DOCTOR OF PHILOSOPHY

Food selection by the rabbit.

Soane, G.A.

Award date:
1980

Awarding institution:
Bangor University

FOOD SELECTION BY THE RABBIT

A thesis submitted for the Degree of Philosophiae Doctor in the University of Wales

by

G.A. Soane

B.Sc. (Hons. Zoology, Edinburgh)

School of Plant Biology,
University College of North Wales,
Bangor,
Gwynedd.

1980.

INDEX

SUMMARY

1. The composition of a sward may reflect the food preferences of grazing animals. These preferences are themselves affected by certain features of the vegetation including the species present, their relative abundance, growth stage and distribution within the sward.
2. Some of these aspects of plant-animal interaction were studied in relation to selection of food by the rabbit (Oryctolagus cuniculus L.).
3. A technique of faecal analysis, based on measurement of areas of epidermal fragments, was used to determine the diets of rabbits feeding on lowland grasslands.
4. Correction factors to allow for the effects of differential digestion of different grass species were derived by feeding captive wild rabbits known proportions of seven common grasses.
5. The relative proportions of different grasses in the faeces of wild rabbits were found to be positively correlated with their relative availabilities at eight differing grassland sites. Distinct preferences resulted in consistent disproportional selection of particular species.
6. Application of correction factors to the faecal analysis data did not alter the results qualitatively due to a positive correlation between palatability and digestibility.
7. The annual grazing pattern of a population of wild rabbits was studied over two successive years by faecal analysis. Consistent seasonal variations in species utilization occurred, including increased selection for certain grasses just prior to flowering, followed by substantial intake of grass seeds.
8. Preferences shown by captive wild rabbits in controlled feeding experiments paralleled the preferences of rabbits in the field.
9. Controlled choice experiments on domestic rabbits indicated that the relative frequencies of two food-types in a mixture, pre-feeding and previous experience affected selection. The behavioural responses were

discussed in terms of conditioning and differential satiation.

10. Computer simulation based on the results of the present study and further experimental work was suggested as a means of further investigating the interaction between vegetation composition and rabbit grazing behaviour.

ACKNOWLEDGEMENTS

I wish to thank my supervisor, Professor J.L. Harper, for his assistance during all stages of this work. I am grateful to the staff at Pen-y-Ffridd Field Station, in particular Mr. E. Williams, for their patient help with housing and feeding numerous rabbits in a somewhat incongruous setting. My thanks are also due to the Department of Zoology for permission to 'borrow' a number of experimental animals and to the staff of the animal house for their help. Valuable assistance with field work and collection of vegetation was received from Mr. D. Shaw. I am indebted to Mr. Ian Soane for his support and useful criticism throughout this study, to Mrs. M. Thomas for typing the manuscript and to Mr. W. Neville for developing and printing the photcgraphs.

GENERAL INTRODUCTION

GENERAL INTRODUCTION

It has long been accepted that grazing by herbivores may determine the relative abundance of species within a sward as well as affecting physical characteristics of the vegetation such as height and density. Early work by Tansley and Adamson (1925) on chalk downlands grazed by rabbits demonstrated that grazing had a marked effect on the botanical composition of the sward. Jones (1933) concluded from his extensive work on controlled grazing of sheep on experimental plots that grazing affected the composition of the sward both directly by differential selection of plant species and indirectly by altering the balance of competitive interactions between plants. There is a great deal of evidence that herbivores are highly selective in their choice of food and that this plays an important role in determining the composition of grazed vegetation. The present study concentrates on a number of aspects of food selection with reference to the grazing rabbit (Oryctolagus cuniculus L.). Choice of food is affected both by its innate characteristics (palatability) and by external factors such as its availability. Particular emphasis is given to this latter aspect of food selection.

In view of the conflict between rabbit grazing and agriculture it is surprising that little attention has been paid to the relationship between rabbit feeding behaviour and vegetation composition. In Britain the emphasis has been mainly on the analysis of grazed vegetation. In Australia where damage by rabbits to sheep pasture has been devastating, there has been a great deal of research into the biology and ecology of Oryctolagus, coincidental with the development of control measures. The Wildlife Division of the Commonwealth Scientific and Industrial Research Organisation has been particularly active in this field and a number of papers have appeared in its journals over the past 20-30 years (e.g. Myers and Poole 1959, 1961; Mykytowycz 1958, 1959, 1960). However, only a small proportion of this work

is concerned with feeding behaviour and food selection.

In the present work emphasis was placed on investigating those factors which might influence the response of the rabbit to the species which formed a major part of its diet. In order to establish these the first requirement was to find a method of accurately determining the composition of the natural diet of the wild rabbit. In the literature a number of methods are described which have been used to obtain information on the selection of food species by rabbits and other lagomorphs. Direct observation of feeding rabbits has yielded some data on preferred plant species (Southern 1940; Thompson 1953; Mykytowycz and Rowley 1958; Myers and Poole 1961; Lockley 1964). Following tracks in snow and observing the species eaten en route, has also been used as a method of diet determination (Dalke and Sime 1941; Flux 1967; Hansson 1970).

A great deal of information on selection of food plants has been obtained indirectly from examination of vegetation which has been grazed by rabbits. Some of this work is reviewed by Thompson and Worden (1956) and Sheail (1971). Some such observations have been made during large-scale vegetation surveys and in effect form catalogues of the species grazed, (e.g. Farrow 1917; Tansley and Adamson 1925; Cameron 1935; Fenton 1940). Vegetation surveys which have been carried out specifically to investigate effects of rabbit grazing include work by Gillham (1955, 1963) on a number of Pembrokeshire and South African islands. After the introduction of myxomatosis into Britain in 1954 several vegetation surveys were carried out to measure the effects of the resultant decline in grazing (Ranwell 1960; White 1961; Thomas 1960, 1963). Similarly, indirect evidence on the food preferences of rabbits has been gathered by artifically excluding rabbits from plots within heavily grazed areas (e.g. Watts 1957, 1960). Further indications of preferences have been obtained from recording successive

changes in the composition of the vegetation of artificially sown pastures consistently grazed by rabbits (Thomas 1937; Phillips 1953; Myers and Poole 1963). Studies on crop damage caused by grazing rabbits, mostly prior to 1954, provide further information on feeding behaviour (Gough and Dunnett 1950; Church et al 1953; Thompson and Worden 1956; Sheail 1971).

More direct information on food selection by lagomorphs has been obtained by analysis of stomach contents and faeces, mainly by identification of undigested epidermal fragments. Analyses of stomach contents have been carried out by Dalke and Sime (1941) in the study of Sylvilagus spp., Hayden (1966) on Lepus californicus deserticola, Bear and Hansen (1966) on Lepus townsendii, and Flux (1970) on Lepus europaeus. Faecal analysis has been used in the study of lagomorph diets by Riegel (1942) on Lepus californicus melanotis and Sylvilagus spp., Dusi (1949 a and b, 1952) on Silvilagus floridanus mearnsi, Flux (1967) and Hansson (1970) on Lepus europaeus and Stewart (1971) on Lepus and Pronolagus spp.

In Britain preliminary studies on the diet of Oryctolagus cuniculus using faecal analysis, have been described by Metcalfe (unpublished) and Oldham (1971). Some quantitative data have been obtained by means of faecal analysis in a study of seasonal changes in cattle and rabbit grazing on Wood Walton Fen National Nature Reserve (Williams et al 1974). The technique has similarly been used by Bhadresa (1977) to study quantitatively the food preferences of rabbits on sand dunes at Holkham National Nature Reserve, Norfolk.

In the present study faecal analysis was chosen as the method of diet analysis most suited to providing some quantitative measure of the relative importance of different species in the diets of wild rabbits, in addition to qualitative data. This method of diet analysis also has the advantage of not causing any interference to the animals themselves.

Part 1 of this thesis describes the application of faecal analysis to determining the importance of some of the factors influencing the selection of species forming major components in the diets of wild rabbits feeding on various types of lowland grassland. This analysis was simplified by the fact that grasses formed the major part of the diet since there was a low diversity of grass species relative to abundance in contrast with the high diversity of herb species. By studying the diets of rabbits in relation to species availability, answers to the following questions were sought:

1. Which species form the main dietary components?
2. How does the intake of these species relate to their availability?
3. Do seasonal changes in availability and/or nutritional quality affect food selection?

The resultant analyses are described in Chapters 4 and 5.

Since the validity of the results obtained by faecal analysis is entirely dependent on an accurate methodology, the reasons for adopting the techniques used in preference to various alternative methods are considered in some detail in Chapter 1. Technical details of the procedures developed are given in Chapter 2.

The practicability of calibrating ingested proportions of species with faecal proportions was determined by controlled feeding of captive wild rabbits (Chapter 3). This was to allow for possible discrepancies caused by differential digestion.

Part 2 of this thesis describes experimental work on food selection by the rabbit in which various factors affecting choice were investigated using domestic and captive wild rabbits. Few studies of food selection by lagomorphs have involved experimental choice trials, although the method has been used extensively with other animals, particularly domestic stock. This

is probably because rabbits and hares do not adjust easily to conditions of close captivity. Choice trials have been used to a limited extent by Dalke and Sime (1941) on a few captive cottontails (Sylvilagus floridanus) and by Hewson (1976) on captive mountain hares (Lepus timidus scoticus). The preferences of wild rabbits (Oryctolagus cuniculus) for certain grass species were investigated by Oldham (1971) who left boxes containing grass swards at a site grazed by rabbits, and measured the quantities eaten. Miller (1968) indirectly measured grazing selectivity on a series of experimental plots by counting the numbers of rabbit droppings left behind on each plot. In the present study overall species preferences indicated by the faecal analysis data in Part 1 were tested by means of choice trials using captive wild rabbits (Chapter 6).

Some of the concepts relating to the study of food preferences are discussed in Part 2, together with a brief theoretical consideration of the factors affecting preferences. Although much has been written about the environmental factors affecting choice of food by herbivores, there is little but circumstantial evidence to substantiate these ideas. A group of experiments was devised to act as a preliminary investigation into the effects of manipulating some of the factors affecting selection. The experiments described in Chapter 7 were specifically designed to investigate the effects of controlling the relative abundances of food-types within mixtures.

In summary, the presentation of this thesis is in two sections. The first section deals with the collection and analysis of data relating to food selection by wild rabbits under natural conditions and the second with the isolation and experimental manipulation of some of the factors involved in the process of selection.

PART 1

THE APPLICATION OF FAECAL ANALYSIS TO INVESTIGATE FOOD SELECTION BY WILD RABBITS UNDER NATURAL CONDITIONS

CHAPTER 1

FAECAL ANALYSIS AND RELATED FORMS OF QUANTITATIVE DIET ANALYSIS: REVIEW AND CONSIDERATIONS TOWARDS ADOPTING AN ACCURATE QUANTITATIVE METHOD.

CHAPTER 1

FAECAL ANALYSIS AND RELATED FORMS OF QUANTITATIVE DIET ANALYSIS: REVIEW AND CONSIDERATIONS TOWARDS ADOPTING AN ACCURATE QUANTITATIVE METHOD.

The technique of faecal analysis has been used to investigate the diets of a wide range of plant-eating animals as is indicated by the selected list of references below.

East African large mammals: Lamprey (1963); Kiley (1966); Stewart and Stewart (1970, 1971). Further examples given in the bibliography of Petersen and Casebear (1971).
Sheep: Croker (1959); Hercus (1960); Martin (1955, 1962); Armitage (1972).
Horses: Stainsby (1973).
Rodents: Hansson (1970); Watts (1968, 1977); Ferns (1976).
Gamebirds: Peters (1958); Eastman and Jenkins (1970).
Slugs: Pallant (1969); Jennings and Barkham (1975, 1976).
Snails: Williamson and Cameron (1976).
Many additional examples are given in the review by Ward (1970).

Analysis of faecal samples is based on the recognition and identification of particles which have survived the process of digestion. The principle structurally identifiable component of herbivore faeces consists of fragments of undigested plant epidermis whose cutin-impregnated cell walls render it to a large extent resistant to the action of digestive enzymes. The structure and composition of plant cuticle has been described by Skoss (1955). The cell

configuration of the epidermis is more or less characteristic for each plant species thus enabling the fragments to be identified with the aid of reference slides prepared from known fresh material.

Perennial grasses are particularly suited to this method of analysis as the cuticle tends to be relatively indestructable compared with annual and herbaceous dicots, many of which have a thin, easily distorted epidermis. The diagnostic features useful for identification include the shape and size of the undifferentiated epidermal cells, the pattern of lignification of the cell walls, the range, distribution and structure of the differentiated cells such as stomatal guard cells, silica cells, hairs and prickles. The taxonomic importance of these epidermal characters has been demonstrated by Prat (1932) and Metcalfe (1960) and their use in the identification of unknown plant material described in some detail by Davies (1959), Martin (1955, 1962) and Stewart (1965).

Microscopic examinations of epidermal fragments in herbivore faeces can therefore provide qualitative information on diet composition. Quantitative data can also be obtained provided that effective methods of sampling are employed. The following review and discussion describes in some detail the various methods of quantitative analysis of diet together with their limitations. The review includes examples of gut-content as well as faecal analysis and is not strictly limited to herbivores in order to give a more comprehensive picture of the uses and limitations of quantitative analysis. Methods of obtaining quantitative data depend a great deal on the availability of time and material as well as the nature of the material and information required.

1. Frequency of occurrence and relative occurrence

Individual gut contents or faecal samples from a number of animals may be scored for the presence or absence of each identifiable food item. The

data are then presented as the percentage (or number) of samples containing each item (frequency of occurrence). The relative occurrence of a particular item is the number of samples in which it is present, expressed as a percentage of the total of all samples containing each identified component.

The method usually involves two sampling stages. Firstly a sufficiently representative sample of animals or faeces must be obtained. The statistical requirements of this sample size are discussed by Hanson and Graybill (1956). Secondly, if each of these samples has to be subsampled to carry out detailed microscopic examination, the minimum subsample size containing all (except trace) food items must be determined.

Such frequency data have been obtained in studies of the diet of Microtus agrestis from faecal samples (Godfrey 1953) and gut contents (Williams 1962; Evans 1973), of Lepus californicus deserticola from stomach contents (Hayden 1966), of Sylvilagus floridanus mearnsi from faecal analysis (Dusi 1949, 1952), of Agrolimax reticulatus from both crop and faeces samples (Pallant 1969, 1972), of red locusts from crop contents (Chapman 1957) and of Chorthippus parallelus also from gut contents (Bernays and Chapman 1970).

Quantitative estimates of diet expressed as frequency of occurrence must be treated with caution since, in terms of overall food selection, they invariably underestimate major components in relation to the lesser items. Such deviations will be particularly marked in the case of herbivores inhabiting relative homogeneous areas of vegetation and whose ability to select food species is not limited by inherent physical restrictions. The method therefore appears to be most applicable to the determination of food selection by relatively immobile invertebrates. Relative occurrence data is useful for detecting seasonal changes and inter-species or population differences, but has the disadvantage of producing apparent changes in the occurrence of a single item which is constant in absolute terms, but changes relative to other food items.

Some experimental assessment of the method has been made. Scott (1941) obtained a rough agreement between the composition of food eaten by captive red foxes and frequency estimations of faecal composition. Hansson (1970) performed a more thorough examination of the method by analysing stomach and caecal contents of trapped Apodemus sylvaticus and Microtus agrestis and comparing volume estimates (assumed to be more accurate) with frequency data. The latter were found to give wide deviations of the type described above.

2. Direct volume and weight measurement

Methods of quantitative analysis involving direct measurement are of limited applicability due to difficulties in separating small fragments of food in gut contents and faeces, and the problems associated with making measurements under constant conditions. The method has proved to be of most use for the macroscopic determination of the composition of crop contents in granivorous birds (e.g. Davison 1940; Peters 1958), but less successful for quantification of their faecal content (Swanson 1940).

The accuracy of such measurements was investigated by Jensen and Korschgen (1947) who fed bob-white quail known mixtures of seeds and subsequently compared the percentage composition of the crop and gizzard contents and faeces. A good correlation was obtained between the measured composition of the crop contents and the ingested mixture, but gizzard and faecal analyses gave a strongly biased estimate of the fed diet. Norris (1943) tried to quantify the stomach contents of sheep fed known diets, by separating and weighing only the larger identifiable fragments from small subsamples. The results were unsatisfactory since the composition of this fraction was not representative of the unanalysed fraction consisting of smaller unidentified fragments which made up over half of the contents. Bergerud and Russell (1964) separated and measured the composition by volume of different sized particles

in the rumen contents of Newfoundland caribou. They found substantial differences in composition between the fractions.

Methods of volume and weight measurement are discussed in more detail in the reviews by Medin (1970) and Ward (1970).

3. Indirect volume estimates

Estimates of percentage volume are the most effective form of diet analysis for animals such as small rodents with mixed diets including seeds, fruit, green plants and insects. The most commonly used method (e.g. Williams 1955, 1962; Watts 1968) is microscopic examination of subsamples of homogenized stomach contents. The percentage of each item is estimated by counting or measuring the area of fragments within a predetermined number of random fields of view at the lowest possible magnification. Since the fragments are flattened beneath coverslips, such measures are assumed to be valid estimates of proportions by volume. Mean percentages can be calculated for a number of animals either directly or by weighting each result according to the volume of the stomach contents from which it was obtained.

Sources of inaccuracy within this method derive from sampling and sub-sampling (which can be quantified and minimised), and from the assumed similarity in relationship between the number or area of fragments of widely differing compositions and volume. An *in vitro* assessment of the method has been carried out by Gebczynska and Myrcha (1966) who made up a series of known mixtures of various plants and seeds which were stained, dried and ground together. The proportions of the components as estimated by area covered within 100 microscope fields of view, did not deviate significantly from the known proportions by dry weight. Hansson (1970) however, found that widely differing food items differed greatly in ease of recognition in the stomach contents of *Microtus*, thus making accurate volume estimates impossible.

Nevertheless further experiments in which Apodemus were fed dried cakes composed of known weights of nuts, grasses, herbs and moss resulted in reasonable agreement between proportions estimated from the analysis of stomach contents and proportions by weight and volume in the rehydrated cakes.

4. Epidermal fragment counts and densities

Epidermal fragments have frequently been used to determine and quantify food species in gut contents and faeces.

(i) Fragment counts. Subsamples of suspended material are mounted on slides and fragments of each identifiable species are counted. To assist computation and statistical analysis the total number counted per slide is usually limited to 100, and replicate slides are analysed. The relative proportions of species are taken as estimates of diet composition.

Fragment counts have been used in the analysis of sheep rumen contents and faeces (Martin 1955, 1962; Hercus 1960), kangaroo stomach contents (Bailey et al 1971), hare stomach contents (Flux 1970) and grouse faeces (Eastman and Jenkins 1970). The diets of sheep and kangaroos have been compared using this method for the analysis of rumen and stomach contents (Griffiths and Barker 1966) and similarly the diets of sheep, kangaroos and goats have been compared (Dawson et al 1975). Compositions of faeces from a number of ungulates from East African plains have also been compared by means of epidermal fragments counts (Stewart and Stewart 1970, 1971).

Apart from the usual sampling variation which can be measured and reduced, the accuracy of the method relies on the assumption that the epidermis of each food species is represented by similar ranges of fragment sizes. Stewart (1967), in a series of experiments to test and compare various methods of quantitative faecal analysis (for herbivores of the East African plains), found that the cuticles of different grasses fragmented into particles which

varied significantly in size. These size differences varied both between species of grass and between animal species, and probably also depended on the degree of maturity of the plant and the age of the animals. By counting fragments, in many instances Stewart found significant differences between the proportions of grasses in faeces, and the freshweight composition of the feed: other methods of estimation gave results in close agreement.

However, the method is relatively rapid which probably accounts for its widespread use, and also gives the illusion of great accuracy since sampling variation is usually low.

(ii) Fragment density. The assumption that all epidermal fragments are of similar size is also made in the method described and experimentally tested (using artificially produced samples) by Sparks and Malechek (1968). On each of five subsample slides, twenty fields of viewwere observed and scored for the presence or absence of each identified species. The frequency figures thus obtained were converted to relative densities using tables. This conversion could only be made if the commonest species occurred at less than 86% of the locations, and was based on the assumption that the fragments were of uniform size and randomly distributed on the slide. The method was tested by making several artificial mixtures containing known weights of grass and herb species which were ground together through a 1 mm screen to produce uniform particles. Very small particles were removed by sifting. The resulting mixtures were sampled as described and good agreement was obtained between the estimated relative density and known composition.

The method of analysis by quantitative measurement of fragment density has been used by Free et al (1970) in the examination of pre-ground and sifted oesophageal and faecal samples from cattle, and by Hansen et al (1973) for the estimation of dietaryoverlap in sheep and cattle. However, no experimental test of the method has been made in vivo.

5. Area of fragments and point quadrats

(i) Area measurement. Errors attributable to differences in fragment sizes between plant species, animals, etc. can be eliminated by measuring areas instead of by counting. Areas are estimated using microscope eyepiece adaptors which are graduated into grid squares, and from these measurements the relative cover of each species is determined.

Storr (1961) experimented with this method by feeding captive quokkas (Setonix brachyrus) a finely chopped mixture of four herb species for five days, and analysing both the feed and faecal samples on the sixth day. Close correspondence was obtained between the ingested and faecal proportions. Although the samples were ground prior to analysis, there were occasional large fragments which resulted in high variances between slides. Standard errors could be reduced to acceptable levels by analysing two slides from a single dropping from each of 20 animals. Area measurement of epidermal fragments has been used by Chippendale (1962) in the analysis of stomach and rumen contents of kangaroos and cattle.

Further experimental tests on the accuracy of the method have been made by Stewart (1967) who compared the results from area measurement with fragment counts. The former method gave the closer agreement between estimated faecal composition and proportion by fresh weight of ingested grasses. It was found necessary to analyse a larger number of subsamples using area measurements, to reduce standard errors to acceptable levels. This was far more time-consuming than analysis by fragment counts. Stewart noted that a certain degree of subjectivity was involved in the measurement of irregularly shaped fragments using grid squares.

(ii) Point quadrats. The point quadrat or microscopic point method of estimating relative cover eliminates the subjectivity involved in measuring areas. Fragments are identified and recorded at random points within

subsamples and the relative percentage cover of each species calculated. The method however, has the disadvantage of introducing yet another sampling stage into the analysis, compared with direct area management.

The method has been used not only to analyse epidermal fragments, but also in the larger scale analysis of samples from oesophageal fistulae (Heady and Torrell 1959; Van Dyne and Torrell 1964). Subsamples contained in a tray which could be moved in fixed stages, were examined under a binocular microscope fitted with eye piece crosshairs. Fragments lying beneath or nearest to the centre point of the crosshairs at each fixed stage were scored. The proportions of different plant parts (e.g. leaf, stem, flower etc.) in ungulate stomach samples were determined in a similar manner using a perspex overlay marked with a 1 cm grid with 100 intersection points (Gwynne and Bell 1968; Bell 1969). Buffalo rumen contents have also been analysed using this method (Sinclair and Gwynne 1972).

During studies of food preferences in impala subsamples of faecal epidermal fragments were mounted on slides marked with a 1mm grid with 836 intersection points (Stewart 1971). The point quadrat method has been adopted for use with a projection microscope for analysis of cattle and rabbit faeces (Williams et al 1974).

An experimental study of the point quadrat method was made by Stewart (1967). Since the placing of a single point marked on a microscope eyepiece at random positions on a slide was extremely laborious due to the low percentage of 'hits', he experimented with groups of 5 and 25 points. In both cases the variance between subsamples was high due to the low number of 'hits' per slide. However, he found no significant differences between the use of 5 and 25 points, indicating that occasional large fragments were not biasing the results. As with the area estimates he obtained close correspondence between the proportions estimated in the faeces and the proportions ingested.

Discussion

The main problem with all methods of analysis of gut contents or faeces is that of testing the validity of the assumption that the measurements obtained are accurate quantitative estimates of the food species ingested. The repeatability of the sampling methods can readily be measured and standard errors reduced to reasonable levels. However, different methods of analysis applied to the same sample may yield different results, even when sampling variance is low (e.g. Stewart 1967). Test analyses of experimental mixtures containing known proportions prepared *in vitro* are of limited usefulness since these mixtures may not fully reflect conditions found within samples from animals. For example, Sparks and Malechek (1968) artifically reduced all fragments in test mixtures to similar sizes whereas wide variations in sizes of particles occur in samples of gut contents and faeces.

Analyses of samples from animals which have been fed known mixtures have resulted in estimations which have been regarded as valid or invalid depending on the degree of correspondence between ingested proportions and sample estimates (e.g. Hansson 1970; Storr 1961; Stewart 1967). There are, however, reasons why the estimated proportions from gut contents or faeces would not be expected to reflect the ingested proportions. Firstly different species may be digested to different extents resulting in altered proportions in the samples analysed. Secondly the relationship between residue proportion measured by volume, fragment number or area, and dry or fresh weight proportions in the ingested mixture is not constant for different plant species, nor even within single species. On theoretical grounds therefore, sample estimates would not be expected to correlate with ingested proportions and methods of assessment in which close agreement is found may not be accurate under all conditions.

Since experimental testing of the validity of estimations made using different methods of analysis is not completely reliable, the best approach to selecting a method most suited to a particular situation would seem to be based on theoretical considerations. Analysis of epidermal fragments in faeces by area measurement combines the least number of assumptions with the least number of sampling stages, of the four methods available. Theoretically this method should result in accurate estimates of relative proportions of species within faeces if the following conditions are applied:

1. The faecal samples are broken up by a method which reduces large disparities in fragment sizes, so that sampling variances will not be excessively high.
2. Sufficient samples are analysed to reduce the standard errors to low levels. Two sampling stages are involved. Firstly representative samples of faeces must be obtained. Secondly these samples must be subsampled for microscopic analysis. Variation at both these stages can be measured and reduced by increasing the sample size.
3. Subjectivity in area measurement is minimized by using a grid of squares which are small enough to enable measurement of irregular shaped fragments.
4. Allowance is made for differences in the ratios of surface area:weight for different plant species.

For the reasons formulated above the method of quantitative analysis of rabbit faeces chosen and adapted for use in the present study was by measurement of areas of epidermal fragments. The conditions which would theoretically improve the accuracy of the estimates as outlined, were taken into consideration in the development of this method.

CHAPTER 2

METHODS USED IN THE ANALYSIS OF RABBIT FAECES IN THE PRESENT STUDY

CHAPTER 2

METHODS USED IN THE ANALYSIS OF RABBIT FAECES IN THE PRESENT STUDY

Preparation of reference material

Permanent reference slides were made of the epidermis of some twenty grasses and a number of other species. Black and white photomicrographs were taken at low (x 100) and high (x 400) power magnification to assist recognition in the faecal samples. 35 mm contact prints were found to be adequate for this purpose when used in conjunction with the slides themselves. Enlarged photomicrographs of the epidermis of several species are given in the Appendix. Numerous temporary preparations were made of less common species and the species frequently encountered at different times of year and from different sites. The reference material was prepared using the following methods.

1. For the majority of species the best permanent slides were obtained using the method described by Martin (1955). Short lengths of about 1 cm from the middle and upper parts of a few leaf blades were placed, with a known surface uppermost, in 50% nitric acid surrounded by a cooling water bath. It was occasionally found necessary to remove narrow strips from the leaf edges before detaching the epidermis, but usually these were retained since they frequently bore characteristic prickles or spines. Two to four hours maceration was usually sufficient to soften the tissue, after which the segments were washed and transferred individually to microscope slides where they were immersed in water. The required epidermis (i.e. abaxial or adaxial) was then scraped gently to remove any adhering mesophyl cells and fibres with the aid of a needle, sharp blade and fine paintbrush.

The cleared fragments of epidermis were transferred through an alcohol series to a stain consisting of acid fuchsin in equal parts of 95% ethyl and butyl alcohols. This process had to be carried out slowly or the fragments tended to become inextricably rolled. Staining usually took about 30 minutes after which the fragments were washed in 95% butyl alcohol and mounted in Euparal.

2. When a large number of epidermal fragments were required (for example to look for variations within species), the following method was used, based on that described by Storr (1961). Leaf sections were boiled under reflux in 20 ml of an equal mixture of 10 % nitric and chromic acids, for a few minutes until the epidermis became detached. The fragments were washed, stained and mounted as described above, or, more commonly, temporary preparations were made by staining with aqueous gentian violet and mounting in gum chloral.

This method was found useful for preparing large numbers of fragments simultaneously, but these were usually incompletely cleared and therefore not suitable for permanent slides unless they were first scraped. A further disadvantage of the method was the problem of identifying abaxial and adaxial fragments without prior knowledge.

3. In some cases identified fragments of epidermis from the faeces of rabbits which had been fed known species were used as additional reference material.

4. The epidermal characteristics of many less commonly occurring grasses and a number of non-grasses were determined from cellulose acetate imprints. The required leaf surface was painted with acetone and quickly pressed on to a strip of cellulose acetate. The resulting imprints were distinct enough

for most identification purposes, but were not suitable for photomicrographs. The method had the advantage of being quick and easy and was valuable for checking the epidermal structure of a species at different times of year and at different sites.

Preparation and quantitative analysis of faecal samples

Preparation of material for analysis

The following method of preparation and analysis was used throughout the study and applied both to samples of faeces from individual captive rabbits (Chapter 3) and samples collected in the field which contained droppings from many individuals (Chapters 4 and 5). Prior to analysis the samples were stored in formalin - acetic - alcohol (5 parts formaldehyde: 5 parts glacial acetic acid:100 parts 60% alcohol).

Inspection of the components of droppings teased apart in water revealed considerable variation in the sizes of epidermal fragments. In order to reduce the fragments to approximately similar sizes each sample of droppings was mixed with water, and ground in a Wareing blender for one minute. This also served to mix the individual droppings, thus forming a homogeneous suspension from which a subsample could be withdrawn. A suitable particle density was usually obtained by adding about 6 ml of water per dropping in each sample, but this volume varied depending on the sizes and textures of the droppings. A few drops of aqueous gentian violet were added to each sample to increase the contrast between the epidermal structures. The prepared material was usually analysed immediately, but could be kept in airtight containers for several months at least, with no apparent deterioration.

Temporary microscope slides were made from a few drops of suspension withdrawn after thoroughly mixing the sample, using a pipette consisting of a tube with a 5 mm bore. A drop or two of gum chloral was mixed with the material on the slide before covering with a large rectangular glass coverslip (22 x 50 mm).

Identification and scoring of epidermal fragments

Experience in identification was gained at the outset of the study by analysis of the samples obtained when known grasses were fed in paired mixtures to captive wild rabbits (Chapter 3). In the analysis of samples from populations of wild rabbits (Chapters 4 and 5) distinctions were made usually only between different species of grass. Grasses which could not be identified were scored as a separate category which as a rule formed only 0-3% of the epidermal fragments with a maximum of 6%. Sedges, when they occurred, were distinguishable as a single separate group. Epidermal fragments of all other non-grass species were usually scored as a single group unless one species occupied a large proportion of the sample and could be identified. Grass seed remains consisting of fragments of the scales which surround the seed grain were recognisable and scored as a separate category whenever they occurred.

The identities of epidermal fragments of certain pairs and groups of grass species could not always be separated with certainty and eventually it was decided to score the following species together:

Lolium perenne and Cynosurus cristatus;

Dactylis glomerata, Poa annua and P. trivialis;

Festuca rubra and Poa pratensis;

Briza media, Koeleria cristata and Sieglingia decumbens.

Although the epidermal characters of these species appear distinct in preparations made for reference purposes, variability resulted in overlapping of some of the features such as cell shape and cell wall structure. It was therefore not possible to distinguish between small fragments consisting of a few cell walls with no consistent diagnostic characters such as silica bodies or prickle cells.

The following abbreviations of species names and categories are used in the tables in the following chapters.

F/P Festuca rubra / Poa pratensis

Ag Agrostis tenuis

H Holcus lanatus

L/C Lolium perenne / Cynosurus cristatus

D/P Dactylis glomerata / Poa annua / P. trivialis

An Anthoxanthum odoratum

Arrh Arrhenatherum elatius

U.G. Unidentified grasses

U.O. Unidentified species other than grasses.

Other species of Festuca, Agrostis and Lolium are indicated when appropriate.

Quantitative analysis

Estimates of the relative proportions of epidermal fragments in each category were made by area measurement. The material on a slide was scanned at a magnification of x 100 using a monocular microscope with a movable stage. Parallel traverses were made across each slide leaving strips of about a tenth of the diameter of a field of view between traverses to avoid duplicating measurements. The area of each epidermal fragment encountered was measured using a grid of small squares engraved on a graticule placed in the eyepiece of the microscope. Relative proportions were calculated from the total number of grid squares measured for each category. Very small fragments of less than half a square in area were ignored, but all other fragments were assigned to the various categories.

Data obtained in this form could be treated by analysis of variance after transforming percentages to degrees. Values of the standard error of the mean of a number of estimations, within 95% confidence limits ($S_{\bar{x}}\, t_{0.05}$),

were frequently calculated as a guide to the accuracy of the sampling methods and in drawing comparisons between samples.

CHAPTER 3

MEASUREMENT OF THE RELATIVE EFFECTS OF DIGESTION ON THE EPIDERMIS OF SEVEN GRASS SPECIES

CHAPTER 3

MEASUREMENT OF THE RELATIVE EFFECTS OF DIGESTION ON THE EPIDERMIS OF SEVEN GRASS SPECIES

Introduction

Although proportions of different food items in samples of faeces can be measured accurately, these values may not correspond with proportions ingested due to the intervening action of digestion. Early work on the practicability of determining correction factors to allow for this was restricted to diet analyses involving relatively large remains in faeces, which required little or no magnification to identify and could be measured by counting or volume. (Scott 1941; Jensen and Korschgen 1947; Adams 1957; Adams et al 1962).

When faecal analysis is carried out by identification of epidermal fragments, quantitative estimates of ingested proportions are further complicated by the fact that the proportions of species in the faeces are derived in terms of epidermal area ratios, whilst food intake is usually measured as ratios by weight. Some preliminary work by Hercus (1960) on a sheep fed fixed proportions by weight of clover and grass, indicated that correction factors may be necessary to relate proportions of epidermal fragments in faeces with ingested proportions. However, Storr (1961) found no significant differences between the ingested and resulting faecal proportions, expressed in both cases as ratios by area, of four herb species fed to quokkas (Setonix brachyrus). Stewart (1970) found that the epidermis of some East African grasses may be destroyed completely during digestion by certain ungulates. However during earlier work (Stewart 1967) on quantitative analysis of faeces from animals fed known diets, he concluded that it may be possible, although time-consuming, to establish correction factors to

allow for differential digestion of epidermis from different species.

The following experiment was carried out to investigate the role of differential digestion of grass epidermis in the determination of the diets of wild rabbits by faecal analysis, and to try to establish correction factors for a number of the commoner grasses which were involved in subsequent analyses.

The term digestibility is used throughout in the sense of organic matter surviving the process of digestion unchanged. As such it does not incorporate any implications of other nutritional values i.e. protein, soluble carbohydrate content etc.

Materials and Methods

Summary of experimental design

Captive wild rabbits were fed mixtures of pairs of grasses, containing equal proportions by surface area. The corresponding faeces were analysed to detect changes in these proportions by measuring the areas of epidermal fragments recognizable after digestion had taken place.

Preparation of the mixtures

The following grass species were included in the mixtures: Festuca rubra, Anthoxanthum odoratum, Agrostis tenuis, Dactylis glomerata, Arrhenatherum elatius, Holcus lanatus, Lolium perenne. The experiment was carried out during July when vegetative growth had slowed down and many of the grasses were flowering. The grass samples were obtained locally from selected areas of grassland wherever they could be found in almost pure stands. The limited availability of some of the species influenced the number of times they were used in the feeding experiments.

Surface area:fresh weight calibrations for the leaf blades of each species were made using photo-sensitive 'Ammonax' paper. For each species

the surface areas of four groups, each comprising six previously weighed complete laminae of random size, were determined. The mean weights per unit area of leaf (one surface) were calculated (Table 3.1A). All weighings were standardized by prior treatment of the grasses, firstly by totally immersing in water, then shaking and draining for 20 minutes and completely drying with paper tissue.

The individual weights of pairs of species making up a mixture weighing 15 g were calculated from the values of weight per unit area, so that each species in the mixture was represented by the same surface area (Table 3.1B). Only the leaves of the grasses were included in the mixtures, which were also free from any other contaminating species.

Experimental animals and feeding sequence

Five sibling wild rabbits were hand reared from the age of approximately 20 days. At the time of the experiment they were 3-4 months old and fairly accustomed to handling. The group consisted of three bucks (1, 2, 3) and two does (4, 5), individually housed in metal cages of dimensions 76 x 36 x 45 cm, each divided into an open and a closed compartment. The diet of the rabbits prior to the experiment was composed mainly of commercial rabbit pellets (which included compressed vegetation) together with small quantities of fresh vegetation.

For six days prior to the experiment and during the 26 days of controlled feeding, commercial rabbit pellets and all other foods containing grasses were withheld. Additional food during this time consisted of grain and vegetables.

Each rabbit was fed 15 g of prepared grass mixture a day. This quantity was usually completely eaten over a fairly short period of time, thus preference factors did not affect the proportions being digested at any one time. Each mixture was fed daily, in the early afternoon, to all five rabbits simultaneously, for four consecutive days. Intervals of two to four days were left between

TABLE 3.1A

Surface area/weight calibrations for laminae of grasses fed to rabbits in the digestion experiment.

	Mean weight/unit area of leaf (one surface) g/cm^2	Range (4 measurements)
Festuca rubra	0.0267	0.0210–0.0316
Anthoxanthum odoratum	0.0137	0.0133–0.0146
Agrostis tenuis	0.0200	0.0181–0.0232
Dactylis glomerata	0.0127	0.0114–0.0137
Arrhenatherum elatius	0.0125	0.0090–0.0153
Holcus lanatus	0.0132	0.0125–0.0137
Lolium perenne	0.0172	0.0162–0.0185

TABLE 3.1B

Weights of component species required in mixtures to give equal surface areas of the paired species

Festuca/Anthoxanthum	9.91/5.09 g
Agrostis/Dactylis	9.17/5.83
Holcus/Arrhenatherum	7.70/7.30
Anthoxanthum/Arrhenatherum	7.84/7.16
Anthoxanthum/Lolium	6.65/8.35
Anthoxanthum/Dactylis	7.78/7.22

Total weight of each mixture - 15 g.

offering different mixtures, during which no grasses were fed. The feeding sequence is given in Table 3.2. Since Rabbits 1, 2 and 5 refused most of the Holcus fed on the first day of the Holcus / Arrhenatherum series, this species was replaced by Anthoxanthum, and Holcus was subsequently fed only to Rabbits 3 and 4. During the final series of tests (4A and 4B), these two groups of rabbits were fed different pair mixtures in order to terminate the experiment. This had to be done earlier than intended as the rabbits were losing weight due to the limited diet they were receiving, which was probably beginning to affect the process of digestion.

Collection and analysis of faecal samples

Collections of droppings for analysis were made once or twice a day from each rabbit on the dates shown in Table 3.2. To ensure that these had been produced during the preceding time interval, all droppings were removed at the collection times. Samples were stored in formalin - acetic - alcohol before analysis.

Ten droppings from each collection were prepared as described in Chapter 2. Subsamples each consisting of four slides were analysed to find the relative proportions of each species by identifying and measuring the areas of epidermal fragments (See Chapter 2).

Results and Discussion

Analyses of the percentage composition of the faecal samples are given in Table 3.3. These values have been calculated from the total fragment measurements for the four slides in each subsample. The separate results for each rabbit are shown diagramatically in Figs. 3.1-3.5. Gaps in the tables are due to insufficient droppings having been produced to make up large enough samples. Values for the percentage composition of each sample from Rabbit 5 are also given as the means of the individual slide percentages to show

TABLE 3.2 Sequence of feeding and faecal collections

RABBIT	EXPT. SERIES NO.	GRASS SPECIES PAIR	DAYS FED	DATE	FAECES COLLECTION NO.
				JULY	
1 - 5	1	Anthoxanthum/Festuca	↑	10	
				11	1
				12	2, 3
				13	4, 5
				14	6
			↓	15	7
			↑	16	
1 - 5	2	Dactylis/Agrostis		17	1
				18	2, 3
				19	4
				20	5, 6
				21	7
				22	
				23	
1 - 5		Holcus/Arrhenatherum	↕	24	
3, 4	3A	Holcus/Arrhenatherum	↑	25	1 Results not recorded as insufficient Holcus eaten
1, 2, 5	3B	Anthoxanthum/Arrhenatherum		26	2, 3
				27	4, 5
				28	6
			↓	29	7
3, 4	4A	Dactylis/Anthoxanthum	↑	30	
1, 2, 5	4B	Lolium/Anthoxanthum		31	1, 2
				AUG.	
				1	3, 4
				2	5, 6
				3	7, 8
			↓	4	9

The results of the faeces collections which are underlined were used in the determination of relative digestibilities (Table 3.8A).

TABLE 3.3

Percentage Composition of Faecal Samples

SERIES 1. ANTHOXANTHUM and FESTUCA (Fed 10-13 July).

RABBIT		1		2		3		4		5	
Faeces Collection No.	Date	An	F	An	F	An	F	An	F	An	F
1	JULY 11 pm	32.9	67.1	-	-	33.3	66.7	-	-	30.9	69.1
2	12 am	41.0	59.0	13.7	86.3	-	-	19.6	80.4	16.2	83.8
3	pm	38.1	61.9	-	-	-	-	-	-	40.8	59.2
4	13 am	30.6	69.4	15.2	84.8	16.9	83.1	18.9	81.1	28.0	72.0
5	pm	35.2	64.8	-	-	35.7	64.3	-	-	36.1	63.9
6	14	28.1	71.9	24.5	75.5	37.1	62.9	37.4	62.6	29.1	70.9
7	15	32.7	67.3	35.7	64.3	-	-	42.2	57.8	29.1	70.9

SERIES 2. AGROSTIS and DACTYLIS (Fed 16-19 July).

RABBIT		1				2				3				4				5			
		An	F	Ag	D	An	F	Ag	D	An	F	Ag	D	An	F	Ag	D	An	F	Ag	D
1	17 pm	21.2	32.3	32.1	14.4	-	-	-	-	-	-	-	-	-	-	-	-	25.8	36.8	31.7	5.7
2	18 am	16.6	29.1	39.2	15.1	8.9	17.0	61.4	12.7	2.8	3.7	70.3	23.2	2.5	5.8	49.2	42.5	3.3	4.4	56.2	36.1
3	pm	5.7	8.2	63.7	22.4	-	-	-	-	-	-	-	-	-	-	-	-	2.0	2.9	69.5	25.7
4	19 am	3.7	8.6	69.8	17.8	3.5	5.4	73.1	18.0	1.2	2.3	71.7	24.8	2.1	5.4	75.6	17.0	0.8	2.6	73.8	22.8
5	20 am	1.3	5.1	75.3	18.3	2.8	5.1	76.2	13.6	0.5	1.5	85.7	12.2	1.0	2.6	81.6	14.0	0	3.3	75.5	21.2
6	pm	0.8	6.8	73.2	19.3	0.1	2.2	73.5	24.1	0	0.6	76.3	23.1	-	-	-	-	0	0.6	73.7	25.7
7	21 am	-	-	-	-	-	-	-	-	0	0.6	81.8	17.5	0	2.0	87.9	10.1	0	1.9	75.7	22.4

TABLE 3.3 continued.

SERIES 3A. HOLCUS and ARRHENATHERUM (Fed 24-27 July)

Faeces Collection No.	RABBIT Date	1	2	3 H	3 Arrh.	4 H	4 Arrh	5
1	JULY 25 pm			-	-	-	-	
2	26 am			64.4	35.6	51.1	48.9	
3	pm			-	-	62.7	37.3	
4	27 am			62.8	37.2	81.4	18.6	
5	pm			60.1	39.9	53.8	46.2	
6	28 am			62.8	37.2	-	-	
7	29			75.3	24.7	64.7	35.3	

SERIES 3B. HOLCUS and ARRHENATHERUM (Fed 24 July)

ANTHOXANTHUM and ARRHENATHERUM (Fed 25-27 July)

	RABBIT	1 H	1 An	1 Arrh	2 H	2 An	2 Arrh	3	4	5 H	5 An	5 Arrh
1*	25 pm	-	-	-	-	-	-			-	-	-
2	26 am	17.6	22.2	60.2	18.4	8.2	73.4			11.1	39.7	49.2
3	pm	3.9	71.0	25.1	-	-	-			9.7	50.9	39.4
4	27 am	2.4	78.2	19.4	7.4	64.7	27.9			5.0	56.0	39.0
5	pm	0	79.5	20.5	3.2	74.5	22.3			1.2	61.8	37.0
6	28 am	0	75.3	24.7	1.8	72.2	26.0			0.3	58.8	40.9
7	29	0	92.2	7.8	0	74.5	25.5			0	59.9	40.1

* Results not recorded as insufficient Holcus eaten.

TABLE 3.3 continued. SERIES 4A. ANTHOXANTHUM and DACTYLIS (Fed 30 July - 2 Aug.)

RABBIT		1	2	3				4				5
Faeces Collection No.	Date			H	Arrh	An	D	H	Arrh	An	D	
1	JULY 31 am			-	-	-	-	14.7	9.8	37.1	38.3	
2	pm			-	-	-	-	-	-	-	-	
3	AUG. 1 am			0.5	0	53.4	46.1	0	0	53.6	46.4	
4	pm			-	-	-	-	-	-	-	-	
5	2 am			0	0	71.3	28.7	0	0	58.4	41.6	
6	pm			-	-	-	-	-	-	-	-	
7	3 am			0	0	68.5	31.5	0	0	57.7	42.3	
8	pm			0	0	79.6	20.4	-	-	-	-	
9	4 pm			0	0	86.1	13.9	0	0	78.8	21.2	

SERIES 4B. ANTHOXANTHUM and LOLIUM (Fed 30 July - 2 Aug.)

RABBIT		1			2			3	4	5		
		Arrh	An	L	Arrh	An	L			Arrh	An	L
1	JULY 31 am	11.3	66.6	22.1	-	-	-			-	-	-
2	pm	0	52.2	47.8	0	43.2	56.8			0	71.5	28.5
3	AUG. 1 am	-	-	-	0	56.4	43.6			0	55.9	44.1
4	pm	0	80.3	19.7	0	51.2	48.8			0	73.1	26.9
5	2 am	-	-	-	0	63.6	36.4			0	64.0	36.0
6	pm	0	72.5	27.5	-	-	-			0	56.2	43.8
7	3 am	-	-	-	-	-	-			0	67.7	32.3
8	pm	-	-	-	0	84.2	15.8			-	-	-
9	4 pm	-	-	-	0	86.1	13.9			0	91.4	8.6

FIG. 3.1. Sequence of composition of faecal samples - RABBIT 1.

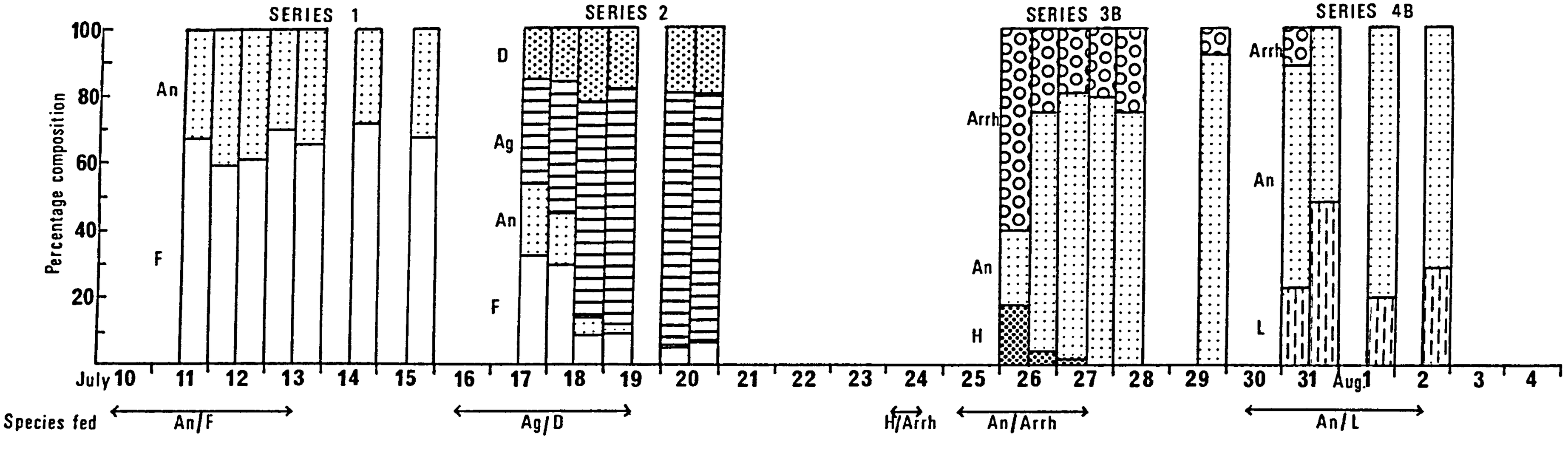


FIG. 3.2. Sequence of composition of faecal samples - RABBIT 2.

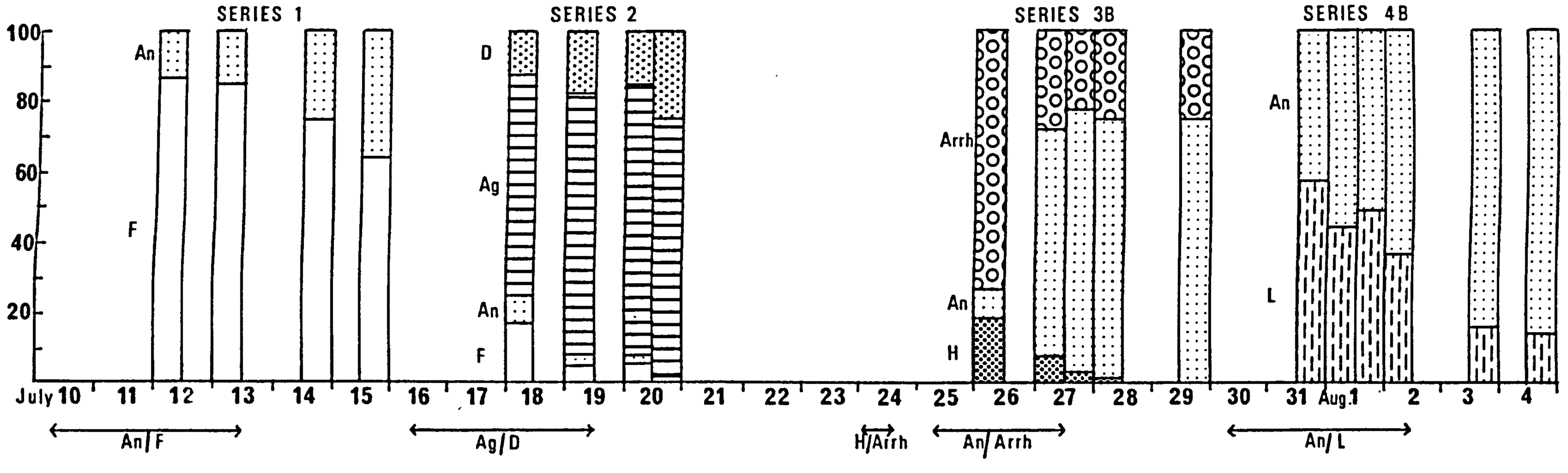


FIG. 3.3. Sequence of composition of faecal samples - RABBIT 3.

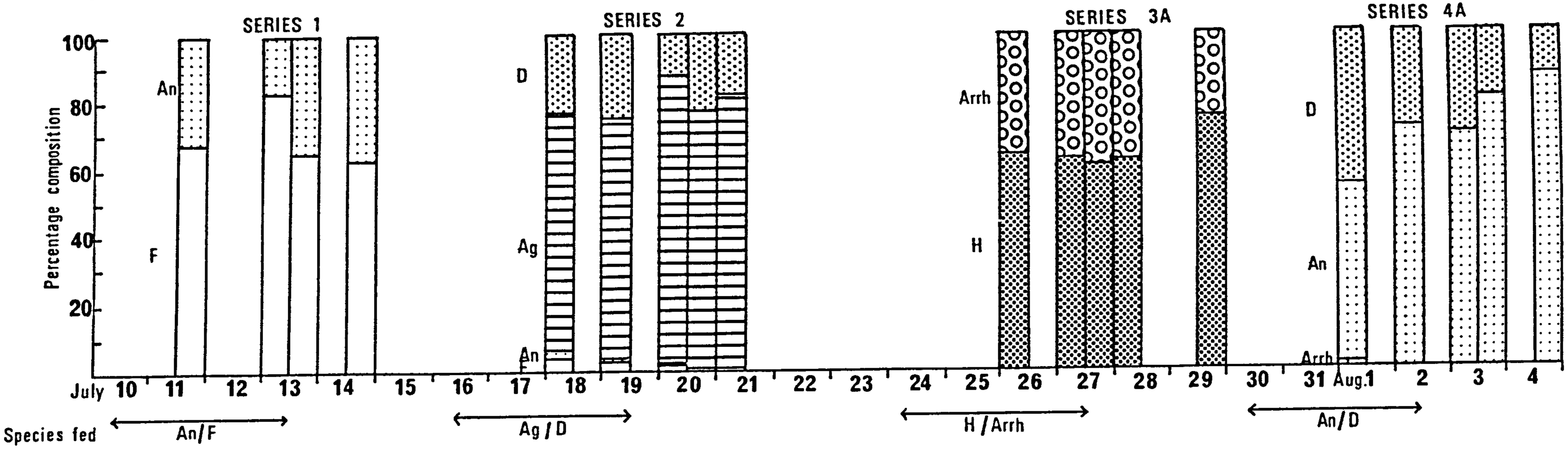


FIG. 3.4. Sequence of composition of faecal samples - RABBIT 4.

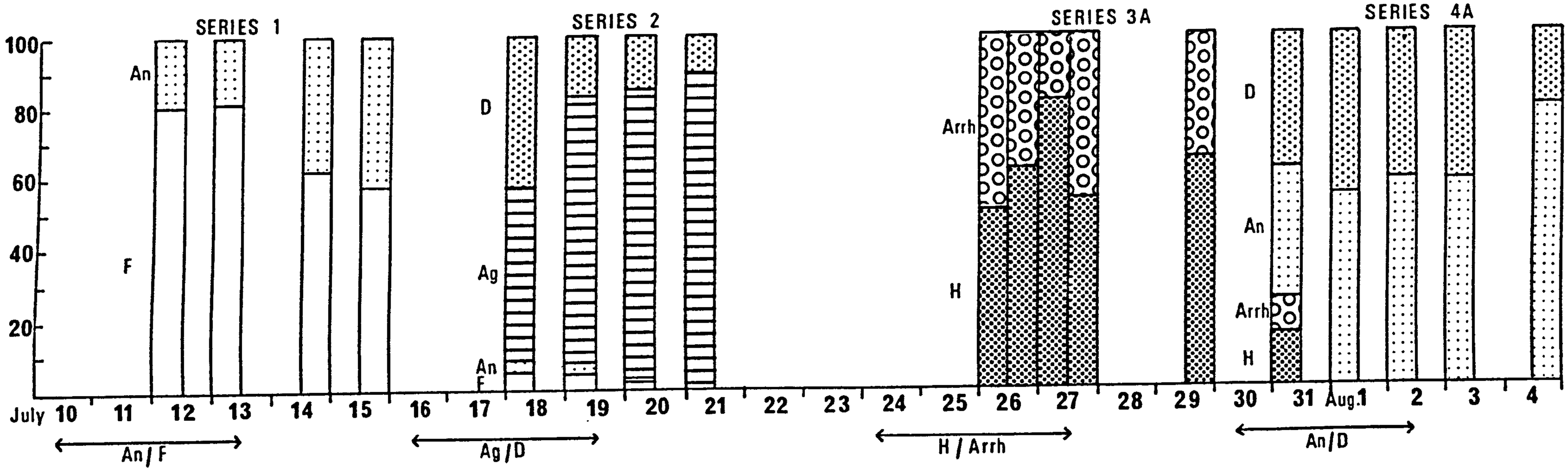
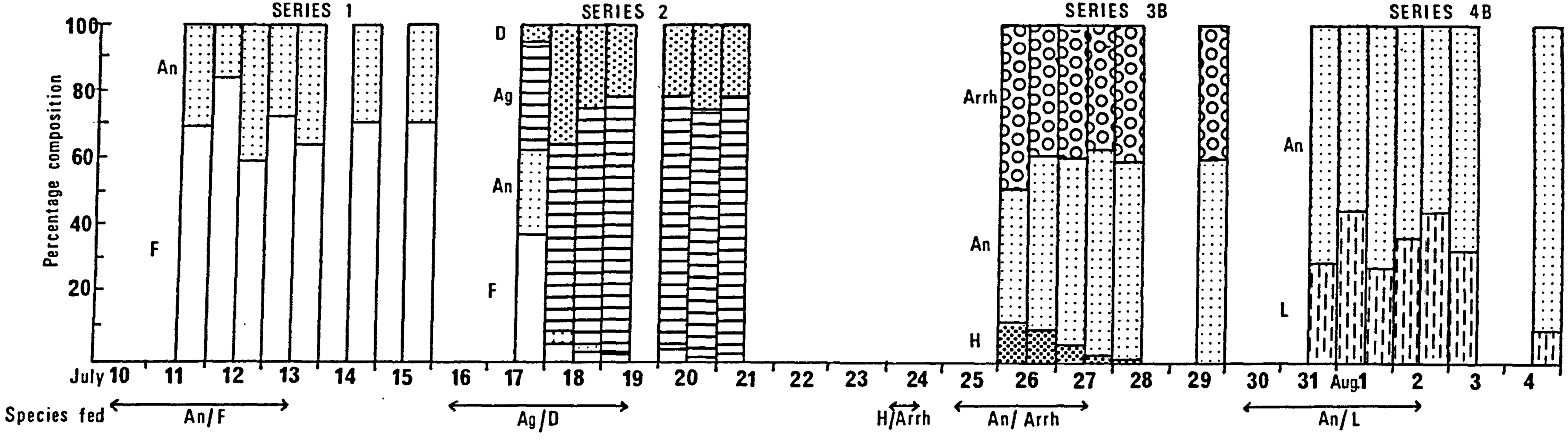


FIG. 3.5. Sequence of composition of faecal samples - RABBIT 5.



An Anthoxanthum odoratum

F Festuca rubra

D Dactylis glomerata

Ag Agrostis tenuis

Arrh Arrhenatherum elatius

H Holcus lanatus

TABLE 3.4 Percentage composition of faecal samples from Rabbit 5 comparing the total fragment counts from four slides with the means of the same four slides (± s.e.).

SERIES 1

SAMPLE	Anthoxanthum			Festuca		
	Total of 4 slides	Mean of 4 slides	s.e.			
1	30.9	31.1	± 1.1	69.1	68.9	± 1.1
2	16.2	16.3	1.6	83.8	83.7	1.6
3	40.8	41.8	3.4	59.2	58.2	3.4
4	28.0	27.9	2.2	72.0	72.1	2.2
5	36.1	37.4	5.3	63.9	62.6	5.3
6	29.1	27.9	2.8	70.9	72.1	2.8
7	29.1	29.8	5.3	70.9	70.2	5.3

SERIES 2

SAMPLE	Anthoxanthum			Festuca			Agrostis			Dactylis		
	Total	Mean	s.e.									
1	25.8	25.9	± 1.7	36.8	37.2	± 1.3	31.7	31.5	± 1.1	5.7	5.4	± 1.0
2	3.3	3.2	1.0	4.4	4.5	0.4	56.2	56.1	1.5	36.1	36.2	1.4
3	2.0	1.9	0.7	2.9	2.88	0.3	69.5	70.0	2.9	25.7	25.3	2.7
4	0.8	0.8	0.3	2.6	2.6	1.2	73.8	73.8	2.0	22.8	22.9	0.9
5	0	0		3.3	3.4	1.5	75.5	75.4	0.6	21.2	21.2	1.4
6	0	0		0.6	0.6	0.5	73.7	73.7	0.8	25.7	25.7	1.3
7	0	0		1.9	1.9	1.1	75.7	75.6	0.8	22.4	22.5	1.2

SERIES 3B

SAMPLE	Holcus			Anthoxanthum			Arrheratherum		
	Total	Mean	s.e.						
1	-	-		-	-		-	-	
2	11.1	10.8	± 0.8	39.7	40.3	± 3.6	49.2	48.9	± 3.3
3	9.7	9.8	1.4	50.9	51.2	2.6	39.4	39.0	3.0
4	5.0	4.9	0.9	56.0	56.1	0.8	39.0	39.0	1.5
5	1.2	1.2	0.7	61.8	61.7	1.3	37.0	37.1	1.7
6	0.3	0.4	0.2	58.8	58.8	4.0	40.9	40.8	3.9
7	0	0		59.9	60.9	5.7	40.1	39.1	5.7

SERIES 4B

SAMPLE	Anthoxanthum			Lolium		
	Total	Mean	s.e.			
1	-	-		-	-	
2	71.5	71.9	± 3.5	28.5	28.1	± 3.5
3	55.9	54.6	3.9	44.1	45.4	3.9
4	73.1	73.2	0.4	26.9	26.8	0.4
5	64.0	63.8	1.0	36.0	36.2	1.0
6	56.2	56.4	2.6	43.8	43.6	2.6
7	67.7	67.3	3.9	32.3	32.7	3.9
8	-	-		-	-	
9	91.4	92.4	3.0	8.6	7.6	3.0

variation between slides (Table 3.4). The differences in sampling variance were thought to be due to the occurrence of occasional large fragments rather than to insufficient mixing of the particle suspensions before withdrawing the subsamples. It was for this reason that the estimates of percentage composition were calculated from the total fragment counts over the four slides in each subsample, rather than from the means of the individual slide results. However, the two sets of results for Rabbit 5 calculated both as mean of four slides and total, corresponded closely (Table 3.4).

Elimination time

The first samples of each series were usually collected approximately 30 hours after the first feed of a particular grass mixture. By this time the species in the mixture were always recorded in the faeces. The approximate time intervals between the last feed of a species and its final appearance in the faeces can be determined from the data in Table 3.3. Festuca was still present in the samples 7 or 8 days after feeding, but had disappeared by the beginning of the following series, five days later, when the next faecal samples were collected. Anthoxanthum disappeared from the faeces of Rabbits 3, 4 and 5 after 6 or 7 days, but was still present in the last sample of the series (Day 7) from Rabbits 1 and 2. There is little information on the persistence of Agrostis and Dactylis, fed in Series 2. Agrostis, but not Dactylis, was still present in the two unrecorded first samples of Series 3, from Rabbits 1 and 5, i.e. six days after feeding. Neither species was recorded in any of the samples on the following day. Holcus persisted for 3 to 5 days after the last feed, and Arrhenatherum for less than 4, to 5 days. Lolium persisted for at least 2 days after the final feed in Series 4B, but further information was not obtained.

The species, listed in order of elimination time are summarized over-leaf;

	Festuca	7+ to 8+ days
	Anthoxanthum	6 to 7+
	Agrostis	? to 6
	Holcus	3 to 5
	Arrhenathemum	<4 to 5
{	Dactylis	<6 }
{	Lolium	>2 }

These results appear to be positively correlated with the digestibility of the epidermis (as shown later in this chapter), Festuca epidermis being affected least by digestion and Arrhenatherum most. Since rabbits are coprophagous (Madsen 1939; Eden 1940) a proportion of the relatively indigestible food particles, such as leaf epidermis, may under natural conditions pass several times through the digestive system before appearing in the faeces. The caecotrophes or reingested pellets have been shown to contain about 50% undigested plant material, the remainder consisting of microorganisms derived from the caecum which are a source of aminoacids and other bacterial products (Eden 1940; Griffiths and Davies 1963; Herning and Hird 1972). Presumably after a certain length of time any remaining epidermis is completely broken down or becomes unidentifiable. This would explain the correlation between elimination time and digestibility.

Eden (1940) found that when copper was included in the feed it was eliminated by normal rabbits over a period of about five weeks, but this time was considerably reduced in rabbits prevented from carrying out the reingestion process. However, Piekarz (1963) found that the elimination time of a dye used to stain the feed, was only two to three days. There appear to be no published measurements of elimination time in normal rabbits using radioactive tracers. Stewart (1967) recorded the presence of identified grass epidermis in the faeces of East African herbivores for about three days after

feeding the grasses to non-ruminants, and five to six days in ruminants. Since ruminants can retain certain undigested food items for 12-13 days before elimination (McAnally and Phillipson 1944) a similar situation to that in the rabbit appears to exist with regard to digestion and elimination of plant epidermis.

Elimination time is an important factor to be considered if the diet of animals feeding on a sequence of vegetation types is studied by faecal analysis. In the present study this consideration did not arise since the diets of rabbits inhabiting and feeding on particular vegetation types were being investigated.

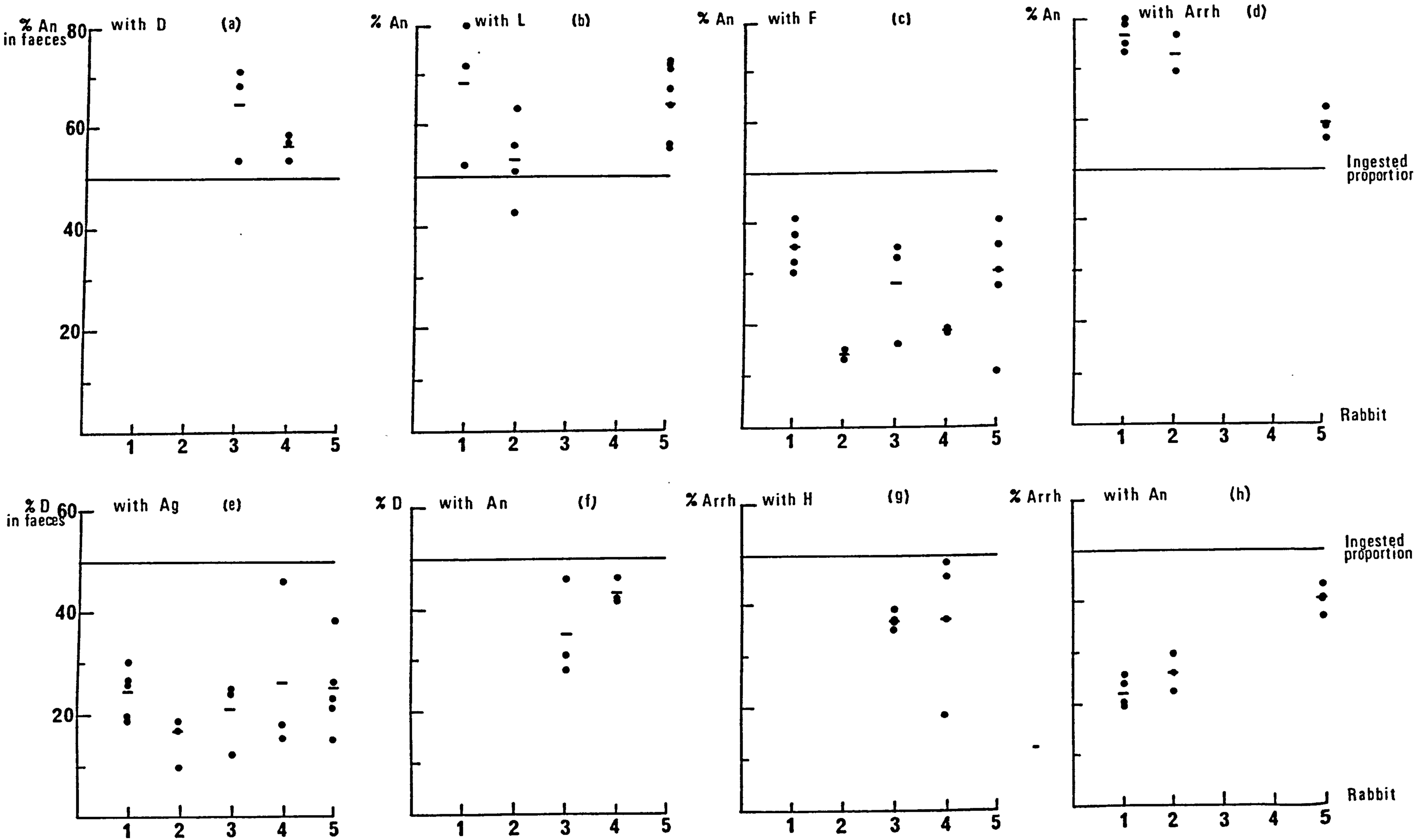
Relative digestion of epidermis

Because of the differences in elimination rates between species, only the results for the samples collected during the feeding period and the morning immediately following were used in the determination of relative digestibilities. Relative proportions (%) of the two components of each mixture were recalculated where necessary, omitting other species still present in the faeces from previous trials. The appropriate collections of faeces (samples) are underlined in Table 3.2. The individual sample results for each rabbit and each pair of grasses fed are shown diagramatically in Fig. 3.6.

Since all the results are in the form of relative proportions, the greatest number of comparisons between species can be made from the data in the form shown in these diagrams. Fig.3.6(a) - (d) illustrate the percentages of Anthoxanthum in the faeces when it was fed with four other species, (e) and (f) the percentages of Dactylis with two other species and (g) and (h) Arrhenatherum with two species. If the epidermis of each species was digested to the same extent, the expected results would be scattered around a mean of 50% in each case. However, the measurements show considerable deviations from

FIG. 3.6 Proportions, by area measurements, of Anthoxanthum, Dactylis and Arrhenatherum, expressed as percentages, after feeding equal proportions (by surface area) of pair mixtures. Only those samples collected during the feeding period are included.

• Individual samples – Mean



this proportion, indicating that differential digestion had taken place.

The results were analysed by analysis of variance after converting percentages to degrees using the angular transformation tables in Bliss (1970), (Tables 3.5,3.6 & 3.7).The faecal proportion of each grass in each pair mixture was found to deviate significantly ($p < 0.05$) from the ingested proportion of 50% (45 degrees).

Hypothetical values for the relative digestibilities of pairs of grasses which had not been fed together in mixtures were calculated using the faecal proportions obtained when they had been ingested with the same partner species (i.e. Anthoxanthum, Dactylis or Arrhenatherum). This was based on the assumption that there were no interaction effects between different pairs of species. The results of statistical analysis are given in Tables 3.5, 3.6 and 3.7, and the comparisons are summarized below.

Comparing spp. fed	Digestibilities of
1. with Anthoxanthum	Dactylis and Lolium do not differ significantly ($p > 0.05$) (Dactylis + Lolium), Festuca and Arrhenatherum all differ significantly ($p < 0.05$).
2. with Dactylis	Agrostis and Anthoxanthum differ significantly ($p < 0.001$).
3. with Arrhenatherum	Holcus and Anthoxanthum do not differ significantly ($p > 0.05$).

Significant differences were found between the results from individual rabbits within each series (see Tables 3.5 & 3.7). In spite of these variations, the individual rabbit means for each series were used to calculate overall mean (%) values of the faecal proportions of each species within each experimental series (Table 3.8A) .From these values was derived the relative

TABLE 3.5

Proportions by area measurements, of Anthoxanthum in faeces samples, expressed in degrees, after feeding equal proportions (by surface area) of pair mixtures. Only those samples collected during the feeding period are included.

ANTHOXANTHUM WITH	(a) DACTYLIS		(b) LOLIUM			(c) FESTUCA					(d) ARRHENATHERUM			see Fig. 3.6
RABBIT	3	4	1	2	5	1	2	3	4	5	1	2	5	
	47.12	47.06	46.26	41.09	57.73	35.00	21.72	35.24	26.28	33.77	59.28	56.66	48.68	
	57.61	49.84	63.65	48.68	48.39	39.82	22.95	24.27	25.77	23.73	63.51	61.34	50.18	
	55.86	49.43	58.37	45.69	58.76	38.12		36.39		39.70	63.08	59.02	52.30	
				52.89	53.13	33.58				31.95	60.20		50.18	
					48.56	36.39				36.93				
					55.37									
Rabbit mean	53.53	48.78	56.09	47.09	53.66	36.58	22.34	31.97	26.03	33.22	61.52	59.00	50.34	

Deviation of faecal proportions from ingested proportions

Observed grass mean	51.15		52.20			31.86					56.77			
Expected grass mean	45.00		45.00			45.00					45.00			
Difference (O-E)	6.15		7.20			13.14					11.77			
$S_{\bar{X}}t_{0.05}$	4.91		$S_{\bar{X}}t_{0.001}$	5.84		$S_{\bar{X}}t_{0.001}$	5.11				$S_{\bar{X}}t_{0.001}$	6.36		
Probability	<0.05		<0.001			<0.001					<0.001			

TABLE 3.5 continued.

Analysis of variance

	d.f.	Sum of squares	Mean square	F	p
Between grasses	3	5330.67	1776.89	19.09	< 0.001
Within grasses					
Between rabbits	9	837.71	93.08	4.56	< 0.01
Within rabbits					
Between samples	34	683.63	20.40		
Total	46	6862.01			

Differences between grasses

	mean	no. of observations		mean	n
Dactylis	51.15	6	Dactylis	51.15	6
Lolium	52.20	13	Arrhenatherum	56.77	11
Difference	1.05	($p < 0.05$)	Difference	5.62	($p < 0.05$)
Least sig. diff. $t_{0.05}$	4.49		L.S.D. $t_{0.05}$	4.68	
Dactylis	51.15	6	Lolium	52.20	13
Festuca	31.86	17	Arrhenatherum	56.77	11
Difference	19.29	($p < 0.001$)	Difference	4.57	($p < 0.05$)
L.S.D. $t_{0.001}$	7.66		L.S.D. $t_{0.05}$	3.76	

TABLE 3.6

Proportions, by area measurements, of Dactylis in faeces samples, expressed in degrees, after feeding equal proportions (by surface area) of pair mixtures. Only those samples collected during the feeding period are included.

DACTYLIS WITH	(e) AGROSTIS					(f) ANTHOXANTHUM		see Fig. 3.6.
RABBIT	1	2	3	4	5	3	4	
	33.83	24.50	29.87	42.88	23.03	42.88	49.94	
	31.82	26.35	30.46	25.33	38.70	32.39	40.16	
	30.72	22.87	20.70	23.11	31.31	34.14	40.57	
	26.78				29.06			
	26.21				27.90			

Deviation of faecal proportions from ingested proportions

Rabbit mean	29.87	24.57	27.01	30.44	30.00	36.47	41.22
Observed grass mean			28.71			38.85	
Expected grass mean			45.00			45.00	
Difference (O-E)			16.29			6.15	
		$S_{\bar{x}}t_{0.001}$	4.67			$S_{\bar{x}}t_{0.01}$	6.13
p			<0.001				<0.01

Analysis of variance

	d.f.	Sum of squares	Mean square	F	p
Between grasses	1	468.84	468.84	19.87	<0.001
Within grasses					
Between rabbits	5	117.95	23.59	0.78	<0.05
Within rabbits					
Between samples	18	542.65	30.15		
Total	24	1129.44			

TABLE 3.7.

Proportions, by area measurements, of Arrhenatherum in faeces samples, expressed in degrees, after feeding equal proportions (by surface area) of pair mixtures. Only those samples collected during the feeding period are included.

ARRHENATHERUM WITH	HOLCUS		ANTHOXANTHUM		
RABBIT	3	4	1	2	5
	36.63	44.37	30.72	33.34	41.32
	37.58	37.64	26.49	28.66	39.82
	39.17	25.55	26.92	30.98	37.70
	37.58	42.82	29.80		39.82

Deviation of faecal proportions from ingested proportions

Rabbit mean	37.74	37.60	28.48	31.00	39.67
Observed grass mean	37.67		33.23		
Expected grass mean	45.00		45.00		
Difference (O-E)	7.33		11.77		
$S_{\bar{x}}t_{0.01}$	5.68		$S_{\bar{x}}t_{0.001}$	6.63	
p	<0.01		<0.001		

Analysis of variance

	d.f.	Sum of squares	Mean Square	F	p
Between grasses	1	91.05	91.05	1.01	<0.05
Within grasses					
Between rabbits	3	270.85	90.28	5.01	<0.05
Within rabbits					
Between samples	14	252.37	18.03		
Total	18	614.26			

TABLE 3.8A.

Mean proportions of grass species estimated in faeces after feeding different mixtures (calculated from mean values for individual rabbits). Faeces collected during the feeding period.

(a)	Anthoxanthum	with	Dactylis
	60.5		39.5 %
(b)	Anthoxanthum	with	Lolium
	62.1		37.9
(c)	Anthoxanthum	with	Festuca
	28.4		71.6
(d)	Anthoxanthum	with	Arrhenatherum
	69.6		30.4
(e)	Dactylis	with	Agrostis
	23.5		76.5
(v)	Arrhenatherum	with	Holcus
	37.6		62.4

TABLE 3.8B.

Hypothetical values for relative proportions of species expected in faeces if all seven were fed simultaneously in equal proportions. These figures are derived from the values given in Table 3.8A.

	percentage by surface area	percentage by fresh weight
Festuca	31.2	21.0
Agrostis	26.5	23.8
Anthoxanthum	12.4	16.2
Holcus	8.9	12.1
Dactylis	8.0	11.3
Lolium	7.5	7.8
Arrhenatherum	5.4	7.8

proportion of each species expected in the faeces if all seven were fed simultaneously in equal proportions by surface area. In order to calculate these proportions the assumption was made that no species interaction effects occur during the process of digestion. The validity of this assumption was not tested, but it seems unlikely that such interactions do occur under normal conditicns.

These expected proportions are direct estimates of the relative survival of the seven species during digestion, since the ingested proportions were equal by surface area, and the faecal proportions of epidermis were obtained by area measurement. However, to be of practical use in the subsequent investigations into diets of wild rabbits by faecal analysis, the estimates were converted to expected faecal proportions resulting from the ingestion of equal proportions by fresh weight instead of surface area (Table 3.8B). The original calibrations of weight/unit area (Table3.1)were used for this conversion.

Conclusions

The results of these controlled feeding experiments suggest that differential digestion of grass epidermis is a factor to be taken into consideration in the study of food preferences of the rabbit by faecal analysis. The differences measured in the experiments were reduced to a certain extent by conversion of the ingested proportions from ratios of surface area to ratios by fresh weight, as shown in Table 3.8B.Even so, there was a difference of a factor of x3 between the survival of epidermis from those species with the most digestible epidermis (Arrhenatherum and Lolium) and those with the least digestible (Agrostis and Festuca).

The correction factors calculated from the experiments reflect the trend of values obtained from a number of measurements of Dry Matter

Digestibility of these grass species (see Spedding 1972). Dry or Organic Matter Digestibility, a measure frequently used in work on the nutrition of agricultural stock, relates the proportion of indigestible to digestible material in a given foodstuff. In the case of the leaves of grasses, the indigestible fraction is composed of vascular bundles, sclerenchyma and epidermis and the individual quantities of these components tend to be directly correlated (Regel 1960; Osbourn et al 1974). This suggests that the correction factors determined in this experiment are valid estimates of epidermal digestibilities and consequently the values were used to calibrate subsequent analyses of wild rabbit droppings.

The prolonging, by coprophagy, of the process of digestion on a proportion of the food ingested by the rabbit appears to be responsible for the correspondence which was found between the digestibility of epidermis and elimination time of the different grasses. Epidermis of lower digestibility is likely to survive the process for longer than more fragile epidermis of greater digestibility.

CHAPTER 4

FOOD SELECTION BY RABBITS ON DIFFERENT TYPES OF GRASSLAND

CHAPTER 4

FOOD SELECTION BY RABBITS ON DIFFERENT TYPES OF GRASSLAND

Introduction

Food selection by grazing animals depends on a number of factors which could invalidate generalizations on palatability and preferences made from observations on a single population of animals grazing on one particular type of vegetation. The following study was made to investigate whether any consistent trends in selection could be detected by comparing the relationships between species available on a number of widely differing grasslands and their selection by resident rabbit populations, as measured by faecal analysis.

MAP 1 Location of sampling sites.

Methods

Summary of methods

The diets of rabbits inhabiting eight contrasting lowland grassland sites in North Wales were investigated by analysis of samples of faeces collected during the months of July and August. The species composition of the vegetation at each site was measured and the relative proportions of identifiable species in corresponding samples of faeces were compared.

Site descriptions

The locations of the sites are shown on Map 1. Photographs of each site are given in Appendix 4.

SITE 1 Permanent pasture, College Farm, Aber.

Grazing, mainly along the northern edge of the field, was carried out by rabbits inhabiting brambles and scrub on an adjacent railway embankment. The field was also sporadically grazed by sheep, cattle and horses. The most consistent components of the vegetation were Agrostis tenuis, Holcus lanatus and Lolium perenne.

SITE 2 Sown pasture, College Farm, Aber.

The sampling site consisted of well-grazed vegetation within a meteorological enclosure, easily accessible to rabbits inhabiting the hedge boundary of the surrounding field. The site and field had been ploughed and seeded with various agricultural grasses and clover. The site contained open plots of pure swards of Festuca arundinacea and L. perenne, surrounded by areas of Poa annua and Trifolium repens. The surrounding sward consisted mainly of L. multiflorum.

SITE 3 Conwy - rough grassland on flat sandy area adjacent to river estuary.

The area was composed of a mosaic of short turf grazed by rabbits, and taller, rank grass, with thick brambles. The main components of the well-grazed areas were A. tenuis, Festuca rubra and Poa pratensis.

SITE 4 Newborough Warren - sand-dune system.

This extensive area supported a high density of rabbits, reflecting its previous use for active rabbit farming which was started some 200-300 years ago. Grazing took place mainly in the dune slacks where the predominant vegetation was Salix repens, Equisetum variegetum and Carex spp., with very low frequencies of grasses.

SITE 5 Penmon - limestone grassland.

The sampling site consisted of a raised hummock of short, well-grazed vegetation, dominated by F. rubra and F. ovina, with a high proportion of herb species. The area was almost completely surrounded by brambles inhabited by a number of rabbits.

SITE 6 Benllech - limestone grassland.

The vegetation was similar to that at Site 5, but the area was more extensive with many gorse thickets inhabited by a large population of rabbits. The area had also been grazed by cattle.

SITE 7 Bangor Normal College grounds.

Sampling was carried out on a well mown lawn which showed the greatest evidence of rabbit grazing when compared with areas of rough grassland nearby. The main components of the lawn were H. lanatus and L. perenne.

SITE 8 Bangor Ancient Camp.

This area of rabbit grazed Agrostis-fescue grassland is described fully

in Chapter 5 in which seasonal changes in diet are described for this site. The results of Sample 10 (see Chapter 5) are included in this section for comparison with those of the other seven sites.

Estimation of vegetation composition

The species composition of the most heavily grazed areas at each of the eight sites was determined by means of point transects. At each site these grazed areas were very distinctive and fairly small in relation to the total rabbits' range over most of which the vegetation was apparently only sporadically or not at all grazed. The transects were made across representative portions of the close-grazed turf covering distances of between 10 and 40 m depending on its area. The point transect method aided identification of species, particularly grasses, which might otherwise have been overlooked, and provided rough estimates of their relative abundances. The repeatability of the method was tested at Site 3 where two separate transects were made across the same well-grazed area. Grasses not occurring along the transects, but locally abundance elsewhere on the sites were also noted. These measurements were made at the same time as the samples of droppings were collected.

Collection and analysis of faecal samples

At each site a representative mixture of droppings was collected by removing single specimens from several dung heaps and scatterings over the whole area. Only fresh droppings were included in the samples, which were stored in formalin-acetic-alcohol until analysed.

At Site 3, three separate collections were made from three different well-grazed areas to test whether there were major differences caused by the samples containing insufficient droppings to be representative, and/or the location of the samples. Each of the seven other samples was composed of droppings collected from over the whole of each site.

Before carrying out the main analysis of each sample a trial analysis was performed using a suspension made up of 5 or 6 droppings prepared in the same way as for the main analysis. Several slides had to be examined in order to become accustomed to identifying epidermal fragments of the different species and different combinations of species, using reference slides for comparison.

The final analysis of each sample was made using twenty droppings which were cut in half to reduce the bulk of material and so that an identical sample could be stored for future analysis if required. The samples were prepared for analysis as described in Chapter 2. The relative proportions by area of cuticle fragments were calculated from the total area counts of each species or species group from five slides in most cases. The accuracy of this subsample size was investigated by repeat sampling of the suspension of faeces from Site 1.

Results and Discussion

Estimates of the composition of vegetation and the results of faecal analysis are given in Tables 4.1 & 4.2. Discussion of the results from the individual sites precedes overall comparisons between the sites.

Size of subsample analysed

The relative proportions of plant species present in the faeces estimated from the two subsamples, each of five slides from the Site 1 sample, are given in Table 4.2. These proportions were calculated from the total areas of epidermis measured, rather than the means of the results from individual slides (for the reasons given in Chapter 3). The maximum difference between subsamples was 4.8% (*Holcus*). Statistical analysis (Table 4.3) of the individual slide

TABLE 4.1

Species composition at the eight sampling sites estimated by point sampling.

SITE 1 Aber, permanent pasture. 1.8.73 (200 points at 10 cm intervals).

	% Composition
Holcus lanatus	27.5
Agrostis tenuis	20.0
Lolium perenne	19.0
Anthoxanthum odoratum	6.5
Poa pratensis	4.0
Arrhenatherum elatius	3.0
Festuca rubra	2.0
Cynosurus cristatus	1.5
Dactylis glomerata	0.5
Trifolium repens	5.5
Leontodon autumnalis	3.0
Ranunculus acris	0.5
Achillea millefolium	0.5
Luzula campestris	0.5
Dead vegetation	6.0

SITE 2 Aber, meteorological enclosure. 3.7.73

1. Within enclosure (200 points at 15 cm intervals)

(a) vegetation between plots.

Poa annua	44.0
Festuca arundinacea	28.0
Lolium multiflorum	1.0
Bromus mollis	0.5
Trifolium repens	19.5
Ranunculus spp.	4.0
Bellis perennis	1.5
Bare ground	1.5

(b) Plots - Festuca arundinacea
Lolium perenne

2. Outside enclosure.

(a) Pure Lolium multiflorum sward

(b) Edge of field (well-grazed area) Poa annua
Phleum pratense
Agrostis tenuis
Dactylis glomerata
Trifolium repens
Ranunculus repens
Matricaria matricarioides

TABLE 4.1 continued.

SITE 3 Conwy. 28-29.6.73 (Transect 1 - 200 points at 15 cm intervals
Two contrasting grazed areas. (Transects 2 and 3 - 100 points at 15 cm intervals)

	Area 1 Transect 1	Area 3 Transect 2	Transect 3
Festuca rubra	33.0	26	24
Poa pratensis	9.5	17	16
Agrostis tenuis	8.0	4	4
Dactylis glomerata	1.5	3	12
Lolium perenne	0	2	3
Aira praecox	3.5	0	0
Bromus mollis	1.5	1	0
Cynosurus cristatus	0.5	0	0
Agropyron junceiforme	0	1	0
Trisetum flavescens	0	0	1
Thymus drucei	8.5	2	0
Lotus corniculatus	4.5	7	3
Trifolium spp.	4.5	8	8
Hieracium spp.	3.0	0	0
Plantago lanceolata	1.0	3	9
Potentilla reptans	0	0	2
Carex spp.	0.5	3	1
Bryophytes	14.5	16	2
Bare ground	6.0	7	15

Patches of other grasses available -
Anthoxanthum odoratum
Holcus lanatus
Arrhenatherum elatius
Ammophila arenaria

SITE 4 Newborough. 5.7.73 (Each transect - 100 points at 20 cm intervals)
Transect 1 across wet slack. Transect 2 across small dry slack.

	Transect 1	Transect 2
Agrostis stolonifera	8	0
A. tenuis	0	1
Poa pratensis	0	11
Holcus lanatus	0	5
Salix repens	32	15
Equisetum variegatum	9	0
Anagallis tenella	5	0
Euphrasia agg.	1	0
Lotus corniculatus	2	19
Hydrocotyle vulgaris	3	4
Trifolium repens	0	6
Leontodon autumnalis	2	7
Potentilla anserina	0	7
Linum catharticum	0	1
Polygala vulgaris	0	1
Ranunculus flammula	1	0
Carex spp.	28	18
Taraxacum officinale	0	5
Bryophytes	2	0
Bare ground	7	0

Other available grasses -
Anthoxanthum odoratum
Festuca rubra
Phleum arenaria
Sieglingia decumbens
Cynosurus cristatus

TABLE 4.1 continued.

SITE 5 Penmon. 2.7.73 (100 points at 10 cm intervals - area appeared fairly homogeneous).

	% Composition
Festuca ovina	19
F. rubra	7
Koeleria cristata	7
Sieglingia decumbens	5
Briza media	3
Agrostis tenuis	1
Thymus drucei	11
Helianthemum chamaecistus	21
Poterium sanguisorba	3
Carex flacca	8
Trifolium repens	1
Bryophytes	8
Bare soil/rock	6

SITE 6 Benllech. 4.7.73 (200 points at 20 cm intervals)

Festuca ovina	14.0
F. rubra	1.5
Sieglingia decumbens	7.0
Briza media	9.5
Koeleria cristata	5.0
Agrostis stolonifera	5.0
A. tenuis	4.0
Anthoxanthum odoratum	1.0
Dactylis glomerata	1.0
Thymus drucei	7.0
Helianthemum chamaecistus	8.5
Poterium sanguisorba	4.0
Galium verum	3.0
Lotus corniculatus	3.0
Plantago lanceolata	2.5
Hieraceum pilosella	1.0
Carex flacca	13.5
Bryophytes	6.0
Bare soil/rock	3.5

TABLE 4.1 continued.

SITE 7 Bangor Normal College 20.7.73 (100 points at 15 cm intervals - vegetation appeared fairly homogeneous)

	% Composition
Holcus lanatus	35
Lolium perenne	12
Anthoxanthum odoratum	9
Agrostis stolonifera	6
A. tenuis	5
Cynosurus cristatus	5
Festuca rubra	2
Dactylis glomerata	2
Poa pratensis	1
Trifolium repens	8
Bellis perennis	4
Achillea millefolium	3
Ranunculus repens	3
Prunella vulgaris	3
Luzula campestris	1
Bryophytes	1

SITE 8 Bangor Ancient Camp 27.6.73 (250 points at 5 cm intervals)

Agrostis tenuis	60.8
Festuca rubra	8.4
Anthoxanthum odoratum	5.2
Dactylis glomerata	3.6
Holcus lanatus	4.8
Arrhenatherum elatius	1.2
Plantago lanceolata	2.4
Lotus corniculatus	0.4
Conopodium majus	1.2
Bryophytes	7.2
Dead vegetation	4.8

TABLE 4.2 Analysis of Faecal Samples

SITE 1 Aber, permanent pasture.

Slides	% composition 1-5	6-10	1-10	% composition (1-10) omitting seed
Lolium/Cynosurus	44.6	40.5	42.5	51.6
Holcus lanatus	11.0	15.8	13.5	16.4
Festuca arundinacea	6.0	4.0	5.0	6.1
F. rubra	0.7	0.7	0.7	0.9
Agrostis tenuis	0	3.1	1.6	1.9
Anthoxanthum odoratum	0.3	2.0	1.1	1.4
Arrhenatherum elatius	0.9	0.5	0.7	0.8
Dactylis glomerata	0.9	0.4	0.7	0.8
Seed	16.8	18.6	17.8	-
Unidentified grasses	4.2	3.7	3.9	4.8
All other spp.	14.6	10.7	12.5	15.3
Total area measured (microscopic grid squares, see text)	2538	2720	5258	

SITE 2 Aber, meteorological enclosure.

	% composition from total of 5 slides	% composition omitting seed
Lolium multiflorum	44.7	49.1
Festuca arundinacea	36.7	40.2
Poa annua	2.3	2.5
Seed	8.9	-
Unidentified grasses	1.2	1.4
All other spp.	6.2	6.8
Total area measured in grid squares on 5 slides	7135	

SITE 3 Conwy.

	% composition from total of 5 slides				% composition omitting seed (mean)
	Area 1	Area 2	Area 3	Mean	
Festuca rubra/ Poa pratensis	25.8	16.1	14.2	18.7	41.4
Lolium/Cynosurus	5.2	2.8	4.1	4.0	8.7
Agrostis tenuis	1.0	0	0.1	0.4	0.8
Anthoxanthum odoratum	+	0	0	+	+
Arrhenatherum elatius	0.1	0	0	+	0.1
Dactylis glomerata	0	0	0.4	0.1	0.3
Seed	52.0	52.8	57.2	54.0	-
Unidentified grasses	1.9	1.0	0.7	1.3	2.7
All other spp.	14.0	27.3	23.3	21.5	46.0
Total area measured *grid squares on 5 slides*	7660	7442	5600		

SITE 4 Newborough.

	% composition from total of 5 slides	% composition omitting seed
Festuca rubra/ Poa pratensis	8.1	12.7
Lolium/Cynosurus	0.2	0.4
Holcus lanatus	2.8	4.4
Agrostis spp.	0.1	0.1
Anthoxanthum odoratum	0.2	0.4
Seed/other thick tissue	36.1	-
Unidentified grasses	0.3	0.1
Equisetum variegatum	36.7	57.4
Carex spp.	0.1	0.1
All other species	15.6	24.4
Total area measured in grid squares on 5 slides	8575	

TABLE 4.2 continued.

SITE 5 Penmon	% composition from total of 6 slides	% composition omitting seed
Festuca rubra/ovina/Poa pratensis	30.2	51.1
Sieglingia /Koeleria /Briza	3.0	5.1
Agrostis spp.	0.3	0.4
Seed	40.9	-
Unidentified grasses	2.3	3.8
Carex spp.	+	0.1
All other spp.	23.3	39.5
Total area measured in grid squares on 6 sides	4185	

SITE 6 Benllech	% composition from total of 5 slides	% composition omitting seed
Festuca spp./Poa pratensis	11.6	22.5
Koeleria/Briza	18.4	35.7
Sieglingia decumbens	0	0
Anthoxanthum odoratum	0.2	0.4
Agrostis spp.	0.2	0.4
Holcus lanatus	0.1	0.2
Seed	48.5	-
Unidentified grasses	1.3	2.6
Carex spp.	6.1	11.8
All other spp.	13.6	26.4
Total area measured in grid squares on 5 sides	7636	

SITE 7 Normal College	% composition from total of 5 slides	% composition omitting seed
Lolium/Cynosurus	15.8	29.1
Holcus lanatus	14.5	26.8
Festuca rubra/Poa pratensis	6.2	11.4
Dactylis glomerata	0.6	1.1
Agrostis spp.	2.3	4.3
Bromus sp.	1.9	3.5
Poa annua	0.6	1.0
Seed	45.7	-
Unidentified grasses	3.1	5.7
All other spp.	9.3	17.1
Total area measured in grid squares on 5 slides.	5050	

SITE 8 Ancient Camp	% composition from total of 8 slides	% composition omitting seed
Dactylis/Poa annua	9.6	21.9
Holcus lanatus	7.8	18.0
Agrostis tenuis	5.1	11.0
Lolium/Cynosurus	2.9	6.6
Festuca rubra/Poa pratensis	1.6	3.6
Anthoxanthum	0.1	0.3
Arrhenatherum	1.5	3.4
Seed	56.4	-
Unidentified grasses	1.7	4.0
All other spp.	13.3	30.4
Total area measured in grid squares on 8 slides.	4101	

TABLE 4.3

Variance between subsamples from Site 1 sample.

RELATIVE PROPORTIONS IN DEGREES

	L/C	H	F.aru	F.rub	Ag	An	Arrh	D	Seed	U.G.	U.O.
SUBSAMPLE 1											
SLIDE 1	35.5	17.3	20.1	3.6	0	7.0	5.4	6.5	24.7	15.9	23.9
2	30.5	23.7	13.7	3.1	0	0	6.3	7.0	34.4	15.1	19.3
3	46.0	17.3	13.8	5.4	0.	0	3.1	4.1	21.0	10.5	23.6
4	40.2	23.7	10.8	5.1	0	0	10.3	6.5	21.0	9.5	25.1
5	49.0	17.9	10.5	5.7	0	0	0	5.1	23.5	8.5	18.8
SUBSAMPLE 2											
SLIDE 6	45.2	20.2	12.0	5.4	0	8.9	0	0	19.9	6.8	24.5
7	37.9	22.0	14.2	2.6	14.2	11.5	8.5	8.3	21.2	12.7	18.1
8	45.3	19.9	7.9	0	13.6	7.5	0	0	19.6	12.8	20.8
9	34.8	27.8	14.7	5.7	9.6	5.7	0	0	30.4	6.0	16.1
10	33.0	26.8	2.6	7.5	4.1	2.6	0	0	36.4	16.0	12.5
Difference between Sample 1 and 2 means	1.0	3.4	3.5	0.3	8.3*	5.8*	3.3	4.2*	0.6	1.0	3.7

* $p < 0.05$

	d.f.	Sum of squares	Mean square	F
Between grasses	10	13966.53		
Within grasses				
Between subsamples	11	428.88	38.99	2.06 $p < 0.05$
Within subsamples				
Between slides	88	1662.03	18.89	
Total	109	16057.44		

$S_{\bar{x}} t_{0.05} = 3.89$

percentages transformed into degrees (tables in Bliss 1970) indicated that the subsamples differed significantly ($F_{88}^{11} = 2.06$; $p < 0.05$). However these differences were significant for only three out of the eleven species groups and the actual differences in percentages were: Agrostis 0 - 3.1%, Anthoxanthum 0.3 - 2.0%, and Dactylis 0.4 - 0.9%. Sampling error of this magnitude was not considered large enough (especially when occurring at the lower end of the percentage scale) to obscure important differences between diets at eight such widely differing sites. Analysis of a subsample of ten slides instead of five would have reduced the standard error of the mean within 95% confidence limits ($S_{\bar{x}}\ t_{0.05}$) from 3.89 to 2.75 degrees. This represents only a relatively small increase in accuracy and a five slide subsample was considered sufficiently representative for the purpose of this study.

SITE 1 (Permanent pasture, Aber)

The category Lolium/Cynosurus includes some L. multiflorum in addition to L. perenne. The presence of both L. multiflorum and Festuca arundinacea in the faeces indicates that the rabbits were feeding not only in the field where sampling took place (where neither of these species were recorded) but were also moving some distance away to reseeded fields on the opposite side of the railway line.

SITE 2 (Sown pasture, Aber)

The droppings collected from the site contained predominantly L. multiflorum and F. arundinacea with no sign of any of the non-agricultural species found in the vicinity of the seeded areas. Very little clover was identified in the sample in spite of its abundance in the area, and obvious signs of grazing. This must be accounted for by extreme digestibility of the epidermis. This hypothesis was supported by the fact that very little

clover was identified in the faeces of domestic rabbits after feeding mixtures of grasses and clover.

The samples from Site 1 and Site 2 indicate that L. multiflorum and F. arundinacea were actively sought after when they were available.

SITE 3 (Rough grassland, Conwy)

The frequencies of species along Transects 2 and 3 over the same grazed area of vegetation (Area 3) correspond fairly closely, and do not differ substantially from the measurements along Transect 1 across Area 1 (considering grass species separately and herb species as a single group). See Table 4.1.

Similarly the separate faecal samples from the three different well-grazed areas contained comparable proportions (Table 4.2) particularly the samples from Areas 2 and 3. The sampling methods were not considered accurate enough to make small-scale comparisons between vegetation and faecal composition at Areas 1 and 3, although the results seem to indicate some correlations. The reasonably consistent results from the three separate sample analyses were taken as evidence that the experimental method produced samples sufficiently representative to enable detection of important differences between the eight sites.

SITE 4 (Sand-dunes, Newborough)

Unlike the other sites, the transect measurements are by no means representative of the species composition available to grazing rabbits, since vegetation cover on the dunes was patchy, variable and frequently sparse or absent altogether. The presence of numerous well-worn rabbit tracks, often continuing for long distances, indicated that the rabbits at this site moved over much larger areas to feed than at the other sites.

A very high proportion of Equisetum variegatum was found in the faecal

sample indicating that much of the grazing took place in the wet slacks where this species occurred. Equisetum was probably somewhat over represented in the faeces due to the indestructability of the silica impregnated epidermis. The discovery that Equisetum was forming an important component of the diet was supported by the observed effects of enclosing a small area of wet slack within a rabbit-proof fence. Within this exclosure there was a lush growth of fruiting Equisetum contrasting with the surrounding well-grazed, non-fruiting plants.

A thick tissue present in the faeces was thought to be undigested fragments of the leaves of Salix repens. In spite of the high frequencies of Carex flacca, and C. arenaria plus other less common sedges on the site, very little Carex epidermis was found in the faecal sample.

The Newborough results contrast with the composition of rabbit faeces from a sand dune system in Dyfed as analysed by Oldham (1971). His samples were collected in spring when the dune slacks were flooded and the rabbits were feeding mainly on the mature dunes. The samples contained high proportions of grasses in the following order of frequency: F. rubra, then H. lanatus, Poa spp., Ammophila arenaria, with Agrostis spp. at a very low frequency. Low proportions of Carex spp. were also present.

SITES 5 and 6 (Limestone grassland, Penmon and Benllech)

Although the vegetation composition at these two limestone sites was similar, the composition of the corresponding faecal samples differed widely in the proportions of Festuca spp. and the class of fragments containing Briza media, Koeleria cristata and Sieglingia decumbens. (In spite of the apparent differences in the epidermal structures of the latter three species, they were not always distinguishable in the faecal samples and were therefore scored as a group). A much higher proportion of Carex (flacca) was found in the Site 6 sample than at any other site, although sedges including

C. flacca were extremely abundant at Site 4. The reasons for the differences between the samples from sites 5 and 6 were not apparent, although the extreme shortness of the vegetation at Site 5, in contrast to that at Site 6 may have contributed in some way.

SITES 7 and 8 (Normal College lawn and Bangor Ancient Camp)

Both faecal samples contained large proportions of seed remains and leaf epidermis, predominantly of grasses.

Comparison between availability and faecal content of species and components common to a number of sites

Direct comparisons between samples containing different combinations of species can only be approximate because of discrepancies between ingested and faecal proportions of different species caused by differential digestion and variations in the ratio of epidermis to leaf weight.

GRASS SEED

The fragments in the faecal samples consisted of sections of the various scales which surround the seeds of grasses. Although the relative proportion of the seed category within each sample is not a comparable estimate of the relative proportion ingested, as is roughly the case with the other proportions consisting of leaf epidermis, these values can be compared between samples.

The following percentages of seed (i.e. expressed as % of total seed and leaf) were found in the faecal samples.

SITE	% SEED
1	17.8
2	8.9
3	54.0
4	36.1 (also includes leaf epidermis of Salix repens)
5	40.9
6	48.5
7	45.7
8	56.4

The comparatively low proportion at Site 1 is explained by the fact that the field had been mown a few days prior to sampling. The area within the meteorological enclosure at Site 2 was regularly mown, and the L. multiflorum in the surrounding field was not in flower when sampling took place, thus explaining the low value at this site. Samples from the Agrostis-fescue grassland sites (3 and 8) and those from the two limestone grassland sites (5 and 6) all contained high proportions of grass seed. None of these sites had been cut. Sample 7 from the lawn also contained a high proportion of seed which suggested that the rabbits had also been feeding elsewhere, since flowering grasses were only present on the uncut banks.

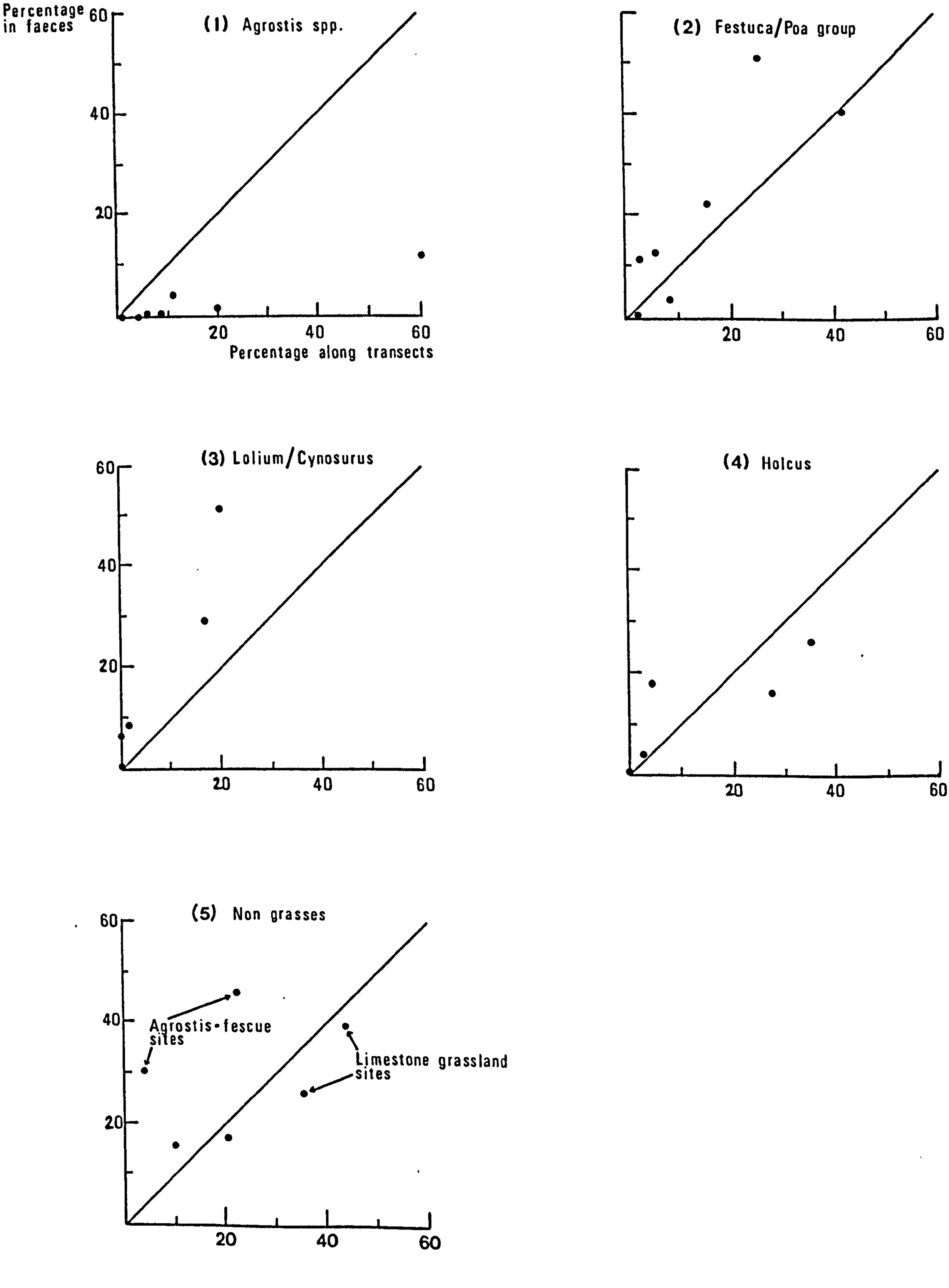
These results indicate that grass seed forms an important component of the diet when it is available. Myers and Poole (1963) in a study of the effects of rabbit grazing on sown pastures in Australia, observed that grass seed heads were used as a major food source in the spring. They also found that in late spring, as grass seed became less available, weed seed heads were consumed in quantity. However, dicot seeds were not detected by faecal analysis in the present study.

HERB SPECIES

Although the proportions of epidermal fragments within this category are not strictly comparable between sites since different herb species occur at each site, the relationship between availability and measured faecal content has been plotted in Fig.4.1(5). Sites 2 and 4 (sown pasture, Aber and sand dunes, Newborough) are omitted because of the extreme heterogeneity of the vegetation and the resulting inaccuracy of the quantitative measurements of availability.

FIG. 4.1

Relationship between availability of species and composition of faecal samples.



Proportionately more herbs appeared to be eaten at the fescue and Agrostis-fescue grassland sites (1, 3 and 8) than at the two limestone grassland sites (5 and 6) in spite of their greater availability at these latter sites. The differing composition of the herb species probably accounts for this. The predominant species at the limestone grassland sites were Thymus drucei and Helianthemum chamaecistus, both of which have small, tough, dry leaves which may well be unpalatable.

Herbs apparently form an important component of the diet of rabbits when they are available. The extent to which differential digestion of epidermis affects the faecal proportions has not been determined however, so the accuracy of the measured relationship between availability and ingestion is unknown. Similar correspondence between availability and uncorrected faecal proportions of herbs has also been found in studies on rabbits grazing on two National Nature Reserves (Williams, Wells and Wells 1974; Bhadresa 1977).

AGROSTIS, FESTUCA, LOLIUM AND HOLCUS

The data in Tables 4.1 & 4.2 were used to compare the relative availabilities of food plants in the field and the proportions found in the corresponding faecal samples, for the four most universally occurring groups of species - Agrostis spp., Festuca spp / Poa pratensis, Lolium / Cynosurus and Holcus (Fig.4.1(1) - (4)). Data from Site 2 (sown pasture, Aber) could not be used since none of these species occurred either in the field or the faecal samples. The relative proportions of all the categories of epidermal fragments in the faeces were recalculated excluding grass seed so that comparisons between sites could be made (Table 4.2).

The availability of species in the field and proportions present in the faeces were positively correlated, but the relationships were

not simple and linear as would be expected if the rabbits were not selective. The results indicate a marked preference for Lolium and avoidance of Agrostis, but no consistent selection either way for Festuca and Holcus.

Adjustment of the data to compensate for differential digestion of epidermis

The measurements of faecal proportions were adjusted using values for the relative digestibilities of the epidermis of seven grasses which had been determined by means of controlled feeding experiments (Chapter 3). The species involved were Festuca rubra, Anthoxanthum odoratum, Agrostis tenuis, Dactylis glomerata, Arrhenatherum elatius, Holcus lanatus and Lolium perenne, The assumption had to be made that epidermis from each of the following species categories had the same digestbility as, or was mainly composed of, the appropriate single species, i.e.

Festuca spp. / Poa pratensis	=	F. rubra
Agrostis spp.	=	A. tenuis
Lolium / Cynosurus	=	L. perenne
Dactylis / Poa annua / P. trivalis	=	D. glomerata

Since the correction factors determined in Chapter 3 are relative, the calibrations have been made considering the seven species involved, in isolation whenever they occurred at the sites and in the faeces (i.e. totalling 100%). The inclusion of other species of unknown digestibilities would invalidate accurate comparisons between sites. Similarly the availabilities of these seven species at the sites were recalculated relative only to each other in order to be comparable with the adjusted faecal proportions. The calibrated values are given in

Table 4.4 and the relationships between availabilities and corrected faecal proportions illustrated in Fig. 4.2(1) - (4). The uncorrected proportions are also shown for direct comparison in Table 4.4.

Correction of the data accentuated the preference factors involving Lolium and Agrostis by increasing the degree of selection for Lolium and against Agrostis. The adjusted proportions of Festuca continued to indicate that it was apparently eaten roughly in the proportions available. There appeared to be an inverse relationship between the availability of Lolium (and hence the proportion eaten) and the relative amount of Holcus in the diet. At Sites 1 and 7, where Lolium was readily available, Holcus was eaten proportionately less than at Sites 2 and 8 where Lolium was scarce.

Anthoxanthum and Arrhenatherum when present at all were only available in small amounts and were correspondingly eaten at low frequencies (Table 4.4). This also applied to Dactylis, with the exception of Site 8 where an unaccountably high proportion of the epidermal fragments belonged to the Dactylis / Poa category.

These direct measurements of food availability and intake by rabbits reinforce the inferences drawn in the past from indirect measurements of vegetation grazed by rabbits. Some such analyses have indicated that rabbits have a considerable preference for Lolium spp. and dislike of Agrostis spp. (Thomas 1937; Phillips 1953; Myers and Poole 1963). In addition, surveys of vegetation composition before and after the outbreak of myxomatosis in Britain indicated that Agrostis spp. tended to become less abundant when grazing intensity was greatly reduced. (Ranwell 1960; Thomas 1960, 1963; White 1961). A similar effect was found by Watt (1957, 1960) when rabbits were artificially excluded from certain areas of Breckland grassland.

TABLE 4.4

Correction factors for differential digestion of epidermal fragments of seven grass species. Relative proportions (%) expected in faeces if ingested in equal proportions by fresh weight. (Figures derived experimentally by controlled feeding of captive wild rabbits. See Chapter 3, Table 3.8B).

Agrostis tenuis	23.8
Festuca rubra	21.0
Anthoxanthum odoratum	16.2
Holcus lanatus	12.1
Dactylis glomerata	11.3
Lolium perenne	7.8
Arrhenatherum elatius	7.8

Recalculation of relative availabilities and faecal proportions in terms of the above seven species only, correcting for the effects of digestion.

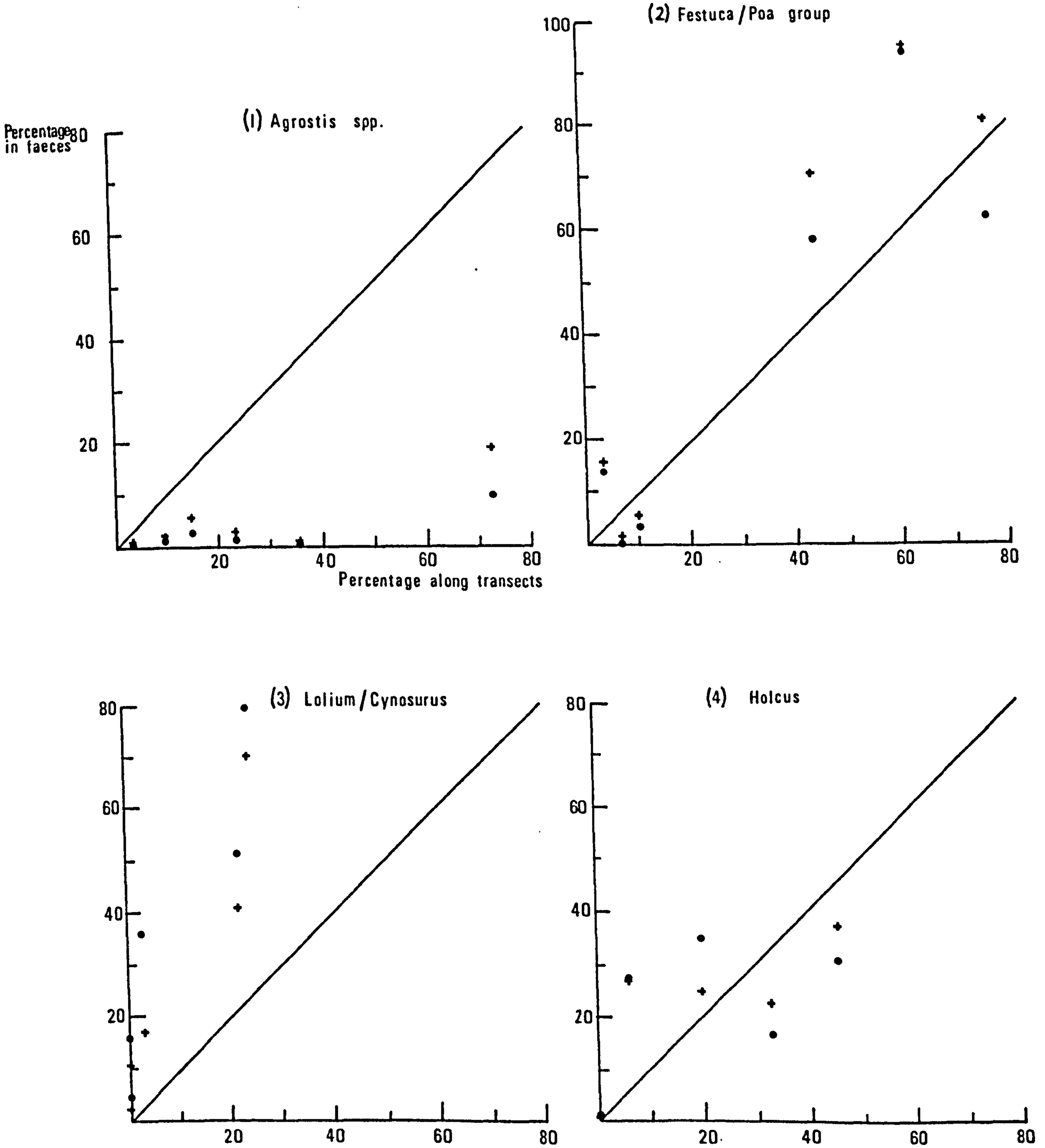
	AVAILABILITY	PROPORTION IN FAECES	
	% along transect (7 spp. only)	% in faeces (7 spp. only)	% corrected for digestion
SITE 1 (ABER, PERMANENT PASTURE)			
F/P	7.1	1.2	0.5
Ag	23.8	2.6	1.0
An	7.7	1.9	1.0
H	32.8	22.2	16.2
D	0.6	1.1	0.9
L/C	24.4	69.9	79.1
Arrh	3.6	1.1	1.3
SITE 3 (CONWY)			
F/P	77.0	80.8	62.6
Ag	9.8	1.6	1.1
An	0	0.1	0.1
D	10.1	0.5	0.7
L/C	3.1	16.9	35.3
Arrh	0	0.1	0.2
SITE 4 (NEWBOROUGH)			
F/P	44.0	70.8	58.0
Ag	36.0	0.6	0.4
An	0	2.0	2.1
H	20.0	24.6	35.0
L/C	0	2.0	4.5
SITE 5 (PENMON)			
F/P	96.3	99.1	99.2
Ag	3.7	0.9	0.8

TABLE 4.4 continued

	AVAILABILITY	PROPORTION IN FAECES	
	% along transect (7 spp. only)	% in faeces (7 spp. only)	% corrected for digestion
SITE 6 (BENLLECH)			
F/P	60.8	95.4	94.4
Ag	35.3	1.8	1.6
An	3.9	1.8	2.3
H	0	1.0	1.7
SITE 7 (NORMAL COLLEGE)			
F/P	3.9	15.6	13.9
Ag	14.3	5.9	2.5
H	45.5	36.8	30.6
D	2.6	1.6	1.4
L/C	22.0	40.1	51.6
SITE 8 (BANGOR ANCIENT CAMP)			
F/P	10.0	5.5	3.2
Ag	72.4	18.0	9.2
An	6.2	0.5	0.4
H	5.7	27.4	27.5
D/P	4.3	33.4	36.0
L/C	+	10.1	15.7
Arrh	1.4	5.1	8.0

FIG. 4.2

Relationship between availability of species and composition of faecal samples before and after correcting data to accountfor the effects of digestion.



Not only is the active avoidance of Agrostis by rabbits responsible for its success in heavily grazed swards, but also the fact that it does not rely exclusively on seed for its spread, but is stoloniferous (A. stolonifera) or rhizomatous (A. tenuis). Species which do rely on seed production for their dispersal must be at a distinct disadvantage in rabbit grazed grassland since seeds appear to form a large component of the diet of rabbits when available. Myers and Poole (1963) have stated that 'rabbits seem to be more competent than sheep in selecting from the pasture those items of food (seedlings, seeds, roots) which are intimately related to the ability of the pasture to maintain itself.'

Under certain conditions therefore, rabbit grazing must play an important part in determining the abundance of Agrostis by means of the following interacting processes: avoidance of the species in preference for other more palatable items, the ability of Agrostis to spread by stolons or rhizomes and the preferential grazing of competitive species which are more palatable. It would be interesting to determine the extent to which Agrostis is eaten by rabbits on upland pastures where there are few alternative grasses available. In such situations it has been shown to form an important component of the diet of sheep (Martin 1962; Spedding 1972).

Discussion on the value of correcting for differential digestion of grass epidermis

Although the digestibility of leaf epidermis differed considerably between each of the seven grass species studied, calibration of the measured faecal proportions did not alter the rank order of the preferences revealed. This was partly due to (a) the wide differences

in species intake caused by differences in both availability and preference and (b) the fact that the greater the number of species involved in the calibration, the less the change to the faecal proportions in absolute terms.

In the case of Lolium and Agrostis, application of the correction factors accentuated the already considerable degree of selection apparent in the uncorrected data. In addition to the difference in digestibility of the epidermis of these two species, the Dry Matter Digestibility (DMD) of Agrostis is less than that of Lolium (see Spedding 1972). In a number of instances a direct correlation has been established between DMD and the palatability of grass species to domestic stock (e.g. Osbourne et al 1974; Simon 1974), although many other factors such as season, species variety, flower production and type of animal affect digestibility (Ivins 1960; Mowat et al 1965). If approximate correlations between epidermal digestibility and DMD (see Chapter 3) and between DMD and palatability exist generally within grasses, the application of correction factors to observed faecal proportions will serve mainly to further differentiate preferences which are already apparent.

In situations where there are wide differences in availabilities and preferences, the disadvantage of the length of time required to determine correction factors may outweigh the additional information obtained by their use. On the other hand, if species with similar availabilities and faecal proportions are involved, calibration of the data may reveal or clarify differences in preferences which were not otherwise obvious.

Conclusions

In spite of the qualitative and quantitative differences in species composition at the eight sites, a number of consistencies in the selection of food by grazing rabbits emerged. In general the leaves of grasses formed the main dietary component, but at the time of year of the study, grass seed and herb species were eaten in quantities which depended on their availabilities. The consistent and pronounced preference for Lolium spp. and dislike of Agrostis spp. which were revealed, substantiate inferences previously drawn from indirect measurements on pasture grazed by rabbits. The extreme unpalatability of Agrostis to rabbits is probably an important factor in determining its abundance in certain types of well-grazed grassland. Selection amongst other species of grasses was much less marked than in the case of Lolium and Agrostis.

The accuracy of the method of measurement of species availabilities by means of point trasnects across grazed areas and of food selection by faecal analysis, was sufficient to reveal these consistent features. Calibration of faecal proportions to compensate for the effects of differential digestion of the epidermis of certain grass species did not substantially alter the measured ratios and under these circumstances was considered to be an unnecessary refinement of experimental technique.

CHAPTER 5

SEASONAL CHANGES IN DIET

CHAPTER 5

SEASONAL CHANGES IN DIET

Introduction

The following chapter describes the study of food preferences of wild rabbits measured by faecal analysis throughout two consecutive years. Collections of droppings were made at monthly intervals over two years from an area of Agrostis - fescue grassland supporting a large, isolated population of rabbits. The species composition of the vegetation was estimated, concentrating mainly on the grasses. Each faecal sample was analysed quantitatively to determine the composition of the diet throughout the two years.

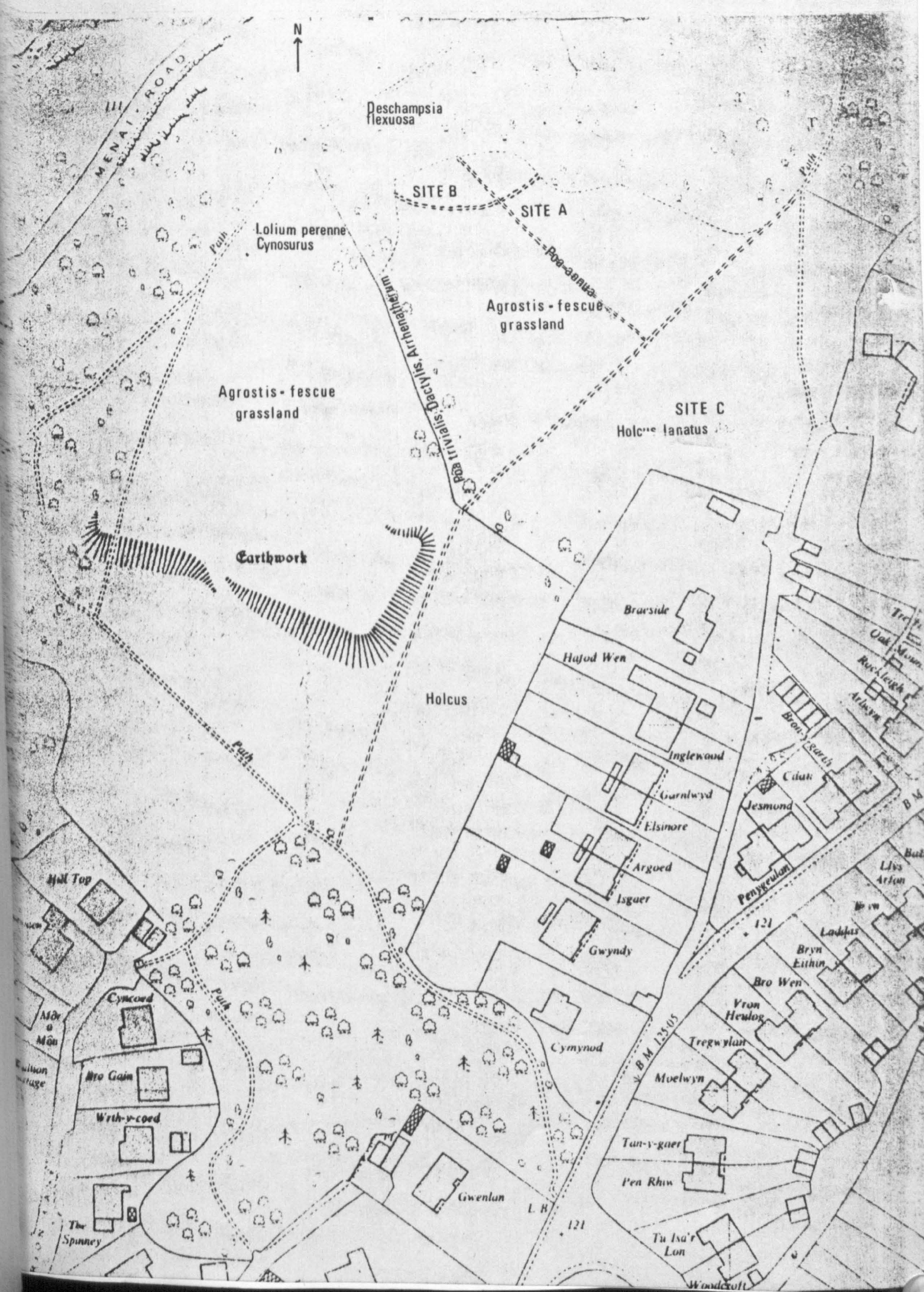
Methods

Area description and sampling site

The Ancient Camp, Bangor, is a public open space comprising about 3 ha of Agrostis - fescue grassland bordered by scrub, woodland and gardens (see Map 2). The turf remained fairly short throughout the year due to a combination of rabbit grazing, human trampling and an annual mowing in July or August. The large rabbit population was isolated from other populations by the surrounding houses and the sea. The colony appeared to be free of myxomatosis during the time of study.

Ideal conditions for determining the average diet of members of such a population would involve a situation in which either the composition of all the grassland was homogeneous at any one time of year, or all points were equally accessible for feeding to all rabbits. However neither of these situations, nor their opposite extremes, was the case. In order to reduce the heterogeneity of

Map 2
Location of Sampling Sites at Bangor Ancient Camp
Scale 1:1250



sampling over the whole area, all the faecal samples were collected from a single, well-grazed site (Site A, Map 2). It was hoped that the composition of the samples would, to some extent, reflect the diet of the rabbits which tended to feed most in that area because they inhabited the adjacent scrub. Observation showed that several rabbits grazed this site: on one occasion nine were observed simultaneously.

Estimation of vegetation composition

The most heavily grazed areas consisted predominantly of an Agrostis tenuis dominated sward with Festuca rubra present at a fairly consistent, but lower frequency. Other species are listed and the map shows the approximate distribution of grass species in relation to the chief grazing areas.

The relative proportions of species present on the sampling site were determined from a series of line transects, recording species at points 2 or 5 cm apart, using a frame of vertical pins. Initially two consecutive estimations were made to determine the repeatability of the method. Consistent results were obtained from a different series of transects, so this method was used to obtain summer and winter estimates of the relative availability of species during the two years of study. Similar recordings were made at two additional well-grazed sites (B and C, Map 2) nearby, for comparison.

Collection and analysis of faecal samples

Faeces were collected from the sampling site at approximately monthly intervals over the two year period. The collections were made up of only fresh droppings which were distinguishable in dry weather conditions by colour and a slight mucous coating. In wet weather samples were not taken as all ages of droppings tended to be similar in appearance. An attempt was made to represent as large a number of individual rabbits as possible by selecting faeces varying in size, shape and colour, collected from different dung heaps and scatterings

over the site. A total of 50-60 droppings were collected on each occasion and were stored in formalin-acetic-alcohol until analysed.

Twenty droppings were removed from each monthly sample for analysis. Each of these was cut in half to reduce the bulk of material. Half the sample was stored in case it was required for further analysis. The samples were prepared and analysed as described in Chapter 2. The relative proportions of cuticle fragments of each grass, or group of indistinguishable species were estimated from a total of eight subsample slides for each sample.

Sources of inaccuracy

Since the methods of collection and analysis of faeces involved a number of sampling stages, the following sources of variation were investigated.

1. Subsampling for microscopic analysis

At the outset of the analyses, twelve slides made from the stained suspension of the twenty half-droppings of Sample 1, were scanned and the areas of cuticle fragments of each species or group measured. Relative proportions were calculated from the results for the individual slides and similarly for groups of increasing numbers of slides using the cumulative area totals. From these results eight slides was estimated to be the optimum number forming a representative subsample from the suspension, in view of the time-consuming nature of the analysis.

The accuracy of the procedure was checked by analysing a further group of eight slides made from the suspension of the other half of the 20 droppings of Sample 1. The results were compared by analysis of variance. Subsequent checks were made by carrying out repeat sampling of Samples 9, 10 and 11.

2. Personal Error

Misidentification and changes in identification with the progress of time and increasing experience are the most likely sources of personal error involved in faecal analysis. Any disparity in the results obtained from repeat

sampling are a compound of sampling variance and such personal errors. In the case of Samples 9, 10 and 11, repeat sampling was carried out three weeks after the first analysis.

3. Variation between individual rabbits

On two sampling dates (Samples 2 and 6) droppings from each of two rabbits were collected and analysed separately. The extent to which the individual results differed was determined by analysis of variance. These individual collections were made up of only 4 or 5 droppings as there was always some uncertainty as to the origin of larger heaps.

4. Collection of a representative sample

The validity of regarding the method of sampling in the field and the number of droppings used in the analyses as representative of the rabbit population in that area was tested. Different samples were collected on two consecutive days (Samples 6 and 6B) from the site and the results of faecal analysis compared by analysis of variance.

Results and Discussion

Composition of the Vegetation

No apparently significant differences occurred between the relative proportions of species present on any of the five sampling occasions at Site A (Table 5.1). The method of measurement used, although rough, appeared to give adequate representation of the relative abundances of species at the sampling site. Further detail was considered unecessary in view of the large area and heterogeneity of the Ancient Camp as a whole. The species list and map illustrate this heterogeneity. The relative abundances of the grass species did not appear to be greatly affected by season, which probably influences quality rather than availability in this type of grassland.

TABLE 5.1

Species composition of grassland at Bangor Ancient Camp (Point samples along line transects).

% COMPOSITION	SITE A					SITE B	SITE C
	JULY 1972	AUG. 1972	DEC. 1972	JUNE 1973	JAN. 1974	JUNE 1973	AUG. 1973
	1	2	3	4	5		
Agrostis tenuis	59.3	53.3	54.7	60.8	51.1	69.5	58.0
Festuca rubra	8.3	10.8	6.0	8.4	14.7	7.5	5.0
Anthoxanthum odoratum	5.4	4.0	10.0	5.2	1.9	2.5	7.0
Dactylis glomerata	4.0	3.9	6.4	3.6	0.8	2.5	6.0
Holcus lanatus	4.7	5.8	3.6	4.8	4.8	0.5	10.0
Arrhenatherum elatius	2.3	1.3	0.8	1.2	2.3	6.0	2.0
Lolium perenne	0.1	0	1.6	0.	0	0	0
Cynosurus cristatus	0.1	0	2.4	0	0.2	0	0
Poa pratensis	0	0	0	0	0.9	3.0	1.0
Luzula campestris	0.4	0.1	1.2	0	0.4	0.5	0
Plantago lanceolata	2.0	2.0	2.8	2.4	0.9	1.0	4.0
Achillea millefolium	0.5	0.8	0	0	0.8	0	0
Lotus corniculatus	0.1	0.3	0	0.4	0	0	0
Ranunculus repens	0.1	0.1	0.4	0	0.2	0	0
Veronica chamaedrys	0.6	0.2	0.4	0	0	1.5	0
Conopodium majus	0.4	0.2	0	1.2	6.0	0.5	0
Endymion non-scriptus	0	0	0	0	0.2	0.5	0
Rumex acetosa	0	0	0	0	0.2	0.5	0
Trifolium repens	0	0.3	0.4	0	0	0	1.0
TOTAL NON-GRASSES	4.1	4.0	5.2	4.0	8.7	4.5	5.0
Bryophytes	4.4	7.7	8.9	7.2	7.3	0.5	3.0
Dead vegetation	7.3	9.2	10.4	4.8	7.2	3.5	3.0
No. of points along transect	800	960	250	250	200	200	100

Similar proportions of species were also found at Sites B and C (Table 5.1), showing that the composition of the short grazed turf was fairly consistent.

The components of the grazed vegetation at sampling site A and areas B and C, listed and grouped in order of abundance, were as follows :-

	Range of percentage abundance - 7 samples
Agrostis tenuis	50 - 70
Festuca rubra	5 - 15
Anthoxanthum odoratum	1 - 10
Holcus lanatus	1 - 10
Dactylis glomerata	1 - 7
Arrhenatherum elatius	1 - 6
Poa pratensis	0 - 3
Cynosurus cristatus	0 - 3
Lolium perenne	0 - 2
All other non-grass species	4 - 9

Faecal composition

Analysis of sampling variances

1. Subsampling and 2. Personal error

The results of the analyses of twelve slides from Sample 1 show wide variation between individual slides (See Appendix 1, Table A1). This variation decreased as the subsample size was increased by calculating relative proportions from the cumulative totals from increasing numbers of slides. Fig.5.1 illustrates the effect of increasing subsample size on the estimated proportion of Holcus which showed the most variation in the individual

Effect of increasing sample size

FIG. 5.1

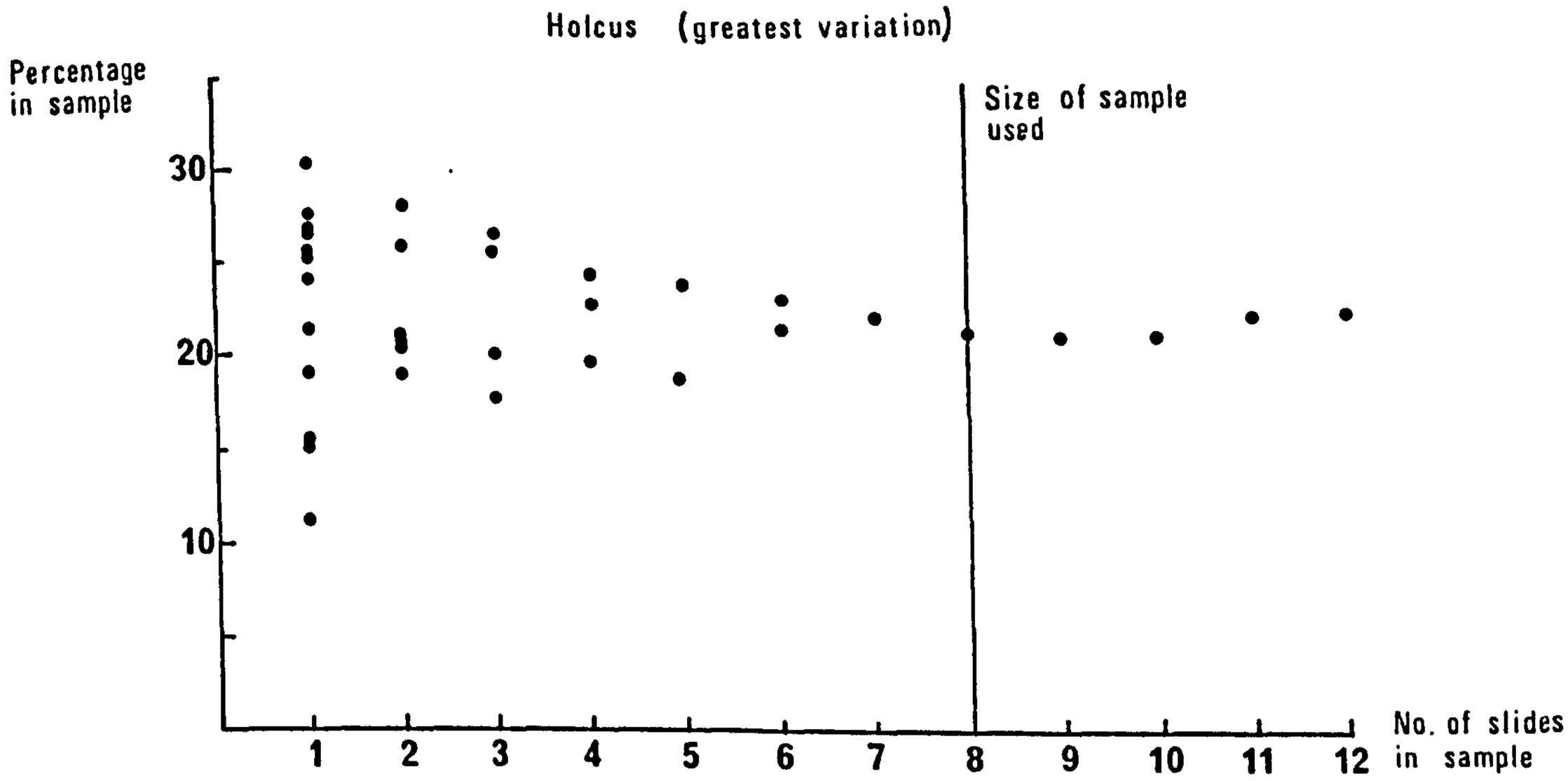
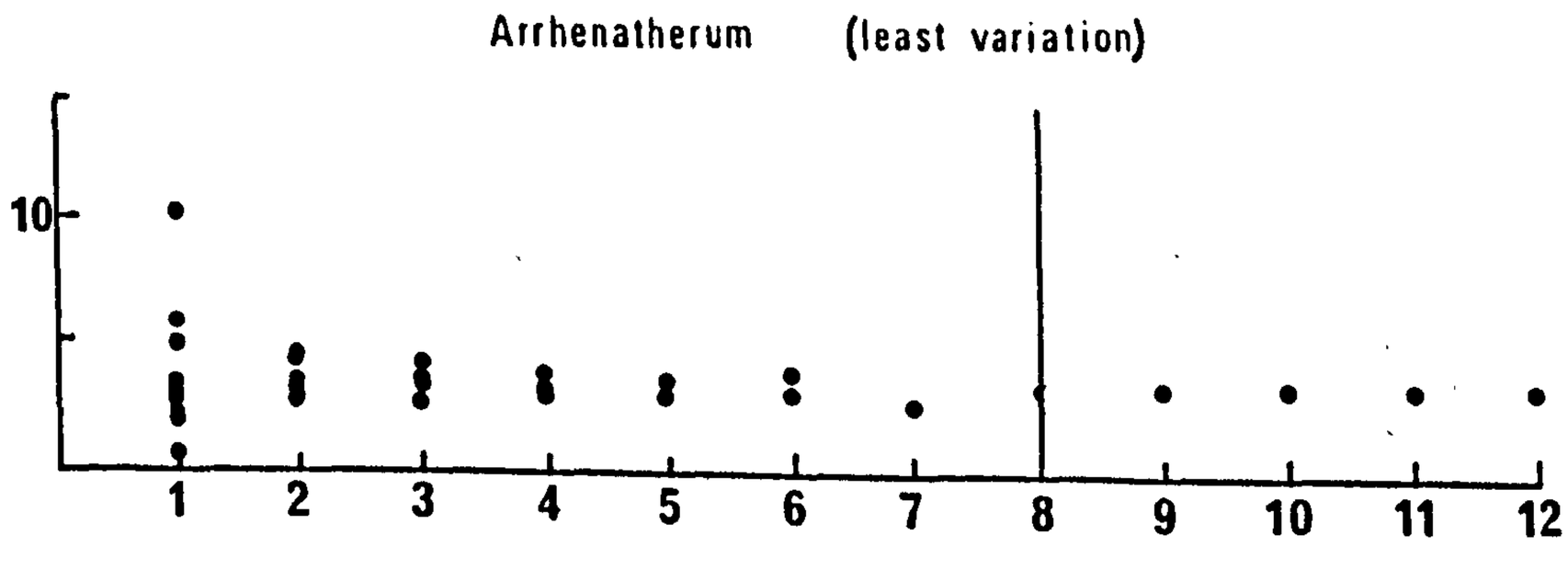


FIG. 5.2



slide analyses, and Fig. 5.2 similarly of Arrhenatherum which showed the least variation. A subsample size of eight slides was selected as being sufficiently representative of the composition of the whole sample.

The results for the first eight slides analysed were used to calculate the combined standard error of the group means (assuming the inter-group variances equal). Percentages were converted into degrees by angular transformation (Bliss 1970) and the between group (i.e. grass) and between slide variances partitioned. The latter estimate was used to calculate the standard error of the means within 95% confidence limits, $S_{\bar{x}}t_{0.05}$ (see Appendix 1, Table A2). As the subsample size is increased $S_{\bar{x}}t_{0.05}$ decreases by progressively smaller amounts so that, for example, doubling from 4 to 8 slides reduces the standard error by a greater amount than by doubling from 10 to 20 slides. Increasing the subsample beyond the selected size of 8 slides would have resulted in only a small increase in accuracy in contrast with the length of time required to analyse further slides. (See Appendix 1, Table A3).

The results of replicate subsamples from Samples 1, 9, 10 and 11 are shown in Table 5.2. Here the relative proportions have been calculated from the area totals from all the slides in each subsample. The estimates appear fairly consistent, the maximum difference between replicate measurements being 6.3%, but generally differences were of the order of 1% - 3%.

The two replicate subsamples, each of eight slides from Sample 1 were compared by analysis of variance. The variance between the subsamples was not significantly higher than the variance within the subsamples (i.e. between individual slides) ($F^{9}_{126} = 1.41$; $p > 0.05$. See Appendix 1, Table A4).

All the results for Samples 9, 10 and 11 were analysed together to determine whether the measured differences between these three consecutive samples were statistically valid. (See Appendix 1, Table A5). The variance

between the replicate subsamples within each sample was not significantly higher than the variance within the subsamples ($F_{270}^{27} = 1.16$; $p > 0.05$), but the three samples differed significantly from each other ($F_{270}^{18} = 15.17$; $p < 0.001$). The experimental error variance (i.e. between slide variance) did not approach anywhere near a magnitude great enough to obscure overall differences between these samples.

As a result of these analyses the method of subsampling, using eight slides, was considered sufficiently accurate to detect fairly small differences between samples.

3. Variation between individual rabbits

The results show differences between faeces from the two individual rabbits and the whole sample estimate in each case (Table 5.3). Analysis of variance indicated significant differences both between rabbits within samples ($F_{194}^{22} = 7.70$; $p < 0.001$) and between the two samples ($F_{194}^{9} = 3.61$; $P < 0.01$) (See Appendix 1, Table A6).

4. Collection of a representative samples of faeces

There appears to be close correspondence between the composition of the two collections (Samples 6 and 6B) made on consecutive days (Table 5.4). The maximum difference was 2.6% for Lolium/Cynosurus. However, when the individual slide results, transformed into degrees, were analysed statistically, significant differences ($p < 0.05$) were found between the proportions of Lolium/Cynosurus, Arrhenatherum and unidentified grasses in the two samples (See Appendix 1, Table A7). However, transformation of the data from percentages to degrees emphasizes variation at the lower end of the scale which partly accounts for these significant differences. Sampling error of this magnitude was not considered sufficient to obscure important seasonal changes in diet and the method of collection of the monthly samples was considered adequate for the purposes of the study.

TABLE 5.2

Results of faecal analysis on replicate subsamples

% COMPOSITION		F/P	Ag	H	L/C	D/P	An	Arrh	U.G.	U.O.	Total area measured (Grid squares)
SAMPLE	1A	11.0	5.5	26.3	16.9	22.3	1.9	3.9	6.6	5.6	2612
	1B	11.7	8.4	21.3	16.3	23.2	4.6	3.6	4.5	6.4	1908
	9A	17.5	18.9	6.1	10.8	22.7	1.9	2.7	3.9	15.5	2021
	9B	16.8	17.2	8.5	10.9	16.5	2.6	4.9	7.1	15.5	2226
	10A	3.6	11.8	18.0	6.6	21.9	0.3	3.4	4.0	30.4	1788
	10B	4.0	8.8	14.3	5.9	22.5	0.8	5.2	1.8	36.7	1976
	11A	26.3	7.1	3.3	16.7	29.1	1.2	1.3	1.4	13.6	3674
	11B	27.1	3.6	4.5	13.2	30.0	0.6	1.1	0.6	19.3	3184

TABLE 5.3

Analysis of faecal samples from two individual rabbits at two different times of year and comparison with the corresponding total sample results.

% COMPOSITION	F/P	Ag	H	L/C	D/P	An	Arrh	U.G.	U.O.	Total area measured (grid squares)
Sample 2										
INDIVIDUAL A (4 droppings)	9.1	13.1	12.4	11.1	20.4	2.9	5.3	11.5	14.2	2085
INDIVIDUAL B (4 droppings)	29.3	13.2	0	7.8	13.1	0	0.5	3.3	32.8	1944
TOTAL SAMPLE (20 x $\frac{1}{2}$ droppings)	18.3	13.0	5.1	9.7	15.5	6.5	1.7	5.3	24.9	2853
Sample 6										
INDIVIDUAL C (5 droppings)	19.5	2.8	15.1	12.2	40.3	1.2	0.4	3.0	5.5	2061
INDIVIDUAL D (4 droppings)	27.9	1.8	4.6	7.3	47.4	1.5	0.1	5.2	4.2	2371
TOTAL SAMPLE (20 x $\frac{1}{2}$ droppings)	20.0	0.1	5.0	5.3	60.5	2.5	0.9	4.1	1.6	4354

TABLE 5.4

Analysis of faecal samples taken on two consecutive days

% COMPOSITION	F/P	Ag	H	L/C	D/P	An	Arrh	U.G.	U.O.	Total area measured (grid squares)
SAMPLE 6 (26th February)	20.0	0.1	5.0	5.3	60.5	2.5	0.9	4.1	1.6	4354
SAMPLE 6B (27th February)	20.8	0.6	4.6	2.7	61.0	3.4	2.2	2.5	2.2	4196

Seasonal variations in faecal composition

The seasonal variations in the relative proportions of fragments within each of the ten non-seed categories of identified species groups are illustrated in Fig.5.3 (a - h), in which the results for the two years are super-imposed on each other. (Data in Appendix 1, Table A8). These percentages have been compiled excluding the seed category since this fraction of the fragment counts bears a relationship within the quantities ingested which is completely different from that of the rest of the diet, composed mainly of leaves.

In general, monthly variations in composition of the diet corresponded closely between the two years, considering that the figures represent relative proportions rather than absolute measures. This suggests an annual cycle of changing grazing behaviour during which the relative utilization of different species fluctuates in a set pattern.

Variations in the proportion of grasses relative to non-grasses (Fig.5.3h) corresponded very closely between the two years, with maximum utilization of herbs in June, and then later in the year in September and October. The temporary increase of grasses in the diet during July and August was a recurring feature. This change did not correspond with the annual mowing of the vegetation in July 1972 and August 1973 (from which the herb species recovered more slowly than the grasses) since on each occasion the drop in herb intake was recorded just before mowing took place. The two peaks in herb intake probably correspond with two separate growth seasons of different food species (late spring and autumn) with a relatively quiescent intervening period. However, not enough information was available on the dicot species eaten at different times of year and their seasonal patterns of growth to test this suggestion.

The three grass categories Festuca rubra / Poa pratensis, Agrostis tenuis and Holcus lanatus each showed a consistent spring / early summer peak in utilization, during which each was represented by 20 - 30% of the faecal

FIG. 5.3

Seasonal changes in proportions of individual dietary components determined as the percentage present in faecal samples (excluding seed). The results are shown for two successive years.

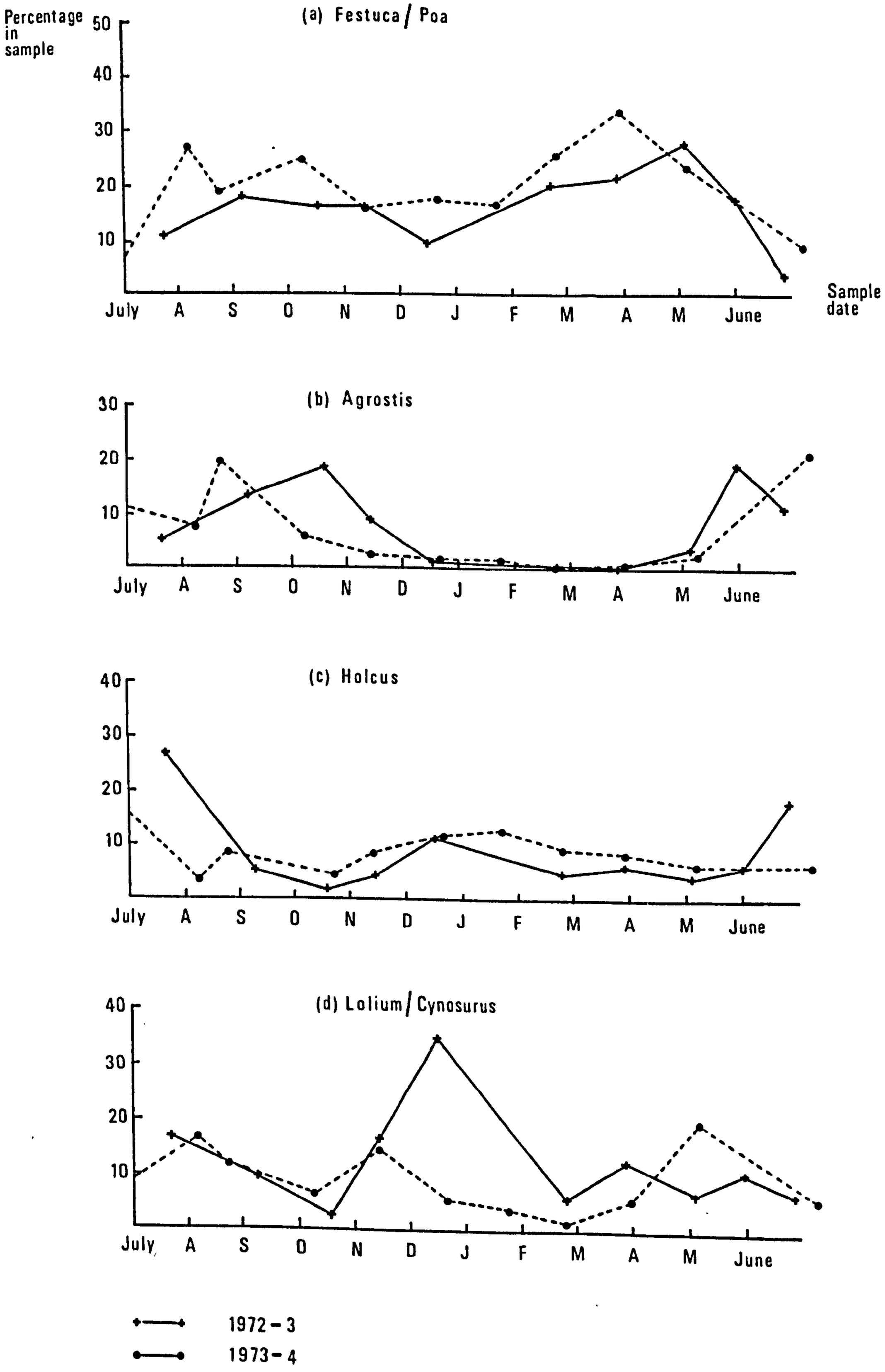
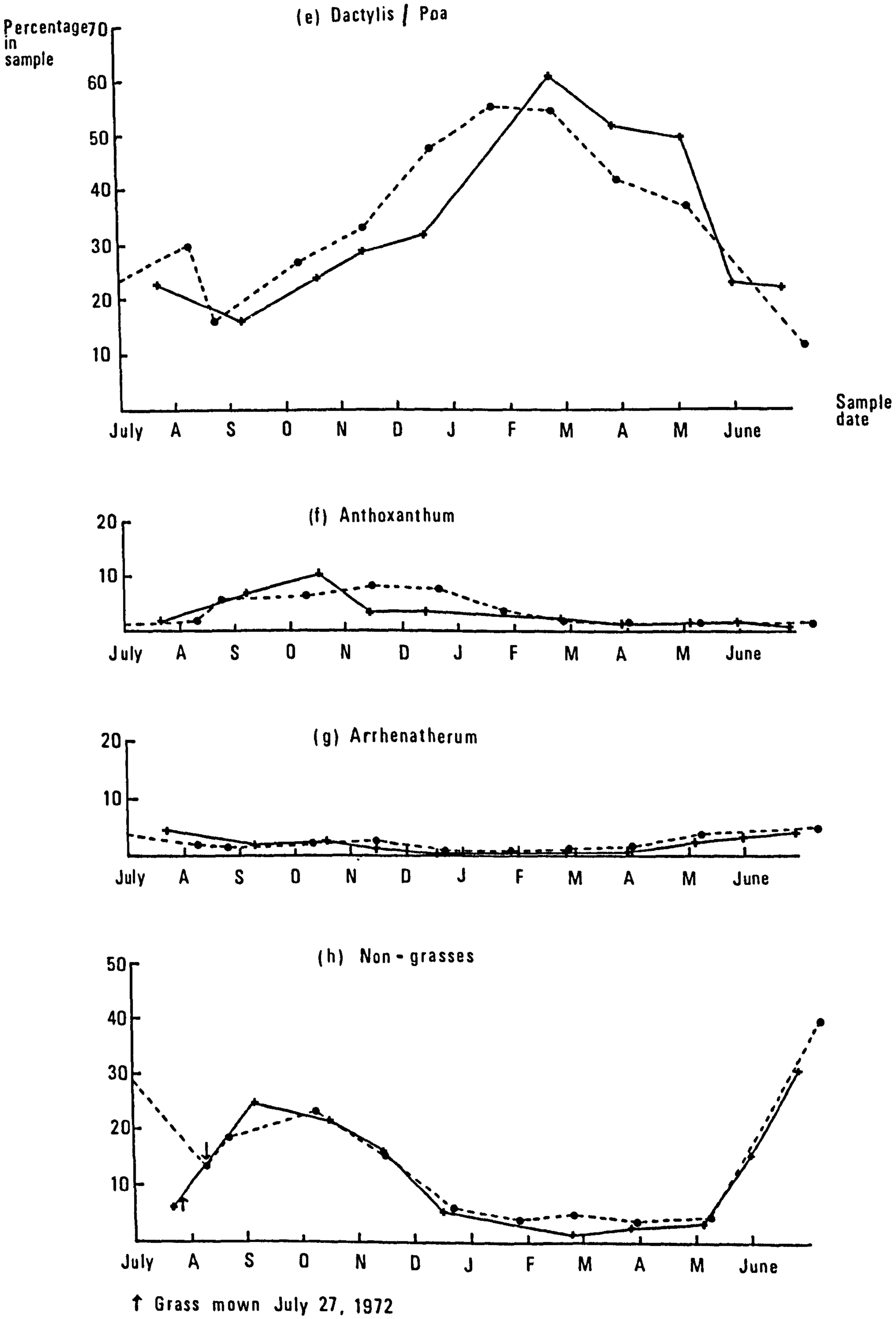


FIG. 5.3 continued



fragments. These peaks occurred successively, with intake of Festuca / Poa at a maximum during April and May, Agrostis throughout June and Holcus from the end of June into July. Assuming that the Festuca / Poa category was composed mainly of F. rubra (which was likely considering the low availability of P. pratensis in comparison with F. rubra), the successive order of utilization of the three species corresponds with the order of the onset of flowering in these species. The differences in the main flowering periods of the species was observed to be well differentiated at the site during both years of the study. The peaks in utilization occurred slightly before the onset of flowering in each case suggesting that palatability is at a maximum at this stage in growth. In the case of Festuca / Poa and Agrostis, utilization increased again during September and October, perhaps corresponding with a late season growth period.

The only other species showing a consistent and distinctive increase in utilization in spring and early summer was Arrhenatherum (Fig.5.3g). However, this species was detected only in very low proportions in the faeces (up to a maximum of 5%). Again the peak in utilization occurred just prior to the onset of flowering in late July and August.

There was no correlation between intake and onset of flowering in Anthoxanthum (Fig.5.3f), although the early (April) peak flowering period was very distinctive in this species. The maximum proportions ingested were recorded between September and December in both years.

The proportion of epidermal fragments in the Lolium / Cynosurus category fluctuated throughout the year with no evident pattern detected in the two years. The fact that neither species was homogeneously distributed throughout the sward, but occurred in only a few widely dispersed patches, could account for these apparently random fluctuations if grazing occurred sporadically and not as a part of a regular pattern. The fact that this category occurred in

the faeces to such an extent in spite of the apparently limited availability of both species, must indicate high palatability which was responsible for attracting the rabbits to these patches.

Seasonal variations in utilization of Dactylis and/or P. trivialis / P. annua showed the reverse of the patterns exhibited by other species. The group comprised 40 - 60% of the epidermal fragments between November and April, and subsequently the proportions fell to around 20% during the remaining months of the year. Since the actual species composition of this group could not be determined in the faecal analyses, reasons suggested for this pattern can only be hypothetical. If the main constituent of the fraction was P. annua the high winter intake could be explained by the continual germination and growth of this species throughout the winter months, contrasting with the coarser, more dormant perennial species. P. annua occurred fairly abundantly on the paths and other disturbed areas at the site and there were signs of grazing at these locations. This theory however is possibly invalidated by the observation that the epidermis of P. annua is very thin and with artificial maceration in acid, tended to disintegrate and become unrecognizable. This may well be the consequence of digestion by the rabbit since close correspondence between the effects of artificial maceration and digestion were found for several other grass species. Unfortunately this correspondence was not tested and demonstrated for P. annua leaving the situation unresolved.

P.trivialis did not occur in the frequently grazed areas and was present only at the bases of the hedges where there was little sign of grazing. It was therefore unlikely that this species was contributing to the major Dactylis / Poa fraction in the faeces. This category was probably composed mainly of Dactylis which was abundant throughout the grazed turf. The reason for its high utilization during the winter was unclear as this species has a generally recognized tendency to become coarse and unpalatable to stock with increasing age.

However, close grazing could possibly prevent this as does regular cutting throughout the growing season (Reid and Jung 1965; Mowat et al 1965) and its high intake could be explained by a higher palatability in relation to other species at this time of year. Dactylis when young is regarded by agronomists as having a similar palatability to Lolium. During winter 1973, Lolium was recorded at its maximum level in the faeces, suggesting that it is even more acceptable in relation to other species at this time of year. The same situation may well apply in the case of Dactylis.

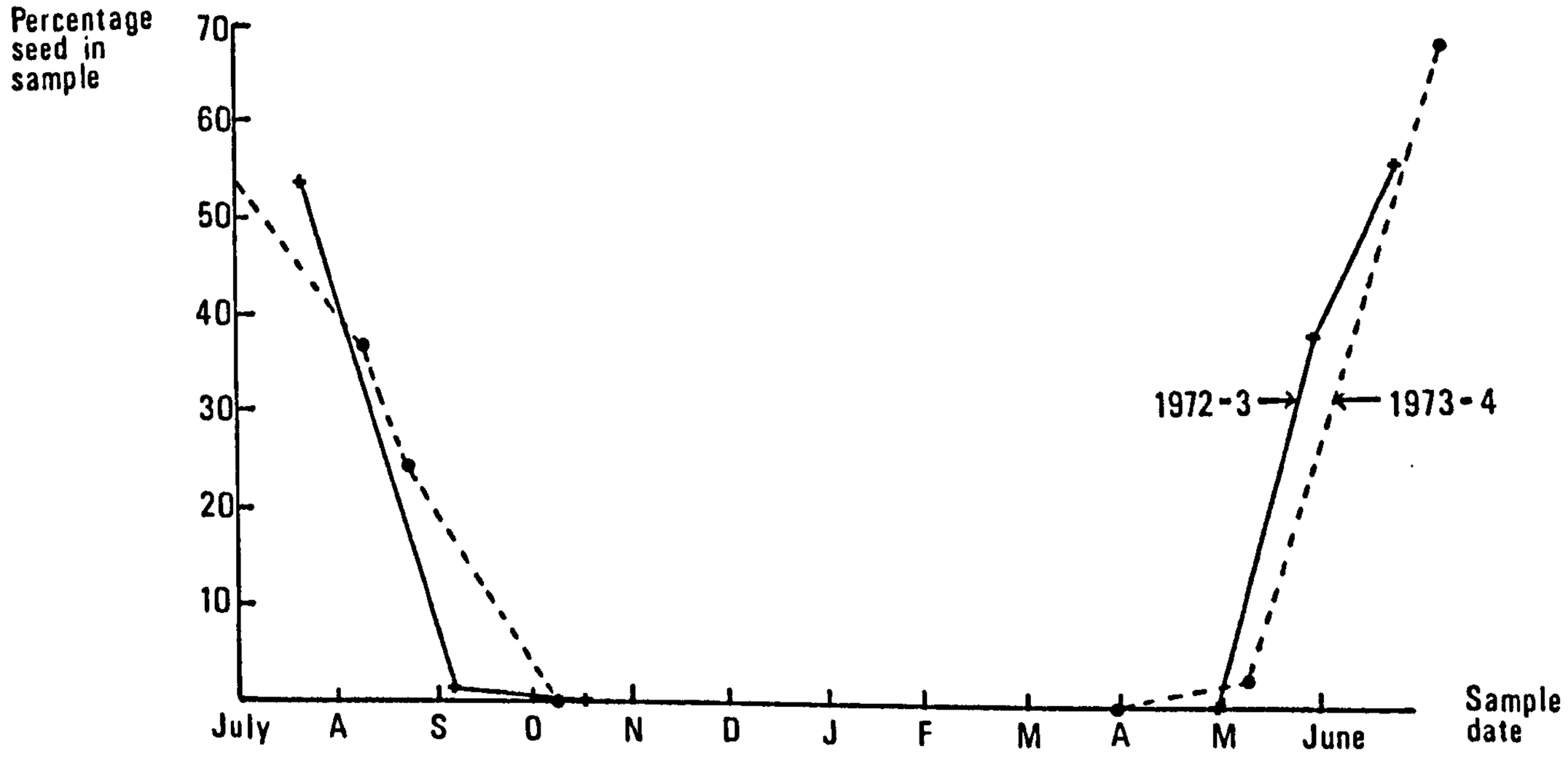
The seasonal variations in seed consumption are shown in Fig. 5.4, in which the seed component has been calculated as a proportion of the total faecal fragment count. This shows that grass seed forms a regular component of the diet between May and August. Its dietary importance relative to plant leaves could not be determined by faecal analysis without considerable calibration because of the large difference in the relationship between proportional intake and faecal occurrence.

General relationships between availability and intake

The estimated availabilities of the components of the vegetation are given in the list on page 54 in rank order of abundance on the grazed areas where the measurements were made. The dominant grass, A. tenuis appeared to be consistently under-grazed in relation to its abundance. When allowance is made for the relatively low digestibility of the epidermis of this species (Chapter 3), this further reduces the already low preference rating. F. rubra occurred consistently throughout the vegetation at a lower level of abundance than A. tenuis. The proportional occurrence in the faeces of F. rubra / P. pratensis tended to slightly exceed their combined availabilities. The relative digestibility of F. rubra epidermis has been found to be even lower than that of A. tenuis which probably results in an approximate correspondence

FIG. 5.4

Proportion of seed in faecal samples from Bangor Ancient Camp.



Month	Sample	% Seed
July	1	53.9
Sept.	2	1.4
Oct. - May	3-8	0
June	9	38.8
June	10	56.4
Aug.	11	37.3
Aug.	12	24.9
Oct. - March	13-18	0
May	19	2.4
July	20	68.5

between the average faecal proportion after correction, and availability.

H. lanatus normally appeared in the faeces in proportions paralelling its availability, except for the increase in intake in July when it appeared to be actively sought. The relationship between the availability and faecal occurrence of Lolium and Cynosurus has already been described in the preceding section. The evident preference for one or both species is further increased when the high digestibility of the epidermis of Lolium is taken into consideration. The consistently high faecal levels of Dactylis / Poa in relation to abundance, indicate a considerable preference for one, two or all of these species. If, as has already been suggested, the main constituent of this fraction was Dactylis, a very high level of preference is indicated since the epidermis has a relatively high digestibility.

Anthoxanthum and Arrhenatherum both occurred in the faeces and in the available vegetation at low levels, so it was concluded that there was no preferential selection of these species.

General Discussion and Conclusions

The annual grazing pattern of rabbits at Bangor Ancient Camp, exhibiting both seasonal and overall selection for certain components of the vegetation, corresponds ingeneral features with the findings of Martin (1962) in a four year study on seasonal changes in the diet of hill sheep in Scotland. Seasonal changes in species utilization by rabbits and cattle have been detected in a one year study by Williams et al (1974) at Wood Walton Fen. In this study however, variations in diet were largely related to changes in species availabilities rather than changes in the palatability of different species throughout the year.

The increased intake of F. rubra (+ P. pratensis), A. tenuis and H. lanatus prior to flowering represents a phenomenon which does not previously seem to

have been detected in herbivores feeding under natural conditions. Experimental work however has indicated that advancing growth stage in plants is a factor frequently correlated with decreasing preference (Heady 1964). For example Reid and Jung (1965) found that the intake of various forage species by sheep declined with increasing age of the plants. Ageing of a plant is associated with a decrease in protein content and an increase in fibre which leads to a fall off in Dry Matter Digestibility (see Chapter 3). This has been illustrated by successive measurements of DMD for various grass species, in which the decrease was found to take place between May and July (Mowat et al 1965; Chenost et al 1970). The lowest values of DMD are reached when the grass is flowering (Spedding 1972). In some way these changes must affect palatability so that a species is most acceptable during the maximum growth period which has been shown to occur just before flowering commences (e.g. Morris and Thomas 1972).

Since different species mature at different rates, relative preferences change with time; thus measurements of species utilization at a particular time of year may give a misleading picture of the overall situation. In the present study however, selection between the particular species of grass available was so marked that seasonal changes in diet only infrequently involved changes in the general overall relationship between intake and availability (i,e. the ratio of proportional intake: proportional availability tended to remain approximately equal to unity, very much greater than unity or very much less).

The overall preferences determined in this study correspond with those concluded in the previous chapter, which were obtained by comparing food selection by rabbits at a number of different sites at one particular time of year.

In conclusion therefore it was found that distinct patterns of seasonal utilization of food species were exhibited by rabbits feeding at the Agrostis - fescue grassland site. These variations were to a large extent attributed to changes in palatability at different growth stages of the vegetation, rather

than seasonal changes in availability. Although these seasonal variations were sometimes very marked, they were not sufficient to obscure overal preferences of the rabbits for particular grasses. In situations where there are only small differences in proportional availabilities and palatabilities of species, seasonal changes in preferences could well be of greater magnitude than overall differences in preferences.

PART 2

EXPERIMENTS ON FOOD SELECTION BY RABBITS UNDER CONTROLLED CONDITIONS

INTRODUCTION TO PART 2 :

FACTORS AFFECTING FOOD SELECTION BY HERBIVORES,

AND DEFINITION OF TERMS

INTRODUCTION TO PART 2: EXPERIMENTS ON FOOD SELECTION BY RABBITS UNDER CONTROLLED CONDITIONS

Factors affecting food selection by herbivores, and definition of terms.

The term preference is used throughout this study as a description or measurement of the behaviour of the animal(s) in selecting food items under any particular set of conditions at any point in time. Palatability, a concept which is sometimes confused with preference, is used to describe the acceptability of a food type as determined by those characteristics of the food itself which stimulate a selective response by the animal. This follows the terminology of Young (1948) and Heady (1964).

Food preferences are dependent on a number of factors which are discussed fully by several authors working in converging fields of psychology, animal behaviour, nutrition, agriculture and range management (e.g. Young 1948; Tribe 1950; Barnett and Spencer 1953; Arnold 1962; Heady 1964). The complexity of these factors and their interrelationships are briefly indicated in the following summary. The factors affecting food preferences appear to fall into four categories:

1. Palatability - theoretically this is a function of the physical and chemical characteristics of the food type, but in practice varying degrees of difficulty have been encountered in attempts to relate chemical composition with palatability (e.g. Arnold and Hill 1972).

2. History of the animal - (a) evolutionary 'history' i.e. species, breed or variety, genotype of individual.
   (b) previous experience of the individual animal, learning.

3. Physiological state of the animal - includes factors such as nutritional state, 'hunger', age, size, pregnancy, lactation.

4. Environmental conditions - includes overlapping factors which affect palatability of the food (.e.g. climate, topography, soil) and modify animal behaviour (e.g. relative abundances and mixtures of available food types, social factors,locality).

Food preferences of animals measured under natural conditions are therefore the resultant responses in terms of food selection elicited by the interacting effects of these factors. Under natural conditions it is difficult to isolate the contribution of individual factors to the preferences exhibited. However, some of these factors can be investigated by means of controlled feeding experiments as described in the following chapters.

CHAPTER 6

PREFERENCE TESTS ON CAPTIVE WILD RABBITS

CHAPTER 6
PREFERENCE TESTS ON CAPTIVE WILD RABBITS

Introduction

Controlled feeding experiments on captive animals have long been used as a method of measuring preferences. Linnaeus (1749) determined the acceptability to sheep of 618 plant species by feeding them singly and in mixtures. A commonly used method of ranking preferences is by means of 'cafeteria' experiments in which animals are given access to a number of different types of food and the relative quantities eaten or relative feeding times are computed. Numerous applications of this method are described in the literature of various fields of zoology, animal behaviour, ecology and agriculture.

The present experiment was designed to investigate whether differential selection of vegetation species by wild rabbits feeding on natural grasslands was paralleled by preferences shown by captive wild rabbits involved in controlled choice experiments. Preferences between a number of species of grasses have been determined in this study by relating proportions ingested, as measured by faecal analysis, to their availabilities in various types of grasslands (Chapters 4 and 5).

In the controlled choice experiment described here, it was impracticable to offer a number of species simultaneously due to lack of space, since the captive wild rabbits were too nervous to be moved from their customary surroundings to a larger experimental area. Consequently a number of choice trials were made involving a number of grass species which were offered in all possible combinations of pairs. This design of experiment, in addition to providing an indication of preference trends, should reveal any major effect due to different associations of species involved in the mixtures since this would disrupt the consistency of the preference ranking.

Materials and Methods

Summary of Experimental Design

Captive wild rabbits were offered choices between pairs of species of grass growing in separate, adjacent swards. Their preferences were determined (a) by the relative times spent eating each species during an observation period and (b) by measuring the relative quantities of each species eaten. Five species were offered in all ten possible pair combinations.

Preparation of the grass swards

Wooden tomato boxes (40 x 30 cm), containing John Innes seed compost, were divided into two equal compartments (20 x 30 cm) by means of strips of perspex projecting about 1 cm above soil level. Seeds of five commercially obtained grasses were sown at the beginning of August (approximately 3 g per compartment), so that each box contained a contrasting pair of species separated by the perspex. The following species were used - Agrostis tenuis (Ag), Anthoxanthum odoratum (An), Dactylis glomerata (D), Festuca rubra (F) and S23 Lolium perenne (L).

Eight replicate boxes were sown with each of the ten possible pair combinations, making a total of 80 boxes in all. The boxes of grass were maintained in a cold greenhouse for the following 9 months, watering daily and cutting when necessary, so that mature, densely growing swards were eventually produced. The perspex dividers were removed prior to offering the swards to rabbits.

Experimental animals

Five sibling wild rabbits, three bucks (1, 2, 3) and two does (4, 5) had been hand reared from the age of three weeks and were approximately one year old at the time of the experiment. The rabbits were individually housed in metal cages (76 x 36 x 45 cm) each divided into an open and closed compartment,

and had adapted fairly well to being handled and observed during the day. The feeding trials were carried out in these cages so that the rabbits remained in their accustomed environment.

The rabbits had previously encountered all the grass species involved in the tests, during the experiment described in Chapter 3, and subsequently as additions to their normal diet of commercial rabbit pellets. During the five weeks of preference testing the rabbits were fed only pellets in addition to the grasses involved in the trials. It was found unnecessary to withhold the pellets before each trial to ensure that sufficient grass was eaten.

Attempts were also made to measure the preferences of two wild rabbits, captured as adults, which were housed in large enclosures. It was found however that observations could not be made on these rabbits while they were feeding and when the boxes containing grass swards were left in the enclosures either all the grass was completely eaten and the soil excavated or the swards were completely untouched.

Preference Testing

One week prior to the start of the experiment a box containing a sward of Lolium perenne was left in the open compartment of each cage for 24 hours to accustom the rabbits to eating in this new situation. Each rabbit readily adapted to these conditions.

The ten choice trials were carried out at intervals over a period of about five weeks. All the rabbits were offered the same pair of species once, on the same day. The pairs were offered in the following sequence, so that no single species occurred in any two consecutive tests:

F/D; Ag/An; F/L; Ag/D; L/An; F/Ag; D/An; Ag/L; F/An; L/D.

For each trial five boxes containing swards with similar densities were selected and the grass in each of the two compartments was cut with scissors to approximately even heights (usually between 5 and 8 cm). The mean height of each species sward before a trial was found by measuring the grass at 16 points (4 rows of 4 points) using a graduated wire rod.

The trials were carried out between 1800 and 2100 hours which was the normal start of a period of feeding and activity. The boxes of grass almost completely occupied the open compartments of the cages and the rabbits had to stand on the swards to feed. Any resulting position effects in the measurement of preferences between the species pairs were accounted for in each trial by randomly assigning one of the species to the end nearest the entrance to the closed compartment in three of the cages, and to the opposite end in the other two cages. Each rabbit was individually tested by measuring the time (in secs.) spent actually eating each species after a box had been introduced into the cage. If it was considered that sufficient grass had been eaten to give a measure of preference, the mean heights of the remaining swards were determined, and hence measures of the quantities eaten were obtained. However, if little or no feeding had taken place after 20 minutes of observation, the box was left in the cage overnight and the sward measured the following morning. Rabbits 2 and 3 could not be timed eating during any of the trials since they rarely emerged even to investigate the boxes of grass. However, they always ate sufficient grass during the night for measurements of preferences to be made from the amounts eaten.

Results

The lengths of time spent by the individual rabbits in eating each species offered and the quantities eaten are given in Table 6.1, together with their relative values (%) for each pair of grasses. There was reasonable correspondence between the relative eating times and the quantities eaten, and in only one instance were conflicting measures of preference obtained (Rabbit 1, D/F). Since this occurred during the first trial, it was possibly due to observer error in failing to distinguish between mere investigation of the grasses and actual eating. No detectable effect was observed which was attributable to the relative positions of the two species

TABLE 6.1

Results of preference tests on captive wild rabbits offered grass species in various pair combinations.

Rabbit	Eating time (secs) during observation period.		Difference in mean height of sward before and after eating (cm).		% time		% eaten	
	D	F	D	F	D	F	D	F
1	145	305	1.93	1.06	32	68	65	35
2	5	0	1.63	0.50	-	-	77	23*
3	0	0	2.44	2.37	-	-	51	49*
4	535	40	2.43	0.06	93	7	98	2
5	490	120	2.31	0.32	80	20	88	12
	Ag	An	Ag	An	Ag	An	Ag	An
1	190	170	2.25	2.57	53	47	47	53
2	10	15	1.19	0.94	-	-	56	44*
3	310	10	2.68	1.63	97	3	62	38*
4	550	155	1.13	0.31	78	22	78	22
5	825	0	2.19	0	100	0	100	0
			(3.56)	(2.57)			(58)	(42)
	F	L	F	L	F	L	F	L
1	290	585	0.50	2.00	33	67	20	80
2	0	150	1.00	2.25	0	100	31	69*
3	7	0	3.18	4.75	-	-	40	60*
4	120	495	0.38	1.69	20	80	18	82
5	60	515	0.12	2.31	10	90	5	95
	D	Ag	D	Ag	D	Ag	D	Ag
1	600	260	1.94	1.44	70	30	57	43
2	0	0	0.88	0.12	-	-	88	12*
3	55	5	2.69	1.18	-	-	70	30*
4	320	275	0.69	0.38	54	46	64	36
5	485	235	2.25	0.81	67	33	74	26
	L	An	L	An	L	An	L	An
1	555	350	1.19	0.68	61	39	64	36
2	0	0	0.63	0.50	-	-	56	44*
3	0	0	1.68	2.69	-	-	38	62*
4	520	100	0.63	0.43	84	16	59	41
5	600	280	1.75	1.19	68	32	60	40

TABLE 6.1 continued

Rabbit	Eating time (secs) during observation period.		Difference in mean height of sward before and after eating (cm).		% time		% eaten	
	Ag	F	Ag	F	Ag	F	Ag	F
1	245	695	0.69	1.38	26	74	33	67
2	0	0	0	0	-	-	-	-*
3	0	0	0.63	1.31	-	-	32	68*
4	370	460	0.69	1.31	45	55	35	65
5	420	520	1.00	1.88	45	55	35	65
	D	An	D	An	D	An	D	An
1	450	330	1.81	0.75	58	42	71	29
2	0	0	0.81	0.43	-	-	65	35*
3	0	0	1.32	0.31	-	-	81	19*
4	415	220	1.88	0.69	65	35	73	27
5	595	15	3.12	0.13	98	2	96	4
	L	Ag	L	Ag	L	Ag	L	Ag
1	205	485	0.31	1.56	30	70	17	83
2	0	0	1.63	0.93	-	-	64	36*
3	0	0	2.56	0.06	-	-	98	2*
4	350	300	1.37	0.75	54	46	65	35
5	605	210	2.12	0.62	74	26	77	23
	F	An	F	An	F	An	F	An
1	480	185	0.50	0.37	72	28	57	43
2	0	0	0.75	0.50	-	-	60	40*
3	0	0	0.19	0.94	-	-	17	83*
4	65	385	0.12	0.81	14	86	13	87
5	510	190	0.94	0.56	73	27	63	37
	D	L	D	L	D	L	D	L
1	480	245	1.06	0.13	66	34	89	11
2	0	0	2.69	0.37	-	-	88	12*
3	0	0	2.37	0.88	-	-	73	27*
4	370	250	0.87	0.68	60	40	56	44
5	550	110	1.63	0.18	83	17	90	10

* Boxes of grass left in cages overnight because insufficient or no grass eaten during observation time.

L Lolium perenne

An Anthoxanthum odoratum

Ag Agrostis tenuis

F Festuca rubra

D Dactylis glomerata

within the cages. The rabbits always investigated the whole of each box of grass thoroughly before starting to eat and subsequently tended to shift their position frequently and intersperse short periods of eating with other activities such as investigation of the boxes, washing and scrutiny of the observer.

The mean of the results for the five rabbits in each trial are given in Table 6.2 and illustrated in Fig. 6.1. The data are arranged to show the order of preference of the five species as indicated by the results of the trials. The species were ranked in order of preference as follows :

Dactylis > Lolium > Festuca > Agrostis > Anthoxanthum.

There was remarkable overall consistency in the qualitative preferences exhibited by the individual rabbits in each trial, apart from one major discrepancy which had the effect of disrupting the order of preferences as indicated by all the other trials. Two rabbits exhibited a marked preference for Anthoxanthum when it was offered with Festuca, which conflicted with the results obtained when Anthoxanthum and Festuca were paired with the other species. Two out of the only three other deviations in preferences of individual rabbits also involved Anthoxanthum, this time paired with Lolium and Agrostis. One of the previously deviant rabbits plus an additional individual were involved in these two additional exceptions.

The data show no indications of any corresponding quantitative grading within the qualitative preferences themselves, e.g. that proportionately more Dactylis was eaten when it was paired with Anthoxanthum than when paired with the less palatable Lolium. This seems to be a general result of measuring preferences by offering only pairs of species on different occasions rather than measuring the relative quantities eaten when all the species are offered simultaneously (Young 1948).

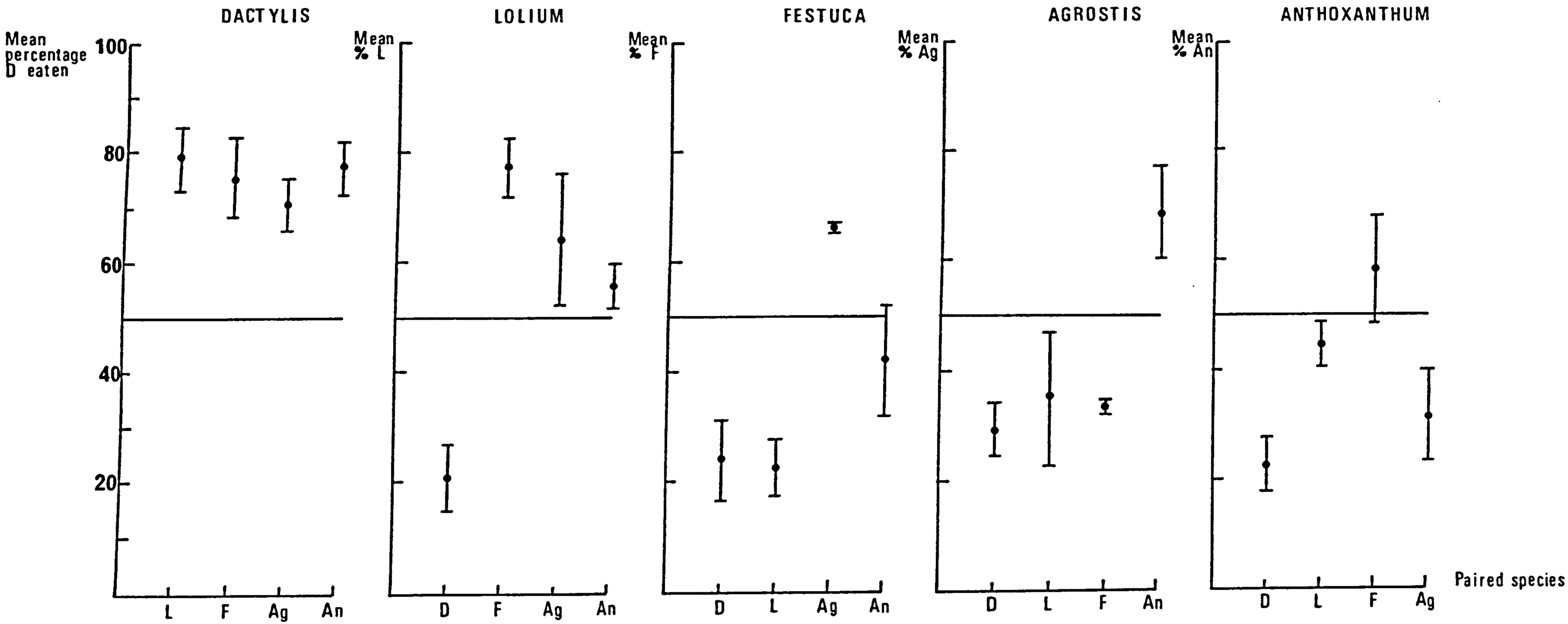
TABLE 6.2

Mean results of preference tests on captive wild rabbits fed grasses in various pair combinations.

Means of results for 5 rabbits.

	Mean % eaten		s.e.
D		F	
75.8		24.2	7.4
Ag		An	
68.6		31.4	8.4
F		L	
22.8		77.2	5.3
D		Ag	
70.6		29.4	4.7
L		An	
55.4		44.6	4.1
Ag		F	
33.8		66.2	0.7
D		An	
77.2		22.8	4.8
L		Ag	
64.2		35.8	11.9
F		An	
42.0		58.0	9.9
D		L	
79.2		20.8	5.9

FIG. 6.1 Preferences of captive wild rabbits fed grasses in various pair combinations.



Means of results for 5 rabbits ± 1 s.e.

Discussion and Conclusions

The rank order of preferences of the three species Lolium, Festuca and Agrostis determined in these trials corresponds with the preferences of wild rabbits in natural conditions previously determined by means of faecal analysis (Chapters 4 and 5). The position of Dactylis in the preferences shown by the wild rabbit populations had not been clearly defined in the earlier faecal studies because the epidermal fragments could not be distinguished from those of Poa trivialis and P. annua. However the high faecal proportions of the Dactylis/Poa category in relation to their combined availabilities had indicated a strong preference for one or more of these species at the Bangor Ancient Camp site (Chapter 5). It seems likely that this preference was for Dactylis since this was the only one of the three species normally present in the regularly grazed areas, and the field choice would be in agreement with the results of the choice trials in which Datcylis ranked highest in preference.

The least preferred species in the trials, Anthoxanthum, was only ever present at low frequencies in the natural grasslands investigated when it appeared from faecal analysis to be eaten roughly in the proportions available. These results appeared to indicate that it ranked higher in preference than Agrostis which was eaten in much lower proportions than its relative availability, (which in some of the grasslands studied was fairly high). These results may therefore indicate that preferences are modified by relative availabilities, and apparently unpalatable species may be more acceptable when they are present only at low frequencies in mixtures with more palatable species. Some corresponding instances of species being heavily grazed when occurring in small quantities amongst more palatable species, but eaten very little when in dense stands, are mentioned in the review by Heady (1964). The situation is further complicated in the case of Anthoxanthum since variation was found in the response to the species by individual rabbits

when it was presented both with the same and with different alternative species in the choice trials. The response of agricultural stock to Anthoxanthum appears equally indeterminate. Spedding (1972) cites conflicting reports on its palatability, varying between low and high according to different investigators.

It is concluded from this study that preferences measured by simple choice experiments tend to mirror the preferences exhibited by animals in their natural environment. The combined results of the field measurements and controlled choice experiments in the present study demonstrate fairly conclusively that Lolium, Festuca and Agrostis are ranked in order of preference, with Dactylis probably heading the rank order. The indeterminancy of the position of Anthoxanthum in the ranking may indicate that in the field preferences can be modified by relative availabilities and differing species mixtures in such a way that they do not entirely correspond with experimental measurements.

CHAPTER 7

CONTROLLED CHOICE EXPERIMENTS ON DOMESTIC RABBITS INVOLVING TWO FOOD-TYPES ONLY

CHAPTER 7

CONTROLLED CHOICE EXPERIMENTS ON DOMESTIC RABBITS INVOLVING TWO FOOD-TYPES ONLY

Introduction

Selective choice of food has been studied extensively in both predator (carnivore) - prey and parasite - host interactions. Not only has selection of food been shown to be a function of its innate acceptability or palatability to the predator, but preference sometimes depends also on the density or relative frequencies of prey available to the predator (Holling 1965; Tinbergen 1960). As early as 1927 it was postulated that stability in prey populations could be maintained if predators tended to feed more heavily on the most abundant type of prey, switching as relative prey densities changed (Elton 1927). That such a mechanism can exist has been partially demonstrated by Murdoch (1969) in a simplified experimental simulation involving the predation of mussels and barnacles by sea-shore snails.

Frequency-dependent food selection involving disproportionately high predation of the most abundant form ('apostatic' selection, Clarke 1962) has also been postulated as a mechanism that maintains genetic polymorphism in prey populations and has been the subject of experimental modelling (Allen and Clarke 1968).

Frequency-dependent selection has also been reported in the choice of seed taken by a granivorous 'predator', the wood pigeon (Murton et al 1963). In this instance (and others, e.g. Tullock 1971) the behaviour was discussed in terms of value to the predator rather than its long-term effects on the prey.

If frequency-dependent selection can play an important role in

stabilizing prey populations and genetic polymorphisms within prey populations, it can similarly be argued that grazing herbivores, selecting in a frequency-dependent manner, could affect the composition of a grazed sward (Harper 1969). Comparatively little attention has been paid to the consequences of such grazing behaviour and probably still less to determining whether indeed herbivores ever do select in a frequency-dependent manner. It was to investigate this latter point in relation to the rabbit that the following experiments were performed.

Wheat and barley grains were used as a substitute for vegetation in all but one experiment in order to provide two comparable types of discrete, measurable food items.

I

The effect on preference of the relative availabilities of two food-types in a mixture.

Materials and methods

Summary of experimental design

Mixtures of wheat (W) and barley (B) were offered to individual domestic rabbits in the proportions (by number of grains) 90%W:10%B, 90%B:10%W and 50%W:50%B. Four trials with each mixture were carried out on consecutive days. The proportions eaten by each rabbit at 90%:10% were compared with (1) the proportion offered and (2) the proportion offered but adjusted to take account of the individual preferences shown at 50%:50%.

Experimental animals

All rabbits used in Experiments I - III were selected from stock in the Department of Zoology, U.C.N.W. Bangor, maintained primarily for use in immunology experiments. However, none of the animals used in the feeding experiments had been involved in any previous experiments.

Twelve Dutch rabbits of varying ages were housed in individual wire cages. They were divided into two equal groups each composed of 3 bucks and 3 does, Group 1 numbered ♂1.1, ♂1.2, ♂1.3, ♀1.1 .... and Group 2 ♂2.1 ...., ♀2.1 .... . The normal diet consisted of commercial rabbit pellets (B.O.C.M. Silcocks Coneybrand) which supplemented the experimental food throughout the several weeks of trials.

Mixtures of wheat and barley

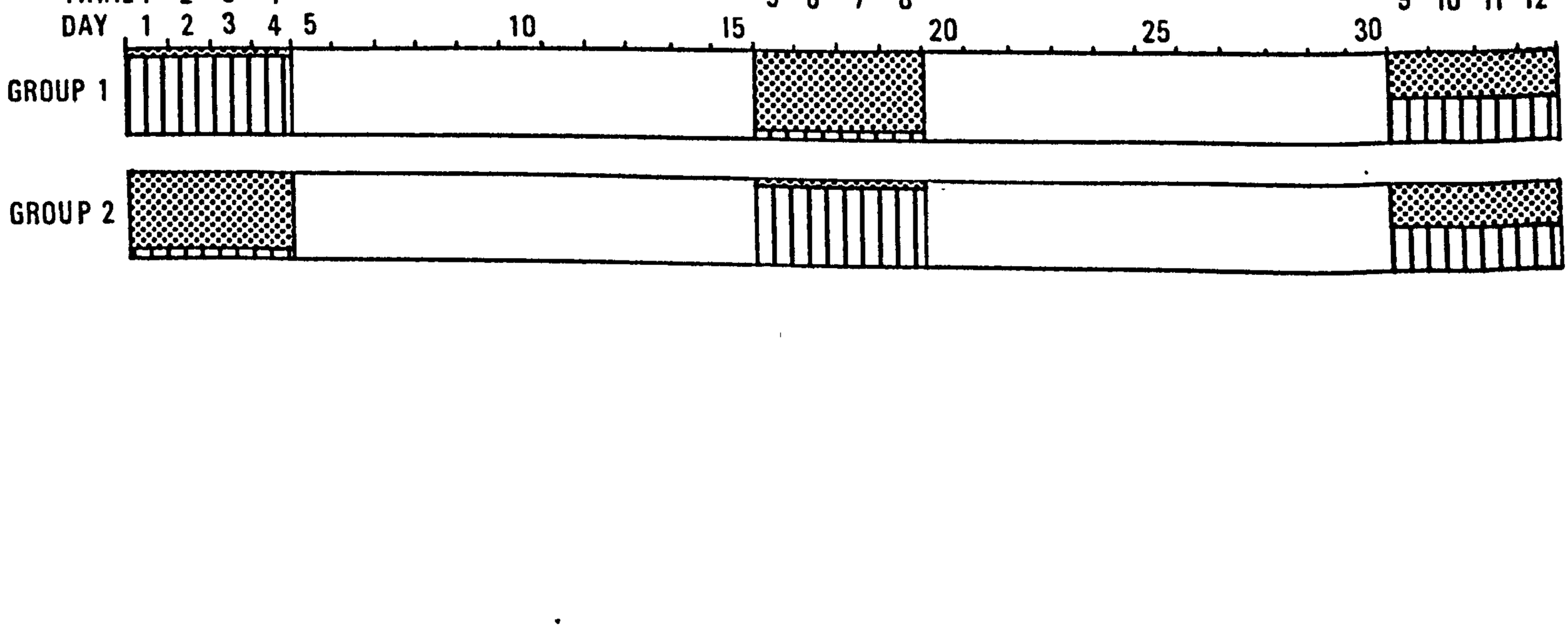
Wheat used in the experiment was cleaned by immersion in water to

float off the accompanying fragments of chaff. The grain was then sifted and dried for 24 hours at 60°C. The barley, which consisted of whole florets, was cleaned by sifting to remove dust. Accurate determinations of mean weight per grain were made for both types of seed, so that large numbers of grains could be obtained rapidly by weighing. During each trial each rabbit was offered a mixture containing a total of 1,500 grains which were loosely scattered in shallow wooden trays of dimensions 31 x 34 cm with rims 4.5 cm high. These trays occupied approximately half the floor space of the cages in which the feeding trials took place.

Feeding trials

During the first set of trials, Group 1 rabbits were offered mixtures containing 90%W:10%B and Group 2 the reverse mixture. Each rabbit was given this mixture during a single trial on each of four consecutive days. Eleven days after the fourth trial, each group was tested with the reverse of the proportions previously offered, and after a further gap of eleven days, both groups were given mixtures of 50%W:50%B. The design of the experiment is outlined in Fig. 7.1 below.

FIG. 7.1. Diagram showing the temporal sequence of trials in Expt. I and the relative proportions of wheat ▯ and barley ▩ fed to each group of rabbits, supplementary to the normal diet of rabbit pellets.



Each rabbit was allowed to eat a maximum of approximately 400 grains during a single trial, or as was usually the case, up to a time limit of one hour. The seeds remaining were separated and the numbers of each type determined by weighing the large fraction and counting the small fraction. From this the numbers eaten were calculated.

Results

The numbers of grains eaten by each rabbit during the trials are given in Appendix 2, Table A9. Much of the heterogeneity shown by individual rabbits within the sets of similar trials was attributable to small numbers of grains being eaten on several occasions. The results for the four similar trials were pooled for each rabbit and these pooled results analysed (Table 7.1). The results for ♂1.2 were omitted since very few grains were eaten during all the series of trials.

Feeding preferences at 50W:50B (After previous experience of different proportions)

The proportions eaten by nine of the eleven rabbits indicated significant preferences ($p < 0.05$) for one type of grain. In eight cases this was for barley, wheat being selectively eaten by only one rabbit. The remaining two rabbits exhibited no preference and ate the grain in the proportion offered.

Although there was variation in the proportions eaten by some individuals during the four trials, the results for each trial show consistency in the direction of preference, if not its magnitude, in almost every case.

There were no consistent differences in preferences at 50:50 between the two groups of rabbits implying that there were no residual effects from the set of trials 11 days before in which Group 1 had received 90%B:10%W and Group 2 90%W:10%B.

TABLE 7.1

Analysis of numbers of wheat and barley grains eaten by individual rabbits when offered at three different relative frequencies. Results are pooled for each set of 4 similar trials. χ^2 values are shown for departures from two hypothetical expectations (see footnote).

		PROPORTIONS OF WHEAT/BARLEY OFFERED								
		9W:1B			9B:1W			1W:1B		
RABBIT		W	B	$\chi^2_{(1)}$	W	B	$\chi^2_{(1)}$	W	B	$\chi^2_{(1)}$ preference
♂ 1.1	O_{np}	352	54		63	668		311	374	
	E^{np}	365	41	4.58*(B)	73	658	1.52	342.5	342.5	5.80*
	E^{p}	358	48	0.85	62	669	0.02			Barley
♂ 1.3	O_{np}	207	39		2	220		67	136	
	E^{np}	221	25	8.73*(B)	22	200	20.18*(B)	101.5	101.5	23.46*
	E^{p}	201	45	0.98	12	210	8.81*(B)			Barley
♀ 1.1	O_{np}	689	68		145	1414		669	687	
	E^{np}	681	76	0.94	156	1403	0.86	678	678	0.24
	E^{p}	679	78	1.43	152	1407	0.36			
♀ 1.2	O_{np}	729	79		50	648		245	317	
	E^{np}	727	81	0.25*	70	628	6.35*(B)	281	218	9.22*
	E^{p}	706	102	5.94*(W)	55	643	0.49			Barley
♀ 1.3	O_{np}	547	62		61	741		361	356	
	E^{np}	548	61	0.02	80	722	5.01*(B)	358.5	358.5	0.04
	E^{p}	549	60	0.08	81	721	5.49*(B)			
♂ 2.1	O_{np}	421	37		55	489		284	361	
	E^{np}	412	46	1.96*	54	490	0.02	322.5	322.5	9.22*
	E^{p}	401	57	8.02*(W)	44	500	2.99			Barley
♂ 2.2	O_{np}	551	56		22	370		166	224	
	E^{np}	546	61	0.46*	39	353	8.23*(B)	195	195	8.62*
	E^{p}	528	79	7.70*(W)	30	362	2.31			Barley
♂ 2.3	O_{np}	546	54		47	399		390	458	
	E^{np}	540	60	0.67	45	401	0.10	424	424	5.46*
	E^{p}	531	69	3.68	39	407	1.80			Barley
♀ 2.1	O_{np}	512	56		39	696		153	197	
	E^{np}	511	57	0.02	74	662	18.40*(B)	175	175	5.54*
	E^{p}	497	71	3.62	58	677	6.76*(B)			Barley
♀ 2.2	O_{np}	521	67		105	804		215	279	
	E^{np}	529	59	1.20	91	818	2.39*	247	247	8.30*
	E^{p}	514	74	0.76	72	837	16.41*(W)			Barley
♀ 2.3	O_{np}	1009	128		60	563		500	399	
	E^{np}	1023	114	1.91*	62	561	0.07*	449.5	449.5	11.34*
	E^{p}	1044	93	14.34*(B)	76	547	3.84*(B)			Wheat

O_{np} Observed number of grains eaten during 4 trials
E^{np} Expected number eaten at 9:1 and 1:1 if no preference
E^{p} Expected number eaten at 9:1 if preference is similar to that at 1:1
* $p < 0.05$. Disproportionately more of the grain-type in brackets was eaten.

Feeding preferences on mixtures containing 90%:10% of the two grain types

The pooled numbers of each type of grain eaten by each rabbit during the two alternate sets of 90:10 trials were tested for deviations from the ratio offered (Table 7.1). In 15 out of the total of 22 results the proportions eaten did not deviate significantly ($p > 0.05$) from the proportion offered. Four of the seven deviant sets of results corresponded with the expected 90:10 ratio weighted with the preferences exhibited at 50:50. The remaining three deviant results were each obtained when the ratio offered was 90B:10W (♂1.3, ♀1.3, ♀2.1). In all three cases significantly more barley was eaten than expected from either the actual or preference-weighted proportion offered. These deviations could be explained either by the preference for barley being greater than that actually measured (in only two cases was this preference at 50:50 significant at $p < 0.05$), or by frequency-dependent selection involving selection of the commoner grain type. However, if preference factors for barley which are high enough to account for the deviations at 90B:10W were to be substituted, the corresponding expected proportions of barley at the opposite frequency of 90W:10B would be shifted well above the proportions actually eaten.

Table 7.2 categorises both the significant and non-significant deviations from the ratios expected if the grains were eaten in the proportions offered and if these were weighted with the preferences shown at 50:50. The trend of deviations from the weighted ratios suggests that there was a tendency for more of the commoner type of grain to be eaten irrespective of whether it was wheat or barley.

TABLE 7.2

Expected ratio	Correspondence with/or deviation from expected ratio.	No.of rabbits exhibiting behaviour	No. of significant deviations ($p < 0.05$)
90W:10B Grain eaten in proportion offered.	1.More wheat, less barley eaten.	4	0
	2.Eaten in expected propn. ($\pm$ 2 grains)	3	
	3.Less wheat, more barley eaten.	4	2
90B:10W Grain eaten in proportion offered.	1.More barley, less wheat eaten.	7	5
	2.Eaten in expected propn. ($\pm$ 2 grains)	3	
	3.Less barley, more wheat eaten.	1	0
90W:10B Proportion offered weighted with preference at 50:50.	1.More wheat, less barley eaten.	8	3
	2.Eaten in expected propn. ($\pm$ 2 grains)	1	
	3.Less wheat, more barley eaten.	2	1.
90B:10W Proportion offered weighted with preference at 50:50.	1.More barley, less wheat eaten.	7	4
	2.Eaten in expected propn. ($\pm$ 2 grains)	1	
	3.Less barley, more wheat eaten.	3	1

Discussion

Under natural conditions the grazing rabbit is usually faced with a continuous sward from which it selects items to eat. Although green vegetation was not used in this experiment, the mixtures of wheat and barley formed an analogous continuum of food. Statistical treatment of the relative numbers of grains eaten can be justified partially on the basis that for each grain eaten a separate act of choice or selection was involved. A more satisfactory method

of analysing results from this type of experiment is in terms of the number of rabbits exhibiting different types of selective behaviour. However, the limited number of rabbits which it was possible to use in this experiment was well below that required for such statistical treatment.

It must be noted that although the weights of individual wheat and barley grains were not identical, the generally preferred type, barley, weighed slightly more than wheat (the mean weight of 1 grain of barley was 0.0305 g and of wheat 0.0273g). Consequently if relative preference is based on intake by weight, both the overall preference for barley at 50:50 and the disproportionate selection of barley when at high frequency becomes more pronounced.

Although some of the rabbits ate the grain in the proportions offered, statistically significant patterns of selective behaviour were exhibited by some individuals:

1. Some rabbits maintained consistent preferences regardless of grain proportion. This could be attributed to the preferred item being specifically searched for, or alternatively the probability of a particular grain being eaten when encountered at random could be greater for the preferred type.

2. Other rabbits showed frequency-dependent selection of food type with disproportionately more of the commoner type of grain being selected. The behavioural response resulting in this form of selection could have been caused by conditioning to the commoner item as a consequence of continually eating more of this type because of the disparity in frequencies. This would have to involve a change in selective response during the course of the experiment, but there was no indication of this occurring progressively throughout each set of feeding trials. However, the following experiment (II) was performed to see if such a response could build up as a result of frequently repeated encounters with the same 90:10 mixture.

II

The effect on preference of repeated encounters with the same 90:10 mixture of two types of grain

---

Materials and methods

Summary of experimental design

Mixtures of wheat and barley were offered to individuals within two groups of domestic rabbits in the proportions (by numbers of grains) 90%W:10%B and 90%B:10%W respectively. Ten consecutive trials were carried out at the rate of two per day over five days (Expt. IIA). The proportions eaten at the beginning of the experimental period were compared with those eaten during the final trials.

The experiment was repeated later using a different set of rabbits and continuing for fifteen trials (Expt. IIB).

Experimental animals

In the initial set of trials the same twelve rabbits were used in the same groupings 1 and 2 as in the previous experiment. The trials commenced 10 days after the termination of the previous series (I).

Twelve different Dutch rabbits were used in the repeat experiment and were similarly divided into two equal groups (3 and 4) each containing three bucks and three does.

The normal diet of rabbit pellets was fed throughout the experimental period.

Mixtures of wheat and barley

The mixtures of wheat and barley were prepared as for the previous experiment and the total number of grains offered to each rabbit in each trial was again 1,500.

Feeding trials

In the first series of trials (IIA) each rabbit was offered the same mixture of grain in the early morning and late afternoon during five consecutive days. Group 1 rabbits were given a mixture of 1,350 wheat and 150 barley grains, and Group 2, 1,350 barley and 150 wheat. The eating time was limited to one hour or until a maximum of approximately 400 grains had been eaten. The seeds remaining were separated and the numbers eaten determined.

The repeat experiment(IIB) was continued for fifteen consecutive trials over 8 days. Group 3 rabbits were offered 1,350 wheat and 150 barley grains throughout these trials and Group 4, 1,350 barley and 150 wheat.

Results

Expt. IIA

The numbers of grains eaten by the individual rabbits during each trial are given in Appendix 2, Table A10. The results for ♂2.1 and ♂1.3 have been omitted since these rabbits ate very few grains during any of the trials. The numbers eaten by each individual during the first three trials were compared with the total numbers eaten during the comparable section of Expt. I when the same mixtures had been offered in four consecutive trials (Table 7.3). In only one case (♀2.1) was there significant heterogeneity ($p < 0.05$) between the results obtained in the two experiments, thus suggesting that any further significant heterogeneity measured between the proportions eaten at the beginning and end of the repeated feeding sequence resulted from the feeding regime itself.

The total numbers of seeds eaten by each rabbit during the first and last three trials are compared in Table 7.4. The proportions of grains eaten by five out of the ten rabbits at the end of the experiment differed significantly

TABLE 7.3

Comparison between results obtained in Experiment I and at the outset of the repeated feeding trials (IIA).

		W	B	heterogeneity χ^2
♂ 1.1	a	352	54	
	b	222	37	0.14
♀ 1.1	a	689	68	
	b	794	83	0.12
♀ 1.2	a	729	79	
	b	397	43	0.05
♀ 1.3	a	547	62	
	b	239	32	0.54
♂ 2.1	a	55	489	
	b	24	341	3.42
♂ 2.2	a	22	370	
	b	14	225	0.02
♂ 2.3	a	47	399	
	b	37	334	0.06
♀ 2.1	a	39	696	
	b	34	335	6.08*
♀ 2.2	a	105	804	
	b	34	305	0.59
♀ 2.3	a	60	563	
	b	60	487	0.57

a - pooled results of the four comparable trials in the previous experiment.

b - pooled results of the first three trials of repeated feeding.

* - $p < 0.05$.

TABLE 7.4

Comparison between the pooled results of the first three and last three trials of the repeated feeding series (IIA).

		W	B	heterogeneity χ^2
♂ 1.1	b	222	37	
	c	507	32	15.42*(W)
♀ 1.1	b	794	83	
	c	954	98	0.01
♀ 1.2	b	379	43	
	c	555	35	6.30*(W)
♀ 1.3	b	239	32	
	c	281	11	12.85*(W)
♂ 2.1	b	24	341	
	c	43	591	0.02
♂ 2.2	b	14	225	
	c	17	328	0.24
♂ 2.3	b	37	334	
	c	39	470	1.48
♀ 2.1	b	34	335	
	c	16	361	7.42*(B)
♀ 2.2	b	34	305	
	c	21	363	5.31*(B)
♀ 2.3	b	60	487	
	c	38	461	3.49

b - pooled results of first three trials.

c - pooled results of final three trials.

* - $p < 0.05$.

The grain type of which significantly more was eaten during the final trials is indicated in brackets.

from those eaten at the outset. Two of the six rabbits offered 90%B ate significantly more barley at the end of the experiment (these included ♀ 2.1 which was the only rabbit to show significant heterogeneity in preference between the previous experiment and the beginning of the repeated feeding trials). Three of the four Group 1 rabbits offered 90%W ate significantly more wheat during the final trials. Thus in each case significantly more of the most frequent grain-type was eaten at the end of the experiment. The non-significant results show a similar trend towards more of the commoner grain-type being eaten by the end of the series of trials.

Expt. IIB

Figures are given only for the numbers of grains eaten during trials 1-3, 8 and 10 and 13-15 (Appendix 2, Table A.11). For each rabbit the degree of heterogeneity between the pooled results for each of these groups of trials has been determined by χ^2 tests (Table 7.5).

When the proportions eaten during trials 8 and 10 were compared with those eaten during the first three trials (as in Expt. IIA), three Group 4 rabbits showed significant differences. However, these differences in each case indicated a change in preference to wheat, the less frequent grain-type. Furthermore the additional heterogeneity between initial, intermediate and final preferences produced by continuing the trials, reveals significant changes in the preferences of both Group 3 and 4 individuals, both towards the more frequent and to the less frequent grain-type.

Discussion

The results of Experiment IIA indicated a definite trend towards selection of the commoner item of diet but the repeat experiment did not

TABLE 7.5

Comparison between the numbers of wheat and barley grains eaten at the start, during the middle and at the end of the 15 consecutive trials during which the same 90:10 mixture was offered to each rabbit (Repeat Experiment IIB).

			W	B	Heterogeneity a:b	Heterogeneity b:c	Heterogeneity $\chi^2_{(1)}$ a:c
Group 3 rabbits offered 90%W:10%B	♂ 3.1	a	395	55	0.02	1.19	1.46
		b	290	40			
		c	313	33			
	♂ 3.2	a	516	50	2.42	8.38*(W)	1.61
		b	429	57			
		c	696	52			
	♂ 3.3	a	540	63	1.10	2.08	0.12
		b	527	50			
		c	701	87			
	♀ 3.1	a	172	34	0.33	4.32*(B)	1.28
		b	331	57			
		c	330	84			
	♀ 3.2	a	356	66	3.72	1.72	11.07*(W)
		b	404	51			
		c	512	49			
	♀ 3.3	a	527	62	0.55	2.98	1.66
		b	237	23			
		c	369	56			
Group 4 rabbits offered 90%B:10%W	♂ 4.1	a	31	277	0.07	5.06*(B)	5.45*(B)
		b	21	181			
		c	22	382			
	♂ 4.2	a	17	252	12.79*(W)	2.79	3.70
		b	52	280			
		c	30	243			
	♂ 4.3	a	25	220	1.49	10.33*(B)	4.99*(B)
		b	25	152			
		c	8	175			
	♀ 4.1	a	33	404	7.87*(W)	0.96	4.64*(W)
		b	53	340			
		c	81	625			
	♀ 4.2	a	34	420	7.49*(W)	0.69	5.23*(W)
		b	38	240			
		c	76	574			
	♀ 4.3	a	41	444	2.80	0.07	3.79
		b	38	277			
		c	99	735			

a - total numbers of grains eaten during trials 1-3
b - total numbers of grains eaten during trials 8 and 10
c - total numbers of grains eaten during trials 13-15

* $p < 0.05$ The grain-type of which significantly more was eaten during the later set of trials is indicated in brackets.

confirm this. The only difference between the two experimental sequences was that the rabbits in Experiment IIB (Groups 3 and 4) had never encountered wheat and barley before the experiment. Consequently during the first three trials their individual reactions to the two grain-types may not have had time to stabilize. The same argument could also apply to the initial results obtained in Experiment I when the Group 1 and 2 rabbits were also naive. The results of Expt. I might therefore have been more clear-cut if the rabbits had been familiar with the grain at the outset.

The results of Expt. IIA indicate that conditioning towards the commoner food-type does indeed build up as a result of repeated encounters, as predicted from the results of Expt. I. That such conditioning can occur when an animal has been pre-trained on one type of food only has been demonstrated by Young (1944), Barnett and Spencer (1953), Murdoch (1969), Allen and Clarke (1968) and Soane and Clarke (1973). The concept of specific search-images (Tinbergen, 1960) has been used to explain this type of frequency-dependent selection.

If conditioning did occur in Expts. I and IIA, the response must have built up during the time the rabbits were confronted with the unequal mixtures, because there was no pre-training period as given in the experiments of the authors cited above. The effect of 'pre-feeding' (Young 1940) only one grain-type was tested in the following experiments (III A and B).

III

The effect of pre-feeding on subsequent choice

Materials and methods

Summary of experimental design

Individual rabbits were fed with either wheat or barley before testing their subsequent preferences with equal mixtures of the two types of grain.

In the first experiment (IIIA) rabbits were pre-fed immediately before each preference test. Single trials were carried out on two consecutive days, reversing the type of grain pre-fed on the second day. The results of these preference tests were compared with preference measured on the day before the commencement of pre-feeding.

In the second experiment (IIIB) rabbits were fed one of the grain-types for limited periods of time for three days before testing preference on the fourth day.

In each experiment both rabbits with experience of eating wheat and barley in previous experiments and rabbits which had never before encountered grain were used, and their reactions to pre-feeding compared.

Experimental animals

In each experiment 12 Dutch rabbits were used, 6 of which had been involved in previous feeding trials. The experienced rabbits consisted of Group 1 (prefix 1) and Group 2 (prefix 2) from experiments I and IIA. The 6 individuals in the immediate pre-feeding experiment (IIIA) with no previous experience of grains consisted of 3 bucks and 3 does, forming Group 5. Similarly those in the prolonged pre-feeding experiment (IIIB) formed Group 6.

Wheat and Barley

The grain was prepared as described in Expt. I. The preference tests were similarly carried out using the same wooden trays containing equal mixtures of wheat and barley, totalling 1,500 grains.

Pre-feeding and preference testing

IIIA Immediate pre-feeding

The 12 rabbits were divided into two groups A and B, each containing equal representations of experienced and non-experienced individuals, and of bucks and does. The composition of each group was as follows :

Group A ♂1.1, ♀1.1, ♀1.3, ♂5.2, ♂5.3, ♀5.3

Group B ♂2,1, ♂2.2, ♀2.3, ♂5.1, ♀5.1, ♀5.2

Day 1: each rabbit was offered an equal mixture of wheat and barley. The numbers of grains of each type eaten after a time limit of one hour were determined.

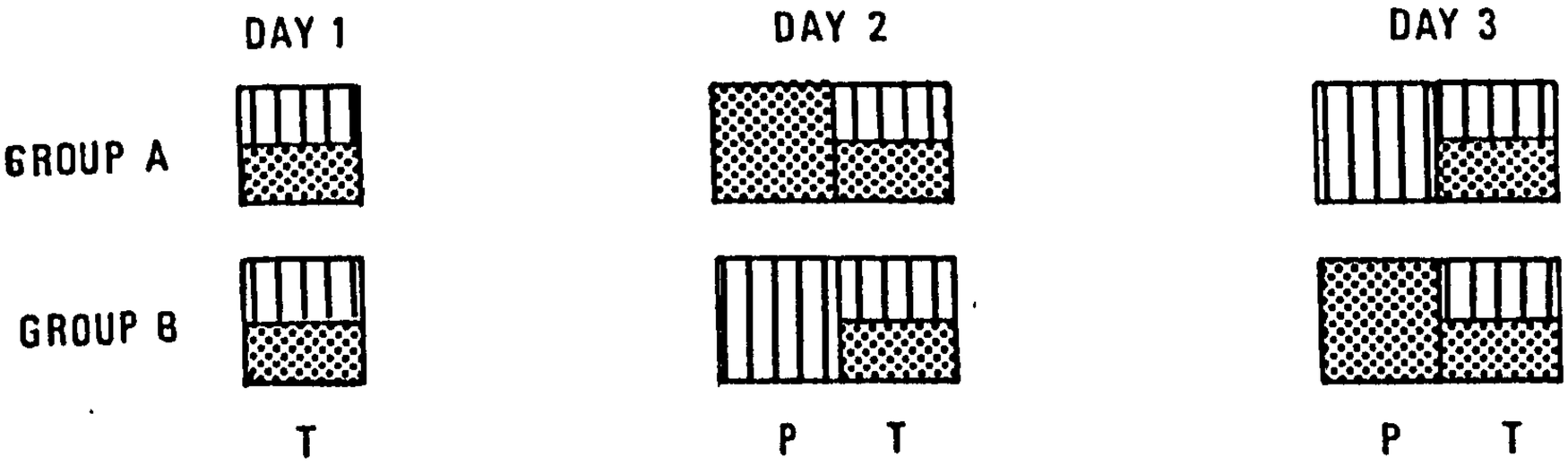
Day 2: Group A individuals were each offered 1,500 barley grains and allowed to eat for one hour or up to a maximum of approximately one fifth of the grain. Similarly Group B were given wheat only. The numbers of grains eaten by each rabbit were determined.

The trays containing the single grain-type were then replaced immediately with trays containing equal mixtures of wheat and barley, and preferences determined as on Day 1.

Day 3: The pre-fed grain-type was reversed so that Group A received wheat and Group B, barley. Preferences were tested subsequently as on Day 2.

The experimental design is summarized below in Fig. 7.2.

FIG. 7.2. Expt. IIIA. Sequence of pre-feeding (P) groups A and B with wheat ▯ and barley ▩ , and trials (T) with equal proportions of grain.



IIIB Prolonged pre-feeding

The 12 rabbits were divided into two groups, C and D, as in the immediate pre-feeding experiment. The composition of each group was as follows :

Group C ♂1.2, ♂1.3, ♀1.2, ♂6.3, ♀6.2, ♀6.3

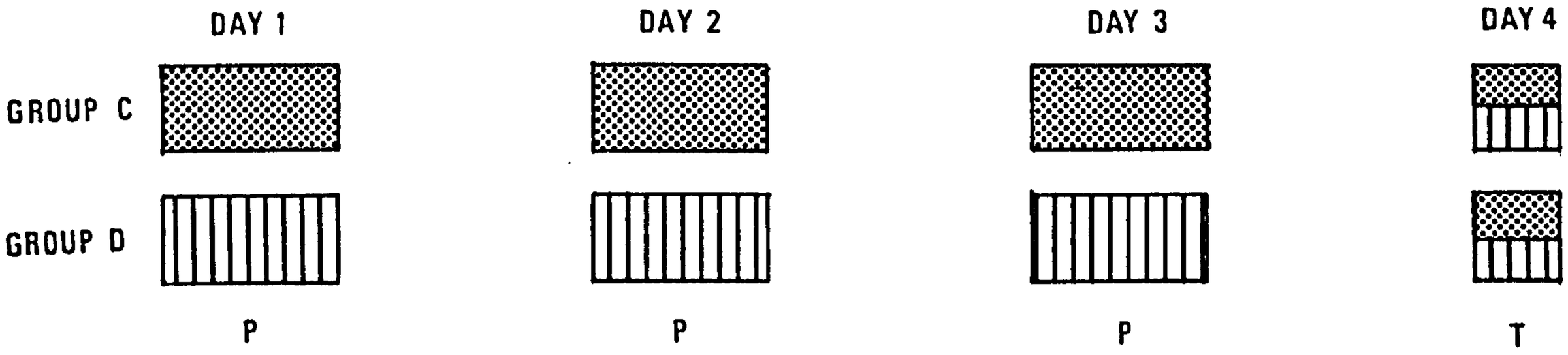
Group D ♂2.3, ♀2.1, ♀2.2, ♂6.1, ♂6.2, ♀6.1

For two hours during each of three consecutive days each rabbit was given 120 g of one type of grain only, contained in an untippable pot. The weights of grain eaten during this time were measured. Group C individuals were pre-fed barley and Group D, wheat.

On the fourth day each rabbit was tested with an equal mixture of wheat and barley as in IIIA.

The experimental design is summarized in Fig. 7.3 below.

FIG. 7.3. Expt. IIIB. Sequence of pre-feeding (P) Groups C and D with wheat ▯ and barley ▩ , and subsequent trial (T) with equal proportions of grain.



Results

IIIA Immediate pre-feeding

The numbers of wheat and barley grains eaten by individual rabbits during the preference tests on Days 1, 2 and 3 are given in Table 7.6, together with the numbers of grains eaten during pre-feeding. No results are given for ♀ 1.3 which was removed from the feeding experiment for use in an immunology experiment.

The numbers of rabbits in each group exhibiting significant changes in preference ($p < 0.05$) from day to day (see Table 7.6) and those showing no change are given in Table 7.7. On Day 2 two of the Group A rabbits which

TABLE 7.6

Numbers of grains eaten during preference testing with equal numbers of wheat and barley grains on the three consecutive days of Expt. IIIA and comparison between the proportions eaten from day to day. The numbers of grains eaten during pre-feeding are given in brackets. (The feeding sequences are shown in Fig. 7.2)

				Heterogeneity $\chi^2(1)$		
	Day	W	B	1-2	2-3	1-3
GROUP A						
	1	23	52			
♂ 1.1	2	83	93(194)	5.86* (W)	0.01	6.47* (W)
	3	95(191)	104			
	1	177	158			
♀ 1.1	2	38	50(234)	3.00	1.89	0.05
	3	110(322)	102			
	1	27	39			
♂ 5.2	2	21	30(127)	0.00	0.00	0.00
	3	37(168)	54			
	1	37	49			
♂ 5.3	2	47	66(156)	0.04	0.11	0.01
	3	49(270)	63			
	1	112	110			
♀ 5.3	2	115	62(220)	8.47* (W)	1.19	4.09* (W)
	3	147(362)	99			
GROUP B						
	1	37	52			
♂ 2.1	2	1(231)	57	29.1* (B)	18.4* (W)	1.68
	3	19	42(188)			
	1	64	61			
♂ 2.2	2	34(153)	34	0.03	1.37	2.09
	3	20	31(71)			
	1	68	77			
♀ 2.3	2	41(261)	32	1.67	4.92* (B)	1.44
	3	40	62(230)			
	1	128	108			
♂ 5.1	2	7(325)	28	14.4* (B)	6.30* (W)	4.65* (B)
	3	64	85(320)			
	1	41	60			
♀ 5.1	2	20(231)	55	3.69 NS (B)	5.61* (W)	0.32
	3	38	47(229)			
	1	60	71			
♀ 5.2	2	20(392)	22	0.04	0.67	2.16
	3	74	61(142)			

* $p < 0.05$ The grain-type of which significantly more was eaten during the later trial is indicated in brackets.

TABLE 7.7

Numbers of rabbits in each group showing significant deviations from the initial preference of Day 1 after immediate pre-feeding on Days 2 and 3 (Expt. IIIA).

	Significantly more wheat eaten ($p < 0.05$)	No significant preference ($p < 0.05$)	Significantly more barley eaten ($p < 0.05$)
DAY 1-2			
Group A, pre-fed B	2 (1E+1N)	3	0
Group B, pre-fed W	0	4	2 (1E+1N)
DAY 2-3			
Group A, pre-fed W	0	5	0
Group B, pre-fed B	3 (1E+2N)	2	1 (E)
DAY 1-3			
Group A, pre-fed W	2 (1E+1N)	3	0
Group B, pre-fed B	0	4	1 (E)

E - rabbits with previous experience of wheat and barley

N - rabbits with no previous experience of wheat and barley

had been pre-fed barley, ate significantly more wheat, but none of the group ate significantly more barley. Conversely on Day 2, two Group B rabbits, pre-fed wheat, ate significantly more barley, and similarly none ate significantly more wheat. Thus the only significant effect of pre-feeding was to decrease the subsequent preference for the pre-fed grain type.

However, on Day 3 after reversing the pre-fed grain, conflicting results were obtained. None of the Group A rabbits ate significantly different proportions on Days 2 and 3, whereas three Group B individuals, pre-fed barley on Day 3, ate significantly more wheat and one ate significantly more barley. Comparison between the results of Days 1 and 3 still indicated similar trends in preference differences to those between Days 1 and 2.

In this experiment there appeared to be no differences between the responses of the experienced (prefix 1 and 2) rabbits and naive (prefix 5) rabbits.

IIIB Prolonged pre-feeding

The numbers of wheat and barley grains eaten by each rabbit during preference testing are given in Table 7.8, together with the weights (to the nearest g) of grain eaten during the preceeding pre-feeding periods.

The numbers of rabbits in each group showing significant preferences ($p < 0.05$) after pre-feeding, for either wheat or barley, and those showing no significant preference are shown in Table 7.9.

Since initial preferences before pre-feeding were not tested (so that naive rabbits would first encounter the non pre-fed grain-type in the mixture presented on Day 4) there was no indication of whether the preferences shown in Table 7.9 developed as a result of pre-feeding. Consequently the numbers of grains eaten by individuals within each group were pooled and compared to see if there were differences between the two groups. There was found to be no significant difference between the proportions eaten by each group on Day 4

TABLE 7.8

Weights and numbers of wheat and barley grains eaten during pre-feeding and preference testing respectively in Expt. IIIB.

	Day	W	B	Preference χ^2
GROUP C				
♂1.2	1-3		6g (4,2,0)	
	4	5	22	10.7 *(B)
♂1.3	1-3		?	
	4	0	23	*(B)
♀1.2	1-3		14g (4,4,6)	
	4	107	55	16.7 *(W)
♂4.3	1-3		31g (10,11,10)	
	4	103	102	
♀4.2	1-3		37g (13,12,12)	
	4	101	74	4.2 *(W)
♀4.3	1-3		11g (2,2,7)	
	4	50	70	
GROUP D				
♂2.3	1-3	18g (6,6,6)		
	4	41	72	8.5 *(B)
♀2.1	1-3	19g (8,5,6)		
	4	82	61	
♀2.2	1-3	31g (8,16,7)		
	4	92	89	
♂4.1	1-3	24g (6,8,10)		
	4	114	117	
♂4.2	1-3	38g (12,11,15)		
	4	74	69	
♀4.1	1-3	36g (6,13,17)		
	4	144	117	

* p <0.05 The grain-type of which significantly more was eaten is indicated in brackets.

TABLE 7.9

Numbers of rabbits in each group showing significant preferences after pre-feeding one grain-type on three previous days. (Expt. IIIB)

	Significant preference for wheat (p <0.05)	No significant preference (p <0.05)	Significant preference for barley (p <0.05)
Group C, pre-fed barley	2 (1E+1N)	2	2 (1E+1N)
Group D, pre-fed wheat	-	5	1 (E)

E - rabbits with previous experience of wheat and barley
N - rabbits with no previous experience of wheat and barley

($\chi^2 = 0.02$). Comparison between the proportions eaten by the experienced and naive rabbits within each group also demonstrated no significant heterogeneity (Group C; $\chi^2 = 0.24$; Group D, $\chi^2 = 0.99$).

Since widely differing weights of grain (between 6g and 38g) were eaten by the individual rabbits during pre-feeding, these weights were compared with the respective proportions of wheat and barley eaten during the preference test. The results of this comparison are illustrated in Fig. 7.4.(excluding ♂1.3 which invariably emptied the grain over the wire mesh floor of the cage during pre-feeding). There was no evidence of any correlation between the weight of the one type of grain eaten and subsequent choice.

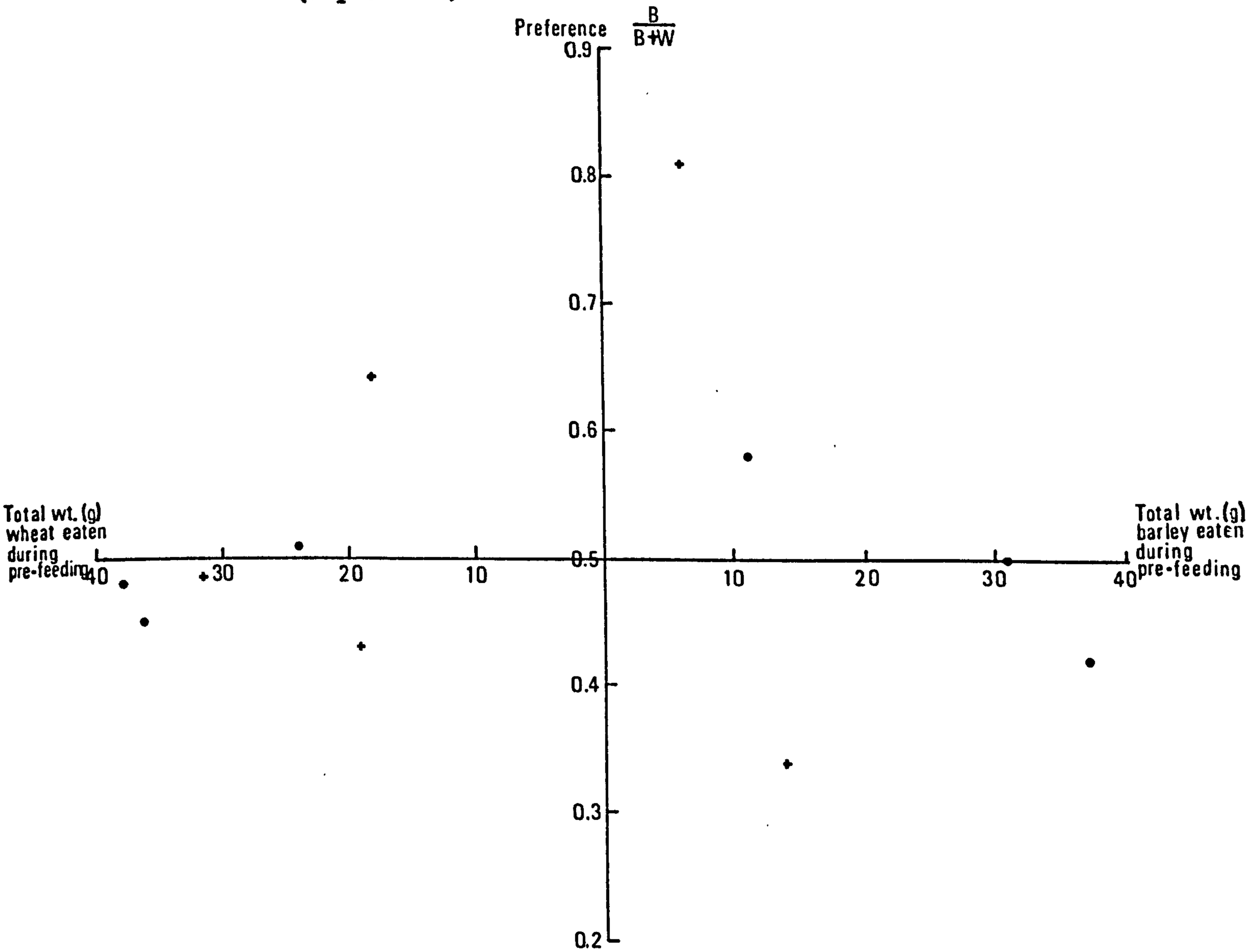
Discussion - Expts. III A and B

The results of the pre-feeding experiments show that, although there was no demonstrable response to prolonged pre-feeding, immediate pre-feeding did affect subsequent choice. However, the change in preference which occurred after the first period of pre-feeding was the reverse of that predicted from the results of Expts. I and IIA, when conditioning occurred. In Expt. IIIA the rabbits exhibited a change in selective behaviour after pre-feeding which corresponded to that described by Young (1940) in laboratory rats, i.e. temporary aversion towards a previously acceptable type of food.

The results obtained after reversing the pre-fed grain in Expt. IIIA suggested that there was a residual effect from pre-feeding on the previous day, the extent of which varied between individual rabbits. This longer term effect was also described by Young (1940). This result appears to contradict that from prolonged pre-feeding which had no demonstrable effect on subsequent choice.

Pre-feeding rabbits can therefore result in entirely the opposite selective response to that of conditioning or training. The conditions giving rise to either type of behaviour are examined more fully in the general discussion at the end of the chapter.

FIG. 7.4 Relationship between total weight of grain eaten by individual rabbits during pre-feeding, and subsequent preference (Expt. IIIB)



+ Rabbits with previous experience of wheat and barley.
• Rabbits with no previous experience of wheat and barley.

	Total weight of grain eaten during pre-feeding (g)	Preference $\frac{B}{B+W}$
♂1.2	6	0.81
♀1.2	14	0.34
♂4.3	31	0.50
♀4.2	37	0.42
♀4.3	11	0.58
♂2.3	18	0.64
♀2.1	19	0.43
♀2.2	31	0.49
♂4.1	24	0.51
♂4.2	38	0.48
♀4.1	36	0.45

IV

Choice experiments involving fresh vegetation

In the following feeding trials 10 four-month old Californian rabbits were used. Two separate groups consisting of 5 bucks and 5 does were housed in outdoor runs approximately 2 x 1m in which the experiments were carried out.

The preference tests involved freshly cut Lolium perenne and Trifolium repens, both of which included flowering heads. The cut vegetation was distributed evenly between six plastic pots embedded in the ground in a single straight line within each enclosure. When preference tests were performed using equal fresh weights of both species, the two species were arranged in alternate pots.

IVA The effect of previous experience on subsequent choice

Prior to this experiment the rabbits had no previous experience of eating either Lolium or Trifolium . 120g of Lolium was fed to each group on three consecutive days prior to offering a choice of Lolium and Trifolium. Equal weights of the two species were then offered on five consecutive days to see whether there were any changes in preference over this time. The rabbits were allowed to eat up to a maximum of approximately one third of the vegetation before the relative weights eaten were determined. The remaining vegetation was then left in the enclosures and was always eaten in the next few hours, well before the commencement of the subsequent day's preference tests.

The weights of Lolium and Trifolium eaten during the five preference tests are given in Table 7.10. On the first day a very low proportion of Trifolium was eaten by each group, but on subsequent days it was always selected in

TABLE 7.10

Weights (g) of Lolium and Trifolium eaten during preference testing after previously encountering Lolium only. (Experiment IVA).

Day	Group	Lolium	Trifolium
1	♂♂	59	4
	♀♀	20	8
2	♂♂	45	65
	♀♀	6	32
3	♂♂	15	50
	♀♀	5	54
4	♂♂	35	50
	♀♀	28	68
5	♂♂	45	72
	♀♀	44	66

TABLE 7.11

Weights (g) of Lolium and Trifolium eaten during the three successive phases of each feeding trial. (Experiment IVB).

		DAY 1		DAY 2		DAY 3	
Phase	Group	Lolium	Trifolium	Lolium	Trifolium	Lolium	Trifolium
1	♂♂	28	45	22	58	30	40
	♀♀	18	46	12	42	22	52
2	♂♂	20	25	26	30	26	18
	♀♀	15	42	14	22	22	22
3	♂♂	25	20	30	14	28	16
	♀♀	23	14	28	30	24	20

FIG. 7.5 Relationship between total weight of vegetation eaten by each group of rabbits on each day, and percentage of Trifolium eaten (Expt. IVA).

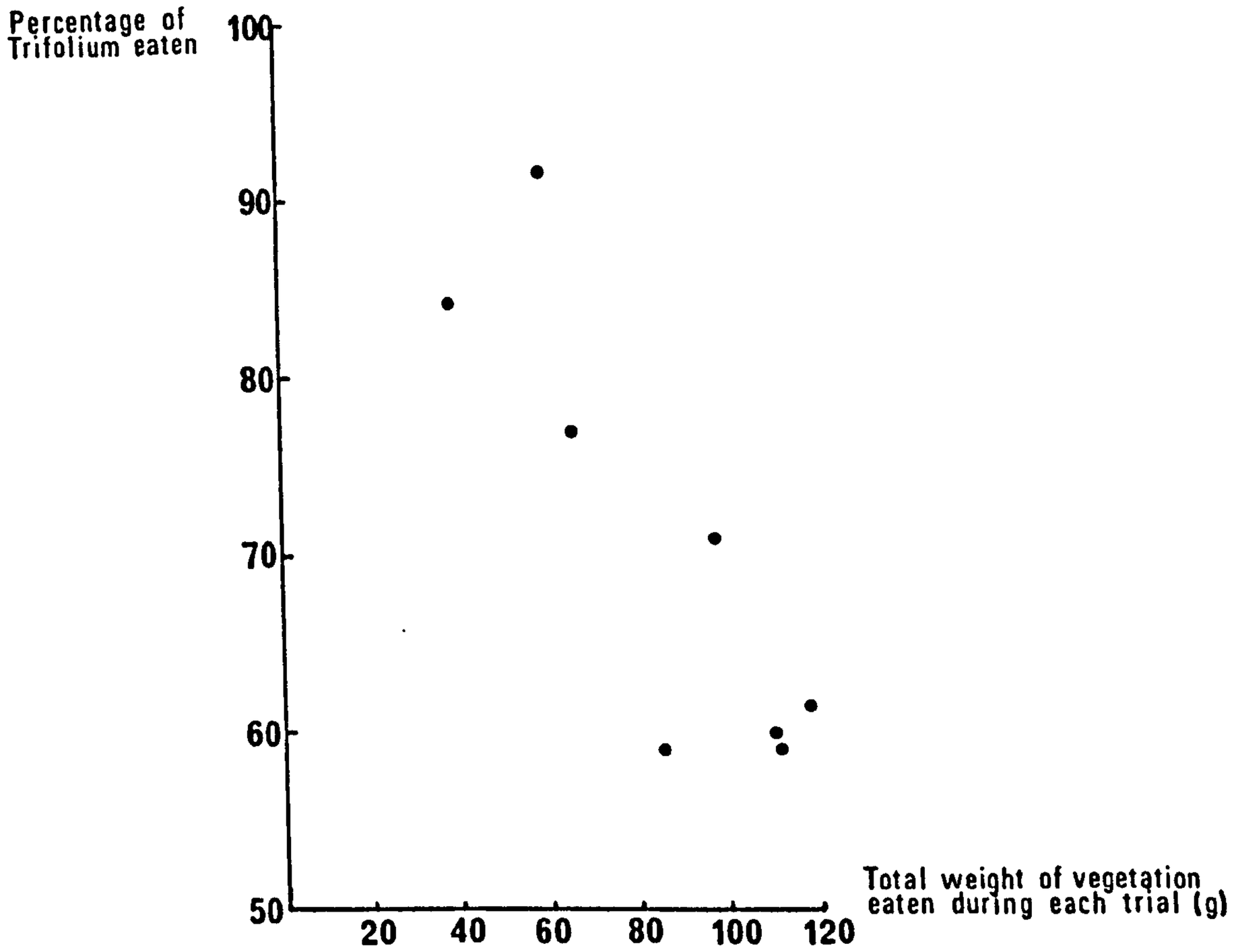
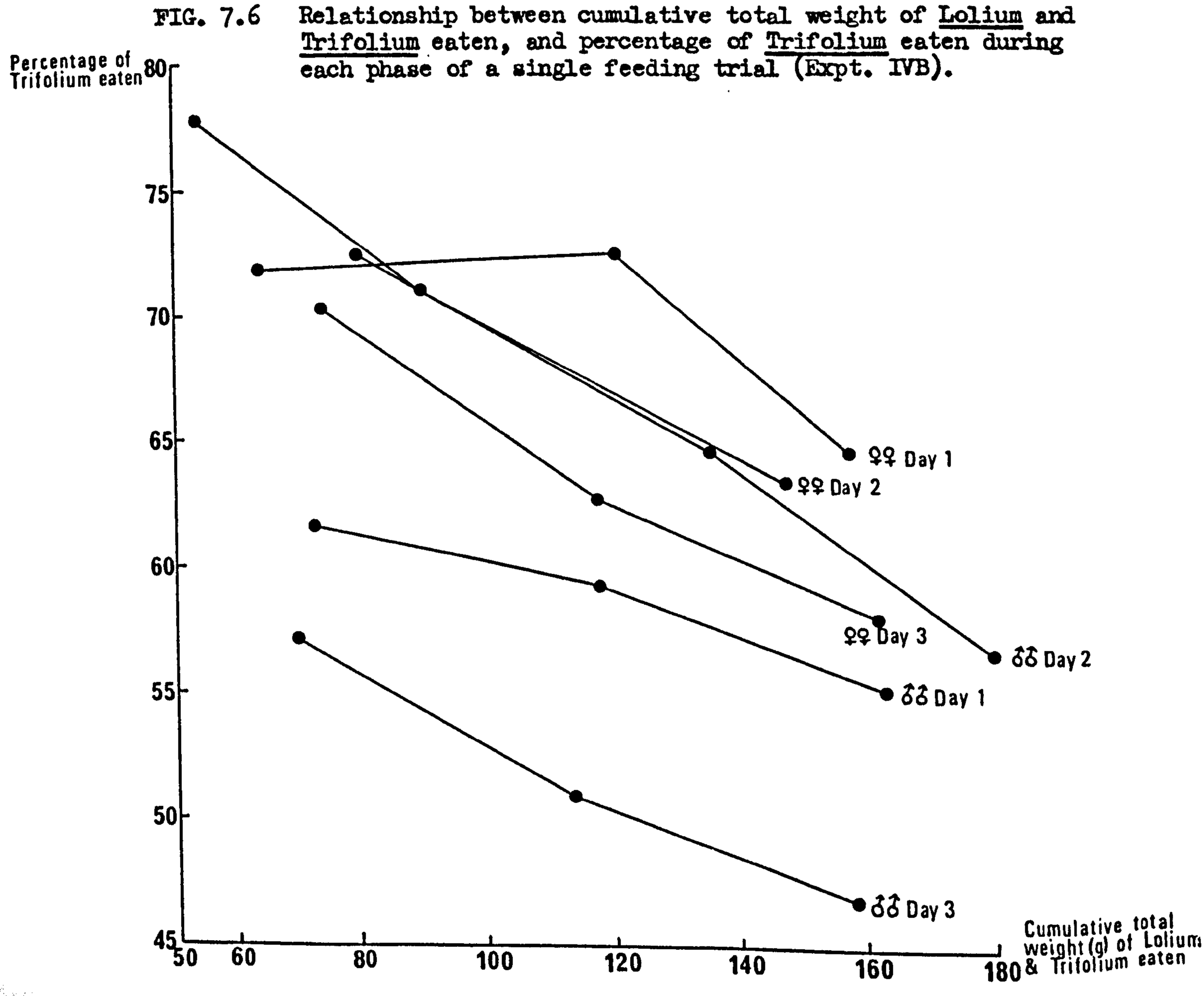


FIG. 7.6 Relationship between cumulative total weight of Lolium and Trifolium eaten, and percentage of Trifolium eaten during each phase of a single feeding trial (Expt. IVB).



preference to Lolium. In contrast with the results of the prolonged pre-feeding experiment (IIIB) this suggests that in the short-term, previous experience or conditioning on one type of food can determine subsequent choice between this and a food-type not previously encountered. Evidence of a similar reaction to 'preconditioning' on one plant species is described by Marten and Jordan (1974) in relation to selection by sheep between three grass species, Phalaris arundinacea, Bromus inermis and Dactylis glomerata.

Fig. 7.5 illustrates that the magnitude of the preference for Trifolium exhibited by each group on each of Days 2 - 5 was negatively correlated with the total weight of vegetation eaten. This may have reflected changes in preference during a single feeding period or could have been due to the relative decrease in availability of Trifolium with respect to Lolium. The following preference tests were carried out to resolve this.

IVB Change in preference during a single feeding period

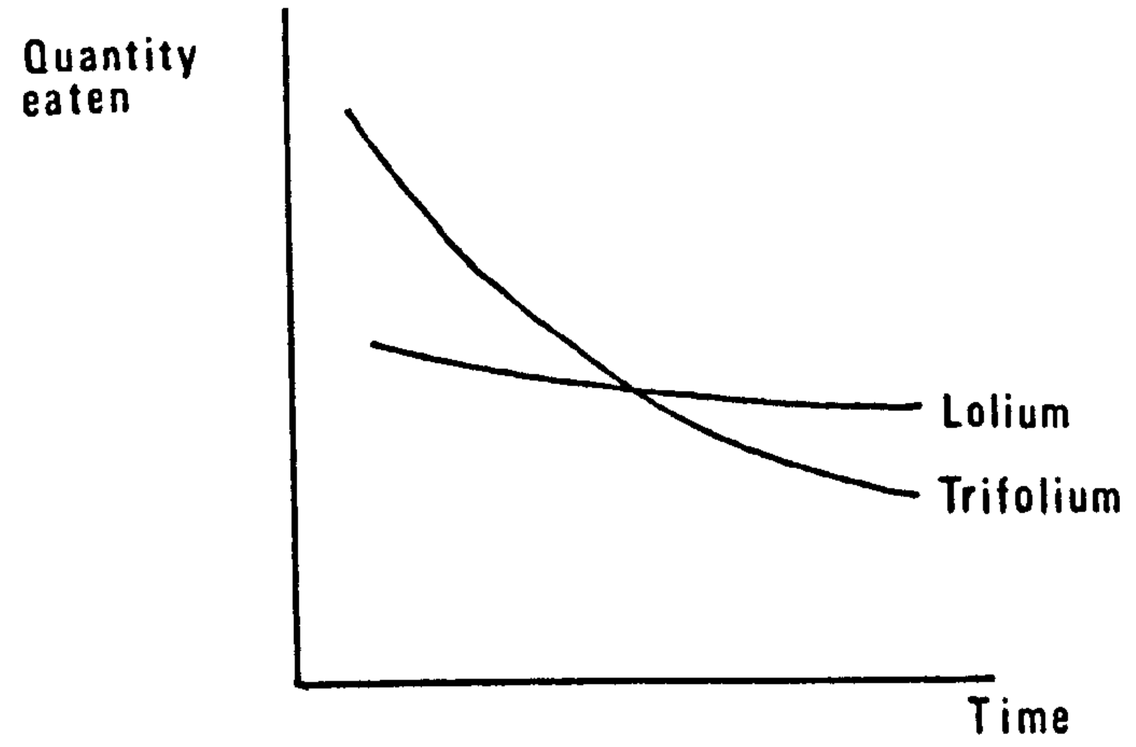
The two groups of rabbits were each offered equal amounts of Lolium and Trifolium (120g of each by fresh weight) as in the previous trials. After approximately a quarter had been eaten the pots were emptied, and refilled with the original weights of fresh vegetation and the rabbits allowed to eat as before. The procedure was repeated a third time and the weights of Lolium and Trifolium eaten during each phase were determined. The whole experiment was repeated on two subsequent days. The results are shown in Table 7.11.

Fig. 7.6 shows that there was a decline in preference for Trifolium over the successive phases of each feeding period. In this experiment the pots were repeatedly refilled so the decline in preference for Trifolium could not have been due to its decreasing availability.

In general the change in the pattern of selection was as illustrated in Fig. 7.7 below.

Fig. 7.7

Changing demand for Lolium and Trifolium with time during a single feeding period.



This fits the response predicted by Young (1940) on the assumption that demands or appetites for different food types are to some extent independent. As time progressed the demand for Trifolium decreased as the rabbits became more rapidly satiated with this species than with Lolium which was eaten at a relatively constant rate throughout each feeding period. This response is discussed in context with the results of the previous experiments in the general discussion at the end of the chapter.

Summary of results of Experiments I - IV

Wheat and Barley

I Comparison of the proportions of wheat and barley eaten at 90:10 frequencies with respective proportions eaten at 50:50 gave some indications of frequency-dependent selection for the commoner seed-type.

IIA Repeated encounters with a 90:10 mixture of the two grain-types for ten consecutive trials over 5 days led to the development of selection for the commoner seed-type.

B Experiment IIA repeated with naive rabbits over a longer period (fifteen trials over 8 days) resulted in significant changes in preference but these were in some cases for the high and in others for the low frequency grain-type.

IIIA Pre-feeding immediately before preference testing resulted in significantly less of the pre-fed grain-type being eaten. Reversal of the pre-fed grain a day later seemed to indicate a residual effect from the previous day.

B Pre-feeding one grain-type for 3 days prior to preference testing had no definite effect on selection.

Trifolium and Lolium

IVA Three days of feeding naive rabbits on Lolium alone had an initial effect on the selection made from a mixture of Lolium and Trifolium, but overall preference for Trifolium developed with increasing experience.

B Relative decrease in preference for Trifolium with respect to Lolium developed with time during a single feeding period.

General Discussion of the results presented in Chapter 7

The results of the preceeding experiments involving selection between two food-types, indicate that preference is not a constant factor determined only by the reation of an individual animal at any given time to the relative physical characteristics (odour, taste, texture etc.) of the food-types, but is also affected by factors such as previous experience, food recently eaten and relative frequency.

Two contrasting types of selective behaviour were demonstrated in the experiments; that of conditioning to select a particular food-type and that involving a decreasing preference after disproportionately high intake of one food-type. The balance of factors causing one or other of these responses is apparently critical and will be considered in further detail.

The term conditioning is used here to describe the development of a new behaviour pattern as a result of previously carrying out a repeated action (in this context eating more of one food-type), regardless of the mechanisms involved. This could embody the concepts of training and specific search image used by Young (1946) and Tinbergen (1960) respectively. In Experiment I there was some evidence that conditioning occurred when rabbits were selecting from unequal mixtures, and further evidence in Experiment IIA for the build up of such a response through repeated encounters with the same unequal mixture. Pre-feeding (i.e. feeding one food-type prior to preference testing) however resulted in this type of response only when rabbits with previous experience of Lolium (but not Trifolium) initially selected Lolium from a mixture of the two species (IVA). When a similar pre-feeding experiment was performed with wheat and barley on both experienced and naive rabbits (IIIB) no such response was detected; moreover when rabbits were pre-fed immediately before preference testing (IIIA) the opposite response to conditioning occurred and less of the pre-fed item was selected. In the pre-feeding experiments however, considerably

more of the pre-fed grain-type was eaten than the higher frequency grain in those experiments in which conditioning occurred.

The change in preference from Trifolium to Lolium occurring within a single feeding period in Experiment IVB supports the theory of Young (1940) that the demand and satiation levels for different food items are to some extent independently determined. In Experiment IVB there was a more rapid reduction in the demand for Trifolium relative to Lolium although initially more Trifolium was eaten. Similarly in Experiment IIIA pre-feeding resulted in more rapid satiation on the pre-fed item. If there is little difference in the palatabilities of two food-types and they occur together in widely differing frequencies, more of the commoner item will be eaten in a given time. It could therefore be predicted that this could have the same effect as pre-feeding and lead to more rapid satiation on the commoner item. This could perhaps explain the conflicting results of Experiments IIA and IIB. In IIA repeated encounters with the same 90:10 mixture resulted in conditioning on the commoner item, whereas in IIB both significantly more and less of the commoner item were eaten by different individual rabbits after varying numbers of encounters. If both conditioning and the relative satiation response were elicited by repeated encounters in IIB there must have been a carry over of the satiation response from day to day as, more predictably, was demonstrated with the conditioning response in IIA. There is evidence of this phenomenon in Experiment IIIA in which the effect of immediate pre-feeding on one day appeared to carry over on to the following day, even after the pre-fed item was reversed.

The extent to which relative satiation levels, and consequently preference, reflect the nutritive condition of the animal has long been a subject of controversy both in theoretical terms and over conflicting conclusions drawn from experiments (Richter 1942, Young 1948, Tribe 1952, Arnold and Hill 1972).

The following table summarizes the factors resulting in the responses of conditioning and differential satiation demonstrated in the preceeding experiments, together with the time scale involved in the development and retention of the response.

Response	Experimental conditions	Time Scale	Expt.
Conditioning	Higher frequency of one food-type in mixture.	Possibly during single feeding period.	I
		Build up during successive feeding periods.	IIA
	Previous experience of one food-type only.	Response soon lost.	IVA
Differential Satiation	Pre-feeding.	Pre-feeding immediately before preference test. Appeared to have a residual effect on subsequent day.	IIIA
	Greater palatability of one food-type resulting in more eaten in given time.	Single feeding period.	IVB

The results of these experiments indicate that the interpretation of preference tests must be undertaken with great caution when comparisons between different food-types are being sought.

An understanding of the mechanisms by which various factors can affect preference is essential if it is to be possible to predict with any accuracy, selective feeding behaviour and hence its bearing on the composition of vegetation. Determination of the contribution of individual variables within the complex of factors affecting food selection by the grazing herbivore and its effect on vegetation composition, lends itself to an approach by computer modelling. Although a mathematical approach has frequently been used to simulate predator-prey systems (e.g. Rapport and Turner 1970, 1975, Rapport 1971, Pulham 1974), this work is not directly applicable to herbivore-plant systems due to the different properties of the 'prey'. For example 'predation' by the herbivore does not necessarily lead to the death of an individual prey item,

but usually a setback in growth. This in turn may affect flowering and seed production, general morphology and competitive interactions between plants in the grazed sward.

Computer simulation of grazing using a fairly simple model involving a limited number of variables relating both to the animal and the vegetation has been pioneered by Goodall (1967, 1969). This method could be adapted to predict the short and long-term effects on vegetation of the various individual factors involved in food selection by the rabbit, based on the results of the preceeding experiments and further laboratory choice tests involving fresh vegetation.

GENERAL SUMMARY AND CONCLUSIONS

GENERAL SUMMARY AND CONCLUSIONS

The aim of this section is to draw together the results and briefly discuss the general implications of this study.

In the first part of the study it was demonstrated that food selection by grazing rabbits is affected by a number of properties of the grazed sward. It was found by faecal analysis that differences in relative availabilities of plant species were reflected in the proportional composition of the diet of wild rabbits under natural conditions. Superimposed on this were distinct preferences which resulted in consistent disproportional selection of particular species. That such preferences are to a large extent innate was indicated by controlled choice experiments with naive captive wild rabbits which exhibited a similar rank order of preferences. Although certain species were consistently preferred and others avoided, variations in preference occurred, corresponding with seasonal changes in the vegetation. The variations were mainly attributed to changes in relative palatability of different species rather than changes in their availability. This was particularly indicated by the fact that certain grasses were selected in sequence, each during the short period of time just prior to flowering, when it has been shown that digestibility generally reaches a maximum.

Experiments described in Part 2 indicated that additional factors besides the range and relative availabilities of species and variations in growth stage, could also be involved in food selection by rabbits. Controlled feeding experiments on domestic rabbits suggested that under certain conditions disproportionate selection of the most frequent food-type could occur. Further experiments revealed that, even in the short term, preferences are not constant, but are affected both by previous experience and by what the rabbit has just eaten. It was found that the response to a species which had never been encountered previously took some time to develop, even if that species

eventually rated high in rank preference. Temporary differential satiation was also found to develop under certain circumstances, causing preference for a normally palatable food temporarily to diminish.

Although faecal analysis was not a suitable method for estimating the influence of individual factors such as frequency-dependent selection and other short term changes in preference, it proved to be a satisfactory method of obtaining sufficient qualitative and quantitative data on dietary selection. In addition to providing quantitative data, the main advantage of the method is that it does not interfere at all with either the animals or the grazed vegetation. Destructive sampling methods such as analysis of stomach contents require large sample numbers and are therefore unsuitable for prolonged investigations into seasonal changes in diet. Disadvantages common to both stomach content and faecal analysis are the lengthy and laborious nature of the analysis, the difficulty in distinguishing the epidermal configurations of certain closely related species and the differential action of digestion on epidermis of different species. However, although controlled experimental feeding of captive wild rabbits showed that differential digestion of grass epidermis occurred, application of correction factors to the field data made no qualitative difference to the conclusions drawn from the analysis. Since this was attributed to a correlation between palatability and digestibility, it seems likely that correction of faecal analysis data is unnecessary when the food items involved are closely related (e.g. all grasses, as in the present study). However if comparable results for dissimilar food-types are required (e.g. grasses and dicots, grass seeds and leaves, annuals and perennials) some calibration of the data must be made.

Selective behaviour by feeding rabbits must in turn partly determine the composition of the grazed vegetation. Constant selection of palatable species and avoidance of others could lead to dominance of the least grazed

species and virtual elimination of the most grazed. Selection of seeds would favour species with the ability to spread clonally. These predictions are consistent for example, with the observation that on certain acid-neutral grasslands, Agrostis tenuis (which is unpalatable to rabbits and spreads by means of rhizomes) tends to be dominant on persistently rabbit-grazed areas. Seasonal sequences in utilization of species, particularly at the stage just prior to flowering and of the seeds after flowering, would conversely have a diversifying effect. In the present study little mention has been made of the fact that an important feature of rabbit grazing behaviour is that certain swards become favoured grazing sites even though they may contain an abundance of less palatable species. The faecal analysis data indicated that highly palatable species such as Lolium perenne, although present in patches in areas occupied by rabbits, were not necessarily included among the favoured grazing areas, presumably due to territorial and social factors as well as geographical position. Such rigorous selection of feeding sites means that not only is the relative abundance of species within a site affected, but also on a larger scale, the pattern of species associations over a wide area, is modified.

It could be predicted by inference from comparable concepts within the field of quantitative genetics, that frequency-dependent selection of species by grazing animals could play an important role in maintaining vegetational diversity. Problems (similar to those in genetics) are encountered in determining both the existence and importance of frequency-dependent feeding behaviour under natural conditions, and further evidence is needed to discover to what extent grazing rabbits exhibit this form of dietary selection.

It has been suggested in conclusion to Chapter 7 that computer modelling techniques could be used to simulate various aspects of rabbit grazing in order to investigate some of the questions raised by the present study. A simple predator-prey model could be adapted employing varying levels of complexity

to predict the effects of rabbit grazing on the composition of vegetation under different conditions. Both qualitative and quantitative data have been presented on the effects on food selection of the following environmental factors, which could be incorporated into such a model:

1. Availability of plant species and their different growth stages.
2. Relative palatabilities of different species and seasonal modifications of these palatabilities.
3. Relative abundance of species.

In addition to these factors, hypothetical cases representing various generalized aspects of vegetation growth and species interaction could be incorporated - e.g. relative growth rates of different species, recovery from grazing, method and rate of spread (seed/stolon production), phenology, competitive effects at different growth stages.

Although such a model would necessarily involve much simplification it would be of value in formulating hypothetical answers to a number of questions including the following:

1. Under what conditions can rabbit grazing contribute to the stability of a sward in terms of species composition?
2. How do the annual growth patterns of different species contribute to the long-term effects of grazing on the composition of vegetation?
3. What types of plants are most likely to be adversely or beneficially affected by rabbit grazing, and under what conditions?
4. Under what conditions could frequency-dependent selection of food affect the relative abundance of species within a sward?

Predictions drawn from such a model could be tested by controlled field experiments and, conversely, existing data on the composition of grazed vegetation could be related to feeding behaviour. By means of further hypothesis construction and experimental work on individual aspects of grazing, an

increasingly complex picture can be built up of the inter-relationships between grazing behaviour and the composition of vegetation.

REFERENCES

REFERENCES

ADAMS, L. (1957). A way to analyse herbivore food habits by faecal examination. N. Amer. Wildlife Conf. Trans. 22, 152-159.

ADAMS, L., O'REGAN, W.G. AND DUNAWAY, D.J. (1962). Analysis of forage consumption by faecal examination. J. Wildl. Mgmt. 26, 108-111.

ALLEN, J. AND CLARKE, B.C. (1968). Evidence for apostatic selection by wild passerines. Nature, Lond. 220, 501-502.

ARMITAGE, P.L. (1972). The significance of mountain grazing in Wales. B.Sc. Thesis, U.C.N.W., Bangor.

ARNOLD, G.W. (1962). Factors within plant associations affecting the behaviour and performance of grazing animals. In Grazing in Terrestrial and Marine Environments. (Ed. by D.J. Crisp). 4th Symp. Brit. Ecol. Soc.

ARNOLD, G.W. AND HILL, J.L. (1972). Chemical factors affecting selection of food plants by ruminants. Phytochemical Ecology (Ed. by J.B. Harborne).

BAILEY, P.T., MARTENSZ, P.N. AND BARKER, R. (1971). The red kangaroo, Megaleia rufa (Desmarest), in north-western New South Wales. C.S.I.R.O. Wildl. Res. 16, 29-39.

BARNETT, S.A. AND SPENCER, M.M. (1953). Experiments on the food preferences of wild rats. (Rattus norvegicus). J. Hyg. Camb. 51, 16-38.

BEAR, G.D. AND HANSEN, R.M. (1966). Food habits, growth and reproduction of white-tailed jackrabbits in Southern Colorado. Colorado State Univ. Agric. Expt. Sta. Tech. Bull. 90.

BELL, R.H.V. (1969). The use of the herb layer by grazing ungulates in the Serengeti National Park, Tanzania. Ph.D. Thesis, University of Manchester.

BERGERUD, A.T. AND RUSSELL, L. (1964). Evaluation of rumen food analysis for Newfoundland Caribou. J. Wildl. Mgmt. 28, 809-814.

BERNAYS, E.A. AND CHAPMAN, R.F. (1970). Food selection by Chorthippus parallelus in the field. J. Anim. Ecol. 39, 383-394.

BHADRESA, R. (1977). Food preferences of rabbits Oryctolagus cuniculus L. at Holkham sand dunes, Norfolk. J. appl. Ecol. 14, 287-293.

BLISS, C.I. (1970). Statistics in Biology. McGraw-Hill, New York.

CAMERON, E. (1935). A study of the natural control of ragwort (Senecio jacobaea L.). J. Ecol. 23, 265-322.

CHAPMAN, R.F. (1957). Observations on the feeding of adults of the red locust. Br. J. Anim. Behav. 5, 60-75.

CHENOST, M., GRENET, E., DEMARQUILLY, C. AND JARRIGE, R. (1970). The use of the nylon bag technique for the study of forage digestion in the rumen and for predicting feed value. Proc. XI Int. Grassld. Congr., 697-701.

CHIPPENDALE, G. (1962). Botanical examination of kangaroo stomach contents and cattle rumen contents. Aust. J. Sci. 25, 21-22.

CHURCH, B.M., JACOB, F.H. AND THOMPSON, H.V. (1953). Surveys of rabbit damage to wheat in England and Wales, 1950-52. Pl. Path. 2, 107-12.

CLARKE, B.C. (1962). Balanced polymorphism and the diversity of sympatric species. Taxonomy and Geography Syst. Ass. Publ. 4, 47-70.

CROKER, B.H. (1959). A method of estimating the botanical composition of the diet of sheep. N.Z. Jl. agric. Res. 2, 72-85.

DALKE, P.D. AND SIME, P.R. (1941). Food habbits of the eastern and New England cottontails. J. Wildl. Mgmt. 5, 216-228.

DAVISON, V.E. (1940). A field method of analysing game bird foods. J. Wildl. Mgmt. 4, 105-116.

DAWSON, T.J., DENNY, M.J.S., RUSSELL, E.M. AND ELLIS, B. (1975). Water usage and diet preferences of free ranging kangaroos, sheep and feral goats in the Australian arid zone during summer. J. Zool. 177, 1-23.

DAVIES, I. (1959). The use of epidermal characteristics for the identification of grasses in the leafy stage. J. Br. Grassld. Soc. 14, 7-16.

DUSI, J.L. (1949a). The food habits of some populations of cottontail rabbits in Ohio. Abstracts Doctoral Dissertations, Ohio State Univ. 61, 131-32.

DUSI, J.L. (1949b). Methods for the determination of food habits by plant microtechniques and histology, and their application to cottontail rabbit food habits. J. Wildl. Mgmt. 13, 295-298.

DUSI, J.L. (1952). The food habits of several populations of cottontail rabbits in Ohio. J. Wildl. Mgmt. 16, 180-186.

EASTMAN, D. AND JENKINS, D. (1970). Comparative food habits of red grouse in northeast Scotland, using faecal analysis. J. Wildl. Mgmt. 34, 610-620.

EDEN, A. (1940). Coprophagy in the rabbit. Nature. Lond. 145, 36.

ELTON, C.S. (1927). Animal Ecology. Sidgwick and Jackson.

EVANS, D.M. (1973). Seasonal variations in the body composition and nutrition of the vole, Microtus agrestis. J. Anim. Ecol. 42, 1-18.

FARROW, E.P. (1917). General effects of rabbits on vegetation. J. Ecol. 45, 1-18.

FENTON, E.W. (1940). The influence of rabbits on the vegetation of certain hill-grazing districts of Scotland. J. Ecol. 28, 438-449.

FERNS, P.N. (1976). Diet of a Microtus agrestis population in south west Britain. Oikos 27, 506-511.

FLUX, J.E.C. (1967). Hare numbers and diet in an Alpine basin in New Zealand. Proc. N.Z. ecol. Soc. 14, 27-34.

FLUX, J.E.C. (1970). Life history of the mountain hare (Lepus timidus scoticus) in north-east Scotland. J. Zool. 161, 75-123.

FREE, J.C. et al.(1970). Estimating dryweights of food plants in faeces of herbivores. J. Range Mgmt. 23, 300-302.

GEBCZYNSKA, Z. AND MYRCHA, A. (1966). The method of quantitative determining of the food composition of rodents. Acta theriol. 11, 385-390.

GILLHAM, M.E. (1955). Ecology of the Pembrokeshire Islands III. The effect of grazing on the vegetation. J. Ecol. 43, 172-206.

GILLHAM, M.E. (1963). Some interactions of plants, rabbits and sea-birds on South African islands. J. Ecol. 51, 275-294.

GODFREY, G.K. (1953). The food of Microtus agrestis hirtus in Wytham, Berkshire. Saugetierkunde Mitteilungen. 1, 148-151.

GOODALL, D.W. (1967). Computer simulations of changes in vegetation subject to grazing. J. Indian bot. Soc. 46, 356-362.

GOODALL, D.W. (1969). Simulating the grazing situation. In Biomathematics Vol. 1. Concepts and Models of Biomathematics. (Ed. by F. Heinmetz). pp. 211-236. Dekker, New York.

GOUGH, H.C. AND DUNNETT, F.W. (1950). Rabbit damage to winter corn. Agriculture, Lond. 57, 374-378.

GRIFFITHS, M. AND BARKER, R. (1966). The plants eaten by sheep and by kangaroos grazing together in a paddock in S.W. Queensland. C.S.I.R.O. Wildl. Res. 11, 145-167.

GRIFFITHS, M. AND DAVIES, D. (1963). The role of the soft pellets in the production of lactic acid in the rabbit stomach. J. Nutr. 55, 171-180.

GWYNNE, M.D. AND BELL, R.H.V. (1968). Selection of vegetation components by grazing ungulates in the Serengeti National Park. Nature, Lond. 220, 390-393.

HANSEN, R.M., PEDEN, D.G. AND RICE, R.W. (1973). Discerned fragments in faeces indicates diet overlap. J. Range Mgmt. 26, 103-105.

HANSON, W.R. AND GRAYBILL, F. (1956). Sample size in food-habits analyses. J. Wildl. Mgmt. 20, 64-68.

HANSSON, L. (1970). Methods of morphological diet micro-analysis in rodents. Oikos 21, 255-266.

HARPER, J.L. (1969). The role of predation in vegetational diversity. In Diversity and Stability in Ecological Systems (Ed. by Woodwell, G.M. and Smith, H.M.) pp 48-61. Brookhaven Symp. in Biol. 22.

HAYDEN, P. (1966). Food habits of black-tailed jack-rabbits in Southern Nevada. J. Mammal. 47, 42-46.

HEADY, H.F. (1964). Palatibility of herbage and animal preference. J. Range Mgmt. 17, 76-82.

HEADY, H.F. AND TORRELL, D.T. (1959). Forage preferences exhibited by sheep with esophageal fistulas. J. Range Mgmt. 12, 28-34.

HENNING, S.J. AND HIRD, F.J.R. (1972). Diurnal variations in the concentrations of volatile fatty acids in the alimentary tracts of wild rabbits. Br. J. Nutr. 27, 57-64.

HERCUS, B.H. (1960). Plant cuticle as an aid to determining the diet of grazing animals. Proc. VIII Int. Grassld. Congr. 443-447.

HEWSON, R. (1976). Browsing by mountain hares, Lepus timidus, on trees and shrubs in north-east Scotland. J. Zool. 182, 168-171.

HOLLING, C.S. (1965). The functional response of predators to prey density and its role in mimicry and population regulation. Mem. Ent. Soc. Canada. 45, 1-60.

IVINS, J.D. (1960). The digestibility of herbage by ruminants. Proc. VIII Int. Grassld. Congr. 459-461.

JENNINGS, T.J. AND BARKHAM, J.P. (1975). Food of slugs in mixed deciduous woodland. Oikos 26, 211-221.

JENNINGS, T.J. AND BARKHAM, J.P. (1976). Quantitative study of feeding in woodland by the slug Arion ater. Oikos 27, 168-173.

JENSEN, G.H. AND KORSCHGEN, L.J. (1947). Contents of crops, gizzards and droppings of bobwhite quail force-fed known kinds and quantities of seeds. J. Wildl. Mgmt. 11, 37-43.

JONES, M.G. (1933). Grassland management and its influence on the sward. Jl. R. agric. Soc. 94, 21-41.

KILEY, M. (1966). A preliminary investigation into the feeding habits of the waterbuck by faecal analysis. E. Afr. Wildlife J. 4,153-157.

LAMPREY, H.F. (1963). Ecological separation of the large mammal species in the Tarangire Game Reserve, Tanganyika. E. Afr. Wildlife J. 1, 63-92.

LINNAEUS, C. (1749). Pan Suecus Amoenitates Academicus. Vol. 2 p. 225.

LOCKLEY, R.M. (1964). The Private Life of the Rabbit. Deutsch.

McANALLY, R.A. AND PHILLIPSON, A.T. (1944). Digestion in the ruminant. Biol. Rev. 19, 41-54.

MADSEN, H. (1939). Does the rabbit chew the cud? Nature, Lond. 143, 981.

MARTEN, G.C. AND JORDAN, R.M. (1974). Significance of palatability differences among Phalaris arundinacea L., Bromus inermis Leyss., and Dactylis glomerata L. grazed by sheep. Proc. XII Int. Grassld. Congr.

MARTIN, D.J. (1955). Features on plant cuticle. An aid to the analysis of the natural diet of grazing animals, with especial reference to Scottish hill sheep. Trans. Proc. bot. Soc. Edin. 36, 278-288.

MARTIN, D.J. (1962). Analysis of sheep diet utilizing plant epidermal fragments in faeces samples. In Grazing in Terrestrial and Marine Environments (Ed. by D.J. Crisp). 4th Symp. Brit. Ecol. Soc.

MEDIN, D.E. (1970). Stomach content analyses: collections from wild herbivores and birds. Range and Wildlife Habitat Evaluation - A Research Symposium, pp 133-145. U.S.D.A. Misc. Pub. No. 1147.

METCALFE, C.R. (1960). Anatomy of the Monecatyledons. I. Gramineae. Clarendon, Oxford.

MILLER, G.R. (1968). Evidence for selective feeding on fertilized plots by red grouse, hares and rabbits. J. Wildl. Mgmt. 32, 849-853.

MORRIS, R.M. AND THOMAS, J.G. (1972). The seasonal pattern of dry-matter production of grasses in the North Pennines. J. Br. Grassld. Soc. 27, 163-172.

MOWAT, D.N., FULKERSON, R.S., TOSSELL, W.E. AND WINCH, J.E. (1965). The in vitro dry matter digestibility of several species and varieties and their plant parts with advancing ages of maturity. Proc. IX Int. Grassld. Congr. 801-806.

MURDOCH, W.M. (1969). Switching in general predators: experiments in predator specificity and stability of prey populations. Ecol. Monogr. 39, 335-354.

MURTON, R.K., ISAACSON, A.J. AND WESTWOOD, N.J. (1963). The use of baits treated with -chloralose to catch wood-pigeons. Ann. appl. Biol. 52, 271-293.

MYERS, K. AND POOLE, W.E. (1959). A study of the biology of the wild rabbit, Oryctolagus cuniculus (L) in confined populations. I. The effects of density on home range and the formation of breeding groups. C.S.I.R.O. Wildl. Res. 4, 14-26.

MYERS, K. AND POOLE, W.E. (1961).
II. The effects of season and population increase on behaviour. C.S.I.R.O. Wildl. Res. 6, 1-41.
III. Reproduction. Aust. J. Zool. 10, 225-267.

MYERS, K. AND POOLE, W.E. (1963). The effects of rabbit grazing on sown pasture. J. Ecol. 51, 435-451.

MYKYTOWYCZ, R. (1958). Social behaviour of an experimental colony of wild rabbits, Oryctolagus cuniculus (L).
I. Establishment of the colony. C.S.I.R.O. Wildl. Res. 3, 7-35.

MYKYTOWYCZ, R. (1959).
II. First breeding season. C.S.I.R.O. Wildl. Res. 4, 1-13.
III. Second breeding season. C.S.I.R.O. Wildl. Res. 5, 1-20.

MYKYTOWYCZ, R. AND ROWLEY, I. (1958). Continuous observations on the activity of the wild rabbit, Oryctolagus cuniculus (L.) During 24 hour periods. C.S.I.R.O. Wildl. Res. 2, 26-31.

NORRIS, J.J. (1943). Botanical analyses of stomach contents as a method of determining forage consumption of range sheep. Ecology. 24, 244-251.

OLDHAM, J.H. (1971). Investigations into the grazing by the wild rabbit at Ynyslas, Cardiganshire. B.Sc. Thesis, U.C.W. Aberystwyth.

OSBOURNE, D.F. et al (1974). The significance of a determination of cell walls as the rational basis for the nutritive evaluation of forages. Proc. VII Int. Grassld. Congr.

PALLANT, D. (1969). The food of the grey field slug (Agrolimax reticulatus (Muller)) in woodland. J. Anim. Ecol. 38, 391-397.

PALLANT, D. (1972). The food of the grey field slug, Agrolimax reticulatus (Muller) on grassland. J. Anim. Ecol. 41, 761-769.

PETERS, S.S. (1958). Food habits of the Newfoundland Ptarmigan. J. Wildl. Mgmt. 22, 384-394.

PETERSON, J.C.B. AND CASEBEER, R.L. (1971). A bibliography relating to the ecology and energetics of East African large Mammals. E. Afr. Wildlife J. 9, 1-23.

PHILLIPS, W.M. (1953). The effect of rabbit grazing on reseeded pasture. J. Br. Grassld. Soc. 8, 169-181.

PIEKARZ, R. (1963). The influence of coprophagy on the time of food passage through the alimentary tract in domestic rabbits. Acta physiol. pol. 14, 359.

PRAT, H. (1932). L'epiderme des graminées. Ann. Sci. 10, 14.

PULHAM, H.R. (1974). On the theory of optimal diets. Am. Nat. 108, 59-74.

RANWELL, D.S. (1960). Newborough Warren, Anglesey. III. Changes in the vegetation on parts of the dune system after the loss of rabbits by myxomatosis. J. Ecol. 48, 385-395.

RAPPORT, D.J. (1971). An optimization model of food selection. Am. Nat. 105, 575-87.

RAPPORT, D.J. AND TURNER, J.E. (1970). Determination of predator food preferences. J. Theor. Biol. 26, 365-372.

RAPPORT, D.J. AND TURNER, J.E. (1975). Feeding rates and population growth. Ecology 56, 942-949.

REGEL, V. (1960). Evaluation of the quality of pasture grasses by the microscopic method. Proc. VIII Int. Grassld. Congr. 522-524.

REID, R.L. AND JUNG, G.A. (1965). Factors affecting the intake and palatability of forages by sheep. Proc. IX Int. Grassld. Congr. 863-869.

RICHTER, C.P. (1942). Total self regulatory functions in animals and human beings. The Harvey Lecture Series. 38, 63-103.

RIEGEL, A. (1942). Some observations on the food coactions of rabbits in Western Kansas during periods of stress. Trans. Kans. Acad. Sci. 45, 369-375.

SCOTT, T.G. (1941). Methods and computation in faecal analysis with reference to the red fox. Iowa St. Coll. J. Sci. 15, 279-285.

SHEAIL, J. (1971). Rabbits and Their History. David and Charles.

SIMON, U. (1974). Palatability and voluntary intake of contrasting legume and grass varieties by grazing sheep. Proc. XII Int. Grassld. Congr.

SINCLAIR, A.R.E. AND GWYNNE, M.D. (1972). Food selection and competition in the East African buffalo. E. Afr. Wildlife J. 10, 77-89.

SKOSS, J.D. (1955). Structure and composition of plant cuticle in relation to environmental factors and permeability. Bot. Gaz. 117, 55-72.

SOANE, I.D. AND CLARKE, B.C. (1973). Evidence for apostatic selection by predators using olfactory cues. Nature, Lond. 241, 62-64.

SOUTHERN, H.N. (1940). The ecology and population dynamics of the wild rabbit (Oryctotagus cuniculus). Ann. appl. Biol. 27, 509-526.

SPARKS, D.R. AND MALACHEK, J.C. (1968). Estimating percentage dryweights in diets using a microscopic technique. J. Range Mgmt. 21, 264-265.

SPEDDING, C.R.W. AND DIEKMANNS, E.C. (Ed.) (1972). Grasses and legumes in British Agriculture. Bull. 49 Commonwealth Bureau of Pasture and Crops.

STAINSBY, C.B. (1973). Carneddau Ponies. M.Sc. Thesis. U.C.N.W. Bangor.

STEWART, D.R.M. (1965). The epidermal characters of grasses, with special reference to East African plains species. Bot. Jb. 84, 63-116.

STEWART, D.R.M. (1967). Analysis of plant epidermis in faeces. A technique for studying the food preferences of grazing herbivores. J. appl. Ecol. 4, 83-111.

STEWART, D.R.M. (1970). Survival during digestion of epidermis from plants eaten by ungulates. Revue Zool. Bot. afr. 82, 343-348.

STEWART, D.R.M. (1971). Food preferences of an impala herd. J. Wildl.Mgmt. 35, 86-93.

STEWART, D.R.M. (1971). Diet of Lepus capensis and L. crawshayi. Food preferences of Pronolagus. E. Afr. Wildlife J. 9, 161-166.

STEWART, D.R.M. AND STEWART, J. (1970). Food preference data by faecal analysis for African plains ungulates. Zoologica Africana. 5, 115-129.

STEWART, D.R.M. AND STEWART, J. (1971). Comparative food preferences of five E. African ungulates at different seasons. 11th Symp. Brit.Ecol.Soc.

STORR, G.M. (1961). Microscopic analysis of faeces, a technique for ascertaining the diet of herbivorous mammals. Aust. J. biol. Sci. 14, 157-164.

SWANSON, G. (1940). Food habits of the sharp-tailed grouse by analysis of droppings. J. Wildl. Mgmt. 4, 432-436.

TANSLEY, A.G. AND ADAMSON, R.S. (1925). Studies of the vegetation of the English chalk. III. The chalk grasslands of the Hampshire-Sussex border. J. Ecol. 13, 177-223.

THOMAS, A.S. (1960). Changes in vegetation since the advent of myxomatosis. J. Ecol. 48, 287-306.

THOMAS, A.S. (1963). Further changes in vegetation since the advent of myxomatosis. J. Ecol. 51, 151-183.

THOMAS, J.O. (1937). Effect of rabbits on pasture. Rural Studies at Dauntsey's School: Report of Experiments.

THOMPSON, H.V. (1953). The grazing behaviour of the wild rabbit, Oryctolagus cuniculus (L.). Br. J. Anim. Behav. 1, 16-19.

THOMPSON, H.V. AND WORDEN, A.N. (1956). The Rabbit. Collins, London.

TINBERGEN, L. (1960). The natural control of insects in pine-woods. I. Factors influencing the intensity of predation by song-birds. Archs. neerl. Zool. 13, 265-336.

TRIBE, D.E. (1950). The behaviour of the grazing animal: a critical review of present knowledge. J. Br. Grassld. Soc. 5, 209-224.

TRIBE, D.E. (1952). Proc. VI Int. Grassld. Congr. 1265.

TULLOCK, G. (1971). The coal tit as a careful shopper. Am. Nat. 105,77-80.

VAN DYNE, G.M. AND TORRELL, D.T. (1964). Development and use of the oesophageal fistula: A review. J. Range Mgmt. 17, 7-19.

WARD, L.A. (1970). Stomach content and faecal analysis: Methods of forage identification. Range and Wildlife Habitat Evaluation - A Research Symposium, pp. 146-158. U.S.D.A. Misc. Pub. No. 1147.

WATTS, A.S. (1957). The effect of excluding rabbits from Grassland B (Mesobrometum) in Breckland. J. Ecol. 45, 861-878.

WATTS, A.S. (1960). The effect of excluding rabbits from acidophilous grassland in Breckland. J. Ecol. 48, 601-604.

WATTS, C.H.S. (1968). The food eaten by wood mice (Apodemus sylvaticus) and bank voles (Clethrionomys glareolus) In Wytham Woods, Berkshire. J. Anim. Ecol. 37, 25-41.

WATTS, C.H.S. (1977). The foods eaten by some Australian rodents (Muridae). Aust. Wildl. Res. 4, 151-157.

WHITE, D.J.B. (1961). Some observations on the vegetation of Blakeney Point, Norfolk, following the disappearance of the rabbits in 1954. J. Ecol. 49, 113-118.

WILLIAMS, O. (1955). The food of mice and shrews in a Colorado montane forest. Univ. Colorado Studies Series. Biology, No. 3.

WILLIAMS, O. (1962). A technique for studying microtine food-habits. J. Mammal. 43, 365-368.

WILLIAMS, O.B., WILLS, T.G.E. AND WELLS, D.A. (1974). Grazing management of Wood Walton Fen. Seasonal changes in the diet of cattle and rabbits. J. appl. Ecol. 11, 499-516.

WILLIAMSON, P. AND CAMERON, R.A.D. (1976). Natural diet of the landsnail Cepaea nemoralis. Oikos 27, 493-500.

YOUNG, P.T. (1940). Reversal of food preferences of the white rat through controlled pre-feeding. J. gen. Psychol. 22, 33-66.

YOUNG, P.T. (1944). Studies of food preference, appetite and dietary habbit. I. Running activity and dietary habit of the rat in relation to food preference. J. comp. Psychol. 37, 327-370.

YOUNG, P.T. (1946). Studies of food preference, appetite and dietary habit. VI. Habit, palatability and the diet as factors regulating the selection of food by the rat. J. comp. Psychol. 39, 139-176.

YOUNG, P.T. (1948). Appetite, palatability and feeding habit: a critical review. Psychol. Bull. 45, 289-320.

APPENDIX 1

ADDITIONAL TABLES FROM CHAPTER 5

TABLE A1

Analysis of twelve slides from Sample 1 to find optimum number representative of sample

% COMPOSITION

SLIDE	F/P	Ag	H	L/C	D/P	An	Arrh	U.G.	U.O.	Total area measured (grid squares)
1	13.1	18.0	25.2	11.2	22.8	1.5	1.9	1.9	4.4	206
2	8.1	3.5	26.8	22.2	28.3	1.0	5.1	3.0	2.0	198
3	11.1	8.6	27.8	10.6	25.3	7.6	3.5	4.5	1.0	198
4	18.1	7.2	15.7	13.4	20.2	8.9	2.4	3.1	11.0	292
5	8.0	2.1	26.8	18.8	18.4	6.3	3.3	4.2	12.1	239
6	6.7	16.8	11.4	11.8	26.1	6.7	2.9	9.2	8.4	238
7	15.6	6.8	24.4	19.5	22.4	0	0.6	4.9	5.8	308
8	10.0	5.2	15.3	21.8	24.5	4.8	10.5	4.4	3.5	229
9	16.4	4.1	19.2	26.5	22.4	0	3.6	2.3	5.5	219
10	12.8	14.6	21.5	14.9	20.1	4.9	2.5	4.9	3.8	288
11	14.5	5.5	30.5	14.2	20.4	0.7	5.8	5.1	3.3	275.
12	8.2	13.0	25.7	16.7	26.8	3.7	3.3	0.7	1.9	269
1-2	10.6	10.9	26.0	16.6	25.5	1.2	3.5	2.5	3.2	404
3-4	15.3	7.8	20.6	12.2	22.2	8.4	2.9	3.7	6.9	490
5-6	7.3	9.5	19.1	15.3	22.2	6.5	3.1	6.7	10.3	477
7-8	13.2	6.2	20.5	20.5	23.3	2.0	4.8	4.7	4.8	537
9-10	14.4	10.1	20.5	19.9	21.1	2.8	3.0	3.7	4.5	507
11-12	11.4	9.2	28.1	15.4	23.5	2.2	4.6	3.0	2.6	544
1-3	10.8	10.1	26.6	14.6	25.4	3.3	3.5	3.2	2.5	602
4-6	11.4	8.6	17.8	14.6	21.5	7.4	2.9	5.3	10.5	769
7-9	14.1	5.6	20.1	22.2	23.0	1.5	4.5	4.0	5.0	756
10-12	12.0	11.1	25.8	15.3	22.3	3.1	3.8	3.6	3.0	832
1-4	13.2	9.2	23.0	14.2	23.7	5.2	3.1	3.1	5.3	894
5-8	10.5	7.7	19.9	18.0	22.8	4.1	4.0	5.6	7.4	1014
9-12	12.8	9.6	24.5	17.6	22.4	2.5	3.8	3.3	3.5	1051
1-5	12.1	7.7	23.8	15.2	22.6	5.4	3.1	3.4	6.7	1133
6-10	12.5	9.7	18.8	18.7	22.9	3.2	3.7	5.1	5.4	1282
1-6	11.2	9.2	21.7	14.6	23.2	5.6	3.1	4.4	7.0	1371
7-12	13.0	8.4	23.1	18.5	22.7	2.3	4.2	3.8	4.0	1588
1-7	12.0	8.8	22.1	15.5	23.0	4.6	2.7	4.5	6.8	1679
1-8	11.7	8.4	21.3	16.3	23.2	4.6	3.6	4.5	6.4	1908
1-9	12.2	8.0	21.1	17.3	23.2	4.1	3.6	4.2	6.3	2127
1-10	12.3	8.7	21.2	17.0	22.8	4.2	3.5	4.3	6.0	2415
1-11	12.5	8.4	22.1	16.7	22.5	3.9	3.7	4.4	5.8	2690
1-12	12.1	8.8	22.4	16.7	22.9	3.9	3.7	4.1	5.4	2959

TABLE A2

Analysis of eight slides from Sample 1 to determine the variance between slides.

RELATIVE PROPORTIONS IN DEGREES

SLIDE	F/P	Ag	H	L/C	D/P	An	Arrh	U.G.	U.O.
1	21.2	25.1	30.1	19.6	28.5	7.0	7.9	7.9	12.1
2	16.5	10.8	31.2	28.1	32.1	5.7	13.1	10.0	8.1
3	19.5	17.1	31.8	19.0	30.2	16.0	10.8	12.2	5.7
4	25.2	15.6	23.3	21.5	26.7	17.4	8.9	10.1	19.4
5	16.4	8.3	31.2	25.7	25.4	14.5	10.5	11.8	20.4
6	15.0	24.2	19.7	20.1	30.7	15.0	9.8	17.7	16.8
7	23.3	15.1	29.6	26.2	28.2	0	4.4	12.8	13.9
8	18.4	13.2	23.0	27.8	29.7	12.7	18.9	12.1	10.8

	d.f.	Sum of Squares	Mean Square
Between grasses	8	3269.9	
Within grasses			
Between slides	63	1264.8	20.1
Total	71	4534.7	

$S_{\bar{x}} t_{0.05} = 3.17$

TABLE A3

Effect of increasing subsample size on $S_{\bar{x}} t_{0.05}$ (Assuming constant variances)

No. of slides in subsample	$S_{\bar{x}} t_{0.05}$
4	4.48
5	4.01
6	3.66
7	3.39
8	3.17
9	2.99
10	2.84
11	2.70
12	2.59
15	2.32
20	2.00
30	1.64

TABLE A4

Variance between subsamples from Sample 1 of rabbit faeces from Bangor Ancient Camp.

RELATIVE PROPORTIONS IN DEGREES

		F/P	Ag	H	L/C	D/P	An	Arrh	U.G.	U.O.
SUBSAMPLE 1A										
(DAY 1) SLIDE	1	18.5	12.7	31.6	20.7	34.0	10.0	9.5	10.9	12.2
	2	18.8	19.3	34.5	23.9	25.2	0	0	16.1	12.1
	3	18.5	12.7	28.0	21.5	29.6	17.2	8.9	16.8	13.8
	4	18.6	12.7	30.1	22.8	24.9	0	0	18.0	24.8
	5	24.0	9.3	33.0	21.6	23.8	0	18.3	14.3	13.4
	6	15.9	10.3	31.6	29.9	26.9	9.1	8.5	18.0	9.1
	7	13.8	14.5	26.0	30.6	32.6	0	13.8	14.2	8.5
	8	24.8	15.1	30.1	19.8	27.1	3.1	15.9	7.7	17.1
SUBSAMPLE 1B										
(DAY 2) SLIDE	1	21.2	25.1	30.1	19.6	28.5	7.0	7.9	7.9	12.1
	2	16.5	10.8	31.2	28.1	32.1	5.7	13.1	10.0	8.1
	3	19.5	17.1	31.8	19.0	30.2	16.0	10.8	12.2	5.7
	4	25.2	15.6	23.3	21.5	26.7	17.4	8.9	10.1	19.4
	5	16.4	8.3	31.2	25.7	25.4	14.5	10.5	11.8	20.4
	6	15.0	24.2	19.7	20.1	30.7	15.0	9.8	17.7	16.8
	7	25.3	15.1	29.6	26.2	28.2	0	4.4	12.8	13.9
	8	18.4	13.2	23.0	27.8	29.7	12.7	18.9	12.1	10.8
Difference between Day 1 and Day 2 means		1.1	2.3	1.0	4.0*	0.2	1.0	7.6*	3.3*	2.0

* $p < 0.05$

	d.f.	Sum of squares	Mean Square	F
Between grasses	8	7733.89		
Within grasses				
Between subsamples	9	260.39	28.93	1.41 $p > 0.05$
Within subsamples				
Between slides	126	2588.01	20.54	
Total	143	10582.29		

$S_{\bar{x}} t_{0.05} = 2.44$

TABLE A5

Variance between faecal samples; within samples and between subsamples; within subsamples and between slides.

RELATIVE PROPORTIONS IN DEGREES

		F/P	Ag	H	L/C	D/P	An	Arrh	U.G.	U.O.
SAMPLE 9										
Subsample 9A										
SLIDE	1	29.1	16.2	18.3	22.1	27.3	7.5	12.7	12.0	20.7
	2	26.3	21.6	15.8	16.7	29.7	0	7.5	12.1	26.9
	3	19.4	23.5	15.7	27.5	27.0	12.5	5.7	10.5	22.7
	4	31.9	19.0	13.3	15.8	29.1	12.9	11.8	10.8	20.6
	5	17.3	36.2	17.8	14.1	26.3	0	14.1	12.8	19.2
	6	29.9	27.6	0	14.7	29.2	4.8	10.3	10.9	23.6
	7	18.8	27.5	12.9	12.9	36.9	0	0	12.8	24.6
	8	19.5	32.2	14.1	22.4	22.9	6.8	5.7	10.8	25.8
Subsample 9B										
SLIDE	1	22.3	33.7	12.4	14.8	22.1	0	15.6	16.0	22.6
	2	26.6	16.5	17.9	12.8	22.5	14.5	9.6	11.2	27.3
	3	20.1	25.7	20.4	21.3	18.8	0	16.7	18.8	22.7
	4	27.3	20.5	15.8	17.6	32.2	11.1	6.3	15.3	18.3
SAMPLE 10										
Subsample 10A										
SLIDE	1	10.1	17.8	22.2	15.7	28.2	8.7	8.7	16.1	34.0
	2	6.8	22.7	31.7	17.5	26.9	0	15.6	8.7	24.5
	3	12.4	22.5	25.1	11.2	29.9	0	0	18.0	29.7
	4	20.7	23.1	19.6	12.8	27.3	0	6.3	8.9	34.0
	5	6.8	24.8	22.6	19.6	22.6	0	0	13.7	36.0
	6	9.3	10.5	29.5	0	28.5	0	4.8	7.0	42.0
	7	6.3	25.5	26.3	15.5	29.6	0	7.7	7.7	30.4
	8	0	13.9	21.2	21.2	28.0	0	21.6	0	34.7
Subsample 10B										
SLIDE	1	14.5	6.8	26.9	23.2	18.3	0	10.8	11.8	38.5
	2	6.0	15.2	22.8	13.7	29.1	6.8	12.9	10.5	38.1
	3	12.0	14.3	21.9	10.8	36.7	6.5	12.7	0	33.5
	4	14.9	25.1	18.6	10.1	23.6	0	14.9	0	38.9
SAMPLE 11										
Subsample 11A										
SLIDE	1	36.6	16.4	9.3	26.1	28.9	7.0	0	4.1	17.7
	2	24.7	16.2	11.2	31.1	31.4	5.4	8.3	4.1	21.7
	3	34.4	18.9	7.9	15.8	34.0	8.3	4.4	6.5	21.2
	4	24.0	14.3	15.1	22.2	36.0	6.8	5.1	0	26.3
	5	34.9	18.1	12.1	24.7	26.4	0	0	8.9	21.6
	6	29.6	16.2	7.0	18.6	36.5	0	12.0	7.5	22.5
	7	31.6	8.1	10.5	28.2	32.8	9.6	7.0	8.9	17.4
	8	30.9	13.6	6.3	24.1	33.5	0	6.3	9.3	23.5
Subsample 11B										
SLIDE	1	28.0	8.9	12.9	20.6	34.2	0	4.1	0	30.7
	2	36.7	9.1	13.3	15.3	31.8	0	7.9	0	26.4
	3	33.3	8.5	4.8	24.0	31.6	0	5.7	0	27.9
	4	26.3	14.8	14.8	22.6	33.0	8.5	5.4	8.1	24.9

TABLE A5 continued

Analysis of Variance

	d.f.	Sum of Squares	Mean Square	F
Between grasses	8	21188.45		
Within grasses				
Between samples	18	7005.53	389.20	15.17 p <0.001
Within samples				
Between subsamples	27	692.44	25.65	1.16 p >0.05
Within subsamples				
Between slides	270	5963.32	22.09	
Total	323	34849.74		

TABLE A6

Variance between faeces from individual rabbits on two sampling occasions.

RELATIVE PROPORTIONS IN DEGREES

		F/P	Ag	H	L/C	D/P	An	Arrh	U.G.	U.O.
SAMPLE 2										
RABBIT A										
SLIDE	1	16.1	21.9	26.3	9.3	27.2	5.4	12.7	25.3	19.6
	2	19.4	24.8	18.3	25.3	29.2	7.0	7.0	16.8	16.4
	3	21.2	25.9	19.9	15.3	24.8	10.8	17.5	17.0	19.0
	4	12.5	18.4	21.4	20.4	28.6	11.4	14.8	17.8	24.5
	5	18.3	16.1	17.8	20.9	24.2	11.5	13.7	20.9	27.3
RABBIT B										
	1	26.6	27.3	0	16.1	25.2	0	0	13.8	31.5
	2	33.8	23.3	0	11.1	17.1	0	8.7	7.0	37.6
	3	36.8	14.8	0	17.1	19.2	0	0	14.1	34.7
	4	31.6	22.9	0	20.2	21.7	0	0	9.5	32.7
	5	31.7	19.8	0	16.0	24.4	0	0	0	37.0
SAMPLE 6										
RABBIT C										
	1	12.4	11.2	22.0	34.3	36.4	7.9	0	17.2	0
	2	23.6	14.1	25.3	14.7	40.4	7.7	0	8.7	15.7
	3	26.2	13.8	17.2	23.0	34.8	9.5	0	11.4	20.0
	4	23.9	0	24.7	19.0	38.8	5.1	6.0	13.3	17.6
	5	24.0	0	22.3	19.4	47.2	5.4	0	0	10.6
	6	27.3	13.9	29.3	11.5	37.1	6.0	8.5	9.1	9.8
	7	36.6	0	20.4	22.6	35.6	0	0	0	10.8
	8	31.8	0	20.6	7.5	47.0	0	0	0	12.4
RABBIT D										
	1	29.1	14.3	15.6	15.1	43.7	6.0	0	11.4	10.9
	2	32.5	6.3	14.1	16.1	44.3	5.4	5.4	7.7	11.5
	3	27.1	0	19.7	16.4	44.9	11.1	0	11.5	8.7
	4	40.5	0	9.8	18.1	38.1	5.7	0	13.9	3.1
	5	27.6	11.8	13.2	16.4	44.1	13.2	0	14.9	5.7
	6	33.3	9.1	4.4	13.8	47.5	0	0	11.2	9.6
	7	29.6	0	5.7	11.8	46.3	0	0	19.4	15.4
	8	34.1	6.3	9.1	17.2	37.6	0	0	12.2	22.4

Analysis of Variance

	d.f.	Sum of Squares	Mean Square	F
Between grasses	8	20684.40	2585.55	
Within grasses				
Between samples	9	5666.55	629.62	3.61 p < 0.01
Within samples				
Between rabbits	22	3834.20	174.28	7.70 p < 0.001
Within rabbits				
Between slides	194	4387.31	22.62	
Total	233	34572.46		

TABLE A7

Analysis of variance within and between faecal samples collected on two consecutive days.

RELATIVE PROPORTIONS IN DEGREES

		F/P	Ag	H	L/C	D/P	An	Arrh	U.G.	U.O.
SAMPLE 6	DAY 1 SLIDE 1	27.3	2.6	11.7	19.0	47.0	4.1	0	14.1	11.8
	2	25.4	0	18.1	14.5	49.7	5.7	0	12.1	8.3
	3	27.1	0.4	12.7	10.1	52.2	12.9	0	7.0	7.5
	4	28.5	0	10.1	11.5	49.9	9.5	5.7	15.5	5.1
	5	31.9	0	5.4	12.1	51.2	8.1	4.8	10.0	3.1
	6	22.6	0	8.7	6.8	56.2	10.8	13.3	9.3	5.7
	7	26.1	0	15.0	13.3	52.7	8.5	0	10.1	0
	8	19.0	0	15.9	15.1	51.6	15.1	0	13.6	6.8
SAMPLE 6B	DAY 2 1	26.1	4.1	11.4	8.5	49.9	16.3	10.6	10.3	5.7
	2	28.7	3.1	10.3	10.8	47.8	15.2	5.4	8.9	12.7
	3	28.6	0	5.1	9.8	47.5	10.6	14.2	13.3	12.0
	4	23.8	0	16.1	3.1	56.0	7.7	7.9	9.3	4.8
	5	26.9	7.9	15.6	15.8	48.9	7.9	8.5	7.5	4.1
	6	29.1	0	14.1	6.5	54.2	7.9	0	0	7.0
	7	27.4	4.8	13.1	6.0	53.4	8.5	8.1	8.1	6.5
	8	26.2	6.0	8.9	8.1	54.4	8.5	6.0	8.1	10.8
Difference between Day 1 and Day 2 means		1.1	2.3	1.0	4.0*	0.2	1.0	7.6*	3.3*	2.0

* $p < 0.05$

	d.f.	Sum of squares	Mean square	F
Between grasses	8	29859.65		
Within grasses				
Between days	9	245.95	27.33	2.25 $p < 0.05$
Within days				
Between slides	126	1526.49	12.12	
Total	143	31632.08		

$S_{\bar{X}} t_{0.05} = 2.44$

TABLE A8

Analysis of monthly samples of rabbit faeces from Bangor Ancient Camp

% COMPOSITION		F/P	Ag	H	L/C	D/P	An	Arrh	U.G.	U.O.	Total area measured
SAMPLE	DATE										(grid squares)
1	19. 7.72	11.0	5.5	26.3	16.9	22.3	1.9	3.9	6.6	5.6	2612.
2	6. 9.72	18.3	13.0	5.1	9.7	15.5	6.5	1.7	5.3	24.9	2853
3	16.10.72	16.3	18.0	2.0	2.6	23.8	10.1	2.0	3.2	22.0	4293
4	13.11.72	16.2	8.8	4.1	16.7	28.3	3.0	0.5	6.2	16.2	4620
5	15.12.72	9.9	1.4	11.1	34.8	31.5	3.1	0	2.8	5.4	5606
6	26. 2.73	20.0	0.1	5.0	5.3	60.5	2.5	0.9	4.1	1.6	4354
7	28. 3.73	21.7	0	6.5	12.5	51.5	1.1	0.4	3.7	2.6	3750
8	2. 5.73	27.5	3.4	4.2	6.5	49.0	1.3	2.0	3.0	3.1	3324
9	1. 6.73	17.5	18.9	6.1	10.8	22.7	1.9	2.7	3.9	15.5	2021
10	27. 6.73	3.6	11.8	18.0	6.6	21.9	0.3	3.4	4.0	30.4	1788
11	8. 8.73	26.3	7.1	3.3	16.7	29.1	1.2	1.3	1.4	13.6	3674
12	21. 8.73	19.1	19.7	8.0	11.5	15.4	5.5	0.9	1.4	18.5	4710
13	8.10.73	24.2	5.8	4.1	6.5	26.5	6.4	1.3	2.0	23.2	3682
14	14.11.73	14.8	2.1	8.0	14.4	32.7	8.2	2.1	1.8	15.9	4400
15	17.12.73	17.2	1.7	11.7	5.2	47.3	7.6	0.6	2.6	6.1	4939
16	23. 1.74	16.1	1.9	12.4	3.3	54.9	3.1	1.9	2.4	4.0	2566
17	27. 2.74	25.4	0.8	9.0	1.2	53.8	1.6	0.8	2.4	5.0	3226
18	30. 3.74	33.7	0.5	8.0	5.7	41.3	1.6	1.8	3.5	3.9	3197
19	6. 5.74	23.2	2.1	6.1	19.8	36.4	1.4	3.7	2.9	4.4	3829
20	8. 7.74	8.5	20.7	6.1	5.8	10.2	1.2	4.1	4.1	39.3	1848

APPENDIX 2

ADDITIONAL TABLES FROM CHAPTER 7

TABLE A9

Numbers of wheat and barley grains eaten by individual rabbits when offered at three different relative frequencies. Each frequency offered during 4 trials on consecutive days. Total number of grains offered in each trial - 1,500.

TRIAL		♂1.1		♂1.3		♀1.1		♀1.2		♀1.3			♂2.1		♂2.2		♂2.3		♀2.1		♀2.2		♀2.3	
		W	B	W	B	W	B	W	B	W	B		W	B	W	B	W	B	W	B	W	B	W	B
1		-	-	60	12	96	11	108	19	109	22		21	220	0	103	0	22	0	92	30	141	0	29
2	GROUP 1	132	22	70	14	149	11	178	21	80	14	GROUP 2	-	-	9	82	25	207	24	318	27	233	18	93
3	9W:1B	127	20	63	7	207	16	196	16	176	16	9B:1W	26	225	13	144	12	83	14	223	29	203	21	272
4		93	12	14	6	237	30	247	23	182	10		8	44	0	41	10	87	1	63	19	227	21	169
TOTAL 1-4		352	54	207	39	689	68	729	79	547	62		55	489	22	370	47	399	39	696	105	804	60	563
5		17	155	0	37	24	326	17	165	11	136		101	16	120	20	138	14	108	13	198	32	140	25
6	GROUP 1	10	190	1	70	50	394	9	141	6	181	GROUP 2	139	12	183	14	225	15	135	18	79	10	269	26
7	9B:1W	22	165	0	63	34	303	12	225	17	186	9W:1B	98	7	131	14	-	-	158	11	149	9	321	40
8		14	158	1	50	37	391	12	117	27	238		83	2	117	8	183	25	111	14	95	16	279	37
TOTAL 5-8		63	668	2	220	145	1414	50	648	61	741		421	37	551	56	546	54	512	56	521	67	1009	128
9		102	121	11	27	199	218	52	97	81	83		75	58	50	55	87	128	44	63	65	80	141	127
10	GROUP 1	124	124	34	38	154	148	64	71	96	98	GROUP 2	41	50	66	83	87	104	42	49	65	71	121	108
11	1W:1B	43	62	21	38	161	162	84	96	83	50	1W:1B	35	86	43	50	97	105	40	48	47	69	122	61
12		42	67	1	33	155	159	45	53	101	125		133	167	7	36	119	121	27	37	38	59	116	103
TOTAL 9-12		311	374	67	136	669	687	245	317	361	356		284	361	166	224	390	458	153	197	215	279	500	399

TABLE A 10

Numbers of wheat and barley grains eaten by individual rabbits (Groups 1 and 2) when offered in the same 90:10 proportion for ten consecutive trials. (IIA)

DAY	TRIAL	♂1.1		♀1.1		♀1.2		♀1.3		♂2.1		♂2.2		♂2.3		♀2.1		♀2.2		♀2.3	
		W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B
1	1	43	16	254	25	107	15	40	7	8	110	8	92	17	175	19	165	8	64	18	128
	2	65	8	267	33	112	11	127	15	6	109	3	51	8	76	9	113	12	119	26	223
2	3	114	13	273	25	160	17	72	10	10	122	3	82	12	83	6	57	14	122	16	136
	4	115	23	108	4	101	7	117	7	3	20	2	21	18	145	5	102	6	76	36	220
3	5	157	17	215	24	205	19	59	9	18	121	9	84	16	155	12	106	17	101	18	103
	6	184	26	273	33	130	20	118	9	18	229	21	80	16	153	6	141	8	102	17	162
4	7	131	19	257	26	169	18	4	0	13	104	15	45	7	76	7	49	5	82	25	172
	8	177	16	351	33	153	13	81	3	22	245	9	113	21	139	0	54	5	56	9	102
5	9	159	12	347	37	190	14	71	8	21	235	6	107	7	175	14	185	7	155	7	150
	10	171	4	256	28	212	8	129	0	0	111	2	108	11	156	2	122	9	152	22	209

Total number of grains offered in each trial - 1,500

TABLE A 11

Numbers of wheat and barley grains eaten by individual rabbits (Groups 3 and 4) when offered in the same 90:10 proportion for fifteen consecutive trials (Repeat Experiment IIB).

DAY	TRIAL	♂3.1		♂3.2		♂3.3		♀3.1		♀3.2		♀3.3		♂4.1		♂4.2		♂4.3		♀4.1		♀4.2		♀4.3	
		W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B
1	1	30	6	47	7	88	10	0	0	42	11	141	21	4	52	5	43	5	51	7	111	12	114	14	103
	2	175	18	234	25	255	24	77	15	153	25	266	28	11	117	10	188	12	116	9	149	11	177	19	213
2	3	190	31	235	18	197	29	95	19	161	30	120	13	16	108	2	21	8	53	17	144	11	129	8	128
	4																								
3	5																								
	6																								
4	7																								
	8	114	16	182	19	206	18	135	35	285	33	124	8	10	97	20	99	13	60	33	240	16	82	14	79
5	9																								
	10	176	24	247	38	321	32	196	22	119	18	113	15	11	84	32	181	12	92	20	100	22	158	24	198
6	11																								
	12																								
7	13	57	6	175	20	236	31	43	17	160	16	150	30	13	130	5	52	3	48	26	192	35	233	32	221
	14	130	10	285	20	265	31	177	46	154	9	84	15	11	131	3	59	4	70	21	183	31	205	48	280
8	15	126	17	236	12	200	25	110	21	198	24	135	11	11	121	22	132	1	57	34	250	10	136	19	234

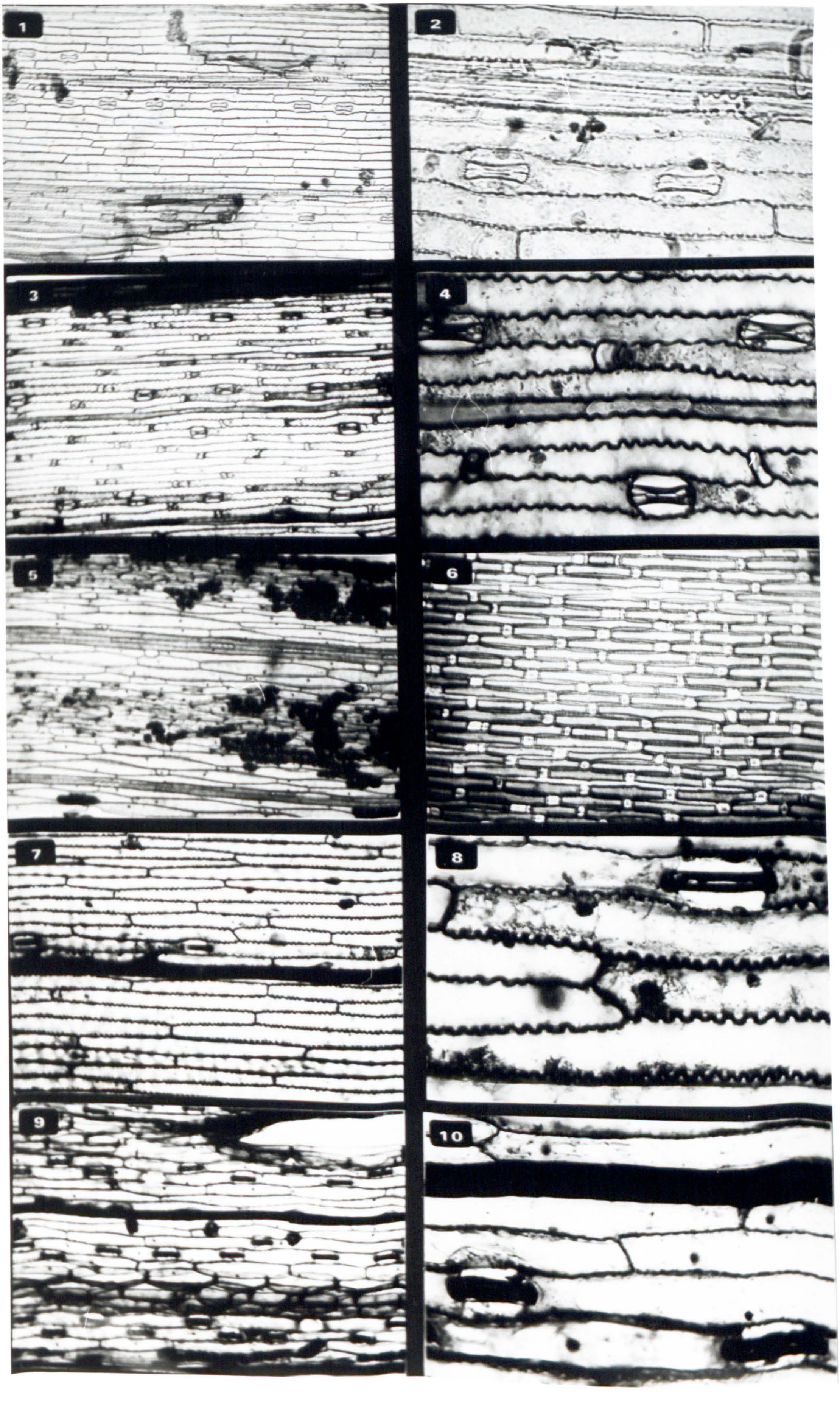
Total number of grains offered to each rabbit in each trial - 1,500

APPENDIX 3

PHOTOMICROGRAPHS OF EPIDERMAL FRAGMENTS

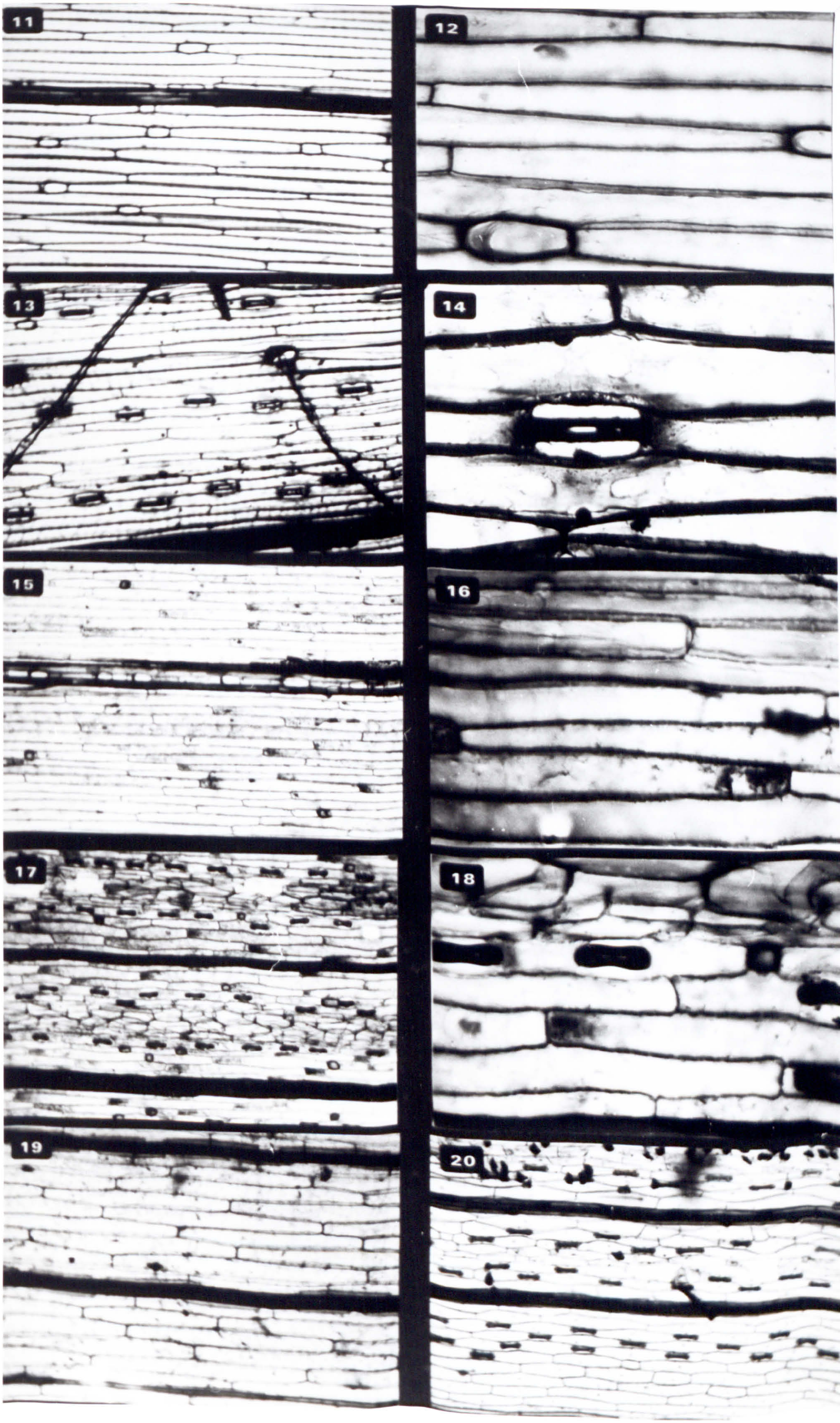
Photomicrographs of permanent preparations of leaf epidermis stained with acid fuchsin.

1.	<u>Agrostis canina</u>	Abaxial	leaf	epidermis	x 215
2.	" "	"	"	"	x 860
3.	<u>A. tenuis</u>	"	"	"	x 215
4.	"	"	"	"	x 860
5.	"	Adaxial	"	"	x 215
6.	<u>Ammophila arenaria</u>	Abaxial	"	"	x 215
7.	<u>Anthoxanthum odoratum</u>	"	"	"	x 215
8.	" "	"	"	"	x 860
9.	" "	Adaxial	"	"	x 215
10.	" "	"	"	"	x 860


1
2
3
4
5
6
7
8
9
10

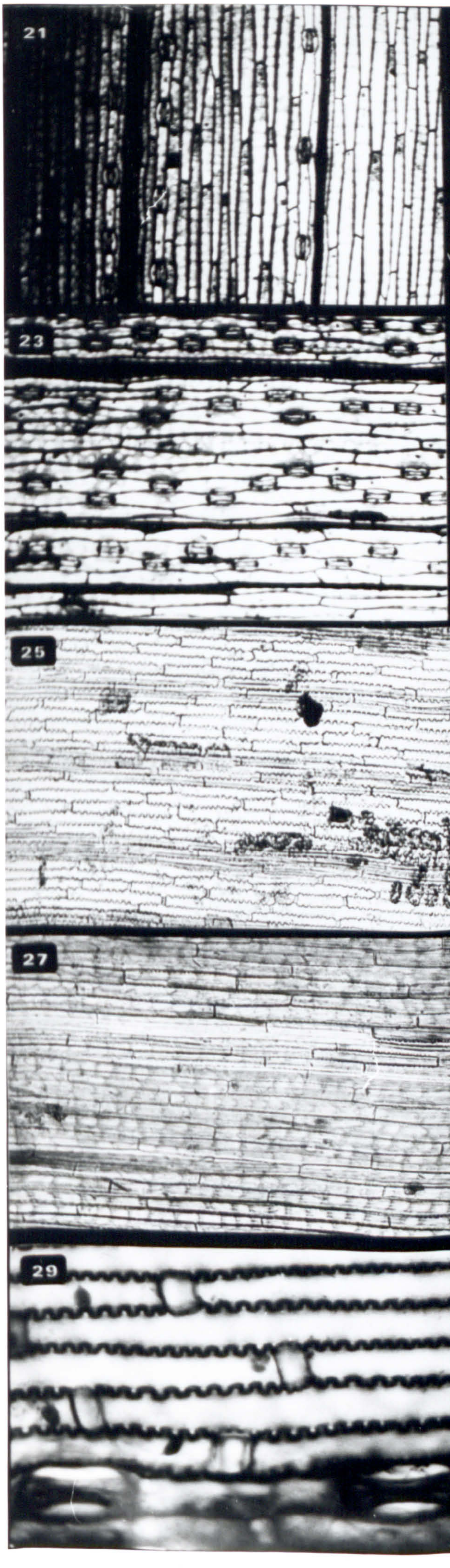
Photomicrographs of permanent preparations of leaf epidermis stained with acid fuchsin.

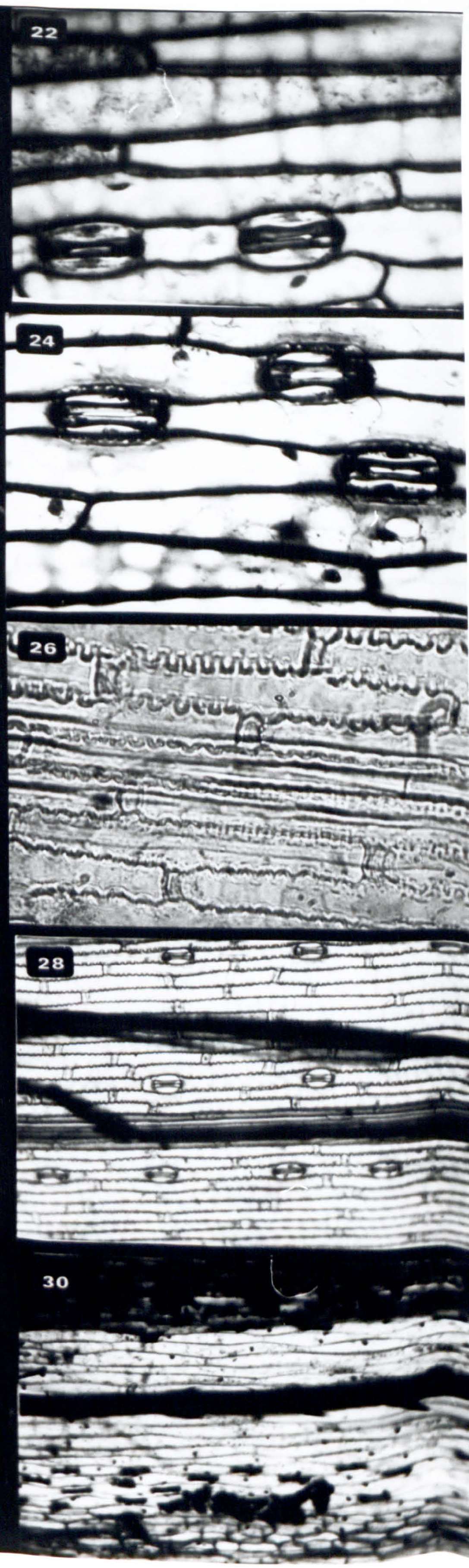
11.	Arrhenatherum elatius	Abaxial	leaf	epidermis	x 215
12.	" "	"	"	"	x 860
13.	" "	Adaxial	"	"	x 215
14.	" "	"	"	"	x 860
15.	Briza media	Abaxial	"	"	x 215
16.	" "	"	"	"	x 860
17.	" "	Adaxial	"	"	x 215
18.	" "	"	"	"	x 860
19.	Cynosurus cristatus	Abaxial	"	"	x 215
20.	" "	Adaxial	"	"	x 215


11
12
13
14
15
16
17
18
19
20

Photomicrographs of permanent preparations of leaf epidermis stained with acid fuchsin

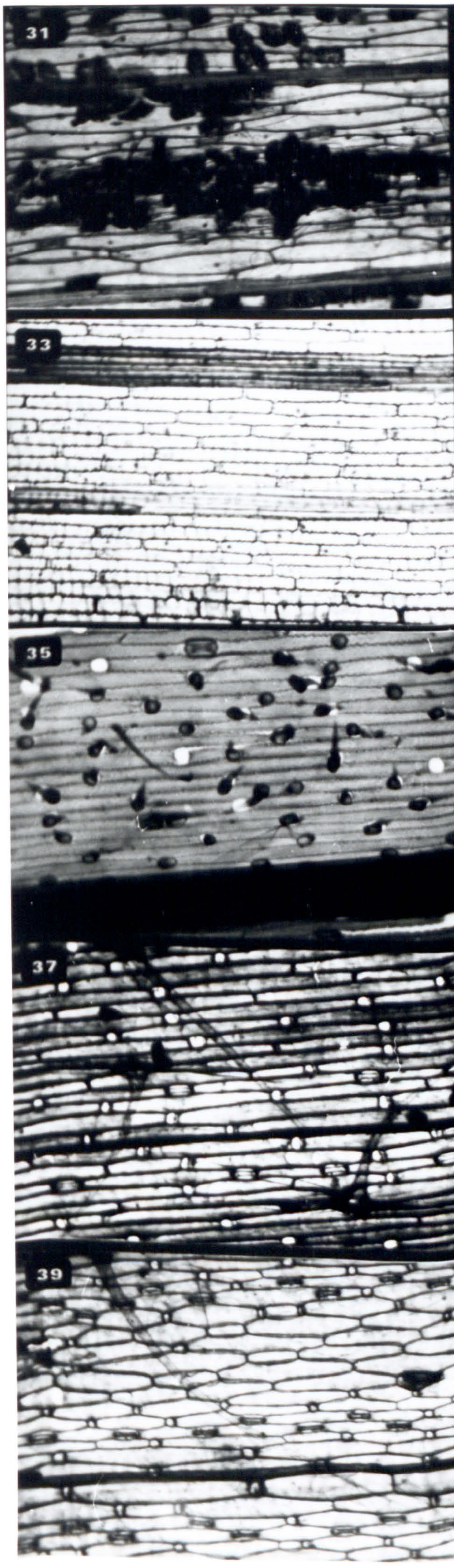
21.	Dactylis glomerata	Abaxial leaf epidermis	x 215
22.	" "	" " "	x 860
23.	" "	Adaxial " "	x 215
24.	" "	" " "	x 860
25.	Deschampsia cespitosa	Abaxial " "	x 215
26.	" "	" " "	x 860
27.	D. flexuosa	" " "	x 215
28.	Festuca arundinacea	" " "	x 215
29.	" "	" " "	x 860
30.	" "	Adaxial " "	x 215

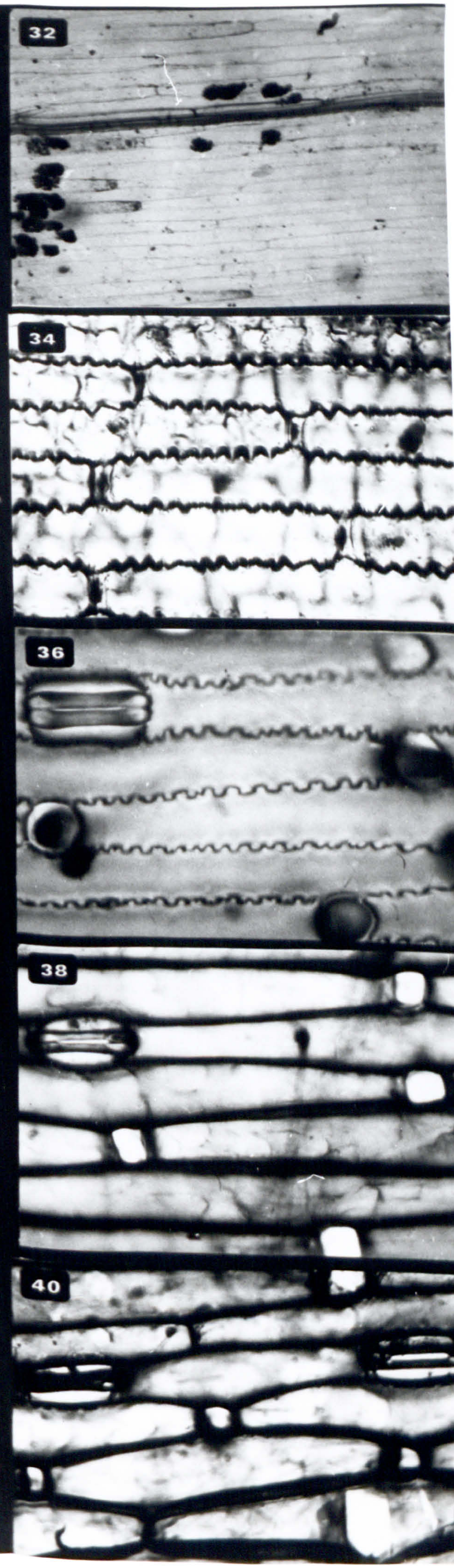

21
23
25
27
29


22
24
26
28
30

Photomicrographs of permanent preparations of leaf epidermis stained with acid fuchsin.

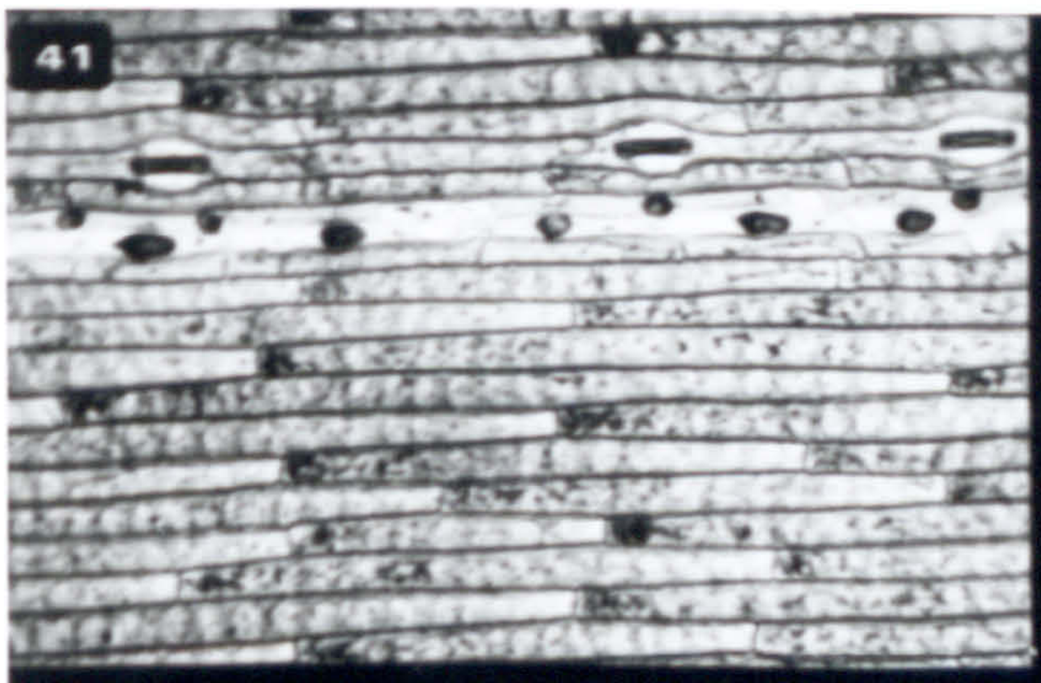
31.	Festuca gigantea	Abaxial leaf epidermis	x 215
32.	" "	Adaxial " "	x 215
33.	F. rubra	Abaxial " "	x 215
34.	"	" " "	x 860
35.	"	Adaxial " "	x 215
36.	"	" " "	x 860
37.	Holcus lanatus	Abaxial " "	x 215
38.	" "	" " "	x 860
39.	" "	Adaxial " "	x 215
40.	" "	" " "	x 860

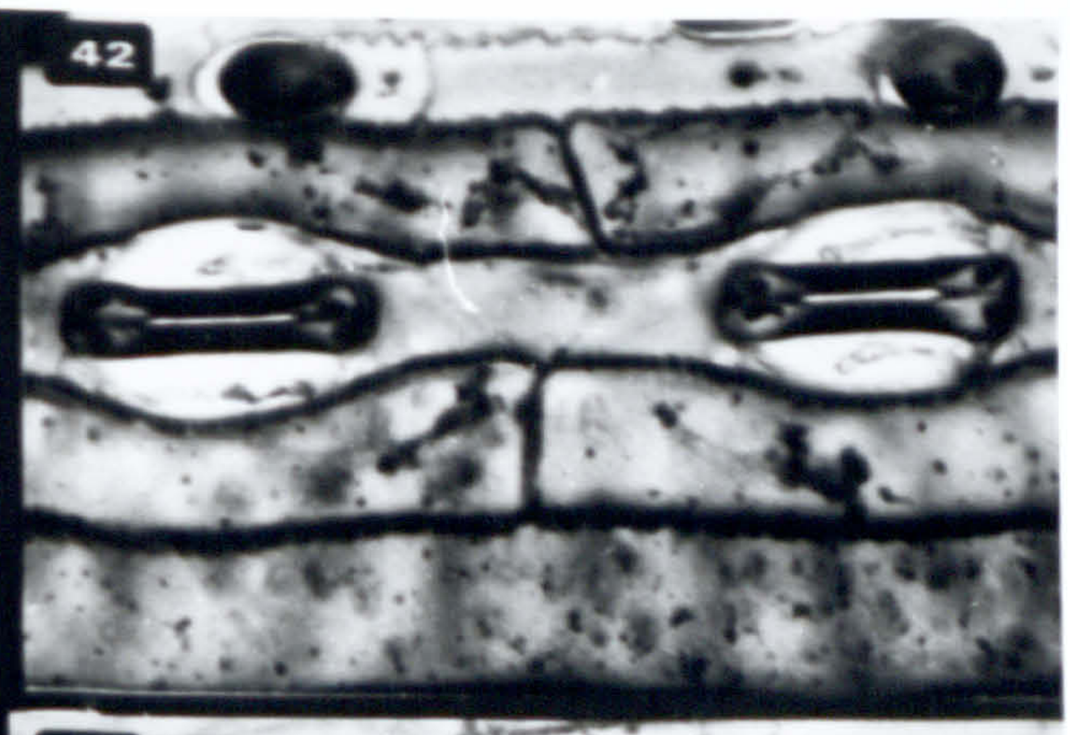

31
33
35
37
39

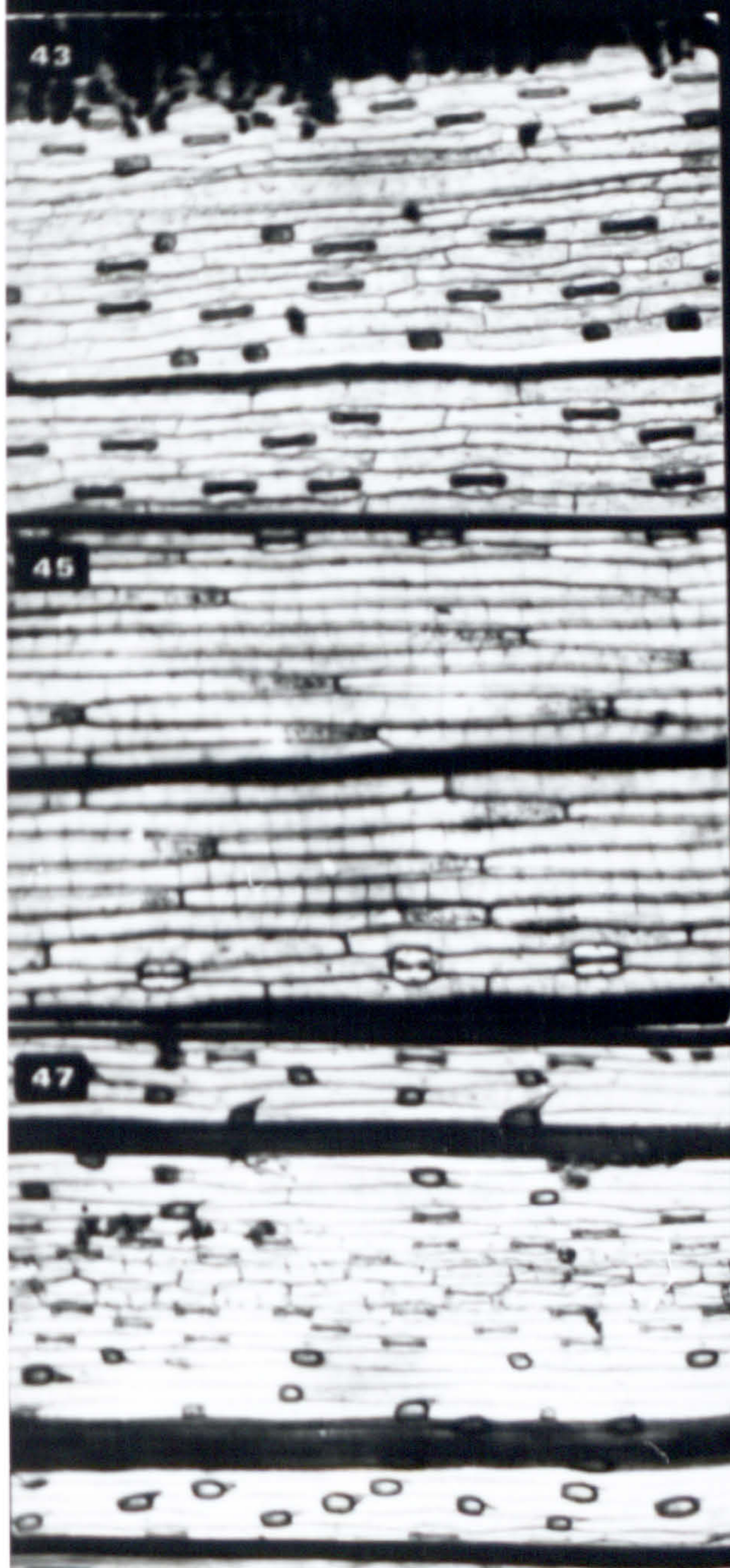

32
34
36
38
40

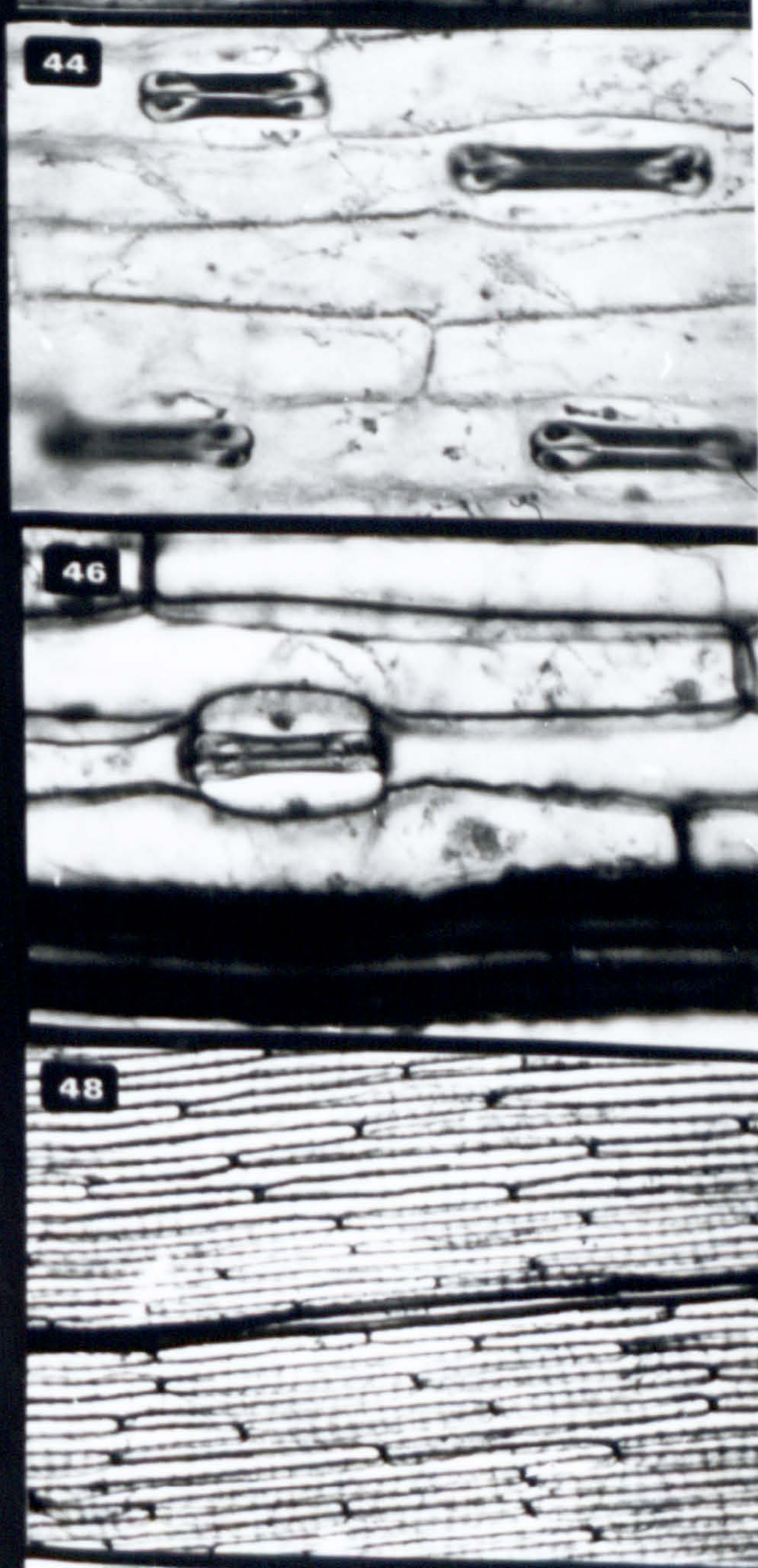
Photomicrographs of permanent preparations of leaf epidermis stained with acid fuchsin.

41.	Koeleria cristata	Abaxial	leaf	epidermis	x 215
42.	" "	"	"	"	x 860
43.	" "	Adaxial	"	"	x 215
44.	" "	"	"	"	x 860
45.	Lolium multiflorum	Abaxial	"	"	x 215
46.	" "	"	"	"	x 860
47.	" "	Adaxial	"	"	x 215
48.	L. perenne	Abaxial	"	"	x 215
49.	"	"	"	"	x 860
50.	"	Adaxial	"	"	x 215


41


42


43


44

45

46

47

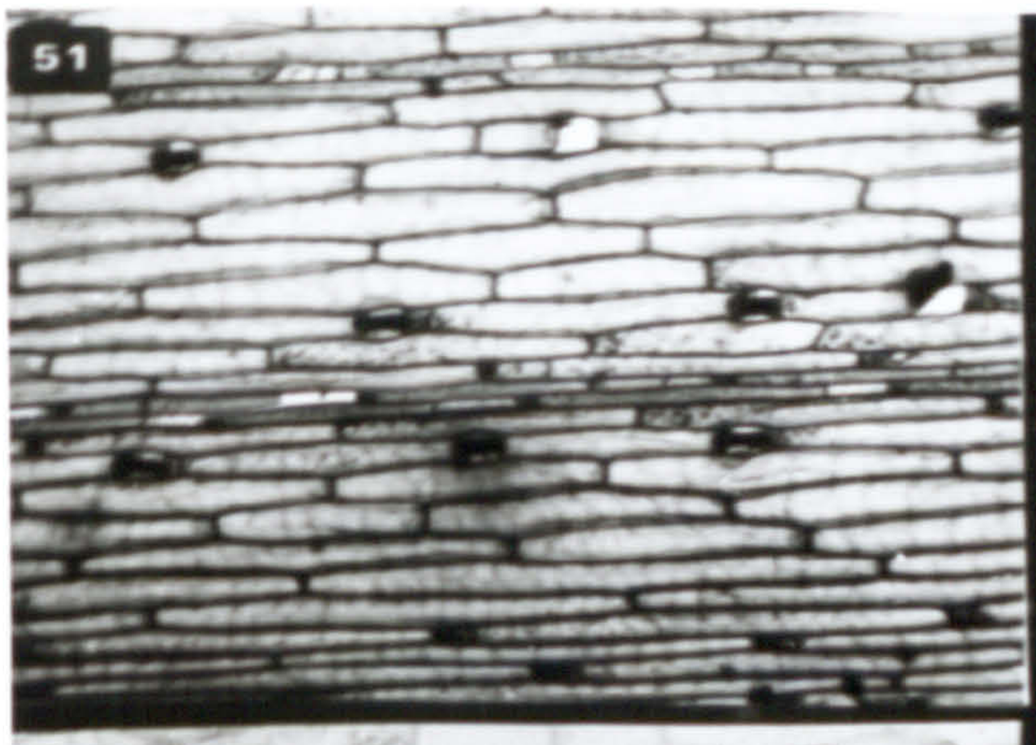
48

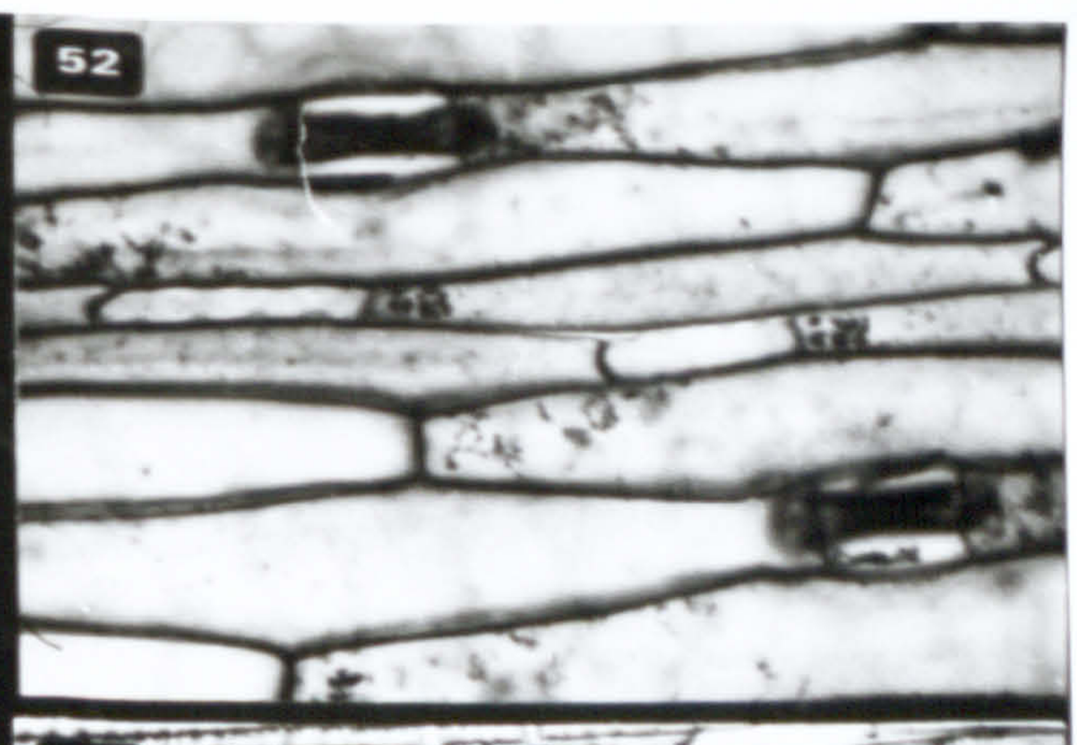
49

50

Photomicrographs of permanent preparations of leaf epidermis stained with acid fuchsin.

51.	Poa annua	Abaxial	leaf	epidermis	x 215
52.	" "	"	"	"	x 860
53.	" "	Adaxial	"	"	x 215
54.	P. pratensis	Abaxial	"	"	x 215
55.	"	"	"	"	x 860
56.	"	Adaxial	"	"	x 215
57.	"	"	"	"	x 860
58.	P. trivialis	Abaxial	"	"	x 215
59.	"	"	"	"	x 860
60.	"	Adaxial	"	"	x 215


51


52


53


54

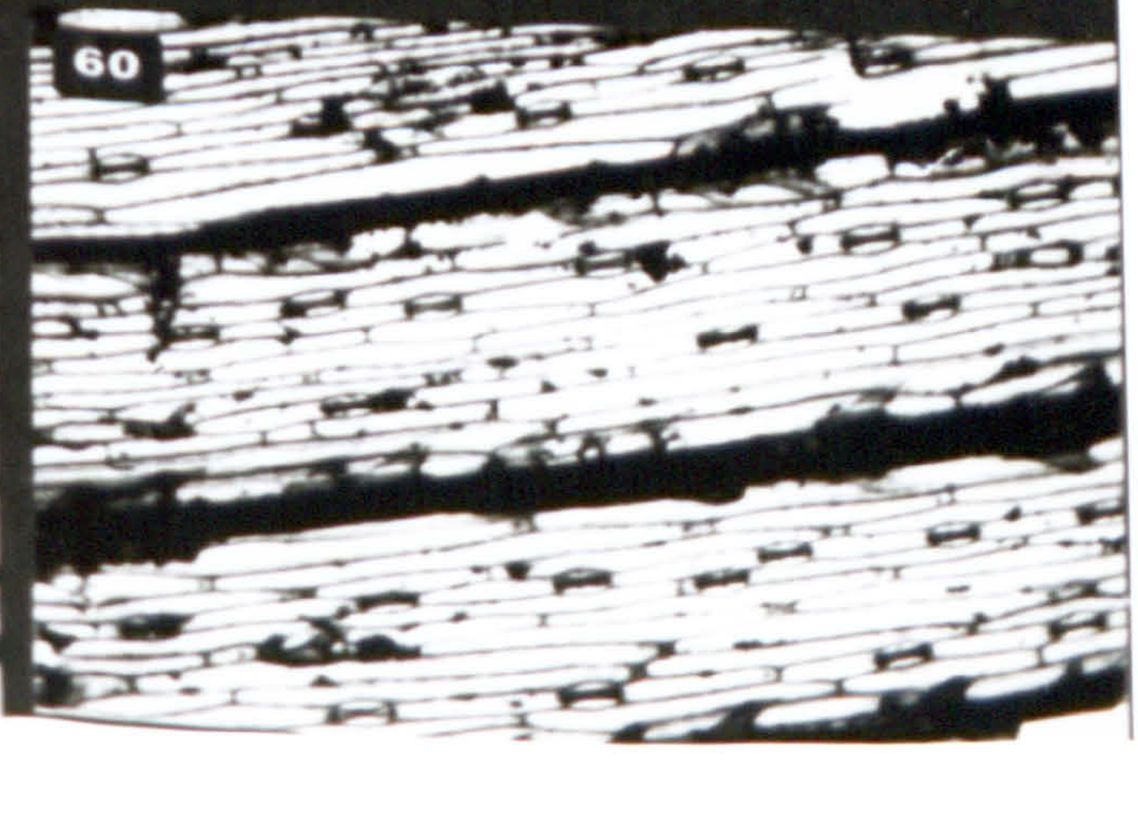

55


56

57

58


59


60

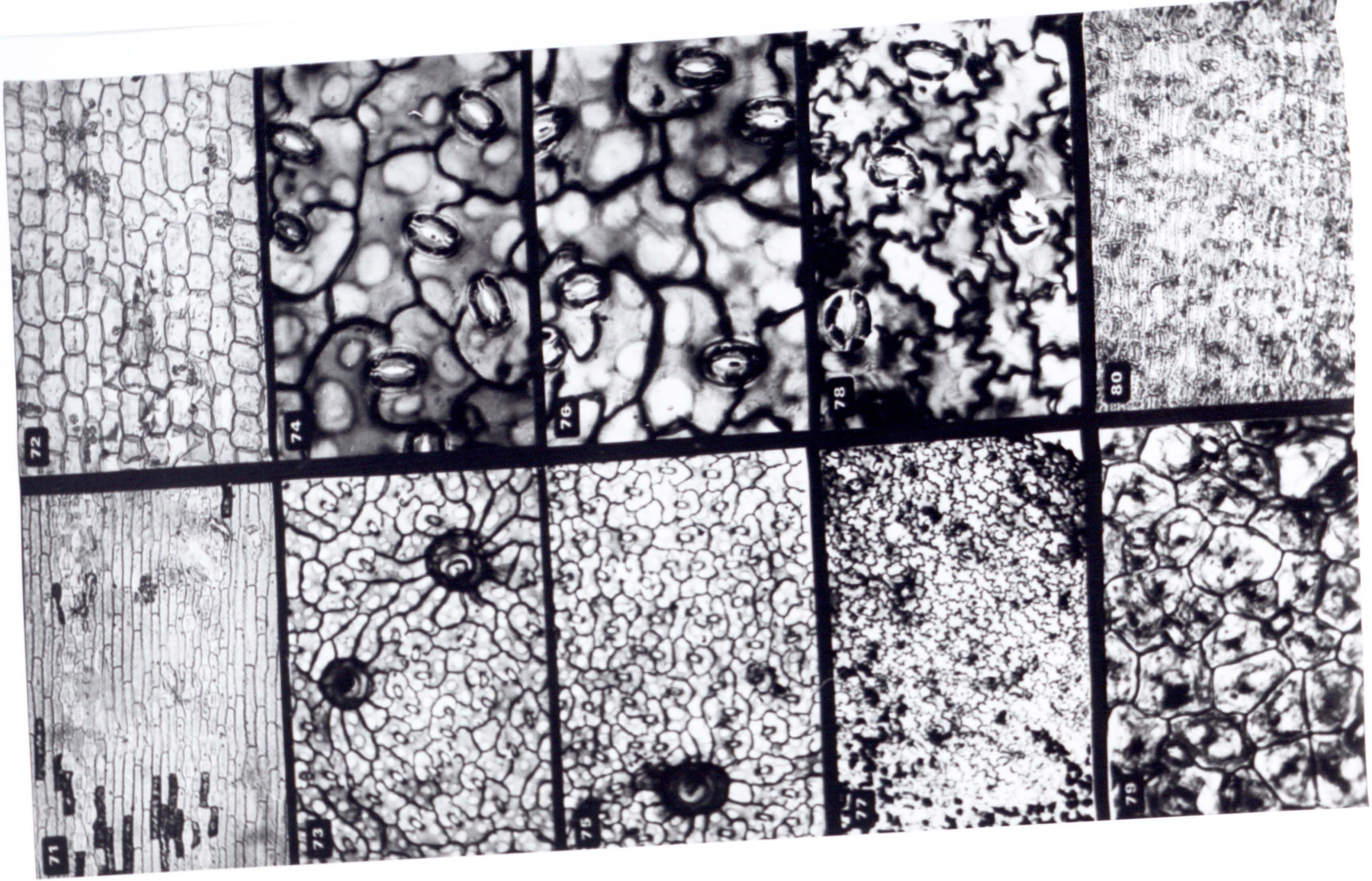
Photomicrographs of permanent preparations of leaf epidermis stained with acid fuchsin.

61.	Poa trivialis	Adaxial	leaf	epidermis	x 860
62.	Sieglingia decumbens	Abaxial	"	"	x 215
63.	"	"	"	"	"	x 860
64.	"	"	Adaxial	"	"	x 215
65.	"	"	"	"	"	x 860
66.	Carex flacca	"	"	"	x 215
67.	C. serotina	Abaxial	"	"	x 215
68.	"	"	"	"	x 860
69.	"	Adaxial	"	"	x 215
70.	"	"	"	"	x 860

61
62
63
64
65
66
67
68
69
70

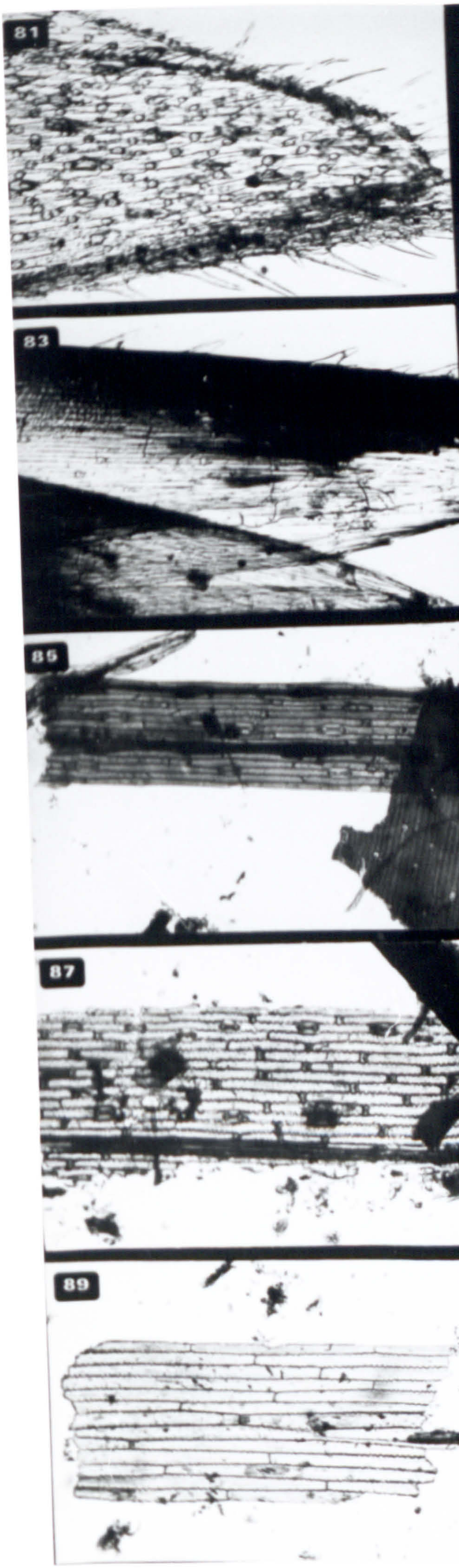
Photomicrographs of permanent preparations of epidermis stained with acid fuchsin.

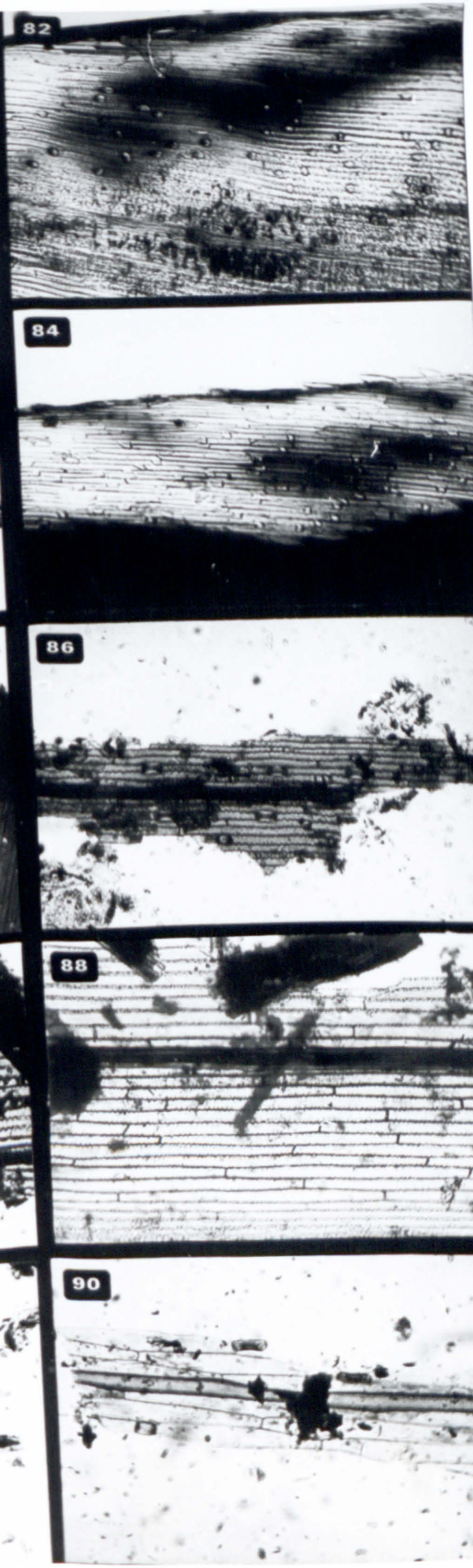
71.	<u>Luzula campestris</u>	Abaxial	leaf	epidermis	x 215
72.	" "	Adaxial	"	"	x 215
73.	<u>Plantago lanceolata</u>	Abaxial	"	"	x 215
74.	" "	"	"	"	x 860
75.	" "	Adaxial	"	"	x 215
76.	" "	"	"	"	x 860
77.	<u>Trifolium repens</u>	Abaxial	"	"	x 215
78.	" "	"	"	"	x 860
79.	" "	Adaxial	"	"	x 860
80.	<u>Equisetum variegatum</u>	Stem epidermis			x 215


72
74
76
78
80
71
73
75
77
79

Photomicrographs of permanent preparations of grass flower glumes stained with acid fuchsin and temporary mounts of faecal fragments of leaf epidermis stained with gentian violet.

81. <u>Holcus lanatus</u>	Glumes	x 215
82. <u>Poa trivialis</u>	"	x 215
83. <u>Agrostis tenuis</u>	"	x 215
84. <u>Dactylis glomerata</u>	"	x 215
85. <u>Agrostis tenuis</u>	Fragments of leaf epidermis (x 215) from samples of faeces from Bangor Ancient Camp.	
86. " "		
87. " "	Fragments of leaf epidermis (x 215) from sample of faeces from captive wild rabbits in digestion experiment.	
88. <u>Anthoxanthum odoratum</u>		
89. " "		
90. <u>Arrhenatherum elatius</u>	Fragment of leaf epidermis (x 215) from sample of faeces from Bangor Ancient Camp.	


81


82

83

84

85

86

87

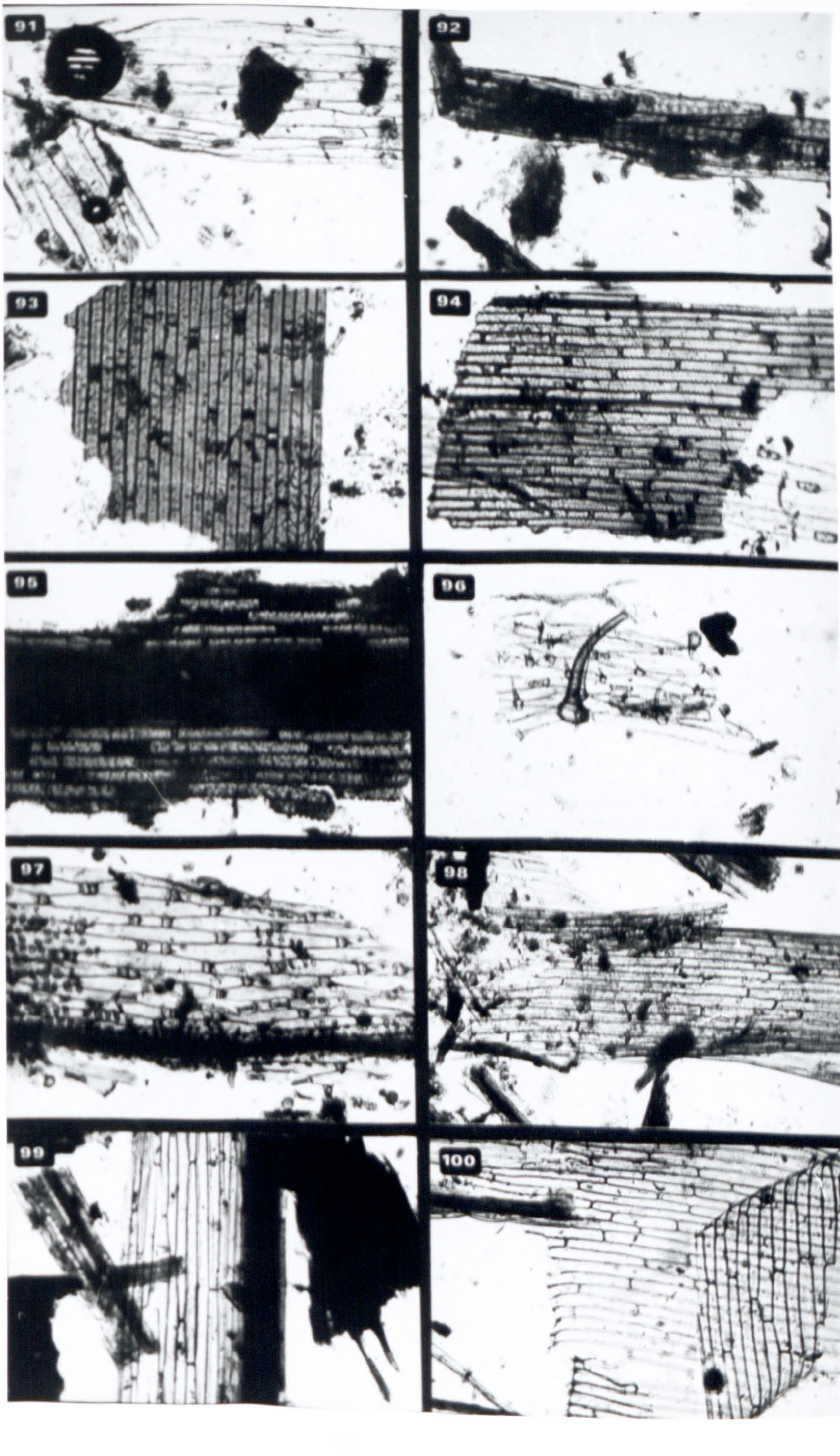
88

89

90

Photomicrographs of temporary mounts of faecal fragments of leaf epidermis stained with gentian violet.

91. Dactylis glomerata	Fragments of leaf epidermis (x 215) from samples of faeces in digestion experiment.
92. " "	
93. Festuca rubra	Fragments of leaf epidermis (x 215) from samples of faeces from Bangor Ancient Camp.
94. F. rubra & A. elatius	
95. F. rubra	Fragments of leaf epidermis (x 215) from samples of faeces in digestion experiment.
96. Holcus lanatus	
97. " "	Fragments of leaf epidermis (x 215) from samples of faeces from Bangor Ancient Camp.
98. Lolium perenne	
99. " "	Fragments (x 215) from digestion experiment.
100. " "	Fragments (x 215) from Bangor Ancient Camp.

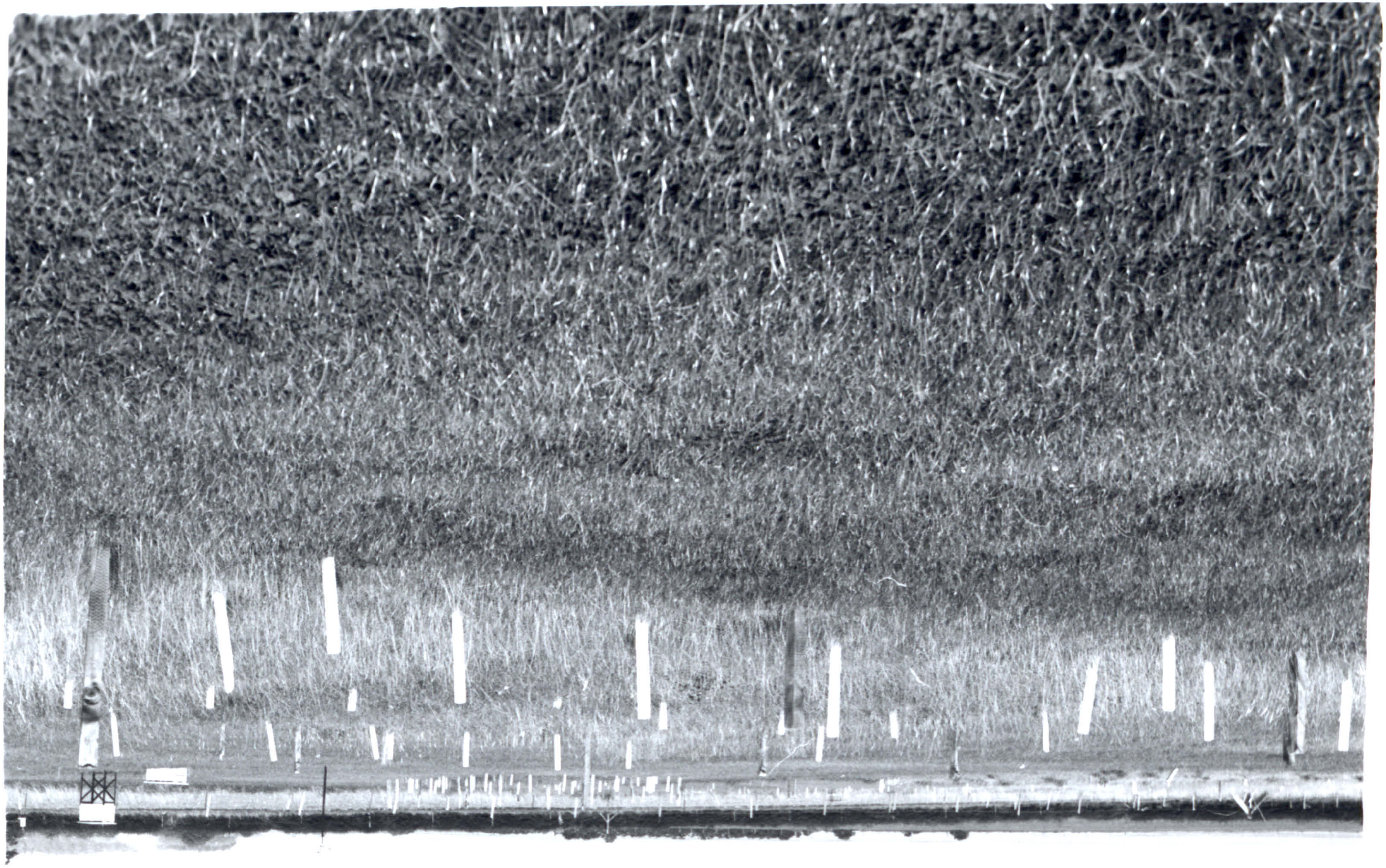

91
92
93
94
95
96
97
98
99
100

APPENDIX 4

PHOTOGRAPHS OF SAMPLING SITES (CHAPTER 4)

Photographs of sites described in Chapter 4.

1. Site 1. Permanent pasture, College Farm, Aber.
2. Site 2. Sown pasture, College Farm, Aber.
3. Site 3. Conwy - rough grassland adjacent to river estuary.
4. Site 4. Newborough - sand-dune system.
5. Site 5. Penmon - limestone grassland.
6. Site 6. Benllech - limestone grassland.
7. Site 7. Bangor Normal College grounds.
8. Site 8. Bangor Ancient Camp.



2