

## Rhizome Growth and Clone Development in *Anemone nemorosa* L.

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### ABSTRACT

Studies of natural populations indicate that clones grow radially, soon developing a hollow centre which may be invaded by new genotypes.

In the populations studied, individual rhizomes elongate by an average of 2.5 cm per year. Growth is sympodial, the terminal bud forming an aerial shoot and further elongation occurring from an axillary bud.

The oldest rhizome segment found was 7 years with most rhizomes being 3 or 4 years old. Because there is no apparent senescence, individual clones may persist for very long periods, possibly hundreds of years.

A computer model of rhizome growth was used to postulate the extent of intermixing of old clones. It indicated that old clones become very fragmentary and totally inmixed with their neighbours, one 'clump' often representing several clones.

To supplement the model, plants from a woodland with an unbroken carpet of *Anemone nemorosa* were collected, 27 morphological characters measured and the data analysed using principal components analysis and cluster analysis. These supported the hypothesis that old clones are large and fragmentary with complete intermixing.

The possible role of soil factors in influencing rhizome extension is discussed and the strategy of *A. nemorosa* is compared with those of its close associates in the pre-vernal woodland herb layer.

Key words: *Anemone nemorosa* L., wood anemone, rhizome growth, clone development, computer model, vegetative reproduction.

### INTRODUCTION

Although *Anemone nemorosa* L. is a common member of the pre-vernal ground flora in British deciduous woodlands, the growth of the rhizome and the development of clones have not previously been studied in depth. In this study rhizome growth was investigated by means of soil excavations, and clone development was determined by observation and measurement of distinct clones. In mature woodland *A. nemorosa* tends to grow in an unbroken carpet in which it is almost impossible to identify individual clones by eye and therefore other methods were necessary to map the clones. A computer model of rhizome growth was useful in indicating not only the extent and spatial pattern of older clones, but also the intermingling of different clones. This was supplemented by morphological analyses of a mature population of *A. nemorosa*.

### MATERIALS AND METHODS

#### *General morphology and clone development*

The annual increment was determined by excavating a 0.25 m<sup>2</sup> area of ground at Tilbouries Wood near Aberdeen (G.R. NO/830983) in late July of 1978, collecting all

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the rhizomes and measuring the new growth. The new portions of rhizome were distinguished by their swollen bases and lighter colour. Late July was chosen for the excavation as the rhizomes have completed their elongation by this time. The total length of rhizomes collected from the excavated area was also measured. In late July, 1979, a fresh area of 0.25 m<sup>2</sup> was excavated at the same wood.

At Tilbouries an identifiable clone was excavated to determine the age structure of rhizomes within a clone and a similar excavation was also carried out on a clone at Carnoustie (G.R. NO/563368).

The size of isolated clones from various British sites was measured to give an indication of clone size and these clones were also mapped to determine the pattern of clone development.

### *Rhizome modelling*

As stated in the introduction, in most populations of *A. nemorosa* the clones merge to form a carpet in which it is difficult to determine the full extent of individual clones and the degree of intermixing. Rhizome modelling presented a method for investigating possible patterns of clone growth and postulating merging in mixed clones which might be verified by other means. The programme used to create the model, RHIZOM, has already been described by Bell, Roberts & Smith (1979).

Details of rhizome growth and pattern were obtained from excavations at several sites in Tilbouries Wood. The following parameters were measured: (1) percentage of annual increments where one bud extends; (2) percentage of annual increments where two buds extend, giving rise to a forked rhizome, and the angle of the branches measured as the deviation from the straight ahead line; (3) percentage of annual increments where three buds extend, and the angle of deviation of these branches from the straight ahead; (4) percentage of cases where the terminal bud and its associated buds are destroyed, allowing a lateral bud from further down the most recent annual increment to develop and continue the rhizome. The angle at which it extends was also noted.

The rhizome durability (maximum age) and the average annual increment were also determined.

The programme uses these probabilities and angles to build up a simulated rhizome system by the progressive yearly accumulation of shoot units. The following simulations were performed: (1) growth of one propagule after 20 years; (2) growth of one propagule after 66 years; (3) growth of two starter propagules after 66 years, initially 60 cm apart and heading towards each other; (4) growth of three starter propagules after 66 years, initially 60 cm apart and heading towards each other.

### *Determining clone mixing using morphological variation*

Two methods were used to try to identify the extent of clone mixing and the size of individual clones in the unbroken carpet of *A. nemorosa* in Tilbouries. In the first of these, 289 plants were collected on a 17 × 17 grid of 30 cm squares, each plant being labelled with its location before pressing. Once pressed, the plants were measured for 27 morphological characters, both floral and vegetative, and the data analysed using principal components analysis (PCA) of all and selected characters.

The plants in the grid were 30 cm apart and a second analysis was performed to ensure that this collecting distance was not too large for clone identification. In this, all the flowers within a 1 m<sup>2</sup> quadrat were mapped before collection. After pressing, the same 27 characters used in the grid analysis were measured and the data analysed using PCA and cluster analysis, with the aim of identifying clones which could then be transferred to the map.

## RESULTS

*General morphology and clone development*

*Anemone nemorosa* spreads vegetatively by means of thin, brittle, brown rhizomes approximately 0.5 cm in diameter. These elongate each year between April and July after flowering.

The excavations at Tilbouries Wood showed that the rhizomes tend to grow in three layers—one on the soil surface covered only by moss and leaf litter, one layer at 1.5 cm depth and a third at 4–5 cm depth. In 1978 the total length of rhizome collected from 0.25 m<sup>2</sup> was 13.71 m, indicating how well *A. nemorosa* exploits its habitat. The following year a total length of 12.1 m was collected from a fresh 0.25 m<sup>2</sup> excavation. At this wood, *A. nemorosa* grows as in a typical coppiced woodland and there is no reason to think that these figures are higher than would be found in other similar sites. Indeed they fit very well with the figure of 48.76 m in 1 m<sup>2</sup> obtained by Tumidajowicz (1975) in Southern Poland.

The average length of new growth at Tilbouries was 2.53 cm in 1978 and 2.50 cm in 1979, although some rhizomes elongated by 4.80 cm in 1 year. A histogram of percentage frequency of annual increment is given in Fig. 1.

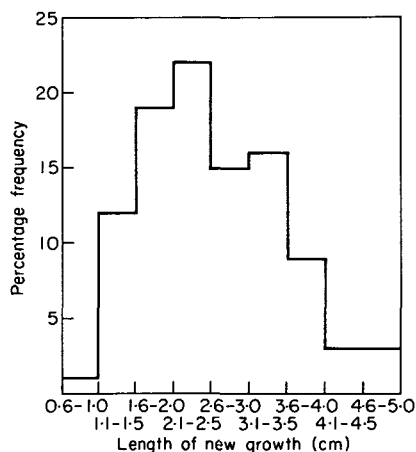


FIG. 1.

FIG. 1. A histogram showing the annual extension of the rhizome of *Anemone nemorosa* at Tilbouries Wood in 1978.

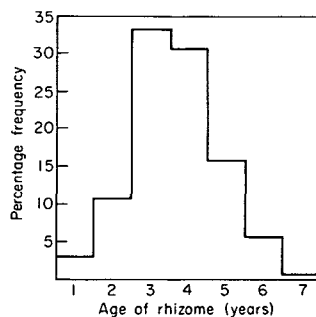


FIG. 2.

FIG. 2. A histogram of the age structure of *Anemone nemorosa* rhizomes at Tilbouries Wood.

The discrete clone excavated at Tilbouries Wood had a maximum rhizome age of 7 years, with most rhizomes being 3 or 4 years old (Fig. 2). At Carnoustie the maximum age obtained was 6 years. However, it is very difficult to excavate the rhizomes intact and this may have affected the result. Tumidajowicz (1975) thought the rhizomes had an average turnover of 3–4 years, with 20 per cent of the rhizomes being 6–9 years old. At Carnoustie the degree of rhizome branching varied greatly, from highly branched to unbranched, even within this single clone.

The buds on the rhizome are of two kinds, leaf buds and flower buds. Most flower buds occur terminally on the new growth but lateral flower buds may develop on older portions from the axils of scale leaves, which are spirally arranged along the rhizome.

The rhizome grows sympodially, the terminal bud becoming a shoot and growth continuing from an axillary bud. Older rhizome portions decay from the inside, leaving the brown skin which eventually disintegrates. Decay may also separate portions of healthy rhizomes. If a budless portion is separated it usually decays, although occasionally a new bud may originate from the axil of a scale leaf.

Initially rhizomes grow radially outward, forming a circular clone. As the older rhizomes decay, the clone becomes hollow-centred with most plants concentrated within 30 cm of the advancing edge. In normal populations this hollow could be filled by new propagules: either achenes or animal-dispersed rhizome portions. In woodland *A. nemorosa* tends to form unbroken carpets, the clones merging into each other.

Of the discrete clones measured to give an indication of clone size, the largest was  $180.0 \times 117.5$  cm, but this must be regarded as a minimum size because the size of old clones is immeasurable due to the difficulty of separating intermingled clones. As the rhizome is constantly renewing itself at the growing end and is only a maximum of 7 years old, there is no senescence, and there seems no reason why a clone could not survive for hundreds of years, a conclusion also reached by Harper and White (1974). There may thus be *Anemone* clones comparable to the huge fungal 'fairy rings' visible in aerial photographs of Salisbury Plain (Ramsbottom, 1953).

### Rhizome modelling

For the purpose of the model, the rhizome system of *A. nemorosa* can be described as follows:

The terminal bud becomes the flowering shoot and further growth can take place by: (1) a sympodial bud extending, continuing the rhizome in a straight line. This occurs in 77.3 per cent of annual increments; (2) two sympodial buds extending, giving rise to a forked rhizome. This occurs in 17.9 per cent of increments, with the branches being  $39.3^\circ$  to right and left of the straight ahead line; (3) three buds extending. This is a very rare occurrence and was ignored for the purpose of modelling; (4) a lateral bud from further down the rhizome can grow out at  $28^\circ$  if the terminal bud and its associated buds are destroyed. This occurs in 4.8 per cent of increments. Four evenly spaced buds per annual increment were included all with an equal chance of being the one to develop if the terminal bud was destroyed.

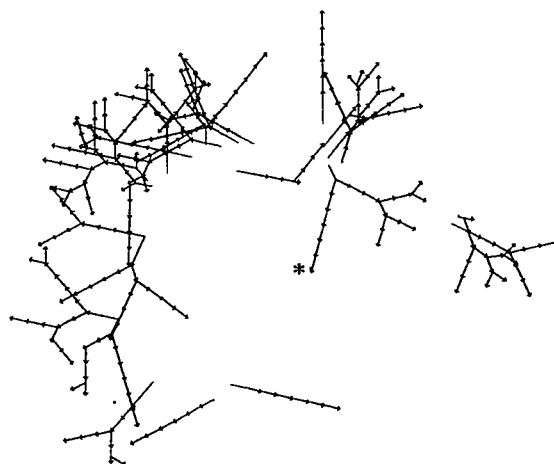


FIG. 3. A computer simulation of a clone of *Anemone nemorosa* after 20 years. The starting position is marked by \*.

The assumption was made that the rhizome durability is 6 years and the average annual increment is 2.5 cm. The computer plot of one propagule after 20 years growth is given in Fig. 3. The starting point is represented by \* in the centre of the plot. The distance between arrowheads represents 1 year's growth, each arrowhead representing a potential aerial shoot. The oldest increments are 'dead' and about to 'rot'. This clone has a maximum diameter of 83 cm. The clone is hollow-centred and the radial advancing edge is incomplete with several gaps. A second model of this data showed a similar pattern of growth. This is in complete accordance with identifiable clones observed in the field. This indicates that the model is a realistic picture of rhizome growth; thus further simulations may also be regarded as accurate representations.

Figure 4 illustrates a model rhizome system after 66 years. The clone is now breaking

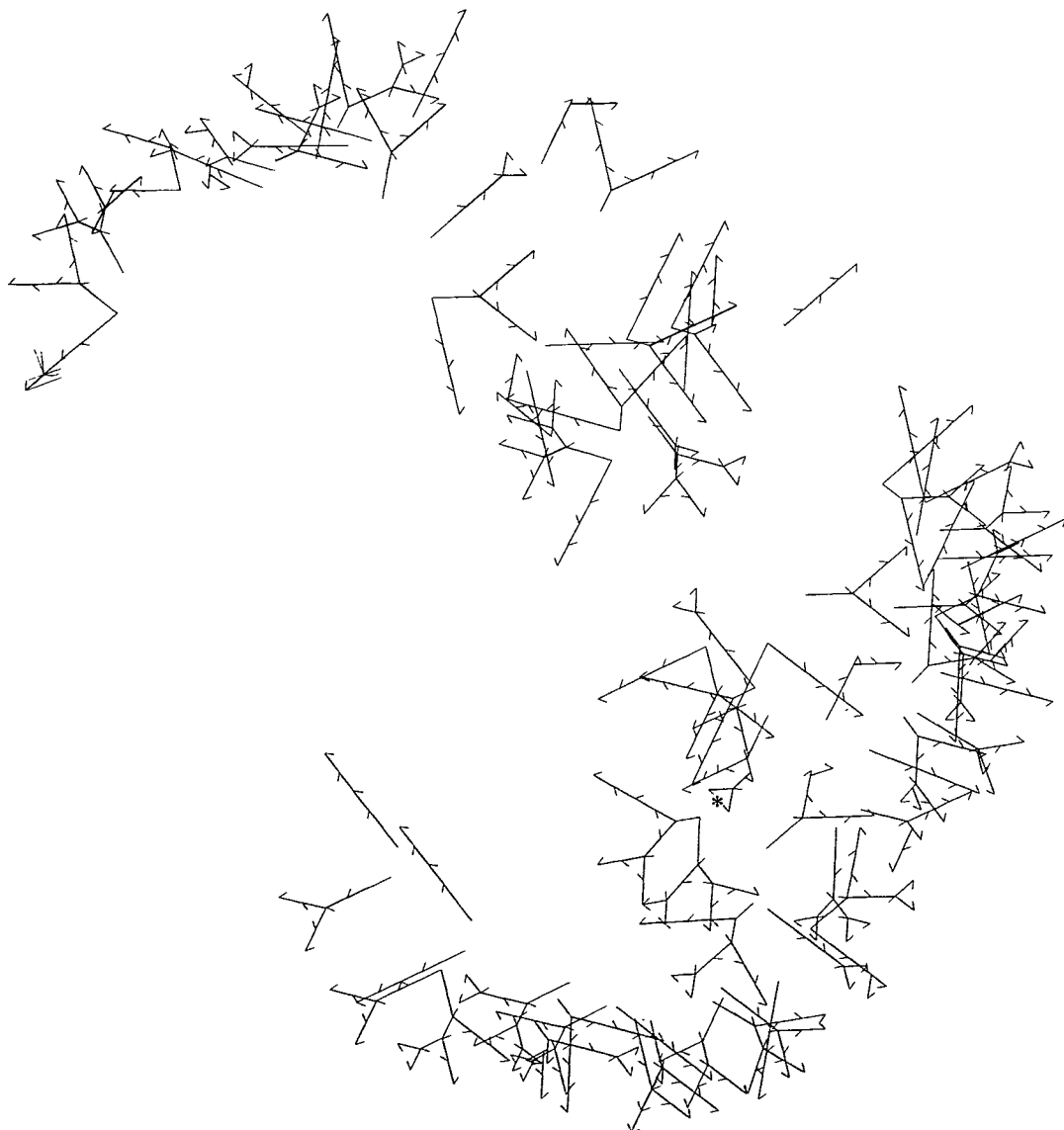


FIG. 4. A computer simulation of a clone of *Anemone nemorosa* after 66 years. \* As in Fig. 3.

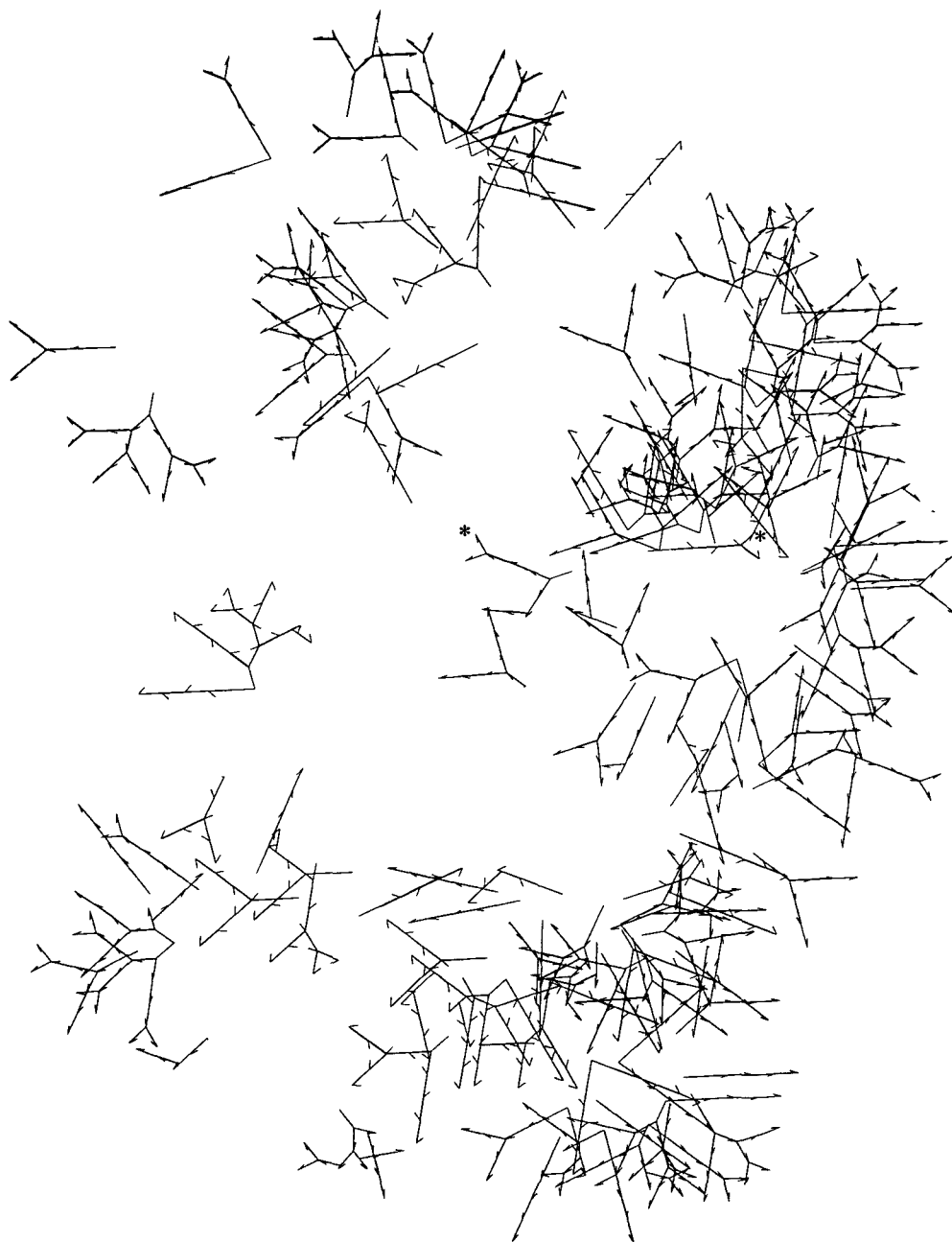


FIG. 5. A computer simulation of the intermingling of two clones of *Anemone nemorosa*, initially 60 cm apart and heading together, after 66 years. \* As in Fig. 3. †, Clone A; ‡, clone B.

into several distinct areas, each area containing mixed directions of growth and in places the clone is growing back in on itself. This lack of direction had previously been observed in natural populations. The total diameter of the clone is now 127 cm. Unfortunately the computer cannot deal with further years, as it would have been interesting to see how fragmented the clone became after 100 years or longer. Many natural clones of *A. nemorosa* are probably at least that old and perhaps much older.

The growth and mixing of two separate starter propagules after 66 years are shown in Fig. 5. The two propagules, which may be distinguished by the different angles of the arrowheads, were initially 60 cm apart and heading towards each other. The simulation shows a high degree of mixing, with some distinct clumps containing both genotypes and in nature it would be very difficult to separate these genotypes on a purely spatial basis.

When three starters are used, heading towards each other, the resultant simulation after 66 years (Fig. 6) shows a densely covered area with few gaps and no distinct clumps. The three clones are totally mixed. Clone C is an example of a clone which, by chance, branched repeatedly in its early years and is now overpreponderant in this simulation.

Thus simulations of rhizome growth indicate that after only 66 years it is possible for clones to be totally mixed with distinct clumps representing several clones. (Of course, the time which elapsed before the clones met would depend on the initial distance apart.)

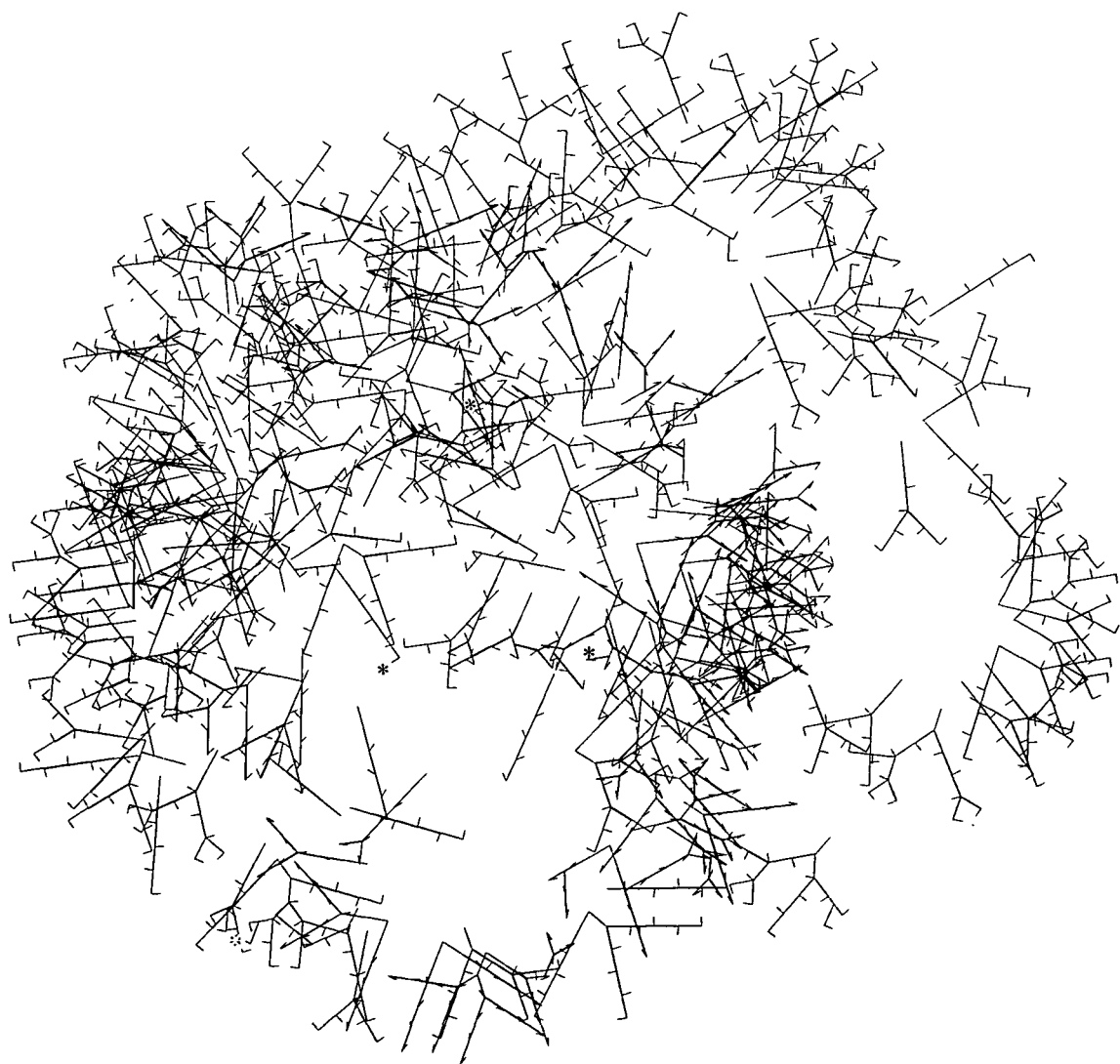


FIG. 6. A computer simulation of the intermingling of three clones of *Anemone nemorosa*, initially growing together, after 66 years. \* As in Fig. 3. †, Clone A; ‡, clone B; ¶, clone C.



As Tilbouries Wood has probably been in existence for several hundred years, we could expect far more mixing and fragmentation of clones than demonstrated by a model representing only 66 years growth.

#### *Determining clone mixing using morphological variation*

In the analysis of the plants collected from the grid, neither the PCA of all the characters measured nor the one using only selected characters placed the plants into discrete groups which could be identified as clones. Adjacent plants were often widely separated by the PCAs as illustrated by Fig. 7. Conversely, plants grouped as morphologically close by the PCAs were often from distant points on the grid.

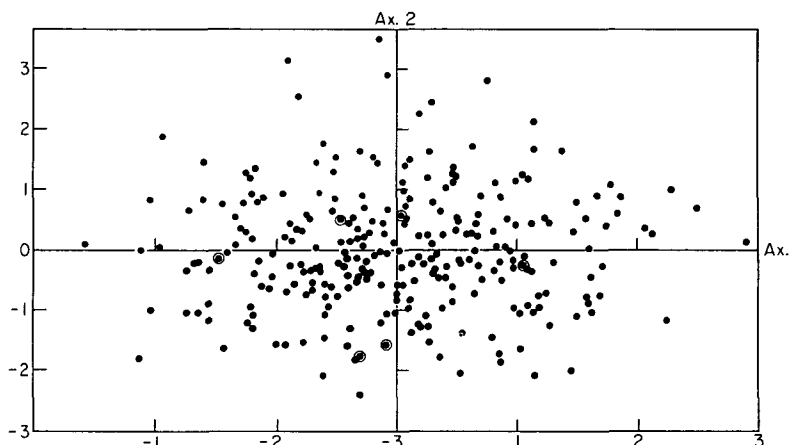


FIG. 7. A principal components analysis of the individual plants of the 17 × 17 grid collected at Tilbouries Wood, based on 29 morphological characters. The circled points show a typical example of the morphological variation between adjacent plants in the grid.

The conclusion drawn from this was that the grid collection was composed of several totally mixed clones. If morphological closeness is a measure of genetic similarity, some clones in the grid are very fragmentary and over 5 m across.

Principal components analysis and cluster analysis also failed to recognize discrete groups when the plants collected from within a 1 m<sup>2</sup> quadrat were analysed. The high variability of the plants in this small area indicates that several clones are present and the analyses reveal that these clones are fragmentary and totally mixed.

A similar PCA of six distinct clones at another wood (Kincardine O'Neil; G.R. NO/605990) was successful in grouping the clones, indicating that the analysis is competent at distinguishing between different clones, and its failure to separate clones at Tilbouries Wood is probably due to the higher number of clones involved and therefore more overlap.

Both these analyses at Tilbouries Wood indicate that the clones in a mature population are very fragmentary, cover extensive areas and are completely mixed with other clones. This is in agreement with the computer model of the rhizome system.

#### DISCUSSION

In *A. nemorosa*, the annual rhizome extension of 2.5 cm is sufficient to allow the species to colonize extensive areas fairly rapidly, whilst still forming a clone which is dense enough to discourage invasion by competitors. As previously mentioned, Tilbouries Wood is a



typical coppiced wood and the observed rate of extension is probably also representative of other similar locations. Environmental factors, however, would obviously affect extension rate and this may account for the lower figure of 1.25 cm per year quoted by Knight (1963).

Shirreffs (1981) found that the cover value of *A. nemorosa* was less when growing on soils with a high bulk density. The increased resistance the growing point meets when pushing through denser soils may result in a smaller annual extension. Prime (1960) thought soil factors affected the movement of *Arum maculatum* tubers through the soil, and noted that *Arum* usually failed to grow in heavy clay soils perhaps due to the difficulty of the growing points forcing their way through the soil. Salisbury (1942) compared the annual increment of underground organs in heavy and light soils and noted a considerable difference. Thus it seems likely that soil factors would affect the annual growth of *A. nemorosa* rhizomes; this would be an interesting topic for further investigation.

There was no significant difference between the annual increments of the young clone at Carnoustie and the old one at Tilbouries. This is to be expected as, although the Tilbouries clone is old, the maximum age of the individual rhizomes is only 7 years and there is no apparent senescence.

How does the annual growth of *A. nemorosa* compare with that of its close associates in the pre-vernal woodland herb layer? *Arum maculatum* tubers move an average of 1 cm per year when mature (Prime, 1960). However many rhizomatous species elongate more rapidly than *A. nemorosa*. Watt (1940) reports that bracken (*Pteridium aquilinum*) can move up to 112 cm per year in Cambridgeshire, quoting even higher figures from the Continent, and Salisbury (1976) states that *Oxalis acetosella* extends by 10 cm per year. *Mercurialis perennis* rhizomes cover 10–15 cm per year and, exceptionally, 40 cm per year (Mukerji, 1936).

Thus the annual extension of *A. nemorosa*, while greater than that of tuberous or bulbous species, is small compared to its rhizomatous associates. However, although small, it is still sufficient to allow *A. nemorosa* to form a carpet if the conditions are suitable.

The rhizome modelling indicated that a mature population of *A. nemorosa* consists of many large, very fragmented clones totally intermixed, a hypothesis supported by the morphological analyses of such a population. In this respect *A. nemorosa* is intermediate between the bulbous populations of *Narcissus pseudonarcissus* (Barkham and Hance, 1982) and *Endymion non-scriptus* (Wilson, 1959), in which there are many clones each with a relatively small number of individuals, and rhizomatous populations of *M. perennis* (Hutchings, 1975) with few clones each with many members. This continuum of strategies is probably a direct result of the capacity for annual extension of clones, combined with the efficiency of reproduction by seed, i.e. small annual extension and high seedling establishment lead to many small clones whereas large annual extension and low seedling establishment result in a few large clones.

What are the ecological implications of an 'everlasting' perennial? To be successful it must be able to cope with environmental changes. *Anemone nemorosa* maintains variability by high phenotypic plasticity and outbreeding. Fertilization is facilitated by the total intermixing of clones (Shirreffs, 1981). Although seed fertility is high, seedling establishment is rare. Ernst (1983) quotes a maximum germination in the field of 5–35 per cent, of which nearly 90 per cent die in the first and second years. However, in a mature population of several hundred years old, infrequent seedling establishment would probably still be sufficient to maintain genetic variability, as suggested by Soane and Watkinson (1979).

Thus the growth pattern of *A. nemorosa* enables it to colonize an area fairly rapidly while still retaining a high degree of morphological and genetical variability.

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