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# RABBIT GRAZING

# STUDIES IN A GRASSLAND COMMUNITY USING FAECAL ANALYSIS AND EXCLOSURES

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ABSTRACT

This paper considers the results of an investigation by 6th form students on the effects of rabbit grazing on a grassland community at Flatford, Suffolk. A key (with illustrations) to the leaf epidermises of the major plant species found in the study area is presented in Appendix 1. Refinements to the technique of faecal analysis, and exclosure studies are reviewed in Appendix 2.

## INTRODUCTION

PLANT-ANIMAL interactions are an important aspect of ècology; both from the point of view of the ways in which animals affect the structure of plant communities in space and in time, as well as to the varied ways in which plants respond to herbivores. Besides being inherently interesting, the study of such relationships is also pertinent to understanding successional development in plant communities, the carrying capacity of particular pastures and energy transfers and nutrient budgets in ecosystems.

This paper concentrates on the european rabbit, *Oryctolagus cuniculus* L., which was introduced to Britain in the twelfth century (Sheail, 1971). They were kept in confinement and bred for fur and meat. It is uncertain when they became feral on a large scale but their numbers started to increase dramatically at the time of the agricultural revolution (c.200 yrs. BP). This was a direct result of clearance of scrub and wood for pasture, the planting of winter crops and the destruction of its predators to protect game birds for sport. The introduction of rabbits to Australia constituted a major ecological disaster (see Pemberton, 1950; Tomlinson, 1959). With the advent of myxomatosis in the 1950's, attempts at controlling the rabbit, regarded by this time as a serious agricultural pest, were finally fruitful. But with the disappearance of rabbits, there were also many irreversible changes in natural vegetation, for example, the floristically-rich short-turf grassland on Downland rapidly became scrub (see Thomas, 1956, 1960, 1963). However, after the initial epidemic, which killed more than 99% of all rabbits (Mead-Briggs, 1977), the national population has gradually risen, despite repeated outbreaks. The population is currently estimated at 20% of the pre-myxomatosis level (of c. 60–70 million) and the annual damage to crops is put at c. £100 million (see Mills, 1986)! Thus studies on plant-rabbit relationships may also be of economic importance.

The paper examines the effects of rabbit grazing using the technique of faecal analysis for dietary assessments, and exclosures to study effects of grazing on vegetation succession.

Faecal analysis is essentially the identification of cuticular plant fragments in faeces. Dietary assessments are possible because the epidermal pattern of each plant species is

unique and plant cuticle is retained in faeces after digestion of the edible component. The main advantage of faecal analysis is that the same herbivore population can be repeatedly sampled without direct interference. However, the method has one serious disadvantage. The proportions of plant fragments in faeces may not necessarily represent the proportions of species consumed. This is due both to differential digestion of the plants (Stewart, 1967) as well as to differential deposition of cutin within them (Bhadresa, 1982). The problem can, however, be tackled by conducting controlled feeding trials in the laboratory where the actual amounts of the species consumed can be carefully monitored and accurately related to the proportions of plant cuticles in faeces.

Dietary studies are important in understanding the grazed plant community but cannot be used to predict changes in the vegetation if grazing were to stop. Thus a useful extension is to fence off areas and exclude the herbivore. These exclosures can provide very valuable information on changes in the plant community following the cessation of grazing. Whether a particular plant species will benefit or not from being protected depends not only on whether it is preferred or avoided by the herbivore but also on its own phenology and competitive abilities together with other factors like the amount of light, changes in the nutrient status and allelopathic effects within the exclosures.

The paper considers the results of a class investigation conducted during July 1984 by 6th form students on a grassland community at Flatford Mill Field Centre. A key (with illustrations) to the leaf epidermises of the major plant species found in the grassland was provided for identification of cuticles in faeces (Appendix 1). By comparing the proportions of plant species in rabbit droppings with the proportions of food plants available to the rabbits, the food preferences of these rabbits were deduced. Comparisons between grazed and ungrazed (fenced plots) areas were also made to examine changes in the vegetation. Refinements to the techniques are reviewed in Appendix 2.

## INVESTIGATION OF THE EFFECTS OF RABBIT GRAZING ON A GRASSLAND COMMUNITY AT FLATFORD MILL FIELD CENTRE, SUFFOLK

The aims of the investigation were two-fold, 1. to compare the proportions of species in the diet of rabbits with the vegetation on offer in order to determine food preferences of rabbits and, subsequently, to consider how both the diet and food preferences affect the grazed plant community. 2. to compare the composition and abundance of plant species in grazed and ungrazed (fenced) areas and to consider how the community develops on the cessation of grazing.

### THE STUDY AREA

The study area (grid ref. TM 077333), a short-turf grassland at Flatford, dominated by *Agrostis tenuis* (common bent grass), is shown in Fig. 1. (Nomenclature of plants follows Clapham, Tutin and Warburg 1962). The area is surrounded by scrub dominated by blackthorn (*Prunus spinosa*) on all sides except for the steep sided flooded gravel pit on the western edge. Some patches of tall grasses also occur on the edges of the grazed area. Mole activity has created an uneven surface and on mounds, rabbit faecal densities are usually high. Mole-hill vegetation consists of low-growing (hemi-cryptophytic) species, e.g. *Ranunculus repens, Glechoma hederacea*. Rabbits live protected amongst the blackthorn thickets and burrows are abundant to the south of the grazed area. The two rabbit

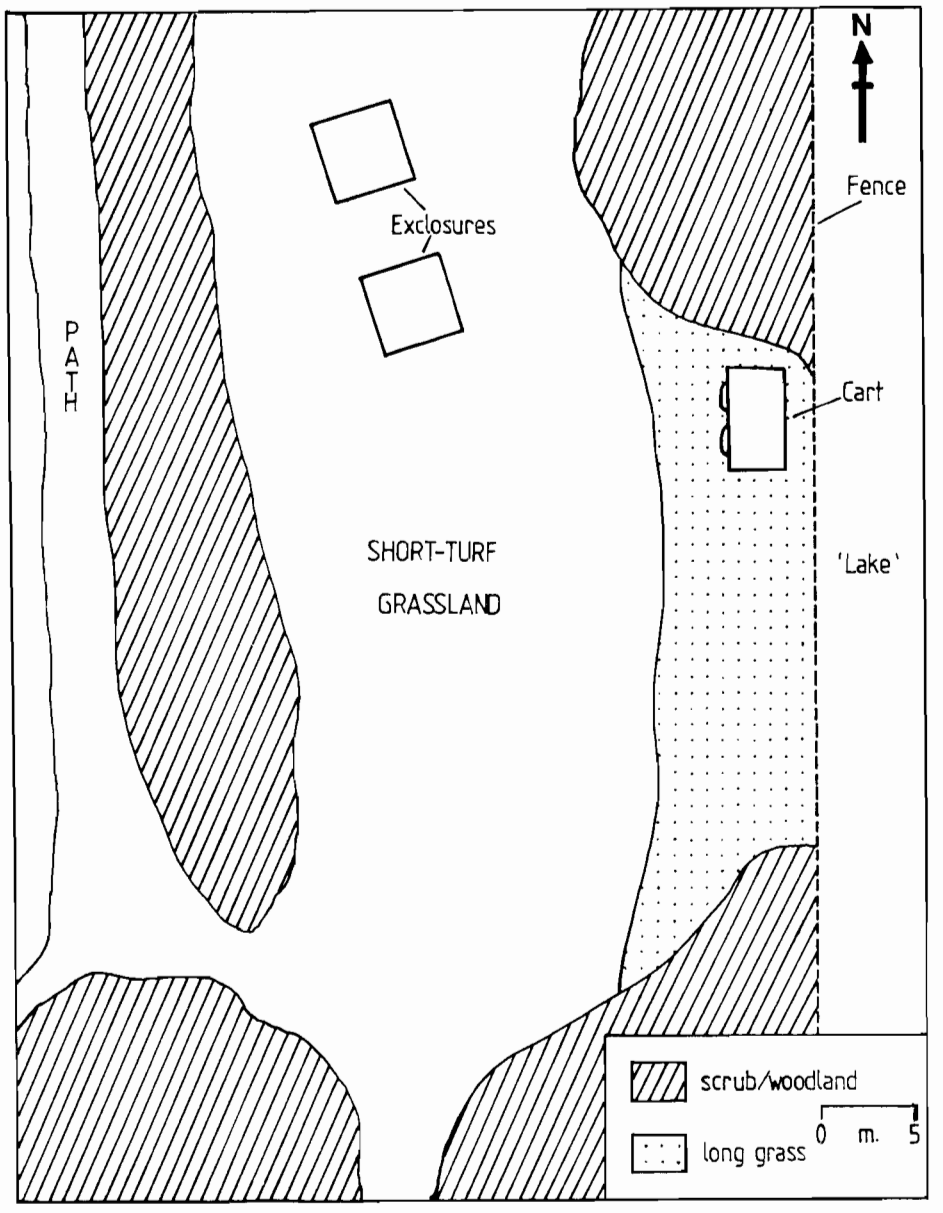



FIG. 1.
The study area showing predominant vegetation types and siting of exclosures.

exclosures (5 × 5m squares, 1m high) were erected in March 1983 and have since provided valuable comparisons with the grazed area surrounding them.

## METHODS

The investigation was conducted on 18 July, 1984. Fresh (green and shiny) faeces (one per student) were collected randomly from the grazed area. The faeces were soaked in

Table 1. *Percentage frequency contributions of plant species to rabbit faeces and to vegetation on offer, together with the rank orders and preference values (PV = rank order in vegetation minus rank order in faeces)*

| Species | Vegetation on offer | Faeces | Rank orders vegtn. | faeces | PV |
|---|---|---|---|---|---|
| *Achillea millefolium* | 12 | 1 | 9 | 13 | −4 |
| *Agrostis tenuis* | 93 | 73 | 1 | 1 | 0 |
| *Cerastium holosteoides* | 11 | 14 | 10 | 7 | +3 |
| *Dactylis glomerata* | — | 1 | ? | 13 | ? |
| *Festuca rubra* | 41 | 68 | 5 | 2 | +3 |
| *Glechoma hederacea* | 34 | 8 | 7 | 9 | 2 |
| *Holcus lanatus* | 32 | 24 | 8 | 3 | +5 |
| *Luzula campestris* | 7 | 17 | 12 | 6 | +6 |
| *Poa pratensis* | 2 | 8 | 13 | 9 | +4 |
| *Plantago lanceolata* | 8 | 8 | 11 | 9 | +2 |
| *Potentilla reptans* | 1 | — | 14 | — | ? |
| *Ranunculus repens* | 46 | 1 | 3 | 13 | −10 |
| *Rumex acetosa* | 40 | 11 | 6 | 8 | −2 |
| *Taraxacum officinale* | 1 | — | 14 | — | ? |
| *Trifolium repens* | 45 | 20 | 4 | 4 | 0 |
| *Veronica chamaedrys* | 71 | 18 | 2 | 5 | −3 |
| Moss | — | 4 | — | 12 | ? |

water, macerated and mounted onto slides. The epidermal fragments in the faeces were identified using the key provided (Appendix 1). Quantification was carried out using the frequency method where the microscope's field of view at × 100 magnification acted as the quadrat (see Appendix 2). Ten quadrats were recorded for an individual subsample from each dropping. The results for 20 droppings (200 quadrats) were pooled and the percentage frequencies of different plant cuticles computed.

The frequency method was also used to record the vegetation on offer in the grazed area and inside the exclosures. Plant identification and recording sheets were provided for this purpose. In the grazed area, each pair of students recorded presence of species in 100 mm × 100 mm quadrats located at 0.5 m intervals along straight lines placed parallel to each other. In all, 200 quadrats were recorded. Within the exclosures, quadrats were dropped at random and in all, 100 quadrats were recorded. The occurrences of species were subsequently converted into percentage frequencies.

Food preferences of rabbits were computed by comparing the rankings of species in the vegetation on offer and in the faecal samples. Thus, if a species ranked high in the grazed area but low in the faeces, it was assumed to be disliked. Conversely, if it ranked low in the grazed area but high in the faeces, it was assumed to be preferred. The order of food preferences was determined by subtracting the ranking of species in the grazed area from their ranking in the faeces.

## RESULTS

*Dietary and Food preference studies*

The results are presented in Table 1. It is evident from the results that grasses (*Agrostis tenuis*, *Festuca rubra* and *Holcus lanatus*) are major contributors to the diet of rabbits, while

Table 2. *Percentage frequency contributions of plant species to grazed and ungrazed areas. Quadrat size 0.01 m*

| Species | Grazed vegetation | Vegetation in exclosures |
|---|---|---|
| *Achillea millefolium* | 12 | 32 |
| *Agrostis tenuis* | 93 | 64 |
| *Anthoxanthum odoratum* | — | 4 |
| *Arrhenatherum elatius* | — | 60 |
| *Cerastium holosteoides* | 11 | 16 |
| *Dactylis glomerata* | — | 10 |
| *Festuca rubra* | 41 | 11 |
| *Glechoma hederacea* | 34 | 10 |
| *Heracleum sphondyllium* | — | 1 |
| *Holcus lanatus* | 32 | 48 |
| *Lathyrus pratensis* | — | 1 |
| *Luzula campestris* | 7 | 2 |
| *Poa pratensis* | 2 | — |
| *Plantago lanceolata* | 8 | 17 |
| *Potentilla reptans* | 1 | – |
| *Ranunculus repens* | 46 | 32 |
| *Rumex acetosa* | 40 | 26 |
| *Taraxacum officinale* | 1 | 6 |
| *Trifolium repens* | 45 | 79 |
| *Veronica chamaedrys* | 71 | 36 |

most of the dicotyledonous species are minor contributors. The order of food preferences, derived from Table 1, is as follows:

| Species | Common name | Preference value |
|---|---|---|
| *Luzula campestris* | Field wood rush | +6 |
| *Holcus lanatus* | Yorkshire fog | +5 |
| *Poa pratensis* | Annual meadow grass | +4 |
| *Cerastium holosteoides* | Common mouse ear | +3 |
| *Festca rubra* | Red fescue | +3 |
| *Plantago lanceolata* | Ribwort plantain | +2 |
| *Agrostis tenuis* | Common bent | 0 |
| *Trifolium repens* | White clover | 0 |
| *Glechoma hederacea* | Ground ivy | −2 |
| *Rumex acetosa* | Common sorrel | −2 |
| *Veronica chamaedrys* | Germander speedwell | −3 |
| *Achillea millefolium* | Yarrow | −4 |
| *Ranunculus repens* | Creeping buttercup | −10 |

*Dactylis glomerata* (cocksfoot), *Potentilla reptans* (creeping cinquefoil), *Taraxacum officinale* (dandelion) and all mosses, are not listed because they were either absent from the vegetation sampled or not recognised in the faeces.

The food preference order indicates that although species may be important contributors to the diet, it does not necessarily mean that they are high on the preference list and, vice

versa, if they are minor contributors, they are not necessarily disliked by rabbits. Food selection appears to be related both to the abundance of particular species and to the rabbit's likes and dislikes. With such a strategy, it is conceivable that the rabbit maximises its intake of good quality food plants.

*Exclosure studies*

Percentage frequencies of species within the exclosures and for comparison in the grazed area, are presented in Table 2.

Startling differences, both in the species abundance and composition are immediately evident between grazed and ungrazed areas. There are more species recorded from within the exclosures (18 compared with 15) of which only 13 are found in both. Also, there are large differences in the levels of abundance of various species common to both areas. The results show rapid changes in the plant community on the cessation of grazing. The species are grouped below under those that have increased and those that have decreased within the exclosures. In this way it would be simpler to identify any common intra-group features. Their respective frequencies in the grazed and ungrazed areas are also shown.

| | % frequencies in | |
|---|---|---|
| | grazed area | exclosures |
| Species that have increased: | | |
| *Achillea millefolium* | 12 | 32 |
| *Cerastium holosteoides* | 11 | 16 |
| *Holcus lanatus* | 32 | 48 |
| *Plantago lanceolata* | 8 | 17 |
| *Taraxacum officinale* | 1 | 6 |
| *Trifolium repens* | 45 | 79 |
| *Anthoxanthum odoratum* | — | 4 |
| *Arrhenatherum elatius* | — | 60 |
| *Dactylis glomerata* | — | 10 |
| *Heracleum sphondylium* | — | 1 |
| *Lathyrus pratensis* | — | 1 |
| Species that have decreased: | | |
| *Agrostis tenuis* | 93 | 64 |
| *Festuca rubra* | 41 | 11 |
| *Glechoma hederacea* | 34 | 10 |
| *Luzula campestris* | 7 | 2 |
| *Ranunculus repens* | 46 | 32 |
| *Rumex acetosa* | 40 | 26 |
| *Veronica chamaedrys* | 71 | 36 |
| *Poa pratensis* | 2 | — |
| *Potentilla reptans* | 1 | — |

It can be seen from the results that it is the tall-growing species, for example, *Arrhenatherum*, *Dactylis* and *Holcus* that have increased while low-growing species (*Agrostis*, *Festuca*, *Glechoma* and *Ranunculus*) have declined. It appears, therefore, that the taller species are kept down, either directly or indirectly by rabbit grazing. However, there are exceptions to this general rule. *Trifolium* and *Plantago*, for example, although low-

growing, have increased in abundance within the exclosures. This rather suggests that other factors, both biotic (competition, phenology, growth patterns) and abiotic (light, microclimate, edaphic) might also play a role.

## Discussion

Faecal analysis of rabbit droppings has shown that grasses are by far the major contributors to the diet of rabbits. One of these species, namely *Agrostis tenuis*, is also the most abundant plant in the grazed area (frequency 93%). Thus, heavy grazing on this species, rather than acting as a deterrent, appears to promote its spread! This is most probably related to its phenology. Having its meristematic tissue close to the ground and therefore generally untouched by rabbits, grazing (not unlike mowing) induces growth of new tillers. This must also enhance the spread of its rhizomatous roots which in turn compete favourably for the nutrients and water in the soil. Nutrients are being continually removed by rabbit grazing and it is conceivable that nutrient levels decrease sharply with soil depth. Thus, only species that can compete favourably for nutrients are likely to be successful in the grazed area and plants with higher meristems and deeper roots are likely to suffer from direct grazing and low nutrient levels respectively. Certainly, there are species in the grazed area which can compete favourably for nutrients. These perennial plants can spread vegetatively by either the possession of strong stolons (*Ranunculus repens*), prostrate stems that root at the nodes (*Glechoma hederacea, Veronica chamaedrys*) or adventitious buds on horizontal roots (*Rumex acetosa*). These species, as the results have shown, are disliked by rabbits which contributes to their success in the grazed area. One very obvious feature of the grazed area is the almost total lack of flowering. Thus the only species which can survive in there are perennials which can spread vegetatively and/or have other means of obtaining nutrients, such as the nitrogen-fixing root nodules in *Trifolium repens*. In fact, all the species found in the grazed area can spread vegetatively but it appears that their degree of success depends on the food preferences of rabbits. Thus, the rabbit-preferred species *Luzula, Holcus, Festuca, Poa, Cerastium and Plantago*, all have a lower abundance.

Another way in which rabbits indirectly affect the abundance and distribution of species in the grazed area is by their regular use of faecal deposition sites (formerly mole hills) for marking their territories. Faeces and urine deposited onto latrine sites appear to destroy the meristems of grasses and this, together with the extra input of nutrients by way of faeces, allows other species to invade.

Overall, grazing reduces flowering and increases the spread of low-growing species able to propagate vegetatively, with their success related to varying degrees of grazing on them.

The changes in the vegetation within the exclosures clearly indicated the rapid increase of tall grasses (*Dactylis, Holcus* and especially *Arrhenatherum*) which, it appears, are kept in abeyance in the grazed area not because they are consumed by rabbits but because they are not able to compete for nutrients. Thus, as soon as vegetation is protected from grazing and the competition for nutrients is greatly reduced, these species become dominant. These common perennial species of rough grassland are stoutly erect and tufted and their rapid growth and deep roots make them particularly suited to ungrazed grasslands. With an increase in dominance of these species, most of the low-growing plants, for example, *Agrostis, Glechoma, Ranunculus, Veronica* and *Rumex*, all species successful in the grazed area, decline as competition for light increases. Of the other species that have increased within the exclosures, *Trifolium*, generally associated with short-turf vegetation, has simply 'exploded'. This increase must be the result of the extra nutrients available, its

nitrogen-fixing facility and creeping habit, and possibly its phenotypically plastic response (of growing taller and expanding its leaves) to low light intensities. Similarly, the increase in *Achillea*, a herb with erect stems, must be due to the extra nutrients and its far creeping stoloniferous habit. In the case of *Plantago* and *Taraxacum*, their deeper roots (tap roots in the latter) make them particularly suitable to the changed environment. *Cerastium*, on the other hand, has managed to maintain its hold possibly as a result of its creeping stock and an increase in inter-nodal distances allowing it to lean procumbently on other species. Two important arrivals in the exclosures are *Lathyrus pratensis* and *Heracleum sphondylium*, the former understandably because of its scrambling habit helped by leaf tendrils and the latter, incidentally a species highly esteemed by herbivores, because of its deep roots and erect habit.

Thus changes in the plant community on the cessation of grazing can be readily explained in terms of both the peculiar growth habits of plants and the changes in microclimatic and edaphic factors. However, the dynamic nature of the vegetation must not be forgotten and only records of long-term changes together with detailed phenological studies on plants can reveal the survival strategies employed by different species.

Two features stand out distinctly within the exclosures; 1. the abundant flowering and 2. the enhanced growth of all the species. These changes are the consequence of the release from constraints, both direct and indirect, imposed on the plants by rabbit grazing. More importantly, they represent the seeds of future changes and successional development of vegetation within the exclosures. Subsequently the differential seed production and growth strategies of the various plant species together with microclimatic and edaphic changes they produce will become increasingly important in governing the fate of the vegetation.

## CONCLUSIONS

Rabbit grazing imposes both direct and indirect pressure on the vegetation and as a consequence only species that can spread vegetatively by either stolons or rhizomes are successful. Their degree of success appears to be related to the varying degree of grazing on them. On the cessation of grazing and the constraints that this imposes on plants, flowering is greatly increased and growth is enhanced by an increase in nutrient availability. The taller grasses invade and become dominant at the expense of low-growing species.

## SUGGESTIONS FOR FURTHER WORK

1. Construction of a hide suitably placed to observe rabbit behaviour and for estimating population size.
2. Mini-transects (together with soil studies) across latrine sites of different ages, to identify the effects of faeces and urine on the distribution and abundance of plants.
3. Comparative studies on the performance of plant species in grazed and ungrazed areas; measurement of heights, inter-nodal distances, leaf surface areas.
4. Soil studies; measurement of soil moisture, pH, organic matter content at different depths in the grazed and ungrazed areas.
5. Microclimatic studies; measurement of light, wind velocity, humidity, temperature at different levels in the herb layer in grazed and ungrazed areas.
6. Study of growth strategies of plants including the behaviour of roots, stolons and rhizomes.
7. Feeding experiments on rabbits to determine exact relationships between proportions of different species consumed to their proportions in faeces.

## Summary

1. The case study presents the results of a class investigation conducted on 18th July 1984 on a grassland community at Flatford Mill Field Centre, Suffolk.

2. Food preferences of rabbits were deduced by comparing the proportions of leaf epidermises in faeces with the proportions of plant species available to the rabbits. The composition and abundance of species in grazed and ungrazed (fenced plots) areas was also compared.

3. The composition and abundance of species in the grazed area appears to be the result of a number of factors, predominantly varying degrees of grazing on particular species, competition for nutrients and the inherent growth strategies of plants. Although important constituents of the diet, because their meristems are close to the ground surface, short grasses, *Agrostis* and *Festuca*, benefit from grazing. As a result of competition for nutrients, other species decline. However, the little-grazed low growing species with a creeping habit, for example, *Ranunculus, Glechoma* and *Rumex*, flourish in the grazed area.

4. By the 2nd year, within exclosures, rank grasses, *Arrehenatherum, Holcus*, and *Dactylis* were dominant and as a result of competition for light, low growing species had declined. Species with deeper roots, for example, *Plantago* and *Heracleum*, were also successful.



## Acknowledgements

Many thanks are due to the students of Wycombe Abbey School (Bucks) associated with the field and laboratory work necessary to produce the case study. Thanks are also due to Messrs. Tom Mercer, Andy Gibbons and Andy Partridge for their help in executing some of the artwork.

## APPENDIX 1

### KEY TO THE EPIDERMISES OF THE MAJOR PLANT SPECIES ON THE GRASSLAND AT FLATFORD*

*A note to identification of epidermal fragments in faeces:*

Variations in epidermal pattern can exist even on the same leaf. The key does not cover all the variations present and relies mainly on the cell patterning found towards the middle of a leaf. Because of its wide cover over the leaf surface, this pattern is expected to be well represented in the faeces. Doubtful identifications can only be verified from a good reference collection of epidermal types.

*A short glossary of terms*

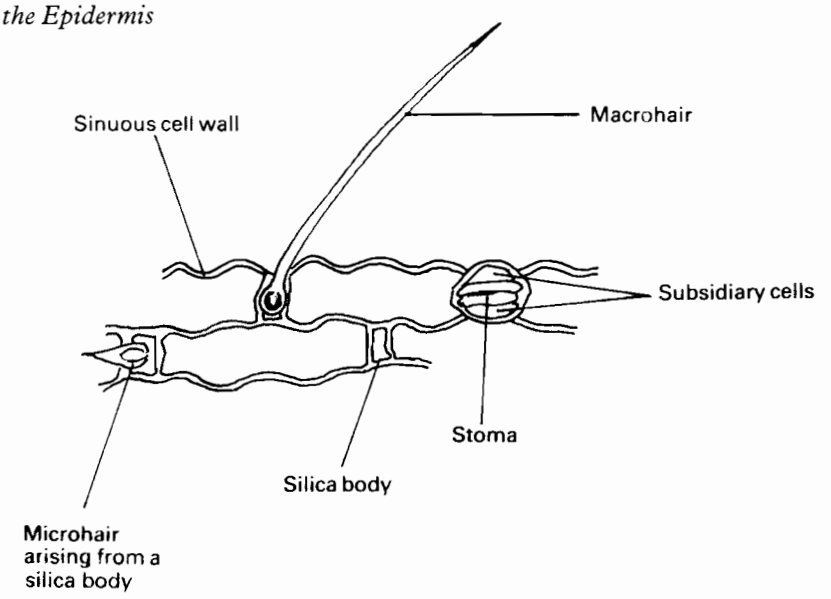
amocytic = pattern of arrangement of epidermal cells, where FOUR equal-sized cells surround the stoma.

anisocytic = pattern of arrangement of epidermal cells, where THREE cells (one smaller than the other two) surround the stoma.

diacytic = pattern of arrangement of epidermal cells, where TWO cells surround the stoma.

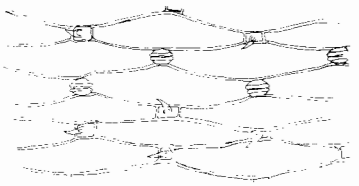
isodiametric = epidermal cells with equal or nearly equal diameters.

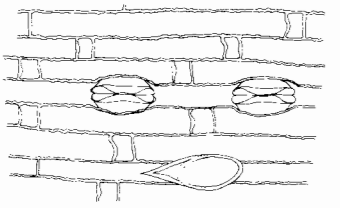
*Parts of the Epidermis*



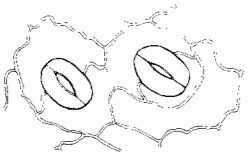

*Editor's note: This is the key used to identify plants epidermises found in rabbit faeces *at Flatford*. Other species may be found in other places. It is printed here as an example of the type of key required: not as the final version of a key to all plants eaten by rabbits.

1a Epidermal cells long and narrow, length:breadth ratio always greater than 4:1. 2

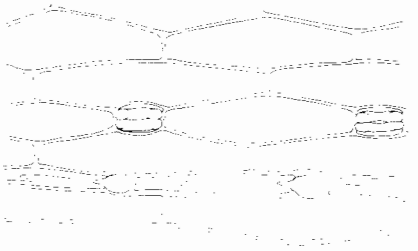


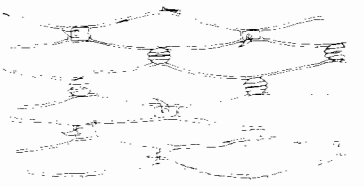


1b Epidermal cells generally with equal or nearly equal diameters (isodiametric): never long and narrow. Length:breadth ratios less than 2:1 . . . . . 9

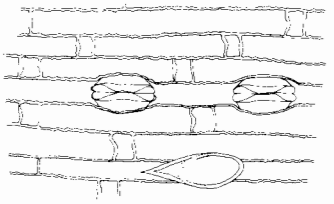


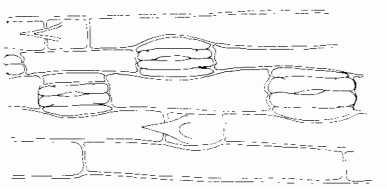
2a Epidermal cells coffin-shaped (i.e. bulging in the middle) . . . . . 3



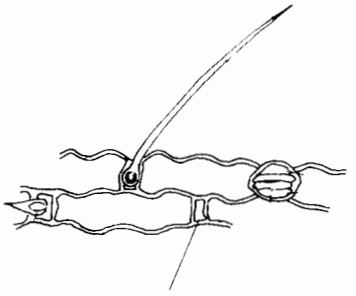


2b Epidermal cells rectangular (parallel-sided) . . . . . 6



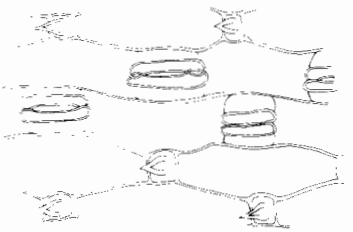


3a Silica bodies obviously present between epidermal cells . . . . . 4

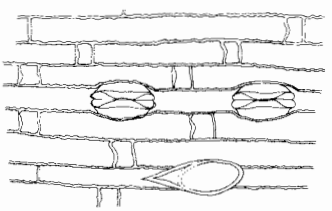


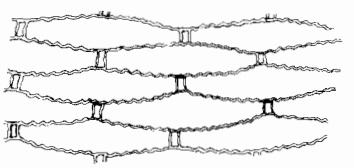

3b Silica bodies absent. Microhairs (with bulbous bases) and stomata are present in rows. Length:breadth ratio 7:1 . . . . . ***Dactylis glomerata***


4a Epidermal cell walls smooth and straight. Microhairs, if present, arising from silica bodies . . . . . 5

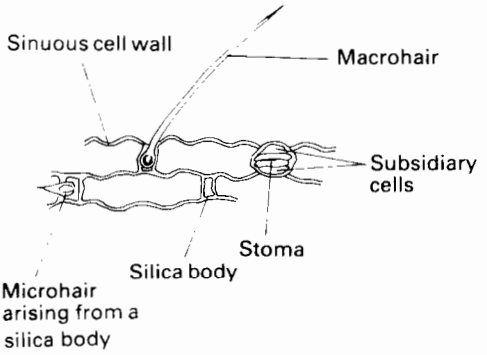


4b Epidermal cell walls sinuous. . . . . 8

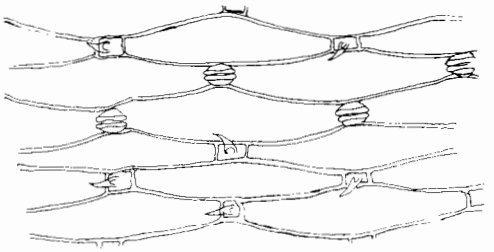




5a Length to breadth ratio of the epidermal cells = 7:1. Microhairs arising from rectangular silica bodies. Stomata in double rows. Stomata and silica bodies of comparable size. . . . . .

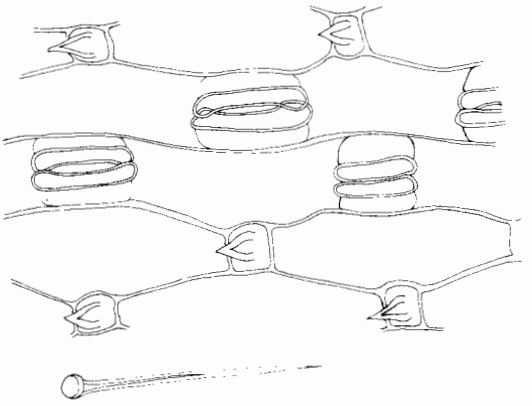



***Poa pratensis***



5b Length to breadth ratio of the epidermal cells = 4:1. Microhairs unicellular with bulbous bases arising from square-shaped silica bodies. Stomata placed close together in double rows. Stomata more than twice the size of silica bodies.

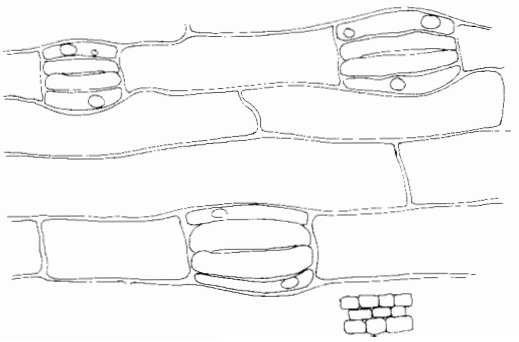
***Holcus lanatus***



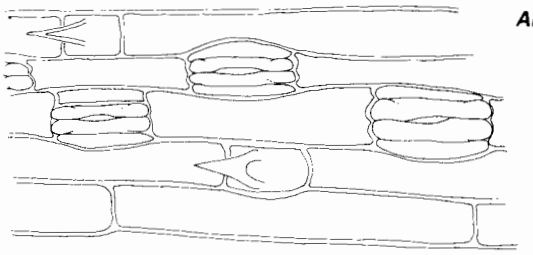
6a Silica bodies present . . . . . 7

6b Silica bodies absent. Epidermal cells rectangular, length:breadth ratio = 7:1. Subsidiary cells rectangular. . . . . .

***Luzula campestris***

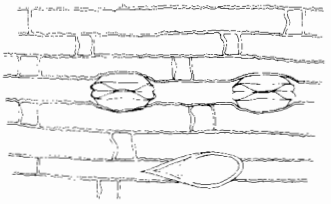


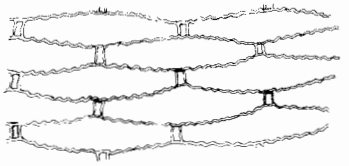
7a Epidermal cell walls smooth and straight. Epidermal cell length:breadth ratio = 15:1. Microhairs arising from oblong silica bodies. . . . . .



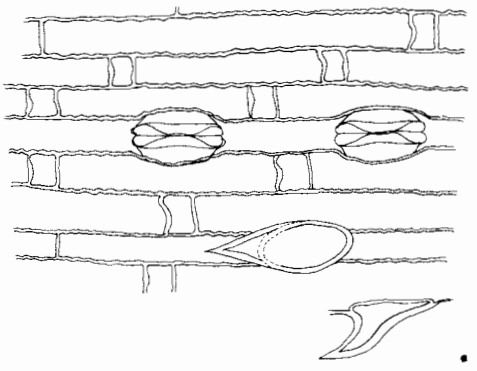
***Arrhenatherum elatius***

7b Epidermal cell walls sinuous. . . . . 8



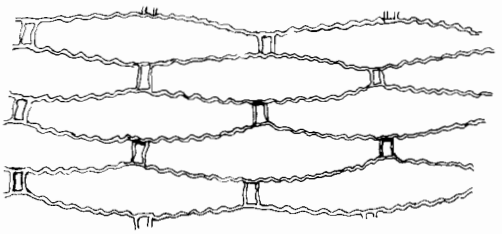


8a Epidermal cell walls moderately sinuous, the length:breadth ratio = 15:1. Stoma/subsidiary cells unit oblong in shape and broader than the epidermal cells. . . . . .



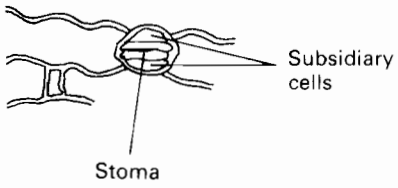
***Agrostis tenuis***

8b Epidermal cell walls extremely sinuous, the length:breadth ratio = 10:1. Stomata apparently absent. . . . . .



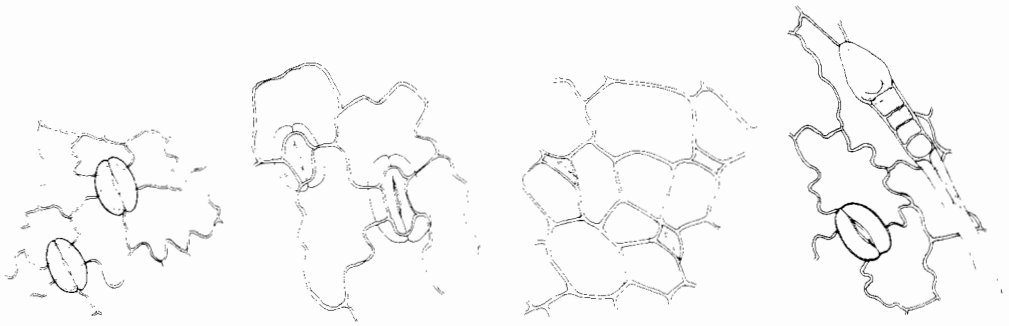
***Festuca rubra***

9a Stomata present . . . . . 10

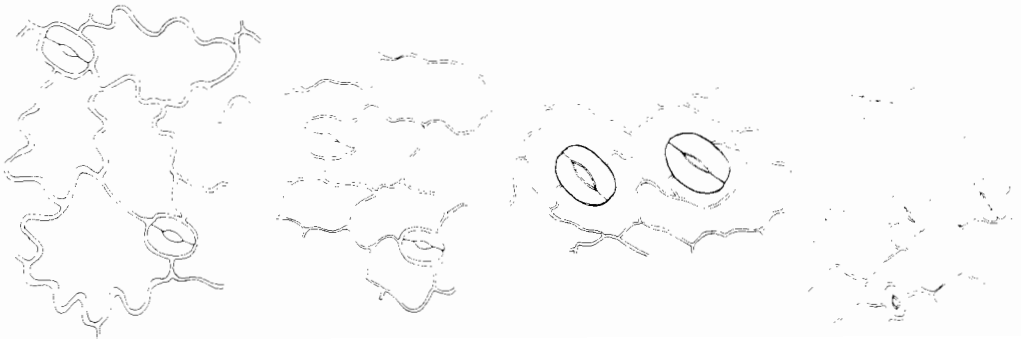



9b Stomata absent . . . . . 18

10a Four equal sized cells surround each stoma (amocytic stomatal pattern) . . 11

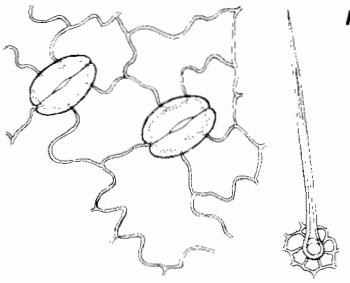


10b Not as above. Either two (diacytic) or three (anisocytic) cells surround the stoma. In the anisocytic pattern, one of the cells will be smaller than the other two . . . . . 16



11a Stomata sunken or flush with epidermal cells . . . . . 12

11b Stomata raised. Macrohairs, if present, unicellular . . . . . .



***Ranunculus repens***

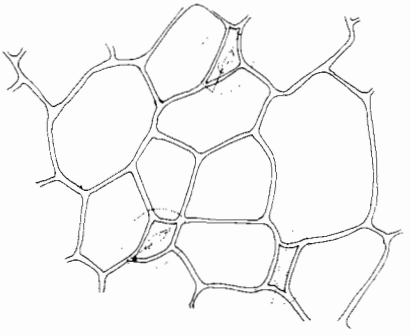
12a Stomata sunken . . . . . 13

12b Stomata flush with epidermal cells . . . . . 14

13a Cell walls sinuous. Macrohairs present or absent. If present, they are 4 or 5 celled, with the basal cell much smaller than the rest. . . . . .

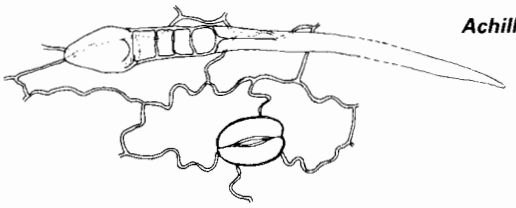
***Cerastium holosteoides***

13b Cell walls angular, hairs absent . . . . . .



***Trifolium repens***
(upper epidermis)

14a Cell walls very sinuous. Macrohairs usually present, very long, 4 or 5 celled with the distal cell extremely long . . . . . .



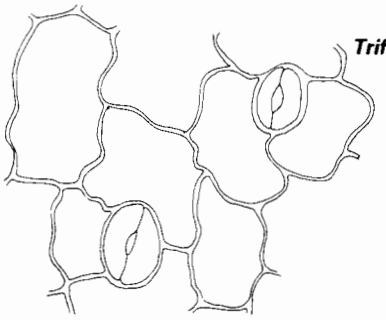
***Achillea millefolium***

14b Cell walls gently undulating . . . . . 15

15a Macrohairs present. Macrohairs composed of 4 or 5 equal-lengthed cells . .

***Veronica chamaedrys***

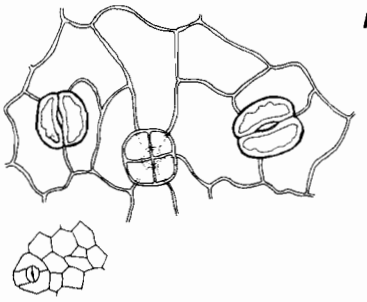
15b Hairs absent. Separation of hairless *Veronica chamaedrys* from clovers (*Trifolium* sp) is very difficult. . . . . .



***Trifolium repens*** (lower epidermis)

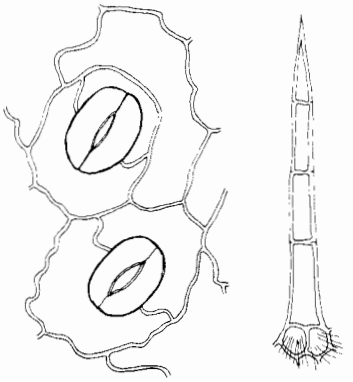
16a Two cells surround almost every stoma (diacytic pattern) . . . . . 17

16b Three cells (one smaller than the other two) surround the stoma. Stomata raised. 4-celled glands may be present . . . . . .



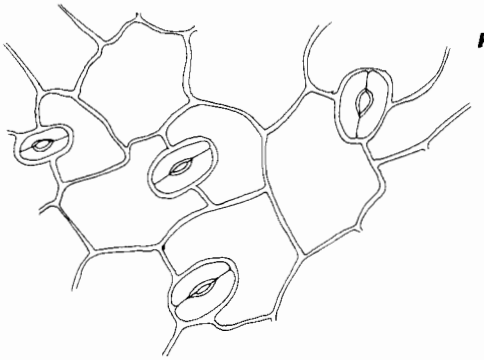
***Rumex acetosa***

17a Cell walls sinuous. Macrohairs present or absent. Macrohairs 4–5 celled with cells of equal length. . . . . .



***Glechoma hederacea***

17b Cell walls smooth and often angular. Macrohairs absent.. . . . . .

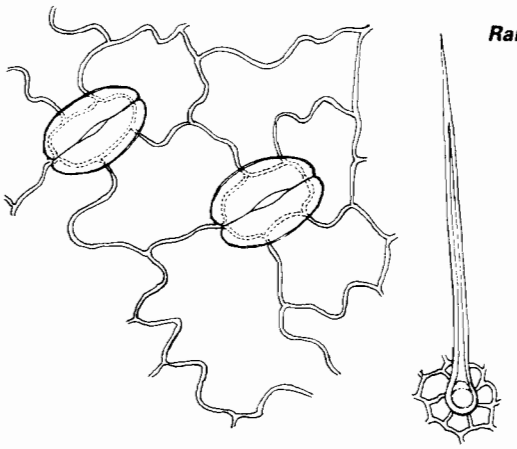


***Plantago lanceolata***

18a Macrohairs present . . . . . 19

18b Macrohairs absent . . . . . 22

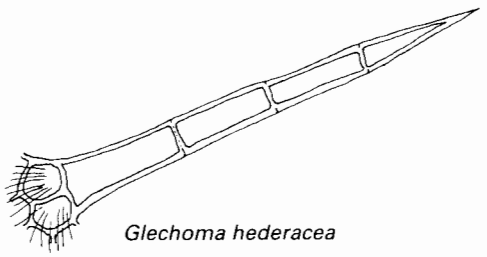
19a Macrohairs unicellular . . . . . .



***Ranunculus repens***

19b Macrohairs 4–5 cells long . . . . . 20

20a Macrohairs with approximately equal-lengthed cells . . . . . .

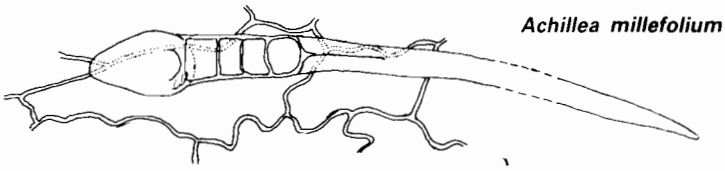


*Glechoma hederacea*

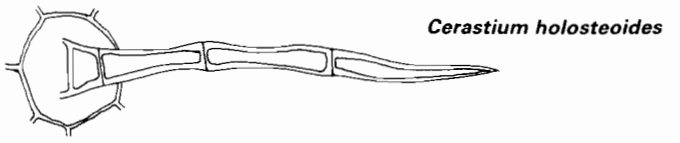
*Veronica chamaedrys*

20b Macrohairs with one cell much larger or much smaller than the others . . . 21

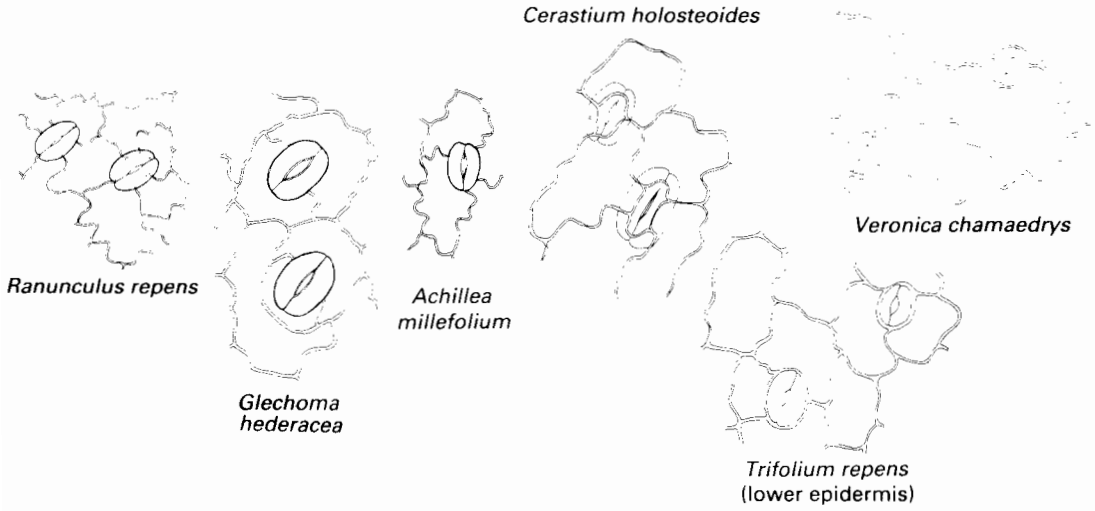
21a Apical cell much bigger than the others . . . . . .



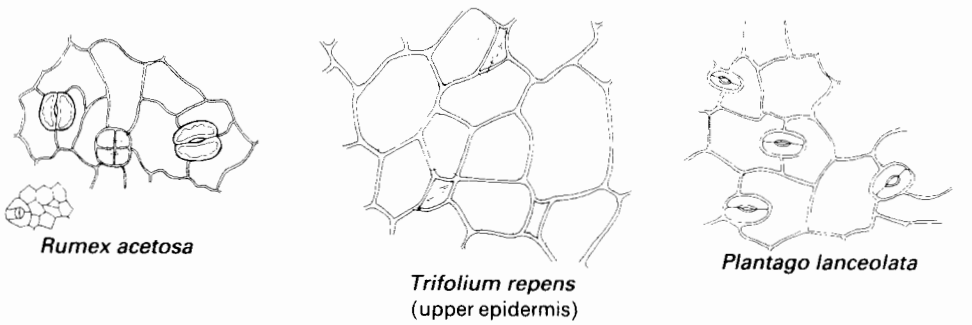

21b Basal cell much smaller than the others. . . . . .




22a Cell walls sinuous. . . . . .




22b Cell walls smooth . . . . . .



APPENDIX 2

## 1. **Faecal Analysis**

It is the uniqueness of the epidermal pattern of each plant species and the retention, after digestion, of the cuticle in faeces, which makes faecal analysis useful in both qualitative and quantitative determinations of the diet of herbivores. The method depends on making thorough observations of a reference (or type) collection of epidermises of the plant species found on each study area and using this information for identifying cuticles present in faecal samples. The collected faeces are subjected to a non-destructive separation of the cuticular fragments which are then mounted onto slides and examined under a microscope.

*Making a reference collection of epidermal types*

The leaf cuticle of potential food-plant species may be peeled off, scraped off with a blade or, in cases where the leaves are very small, gently macerated. The epidermises may then be mounted in glycerol jelly on microscope slides. For making permanent stained mounts, the process of dehydration in alcohol of increasing strengths, final replacement by xylol and mounting in Canada Balsam or Euparol is necessary. Staining in safranin, acid fuchsin and gentian violet is conducted at the 70% alcohol stage.

The leaf epidermis is best studied by making detailed drawings and/or taking photomicrographs, and noting down the size and shape of various epidermal characteristics, e.g. epidermal cells, cell walls, stomata, trichomes and silica bodies. The stomatal patterns (amocytic, anisocytic, diacytic etc.), density of stomata and length to breadth ratios of epidermal cells can often be useful. Where possible, both the abaxial and adaxial ("upper" and "lower") surfaces of leaves should be studied. The characteristics can subsequently be used to construct a dichotomous key to aid the identification of epidermal fragments in faeces (Appendix 1).

*Collection of faecal samples*

It is useful to know the time at which the faeces were deposited so that a comparison between the proportions of plant cuticles in faeces and the vegetation on offer at a particular time can be made. It is necessary, therefore, to clear plots of all previous faeces in advance, thus ensuring the subsequent collection of fresh faeces only. The size of the plots will depend upon the size and habits of the herbivore concerned, for example, $4 \times 4$ m square plots are quite adequate for rabbits but larger areas should be cleared for larger mammals. To reduce edge effects, and to ensure collection of only those faeces deposited since the plots were cleared, it is advisable not to collect faeces from within, say, a metre of the outer margin of the cleared plots. This method of collection is particularly relevant to periodic (weekly, monthly) collections for assessing the seasonal changes in the diet of the herbivore. Although less satisfactory, a knowledge of the colour changes associated with ageing and weathering of faeces can also be used for the estimation of their degree of freshness (or otherwise) and, in this respect, a Munsell soil colour chart is useful. Since areas are often inhabited by more than one species of herbivore, a knowledge of the characteristics of faeces of each species is essential for accurate identification.

The collected faeces are dried and stored in a dry place until required for analysis. Faeces can be kept for long periods in the dried state without deterioration. The faeces may also be stored in a formalin-acetic acid-alcohol mixture in the ratio 10:5:50:35 (of water) respectively.

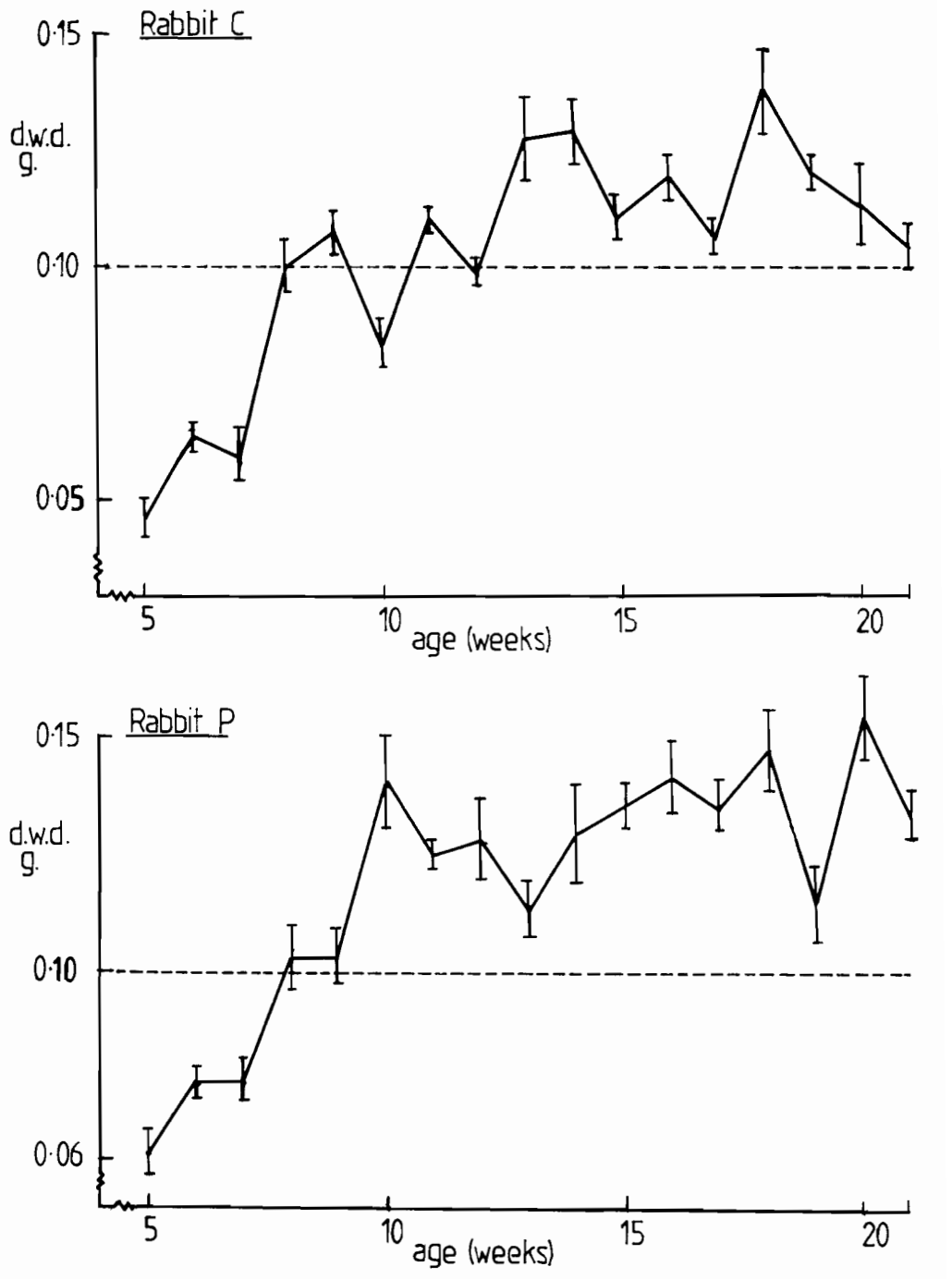



FIG. 2.

Relationship between dry weight per dropping (d.w.d) and age, for two domestic New Zealand White rabbits; 95% confidence limits are presented. The broken line shows the value used to separate droppings belonging to juvenile rabbits ($<0.10$ g) and adult rabbits ($>0.10$ g) (from Bhadresa 1982).

*Measurement of the size of faeces*

Differences in the size of faeces can provide a useful way of comparing the diets of juvenile and adult animals and changes in, for example, the monthly frequencies of different-sized droppings may reflect changes in the age structure of the herbivore population. Bhadresa (1982) ascertained the change in the size of droppings with age in the rabbit (see Fig. 2) and used the relationship to distinguish between rabbit droppings belonging to juvenile and adult rabbits.

An indication of size may be obtained either by weighing or estimating the volume of dried individual faeces. It is also useful to calculate the weight to volume ratio (or density). Bhadresa (1982), for instance, has found that in the european rabbit, a fibrous and/or a monocotyledonous diet produces less dense faecal pellets while a dicotyledonous diet produces dense ones. The density of faeces thus has the potential of providing a useful insight into the dietary habits of herbivores.

*Preparation of faeces for analysis*

Various techniques have been used in the preparation of epidermal fragments in faecal samples, the simplest being that described by Croker (1959). He dispersed the faeces in water. Zyznar and Urness (1969) recommend soaking in 10% sodium hydroxide to remove the mucous coating to aid dispersal of fragments. The method developed by Williams (1969) includes dispersing the faecal samples in 70% alcohol to extract the chlorophyll before placing them in boiling water and sodium hypochlorite. Stewart (1967), on the other hand, used nitric acid and potassium hydroxide for dispersing the fragments. The method described here is a modification of Stewart's and has been used extensively by the author for rabbit faeces. It applies to small (*c.* 0.1 g each) droppings. The amounts of reagents used should be proportionately altered for larger or smaller faeces. It is more practical to use smaller subsamples for very large faeces.

The dried faecal sample is inserted into a 50 ml conical flask and soaked in 2 ml distilled water, followed after 5 minutes by 2 ml concentrated nitric acid. The acid loosens the faecal sample by dissolving away the mucous. The flask is warmed in a water bath set at 50–60°C for 5 minutes and dispersal of fragments is achieved by gentle teasing with a glass rod; 10 ml of potassium hydroxide are then added to neutralise the acid and stop further reaction. Using the wide end of a Pasteur pipette, a 1 ml sample is removed while the flask is being shaken (using a flask shaker, if available) so that the subsample is representative of the entire faecal sample. The subsample is placed in a crucible and, after a settling period of about 5 minutes, the supernatant is removed using a Pasteur pipette. Distilled water (2–3 ml) is then added to wash the fragments; 2–3 drops are left with the sample while the rest is pipetted off. Using a spatula, the fragments are transferred onto a labelled microscope slide and allowed to dry. The drying might be assisted by the use of a slide-drying oven or plate. After the fragments have dried, they are mounted in glycerol jelly. If the density of the fragments on the slide is high and there is much overlapping of fragments, it is necessary to dilute the sample and remove a subsample for analysis.

*Identification of the fragments in the faecal subsamples*

Identification of cuticular fragments in faeces is conducted with the aid of a key to the reference collection, illustrations and photomicrographs. In most circumstances, × 100 magnification is adequate for this purpose but for critical assessments, a higher magnification is sometimes necessary. The majority of fragments can be identified down to the species level. Even when this is not possible, fragments may be classified as belonging to a

dicotyledonous or monocotyledonous species respectively on the basis of the epidermal pattern of random arrangement of isodiametric epidermal cells or long, narrow cells placed parallel to each other. Similar criteria—namely, small cells with thick, smooth, straight cell walls—can be used to group the mosses. The faecal sample may yet contain fragments that cannot be put into any category, including those that are either too deteriorated to show any cuticular pattern or those that are hardly digested, thus making them opaque. They also include fragments of fibrous and vascular plant tissues. This disadvantage, however, cannot be overcome and it must be assumed that the unidentifiable fragments represent similar proportions of species in the identifiable portion of the sample.

*Quantification of the fragments in the subsamples*

Four methods are available for quantifying proportions of different cuticles in faeces (see Bhadresa 1981):

(a) Counting the number of fragments of different species in the entire subsample.
(b) Frequency counts in microscope fields, at a particular magnification.
(c) Direct estimation of surface areas of fragments.
(d) Estimation of proportions using the point quadrat principle.

There is usually a great variation in the size of fragments in the faecal samples and for this reason, numerical counts of fragments would be biased towards the species which break down into smaller fragments. Frequency counts also suffer from similar considerations but at the right magnification, they do indicate relative importance of different cuticles in the faeces. Direct estimation of surface areas of fragments is tedious and time-consuming (and somewhat impractical) since fragments are variously shaped. Estimation of cover of fragments using the point quadrat principle is considered the best method for assessing proportions of different cuticles in faeces. The method bears close similarities to point quadrats employed in vegetation analysis (Chalmers & Parker, 1986); however, because of the two-dimensional, static nature of the material for examination, faecal analysis using point quadrats is less tedious and more accurate than analysing vegetation in the same way.

The point 'frame' is placed in the eyepiece and may take the form of a micrometer/crosswires or one made by marking a round coverslip with a grid of equidistant dots. Bhadresa (1982), for example, used one with nine dots placed 5 mm apart in a square grid. The size of the dots (0.5 mm) correlated with the size of the smallest identifiable fragment at $\times 100$ magnification. When recording point 'hits', the points (dots) are orientated parallel to the sides of the microscope slide. Hits on cuticles belonging to different species are recorded in straight line transects across the slide or by random location of microscopic fields. To reduce edge effects, a margin of $> 2$ mm of the coverslip is avoided when recording and the distance between microscopic fields in the case of straight lined transects, should be large enough to ensure that there is no overlap between consecutive microscopic fields.

Tests should be carried out to determine the minimum number of hits required for estimating percentage cover of different cuticles; the number of hits required depending on the diversity of species in the sample (Chalmers & Parker, 1986). Running percentage means are recorded at, for example, 10-hits intervals (for a number of samples) until the values of the major components become stable. The percentage means of species with sporadic occurences may continue to vary, however, so a compromise is needed when deciding upon the number of points required, so that estimates of the percentage cover of different cuticles give an acceptable representation of the rarer components and a good representation of the commoner ones.

The methods outlined above should enable accurate estimates to be made of the proportions of cuticles in faeces but these may not necessarily represent the actual proportions of food species consumed. The amount of cuticle of any particular species in the faeces depends on two major factors: (i) the surface area of epidermis per unit weight of species and (ii) the amount of cuticular material surviving digestion. In order to overcome these disadvantages, it is necessary to conduct controlled feeding trials on the herbivore concerned to ascertain the relationship between the amounts of food plants consumed to proportions of cuticles of these plants in the faeces. For further details refer to Bhadresa (1986).

## 2. **Exclosure studies**

The main effect of grazing on the vegetation is to change the course of succession. Directly, it can lead to an increase in 'rejected' species and, by keeping the vegetation down, favour the spread of hemicryptophytes. Conversely, grazing can increase the competitive abilities of selected species (e.g. increased shoot and root growth) to the detriment of unselected ones. Vegetation may also be affected by trampling and faecal deposition. The sum effect of these various factors on the plant community can be more readily understood by erecting exclosures designed to keep grazing animals out.

### *Siting of exclosures*

Exclosures should be erected in fairly homogeneous areas in terms of the vegetation, soil characteristics and topography. This is to ensure that future differences in the vegetation between grazed and ungrazed areas are mainly attributable to grazing. Similar-sized grazed plots may be marked out at the same time for comparative studies.

### *Design of exclosures*

The size of the exclosures and the type of fencing used will depend on the size of the herbivore. Thus, for large mammals (e.g. deer, sheep, cows) barbed wire fences would be adequate, and the size of exclosures can vary in the range of 10–100 × 10–100 m squares or rectangles. Stakes for securing the wire should be erected at 5 m intervals.

For smaller mammals, e.g. rabbits, exclosures need not be too large and 5 × 5 m plots are adequate. The type of fencing used is, however, very important and wire-netting with a mesh size of 3–4 cm for rabbits and 0.5 cm for voles and mice, is recommended. Many small mammals are habitual burrowers and therefore the wire-netting should be buried in the soil, with the lower edge bent outwards.

### *Vegetation recording*

Vegetation recording should be carried out at regular intervals and at similar times for assessing long-term (e.g. seasonal, annual) changes. Various methods (see Chalmers & Parker 1986, Goldsmith, Harrison & Morton 1986) may be used to record the vegetation. Often, a combination of methods may be necessary. To avoid the problems of edge effects, e.g. from rain splash, bird droppings, recording from within a small margin immediately inside and next to the fencing of the exclosures should be avoided. Various methods are listed below. It is essential that quadrat sizes be accurately determined before doing any recording.

(a) A species list.

This is very useful since other sampling methods may fail to record uncommon species.

(b) i. Percentage cover of species.

This may be determined by locating quadrats on a grid, selecting sites in a regular or random manner. In man-managed habitats it is usually preferable to use a random distribution, and many statistical tests for analysis of the results require this (see Chalmers & Parker, 1986).

ii. Cover repetition

For this, a variant of the point quadrat that allows a three-dimensional measure of the vegetation cover may be contrived. This is achieved by marking the pins with vertical (height) intervals. The pins are lowered down to the soil level and vegetational contacts at different heights are recorded. The method allows a measure of both cover and vertical spread. The latter is a useful measure and when used separately will identify changes in the canopy due to grazing. The vertical measures can also be used together with the cover of species in estimating the biomass of different species (see (d) below).

iii. Cover map charts

These are very appropriate for very long-term studies. Small plots are permanently pegged-out within and outside the exclosures and the vegetation detail mapped out on graph paper. A gridded quadrat is used for the purpose. These are ideal for showing sequential changes in the cover of different species (Fig. 3).

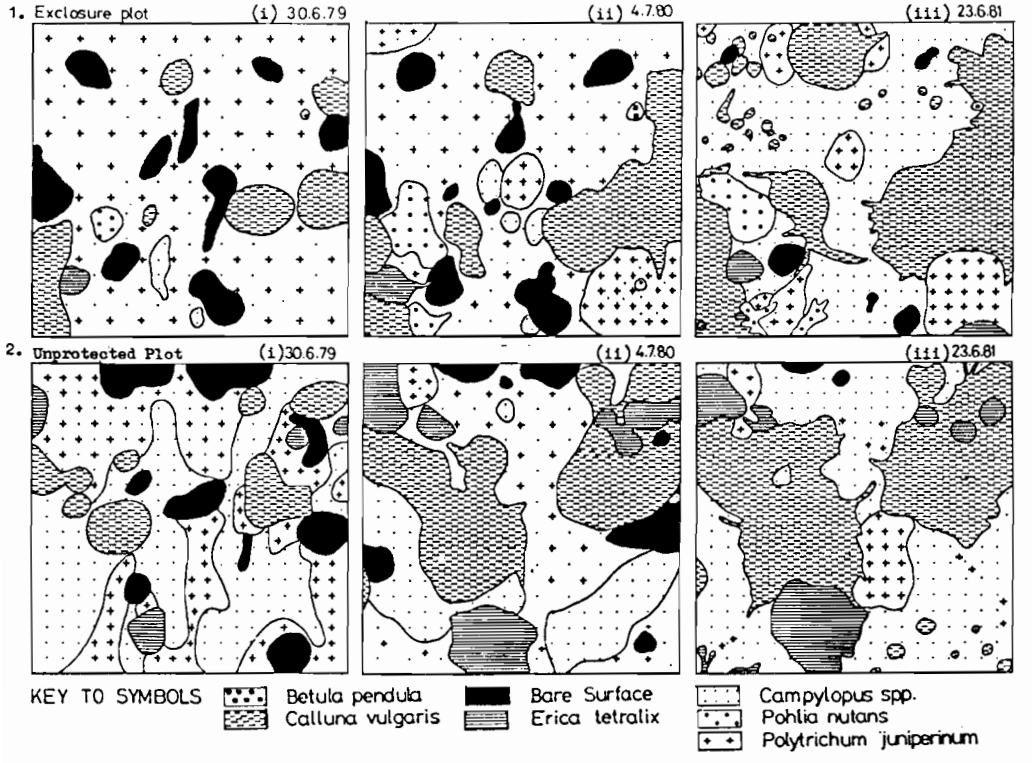



FIG. 3.
Cover map charts showing changes in cover in 1 × 1 m permanent plots within and outside exclosures (from Bhadresa 1982).

(c) Percentage frequency of species

This is determined by recording the presence or absence of species in a number of quadrats placed either randomly or regularly. This method is by far the most rapid if a great deal of sampling is required.

(d) Biomass

Unless exclosures are very large and a certain amount of destructive sampling is permissible, biomass has to be determined indirectly. This can be determined by harvesting vegetation from small plots outside the exclosure after first recording the cover (surface area of ground covered) of the different species in individual plots and using marked point quadrat pins (as in (b) ii) to determine the mean height for the different species. The vegetation is subsequently separated into species, dried and weighed. These weights may now be related to a product of cover (surface area) and mean height—effectively the volume—of the various species. This should be done for a number of plots for accurate volume to biomass conversions for the different species. These conversion factors may now be used to convert similar 'volume' measures for different species in the exclosures as well as in grazed areas. Differences in biomass could be directly related to grazing pressures.

The various comparisons between the grazed and ungrazed areas should provide very useful information, both qualitative and quantitative, on the effects of grazing on the plant community.

In conclusion, although the study of the diet of grazing animals can provide a valuable insight into the existing pattern in grazed vegetation, it is unable to predict the outcome of vegetation changes if grazing were to cease. Thus, whenever possible, exclosure studies, should be performed concurrently with studies on the dietary habits of grazing animals.

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