relatively high temperature of 400°C, small blisters may be produced over all cell surfaces as illustrated on the external testa surface of Lathyrus clymenum (Plate 58, Fig. 9) and on the broken hourglass cells in Lathyrus pratensis (Plate 73, Fig. 12).

The inner surface of the testa carries an imprint of the surface of the cotyledons, which is preserved after

charring. This is exemplified by the imprints of the petioles of the cotyledons, lying on either side of the embryonic axis, described above in fresh seeds of Vicia narbonensis (Plate 15, Fig. 7, 8), and illustrated on a charred detached piece of testa in Lens nigricans (Plate 43, Fig. 4). Such features can be used to identify the original position and orientation of a testa fragment on the seed, and this can be important when comparing the external testa surfaces of archaeobotanical and recent seeds for identification.

#### 4.4.4. Micromorphology of the charred cotyledons.

The cell structure of the cotyledons becomes altered on charring, in two respects readily observable under SEM. Firstly, the large storage cells which contain starch grains and which comprise the major part of the cotyledon tissues, lose their cell contents, and appear as rounded hollow vacuoles. The characteristic appearance of this charred tissue is also readily recognised under the low-power dissecting microscope, during the initial sorting of plant macroremains from archaeological contexts. Secondly, small parenchymatous cells lose definition, through adjacent cell walls breaking down and fusing. Thus some tissues in the vascular strands may appear as solid tracts of tissue; epidermal cells, together with their cuticles also become fused, such that the cotyledons adhere together with a physical and chemical bonding of their tissues. This is illustrated in Vicia

ervilia (Plate 7, Fig. 3, 5), V. melanops (Plate 14, Fig. 8), Lathyrus cicera (Plate 55, Fig. 7, 8), L. sativus (Plate 77, Fig. 11), and Pisum sativum sativum sativum (Plate 87, Fig. 3). Similar phenomena have been recorded in the phloem cells of roots and tubers which may fuse on charring (Hather, 1989, p. 157).

#### 4.4.5. Micromorphology of the charred hilar region.

The morphology of the hilar region in transection appears little altered following charring (Vicia faba, Plate 8, Fig. 2; Lathyrus cicera, Plate 55, Fig. 2; Lens odemensis, Plate 46, Fig. 5). However the anatomy of the tracheoids tends to be altered. Pit ornamentation always becomes reduced, but to varying extents. Rarely the pit borders are little changed (Vicia faba, Plate 10, Fig. 4, 6); more commonly the warts and vestures become indistinct or are even lost (Vicia sativa nigra, Plate 25, Fig. 5; Lathyrus cicera, Plate 55, Fig. 3; L. pratensis, Plate 74, Fig. 4, 7). Some tracheoids develop artifactual structures in the form of bars that traverse the pit apertures (Lathyrus cicera, Plate 55, Fig. 3; L. pratensis, Plate 74, Fig. 4, 7; Lens culinaris, Plate 40, Fig. 8); this phenomenon follows charring at temperatures over 300°C, having been recorded after charring at 400°C.

#### 4.4.6. Micromorphology of the charred testa layers.

The testa most typically does not shrink in thickness to

an observable extent under the charring regimes employed, as exemplified by representative specimens of a wide range of members of the Viciaeae listed in Table 12.

Table 12. Comparison of Testa Thickness between Fresh and Charred Seeds, Following Charring at 300C.

Species	Plate (P) and Figure (F)			
	Fresh		Charred	
<u>Vicia ervilia</u>	P7	F7	P7	F8
<u>V.faba</u>	P10	F7	P10	F8
<u>V.melanops</u>	P14	F6	P14	F7
<u>V.narbonensis</u>	P16	F9	P16	F10
<u>V.villosa</u>	P37	F8	P37	F10
<u>Lathyrus aphaca</u>	P52	F8	P52	F9
<u>L.niger</u>	P68	F5,6,7	P68	F8,9,10
<u>L.sativus</u>	P77	F9	P77	F10
<u>Pisum sativum sativum sativum</u>	P86	F8	P86	F9

The palisade cells show variable changes in texture, but not in dimensions. The hourglass cells typically become angular, rather than fluted and circular, in plan along their length. The remnant layer appears unchanged.

The crystals recorded in one population of Lathyrus aphaca appear to be unchanged by charring at 300C for 2 hours (Plate 52, Fig,8).

The following paragraphs in this chapter describe the observations of charred ancient seeds from archaeological contexts; the features recorded are the same as those in modern seeds.

#### 4.5. Observations on archaeological seed remains.

##### 4.5.1. Introduction.

The charred remains of seeds from archaeological contexts were investigated throughout the survey of modern seeds. The purpose of these observations was to explore the possibility that the micromorphology seen in the fresh material is potentially visible in well-preserved ancient charred seeds and that the characters of testa micromorphology and anatomy that have been recorded in this project by SEM have a relevance to archaeobotany. The data that have been recorded from the ancient seeds and their fragments, and the manner of their expression, reflect this objective.

The ancient seeds have been selected to represent examples of the major pulse crop species. These remains are discussed in alphabetical order of the sites. The sites are those with identified storage or burial contexts, where the stored material could reasonably be interpreted as food resources, and where there was good preservation of the plant material.

Characters in the archaeological charred seed specimens are described in a sequence similar to that used for modern material (see 4.2.1.1, above), following a brief initial consideration of the physical state of the specimens, with reference to the relevant plates and

figures. The identifications supplied with the specimens are initially used; their validity is discussed at the end of each description.

#### 4.5.2. The state of preservation of ancient charred legume seeds.

The remains of legume seeds from archaeological contexts most commonly have lost their testas, including the hilum. Frequently they consist of separate cotyledons. However, occasionally whole seeds are recovered, that have retained the hilum and a proportion of the testa. The most well-preserved entire seeds tend to be those from storage contexts. It is these that have provided the specimens used in this project. If well-preserved, however, even fragments of ancient seeds may sometimes carry sufficient diagnostic features to allow for the observation and recording of most relevant characters. In situations where such stored seeds have lost their hila, the detached hila may sometimes be found amongst the small seed fragments at the bottom of the storage vessels; commonly the radicle is attached. As has been described above (section 4.4.2.), the aerenchymatous tissue beneath the hilum, and the tracheid bar are usually preserved as part of the hilum. Thus detached hila tend to carry the majority of salient characters that have been recorded in this project.

##### 4.5.2.1. Morphology and micromorphology of charred

archaeological seed material.

Assiros. Bronze Age, Greece (Jones, 1981; Jones et al., 1986). Vicia ervilia. (Plate 88. Number of specimens studied: 2.)

i. Gross seed morphology. The seeds are bluntly triangular and isodiametric in shape. The testa is largely retained and is smooth. The hilum has survived entirely in one specimen (Plate 88, Fig. 1, 3), and half is still present in the other (Plate 88, Fig. 2, 4). It is oval to wedge-shaped (Plate 88, Fig. 3). The micropyle is difficult to discern, due to splitting of the testa along the line of the hilar groove.

ii. Micromorphology of the testa surface. The testa is cracked in both specimens. The palisade layer is missing adjacent to the hilum, revealing broken hourglass cells, which appear to look like papillae (Plate 88, Fig. 4, 5) (as described in section 4.3.2., above). The external surface of the palisade layer is papillose, and blunt papillae are seen over both seeds, adjacent to the hilum (Plate 88, Fig. 6, 8). Lateral to the hilum in one specimen, ridges can be clearly discerned intercalating between adjacent cells (Plate 88, Fig. 7).

The shape of the seed, the gross morphology of the hilum and the patterning of the testa surface compare well with those of the fresh seeds of Vicia ervilia.

Catal Hüyük. Early Ceramic Neolithic, Anatolia  
(Helbaek, 1970). Species A. Pisum sativum elatius.  
(Plates 89, 90. Number of specimens studied: 4.)

i. Gross seed morphology. The seeds are subspherical in shape, with fragments of rugose testa adherent to the cotyledons (Plate 89, Fig. 1, 2). The hilum is wedge-shaped. The micropyle is indistinct and therefore cannot be classified.

ii. Micromorphology of the testa surface. The testa surface is covered in low mounds (Plate 89, Fig. 2), and is papillose. Both between the mounds (Plate 89, Fig. 3, 4), and upon the mounds (Plate 89, Fig. 5), the papillae appear low, and surrounded by a series of ridges, bundles of which intercalate between those from adjacent cells (Plate 89, Fig. 3, 4).

iii. Micromorphology of the internal hilar region. The detached hilum (Plate 90, Fig. 1) shows protruding lips. The tracheid bar is round (Plate 90, Fig. 1, 3, 4), and at least seven tracheoids in width. The tracheoid walls are blistered (Plate 90, Fig. 5). Tracheoid pits are frequently obliterated (Plate 90, Fig. 2), but some specimens show evenly distributed regularly-arranged alternate pitting, of slit shape with plain borders (Plate 89, Fig. 6; Plate 90, Fig. 5, 6). Some pits have barred apertures, similar to the artifacts created by charring fresh seeds at high temperatures (see section 4.3.5. above; Plate



90, Fig. 5, 6).

The altered pits (Plate 90, Fig. 2) appear similar to those seen in seed specimens of Pisum sativum elatius which has been infested with an insect predator (Plate 83, Fig. 6). The fragmented specimen did not contain any cotyledon tissue, and thus it is not possible to speculate further on the likelihood of insect invasion of the specimen in antiquity.

iv. Micromorphology of the internal testa layers. The palisade cells are of the uneven length expected in a rugose testa. The hourglass cells are relatively short adjacent to the hilum (Plate 89, Fig. 7, 8; Plate 90, Fig. 8, 9), and not discernible at other regions (Plate 90, Fig. 8, 9). The remnant layer is moderately thick.

All features observed compare well with those of seeds of recent Pisum sativum elatius (Plates 81-83). The altered form of the pit anatomy in one specimen (Plate 90, Fig. 5, 6) suggests that it was charred at a temperature over 300C.

Çatal Hüyük. Early Ceramic Neolithic, Anatolia (Helbaek, 1970). Species B. Pisum sativum sativum. (Plate 91. Number of specimens studied: 4.)

i. Gross seed morphology. The seeds are subspherical, with smooth testas (Plate 91, Fig. 1, 2). The hila are oval.

The micropyles are indiscernible.

ii. Micromorphology of the testa surface. The testa is papillose. Adjacent to the hilum the papillae are blunt with ridges radiating from the apices (Plate 91, Fig. 3). Lateral to the hilum, the papillae are much reduced, with a stellate arrangement of ridges, which have very acute edges (Plate 91, Fig. 4).

iii. Micromorphology of the internal hilar region. The hilar lips protrude (Plate 91, Fig. 5). The tracheid bar is round, and at least eight tracheoids in width (Plate 91, Fig. 5). The tracheoid pits are evenly distributed in a regular alternate arrangement. Pit shape is a slit, oval, round or subrectangular. Pit borders are mostly plain, but a few warts have been recorded (Plate 91, Fig. 7, 8).

iv. Micromorphology of the internal testa layers. The palisade cells are relatively short. Hourglass cells are short even adjacent to the hilum (Plate 91, Fig. 6). The remnant layer is thin.

The micromorphology compares well with that of fresh specimens of Pisum sativum sativum. There is no evidence of the charring at high temperatures recorded in species A, but since species B was recovered from a different context on the site, this is unsurprising.

Gordion. Iron Age, Anatolia (de Vries, 1980). Lens cf. culinaris. Viciaeae species. (Plates 92, 93. Number of specimens studied: 6 seeds, and 2 hilum fragments.)

i. Gross seed morphology. The seed shape is round in outline, and lenticular in transection (Plate 92, Fig. 1, 5; Plate 93, Fig. 3). The testa is smooth. A single specimen retains a complete wedge-shaped hilum. A second specimen has part of the hilum retained (Plate 93, Fig. 1).

ii. Micromorphology of the testa surface. The seeds retain fragments of the testa in varying amounts. In most specimens the different testa layers can be discerned (Plate 92, Fig. 2). In some specimens most of the testa has been lost (Plate 93, Fig. 1). The external testa surface is papillose (Plate 92, Fig. 3, 4, 6, 7; Plate 93, Fig. 2, 4), with low irregular papillae surrounded by ridges at the base (Plate 92, Fig. 4, 7; Plate 93, Fig. 2). One specimen has a testa surface with reticulate topography (Plate 93, Fig. 8), showing it to belong to a separate taxon, probably Lathyrus, being most comparable with L. aphaca, although, due to its gross morphological characters of seed shape and size, originally it had been grouped with the others.

iii. Micromorphology of the internal hilar region. The lips of the hilum protrude (Plate 93, Fig. 5). The tracheid bar is narrow. The tracheoid pits are slit-like and oval. Pit borders are indistinct, but barred apertures,

described above as artifacts of charring at high temperatures (paragraph 4.3.5.), can occasionally be seen (Plate 93, Fig.7).

iv. Micromorphology of the internal testa layers. The palisade cells are short. The hourglass cells can be seen in transection. The remnant layer is moderately thick.

All the specimens except the reticulate seed compare well with Lens. The hilar gross morphology excludes L.ervoides. The testa surface micromorphology excludes L.odemensis. It is not possible to distinguish between the species L.culinaris, L.nigricans or L.orientalis; there is an overlap in the range of seed size between these wild species and the small-seeded forms of the cultigen; these specimens fall within this size range. The seed that is not lentil is tentatively identified as Lathyrus cf.aphaca.

Manerba. Bronze Age, Italy (Colledge, in prep.). Pisum sativum. (Plate 94. Number of specimens studied: 1 seed, 1 hilar fragment.)

i. Gross seed morphology. The seed is subspherical (Plate 94, Fig.1). The testa is smooth. The hilum is oval or wedge-shaped (Plate 94, Fig.2). The micropyle is indiscernible.

ii. Micromorphology of the testa surface. The testa

surface is covered with very low mounds, seen adjacent to the hilum in Plate 94, Fig. 4. The surface is papillose, with low papillae surrounded by ridges that intercalate with those from adjacent cells in both specimens (Plate 94, Fig. 5-7). The surface is comparable with that of recent seeds of Pisum sativum sativum arvense, however the very low mounds are more distinct than those observed in the modern populations in this study (Plate 84, Fig. 3, 4). This may possibly be an indication of an earlier stage in the development of the cultigen, when it was nearer in form to the wild progenitor, P. sativum elatius; or it may merely be a variant that has not been observed in the recent cultigen.

iii. Micromorphology of the internal hilar region. The hilar lips protrude (Plate 94, Fig. 3). The tracheid bar is preserved only in part, and appears round. It is impossible to estimate the number of tracheoids in the width. Tracheoid pits appear to be evenly distributed in a regular manner, and those observed are oval, round or subrectangular in shape. Pit borders are plain.

All the features observed equate well with the identification of Pisum sativum sativum arvense.

Pella. Bronze Age, Jordan (Colledge, in prep.). Vicia faba. (Plate 95. Number of specimens studied: 3.)

i. Gross seed morphology. The seed is obovate to

subrectangular in shape. Fragments of the testa adhere to the cotyledons (Plate 95, Fig. 1, 2). One specimen retains most of the hilum (Plate 95, Fig. 2), which is situated along the shorter edge of the seed. The hilum appears to be oval to wedge-shaped (Plate 95, Fig. 1). No micropyle can be seen.

ii. Micromorphology of the testa surface. The testa is smooth. No papillae can be seen, but the surface is slightly domed (Plate 95, Fig. 4-6).

iii. Micromorphology of the internal hilar region. The hilum is flat. The tracheid bar is rounded with at least eight tracheoids in its width (Plate 95, Fig. 7). The tracheid pits are evenly distributed in a regular manner, and are mainly oval in shape. Pit borders are plain.

iv. Micromorphology of the internal testa layers. The palisade layer is moderately thick. The hourglass cells lateral to the hilum cannot be discerned. No remnant layer can be seen (Plate 95, Fig. 8).

All features observed equate with the identification of Vicia faba.

Servia. Bronze Age, Greece (Hubbard, 1979). Lathyrus cf. sativus. (Plate 96. Number of specimens studied: 2 seeds, 4 fragments of hilum and 2 testa fragments.)

i. Gross seed morphology. The seeds are rhombic in outline, and rounded-triangular in transection (Plate 96, Fig. 1, 2). The testa is smooth. The hilum is oval, and the micropyle is a slit.

ii. Micromorphology of the testa surface. The testa is smooth and papillose. Details of the testa ridging cannot be discerned (Plate 96, Fig. 4, 5).

iii. Micromorphology of the internal hilar region. The hilar lips protrude. The tracheid bar is missing in one transection (Plate 96, Fig. 6), but the evacuated area retains the impression of the outline of the bar, which was round. Some tracheoids have been retained in one specimen, which show evenly-distributed pits arranged irregularly. The pits are oval, round and subrectangular in shape. Pit borders are plain (Plate 96, Fig. 7, 8).

iv. Micromorphology of the internal testa layers. The palisade cells are moderately short. The hourglass cells and the remnant layer have been indiscernible.

On the basis of the seed shape, these specimens can be identified as Lathyrus of the L. sativus group, previously classified as of section Cicerula, and now incorporated into section Lathyrus. It is impossible to identify the seeds to the level of species, since all other observed characters are comparable with those of the cultigen L. sativus and its closest wild relatives, such as

L.cicera, and L.gorgoni. The circumstantial evidence, that had previously been used to identify the seeds as probably those of the cultigen (Hubbard,pers.comm.), remains the basis for an identification of the L.cf.sativus.

Tell Brak. Bronze Age, Mesopotamia (Charles, in prep.).

Pisum sativum sativum. (Plate 97. Number of specimens studied: 1 seed, 1 testa fragment and 2 detached hila.)

i.Gross morphology. The seed is subspherical (Plate 97, Fig.1). The testa is smooth (Plate 97, Fig.2). The hilum is oval. The micropyle is a slit.

ii.Micromorphology of the testa surface. The testa is papillose, with low papillae surrounded with ridges (Plate 97, Fig.3,4). Bundles of ridges intercalate between those from adjacent cells (Plate 97, Fig.4).

iii.Micromorphology of the internal hilar region. The lips of the hilum protrude slightly (Plate 97, Fig.5). The tracheid bar is rounded, with seven to eight tracheoids in the width. The tracheoid pits are evenly distributed in a regular arrangement. Pit shapes are slit, oval or round. Some show imperforate primary cell walls. Pit borders are plain or have very small warts. Some pits have the barred apertures indicative of a relatively high temperature of charring (see section 4.4.5. above; Plate 97, Fig.5,6,8,9).



iv. Micromorphology of the internal testa layers. The palisade cells are relatively short. The hourglass cells are short adjacent to the hilum and have not been observed at other areas (Plate 97, Fig. 5-7). The remnant layer has not been seen.

All features observed are consistent with the identification of Pisum sativum sativum sativum, comparable with those of the modern specimens (Plates 86, 87), and probably charred at a temperature over 300C.

#### 4.5.2.2. Summary of the results of SEM of ancient charred seeds.

Those ancient charred seed remains that retain the shape of the whole seed, the hilum and most of the testa are likely to manifest all the micromorphological characters that have been recorded in fresh modern seeds.

Amongst the surface features, observations by SEM have shown that the micropyle is frequently impossible to discern where the testa has split. The testa surface, even when appearing to be relatively undamaged to the naked eye, may not portray a sufficiently well-defined patterning for records to be made of its form. Surface debris commonly obscures much detail.

The micromorphology of the hilar region is commonly very well-preserved. The micromorphology of the internal

tissues of the ancient charred seeds, both of the hilar region, and of the testa layers, appears to be little changed from that of modern charred seeds.

Hila that have been detached from the seed surface may bear most of the features that have provided the characters that have been recorded in this survey.

#### 4.6. Further work on the results of microscopy.

In view of the size of the dataset, it was decided to use numerical methods to process the results of the microscopy of the modern seeds, in order to achieve an objective analysis of the data. These analytical methods are next described.

## 5. NUMERICAL METHODS.

### 5.1. Introduction.

At the beginning of this project, in view of published data (see section 1.4.), it had been anticipated that a survey of the surface micromorphology of the testas of the legume seeds would provide criteria that could aid the identification of seeds of members of the Viciaeae. Shortly after the initial results were recorded, it became obvious that a number of taxa have seeds that manifest very similar external features, and that a more elaborate analysis of testa structure was necessary, than the previous purely subjective assessment of a few characters. Thus it was decided to record a larger set of observations and to follow analytical procedures conventional to the field of numerical taxonomy.

### 5.2. Selection of operational taxonomic units (OTU's).

The unit of identity in numerical taxonomy is the operational taxonomic unit (OTU). It might thus be expected that in this study each species investigated would represent a single OTU. However, although this project was designed to examine differences in seed structure between species, as has been explained above, it was considered necessary to take into account intraspecific variation; and thus observations were made on replicate material from several populations of certain

species, and results should be considered as applicable to particular populations. The lowest units of identity of the specimens of fresh seeds of the Viciaeae used throughout this study have been the populations of each species, which consequently have been selected as the OTU's. These are listed in Table 4 (section 3.2.2.).

### 5.3. Selection of characters.

The characters recorded are very largely those of the gross morphology, the micromorphology and anatomy of the testa. They include characters of gross morphology conventionally used in legume seed identification (see section 2.2.2.), micromorphological features of the testa surface that have formed the subject of recent publications (such as Lersten & Gunn, 1982), as well as criteria of the internal testa anatomy that have been used in archaeobotanical identifications (such as those of Hopf, 1986). All these characters are those that are readily observable in well-preserved charred material of archaeological provenance. Two further characters have been incorporated in the dataset: testa colour, and the life habit of each species (whether annual or perennial). These latter data have been included for reference during the evaluation of the analyses, and they also increase the relevance of the data in the more general botanical field.

Altogether a total of 47 characters were recorded for

each population, and formed the complete dataset. This was the basis of the first analyses.

Each unit character is represented by a single attribute for each OTU. Characters have been of both qualitative and quantitative types. They are listed in Table 13.

#### 5.4. Scoring of characters.

##### 5.4.1. Qualitative characters.

These characters have been assessed subjectively and recorded as:

- (i) dichotomous or bistate attributes, scored by presence or absence of a feature, with values of 1 or 0 respectively; certain characters (exemplified by 20,21) may occur in an intermediate state, in which case they are recorded as 0.5;
- (ii) descriptive attributes, assigned coded numerical values, scored as consistently as possible, increasing numerically in value with the increasing prominence or intensity of the feature. The characters are explained in detail where necessary in the following paragraph.

Character 2, testa colour, is graded 1 to 5, based upon

an increasing intensity of tone, from white through greens and browns to black. This character is obviously highly subjectively assessed, but it was attempted to score it as consistently as possible. Character 7, hilum shape, is scored from 1 to 5, based upon an increase in size, and using the 5 categories of Gunn (1970): oval, wedge, oblong, linear and circumlinear (see section 2.2.2.). Character 8, micropyle shape, is based on the size of the aperture and its shape: very small and circular, slit-shaped, oval and triangular. Character 11 refers to the ridging that may or may not intercalate between the papillae of adjacent palisade cells.

#### 5.4.2. Quantitative characters.

These characters have been recorded by the direct measurement of features, each attribute representing a single dimension of a feature or a ratio between two such dimensions, such as the length of the hilum, expressed in millimetres, or the ratio between the depth and the width of the seed. Character 19 is the angle between the hilar slit, and the edge of the aerenchyma on the surface of the cotyledon, against the hourglass cells immediately adjacent to the hilum, as seen in the transverse surface view of the hilum in the bisected seed.

#### 5.4.3. Missing data (NC's).

In some specimens certain features have not been

observable, resulting in missing values, conventionally known in multivariate analysis as no-comparison entries (NC's) (Sneath & Sokal, 1973). Such missing attributes have been scored as 9.99.

### 5.5. Multivariate analysis.

The statistical analyses were carried out following consultations with Dr.D.H.Dalby of the Department of Pure and Applied Biology, Imperial College London. Two analytical procedures were selected: cluster analysis and principal coordinates analysis, that are described in the following section.

#### 5.5.1. Cluster analysis (CA).

This system brings together into clusters the OTU's whose members show the closest similarity. The clusters are arranged in a hierarchic dendrogram based on successive linkages formed between nearest neighbours at decreasing levels of Euclidean distance, with groups, or phenons, defined by an overlay of horizontal lines drawn at the various levels of similarity. Lowest rank units are the OTU's. The abscissa has no meaning except for spacing out the OTU's. The ordinate represents similarity values, the association or resemblance coefficients, correlation coefficients or distances (Sokal & Rohlf, 1962). Of the number of different methods of CA, the unweighted pair-group method using arithmetic averages (UPGMA) was

employed for this project. The values along the ordinate axis are similarities expressed as distances.

The hierarchical structure imposed on the data by CA may be subject to simplification and it may contain distortions which affect the observed proximity of the OTU's. The reliability, or the extent of likelihood that the clusters are non-random, may be evaluated by the cophenetic correlation coefficient. It has been estimated that a coefficient of around 0.8 is sufficient to deny a null hypothesis (Rohlf & Fisher, 1968). The reliability of CA is considered to be greater for revealing fine details at high levels of similarity at the tips of the dendrogram than it is for the definition of major clusters (Sneath & Sokal, 1973).

#### 5.5.2. Principal coordinates analysis.

This method works on a matrix of similarity between OTU's. It utilizes Gower's similarity coefficient to measure the resemblance between the OTU's, which are ordinated in Euclidean space (Gower, 1971). This type of analysis has particular mathematical suitability for data expressed in both binary and multistate terms, unlike principal component analysis (PCA), which has more commonly been used in numerical taxonomy; however, principal coordinates analysis, like PCA, is useful for detecting major clusters and gradients on the dataset (Dalby, pers.comm). A second advantage of this system



over PCA is that it seems to be less disturbed by missing data (NC entries) (Sneath & Sokal, 1973).

#### 5.5.3. Analytical programs.

The two statistical methods differ in their optimal areas of analysis, and thus both were selected and applied to the data. The unpublished principal coordinates analysis program was prepared at Imperial College London by Dr.D.H.Dalby with the assistance of Professor R.G.Davies, who also wrote the cluster analysis program HYCLUS, which was modified for this study. Both analytical programs were converted to Fortran 77 and run on the Cyber 855 computer at the Imperial College Computer Centre by Dr.D.H.Dalby.

#### 5.5.4. Weighting of characters.

The weighting of characters prior to numerical analysis of the data can only be based on intuitive judgements of their relative importance, which can introduce bias (Everitt,1980,p.11). In numerical taxonomy it is usually the practice to treat all characters as of equal significance, at least at the preliminary stages, and to include all data. Should the first analyses indicate a need for some weighting, this should only be employed a posteriori (Stace, 1980,p.14). No weighting of the characters has been employed in this study, as can be inferred from paragraph 5.4.5.1 above.

#### 5.5.5. Preliminary analyses.

A pilot study was designed, using the data recorded from a single genus, to establish at an initial stage the validity of the analytical methods that had been selected. Also, at an intermediate stage during the research period, numerical analyses were made of the data.

##### 5.5.5.1. The pilot study.

The data were initially collected from the seeds of thirteen species of the genus Lathyrus; the number of OTU's was 18 (see Table 3). Eleven characters were recorded (see Table 14). These data were analysed by CA and principal coordinates analysis (Butler, 1986).

Two major clusters resulted from CA; the annual species of section Lathyrus separated from all other taxa. Within the clusters related taxa grouped closely. The cophenetic correlation coefficient of 0.8365 was considered to be a measure of the reliability of the analysis (Dalby, pers. comm.; see paragraph 5.5.6.). The principal coordinates analysis produced similar groupings. The characters responsible for most of the separations related to the size of the hilum and the associated tracheid bar.

On the basis of these results, the decision was made to extend the dataset by increasing the number of both the OTU's and the characters, and to retain the two analytical methods.

#### 5.5.5.2. Interim analyses.

Following the results of the pilot study, observations were made of the seeds of species of Vicia and Lens; the number of OTU's was 61. All 47 characters in the complete dataset were recorded. These data were also analysed by CA and principal coordinates analyses.

The clusters produced by CA largely reflected taxonomic groupings, although there was no clear separation of the three genera. The cophenetic correlation coefficient was 0.7495. Again, the principal coordinates analysis produced similar results.

It was considered that the results were sufficiently encouraging to proceed along this line of research (Butler, in press a; Dalby, pers.comm.). Data were recorded from the seeds of more species, including those of the genus Pisum; these completed the dataset of 106 OTU's (Table 4) and 47 characters (Table 13).

#### 5.5.6. The final dataset.

The complete dataset of values for 106 OTU's and 47

characters is displayed in Table 15 (Appendix 3). Initially this full dataset was analysed by CA (characters 1-47). The cophenetic correlation coefficient for this analysis of 106 OTU's and 47 characters was 0.73. The dataset was revised; the non-morphological and anatomical criteria (characters 1 and 2) and a group of characters relating to features of tracheoid pit anatomy (characters 24-38), and found to be unstable, were removed. This decision is discussed in greater detail below (section 8.). Using this abridged dataset of 31 characters, the cophenetic correlation coefficient increased to 0.79, approximating to the coefficient of 0.8, a figure indicative of non-random clustering (Rohlf & Fisher, 1968); this has supported the reliability of the analysis (Dalby, pers.comm.). It has been found that the value of the coefficient is almost independent of the number of characters recorded (Rohlf & Fisher, 1968), and therefore the reduction in the dataset alone is not likely to have produced this improved result. Subsequently the same dataset was used in all further analyses, including principal component analysis, in order that the results would be directly comparable.

#### 5.5.7. Expression of results.

The results of cluster analysis are listed in Table 16; these data have been expressed as a dendrogram which illustrates the groupings of OTU's resulting from the analysis (Figure 2). The results of principal coordinates

analysis are expressed as a series of eigenvectors along a series of axes (Table 17). In this project, principal coordinates along the five principal axes of variation have been calculated for each OTU. The first three axes showing the highest eigenvalues have been selected and scattergrams have been plotted of the distribution of OTU's on axes I and II, I and III, and II and III (Figures 3-5).

These results are discussed in the following chapter 6.

Table 13. Key of 47 Testa Characters Used as Variables for Multivariate Analysis

(Intermediate states in presence/absence characters 20 and 21 are represented by the value 0.5.)

<u>Number</u>	<u>Character</u>	<u>Type</u>
1	Life cycle annual 1 /perennial 2	Qualitative
2	Testa colour light/dark 1-5 states	Qualitative
3	Hilum length as % of seed circumference	Quantitative
4	Hilum length measured with graticule at x8, mm	Quantitative
5	Hilum width " " " " "	Quantitative
6	Lens-hilum distance, mm	Quantitative
7	Hilum shape (1-5)after Gunn (1970)	Qualitative
8	Micropyle shape (1-4)	Qualitative
9	Testa surface papillose presence 1 / absence 0	Qualitative
10	Epidermal wax presence 1 / absence 0	Qualitative
11	Epidermal cell linking presence 1 / absence 0	Qualitative
12	Deposits overlying testa surface	Qualitative
13	Secondary patterning presence 1 / absence 0	Qualitative
14	No.cells per unit area testa near hilum	Quantitative
15	No.cells per unit area testa lateral to hilum	Quantitative
16	Depth:width seed shape	Quantitative
17	Depth hilum in TS, $\mu\text{m}$	Quantitative
18	Width aerenchyma beneath hilum in TS, $\mu\text{m}$	Quantitative
19	Angle, hilar slit to cotyledon surface at edge	Quantitative
20	Protruding hilum presence 1 / absence 0	Qualitative
21	Sunken hilum presence 1 / absence 0	Qualitative
22	Depth tracheid bar in TS, $\mu\text{m}$	Quantitative
23	Width tracheid bar in TS, $\mu\text{m}$	Quantitative
24	No.tracheoids per width tracheid bar in TS	Quantitative
25	Pit distribution even 1 / uneven 0	Qualitative
26	Pit distribution regular 1 / irregular 0	Qualitative
27	Pit distribution alternate 1 / not alternate 0	Qualitative
28	Pit shape slit 1 / not slit 0	Qualitative
29	Pit shape oval 1 / not oval 0	Qualitative
30	Pit shape round 1 / not round 0	Qualitative
31	Pit shape subrectangular 1 / not subrect.0	Qualitative
32	Pit aperture shape slit 1 / not slit 0	Qualitative
33	Pit aperture shape oval 1 / not oval 0	Qualitative
34	Pit aperture shape round 1 / not round 0	Qualitative
35	Pit aperture shape subrectangular 1 /not subrect.0	Qualitative
36	Pit ornamentation plain borders 1-3	Qualitative
37	Pit ornamentation warts 1-3	Qualitative
38	Pit ornamentation vestures 1-3	Qualitative
39	Testa thickness adjacent to hilum. Total, $\mu\text{m}$	Quantitative
40	Testa thickness " " ". Ht.palisade cells, $\mu\text{m}$	Quantitative
41	Testa thickness " " ". Ht.basement layer, $\mu\text{m}$	Quantitative
42	Testa thickness lateral to hilum. Total, $\mu\text{m}$	Quantitative
43	Testa thickness " " ". Ht.palisade cells, $\mu\text{m}$	Quantitative
44	Testa thickness " " ". Ht.basement layer, $\mu\text{m}$	Quantitative
45	Testa thickness opposite hilum. Total, $\mu\text{m}$	Quantitative
46	Testa thickness " " ". Ht.palisade cells, $\mu\text{m}$	Quantitative
47	Testa thickness " " ". Ht.basement layer, $\mu\text{m}$	Quantitative

Table 14. Key of 11 Characters Used as Variables in Multivariate Analysis - Pilot Study. (Explanation of terms as in Table 13)."

<u>Number</u>	<u>Character</u>	<u>Type</u>
1	Hilum length as % of seed circumference	Quantitative
2	Hilum length measured with graticule at x8, mm	Quantitative
3	Hilum width " " " " "	Quantitative
4	Lens-hilum distance, mm	Quantitative
5	No.cells per unit area testa near hilum	Quantitative
6	Depth hilum in TS, $\mu\text{m}$	Quantitative
7	Width aerenchyma beneath hilum in TS, $\mu\text{m}$	Quantitative
8	Angle, hilar slit to cotyledon surface at edge	Quantitative
9	Depth tracheid bar in TS, $\mu\text{m}$	Quantitative
10	Width tracheid bar in TS, $\mu\text{m}$	Quantitative
11	No.tracheoids per width tracheid bar in TS	Quantitative

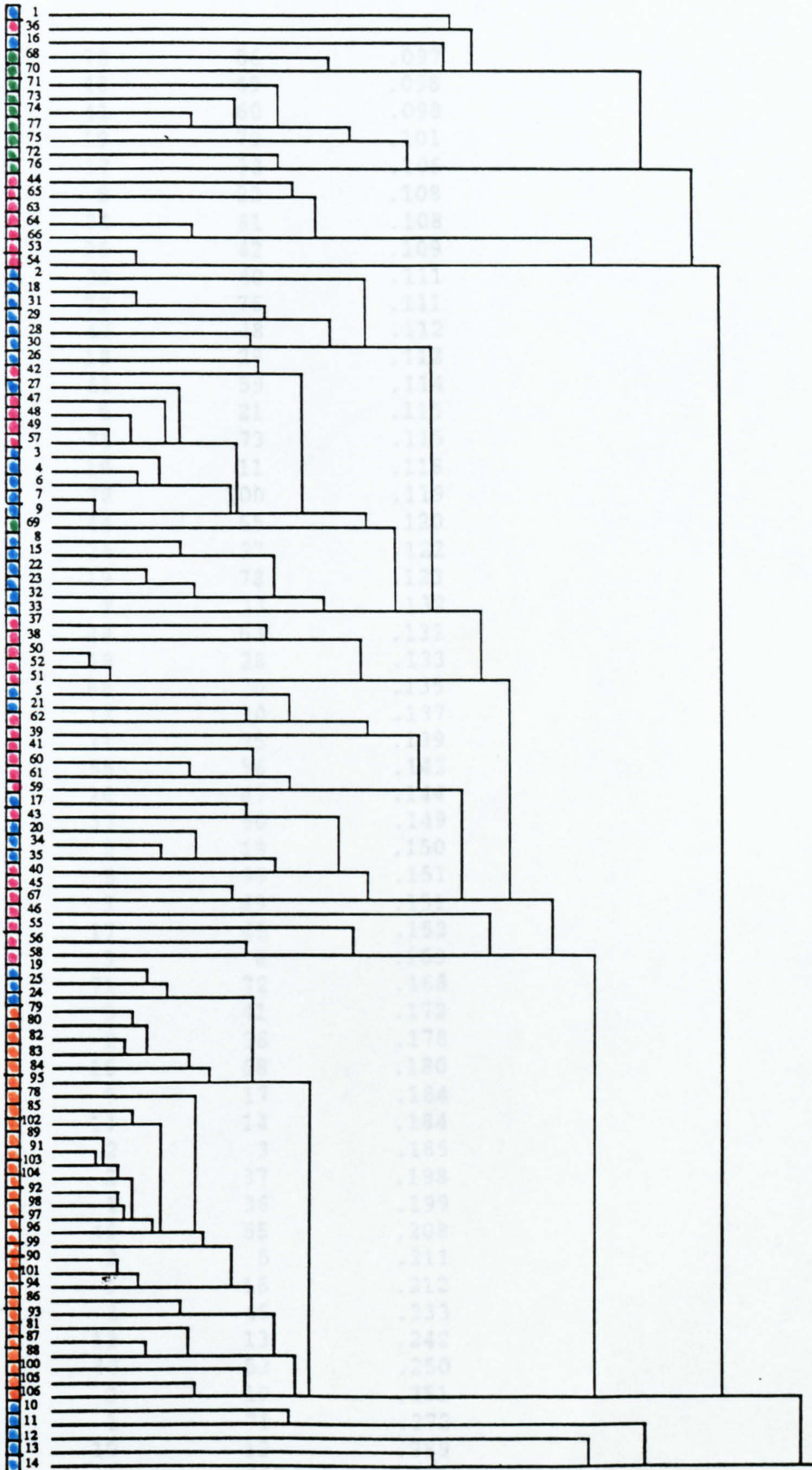
Table 16. Cluster Analysis

(Unweighted Pair-Group Method  
Using Arithmetic Averages)

106 OTU's		31 Characters
<u>Table of Linkage Levels</u>		
OTU's		Linkage Level
50	52	.027
91	103	.031
89	91	.033
7	9	.034
63	64	.037
50	51	.038
89	104	.039
92	98	.041
82	83	.041
90	101	.041
85	102	.045
92	97	.045
79	80	.048
89	92	.048
90	94	.049
4	6	.050
22	23	.051
18	31	.051
53	54	.052
87	88	.054
19	25	.054
89	96	.057
79	82	.058
85	89	.061
34	35	.061
19	24	.063
8	15	.067
79	84	.068
3	4	.068
86	93	.069
81	87	.071
60	61	.072
78	85	.074
63	66	.074
49	57	.074
22	32	.075
20	34	.075
74	77	.076
105	106	.076
79	95	.077
78	99	.079
3	7	.086
73	74	.089
45	67	.090
78	90	.090
21	62	.093
100	105	.093
56	58	.095
17	43	.096
28	30	.097



Figure 2 . Dendrogram of Cluster Analysis.



106 OTU's 31 Characters

<u>Vicia</u>	<span style="color: blue;">■</span>	<u>Lathyrus</u>	<span style="color: pink;">■</span>
<u>Lens</u>	<span style="color: orange;">■</span>	<u>Pisum</u>	<span style="color: green;">■</span>

78	86	.097
48	49	.098
41	60	.098
19	79	.101
37	38	.106
8	22	.108
78	81	.108
26	42	.109
20	40	.111
72	76	.111
47	48	.112
18	29	.112
41	59	.114
5	21	.115
71	73	.115
10	11	.118
78	100	.119
44	65	.120
26	27	.122
19	78	.123
8	33	.132
44	63	.132
18	28	.133
68	70	.135
17	20	.137
71	75	.139
55	56	.142
26	47	.144
37	50	.149
2	18	.150
5	39	.151
3	69	.151
17	45	.152
3	8	.163
71	72	.166
5	41	.172
2	26	.178
16	68	.180
5	17	.184
13	14	.184
2	3	.185
2	37	.198
1	36	.199
46	55	.208
2	5	.211
1	16	.212
2	46	.233
12	13	.242
44	53	.250
2	19	.251
1	71	.272
10	12	.289
1	44	.291
1	2	.306
1	10	.427

Cophenetic Correlation  
Coefficient = .7917

## 6. RESULTS OF NUMERICAL ANALYSIS.

### 6.1. Introduction.

The aims set out for this project (section 1.5) require a number of questions to be addressed via multivariate analyses. These questions are listed below, and answers to these have been sought from each analysis of the data.

#### 6.1.1. Questions that are addressed via the numerical analyses.

##### 6.1.1.1. Identification of taxa.

The identification of the legume seeds has been approached in a conventionally hierarchical regime. The separation of the four genera, the distinguishing of individual species within each genus, and the separation of certain subspecies and even varieties have been sought. Each expression of dataset analysis in this project has been studied to elicit answers to the following questions:

- (i) Is there discrete separation between genera?
- (ii) If so, do the species separate clearly within the generic clusters; if not, is there any specific separation based on particular characters?

(iii) Do populations of the same taxon always group together?

(iv) What other groupings are apparent?

#### 6.1.1.2. Recognition of cultigens.

As has been discussed above (section 2.4.2.), the seeds of cultigens exhibit systematic differences in gross morphology compared with that of their wild relatives. There are also micromorphological features that are reputed to have changed following selection under cultivation (Kislev, 1986). Thus it may be expected that multivariate analyses of the set of testa characters compiled for this project might achieve some separation of the seeds of cultigens from those of the wild taxa. The following questions have thus been asked of the analyses:

(i) Do the cultigens group together with their wild progenitors or at least with their nearest known relatives, or do they separate?

(ii) Do the cultigens as a whole show any common groupings?

(iii) Do any clustering tendencies relate to characters that are known to be associated with cultivation?

## 6.2. Results of Cluster Analysis.

The results of cluster analysis, as stated above (paragraph 5.5.6), are expressed as a dendrogram (Figure 2). The final dataset is comprised of 31 characters, and the cophenetic correlation coefficient of 7.9 indicates a degree of reliability above random clustering in the separations achieved (see section 5.5.1.).

Figure 2 shows the dendrogram; for ease of recognition, the OTU's corresponding to the four genera have been colour coded: Vicia (1-35), blue; Lens (77-106), orange; Lathyrus (36-67), pink; Pisum (68-77), green.

When referring to the different taxa that have been analysed, the OTU's have been given in parentheses after the name.

### 6.2.1. Separation of the genera.

At the most simplistic level, following the colour codings, it can be seen that the taxa in the genus Pisum (68-77) group together, and the Lens species (78-106) also group together. The species and subspecies in the genera Vicia (1-35) and Lathyrus (36-67) are spread across overlapping clusters.

Some species of Vicia, including populations of the highly-developed cultivated species, V.faba, cluster

separately at a high level.

6.2.2. Separation of species and subspecies within a genus.

6.2.2.1. Vicia (1-35).

Vicia faba (10-13) forms a separate cluster from the other species of the Viciaeae, however V.lutea (14) is linked to this cluster at a moderately high level. These two species separate widely from the other taxa in this genus.

The V.sativa group of taxa (22-27) does not form a separate cluster. V.sativa sativa (24-26) is closely linked to Lens (78-106). Unexpectedly the lentil-seeded form of V. sativa sativa (27) is not close to lentil in this analysis, and separates further away from this genus than the more typical forms of Vicia sativa sativa (24-26). The wild subspecies, V. sativa nigra (22,23) separates together with a number of other wild Vicia species (8,15,32,33).

Vicia ervilia (3-9) is largely grouped within a single cluster. One population (8) forms part of an adjacent cluster of Vicia species (15,22,23,32,33) and one Pisum population (69).

The other members of the genus Vicia are scattered

amongst other clusters with members of the genus Lathyrus.

6.2.2.2. Lens (78-106).

The clustering is entirely consistent for the genus, the populations of all five species grouping closely together. However, a small number of Vicia species (19,24,25) are linked with this cluster at a moderately high level.

Separation of the five species within the genus is not achieved. The populations of Lens culinaris (78-84) largely separate together, with one population of Lens nigricans (95). L.orientalis (101-106) is not closely grouped with its relative L.culinaris.

6.2.2.3. Lathyrus (36-67).

Related taxa do not always group closely. Thus Lathyrus clymenum (42,43) and L.ochrus (53,54), of section Clymenum, do not form part of near clusters. However L.cicera (39,41) and L.sativus (59-62), of section Lathyrus, largely group together in a single cluster. One population of L.cicera (40) is found in an adjacent cluster of mixed Vicia and Lathyrus taxa.

Where more than one population of the same species is included in the analysis, usually there is clustering of

members of the same taxon, as with L.nissolia (50-52), L.pratensis (55,56,58) and L.sylvestris (63-66).

6.2.2.4. Pisum (68-77).

The three taxa of Pisum in this study are all members of the same species. Thus perhaps it is unsurprising that they, with a single exception, form a discrete cluster. Three of the four populations included in this study of the taxon Pisum sativum elatius (68-70), which is distinguished by a thick, rough testa, group together. The one population (69) that separates apart, clusters with populations of Vicia ervilia (3-9).

The two varieties of P.sativum sativum (71-75; 76,77) do not separate into different clusters.

Two members of different genera, Vicia bithynica (1), and Lathyrus annuus (36), cluster closely with the peas (68-77).

6.2.3. Separation of cultigens.

The cultigens as a group do not show obvious separation from the wild taxa in this analysis.

Lathyrus sativus (59-62) exhibits in the clustering its known close taxonomic relationship with L.cicera (39-41) in the same section Lathyrus. Vicia faba (11-13),



however, does not group with its closest wild relatives, the members of section Faba, V.bithynica (1) and V.narbonensis (16). Lens culinaris (78-84) also does not separate closely with its wild progenitor, L.orientalis (100-106), although all the lentils in this analysis portray the close relationship of the genus as a whole.

#### 6.2.4. General summary of results.

The groupings achieved by cluster analysis seem to reflect little more than the same separations that might be obtained from considerations of the gross morphological character of the seed shape. The main exception to this generalisation is the lentil-seeded Vicia sativa, which does not cluster with species of Lens (see section 6.2.2.4.).

Replicate populations of the same taxon tend to cluster together.

#### 6.3. Principal coordinates analyses.

The results have been expressed graphically as scattergrams. The principal coordinates along the first three axes have been plotted in the following manner: axis I against axis II, axis I against axis III, and axis II against axis III. The results of these three scattergrams are described in the following sections (6.3.1-6.3.3).

As with the dendrogram, a colour coding has been adopted for the OTU's, using the same colours for each of the four genera: Vicia (1-35), blue; Lens (78-106), orange; Lathyrus, (36-67) pink; Pisum (68-77), green. Thus these colours identify the points representing the different genera amongst the OTU's on the scattergram.

6.3.1. Scattergram 1 (Figure 3), plotting principal coordinates on axes I and II (Table 17).

6.3.1.1. Separation of the genera.

There is a distinct separation of the genus Lens (78-106).

Pisum (68-77) separates in a wide cluster.

Vicia and Lathyrus species are intermixed on the scattergram. There are, however, overall tendencies that can be seen. Members of the genus Vicia occupy positions mainly along the centre of axis II and are spread along axis I. Members of the genus Lathyrus, on the contrary, may be found at the centre of axis I and are spread along axis II. Unfortunately these distributions are not very marked.

6.3.1.2. Separation of the species.

Within its cluster Lens does not group into species.

Pisum is very loosely grouped and the taxa are mingled.

Species of Vicia and Lathyrus are not very discretely defined on this plot and show much overlap.

#### 6.3.1.3. Taxonomic groupings.

It may be seen that usually representatives of the same taxon cluster closely, and that species from the same taxonomic section are adjacent to each other.

#### 6.3.1.4. Separation of the cultigens.

Within the group of Lens species the cultigens (78-84) are not clearly separated.

Amongst the peas (68-77), the most developed cultigens, P.sativum sativum sativum (76,77), are found at the most positive end of axis II, but are also close to some related taxa (74,75,73).

Vicia faba (10-13) is separated from other taxa, but the subspecies considered to be least developed, V.faba paucijuga (13) (Lawes et al.,1983), does not show a greater affinity with any wild species; it is not the subspecies nearest on the plot to the other species from section Faba. V.ervilia (3-9) is diffusely scattered

across the centre of the scattergram. V.sativa sativa (24-26) also is not distributed in a tight group.

Lathyrus sativus (59-62) does not separate from its close wild relative L.cicera (39-41).

For the cultigens as a whole there is a tendency that can be seen on the scattergram: most large-seeded forms seem to be situated further towards the positive end of the axis II on the plot. Thus most of the cultivated lentils (78-84) are at the top of the cluster; the largest peas are at the top of axis II. In the other two genera this is less marked.

#### 6.3.1.5. Summary of Scattergram 1.

It can be seen that clear separation of genera and species and definition of the cultigens has not been achieved, except for the genus Lens and Vicia faba.

#### 6.3.2. Scattergram 2 (Figure 4), plotting principal coordinates on axes I and III. (Table 17).

##### 6.3.2.1. Separation of the genera.

Again there is a distinct separation of the genus Lens (78-106). Pisum (68-77), however, also forms a discrete group, within which are a few Lathyrus and Vicia

representatives (1,36,45).

Vicia and Lathyrus taxa do not appear to group by genus.

#### 6.3.2.2. Separation of the species.

Within its cluster Lens does not group into species. The other genera show varying degrees of infrageneric grouping.

Populations of the species Vicia faba (10-13) separate widely from all other taxa. V.faba paucijuga (13) is closer to the other members of the Vicieae than the more highly-developed cultivated subspecies of this species. The related species, V.narbonensis (16), from the same section Faba, is situated near to this group.

Some of the Vicia sativa group (24,25) are close to Lens, but, interestingly, the lentil-seeded variety, V.sativa sativa lentisperma (27) is further removed from this genus.

Lathyrus shows the greatest intrageneric separation in some of the wild species; the perennial species, L.sylvestris (63-66), occupies the position most remote from the other taxa.

#### 6.3.2.3. Taxonomic groupings.

Replicate populations of the same taxon are usually found adjacent to each other; these are exemplified by Vicia sepium (28-30), V.tetraspermum (32,33), V.villosa (34,35); Lathyrus niger (48,49), L.nissolia (50-52), and L.sylvestris (63-66).

Members of the same taxonomic section similarly may be found close together, exemplified by Lathyrus laxiflorus (47) and L.pratensis (55-58) of section Pratensis, and L.cicera (39-41) and L.sativus (59-62) of section Lathyrus, formerly classified as section Cicercula. However, this does not hold true for L.clymenum (42,43) and L.ochrus (53,54) of section Clymenum.

#### 6.3.2.4. Separation of the cultigens.

The cultivated species, L.culinaris (78-84), occupies a position amongst other species of Lens, and is not clearly separated.

Within the Pisum group, similarly, the most developed cultigens (76,77) do not separate.

In the genus Vicia, the populations of V.faba (10-13) form a distinct cluster. The populations of V.ervilia (3-9) form part of a loose grouping with other Vicia species. As has been described above, the members of the

V.sativa group (22-27) separate loosely, and within that group, the cultigens (25-26) cannot be distinguished.

Lathyrus sativus (59-62) is found with its nearest wild relative, L.cicera (39-41), and does not show any separation from it.

#### 6.3.2.5. Summary of Scattergram 2.

Clear separation is achieved only for the group of lentils (78-106) and for Vicia faba. Cultigens as such cannot be distinguished on this plot.

#### 6.3.3. Scattergram 3 (Figure 5), plotting principal coordinates on axes II and III (Table 17).

##### 6.3.3.1. Separation of the genera.

This scattergram does not show any obvious generic clusters. The overall distribution of members of the four genera is more diffuse than in the other plots.

6.3.3.2. Separation of the species.

Lens species are scattered across the centre of the plot without specific groupings.

Pisum taxa are similarly not separated discretely.

Vicia faba (10-13) separates clearly; the subspecies, V.faba paucijuga (13) here shows a close proximity to the lentil-seeded variety of Vicia sativa (27), which itself is not grouped closely with other members of its own species.

Lathyrus is the genus that shows the most clustering, and can be seen distributed mainly around the periphery of the plot, small groups being formed by members of the same taxon, such as L.nissolia (50-52), L.pratensis (55-58), and L. sylvestris (63-66).

6.3.3.3. Taxonomic groupings.

Members of the same taxonomic section are again often grouped together, as in the other plots.

6.3.3.4. Separation of the cultigens.

Large-seeded cultivated varieties show a tendency to separate towards the positive end of axis II. This can be seen in Pisum where the examples of Pisum sativum sativum



(73-77) are found amongst the peas at the most positive position on that axis. The most highly-developed subspecies of V.faba (10-12) also demonstrate this tendency<sup>on axis I</sup>. This distribution is less clearly apparent in lentil (78-84), and in Lathyrus, nevertheless the general trend can be distinguished. However in the latter genus, one population of the wild species L.cicera (39) is situated in the most positive position amongst the L.sativus group.

By contrast, the periphery of the plot, particularly at the negative end of axis II, is occupied by predominantly wild species, mainly of Lathyrus.

Although these tendencies have been noted, it is not possible readily to distinguish cultigens amongst the taxa as a whole.

#### 6.3.3.5. Summary of Scattergram 3.

An initial scan of the scattergram does not reveal any clear clusters apart from the close groupings of like taxa, and V.faba. The detailed analysis of the plot however shows the tendencies described in the paragraph above.

6.3.4. Analysis of factors that have most influenced the separation.

The eigenvalues for each of the five axes are shown in Table 17. The value of 2.257 for the first axis is far higher than those of the other axes (of .657 and below), and thus the separations achieved along this axis are the most significant. Consequently, distributions of the OTU's in scattergrams 1 and 2, which incorporate axis I, are more important than those of scattergram 3.

Correlation coefficients between the characters and the principal coordinates for the five axes are also listed (Table 18).

Along axis I, the highest correlation coefficients are those of characters 5,14,15 and 18. These characters are responsible for most of the separation in the principal coordinates analysis.

Along axis II, with the lesser eigenvalue of .657, the characters with highest correlation coefficients are numbers 9 and 12.

Along axis III, with an eigenvalue of .563, similarly, the character with highest correlation coefficient is number 13. The characters are listed in Table 19.

Table 19. List of Characters with Highest  
Correlation Coefficients on the First  
Three Axes in Principal Coordinates  
Analysis.

Character	5	Width of the hilum
"	14	Size of palisade cells, external surface, near the hilum
"	15	Size of palisade cells, external surface, at midseed
"	18	Width of the aerenchyma beneath the hilum
"	9	Presence or absence of papillae on testa
"	12	Presence or absence of deposits on testa surface
"	13	Presence or absence of secondary patterning, "mounds"

It can be deduced that the quantitative characters that have been most responsible for the distributions of OTU's in the analysis have been those relating to the hilar width, measured both on the external surface, and as represented internally by the pad of aerenchyma beneath the hilum, and the surface area of the palisade cells, recorded at positions both adjacent to the hilum and at midseed.

The multivariate analyses of the dataset have not achieved separations that distinguish the species of all of the four different genera that have been included in this project. The two genera, Lens and Pisum, that are relatively easily separated by conventional means, retain this property; the two genera, Vicia and Lathyrus, that have overlapping morphological characteristics causing identificatory problems, also show the same problems when

analysed by the methods employed in this study.

During the course of the work for this thesis, a number of investigations and observations have been undertaken, that fall outside the main microscopical study and the numerical analyses of the dataset described above. These miscellaneous aspects of the project form the content of the next chapter 7.

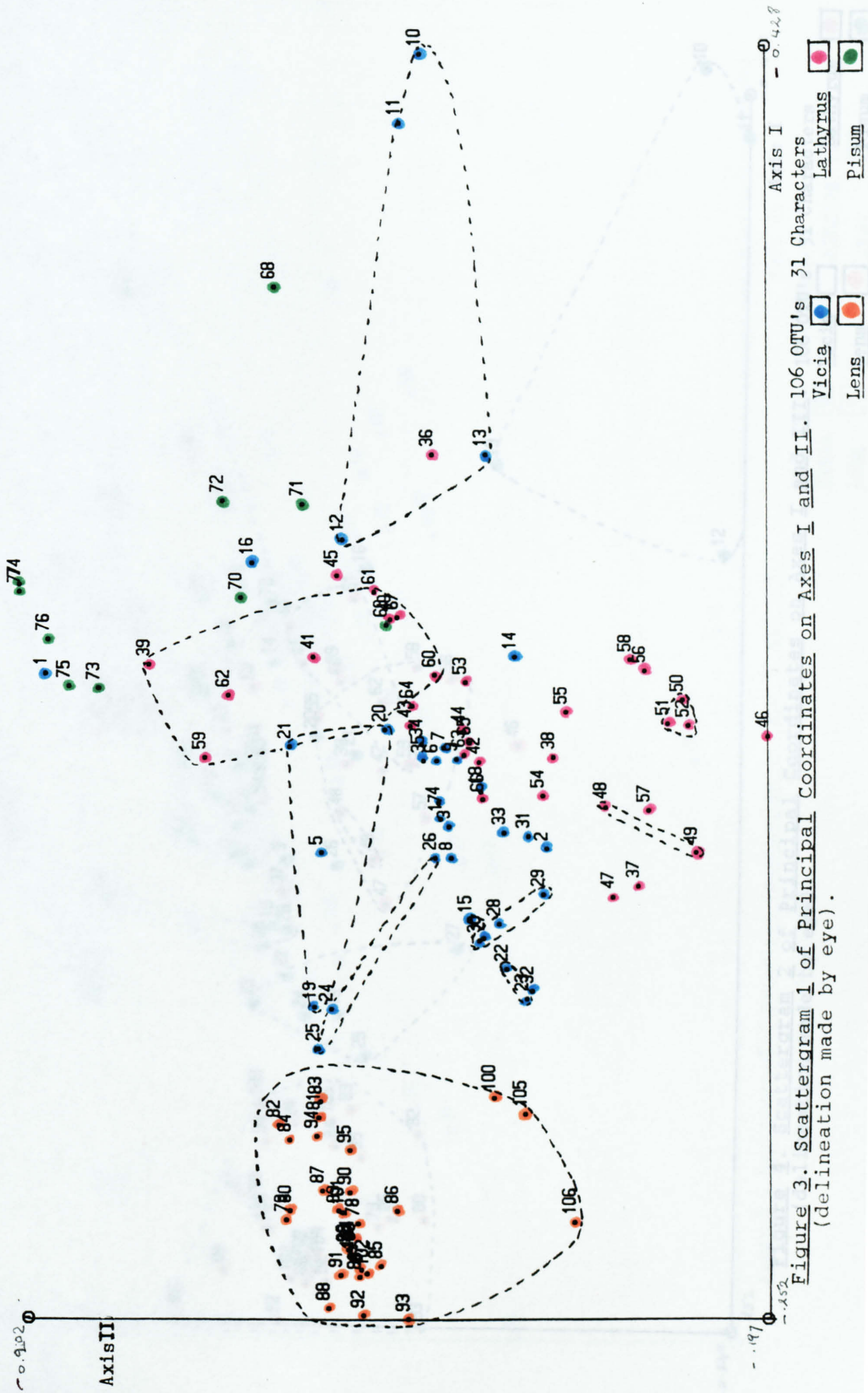


Figure 3. Scattergram 1 of Principal Coordinates on Axes I and II. (delineation made by eye).

106 OTU's 31 Characters

Vicia Lathyrus

Lens Pisum

Axis I - 0.428

Axis II - 0.902

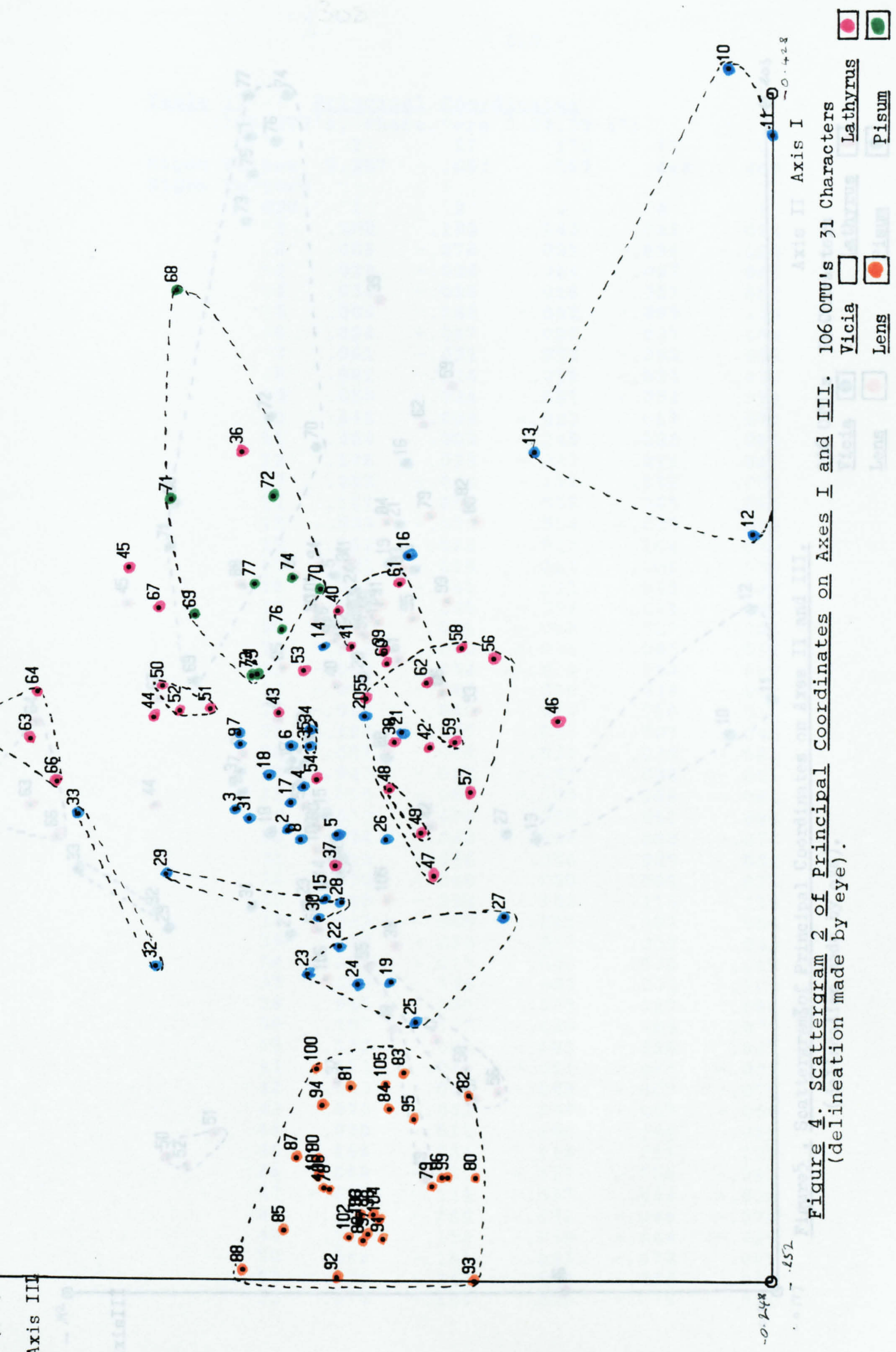


Figure 4. Scattergram 2 of Principal Coordinates on Axes I and III. 106 OTU's 31 Characters (delineation made by eye).

Vicia
  Lens
  Lathyrus
  Pisum

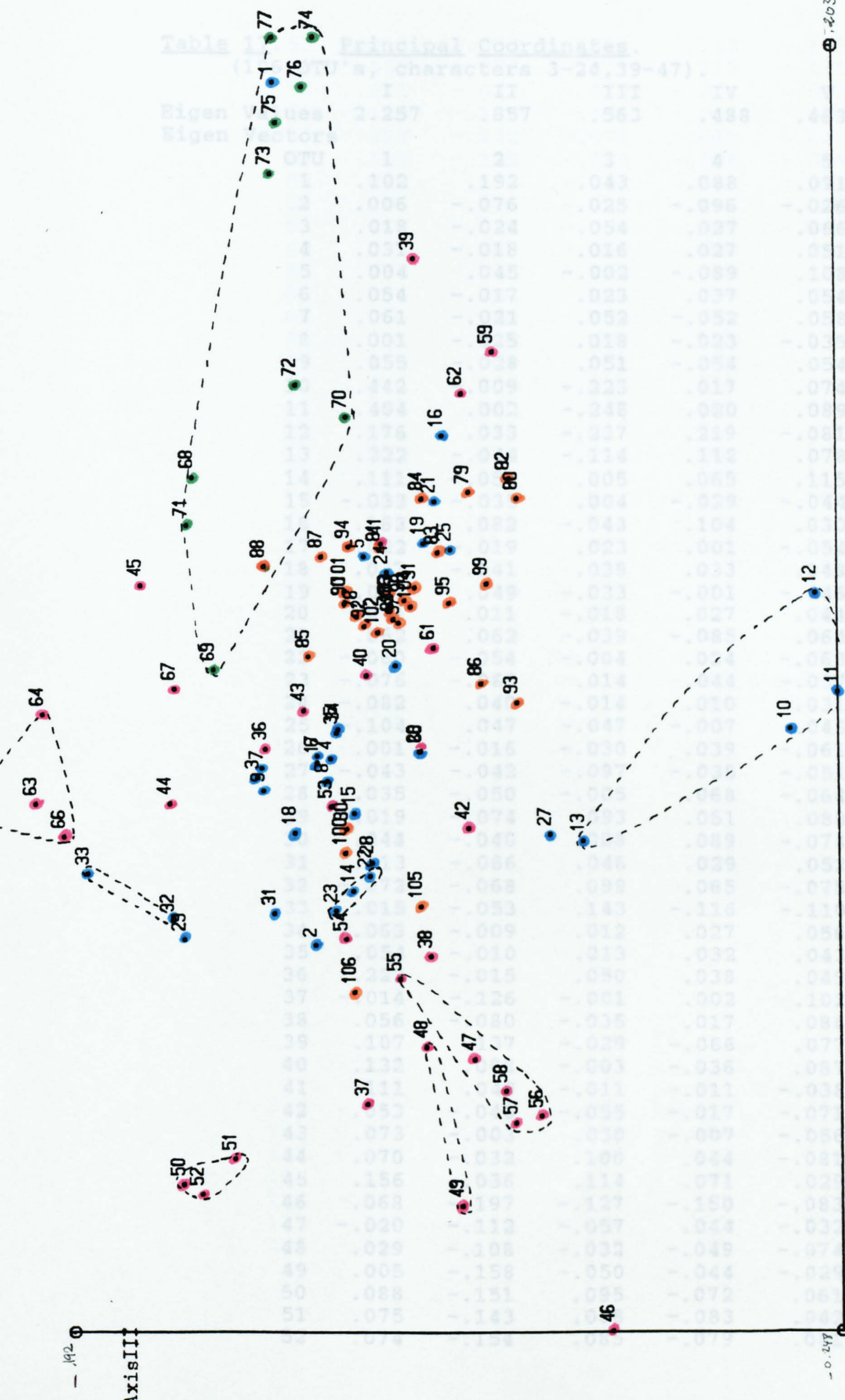


Figure 5. Scattergram of Principal Coordinates on Axes II and III.  
(delineation made by eye).

106 OTU's 31 Characters

Vicia (blue circle)      Lathyrus (pink circle)

Lens (red circle)      Pisum (green circle)

Table 1. Principal Coordinates.

OTU	Character 1	Character 2	Character 3	Character 4	Character 5	Character 6	Character 7	Character 8	Character 9	Character 10	Character 11	Character 12	Character 13	Character 14	Character 15	Character 16	Character 17	Character 18	Character 19	Character 20	Character 21	Character 22	Character 23	Character 24	Character 25	Character 26	Character 27	Character 28	Character 29	Character 30	Character 31				
1	0.102	0.006	0.102	0.006	0.102	0.006	0.102	0.006	0.102	0.006	0.102	0.006	0.102	0.006	0.102	0.006	0.102	0.006	0.102	0.006	0.102	0.006	0.102	0.006	0.102	0.006	0.102	0.006	0.102	0.006	0.102	0.006			
2	0.257	0.857	0.257	0.857	0.257	0.857	0.257	0.857	0.257	0.857	0.257	0.857	0.257	0.857	0.257	0.857	0.257	0.857	0.257	0.857	0.257	0.857	0.257	0.857	0.257	0.857	0.257	0.857	0.257	0.857	0.257	0.857	0.257	0.857	
3	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	
4	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488	0.488

-0.192  
Axis III

-0.247

-0.197

0.203

Table 17.      Principal Coordinates.  
 (106 OTU's, characters 3-24,39-47).

Eigen Values	I	II	III	IV	V
	2.257	.657	.563	.488	.463
Eigen Vectors					
OTU	1	2	3	4	5
1	.102	.192	.043	.088	.091
2	.006	-.076	.025	-.096	-.026
3	.018	-.024	.054	.027	.066
4	.031	-.018	.016	.027	.051
5	.004	.045	-.002	-.089	.108
6	.054	-.017	.023	.037	.054
7	.061	-.021	.052	-.052	.058
8	.001	-.025	.018	-.023	-.035
9	.055	-.028	.051	-.054	.054
10	.442	-.009	-.223	.017	.074
11	.404	.002	-.248	.020	.089
12	.176	.033	-.237	.219	-.081
13	.222	-.044	-.114	.112	.078
14	.111	-.059	.005	.065	.115
15	-.033	-.035	.004	-.029	-.044
16	.163	.082	-.043	.104	.030
17	.022	-.019	.023	.001	-.054
18	.037	-.041	.035	.033	.048
19	-.081	.049	-.033	-.001	-.046
20	.071	.011	-.018	.027	.044
21	.062	.062	-.039	-.085	.064
22	-.060	-.054	-.004	.024	-.063
23	-.076	-.065	.014	.044	-.037
24	-.082	.040	-.014	.010	-.031
25	-.104	.047	-.047	-.007	-.046
26	.001	-.016	-.030	.039	-.061
27	-.043	-.042	-.097	-.035	-.051
28	-.035	-.050	-.005	.068	-.064
29	-.019	-.074	.093	.051	.080
30	-.044	-.040	.008	.089	-.074
31	.013	-.066	.046	.029	.052
32	-.072	-.068	.099	.065	-.075
33	.015	-.053	.143	-.116	-.110
34	.063	-.009	.012	.027	.056
35	.054	-.010	.013	.032	.043
36	.222	-.015	.050	.038	.049
37	-.014	-.126	-.001	.002	.102
38	.056	-.080	-.035	.017	.084
39	.107	.137	-.029	-.066	.077
40	.132	.008	-.003	-.036	.087
41	.111	.049	-.011	-.011	-.038
42	.053	-.040	-.055	-.017	-.071
43	.073	-.003	.030	-.007	-.056
44	.070	-.032	.100	.044	-.081
45	.156	.036	.114	.071	.029
46	.068	-.197	-.127	-.150	-.083
47	-.020	-.112	-.057	.044	-.032
48	.029	-.108	-.032	-.049	-.074
49	.005	-.158	-.050	-.044	-.029
50	.088	-.151	.095	-.072	.061
51	.075	-.143	.068	-.083	.042
52	.074	-.154	.085	-.079	.081



53	.097	-.033	.016	.142	-.197
54	.035	-.074	.009	.119	-.175
55	.081	-.087	-.019	-.024	.023
56	.104	-.130	-.091	-.057	.034
57	.028	-.132	-.078	-.061	-.047
58	.110	-.122	-.073	-.096	-.069
59	.056	.108	-.069	-.149	-.025
60	.101	-.016	-.031	-.102	-.049
61	.147	.016	-.038	-.102	-.046
62	.090	.095	-.053	-.080	.080
63	.057	-.032	.170	.104	.051
64	.084	-.004	.166	.100	.043
65	.064	-.035	.203	-.046	-.088
66	.033	-.042	.155	.080	.029
67	.133	.004	.097	.051	.006
68	.314	.070	.087	.018	.000
69	.129	.010	.077	-.041	.004
70	.144	.088	.007	.148	-.112
71	.195	.055	.090	-.088	-.104
72	.197	.098	.033	.059	.010
73	.094	.164	.045	-.118	-.100
74	.151	.206	.022	-.113	-.086
75	.095	.180	.042	-.029	-.063
76	.121	.191	.028	.019	.059
77	.147	.206	.043	-.115	-.092
78	-.200	.026	.002	.033	-.021
79	-.198	.065	-.056	.028	-.025
80	-.193	.063	-.081	.027	-.018
81	-.142	.047	-.010	-.077	-.007
82	-.146	.069	-.077	.040	-.036
83	-.133	.046	-.040	.026	-.042
84	-.154	.063	-.032	.033	-.020
85	-.223	.014	.027	-.001	.015
86	-.193	.005	-.062	-.095	.026
87	-.182	.045	.020	-.065	.003
88	-.246	.042	.050	-.084	.026
89	-.229	.025	-.017	.027	-.002
90	-.183	.030	.008	.001	.142
91	-.228	.035	-.028	.034	.001
92	-.250	.023	-.002	.027	.007
93	-.252	-.001	-.080	-.089	.022
94	-.152	.048	.006	-.016	.165
95	-.160	.030	-.046	.007	-.024
96	-.214	.030	-.016	.023	.011
97	-.225	.024	-.020	.023	.010
98	-.214	.031	-.023	.026	.009
99	-.193	.036	-.065	.012	-.003
100	-.131	-.047	.009	-.025	.008
101	-.194	.034	.008	.005	.148
102	-.227	.021	-.009	.010	.020
103	-.218	.028	-.016	.029	-.001
104	-.217	.029	-.026	.023	.007
105	-.140	-.064	-.030	.055	-.006
106	-.199	-.091	.005	.081	-.007

Table 18. Correlation Coefficients Between Characters and Principal Coordinates

Character	I	II	III	IV	V
3	.103	-.332	.240	.246	.002
4	.548	-.024	-.161	.240	-.112
5	.856	.292	-.044	.048	.093
6	.576	.382	-.265	.138	.037
7	-.161	-.279	.198	.274	-.053
8	-.652	-.092	.237	.171	.347
9	-.273	.595	.287	.131	-.126
10	.078	-.220	-.080	.093	-.391
11	-.394	.271	.489	-.145	.148
12	-.587	.650	-.174	-.209	.119
13	.387	.035	.645	.157	-.198
14	-.832	.057	-.118	-.084	.241
15	-.825	.104	-.130	.005	.233
16	-.523	.198	-.473	.023	.196
17	.747	.407	-.153	.249	-.033
18	.882	.315	-.080	.095	.040
19	-.595	.181	-.399	.246	-.207
20	-.584	-.004	-.283	.124	-.671
21	.392	-.204	-.175	-.634	.013
22	.742	.382	-.330	.159	-.030
23	.783	.256	-.331	.158	.105
24	.660	.277	-.192	-.124	.225
39	.765	.159	-.206	.274	-.003
40	.767	.174	-.173	.314	.027
41	.651	.163	-.212	.205	-.027
42	.704	.161	-.107	.383	.002
43	.681	.159	-.084	.355	.013
44	.471	.121	-.098	.263	-.067
45	.587	.140	.165	.253	-.006
46	.591	.137	.150	.260	-.015
47	.193	.190	.101	.116	-.019

## 7. MISCELLANEOUS OBSERVATIONS.

### 7.1. Introduction.

During the project the legume seed reference collection was augmented. Seeds of wild taxa were collected from natural habitats. Seeds of cultigens were both harvested from cultivated crops and obtained from commercial sources. Seeds of both wild and cultivated taxa were collected from plants grown on from seed originally collected in the Near East, which had been supplied for the project. Some observations that were made during these procedures have been recorded and added to the data.

#### 7.1.1. Field observations, in Britain, Europe and Western Asia.

Seeds of both wild and cultivated species of the Viciaeae have been collected in north Spain, southern France, Crete, and central and south-eastern Turkey. Also populations of some wild species native to Britain have provided supplies of fresh seed during the period of the research. Records have been made of some observations both of direct relevance to this project, and of more general botanical interest. Some stages in the cultivation of certain species have also been observed in Britain, southeastern Europe and Turkey and have provided additional data.

7.1.1.1. Seed collection from the wild in Britain.  
Observations of a change in seed shape.

Wild populations in the Viciaeae have explosively dehiscent pods. When the pods are mature and fully dried, the ripe seeds are shed. In order to collect seed it is necessary either to attempt to retrieve it after dissemination by sieving the soil surrounding each plant, which may introduce seed material of unknown origin, or the seed may be collected from pods prior to dehiscence, at a stage judged to be immediately before the pod has reached the dry ripe stage. Commonly at this time the seed will not have reached its final state of dryness.

The perennial species Vicia sepium, and the annual V. sativa nigra have both been harvested from the wild in this slightly immature state during the project.

The seeds of both species when gathered were of the characteristic subspherical shape. They were placed on filter paper in open petri dishes to dry in the air before being added to the reference collection. After one hour at room temperature, the seed shape in both species had altered. The seeds now were approximately spherical in shape. The seeds of Vicia sativa showed the greatest rotundity. V. sepium seeds retained a slightly subspherical shape. This change in shape remained constant after the seeds had fully dried.

7.1.1.2. Cultivation of Pisum sativum and Vicia faba as animal feed in Britain: the retention of some dehiscence in cultigens. Farming attitudes.

Faba bean and field pea are cultivated in Britain as pulses for animal feed. Commercial cultivation of both these species has been observed in southern England over several years, and seeds from both crop plants have been added to the reference collection used in this project.

It was observed that a proportion of the plants of both species always retained some dehiscence, with a proportion of seed loss from the harvest. The farmer acknowledged the desirability of harvesting early in the morning or during damp weather to achieve the maximum yield, by decreasing the extent of seed loss from dehiscence. However, he stated that in practice harvesting occurred when the machinery and the labour force were available. This usually had to be planned ahead, regardless of the prevailing climatic conditions. The economics of the harvesting operation favoured this order of priority rather than planning for maximum yield.

Such evidence of the operation of economic priorities over what may appear as the conditions for achieving greatest productivity is an important instance where practice differs from theory, and one which has to be borne in mind when making and applying models of ancient agriculture to the reconstruction of past societies.

## 7.2. Growing trials.

The experimental growing of certain species has been carried out on a small scale as part of the project. In all cases to ensure rapid germination, imbibition was facilitated: the testas were scarified with a sharp object, and the seeds were soaked overnight in water at room temperature. They were then planted in a peat-based compost and perlite mixture, five to one by volume, in five inch pots. These were placed in an unheated greenhouse, or on a windowsill at room temperature for the whole growing period.

### 7.2.1. Seed yield of wild lentils.

Five seed specimens of single populations of Lens nigricans and L.odemensis from Israel germinated in June in an unheated cool greenhouse in the south of England. In August, the resultant plants produced between nine and twenty four pods per plant averaging two seeds per pod. This yield exceeds that reported by Ladizinsky (1987) of an average of 10 seeds per plant for the wild lentil, Lens orientalis, and supports his observation that different growing conditions can produce varying harvests.

7.2.2. Observations on the fruits and seeds of some Lathyrus species from section Lathyrus.

Five seeds of each of the following species collected from Syria were grown in positions of maximum light intensity on windowsills in the south of England during the summer: Lathyrus blepharicarpus, L.cicera, L.pseudocicera, and L.sativus. All the seeds germinated and fruited,; the seeds harvested from these plants had the sharply angular "chisel" shape characteristic of this group. The shape of the fruits varied. While L.sativus, L. cicera and L.pseudocicera had fruits that were "chisel"-shaped in section, the fruits of L.blepharicarpus were rounded in section, and appeared not to be in immediate contact with the seeds within.

7.2.3. The crop-mimicking lentil-seeded vetch.

In a commercially-obtained sample of Lens culinaris produced in Puy, France, were found some seed contaminants that, although being of the same size and approximate shape as the lentils, were provisionally identified as Vicia sativa on the basis of the hilum length, and the position of the lens.

Seeds of both the lentils and the contaminant species were planted and grown on a windowsill during the late summer. Both species germinated at approximately the same time, and came to fruition over the same period. The

contaminant was identified as Vicia sativa sativa on the basis of its general morphology and its characteristic torulose pods. The plants were large with fruits typical in shape of the normal V. sativa sativa, and not flattened; however the seeds were lenticular in shape. The cotyledons were found to be orange in colour, similar to those of the lentil with which it was associated and unlike those of the normally yellow-coloured cotyledons of Vicia sativa.

### 7.3. Discussion of the results.

The results of all the various observations above, together with the analyses of the main dataset are discussed in the following chapter 8, with reference to earlier published work.



## 8. DISCUSSION.

### 8.1. Introduction.

The publications relating to seed surface morphology and micromorphology in the Viciaeae draw two main conclusions: (i) the seeds of members of this tribe have a number of distinguishing features of the testa, making them "obviously different from other legumes" (Lersten, 1981, p.184); and (ii) they also exhibit interspecific differences in the testa surface observed by SEM (Lersten & Gunn, 1982). It was thus initially anticipated that, during the research for this thesis, criteria of the testa surface would be isolated that could be applied in the identification of seeds of the Viciaeae; it was also expected that some of these criteria could be employed to help distinguish cultigens from the wild species.

During the research for this project, limitations have been found in the extent to which testa surface micromorphology can provide diagnostic criteria. This result is illustrated by the key for the identification of seeds of the Viciaeae using characters of the surface micromorphology (4.3.2). As a consequence of the limitations, an extended list of testa characters was recorded and the final dataset was analysed statistically.

The first part of this discussion is a comparison of the testa surface micromorphology, as recorded in this survey, with that described in the literature; a number of features that have been observed on the testa surface are examined and their possible significance is considered. The second part comprises an evaluation of the extended list of characters based on the gross morphology and from the internal micromorphology of the testa. This is followed by a discussion of the results of multivariate analyses.

The effects of charring are considered as each feature is discussed, and their significance is examined in relation to archaeological seed specimens.

## 8.2. Micromorphology of the testa surface in seeds of the Viciae.

An important aspect of this research is the detailed comparison of the data with the published accounts of other workers. Most previous work has focussed upon the descriptions and illustrations of one or two seeds of single populations of each species. The data have often been presented as generalised examples of specific micromorphologies, and the stability of certain features has not been ascertained, either in terms of general phenotypic plasticity, or genotypically conditioned variation within species. The observations made during this project do not agree with some previously-published

results. It has been necessary to highlight these differences, because archaeobotanists routinely depend upon such literature to provide information to aid their interpretations. In the research for this thesis, intraspecific variation in testa micromorphology has emerged as an important aspect, and has therefore been considered in some detail. This emphasis seems to differ from that of most published work, and probably underlies the lack of agreement.

#### 8.2.1. The basic patterning of the testa surface in the Viciaeae.

The papillose patterning of the testa surface in the Viciaeae observed in this project is typified by that seen in Vicia sativa. This type of surface micromorphology has been recorded in most of the taxa examined. It fits well with the descriptions given by other authors, and agrees with the statements of Lersten (1981) and Lersten & Gunn (1982,p.4,5) that the characteristic testa in the Viciaeae is papillose.

However, as well as the papillose type, a wider range of surface micromorphology has been found in the Viciaeae, mainly in the genus Lathyrus, which has extended the potential for finding identificatory testa patterning. This observation is in agreement with the work of Saint-Martin (1978), and of Roti-Michelozzi & Serrato-Valenti (1986), who have referred to the testa in Lathyrus aphaca

as folded, and in four species of Vicia variously as lophate, rugulate and polygonally ornated. Lersten (1981) has described eight different non-papillose forms of testa topography in species from a number of tribes other than the Viciaeae, but not in the Viciaeae. Yet, strangely, Lersten & Gunn (1982, p.25) have clearly illustrated the non-papillose testa of Lathyrus inconspicuus in their atlas, without any acknowledgement of its lack of papillae, as has been remarked by Roti-Michelozzi & Serrato-Valenti (1986). It appears that the papillose testa is variably interpreted. This subjective aspect of data recording underlines the value of the inclusion of the electron micrographs in publications to minimise the possibility of interpretative misunderstandings, a strategy recommended by Heywood (1971).

It has been recorded that testa surface micromorphology varies within some individual taxa. Also, close similarities in the testa surface between a number of different taxa have been found; in this survey this has been most apparent in the seeds with papillose testas. These two observations are discussed below.

8.2.2. The variation of the patterning with its position on the seed surface.

Lersten (1981) noted that in 90% of leguminous species, a discernible testa pattern is found only around the hilum, and that this topography becomes attenuated, or

occasionally changed, at midseed. Roti-Michelozzi & Serrato-Valenti (1986) cite these observations as the reason for restricting their records of testa patterns to the midseed region, which they consider to manifest the most characteristic topography. Saint-Martin (1978) found that the ridges on palisade cells around the hilum tend to be aligned with the hilum, causing a pattern variation.

The results of this project have borne out all these observations, and disagree with the statement of Trivedi et al. (1978,p.410) that, in the Viciaeae, "the basic spermoderm (testa) pattern does not vary from place to place on the seed". In a few species the palisade cells may manifest different pattern types on different regions of the surface of single seeds; such species are Lathyrus niger, and L.pratensis, which have a basic non-papillose, reticular patterning, but manifest "domes" and "dimples" near the hilum. Lersten & Gunn (1982,p.9) observed six species of Vicia in which the papillae were restricted to the hilar region. In no species of Vicia examined in this project was this particular feature observed.

The regional differences in micromorphology reported here largely concern the degree of expression rather than the form of the topography. They tend to blur the minor features that could possibly help to distinguish some taxa.

Some possible explanations for regional variation in patterning have been considered. Differences may occur in the rate of development of certain areas of the testa. It has been shown in Ononis, a genus from a related papilionaceous tribe, that the testa pattern starts to develop only a few days after anthesis, and that it develops at different rates over the seed. It reaches its final form first on the region opposite the hilum; and the area around the hilum is the last region of the testa to reach maturity; in this area the pattern density is usually greatest (LaSota et al., 1979). Following these observations, it has been suggested that the "pristine" patterning adjacent to the hilum in seeds of the Viciaeae is the manifestation of its later development (Lersten, 1981, p.189). A second possible cause of regional variation of the testa could relate to the funicle; this is interposed between the seed and the fruit wall; it prevents the pressure upon the testa surface near the hilum that in other regions might modify the micromorphology. Observations by SEM of the surface of immature seeds would help clarify these explanations, but have not been possible during this project.

On different regions of most seeds, the density of the surface patterning differs; this is a reflection of the varying sizes of the palisade cells and might be related to the shape of the seed. Spherical seeds, such as those of Lathyrus ochrus and Pisum, have palisade cells that appear consistently to be approximately homogeneous in

size. Flat seeds, however, appear to be variable: for example, Lens species have larger cells adjacent to the hilum; but the seeds of the lentil-seeded Vicia sativa, and the flat-seeded populations of Vicia faba faba have smaller palisade cells in this region. Thus there does not seem to be a consistent relationship between the seed shape and the variation in cell size over the seed surface.

Although the possibility of recording palisade cell size on charred ancient seeds is commonly limited by the extent and the fragmentary nature of the retained testa, useful observations can be made providing that the testas of modern seed comparatives are examined in areas similar to those of the ancient seeds.

### 8.2.3. Intraspecific variation in testa micromorphology.

Testa patterning in the Viciaeae varies in seeds from different populations of the certain individual species. References to similar findings in the seeds of other legume genera have not been found; for example, in a key for the identification of ten species of Ononis by their testa morphology and micromorphology, seeds of an unspecified number of different populations of each species have been recorded as anatomically the same (LaSota et al., 1979). Similar literature concerning intraspecific variations in the testa surface in the Viciaeae has not been found; an apparently relevant

reference to the unreliability of the testa pattern as a diagnostic character in L.aphaca, cited in Davis (1970,p.367), refers solely to testa colour (Barnes,1933). In the present study, while variations have been recorded in some papillose-seeded species, such as Lathyrus sativus, the most marked variations have been observed in species with non-papillose testas, such as Lathyrus aphaca.

#### 8.2.3.1. Relation between testa colour and testa surface micromorphology.

The biochemistry of legume seeds may be altered by selection under cultivation (Smartt,1990). Evidence of such changed biochemistry in ancient pulses could provide important evidence to archaeobotanists of reduced dormancy, and thus provide an indication of early cultivation. In the search for evidence of such selection, the possibility of a relationship between the testa patterning and the seed biochemistry has been examined. Since biochemical analyses have been outside the scope of this project, testa colour has been employed as a crude indicator of tannin levels in the seed. In the Viciaeae, it has been shown that a pale testa is correlated with a reduction in seed dormancy and an increased rate of imbibition in Pisum (Marbach & Mayer,1974,1975,1987) and Lens (Vaillancourt et al.,1986). The seeds of highly-developed legume cultigens, which have low dormancy, commonly have light-



coloured testas; this is said to be due to conscious selection by man for tannin-low, more palatable varieties (Dodds in Feenstra,1960). Conversely, dark-coloured testas are associated with wild taxa or relatively undeveloped varieties of cultigens, as has been described in Vicia faba (Cubero & Suso,1981). The genetic control of testa colour and phenolic concentration in seeds of legumes appears to be complex: for example, in Phaseolus vulgaris more than four genes have been implicated (Feenstra,1960). The testa colour in Vicia faba is the product of numerous phenolic compounds; three classes of flavonoids, including tannins, have been analysed in this species; while white seeds contain undetectable tannin levels, coloured seeds contain a range of concentrations of different compounds controlled by a number of genes (Nozzolillo et al.,1989) (see 2.2.2.). In summary, while it is impossible to predict from testa colour the levels of these compounds, and hence the possible extent of dormancy, in darker-coloured seeds, light-coloured seeds appear to be low or lacking in tannins, and thus should exhibit reduced dormancy.

Seeds of the same species with testas of different colours were included in this survey, and their surface micromorphologies were compared. In particular, white and brown seeds of Lathyrus sativus, and white, brown and black seeds of Vicia sativa were examined.

It was found that intraspecific variations in the primary

patterning of the surface micromorphology are not correlated with the colour of the testa in either species. This result is similar to that reported in the seeds of some Vigna hybrids, in which brown and black testas do not appear to correlate with either rough or smooth testa micromorphologies (Rajendra et al., 1979).

Similarly, the surface deposits on the testa, that appear to occur most commonly on the seeds of cultigens, are no heavier in light-seeded populations. Indeed, in Vicia sativa, the black-seeded population has more deposit on the testa than lighter-coloured populations.

In summary, no correlations between testa colour and testa characters have been found. It is, however, appreciated that these conclusions are of limited validity, because most seeds in the reference collections used in the project have medium brown-coloured testas, and only single populations with either very dark or light seeds have been available from any one taxon.

Thus the potentially-useful indicator of cultivation, the changed biochemistry associated with a reduction in dormancy, which could be important evidence in archaeobotanical seed remains, appears not to be reflected in the micromorphology of the testa.

8.2.3.2. Comparisons of the testa surface between highly-developed and less-developed cultigens in the four genera and their wild relatives.

The major problem concerning the remains of ancient pulses, that is identified by archaeobotanists, relates to their cultivated or wild status. The separation between the different wild species or subspecies is often of lesser importance in answering questions concerning ancient agriculture, although it can sometimes provide useful environmental data. Hence the main focus of the project has concerned the extent to which the cultigens can be distinguished from related wild taxa.

The testa surface has been examined for differences between the patterning in seeds of cultigens and related species. The testa patterns of the four genera studied for this thesis are discussed in the conventional taxonomic sequence.

(i) Vicia. The most highly-developed cultigen in this genus, Vicia faba, has no known progenitor or very close relative. Primitive small-seeded populations of Vicia faba, such as those that have been identified from Pre-Pottery Neolithic B levels at Jericho (Hopf, 1983), have rounded seeds very similar in gross morphology to those of some of the wild species in section Faba. The representatives of this section studied in this project, V. bithynica and V. narbonensis, both have the typically

papillose testas that can be readily distinguished from those of V.faba.

Seeds from the cultivated populations of Vicia ervilia are not readily distinguished from those of wild forms, except on the basis of seed size. The seven populations studied in this project have included cultivated varieties and segetal forms; seeds from all these populations have been very similar in all respects. However, since this species is a relatively undeveloped cultigen, that today is grown for animal feed, perhaps this is unsurprising. Indeed, Barulina (1930) commented on the low variation in the gross morphology of the seeds of bitter vetch, and linked this with its exploitation as an animal fodder; Ladizinsky & van Oss (1984) have demonstrated by hybridisation studies a close relationship between wild and cultivated varieties of this species.

The cultigen Vicia sativa sativa is another relatively undeveloped pulse, also now mainly cultivated as a grain for animal feed; it has not been possible to distinguish its testa surface from that of the wild subspecies, V.sativa nigra. The genetics of the Vicia sativa complex has been investigated by Zohary & Plitmann (1979) and Hanelt (1986); in contrast to some other workers, and because of numerous morphological intergradations found between the subspecies, they regard the group as a single species complex that is still evolving. Certainly the

representatives of the two subspecies studied for this project, which were collected from regions as far apart as the Near East and Britain, have, with a single exception, not demonstrated any marked dissimilarities apart from a larger seed in the cultigens. The exception, as has been described above (section 4.2.1.2.), is the lentil-seeded variety, in which the testa micromorphology differs from the typical pattern. It is unlikely that this variety of vetch would be confused with lentil in archaeobotanical seed remains, since their hila differ. Also, since the existence of this lentil-seeded vetch depends upon selective processes connected with the cultivated lentil, which would presumably be present in the same assemblage in far greater amounts, its presence would be of limited significance. The main interest in its surface micromorphology stems from its similarities to the reduced form of patterning seen in Vicia faba. It has not been possible to investigate whether this patterning is correlated with the narrow shape of the seed, whether the association is coincidental, or whether it is an expression of either genotypic or phenotypic variation; thus any significance in these similarities remains unknown.

(ii) Lens. In this genus the testa patterning is very similar in all species except for L.odemensis. It is impossible to distinguish by the testa surface patterning even the most highly-developed macrosperma varieties of the cultigen, L.culinaris, from its wild progenitor,

L.orientalis.

(iii) Lathyrus. The testa pattern in Lathyrus sativus has been compared with that of L.cicera and other closely-related species from the same taxonomic section in the genus. Plitmann et al. (1986) have attempted to clarify the taxonomic affinities in this group, employing the testa micromorphology together with vegetative and other characters, biochemical and karyotype analysis and hybridisation trials. They suggested that parallel homologous variation is responsible for most of the interspecific similarities that they recorded in up to four populations of each taxon; they observed, however, that the testa surface patterning is distinctive in L.sativus, and that the papillae are flatter and more dispersed than those in seeds of the other taxa.

Kislev (1986,1989) and Kislev & Hopf (1985) have described in greater detail this typical testa pattern of L.sativus, observed in the seeds from five populations; they illustrated the blunt and wide-spaced papillae separated by a reticulum that usually distinguish seeds of this species from those of the closely-related species L.cicera, in which the papilla are acute and more densely distributed with a less-developed reticulum.

While the pattern characteristic of seeds of L.sativus described by these other workers has been recorded in this survey, some populations of this species, including

both that of the largest white-seeded cultigen, and that of a less highly-developed small and dark-seeded variety, have a testa pattern indistinguishable from that of wild species such as L.cicera and L.gorgoni; this type of micromorphology occurs commonly in both Lathyrus and Vicia. It appears that this pattern variation is unrelated to seed size, seed colour or whether or not the taxon is a highly-developed cultigen. While it may be possible to identify seeds of L.sativus when they exhibit their "typical" testa pattern, seeds with the variant type of testa cannot be identified on the basis of surface micromorphology to the level of species or even to genus.

(iv) Pisum sativum. The marked difference in testa topography between the cultivated and the wild subspecies of Pisum sativum has been described above (section 4.3.1.); it involves the presence or absence of secondary patterning; this difference seems to be unique to this species in the Viciaeae; although, in the Cicerae, Cicer arietinum and its wild progenitor display similar properties of the testa surface (Zohary & Hopf, 1988, p.99).

In summary, amongst the Viciaeae, only in Pisum sativum can the seeds of the cultigen be distinguished from those of its wild progenitor on the basis of testa surface micromorphology; Vicia faba can be readily separated from its closest wild relatives; and the seeds of certain

populations of Lathyrus sativus can be separated from those of related species.

#### 8.2.3.3. Possible causes of infraspecific variation in testa patterning.

It is difficult to assess the factors that might be responsible for infraspecific variation in testa patterning (Snaydon, 1984). During this project, constraints imposed by the research period have precluded any experimental work to investigate the possible causes. However some relevant research has been reported in the literature.

(i) Micromorphological variations within species due to genotypic variation.

The testa is an organ derived from the integuments of the parent plant (*i.e.* from sporophyte tissue). All the seeds from a single plant have a testa pattern that is the expression of the parent genotype. The best evidence for genotypic variation is said to be provided by hybridisation studies; work on leaf surfaces has shown that, in general, the micromorphological characters of the epidermis that are under genetic control outnumber those influenced by environmental conditions (Stace, 1984). Evidence for the genetic control of the variation in the morphology of seedcoat surfaces has been investigated in pea, and the genetics of the



micromorphology of seed surfaces has also been investigated in some legumes; certain characters have been studied by SEM in hybrids of Vigna sinensis and V. sesquipedalis, in the tribe Phaseolae, in which it appears that a single gene controls the rough/smooth topography (Rajendra et al., 1979). The extent of genetic control of testa surface micromorphology in the Viciae awaits detailed investigation.

(ii) Micromorphological variations due to environmental conditions.

Research has been undertaken on legume seeds to ascertain the variation in surface micromorphology due to a number of environmental factors. While it has been pointed out that it may be difficult to separate the existence of phenotypic plasticity from genotypic adaptation (Stace, 1984), the following observations appear to result from phenotypic responses to environmental conditions. The effects of certain environmental factors on the seeds of certain forage legumes and some pulses from various tribes are listed below: (i) photoperiod: Ononis sicula has a papillose testa on which the ridges between the papillae are larger and more prominent on seeds from plants grown under long-day conditions (Gutterman & Heydecker, 1973; LaSota et al., 1979); (ii) temperature: in Stylosanthes hamata the testa pattern varies with the

hardseededness of the seed, which is influenced by the temperature during seed maturation: cool conditions produce soft seeds with an indistinct testa topography, while warmer conditions produce hard seeds with a clearly defined testa pattern (Argel & Humphreys, 1983c).

While seeds from genera in the Viciaeae may well react differently from those of these other taxa under the same conditions, it seems likely that environmental conditions could play some part in influencing the extent of expression of testa patterning in this tribe as well. There is, however, no evidence that environmental factors control the basic micromorphology of the surface of the testa (*i.e.* for example, whether or not it is papillose).

#### 8.2.4. Intergeneric similarities in testa micromorphology.

Saint-Martin (1978) has described the structural homogeneity of the testa at tribal level in the papilionaceous legumes, referring to the typically papillose micromorphology in the Viciaeae. The similarity of the testa surface patterning between different genera has shown that species of some different genera can be indistinguishable by characters of the surface testa micromorphology. In the genera surveyed here, this is illustrated most strikingly in Vicia ervilia and Lathyrus cicera. This highlights the limitations of relying on

testa surface micromorphology as an exclusive basis for the separation of either genera or species.

#### 8.2.5. Intertribal similarities in testa surface micromorphology.

A remarkable observation in this study has been that, in the Viciaeae, the testa micromorphology may not only be non-specific to species or even to genus, but can also have a close similarity to that of taxa from other tribes. A similar range of testa patterning, both primary and secondary, can be found in the published illustrations of other legume testas, for example in Ononis, where species with "mounds", papillae, both freely-arranged, and linked in chains, "domes" and "dimples" and non-papillose testas occur (LaSota et al, 1979). Cicer includes some species with seeds with a papillose topography reminiscent of seeds of the Viciaeae (Lersten & Gunn, 1981). In Sophora certain species have seeds that are variously papillose and non-papillose (Bridges & Bragg, 1983). Illustrations of the testas in some species of Vigna (Gopinathan & Babu, 1985), of Lupinus (Bragg, 1983; Heyn & Herrnstadt, 1977), of Hypocalyptus and also of other genera (Lersten, 1981) appear virtually indistinguishable at high magnifications made with SEM from the testas of some of the species of Lathyrus in this survey, particularly those of the non-papillose forms, such as L.pratensis.

Despite the overlap in seed micromorphology between taxa, some authors consider that it can have taxonomic applications. Prakash (1987,p.260) has stated that "although the the anatomy of the seed-coat is very uniform throughout the family, there is enough variation in micromorphological characters to be useful in legume classification"; and in the Viciaeae testa patterning has indeed been used as one of a small number of characters as the basis for suggesting the reclassification of a group of four species of Vicia (Roti-Michelozzi & Serrato-Valenti,1986).

#### 8.2.6. Features overlying the testa patterning in the Viciaeae.

On many of the seeds examined in this project, the patterning of the testa surface has been obscured by different materials. It has been sometimes been necessary to treat the seed surface to remove these (see section 3.3.2., above). The nature of these substances is discussed in the following paragraphs.

##### 8.2.6.1. Epicuticular wax.

Wax crystals of two different forms have been recorded on the surface of seeds of two species in this survey: Lathyrus inconspicuus and L.ochrus (Butler,1986). They seem not to have been recorded previously on seeds in this tribe. Descriptions of wax depositions on the testa

of six other species in the Viciaeae (Trivedi et al., 1978) appear to refer to topographic features of the palisade cells.

Epicuticular secretions, termed "tertiary sculpture", although common on the surfaces of leaves, stems and fruits (Caldicott & Eglinton, 1973), are said to be rarely exhibited on seeds (Barthlott, 1984).

The presence of epicuticular wax is under genetic control, and the structure of the wax crystals is said to be related to their individual chemistry. Thus the form of the crystals may be of taxonomic significance. Environmental factors influence the amount of wax: a high humidity and high temperatures during plant development tend to increase wax production (Juniper & Jeffree, 1983, pp. 19, 20). However, light intensity is the variable that has the greatest influence: normal wax synthesis requires at least 20% full daylight (Juniper, 1960). Baker (1982) has investigated the mechanisms of wax production through the cuticle.

It has been recorded that the technique of SEM tends to destroy the crystals, and that the waxes tend to melt at accelerating voltages in excess of 15 kv and at magnifications above x2000 (Baker & Holloway, 1971). During this study the wax crystals on the testa surface of seeds of both species of Lathyrus have survived an accelerating voltage of 25 kv, and magnifications of

x5000 during scanning. However, the possibility arises that some other taxa may also have epicuticular wax crystals on the testa under certain environmental conditions; these may have been originally present on seeds investigated in this survey, but have been destroyed during SEM, the remnants of the crystals adding to the surface deposits that occasionally are found on the seeds (see 8.2.6.2. below). The fragility of the crystals also may explain the lack of published observations by other workers of the presence of epicuticular waxes on seeds of members of the Viciaeae.

The presence of epicuticular wax on the seeds is of general botanical interest, and may be an additional character that could be used in the identification of fresh seeds. However, since the process of charring destroys the crystals, they cannot be used to further the identificatory process of charred archaeobotanical seed remains. The attributes of their presence or absence are included in the dataset (Table 15) (see 8.5.1.1., below).

#### 8.2.6.2. Testa surface deposits of extraneous origin.

As well as the wax crystals described above, other types of material have been recorded which overlie the testa patterning. Occasionally a very thin, even layer can be observed lying loosely across the papillae. This has been illustrated by other workers, such as Stocks (1980). It appears to arise from the surface of the testa (Plate

26, Fig. 6), and is thought to be of cuticular derivation and an artefact of the SEM. It has therefore not been recorded as part of the dataset. Similarly, it should be noted that some deposits might represent the remnants of epicuticular wax crystals, possibly altered during SEM (8.2.6.1. above).

Other deposits can sometimes be seen on the testa surface, that appear not to be of cuticular origin; the fact that they are removed when treated with proteases (3.2.2.a)(ii)), demonstrates their proteinaceous composition. They have previously been recorded on the seeds of a number of taxa, such as lentil in the Viciaeae, in which species they have been classified into four types, based upon their extent (Hughes & Swanson, 1986). It has been suggested that they derive either from the fruit, being a break-down product of the pod wall that attaches to the testa prior to dehiscence, or that they are a product of an organism within the pod, such as a bacterium. In the work for this thesis, the deposits were most commonly found on the seeds of cultivated taxa; it is postulated that some of the deposits might be the result of a reduction in dehiscence, which prolongs the period of contact between the pod wall and the testa. If this is verified, they have a potential use as an indicator of cultivation (Butler, in press a).

Unfortunately there are limitations upon the applicability of this character in archaeobotany. In

archaeological seed remains the testa surface is commonly covered with debris, which tends to obscure surface details. Because of the fragile nature of the ancient remains, it is not possible to treat the seed surfaces by the techniques employed routinely to clean fresh seeds or to investigate their chemistry. Much of the surface debris seems not to be of plant origin, and can be considered as extraneous contamination. As a consequence of these conditions found in ancient charred seeds, the deposits discussed above cannot, at present, be distinguished from those of these artefacts. They have been included as attributes in the final dataset (see 8.4.1.1. below).

#### 8.2.7. Effects of charring on the seed surface.

It has been shown that the charring of seeds of the Viciaeae, when carried out in the routine manner described, tends not to cause appreciable changes to the seed shape or size. Any minor amount of shrinkage that might occur, results in seeds that still tend to lie within the size range of that taxon. Commonly, the testa is at least partially lost; this could be an indication of stresses set up by shrinkage, the possibilities of which are discussed next.

##### 8.2.7.1. Effects of charring on cell size.

The size of the epidermal cells of the testa shows little



change following charring at 300C for two hours or at 400C for one hour. Thus features on the external surface of the palisade cells, such as the papillae, retain their original density of distribution. As a consequence, quantitative data derived from dimensions relating to the seed surface of fresh seeds may be applied to these charred seeds.

This, however, is not usually the case for the qualitative data from the micromorphology of the testa surface. It has been shown for example that, on papillose seeds, the ridges running both longitudinally down the papillae and between the papillae commonly become reduced on charring. This could be due to some shrinkage of the external cell wall of the palisade cells, which is accommodated by expansion across the ridges, thus preserving a constant circumference of the cell wall. Fine details of the ridging thus cannot always be used as criteria by which to identify charred seeds.

The form of the secondary testa patterning, or "mounds", is little affected by charring, and thus remains a good criterion for species separation. "Mounds" have been only recorded in very few species of Vicia (V.lathyroides, V.koeieana) (Gunn, 1970; Lersten & Gunn, 1982;), which were not observed in this project, in the wild <sup>b</sup>species of Pisum sativum, and in a number of Lathyrus species. In this study all such taxa with secondary testa patterning were sufficiently different in the micromorphology of

this feature to be readily separable. However since the seed material studied here comprises specimens from only about 10% of the total number of species in the Viciaeae, many identifications based on the criteria under study will remain insecure, until such time as additional taxa have been incorporated in the analyses.

#### 8.2.8. Limitations of characters of the surface micromorphology of the testa as diagnostic criteria.

It has been shown that the different genera in the Viciaeae do not always have consistently distinctive testa surface micromorphologies; there is intergeneric overlap, and intraspecific variation can also occur often in the same criteria. The published reports of identificatory patterning in the Viciaeae do not appear to have taken these factors into account. Further, charred seeds have altered surface patterning, which reduces potentially useful micromorphological detail. Some species and subspecies, however, do appear to have diagnostic seed topographies, which withstand the effects of charring, and which therefore could be used to identify archaeobotanical seeds. The taxa concerned are mainly wild species with secondary patterning.

A number of species in the Viciaeae, particularly cultigens and their close relatives, cannot be distinguished by the surface micromorphology of the testa, either when fresh or in a charred state. Further

observations in the seeds have therefore been recorded. These have included both some characters of the gross morphology that are used conventionally to identify legume seeds, and the additional characters of the micromorphology and anatomy that have been recorded by SEM. These characters are discussed in the following section.

### 8.3. Further morphological and micromorphological characters in the seeds of the Viciaeae.

The traditional characters of the gross morphology are first discussed, followed by the micromorphology and anatomy.

#### 8.3.1 Gross morphology of the seed.

The gross morphology has supplied most of the characters that have been used conventionally to identify seeds of the Viciaeae. These can be observed at low magnifications or with the naked eye, as described above (section 2.2.2.). Their stability, which is seldom questioned, is examined below, with their applicability for the identification of charred ancient seeds.

#### 8.3.1.1. Seed shape.

The basic seed shapes found in the Viciaeae have been described above (section 2.2.2.). Modifications in seed shape may result from pressure within the fruit (pod) between adjacent seeds, as is exemplified in Lathyrus nissolia and sometimes in Pisum sativum, where the seeds may be drum-shaped, with the flat faces developing between seeds. Variations in the amount of pressure occur as a result of the presence of unfertilised or aborted ovules in the pod (Lersten & Gunn, 1982, p.2). In certain species, exemplified by Lathyrus sativus and its close relatives, the seeds may be of variable shape, depending upon their position in the pod; seeds from the mid-region of the pod typically have two flattened faces resulting from contact from adjacent seeds on both sides; seeds at the ends of the pod have one flattened face and one rounded face. These morphologies have been described by such authors as Helbaek (1965) and Kislev (1986) in charred ancient seeds.

Pressure may also result from compression of the seeds by the pod walls (Lersten & Gunn, 1982, p.2). The characteristic triangular isodiametric shape of the seeds of Vicia ervilia may be associated with their development within the highly torulose pods that typify the species. Similarly, in Lathyrus sativus and its close relatives, lateral pod pressure may explain the chisel-shaped seeds, which are flattened parallel to the plane of the

cotyledons. However some conflicting evidence has been observed regarding the role of the pod in influencing seed shape.

The pods of Lathyrus blepharicarpus, of the L.sativus group of species in section Lathyrus, observed during development (section 7.2.1.2., above), never displayed any evidence of compression upon the developing seeds; at maturity the pod walls were even slightly inflated away from the seeds. Yet the harvested seeds were of the characteristic sharp angular shape.

In another experiment with growing plants, seeds of the flat-seeded vetch, Vicia sativa sativa lentisperma, were planted (section 7.2.1.3., above). The resultant plants were typical of Vicia sativa sativa in morphology; the pods were long, straight and slightly torulose, with no sign of any atypical lateral compression. Yet the seeds when harvested exhibited the lenticular form of this variety.

In these two examples the seeds were flattened in shape, and yet did not develop in compressed pods.

Conversely, it has been observed that the seed, prior to its drying-out at full maturity, has some plasticity, and, in some species at least, its shape may be influenced by the pod. The seeds of the wild species Vicia sativa nigra when collected from ripe pods that are

judged to be just prior to the point of dehiscence, have the slightly flattened subspherical shape, characteristic of the seeds of many Viciaeae species; when allowed to dry free from any pressure from the pod, they acquire an approximately spherical shape, which is subsequently retained. This suggests that, in this species, pressure from the pod upon the developing seed contributes to the shape of the seed at maturity.

The influence of external forces acting upon the developing seed could be of importance for the identification of seeds using conventional characters of gross morphology. Associated with a change in seed shape, described above, and caused by the collection of seeds prior to ripeness, is a change in the ratio between the length of the hilum and the seed circumference; this character has been used by Gunn (1970) and others to separate species of Vicia (see section 2.2.2. above). Although seeds that have disseminated naturally would not be expected to demonstrate this phenomenon, it could be a concomitant of the gathering by man of the seeds of dehiscent species of the Viciaeae, prior to full maturity. These might be from wild or relatively undeveloped cultivated species, which are the types of species that occur in early archaeological contexts. The criterion of the hilar length expressed as a percentage of the circumference can be unstable in seeds from these species. Consequently, there are limitations to the applicability of this character for the identification of

ancient legume seeds.

#### 8.3.1.2. Seed size.

Seed size has been a character that has been used particularly in agricultural contexts to delimit varieties of cultigens; for example in Lens culinaris, seeds less than six millimetres in diameter are classified as lentils of the microsperma group, and those over six millimetres are from the macrosperma group (Barulina, 1930). Modern populations of this species vary from three to nine millimetres across the greatest diameter, and overlap in size with the wild species L.nigricans and L.orientalis, both of which species have seeds which can be up to 3.5 mm in diameter (Cubero, 1981). For certain taxa, seed size can be of diagnostic relevance.

During this project the variations in dimensions of fresh seeds from single taxa have been evident. This can sometimes be explained by differences in the developmental state of the seeds.

##### 8.3.1.2.1. Indeterminacy and seed size.

As has been described above (section 2.3.), the the growth habit of species in the Viciaeae is characteristically indeterminate; thus a single plant may bear flowers and fruit in varying states of maturity at

the same time, the lowest nodes being the most mature. The growing trials in this project have shown that it is unusual for more than very few nodes to bear flowers or fruits at one time on any branch, but that if damage occurs to one of these, or following seed maturity, then further nodes become activated. If harvesting is confined to one episode, the seeds constituting the crop are not all in the same state of maturity. This may be reflected in their size; smaller, slightly immature seeds may be present together with the full-sized ripe ones. Commonly, it seems, the number of fruiting nodes per branch is two, which bear seeds at two stages of maturity at any one time. If these are harvested together, the impression may be formed that the seeds have been derived from two different populations or even different taxa. This could lead to erroneous identifications such as the seeds of a cultigen occurring together with those of its wild relative. This observation might account for the two size populations of Vicia ervilia seeds at Mycenae (Hillman, pers.comm.).

#### 8.3.1.2.2. Charring and seed size.

The effects of charring have been observed to produce variable effects upon seed size, both in different taxa and under different regimes of charring. Since at the present time it is impossible to deduce the conditions of the charring episodes in antiquity, it is also impossible to tell whether the remains of any ancient charred seeds



are either larger or smaller than they were in their original fresh state.

van Zeist & Bakker-Heeres (1982), in the absence of information about the effects of charring, have assumed that shrinkage occurs; they estimated that in lentil this results in a decrease in diameter of about 10 per cent. Helbaek (1970, p.226) referred to reports of the charring of pulses, which were "divorced from reality", in which lentil seeds increase in volume on carbonisation. His own experiments on lentils, using a charring temperature of 400°C, resulted in shrinkage of 16%. Helbaek considered this size decrease to be the same as that recorded for the seeds of other species.

The results of the experimental charring of legume seeds undertaken for this thesis demonstrate that such assumptions are not always valid: shrinkage is not the inevitable result of charring. It has been concluded that the temperature of 300°C, applied for two hours, does not appear to result in more than possibly very slight changes in dimensions in any taxon examined. The difference from Helbaek's data may possibly be explained by the different heating regimes that have been employed. The following example should be considered.

The episode of accidental charring at a higher temperature, described above (paragraph 4.3.1.1.), caused different results in different genera. In pea, there was

no change in size. In lentil, by contrast, the charred seeds appeared to be larger than the fresh specimens; and measurements have shown this to be true. Here, indeed, the unlikely observation has been made that lentil seeds may increase both in diameter and in thickness under certain conditions of charring.

No explanation for this phenomenon has been found. The importance of the observation lies in an increased awareness of the variable response of legume seeds of different species to different conditions of charring. It is concluded that, in the absence of information about the charring conditions, it is impossible to ascertain the original dimensions of ancient charred seeds.

In the archaeobotanical literature, ancient lentils are almost invariably measured, and assigned to species by the size of their maximum diameter. The following two examples illustrate how seed size has been applied to identify such material. (i) The lentils amongst the plant remains from Franchthi Cave, Greece, from the levels prior to the Neolithic, have an average maximum diameter of below 2.5 mm., while those from later levels dating from 8000 BP are over 3 mm. in average maximum diameter; the conclusion has been made that assemblages with the seeds of larger average size represent those from cultivated populations (Hansen, in press). (ii) From the 53 small lentils recovered from the site of Hacilar in Turkey, two populations were separated: one is medium in

diameter, thick with a blunt edge; and the other is of larger diameter, thinner with a sharp edge. They have been assigned to two different species by Helbaek (1970). The results of the present studies suggest that these distinctions must be suspect.

Ancient charred legume seeds, exemplified by those of Vicia ervilia from Cayonu (van Zeist, 1972) are usually much smaller than those seen today; even taking into account the possibility of some shrinkage due to charring, it appears that pulse crops previously had smaller seeds than their modern counterparts, including even the least developed modern landraces.

As a consequence of all these factors, seed size as a direct measurement has not been a variable added to the dataset; it has been incorporated only indirectly, expressed by the length of the hilum as a ratio of the seed circumference.

8.3.1.3. The lens-hilum distance as a criterion for the identification of archaeobotanical seed remains.

Gunn (1970) and others have found that the distance between the lens and the edge of the hilum has been a useful criterion for separating species of the Viciaeae. However, the lens usually cracks during charring, and this area of the testa is often not retained on charred seeds. The lens-hilum distance is thus of limited use in

archaeobotanical identifications.

#### 8.3.1.4. Hilum shape and size.

##### 8.3.1.4.1. The width of the hilum.

The hilum surmounts parenchymatous tissue that surrounds the tracheid bar, as has been described above (section 2.2.5.). The aerenchyma is closely associated with the hilum, and remains attached as an integral part of the fragment when the hilum becomes detached from the seed during charring. The width of this parenchyma as viewed transversely across the hilum has been recorded, as well as the width of the hilum as measured on the seed surface. These two expressions of hilar width may be related to the thickness of the seed; both have been incorporated in the dataset. It appears that hilar width is not a criterion that has been used in the separation of seeds of the Viciaeae, except as a factor in the shape of the hilum, as employed by Gunn (1970). Yet this dimension is readily recordable; it can be measured in charred ancient seeds and it even can be accurately estimated when a proportion of the hilum is retained to half its width.

##### 8.3.1.4.2. The length of the hilum.

The hilum length appears to have taxonomic potential. The ratio between the length of the hilum and the seed

circumference is preferred to the hilar length as a diagnostic character by Gunn (1970), but it has been found that this character is liable to vary with the state of maturity of the seed at harvesting (section 8.2.1.1.).

Gunn (1970) includes the size and shape of the hilum amongst the criteria he employs to separate species of Vicia. The length of the hilum varies most widely in Vicia amongst the genera in the Viciaeae. In Lathyrus, as has been shown in the pilot study (Butler, 1986), and also in Lens and Pisum, the length of the hilum has a narrower range of variation.

#### 8.3.1.5. Micropyle shape.

The shape of the micropyle has been described by Lersten (1982) as linear. It is said to be characteristic of the Viciaeae (Lersten & Gunn, 1981). Lersten & Gunn (1982, p.35) have illustrated a bifurcated micropyle in Vavilovia formosa, but, apart from this one example, there are no published observations of different micropylar shapes in the Viciaeae. Four shapes of the micropyle in fresh seeds have been recorded in this study. Commonly, however, the micropyle is damaged in charred ancient seeds, thus attributes of this feature could be of limited relevance to archaeobotanical diagnoses.

### 8.3.2. Internal features of the testa.

#### 8.3.2.1. Hilum micromorphology.

The profile of the hilum in transection has been illustrated in six species of the four major genera in the Viciaeae by Lersten & Gunn (1982,p.29), and recorded as elevated, raised, level or flat, without further comment. The hilar profile in this study has been recorded in a number of characters; by the subjective description of sunken or projecting, by the relationship between measurements of the hilar width and hilar depth, and by the angle between the surface of the cotyledons and the hilar slit. The profile is not directly related to the shape of the seed, as is shown by the following examples: in Lens species, the hilum is prominent; in the lentil-seeded Vicia sativa it is less so; and the slightly flattened seeds of V.faba have sunken hila.

#### 8.3.2.2. The anatomy of the tracheid bar: criteria that may provide evidence of a changed physiology following cultivation. Comparisons between seed tracheoids and wood elements.

As vascular tissue is a conservative feature, it is considered to have particular value in taxonomy (Bell,1969,p.71), and the tracheid bar has therefore been a particular focus of this research.

The tracheid bar, an extension of the ovule vascular bundle, exists only in the Papilionoideae. It has been described as remarkably uniform in structure for such a large and diverse plant group. A range of different tracheid bar morphologies, pit types and pit ornamentation have been observed in the subfamily (Lersten, 1982). In the Viciaeae, the tracheoid pits, described as surprisingly uniform in size and shape, are held to be of particular interest. In a study of pit ornamentation, 35 species were recorded as having non-vestured pits; 31 species had small warts; and 10 species had some vestures (Lersten & Gunn, 1982, pp. 6, 18-21).

Results of the survey for this thesis have conflicted with those of Lersten (1982) and Lersten & Gunn (1982, p. 6, 18-21) (section 4.2.2.4.); it has been shown that, in seeds of members of the Viciaeae, the pits are not usually plain-bordered; they do not invariably have fully-perforated pit membranes; they are not always evenly distributed or regularly arranged.

In order to achieve more understanding of the possible significance of some of the features of pit anatomy, literature relating to the pits in secondary xylem has been consulted. The pits of dicotyledonous woods have been said to have considerable taxonomic value (van Vliet, 1978). Vesturing on pit walls in wood is characteristic of the Leguminosae; this ornamentation is considered to be of great taxonomic value (Baretta-

Kuipers,1981). The tracheoids in seeds have been compared with the elements of the secondary xylem of legume wood, in which elaborate vestures are common, and the pit membranes are retained to varying extents. In legume seeds it has been suggested that elaborate vestures tend to be associated with more primitive tribes, and it has been recorded that the pit membranes are virtually always lost (Lersten,1982). A hypothesis has been formulated that these conditions support an evolutionary shift away from functions relating to water conduction in the tracheid bar (Zweypeyning,1978). However, it has been stated that interpretations of possible tracheoid functions are still speculative (Lersten,1982).

The structure of pits in cell walls has been investigated by Meylan & Butterfield (1972,p.13). They have compared the vestures with the warts in vessels of leguminous woods. It was originally considered that, while vestures develop in the living cell wall, warts are deposited in dying cytoplasm. Recent work has led to the conclusion that warts and vestures have a similar aetiology, morphology and chemical composition (Ohtani et al.,1984).

It has been postulated that phenolic compounds polymerising in the cell walls and on their surfaces might contribute to the formation of warts (Liese,1965), a view supported by the histochemical work of Scurfield (1972). Pit ornamentation is composed of lignin and hemicelluloses; and it has been suggested that as



lignification appears to be a prerequisite for wart formation, then an oversupply of lignin from the cytoplasm would result in the production of vestures (Ohtani et al., 1983, 1984).

These background investigations into pit anatomy and the chemistry of pit ornamentation prompted investigations of pit anatomy in this project. If warts and vestures were produced under conditions of excess phenolics in wood, then perhaps the same conditions might underlie their production in seeds.

The work of Werker et al. (1979), as mentioned above (section 2.4.2.2.), has associated high levels of phenolics in the seeds of Pisum with seed dormancy; conversely, low phenolic levels have been equated with a reduction in dormancy. If high phenolic levels are also associated with vesturing of the pit walls of the tracheoids, then the presence of this pit anatomy might indicate a seed with a high level of dormancy; conversely, plain bordered pits might provide evidence of reduced dormancy, and hence of having derived from a cultivated taxon. It is suggested that these anatomical criteria might provide some indirect evidence for the altered physiology that follows selection under cultivation (Butler, 1989).

The first observations made during this study of wild and cultivated species of Lens, and the species of Vicia from

section Faba, appeared to support this hypothesis; thus variables relating to pit anatomy as well as pit micromorphology were recorded and added to the dataset. However, subsequently, a population of Vicia faba with marked pit ornamentation was seen, followed by more numerous examples of the seeds of cultigens showing this character. It has now been accepted that the extent of pit ornamentation cannot be used as a reliable criterion to provide evidence for or against cultivation.

The data derived from the tracheid bar has typified the results of the survey as a whole. The first observations on single or very few populations of a species or subspecies have led to hypotheses that data from more populations have subsequently disproved.

Charring may change the pit anatomy. At 400°C, the pit apertures may lose definition, and the ornamentation becomes altered or disappears; artifactual "bars" may appear across the apertures (section 4.4.5.). Research on legume wood charcoals has suggested that the pit anatomy becomes obscured by gums which fuse with the pit walls. It has been suggested that this phenomenon is peculiar to the legumes; it has not been observed in charcoal prepared from trees of other families, in which most anatomical details commonly survive high temperatures (Prior & Alvin, 1983).

#### 8.3.2.3. Testa thickness.

It appears to be generally accepted amongst both botanists (such as Smartt & Hymowitz, 1985), and archaeobotanists (such as Hopf, 1986), that the testa in leguminous cultigens is thinner and smoother than that of wild legume species, and that this reduction follows selection under cultivation; this view has been stated by Zohary & Hopf (1988, p.84): "Smooth, thin seed coats evolved under domestication..... the reduced seed coat is more permeable to water. A thin coat may thus reflect the breakdown of the wild-type germination regulation". Examples have been published of the absolute height of the cells of both the palisade layer and the hourglass cell layer, as well as their relative proportions, their structure and their arrangement (Hopf, 1986, p.38). However, in this article, these characters of the testa in longitudinal view were illustrated in only five species in the Viciaeae: four cultigens, including the rarely-cultivated species Vicia narbonensis, and the wild species Lathyrus tingitanus.

Some work has been published on the genetics of testa thickness in legumes. The inheritance of testa thickness in the Phaseolae has been investigated by Ojomo (1972) working on Vigna unguiculata; he has found evidence of two major and possibly several minor genes controlling this character. Rajendra et al. (1979), working on hybrids of Vigna, recorded a correlation between testa

thickness and seed size, thicker testas being associated with larger seeds; however these workers did not record the regions of the seeds from which the measurements were taken. Literature relating to similar research in the Viciaeae is not available.

As has been described above (section 4.2.2.5.) during this survey, in Pisum sativum, a thinner testa has been recorded in seeds of the cultigen, which has larger seeds than the other subspecies in this species. This supports the results of Werker et al. (1979). Marbach & Mayer (1974) showed that the permeability of the testa in peas is increased in thin-coated seeds, however it was found that this is associated with lower levels of phenolics in the testa; these compounds occur both in the palisade layer and in the hourglass cells; the site of the phenolics appears to be immaterial to their effect on seed coat permeability. The palisade cells are said to have pectinaceous outer surfaces which also play a role in testa permeability (Werker et al., 1979). There appears to be no evidence to suggest that the thickness of the testa is correlated with testa permeability either by containing higher levels of phenolics, or by providing a mechanical barrier to water penetration.

In the genera other than Pisum that have been examined in this project, thin testas are not necessarily associated with seeds of cultigens. Some examples can support this assertion: in Lathyrus, some wild species, such as

L.aphaca and L.pratensis, have thin-coated seeds; in Lens, all species have thin testas. These results conflict with the statement of Zohary and Hopf (1988,p.84): "Seeds of wild pulses have a thick and coarse testa which serves as a mechanical protection and for the delay of germination".

Similarly, smooth testas occur in wild taxa; indeed they are found more commonly than rough testas (see paragraph above). Again, this observation conflicts with the statement of Zohary and Hopf (1988,p.84) that "smooth, thin seed coats evolved under domestication".

The trait of testa roughness, or the presence of secondary patterning, appears to be correlated with testa thickness; and the thickest-coated seeds, and those with rugose testas that have been examined, have all been those of wild taxa.

The generalisation that there has been selection under cultivation for thin testas in the Viciaeae has been confirmed only in Pisum. It has been demonstrated that thin smooth testas are not confined to the cultigens.

In summary, the work for this thesis has not shown a correlation between thin testas and cultigens. Thin testas occur in the cultigens investigated, but also in some wild taxa. Thick testas, however, are invariably found in wild taxa.

8.3.2.3.1. Effect of the environment upon size of palisade cells.

Some published research has demonstrated that certain environmental factors can influence testa dimensions, affecting both the surface area, with the associated density of patterning, and the thickness.

The primary testa pattern, as has been described, is a product of individual palisade cells (Lersten, 1979), and thus the density of its distribution is a reflection of the external surface area of the palisade cells, and is at its lowest when the testa is at full turgor at seed maturity (LaSota et al., 1979).

The size of the palisade cells, both adjacent to the hilum, and at midseed, may be correlated with the overall size of the mature seed, in any species. Kislev & Hopf (1985) have inferred that the density of testa patterning is a function of seed size in Lathyrus sativus. As has been discussed above (8.3.1.2.), direct measurements of seed size, such as the maximum diameter, are commonly employed as characters to identify subspecies or separate cultigens from their wild relatives. Palisade cell size is a criterion that could be similarly employed. This relationship of cell size to the whole seed could help to interpret fragmentary seed remains.

The effects of different environmental conditions on the

dimensions of the cells of the testa have been studied by Argel & Humphreys (1983a,b,c), who have found that, in the forage legume Stylosanthes hamata, seeds that have matured under cool conditions have longer palisade cells, and thus a thicker testa, than those from the same population grown under warmer conditions. It is possible that environmental factors could similarly influence testa cell length, and thus the thickness of the testa, in taxa from the tribe Viciaeae. The lengths of both the palisade cells and the hourglass cells have been recommended by Hopf (1986) as diagnostic characters for seeds of the Viciaeae without reference to their stability, in terms of phenotypic plasticity or genetic variation, or both factors.

#### 8.3.2.3.2. Charring and testa thickness.

In the seeds that were charred at 300°C for two hours that have been prepared as comparatives for this study, no appreciable change in testa thickness was observed. It has not been possible to compare these results with those of other workers, as no literature describing the micromorphology of charred legume testas has been found.

#### 8.3.2.4. Calcium oxalate crystals.

Calcium oxalate crystals are of widespread occurrence amongst the Leguminosae, and they have been used in legume taxonomy. Four crystalline types have been

described; their chemical composition is commonly assumed without chemical analysis, a practice that has been justified by their comparison with crystals of known composition (Zindler-Frank, 1987).

Observations made of members of all three subfamilies have shown that crystals occur mainly in association with the vascular strands, and less commonly in the epidermis, and that in the Papilionoidae these crystals are commonly single or twin crystals. Most relevant research has focussed upon the tribe Phaseoleae; in the seeds of Phaseolus vulgaris crystals are found in the testa, where they have been said to protect the embryo against insect action. There are few data relating to these inclusions in members of the Viciaeae, but crystals have been recorded in the leaves and fruits of a few species, such as Pisum sativum (Zindler-Frank, 1987). Investigations of the effects of charring on calcium oxalate crystals have shown that, in the parenchyma of the leguminous tree species, Dichrostachys cinerea, they survive charring at temperatures up to 700°C, above which most disappear (Prior & Alvin, 1983).

In the present survey, crystals have been recorded within the testa of seeds of a single population of one species. They are situated beneath the hourglass cells surrounding the hilum and appear not to occur elsewhere in the seed. They have survived charring at 300C for 2 hours (section 4.4.6. above).



Despite the reputed taxonomic value of calcium oxalate crystals in the legumes, their presence is not consistent within seeds of taxa in the Viciaeae. Therefore it cannot be used as a criterion for the identification of species.

#### 8.4. Further effects of charring on the anatomy and histology of legume seeds.

No literature concerning the effects of charring on the micromorphology of legume testas has been found, noted above (section 8.3.2.3.). However, some relevant research on the effects of charring on the anatomy of legumes has been undertaken on wood charcoal. It has been observed that in the charred wood of the leguminous tree Dichrostachys cinerea, the anatomy of parenchymatous tissues, such as ray cells and xylem parenchyma, tends not to be altered at temperatures of 300°C or below. At 400°C the cells become rounded. Rupture and disorganisation starts to occur at 700°C, and by 800°C cavities replace the cell groups. Starch grains have disappeared at 300°C. Normal fibres lose all evidence of wall stratification and the pit canals are obliterated at 400°C (Prior & Alvin, 1983).

Cope & Chaloner (1985) have observed by SEM the cell walls in ancient wood charcoals; there is homogenisation of the walls with obliteration of the middle lamella. Similar results of charring have also been recorded by McGinnes et al. (1974) in modern wood tissues

experimentally charred at temperatures in the range 270-400°C.

The charred parenchyma of modern tubers has been investigated by SEM by Hather (1989, pp.134,162-4). He found that charring at 250°C for 2.5 to 4 hours obliterated the middle lamella, and usually destroyed the cell contents.

Comparable anatomical changes produced by charring between 300 and 400°C for one to two hours have been observed in the parenchyma of the cotyledons and the tracheoids of legume seeds in this project. There is obliteration of the middle lamellae, and rounding of parenchyma cells. There is also a loss of the cell contents of the starch cells of the cotyledons. An examination of the starch grains, which has been recommended in some taxonomic studies (Biliardiris et al., 1981), is therefore not possible in charred legume seeds.

The effects of charring on tracheoid anatomy have been discussed above (8.3.2.2.).

A detailed study of the effects of charring legume seeds over a temperature range of perhaps 250°C to over 450°C might make it possible to deduce the conditions of charring of some ancient seed remains. However, as has been described above (3.3.3.b)(ii)), the charring regime,

heating at 300°C for two hours, was selected for producing the charred modern seeds used in this project because, when charred under those conditions, modern legume seeds appear under SEM to be very similar in micromorphology and anatomy to those of the well-preserved remains of ancient pulses that have been examined.

#### 8.5. Multivariate analysis of the dataset.

##### 8.5.1. An explanation of certain characters.

The reasons underlying the treatment of certain characters are discussed in the following paragraphs.

##### 8.5.1.1. Attributes of the micromorphology of the seed surface.

The micromorphology of the seed surface is represented by five characters in the dataset. Variations of patterning within taxa, and the likelihood of reduction in surface detail due to charring have been the reasons underlying the decision to restrict the representation of the patterning to (i) the presence or absence of secondary patterning; (ii) the presence or absence of papillae; (iii) the confluence of patterning between adjacent palisade cells; (iv) and the density of patterning at both the hilum and (v) at midseed. These characters have been observed in well-preserved charred ancient material. Two additional characters, of the presence or absence of

epicuticular wax, and of superficial testa deposits have limited applicability in ancient charred material (see 8.2.6.), but were included to test their relevance as characters for the separation of particular taxa.

8.5.1.2. Internal testa characters omitted from the final dataset.

Anatomical characters of the tracheoid pits were recorded, as has been described, but were not used in the final analyses. This decision was made after it was found that (i) pit ornamentation appears to be an unstable character within single taxa and not associated with their wild or cultivated status; and (ii) that charring changes the form of the ornamentation and creates artefacts in the pits (see section 8.3.2.2).

8.5.2. The separations by multivariate analysis.

The conclusion drawn from the cluster analysis (6.2.4.) is that the shape of the seeds seems to underlie most of the groupings, as shown by the clustering of the round seeds of Pisum and Lathyrus ochrus. However, there are indications that other factors are also responsible: for example, the lentil-seeded Vicia sativa does not cluster with lentil.

The characters that have been implicated in most of the separations achieved by principal coordinates analysis,

listed in Table 18 (section 6.3.4. above), have been those relating to the width of the hilum and the aerenchyma beneath the hilum, the surface area of the palisade cells, the deposits on the testa, and the micromorphology of the testa surface. The first three of these characters, which have the highest correlation coefficients, appear in part to reflect the seed shape and size. These gross morphological characters, together with the presence of secondary patterning of the testa surface, can all be recorded by conventional means, without the need of SEM. From this observation, it might appear that little advantage has been gained by using SEM to attempt to identify seeds of the Viciaeae in the fresh state.

However, SEM offers more obvious advantages when used on archaeological seeds. When ancient charred seeds are too fragmentary for their original shape and size to be deduced, observations made by SEM of internal features in the hilar region can provide useful identificatory data. The characters that have most influenced the separations are largely those of the hilar region; the character of palisade cell surface area at midseed alone falls outside this site. It is of particular importance that the detached hila, which are sometimes recovered from archaeological deposits, and commonly carry a rim of the testa surface (sections 4.4.2., 4.5.2.), are fragments that could provide most of the useful characters that have been employed in these analyses, including most of

those of the surface micromorphology.

The attributes concerning the superficial deposits on the testa surface appear to be useful in separating some taxa. Further research into the nature of these deposits is needed for their significance to be more fully understood. It appears that some of the problems that confront the seed analyst when using conventional characters of the gross morphology of the seed to identify species in the Viciaeae are also present when characters of the micromorphology and anatomy are involved in the analysis. The two genera, Lens and Pisum, which are usually easily separated by conventional means, retain this property; the two genera, Vicia and Lathyrus, which have overlapping morphological vegetative characteristics, have overlapping seed micromorphologies, demonstrated by the methods employed in this study. Neither cluster analysis, nor principal coordinates analysis have discretely separated species of Lathyrus from those of Vicia.

Using the characters compiled in the final dataset, the seeds of the cultigens in the Viciaeae cannot be isolated from those of their wild counterparts in general, or from their wild ancestors, with the exception of Vicia faba.

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## 8.6. Realisation of the aims of the project.

### 8.6.1. Identification of species.

#### 8.6.1.1. Diagnostic potential of testa surface micromorphology.

Brisson & Peterson (1976), reviewing the earliest research using SEM, found that many workers recorded diagnostic differences between taxa on the testa surface. However they reported that the seed surface characters often show polymorphism due to phenotypic plasticity and genotypic flexibility. Barthlott (1984) has observed that surface features of the testa are little affected by environmental factors, and that variation in testa characters seems always to be under genetic control. Recent work has led to the conclusion that there is sufficient variation in micromorphological characters of the testa to be useful in legume classification (Prakash, 1987). Lersten (1981) reported that legume testa patterns seems to be taxonomically significant within and between certain tribes. In various genera from a number of tribes in the Papilionaceae, the testa surface micromorphology has been found to be useful for separating species, as in Phaseolus (Sharma et al., 1977) and Vigna (Gopinathan & Babu, 1985) in the Phaseolae.

In the tribe Viciaeae, Lersten (1981) considers the testa pattern to be distinctive amongst the other legumes.

Trivedi et al. (1978) divided the types of testa patterning in the Viciaeae into three groups: tuberculate, undulate and smooth (this last only found in Abrus, previously classified in the Viciaeae), and found a distinctive testa pattern in all eight species they examined, which led to anticipations of diagnostic significance of the patterning.

As has been described above, while it is acknowledged that different species in the Viciaeae have characteristic types of testa topography, these may exhibit infraspecific variation and intergeneric similarities. Correspondingly, there are limitations to the application of the micromorphology of the testa surface in the identification of species. However while it is often impossible to assign a seed to a particular taxon using testa surface characters, it is usually possible at least to narrow the range of possibilities.

#### 8.6.1.2. Identifications using the extended dataset.

The extended list of characters of the testa has not provided diagnostic criteria that can separate all the taxa that have been examined in this project. It appears that the width of the hilum, which seems to equate with the shape of the seed, in conjunction with the surface micromorphology, is responsible for most of the separations that have been achieved. The limitations to these separations have been described above



(8.2.8.,8.3.1.1.).

Some factors that might explain the results are discussed in the following section.

#### 8.6.1.3. Parallel evolution in the Viciaeae.

The overlapping morphologies, mainly in vegetative and floral structures, observed in Vicia and Lathyrus in particular, but also including the other genera in the tribe, have complicated taxonomy in the Viciaeae. They have been cited as examples of convergence, or parallel evolution, by Kupicha (1981).

Vavilov (1951,pp.viii,88) used forms of vetch and lentil amongst the Viciaeae as examples of the expression of "homologous series of heritable variations", by which he described his Law of Homologous Series. This has recently been re-defined in detail by Jackson (1990,p.18), who stated that closely-related Linnean species are characterised by "similar and parallel series of variations", and quoted Vavilov's second law that "closely related allied genera display similarity in their series of phenotypical, as well as genetical, variability".

The interspecific and intergeneric similarities in seed micromorphology, that have been observed during this project, appear to a manifestation of this phenomenon;

and illustrate that micromorphological characters behave in the same manner as those of larger scale. Intertribal similarities in the micromorphology of seed surfaces appear to be an extension of the same phenomenon.

#### 8.6.2. Identification of the cultigens.

The characters in legume seeds that conventionally have been used as evidence of the cultivation of a taxon have centred upon easily observed criteria of the morphology that reflect the changes that have occurred as a result of selection by man. They have been considered above (1.2.3.2.-1.2.3.3.), and are briefly recapitulated in the following paragraphs.

##### 8.6.2.1. Gigantism.

Gigantism in seeds, the most obvious sign of cultivation in the Viciaeae (Hanelt, 1986), is generally agreed to be a phenomenon that develops at a relatively late stage in the development of the cultigens (Hopf, 1986), and is therefore a character of limited application in the analysis of the charred seed remains from the early potentially-agrarian sites.

##### 8.6.2.2. Hardseededness and testa thickness.

The testa carries the property of hardseededness in legumes that reduces the rate of imbibition, and governs

the rate of germination (Werker, 1980/81). The control of germination is believed to be a prerequisite in the development of the cultigens, and the decrease of the hardseeded property is said to be one of the first changes in cultivated seeds from those in the wild state. Thus evidence of this condition would be useful in the diagnosis of the seed of a cultigen.

The hardseeded property conventionally has been ascribed to the thickness and the texture of the testa (Hopf, 1986). In various legumes that have been investigated, it has been shown that the hardseededness is associated with different layers of the testa, and with the biochemical constituents of the seed. These have been correlated in Pisum with the thickness of the testa (Werker et al., 1979).

During this project, no evidence has been found to correlate the thickness of the testa with exposure to cultivation, except in Pisum sativum.

#### 8.6.2.3. Hardseededness and testa roughness.

As with the thin testas, smooth testas are equated with cultivated taxa in seeds of the Viciaeae (Hopf, 1986).

In this survey, smooth testas have been seen in wild species of all genera except Pisum; in Pisum too, published records show that the seed surface is smooth in

the wild species Pisum fulvum (Lersten & Gunn 1982, pp.19,33), seeds of which were not available during the research period. As stated above (section 8.3.2.3.), although rough seeds are invariably those of wild taxa, smooth seeds are not confined to cultigens.

Thus, with the exception of pea, which readily may be identified to species by gross morphological characters, the testa texture can be used only to eliminate rough-coated specimens from the list of cultigens.

#### 8.6.2.4. Testa colour.

A light-coloured testa is held to be associated with seeds of cultigens. The dark testa is associated with high levels of tannins, and these have been implicated in dormancy due to hardseededness in peas (Marbach & Mayer, 1974; 1975). Thus it has been assumed that soft seeds have low tannin levels, and are light in colour.

Certainly, a feature of the seeds of developed cultigens is the pale testa, evidenced in this project by the very large white seeds of one variety of Lathyrus sativus. However, the seeds of landraces commonly retain the range of testa colours of closely related wild species, and may have very dark or even black testas. Many of these landraces exhibit physiological properties little altered from the wild state and exhibiting little obvious adaptation to cultivation; and thus they may have a high

level of dormancy.

The pigmentation of the testa has been described in Vicia faba (section 2.2.2. above), and this may involve a number of different compounds. It appears that tannin levels may not always be deduced from the testa colour. While light testas have low tannin levels, testas low in tannin may sometimes be deeply pigmented.

Some disadvantages of the light-coloured testa have been examined above (section 2.4.2.3.), from which it can be deduced that the earliest cultigens may well not have been selected for this property. Also, reference has been made to the preference of some societies for seeds that carry an astringent taste, such as is associated with high-tannin pulses.

It appears that a number of variables potentially governed the extent to which the light-coloured testa in members of the *Vicieae* might have been subjected to positive selection by the early agrarian societies. It is impossible to deduce the role they might have played.

8.6.2.5. Characters in legume seeds that might provide indirect evidence of cultivation.

The characters discussed above (sections 8.6.2.2.-8.6.2.4) potentially provide direct evidence on the seed. However, there are further characters that might provide

indirect evidence of cultivation.

One line of indirect evidence for cultivation, based on the levels of phenolics, and possibly reflecting the extent of dormancy in the testa, has been discussed above (section 8.3.2.2.); it remains inconclusive.

A second potential line of indirect evidence concerns the presence of the condition "hollow heart" (recorded above, section 4.3.8.2., and described below, appendix 2, A2.1). This condition is manifested by concavities between the cotyledons. It has been suggested that this results from harvesting pulses in an immature state, a practice which commonly is employed both for wild taxa, and for relatively undeveloped cultigens which retain a high level of dehiscency. This potentially-useful feature awaits verification, prior to its application as evidence of early cultivation or wild plant gathering.

8.6.2.6. The effects of agronomic methods on cultigen development.

It appears that the seeds of cultigens in the Viciaeae, apart from Pisum, do not portray visible changes in their testa micromorphology or anatomy, compared to those of their wild relatives. This observation might be explained, at least in part, by the type and extent of the selection pressures imposed on cultivated members of the Viciaeae as a result of the particular agronomic

systems.

A number of archaic systems of pulse agronomy have been described above (section 2.5.). It has been postulated that mixed cropping is likely to have been important in legume cultivation in antiquity (section 2.5.11.). In such agronomic systems, particularly where there is mixed sowing and hand harvesting (section 2.5.5.5.), the selection pressures favouring loss of dormancy and dehiscence appear to be low. In regions practising such methods landraces are commonly cultivated (section 2.5.10); these varieties tend to have seeds which are small, often dark in colour and little changed in physiology: there is often little reduction in dormancy, and dehiscence is retained to appreciable extents (Haddad et al., 1988). Advantages, or, at least, relatively few disadvantages, to the farmer may be inferred from the retention of these properties, usually associated with wild species, particularly where there is mixed cultivation (see sections 2.5.5, 2.5.9); for example, a prolonged period of germination can be an insurance against the effects of short-term unfavourable climatic conditions; dehiscence may not be a problem when harvesting is not confined to a single event, or, when the crop is gathered in a semi-ripe or green state; and high phenolic levels in the testa may offer protection to the seed from animal predation (Harborne, 1977, p.135).

Consequently, it perhaps is not surprising to discover a

lack of micromorphological differences between seeds of legume cultigens and wild taxa. The exception of pea might possibly be explained by a longer history of intensive monocropping, perhaps involving small-scale garden cultivation, in some areas; but as yet this is without evidence. At this stage, these suggestions are no more than speculative, but they stimulate ideas for future research.

#### 8.6.3. Evaluation of SEM as a technique applied in archaeobotany.

The potential of the SEM as applied in biology, and particularly in plant taxonomy, has been discussed by Heywood (for example, 1971, 1984, 1985). He highlighted the absence of suitable terminology for many of the features observed. Lersten (1981) reiterated this lack in relation to descriptions of legume testa micromorphology. The problem endures today, and has been encountered during this project.

The role of the SEM in examinations of the testa in seeds of members of the Viciaeae has been described together with the applications in archaeobotany (Butler, 1988). Although there are disadvantages associated with this technique, it remains an ideal method for making observations on charred tissues, as previous publications have demonstrated.



#### 8.6.3.1. Early work on fresh seeds with SEM.

The application of the technique of SEM for the study of seed micromorphology has been reviewed by Brisson & Peterson (1976). Following the initial studies of the testa surface in many taxa, they highlighted the difficulties that can occur when examining seed surfaces by SEM, such as artifacts that can arise under vacuum. Heywood (1985) has stressed the importance of accurate interpretation of the features that may be observed under SEM. However in terms of the high magnifications and the degree of resolution that are possible with this technique, the advantages of SEM over the more conventional methods of optical microscopy are great; they are described below.

#### 8.6.3.2. Advantages of SEM of fresh and charred seeds.

The methods of preparation of specimens of ripe legume seeds for SEM are usually simple (see section 3.3.2. above). Fixing and critical point drying, which are usually practised for soft or wet tissues, are unnecessary. Dry seeds may be affixed to stubs directly, while in a living state. This facilitates the speed and the ease of the operation, and reduces the possibility of the production of artefacts. Specimens can be scanned and viewed in an intact state over a wide range of conditions, which allows for a mapping of the features upon or within each specimen, and ameliorates the

understanding and interpretation of many characters. A wide range of magnifications can be achieved by SEM; this includes very high magnifications not possible by optical microscopy, and also low magnifications comparable with those obtained by the dissecting microscope.

The photomicrographs, which form easily portable and storable expressions of the results of SEM, provide a virtually permanent record of the observations made. The relatively great depth of field provided by SEM gives a sharp focus to these photographic records even with such three-dimensional specimens as whole seeds.

Detailed observations of charred seeds cannot be made by optical microscopy. Thin sectioning is not normally possible. Only the minimal resolution of some surface detail of the opaque specimens can be achieved at low magnifications. With SEM the characteristics can be recorded similarly on charred and on fresh seeds, following the same techniques of specimen preparation. Thus SEM is particularly to be recommended where seeds in both states are to be examined.

#### 8.6.3.3. Disadvantages of SEM of fresh seeds.

Central to this project has been a study of variations in the testa micromorphology and anatomy, for reasons given above (section 8.2.). Thus the scanning of several seed

specimens from replicate populations of certain taxa has been required. SEM itself tends to be a relatively time-consuming technique, in spite of the ease of preparation of specimens, and also expensive. Different makes of machine are capable of different types of performance under various conditions. However SEM is a technique that is difficult to apply to large-scale comparative surveys, which may explain the small samples of seeds that are usually studied, and hence the lack of published observations of infraspecific variation.

Because of the ease of making observations at very high magnifications, it is possible to make errors in the interpretation of features, as noted by Heywood (1985). It is also important to exclude electronic aberrations and artifacts from the characters recorded. Further, it is tempting to concentrate upon the areas on the specimen where the clearest expression of any feature can be observed. These may not always be the most representative or relevant regions, although they may produce the most aesthetically pleasing photographic records. It is often difficult to exclude these results of operator bias. Awareness of such hazards to the collection of accurate data hopefully minimises their occurrence.

#### 8.6.3.4. SEM of charred archaeobotanical seed remains.

Archaeobotanists have now been using SEM in the analysis

of ancient seed remains for well over ten years. This technique is becoming a standard procedure in many archaeological laboratories, where only a few years previously SEM equipment was rarely available on a routine basis. However the applications of SEM in the identification and interpretation of charred seed remains are limited by a number of practical considerations, which are described below, following a very brief outline of some examples of the early use of SEM on charred archaeobotanical remains.

Some of the earliest applications of the SEM in archaeobotany were in studies of ancient wood charcoal. The suitability of this technique for investigating the anatomy of such brittle, opaque material has been described by McGinnes et al., 1974; 1976). Conolly (1976) was one of the earliest workers to describe the good preservation that can remain of the micromorphology and anatomy of charred ancient seeds, observable by SEM. Observations can be made of charred ancient seed fragments without special preparation, because of their good electrical conductivity; and the high quality of resolution of SEM provides electron micrographs of a similar high quality (Feindt & Mesterharm, 1980).

Körber-Grohne & Piening (1980) published the results of some of the first applications of SEM in the identification of ancient crop remains. They scanned the epidermal cells on cereal caryopses, comparing fresh

specimens with the charred archaeobotanical remains. More attempts have been made to separate different species of wheat and rye using characters of the micromorphology of cells of the pericarp and testa, particularly those from caryopses from Near Eastern contexts that might provide evidence of early agriculture (Colledge, 1988; Körber-Grohne, 1981). Further examples of the archaeobotanical applications of SEM are provided by the work of Kislev & Hopf (1985) and Kislev (1986, 1989) on Lathyrus, described above (section 8.2.3.2.). SEM has also been used to examine the disseminules of other early crop species, such as the seeds of Chenopodium from archaeological sites in the New World (Smith, 1988), primarily to seek for evidence of their cultivation as reflected in changes in testa structure.

#### 8.6.3.5. Disadvantages of the use of SEM in archaeobotany.

The glueing of seeds, or other plant material, to stubs for SEM removes the specimens from the plant collections. When large collections of fresh material are involved this may not be a disadvantage, but when the reference collections are very small, and, above all, when archaeological assemblages provide the specimens, the irreversible removal of even small numbers has to be very carefully justified. It is not often possible that replicate material can be spared. Although SEM is not in itself a destructive technique, and the mounted specimens

can endure for a number of repeated scannings, the specimens are irreversibly altered when gold-coated in preparation for SEM. It is theoretically possible to remove such specimens from stubs when cellulose-based glues have been used, but, in practice, it has been found that charred seeds are too fragile to survive such procedures. It is even possible in theory to scan uncoated charred material, as has been advocated by Feindt & Mesterharm (1980); but usually electrical contact is insufficient for clear resolution at high magnifications unless some coating has been applied (Prior, 1988). Thus in effect SEM is a destructive technique, and carries the disadvantages that are associated with this property.

#### 8.6.3.6. Role of SEM in archaeobotany.

Restrictions on the application of SEM in archaeobotany can be deduced from the above paragraph, and relate to both the irreversible removal of specimens from the archaeological record, and the expense in terms of labour and the operation of relatively-sophisticated and previously sparsely-distributed equipment.

While SEM may now be freely available as a technique in archaeology, it cannot be perceived as a routine alternative to optical microscopy. It is a technique which allows charred opaque tissue to be observed at high magnifications, and it is therefore the method of choice

where such observations are essential.

SEM is becoming increasingly available to environmental archaeologists. At this relatively early stage, much reliance tends to be placed by archaeobotanists upon the published observations by SEM. The earliest literature used to assist in the identification of seed remains has mainly focussed on cereals (such as Körber-Grohne, 1981; Körber-Grohne & Piening, 1980). In cereals, as in the pulses described in this thesis, the recent work has revealed that cell patterns are not consistent in different populations of the same species, and that similarities may occur, both between the grains of wild and cultivated forms of the same species, and between grains of different genera. As has been remarked by Colledge (1988, p.236), with reference to cereals, "there needs to be a much more thorough investigation of a range of modern species". Such an investigation has now been undertaken in the pulses, and it has demonstrated that there are limitations in applications of surface micromorphology as diagnostic criteria for the identification of species, and for distinguishing wild and cultivated forms of the same biological species.

## 9. CONCLUSIONS.

### 9.1. Introduction.

The research for this project has isolated criteria that can be used to identify the seeds of certain species of the Viciaeae but no characters have been found that provide firm evidence of their cultivated or wild status. However, the recorded observations have yielded information concerning the properties of pulse seeds that are of value to botanists, both in archaeology and in other fields. These conclusions, together with final observations regarding the usefulness of SEM in the project, are set out in detail below.

#### 9.1.1. The aims of the project.

The project was designed to attempt to find characters of the micromorphology and anatomy of fresh modern seeds of the major pulse crops that can be used (i) to identify the seeds to the level of species if possible, or at least to genus, and (ii) to provide evidence of cultivation. It was essential to select characters that survive and can be recorded in charred ancient seed remains. A supplementary aim (iii) was the evaluation of SEM as the main technique employed.



9.1.2. The approach to the project.

9.1.2.1. The results of the initial survey of the testa surface.

From the published results of observations by SEM of testa micromorphology (Lersten, 1979; 1981; Lersten & Gunn, 1981; 1982a; 1982b; McEwen et al., 1974; Trivedi et al., 1978), initially it <sup>had</sup> been anticipated that the surface micromorphology of the testa of seeds of members of the Viciaeae would be sufficiently different in the different species for the testa characters to be used in identifying most of the taxa that have particular relevance in early Old World agricultural contexts, including the major pulse crops and related wild taxa.

However at an early stage of the project it became apparent that while the seeds of some taxa do appear to be morphologically distinctive, others are not. In the Viciaeae, in some taxa parallel variation is exhibited in the seed micromorphology, as it is in the gross morphology of seeds, and the other organs such as leaves; there is an overlap in micromorphology between the seeds of certain taxa. Closely-related species may be expected to show some similarities; however they are also found between some species of different genera. Evidence from published illustrations even seems to show close similarities in testa surface micromorphology between some members of different papilionate tribes. There is

the added complication that there may be wide variation in micromorphological detail within any one taxon.

#### 9.1.2.2. The survey of internal testa characters.

The conclusion, that characters of the testa surface can separate the seeds of a restricted number of species, has resulted in the collection of data on a broader basis. Characters from both the external and internal testa morphology, micromorphology and anatomy were recorded numerically. These were assembled into a dataset for multivariate analysis. To check the applicability of the characters recorded in fresh seeds to those in archaeobotanical seed remains, charred comparatives were prepared from modern seeds and observed, and charred ancient seeds were also examined. The observations on all these specimens were obtained by SEM.

The extent to which the aims of the project have been fulfilled is considered in the following section beginning with the identification of species, and continuing with the separation of cultigens. The evaluation of the SEM as a technique applied to the analysis of fresh and charred plant remains is finally considered.

#### 9.2. Identification of seeds of the Viciae.

Conclusions relating to SEM-observable criteria which can

be used in identifying seeds of species of the Viciaeae are as follows:

(i) The papillose testa patterning, typically found in the Viciaeae, occurs in all four major genera in the tribe. The most common type is found in some species of both Vicia and Lathyrus, which therefore cannot always be separated from surface characters. Non-papillose testas also occur, most commonly in Lathyrus. In both types of testa micromorphology, interspecific, intergeneric and even intertribal similarities exist, and frequently restrict seed identifications.

Nevertheless, some species can be identified by their testa patterning: these are mainly wild Lathyrus species with secondary patterning; but also included is Vicia faba. The "primitive", small-seeded varieties of this latter species can be readily distinguished by testa surface features from other members of section Faba, such as V.narbonensis, with which they have been confused in the past on the bases of their seed size and shape.

(ii) Intraspecific variations in testa patterning were commonly recorded, upon individual specimens, between seeds of the same population, and between populations of the same taxon.

This observation has prompted caution in the application of micromorphological criteria for identification.

These two conclusions are important in the light of certain publications which are used as laboratory manuals by archaeobotanists, and in which some statements could be misleading.

(iii) Charring may not change the size of the seeds, but it reduces much of the detail that might distinguish the micromorphology of fresh seeds. However, those characters that can be observed in charred fresh seeds are equally visible in well-preserved charred ancient seeds.

(iv) The multivariate analyses of testa characters have indicated that, together with certain features of the testa topography, characters expressing the width of the hilum, and the size of the palisade cells on the testa surface are useful in separating some taxa.

(v) There are particular advantages to the examination by SEM of the micromorphology of the remains of charred ancient pulses. The salient characters observed in this project occur mainly in the hilar region of the testa. This fact has important archaeobotanical implications; detached hila are sometimes recovered from archaeological deposits. It should be possible in some instances to identify the remains of ancient legume seeds from small fragments of hilum.

### 9.3. Separation of cultigens from wild species.

No new criteria have been observed in the testas of the Viciaeae which are correlated with the cultivated status of any of the taxa studied. The wild progenitor of Pisum sativum has secondary patterning and a thicker testa than the cultigen. These distinctions, which were already well-known prior to this study, and are visible at low magnifications by optical microscopy, have been observed here in greater detail than in earlier studies, and verified by SEM. Conversely, it has been found that, in other taxa, published generalisations comparing the micromorphological properties of the testa between wild species and cultigens have not been corroborated.

### 9.4. Role of SEM in the interpretation of legume seeds.

It has been described how SEM facilitates the observation and interpretation of testa micromorphology in charred ancient seeds. Yet, as has also been explained above (9.2), limitations have been found in these applications of SEM in the Viciaeae. These limitations concern the nature of the specimens, rather than the technique per se.

SEM has proved to be an ideal technique for making observations of fresh, charred and ancient charred seed material, using standardised procedures. However, the particular material in this survey has consisted of seeds

of the Viciaeae, and most of the taxa examined, have appeared not to portray features that can be used to distinguish the different taxa. While this conclusion can only have been reached following the use of SEM, it has also demonstrated a limitation for some potential future applications of SEM.

This seemingly negative outcome of the research has some positive advantages. At the present time, when the applications of SEM in archaeobotany are still being explored, the need may be felt to examine the well-preserved remains of ancient seeds by SEM "because it is there", and, being a comparatively newly-introduced technique in this field, is sometimes still regarded as a novelty. Laboratories without SEM facilities often make strenuous efforts to obtain access to them. The appreciation that SEM cannot provide the answers to some specific problems can reduce the unnecessary expenditure of labour and time on a routine basis.

#### 9.5. Suggested directions for future research.

The identification of seeds of the Viciaeae from ancient charred plant material remains problematical (see 9.2), as, indeed, it is in fresh modern seeds. However, the important resolution of the cultivated or wild status of the taxon of legume seeds perhaps can be ascertained by further research via an alternative route.

#### 9.5.1. Biochemical research.

The search for micromorphological characters as evidence of a changed physiology due to selection under cultivation has been unsuccessful (9.3). A potentially more fruitful avenue of exploration towards the same end could be a direct investigation of the biochemistry of the seed. This would involve seeking, primarily in modern legume seeds from wild, segetal and cultivated populations, and subsequently in the charred ancient seeds of putative cultigens, evidence of a systematic decrease in concentration of the tannins that impose seed dormancy. The possibility of the preservation of these polyphenolic compounds in the testa following charring is as yet uninvestigated. It is anticipated that this project will proceed in a second phase along this avenue.

#### 9.5.2. Ethnographic records of mixed cultivation.

A second direction of future research would be towards a more complete understanding of the role of mixed cultivation of pulses; this might help explain some of the properties found in seeds of legume cultigens.

The regions where pulses are commonly grown as components of mixed systems are particularly those in the north of the Indian subcontinent and in areas of Ethiopia. The political unrest prevailing in these regions limits the possibility of field research in the immediate future.

However, it is important that ethnographic records are collected before some of these traditional systems are totally extinguished. The feasibility of undertaking fieldwork in areas such as northern Pakistan, Nepal and the southern states of Russia currently is being explored.

Experimental mixed cropping with pulses has not yet been undertaken. The analysis of crop residues from such systems is highly overdue. It is hoped to design and undertake suitable growing trials in Europe, to provide, together with ethnographic observations, a basis for creating new models of ancient pulse cultivation.

#### 9.6. Concluding remarks.

The initial investigation of legume seed micromorphology by SEM has evolved into a far wider investigation. Many questions have been raised, in particular relating to early systems of pulse cultivation. It is suggested that the time has come to reassess the role of legumes in antiquity.

Investigations along the biochemical, ethnographic and agricultural pathways outlined above (9.5.) will provide a rewarding extension to the research undertaken during this project on the micromorphology of seeds of the Viciae.



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APPENDIX 1. Toxicity of the legumes, and food-processing strategies.

A1. Introduction.

Legumes contain a wide range of components that can have an adverse effect when consumed, ranging in extent from a relatively minor reduction in the nutritional value of the diet, to an acute and lethal toxic reaction. This toxicity is a major consideration to man in the use of this plant group both as animal feed and more directly as a resource for human food.

The following section contains a brief account of some of the toxic and antinutritional constituents found in pulses. Many of these compounds are usually associated with species in tribes other than the Viciaeae; however, some occur at low concentrations in members of the Viciaeae. Short notes are included on some of the medical conditions that are associated with their ingestion by man. Strategies that have been developed to overcome some of these potentially harmful compounds are described.

A1.1. An introduction to legume toxicity.

Much of legume biochemistry is sufficiently distinctive to form the basis for a system of classification of this family (Harborne et al., 1971; Gomes et al., 1981).

However, many classes of compound, although characteristically present in high concentrations in certain taxa, have also been found more widely at lesser concentrations in other taxa (Montgomery, 1969). Further, some species, and even specimens within populations of a species, may contain very different levels of a particular compound (Nowacki, 1980). Consequently, in considerations of the possible effects of plant toxins in the nutrition of past societies, it is necessary to bear in mind that toxicological data relating to one particular population of a species may not necessarily be simply applied to another.

Much of the research into the biochemistry of legumes has centred on compounds in wild rather than cultivated species. However many of these data are of relevance to those working on the early subsistence of man, both because of the relationship of the wild species with the cultigens, and, more directly, because many species now considered wild or, at best, as forage or fodder crops, are known to have once been resources for human nutrition, either as early cultivated plants, or as part of the dietary repertoire of a gathering economy.

Today it is common to regard seeds of members of the Viciaeae, with a few exceptions, as harmless to man. They are said generally to lack "deleterious or inconvenient constituents" (Smartt, 1978, p.191). However this view could be considered to be an oversimplification; often,

potentially harmful compounds are present in these pulses, but in concentrations sufficiently low not to cause pathological symptoms, as is shown below.

A decrease in the toxicity of seeds is one of the changes that is said to have resulted from the cultivation of legumes (Smartt & Hymowitz, 1985). It is probable that some of the early cultigens in the Viciaeae would have had seeds that contained higher concentrations of certain toxins than those of modern varieties.

#### A1.2. Antinutritional factors.

The seeds of legumes are rich in carbohydrates, protein and oil. They can provide minerals such as calcium and iron, and vitamins such as thiamin and nicotinic acid. They are well-known sources of certain essential amino acids, particularly lysine, that complement those that are in low concentrations in cereals (Aykroyd & Doughty, 1982, pp. 82, 108, 109). The reputation of the nutrient value of pulses tends to overshadow their lesser-known antinutritional properties. Some of the groups of compounds that are responsible for the deleterious effects of pulses are described below.

##### A1.2.1. Lectins (haemagglutinins).

Some proteins, the lectins, can agglutinate blood cells,

and impair nutrient absorption across the intestinal mucosa; they may cause acute gastroenteritis (Aykroyd & Doughty, 1982, p.38). They commonly occur in the seeds of species of Phaseolus and soybeans, but are also found in lower concentrations in lentils and peas, at levels considered to be non-toxic. Germinating seedlings also contain low levels. Lectins may be deactivated by heat, but may prove relatively resistant below 100C (Liener, 1986).

#### A1.2.2. Toxic amino acids.

A number of toxic non-protein amino acids are known to occur in legumes. Many are heat-stable and most remain to be fully investigated (Nowacki, 1986).

A particular group, the isoxazolinone derivatives, has been found only in members of the Viciaeae. These compounds are unstable, and can give rise to lathrotoxins in the plant; an example is B-aminopropionitrile (BAPN); this toxin is responsible for osteolathyrism, a condition of skeletal deformation, in animals that have ingested Lathyrus odoratus, but not reported in man. Levels of these toxins vary throughout the plant, and with the stage of maturity. For example, dry mature seeds contain low concentrations of BAPN; higher levels are found in immature seeds; particularly high levels occur in young germinating seedlings.

All pea and lentil species, most Lathyrus and some Vicia species contain several examples of isoxazolinone derivatives. Fortunately they can easily be removed; they are readily soluble in water, and tend to leach out of seeds during imbibition (Lambein et al., 1986).

### Lathyrism.

Perhaps the best-known effect of toxic amino acids in legume seeds is the condition of lathyrism, a progressive and irreversible spastic paralysis of the legs caused by eating certain species of Lathyrus, predominantly the crop plant L.sativus. The compound involved is beta-N oxalyl amino-L-alpha,beta-diamino propionic acid (ODAP). The pathological condition arises when more than a third of the diet consists of Lathyrus seeds. While this may seem to be a relatively unlikely eventuality, the poorest class of farm labourer traditionally has been paid in these pulses in certain regions. Sometimes in this section of the community the food is reduced to bread made from a dough of salted pulse flour and water (Kaul, pers.comm.).

In spite of the toxicity of Lathyrus sativus, it is a popular crop, having the desirable properties of drought tolerance and very low cultivation requirements. Today it is most commonly grown in the Indian subcontinent, being the largest source of grain legumes in Bangladesh (Kaul et al., 1986), occupying the largest area of any

crop in Nepal (Bharati,1986), and being the third most widely cultivated species in India (Lal et al.,1986).

Although the Indian subcontinent is undoubtedly the region most associated with the occurrence of lathyrism today, historical records illustrate the widespread distribution of that condition in the recent past. Outbreaks have been reported for example from Italy in the seventeenth century, and France and Algeria in the nineteenth century (Stockman,1917).

While Lathyrus sativus is the major cause of lathyrism, Lathyrus cicera and L.clymenum have also been incriminated for causing this condition. These species are much less commonly used as food resources for man, but in the past they have been important in nutrition in some areas, such as North Africa (Stockman,1917). ODAP is now known to occur more widely than previously thought, having been found in members of all three leguminous subfamilies. Recent surveys of Lathyrus have shown that high concentrations exist in 15 species, low concentrations in 6 species, while 20 species have undetectable amounts (Quereshi et al.,1977).

#### A1.2.3. Cyanogens.

The glucoside phaseolutin, or linamarin, is the constituent of the lima bean (Phaseolus lunatus L.) that is responsible for its well-known toxicity. In the



intact plant, this compound occurs harmlessly in cells in all organs. However, should damage cause cell rupture, the cell contents can come into contact with the extracellular glucosidase enzyme, with the consequent release of poisonous hydrogen cyanide.

Cyanogens are not normally considered to be associated with other species of grain legume. However, such innocuous pulses as the garden pea (Pisum sativum sativum sativum) and chickpea (Cicer arietinum) also contain low levels of these toxins. Similarly, some varieties of the common vetch (Vicia sativa), a species rarely used as a dietary component by man, and more commonly grown for animal feed, may contain significant amounts of the related glucoside vicianin (Liener, 1977; Montgomery, 1964; Nowacki, 1980).

Cyanogens are heat stable, and remain inactive at the pH of saliva and gastric juices, and in the presence of cellulose or glucose. The enzyme is thermolabile, being destroyed by heat. Cooking will not remove the cyanogens, but will deactivate the enzyme. Toxicity can arise on mixing cooked beans with other vegetables that contain active glucosidase enzymes, and thus release cyanide (Montgomery, 1969).

Acute poisoning usually leads to severe abdominal pain and vomiting, and even rarely to death. Prolonged exposure to low concentrations of cyanide has been

implicated in the aetiology of some goitres, following the conversion of the toxin to thiocyanate in the gut (Aykroyd & Doughty, 1982, p.37).

Strategies to avoid the toxic effects of cyanogens primarily centre upon the selection and cultivation of low-toxin varieties of pulses, and avoidance of the toxic strains. Sometimes these may be readily recognisable; seeds with black testas appear to contain the highest concentrations of the toxin (Aykroyd & Doughty, 1982, p.37). Recommended food-processing techniques include the washing of broken plant surfaces, discarding of the water following soaking and boiling, cooking in open containers to allow the easy escape of volatilising cyanide gas, and the consumption of cooked beans unmixed with fresh vegetables (Montgomery, 1969).

#### A1.2.4. Polyphenolic compounds.

Polyphenolics, such as the condensed tannins, inhibit the activity of enzymes such as amylase, trypsin and cellulase (Lawes, 1980). They reduce the amount of available nitrogen in the diet by binding with proteins (Bressani & Elias, 1980). They are also known to depress the growth rate by further mechanisms that remain to be investigated (Liener, 1980).

In seeds they are concentrated in the testa, being responsible for the black and brown pigmentation. Removal

of the testa, hulling, is the most usual method of reducing tannin levels.

#### A1.2.5. Goitrogens.

Metabolites of the phenolic compounds, such as tannin, may be found in varying concentrations in different populations of seeds of all members of the Viciaeae. They are particularly associated with the testa. They tend to be preferentially bound with iodine, limiting the availability of iodine for the production of thyroxine. This can result in goitres (Van Etten, 1969). Many food-processing strategies include the de-hulling of pulses, which removes most of the tannin.

#### A1.2.6. Saponins.

These compounds are believed to be present in all plants. In legumes they tend to be found in relatively high concentrations; most research has been undertaken on Glycine max. They are toxic to fish, since they lower surface tension, which damages the gills. Although in vitro experiments on rats have shown that saponins can haemolyse red cells, they are not deleterious to man in the concentrations encountered in vivo. They can have an important economic impact on farming; they can cause bloat in calves and inhibit the growth of farm animals, when fed on such legume forage crop species as Vicia sativa, alfalfa and clovers, due to their action on the

gut flora (Birk,1969).

#### A1.2.7. Protease inhibitors.

These compounds inhibit the proteolytic activity of certain enzymes, and thus reduce the availability of nutrients in the diet; if taken repeatedly in high concentrations in the diet, they can inhibit growth. They are contained in all parts of the plant, but may be concentrated in certain organs during development. Although they tend to be relatively stable to heat, acid and alkali, they can be destroyed by wet heat. Table 21 lists the members the Viciaeae known to contain protease inhibitors.

Table 21. Species of the Viciaeae  
Containing Protease Inhibitors.

<u>Species.</u>	<u>Compound Inhibited.</u>	<u>Organ (where known).</u>
<u>Vicia cracca</u>	trypsin chymotrypsin	
<u>Vicia faba</u>	papain trypsin chymotrypsin	embryo all parts
<u>Lens culinaris</u>	trypsin	seed
<u>Lathyrus latifolius</u>	trypsin chymotrypsin	
<u>Lathyrus odoratus</u>	trypsin chymotrypsin	
<u>Lathyrus sativus</u>	trypsin	
<u>Lathyrus sylvestris</u>	trypsin chymotrypsin	
<u>Pisum sativum</u>	papain trypsin chymotrypsin	embryo

The effect of increasing the digestibility of proteins in legumes that is produced by heating, is thought to be due

largely to the destruction of the protease inhibitors (Liener & Kakade, 1969; Weder, 1981).

#### A1.2.8. Favism.

Favism occurs as a result of the ingestion of the seeds, or the inhalation of the pollen from Vicia faba. It manifests itself by haemaglobinuria and jaundice, resulting from the haemolysis of red cells, which, however, is generally reversible after one or two days. It is due to a genetic defect on the X-chromosome in some individuals. This can produce a deficiency in glutathione (GSH) and reduced levels of glucose-6-phosphate dehydrogenase (G6PD) in the red cells, acting on the pyrimidine derivatives vicine and convicine. These are found in varying concentrations in different plants, which explains the varying responses of G6PD-reduced people to broad beans. Pisum sativum is known to have a similar effect on some individuals. The condition of favism is mainly associated with peri-Mediterranean populations. Avoidance of the pulse is the method of prevention (Mager et al., 1969).

Favism is believed to be the basis for the well-known taboo on broad beans that has been described and explained by a number of classical authors. Herodotus (Book II:37) reported that in Egypt the priests looked upon beans (Vicia faba) as unclean pulses. Pythagorus,

himself a sufferer from favism, recorded that this taboo was due to the belief that the beans were composed of the same matter as man; Diodorus ascribed it to self-denial on the part of the priests (cited by Darby et al., 1977, p.683).

#### A1.2.9. Phytates.

Most legumes, especially Pisum sativum, contain the anti-nutritional factors phytates. These form complexes with metal ions, particularly zinc, which can result in mineral deficiencies in the diet (Aykroyd & Doughty, 1982, p.40; Bender, 1987).

#### A1.2.10. Oligosaccharides.

These are the compounds that are chiefly responsible for the flatus-inducing properties of pulses, and are found in the ripe seeds of all food legumes. They include the compounds raffinose, stachyose and verbascose. These carbohydrates cannot be digested by direct enzymic action in the gut, but are metabolized by species of Clostridium bacteria in the lower intestine. These produce methane, carbon dioxide and hydrogen in the lower gut (Aykroyd & Doughty, 1982, p.38; Norton et al., 1985).

#### A1.2.11. Antivitamins.

Factors have been discovered in some pulses, such as

Pisum sativum, that interfere with the activity of vitamin E. This can limit the availability of zinc (Liener,1969).

#### A1.2.12. Oestrogenic substances.

Compounds, such as the isoflavone glucosides, commonly present in a number of legume species, are oestrogenic. They usually occur at low concentrations and thus are not normally considered harmful (Bender,1987). However they may cause problems when fed to breeding animals; Trifolium subterraneum can cause "clover disease", which causes lambing failure (Harborne,1988,p.124). Sometimes the oestrogenic properties are deliberately exploited; in Turkey, for example, Lathyrus sativus seeds are soaked for 24 hours, cracked and fed to cattle to increase the milk yield (Hillman pers.comm.).

#### A1.2.13. Alkaloids.

These have been described as possibly the most toxic group of biochemicals in the legumes; for example, they are the compounds associated with the highly poisonous properties of Laburnum seeds (Smartt & Hymowitz,1985). They are not normally associated with cultivated species, and indeed commonly are not mentioned in the literature concerning legume toxicity. However amongst the Viciaeae, Vicia ervilia, owes its common name, bitter vetch, to the alkaloids in the seeds. These toxins may be removed by

leaching.

#### A1.2.14. Summary.

There is some justification for the reputation of low-toxicity of majority of pulses in the tribe Viciaeae. However most species do contain low levels of toxic compounds. It is probable that, in the past, during the earlier stages of their cultivation, when they were less developed from their wild progenitors, the seeds of some pulses would have been more toxic to man than they are today.

#### A1.3. Food processing of toxic legume crops.

The toxins described above are largely secondary metabolites that accumulate in certain organs at different stages of maturity of the plant. The presence of toxins and antimetabolic factors in plants acts as a mechanism of defence against the predation of herbivores, an interesting account of which is given by Harborne (1988, pp.82-114), and it is an adaptation which appears to be particularly successful in legumes. Stahl (1984), in a study of hominid dietary selection before the use of fire, considers that generally the toxicity of legumes precluded their inclusion as major suppliers of protein in primate nutrition, especially in hominid species that lacked specialised detoxification mechanisms. However maintaining potentially toxic resources at a low level in



the diet may allow their consumption to be relatively harmless. As stated by Stahl(1984), a generalist feeding strategy would tend to minimise the amount consumed of any single toxin. Further, as has been described above, most legumes, including members of the Viciaeae, have populations containing a range of concentrations of the various toxins, some of which may signal their relative abundance or virtual absence by correlation with certain characters; thus the more toxic strains may sometimes be readily recognisable. The primary strategy for minimising the toxicity of food plants would be that of avoidance, by the selection of the strains with lowest toxin levels.

Little is known about the initial development of the different techniques of processing plant foods (Stahl,1989). It has been suggested that the use of heat for roasting probably began in the Upper Palaeolithic, around 20,000 BP. Boiling is only used by hunter-gatherers in Arctic zones, or after contact with cultivators or pastoralists. The evidence is said to indicate that cooking techniques are relatively recent innovations (Hayden,1981).

It is not generally recognised that the seeds of some members of the Viciaeae may have, or once have had, toxic properties, as can be deduced from some accounts of the food-processing techniques of early societies in western Asia (Hersh,1981,p.400). In their ethnobotanical survey of techniques employed by foragers and farmers around the

world for detoxification of plant foods, Johns & Kubo (1988,p.83) exclude beans, peas and lentils, described as "major exploited legumes" from their records, stating that these crops require no detoxification other than cooking. Similarly Hersh (1981), in her discussions on possible food-processing techniques in Neolithic Turkey and Greece, does not consider pulses to have had toxic properties. While this is certainly true in the most general terms and when considering many highly-evolved pulses commonly cultivated today in Europe, such a view may not necessarily be applicable to crops in some regions or to some species that are no longer part of the resource base for human food. Some stages in the processing for food of non-toxic pulses may retain evidence of a past need for detoxification.

Described below are some processes used in the preparation of the bean, pea, lentil and chickpea group of pulses, that are employed by societies practising traditional agriculture. Often a particular procedure may be adapted for different species of pulse or mixtures of grains, depending upon their local or seasonal availability.

#### A1.3.1. Dehulling.

The dehulling, dehusking or decortication of legume seeds to reduce pulses to the two cotyledons, known as dhal in India, is a common procedure. The seedcoats are often

tough and indigestible, and may also contain antinutritional substances, such as tannins (Liener,1980), that, apart from having antimetabolic properties, also have a bitter astringency. They can be found in high concentrations particularly in dark-coloured seeds. Such seeds may be particularly sought-after, since, apparently, regardless of the antidigestive properties, the taste of the dark varieties of seeds is preferred by some communities (Bressani & Elias,1980a). Removal of the seedcoat is the simplest method of reducing the concentration of tannins in pulses.

In India, where pulses are commonly consumed as dhal, the whole grains of each species may be submitted to one of two basic dehulling procedures, depending upon the geographical region (Kurien & Parpia,1968; Chakraverty & De,1981). In the north and central parts of the subcontinent, where the climate following the harvest is predictably sunny and hot, dry processing is usual. The legume seeds are spread in thin layers to dry in the sun, sometimes being mixed with linseed or other oil, in order to prevent excessive drying-out. After one or two days, they are sprinkled with water, heaped and allowed to stand overnight, before being milled in a mortar, or chakki, a grinder of stone or wood. The seedcoats crack and are removed, and the two cotyledons separate. For complete hulling it may be necessary to repeat the process a number of times. It has been estimated that a man can mill 30-40 kg. of pulses in eight hours.

The wet process, more common in southern India, involves the steeping of grains in water for a few hours, draining and then treatment with red earth. This latter process, where the pulses are coated with a paste of earth and water and allowed to stand overnight, is said to colour pale cotyledons a deep yellow, and also impart certain abrasive qualities which facilitate the seedcoat removal. Following this, the seeds are spread in the sun for two to four days and subsequently milled, as in the dry process, in chakkis. A man may mill 60-75 kg. of these pulses in eight hours, about twice the amount possible than after the dry method. Wet processing is less common, and the end-product apparently takes longer to become tender on cooking. However it is considered to have an improved taste. Some pulses with highly resistant seedcoats may also be treated with sodium hydroxide or sodium carbonate during the soaking period to further soften the seedcoat.

#### A1.3.2. Leaching.

Water-soluble toxins and antinutritional factors may be leached from legume seeds by soaking in water. The isoxazolinone compounds that can give rise to the lathyrogens such as BAPN and ODAP, described above, are readily water-soluble, and can be reduced in concentration in this way (Lambein et al., 1986).

The recommended treatment for detoxification of the seeds

of Lathyrus sativus is to employ two stages of leaching: (i) cold-water soaking overnight, followed where possible by (ii) steeping for several hours in hot water, prior to drying and grinding into flour, usually used to make chapattis. Boiling in water and discarding the liquor is a less-efficient alternative for removal of the lathyrigen (Mohan et al,1966; Anon,1967).

Following such treatments, the nutrient and cooking properties of the seeds may be altered, resulting in a loss of some vitamins, for instance vitamin B, and also in a reduction of the binding power essential for making bread (Padmanaban,1980).

The Neolithic site of Servia in west Macedonia possibly provides evidence for the detoxification of Lathyrus sativus in antiquity. During excavation, charred seeds of this species were found distributed over the floor in a pattern which indicated that they might have been stored in water in a jar; this has been interpreted as evidence of a possible leaching process (Hubbard,1979).

There are records of the cultural control of leaching techniques. In India, on Hindu feast days, pulses are soaked and ground into flour for a special dish. Grinding the pulses prior to soaking is not permitted (Katona-Apte,1975).

Bitter vetch, Vicia ervilia, well-known as an animal

feed, is less commonly thought of as human food. However this species is documented as a food resource. The Hebrew text, the Mishnah, for example, records the process of soaking these vetch seeds to remove the alkaloids and make them palatable (Danby, 1933, p.101). Pliny (cited by Southall, 1879) ascribes twenty virtues to bitter vetch as a medicine, including the cure of snakebite; he states that autumn-sown crops are injurious to animals and man, but spring-sown crops are harmless. Turner (1551, cited by Southall, 1879, p.481) describes the side-effects of the medicinal use of this pulse: "Bitter fische burdeneth the hede mych, the same troubleth the belly. It draweth out bloude by the water". Some toxic effects of untreated bitter vetch have been described by Southall (1879) when seeds imported from Turkey were fed to pigs with fatal results. Gerarde's notes in his Herballe of the seventeenth century, cited by Southall, state that "men do altogether abstain from the bitter vetch for it hath a very unpleasant taste and naughty juice; but kine in Asia and most other countries do eat thereof, being made sweet by steeping in water", which is an interesting reference to the detoxification of animal food.

The peas and beans that formed a large proportion of the diet in medieval Europe are known to have had different properties from the varieties familiar today. Historical records, exemplified by the sixteenth century writings of Platt (cited by Drummond & Wilbraham, 1939, p.51),

describe their processing : " Boile your beanes, pease, and beechmast etc. in fair water...and the second or third boyling, you shall finde greatest part of their ranknesse, then muste you drie them...and make bread thereof". At that time, these legumes were entirely used as dried pulses as flour for baking, or as thickenings in stews, or for such staples as pease pudding.

#### A1.3.3. Fermentation.

The employment of the natural agencies of microorganisms to alter the properties of foodstuffs is an ancient technique, and is practised throughout the world. The chemical changes induced by fermentation of pulses include the breaking down of some large carbohydrate and protein molecules, increasing the availability of free sugars and amino acids and reducing the levels of oligosaccharides, lectins and protease inhibitors, which enhances digestibility. Fermentation, commonly used in the preparation of species of Phaseolus and Vigna, is also sometimes a method applied to lentils, peas and chickpeas as is illustrated in the following examples:

Dhokla is a western Indian dish of rice and chickpeas, that are coarsely ground, made into a batter and fermented overnight. Large pancakes are steamed, and cut into diamonds and spiced for

eating (Ramakrishnan,1979).

Dhosa, a pancake eaten in southern India, consists of rice and lentils, soaked for four to six hours prior to grinding, diluting, flavouring with salt and fenugreek, followed by fermentation overnight.

60-80ml. batter are fried in a hot pan

(Campbell-Platt,1987).

Idli is a Gujarati dish made from chickpea with or without rice, which is soaked in water for five to ten hours, ground and mixed with water to a coarse paste, prior to further dilution and seasoning with salt. After an overnight fermentation, ladlefuls of batter are steamed in pans for 10-20 minutes

(Campbell-Platt,1987).

Katatakarna is made from ground and spiced peas to which water is added; following fermentation, it is fried in butter (Prakash,1961).

As stated above, fermentation is most associated with the species that are members of the Phaseolae, such as Phaseolus vulgaris, that are relatively toxic and were introduced from the New World post-Conquest. Yet this process is documented for the preparation of legume foods in India at least since the 8th.century AD (Ramakrishnan,1979), presumably originally being applied to Old World species of Phaseolae, such as Vigna species, and also probably species of the Viciaeae.



#### A1.2.4. Parching.

Dry-heating legume seeds destroys and thus removes the toxic effect of the heat-labile compounds. Chickpea, lentil, pea and broad bean are parched in many regions of India. A common procedure for parching involves the use of heated sand. The grains are sprinkled with water and seasoned with salt; they are then mixed with an equal volume of sand preheated in a metal pan to a temperature of 235-240C. The final temperature of the pulses rises from 26-29C to 132-136C. After 2-3 minutes, the seeds are sieved free of sand, and are ready to cook, for example by frying. Bhrstadhanya, a lentil dish, is one traditional Indian example prepared in this way (Prakash, 1961). Also, ata, a coarse flour ground from mixed parched grains, is used to make roti (breads) (Anderson et al., 1925). Chabena is a convenience food prepared from parched pulses, such as peas or chickpeas, in Northern India and traditionally used by persons going on a journey (Atkinson, 1980, p.693). Parched pulses are ground into flour, mixed with water, and eaten uncooked as sattu in parts of India (Anderson et al., 1925).

Chickpeas and peas may also be puffed by dry heat to be eaten as snacks with or without the addition of seasonings and spices in India, parts of North Africa and the Near East (Kurien et al., 1972).

The result of parching has been investigated by Acharya

et al. (1942), who were concerned that such treatment might reduce the food value of the pulses together with the toxins. Surprisingly, although in chickpea the biological value of the protein content is reduced, in broad bean and pea it appears to be increased.

#### A1.3.5. Baking and boiling.

Dry heat is applied to pulses in bread baking. Sometimes pulse meal may be added to cereal flour (Aykroyd & Doughty, 1982, p.68). In the Yemen, loaves are made of sorghum or millet, mixed with ground lentils, peas or beans, and baked in a tannur to form qafue (Borsten-Johanssen, 1975). Pulses are also commonly boiled and consumed in the form of soups, such as the lentil dish, mercimek corba in Turkey, or stews exemplified by the Egyptian bean stew (Tannous et al., 1979).

Heat deactivates enzyme inhibitors and denatures most toxic proteins. However it has been found that although lectins are generally rendered inactive by cooking, those in Vicia faba, for example, are relatively heat-resistant (Jaffe, 1980). Roasting has been found to be an alternative to leaching for the detoxification of Lathyrus sativus seeds; heating for 15-20 minutes at 140C has been found to reduce the toxin ODAP by 80-90% (Rao et al., 1969).

#### A1.3.6. Sprouting.

A number of grain legumes are commonly allowed to germinate, and are eaten as young seedlings. Most dry pulses contain very low levels of vitamin C, but this essential nutrient increases in concentration during germination. In Coonor, south India, for example, chickpeas may be soaked for twelve hours in water, spread on a moistened cloth in diffused sunlight at about 21-25C, and encouraged to sprout for two to four days prior to consumption. Pea and Lathyrus sativus are also eaten as sprouts (Bhagvat & Rao, 1942). In Nepal, the sprouts of mixed pulses are known as kwanti (Anon, 1976). In Egypt, broad bean sprouts are boiled for soup (Simpson, 1983).

Some toxins, for example the lectins, as described above, are reduced during germination. However some potentially poisonous compounds are known to increase in concentration during germination, as is described for some lathyrogens, which would restrict sprouting to particular species.

An example of the food prepared from germinated seeds is found in Egypt, where a spiced puree of Vicia faba sprouts, made into balls and fried, is known as taamiya; this concoction has given rise to the proverb: "who tastes of taamiya would sell his bonnet for it" (Darby et al., 1977, p.683).

A1.4. Food processing or detoxification?

The techniques commonly employed in the preparation of pulses incorporate many procedures that may be considered to be detoxification processes; yet they are applied to the seeds of cultigens that are relatively non-toxic. From this a number of interpretations can be made: (i) The techniques were originally devised for other, more toxic seeds from different species. (ii) The toxin-free seeds are those from varieties of a species that previously had toxic seeds. (iii) The techniques only coincidentally are detoxification processes. The rationale underlying many of the procedures is unknown, and, at this stage, most interpretations for their use would be speculative.

The development of food-processing appears to be a subject that has attracted little research. A study of this subject could augment an understanding of the selection of some of the early food plants.

APPENDIX 2. Evidence of physiological disorders and pathological conditions in seeds of the Viciaeae.

A2. Introduction.

Reference has been made (section 4.3.8. above) to possible physiological disorders and pathological conditions that might explain some of the features that have been observed in some of the seed specimens in this project. These seeds have micromorphological features that are obviously unstable in occurrence; they cannot be used for seed identification. However above the general botanical and agricultural interest that they hold, sometimes some of them also could be of significance in the greater understanding of seeds from archaeological contexts, and provide evidence that may increase the depth of interpretation.

A2.1. Cavities between the cotyledons: "hollow heart".

Cavities that sometimes are recorded in fresh seeds of all four genera in this project have been described above (paragraph 4.3.8.2.). The seeds of Pisum sativum can exhibit a condition known as "hollow heart", or "cavitation" (Heydecker & Kohistani, 1969). This presents as a hollowness of the seeds, due to concave depressions in the adaxial surface of the cotyledons. The cause has been suggested variously as water deficiency or excessive temperatures during germination, or a bacterial agent.

However current views hold that a predisposition to the condition may result from the premature removal of the seeds from the pod, prior to the dry-ripe stage (Perry & Harrison, 1973).

The seeds of Vicia sativa nigra, that were harvested at an immature stage (see 7.1.2.1. above), were bisected to check for the presence of "hollow heart"; no instances of the condition were recorded. Thus "hollow heart" is not an invariable result of early harvesting, and indeed may not occur in all species of the Viciaeae.

Nevertheless, it is suggested that the hollow seeds observed in this survey have "hollow heart", since they fit well the descriptions of this condition. If this is so, the presence of "hollow heart" in seeds of the Viciaeae may be associated with the gathering prior to full maturity of seeds of wild species or the early harvesting of relatively undeveloped cultigens in which a high degree of dehiscency is retained. As a consequence "hollow heart" can be added to the evidence that could be useful in the interpretation of ancient seed remains.

Evidence has indeed been found where these observations may apply. Some charred legume seeds recovered from two archaeological sites in Lower Austria appear to exhibit "hollow heart". Lentils both from the Bronze Age site of Stillfried, dating from 800 BC, and from the ninth century site of Gars-Thurau appear to have this seed

morphology (Schneider, pers. comm.). It has also been recorded in lentils from the Roman site of Hooper Street in London (de Moulins, pers. comm.).

#### A2.2. Testa pits.

Pits in the surface of the testa have been recorded in a number of genera and species in the Viciaeae. Lersten & Gunn (1982, p.18,19) observed testa pits in Lathyrus gorgoni, L.nissolia and L.numidicus. They have been recorded in this survey in six different species, and not in L.nissolia.

Such pits have been recorded in the testas of seeds from species representative of other tribes in the Papilionoideae; these include Cercis siliquastrum (Riggio Bevilacqua et al., 1984), Sophora secundiflora (Bridges & Bragg, 1983), Glycine max (Wolf et al., 1981; Yaklich et al., 1984), and Cajanus cajan and species that are members of five other tribes (Lersten, 1981); Riggio Bevilacqua et al. (1984) found that the pits in Cercis penetrate the full testa thickness, and that the endosperm beneath each pit projects as a cone; they postulated an association between the pits and imbibition. Wolf and his co-workers (1981) have stated that the significance or function of these pits is unknown, and that there is no evidence to support their role in imbibition, but they have postulated that they have a function during seed development or germination. Yaklich et al. (1984) consider

that the pits in Glycine, which penetrate to the cotyledon surface, might be concerned in nutrition.

There is no evidence that the testa pits recorded in all the Papilionate tribes have the same aetiology, possess the same morphology or perform the same function. However, generally it has been observed that the pits are of a similar irregular occurrence and distribution. The possibility that some of them might have a pathological aetiology has not been discussed. Lersten (1981) reports that the cuticle over the surface of the pit is unbroken, and that the cell surface in pits does not differ from the cell surface of the surrounding testa. Although this may appear to be evidence against invasion from external sources, it was decided to make some investigations into the possibility of a pathological aetiology of the pits.

Some pitted seeds of Lathyrus niger and L.pratensis were sent to Dr. Alan Cockbain of the Crop Protection Division at Rothamstead Experimental Station for an investigation into the aetiology of the pits. It was reported that it was unlikely that they had been caused by either bacterial or viral infections. Similar specimens sent to Professor H.F. van Emden in the Department of Agriculture and Horticulture, University of Reading, were reported as probably having become pitted as a result of the actions of sap-sucking insects, such as aphids, during the green immature stage of the seeds. The higher density of



distribution of the pits around the hilum would support this view. Although there appears to be no disruption to the cells on the testa surface, if the pits were the result of the actions of sap-sucking insects during the immature green stages of the seeds, by the time they have developed to a mature and dry state, any damaged cell and cuticle surfaces may have been regenerated. van Emden (pers. comm.) considers that the slightly altered state of the tissues surrounding the pits in some of the seeds of the two species of Lathyrus he investigated, is compatible with the effects of aphid saliva. It has been outside the scope of this project to investigate these pits further; however it is suggested that they have a pathological aetiology.

The importance of the presence of testa pits in the species observed in this survey mainly lies in the fact that it is not a stable character, and cannot be used for the identification of species.

### A2.3. Invasion of seeds by insects.

As described above (section 4.3.8.4.), evidence has been found in this survey of the actions of a second group of insect predators upon seeds of the Viciaeae.

The observation that the mature seeds when infested with insects have appeared to be normal, with no external sign of their infestation, supports the interpretation that

the seeds were invaded by the predators while in an immature state, rather than post-harvest, and that the insect died prior to completion of its life cycle. Such a predatory insect species is exemplified by the bruchid beetle.

Some Bruchis species lay their eggs on the immature pods of species of the Viciaeae. Different species of this beetle select only particular leguminous species, and are usually specific to them. The hatched bruchid larvae burrow into the developing ovules and feed on the cotyledons in the developing seed. The activities of B.affinis have been investigated on Lathyrus latifolius and L.sylvestris by Bashar et al. (1986), who recorded that the bruchids emerge from mature seeds as adult beetles and hibernate in the soil until the spring flowering of the host plants. The beetles may consume the pollen from flowers of a wider group of species, for example L.pratensis, prior to the onset of flowering in the preferred host species; ovogenesis is stimulated by chemical compounds within the flowers of the preferred hosts, thus perpetuating the cycle.

An infested seed bears no evidence externally of the beetle within until that organism burrows to the surface. However following the emergence of the predator, it might well be difficult to distinguish in the mature seed between any damage due to an infestation at the ovular stage from similar damage inflicted by different insects

such as weevils after harvest and during storage. The changed anatomy of the tracheoids, described above (section 4.3.8.4.), that seems to be associated with beetle infestation, could be one indication of the type of infestation.

While not being a factor that could be used in seed identification or that could be employed to interpret the cultivated or wild nature of the taxon involved, the observation of evidence in seeds of insect infestation could be of relevance in archaeobotany, particularly as an indication of possible storage conditions.

APPENDIX 3.

Table 15. The Complete Dataset.

The following table is comprised of numerical values recorded for the 106 OTU's (as listed in Table 4) for each of the 47 characters (as listed in Table 13). Column 1 lists the OTU's. The characters are numbered across Row 1.

Missing values are represented by \*.

<u>OTU</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>11</u>	<u>12</u>
1	1	4.0	14.1	1.9	1.1	1.4	1.0	2.5	1.0	0	1.0	1.0
2	0	4.0	35.7	2.9	0.6	0.7	3.0	3.0	1.0	0	1.0	0.0
3	1	2.0	9.1	1.0	0.5	2.2	1.0	3.5	1.0	0	1.0	0.0
4	1	2.0	6.5	1.0	0.5	2.2	2.0	2.0	1.0	0	1.0	0.0
5	1	1.5	7.9	0.9	0.5	1.9	2.0	3.0	1.0	0	1.0	1.0
6	1	3.0	8.9	1.0	0.5	2.3	3.0	2.0	1.0	0	1.0	0.0
7	1	3.0	8.7	1.0	0.5	2.3	1.0	3.0	1.0	0	1.0	0.0
8	1	3.0	9.1	0.9	0.5	1.8	2.0	2.0	1.0	0	1.0	0.0
9	1	3.0	9.1	1.0	0.5	2.3	1.0	3.0	1.0	0	1.0	0.0
10	1	2.0	16.8	4.7	1.3	3.5	2.0	1.0	0.5	0	0.0	0.0
11	1	1.5	16.3	5.8	1.5	5.0	2.0	1.0	0.5	0	0.0	0.0
12	1	4.0	12.1	3.3	0.9	3.5	2.0	2.0	0.5	0	0.0	0.0
13	1	5.0	15.6	3.0	0.8	2.5	2.0	2.0	0.0	0	0.0	0.0
14	1	4.0	22.4	3.5	0.9	*	3.0	3.0	0.0	0	1.0	0.0
15	1	5.0	13.7	1.8	0.4	0.9	2.0	1.5	1.0	0	1.0	0.0
16	1	4.5	11.8	2.0	1.0	2.3	1.0	1.0	1.0	0	1.0	0.0
17	1	4.5	11.2	1.8	0.4	*	2.0	2.0	1.0	0	1.0	0.0
18	0	3.0	42.4	4.4	0.5	1.8	4.0	2.0	1.0	0	1.0	0.0
19	1	5.0	15.6	2.0	0.5	1.0	2.0	1.0	1.0	0	1.0	1.0
20	1	3.0	9.1	1.5	0.7	1.5	2.0	1.0	1.0	0	1.0	0.0
21	1	2.0	7.9	1.2	0.6	1.3	2.0	1.0	1.0	0	1.0	1.0
22	1	3.0	18.7	1.6	0.4	0.8	2.0	1.0	1.0	0	1.0	0.0
23	1	3.0	18.1	1.6	0.4	0.9	2.0	3.0	1.0	0	1.0	0.0
24	1	5.0	16.7	2.2	0.4	0.9	3.0	2.0	1.0	0	1.0	1.0
25	1	1.0	16.2	1.9	0.4	0.9	2.0	1.0	1.0	0	1.0	1.0
26	1	3.0	16.1	2.5	0.5	0.8	2.0	1.0	1.0	0	1.0	0.0
27	1	1.5	13.7	2.0	0.4	1.0	2.0	1.0	1.0	0	1.0	0.0
28	0	3.0	61.7	*	0.3	*	3.0	2.0	1.0	0	1.0	0.0
29	0	3.0	61.8	*	0.7	*	5.0	4.0	1.0	0	1.0	0.0
30	0	2.0	56.6	*	0.4	*	5.0	*	1.0	0	1.0	0.0
31	0	2.5	32.0	*	0.5	0.7	4.0	2.0	1.0	0	1.0	0.0
32	1	4.0	19.0	1.1	0.3	0.5	2.0	2.5	1.0	0	1.0	0.0
33	1	4.5	19.7	1.3	0.4	0.5	2.0	1.0	1.0	0	1.0	0.0
34	1	4.0	12.1	1.6	0.6	1.4	1.5	2.0	1.0	0	1.0	0.0
35	1	4.0	14.0	1.6	0.6	1.0	1.5	2.0	1.0	0	1.0	0.0
36	1	3.0	9.0	2.3	0.9	0.8	2.0	2.0	1.0	0	*	0.0
37	1	3.5	11.7	1.0	0.5	0.3	1.0	2.0	0.0	0	1.0	0.0
38	1	5.0	84.0	1.0	0.5	0.3	1.0	2.0	0.0	0	1.0	0.0
39	1	3.0	8.9	1.6	0.8	1.1	1.5	1.5	1.0	0	1.0	1.0
40	1	2.0	10.0	1.4	0.6	1.4	1.0	*	1.0	0	1.0	0.0
41	1	2.0	7.3	1.2	0.7	1.5	1.0	1.0	1.0	0	1.0	0.0
42	1	3.0	18.8	4.0	0.5	0.6	3.0	1.0	1.0	0	1.0	0.0
43	1	4.0	14.3	2.4	0.6	0.4	3.0	1.0	1.0	0	1.0	0.0
44	0	5.0	34.0	4.7	0.8	1.3	4.0	4.0	1.0	0	1.0	0.0
45	1	3.0	17.6	1.8	0.9	0.6	1.0	1.5	1.0	0	1.0	0.0
46	1	2.0	5.2	0.4	*	0.9	1.0	1.5	0.0	1	0.0	0.0
47	0	5.0	15.5	1.6	0.5	3.5	2.0	2.0	0.0	0	1.0	0.0
48	0	3.0	24.3	2.3	0.6	0.6	2.0	1.5	0.0	0	1.0	0.0
49	0	4.0	25.6	2.8	0.5	0.9	3.0	2.0	0.0	0	1.0	0.0
50	1	4.0	11.9	1.1	0.5	0.3	1.5	*	0.0	0	1.0	0.0
51	1	5.0	9.1	0.9	0.4	0.3	1.0	1.0	0.0	0	1.0	0.0
52	1	3.0	11.9	1.0	0.5	0.4	1.0	2.0	0.0	0	1.0	0.0
53	1	2.0	16.8	2.3	0.6	1.2	2.5	1.0	1.0	1	0.0	0.0

<u>OTU</u>	<u>13</u>	<u>14</u>	<u>15</u>	<u>16</u>	<u>17</u>	<u>18</u>	<u>19</u>	<u>20</u>	<u>21</u>	<u>22</u>	<u>23</u>	<u>24</u>
1	1.0	11.0	15.0	1.7	720.0	1640.0	48.0	0.0	0.0	320.0	200.0	6.5
2	0.0	12.0	11.0	1.2	290.0	920.0	40.0	0.5	1.0	180.0	100.0	3.0
3	0.0	12.0	12.0	1.0	355.0	1120.0	34.0	0.0	0.0	185.0	100.0	4.5
4	0.0	26.0	17.0	1.0	444.0	1180.0	36.0	0.0	0.0	160.0	110.0	5.0
5	0.0	24.0	19.0	0.8	440.0	1400.0	36.0	0.0	1.0	184.0	96.0	6.0
6	0.0	13.0	15.0	1.0	420.0	1260.0	34.0	0.0	0.0	178.0	113.0	7.0
7	0.0	18.0	11.0	1.0	420.0	1090.0	37.0	0.0	0.5	195.0	105.0	6.0
8	0.0	18.0	16.0	1.0	240.0	900.0	43.0	0.5	0.0	140.0	95.0	5.0
9	0.0	12.0	10.0	1.0	350.0	1100.0	41.0	0.0	0.5	115.0	85.0	6.0
10	0.0	5.0	3.0	1.4	850.0	2800.0	44.0	0.0	1.0	809.0	547.0	8.5
11	0.0	2.0	2.2	2.0	900.0	3550.0	44.0	0.0	1.0	540.0	450.0	8.5
12	0.0	4.5	2.3	1.3	720.0	1840.0	70.0	1.0	0.0	500.0	300.0	7.0
13	0.0	3.0	3.0	*	502.0	1640.0	47.0	0.0	0.0	350.0	290.0	7.0
14	0.0	24.0	24.0	1.1	480.0	1500.0	32.0	0.0	0.0	210.0	170.0	6.0
15	0.0	24.0	24.0	1.3	230.0	720.0	45.0	0.5	0.0	165.0	100.0	3.0
16	0.0	6.0	5.0	1.2	740.0	1480.0	64.0	0.0	0.0	500.0	320.0	5.0
17	0.0	16.0	10.0	1.0	368.0	1074.0	39.0	0.5	0.0	188.0	113.0	3.0
18	0.0	15.0	12.0	1.2	306.0	1035.0	32.0	0.0	0.0	169.0	65.0	5.0
19	0.0	22.0	39.0	1.1	330.0	880.0	50.0	1.0	0.0	180.0	100.0	4.0
20	0.0	24.0	20.0	1.4	365.0	976.0	40.0	0.0	0.0	259.0	177.0	6.0
21	0.0	14.5	22.0	1.1	380.0	1000.0	40.0	0.0	1.0	250.0	200.0	6.5
22	0.0	19.0	18.0	1.0	180.0	550.0	44.0	1.0	0.0	90.0	50.0	3.0
23	0.0	18.0	21.0	1.0	250.0	760.0	45.0	1.0	0.0	76.0	32.0	3.0
24	0.0	23.0	27.0	1.0	315.0	820.0	45.0	1.0	0.0	260.0	115.0	5.0
25	0.0	27.0	27.0	1.3	222.0	585.0	53.0	1.0	0.0	215.0	107.0	4.0
26	0.0	18.0	16.0	1.2	350.0	940.0	45.0	1.0	0.0	280.0	160.0	4.0
27	0.0	36.0	31.0	2.0	390.0	720.0	58.0	1.0	1.0	250.0	110.0	3.5
28	0.0	10.0	10.0	1.1	278.0	1008.0	48.0	1.0	0.0	140.0	88.0	2.5
29	0.0	13.0	12.0	1.0	200.0	870.0	36.0	0.0	0.0	93.0	53.0	3.0
30	0.0	11.0	11.0	1.2	470.0	860.0	54.0	1.0	0.0	180.0	95.0	3.0
31	0.0	13.0	12.0	1.1	254.0	795.0	34.0	0.0	0.0	147.0	76.0	*
32	1.0	18.0	15.0	1.1	190.0	480.0	45.0	1.0	0.0	115.0	45.0	3.0
33	1.0	18.0	14.0	1.0	158.0	478.0	39.0	0.5	0.5	93.0	40.0	4.0
34	0.0	20.0	16.0	1.0	310.0	1105.0	34.0	0.0	0.0	270.0	180.0	7.0
35	0.0	22.0	19.0	1.0	370.0	1000.0	41.0	0.0	0.0	235.0	155.0	5.5
36	1.0	*	*	1.1	700.0	2120.0	38.0	0.0	1.0	400.0	220.0	3.5
37	0.0	13.3	*	1.5	194.0	564.0	32.0	0.0	0.0	128.0	70.0	3.5
38	0.0	9.0	*	1.3	370.0	870.0	47.0	0.0	0.0	230.0	130.0	7.5
39	0.0	13.0	13.5	1.5	610.0	1740.0	35.0	0.0	0.5	245.0	230.0	8.5
40	0.0	17.0	*	1.2	770.0	1760.0	37.0	0.0	1.0	315.0	193.0	6.0
41	0.0	12.0	10.0	1.3	450.0	1650.0	33.0	0.5	0.0	360.0	285.0	8.0
42	0.0	4.0	4.0	1.2	360.0	1100.0	53.0	1.0	1.0	240.0	120.0	6.0
43	0.0	12.0	11.0	1.0	330.0	1250.0	36.0	0.5	0.0	235.0	130.0	6.0
44	1.0	4.5	4.5	1.0	560.0	1420.0	42.0	1.0	1.0	220.0	125.0	3.0
45	1.0	4.3	*	1.0	580.0	1660.0	33.0	0.0	0.0	355.0	218.0	6.0
46	0.0	16.0	11.0	1.3	260.0	520.0	48.0	0.5	1.0	135.0	85.0	4.5
47	0.0	20.0	18.0	1.2	245.0	729.0	39.0	1.0	0.0	135.0	84.0	3.0
48	0.0	15.0	8.5	1.1	320.0	935.0	40.0	1.0	0.5	265.0	100.0	3.5
49	0.0	17.5	20.5	1.1	310.0	920.0	34.0	1.0	1.0	180.0	95.0	3.5
50	1.0	14.0	15.3	1.0	215.0	670.0	34.0	0.0	1.0	150.0	105.0	5.0
51	1.0	*	*	1.1	250.0	630.0	43.0	0.0	1.0	189.0	125.0	4.0
52	1.0	*	*	1.1	220.0	800.0	36.0	0.0	1.0	180.0	113.0	5.0
53	1.0	7.0	10.5	1.0	600.0	1400.0	54.0	1.0	0.0	320.0	137.0	2.5

<u>OTU</u>	<u>25</u>	<u>26</u>	<u>27</u>	<u>28</u>	<u>29</u>	<u>30</u>	<u>31</u>	<u>32</u>	<u>33</u>	<u>34</u>	<u>35</u>	<u>36</u>	
1	1	1	1	1	1	1	1	0	1	1	1	0	1.0
2	0	0	0	0	0	1	1	0	1	1	0	0	1.0
3	1	0	0	0	0	1	1	1	0	1	1	1	3.0
4	1	0	0	1	1	1	1	1	1	1	1	1	2.0
5	1	0	0	1	1	1	1	0	1	1	1	0	1.0
6	1	0	0	1	1	1	1	0	0	1	1	0	1.0
7	1	0	0	0	1	1	1	0	1	1	0	0	3.0
8	0	0	0	1	1	1	1	1	1	1	1	1	3.0
9	0	0	0	1	1	1	1	1	1	1	1	1	2.0
10	1	1	1	1	1	1	1	1	1	1	1	1	2.0
11	1	1	1	1	1	1	0	1	1	1	0	0	0.0
12	1	1	1	1	1	1	1	1	1	1	1	1	2.0
13	1	1	1	1	1	1	1	0	1	1	1	0	2.0
14	1	0	0	1	1	1	1	0	1	1	0	0	2.0
15	1	1	1	1	1	1	1	1	1	1	1	1	1.0
16	1	1	1	1	1	1	1	1	1	1	1	1	1.0
17	1	1	1	1	1	1	1	1	1	1	1	1	3.0
18	1	1	1	1	1	1	1	0	1	1	1	0	2.0
19	0	0	0	1	1	1	1	1	1	1	1	1	2.0
20	1	1	1	1	1	1	1	1	1	1	1	1	2.0
21	1	1	1	1	1	1	0	1	1	1	0	0	2.0
22	0	0	0	1	1	1	1	0	1	1	1	*	*
23	0	0	0	0	1	1	1	0	0	1	1	0	3.0
24	1	1	1	1	1	1	1	1	1	1	1	1	2.0
25	1	1	1	1	1	1	1	0	1	1	0	0	2.0
26	0	0	0	1	1	1	1	1	1	1	1	1	2.0
27	0	0	0	1	1	1	1	1	1	1	1	1	3.0
28	0	0	0	1	1	1	1	0	1	1	1	0	0.0
29	0	0	0	1	1	1	1	1	1	1	1	1	1.0
30	0	0	0	1	1	1	1	0	1	1	1	0	0.0
31	1	0	0	1	1	1	1	1	1	1	1	1	2.0
32	0	0	0	0	1	1	1	0	0	1	1	0	2.0
33	0	0	0	1	1	1	0	0	1	1	0	0	0.0
34	1	1	1	1	1	1	1	1	1	1	1	0	1.0
35	0	0	1	1	1	1	1	1	1	1	1	1	3.0
36	0	0	0	1	1	1	1	0	1	1	1	0	3.0
37	1	0	1	1	1	1	1	0	1	1	1	0	1.0
38	1	0	0	1	1	1	1	0	1	1	1	0	1.5
39	1	1	1	1	1	1	1	0	1	1	1	0	2.0
40	1	0	1	0	1	1	1	1	0	1	1	1	1.5
41	1	1	1	1	1	1	1	0	1	1	1	0	0.0
42	1	1	1	0	1	1	1	0	0	1	1	0	*
43	1	1	1	1	1	1	1	1	1	1	1	1	3.0
44	1	1	1	1	1	1	1	1	1	1	1	1	2.0
45	1	1	1	1	1	1	1	1	1	1	1	1	2.0
46	0	0	0	0	1	1	1	0	1	1	1	0	2.0
47	0	0	0	1	0	1	1	0	1	1	1	0	1.0
48	0	0	1	0	1	1	1	0	1	1	1	0	1.0
49	0	0	0	1	1	1	1	0	1	1	1	0	2.0
50	0	0	0	1	1	1	1	0	1	1	1	0	0.0
51	0	0	0	1	1	1	1	0	1	1	1	0	1.0
52	0	0	0	1	1	1	1	1	1	1	1	1	2.0
53	1	0	0	1	1	1	1	0	1	1	1	0	0.0

<u>OTU</u>	<u>37</u>	<u>38</u>	<u>39</u>	<u>41</u>	<u>41</u>	<u>42</u>	<u>43</u>	<u>44</u>	<u>45</u>	<u>46</u>	<u>47</u>
1	3.0	1.0	260.0	165.0	40.0	133.0	103.0	13.0	155.0	132.0	30.0
2	1.0	3.0	110.0	53.0	11.0	70.0	58.0	13.0	91.0	60.0	14.0
3	3.0	1.0	100.0	20.0	10.0	53.0	35.0	8.0	45.0	35.0	5.0
4	1.5	1.5	90.0	50.0	15.0	38.0	24.0	9.0	32.0	20.0	6.0
5	3.0	0.0	105.0	51.0	20.0	49.0	36.0	8.0	*	*	*
6	3.0	0.0	70.0	40.0	24.0	45.0	30.0	10.0	49.0	30.0	15.0
7	1.5	1.0	59.0	32.0	11.0	40.0	28.0	8.0	47.0	24.0	15.0
8	2.0	1.0	100.0	65.0	19.0	46.0	33.0	10.0	44.0	40.0	5.0
9	1.0	3.0	65.0	43.0	10.0	50.0	30.0	10.0	70.0	48.0	10.0
10	1.0	1.0	440.0	240.0	80.0	180.0	135.0	20.0	*	*	*
11	0.0	3.0	257.0	189.0	48.0	195.0	120.0	23.0	*	*	*
12	1.0	0.0	320.0	180.0	100.0	160.0	115.0	30.0	*	*	*
13	2.0	2.0	260.0	170.0	40.0	160.0	113.0	8.0	200.0	130.0	40.0
14	2.0	0.0	200.0	136.0	45.0	175.0	147.0	23.0	145.0	115.0	15.0
15	3.0	1.0	104.0	55.0	10.0	55.0	43.0	6.0	*	*	*
16	3.0	1.0	210.0	145.0	60.0	170.0	125.0	20.0	*	*	*
17	1.0	0.0	189.0	86.0	23.0	112.0	69.0	19.0	120.0	66.0	26.0
18	1.0	3.0	130.0	73.0	15.0	82.0	54.0	14.0	85.0	52.0	5.0
19	3.0	1.0	100.0	55.0	20.0	60.0	48.0	12.0	56.0	44.0	7.0
20	2.0	1.0	177.0	82.0	41.0	111.0	70.0	10.0	108.0	74.0	36.0
21	2.0	2.0	143.0	90.0	23.0	88.0	70.0	8.0	84.0	69.0	3.0
22	0.0	1.0	70.0	33.0	5.0	62.0	36.0	6.0	53.0	39.0	4.0
23	1.0	1.0	85.0	60.0	10.0	69.0	57.0	4.0	56.0	49.0	6.0
24	2.0	2.0	106.0	50.0	16.0	61.0	43.0	5.0	51.0	42.0	8.0
25	2.0	1.0	69.0	41.0	9.0	38.0	33.0	7.0	42.0	33.0	4.0
26	2.0	3.0	123.0	73.0	20.0	80.0	54.0	16.0	83.0	64.0	9.0
27	1.0	0.0	123.0	58.0	20.0	67.0	36.0	10.0	89.0	53.0	17.0
28	3.0	1.0	108.0	84.0	27.0	60.0	36.0	2.0	93.0	50.0	12.0
29	2.0	1.0	130.0	60.0	10.0	80.0	56.0	9.0	145.0	64.0	53.0
30	2.0	3.0	120.0	70.0	20.0	70.0	50.0	11.0	130.0	70.0	40.0
31	1.0	3.0	87.0	62.0	10.0	75.0	57.0	11.0	104.0	66.0	17.0
32	3.0	1.0	68.0	49.0	2.0	53.0	49.0	5.0	46.0	37.0	6.0
33	3.0	0.0	68.0	43.0	4.0	49.0	41.0	4.0	49.0	34.0	6.0
34	1.0	3.0	112.0	72.0	12.0	100.0	70.0	18.0	91.0	71.0	11.0
35	2.0	0.0	165.0	95.0	20.0	103.0	80.0	10.0	125.0	80.0	25.0
36	0.0	0.0	260.0	210.0	40.0	*	*	*	*	*	*
37	3.0	1.0	100.0	50.0	30.0	70.0	55.0	9.0	70.0	45.0	8.0
38	1.5	1.5	135.0	60.0	11.0	80.0	55.0	6.0	63.0	49.0	4.0
39	2.0	1.0	185.0	95.0	35.0	113.0	95.0	5.0	135.0	55.0	15.0
40	1.5	1.5	150.0	95.0	15.0	104.0	70.0	15.0	108.0	70.0	20.0
41	1.0	3.0	125.0	55.0	24.0	74.0	55.0	10.0	70.0	58.0	8.0
42	2.0	2.0	155.0	85.0	23.0	108.0	80.0	15.0	105.0	75.0	10.0
43	1.0	1.0	195.0	110.0	40.0	115.0	91.0	23.0	140.0	111.0	14.0
44	2.0	1.0	200.0	105.0	37.0	113.0	85.0	15.0	125.0	90.0	15.0
45	2.0	1.0	140.0	95.0	35.0	96.0	80.0	10.0	*	*	*
46	2.0	0.0	85.0	50.0	20.0	55.0	42.0	5.0	49.0	39.0	0.0
47	2.0	0.0	132.0	66.0	15.0	66.0	50.0	8.0	66.0	55.0	9.0
48	1.0	3.0	95.0	68.0	15.0	88.0	59.0	13.0	103.0	75.0	20.0
49	1.0	3.0	110.0	63.0	20.0	60.0	35.0	13.0	61.0	48.0	6.0
50	2.0	2.0	110.0	60.0	25.0	68.0	43.0	5.0	56.0	35.0	11.0
51	2.0	2.0	110.0	58.0	25.0	53.0	45.0	5.0	56.0	33.0	15.0
52	2.0	2.0	105.0	53.0	25.0	50.0	38.0	5.0	68.0	43.0	15.0
53	2.0	1.0	149.0	84.0	20.0	127.0	83.0	16.0	*	*	*



<u>OTU</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>11</u>	<u>12</u>
54	1	1.5	16.5	2.6	0.5	1.0	2.0	1.0	1.0	1	0.0	0.0
55	0	4.0	17.8	1.8	0.5	0.7	2.0	2.0	1.0	0	0.0	0.0
56	0	3.0	12.1	1.3	0.4	0.8	2.0	1.0	0.0	0	0.0	0.0
57	0	2.5	13.8	2.3	0.5	0.6	2.0	1.0	0.0	0	1.0	0.0
58	0	3.0	17.2	1.8	0.5	0.4	2.0	2.0	0.0	0	0.0	0.0
59	1	1.0	5.2	2.8	0.7	1.9	2.0	1.0	1.0	0	1.0	1.0
60	1	2.0	3.7	1.3	0.5	1.2	2.0	1.0	1.0	0	1.0	0.0
61	1	2.0	6.0	1.5	0.7	1.5	1.0	1.0	1.0	0	1.0	0.0
62	1	2.0	6.8	1.2	0.7	1.6	2.0	1.0	1.0	0	1.0	1.0
63	0	4.0	36.2	3.0	0.7	1.3	4.0	4.0	1.0	0	1.0	0.0
64	0	3.5	36.0	3.1	0.7	1.7	4.0	4.0	1.0	0	1.0	0.0
65	0	3.0	37.7	3.2	0.6	0.8	4.0	4.0	1.0	0	1.0	0.0
66	0	3.0	34.5	*	0.4	1.0	4.0	4.0	1.0	0	1.0	0.0
67	0	3.0	16.2	2.0	0.9	0.8	2.0	1.0	1.0	0	1.0	0.0
68	1	4.0	8.8	1.4	1.0	2.0	1.0	1.0	1.0	0	1.0	0.0
69	1	3.5	8.4	1.0	0.6	1.4	1.0	1.0	1.0	0	1.0	0.0
70	1	4.0	7.6	1.4	0.2	1.9	1.0	1.0	1.0	0	1.0	0.0
71	1	4.0	8.2	1.2	0.7	2.0	1.0	1.0	1.0	0	1.0	0.0
72	1	4.0	7.9	1.8	1.0	2.6	1.0	1.0	1.0	0	1.0	0.0
73	1	3.5	7.2	1.6	0.9	2.5	1.5	1.0	1.0	0	1.0	1.0
74	1	4.0	6.9	1.7	1.0	2.4	1.0	1.0	1.0	0	1.0	1.0
75	1	1.0	9.0	2.4	1.0	3.4	1.0	1.0	1.0	0	1.0	1.0
76	1	1.0	11.0	2.8	1.1	2.7	1.0	1.0	1.0	0	1.0	1.0
77	1	3.5	7.6	1.6	0.9	2.5	1.0	1.0	1.0	0	1.0	1.0
78	1	3.5	8.2	1.3	0.3	1.0	2.0	4.0	1.0	0	1.0	1.0
79	1	2.0	6.8	1.7	0.3	1.1	2.0	*	1.0	0	1.0	1.0
80	1	1.0	8.4	1.8	0.3	1.0	2.0	2.0	1.0	0	1.0	1.0
81	1	4.0	6.9	1.0	0.3	0.9	2.0	2.0	1.0	0	1.0	1.0
82	1	2.0	6.5	1.3	0.3	1.0	2.0	2.0	1.0	0	1.0	1.0
83	1	3.0	8.0	1.1	0.3	0.8	2.0	2.0	1.0	0	1.0	1.0
84	1	2.5	8.1	1.0	0.3	0.9	2.0	3.0	1.0	0	1.0	1.0
85	1	3.0	14.8	1.2	0.2	0.8	2.0	4.0	1.0	0	1.0	1.0
86	1	3.0	12.5	0.9	0.3	0.6	2.0	4.0	1.0	0	1.0	1.0
87	1	3.0	14.3	1.1	0.2	0.8	2.0	4.0	1.0	0	1.0	1.0
88	1	3.0	13.8	1.1	0.2	0.9	2.0	4.0	1.0	0	1.0	1.0
89	1	3.0	12.7	1.1	0.2	0.7	2.0	4.0	1.0	0	1.0	1.0
90	1	3.5	7.7	0.8	0.3	0.7	2.0	4.0	1.0	0	1.0	1.0
91	1	3.0	10.1	1.0	0.2	0.9	2.0	4.0	1.0	0	1.0	1.0
92	1	3.5	9.8	0.8	0.3	0.7	2.0	4.0	1.0	0	1.0	1.0
93	1	3.0	8.9	0.8	0.2	0.8	2.0	4.0	1.0	0	1.0	1.0
94	1	4.0	8.9	0.8	0.3	0.8	2.0	4.0	1.0	0	1.0	1.0
95	1	5.0	7.8	1.0	0.2	0.8	2.0	2.0	1.0	0	1.0	1.0
96	1	4.0	7.8	0.8	0.3	0.6	2.0	4.0	1.0	0	1.0	1.0
97	1	4.0	7.4	0.8	0.3	0.7	2.0	4.0	1.0	0	1.0	1.0
98	1	4.0	7.4	0.7	0.3	0.6	2.0	4.0	1.0	0	1.0	1.0
99	1	4.0	7.8	0.7	0.3	0.6	2.0	2.0	1.0	0	1.0	1.0
100	1	3.5	6.7	0.7	0.2	0.6	2.0	3.0	1.0	0	1.0	0.0
101	1	3.0	9.5	0.9	0.2	0.7	2.0	4.0	1.0	0	1.0	1.0
102	1	3.0	7.9	0.9	0.2	0.8	2.0	4.0	1.0	0	1.0	1.0
103	1	3.5	6.7	0.8	0.2	0.7	2.0	4.0	1.0	0	1.0	1.0
104	1	3.5	7.1	0.7	0.2	0.7	2.0	4.0	1.0	0	1.0	1.0
105	1	3.0	7.6	0.7	0.2	0.7	2.0	3.0	1.0	0	1.0	0.0
106	1	2.5	6.9	0.7	0.2	0.6	2.0	4.0	1.0	0	1.0	0.0

<u>OTU</u>	<u>13</u>	<u>14</u>	<u>15</u>	<u>16</u>	<u>17</u>	<u>18</u>	<u>19</u>	<u>20</u>	<u>21</u>	<u>22</u>	<u>23</u>	<u>24</u>
54	1.0	10.4	10.2	1.0	380.0	840.0	62.0	1.0	0.0	185.0	100.0	4.0
55	0.0	8.0	5.3	1.3	258.0	667.0	35.0	0.0	0.5	164.0	78.0	4.0
56	0.0	*	12.0	1.4	350.0	840.0	50.0	0.0	0.5	175.0	130.0	5.5
57	0.0	11.0	17.0	1.2	235.0	765.0	41.0	1.0	1.0	128.0	73.0	4.5
58	0.0	6.0	4.0	1.4	371.0	990.0	45.0	0.5	0.5	205.0	145.0	5.0
59	0.0	8.6	8.0	*	355.0	1750.0	43.0	0.5	1.0	260.0	135.0	9.0
60	0.0	16.4	11.8	1.1	390.0	1150.0	42.0	0.5	1.0	305.0	175.0	9.0
61	0.0	11.1	7.2	1.3	510.0	1320.0	42.0	0.5	1.0	290.0	265.0	9.5
62	0.0	20.0	18.0	1.2	460.0	1530.0	30.0	0.0	1.0	360.0	250.0	7.0
63	1.0	6.0	8.5	1.1	425.0	1213.0	39.0	0.0	0.0	159.0	97.0	4.0
64	1.0	9.0	7.0	1.0	480.0	1700.0	35.0	0.0	0.0	140.0	123.0	4.0
65	1.0	8.0	5.0	0.9	440.0	1280.0	37.0	0.5	0.5	130.0	93.0	3.0
66	1.0	7.0	5.0	1.0	360.0	1120.0	42.0	0.0	0.0	173.0	115.0	4.0
67	1.0	7.0	8.0	1.0	333.0	990.0	30.0	0.0	0.0	293.0	180.0	6.0
68	1.0	10.0	10.0	1.1	800.0	2200.0	43.0	0.0	0.5	495.0	350.0	8.0
69	1.0	20.0	15.0	1.5	390.0	1220.0	49.0	0.0	0.5	260.0	155.0	7.5
70	1.0	12.0	12.0	1.1	800.0	1840.0	54.0	1.0	0.0	420.0	300.0	9.0
71	0.5	10.0	5.0	1.0	510.0	1500.0	35.0	0.5	0.5	340.0	210.0	6.0
72	0.5	8.0	7.0	1.2	610.0	1880.0	49.0	0.0	0.0	395.0	205.0	9.0
73	0.5	9.0	11.0	0.9	480.0	1480.0	50.0	0.5	0.5	330.0	130.0	5.0
74	0.5	8.0	5.0	1.3	660.0	1900.0	42.0	0.5	0.5	420.0	215.0	5.0
75	0.5	5.0	4.0	1.2	440.0	1640.0	34.0	0.5	0.0	280.0	150.0	5.0
76	0.5	6.0	5.0	1.5	480.0	1800.0	32.0	0.0	0.0	365.0	190.0	5.5
77	0.5	8.0	7.0	1.0	630.0	2000.0	45.0	0.5	0.5	340.0	108.0	6.0
78	0.0	30.0	50.0	1.1	230.0	420.0	76.0	1.0	0.0	168.0	63.0	3.0
79	0.0	27.0	72.0	2.3	360.0	450.0	74.0	1.0	0.0	200.0	90.0	3.0
80	0.0	52.0	52.0	2.4	379.0	474.0	76.0	1.0	0.0	214.0	100.0	3.5
81	0.0	34.0	47.0	1.7	200.0	430.0	45.0	0.5	0.0	114.0	51.0	3.0
82	0.0	27.0	32.0	2.3	490.0	540.0	75.0	1.0	0.0	189.0	79.0	3.0
83	0.0	22.0	33.0	*	405.0	584.0	64.0	1.0	0.0	195.0	88.0	3.0
84	0.0	32.0	42.0	*	432.0	520.0	64.0	1.0	0.0	290.0	95.0	4.0
85	0.0	46.0	60.0	*	143.0	343.0	40.0	1.0	0.0	94.0	51.0	3.5
86	0.0	46.0	61.0	1.6	190.0	330.0	60.0	1.0	1.0	128.0	60.0	5.0
87	0.0	49.0	21.0	1.7	200.0	440.0	52.0	0.5	0.0	100.0	50.0	3.0
88	0.0	68.0	98.0	*	125.0	270.0	51.0	0.5	0.0	74.0	43.0	3.5
89	0.0	41.0	57.0	1.7	170.0	320.0	65.0	1.0	0.0	115.0	44.0	3.0
90	0.0	56.0	64.0	2.0	190.0	300.0	56.0	0.0	0.0	125.0	50.0	3.5
91	0.0	45.0	64.0	1.9	300.0	550.0	69.0	1.0	0.0	113.0	55.0	3.0
92	0.0	57.0	84.0	*	249.0	330.0	70.0	1.0	0.0	119.0	47.0	4.0
93	0.0	60.0	68.0	2.1	181.0	248.0	75.0	1.0	1.0	110.0	60.0	3.0
94	0.0	60.0	72.0	1.8	229.0	522.0	47.0	0.0	0.0	157.0	100.0	6.0
95	0.0	35.0	42.0	1.6	230.0	400.0	55.0	1.0	0.0	165.0	83.0	3.0
96	0.0	54.0	90.0	1.5	290.0	460.0	63.0	1.0	0.0	190.0	90.0	4.0
97	0.0	53.0	70.0	1.8	202.0	355.0	62.0	1.0	0.0	138.0	62.0	3.5
98	0.0	32.0	88.0	1.8	264.0	330.0	66.0	1.0	0.0	161.0	79.0	4.0
99	0.0	45.0	110.0	1.9	260.0	380.0	65.0	1.0	0.0	185.0	80.0	4.0
100	0.0	42.0	85.0	1.7	202.0	406.0	53.0	0.5	0.0	139.0	87.0	4.5
101	0.0	56.0	80.0	1.8	215.0	350.0	64.0	0.0	0.0	117.0	67.0	4.0
102	0.0	49.0	89.0	1.8	180.0	390.0	50.0	1.0	0.0	105.0	63.0	3.5
103	0.0	41.0	74.0	1.6	305.0	470.0	65.0	1.0	0.0	120.0	70.0	3.0
104	0.0	41.0	63.0	2.0	240.0	385.0	59.0	1.0	0.0	168.0	79.0	3.5
105	0.0	28.0	57.0	2.4	210.0	440.0	51.0	1.0	0.0	183.0	84.0	4.0
106	0.0	68.0	59.0	*	220.0	300.0	69.0	1.0	0.0	133.0	125.0	3.5

<u>OTU</u>	<u>25</u>	<u>26</u>	<u>27</u>	<u>28</u>	<u>29</u>	<u>30</u>	<u>31</u>	<u>32</u>	<u>33</u>	<u>34</u>	<u>35</u>	<u>36</u>	
54	0	0	0	1	1	1	1	0	1	1	1	0	2.0
55	0	0	0	1	1	1	1	1	1	1	1	1	1.0
56	0	0	1	0	1	1	1	0	0	1	1	0	*
57	0	0	1	1	1	1	1	0	1	1	1	0	2.0
58	0	0	1	0	1	1	1	1	1	1	1	1	2.0
59	1	1	1	1	0	1	1	0	1	1	1	0	0.0
60	1	1	1	1	0	1	1	0	1	1	1	0	1.0
61	1	0	1	1	1	1	1	1	1	1	1	1	2.0
62	1	1	1	1	1	1	1	1	1	1	1	1	1.5
63	0	0	0	1	0	1	1	0	1	1	1	0	2.0
64	1	0	0	1	1	1	1	0	1	1	1	0	2.0
65	0	0	0	1	1	1	1	0	1	1	1	0	3.0
66	1	0	1	1	1	1	1	1	1	1	1	1	3.0
67	1	0	0	1	1	1	1	0	1	1	1	0	2.0
68	1	0	0	1	1	1	1	0	1	1	1	0	3.0
69	1	0	0	1	1	1	1	0	1	1	1	0	3.0
70	1	0	1	1	1	1	1	0	1	1	1	0	3.0
71	1	0	1	1	1	1	1	0	1	1	1	0	3.0
72	1	0	1	1	1	1	1	0	1	1	1	0	3.0
73	1	1	1	1	1	1	1	1	1	1	1	1	3.0
74	1	1	0	1	1	1	1	1	1	1	1	1	2.0
75	1	0	0	1	1	1	1	1	1	1	1	1	1.0
76	0	0	0	1	1	1	1	0	1	1	1	0	0.0
77	0	1	1	1	1	1	1	0	1	1	1	0	3.0
78	0	0	0	1	1	1	1	1	1	1	1	1	3.0
79	0	0	0	1	1	1	1	1	1	1	1	1	3.0
80	0	0	0	1	1	1	1	1	1	1	1	1	3.0
81	0	0	0	0	1	1	1	1	0	1	1	1	3.0
82	0	0	0	0	1	1	1	1	0	1	1	1	3.0
83	0	0	0	0	1	1	1	0	0	1	1	0	3.0
84	0	0	0	0	1	1	1	0	0	1	1	0	2.0
85	1	0	0	0	1	1	1	1	0	1	1	0	3.0
86	0	0	0	1	1	1	1	1	1	1	1	1	3.0
87	0	0	0	0	1	1	1	0	0	1	1	0	3.0
88	0	0	1	0	1	1	1	0	0	1	1	0	0.0
89	0	0	0	0	1	1	1	1	0	1	1	1	1.0
90	0	0	0	0	1	1	1	1	0	1	1	1	1.0
91	1	1	1	1	1	1	1	1	1	1	1	1	1.0
92	0	0	1	0	1	1	1	1	0	1	1	1	1.0
93	1	1	1	0	1	1	1	1	0	1	1	1	3.0
94	0	0	1	0	1	1	1	1	0	1	1	1	1.0
95	1	1	1	1	1	1	1	1	1	1	1	1	3.0
96	1	1	1	1	1	1	1	1	0	1	1	0	3.0
97	0	0	0	1	1	1	1	1	1	1	1	1	3.0
98	0	0	1	1	1	1	1	1	1	1	1	1	3.0
99	0	0	0	1	1	1	1	1	1	1	1	1	1.0
100	0	0	1	1	1	1	1	1	1	1	1	1	3.0
101	0	0	0	1	1	1	1	1	0	1	1	1	3.0
102	0	0	0	1	1	1	1	0	1	1	1	0	3.0
103	0	0	0	0	1	1	1	0	0	1	1	0	3.0
104	0	0	0	0	1	1	1	0	0	1	1	0	3.0
105	0	0	0	0	1	1	1	1	0	1	1	1	3.0
106	0	0	0	0	1	1	1	0	0	1	1	0	3.0

<u>OTU</u>	<u>37</u>	<u>38</u>	<u>39</u>	<u>41</u>	<u>41</u>	<u>42</u>	<u>43</u>	<u>44</u>	<u>45</u>	<u>46</u>	<u>47</u>
54	1.5	0.0	149.0	84.0	20.0	127.0	83.0	16.0	*	*	*
55	1.0	3.0	175.0	88.0	25.0	103.0	71.0	19.0	109.0	63.0	36.0
56	*	3.0	140.0	75.0	15.0	69.0	55.0	9.0	79.0	55.0	5.0
57	0.0	3.0	156.0	73.0	20.0	74.0	53.0	15.0	*	*	*
58	1.0	3.0	173.0	70.0	38.0	71.0	59.0	5.0	93.0	70.0	10.0
59	2.0	3.0	115.0	65.0	10.0	75.0	59.0	11.0	81.0	57.0	11.0
60	2.0	2.0	130.0	65.0	30.0	55.0	43.0	7.0	100.0	60.0	30.0
61	2.0	1.0	127.0	65.0	20.0	83.0	75.0	10.0	98.0	78.0	15.0
62	1.5	3.0	130.0	75.0	32.0	126.0	94.0	18.0	137.0	100.0	20.0
63	1.0	1.0	*	*	*	137.0	73.0	18.0	*	*	*
64	1.0	1.0	190.0	113.0	43.0	125.0	95.0	20.0	168.0	106.0	28.0
65	1.0	1.0	195.0	105.0	25.0	105.0	75.0	13.0	133.0	100.0	20.0
66	1.0	1.0	170.0	85.0	50.0	103.0	78.0	8.0	90.0	65.0	13.0
67	2.0	0.0	146.0	102.0	19.0	122.0	82.0	32.0	*	*	*
68	1.0	0.0	215.0	155.0	25.0	175.0	155.0	8.0	185.0	155.0	15.0
69	0.0	0.0	108.0	55.0	25.0	65.0	55.0	8.0	53.0	41.0	10.0
70	1.0	0.0	265.0	158.0	15.0	185.0	160.0	15.0	200.0	150.0	10.0
71	1.0	0.0	168.0	88.0	45.0	69.0	48.0	10.0	61.0	43.0	10.0
72	0.5	0.0	125.0	85.0	13.0	70.0	46.0	10.0	68.0	51.0	11.0
73	1.0	0.0	170.0	75.0	40.0	110.0	58.0	23.0	59.0	47.0	7.0
74	1.0	0.0	185.0	100.0	40.0	93.0	55.0	15.0	117.0	55.0	50.0
75	3.0	0.0	190.0	100.0	50.0	111.0	63.0	11.0	131.0	52.0	66.0
76	3.0	0.0	180.0	85.0	20.0	75.0	58.0	15.0	85.0	70.0	10.0
77	1.0	0.0	*	120.0	35.0	100.0	79.0	10.0	107.0	83.0	14.0
78	0.0	0.0	71.0	40.0	8.0	49.0	30.0	3.0	44.0	36.0	4.0
79	0.0	0.0	57.0	40.0	9.0	41.0	29.0	4.0	63.0	35.0	21.0
80	0.0	0.0	59.0	33.0	8.0	49.0	37.0	4.0	43.0	37.0	3.0
81	0.0	0.0	73.0	38.0	9.0	38.0	29.0	7.0	43.0	27.0	13.0
82	1.0	0.0	123.0	57.0	29.0	*	*	*	60.0	37.0	27.0
83	0.0	0.0	110.0	50.0	16.0	44.0	35.0	4.0	54.0	38.0	7.0
84	1.0	2.0	95.0	37.0	21.0	39.0	29.0	8.0	30.0	26.0	4.0
85	1.0	0.0	60.0	34.0	6.0	43.0	29.0	2.0	*	*	*
86	1.0	0.0	77.0	37.0	7.0	43.0	32.0	8.0	45.0	35.0	7.0
87	1.0	0.0	81.0	40.0	13.0	54.0	41.0	7.0	47.0	33.0	7.0
88	3.0	0.0	73.0	36.0	11.0	41.0	32.0	7.0	42.0	30.0	9.0
89	3.0	0.0	71.0	40.0	8.0	45.0	33.0	9.0	52.0	29.0	20.0
90	3.0	0.0	100.0	50.0	4.0	56.0	43.0	8.0	57.0	49.0	5.0
91	1.0	3.0	77.0	43.0	15.0	53.0	35.0	9.0	67.0	39.0	16.0
92	3.0	1.0	82.0	43.0	10.0	52.0	36.0	8.0	64.0	40.0	17.0
93	1.0	0.0	94.0	41.0	10.0	47.0	30.0	13.0	58.0	44.0	23.0
94	1.0	3.0	64.0	38.0	8.0	57.0	40.0	6.0	53.0	40.0	1.0
95	0.0	0.0	71.0	34.0	13.0	35.0	30.0	2.0	73.0	42.0	29.0
96	1.0	0.0	71.0	40.0	5.0	71.0	48.0	18.0	63.0	43.0	23.0
97	1.0	0.0	90.0	50.0	23.0	81.0	59.0	9.0	61.0	47.0	11.0
98	2.0	0.0	71.0	44.0	2.0	62.0	53.0	8.0	69.0	46.0	7.0
99	2.0	1.0	93.0	48.0	13.0	73.0	44.0	9.0	55.0	43.0	5.0
100	1.0	0.0	63.0	36.0	5.0	60.0	45.0	5.0	61.0	47.0	11.0
101	1.0	0.0	80.0	43.0	12.0	56.0	32.0	5.0	49.0	37.0	10.0
102	1.0	0.0	73.0	43.0	11.0	50.0	42.0	7.0	51.0	31.0	7.0
103	1.0	0.0	72.0	45.0	4.0	60.0	46.0	9.0	*	*	*
104	1.0	0.0	67.0	40.0	13.0	44.0	32.0	7.0	63.0	37.0	24.0
105	1.0	0.0	68.0	38.0	9.0	48.0	37.0	5.0	56.0	40.0	7.0
106	0.0	0.0	72.0	39.0	13.0	55.0	37.0	11.0	70.0	47.0	20.0