DOCTOR OF PHILOSOPHY

Ecological studies on the tick Dermacentor reticulatis.

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ECOLOGICAL STUDIES ON THE TICK *DERMACENTOR RETICULATUS*

A thesis submitted for the degree of

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by

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To my parents and my brother Mike

SUMMARY

The distribution and the activity patterns of *Dermacentor reticulatus* in Wales were investigated.

Two different methods of geographic variation analysis were employed. A morphometric study of variation in the scutal shape and base-pattern using discriminant analysis revealed a north-south cline running from Wales through Devon down to France. The similarity of the Welsh populations to one another suggest a common origin. A genetic analysis revealed little allozyme variation in the enzymes isocitrate dehydrogenase (ICD) and phosphoglucomutase (PGM), though a fast allele (ICD^f) was found in some Welsh specimens and a slow allele (ICD^s) in a few French specimens.

The seasonal activity, habitat and host associations of *D. reticulatus* was investigated at Morfa Harlech. Activity began in late August and lasted through to the following May with two peaks in activity, a marked peak in March-April and a more diffuse and reduced peak between September and November. There was no winter diapause. There were significant variations in activity from year to year and between sites (ungrazed, grazed and marsh) within the Morfa Harlech area. Macroclimatic temperature had a significant effect on activity at the ungrazed and grazed sites whereas photoperiod had a significant effect on activity at the marsh site. Microclimatic temperature had a significant effect on activity at the grazed and marsh sites and the microclimatic humidity also had a significant effect on activity at the grazed site. At Morfa Harlech ticks were found in a number of vegetation sub-communities within the dunes. Seasonal sex-ratio variation was also observed; in the autumn males appeared earlier than females, but females predominated for much of the year and were numerically dominant. Adult ticks were found on Welsh black cattle but not on rabbits. Larvae and nymphs were found on four small mammal hosts at the marsh site, *Clethrionomys glareolus*, *Apodemus sylvaticus*, *Sorex araneus* and *Sorex minutus*. *C. glareolus* was the most important host species. Larval activity appeared to be over by mid-July and nymphal activity peaked during July.

The more common and widespread *Ixodes ricinus* showed a bi-modal pattern of activity with spring/early summer and autumn peaks and was active during the summer diapause of the rarer *D. reticulatus* and inactive during the period of winter activity in *D. reticulatus*. Experiments on the cold-hardiness in the two species showed no significant differences in their supercooling points (SCP's) and there were no marked differences in the SCP of upland and lowland populations of *I. ricinus*. The critical equilibrium humidity (CEH) of Welsh and French *D. reticulatus* (86-92% RH) is similar to that published for *I. ricinus* (86%).

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CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

Ticks are arthropods, members of the Order Acarina (ticks and mites), sub-order Metastigmata which is divided into three families, the Argasidae (soft ticks), the Ixodidae (hard ticks) and the Nuttalliellidae which is represented by just one species, *Nuttalliella namaqua*. There are approximately 825 tick species worldwide, most belonging to the Ixodidae (approximately 643 spp.) with around 167 spp. belonging to the Argasidae. All ticks are obligate blood-feeders and as such act as vectors of disease. Most tick species (90%) are specific for hosts that do not normally include humans and their livestock (Hoogstraal and Aeschlimann 1982). However, the remaining 10% of tick species pose immense medical and veterinary problems to mankind and it is a little known fact that ticks act as vectors of more kinds of microorganisms than any other single arthropod taxon, including mosquitoes (Hoogstraal 1985).

The two major families of tick are structurally dissimilar and exhibit different feeding and development strategies. The Argasids have a smooth, leathery cuticle and lack a scutum in all stages. The capitulum is usually sub-terminal and the spiracles located between the 3rd coxae. The general life cycle consists of egg, larval, several nymphal stages and male and female stage. The nymphs and adults feed several times, usually for a couple of minutes at a time. The larvae, however, feed over a period of several days. There are usually two nymphal stages, though there may be up to eight in some species. The adults can mate on several occasions and in general lay several hundred eggs. They usually frequent the nests and burrows of their hosts (including human dwellings). They are important vectors of disease including tick-borne relapsing fever transmitted to humans by *Ornithodoros spp.* and fowl spirochaetosis, transmitted to poultry by *Argas persicus*. Hoogstraal (1985) has given a full account of disease transmission by the Argasids.

The Ixodids have a relatively hard cuticle. A scutum is present in all developmental stages, covering the whole dorsal region of adult males, but only a small prodosomal zone region of larvae, nymphs and adult females. The capitulum is anterior and always visible

from the dorsal view and the spiracles are located behind the 4th coxae. The general life cycle consists of egg, larval, nymphal and adult stages. The larvae, nymphs and adults feed only once and for several days. The males die after copulation which generally occurs during the feeding act of the female. The females die after laying their eggs in the vegetation (3,000 in *Ixodes*, 6,000 in *Dermacentor* and 15,000 in *Amblyomma)*. During its lifespan a species may attach consecutively to one to three hosts. The 3-host cycle is the most common in the Ixodidae, in which the larval, nymphal and adult phase all seek a host, feed and then disengage from the host before metamorphosing into the next stage. In the one-host cycle the larval stage seeks a host, engorges and undergoes ecdysis on the host. The resulting nymphal stage emerges on the host, feeds and then ecdyses on the same host. It is only the adult female stage which leaves the host to lay it s eggs in the vegetation. There is also a 2-host cycle found in some species in which the larval stage seeks a host, feeds and ecdyses on a host, but the resulting nymphal stage, once replete leaves the host so that the adult stage must also seek a passing host. The one and 2-host cycles have developed in a few species of the families *Hyalomminae* and *Rhipicephalinae* and these life cycles are thought to be adaptations to either feeding on large mammal host with extensive home ranges, or to environments with long and hot dry seasons, or to feeding during winter when the small mammal hosts of the immature stages are moving under the snow and are therefore unavailable to species that do not inhabit burrows (Hoogstraal and Kim 1985).

The Ixodidae is the dominant tick family with respect to both number of species and their medical and veterinary importance. Many tick-borne diseases are prevalent today. Examples affecting humans include; Lyme disease, a spirochaetal infection which is, perhaps, the most important vector-borne disease in the USA and Europe, Rocky Mountain Spotted Fever (a rickettsial infection) affecting areas of the USA and Tick-borne encephalitis (TBE) a viral disease occurring throughout Europe and Asia. Many diseases of veterinary importance are also transmitted to livestock including Heart water, East coast

fever, louping ill and cattle fever. In addition to the pathogens they transmit, ticks may also induce lethal paralysis or severe toxemia and severe anaemia through blood loss as a result of their bites (Sonenshine 1991).

In terms of the evolutionary history of tick s, the time at which a genus evolved will influence its' present day distribution. Hoogstraal (1978) postulated that the hard ticks, the Ixodoidea had "..evolved as obligate parasites of Reptilia in the warm, humid climate of the late Paleozoic or early Mesozoic era". It is possible that ticks evolved even earlier, Oliver (1989) suggested that they had evolved in the Devonian period where they fed on the great variety of Amphibia. Seventy million years ago, in the early Tertiary or late Cretaceous period, the primitive bird and mammal lines underwent explosive radiation replacing reptiles as the dominant terrestrial vertebrates. Those tick groups which were able to adapt to their new hosts would have undergone a parallel adaptive radiation.

One of the groups to evolve after the evolution and subsequent dominance of the birds and mammals was the sub-family Rhipicephalinae which includes the genera *Dermacentor*, *Rhipicephalus* and *Boophilus*. The genus *Dermacentor* is thought to have evolved in the Oligocene in the steppes of Asia. From there it spread to Europe and America (via the Bering land bridge) in the Miocene and it eventually reached Africa in Miocene-Pliocene times (Berdyev 1989).

In Britain we have a single representative of the genus *Dermacentor*, *Dermacentor reticulatus* (Fabricius, 1794). Its distribution in Britain is shown in fig 1.1 and in the Palaearctic in fig. 1.2 The distribution is described by Immler (1973) as being sporadic over a cool climate zone, the southern limit of it's distribution corresponding to the 20° C July isotherm in W.Europe and with the 0° C January isotherm in Eastern Europe and Asia. Throughout this range it exists in a number of different habitats and feeds on a range of different hosts. It has been recorded in a variety of habitats e.g. meadow and oak forest in Czechoslovakia (Cerny *et al.* 1982), river basins, swampy mixed woods, shrub pasture and lakeshore vegetation in Hungary (Nosek 1972), lowland forest in

Fig. 1.1: Distribution of *D. reticulatus* in Britain (redrawn from Martyn 1988).

Key:

h - Morfa Harlech
da - Dyffryn ardudwy
t - Tywyn
ab - Aberdyfi
r - Revelstoke / Newton Abbot
p - Paignton

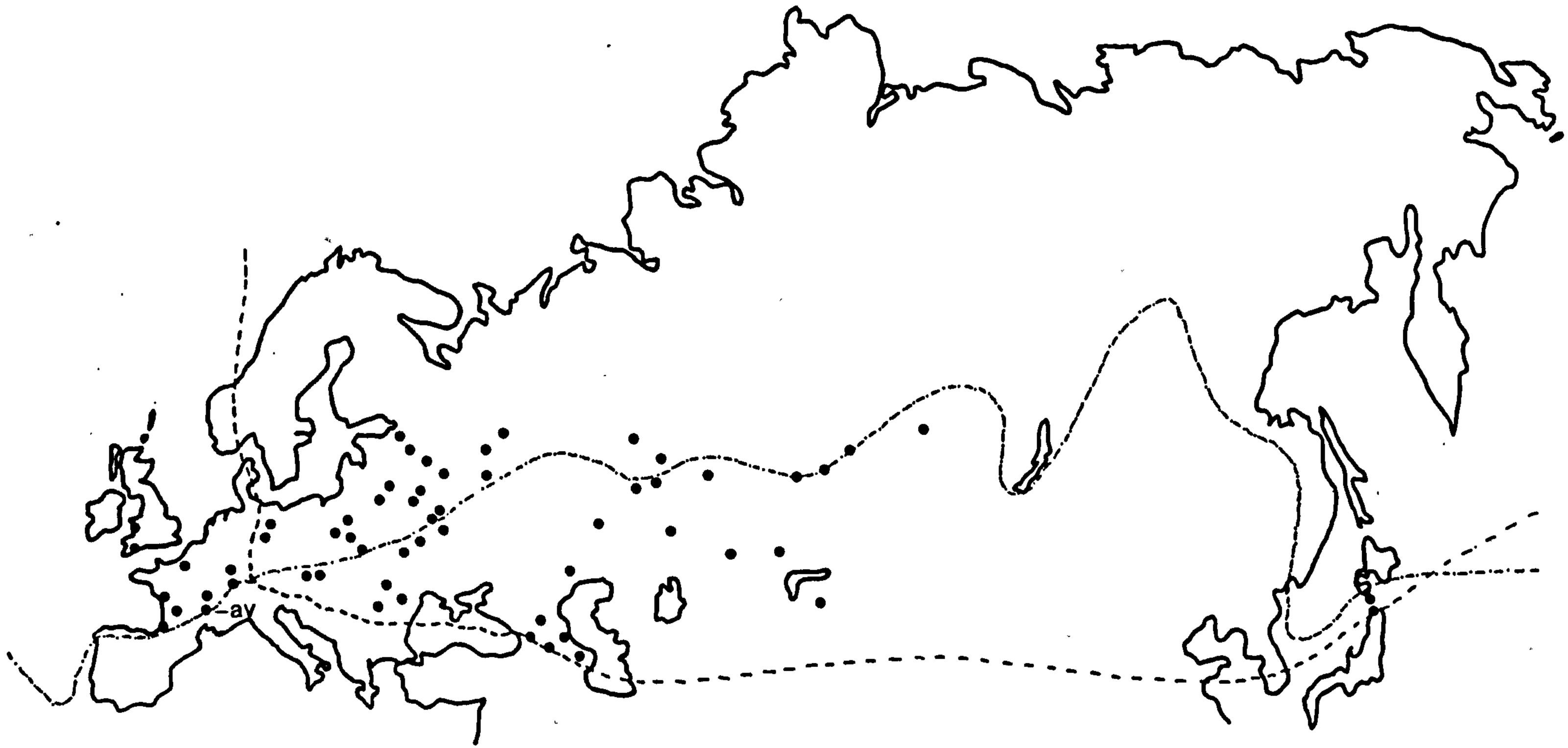


Fig. 1.2: Distribution of *D. reticulatus* in the Palaearctic region (redrawn from Immler 1973).

Switzerland (Immler *et al.* 1970), riverine scrub (Immler 1973), pastured land, formerly mowed meadows, heath, scattered scrub and suburban wasteland in France (Gilot *et al.* 1973,1974) , river basin and forest areas in Poland (Szymanski 1986, 1987a) and forest areas in Germany (Liebisch and Rahman 1976). In Britain, it has been recorded along river banks (Hirst 1916) and on clifftops in Devon (Thompson 1967) , on rough hill land near Aberystwyth (Hills 1957) and in the dune systems along the west coast of Wales from Borth bog (Evans 1951a) up to Harlech (Miles 1970 for Tywyn, pers.obs for Harlech, Dyfrynn ardudwy and Aberdyfi). In these dune systems the tick has been recorded on the dry fixed dunes and in adjacent wet slack areas.

The hosts of adult *D. reticulatus* are listed by Arthur (1963) as cattle, horse, sheep, goat, pig, dog, wolf,hares and hedgehog. Nymphs have been recorded on horses, small mammals and occasionally birds, hedgehogs and hares. Larvae have been recorded on small mammals such as field mice, voles, shrew and also on hedgehog and hare. Immler (1973) recorded most of the immatures on the bank vole (*Clethrionomys glareolus*).

Diseases known to be transmitted by *D. reticulatus* include the tick-borne encephalitis virus (though it's role as a vector in Central Europe is relatively small (Nosek 1972)), tick-borne rickettsia, *Babesia bovis*, *B. equii* and *B. canis*, the latter being of veterinary importance in southern France (Gilot *et al.* 1974, Martinod and Gilot 1991). Little is known of it's role in disease transmission in Britain, Arthur (1963) had suggested that it may have been involved in the transmission of *B. bovis* but that there was no direct evidence of this. Also, there are, as far as I know, no records of *B. canis* in Britain, but it obviously has potential for creating important veterinary problems.

Besides it's distribution (Martyn 1988), little was known about *D. reticulatus* in Britain. It has been found in Somerset, S.Devon, N and SE Cornwall, Pembrokeshire (Dyfed) and Cardiganshire (Ceredigion) (Thompson 1967) and Merionethshire (Gwynedd) (Miles 1970). Most of the records for SW.England are old, though there are record from the 1970's for Devon, Cornwall, Dorset and Somerset. There are no recent records of the tick though it is still assumed to be extant in this area (A.D.A.S pers.comm). Other records

from Surrey, Essex and Lancashire (fig 1.1) are reports of the tick on either humans and dogs and I have assumed that they have been picked up in either Wales or S.W.England and do not represent extant populations in these areas (records courtesy of BRC, Monk's Wood).Prior to this study this tick had been found by workers from Bangor at Morfa Harlech and Aberdyfi and also recorded at Dyfrynn Ardudwy and Tywyn in Gwynedd (Mathias 1985). The distribution in Wales is shown in fig. 1.3. It was thought that their activity began early in spring (February-March) which was earlier than that recorded for the more widespread *Ixodes ricinus* and there were no records of activity in the autumn (Mathias 1985).

The objectives of this study were:-

1) To try and account for the current distribution of *D. reticulatus* in Britain, particularly the Welsh populations (Chapters 2 and 3).

2) To investigate the activity, habitat and host associations of the ticks in order to determine how it persists in the Welsh populations and to compare it's activity, habitat and host associations to those found in Europe (Chapter 4).

3) To compare the seasonal activity of *D. reticulatus* with that of the more common and widespread tick, *I. ricinus*, and to investigate physiological factors which may account for differences in their activity and distribution.

To achieve the first objective I conducted two types of geographic variation analysis, one morphometric and one genetic. As stated by Mayr (1963), geographic variation analysis is the ".. causal analysis of the geographic variation patterns in order to interpret these adaptations to variation in known environmental factors such as climatic, topographic or edaphic variables or differences in distributional, reproductive or ecological patterns within the populations. Finally, geographic analysis may lead to the allocation of unknown specimens to a given population or a geographic locality with a stated probability of success". The first analysis was a morphometric study of the geographic variation in the scutal pattern of female ticks from four Welsh populations, one population from

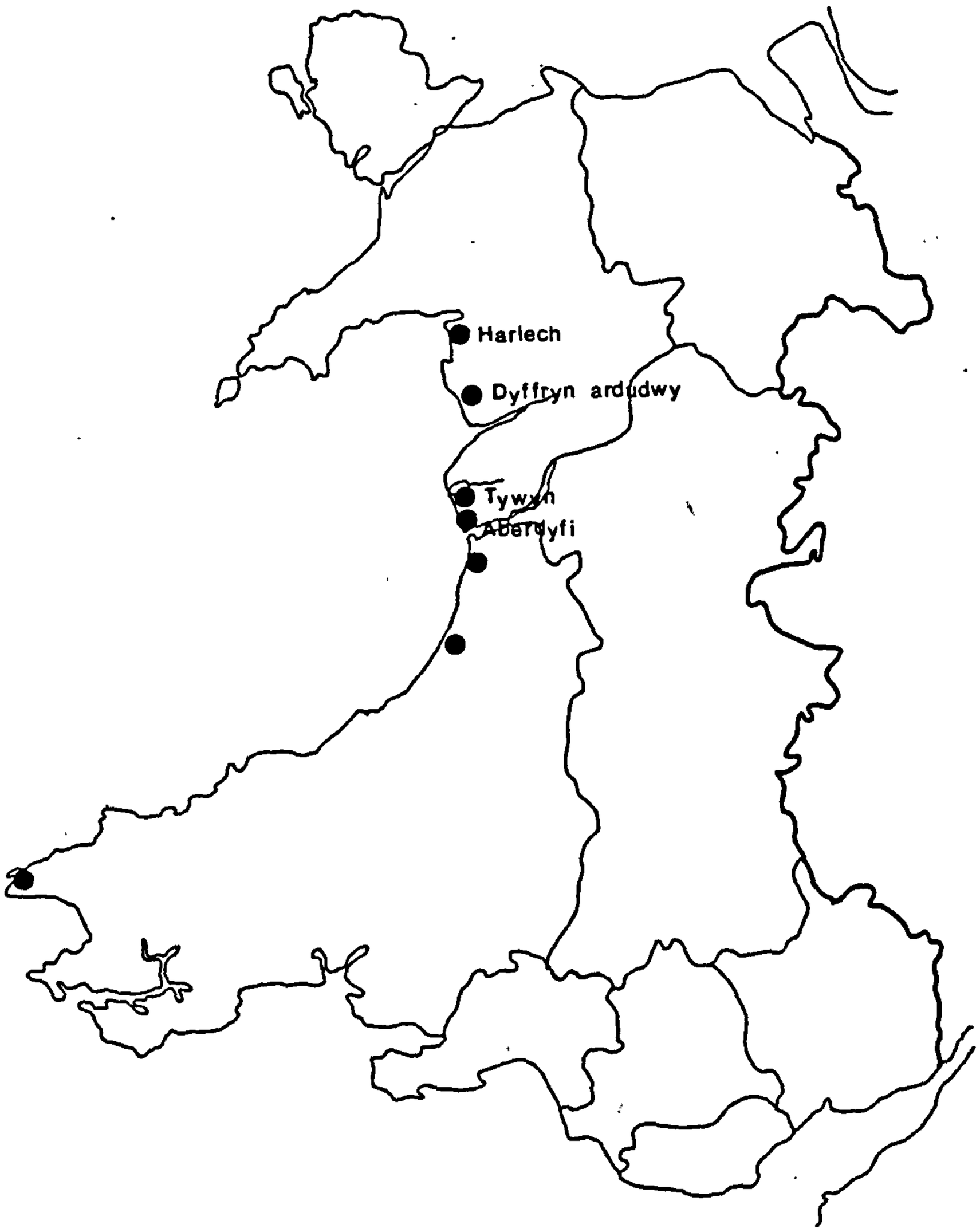


Fig. 1.3: Distribution of *D. reticulatus* in Wales (after Mathias 1985).

S.W.England and one population from S.E.France (Chapter 2). The second was a genetic study of allozyme variation of the same four Welsh populations and the same French population (Chapter 3). This approach of combined morphological and electrophoretic analysis of geographic variation has been put to good effect in a range of organisms including Crustacea (Weber and Galleguillos 1991), mites (Messing and Croft 1991) and mosquitoes (*Aedes spp.*) (Kambhampati and Rai 1991a). However, the genetic study, because of restrictions outlined later, was a preliminary analysis to identify possible sources of variation and to compare any variation found with that found in other studies on genetic variation in ticks.

To achieve the second objective, I conducted a field study at Morfa Harlech between 1987-1990. The activity of the off-host and on-host phase of adults and immatures was to be monitored. The habitats in which the ticks occurred were also to be quantified. Macro- and microclimatic data was also to be collected to investigate climatic effects on activity (Chapter 4).

To achieve the third objective I compared the activity of *D. reticulatus* at Morfa Harlech with that of *I. ricinus* at a site at sea-level on Anglesey. Laboratory experiments were to be conducted to investigate the cold-hardiness of the two species to try and account for the winter activity of *D. reticulatus* and the absence of winter activity in *I. ricinus*. Experiments were also to be carried out to determine the Critical Equilibrium Humidity (CEH) of *D. reticulatus* to see whether this species is physiologically adapted to survive in more xeric environments, such as dune habitats, than *I. ricinus* which appears to be absent from these habitats. The CEH value for *I.ricinus* was taken from the literature (Lees 1946). The results for this section are presented in Chapter 5.

There was one other initial objective of this study, to investigate any competitive interactions between *D. reticulatus* and *I. ricinus*. The study was designed to see whether the presence of *I. ricinus* feeding on the same host as *D. reticulatus* had a significantly detrimental effect on the fecundity of *D. reticulatus*. My hypothesis was that such a competitive interaction acting through host resistance could exclude *D. reticulatus* from

habitats favourable to *I. ricinus* and thus restrict *D. reticulatus* to habitats unfavourable to *I. ricinus* but favourable to itself. Unfortunately, this experiment was not completed due to reasons beyond my control. However, sufficient data was collected to give an indication of the effects of host resistance on the feeding success and fecundity of *D. reticulatus* and these results are presented in the appendix.

CHAPTER 2

AN ANALYSIS OF THE GEOGRAPHICAL VARIATION IN THE SCUTUM AND SCUTAL PATTERN OF THE TICK *DERMACENTOR RETICULATUS*

INTRODUCTION

The biogeography of a species is it's distribution in time and space and the factors causing this distribution (Barry Cox and Moore 1980). During it's evolution, a species will have developed in terms of its' morphology, physiology and behaviour to attune it to a particular environment. Through the interaction of biotic factors e.g competition,predation and parasitism and abiotic factors e.g climate,soil type it will have evolved to occupy a particular niche (Hutchinson 1957). The resulting distribution of a species will depend on a complex interaction through evolutionary history of abiotic and biotic factors which may include large scale physical factors such as geographic barriers and random effects such as chance introductions.

Parasitic species tend to occupy specialised niches. Their distribution will depend on the availability of their niche and their ability to occupy that niche. For a parasite, factors influencing this include host availability and distribution (which may in turn be affected by the parasite distribution) and the availability of a suitable microclimate/habitat should the parasite have a free-living stage.

Ticks are a group of ectoparasites which are unusual in that they have an obligatory period off the host, on three occasions for a three-host tick and one occasion for a one-host tick, only to return to another or the same host. Therefore, tick distribution is influenced by the on-host phase (host specificity, host availability and host movements) and the off-host phase (availability and distribution of suitable microclimate/habitat). According to Hoogstraal and Kim (1985) an important biological factor in determining the ecological and geographical distribution and population densities of most ticks is the high degree of strict host specificity found in the Ixodoidea. However, this pattern may be altered with the introduction of physiologically acceptable domestic or feral animals . These secondary hosts can often replace the primary hosts in the host-parasite association. As I have already mentioned, the time at which a genus evolved will influence it's present

day distribution. To reiterate, the genus *Dermacentor* was thought to have evolved in the Oligocene (37 - 22.5 million years B.P) in the steppes of Asia from where it spread to Europe and Asia in the Miocene (22.5 - 5 million years B.P) and eventually it reached Africa in Miocene-Pliocene times (≈5 million years B.P) (Berdyev 1989). It is interesting to note that a specimen of *D. reticulatus* was collected from a fossil Woolly rhino from the Pliocene (5 - 1.6 million years B.P.)(Sonenshine 1991). Thus, we see that *Dermacentor* ticks, and *D. reticulatus* in particular, have been present in Europe for a considerable amount of time. *Dermacentor spp.* in N.America show considerable diversity in the number of species and in the life history despite a short evolutionary history and moderate host specificity (mainly restricted to mammals) (Gunn and Hilburn 1990). A similar process has probably occurred in Europe, perhaps to a greater extent given that *Dermacentor* has been present here for a longer period. Thus, we might expect that the different species and local populations of *Dermacentor* to have become adapted to varying climatic conditions and possibly to a range of hosts in this period. It is likely that the distribution patterns of the evolving species of *Dermacentor* will have shifted over this time and in more recent times mankind is likely to have had a profound effect on the distribution of ticks like *D. reticulatus* through the introduction of domestic animals and the alteration of the environment. There is some evidence for the effect of agricultural improvements on the distribution of *D. reticulatus* in Czechoslovakia and Poland (Daniel *et al.* 1986).

The aim of this study was to investigate the geographic variation of *D. reticulatus* to account for it's distribution, particularly in Britain. To do this we have used morphometric data taken from the scutum and the ornate scutal pattern (fig. 2.1 and plate 2.1). It had previously been noted that there was much variation in the scutal pattern of *D. reticulatus* (Arthur 1960a) and much variation was also observed in the North American species of *Dermacentor* by Cooley (1938). A multivariate statistical technique , discriminant analysis has been employed. In this study we have compared four populations from the west coast

Plate 2.1: Unfed female (a) and male (b) *Dermacentor reticulatus*. Specimens from Morfa Harlech. (Magnification approximately x 20).

a

b

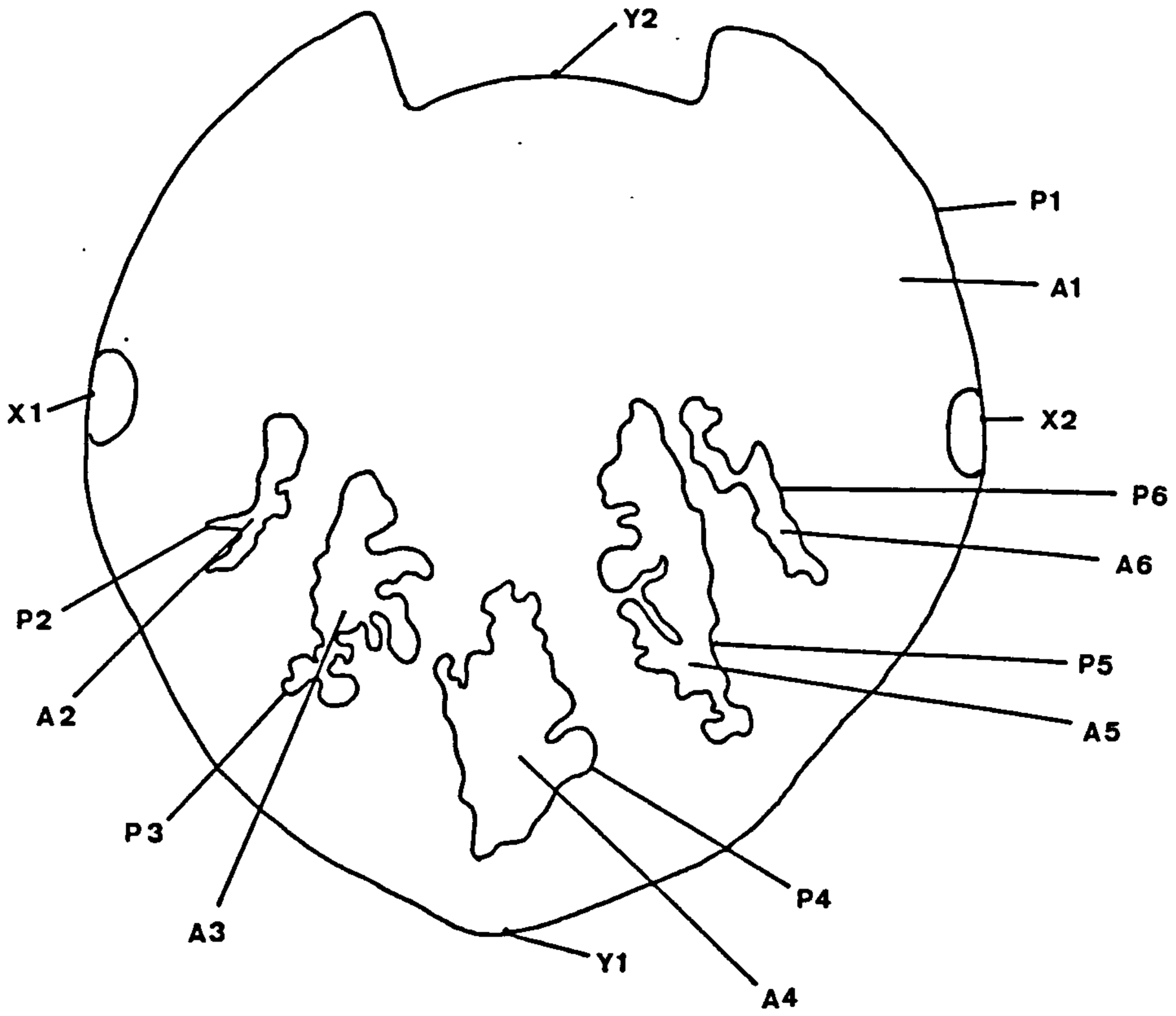


Fig. 2.1: Parameters measured for the determination of the scutal area and scutal pattern of female *D. reticulatus*. For an explanation of the parameter labels see table 2.2.

of Wales , one population from Devon and one population from the Aveyron region of France. This study could indicate whether the scutal pattern is of use in analysing the geographic distribution of *D. reticulatus*.

MATERIALS AND METHODS

Material:

Questing adult *D. reticulatus* were collected by blanket dragging from the 4 Welsh sites during 1988-1989. Ticks from the Aveyron region of France were kindly supplied by Dr B.Gilot. A number of specimens were also obtained on loan courtesy of the British museum (Natural History). They included 10 specimens from S.W. England (1 from Newton Abbot, 7 from Revelstoke, N.Devon and 2 from Paignton,Devon). The Welsh sites were as follows:-

Morfa Harlech, Gwynedd. Map ref: SH 5831

Extensive dune system backed by large dune slack areas with a saltmarsh to the north. Most of the area is grazed by Welsh black cattle and sheep.

Dyffryn ardudwy, Gwynedd. Map ref: SH 5822

Area where ticks found form a small part of the extensive dune system running from Mochras to Afon Ysgethin. The site is adjacent to the Ysgethin estuary and consists of a dune system backed by an area with large stands of *Juncus acutus*. The area is grazed by Welsh black cattle.

Tywyn, Gwynedd. Map ref: SH 5800

Juncus spp. dominated marsh bordered by embankment to the Dysynni estuary. Area grazed by sheep.

Aberdyfi, Gwynedd. Map ref: SH 6196

Dune system running parallel to golf course (grazed by sheep) and back area of *Juncus spp.* marsh (grazed by Welsh blacks).

The map references for the Devon samples are Newton Abbot SX 8671 and Paignton SX 8960. The Aveyron region of France is approximately at latitude 44°N and longitude 2°E. The number of ticks analysed from each population is shown in table 2.1. The small sample sizes reflect the difficulty in collecting *D. reticulatus* from a number of localities. The specimens from Wales and France were frozen in liquid nitrogen and stored at -40°C. These individuals were also used in the genetic study (Chapter 3).

Measurements:

The ticks were photographed using a Zeiss Ultraphot producing x 6.3 mag.negatives. 5"x4" prints (x3 mag.negatives) were also produced to aid in visual analysis. The negative images were projected onto a transparent screen producing a x11.4 mag.image of the negative. The final images were then 6.3 x 11.4= 71.82 x the actual tick. These traced images were then digitised, the digitiser programme calculating the perimeter and area of the patterns marked out. The patterns referred to here are the base patterns i.e. these dark basal patches are the base colour (chitin) on which the colour (paler areas) is superimposed (Cooley 1938). Reference points were also digitised (x1, x2, y1, y2) so that the scutal length and width could be calculated. Table 2.2 shows the parameters measured for each tick. Males were not included in the analysis as their scutal patterns were more complicated and difficult to trace. A more complicated pattern generates more variables for analysis, but if multivariate analysis is to be used it is important that the number of specimens per group is at least three to four times that of the number variables (Flury and Reidwyl 1988). In this case a balance has been struck so as to retain as many

Table 2.1: Habitat type and number of specimens used from each site.

Site	Habitat	No.
Harlech	Dune slack	18
D.ardudwy	Fixed dune	18
Tywyn	Juncus sp. marsh	18
Aberdyfi	Fixed dune	18
Devon	unknown	18
France	Scrub	18

Table 2.2: Parameters measured.

Parameter	Abbreviation
Scutum length	SL ($y_1 \rightarrow y_2$)
Scutum width	SW ($x_1 \rightarrow x_2$)
Scutal perimeter	P1
Scutal area	A1
Perimeter pattern 2	P2
Area pattern 2	A2
Perimeter pattern 3	P3
Area pattern 3	A3
Perimeter pattern 4	P4
Area pattern 4	A4
Perimeter pattern 5	P5
Area pattern 5	A5
Perimeter pattern 6	P6
Area pattern 6	A6

variables as possible.

A data file consisting of relative not absolute values for each individual tick was then created on the VAX mainframe computer.

Statistical techniques:

a).Ordination techniques:

Ordination techniques can be used to express the variation between Operational Taxonomic Units (OTU's) where an OTU is a specimen, population etc. Ordination serves to summarise data by producing a low- dimension space in which similar samples are close together and dissimilar samples far apart (Gauch 1982). Considering a pair of characters , the relationship between the OTU's can be shown by a simple scatter plot. If we now consider the variation in several characters , we can imagine the OTU's dispersed in a multidimensional space with one dimension or axis for each character. The distance between the OTU's is equivalent to their similarity. In ordination, the first axis or eigenvector goes through the scatter of OTU's along the dimension expressing the greatest amount of variation. The next eigenvector accounts for the next greatest amount of variation and so on. In the same way that an OTU has a character state for each character, each OTU has a score for each eigenvector. A large proportion of the variation in many characters can be summarised in a lesser number of vectors, each vector expressing a known proportion of the variation. For a fuller account of ordination techniques see Thorpe(1976) and Gauch(1982) and references therein.

b).Canonical (Discriminant) analysis:

Canonical analysis ordinates groups of specimens rather than individuals. Canonical or multiple discriminant function analysis (from herein referred to as discriminant analysis) ordinates more than two groups. Single function discriminant analysis ordinates two

groups. The techniques maximise the separation between the pairs or series of groups (Thorpe 1976). The data used are the values of the variables for cases whose group membership is known. Linear combinations of these independent variables are formed and serve as the basis for classifying cases into one of the groups. The coefficients for the linear combinations are chosen to give the "best" separation amongst the groups. The accuracy of the classification can be estimated by applying the model to cases for whom group membership is known and comparing group membership to actual membership. The technique used here is known as jackknifing or the leave-one out method (see Norusis 1985).

Discriminant analysis aims to :-

i).Classify cases into one of several mutually exclusive groups on the basis of various characteristics.

ii).Establish which characteristics are important for distinguishing among groups.

iii).To evaluate the accuracy of the classification.

For a review of discriminant analysis and its' ecological applications see Thorpe (1976) and for a thorough account see Gittins (1985) and Sneath and Sokal (1973). Discriminant analysis of morphometric data has been used to distinguish between populations in a variety of organisms including plants (*Lotus sp.* Steppan 1991), insects (*Aedes sp.* Kambhampati and Rai 1991a), crustaceae (*Liopetrolisthes sp.* Weber and Galleguillos 1991), acarines (*Amblyseius sp.* Messing and Croft 1991, *Varroa sp.* Delfinado-Baker and Houck 1989) and reptiles (*Natrix natrix* Thorpe 1989).

Analysis:

a).Creating residuals:

Morphometric data consists of absolute measures of body size. If the size of adults is not fixed and there is an absence of well-defined stages of growth , then it is necessary to transform the absolute size to some estimate of relative size (shape). Size and shape are known to covary and this implies a changing relationship between size and shape (Gould 1966). Heterogeneity in size and shape between samples can increase due to sampling biases or differences in growth patterns. Newson and Chiera (1987) found in *Rhipicephalus appendiculatus* that the scutal size varied inversely with the degree of host resistance. Therefore, given the differences in host resistance experienced by individuals both within and between populations,it was important that this effect was removed. Comparison of samples should ideally be in terms free from magnitude effects such as size (Reist 1986). To free the data from magnitude effects we used the residuals derived from the original data (Turner 1990, Reist 1986). Here , each original variable was regressed on the scutum length(SL) for all populations combined. The residuals are determined using a common within-groups line in which all within-group lines are adjusted to a common slope , but each within groups line may differ with respect to intercept. The residuals are the deviations of each individual from this common within-groups line. This part of the analysis was conducted using the Minitab package.

b).Selecting variables:

The data base here consists of the residuals of 13 variables. As mentioned previously we need at least twice the number of specimens per group as variables. Therefore, the number of variables to be included in the analysis had to be reduced. I decided to reduce the variable number down to 6. This gave three times the

number of specimens:variables in the Welsh and French populations and just under twice the number of specimens:variables in the Devon population. The variables were selected using a combination of the following methods.

i) Test for normality.

Each variable was tested for normality using the Kolmogorov-Smirnov goodness of fit test. If most of the variables have non-normal distributions then the data may be normalised by taking logs for example. If there are only a few variables with non-normal distributions then they will be removed from the analysis at this stage.

ii) Stepwise selection using minimisation of Wilks' lambda.

In a stepwise method the first variable included in the analysis has the largest acceptable value for the selection criterion. After the first variable is entered, the value of the criterion is reevaluated for all variables not in the model, and the variable with the largest acceptable criterion is entered next. At this point, the variable entered first is reevaluated to determine whether it meets the removal criterion. If it does it is removed from the model. The next step is to examine the variables not in the equation for entry , followed by examination of the variables in the equation for removal. Variables are removed until none remains that meets the removal criterion.

The selection procedure here used minimisation of Wilks's lambda. Wilks' lambda is the ratio of the within-groups sum of squares to the total sum of squares. A lambda of 1 occurs when all observed group means are equal. Values close to 0 occur when within-groups variability is small compared to the total variability i.e. when most of the variability is attributable to differences between the group means . Thus , at each step the variable that results in the smallest Wilks's lambda for the discriminant function is selected for entry (Norusis 1985).

iii) Information redundancy.

Information redundancy occurs where two characters have high intra-locality correlations as they reflect the same epigenetic information. The redundancy of information in a series of characters can be assessed by investigating the elements in a matrix of the intra-locality correlations between characters e.g. Groves (1970), Thorpe (1976), Turner (1990).The magnitude of the correlation coefficients is important rather than their sign or statistical significance. In this study correlations >0.8 were taken to indicate redundancy. In these cases the variance is at least 0.64 i.e. 64% of the variance in a character is accounted for by the other correlated character. The significant correlations are shown in table 2.4.

c) Test for violation of assumptions:

The two important underlying assumptions are that:-

i).The variables are from multivariate normal distributions.

ii).The covariance matrices for all groups are equal.

A simple test for the first assumption is to examine the distributions of each of the variables individually. The second assumption may be tested using Box's M test, which is based on the determinants of the group covariance matrices. The significance probability is based on the F- transformation. A small probability ($p<0.05$) will mean rejecting the null hypothesis that the group covariance matrices are equal.

d) Running the procedure:

The discriminant analysis was conducted on the VAX mainframe computer at Bangor using the DISCRIMINANT package on the SPSSX programme.

e) Discriminant output:

The analysis provides information on the following;-

i) The predicted membership(classification) of individuals from the known populations based on the derived discriminant functions.

ii) A test of the above classification by jackknifing(see results section).

iii) A test of the significance of the discriminant functions.

iv) Plots of the populations along the important discriminant functions.

v).Population means (centroids) of the discriminant functions and a test of the significance of the distance between group means.

vi).Correlations between the variables and the discriminant functions.

On the basis of the above analysis the mean values for each population of the important variables was given. Also, the degree to which the scutum was patterned expressed as percentage of scutal area patterned was determined for each of the populations.

RESULTS

Selecting variables

a) Test for normality:

Only one of the variables (residuals) was shown to have a non-normal distribution using the Kolmogorov-Smirnov goodness of fit test. This was the variable P1, the perimeter of the scutum. Since it was the only variable with a non-normal distribution it was removed from the analysis at this point.

b) Minimising Wilk's lambda:

Table 2.3 shows the results of the stepwise procedure of minimising Wilk's lambda. Eight variables met the entry requirement and did not meet the removal criterion and , therefore, remained in the model. Four variables did not meet the entry requirement. At step 1, A1 had the smallest Wilk's lambda and was thus entered. At step 2, of the variables not in the model, A5 had the smallest Wilk's lambda and was entered, A1 was then reassessed to see if it met the removal criterion. It did not and so remained in the model. The procedure continued until the last variable that met the entry requirement, SW was entered.The following variables met the entry criterion, A1 A2 P3 A4 P5 A5 A6 and SW and remained in the model. P2 A3 P4 and P6 were not entered.

c) Information redundancy:

The summary of results of the intra-locality correlation matrices for each population is given in table 2.4. The important elements of the matrix in table 2.4 are given below:-

Table 2.3: Selection of variables using minimisation of Wilk's lambda.

Step	Variables entered	Variables removed	No. of vars. in	Wilk's lamda	Sig.
1	A1	-	1	0.70479	0.000
2	A5	-	2	0.49388	0.000
3	P5	-	3	0.37720	0.000
4	A2	-	4	0.29784	0.000
5	P3	-	5	0.25286	0.000
6	A4	-	6	0.21701	0.000
7	A6	-	7	0.19041	0.000
8	SW	-	8	0.17640	0.000

Variables not meeting entry requirements.

Variables	Wilk's lamda
P2	0.17219
A3	0.16772
P4	0.16849
P6	0.16676

Table 2.4: Test for redundancy - significant correlations between variables.

Population	Significant correaltions
Harlech	P1:A1, P4:A4
D.ardudwy	SW:A1, P1:A1, P2:A2, P3:A3, P5:A5
Tywyn	SW:A1, P1:A1, P2:A2, P3:A3, P4:A4, P5:A5, P6:A6, A3:A5
Aberdyfi	SW:A1, P3:A3, P5:A5
Devon	SW:P1, SW:A1, SW:A5, P1:A1, P2:A2, P4:A4, P6:A6
France	SW:A1, P4:A4

* significant correlations where Pearson correlation coefficient (r)>0.8

Table 2.5: Test for equality of group covariance matrices using Box's M.

Box's M	Approx. F	d.f		sig.
156.90	1.227	105	9376.3	0.0578

SW was significantly correlated with A1 in 5 populations

SW was " " " " with A5 in 1 population

P2 was " " " " with A2 in 3 populations

P3 was " " " " with A3 in 4 populations

A3 was " " " " with A5 in 1 population

P4 was " " " " with A4 in 4 populations

P5 was " " " " with A5 in 2 populations

P6 was " " " " with A6 in 2 populations

As a result of the above tests the following variables were de-selected. P2, A2, P4 and P6 on the basis of the stepwise procedure. SW was also de-selected as it was correlated with A1 in 5 populations. A1 was judged to account for more variability between group means on the basis of its' position of entry into the model in the stepwise procedure. Likewise P5 was de-selected because it was significantly correlated with A5 in 3 populations. A5 was selected for entry before P5 and ,therefore, accounted for more variability between group means.

Thus, the variables entered were A1 A2 P3 A4 A5 and A6 (fig. 2.1).

Test for violation of assumptions

The results from the test for normality show that all the variables included in the analysis had normal distributions. This was taken as satisfying the multivariate-normal distribution.

The results of the test for equality of the group covariance matrices is shown in table 2.5 . The result of Box's M test based on the determinants of the group covariance matrices gave a significance probability value of p=0.0578. Therefore, the group covariance matrices were assumed to be equal.

Discriminant output:

a) Classification:

The diagonal elements in table 2.6 show the number of cases correctly classified into groups. The total values are given in the corner of each box. Thus, for Harlech 61.1% (11 inds.) were correctly classified, 22.2%(4 inds.) were classified as being from the Dyffryn ardudwy population and 5.6%(1 ind.each) were classified into the Aberdyfi, Devon and French populations respectively. The overall percentage of cases classified correctly is the sum of the number of cases correctly classified in each group divided by the total number of cases.The percentage of cases correctly classified is often taken as an index of the effectiveness of the discriminant function. However, it is important to compare the observed misclassification rate to that expected by chance alone.For example, given two groups with equal prior probabilities , say 50 individuals in group 1 and 50 individuals in group 2, then assigning cases based on chance alone we would expect a 50% misclassification rate. Therefore, a discriminant function with an observed misclassification rate of 50% is performing no better than chance alone. As the number of cases with equal prior probabilities increases so the percentage of cases that can be classified correctly by chance alone decreases. In this case the prior probabilities are 18% for Harlech, D.ardudwy, Tywyn, Aberdyfi and France (18 inds. per group) and 10% for Devon (10 inds.). We can see that the discrimination is performing much better than by chance alone in classifying the Harlech, D.ardudwy, Tywyn and French populations. However, it is a poor performer in classifying Aberdyfi and Devon specimens.

From the above classification table we can extrapolate how well the discrimination functions when comparing all the Welsh populations combined with those of Devon and France. For all Welsh populations, 5 are misclassified as being from Devon and 4 are misclassified as being from France.

Therefore, (4x18)-9 = 63 individuals are classified as Welsh i.e. 87.5%. The results of

Table 2.6: Classification table.

Actual popn.	Predicted population membership (%)					
	Harlech	D.ardudwy	Tywyn	Aberdyfi	Devon	France
Harlech	61.1	22.2	0.0	5.6	5.6	5.6
D.ardudwy	16.7	61.1	0.0	11.1	11.1	0.0
Tywyn	0.0	0.0	50.0	27.8	11.1	11.1
Aberdyfi	33.3	33.3	5.6	22.2	0.0	5.6
Devon	10.0	10.0	10.0	10.0	20.0	40.0
France	0.0	0.0	5.6	5.6	11.1	77.8

% of grouped cases correctly classified = 51.0 %

Table 2.7: Classification of the combined Welsh populations compared to the populations from Devon and France.

Actual popn.	Predicted population membership (%)		
	Wales	Devon	France
Wales	87.5	6.9	5.6
Devon	40.0	20.0	40.0
France	11.1	11.1	77.8

* Data extrapolated from table 2.6

% of grouped cases correctly classified = 79.0 %

this classification are given in table 2.7. The classification performs well at identifying Welsh and French individuals but performs poorly with those from Devon. The percentage of group cases correctly classified increases from 51% (table 2.6) to 79% (table 2.7). We did not run a comparison of all the Welsh populations, clumped as one population, with the populations from Devon and France as this is in effect a completely different classification with different discriminant scores derived, so that any comparisons with the rest of this discrimination are invalid.

b) Test of the classification:

As mentioned this uses the test known as jackknifing. A test is required because a model usually fits the sample from which it is derived better than it will fit another sample. Therefore, the percentage of cases correctly classified by the discriminant function is an inflated estimate of the true performance in the population. To obtain an improved estimate of the misclassification rate we use the jackknife technique. One case was left out in turn and the discriminant function was calculated on the n-1 cases remaining, the left out case was then classified. Since the case being classified is not included in the calculation of the function, the observed misclassification rate is a less biased estimate of the true one (Norusis 1985). By comparing the results of the jackknife procedure to those obtained in the original classification (table 2.6), we see in table 2.8 that the classification can be considered as unbiased as the pairs of results do not differ to a great degree for any population.

Table 2.8: Test of the classification.

Population	Reference "known" classification	Test "unknown" classification (jackknifing)
Harlech	61.1	50.0
D.ardudwy	61.1	55.6
Tywyn	50.0	44.4
Aberdyfi	22.2	16.7
Devon	20.0	20.0
France	77.8	72.2

* values are the percent of cases correctly classified.

Table 2.9: Test of the significance of the discriminant functions.

Function	Eigen value	% of var.	canonical corr.	After func.	Wilk's lamda	sig.
				0	0.228	0.0000
1	1.34	65.0	0.76	1	0.532	0.0000
2	0.41	20.0	0.54	2	0.751	0.0000
3	0.20	9.9	0.41	3	0.904	0.0090
4	0.09	4.5	0.29	4	0.988	0.1553
5	0.01	0.6	0.11	5		

c) Test of significance of the discriminant functions:

Table 2.9 provides information on the significance of the discriminant functions and the variability that they account for. The eigenvalues are the ratio of the between-groups to within-groups sums of squares. Large eigenvalues are associated with "good" functions. The total between-groups variability for each function was calculated from the canonical correlations. The canonical correlation is the ratio of the between groups sum of squares to the total sum of squares. Variability in the discriminant scores due to between group differences was calculated by obtaining the squared value of the canonical correlation and multiplying by 100 to produce the percentage of variance. From table 2.9 we see that function 1 accounts for 65% of the between- groups variability and function 2 accounts for 20% of the between-groups variability.

When there are no differences among the populations from which the samples are selected, the discriminant functions reflect only the sampling variability. The test of the null hypothesis that in the populations the means of all discriminant functions in all groups are equal was based on Wilk's lambda. The significance level was based on the chi-squared transformation. If the observed significance is $p < 0.00005$, the null hypothesis that the means of both functions are equal in the six populations can be rejected. The means of the functions were tested in succession by first testing all means simultaneously and then excluding one function at a time and testing the mean of the remaining functions at each step. Using these successive tests it was possible to determine which discriminant function accounted for true population differences and which functions accounted for random variation only. From table 2.9 we see that the first two functions account for a cumulative 85% of the variability between group means and the first two functions are the only functions accounting for significant, real differences.

d) Plots of the populations scores for the important discriminant functions.

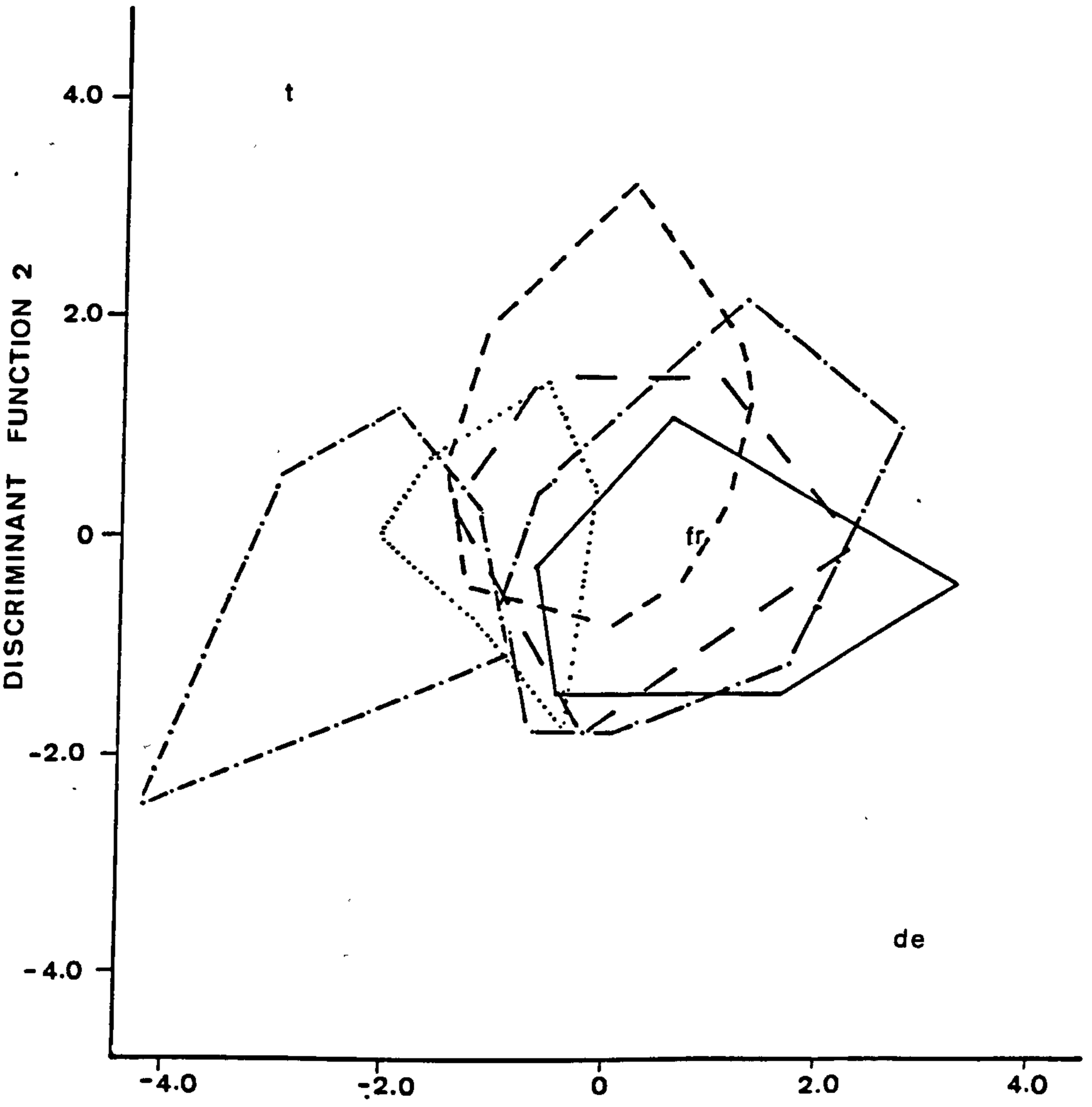
Having determined that the first two functions were the only ones of significance, the populations were plotted along these two functions as shown in fig. 2.2. The boundaries mark out the area occupied by the individuals of a given population except for outliers. The outliers are plotted outside the boundaries of their respective populations and are marked by their population code. From fig. 2.2 we see that most of the separation is along the first discriminant function, the Welsh populations overlap to a large degree with the Aberdyfi population bound within the other Welsh populations (see also table 2.6)

The French population slightly overlaps the Welsh populations but is effectively separate. The population from Devon is overlapped by both the Welsh and French populations. There is little separation along the second axis except perhaps for the Tywyn population (see later).

The outliers were left in the analysis as tests showed that their removal did not significantly improve the performance of the discrimination, the percent of grouped cases being correctly classified increasing from 51.0 to 52.58. Given the small sample sizes and perhaps the teleological nature of leaving outliers out to "improve" the discrimination, it was decided that they would remain in the analysis.

e) Population means (centroids) of the discriminant functions:

The mean values (centroids) for each population of the first two discriminant functions are given in table 2.10. They are also shown graphically in fig. 2.3, which, perhaps, shows the separation between the groups more clearly than fig. 2.2 (but note different axes). We see from fig. 2.3 that three of the Welsh populations, Harlech, D. ardudwy and Aberdyfi are separated out along the first discriminant function from the Devonian and French populations, with Tywyn falling between the two groups. However, if we consider the cline shown on fig. 2.3 the Welsh populations are all approximately equidistant from the French population with the Devonian population lying intermediate. Tywyn is separated



Key:

—·—	h	(Morfa Harlech)
———	da	(Dyffryn ardudwy)
- - - -	t	(Tywyn)
— —	ab	(Aberdyfi)
.....	de	(Devon)
—·—·—	fr	(France, Aveyron)

Fig. 2.2: Outlines of areas occupied by the scores for all ticks from the different populations for discriminant functions 1 and 2. Outlying individuals are shown by their respective population label (e.g. fr).

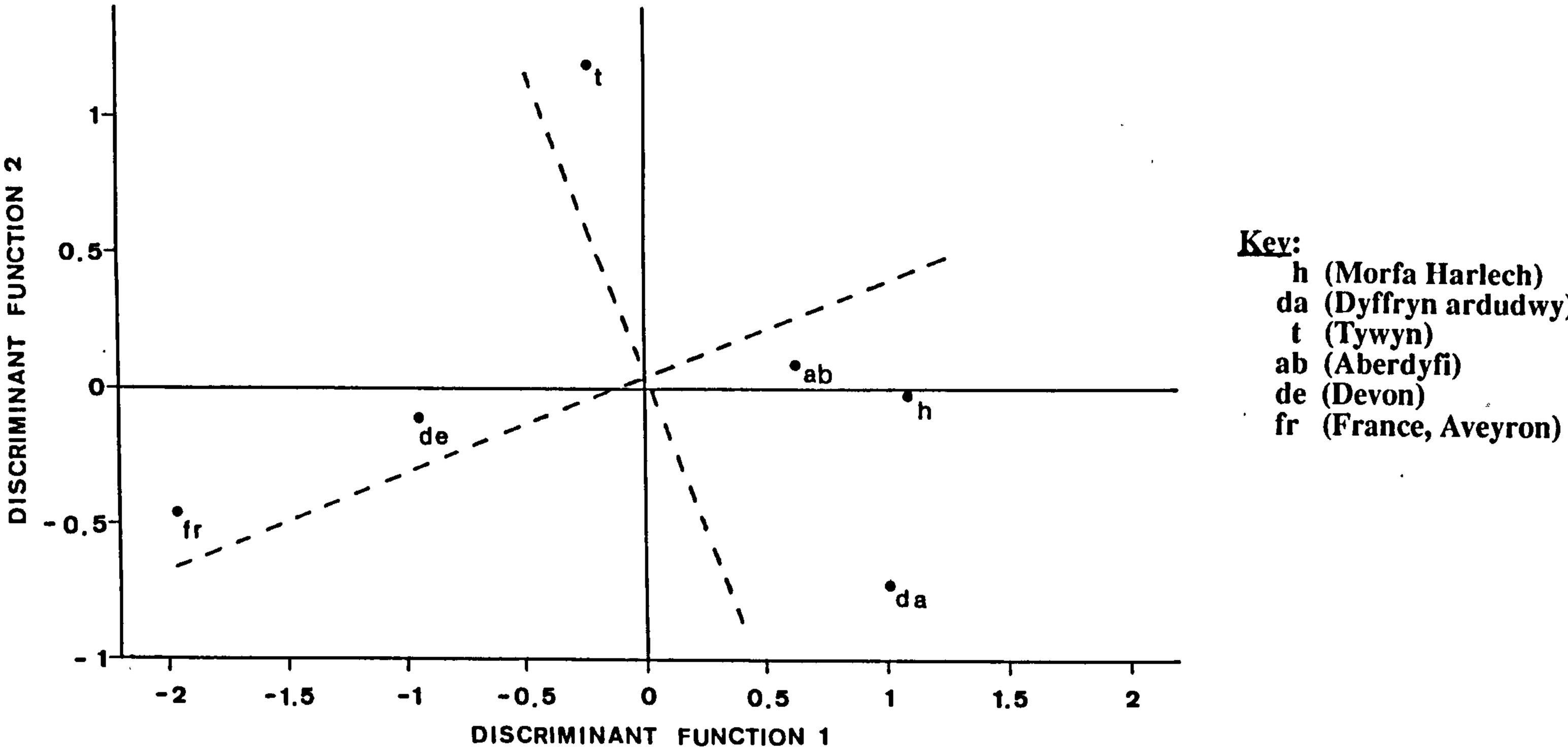
Table 2.10: Group centroids - discriminant functions evaluated at group means.

Population	Function 1	Function 2
Harlech	1.085	-0.027
D.ardudwy	1.006	-0.717
Tywyn	-0.229	1.190
Aberdyfi	0.624	0.085
Devon	-0.940	-0.119
France	-1.965	-0.462

Table 2.11: Test of significance of the between-groups means based on the derived F-statistic.

Population	Harlech	D.ardudwy	Tywyn	Aberdyfi	Devon	France
Harlech	-	**	***	ns	***	***
D.ardudwy		-	***	ns	***	***
Tywyn			-	**	*	***
Aberdyfi				-	**	***
Devon					-	***
France						-

*** p<0.001
** p<0.01
* p<0.05
ns non significant



Key:

h (Morfa Harlech)
da (Dyffryn ardudwy)
t (Tywyn)
ab (Aberdyfi)
de (Devon)
fr (France, Aveyron)

Fig. 2.3: Plots of the population centroid scores for discriminant functions 1 and 2. The north - south cline indicated by the line "--" was drawn by eye. It shows the Welsh populations approximately equidistant from the French ticks with the Devon ticks in an intermediate position. The Tywyn population is distinct from the other Welsh populations along a secondary cline.

out along the second discriminant function from the other populations.

We tested the significance of the differences between the means using the derived F-statistic based on Mahalanobis' distance where Mahalanobis' distance is a generalised measure of distance between the groups. The matrix of the F-ratio for each pair of groups is shown in table 2.11. All populations show significant differences between each other except for Aberdyfi with both Harlech and D.ardudwy. The French population was highly significantly different from all the other populations which bears out the separation along the discriminant function 1. Other highly significant differences were between Harlech : Tywyn and Harlech : Devon and between D.ardudwy : Tywyn and D. ardudwy : Devon. Thus, there appeared to significant differences between the population means based on the variables selected.

f) Correlations between the variables and the discriminant functions:

I then wished to determine which variables were contributing to these significant differences between the groups. This was done by examining the correlations between the values of the functions and the values of the variables. for each case the value of the discriminant function was computed and the Pearson correlation coefficient between it and the original variables was obtained. Separate correlation matrices were calculated for each group and the results combined to produce a pooled within-groups correlation matrix (table 2.12). From this we can deduce that the separation along DF1 is due to the range in size of A1 (positively correlated with DF1) or in the range in size of A2,A5 and A6 (positively correlated with DF1), or a combination of the above. The separation along DF2 is positively correlated with A6 and P3 and negatively with A4. The mean values for each population of the variables A1, A2, P3, A4, A5 and A6 are given in table 2.13.

Table 2.12: Pooled within-groups correlations between the discriminant variables and discriminant functions.

Variable	Function 1	Function 2
A1	0.49965	0.08836
A5	-0.43372	0.02658
A2	-0.36310	0.21644
A6	-0.34464	0.57676
P3	0.06461	0.56388
A4	0.03503	-0.38673

Table 2.13: Mean and S.E. values for the important discriminating variables.

Population	Variables (mm^2)			
	A1	A5	A2	A6
Harlech	1.85±0.05	0.07±0.004	0.018±0.001	0.020±0.001
D.ardudwy	1.84±0.07	0.05±0.004	0.017±0.001	0.015±0.001
Tywyn	1.96±0.05	0.07±0.005	0.020±0.002	0.025±0.002
Aberdyfi	1.99±0.05	0.07±0.003	0.022±0.001	0.020±0.002
Devon	1.70±0.09	0.08±0.006	0.020±0.002	0.021±0.002
France	1.82±0.09	0.09±0.005	0.026±0.002	0.024±0.002

g) Degree of pattern in the populations:

Table 2.14 shows the degree of scutal patterning in the six populations calculated as the sum of the population mean values of A2, A3, A4, A5 and A6 expressed as a percentage of the scutal area, A1. Though the differences between the populations was small, there appeared to be a north-south cline with the Welsh populations being slightly paler than the Devonian and French populations.

In summary, we can make two points about the discrimination:-

i).There are significant differences between the population means of the discriminant functions(table 2.10).

ii).Though we can see these significant differences at the population level, the results of the classification (table 2.6) show that there is much individual variation and so the discriminant functions are not particularly efficient predictors at the individual level.

Table 2.14: The degree of scutal patterning in the different populations.

Population	P= % area of scutum which is patterned.
Harlech	15.9
D.ardudwy	14.2
Tywyn	14.8
Aberdyfi	14.4
Devon	16.8
France	17.6

where P =[(A2 + A3 + A4 + A5 + A6)/ A1] * 100

Population means of the variables were used.

DISCUSSION

There are significant differences between the population means of the discriminant functions in all but two cases, but there is also much individual variation so that we cannot have a high degree of confidence in identifying an individual as coming from a particular population, except when comparing the combined Welsh populations with the French (table 2.7). There is variation in the overall scutal shape variable A1 and the base pattern variables A2, A4, A5, A6 and P3. A1 is the variable most highly correlated with the first discriminant function. For a given scutal length, ticks from Harlech have a larger area than those from France. There is a latitudinal cline in A1 with larger values in the north and smaller values in the south. Conversely, ticks from Harlech generally have smaller residual values of A2, A5 and A6 than ticks from France. Thus, for a given scutal length, the area of base pattern in A2, A5 and A6 is less in Harlech than in France. From table 2.14 we see that the ticks from Wales have a smaller base patterned area of scutum than those from France i.e. the Welsh ticks are paler. A similar trend was found in *Dermacentor albipictus* in N.America with paler ticks in the north and west and darker (more base patterned area) ticks in the south (Cooley 1938, Ernst and Gladney 1975). The two forms were initially thought to be 2 separate species.

The plots of the group centroids in fig. 2.3 shows a latitudinal morphological cline with the Welsh populations grouped together, clearly separated from the French population with the Devonian population lying in an intermediate position. Clines are the result of either selection or drift. In the case of selection, then different selective regimes may occur in different habitats and if movement between geographic areas is restricted then significant genetic differences may develop between the populations. The alternative process which might generate clinal variation is drift. Drift is a random process which operates especially in small populations. In small populations the gene frequency will drift with time and

genes may be lost leading to a reduction in genetic variability. One aspect of this process is that the gene frequencies of a number of small populations formed from a single large one will diverge due to genetic drift. A special type of drift is known as the founder effect, where founder individuals of a new population are few in number and represent only a limited part of the variation present in the parental population.

Both selection and drift are counteracted by the effects of migration which tends to average out genetic differences between different populations by mixing.

The latitudinal cline shown in fig. 2.3 may be the result of:-

i) an environmental effect which is correlated with latitude e.g. temperature, which has a selective genetic or purely phenotypic effect on morphology.

ii) Different selective regimes acting in different areas of the ticks range so that as the tick spread northwards through host movements a clinal pattern of variation is generated as populations separate off from one another.

iii) The variation may have been produced by genetic drift. Also, if the movement of migratory birds or the shipment of cattle had introduced a small number of ticks into an area, then the founder effect may have operated.

iv) The tick may have a chaotic distribution in which case any patterns of geographic variation are produced by chance.

The data presented do not give a firm indication of which of the above cases is correct. In case i) I would expect the Welsh populations to resemble those of northern and central Europe i.e. populations on the same latitude. In case ii) I might expect the ticks to resemble those in Devon and France particularly those found along the Atlantic coast of France. This is assuming that the species spread into Europe from the east as is suggested by Berdyev (1989). In case iii) if drift has acted it may be difficult to ascertain which were the parental populations though they may remain more similar to their population of origin than to other populations across the ticks range. In case iv) I would not be able to predict which populations the Welsh populations would resemble.

Considering the 4 Welsh populations alone, there appears to be considerable variation,

but mainly in relation to discriminant function 2. Thus, the 4 Welsh populations are approximately equidistant from the Devon and French populations along the cline shown in fig 2.3. This does not give any clear indication of the origin and spread of the Welsh populations and there is no evidence of any of the Welsh populations being a good candidate as a founder site (i.e. closer to Devon or France). However, the overall similarity of the Welsh populations argues against multiple introductions and favours the idea of a single colonisation event. There is no north - south cline within the Welsh populations sampled. The intermediate position of the Devon population between Wales and France suggests perhaps that Devon is the most likely source of origin of the Welsh populations.

Within Britain, there appears to be 2 centres of population, one along the west coast of Wales and one in S.W.England. The other sites can be considered to be the result of cattle droving activities from Wales (Martyn 1988) and S.W.England to markets in London or chance records on humans and dogs, probably picked up in Wales or S.W.England (records courtesy of BRC, Monk's Wood). Having discussed the observed pattern of clinal variation I now offer three alternative hypotheses to account for the occurrence of *D. reticulatus* in Wales as follows.

i) The British distribution, which represents the extreme north-westerly distribution of the species in Europe, has resulted from our past connection with the continent before the land bridge was breached 9,000 years ago. This distribution can be considered similar to that of the Lusitanian floral distribution. These plant species are found in S.W.Britain and Ireland and have affinities with the flora of the Iberian peninsula. It is thought that these plant species spread from Spain and Portugal up the Atlantic seaboard of Europe in post-glacial times and they were subsequently cut-off by rising sea-levels (Barry Cox and Moore 1973). Thus, *D. reticulatus* could be considered to be a relictspecies. It is interesting to note at this point that one of the species with such a Lusitanian distribution is the Sharp rush (*Juncus acutus*) whose most northerly population is found at Morfa Harlech (Perring and Walters 1962). The dune systems along the west coast have been in existence for

several thousand years, though not in their present position. The tick may have spread through host movements across the land bridge, since in general, mammalian ectoparasites depend on their hosts for dispersion (Janzen 1985). Thus, the Devon populations may be related to one another and the Welsh populations may have been established from founders from Devon. The resulting differences between Welsh, Devon and continental populations would then come about by genetic drift.

ii) Britain may have been colonised in more recent times by the movement of migratory birds. Birds are known agents in the establishment of ticks in new biotopes (Daniel et al. 1977a) and the transference of tick-borne diseases to new areas (Kovaleicik 1983). Immatures of *D. reticulatus* have been recorded on migrant birds on Bardsey Is, Skokholm (Thompson 1964) and Lundy Is were nymphs were recorded on a Meadow pipit (Thompson and Arthur 1955). Thompson (1967) suggests that *D. reticulatus* was introduced into Britain by migrant birds. However, Hoogstraal and Aeschlimann (1982) state that immature *Dermacentor* rarely parasitise birds and adults never do so. Having said this I think it is clear from the bird observatory records that birds can act in introducing this species to this country. The tick could then have spread from a number of different sources (different founders) via cattle movements. Trade in cattle between Wales and S.W.England is likely to have been limited as both areas traditionally supplied cattle to the towns and cities of England, especially London. Having said this there may have been a limited exchange of breeding stock. However it is easy to imagine an exchange of cattle between farms in the Cardigan Bay area e.g. the exchange of breeding stock between farms, particularly as the farms on the four Welsh sites in this study all lie in what was once Merionethshire and would use the same market at the county town of Dolgellau. In this case we would expect neighbouring populations to be markedly similar in comparison to more distant populations. That this species is a recent coloniser may be supported by early records of the tick in Britain, the first record being Pocock (1900) who found the tick near Revelstoke, Devon. The author reports that the tick was unknown in the area 15 years earlier. Whelar (1906) considered it to be "most probably an imported species". There is

also evidence that the tick is spreading into new areas on the continent e.g. in the Netherlands (Uilenburg 1984).

iii). There is also the combination of the above two alternatives, whereby the tick is a long established relict species and there is a significant input into the gene pools of various populations via ticks brought in by migratory birds.

It is not possible to draw definite conclusions from the data. However, the Welsh populations are grouped together suggesting a single origin rather than multiple introductions. It cannot be distinguished whether the populations are relict or recent. If there were both relict and recent populations occurring allopatrically in Wales, then we would expect some populations to be dramatically more similar to the French or Devon populations. The data tends to suggest a Devon rather than French origin for the Welsh populations.

If we look at body size variation in the different populations using population mean scutal area (A1) values (table 2.13) as a body size variable, we see that there is no north -south cline. The values of A1 are larger in the Welsh populations than in the Devonian, but smaller than the French. Thomas (1968) found a latitudinal cline in body size in the rabbit tick *Haemaphysalis leporispalustris* with larger body sizes found at higher latitudes (colder climates). This variation was thought to be the result of the ticks need to hibernate in colder climates which requires a larger fat store and so larger body size. That there is no obvious north - south cline in body size may possibly be due to the confounding differences in climate which are not correlated with latitude. The winter climate experienced in S.E.France is more severe than that experienced on the west coast of Wales and it is known that *D. reticulatus* is inactive for a short period in France (Gilot *et al.* 1973), though the ticks may appear in warmer weather in winter (Martinod and Gilot 1991).

Immler (1973) made a study of morphological variation in *D. reticulatus* and found a remarkably uniform morphology, though no multivariate analysis was carried out and no

measurements made of scutal shape and pattern. A recent study has investigated geographic variation in the morphology of *Dermacentor marginatus* (Estrada-Peña and Estrada-Peña 1992). They conducted a Principal axis factor analysis (PAFA) of a range of morphological features including the scutal spots, but direct measurements of the scutal spots were not made, instead they were fitted to different arbitrarily defined categories. Cladistic analysis suggested the existence of 5 different geographic groups, an eastern group (specimens from Iran, Lebanon and the Soviet Union), a central group (Albania, Switzerland, Czechoslovakia, Poland and Romania), a northern group (temperate Europe, France and northern Spain), a Mediterranean group (Italy, E.France and most of Spain) and western group (Africa and W.Spain). There was clinal variation in the relative width of the internal and external spurs of coxa I and in females a comparable clinal variation in the measurements of gnathosoma, revealing a radiating evolution from the centre of Europe. However, the authors make it clear that this species is highly variable and that there was an absence of a clear separation into geographical groups as was shown by the close proximity of specimens from all geographical areas on the 1st principal axis of the PAFA. Thus, the geographic origin and dispersion of the species was highly speculative. The authors also mention that in the past, the dorsal scutal pattern has been used to separate *Dermacentor niveus* and *D. marginatus*. However, in their study the presence and arrangement of spots of the scutal pattern did not follow any geographical trend and was not correlated with other anatomical structures.

Apart from these studies, Bull (1985) investigated geographic variation in *Aponomma hydrosauri* using discriminant analysis and McEnroe (1974a) looked at size variation in *D. variabilis*. Bull (1985) found 3 groupings, ticks from W.Australia being separated from those in S.Eastern and South-Central regions by a discrimination function with a major contribution from a body length factor. The S.Eastern and South-Central regions were separated from each other by a function with a major contribution from a body width factor. Bull suggests that the variation in body size may have been due to adaptations to host species, or climate or environmentally-induced phenotypic differences

with no genetic basis. McEnroe (1974a) found size variation in *D. variabilis* using a scutal index (product of scutal length and width). He found an ecological cline along a gradient from the ticks long established populations on offshore islands to recent inland populations. On finding that female size increases along the cline from offshore to inland with a corresponding decrease in male size, he suggests that this change in sexual dimorphism is due to selective forces rather than random effects. However, McEnroe has assumed that " the ticks requirement for 3 blood meals imposes a uniform nutritional basis for the phenotype and this maximises the genetic expression for morphological characters". The findings of Newson and Chiera (1987) and Koch (1986) differ from this view.

By the use of residuals, environmental influences on the development and the resulting size of ticks will most probably have been removed. In ticks such environmental influences may include host resistance and climate. Newson and Chiera (1987) have shown an inverse relationship between scutal size and host resistance in *Rhipicephalus appendiculatus*. Similarly, Koch (1986) found a close correlation between resulting size and engorged weight of larvae and nymphs of *Amblyomma americanum*. Also, Nosek (1979) reports that in both *Dermacentor marginatus* and *D. reticulatus*, adults which hatched in the spring were smaller in size. This reduction in size was attributed to incomplete engorgement in the nymphal stage. Variation has been observed in the size of ticks of the genera *Rhipicephalus* and *Hyalomma* (Pervomajskij 1953). In this study, although there may possibly be environmental influences on scutal shape and pattern, it is probable that the observed variation has a genetic basis. The observations of Cooley (1938) in other species of *Dermacentor* suggest a genetic basis to the scutal pattern. In adults of *D. andersoni* reared from nymphs that fed on an unsuitable host (chipmunks), the scutal pattern was missing or much reduced. These ticks were also very small. However, the usual colour pattern and morphology was produced within one generation of this strain being reared in the laboratory. This suggests that the patterning has a genetic basis which may be

influenced by environmental effects such as host-mediated responses. The use of residuals would not help if individuals were present whose pattern had been modified in this way. However,such individuals are likely to be very small and so could be discounted from samples at the start, or they would appear as anomalous outlying individuals and discounted as such.

It has not been shown whether this variation is affected by selective forces or is essentially a neutral feature affected by random effects such as founder effects, migration and drift. It is possible that the scutal pattern has adaptive value, for example the dark patterned areas may enhance radiative heat exchange (Hamilton 1973). If this were the case then I would expect to find darker ticks in the north than in the south. It may be that the dark areas offer protection from radiation, in which case I would expect to find darker ticks in the south, but the radiation protection hypothesis is not widely supported (Hamilton 1973). It may well be the case that the colouration is caused by genes linked to some other underlying physiological function which are maintained by selection (Mayr 1963). It is possible that the colouration has a cryptic function , I have certainly noticed that ticks I have observed questing in the field, particularly males, are very difficult to pick out against the vegetation to which they cling. This cryptic function may be particularly important to males which may remain on their hosts for some time and may be vulnerable to predators such as birds. Starlings (*Sturnus vulgaris*) have been observed feeding on ticks on cattle in Australia (Wilkinson 1970), and this species was seen on the backs of cattle at Harlech.

This study has confirmed the existence of shape (as opposed to size) variation in *D. reticulatus*. It has not been possible to account for the distribution through the interpretation of this geographic variation due to the limitations of the study (small sample sizes from a small number of populations). It would be worthwhile to consider larger samples from populations over the range of *D. reticulatus'* distribution to see how shape and pattern varied and from this how the populations relate to one another. I would be particularly interested in comparing specimens from the Atlantic coast of France where the

tick exists in a dune habitat similar to that occupied in Wales. There are potential future applications of this type of study. Ultimately, if one is trying to investigate the distribution of a species particularly in a dynamic situation where the species is undergoing an expansion of range, then direct genetic analysis of the DNA or isoenzyme/allozyme electrophoresis is most applicable, the latter being attempted in the next chapter. However, the costs of these techniques may be prohibitive especially in developing nations, so this type of study of morphometric analysis may provide an adequate alternative. This type of analysis could be conducted on other species of *Dermacentor*. Much variation has been observed in the scutal pattern of N.American *Dermacentor* and *D. reticulatus* is thought to belong to this group (Cooley 1938). Two of these species are important vectors of Rocky Mountain spotted fever, *D. variabilis* in eastern USA and *D. andersoni* in western USA. Similarly, *D. marginatus* in the Palaearctic exhibits variation in the scutal pattern, particularly in males (Estrada-Peña and Estrada-Peña 1991a, b, 1992) and this species is a known reservoir and vector of tick-borne encephalitis (TBE) in Central Europe (Nosek 1972).It need not, of course, be applied to patterns only as it is equally valid for more standard morphometric characters such as limb measurements.

CHAPTER 3

A PRELIMINARY ANALYSIS OF ALLOZYME VARIATION IN *DERMACENTOR RETICULATUS*

INTRODUCTION.

The tick *Dermacentor reticulatus* has a markedly Western distribution in Britain and a disjunct distribution across Europe as far as Western Siberia. The distribution of the tick in Britain is shown in fig. 1.1. To account for this geographic distribution we would have to determine how the populations are related to one another. The last chapter determined the variation and relatedness using the scutal pattern of the tick. In this chapter the genetic relatedness of populations is measured.

The technique most commonly used to measure genetic variation, and thus genetic relatedness, within and between populations is electrophoresis (see materials and methods). This technique can be used to detect the presence of allozymes, allozymes being the forms of an enzyme that are coded for by alternative alleles at the same locus (Prakash *et al.* 1969).

The genetic data obtained can be used to compare the British populations with those on the continent to suggest how the British distribution evolved e.g. whether the present populations are the result of chance introductions or whether they are relic populations from a previously more continuous distribution. Genetic distances between the populations can be calculated using the allelic frequencies at loci in the different populations (Nei 1972). Discriminant analysis of the allelic frequency data in conjunction with a phenetic analysis of the genetic distance data could then be conducted to show the relatedness of the different populations. Unfortunately in this study the sample sizes are small and more importantly the number of loci screened for variation is very small.It has been shown by Gorman and Renzi (1979) that genetic distance estimates are severely affected by the number of loci sampled in particular. Hence, I could not really justify conducting further discriminant or phenetic analysis on this data.

However, it was possible to compare the pattern of variation to that found in other studies

of ticks. Most studies on ticks to date have recorded a low level of genetic variation within and between populations (Hilburn and Sattler 1986a). However, according to Price's (1977) hypothesis, parasite populations should show reduced intrapopulation variation, but greater interpopulation variation than non-parasite populations i.e. show a more marked tendency to speciation.

The aims of this study are more modest than trying to account for the geographic distribution of this tick. This was not deemed possible given the limited availability of samples and the restrictive cost of screening more than a few enzymes. This study can, therefore, be viewed as a preliminary analysis. Given this, the aims of this study were:

i). To identify possible sources of variation.

ii) To compare the results to those obtained in other studies on genetic variation in ticks.

iii). To suggest further studies to elucidate the geographic distribution of *D. reticulatus*.

To do this I compared ticks from four Welsh populations and one population from S.E.France.

MATERIALS AND METHODS

Electrophoresis

I begin this section with a brief introduction to the rationale behind the technique of electrophoresis. Electrophoresis is the movement of charged particles under the influence of an electric field. All proteins have an electric charge determined by their amino-acid composition and the pH of the medium. Electrophoresis is conducted in a buffer or a series of buffers to stabilise the charge. In an electric field, a protein will move towards the oppositely charged pole at a rate proportional to the magnitude of its charge. So proteins with different charges will move at different rates and directions in electrophoresis. Also, the supporting medium (in this case starch) has a sieving effect so that if the proteins have the same charge but different sizes and shapes, then they will move at different rates. Electrophoretic mobility is used as an indicator of similarity of amino-acid composition when proteins are compared between individuals. The character examined is the mobility of a protein under a given set of electrophoretic conditions and if it exists in two states i.e. the same or different. The actual degree of mobility difference is difficult to interpret.

Polymorphism

Organisms which are diploid and sexually reproducing receive one complete set of chromosomes from each parent. The gene (or locus) which directs the synthesis of a polypeptide is composed of two parts or alleles. An allele is the corresponding base sequence on each member of the homologous chromosome pair, one allele comes from each parent. The two alleles code independently for the same polypeptide so that each directs the production of half the amount of that polypeptide. If one of the alleles contains a different codon, then the locus will produce two polypeptides which are

different from one another by an amino-acid substitution. When the most common allele at a locus is of a frequency of < 0.99, then that locus is said to be polymorphic (Ferguson 1980).

Within a species it is possible that there are a large number of alleles differing from each other by one or more bases. In each individual, two conditions are possible:

i) The alleles at a given locus are the same (homozygous).

ii) The alleles at a given locus are different (heterozygous).

So if at a particular locus we have two different alleles A_1 and A_2, then three possible genotypes can be produced A_1A_1, A_1A_2 and A_2A_2 (fig. 3.1 a). We assume that the alleles are co-dominant (both equally expressed). If the proteins coded for by alleles A_1 and A_2 differ by a single amino-acid and this results in a change in electric charge or configuration of the polypeptide, then they will have different electrophoretic mobilities. So electrophoresis provides a convenient method of determining genetic variation at structural loci. The above example is the most simplistic situation. If there are 3 alleles, A_1, A_2 and A_3, then six patterns may be formed corresponding with the A_1A_1A, A_2A_2 and A_3A_3 homozygotes and the A_1A_2, A_1A_3 and A_2A_3 heterozygotes (fig. 3.1 b). If the protein is a monomer i.e. it consists of a single polypeptide subunit, then 2 bands are usually formed in the heterozygote. The enzyme may consist of more than one polypeptide chain which combine to form an enzymatically active multimer. In these multimers the sub-units may be identical in which case the molecule is called a homopolymer or the sub-units may differ (i.e. if they are the product of different alleles or different loci) whereby the molecule produced is known as a heteropolymer. The zones of activity produced on a gel when sub-units of different charges (due to allelic variation) are shown in fig. 3.1 c and d. In a dimer (fig. 3.1c) there are three zones of activity, two homopolymers (A_1A_1 and A_2A_2) and a heteroploymer (A_1A_2). In a trimer, two homopolymers ($A_1A_1A_1$ and $A_2A_2A_2$) and two hetropolymers ($A_1A_1A_2$ and $A_1A_2A_2$) and in a tetramer (fig. 3.1d) there are two homopolymers ($A_1A_1A_1A_1$ and $A_2A_2A_2A_2$) and three heteropolymers ($A_1A_1A_1A_2$,

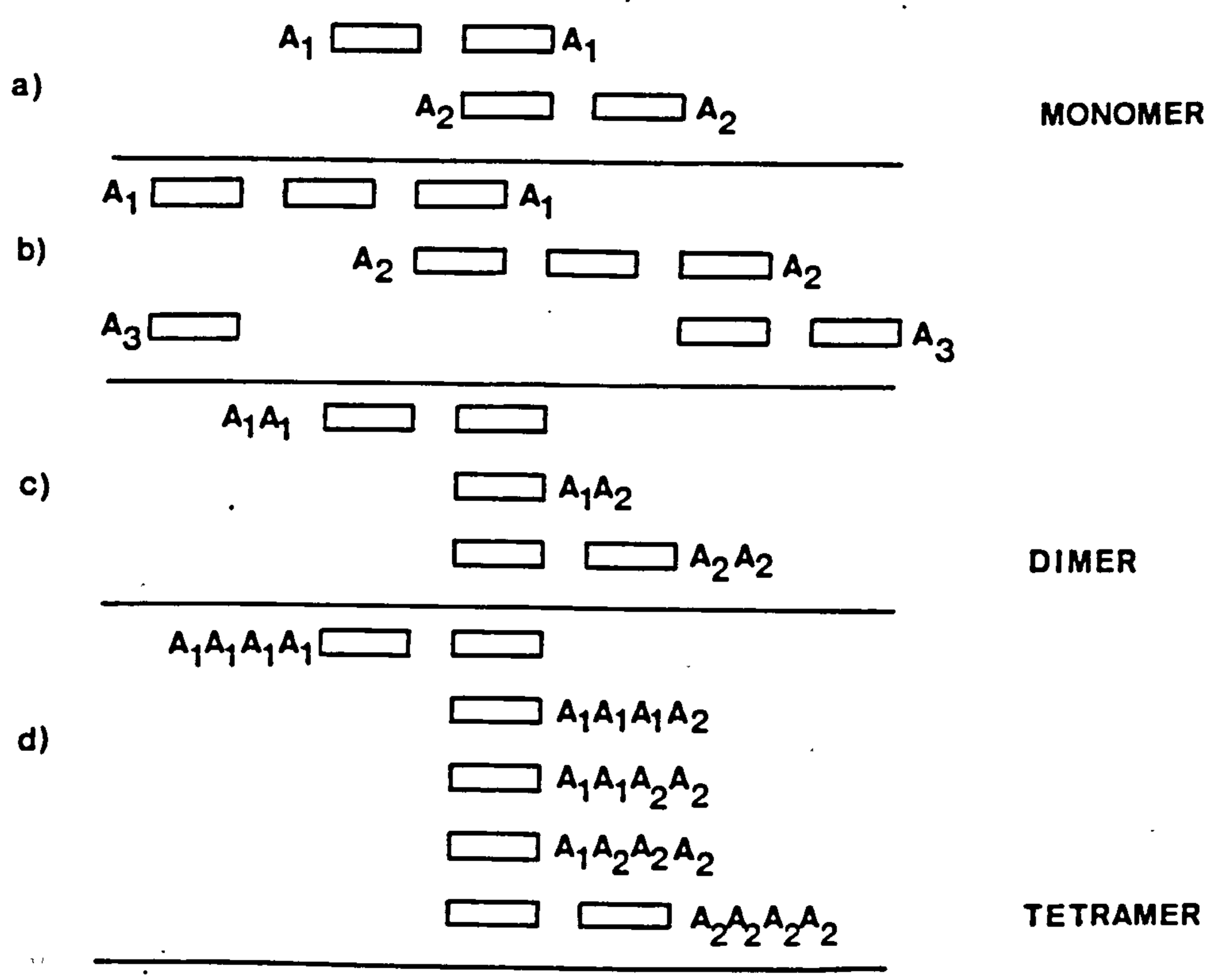


Fig. 3.1: Patterns of protein polymorphism : a) two co-dominant alleles - monomeric protein; b) three co-dominant alleles - monomeric protein; c) two co-dominant alleles - dimeric protein; d) two co-dominant alleles - tetrameric protein (redrawn from Ferguson 1980).

$A_1A_1A_2A_2$ and $A_1A_2A_2A_2$). For a more thorough explanation of electrophoresis and polymorphism see Richardson *et al.* (1986) and Ferguson (1980).

Electrophoretic techniques

a) Preparation of material:

Whole adult male and female ticks were ground up in homogenising buffer (see later for specific buffer system) using a ground glass rod and sterile sand placed in a small depression on a plastic plate. Although some particles of the chitinous exoskeleton remained, sufficient material was extracted from an individual tick. Small strips of chromatography paper were then soaked in the homogenising buffer to absorb the tick extract. Prior to analysis the ticks had been frozen in liquid nitrogen and then stored at -70°C. Each tick was ground up in 15μl of homogenising buffer for buffer systems 1, 4 and 5 see below) where two extracts were taken (for two gels). For buffer systems 1, 2 and 3 (later comparison) and for the final run (system 3) 10μl of homogenising buffer was used as only one extract was taken.

b) Preparation of starch gels:

The procedure used here was starch-gel electrophoresis. This enables several enzymes to be tested (see later). All electrophoresis reported here was conducted on 13% starch gels. The gels were made with 715g hydrolysed starch/ 550 ml gel buffer. The gels were cooked using a heating mantle and stirrer, once prepared they were poured into perspex frames and left to stand for 1 hour before being wrapped in cling-film. They were then allowed to stand for a further 2 hours and were then placed in the fridge (4°C) at least 1 hour before electrophoresis commenced.

c) Insertion of material:

The gels were cut from their perspex frames by running a scalpel around the rim. Then using a scalpel and ruler, a line was cut through the gel towards one end of the gel, this acts as the origin. The two parts of the gel can be teased apart and the strips of chromatography paper containing the tick extracts inserted. In this study up to 15 individuals were put on a single gel.

d) Horizontal starch-gel electrophoresis:

The set up is outlined in fig 3.2. A continuous buffer system was used i.e. the gel buffer was the same as that used as the electrode buffer (though usually more dilute). Sponge wicks connect the electrode buffer to the starch gel. An electric current is run through the system for a particular length of time depending on gel/buffer system being used. The whole procedure was carried out at 4°C. After the electrophoresis run, the inserts are removed and the gel sliced into thinner slices, each of which can be stained for a different enzyme.

e) Preliminary screening:

The following enzymes were investigated:-

α-glycerophosphate dehydrogenase (α-GPDH)

Hexokinase

Isocitrate dehydrogenase (ICD)

Malic dehydrogenase (MDH)

6-Phosphogluconate dehydrogenase (6-PGDH)

Phosphoglucomutase (PGM)

Phosphoglucoisomerase (PGI)

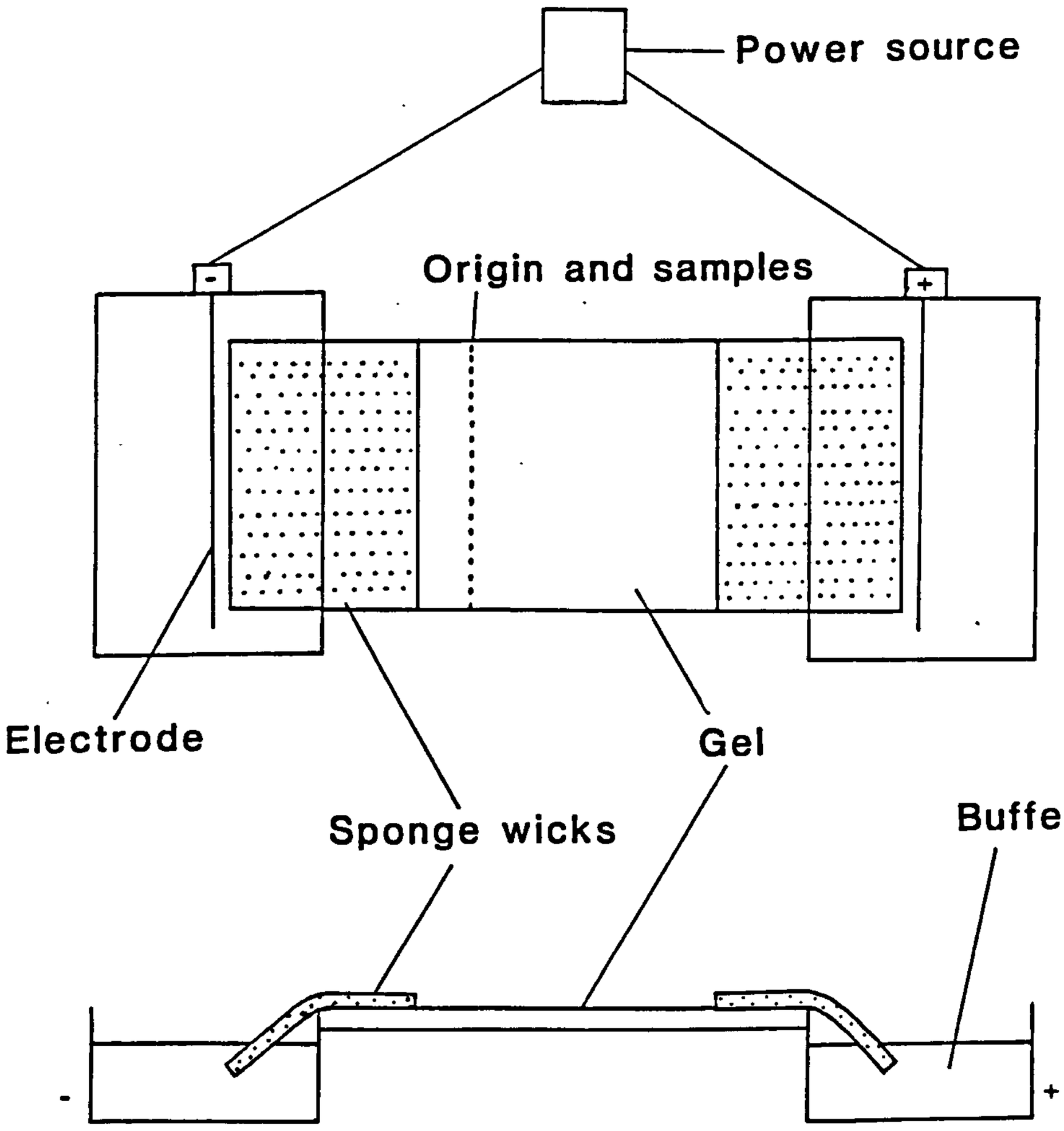


Fig. 3.2: Diagrammatic representation of the equipment for starch-gel electrophoresis.

and the following buffer systems were tried

1. Tris-Maleic pH 7.9

electrode buffer: 0.1M Tris-Maleic pH 7.9

gel buffer: 1:10 electrode buffer

homogenising buffer: 1/10 gel buffer + 10mg/ml NADP

run at 15mA per gel for 16 hours

Enzymes stained for α-GPDH, Hexokinase, ICD, MDH, 6-PGDH, PGM and PGI.

2. 0.1M Tris-maleic pH 6.9

as for 1). except for pH and enzymes stained for were ICD and PGM.

3. 0.1M Tris-maleic pH 8.9

procedure as for 2) except for pH.

4. Citrate morpholine pH 7.4

electrode buffer: Citrate morpholine pH 7.4

gel buffer: 1:9 electrode buffer

homogenising buffer: 50:50 mixture of gel buffer: B-mercaptoethanol / Triton-x-100 + 10mg/ml NADP

run at 35mA per gel for 3 hrs

Enzymes stained for ICD, MDH, 6-PGDH, PGM and PGI

5. Tris-citrate pH 8.0

electrode buffer: Tris-citrate pH 8.0

gel buffer: 1:25 electrode buffer

homogenising buffer: as in system 2

run at 35 mA per gel for 3 hrs

Enzymes stained for as above (system 2).

f) Staining procedure:

The enzymes stained for were listed previously. The recipes for these stains are given in the appendix. Once the gel has run and has been cut into the appropriate slices, the stains are made up. 25 ml of 0.1M Tris-HCl is added to each stain and they are mixed continuously on a magnetic stirrer. To this 25ml of agar (previously kept at 60°C) is added. The mixture is then immediately poured over the gel as evenly as possible. The gel is then placed in the dark to allow the stain to work and the gel is viewed after 1, 2 and 3 hours. Measurements of the distance bands have moved are taken and each gel is photographed.

The 0.1M Tris-maleic system was superior in resolving the bands than both Citrate morpholine and Tris-citrate. Of the enzymes, ICD and PGM looked the most promising in terms of the resolution of the bands and the detection of heterozygotes (ICD). These two enzymes were then stained for using the 0.1M Tris-maleic buffer system at pH 6.9 and pH 8.9. I decided that pH 8.9 gave slightly better resolution than pH 6.9 and 7.9. Therefore, this system was chosen for the main analysis. Individuals in this preliminary screening had come from Harlech, Tywyn and France and a total of 103 individuals were screened.

The results section concentrates wholly on the screening of individuals from 5 populations

for the enzymes ICD and PGM. The 5 populations compared and the number of individuals sampled are shown below:

Population	No. of ticks sampled
Harlech	22 (3 males 19 females)
D.ardudwy	20 (5 m 15 f)
Tywyn	25 (3 m 22 f)
Aberdyfi	22 (5 m 17 f)
Aveyron, (SE.France)	25 (8 m 17 f)

Unfortunately the sample sizes are small and unequal. I was restricted by the costs of the technique and the availability of ticks but had hoped to sample 25 individuals from each population. However, some individuals were lost on failed gels.

A standard was run on each of the gels consisting of an extract from an individual mussel (*Mytilus edulis*)in which all the enzymes studied were present. All bands can then be compared to the standard so that different gels can be compared to each other. Unfortunately, the standard did not stain on all the gels, but did provide a rough marker overall to aid in interpretation.

RESULTS

Isocitrate dehydrogenase (ICD):

Three alleles, a fast allele ICD^f, a medium speed allele ICD^m and a slow allele ICD^s where found in the ticks sampled. Fig. 3.3 gives a diagrammatic representation of the three genotypes. Plate 3.1 a, b are photographs of the gels showing individuals heterozygous for the f and m alleles and the m and s alleles and also individuals homozygous for the medium speed allele (mm). The heterozygotes conform to the typical banding pattern for a dimeric enzyme. ICD usually has a dimeric structure as has been found in other tick species (Healy 1976, Hilburn and Castillo 1986) and other studies (Manwell and Baker 1970 and Ayala *et al.* 1974)

Most of the ticks sampled were homozygous mm at this locus. Two individuals from Harlech and one individual from Aberdovey were fm heterozygotes and two individuals from France were ms heterozygotes. The allelic frequencies for each population are given in Table 3.1.

The genotype frequencies were tested to see if they were in Hardy-Weinberg equilibrium. This tests whether the bands counted can be considered as the product of a single locus. Also, if the populations deviate from the Hardy-Weinberg equilibrium then we can assume that one of the following forces are acting on the population; migration, genetic drift, mating choice, mutation and natural selection. The results for the variable populations are given in table 3.2 indicating that none of the populations differ significantly from the Hardy-Weinberg equilibrium i.e. the alleles observed are the products of a single locus and that none of the above forces are acting on the population.

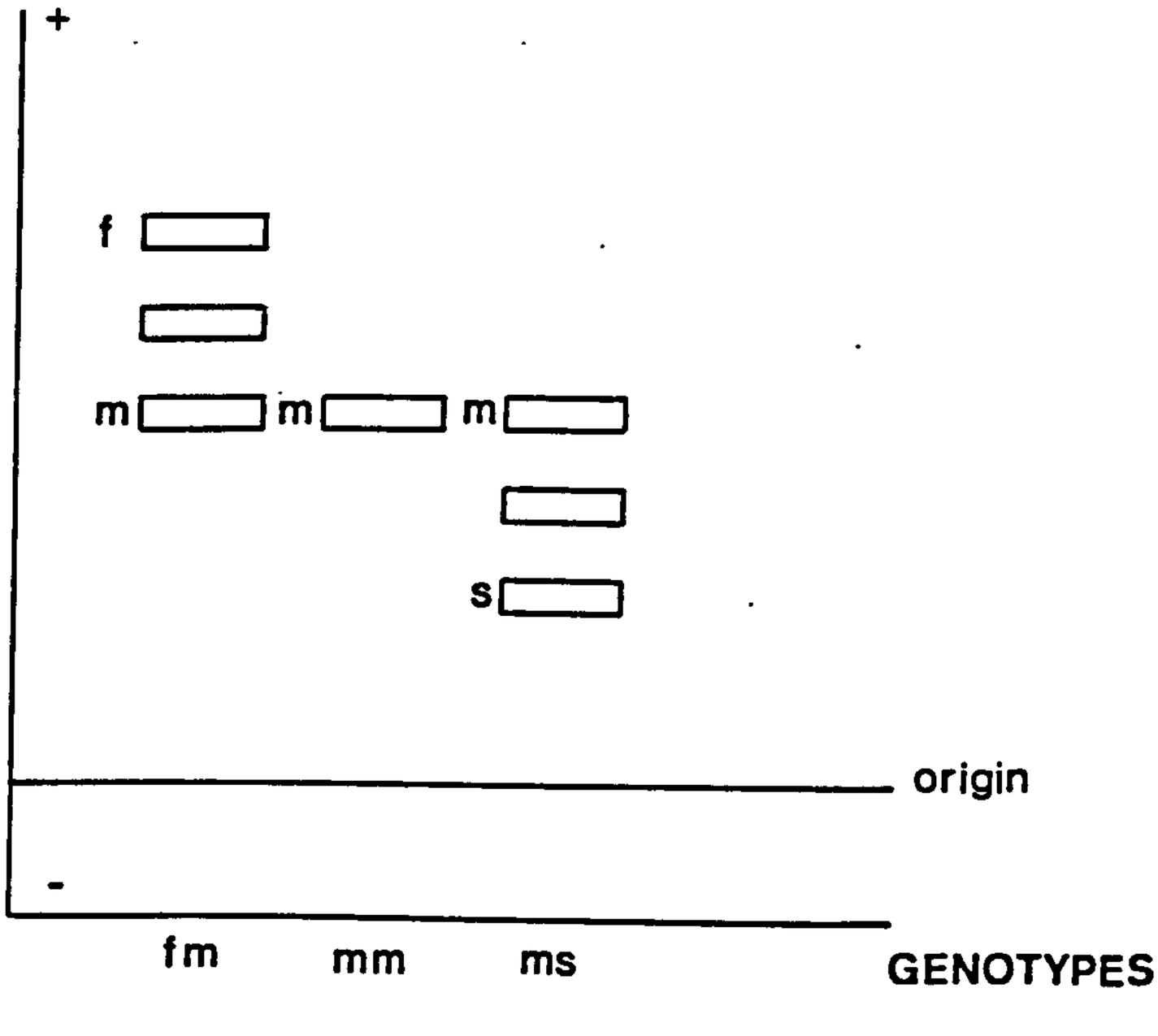
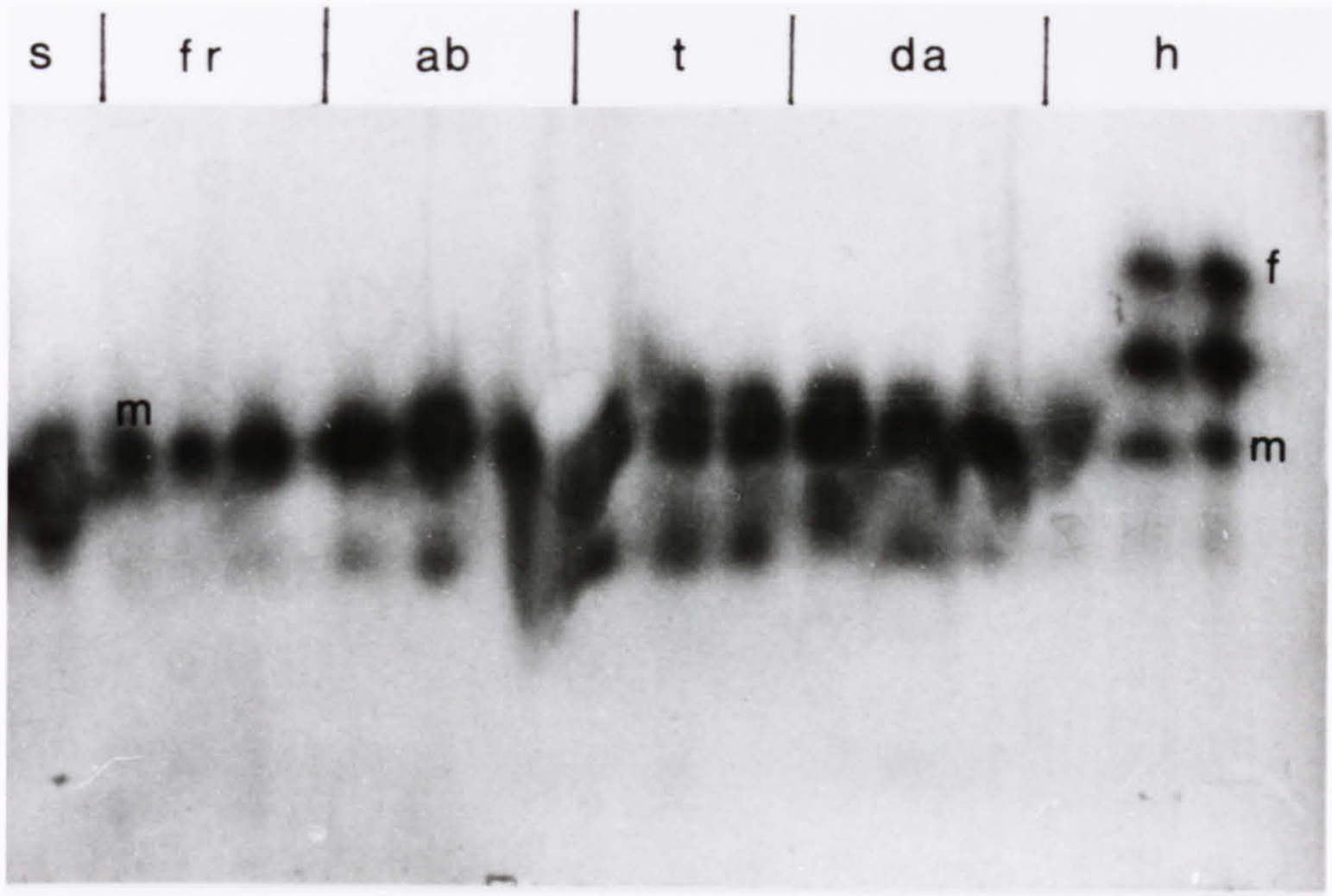


Fig. 3.3: Diagrammatic representation of the patterns produced in starch-gel electrophoresis by the 3 different alleles of Isocitrate dehydrogenase (ICD) found in *D. reticulatus*. The alleles are a fast allele (f) found in a few Welsh individuals (fm heterozygotes), a medium speed allele (m) found in most individuals (mm homozygotes) and a slow allele (s) found in a few French individuals (ms heterozygotes). (Extrapolated from plates 3.1 a, b)

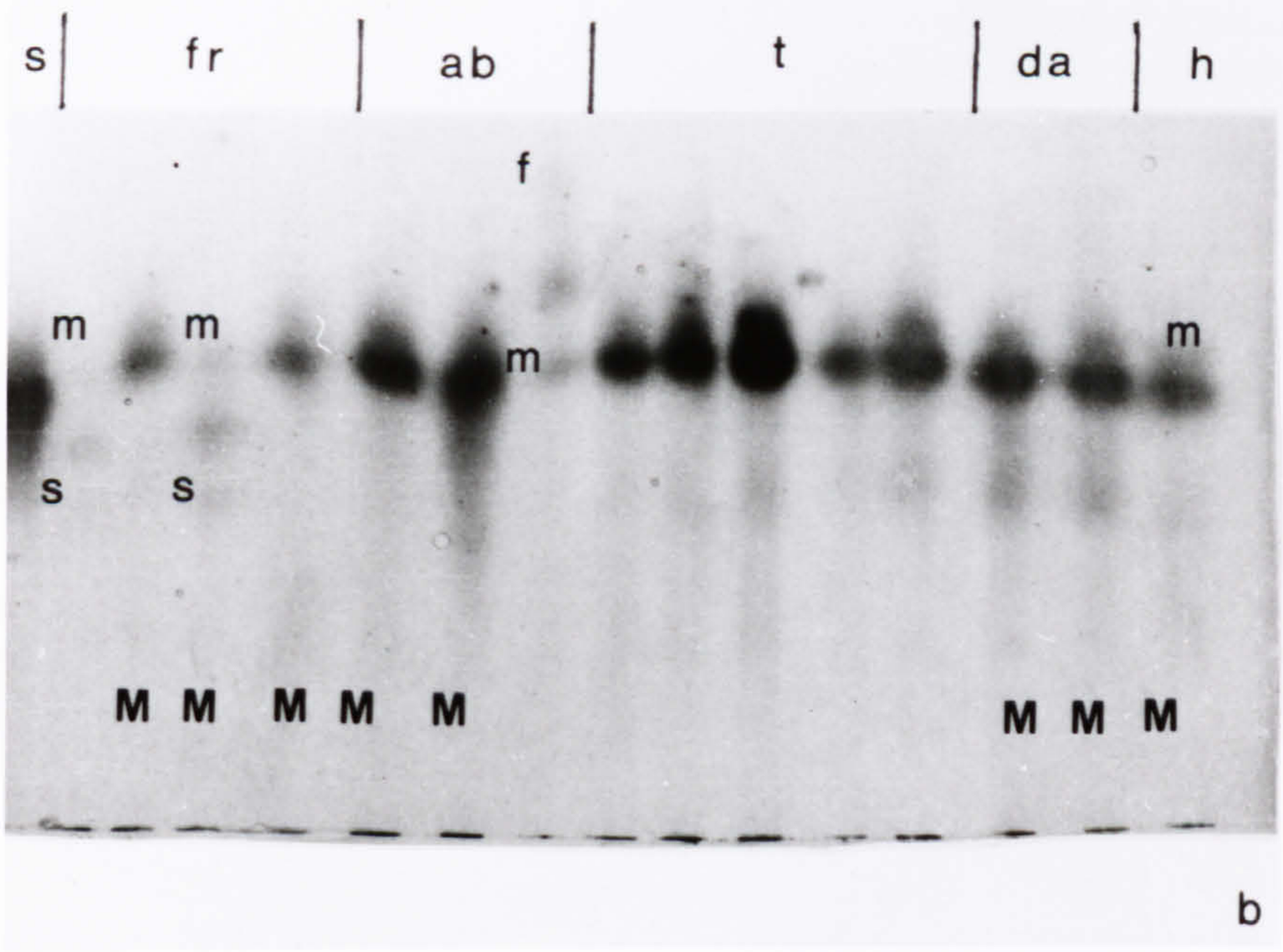
Plate 3.1 a: Gel showing the presence in Isocitrate dehydrogenase (ICD) of f m heterozygotes in two individuals from Harlech and m m homozygotes in all other individuals. All individuals were female.

b: Gel showing the presence in ICD of ms heterozygotes in two French individuals and a f m heterozygote in one individual from Aberdyfi. The other individuals were m m homozygotes. Male individuals are marked M.

Key:
h - Harlech
da - Dyffryn ardudwy
t - Tywyn
ab - Aberdyfi
fr - France
s - Mussel standard



a



b

Table 3.1: Allelic frequencies recorded at an isocitrate dehydrogenase locus in 5 populations of *D.reticulatus*.

	Population				
Allele	H	Da	T	Ab	Fr
ICD^f	0.045	0.0	0.0	0.023	0.0
ICD^m	0.955	1.0	1.0	0.977	0.96
ICD^s	0.0	0.0	0.0	0.0	0.04

Table 3.2 : Test for Hardy-Weinberg equilibrium in the variable populations.

Population	G	d.f	sig.
Harlech	0.254	1	ns
Aberdovey	0.04	1	ns
France	0.08	1	ns

In a two allele system e.g. allele A and allele B, if the frequency of allele A = p, and the frequency of allele B = q, then if the population is in Hardy-Weinberg equilibrium, the frequencies of genotypes AA, AB and BB are :-

$$p^2 + 2pq + q^2 = 1$$
$$(AA)\ (AB)\ (BB)$$

The genotype frequencies expected by Hardy-Weinberg equilibrium can be compared to those observed in the population using the log-likelihood x^2 test (G-test)(Sokal and Rohlf 1969):

$$G = 2\ \Sigma(\ Obs\ ln\ (Obs)/(Exp))$$

$$= 2\ [\ \ \Sigma(Obs\ ln\ Obs) - (2.30259)\ \Sigma(Obs\ log\ Exp)\ ]$$

Phosphoglucomutase (PGM):

The banding pattern for the majority of the ticks sampled is shown in fig. 3.4 (a) and plate 3.2. In some specimens, 1 from Harlech, 8 from D.ardudwy, 8 from Tywyn and 4 from Aberdovey, the banding pattern is that shown in fig. 3.4 (b) and plate 3.2. The differences is the presence of band E in these specimens. Bands C, D and E are likely to be satellite bands (Lush 1969, Markert and Whitt 1969). They are secondary modifications of the enzyme. It is not possible to interpret these banding patterns in genetic terms, but is interesting to note that none of the variants shown in fig. 3.4 (b) occurred in the French population.

It is possible that some of the French ticks do not possess band B, as shown in figs. 3.4 (c) and plate 3.2. If this is the case then it is likely that bands A and B represent two different loci with B (PGM-2) locus being absent from some French individuals. An alternative, more unlikely, scenario is that most of the ticks sampled are heterozygous at this PGM locus with a small number of individuals from France which are homozygous at this locus.

There is insufficient evidence of variation to warrant further analysis of this data. In summary, it is possible that some French specimens lack a locus common to all the Welsh and most of the French ticks. Also, there is variation in satellite bands, with further improvements in the resolution, more variation may be discovered. It is possible that these bands turn out to be other PGM loci. It's worth noting at this point that Healy (1979a) found much variation at a PGM locus in *Ixodes ricinus*, 10 alleles being identified.

All further analysis will be concerned solely with the variation found in ICD.

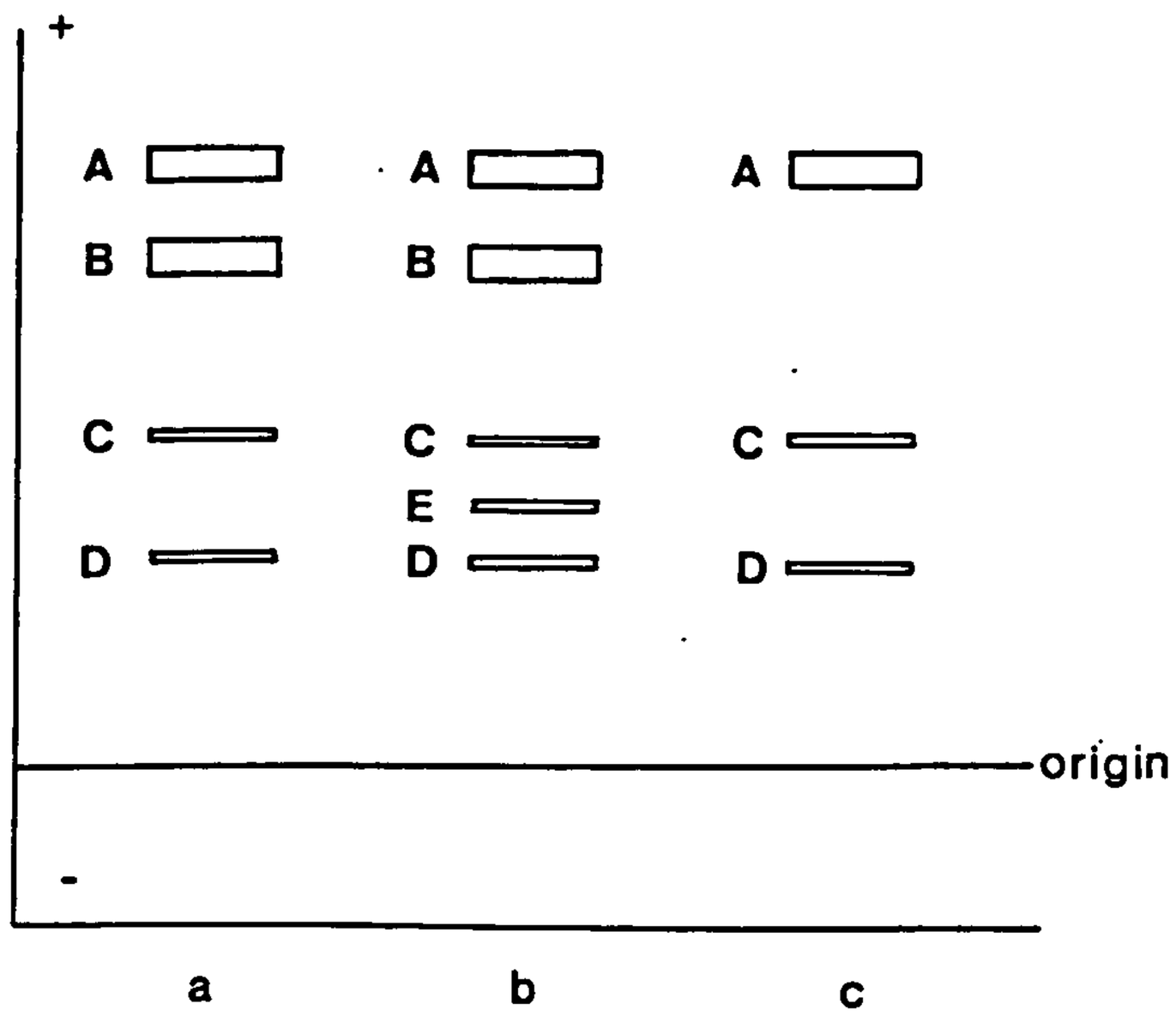
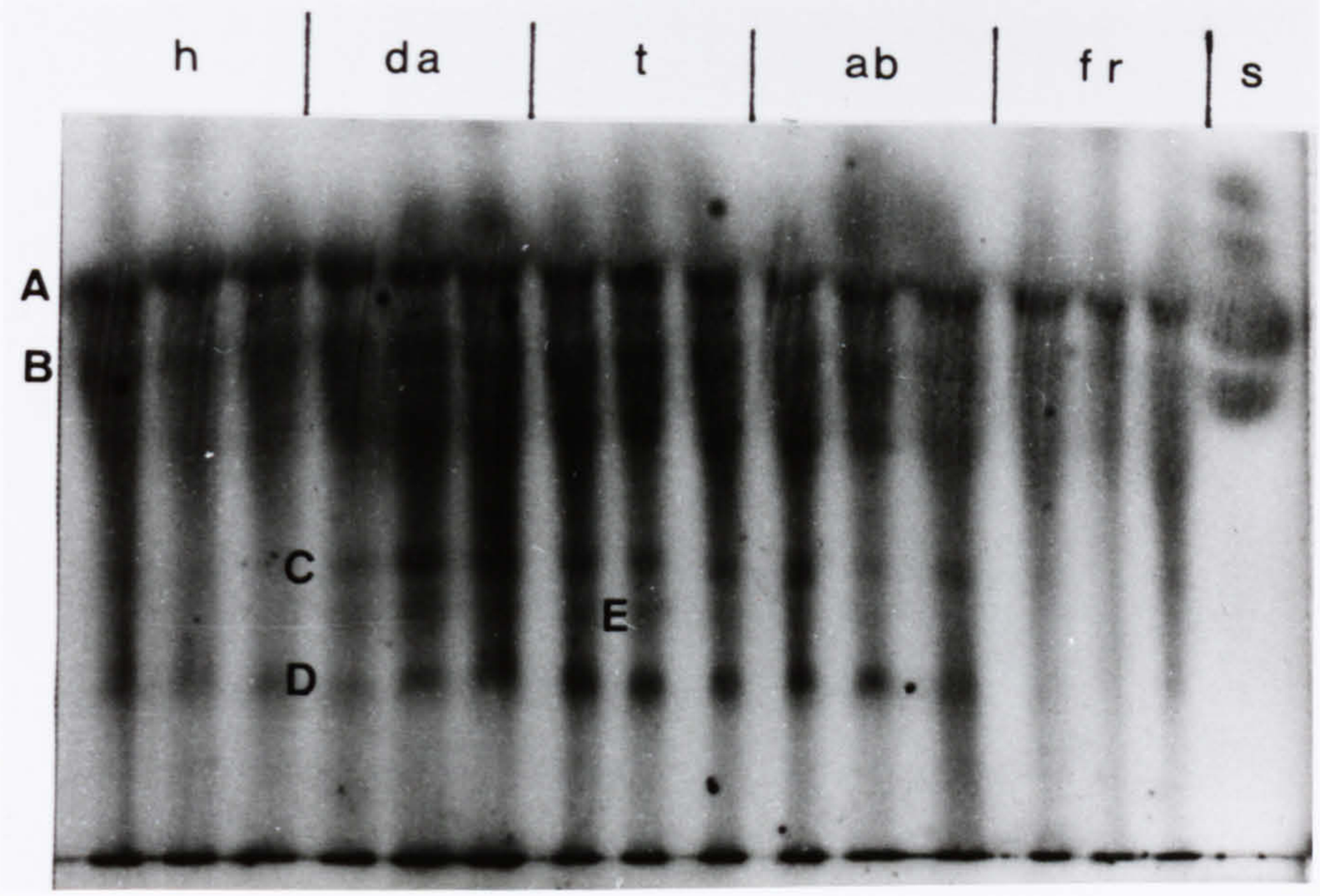


Fig. 3.4: Diagrammatic representation of the patterns produced in starch-gel electrophoresis by 3 possible phenotypes of Phosphoglucomutase (PGM) found in *D. reticulatus*. Most individuals showed the pattern of phenotype (a), but 1 individual from Harlech, 8 from Dyffryn ardudwy, 8 from Tywyn and 4 from Aberdyfi showed the pattern of phenotype (b). No French individual showed the pattern of phenotype (b), most were phenotype (a), but 6 individuals had phenotype c. The most likely explanation of the banding pattern was that bands A and B are two different PGM loci which are present in most individuals with the possible exception of a few French individuals. Bands C, D and E are probably satellite bands which cannot be interpreted genetically but which may be used to identify populations (see text for details).

Plate 3.2: Gel showing the presence in Phosphoglucomutase (PGM) of bands A and B in the Welsh populations and the absence of band B in the French ticks. The gel also shows the presence of satellite bands C, D and E.

Key:

h - Harlech
da - Dyffryn ardudwy
t - Tywyn
ab - Aberdyfi
fr - France
s - Mussel standard


h
da
t
ab
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s
A
B
C
D
E

Intra-population variability:

A measure of the amount of genetic variation in a population is the frequency of heterozygotes. The heterozygosity per locus (H) is calculated as follows:

$H = 1 - \Sigma x_i^2$ where x = the frequency of the i^{th} allele at a locus.

The heterozygosity in the three variable populations is given below:

Harlech	8.6%
Aberdyfi	4.5%
France	7.7%

2 populations were invariant and ,therefore, had heterozygosity levels of 0%.

Mean value for 5 populations = 4.16%

These values will be discussed further.

Inter-population variability:

Samples of a species taken from different areas may differ significantly in their allelic frequency. This could be due to selection for different homozygotes under varying environmental conditions (diversifying selection) or due to genetic drift in isolated populations.

A measure of the genetic variability between populations is given by Nei(1972), as genetic distance(D) and genetic identity(I)(Table 3.3). D gives an estimate of the average number of electrophoretically detectable allele substitutions accumulating in the genome since the two populations began to diverge. D ranges from 0(where populations are identical) to infinity(where populations share no common alleles). The values of D for

Table 3.3: Nei's genetic distance (D) between populations of D.reticulatus.

	H	Da	T	Ab	Fr
Harlech	-	0.001	0.001	0.0003	0.0016
D.ardudwy		-	0.0	0.0002	0.0008
Tywyn			-	0.0002	0.0008
Aberdyfi				-	0.0009
France					-

pairwise comparisons of the populations are given in Table 3.3. It is evident that the genetic distances between the populations are exceedingly small. However, the overall pattern of inter-population variability will be discussed further. The reduction in the number of loci that have identical or even similar allele frequencies is usually seen moving from geographic populations, through semi-species, to full species as shown in the *Drosophila willistoni* group (Ayala *et al.* 1974). The value for geographic populations was 0.031 ± 0.007, clearly the values in this study are considerably smaller.

DISCUSSION

The salient point to be made from the results is that we have evidence of variation in the enzyme Isocitrate dehydrogenase (ICD). Three alleles were detected ICD^f, ICD^m and ICD^s (fig. 3.3 and plate 3.1). The levels of heterozygosity within the populations appear low and the genetic distances between the populations are very low. However, one allele, ICD^f, was found only in Welsh populations (Harlech and Aberdyfi) and the allele ICD^s was found only in the French population. Most individuals from all the populations were homozygous for the ICD^m allele. The enzyme phosphoglucomutase (PGM) showed no obvious variation, though it is possible that one locus (band B, fig. 3.4 and plate 3.2) was lacking in some individuals from the French population. There was evidence of variation in what were probably satellite bands. One satellite band (band E, fig. 3.4 and plate 3.2) was only found in the Welsh populations.

It must be stressed that this study can only constitute a preliminary analysis given the small sample sizes and the small number of enzymes screened. From these results we cannot deduce how the distribution of *D. reticulatus* in Britain came about. Clearly there does not appear to be much variation within or between the 4 Welsh populations nor between these populations and the French. However, the study has been successful as far

as identifying a site of variation. As mentioned, it should have theoretically been possible to have conducted a discriminant analysis using the allelic frequencies of individuals from the different populations as raw data. The results would have been directly comparable with the results of the scutal pattern analysis. Such an analysis could have indicated which isozymes or allozymes were significant in separating the populations (indicated by their correlations with the discriminant functions - see table 2?2). This analysis would have been conducted in conjuntion with a phenetic analysis such as UPGMA (Unweighted pair group method of analysis) or cluster analysis, producing a cladogram. A recent example of the use of discriminant analysis of electrophoretic data is given Pigluicci *et al.* (1990) in a study on the within and among population genetic variability in *Ornithogalum montanum*.

The genetic distance measurements (table 3.3) must be viewed with some caution given the affects of both a small number of loci and small sample sizes (Gorman and Renzi 1979) and I considered that further analysis of this data would only lead to spurious results.

Bearing in mind the limitations of the data (small sample sizes, only two enzymes analysed), we can compare it to that reported in other studies on genetic variation in ticks. This consists of the work of Bull on the Australian reptile ticks (Bull *et al.* 1984), Healy (1976, 1979a, b) on *Ixodes ricinus*, and Hilburn and Sattler on *Boophilus* and *Amblyomma* species in the USA (Hilburn and Sattler 1986a, b, Hilburn, Gunn and Castillo 1989, Sattler et al 1986). The mean value of heterozygosity in 5 populations of *D. reticulatus* was 4.16%. The levels of heterozygosity recorded in other tick species is shown in table 3.4 and the genetic distances between conspecific populations of ticks are shown in table 3.4.

The level of heterozygosity (within population variability) found in *D. reticulatus* is similar to that found in most other studies on ticks with the exception of *Ixodes ricinus* (Healy 1979 a,b). In these studies on *I. ricinus*, though high levels of heterozygosity were recorded, there does not appear to be a heterozygous advantage, in fact in the case of α-GPDH female heterozygotes are more susceptible to desiccation. However, in variable environments heterozygotes, because they possess intermediate characteristics, will have a

Table 3.4: Levels of heterozygosity recorded in other species of tick.

Species	Heterozygosity (h, %)	references
Amblyomma limbatum	1.6	Bull *et al* (1984)
Aponomma undatum	0.0	" "
A.fimbriatum	1.7	" "
A.hydrosauri	2.5	" "
Amblyomma americanum	8.5	Hilburn and Sattler (1986b)
Boophilus microplus	9.2	Sattler *et al* (1986)
Ixodes ricinus	53.3 (α-GPDH) 66.9 (PGM)	Healy (1979a) Healy (1979b)
Ornithodorus erraticus	5.5 (depending 3.3 on enzyme)	Wallis and Miller (1983)
O.sonrai	2.7	" "
Average value for invertebrates	11.2	Nevo (1978)

lower fitness variance and, therefore, a greater geometric mean fitness than homozygotes (Gillespie and Langley 1974). Hilburn and Sattler (1986b) attributed these high levels of heterozygosity to the fact that Healy was dealing with the most polymorphic enzymes.

Considering the measurements of genetic distance (inter-population variability), they were very small in this study and as I have mentioned they must be viewed with caution. In other studies on ticks the genetic distances between conspecific populations were also small (table 3.5), smaller than the typical values recorded for *Drosophila sp.* i.e. 0.031 (Ayala *et al.* 1974). This suggests that there is reduced inter-population variability in ticks. Overall, *D. reticulatus* would appear to conform to the pattern found in the Australian reptile ticks and *Amblyomma* and *Boophilus* ticks in the USA.

How does this study and other studies on ticks conform to Price's (1977) model of genetic variation in parasites? To reiterate, Price predicted that parasite populations would be more likely to diverge from one another than non-parasite species given the low levels of dispersion between populations, the small population size of parasites and the development of reproductive systems that lead to reduced outbreeding. According to, this hypothesis parasites should exhibit low levels of genetic variation within populations and high levels of variation between populations. Though heterozygosity levels appear low (with the exception of Healy 1979a, b but see above), they are not significantly lower than that found in other invertebrates (Hilburn and Sattler 1986a). The levels of variation between populations are low and do not conform to Price's model.

Hilburn and Sattler (1986b) consider it inappropriate to apply Price's model to ticks. They suggest that the host specificity of the ticks, the host mobility and abundance are important factors in determining the genetic variation in ticks. For example, the Australian reptile ticks are highly host specific, have small population sizes and their hosts have small ranges. We might expect such ticks to diverge rapidly but this is not borne out by Bull *et al.* (1984). Their host specificity may impose a strong stabilising selection on their phenotype counteracting the effects of random drift. Ticks with more catholic tastes such as *Ixodes ricinus* are less likely to have the effect of such stabilising selection, but the

Table 3.5: Genetic distances recorded between conspecific populations of ticks.

Species	Genetic distance (D)	reference
Australian reptile ticks (see table 3.4)	0.06	Bull *et al* (1984)
Amblyomma americanum	0.008	Hilburn and Sattler(1986b)
A.cajennense	0.006	Hilburn *et al* (1989)
A.imitator	0.009	" "
Ornithodorus sonrai	0.017	" "
Example of another invertebrate		
Drosophila willistoni	0.031	Ayala *et al* (1974)

abundance and mobility of their hosts would lead to more gene flow between populations. As long as gene flow is not solely between near neighbouring populations, even a rare exchange of genes between populations will prevent significant divergence between populations (Crow and Kimura 1970). Oliver (1983) also suggests that the diversity of hosts and rapid dispersal of sheep and cattle stock allows much greater gene flow in *I. ricinus* than in the slow moving reptile hosts of *A. hydrosauri*. He concludes that ecological differences, host range and dispersal parameters play important roles in determining the levels of genetic variability between populations.

In the case of *D. reticulatus*, the tick has been found on a wide range of hosts as adults including cattle, sheep, goats, dogs and hares (Arthur 1963). However, within a local area, the immatures have been found to occur on one major host species, *Clethrionomys glareolus* (Immler 1973). The distribution from W.Siberia through to Wales is very disjunct, so even though the adults have large mobile hosts, there is a possibility that populations will remain isolated bar the possibility of introductions from migratory birds (though immatures rarely parasitise birds and adults never parasitise birds according to Hoogstraal and Aeschlimann (1982)) . The current distribution may represent a series of relict populations from a previously more continuous distribution or it may represent a more dynamic situation were the tick is undergoing range expansion e.g. in the Netherlands (Uilenburg 1984). The tick transmits *Babesia canis* to domestic dogs in France, so an understanding of the nature of the ticks distribution would be important for any future control strategies.

The potential variable marker identified in this study is ICD. This enzyme is involved in the tri-carboxylic acid cycle (TCA) which is an important oxidative pathway for aerobic organisms. It provides the complete intramitochondrial combustion of pyruvate to CO_2 and H_2O with a greater yield of energy than in glycolysis. The TCA cycle is also linked to lipid and protein metabolism. PGM is involved in the mobilisation of glycogen and in the oxidative reactions associated with glycolysis. Most enzymes studied are involved in

metabolic reaction. Allozymes produce enzymes of different catalytic properties. They be necessary, as are isoenzymes, to catalyse the same reaction under different catalytic conditions (Ferguson 1980). Given this they may be ideal for investigating geographic variation. Allelic variation in relation to environmental factors such as temperature have been shown e.g. Koehn and Rasmussen (1967) found a latitudinal cline in the frequency of an esterase allozyme in the fish *Catastomus clarkii* associated with a north-south temperature gradient. Temporal and spatial variation was found in the frequency of a α-GPDH allele in *Drosophila melanogaster* which was probably correlated with temperature (Berger 1971, Johnson and Schaffer 1973).

However, if ticks are constrained by a narrow range of environmental conditions in which they can exist both on and off-host, then we might not expect much variation in their physiology and thus in their metabolic enzymes. An alternative approach would be to look at variation in mitochondrial DNA (mtDNA) (Avise 1986, Avise *et al.* 1987). mtDNA is inherited almost entirely from the female parent and genetic polymorphism among conspecifics is extensive. It as assumed that mtDNA genotypes are effectively neutral markers of female lineages. This would remove "noise" created by adaptations when evolutionary relationships are being investigated. For many species the geographical spread of mtDNA genotypes by dispersal is not sufficient to mask the historical pattern of population subdivision revealed in mtDNA phylogeny reconstruction (Avise 1986). It may not be surprising that most species are geographically distinct populations, but this has not always been possible to demonstrate. For example, assemblages of the deer mouse *Peromyscus maniculatus* revealed by mtDNA showed no major differences in the frequency of allozymes (Avise et al 1979). Similarly, subgroups of *Aedes spp.* which did not exhibit significant divergence based on an analysis of their morphology and allozymes, did show significant divergence in their mtDNA (Kambhampatis and Rai 1991b).

Thus we see that there is a lot of scope for further work on the geographic distribution of the tick *D. reticulatus* either through allozyme analysis or perhaps more excitingly through mt DNA analysis.

CHAPTER 4

AN INVESTIGATION INTO THE SEASONAL ACTIVITY, HABITAT AND HOST ASSOCIATIONS OF *DERMACENTOR RETICULATUS* AT MORFA HARLECH

INTRODUCTION

In Wales, *Dermacentor reticulatus* is known to occur in the dune systems along the coast of Cardigan Bay (Mathias 1985, Martyn 1988). In the course of this study, adult ticks were found at Morfa Harlech, Morfa Dyfrynn, Tywyn (marsh next to the Dysynni estuary) and Aberdyfi dunes. These populations represent the extreme north-west of the ticks range. This dune habitat struck me as an unusual one in which to find ticks given the extremes in temperature and humidity experienced in the summer months (Ranwell 1972).

Much is known of the tick's seasonal activity throughout its range from France through to western Siberia (Szymanski 1987a) but little is known of its seasonal activity in Britain. Previous studies have shown that the tick exhibited geographic variation in seasonal activity (Szymanski 1987a). Such variation is likely to be correlated with the climatic conditions. At the eastern end of the range the ticks were active only during a brief spring (April-June) with the short autumn activity picking up almost immediately afterwards (July-Sept) with no activity through the rest of the year. At the western end of the range, the ticks were active for most of the year with a short summer diapause (2 months, June-Aug.) and a brief winter period of inactivity (1 month, Dec.-Jan) (Martinod and Gilot 1991) which does not qualify as a true diapause.

The tick also occurs in a range of different habitats such as river basins, swampy mixed woods, shrub pasture and lake side vegetation (Nosek 1972), dry riverine scrub (Immler 1973), alpine biotopes, agricultural land and suburban wasteland (Gilot *et al.* 1973, 1974) and forests and river basins (Szymanski 1986, 1987a). The habitat type will influence the microclimatic conditions and thus the activity and survival of the ticks. In addition, host availability and abundance will also affect tick survival and persistence in an area.

Morfa Harlech was chosen as the study site due to its proximity to Bangor and preliminary sampling suggested that there was a healthy population of *D. reticulatus*.

The initial aim of this study was to investigate the seasonal activity at Morfa Harlech and to relate it to the macro- and microclimatic conditions. The host relationships were also investigated. I hoped that such information would indicate how *D. reticulatus* persists in the dune environment. During the course of the study it transpired that the ticks occurred in a range of sub-habitats within the dune system and what's more they appeared to exhibit different seasonal activity patterns in these sub-habitats. A comparison between the activity patterns in these sub-habitats provided a way of investigating the affect of habitat/ microclimate on activity. I also decided to quantify the vegetation stands in order to identify the vegetation communities in which the tick occurred.

The investigation of the host relations proved more problematic. Cattle hosts were only monitored once as it was difficult to get access. The limited data on the cattle and rabbits on the dunes are presented. The immature stage bore more fruit, but was still somewhat limited in quantity. However, data on their host relations are presented and some indication was given of their preferred hosts and a measure of the immatures activity gained.

A: STUDY SITES AT MORFA HARLECH

GENERAL DESCRIPTION

Morfa Harlech is a dune system at the north end of Cardigan bay in west Wales. The area was not long ago covered by the sea and the land grew from the south in a north-westerly direction as a result of sand deposition by the sea. The northern end of Morfa Harlech is a salt marsh largely dominated by the sea rush, *Juncus maritimus* (fig. 4.1 and plate 4.1). To the south lies a sandy grassland dominated by red fescue, *Festuca rubra*. South of this is a flat marshy area dominated by sharp-flowered rush, *Juncus acutifloris* and adjacent to this area is a permanent swamp. Further south is a dune slack system with drier patches with gorse (*Ulex europea*) present. This area is bound on the landward side by a coniferous plantation and on the coastal side by an extensive fixed dune system. The southern end of this fixed dune is divided by a fence so that the area north of the fence is grazed by Welsh black cattle and sheep, whilst the area south of the fence remains ungrazed by any large mammal, though some rabbits are present. The whole dune system has until recent times been grazed by large numbers of rabbits.

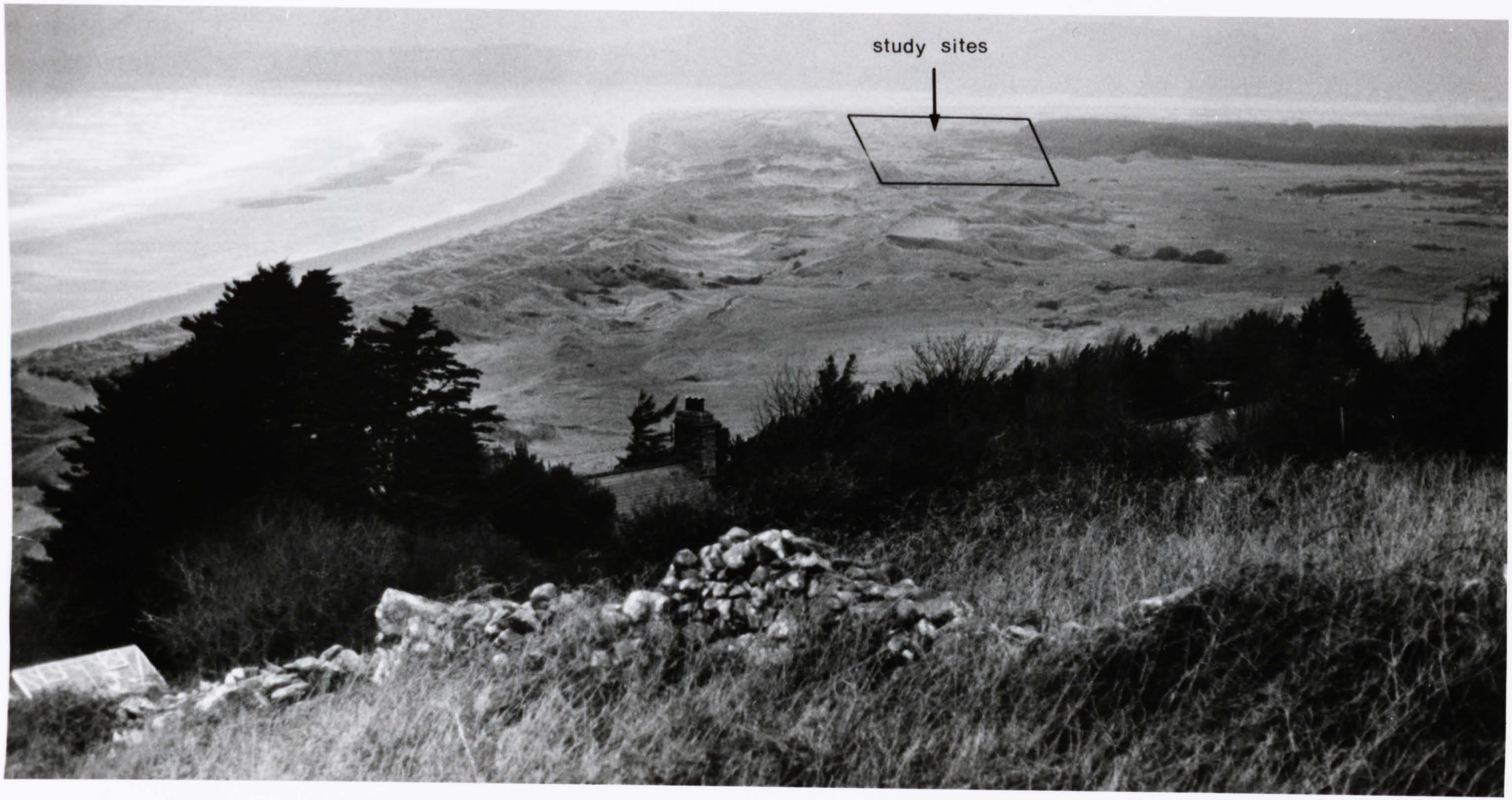
The study sites chosen within the Morfa Harlech system are shown in fig. 4.2 and plates 4.2 - 4.5. They were designated as the **ungrazed**,**grazed** and **marsh** sites on the basis of subjective differences in their vegetation.

The **ungrazed** site is a fixed dune area dominated by dense tussocks of marram, *Ammophila arenaria* (plate 4.2a).

The **grazed** site is also partly a fixed dune area with patches of *Ammophila areneria* and also areas of close-cropped vegetation due to grazing effects of primarily cattle. The grazed site was subdivided further on a subjective level into three habitat types (plate 4.2b):

i). The marram dominated fixed dune, though here the marram patches were much sparser than in the ungrazed site (plate 4.3a).

Plate 4.1: View from the south overlooking Morfa Harlech showing the location of the study sites. The coniferous plantation can be seen to the right (see figs. 4.1 and 4.2)


study sites

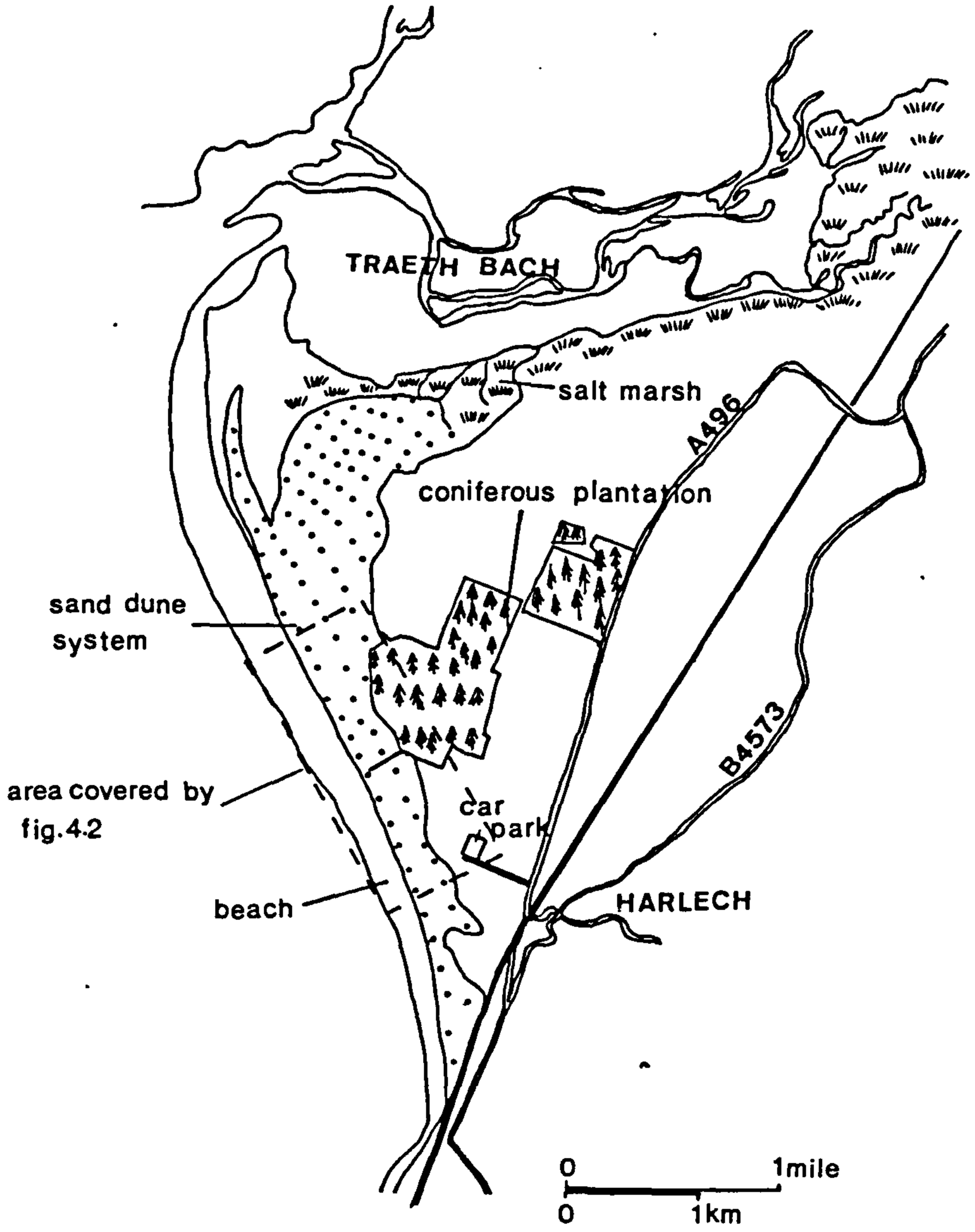


Fig. 4.1: Map showing the major habitat types and study area at Morfa Harlech, Gwynedd.

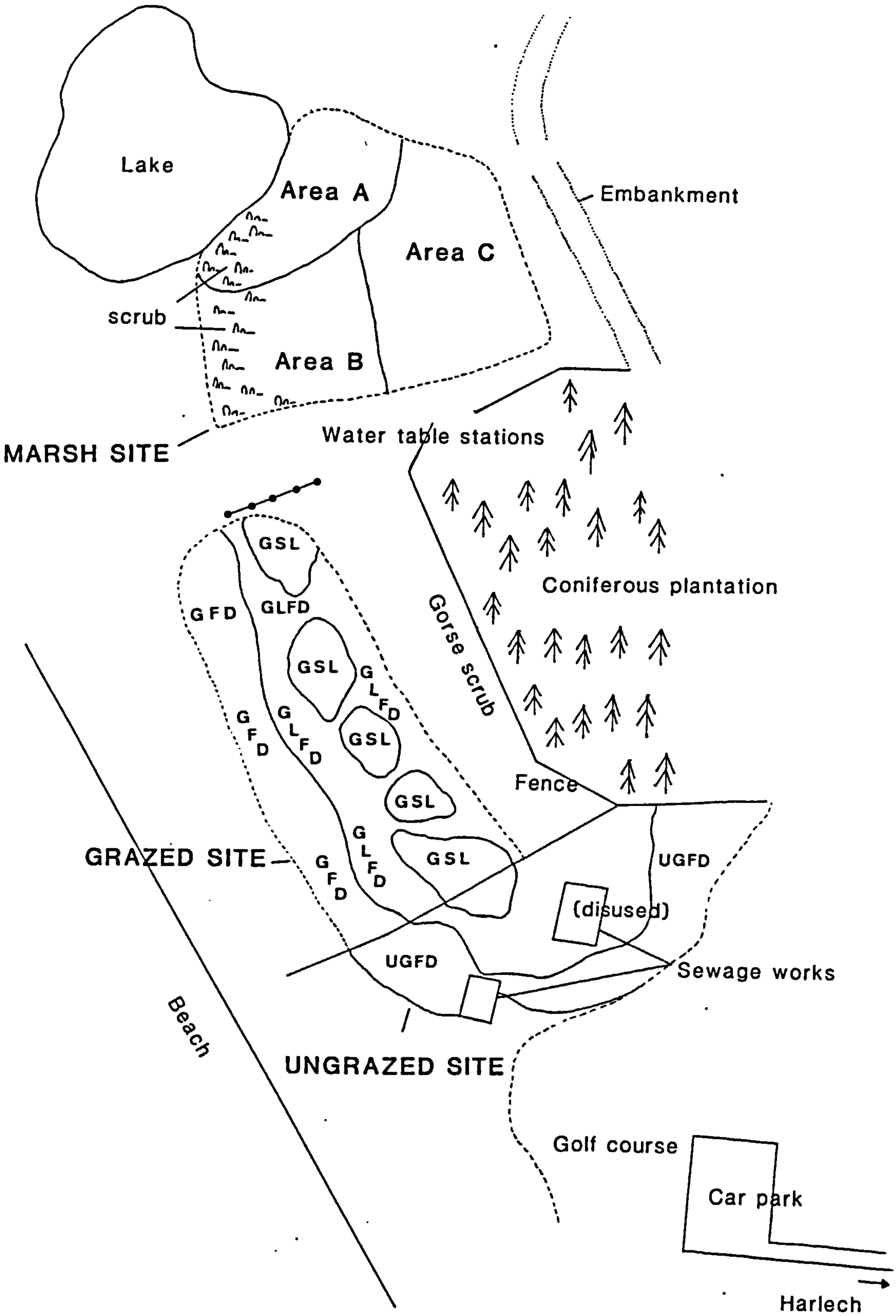


Fig. 4.2: Map showing the study sites and the subjectively defined sub-communities of vegetation at Morfa Harlech.

Plate 4.2 a: The ungrazed site (UGFD) at Morfa Harlech showing the dense clumps of marram grass (*Ammophila arenaria*) found on the fixed dunes.

b: General view looking north over the grazed site at Morfa Harlech showing the grazed fixed dune comunity (GFD), lower fixed dune grassland community (GLFD) and the wetter slack areas (GSL). The ungrazed site lies immediately to the south of this area.

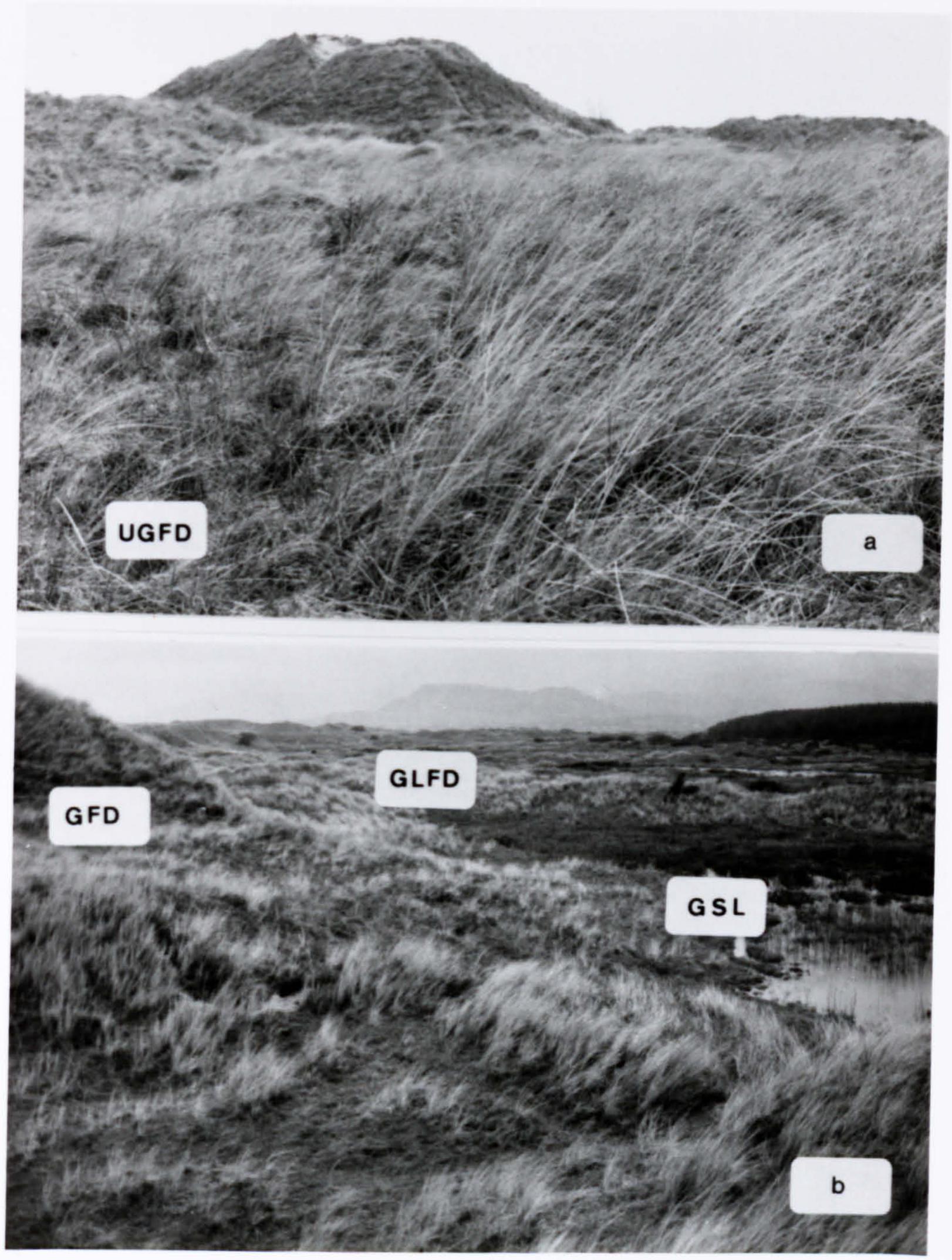
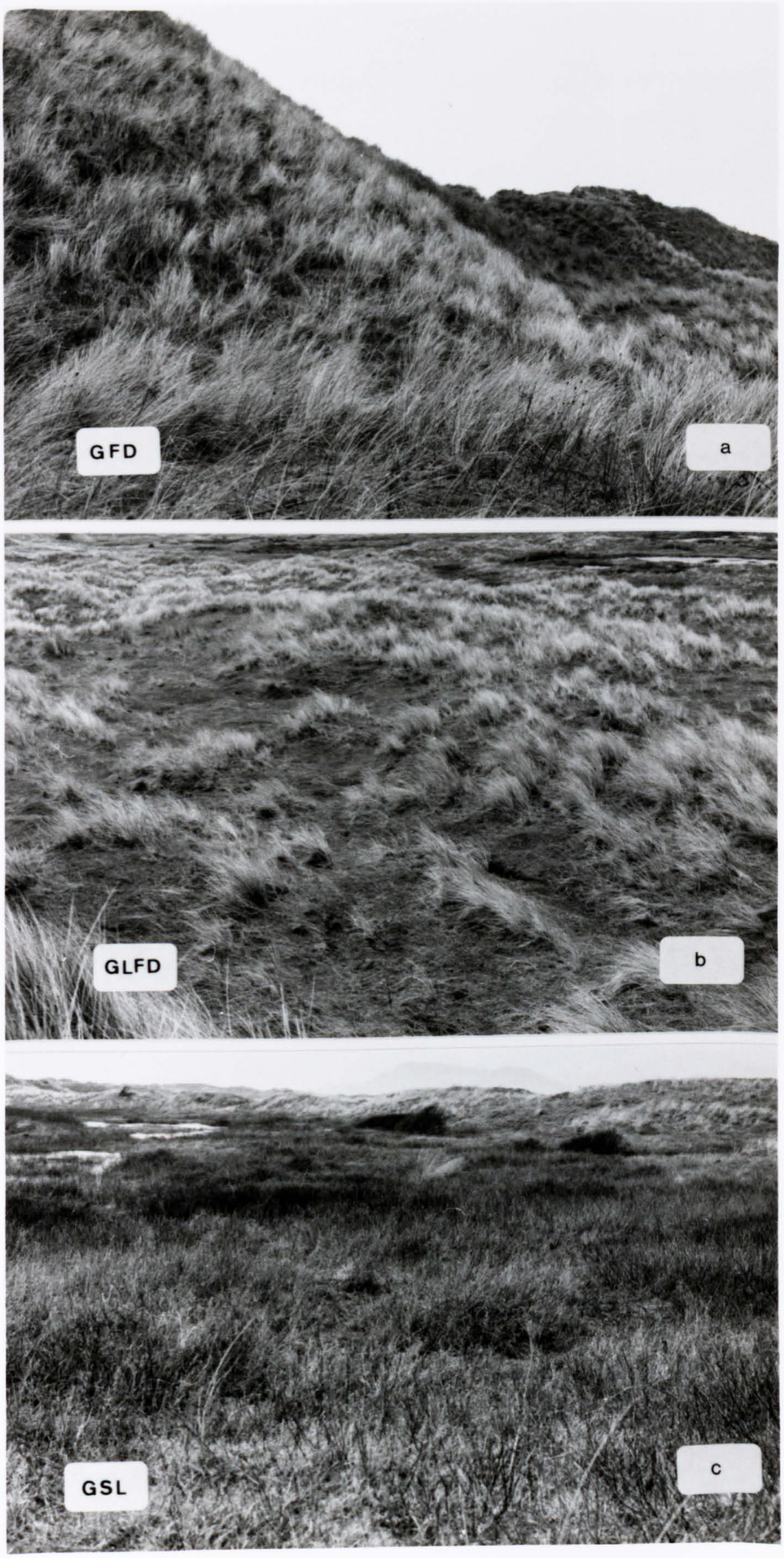

UGFD
a
GFD
GLFD
GSL
b

Plate 4.3: The vegetation sub-communities at the grazed site Morfa Harlech. (a), the grazed fixed dune area (GFD) showing clumps of marram (*Ammophila arenaria*). The slopes of these fixed dunes were criss-crossed by cattle tracks. (b), the grazed lower fixed dune grassland community (GLFD) showing scattered clumps of marram interspersed by close-cropped grassy areas. (c), the slack areas (GSL) dominated by creeping willow (*Salix repens*).


GFD
a
GLFD
b
GSL
c

ii). The *Festuca rubra* dominated dune grassland community , an area criss-crossed by cattle tracks (plate 4.3b).

iii). The area adjacent to the dune slack system dominated by close-cropped *Festuca rubra* and with creeping willow, *Salix repens* present (plate 4.3c).

The marsh site is a flat, marshy area adjacent to a permanent bog. This site was also subjectively subdivided into three further areas (plate 4.4):

i). Area A, a wet area adjacent to the bog and dominated by the jointed rush, *Juncus articulatus* (plate 4.5a).

ii). Area B, a drier area with patches of *Juncus articulatus* and also an area of grassland / alder *Alnus glutinosa* scrub (plate 4.5b).

iii). Area C, a drier/sandy area consisting of dense clumps of the sharp sea rush, *Juncus acutus* interspersed with close- cropped grassland (plate 4.5c).

This marsh site , particularly areas A and B, periodically flood during the winter months (see water table data later).

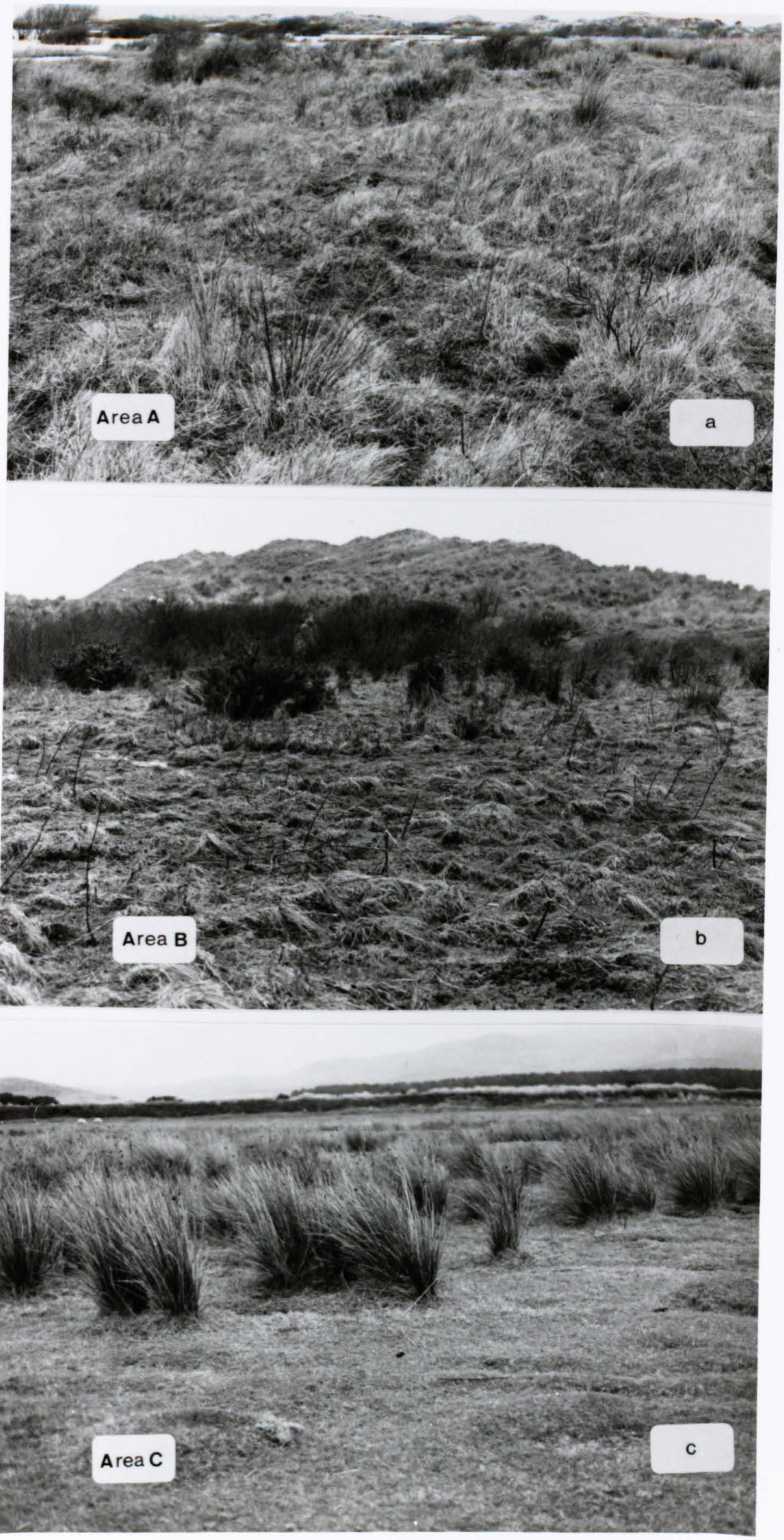
Monitoring of seasonal activity commenced in March 1987 at the ungrazed site, large numbers of ticks being found here on a preliminary visit in February of that year. A survey in the spring of 1987, when ticks were at their peak numbers (see later), found no ticks in the adjacent grazed site nor in the marsh site. These other 2 sites were visited at intermittent intervals during the year and ticks were first recorded from the grazed site in February 1988, from which time a comparative study has been made with the ungrazed site. Ticks were not found at the marsh site until October 1988 when cattle which had recently grazed the area were found to be infested with *D. reticulatus*. The marsh site was included in the comparative study from then on. The tick was also found across much of the Morfa Harlech NNR and even in the saltmarsh at the northern end of the reserve which floods on spring tides.

To quantify the differences in these subjectively defined study sites, quadrat data on the percentage cover of plant species present were taken and analysed using a classification and an ordination technique. The following two sections describe the methods employed

Plate 4.4: General view looking north over the marsh site at Morfa Harlech showing the three vegetation sub-communities Area A, Area B and Area C. Welsh black cattle can be seen in the distance.


Area A
Area B
Area C

Plate 4.5: Vegetation sub-communities at the marsh site, Morfa Harlech. (a) marsh area A showing clumps of jointed rush (*Juncus articulatus*) with a few scattered clumps of sharp sea rush (*Juncus acutus*) and creeping willow (*Salix repens*). (b) marsh area B showing a grassland / *Juncus articulatus* area with alder (*Alnus glutinosa*) scrub in the background. (c) the drier marsh area C showing large clumps of sharp sea rush (*Juncus acutus*) interspersed with close-cropped grassy areas.


Area A
a
Area B
b
Area C
c

and the results obtained from the vegetation analysis.

VEGETATION ANALYSIS

MATERIALS AND METHODS

15x1m quadrats were taken in known tick areas in each of the subjectively defined areas listed below (see study sites and fig. 4.2). For each quadrat the percentage cover of all plant species present was estimated.

UGFD-Ungrazed(by cattle) fixed dune area.

GFD-Grazed fixed dune area.

GLFD-Grazed dune grassland area.

GSL-Grazed area adjacent to dune slack.

MSA-Marsh area A adjacent to bog.

MSB-Marsh area B,wet/scrub type area.

MSC-Marsh area C, drier/sandy area.

The quadrats were taken during the summer of 1989. Identification of the plant species is aided when the plants are in flower. Identification was made using the keys of Clapham *et al.* (1981) and Hubbard (1968) and the field guide of Rose (1981).

The data were analysed using a classification technique, Two-Way Indicator Species Analysis or TWINSPAN (Hill 1979a) and an ordination technique, Detrended Correspondence Analysis or DECORANA (Hill 1979b, Hill and Gauch 1980).

The basis of classification is to assign the samples (quadrats) and species to classes or groups. Ordination arranges samples and species in a low-dimensional space so that similar samples (or species) are close by and dissimilar samples far apart (Gauch 1982). Using the

two techniques in conjunction aids in discerning any vegetative differences between the areas. this follows Gauch (1982) who states " The spatial, graphical output of ordination, the cluster assignments of classification (hierarchical or nonhierarchical), and arranged data matrices are complimentary for elucidating and communicating patterns in community data".

Classification:

TWINSPAN is a classification technique designed primarily for ecologists and phytosociologists who have data on the occurrence of a set of species in a set of samples. The programme first constructs a classification of the samples and then uses this classification to obtain a classification of the species according to their ecological preferences. The two classifications are then used together to form an ordered two-way table that expresses the species synecological relations. The aim of the arrangement is to highlight the major features of the data by grouping like species with like and like samples with like. The basic activity in TWINSPAN is to make a dichotomy. The samples are divided up into groups by repeated dichotomisation and then this is repeated for the species.

Ordination:

DECORANA was used to aid the interpretation of the TWINSPAN hierarchical classification . DECORANA represents samples in an ordination space in which distances have a consistent meaning in terms of the amount of change in species composition. The first axis sample scores summarise data on numerous species into a single number for each sample, thus converting species data into a vegetation variable (Hill and Gauch 1980). The output plots the samples (and species) along orthogonal axes, the first axis accounting for most of the variation in the data. The plots can be considered as showing the position of the samples (quadrats) in an ecological space.

For a more full account of TWINSPAN and DECORANA see Gauch (1982) and Hill and Gauch (1980).

RESULTS

Two-way ordered table:

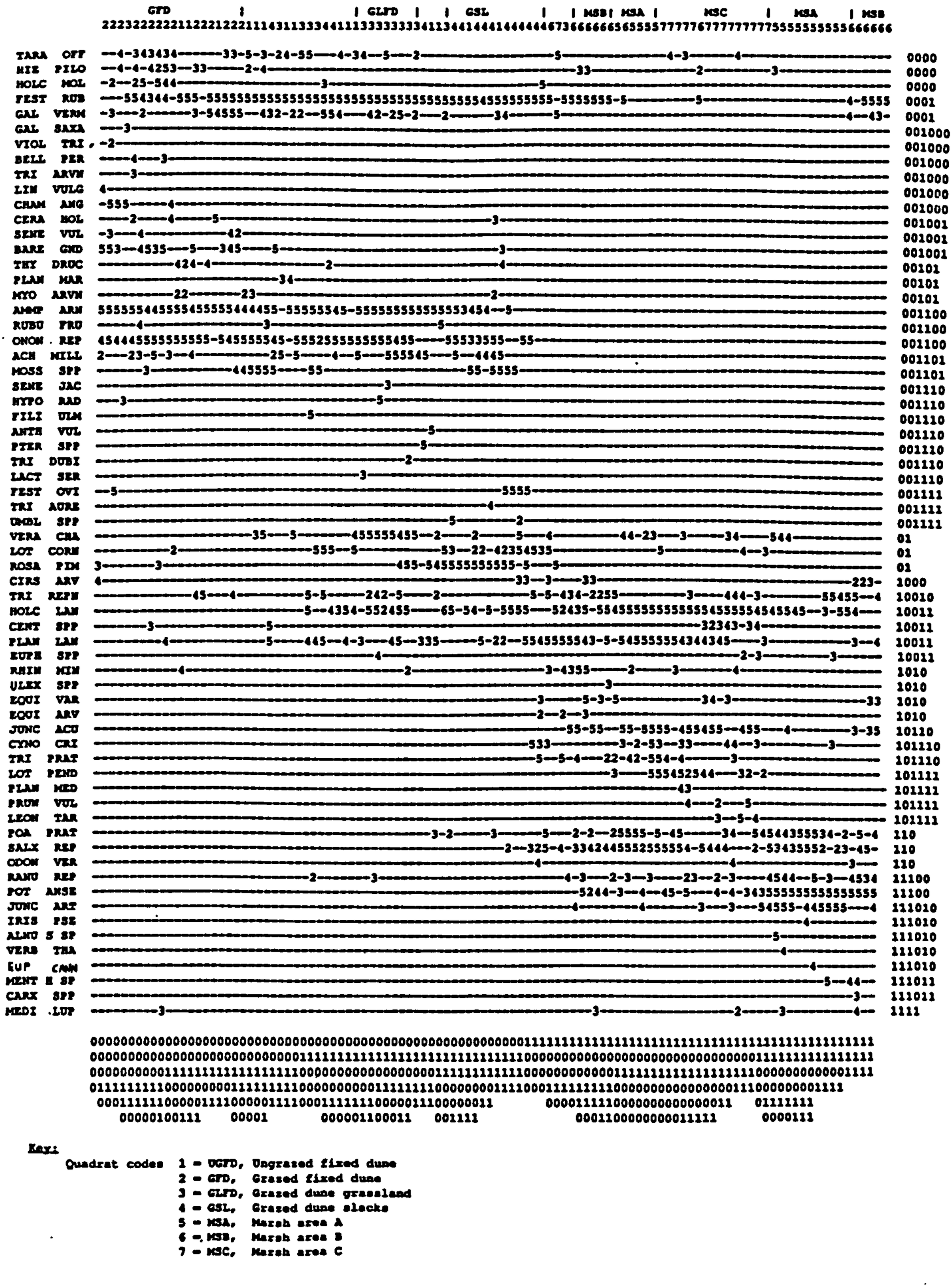
The two-way ordered table (table 4.1), can be interpreted to assess the degree to which particular species groups are characteristic of particular stand groups.

The table shows a major dichotomy between the dune stands and the marsh stands. species characteristic of the dune stands are *Ammophila arenaria* (Marram grass), *Achillea millefolium* (Yarrow), *Ononis repens* (Rest harrow), *Festuca rubra* (Red fescue) and *Rosa pimpinellifolia* (Burnet rose).

Species characteristic of the marsh stands are *Juncus acu tus* (Sharp sea rush), *Juncus articulatus* (Jointed rush), *Salix repens* (Creeping willow), *Lotus pendunculatus* (Greater bird's-foot trefoil), *Trifolium pratense* (Red clover), *Cyanosurus cristatus* (Crested dog's tail) and *Potentilla anserina*(Silverweed) The dune stands can be delineated further, group 2 (the grazed fixed dune) is fairly homogeneous. Species characteristic of this were *Hieracium pilosella* (Mouse-ear hawkweed), *Holcus mollis* (Soft bent grass), *Chamaenerion angustifolium* (Rosebay willow herb) and the presence of bare ground (cattle tracks). There were also smaller homogeneous stands of grazed dune grassland (group 3) and grazed slack (group 4). Species characteristic of the grazed dune grassland were *Veronica chamaedrys* (Germander speedwell) and *Holcus lanatus* (Yorkshire fog). Those characteristic of the grazed slack were *Festuca ovina* (Sheep's fescue) and *Rosa pimpinellifolia* (Burnet rose). The ungrazed fixed dune (group 1) overlaps with all the above dune types.

The marsh stands were also separated into groups of homogeneous stands. Two small

Table 4.1: Two-way ordered table produced by TWINSPAN showing the vegetation associations of the tick habitats. Species names are shown on the left, quadrat numbers at the top. The classification of species and samples are indicated along the right and bottom margins.



Key:

Quadrat codes
1 = UGFD, Ungrazed fixed dune
2 = GFD, Grazed fixed dune
3 = GLFD, Grazed dune grassland
4 = GSL, Grazed dune slacks
5 = MSA, Marsh area A
6 = MSB, Marsh area B
7 = MSC, Marsh area C

Table 4.1 cont'd

Species list for TWINSPAN two-way ordered table.

Abbreviation	Scientific name	English name
TARA OFF	*Taraxacum officinale*	- Dandelion
HIE PILO	*Hieracium pilosella*	- Mouse-eared Hawkweed
HOLC MOL	*Holcus mollis*	- Soft bent grass
FEST RUB	*Festuca rubra*	- Red fescue
GAL VERM	*Galium verum*	- Lady's Bedstraw
GAL SAXA	*Galium saxatile*	- Heath Bedstraw
VIOL TRI	*Viola tricolor*	- Wild Pansy
BELL PER	*Bellis perrennis*	- Daisy
TRI ARVN	*Trifolium arvense*	- Hare's foot Clover
LIN VULG	*Linaria vulgaris*	- Common Toadflax
CHAM ANG	*Chamaenerion angustifolium*	- Rosebay Willowherb
CERA HOL	*Cerastium holosteoides*	- Common Mouse-ear
SENE VUL	*Senecio vulgaris*	- Groundsel
THY DRUC	*Thymus drucei*	- Wild Thyme
PLAN MAR	*Plantago major*	- Greater Plantain
MYO ARVN	*Myosotis arvensis*	- Field Forget-me-not
AMMP ARN	*Ammophila arenaria*	- Marram grass
RUBU FRU	*Rubus fruticosus*	- Bramble
ONON REP	*Ononis repens*	- Restharrow
ACH MILL	*Achillea millefolium*	- Yarrow
SENE JAC	*Senecio jacobea*	- Common Ragwort
HYPO RAD	*Hypochoeris radicata*	- Common Cat's-ear
FILI ULM	*Filipendula ulmaria*	- Meadowsweet
ANTH VUL	*Anthyllis vulneraria*	- Kidney Vetch
TRI DUBI	*Trifolium dubium*	- Lesser trefoil
LACT SER	*Lactuca serriola*	- Prickly Lettuce
FEST OVI	*Festuca ovina*	- Sheep's fescue
TRI AURE	*Trifolium aureum*	- Large Hop Trefoil
VERA CHA	*Veronica chamaedrys*	- Germander Speedwell
LOT CORN	*Lotus corniculatis*	- Common Bird's-foot-trefoil
ROSA PIM	*Rosa pimpinellifolia*	- Burnet Rose
CIRS ARV	*Cirsium arvense*	- Creeping Thistle
TRI REPN	*Trifolium repens*	- White Clover
HOLC LAN	*Holcus lanatus*	- Yorkshire Fog
CENT NIG	*Centaurea nigra*	- Black Knapweed
PLAN LAN	*Plantago lanceolata*	- Ribwort Plantain
RHIN MIN	*Rhinanthus minor*	- Yellow rattle
ULEX EUR	*Ulex europaeus*	- Common gorse
EQUI VAR	*Equisetum variegatum*	
EQUI ARV	*Equisetum arvense*	
JUNC ACU	*Juncus acutus*	- Sharp Sea Rush

Table 4.1 cont'd:

CYNO CRI	*Cynosurus cristatus*	- Crested Dog's-tail
TRI PRAT	*Trifolium pratense*	- Red Clover
LOT PEND	*Lotus pendunculatus*	- Greater Bird's-foot trefoil
PLAN MED	*Plantago media*	- Hoary Plantain
PRUN VUL	*Prunella vulgaris*	- Selfheal
LEON TAR	*Leontodon taraxacoides*	- Lesser Hawkbit
POA PRAT	*Poa pratensis*	-
SAL REPN	*Salix repens*	- Creeping Willow
ODON VER	*Odontites verna*	- Red Bartsia
RANU REP	*Ranunculus repens*	- Creeping Buttercup
POT ANSE	*Potentilla anserina*	- Silverweed
JUNC ART	*Juncus articulatus*	- Jointed Rush
IRIS PSE	*Iris pseudacorus*	- Yellow Iris
ALNU GLU	*Alnus glutinosa*	- Alder
VERB THA	*Verbascum thapsus*	- Great Mullien
EUP CANN	*Eupatorium cannabinum*	- Hemp-agrimony
MEDI LUP	*Medicago lupulina*	- Black Medick
MOSS SPP	*Moss spp.*	- Moss species
PTER SPP	*Pter spp.*	- Fern species
UMBL SPP	*Umbl spp.*	- Umbelliferae spp.
MENT SPP	*Mentha sp.*	- Mint species
CARX SPP	*Carx spp.*	- Carex species (sedge)
BARE GRD	Bare gnd	- Bare ground

homogeneous stands of marsh area B (group 6) and marsh area A (group 5) were separated by a homogeneous stand of marsh C (group 7). The first two stands of marsh areas B and A were characterised by the presence of *Rhinanthus minor* (Hayrattle), *Trifolium repens* (White clover) and *T. pratense*. Marsh area C was characterised by the presence of *J. acutus*, *L. pendunculatus* and *C. cristatus*. The other two stands of marsh A and B contained *J. articulatus*, *Ranunculus repens* (Creeping buttercup) and *P. anserina*.

DECORANA plots:

The relationships between the stands is further elucidated by the ordination plots obtained from DECORANA. Fig. 4.3, shows that axis 1, which accounts for most of the variation in the data, is the primary axis of separation between the stands. There is a clear separation between the dune stands and the marsh stands. Axis 2 offers little separation. The dune stands show a considerable degree of overlap with one another as do the marsh stands.

Fig.4.4 shows the DECORANA plots for the dune stands only. The GFD, UGFD and GSL stands are separated out along axis 1, but the GLFD stand overlaps the GSL and UGFD stands to a large degree. This suggests that the GFD stand has a different vegetation structure to the other stands but the GLFD stand is composed of a range of vegetation types encompassing those found in the UGFD and GSL stands. This may indicate that when the grazed area as a whole is considered (GFD, GLFD and GSL) in comparison to the UGFD, there are no marked differences.

The DECORANA plots of the marsh stands are given in fig. 4.5 areas MSA and MSC are separated along axis 1 and MSB overlaps these two stands along axis 1, but is separated out along axis 2. Axis 1 may represent a water table gradient from the drier MSC area to the wetter MSA area, with MSB having both wet and dry areas. This plot does appear to validate the choice of the subjective areas designated on the marsh for monitoring tick activity.

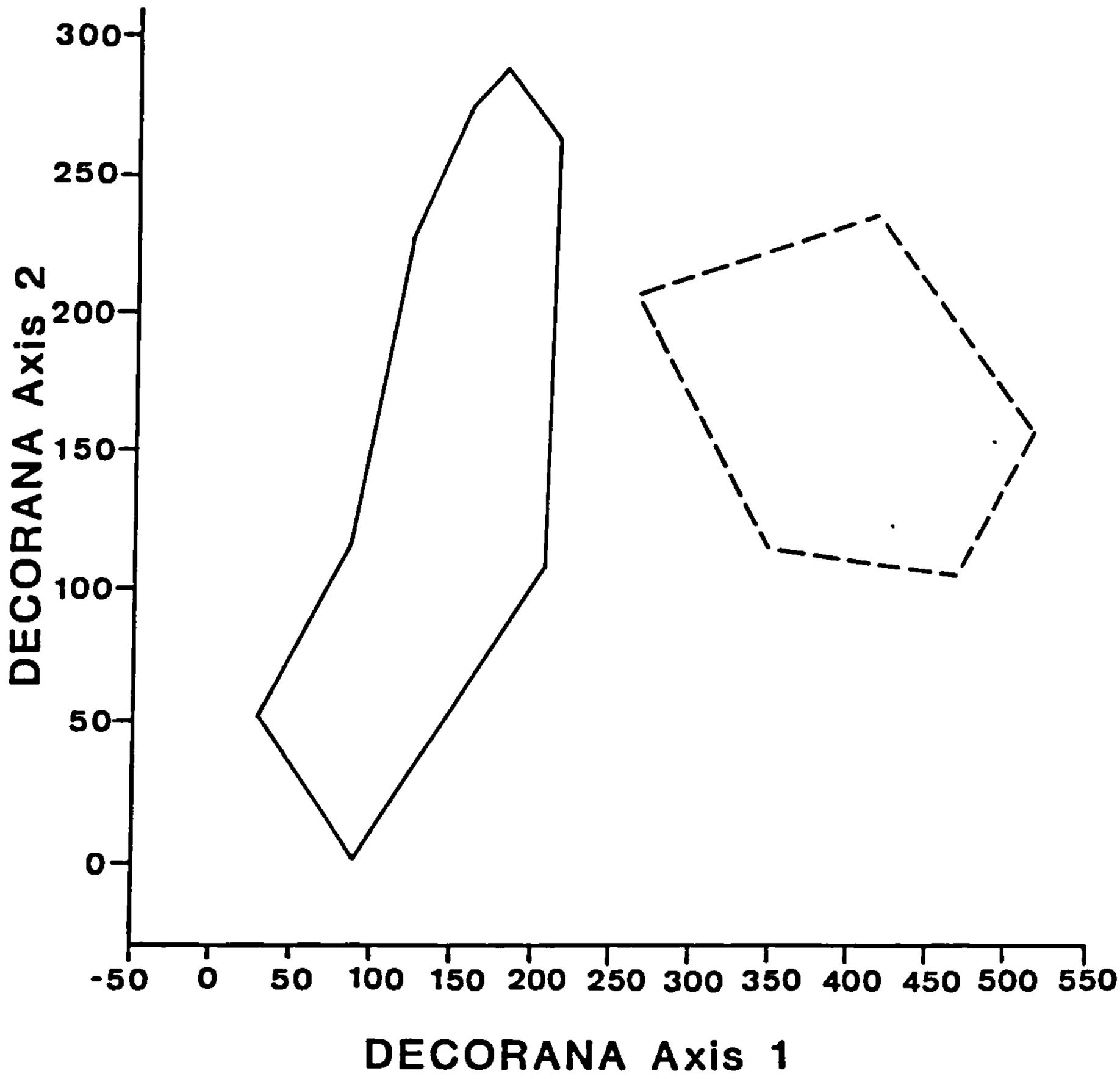


Fig. 4.3: Outlines of the scores for the quadrats from the dune and marsh sites at Morfa Harlech on the DECORANA (Detrended Correspondence Analysis) axes 1 and 2.

Key:

—— Dune Stands

– – Marsh Stands

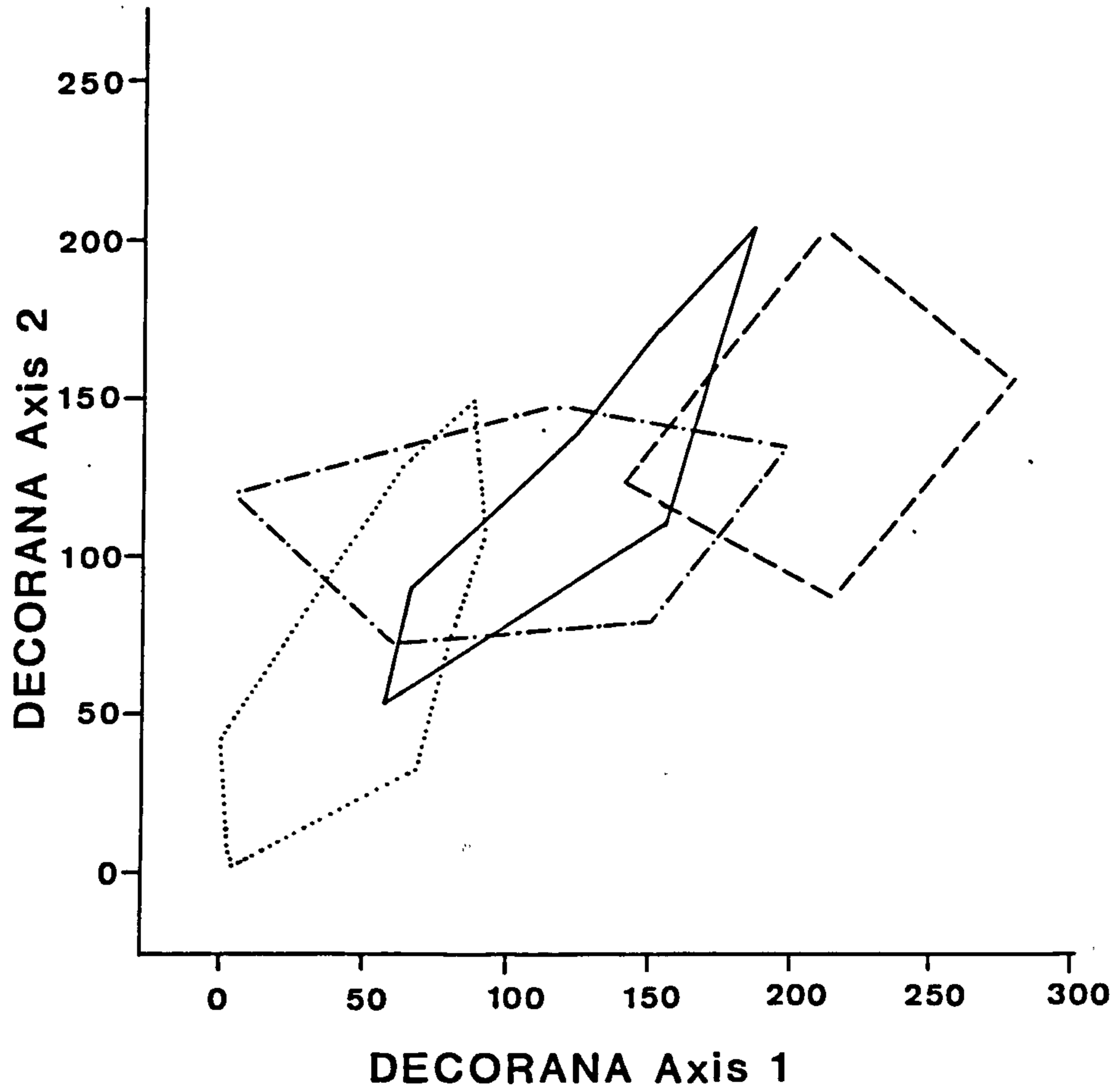


Fig. 4.4: Outlines of the scores for the quadrats from the dune area (UGFD, GFD, GLFD and GSL) on the DECORANA axes 1 and 2.

Key:
—— UGFD
– – GFD
—·— GLFD
····· GSL

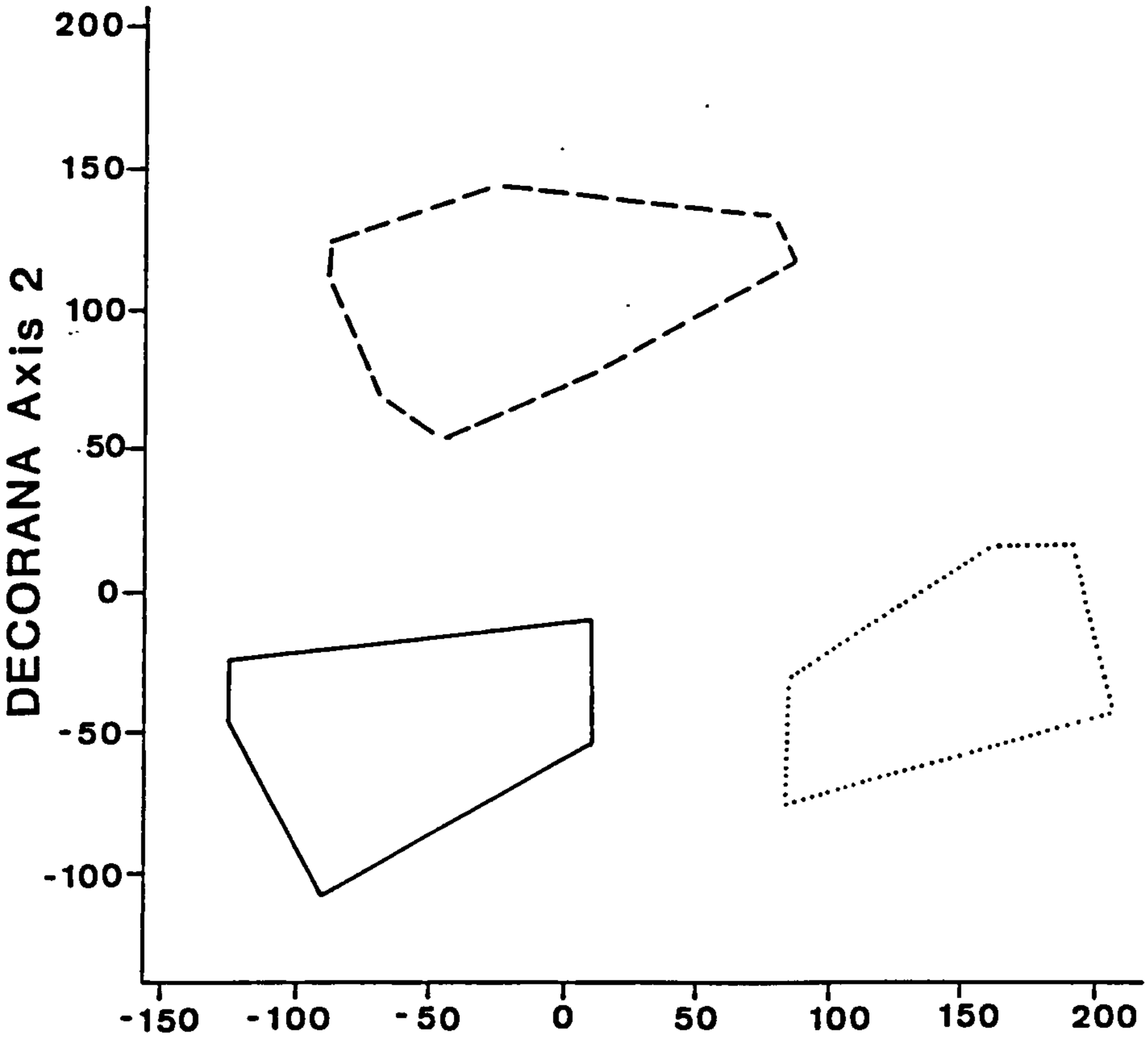


Fig.4.5: Outlines of the scores of the quadrats from the marsh site (marsh area A, area B and area C) on the DECORANA axes 1 and 2.

Key:

—— Marsh area A

– – Marsh area B

····· Marsh area C

The results of the TWINSPAN and DECORANA analyses show that there are marked differences in the plant communities of the dune and marsh sites. Within these two main types there exists a range of habitat types.

Within the dune area the grazed fixed dune area is well defined and the grazed slack area is reasonably well defined. Both the ungrazed fixed dune (table 4.1) and the grazed dune grassland (fig 4.4) tend to overlap the other stands. Data on the vegetation physiognomy may aid in differentiation between the dune stands, particularly between the ungrazed and grazed stands.

Within the marsh site, area C appears to have a very homogeneous vegetation composition. Areas A and B are both composed of two stands straddling the area C type stand. They appear to be composed of a wetter stand to the left of area C and a drier stand to the right (table 4.1).

Thus, the results suggest that there were some quantitative differences in the vegetation composition of the subjectively defined tick habitats. These results are discussed at the end of the next section on the seasonal activity of adult ticks in relation to habitat-type.

B: SEASONAL ACTIVITY AND CLIMATE

MATERIALS AND METHODS

Sampling technique:

Blanket dragging was used to monitor the ticks activity. A 1 x 1.5m woollen blanket with a folding end-frame (to enable it to fit in a car boot) was dragged across the vegetation by means of a cord. After each drag the ticks were counted, sexed and returned to the vegetation along the transect. The term transect is used loosely in that the transects were not clearly delineated and the same area was likely to have been repeatedly sampled particularly at the ungrazed and grazed site.

Though blanket dragging has it's limitations it is the most practical technique for sampling tick populations. It can be considered to be a line transect sampling method(see Southwood 1978) and it gives an estimate of the population from a known width of habitat.

The efficiency of blanket dragging is variable (Milne 1943) and may be affected by:

1) Different habitats or changes in habitat.

2) Changes in the vertical distribution of the ticks.

3) Variation in the weather conditions.

The technique will, therefore, give a relative and not absolute estimate of the population.

Biological interpretation of the population size will be difficult as the estimates are influenced by:

1) Changes in actual numbers.

2) Changes in the number of ticks in a particular phase (e.g numbers questing)

3) Changes in activity following some change in the environment (e.g behaviour of questing *Ixodes ricinus* is known to be affected by temperature and humidity, (Lees 1948 and MacLeod 1935, 1936).

4) Changes in the efficiency of the drag itself.

As a relative method, however, it can give a measure of the availability of the tick population, the availability being the result of the response to the stimuli, the abundance and the activity. It is defined as the ratio of the total catch to the total effort (Southwood 1978). If we assume that the efficiency of the drag does not change, catch per unit effort will provide a measure of availability and, therefore, activity.

It is unrealistic to assume that the efficiency of the drag does not change, but measures can be taken to minimise any changes. For example, each drag was performed at a slow, measured pace. Sampling was carried out between the hours of 1100 and 1500 to minimise variations caused by the diurnal cycle of the ticks. Milne (1943) considered that sampling should occur around midday when meteorological conditions (such as temperature, humidity and solar intensity) were relatively stable. The nature of the technique ensured that sampling did not occur when there were strong winds or heavy rainfall.

Sampling regime:

Sampling was conducted at approximately 2-4 weekly intervals depending on the weather, though more frequent visits were made during periods of peak activity. The ungrazed site was sampled between March 1987 and February 1990, the grazed site between February 1988 and February 1990 and the marsh site between October 1988 and February 1990.

Initial sampling at the ungrazed site employed 20m drags. However, from September 1987 onwards 25m drags were used and a total of 24 drags were made on each sampling occasion. Similarly, at the grazed site 24 x 25m drags were sampled, 8 drags taken per sub-habitat (see study sites). 25 m drags were deemed the most appropriate for the topography and uniformity of habitat found in these dune sites. Milne (1943) regarded 25yd drags as the most efficient for sampling *Ixodes ricinus*. At the marsh site given the large area to sample and the relative homogeneity of the habitat within the three sub-

areas (A, B and C see study sites), 40m drags were employed. In order to validate the comparison in activity between the sites in accordance with Milne (1943), as far as was possible sampling occurred on the same day at the three sites.

Analysis of seasonal activity:

Since there were differences in the size of area sampled at the different sites I could not convert the numbers of ticks into counts per drag (catch per unit effort). However,given that the blanket was 1m wide we know the area covered per drag (25m for the dune sites and 40m for the marsh). Therefore, the count data were converted into density values of ticks/100m^2. A graph showing the seasonal activity patterns of the three sites was produced.

The GLIM (Generalised linear model)(Baker and Nelder 1978) package on the VAX mainframe computer was used to analyse any variation in the seasonal activity patterns within and between sites. The GLIM analysis fits a Poisson error distribution model. The count data contained many zeros and having small counts meant that it was not possible to transform the data and the data is assumed to conform to a Poisson distribution. The GLIM analysis is, in effect an analysis of variance for non- normally distributed data. An analysis of deviance is a more appropriate definition.

Meteorological data:

Meteorological data were taken from the Met.office monthly weather summary reports. The following variables were used:

Mean maximum monthly temperature

Mean minimum monthly temperature

Total monthly rainfall

Photoperiod

Unfortunately, the nearest site to Harlech from which data was available was RAF Valley on Anglesey. However, given that the Valley site is a dune site and when we are considering long term seasonal trends then the conditions at Valley should not be that different from Harlech. In March 1988 a soil thermometer was installed in the ungrazed site and readings were taken on each sampling visit. From May 1988 onwards I was able to use a hand-held temperature and humidity meter (Jenway) and recorded the temperature and relative humidity of the vegetation at the 3 sites on the day of sampling. The photoperiod data was taken from tables in Beck (1980) and is for a site in N.Ireland 55°N. In the spring of 1987 a Grant multimeter was installed to continuously record temperature and humidity at the ungrazed site. Unfortunately the equipment was vandalised within a couple of weeks of instillation and I considered the site unsafe to leave equipment in situ.

Graphs showing the above meteorological data were produced for comparison with the seasonal activity patterns. Also, the activity at the marsh site was compared to the level of the water table. The water table data was kindly provided by the N.C.C. which has been collecting these data in a transect through a dune slack and neighbouring golf course since December 1988. Data from 5 stations just south of the marsh site are used here (see fig. 4.2).

Seasonal variation in sex ratio:

The ticks from each transect were sexed during the monitoring of seasonal activity at each site. Using this data it was possible to look for trends in the seasonal variation of the sex ratio as has been found in previous studies on *D. reticulatus*(Gilot *et al.* 1974, Nosek 1972, Szymanski 1987b and Szymanski and Cerny 1981).

Association of ticks with tracks:

During sampling at the ungrazed site drags were made both along obvious

animal/human tracks and amongst vegetation away from any such tracks. Numbers collected on each sampling occasion (12 drags along tracks / 12 drags off track) were compared using a Wilcoxon signed ranks test. For each pair of data (number on tracks / number off track) the test works out the difference, then works out an average distance and tests whether it is different from zero.

RESULTS

Seasonal activity of questing adults I: within the ungrazed site.

The activity of *D.reticulatus* at the ungrazed site was monitored between March 1987 and February 1990. The seasonal activity pattern for this site is shown in fig. 4.6. It can be seen that there was initially a much higher level of activity (density value) in the spring of 1987 and there was a sharp cut-off in questing activity in May of that year. There followed a period of summer inactivity and then questing commenced in September 1987 with an autumnal peak in November with a sharp decline in activity in December- January followed an equally sharp pick-up of activity in the spring with a peak in March 1988 and a cessation of activity in May. There again followed a period of summer inactivity and activity recommenced in September 1988. Unlike the previous autumn-winter period there was not an autumnal peak followed by a spring peak, instead activity increased gradually following a small peak in September reaching a peak in activity at the end of January 1989 with a fall-off of activity in April and a cessation of activity in May. Activity commenced again in September 1990 with a peak in activity in November and a tail off in activity to February 1990. The smaller number of samples collected in the autumn/winter of 1989-90 must, perhaps, be taken into account. In general, the activity cycle begins in late August/ early September and ends in May with a period of summer inactivity. This is shown in fig. 4.7 which represents the trend in the mean monthly counts at the ungrazed site between

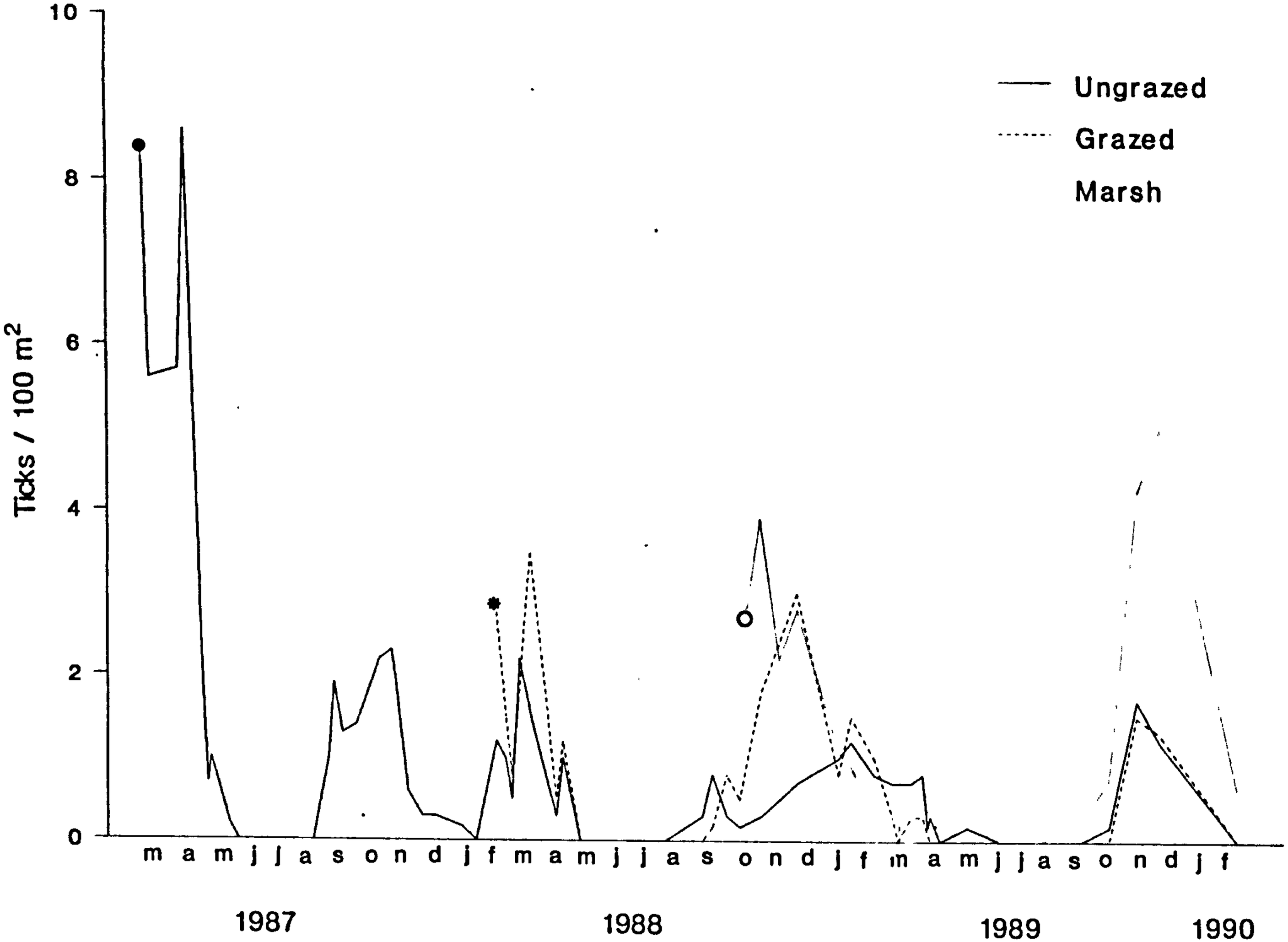


Fig.4.6: Seasonal activity of *D. reticulatus* at the ungrazed, grazed and marsh sites at Morfa Harlech, 1987-90. The values are ticks / 100 m².

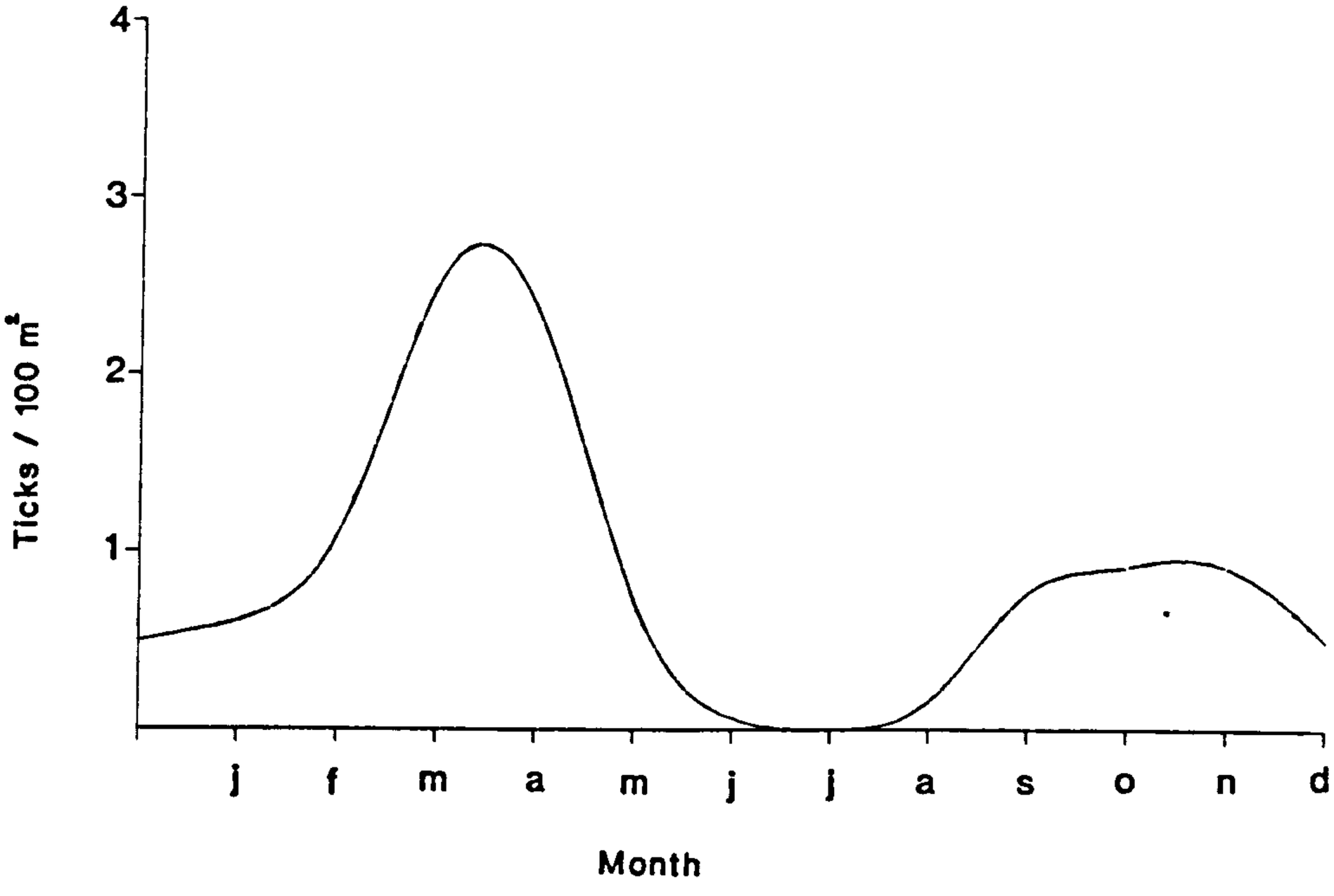


Fig.4.7: General pattern of seasonal activity at the ungrazed site, Morfa Harlech, 1987-90. The *y*-axis values are the mean monthly counts over the three years of study at the ungrazed site.

1987-90.

There appears to be variability in the activity pattern from year to year and this was tested using GLIM analysis (see materials and methods). By comparing months on a year to year basis with the year running from March-February, the result (from table 4.2, chi-squared statistic 90.9, 18 d.f $p<0.001$) suggests that there is a significant difference in the way the months behave (in terms of activity) from year to year i.e there is significant variability in the activity pattern from year to year.

Seasonal activity of questing adults II: between sites

The seasonal activity of questing adult *D.reticulatus* at the three sites at Morfa Harlech is shown in fig. 4.6. The results suggest that there is variation between the sites in the pattern of activity. The marsh site appears to have an earlier autumnal rise and a higher activity level (density value) than the other two sites. The grazed site has a higher activity level than the ungrazed site but these two sites tend to have an end to tick activity at the same time in the spring in 1988, 1989 and 1990. The marsh site had an earlier end to spring activity (March) in 1989 than both the grazed and ungrazed sites. All three sites had a similar pattern of activity in the autumn-winter of 1989-90, though the smaller number of samples taken then must, perhaps, be taken into account. There were clearly greater numbers of ticks active at the marsh site during this period. GLIM analysis was used to determine whether there were significant differences in the activity patterns at the different sites. From table 4.3 we see that the site by month interaction term (si.m) has a significant effect on the model (chi-squared 114.9, 26 d.f, $p<0.001$) and this implies that the activity at the three sites varies significantly with time (months). GLIM analysis was then used to determine which of the sites were significantly different from each other. The results of this analysis are given in table 4.4. The analysis shows that the ungrazed and grazed sites are not significantly different from each other, though they are a borderline case (chi-squared statistic = 21.7, df = 13, $p<0.1$). However, both the ungrazed (chi-squared statistic

Table 4.2: GLIM analysis of variation in seasonal activity patterns at the ungrazed site.

Model	Scaled deviance	degrees of freedom (d.f)	change in deviance (x^2)	change in d.f	
1+a+m	59.2	33			
1+a+yr+nm	150.1	51	90.9	18	p<0.001

Poisson distribution model fitted for count data.

Initial model 1+a+m fitted for 36 months of study where m=months and a=area sampled.

The second model 1+a+yr+nm fitted a new factor of yr=year, 1=year March 1987-Feb 1988, 2=year March 1988-Feb 1989 and 3=year March 1989-Feb 1990.
and a new factor of nm=new month, where nm is 1-12 for each of the years 1, 2 and 3 i.e March is month 1 in year 1, 2 and 3.
The difference in scaled deviance between the two models is due to the interaction between yr and nm. A significant difference implies that the activity pattern is not the same for the three years.

Table 4.3: GLIM analysis of variation in the seasonal activity patterns between the sites I.

Model	Scaled deviance	degrees of freedom (d.f)	change in deviance (x^2)	change in d.f	
1+a	838.8	88			
+m	223.7	73	615.1	15	p<0.001
+si	175.5	71	48.2	2	p<0.001
** +si.m	60.6	45	114.9	26	p<0.001
+sx	55.5	44	5.1	1	p<0.05
+sx.m	13.6	29	41.9	15	p<0.001

Final model= 1 + a + m + si + si.m + sx + sx.m

Legend:

a= area sampled
m= month
si= site
sx= sex
si.m= site by month interaction
sx.m= sex by month interaction

Terms were included if, on being added to the model, they caused a significant change in the deviance. This implies that they had a significant effect on the count (activity).

** si.m is the interaction between the site and month and had a significant effect on the model (count). This implies that the sites differ in terms of count (activity) with respect to time (month) i.e the sites have different activity patterns.

sx.m also has a significant effect i.e the sexes differ in terms of count with respect to time. Thus, the sexes have different activity patterns (see later).

The interaction term si.sx had no significant effect on the model and so was not entered.

Table 4.4: GLIM analysis of variation in the seasonal activity patterns between the sites II.

Original model (see table 4.2):

1 + a + m + si + si.m + sx + sx.m

Site term used	Scaled deviance	degrees of freedom (d.f)	change in deviance	change in d.f	
si	13.6	29			
nsugg	35.2	42	21.6	13	p>0.05
nsugm	128.4	43	114.8	14	p<0.001
nsgm	81.1	42	67.5	13	p<0.001

Legend:
si= original site term (3 sites ungrazed, grazed and marsh)

nsugg= ungrazed and grazed site grouped as one site

nsugm= ungrazed and marsh site grouped as one site

nsgm= grazed and marsh site grouped as one site

In each case the new site term was substituted into the model for the original site factor (si). If the new term had a significant effect on the model it implies that the sites grouped in this new site term should not be grouped together as they are significantly different from each other in terms of count (activity).

The above table shows that the ungrazed and grazed site are not significantly different from each other at the 5% level, but are significantly different at the 10% level. As such they can, perhaps, be thought of as a borderline case. However, both the ungrazed site and the grazed site are significantly different from the marsh site.

= 114.8, df = 14, $p<0.001$) and the grazed site (chi-squared statistic = 67.5, df = 13, $p<0.001$) are significantly different from the marsh site i.e the ungrazed and grazed sites have significantly different activity patterns than the marsh site. Thus, we have evidence of variation in seasonal activity between the sites within the Morfa Harlech area.

The next section investigates the affects of climate on activity. Here I will briefly discuss the possible affects of hosts on the activity pattern. The activity pattern of ticks was thought to be affected by the presence of hosts (Randolph and Steele 1985). In theory, ticks were lost more rapidly from the questing tick population when hosts were present through the losses due to attachment to hosts and to death. In the absence of hosts the losses were due to death alone. The grazed and marsh site are grazed by cattle, the grazed site being most frequently used during windy weather and the marsh site being regularly used. Unfortunately, I have no indication of the hosts maintaining the tick population at the ungrazed site and can only assume that rabbits and possibly dogs play a role. The peaks in activity at the grazed and marsh sites are generally greater than at the ungrazed site (with the exception of spring 1987 when no comparisons were available). This may reflect a larger tick population at the grazed and marsh sites because of the greater abundance of suitable hosts for adults. However, it was not possible to say from these results whether questing ticks are being depleted from the grazed and marsh site at a more rapid rate than at the ungrazed site as this may be masked by the greater recruitment into a larger questing population at these sites. Activity ceased earlier at the marsh site than at the other sites in spring 1989 which may have been the result of depletion by hosts, but other factors cannot be ruled out such as seasonal flooding (see later).

Seasonal activity in relation to climate

Figs. 4.8 a and b show the seasonal activity patterns of the 3 sites at Morfa Harlech in relation to the macroclimate. The only consistent feature in the activity pattern from year to year at the ungrazed site (the longest studied site) is the apparently obligatory period of summer inactivity (diapause) and this corresponds with increasing daylength and an associated rise in temperature. The initiation of activity in the autumn coincides with decreasing day length but not a fall in temperature as this lags behind daylength and only begins a marked decrease at the end of September -early October. The rainfall follows a more erratic path, but in each spring (March-May)during the period of study the rainfall decreased, then generally increased during the summer months. Thus, the rainfall is decreasing when tick activity peaks and then ceases.

GLIM analysis of the affect of macroclimate on the activity of ticks at the 3 sites is shown in tables 4.5-4.7. Considering each site in turn :-

Ungrazed:

The mean monthly maximum temperature was the only macroclimate variable to have a significant effect on activity. The mean monthly minimum temperature, total monthly rainfall and photoperiod do not appear to have a significant effect. The ticks at this site had a spring peak in 1987 and 1988 (but not 1989) which coincided with mean maximum monthly temperatures in the range 8.4-13.4°C. The summer diapause coincided with the highest mean monthly maximum temperatures in the range 14-20.9°C.

Grazed:

As above the mean monthly maximum temperature was the only macroclimate variable to have a significant effect on activity. The ticks here had a peak in activity in the spring of 1988, but also in late autumn (November) in 1988 and only in the autumn of 1989. The mean monthly maximum temperature in spring 1988 was in the range 9.4-11.9°C and the mean monthly maximum monthly temperatures in November 1988 and 1989 were 10.5°C and 11.1°C respectively. The summer diapause coincided with the same

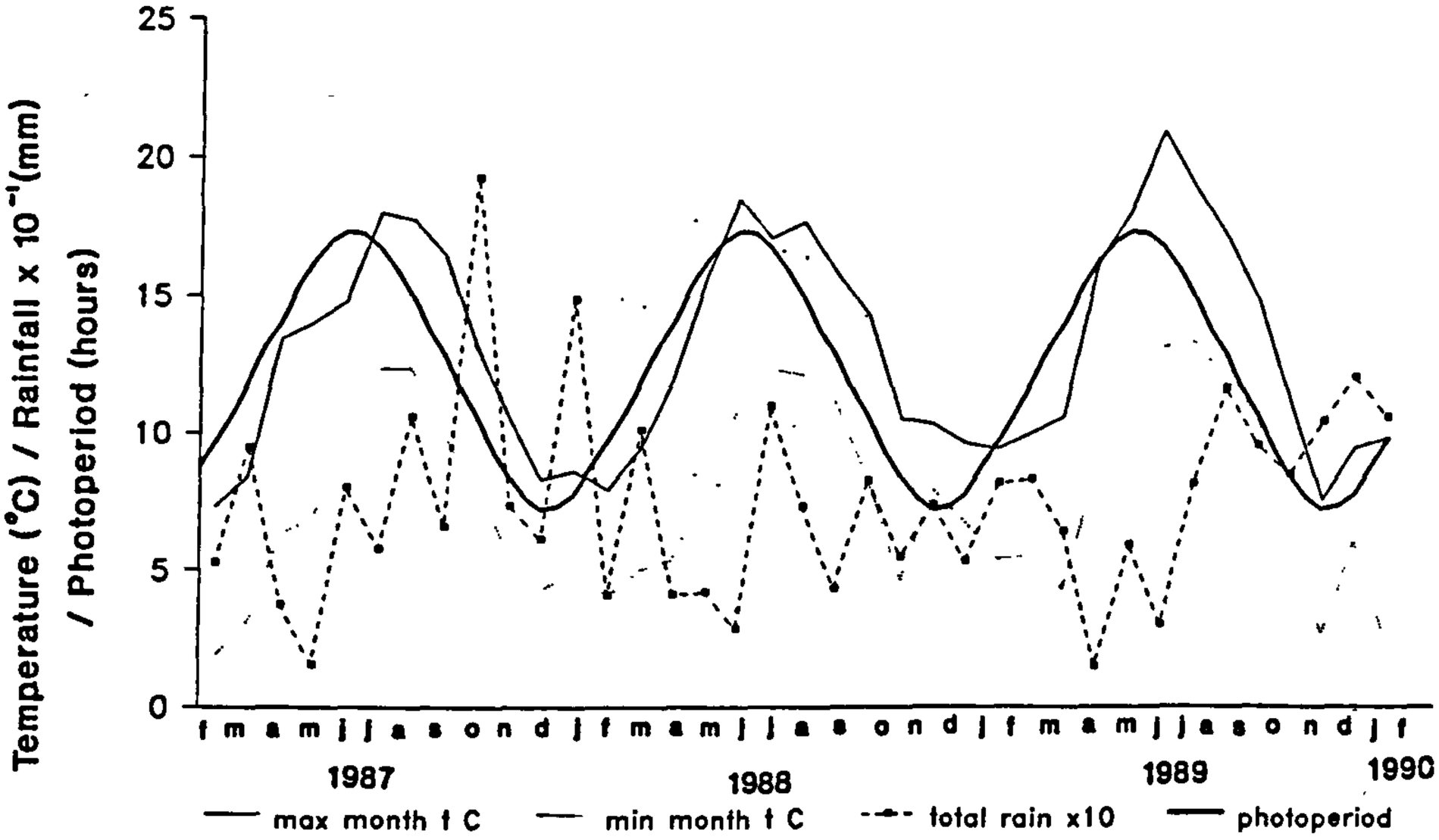


Fig. 4.8a: Macroclimate variables between 1987-90. The temperature and rainfall data are from RAF Valley, Anglesey and the photoperiod data from N. Ireland 55°N (Beck 1980).

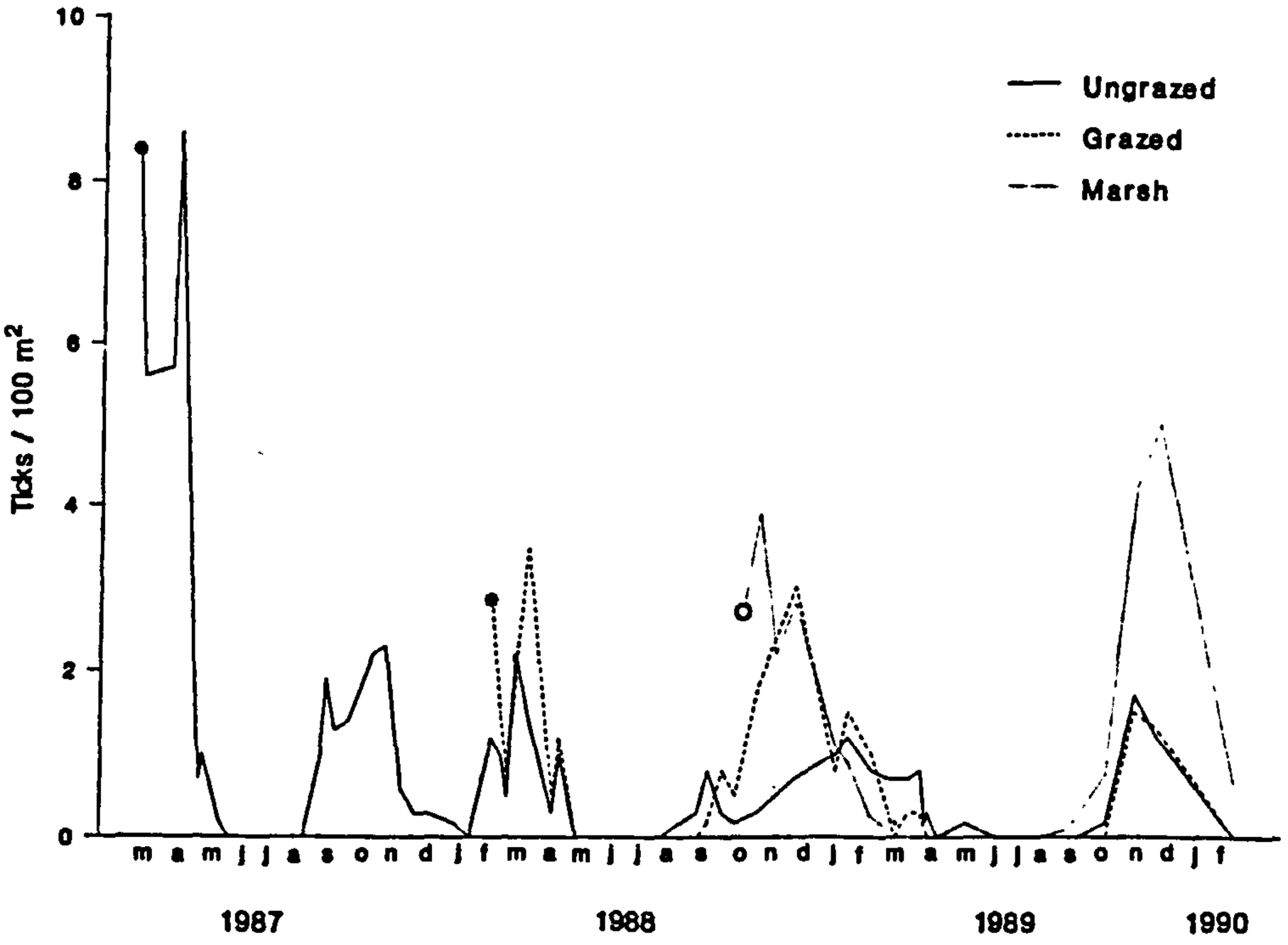


Fig.4.8b: Seasonal activity at three sites within Morfa Harlech (fig.4.5).

Table 4.5 a, b: GLIM analysis of effect of macroclimate on activity at the ungrazed site.

a)

Model	Scaled deviance	df	Change in deviance (x^2)	Change in df	
1	63.6	33			
1+max t	47.4	32	16.2	1	p<0.001
1+min t	50.4	32	13.2	1	p<0.001
1+rain	63.6	32	0.0	1	p=0.999
1+photo	58.9	32	4.7	1	p<0.05

b)

Model	Scaled deviance	df	Change in deviance (x^2)	Change in df	
1+max t	47.4	32			
1+max t + min t	47.3	31	0.1	1	p>0.75
1+max t + rain	45.0	31	2.3	1	p>0.15
1+max t + photo	47.3	31	0.1	1	p>0.75

The results in table 4.5a show that mean maximum monthly temperature has the most significant effect on the activity. This is indicated by the lowest scaled deviance (smallest variance).

The maximum temperature is now incorporated into the model (table 4.5b) and the remaining terms added to see if any have a significant effect on the activity. This procedure ensures that the macroclimate variables have a significant individual effect on the activity. The significant results for mean monthly minimum temperature and photoperiod shown in table 4.5a may be the result of their correlation with mean mohthly maximum temperature.

The results in table 4.5b show that mean maximum monthly temperature is the only macroclimate variable to have a significant effect on activity.

Table 4.5 cont'd:

Key to tables 4.5-4.7:

max t= mean monthly maximum temperature.

min t= mean monthly minimum temperature.

rain = total monthly rainfall.

photo= photoperiod.

Table 4.6 GLIM analysis of effect of macroclimate on activity at the grazed site.

Model	Scaled deviance	df	Change in deviance (x^2)	Change in df	
1	25.5	21			
1+max t	10.2	20	15.3	1	p<0.001
1+min t	13.9	20	11.6	1	p<0.001
1+rain	22.9	20	1.6	1	p>0.15
1+photo	13.3	20	12.2	1	p<0.001

The results above show that the mean monthly maximum temperature has the most significant effect on activity at the grazed site.

Model	Scaled deviance	df	Change in deviance (x^2)	Change in df	
1+max t	10.2	20			
1+max t + min t	10.0	19	0.2	1	p>0.50
1+max t + rain	10.2	19	0.0	1	p=0.99
1+max t + photo	8.1	19	2.1	1	p>0.15

The results show that mean monthly maximum temperature is the only macroclimatic variable to have a significant effect on activity at the grazed site.

Table 4.7: GLIM analysis of effect of macroclimate on activity at the marsh site.

Model	Scaled deviance	df	Change in deviance (x^2)	Change in df	
1	26.1	14			
1+max t	24.8	13	1.3	1	p>0.25
1+min t	23.2	13	2.9	1	p>0.05
1+rain	23.2	13	2.9	1	p>0.05
1+photo	9.8	13	16.3	1	p<0.001

Model	Scaled deviance	df	Change in deviance (x^2)	Change in df	
1+photo	9.8	13			
1+photo+max t	7.7	12	1.1	1	p>0.25
1+photo+min t	9.3	12	0.5	1	p>0.25
1+photo+rain	9.4	12	0.4	1	p>0.25

The results above indicate that photoperiod is the only macroclimatic variable to have a significant affect on activity at the marsh site.

mean monthly maximum temperatures as at the ungrazed site.

Marsh:

The photoperiod is the only macroclimate variable to have a significant effect on activity. Activity peaked in autumn (early November) 1988 and again in November 1989. The daylength is in the range 9.3-7.6 hours during November at this latitude. The summer diapause began with a daylength in the range 10.7-13.1 hours and ended with a daylength in the range 15-13.8 hours.

The macroclimatic data was compared to the 30 year average monthly values (1951-80) in order to see whether there were any anomalous years during the study. The results are shown in figs. 4.9 a, b. The winter, summer and autumn temperatures in 1987 were below average. However, for most of 1988 and 1989 the temperatures were above average (by as much as 4°C in 1989). The exceptions are in late summer/early autumn 1988 and December 1989 when the temperatures are below average. The rainfall pattern varies more erratically, but in general, the rainfall is above average between June 1987 and August 1988, and below average between Sept. 1988 and Nov. 1989. As in all short-term ecological studies there is the problem of deciding whether the observed patterns (in this case of seasonal activity) are typical for the species in question, or whether atypical climatic effects are producing atypical patterns. In this case, 1989 was a much warmer than average year, but there are no obvious effects on the activity patterns, though they do clearly vary from year to year. That the ticks are exhibiting different behaviours at the different sites at Morfa Harlech would seem to indicate that they are experiencing and responding to different microclimatic conditions.

GLIM analysis of the affect of microclimate on activity in the 3 sites is shown in tables 4.8-4.10. The vegetation temperature and humidity had no significant affect on activity at the ungrazed site. However, the vegetation temperature and humidity had a significant

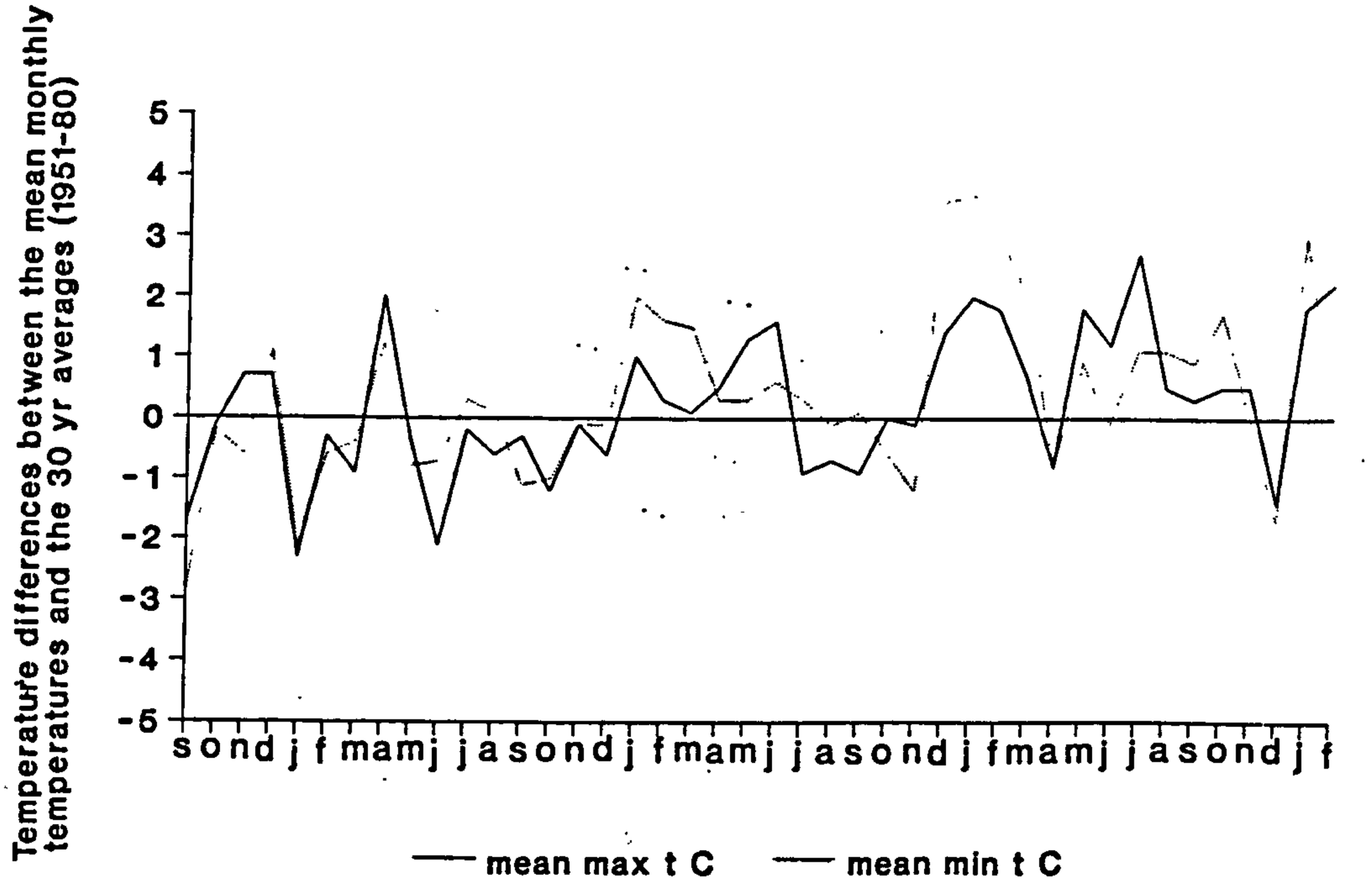


Fig.4.9a: Differences between mean monthly temperatures (1986-90) and the 30 year average monthly values (1951-1980).

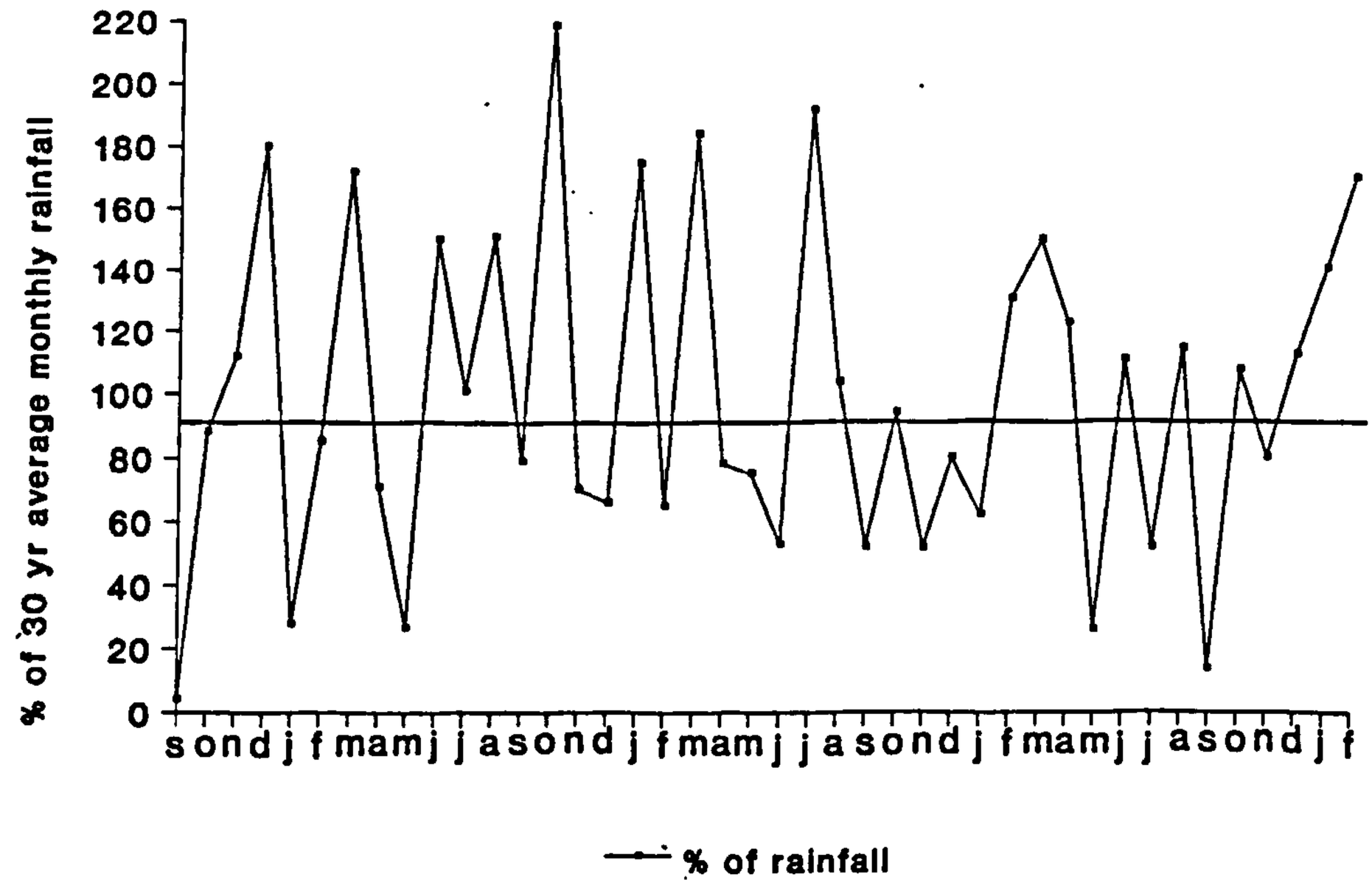


Fig.4.9b: Mean monthly rainfall as a percentage of the 30 year average monthly rainfall (1951-80).

Table 4.8: GLIM analysis of the effect of microclimate on activity at the ungrazed site.

Model	Scaled deviance	df	Change in deviance (x^2)	Change in df	
1	8.4	16			
1+Vt	5.5	15	2.9	1	p>0.05
1+Vrh	8.3	15	0.1	1	p>0.75

The results above indicate that the microclimate has no significant effect on the activity at the ungrazed site.

Key:

Vt= Temperature in the vegetation.

Vrh= Relative humidity in the vegetation.

Table 4.9: GLIM analysis of the effect of the microclimate on activity at the grazed site.

Model	Scaled deviance	df	Change in deviance (x^2)	Change in df	
1	18.6	16			
1+Vt	13.2	15	5.4	1	p<0.05
1+Vrh	17.8	15	0.8	1	p>0.25
1+Vt+Vrh	8.6	14	4.6	1	p<0.05

The results above indicate that the vegetation temperature and vegetation humidity have a significant effect on the activity at the grazed site.

Table 4.10: GLIM analysis of the effect of the microclimate on activity at the marsh site.

Model	Scaled deviance	df	Change in deviance (x^2)	Change in df	
1	23.8	13			
1+Vt	19.7	12	4.1	1	p<0.05
1+Vrh	22.8	12	1.0	1	p>0.25
1+Vt+Vrh	18.9	11	0.8	1	p>0.25

The results above indicate that the vegetation temperature has a significant effect on the activity at the marsh site.

affect on the activity at the grazed site. The vegetation temperature also had a significant affect on activity at the marsh site.

The microclimate data are presented in figs.4.10a and 4.11a. The vegetation temperature shown in fig. 4.10a, b show that there were no marked differences between the 3 sites. The trend follows a similar path to the macroclimate temperature. The summer diapause coincides with the highest temperatures which are slightly higher in the ungrazed site (max. 23.2°C in 1988, 22.9°C in 1989). The soil temperatures at the ungrazed site follow a similar pattern as the vegetation temperature, but the temperatures are not as high as the vegetation temperature. During the winter months the temperature is more stable and remains around 9.5°C (at 5cm).

The vegetation relative humidity (vRH) data are shown in fig. 4.11a. The vRH follows an erratic path and the main differences between the sites were in the autumn of 1988 when the humidity at the grazed and marsh sites was lower than at the ungrazed site and in the summer of 1989 when the humidity was higher in the grazed and marsh sites than in the ungrazed site. The lower humidity which then increases in the autumn of 1988 coincides with the peak activity in the grazed and marsh sites. However, the peak in activity at all 3 sites in the autumn of 1989 is associated with falling humidity.

The ticks at the dune sites (ungrazed and grazed) appear to be responding to the mean monthly maximum temperature and those at the marsh site to photoperiod. Though the mean monthly maximum temperature and photoperiod are highly correlated (Pearson correlation coefficient = 0.79), the ticks at the marsh site appear to be responding to the photoperiod only (table 4.7). There also appears to be a microclimatic affect at the grazed and marsh site, but not at the ungrazed site. Account must be taken of the limitations of the microclimate data in that they are day of sampling data. Ideally, the data would have been collected daily or continuously with meters in situ, unfortunately this was not possible (see materials and methods). It is difficult to infer what the mechanism operating to generate these behavioural differences. However, fig. 4.12 may indicate a possible

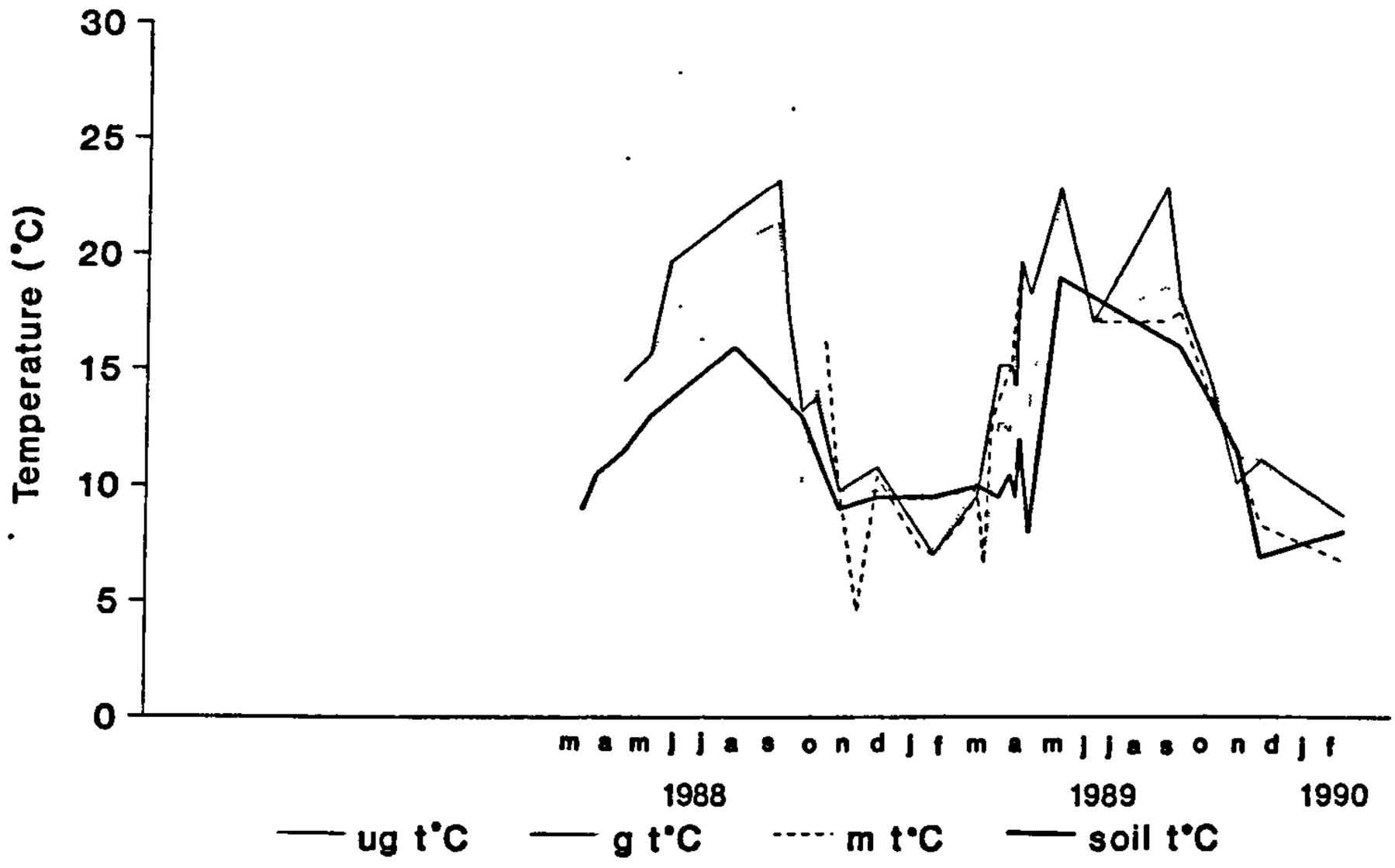


Fig.4.10a: Day of sampling microclimate temperatures at the ungrazed (ug t), grazed (g t), marsh (m t) sites and the soil temperature at the ungrazed site (soil t) at Morfa Harlech.

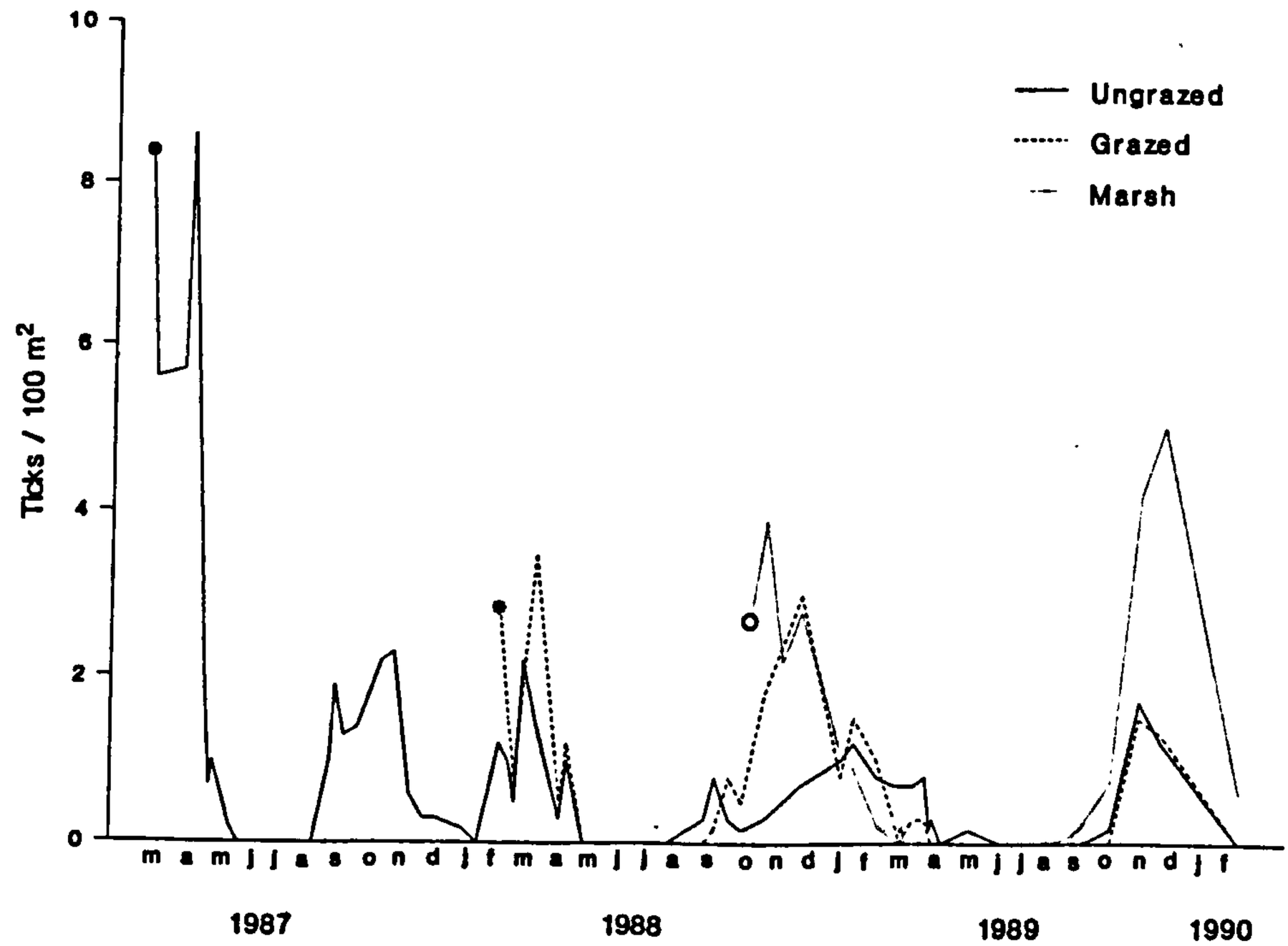


Fig.4.10b: Seasonal activity at three sites at Morfa Harlech (1987-90) (fig.4.5).

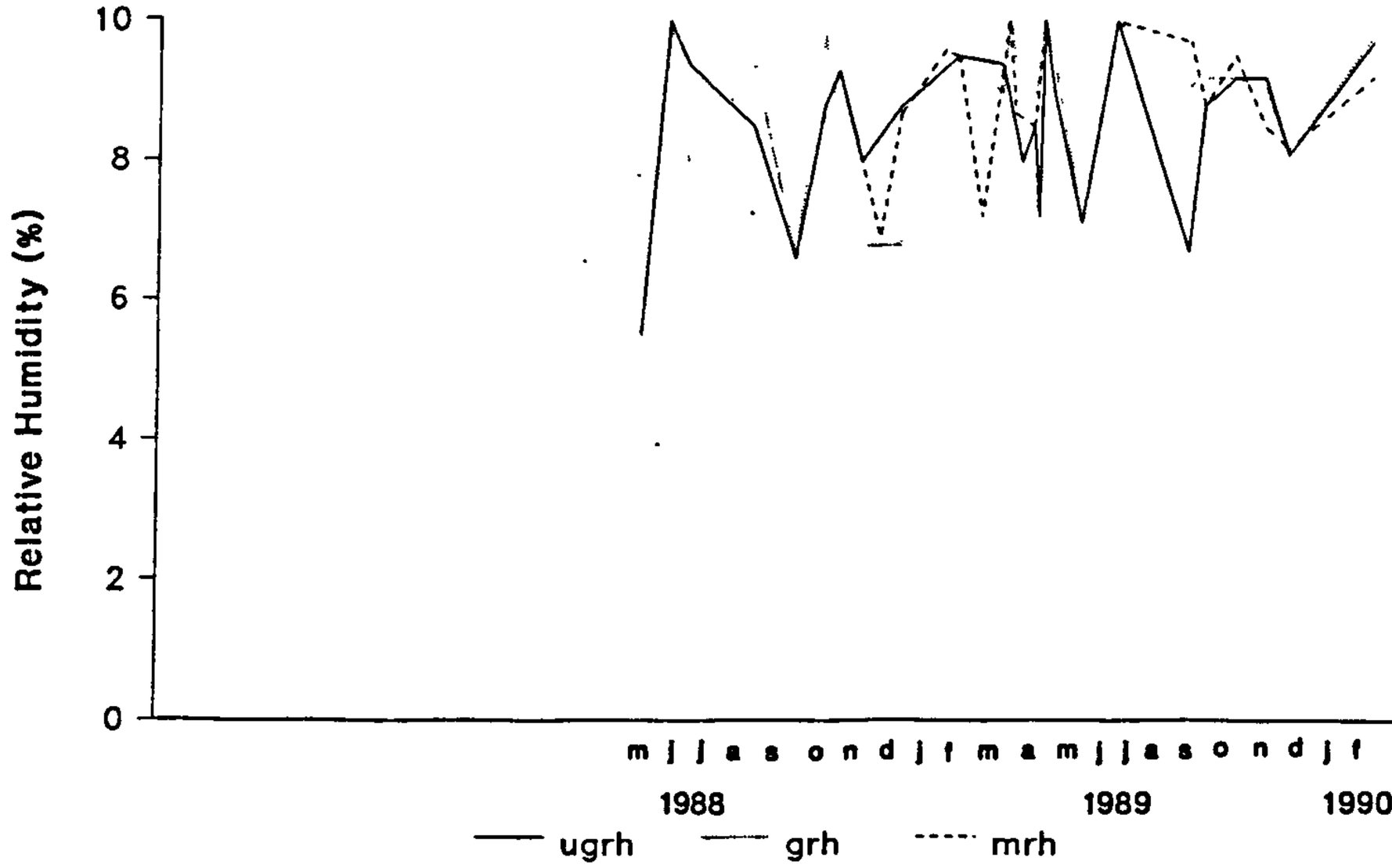


Fig.4.11a: Day of sampling microclimate RH at the ungrazed (ug rh), grazed (g rh) and marsh (m rh) at Morfa Harlech.

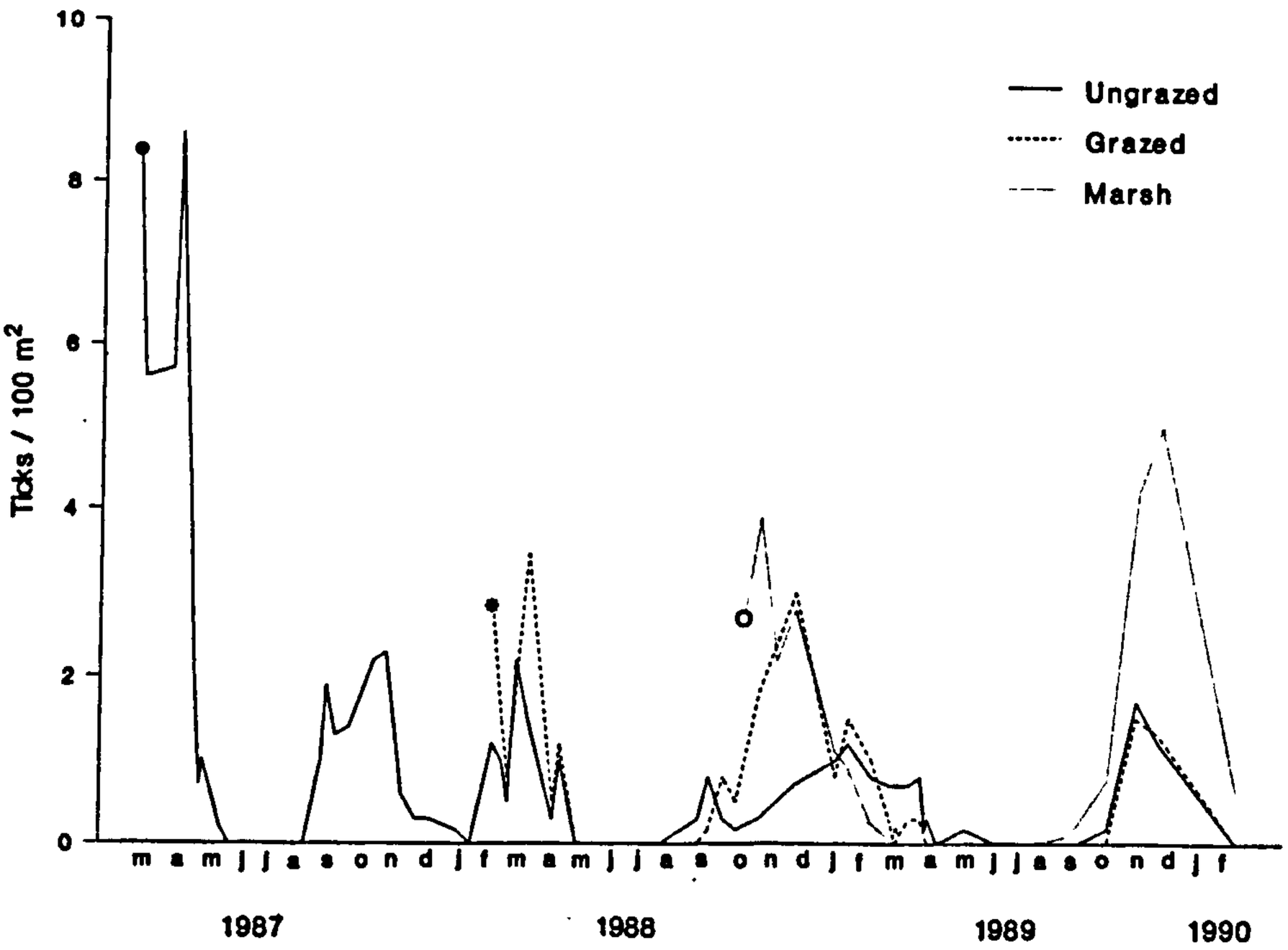


Fig. 4.11b: Seasonal activity at three sites at Morfa Harlech (fig. 4.5).

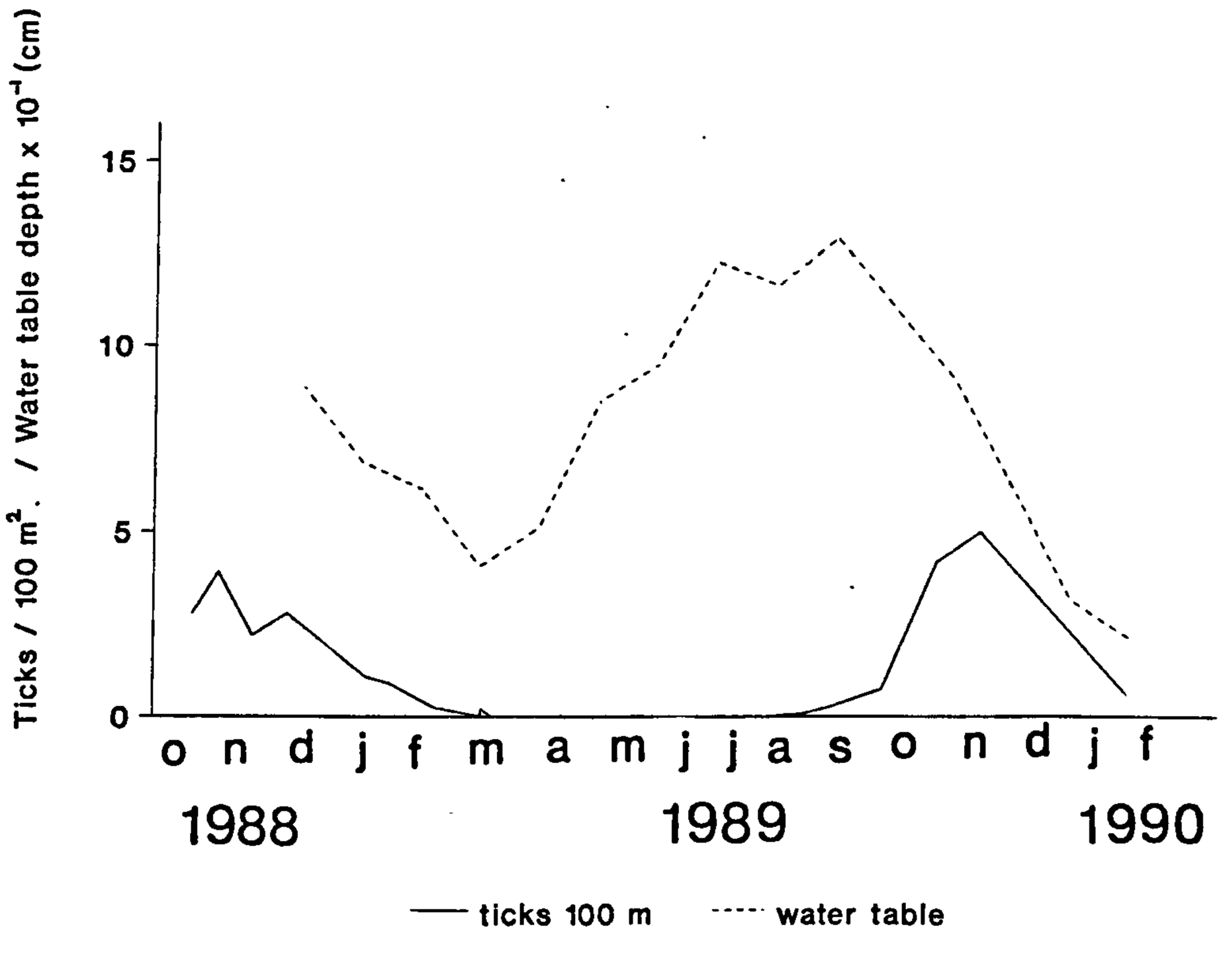


Fig. 4.12: Relationship between the water table depth and seasonal activity of ticks at the marsh site, Morfa Harlech (a water table depth of zero would represent a flooded area).

explanation for the difference in activity at the marsh site. The reduction and cessation of activity in the spring of 1989 corresponds with the highest level of the water table (minimum water table depth) and a similar trend appears to occur in 1990. It must be remembered that the water table data are mean values for 5 stations so that some areas were in fact submerged when the water table was at its highest. These stations were also in a drier area than the actual marsh, so that it is very likely that at the time of minimum water table depth shown here, some areas of the marsh would be submerged. Ideally, data would be collected on the activity and water table levels on the actual marsh over several years.

Seasonal changes in the sex ratio

It was apparent from the data collected on monitoring the seasonal activity at the three sites that there were seasonal changes in the sex ratio. The data are shown in fig 4.13a, b. In the spring of 1987 females predominated comprising 63.2% of ticks collected a ratio of females : males (f : m) of 1.7f : 1m. In the spring of 1988 females formed 64.5% of ticks collected (1.8f :1m) and in the spring of 1989 females comprised 92.6% of ticks collected (12.5f :1m). In the autumn of 1987 males predominated the females comprising only 30.8% of ticks collected (1f :2.2m), but in autumn 1988 females predominated slightly forming 54.4% of ticks collected (1.2f :1m). However, in the autumn of 1989 males again predominated with females comprising 21.4% of ticks collected (1f :3.7m), but note small numbers collected. In the winter months the trend was similar to that of spring with females predominating in 1987/88 and 1988/1989 (69.6%f, 2.3f :1m), and 56.1%f, 1.3f :1m), but in the winter of 1989/90 males just predominated (48.3%f ,1f :1.1m).

In all three sites there is significant variation in the activity of the sexes (table 4.3, Sx.m interaction term has a significant effect on the model, chi-squared statistic $p<0.001$). Fig.4.13b shows the activity patterns of males and females at the ungrazed site. In all years there are more females than males in the spring. The autumnal pattern is not as consistent, in 1987 there are more males than females, in 1988 there are slightly more males than

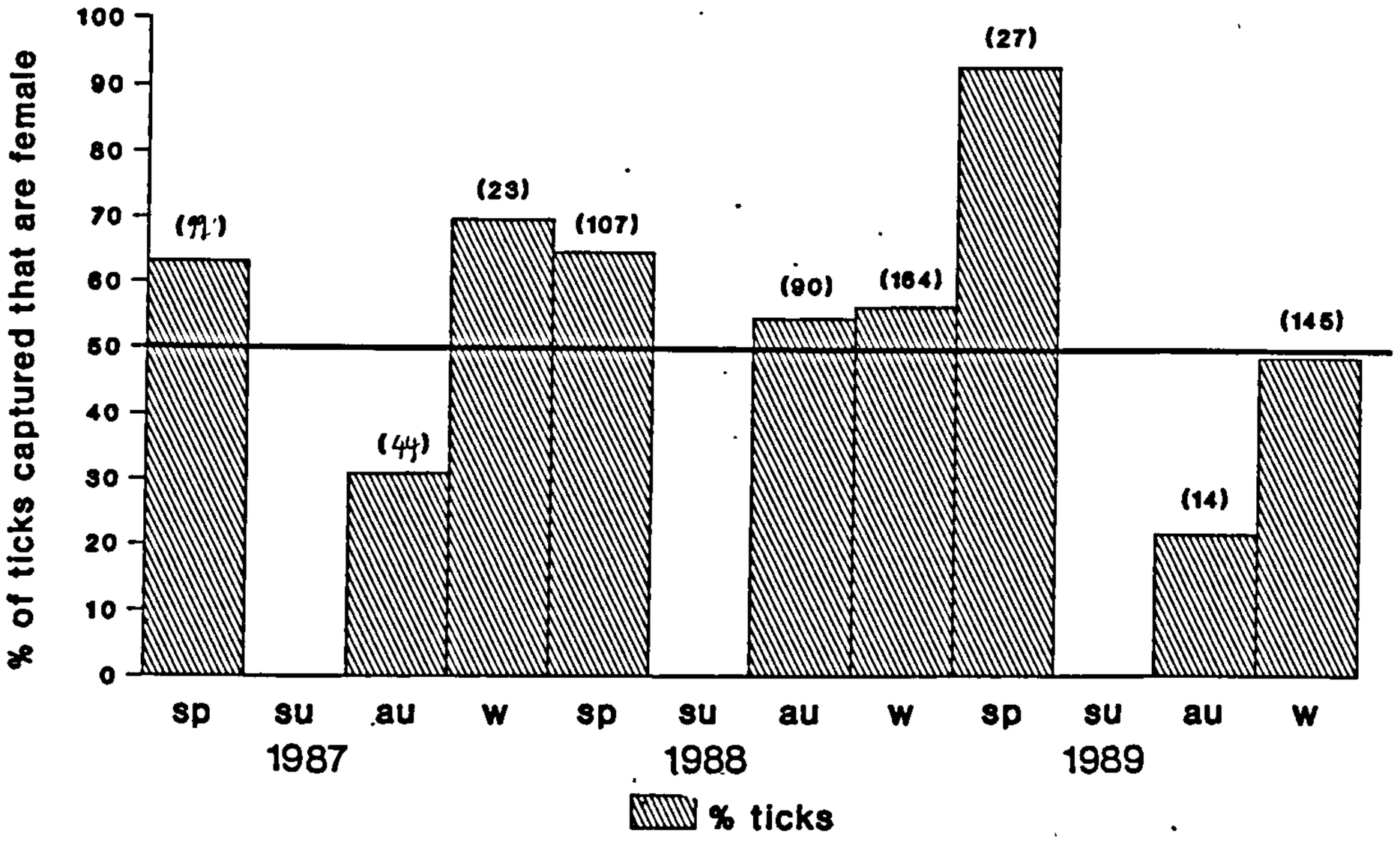


Fig. 4.13a: The percent of ticks captured that are female in spring (sp), summer (su), autumn (au) and winter (w). Numbers in parentheses are total number of ticks captured.

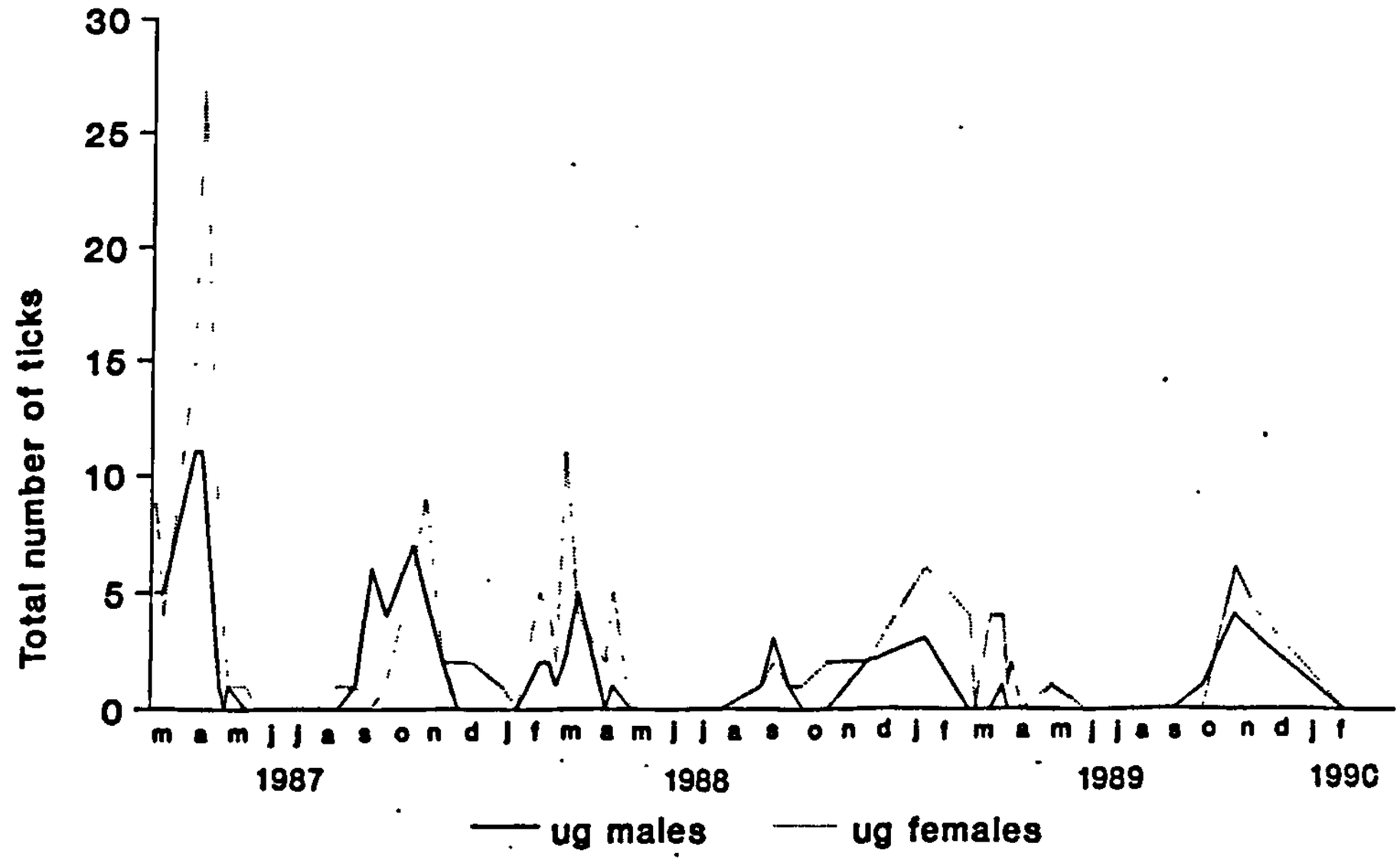


Fig. 4.13b: Total number of males (ug males) and females (ug females) captured at the ungrazed site, 1987-90.

females (very minor peak), but in 1989 though males become active earlier, females predominate quite early on in the autumn. Table 4.11 shows that in all three years of monitoring the activity at the ungrazed site more females were collected than males.

Association of ticks with tracks

Significantly more ticks were found on tracks than amongst vegetation away from the tracks at the ungrazed site (Wilcoxon signed ranks test: $H=291.0$, $n=24$, $p<0.0001$). These results are in agreement with those of Gilot *et al.* (1973) who found that *D. reticulatus* was positively associated with tracks frequented by dogs. Similarly, Carroll *et al.* (1991) found more adult *D. variabilis* along park trails than in the vegetation off them.

Table 4.11: Seasonal changes in numbers of males and females at the ungrazed site.

Season	1987		1988		1989	
	m	f	m	f	m	f
Spring	34	65	9	24	1	16
Autumn	25	19	5	7	8	10
Winter	4	14	5	8	-	-
Total	63	98	19	39	9	26

Numbers are total counts.

Key: Spring= March, April, May.
Autumn= September, October, November.
Winter= December, January, February.
m - males
f - females

DISCUSSION

The results show that the general activity at the ungrazed site as shown by the average monthly counts over the 3 years of study (fig. 4.7) has a long period of activity (late Aug. -May) with a short summer diapause lasting 3 months (late May- late Aug.). The spring peak is much greater than the autumn/winter activity peak. This general model can be compared with Szymanski's (1987a) model of activity patterns in *D. reticulatus*. In West European populations the ticks have a prolonged autumn- winter activity period with a short winter inactivity (not a true diapause), a marked spring peak and a lesser autumnal peak separated by a short summer diapause (1 month, July-August)(Gilot *et al.* 1974, Martinod and Gilot 1991). At the other end of the species range in Western Siberia, the winter diapause is long (>6 months) and the periods of activity are short (just over 2 months). The summer diapause is indistinct as autumn activity begins almost immediately after the drop in spring numbers.

At Morfa Harlech, the winter activity is continuous with no diapause apparent. Given that this is the most westerly site monitored this pattern of activity conforms with the trend shown by Szymanski (1987a). However, the summer diapause lasts 2-3 months and so is longer than that recorded in Western Europe (Gilot *et al.* 1974, Immler 1973, Martinod and Gilot 1991). This may be a response to the dune conditions of high temperatures and low humidities in the summer months. The winter diapause found in Central Europe (Nosek 1972, 1979) and Eastern Europe (Szymanski 1987a) and Western Siberia (Olsurf'ev 1953, as quoted in Szymanski 1987a) is likely to be in response to the harsh winter conditions, such a diapause is not required here on the western edge of the ticks range. It may be that there is an East-West cline in the inductive photoperiod as found in the alfalfa weevil *Hypera postica* (Schroder and Steinhauer 1976a,b). Alternatively, the diapause in Europe may be the result of a temperature/photoperiod interaction (Danks 1987) so that the prevailing mild winter conditions on the West Wales coast are not sufficient to induce

diapause. The seasonal activity pattern and diapause response in *D. reticulatus* shows geographical variation. There are several possible factors which may produce this variation, which can be broadly grouped into genetic, environmental and genotype-environment interactions. Genetic studies have not been undertaken and I have concentrated on environmental factors such as photoperiod, temperature, rainfall and humidity.

Photoperiod:

GLIM analysis (tables 4.5-4.7) shows that the photoperiod has a significant effect on activity at the marsh site but not at the ungrazed and grazed site. The initiation of activity in the autumn may be associated with the corresponding decrease in daylength. Similarly, the cessation of activity in spring may be associated with increasing daylength. In almost all cases of this kind of diapause, where there is a suppression of host-seeking activity (behavioural diapause) it has been shown to be controlled by photoperiod (Belozerov 1982). This diapause is characteristic of a short-day reaction, where cessation in activity and active development is a response to long days. This reaction is common to southerly species which have one generation per year (Belozerov 1982). Szymanski (1987a) considers the end of spring activity in *D. reticulatus* to be a reaction to lengthening day coinciding with the end of May. The initiation of activity in the autumn is thought to depend on daylength and ambient temperatures.

Temperature:

Mean monthly maximum temperatures had significant effect on activity at the ungrazed and grazed sites (table 4.5, 4.6), but not at the marsh site (table 4.7). The cessation of activity in spring may be associated with increasing temperatures but the initiation of activity in the autumn occurs before temperatures fall.

Rainfall:

GLIM analysis shows no significant correlation between rainfall and activity at any of the sites. The rainfall pattern shows a fall in the spring of each year, when activity peaks and then ceases, but there is no significant affect. Rainfall has been shown to affect activity in *Dermacentor occidentalis*, heavy rainfall causing a depression in questing activity (Lane *et al.* 1985).

Microclimate- vegetation temperature and humidity:

The temperature within the vegetation appears to have a significant affect on the activity at the grazed and marsh sites, but not at the ungrazed site. The humidity within the vegetation has a significant affect on the activity at the grazed site only.

In summary, the activity at the dune sites(ungrazed and grazed) was affected by mean monthly maximum temperatures and by photoperiod at the marsh site. This is surprising given that these ticks are likely to be part of one population (see later). A possible explanation for these local behavioural differences may be linked to the level of exposure in the different habitats. The dune types (ungrazed and grazed) have a less continuous cover of vegetation than the marsh site and may be more prone to extremes in temperature. This might be reflected in the significant affect of maximum temperature. The marsh site, having a more continuous cover of vegetation, may be buffered from extremes in temperature. The ticks here appear to be responding to photoperiod. The macroclimate will have a significant affect on the microclimate. Any correlations between activity and macroclimate are likely to reflect the ticks response to the microclimate. There is an apparent microclimatic affect at the grazed and dune sites, but not at the ungrazed. The grazed site is, perhaps, the most exposed and both temperature and humidity in the microenvironment seem to have an important affect here. However, following the above hypothesis on exposure we might expect the ungrazed site to show a significant affect of temperature and humidity. The results show no significant affect of microclimate on

activity at the ungrazed site, but a significant affect of temperature at the marsh. The limitations of this day of sampling data must be taken into account.

Regulation of activity and diapause:

In terrestrial arthropods, photoperiod is thought to be the main factor controlling the induction of diapause (Saunders 1982, Danks 1987). However, temperature has been found to modify the photoperiodic response and may also act independently of photoperiod as a stimulus itself for diapause (Van Houten and Veenendaal 1990).

In general, the regulation of seasonal activity in ticks may depend on the direct response to environmental conditions and also an internal physiological clock controlled by photoperiod (Belozerov 1982). Sonenshine (1988) considers that in ticks, photoperiod is probably the most important environmental factor controlling the induction and termination of diapause, with temperature playing a significant but secondary role. Evidence for the role of photoperiod in ticks was found in *D. variabilis* were daylength had a strong effect on overwintering diapause, seasonal activity and feeding success (Smith and Cole 1941). Also, Goddard (1992) found that the questing activity of *Ixodes scapularis* began at almost exactly the same date each year and that the time of peak activity varied by only one day for both years of study suggesting that there was a photoperiodic effect. There is evidence of the role of neurosecretory substance (NSS) in diapause in *D. reticulatus* (Ioffe 1965). The quantity of NSS doubled from the lowest level in May to peak concentrations in August. This increase corresponded with a gradual increase in daylength with a lag of approximately one month.

Whilst photoperiod may have the most significant effect on the initiation or termination of diapause, other studies have found that temperature has the most significant effect once activity is underway. For example, Hair and Bowman (1986) found in the lone-star tick *Amblyomma americanum* that though photoperiod may have an influence on the cessation (or initiation) of activity, the subsequent activity is predominantly regulated by the

temperature and humidity. Robertson *et al.* (1975) also found that the influence of temperature on the activity of *A. americanum* was greater than that of photoperiod and RH. McEnroe and McEnroe (1973) found variation in the activity of adult *Dermacentor variabilis* which was related to the climatological gradient. Conditions within the soil microclimate were thought to regulate the initiation of activity and the ambient water stress conditions considered to regulate the termination of activity. Also, Harlan and Foster (1990) found that ambient temperature was the best predictor of adult questing in *D. variabilis*. In *I. pacificus* the activity of adults was positively correlated with RH and negatively correlated with ambient temperature (Loye and Lane 1988). In a study on *D. reticulatus* in S.E.France (Martinod and Gilot 1991), environmental temperature was found to have the most significant effect on activity, but photoperiod was assumed to initiate the summer diapause.

The geographic variation in the activity pattern shown by Szymanski (1987a) and including this work indicates that there is no fixed photoperiodic event, though whether this response is thermolabile as in the morphogenetic diapause of *Ixodes ricinus* (Belozerov 1982) needs to be determined by laboratory experiments. The climatic conditions would seem to have an important bearing on this geographic variation in activity. By comparing the activity at Harlech with that in S.E.France it would appear that there are possible strain differences in the populations response to temperature. Both populations show a significant affect of temperature on activity, but at Harlech the peak activity occurs in the range 8-13.4°C, whereas in France peak activity occurs in a much narrower range of temperature (13-15°C).

There is evidence of within area differences at Harlech which appear to be correlated with either mean monthly maximum temperature or photoperiod. This tends to support the idea that there is a climatic and photoperiodic input into the regulation of activity. It is possible that the ticks at the marsh were also responding to the water table level. Other work by Semtner and Hair (1973) found distinct activity patterns for each stage of *A. americanum* depending on habitat type (microclimate). The variations between the sites at

Morfa Harlech indicate that the ticks exhibit flexible behavioural responses which are likely to be dependent on their own specific microenvironment. This is a form of phenotypic plasticity known as polyphenism whereby there are environmentally cued alternative phenotypes in a population (Mayr 1963, West-Eberhard 1989). Martinod and Gilot (1991) had suggested that, because the microenvironments of the various favourable biotopes were different, then the dynamics of each *D. reticulatus* population may vary from one field to another and this seems to be borne out by this study.

Persistence of D.reticulatus at Morfa Harlech:

The Welsh populations may be considered marginal within the context of the geographic distribution of *D. reticulatus*. Within the Harlech site itself, the ungrazed site may be considered marginal in that it is deprived of host cattle and must rely on a more infrequent and unreliable source of hosts in the form of dogs (*Canis familiaris*) and a dwindling rabbit (*Oryctolagus cuniculus*) population (see later).In the years that there was concurrent sampling at the ungrazed and grazed sites there was a greater peak in activity at the grazed site, similarly there was a greater peak at the marsh site than the ungrazed site in 1988 and 1989 (fig. 4.5). However, the greatest activity peak was recorded in 1987 at the ungrazed, but there were no comparisons available with the other two sites. McEnroe (1978) says that marginal areas will be characterised by chaotic population cycles and that climatic regulation will be the dominant regulating factor. However, if MaCleod's (1962) theory that *D. reticulatus* is a cool- temperate species which will find optimal conditions as it moves north is correct then we may consider Harlech to be an optimal site. The optimal conditions for the development of all stages of *D. reticulatus* are 22°C , RH of 80-95% and a 12- 15 hour photoperiod. These conditions resulted in a life- cycle lasting 120-180 days (Stelmaszyk 1979). If the microclimatic conditions have a sufficiently high humidity then these conditions approximate to the mean conditions experienced at Morfa Harlech during the summer months in which case it may

be considered as being a near optimum environment. However, during the winter months when adults are feeding on hosts, then the conditions will be far from optimal and it is likely that winter development is slow. Another *Dermacentor* species *D. variabilis* successfully colonised Nova Scotia as it required no shift in diapause or breeding season (McEnroe 1985) and so was in essence pre-adapted to the area which may well be the case with *D. reticulatus* and Harlech. In this event the fluctuations in tick numbers may be linked to host availability. The role that the availability of hosts played in creating the differences in the activity patterns between the 3 sites studied is not clear from the results of this study. Hosts can in theory alter the activity pattern through the depletion of questing ticks (Randolph and Steele 1985). To unravel the complexities of the tick-host interaction at these sites, sampling of ticks on hosts should be conducted concurrently with the sampling of the off-host phase. Ideally hosts should be sampled throughout the active phase of the adults to determine the seasonal variation in the numbers of ticks on the hosts. As I will discuss later this was not possible at this site.

Persistence in a range of habitats:

The vegetation analysis (see study sites) confirms that within a local area, *D. reticulatus* persists in a range of habitat types. Ticks are generally associated with certain plant communities and these communities reflect the environmental conditions such as temperature, humidity, light and soil type (Nosek 1978). The plant communities themselves will have a direct and indirect effect on the tick populations. The direct effects are how favourable the microclimates are to the ticks survival, the plant litter layer having an important influence on this (Nosek 1978). The microclimatic conditions are known to affect the development, density and behaviour of ticks (Daniel *et al.* 1977, Nosek 1978). Should these habitats be altered through agricultural improvements, then *D. reticulatus* will disappear from these areas (Daniel *et al.* 1986). The indirect effects will be primarily connected with how suitable the habitat is for the ticks hosts, including the availability of food and cover for small mammal hosts and the utilisation of the habitat by large mammal

hosts (cattle).

On a geographic scale *D. reticulatus* exists in a range of habitat types including meadow and oak forest (Cerny *et al.* 1982), river basins, swampy mixed woods, lakeshore vegetation (Nosek 1972), pastured land, heath, scattered scrub and suburban wasteland (Gilot *et al.* 1973, 1974) and here in Wales in the coastal dune systems.

It is interesting to find *D. reticulatus* occurring in a range of habitats within a local area. Whether the microclimates of the different habitats are significantly different is a matter of speculation, but it may be reasonable to assume that the microclimate of the dune habitats will be different from the marsh habitats. Accepting this a number of hypotheses are offered to account for the ticks' persistence in the different habitats:-

a) The ticks have become adapted to the different habitats leading to strain differences.

b) The tick is tolerant of a range of microclimatic conditions.

c) The ticks avoid unfavourable conditions by exhibiting a range of behaviours in the different habitats.

Considering each point in turn, for case a) this would require a strong selection pressure leading to a very fine- grained genetic differentiation within the population. At this site there is likely to be significant gene flow between the dune and marsh areas as ticks(adults) from the dune area are likely to be transferred to the marsh area and vice-versa by the cattle which roam freely over the dune system. Unless the selection pressure is very strong such a situation is unlikely to lead to genetic differentiation within a local area. Strain differences on a geographic scale have been shown in *D. reticulatus* from different biotopes (Daniel *et al.*1980).

For case b) it is known that ticks have particular environmental requirements at the

species level e.g in North America, *Dermacentor variabilis* is confined to moist areas whereas *Dermacentor andersoni* is found in scrub areas where the humidity is low in the summer (Knulle and Randolph 1982). Knulle and R u dolph (1982) suggest that the distribution of a tick species with respect to environmental humidities is primarily determined by the degree of waterproofing of the integument. Given this, it may be unlikely that a tick species will exhibit a range of humidity tolerances.

Case c) may be the more likely scenario in which the ticks avoid unfavourable extremes of the different microclimatic conditions by exhibiting different behaviours in the different habitats. This would require the ticks to respond to short term cues from the local microclimate. This will be a purely phenotypic response to local environmental cues and will lead to differences in seasonal activity patterns between sites if they have significantly different microclimates. This hypothesis may be supported by the significantly different activity patterns shown by the dune areas (ungrazed and grazed) on the one hand and the marsh area on the other.

Seasonal sex ratio variation:

Seasonal differences in the sex ratio were also observed. Similar findings are reported for *D. reticulatus* by Szymanski and Cerny (1981), Szymanski (1987b), Nosek (1972) and Gilot *et al.* (1974). The general trend suggests an earlier onset of male activity in the autumn, then females predominate numerically before the onset of winter and increase in predominance in the spring (see figs.4.13a, b). No adequate explanation has yet been offered to account for the sex ratio differences observed in *D. reticulatus* and other *Dermacentor* species. Fig.4.13b and table 4.11 indicate that there are more females than males and this has also been found by Szymanski (1987b). Behavioural differences may account for the earlier onset of male activity in the autumn whereby males emerge first from the summer diapause (Szymanski and Cerny 1981), the males perhaps, being more resistant to the autumn conditions (high temperatures, low humidities). However, Szymanski (1987b) showed that marked males and females exhibited the same

behaviour in spring and autumn and expressed a roughly equal sex ratio. The numerical predominance of females cannot be accounted for by behavioural means and there does not appear to be any difference in the survivorship of males and females (Szymanski 1987b). Parthenogenesis is known to occur in ticks (Oliver 1971) and could account for the observed differences in the number of males and females, but as yet there is no evidence that it can occur successfully in *D. reticulatus* (Szymanski 1987b). Females have been shown to predominate in artificially bred groups of *D. reticulatus* (Immler 1973) even in lines derived from single, fertilised females suggesting the existence of a genetic mechanism. Davey and Cooksey (1988) found more females reached the adult stage in *Boophilus* ticks. They suggest that the presence of a XX-XO sex determining mechanism accounts for the sex ratio differences, because any recessively lethal mutation occurring on the X-chromosome will be expressed in the male but not in the female unless it is homozygous recessive for the lethal trait. The XX-XO sex determining mechanism has been found in North American species of *Dermacentor* (Gunn and Hilburn 1990).

Association with tracks:

The ticks appear to be associated with tracks at the ungrazed site, the tracks being frequented by humans and dogs and, perhaps, rabbits. Gilot et al. (1973) found a positive association of *D. reticulatus* with tracks used by dogs in a suburban area of Grenoble. Ticks are known to be able to move over some metres horizontally and may be attracted to host odours such as CO_2 . Many people walk their dogs in the ungrazed dune area and many have mentioned to me that their dogs often pick up ticks. Such infestations are unlikely to maintain the tick population as the engorged ticks will be removed to unsuitable environments, though a few may disengage back at the dune site. This species of tick is known to transmit *Babesia canis* to dogs in France (Gilot *et al.* 1974) and it will interesting to see whether this disease turns up in this country.

Further work should try and elucidate the important factors contributing to the observed

differences in behaviour at this site. Ideally, this will involve monitoring microclimatic variables such as the temperature and relative humidity *in situ* on a continuous or frequent level. The activity of caged specimens on site as well as activity levels in the field population would be measured. To supplement this data, the questing activity of adults under different experimental conditions in the laboratory would be monitored using time-lapse videos. Having determined the microclimatic conditions in the field, the effects of the different conditions experienced in the different habitats on the rates of development, activity and mortality in all stages of the tick could be assessed. These experiments will give an indication of the suitability of the different habitats for the survival of *D. reticulatus*. A prerequisite for these experiments is a laboratory culture of the tick to supply adequate numbers for experimentation.

C: ON-HOST PHASE - ADULTS

RESULTS AND DISCUSSION

As mentioned little data was collected on adults feeding on their hosts for various reasons. The data that was collected is presented below:

Cattle:

The main hosts for the adults at the grazed and ungrazed site was assumed to be Welsh Black cattle which roamed over the dune system. Unfortunately, the cattle were only inspected on one occasion (13/10/88) and then only a total of 7 individuals were examined. The cattle belonged to Glan-y-mor farm at Harlech. The farmer was very co-operative about access, but unfortunately was not prepared to round-up his cattle for inspection. This is quite understandable given the large area over which the cattle range. The small amount of data from cattle which had recently been grazing on the marsh area

gave evidence of a simultaneous infestation of *D. reticulatus* and *I. ricinus*. All specimens of *D. reticulatus* were found on the neck and side of the neck, all *I. ricinus* were found on the rear axillae. The mean infestations are given in table 4.12:

Table 4.12: Mean infestations of *D. reticulatus* and *I. ricinus* on Welsh black cattle at Glan-y-mor farm, Morfa Harlech

	Mean no. males	Mean no. females
D.reticulatus	10.0 ± 2.6	7.7 ± 1.6
I.ricinus	0.1 ± 0.3	1.6 ± 0.5

In the years when comparisons were available the peaks in activity of adult ticks were greater at the grazed and marsh site than that at the ungrazed site, possibly suggesting the importance of a cattle population in determining the tick population size. The abundance of an abundant host population is clearly important in maintaining a viable tick population. This has been shown in *D. reticulatus* in Czechoslovakia where ticks were absent from potentially favourable habitats because of the absence of suitable hosts for adults (Daniel *et al.* 1986). The large tick population in the Biebrza river basin in Poland is attributed to the high density of elks (*Alces alces*), a preferred host of adults in this region. The distribution and abundance of *Ixodes dammini* was influenced by the availability of suitable hosts, movement of infested hosts and climate. The abundance of white-tailed deer hosts was directly correlated with that of the tick (Wilson *et al.* 1988). However, contrary to this view, Schulze *et al.* (1984) found no significant relationship between the density of adult *I. dammini* and their major hosts white-tailed deer and Norval (1979) has also shown that the population size of adults was not influenced by the population size of their ungulate hosts. Clearly, the activity levels of adults on their cattle hosts and any possible host-mediated

regulation of tick numbers needs to be investigated.

Rabbits:

At the ungrazed site, I assumed that the rabbit population must play a role in the maintenance of the adult population given the lack of any other obvious hosts with the possible exception of dogs. In the autumn of 1987 I attempted to assess the population size of rabbits along 2 transects, one on the ungrazed side and one on the grazed side. The counts were done after dark using a powerful beamed torch. No more than 2 rabbits were observed in either transect and occasional monitoring by the N.C.C. through the year revealed that the population was at a very low level and was not making any kind of recovery. Myxomatosis appeared to be the culprit.

Two attempts were made to measure the infestations on the rabbits and to get an estimate of the rabbit population size. This involved the use of two expert ferreters. Terrier dogs identified occupied burrows and the rabbits captured provided an estimate of the population size. On the first trapping occasion, on 18/3/88, 6 rabbits were captured in the grazed and ungrazed area but no tick infestations of any species were recorded. On the next trapping occasion, 16/11/89, 9 adult rabbits were captured, 7 of them males indicating that the females were breeding and probably occupying single hole burrows on the edge of the warren which are very difficult to locate. This means that the numbers captured represented an underestimate of the rabbit population. However, the counts reflected a small population size and there was much evidence of myxomatosis still being prevalent. No ticks were found on any rabbits, but 11 adult *D. reticulatus* were found on one of the terrier dogs. This may suggest that rabbits are unsuitable hosts for *D. reticulatus*. However, adult ticks fed successfully on rabbits in the laboratory with large numbers of eggs and larvae being produced. Also, rabbits are known hosts of members of the genus *Dermacentor* (Hoogstraal and Aeschlimann 1982). This mystery may be solved by further trapping in the field and, perhaps, by monitoring the success of ticks feeding on wild as opposed to laboratory rabbits.

D: ON-HOST PHASE: IMMATURES

INTRODUCTION

It has proved extremely difficult to capture larval and nymphal (immature) *D. reticulatus* by means of blanket dragging and flagging in this study and other studies (Gilot pers.comm.). The only records in this study are from Aberdyfi where 2 larvae were recorded in July 1988 and 1 nymph in August 1988. The inability to capture immatures by blanket dragging may be linked to their questing behaviour. It was found in *D. variabilis* that immatures were present on the walkways of their small mammal hosts (Hoogstraal 1978) and were, therefore, likely to be unavailable for capture by blanket dragging. It is also possible that replete immatures drop-off in their hosts resting places as was found in *I. ricinus* (Matuschka *et al.* 1991). The immatures may also quest at heights below that of the main vegetation e.g. in *I. ricinus*, only half the larvae placed in 35cm tubes in the field quested at the top of the tubes and it was suggested that the larvae have adapted to quest at heights suitable for their small rodent hosts (Gray 1981). The only practical way of monitoring the activity in the immature population was to trap their hosts and monitor the levels of infestation. Szymanski (1987c) also considered that the only way to monitor the activity of immature *D. reticulatus* is to monitor the infestations on their hosts, which, as in most of the Palaearctic species of *Dermacentor*, are almost exclusively small mammals. Therefore, the aim of this study was to monitor the infestations on small mammals through the activity period of the immatures. The activity period of the immatures was assumed to correspond with the period of adult summer inactivity as found by Immler (1973) and Syzmanski (1987c).

The infestations of ticks on their small mammal hosts does not give an absolute value of immature activity and density. This would be almost impossible to measure given the

generally highly aggregated distribution of larvae. The parasite-host relationship is complex, but the following measurements on host infestations can be made to monitor the parasite population (Kim 1985) :

1. Infestation rate (Prevalence) - the percentage of host individuals infested.

2. Population rate (Intensity) - mean no. of parasites per host (mean tick burden).

3. Dispersion rate (frequency distribution) - the percentage of hosts infested with a specific no. of parasites.

The first two parameters, infestation rate (prevalence) and population rate (intensity) can give a general description of the parasite population in a local host population (Kim 1985). In general, where parasites are aggregated, the prevalence is relatively low and the intensity relatively high, indicating that the parasite occurs in localised high density patches (Anderson 1982). However, the infestation rate (prevalence) does not provide much information on the parasite population, especially when it is very high and the population rate (intensity) does not give an accurate picture of the parasite population density and distribution for clumped parasite populations without the dispersion rate (Marshall 1981).

In general, three dispersion patterns are recognised in parasites : random, overdispersed (aggregated) and underdispersed (uniformly distributed) (Anderson and Gordon 1982). The degree of dispersion can be measured using the formula s^2/x, where s^2 = the sample variance and x = the sample mean number of parasites per host. This is also known as the Index of Dispersion or I. When I is unity a Poisson distribution is described i.e. the parasite is randomly distributed. When I is >1, then the parasite is overdispersed i.e. the population is aggregated. When I is <1 then the parasite population is underdispersed i.e. the population is uniformly distributed (Southwood 1978).

Parasites are usually overdispersed (Crofton 1971), but these patterns can change in time and space owing to mechanisms that increase or decrease overdispersion. These mechanisms include two types of factors: a). demographic stochasticity involving population growth (natality, mortality, dispersal rates) and b). environmental stochasticity, which includes variable environmental factors such as macroclimate, microclimate, host susceptiblity and behaviour (Anderson and Gordon 1982). Overdispersions of parasitic arthropods are usually the result of heterogeneity in the probability of parasite infestation of a host (Kim 1985). For temporary parasites such as ticks, heterogeneity in climate and demographic parameters are critical (Kennedy 1975, Nelson *et. al.* 1975, 1977, Marshall 1981).

Levels of tick infestation on the host population as measured using the above parameters gives an indication of the immature tick activity in the field. The hosts can be interpreted as acting as continual blanket drags giving a measure of the current activity (recently engorged ticks) and activity several days previously (ticks near to engorgement and stages in between). This is, of course, a simplistic view as the host-parasite interaction is a complex one and will be affected by many variables such as the degree of immunity in an individual host and in different species of host, behavioural differences within a species (age, sex differences) and between species and competition for engorgement sites on the host.

To show the activity patterns of the immature stages, Szymanski (1987c) derived curves of mean no. of larvae and nymphs per host for the important host species. Ideally, a similar study would have been made here with sequential trapping through the immatures'activity period. Unfortunately, for various reasons, mainly logistical, the trapping was far more restricted than this and the first records of immatures on hosts were not made until the summer of 1989. Therefore, the data on infestations are rather limited, but what was obtained is used to provide an assessment of the small mammal-immature tick interaction.

MATERIALS AND METHODS

Small mammals were live trapped using Longworth traps set up in a grid. The grid was 90 x 90m, each trapping station being 15m away from adjacent stations. Two traps were placed at each station to avoid saturation of the trapping point (Andrzejewski *et al.* 1966). Captured small mammals were sexed, weighed and fur-clipped (to record recaptures). They were then anaethestised in the field and examined for ticks using a portable binocular microscope. The traps were inspected between 0800-0900 hrs and 1600-1800 hrs each day over several days. The population densities of the small mammals were estimated using Schnabels' estimate (1938).

A permanent grid was set up at the ungrazed dune site at Morfa Harlech in September 1987. The first trapping occasion was between 4-8/10/87. No ticks were recorded, perhaps not surprisingly given that it was late in the season , although active nymphs have been recorded in Slovakia in winter (Nosek 1979). The next trapping occasion was in July 1988 (11-15/7/88). Again, no ticks were recorded on the small mammals.

In 1989 a temporary grid was set up at the marsh site where a healthy population of adults had been found the previous winter. The grid covering parts of areas A, B and C (see study sites). Trapping occurred between 4-7/7, 18-22/7 and 14- 17/8/89. Immatures were recorded for the first time in the early July sample. Unfortunately there were not many trap records during the early July trapping episode. This was mainly due to the use of inadequate plastic traps, the N.C.C traps being unavailable until later in the month due to their own trapping programmes. The trapping episode later in July and in August were more successful and the results presented here will concentrate on the data collected on these two occasions. Use will be made of the small mammal population estimates obtained at the ungrazed site.

RESULTS

There were no records of immature ticks on their small mammal hosts at the ungrazed site. The population densities of the small mammals found at each site on each trapping occasion are given in table 4.13. There were insufficient trap records in early July 1989 to determine population densities. Host species recorded at both sites were *Clethrionomys glareolus*(bank vole), *Apodemus sylvaticus* (wood mouse), *Sorex araneus* (common shrew) and *Sorex minutus* (pygmy shrew). There was one record of *Neomys fodiens*(water shrew) on 16/08/89 at the marsh site (uninfested). The values for *S. araneus* and *S. minutus* are total captures as they died in the traps. The values represent the number per grid, the area sampled being common to both sites (8,100m).

At the ungrazed site, *A. sylvaticus* was more common than *C. glareolus* in October, but in the following July the reverse was true (Table 4.13). Apart from the population cycles that these small rodents may go through, this change may be explained by the difference in "trappablity" of some species depending on the season. *A. sylvaticus* is known to be more difficult to catch in the summer months (Kikkawa 1964; Tanton 1965, 1969). At the ungrazed site, *A. sylvaticus* was usually found at the tops of the dunes in areas where broken sand was present, whereas *C. glareolus* was mainly found at the base and on the sides of the fixed dunes/ dune grassland.

At the marsh site, *C. glareolus* was clearly the dominant species and the population is comparable to that found at the dune site. The *A. sylvaticus* population was smaller than at the dune site, but showed a marked increase in numbers between July and August 1989. *C. glareolus* was found in parts of the grid characterised by marsh areas A and B, whereas *A. sylvaticus* was mainly found in parts of the grid with the habitat of marsh area C i.e. dry areas of short grass with clumps of *Juncus acutus* .

The few larval records obtained are shown in table 4.14.

Table 4.13: Population size estimates of small mammals trapped at the ungrazed and marsh sites at Morfa Harlech 1987-89.

Site	Date	Species/population per grid			
		Cg	As	Sa	Sm
Ungrazed	5-8/10/87	16(9)	30(10)	7	0
Ungrazed	11-15/7/88	44(20)	17 (8)	25	6
Marsh	18-22/7/89	61(36)	3 (2)	19	5
Marsh	14-17/8/89	44(30)	15 (5)	9	2

Cg = *Clethrionomys glareolus*
As = *Apodemus sylvaticus*
Sa = *Sorex araneus*
Sm = *Sorex minutus*

Cg and As estimates determined by Schnabels' estimate (1938), figures in parentheses are total captures. Values for Sa and Sm are total captures on each occasion.

Schnabels' estimate:

$$N = \Sigma\, ni.Mi / \Sigma\, mi$$

where:

Mi= total number of marked animals in the population before the ith sample.

ni= number in the ith sample

mi= number in the ith sample that are marked.

Table 4.14: Records of larval *D. reticulatus* on small mammals at Morfa Harlech.

Date	Species of host	Sex	no.larvae	no.nymphs
5/07/89	Cg	f	2	2
	Sa	-	2	0
6/07/89	Cg	f	2	1
	Sa	-	3	7
	Sa	-	6	5
19/07/89	Cg	f	4	12
	Cg	m	2	19

Unfortunately, there were few trap records in early July mainly due to the traps used; the plastic traps tripped too easily and so they were closed but empty when inspected, but these records show a simultaneous infestation of larvae and nymphs on the one host. By mid-July there were only 2 hosts recorded with larvae, nymphs clearly predominating (see below) and by August there were no records of larvae. Although limited, these data suggest that larval activity ceases between mid-late July. Monitoring in June may have revealed much more larval activity, but traps were not available at this time.

Table 4.15 shows the nymphal infestations on the four recorded host species in July and August 1989 at the marsh site. In July the prevalence was highest in *C. glareolus* (89.7%)

Table 4.15: Infestations of *D.reticulatus* nymphs on small mammal hosts in July and August 1989 at the marsh site, Morfa Harlech.

July

	Cg	As	Sa	Sm
No. of hosts	39	2	19	5
% infested hosts	89.7	50.0	68.4	40.0
mean no. nymphs/host ($\bar{x}$) ± S.E	10.5 ±1.8	0.5 ±0.5	1.5 ±0.4	0.6 ±0.4
Variance (s^2)	124.3	0.5	3.2	0.8
$s^2/\bar{x}$	11.8	1.0	2.1	1.3

August

	Cg	As	Sa	Sm
No. of hosts	30	5	9	2
% infested hosts	66.7	20.0	33.3	0
mean no. nymphs/host ($\bar{x}$) ± S.E	1.5 ±0.3	0.4 ±0.4	0.7 ±0.4	0
Variance (s^2)	2.5	0.8	1.3	-
$s^2/\bar{x}$	1.7	2.0	1.9	-

Test of the significance of differences in infestations between host species using multiple comparisons following a Kruskal-Wallis test (Siegel and Castellan 1988).

July		August	
Cg-As	ns	Cg-As	ns
Cg-Sa	***	Cg-Sa	ns
Cg-Sm	***	Cg-Sm	ns
As-Sa	ns	As-Sa	ns
As-Sm	ns	As-Sm	ns
Sa-Sm	ns	Sa-Sm	ns

and relatively high in *S. araneus* (68.4%). The intensity (mean no. ticks per host) is high in *C. glareolus* (10.5±1.8). There was a high degree of clumping in the *C. glareolus* and *S. araneus* populations suggesting that a small number of individuals harbour a large number of ticks (see later). To compare the number of ticks carried by the different host species multiple comparisons following a Kruskal-Wallis test (Siegel and Castellan 1986) were conducted. In essence, this test ranks the individuals according to their tick burdens and calculates an average rank for each species. These average ranks are then compared to an overall rank to determine the significance of differences in tick burdens between species. In July, the results show that on average *C. glareolus* are infested with more nymphs than *S. araneus* and *S. minutus* but not *A. sylvaticus*, though the small number of records (n=2) for *A. sylvaticus* must, perhaps, be considered.

In August, *C. glareolus* again predominated in the captures and had the highest prevalence and intensity of infestation. These two parameters were much lower than in the previous month. The prevalence is also much lower in *A. sylvaticus* and *S. araneus* than in July. The ticks show a clumped distribution in *C. glareolus*, *A. sylvaticus* and *S. araneus*. The results of the multiple comparisons test show that there are no significant differences in the tick loads borne by the 4 host species.

C. glareolus appears to be the main host species at the marsh site. The frequency distribution of the nymphs on this species are shown in fig 4.14 a, b. In July, 33.3% of the population had no, or low, tick burdens (0-2), but 44.8% of the population had > 10 ticks per host. In August, 79.9% of the population had low burdens (0-2) and no individual had more than 5 ticks.

The above data indicate that *C. glareolus* is the most important host species at the marsh site and that the infestation rate, population rate and degree of aggregation of the nymph infestation is much reduced between July and August.

Table 4.16 shows the nymphal infestations of sub-adult, male and female *C. glareolus* in July and August 1989. In July, the intensity were higher in males and females than in sub-adults, as are the mean tick burdens. The distribution of the nymphs was highly clumped in

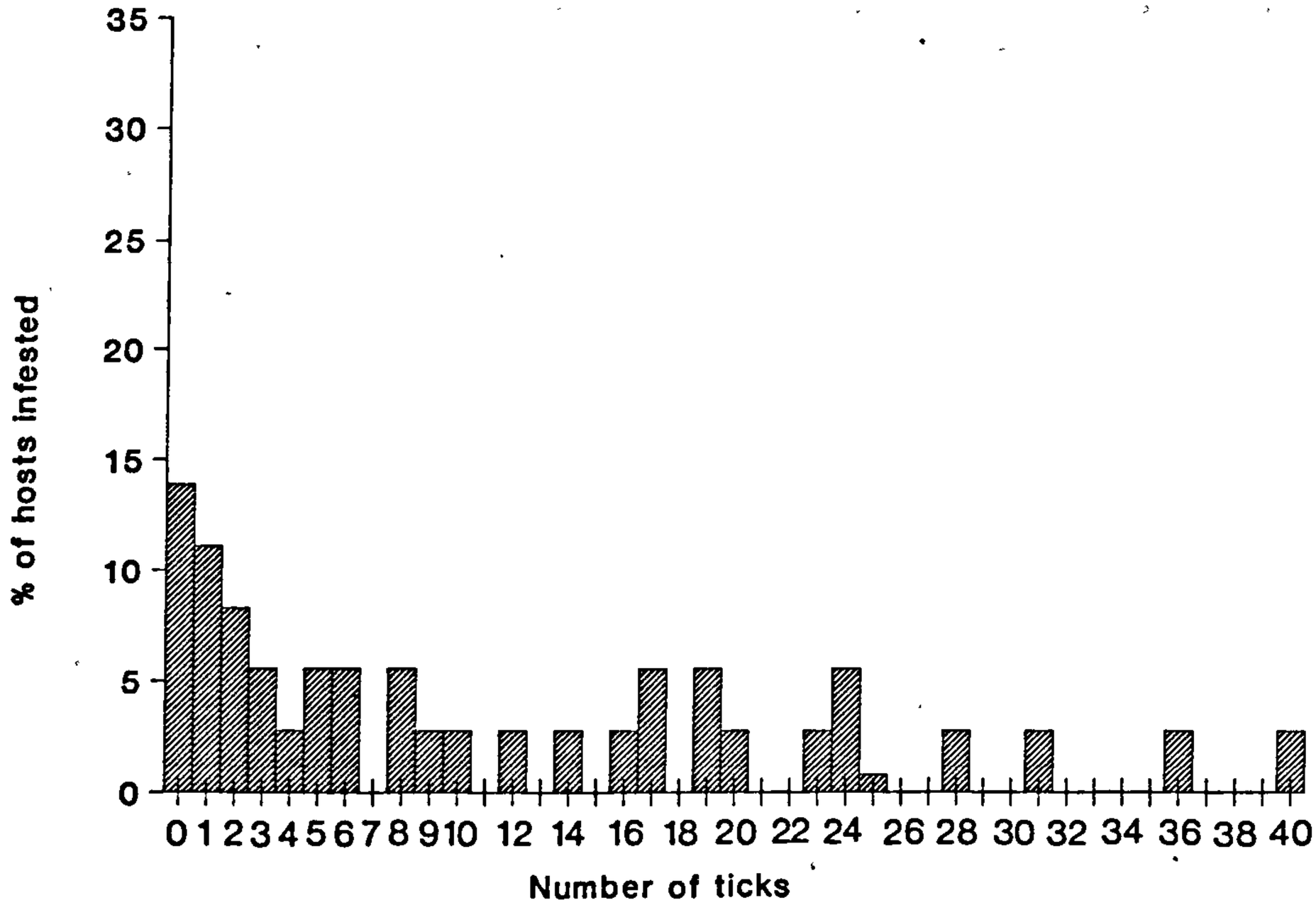


Fig. 4.14a: Frequency distribution of *D. reticulatus* nymphs on *C. glareolus* in July 1989.

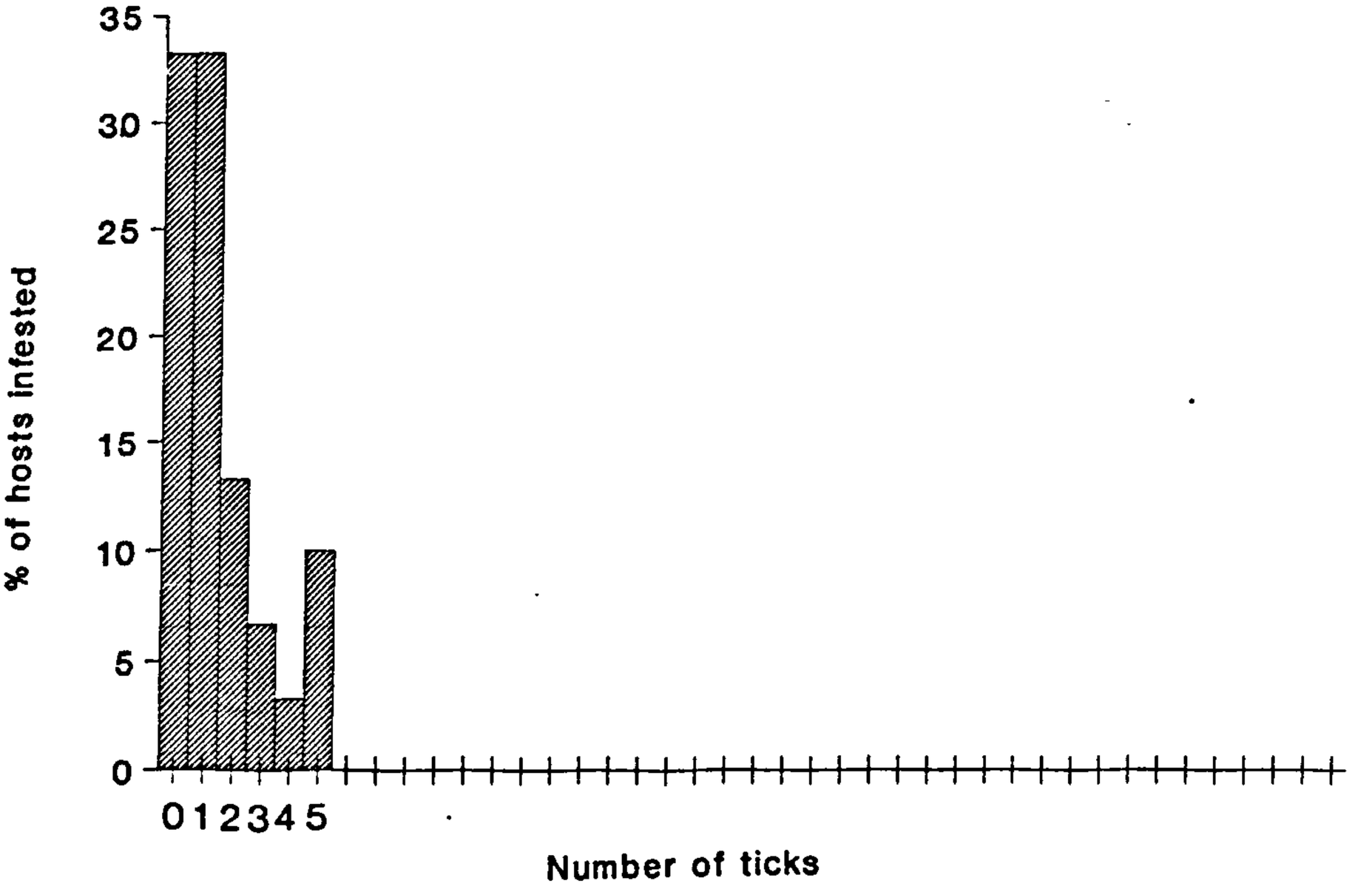


Fig. 4.14b: Frequency distribution of *D. reticulatus* nymphs on *C. glareolus* in August 1989.

Table 4.16: Infestations of *D.reticulatus nymphs* on sub-adult, male and female *Clethrionomys glareolus* in July and August 1989 at the marsh site, Morfa Harlech.

July

	sub-ad	male	female
No. of hosts	14	13	12
% infested hosts	78.6	92.3	91.7
mean no. nymphs/host ($\bar{x}$) ± S.E	3.6 ±1.7	19.9 ±0.5	8.4 ±1.7
Variance (s^2)	40.7	164.1	32.8
$s^2/\bar{x}$	11.2	8.2	3.9

August

	sub-ad	male	female
No. of hosts	8	12	10
% infested hosts	75.0	91.7	30.0
mean no. nymphs/host ($\bar{x}$) ± S.E	1.3 ±0.5	2.5 ±0.5	0.4 ±0.2
Variance (s^2)	1.6	3.0	0.5
$s^2/\bar{x}$	1.3	1.2	1.2

Test of the significance of differences in infestations between sub-adults, male and female C.glareolus using multiple comparisons following a Kruskal-Wallis test (Siegel and Castellan 1988).

July		August	
sub-ad..male	***	sub-ad..male	ns
sub-ad..female	ns	sub-ad..female	ns
male..female	ns	male..female	***

sub-adults, males and females. The multiple comparisons test showed that males had on average higher tick loads than sub-adults, but there were no significant differences between males and females and sub-adults and females.

In August, the infestation rate in males and sub-adults was similar to that in July, but was markedly lower in females. The population rate was much reduced in males and females as compared to July and the degree of clumping of the infestations was much reduced in sub-adults, males and females. Males had significantly more ticks than females, but there were no significant differences between males and sub-adults, and females and sub-adults.

DISCUSSION

From the data collected here, it is not possible to deduce the activity patterns of larval and nymphal *D. reticulatus*. however, it appears that larval activity is over by the end of July, and that the nymphal activity is more pronounced in July, but decreases through to August. With such limited data, it cannot be deduced whether this is a result of the activity cycle of the ticks, or a reaction of the ticks to short-term climatic fluctuations, or to the effects of acquired resistance of the hosts to the ticks. The phenology of the immature stages of the tick is thought to depend on the climate (Szymanski 1987c). At the eastern end of the tick's range (W.Siberia) the activity period of larvae is brief, beginning in mid-June, peaking in early July and ceasing by the end of August. The nymphal activity period begins at the end of July, peaks by mid-August and disappears by the beginning of September. Westwards, the activity period of larvae and nymphs is longer, particularly when influenced by the moist oceanic climate. The Welsh populations are ideal for testing this activity model as they should in theory possess the longest activity phase in the ticks range. Although we cannot draw firm conclusions here, it does appear that larval activity is over by mid-July which is not in accordance with Szymanski's model.

The data do give an indication of the host-tick interaction at the site, particularly for the nymphal stage. No other tick species was recorded at this site. The lack of evidence of immatures on hosts at the ungrazed dune site may reflect a very low population density of immatures. All evidence of immature infestations comes from the marsh site. It is possible that the marsh site and slack areas at Harlech are the only suitable habitats for immatures at Harlech. Adults, which are more tolerant of lower humidities, may be dispersed into the more xeric dune habitats by the movements of small mammal hosts of the nymphal stage. Such a distribution pattern was found in *D. variabilis* where immatures were found concentrated near the forest/field border (higher RH), but the adults were much more widely dispersed and occurred in more xeric habitats (Sonenshine and Stout 1968, Sonenshine and Levy 1972).

There were few records of larval infestation with two host species recorded *C. glareolus* and *S. araneus*. Four species of host were recorded with nymphal infestations, *C. glareolus*, *A. sylvaticus*, *S. araneus* and *S. minutus*. Of these, *C. glareolus* appears to be numerically dominant and to support most of the nymphal population. Immler(1973) found that immature *D. reticulatus* were restricted to a single host species, *C. glareolus*. The restriction to a major host species varies through the tick's range and is dependent on the geographic location and the extent of specific habitats therein (Szymanski 1987c). The restriction of the immature stages to a small number of host species may be due to host specificity or a consequence of the ecological requirements of host and tick. Although the immatures may occur on a range of host species throughout the tick's range, it may be possible for the tick to evolve a specificity within a geographic location. Szymanski (1987c) suggested that different species may act as the main host depending on geographic location and habitat type. In open areas in Siberia, *Microtus gregalis* is the main host, whereas in forest areas, *M. oeconomus*, *Clethrionomys rutilus* and *S. araneus* were the main hosts. He also discovered that the host species was of more importance than host abundance as at study sites in Poland, although *S. araneus* was the most abundant host, *M.*

oeconomus and *M. agrestis* fed most of the nymphs. Larvae of *Ixodes ricinus* have been shown to have a marked preference for *A. sylvaticus* in the laboratory, 53% of introduced larvae engorged on *A. sylvaticus*, with only 8% of introduced larvae engorging on *C. glareolus*. This preference was reflected in the field with much greater larval burdens on *A. sylvaticus* even though it has a shorter daily active period and range than *C. glareolus* (Nilsson and Lindqvist 1978). At this site *C. glareolus* was the most abundant species but also had a markedly higher prevalence and mean intensity of nymphal infestation which may have indicated a host preference for this species by the nymphs of *D. reticulatus*.

The role that *A. sylvaticus* plays in maintaining the immature population may be underestimated, given the difficulty of catching the species in the summer months (Kikkawa 1964, Tanton 1965, 1969). Laboratory experiments would reveal whether the differences observed in the field (Immler 1973) are the result of host specific responses.

The most abundant species may maintain the bulk of the immature population because of the increased probability of infestation. This simplistic situation will be affected by the area of ground covered per unit time by the host and the general habitats of the host (underground/overground dweller). Mohr (1961) found that the infestations of the rabbit tick, *Haemaphysalis leporis-palustris* w ere higher in the snowshoe hare (*Lepus americanus*) than in the cottontail rabbit (*Sylvilagus floridanus*), and this was associated with the larger size and home range size of the hare. This difference was also associated with the different habitat usage of both species, hares selected habitats that were more suitable for ticks than those selected by rabbits. Also, it was found in *Ixodes trianguliceps* infestations that tick loads were not related to home range size alone, but were influenced by factors such as the daily activity periods of hosts (Randolph 1975).

The nymph burdens of the different age-classes and sexes of the main host, *C. glareolus*, were investigated. In July, fewer sub-adults were infested than males or females and the burdens were on average significantly greater in males than sub-adults. In August, fewer sub-adults were infested than males, but even fewer females (30%) were infested. There

were significantly more nymphs on males than females and the degree of aggregation on all classes was much diminished. The overall reduction in nymphal numbers between July and August is likely to reflect the diminishing activity of the nymphs. However, the differences in the observed burdens of the different age classes and sexes of *C. glareolus* must reflect both changes in the number of nymphs active and behavioural changes in the host. The lower infestation rates in sub-adults in July may result from their shorter home range size. The higher burdens in males may be due to their territorial behaviour, and females may be foraging over larger areas when maintaining offspring. The reduction in the infestation rate of the females and the reduction in their nymph burdens in August may be due to a decrease in foraging by the females once they have reared their offspring. Langley and Fairley (1982) found that *I. ricinus* larvae were more abundant on male *A. sylvaticus* than on females, because the males covered more ground than the females. Similarly, Davidar *et al.* (1989) found more and better engorged immatures of *I. dammini* on male than female *Peromyscus leucopus*. Overall, the nymphal infestations on *C. glareolus* in July are of a high prevalence and high intensity and the frequency distribution in fig. 4.14a suggests a high density of nymphs in the field. In August, the prevalence is high but the intensity is low and the frequency distribution suggests a low density of nymphs in the field.

To determine the seasonal activity patterns of the immatures at this site requires an intensive programme of trapping. Now that a suitable site has been located (marsh site), small mammal trapping should occur regularly (bi-weekly) between May and September. Trapping may also be carried out at less frequent intervals during the winter months in case immatures have a prolonged activity period at this site given the mild winter conditions. If this programme is carried out over a number of years, it will be possible to determine the activity pattern of the immatures and also to discover more about the tick-host interaction e.g the effects of fluctuations in the host population size and any effects of acquired resistance. The immature stages may be critical in determining the adult tick population size. In *D. variabilis* there was a strong positive correlation between the adult tick

population and the size of the small mammal population indicating that the feeding success of the immatures was the most important factor in regulating the adult tick population. The population regulation appeared to be determined by the feeding success of the nymphal stage (Smart and Caccarnise 1988). Further work in the laboratory on the host preferences of immatures following Nilsson and Lindqvist (1978) and the effects of acquired host immunity on the ticks could also be carried out to elucidate the tick-host interaction.

CHAPTER 5

A STUDY OF BEHAVIOURAL AND PHYSIOLOGICAL RESPONSES TO ENVIRONMENTAL FACTORS IN *DERMACENTOR RETICULATUS* AND *IXODES RICINUS*

A: FIELD STUDIES:

INTRODUCTION

The seasonal activity pattern of *Ixodes ricinus* in Britain is generally described as being bi-modal e.g. in northern England (Milne 1945, Lees and Milne 1951), in Wales (Arthur 1948, Evans 1951b), Scotland (Evans 1951b) and this has also been recorded in Ireland (Walton and O'Donnell 1967, Gray 1982). In some of these studies a unimodal pattern of activity was found in some areas but was considered to be aberrant. Steele and Randolph (1985), however, confirmed the existence of a uni-modal pattern of activity in an upland area of Wales and this was thought to be climatically controlled.

It was apparent during the first year of the study of *Dermacentor reticulatus* activity that this tick was active during the winter months at a time when the common and more widespread *I. ricinus* was generally assumed to be inactive. In order to confirm the differences in the activity patterns of these two species a comparison was made between the activity pattern of *I. ricinus* at a lowland site on Anglesey and the activity of *D. reticulatus* at the grazed site at Morfa Harlech.

MATERIALS AND METHODS:

A site at Maltraeth marsh adjacent to the forest at Newborough Warren, Anglesey (Grid ref: SH 4265) was selected to monitor the activity of *I. ricinus*. The site is at sea-level and the macro-climatic conditions would have been similar to those experienced at Morfa Harlech. The site was an enclosed paddock owned by the Institute of Terrestrial Ecology which used the area as grazing for a small flock of Soay sheep. The dominant vegetation at the site was a thick covering of rushes (*Juncus spp.*) and the vegetation mat was thick and generally water-logged. A comparison was made with the activity of *D. reticulatus* at

the grazed site at Morfa Harlech as both sites were grazed (cattle at Harlech and sheep at Newborough) and both sites were sampled over approximately the same time period (Feb 1988-March 1990 for *D. reticulatus* at the grazed site, Harlech and March 1988-Nov 1989 for *I. ricinus* at Newborough). Each site was sampled at approximately bi-weekly intervals during the activity period and monthly intervals during the rest of the year. On each sampling occasion the same sized area was covered, 24 x 100 m^2 blanket drags at each site. Adult ticks were counted after each 100m blanket drag and returned to the vegetation along the transect. The data were presented as total monthly counts for both tick species. GLIM analysis of the effect of the macroclimatic variables (mean monthly maximum and minimum temperature, total monthly rainfall and photoperiod) on activity was conducted. The meteorological data were collected at RAF Valley , approximately 10 miles north of this site.

RESULTS

The activity patterns of both species are shown in fig. 5.1. As discussed previously (p73) the activity of *D. reticulatus* begins in late August and finishes in the following May, though there was much within site variation in this pattern. It can be seen from fig. 5.1 that there are marked differences between the activity patterns of these two species.

In 1988 the activity of *I. ricinus* did not begin until March, showed a marked peak in June before dropping-off rapidly in July/August, but picked up immediately, so that autumn activity began immediately that spring activity ended. The autumn activity peaked in September and ended in November. The spring and autumn peaks were of similar size. In 1989 a similar pattern emerged, but peak activity in spring was in May and there was more of an obvious demarcation between the spring and autumn periods of activity. The spring and autumn peaks were again of similar size, but only half the magnitude of the peaks in 1988.

The results of the GLIM analysis of the affect of macroclimatic variables on the activity

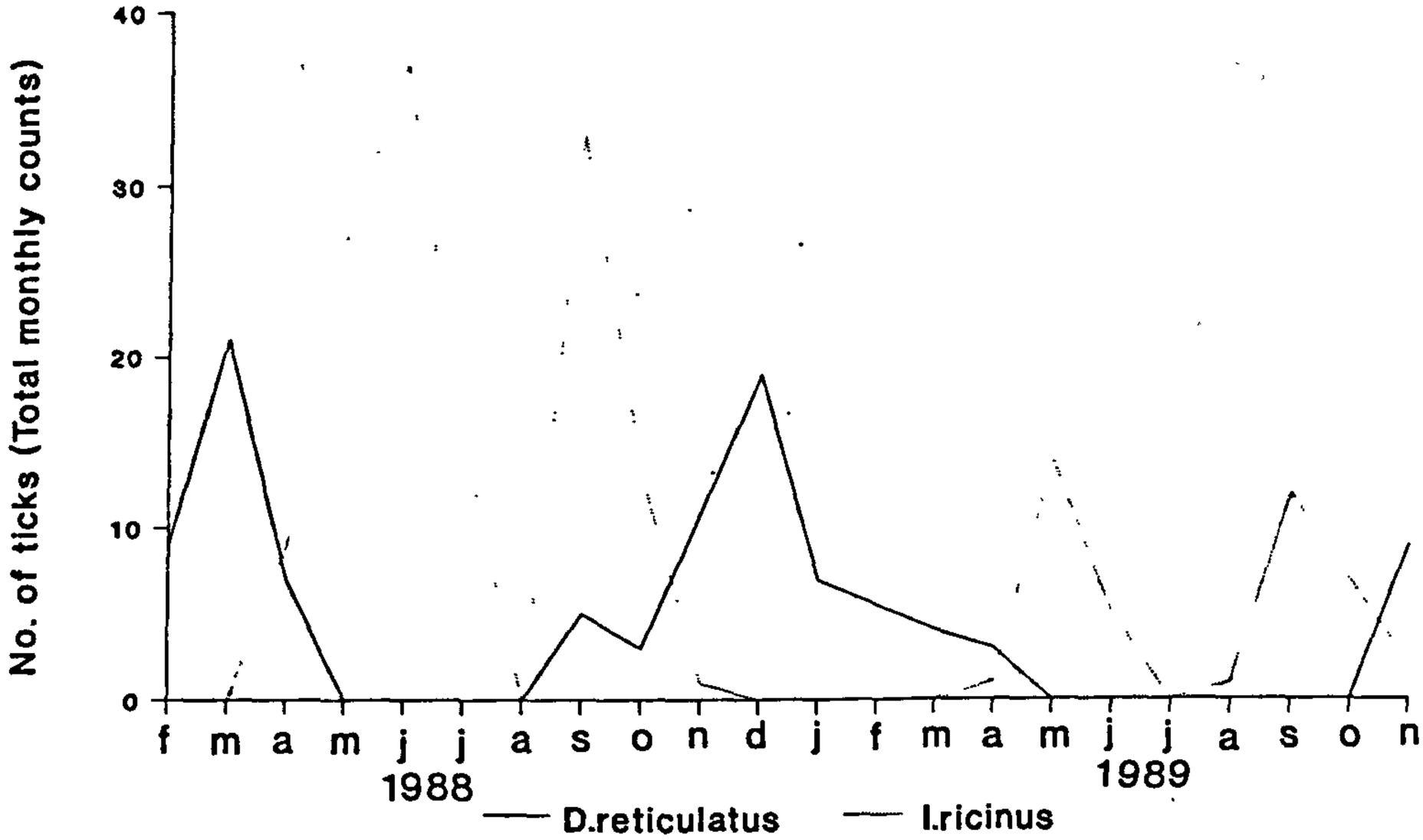


Fig. 5.1: Seasonal activity of adult *Dermacentor reticulatus* at the grazed site, Morfa Harlech (Feb. 1988 - Nov. 1989) and adult *Ixodes ricinus* at Newborough, Anglesey (Mar. 1988 - Nov. 1989). Values are total numbers of ticks captured per month.

of *I. ricinus* are given in table 5.1. The results show that both photoperiod and rainfall have a significant effect on the activity of *I. ricinus*. The only macroclimatic variable to have a significant effect on the activity of *D. reticulatus* at the grazed site was the mean maximum monthly temperature (table 4.5). The photoperiod and temperature data are highly correlated so that any temperature effect may be masked. The significant effect of rainfall is somewhat surprising given that the peaks in tick activity correspond with periods of lowest rainfall which would presumably result in lower humidities in the vegetation.(fig.5.2)

In summary, it is clear that the seasonal activity patterns of *I. ricinus* and *D. reticulatus* are markedly different. *D. reticulatus* was active during the winter months when *I. ricinus* was inactive and was inactive during the spring/summer when *I. ricinus* was active.

DISCUSSION

The activity pattern of *I. ricinus* at Newborough conforms with the bi-modal pattern of activity recorded for this species in Wales (Arthur 1948, Evans 1951b). The pattern of activity at this site differs from the typical bi-modal pattern in that the spring and autumn peaks of activity are of a similar size. In the typical pattern the spring peak is greater than the autumn peak.

There were clear differences in the activity patterns of the 2 species, *D. reticulatus* is active during the winter months and showed peaks of activity in March 1988 and December 1988, *I. ricinus*, in contrast, is active during the period of the summer diapause of *D. reticulatus*. As shown in chapter 4 the activity of *D. reticulatus* at the grazed site was affected by the macroclimatic temperature and the temperature and humidity in the vegetation. The activity of *I. ricinus* was affected by the photoperiod and the degree of rainfall, no data was available on the microclimatic variables. Photoperiodic control of the bi-modal activity pattern of *I. ricinus* was suggested by Arthur (1960) and Belozerov (1973). The similarity of the activity patterns in England and Wales (Milne 1945, Evans

Table 5.1 a, b: GLIM analysis of effect of macroclimate on the activity of *Ixodes ricinus* adult at Newborough, Anglesey.

a)

Model	Scaled deviance	df	Change in deviance (x^2)	Change in df	
1	288.5	20			
1+max t	232.6	19	55.9	1	p<0.001
1+min t	256.5	19	32.0	1	p<0.001
1+rain	235.9	19	52.5	1	p<0.001
1+photo	224.4	19	64.1	1	p<0.001

b)

Model	Scaled deviance	df	Change in deviance (x^2)	Change in df	
1+photo	224.4	19			
1+photo + max t	220.6	18	3.8	1	p>0.05
1+photo + min t	222.3	18	2.1	1	p>0.1
1+photo + rain	203.4	18	21.0	1	p<0.001

The results in table 5.1a show that photoperiod has the most significant effect on the activity. This is indicated by the lowest scaled deviance (smallest variance).

The photoperiod is now incorporated into the model and the remaining terms added to see if any have a significant effect on the activity (table 5.1b). This procedure ensures that the macroclimate variables have a significant individual effect on the activity. The significant results for mean monthly maximum temperature and and mean monthly minimum temperature shown above may be the result of their correlation with photoperiod.

The results show that the photoperiod and the total monthly rainfall have a significant effect on the activity of *I.ricinus*.

Table 5.1 cont'd.

Key: max t= mean monthly maximum temperature.

min t= mean monthly minimum temperature.

rain = total monthly rainfall.

photo= photoperiod.

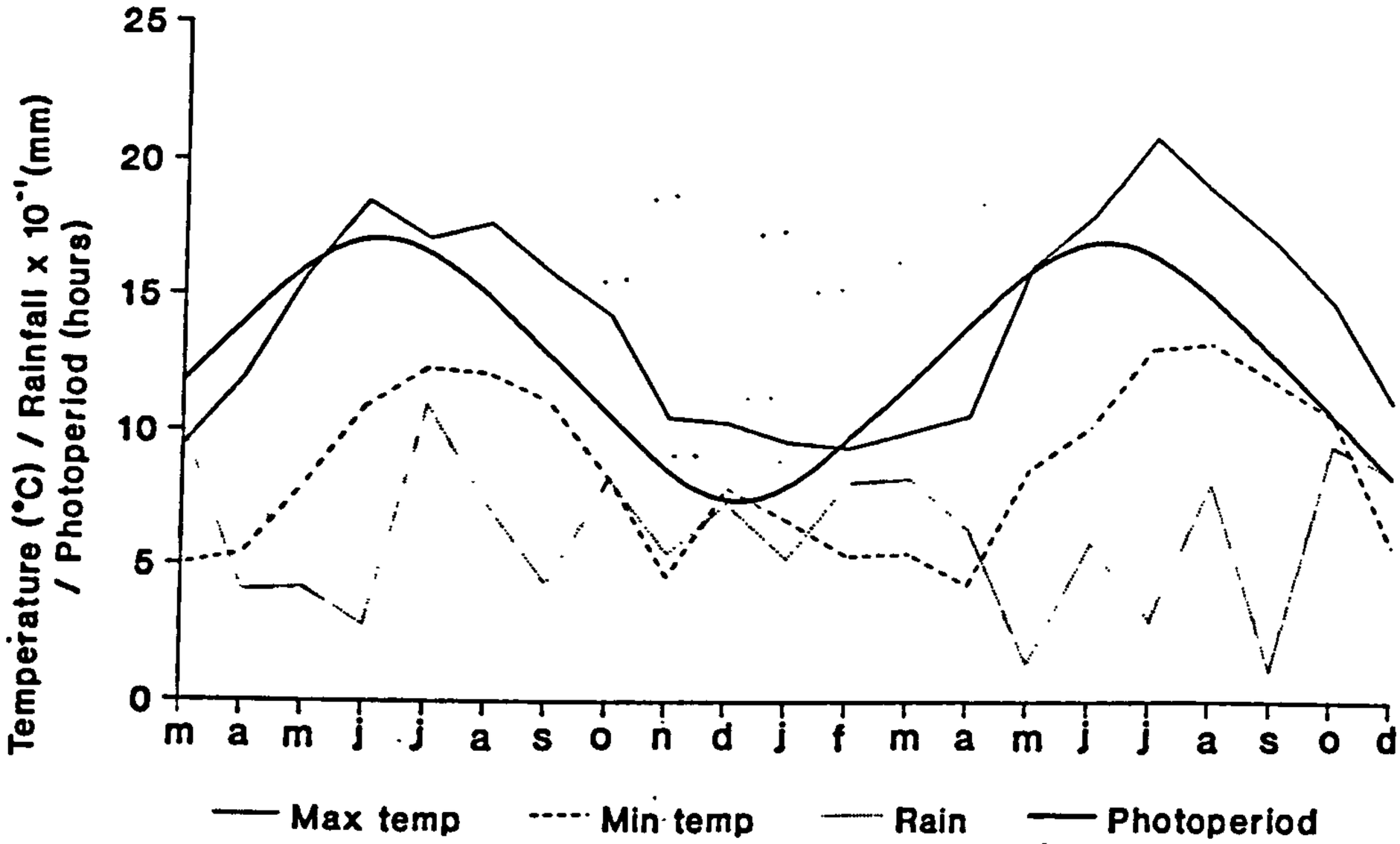


Fig. 5.2a: Macroclimate variables between March 1988 - November 1989. The temperature and rainfall data are from RAF Valley, Anglesey and the photoperiod data from N. Ireland, 55°N (Beck 1980).

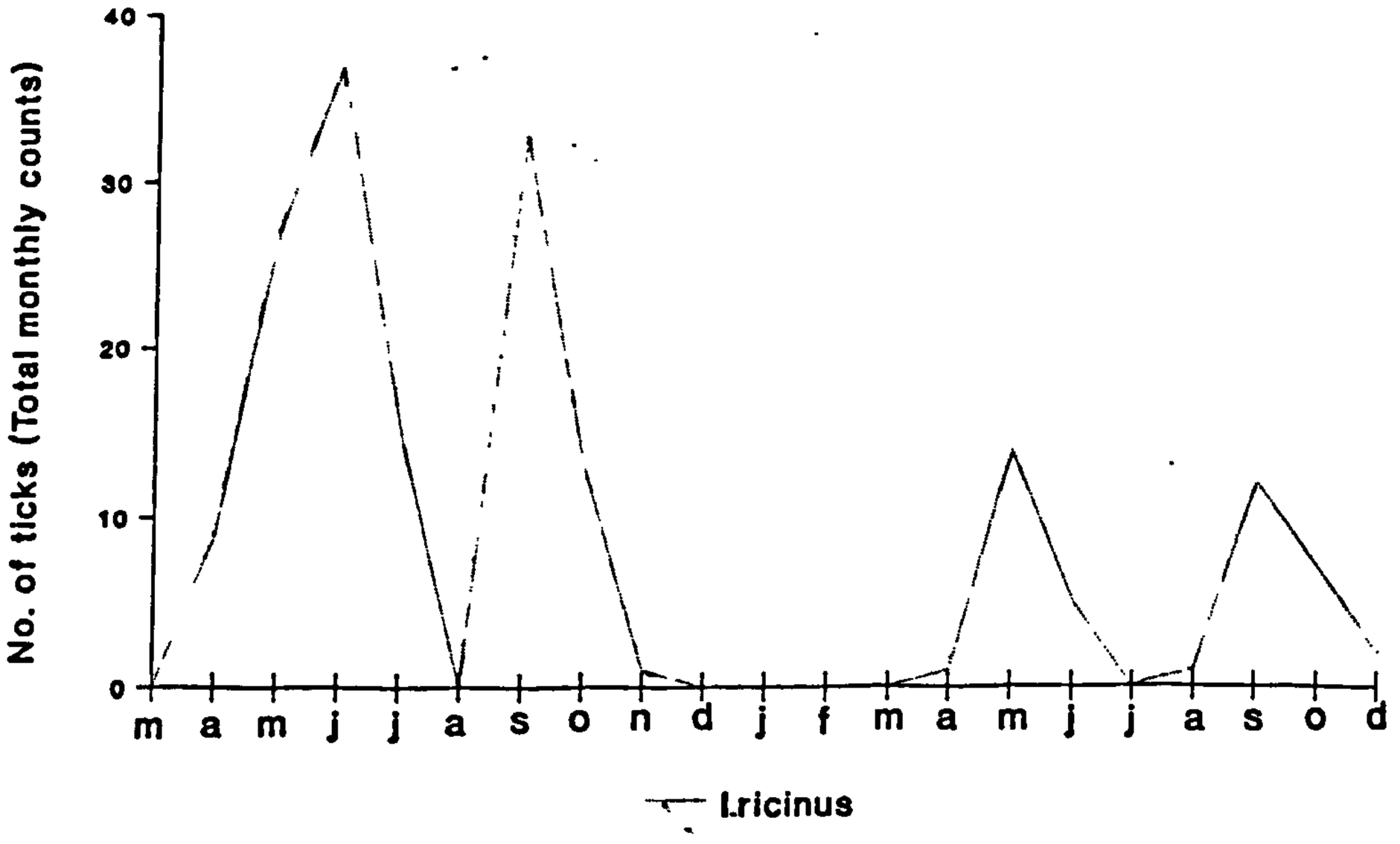


Fig. 5.2b: Seasonal activity of *I. ricinus* adult at Newborough, Anglesey (fig. 5.1).

1951b and Lees and Milne 1951) and in Austria (Pretzmann *et al.* 1964, 1965) despite differences in location, altitude and climate has been cited as evidence of this (Sonenshine 1988). However, variation in the activity pattern is apparent in Britain with uni-modal patterns of activity being found in Scotland (Evans 1951b) and in upland areas of Wales (Steele and Randolph 1985). It is very likely that this variation is under climatic regulation. The mechanism may work by a photoperiod/temperature interaction as discussed in chapter 4. Donnelly and Mackellar (1970) found a strong relationship between air temperature and tick activity (as determined by the incidence of redwater fever). However, Gray *et al.*(1978) and Gray (1980, 1984) found that there was no obvious temperature effect above the threshold but any temperature effect may have been obscured by the overall availability and exhaustion of a population of questing ticks. The significant effect of rainfall is surprising given that the peak activity in spring and autumn coincide with the periods of lowest rainfall in these two years. One might have assumed the opposite, i.e. that higher rainfall led to an increase in tick activity by increasing the humidity in the vegetation. However, heavy rainfall has been shown to depress the questing activity in the tick, *Dermacentor occidentalis* (Lane *et al.* 1985). It is more likely that the activity is governed by a combination of photoperiod and temperature. Activity begins and ends at the same time each year, but there are differences in the time of peak activity and the shape of the activity curve which are presumably temperature effects. Spring and autumn in 1988 and 1989 were drier than average (fig. 4.8,a) so that the normal timing of spring and autumn peak activity may have coincided with a drier than normal period resulting in the association between activity and rainfall. Longer term data would help to discern which factors are having a major effect on the activity of *I. ricinus*.

The two sites, though comparable macroclimatically, would have had very different microclimates. The grazed site consisted of low cropped vegetation with clumps of marram (*Ammophila arenaria*) and the conditions of temperature and humidity on some summer days would have been extreme for tick survival. The site at Newborough consisted of a much denser vegetation with a thick and very moist vegetation mat and the temperature

and humidity conditions at this site are likely to have been much more equable than at the grazed site at Morfa Harlech.

It is clear that *D. reticulatus* is active in winter when exposed to temperatures at which *I. ricinus* is not active. The threshold temperature below which activity ceases in *I. ricinus* is thought to be ≈7°C (Gray 1984) but *D. reticulatus* has been shown to be active at temperatures below this during the course of this study. During a 24 hour monitoring of questing by ticks at the marsh site in March 1989 the minimum temperature at which ticks were recorded active was 3.3°C (0900 hours) and the minimum overnight temperature was -5.4°C. This part of the study has confirmed the differences in the activity patterns of the 2 species. Given these differences I aimed to identify the physiological attributes which enabled *D. reticulatus* i) to remain active in the winter months and ii) to persist in an extreme environment such as the dune habitat at Morfa Harlech. To try and account for this the next two sections aim to investigate differences in both the cold-tolerance and humidity tolerances of the two species.

B: COLD-HARDINESS IN THE TICKS *DERMACENTOR RETICULATUS* AND *IXODES RICINUS*

INTRODUCTION

The results of the field study show that *D. reticulatus* and *I. ricinus* have markedly different activity patterns. During the winter *D. reticulatus* will be subjected to sub-zero temperatures at infrequent intervals. Observations made at Harlech indicate that some adult ticks were active even when the underlying sand surface was frozen. It is unlikely that these ticks would have found a micro-climate that did not experience overnight sub-zero temperatures. To do so they would have had to bury themselves beneath the sand surface on a daily basis. Therefore, I assumed that *D. reticulatus* was able to withstand sub-zero

temperatures, whereas *I. ricinus* which is inactive in the winter months, is less tolerant of sub-zero temperatures than *D. reticulatus*.

In terms of cold-hardiness, insects and other arthropod species fall roughly into two groups, freeze-tolerant species which are able to tolerate the formation of ice in the body fluid and freeze-sensitive species which are intolerant of ice-formation in the body fluid (Salt 1961, Zachariassen 1985). Most insects and other terrestrial arthropods are freeze-sensitive species (Somme 1982) and there are no records as yet of freeze-tolerant acarines (Somme 1981).

Freeze-sensitive species must avoid freezing and they do so by their lesser or greater ability to supercool. In biological systems, supercooling can be defined as the ability to avoid freezing and, therefore, injury at temperatures below freezing (Danks 1978). It is possible to determine the degree of supercooling in a species by measuring it's supercooling point (SCP). The SCP is defined by Zachariassen (1985) as follows " When a sample of water or an aqueous solution is cooled, it will normally not freeze when the melting point is reached, but will remain unfrozen even when cooled far below this temperature. A system that remains unfrozen at temperatures less than it's melting point is said to be "supercooled" and the temperature at which spontaneous freezing occurs in a supercooled system is termed the supercooling point". The principle of supercooling works as follows, as water is supercooled below it's freezing point the size of the molecular aggregates increases as the temperature decreases. Eventually these aggregates will reach a critical size and embryo ice-crystals form in the solution. In biological systems, these embryo crystals form typically around particles e.g. food in the gut, particles in the haemolymph. In the absence of such particles or nucleating agents, pure water supercools to $\approx$ -40°C (Duman and Howarth 1983). Arthropods can be considered as liquid containers in which the conditions for supercooling are exceptionally favourable (Somme 1982). Supercooling is known as a means of winter survival in a large number of freeze-sensitive species of insects and other arthropods (Somme 1982, Bale 1987).

Freeze-tolerant species raise their SCP's to ensure that extracellular freezing occurs, thus

avoiding fatal intracellular freezing (Zachariassen 1980). Extracellular freezing is usually facilitated by the presence of ice-nucleating agents (INA). Most species are considered freeze-tolerant if they survive freezing at temperatures corresponding to their SCP or lower. A few freeze-tolerant species have very low SCP's which are associated with the presence of cryoprotectants e.g. glycerol, and the lack of INA's in their haemolymph.

I had initially hoped to compare the supercooling points of *D. reticulatus* and *I. ricinus* throughout their activity period to see if *D. reticulatus* had a greater ability to supercool (lower SCP) than *I. ricinus*. Unfortunately, due to the restricted availability of ticks and the loan of experimental equipment the study was more limited. Instead, the supercooling points of spring collected *D. reticulatus* and upland and lowland specimens of *I. ricinus* were compared.

MATERIALS AND METHODS

The supercooling point (SCP) was measured using the same principles as those of Bachmetjew (1901), Payne (1927) (both as quoted in Somme 1982) and Salt (1961), and are based on the use of the thermocouple. The apparatus is shown in fig. 5.3. The ticks were cooled using a small semi-conductor Peltier device which acts as a heat pump, the principle of the pump being that the passage of an electric current through the junction of two dissimilar conductors can either cool or heat this junction depending on the direction of the current. The heat absorption rates from the surface of the Peltier device are proportional to the magnitude of the current and also the differential temperature existing across the device. The body heat of the tick is transferred from one side of the plate of the heat pump to the other and this heat is dissipated by a finned aluminium heat sink ensuring that the heat pump functions properly. The body temperature of the tick was monitored using a copper-constantan thermocouple in conjunction with a reference thermocouple maintained at 0°C. The output of the thermocouple measuring circuit was connected to a chart-recorder. The deflection span of the chart recorder was calibrated by immersing the

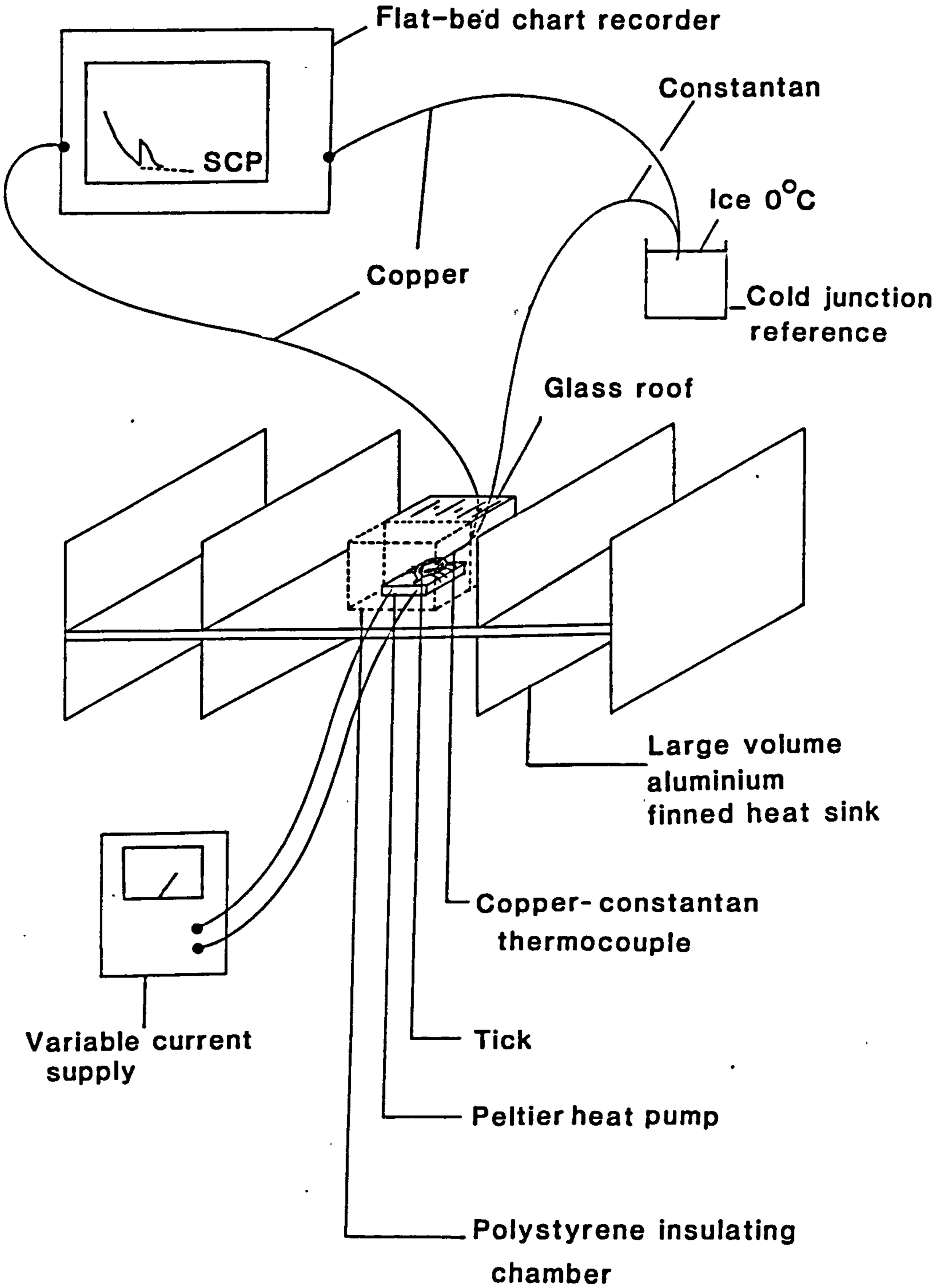


Fig. 5.3: Apparatus to determine the supercooling points of ticks.

measuring thermocouple into 2 water baths of known different temperatures. To increase the thermal conductivity, the ticks were connected to the thermocouple and to the Peltier device via zinc oxide conducting paste and cooled at as near a constant rate as possible. Somme (1982) recommended a rate of 1°C / min for comparative purposes as the rate of cooling will affect the freezing temperatures (Salt 1966). The intention was to cool at this rate but the nature of the equipment did not permit a very precise control of the cooling rate, this being controlled by manually adjusting the amount of current being fed to the Peltier heat pump. The chart recorder plotted the cooling curve of the tick and the SCP was easy to discern, being marked by a small but significant rise in temperature that accompanies the emission of latent heat during freezing.

Ticks were collected in the field at the following sites and were tested on the same day. All specimens of *D. reticulatus* were collected from Harlech (Grid ref: SH 5831 sea-level) with the exception of the individuals from Aveyron, France. Specimens of *I. ricinus* for the lowland site were collected from Llandonna (Grid ref: SH 5779 alt: 100 m), Newborough (Grid ref: SH 4265 alt: sea-level) on Anglesey and Mynydd Mawr, on the Lleyn Peninsula (Grid ref: SH 1525 alt: 50 m). For the upland site, ticks were collected above Dolgarrog in the Conway Valley (Grid ref: SH 7766 alt: 320 m).

RESULTS

The results of the supercooling points determined for *D. reticulatus* and *I. ricinus* are shown in table 5.2. In comparing the SCP of the 2 species, with the exception of the males ticks at the lowland site in March, the mean SCP in *I. ricinus* is consistently lower than that recorded for *D. reticulatus*. The mean and standard deviation are presented, the s.d gives an indication of the individual variation and, therefore, the chances of survival of sub-zero temperatures of individuals in the population (Somme 1982). There was no obvious difference between the mean SCP of the small number of specimens sampled from Harlech and those from Averyron in France.

Table 5.2 The supercooling points recorded in *D.reticulatus* and *I.ricinus* in spring 1989.

D.reticulatus:

Population	N	Sex	Mean SCP ± s.d (°C)	Cooling rate ± s.d (°C)
Harlech	6	♂	-6.34± 4.18	2.53± 0.98
	2	♀	-7.77± 3.59	3.15± 1.21
Aveyron	2	♀	-6.59± 6.10	2.65± 0.50

I.ricinus:

		LOWLAND			UPLAND		
Month	Sex	N	Cooling rate±s.d (°C)	Mean SCP ± s.d (°C)	N	Cooling rate±s.d (°C)	Mean SCP ± s.d (°C)
March	♂	2	2.14 ±0.19	-4.74 ±7.26	-	-	-
	♀	2	2.22 ±0.13	-9.34 ±0.19	-	-	-
April	♂	18	4.68 ±1.06	-11.46 ±4.15	4	3.34 ±0.12	-9.79 ±1.90
	♀	17	3.57 ±1.56	-8.61 ±1.73	1	2.62	-12.80
May	♂	15	4.09 ±0.96	-12.24 ±4.73	10	5.03 ±0.66	-8.65 ±1.53
	♀	15	4.55 ±0.87	-8.85 ±4.19	9	4.49 ±0.58	-10.74 ±4.15

Considering the lowland and upland populations of *I.ricinus*, the mean SCP of males at the lowland sites increases markedly between March and April from -4.74 to -11.46°C, but the small sample size and large s.d. of the March sample suggest that this difference is not significant. The mean SCP of males in April and May are similar at the lowland site, but they are lower than at the upland site (but with more variation). The mean SCP of females are lower at the upland site in April and May, but very small numbers of females were sampled in April, and in May there is so much variation at both sites that the differences do not appear to be significant.

In summary, the mean SCP is marginally lower in *I. ricinus* than in *D. reticulatus* though there is so much individual variation in both species that the differences are unlikely to be significant. There do not appear to be significant differences in the SCP's between upland and lowland populations of *I. ricinus*. Also, there do not appear to be significant changes either in the SCP through the spring, nor are there significant differences between the sexes.

DISCUSSION

The results suggest that the mean SCP is lower in *I. ricinus* than in *D. reticulatus*, though the size of the standard deviations indicate that there is much individual variation in both species. In *I. ricinus* there appeared to be no marked differences between the SCP's of upland and lowland populations. The values of the SCP's of both species indicate that they can withstand moderate sub-zero temperatures. The SCP for *D. reticulatus* is in the range -6 to -8°C and in *I.ricinus* -4 to -12°C. Lee and Baust (1987) determined the SCP of the antarctic tick, *Ixodes uriae* and found levels in the range -7 to -13°C for unfed adult males. The SCP's of the Antarctic dwelling tick are remarkably similar to those found here in these two temperate species. Similarly, the SCP's of summer-active insects from temperate and Arctic regions, and from tropical regions of Kenya were in the range -7 to -12°C (Husby and Zachariassen 1980, Zachariassen 1980). Thus, it appears that insects possess an innate ability to supercool, whether it is necessary or not. Young (1980) determined a

low SCP (-23°C) in the temperate cryptostigmatid mite *Humerobates rostralamellatus* and suggested that temperate species may possess cold-tolerance mechanisms in order to escape the effects of severe winters. Infrequent extreme temperatures in temperate habitats may exert sufficient selection pressure to maintain such adaptations in a population. Of the two species studied here, *I. ricinus* is more likely to seek out a microclimate which may be sheltered from the extremes of atmospheric conditions as it is inactive during the winter months. *D. reticulatus* must be exposed to more severe conditions as it is unlikely to be able to seek out such a sheltered microhabitat and then return to questing during the day. *I. ricinus* is likely to be in a state of behavioural diapause and in more protected microhabitats at this time.

Diapausing and non-diapausing species are able to reduce their SCP's in winter, usually through the accumulation of cryoprotectants such as glycerol (Somme 1982). It would be necessary to determine the seasonal changes in SCP's and in the levels of cryoprotectants to determine cold-hardiness differences between the two species.

It is possible that *D. reticulatus* is a freeze-tolerant species, in which case it's higher SCP is a reflection of the presence of ice-nucleating agents which ensure that freezing is extracellular and not intracellular. As mentioned previously, however, there are as yet no records of freeze-tolerant acarines (Somme 1981) and the lower winter temperatures in SE.France enforce a temporary quiescence so that freeze-tolerance is unlikely. The differences in the SCP's between the two species may be a result of size differences between the two species. In the psychid moth *Clonia variegatum*, the smaller males have lower SCP's than the larger, more cold-hardy females (Kaku 1969). As shown in the next section (p114), male *D. reticulatus* are 5x larger than male *I. ricinus* and female *D. reticulatus* are 3x larger than female *I. ricinus*.

Another possible explanation for the winter activity of *D. reticulatus*, not directly attributable to cold-hardiness,may be connected to the threshold temperature for activity. For *I. ricinus* the threshold temperature below which the tick is inactive has been

determined as 7°C (mean weekly air temperature) for adults active on sheep and 10°C (current air temperature) and 7.1°C (5cm soil depth temperature) for blanket collected adults (Gray 1984). *D. reticulatus* is active at temperatures below this threshold, in a 24 hour monitoring experiment at the marsh site in March 1989 active ticks were collected at a temperature of 3.3°C. It is also possible that *D. reticulatus* is more able to maintain water homeostasis at lower temperatures than *I. ricinus*, in *Dermacentor variabilis* active water uptake was not possible below 5°C (McEnroe 1971) and this was considered an important factor in determining the northern limit of the distribution of this species. Similarly, the active water uptake threshold temperature in *Amblyomma americanum* was 5-9°C (Sauer and Hair 1971).

There were no marked differences in the mean SCP's of male and female *I. ricinus* from the upland and lowland populations. The only peculiar result is that from the lowland site in March when the SCP is much higher than in April and May, but the sample sizes were very small in this month and the individual variation great. The low values of SCP at the lowland site in May, when conditions will not be severe, suggest that the SCP is not a reflection of cold-hardiness in this species, but is indicative of the body chemistry of the tick. Salt (1961) observed that all insects supercool to some extent, regardless of need, and Cloudsley-Thompson (1973) noted that the desert scorpion (*Leirus quinquestriatus*) from Sudan was able to supercool even though winter temperatures seldom fell below 5°C. If the SCP in *I. ricinus* was a reflection of cold-hardiness then one might expect the SCP to rise as spring progresses which does not appear to be the case. One might also have expected the SCP to have been lower at the upland site which will experience lower winter/spring temperatures with an increased frequency of frosts.

The SCP has been shown to be an unreliable indicator of cold-hardiness in a number of other studies. Turnock *et al.* (1983) suggested that three temperature zones should be recognised:

1) Cold injury- temperatures at which survival and development are affected and the effects are accumulated. Cold injury cannot be repaired at any temperature and the lower

boundary of this zone is the SCP.

2) Neutral- temperatures at which no cold injury occurs but at which cold injuries previously incurred cannot be repaired and at which the previous level of cold-hardiness is maintained.

3) Active- temperatures at which no cold injury occurs, previous cold injury may be repaired and changes in cold-hardiness may occur.

Lee *et al.* (1987) found that " the supercooling point is not indicative of the lower lethal temperature: both types of pupae (of *Sarcophaga crassipalpis* and *S. bullata*) die at temperatures far above the supercooling point. This mortality occurring in the absence of tissue ice-formation is known as cold shock, direct chilling injury or thermal shock..". They also suggested that many insects, including those in the non-diapausing state, have the ability to rapidly enhance their cold-tolerance, in response to a rapid temperature drop, by the accumulation of cryoprotective compounds. If the insect is not in diapause then these mechanisms are unlikely to provide protection through prolonged periods of exposure to low temperatures, but should ensure survival through brief periods of exposure. Bennett and Lee (1989) did not consider that the SCP was an indicator of cold-hardiness in the lady-beetle *Hippodamia convergens*, they found that cold-hardiness continues to decrease steadily throughout the acclimation period, despite the fact that SCP's remained constant near -13°C.

The SCP may be affected by a number of factors including the presence of ice-nucleating agents (INA's), thermal hysteresis proteins(THP's), the state of dehydration, accumulation of cryoprotectants and the fed state of the organism (gut and haemolymph contents).

Freeze-tolerant species usually possess INA's in the extracellular body fluid, ensuring a protective extracellular freezing at a few degrees below zero (Zachariassen 1985). Freeze-sensitive species usually remove or inactivate such INA's from their haemolymph. THP's are glycoproteins which have an antifreeze function. These compounds lower the SCP, it is thought, by inhibiting the formation of embryo-crystals by binding to them and thus

lowering the temperature at which spontaneous nucleation occurs. They have been found in insects in winter whilst they are not present, or only at low levels, in the summer (Duman *et al.* 1982). The state of dehydration of the insect may affect the SCP as an increase in solute concentration through dehydration results in a lower SCP. However, Salt (1961) considered that water loss must be extreme before such a depression of SCP occurred, but Young and Block (1980) found that dehydration increased glycerol production in the mite *Alaskozetes antarcticus*, which will depress the SCP. Freeze-sensitive and freeze-tolerant species are known to accumulate cryoprotective substances such as glycerol which act to depress the SCP. In non-diapausing and diapausing states of insects and mites there appears to be seasonal variation in the production of glycerol and other cryoprotectants (Somme 1982). The fed state of the insect may also be significant; the presence of food material in the gut raises the SCP and it is a prerequisite of many diapausing species to evacuate the gut before entering diapause (Somme 1982). The mobilisation of stored energy reserves by ticks may affect the composition of their haemolymph and , therefore, the SCP of the tick. Active ticks will presumably be more prone to raising their SCP and so will be more likely to freeze at higher temperatures.

The results of this study give an indication of the SCP's in these two temperate species of tick, but they do not indicate whether *D. reticulatus* possesses a greater cold-tolerance than *I. ricinus*, which would enable it to be active throughout the winter months when *I. ricinus* is in a state of diapause. To elucidate this, further experiments are required to determine:

i) The seasonal variation in the SCP of both species.

ii) Seasonal variation in cryoprotectants such as glycerol, and the seasonal variation in the concentration of haemolymph constituents such as INA's and THP's.

iii) Temperatures at which cold-injury occurs in both species.

iv) The activity threshold temperature of *D. reticulatus* and the threshold temperature for active water uptake in both species.

Such a series of experiments should reveal the mechanisms by which *D. reticulatus* remains active during the winter months.

C: THE CRITICAL EQUILIBRIUM HUMIDITY IN WELSH AND FRENCH POPULATIONS OF *DERMACENTOR RETICULATUS*

INTRODUCTION

A problem facing all terrestrial arthropods is one of maintaining water homeostasis. This problem is of particular significance to ticks, especially 3-host ixodid ticks which may spend up to 98% of their lives off-host (Norval 1977). Ticks have a number of adaptations to reduce water loss and these include the ability to actively take up water from the environment; an integument to reduce transpirational water loss, spiracular valves, which open only infrequently during inactive periods, to close off the tracheal system; and the tick tissues themselves are able to withstand significant changes in the volume of the haemolymph some species being able to tolerate the loss of 1/2 their body weight (Needham and Teel 1986). The low metabolic rate of ticks also enables them to conserve energy reserves which are important for the active mechanism of water uptake.

It is clear that different species of tick have different geographical distributions and one of the possible reasons to account for these differences is the ability of the tick species to maintain water balance. Species such as *Ixodes ricinus* are restricted to damp, moist habitats, whereas others e.g. *Hyalomma asiaticum* inhabit desert areas (KnÜlle and Rudolph 1982). When exposed to subsaturated atmospheres there is a Relative Humidity (RH) threshold below which ticks continuously lose water, this threshold is known as the Critical Equilibrium Humidity (CEH) (Wharton 1964). Above this threshold the ticks are able to maintain their water balance provided they have sufficient energy reserves. The CEH is sometimes referred to as the Critical Equilibrium Activity (CEA) where activity = RH/100. This is the activity at which water uptake from the environment is equal to the

loss from the body. For this value to be lower than 0.99 (haemolymph activity) the tick must have the ability to actively absorb water from the air (Needham and Teel 1986). The CEH value may give an indication of why certain tick species are restricted to specific habitats. At sites in Wales *D. reticulatus* exists in the relatively xeric environment of a fixed dune and the more widespread species *I. ricinus* appears to be absent from these areas at the sites studied. The aim of this study was to determine the value of the CEH for *D. reticulatus* individuals and to compare these values with those determined for *I. ricinus* by Lees (1946).I was also able to compare ticks from both Harlech and the Aveyron region of France* to look for possible strain differences between these populations. These experiments were completed before the detailed review of Needham and Teel (1991) on the water-balance in off-host ticks became available. The results of the CEH's determined are discussed in the light of this recent work.

* Ticks provided courtesy of Dr B.Gilot.

MATERIALS AND METHODS

5 tanks with different relative humidities (24%, 72%, 85.5%, 89% and 99%) were established with saturated salt solutions (Winston and Bates 1960). The humidities generated by the salt solutions in the experimental tanks were monitored at the beginning and end of the experiment using a humidity probe connected to a Grant tintometer. Live ticks were collected from the marsh site at Harlech and ticks from the Aveyron region were kindly provided by Dr B.Gilot. For both the Welsh and French populations 20 ticks (10 male and 10 female) were tested in each humidity tank. The ticks were predesiccated at 24% RH for 24 hours to remove surface body water following Williams *et al.* (1986). The pre-weight of each individual tick was determined and each tick was placed in an individual 3x1 plastic tube which had an open end covered with a muslin cloth to allow free diffusion of air and to retain the tick. The tubes were then placed in the humidity

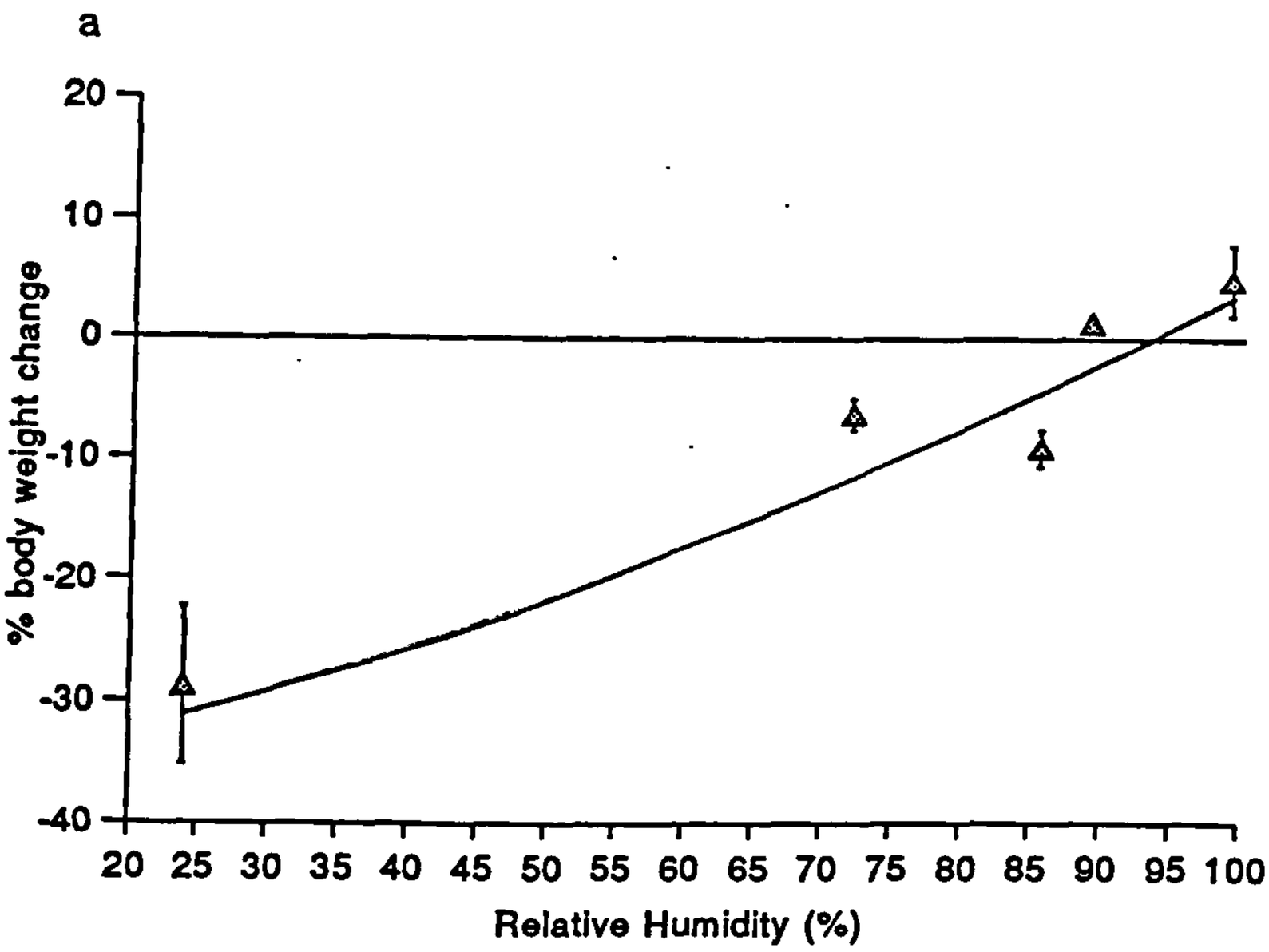
chambers held at 20°C and 12:12 L:D photoperiod. The ticks were exposed to these humidities for 1 week, after which they were removed from the humidity chambers and reweighed. The % body weight change was determined for each individual tick to determine the weight gain/loss at each of the humidities. The initial weights of the individual ticks were used to calculate the mean weight (as a measure of size) of male and female *D. reticulatus* in the Harlech and French populations. The weights of a sample (20 male and 20 female) of *I. ricinus* used in another experiment were used to determine the mean size of males and females from a site at Newborough, Anglesey. The size of ticks may have important bearings on the water loss rate (Edney 1977, Hair *et al.* 1975). The effect of body size (weight), sex and RH on the percentage loss/gain in weight was also investigated by an analysis of covariance using the SPSS package on the VAX computer.

RESULTS

The results of the change in body weight at each of the RH values are shown in figs. 5.4 a, b and 5.5 a, b for the Welsh and French ticks respectively. The lines for each of the graphs were fitted by eye and give an estimate of the CEH value i.e. where the line crosses the zero loss/gain in weight line. The CEH estimates derived from the graphs are given in table 5.3.

Table 5.3: The CEH values for male and female *D.reticulatus* from Harlech, Wales and SE. France.

Harlech male	86%
Harlech female	89%
French male	92%
French female	88%



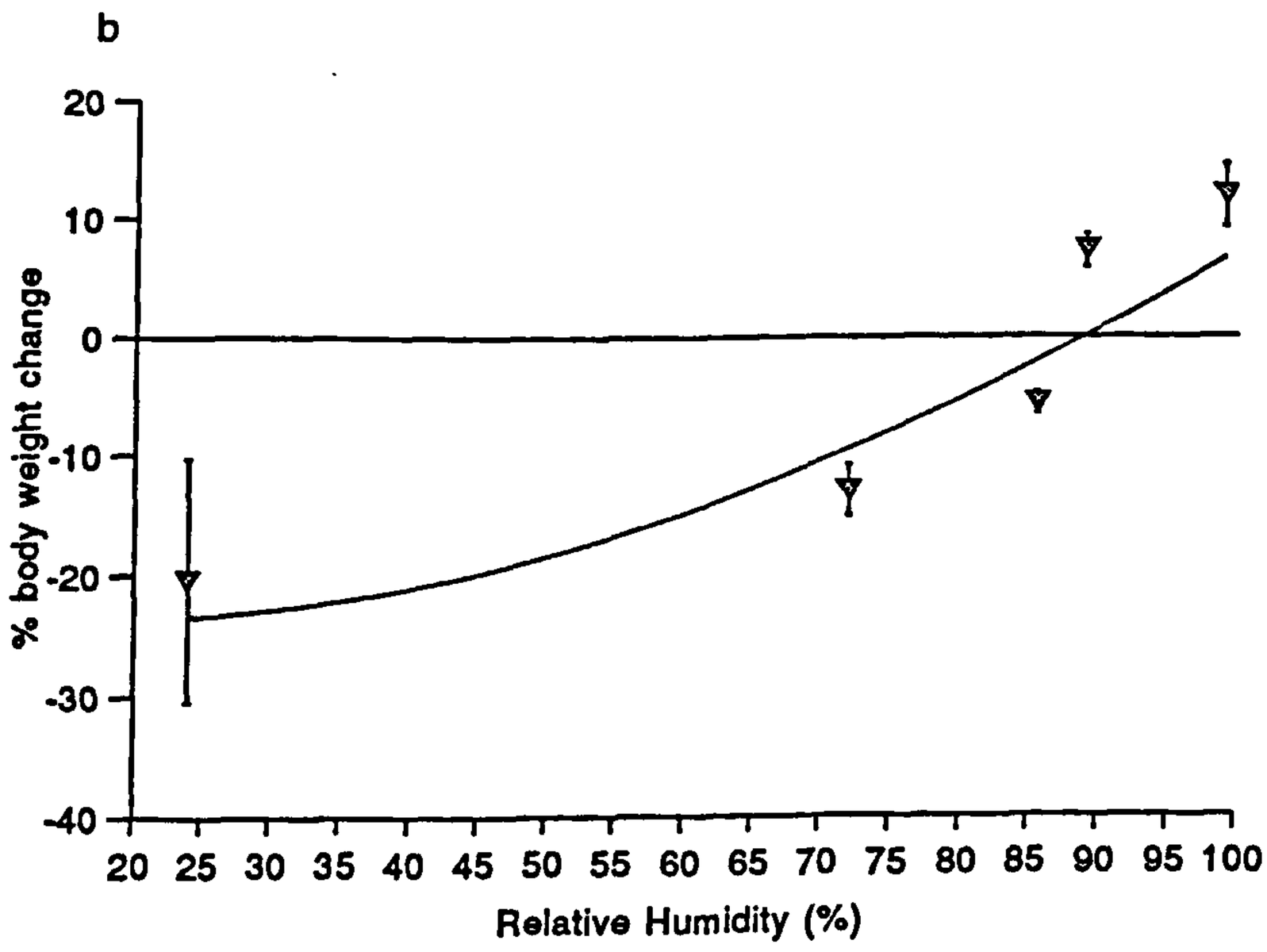


Fig. 5.5a, b: Percent body weight change in male (a) and female (b) *D. reticulatus* from France exposed to a range of humidities. The C.E.H. is the point where there is zero body weight change. The line was drawn by eye.

The 3 unfed females investigated by Lees (1946) had CEH values of 86%, 86% and 89%. The results of this study (for females) are not inconsistent with those of Lees. The CEH value determined for *I. ricinus* by Lees(1946) was in the range 86-96% RH i.e. the CEH values of the 2 species overlap. The French males appear to have a slightly higher CEH of 92% and the Harlech males have a slightly lower one of 86%. It is clear from the results that there is so much individual variation that these results are estimates. Therefore, I do not consider that there are significant differences between the Welsh and French females.

The mean body size of male and female *D. reticulatus* and *I. ricinus* are given in table 5.4.

Table 5.4: Mean body size (mg ± s.d) of male and female *D. reticulatus* and *I. ricinus*.

Species	Sex	Mean wt ± s.d	Site
D. reticulatus	m	5.45 ± 1.6	Harlech
	f	4.96 ± 0.7	
	m	4.92 ± 1.6	France
	f	4.24 ± 0.8	
I. ricinus	m	0.99 ± 0.3	Newborough
	f	1.73 ± 0.4	

An analysis of covariance was conducted to see what affect sex, RH and body weight had on the percentage weight change of ticks in each of the populations. In the first model it was shown that there were no significant differences between the sexes in either of the populations, therefore, a simpler model with RH and body weight as factors was tested combining the data for both sexes. In both populations the results of this model showed

that body weight did not have a significant effect on the percentage weight change. Therefore, a one-way ANOVA with RH was carried out and this showed that there was a significant difference in the percentage weight change at each humidity (Harlech, $F(95,4$ d.f$)= 10.73$, $p<0.001$, France, $F(34,4$ d.f$)= 4.31$, $p=0.006$)

DISCUSSION

It appears from the results of this study and from the findings of Lees(1946) that *D. reticulatus* and *I. ricinus* have similar CEH values and, therefore, this criterion by itself does not provide an indication of whether *D. reticulatus* can persist in more xeric environments than *I. ricinus*. Larger samples were used in this study than in that reported by Lees (1946) but the results for the females were quite similar in both studies and in both Welsh and French ticks. The French males do have an apparently higher CEH than their Welsh contempories, although this difference is unlikely to be significant. If a difference exists it is unlikely that it could be adaptive, as males spend more time on the hosts than females and are able to feed intermittently, so they should not be under water stress on-host. Off-host, as revealed by the seasonal sex ratio data of this study and in other studies, most of the active population in the autumn consists of males and this is a time when temperatures in the dunes are still high and rainfall (and presumably humidity) low. We might expect that this would result in males having a lower CEH than females which is certainly not the case in France. A comparison of the climate in West Wales and S.E. France (Gilot *et al.* 1974, Martinod and Gilot 1991) shows that the temperatures are slightly higher during the summer months and lower in the winter months in S.E.France and that the annual rainfall is slightly higher and the rainfall pattern is less variable, at the higher altitude sites studied by Gilot *et al.* (1974) than at Harlech which is at sea level. Though the rainfall may be higher in SE France it is unlikely that the French ticks are under less stressful conditions of water balance than the Welsh ticks given that they are exposed to higher temperatures in the summer months and lower temperatures in the

winter months. These conditions can also affect the efficiency of the water "pump" as shown by McEnroe (1971) in *D. variabilis* and Sauer and Hair (1971) in *Amblyomma americanum*. In summary, in terms of the CEH there do not appear to be either significant differences between the two species or between the two strains of *D. reticulatus* investigated. The CEH estimates of 3 species of *Amblyomma* in the USA were inversely correlated with the habitat type in which the species were found (Needham and Teel 1991). The three species, *A. americanum*, *A. cajennense* and *A. maculatum* had overlapping geographical distributions but different hydrophilic and vegetative associations. *A. americanum* is typically a woodland species occupying oak-hickory forests, *A. cajennense* is associated with prairie and thorn shrublands and *A. maculatum* is found in xeric grass and scrub habitats.

However, the CEH values of a range of tick species were reviewed by Knulle and Rudolph (1982) who found that the CEH values of different tick species do not differ significantly and that all the CEH values were fairly high in the humidity scale. In other studies of CEA values in ticks (including the CEH values in this study) much individual variation has been shown within and between tick species. Needham and Teel (1991) suggested that, in general, there is a lack of satisfactory association of CEA's with environments for the off-host phase of ticks. The variability may be accounted for by factors of age, inherited variability, prior exposure to extrinsic factors and the immune status of the host fed upon in the previous stage. The age and physiological condition of an individual tick may affect the efficiency of the active water "pump" as this is dependent on the energy reserves of the tick (Needham and Teel 1986). Also, they suggest that the integument may not be as efficient a water barrier as lipids are used up as energy reserves. Lees (1964) found that CEH values increased with age and a similar effect was found in *A. americanum* (Williams *et al.* 1986). Knulle and Rudolph (1982) suggest that the threshold humidity is not the critical factor in determining the habitat requirements of ticks, but that the rate of water-loss at sub-equilibrium humidities is of greater importance.

Hair *et al* (1975) compared the ability of *A. americanum*, *A. maculatum* and *D. variablis* to resist dehydration. *A. maculatum* had the highest CEH (92-93%), then *D. variabilis* (84-86%) and then *A. americanum* (80-82%). However, *A. americanum* was the most susceptible to desiccation at humidities less than it's CEH. This species lost absolute amounts of water as rapidly as *A. maculatum* but is only half the size. An indication of this for the 2 species in the present study might be given by Lees (1946). He measured the water loss and survival of ticks in dry air at 25°C. *I.ricinus* had a 30% weight loss in 1-2 days and all specimens were dead by the third day. *D. reticulatus* on the other hand was able to persist until the 21st day. Hair *et al.* (1975) demonstrated that tick size may be important and as shown in table 5.4 male *D. reticulatus* are approximately 5x bigger than male *I. ricinus* and female *D. reticulatus* are approximately 3x bigger than female *I. ricinus*. However, in *D. reticulatus* there did not appear to be a significant effect of body weight on the percentage weight change as shown by the results of the ANCOVA. The resilience of this species to desiccation may also be important, for example, (Needham and Teel 1991) have shown that *A. americanum* could not recover after desiccation at 0% RH on being returned to 98% RH, whereas, *A. cajennense* was able to recover after the same treatment.

Needham and Teel (1991) consider that " Integumental permeability to water flux and the amount of water in a tick may be of greater value in interpreting survival potential and habitat associations of ixodid ticks than critical equilibrium activity". They recommend that a whole-organism approach should be adopted whereby the whole body permeability, water mass, capability of water-vapour uptake and survivorship are measured and in adopting this approach they found strong associations with life-style and off-host adaptations among adult 1 and 3 host ticks. Whole body permeability was determined by measuring the water-loss rates in a desiccator which estimates water loss via all avenues. The water mass was the difference between the initial "wet" weight and the final dry weight after drying in a desiccator. The capability of water-vapour uptake and survivorship was determined by measuring the survival and water loss of ticks at 75% RH i.e. a

humidity below the CEA at which active uptake was unlikely. They feel that this approach provides a better assessment of hydrophilic associations among off-host adapted 3-host adults than CEA values.

Such experiments should be conducted on *D. reticulatus* and *I. ricinus* to determine whether *D. reticulatus* can indeed persist in drier environments than *I. ricinus*. One of the problems of this study was that the ticks were collected from the field so that their age and physiological condition were unknown. Ideally, laboratory reared ticks should have been used so that their age was known and factors affecting their physiological condition such as temperature, humidity, photoperiod and the immune status of the host animals could all be controlled.

The evidence from the field suggests that *D. reticulatus* occurs in areas of fixed dune from which *I. ricinus* is absent. Though the conditions experienced during the period of adult activity will not be particularly extreme, ticks are active during May, and at the end of August, when conditions can be very dry in this habitat. The ticks must also survive their summer diapause when conditions of high temperature and low humidity must be endured and they will also be exposed to occasional periods of sub-zero temperature in the winter months. *D. reticulatus* may find suitable microclimates in the dune site but there does not appear to be a substantial vegetation mat which may account for the absence of *I. ricinus* in these areas.

CHAPTER 6

GENERAL DISCUSSION

I have attempted to interpret the distributional pattern of *D. reticulatus* in Wales by the use of two different methods of geographic variation analysis. The first method, a morphometric study of the variation in scutal shape and base-pattern, revealed a north-south cline running from Wales through Devon and down to France. There is, however, no evidence of a north-south cline within the Welsh populations. The similarity of the Welsh populations to one another tends to suggest that they had a common origin. The second study of geographic variation analysis using the analysis of allozyme variation was more limited mainly through the prohibitive costs of screening a large number of enzymes. Only two enzymes were analysed isocitrate dehydrogenase (ICD) and phosphoglucomutase (PGM) and little variation was found in specimens from Wales and France. However, a fast allele (ICD^f) was found in a few specimens from Wales and a slow allele (ICD^s) was found in a few French specimens.

The origin of the Welsh populations remains a mystery. There are two major hypotheses, one that *D. reticulatus* is a relict species, the other that the tick is a recently imported species.

The relict theory suggests that after the end of the last ice-age the species spread up the western fringe of Europe, a route favoured by southern, warmth-loving species (Barry Cox and Moore 1980). The tick would have moved northwards via host movements and would have cut off from mainland Europe when the land-bridge was breached by the rising sea-levels. This distribution is similar to that of the Lusitanian flora distribution. The Lusitanian flora show affinities with the flora of the Iberian peninsula. Examples include the strawberry tree (*Arbutus unedo*) which is found in Ireland but not Britain, cornish heath (*Erica vagans*) and pale butterwort (*Pinguicula lusitanica*) both of which are found in SW.England and Ireland. Another example is the sharp sea rush (*Juncus acutus*) which, interestingly, has it's northern limit at Morfa Harlech which is the north-western edge of *D. reticulatus*'s range. That *D. reticulatus* was a southern species that has since moved north may be indicated by it's short-day photoperiodic response which is common to southern

species (Belozerov 1982). The tick has then presumably been restricted to favoured habitats. The north-south cline revealed by the morphometric study with the Devon population occupying an intermediate position between the Welsh and French populations could be the result of the northward spread of *D. reticulatus* populations. The Welsh populations are then likely to have originated from ticks from Devon.

The imported or introduced theory suggests that the tick has been imported in recent times through host movements. Migratory birds are a known source of tick imports (Daniel *et al.*1977a) and specimens of nymphal *D. reticulatus* have been found on birds at Lundy Is (Thompson and Arthur 1955) and Bardsey and Skolkholm (Thompson 1964). Though *Dermacentor* species are not renowned for feeding on birds (Hoogstraal and Aeschlimann 1982) there clearly exists the potential for bird imports and it has been suggested by Thompson (1967) that this is how *D. reticulatus* became established in Britain. We must also take into account the timing of bird migration in Britain i.e. whether it is coincident with the period of immature tick activity. The peak migratory period for birds travelling north from Africa across Europe is April-June. Larvae will be active in this period and nymphal numbers should be picking up towards the end of this time.

There is the possibility that cattle movements have introduced the species to Britain. In Wales the tick appears to be associated with Welsh black cattle. So within Wales it is possible that movements of cattle to and from markets and between farms may have spread the tick from area to area. Considering the four Welsh populations studied, all are in the old county of Merionethshire. Cattle from these farms are likely to have been taken to the same market at the county town of Dolgellau were breeding stock may have been exchanged (Toulson 1982). However, trade between Wales and SW.England is likely to have been limited. Both areas had similar agricultural systems and most of the movement of cattle would have gravitated towards the growing market of London and the peak of this trade was in the 17th and 18th century (Bonser 1970).The absence of *D. reticulatus* along the drover's routes to London from Wales and SW.England suggests i) that the tick was

not present at these sites in Britain, ii) that cattle were not moved from these particular areas to London, iii) the timing of the droving did not coincide with the activity period of adult ticks. Droving was traditionally done in late spring-summer-early autumn i.e. not during the peak period of tick activity in spring iv) that habitats inland along the drover's routes were unsuitable for reasons of microclimate, host availability and interactions with other ticks. If this tick was found on cattle moved to London, then we should expect that replete ticks would drop off at the various grazing sites along the route. The people of Wales, S.W.England and Brittany are of common Celtic stock, but it is unlikely that there were significant exchanges of cattle between these areas as they all traditionally exported cattle to the towns and cities. However, it may have only taken a small number of infested cattle to introduce the tick to new areas. It may be of note that the first definite record of *D. reticulatus* is from the turn of this century (Pocock 1900) and he states that "A farmer tells me they were not known here 15 years ago". The recentness of the first record of this tick in Britain could indicate that either the tick arrived in Britain as recently as the end of last century or that the tick had been previously overlooked by other field biologists and that it had in fact been present for some time.

Further study is required to account for the distribution of *D. reticulatus* in Wales. Samples should be collected from across the range and it would be particularly interesting to compare ticks from Wales and Devon to those thought to occur along the Atlantic coast of France. A morphometric study would have the attraction of lower costs than a genetic study. A problem with this technique is that we cannot be sure that the variation has an underlying genetic basis, though efforts were made in this study to minimise environmental effects by the use of residuals. This type of analysis could also be conducted on other species of *Dermacentor* such as the vectors of Rocky mountain spotted fever in the USA, *D. variabilis* and *D. andersoni*.

Ultimately, if one is trying to account for the distribution of a species, an analysis of genetic variation is required. Such a phylogeographic study would analyse variation in enzymes or if possible variation in the mitochondrial DNA (mtDNA). A preliminary

analysis of enzyme variation was carried out in this study, but the resulting data was rather limited, primarily restricted by the small numbers of enzymes studied. This has been shown to seriously affect estimates of variation (Gorman and Renzi 1979). The prohibitive costs of reagent chemicals prevented me from screening a large number of enzymes in this study. The small sample sizes in themselves are not a major problem, given that in many species, allozymic variation is among rather than within local populations (Selander and Whittam 1983). Therefore, a strategy of collecting small samples throughout the tick's range should encompass more allelic variation than large samples from a small number of populations (Buth 1984). The genetic study did reveal a site of variation at an ICD locus. A fast allele (ICD^f) was found in a few Welsh individuals and a slow allele (ICD^s) was found in a few French specimens. The next step in this study should be to screen a large number of enzymes (20+) in small samples from across the species range. This study should provide a realistic indication of the extent of geographic genetic variation. Discriminant analysis and a phenetic analysis of the genetic distances between the populations may clarify the origin and the spread of the species.

Ultimately, to investigate the phylogeographic distribution of this species, an analysis is required of the mitochondrial DNA (mt DNA) in samples from populations across the tick's distribution. Because of it's maternal, non-recombining mode of inheritance, rapid pace of evolution and extensive intraspecific polymorphism, mtDNA is an ideal tool for investigating micro- and macro- evolutionary processes. It is, therefore, ideal for investigating the evolutionary relationships of populations. This phylogeographic approach has been applied to a range of taxa including fish, amphibians, reptiles, mammals and an invertebrate (*Limulus polyphemus*) (reviewed in Avise *et al.* 1987). mtDNA analysis has shown assemblages of species not revealed by allozyme analysis e.g. in the deer mouse (*Peromyscus maniculatus*) (Avise *et al.* 1979) and *Aedes* sp.(Kambhampati and Rai 1991b). Apart from a pure interest in the evolutionary history of a tick, such a study would

be of value in disease control. Information can be provided on both a macro-scale (geographic) and micro-scale (local) of the degree of genetic exchange and , therefore, immigration between populations. Thus, if a comprehensive disease control strategy is to be devised, incorporating other information on activity, host and habitat associations, it is clearly of underlying importance to identify possible sources of recolonisation into an area and this approach may provide such information. This type of study may be applicable to *D. reticulatus* in Southern France where it is an important vector of *Babesia canis*. Obviously, it is equally applicable to other tick species or disease vector species of medical and veterinary importance.

If we now consider the distribution of *D. reticulatus* across the Palaearctic (fig. 1.2) we see that it is patchily distributed. This distribution is described by Immler (1973) as follows, "The species occurs in several isolated places, situated in the cool climatic zone. The southern limitation of the European distribution is the July-Isotherme of 20°C in Russia and Asia, the limitation coincides with the January-Isotherme of 0°C". There are likely to be climatic limits within which the tick exists, but the patchy nature of the distribution of the tick across climatic zones suggests that other factors such as habitat and host preferences are also determining the distribution. There is also evidence that the current distribution has been affected by man's activities so that the tick may have once been more widely distributed. Agricultural improvements are known to have restricted the distribution of *D.reticulatus* in Czechoslovakia and Poland (Daniel *et al.* 1986). In Czechoslovakia, the nature of the vegetation (specific species or communities) did not seem to be the decisive factor in determining the distribution of *D. reticulatus*. Instead, the absence of suitable hosts seemed to preclude the presence of the tick in apparently suitable habitats. Where ticks were found on grazed pastures, the discontinuation of grazing or improvement of the land resulted in the rapid disappearance of *D. reticulatus*. In mixed areas of grazed land and natural vegetation, the discontinuation of grazing resulted in a reduced number of ticks. Wild areas were the least affected by agricultural activities provided that large areas of suitable habitat were present and that large mammals were

supported in these areas. In Poland, the tick is widespread over the Biebrza river basin , an area characterised by low intensity agriculture and the presence of a high density of suitable hosts , the elk (*Alces alces*). In areas of intensive agriculture in Poland then *D. reticulatus* is restricted to woods and spinneys. The population at Ile du Rhin, France (Immler 1973) was once more widely distributed before alterations to the river course altered the tick habitats. Immler (1973) considered that this species was primarily associated with natural habitats and wild mammal hosts but would become secondarily associated with domestic animals on the intrusion of man into it s habitats.

If I now consider the Welsh populations, as I have mentioned, all the populations appear to be associated with Welsh black cattle which presumably fulfil the large mammal requirement. The dune habitats themselves can be fairly xeric, but as Immler (1973) found in the xeric habitats at Ile du Rhin the ticks can persist in microclimatically suitable areas. It is possible that the dune slack areas play a significant role in maintaining the population at the dune sites and that the more xeric dune areas are supplied by ticks (engorged nymphs) exported from the damper slack areas. The investigation into *D. reticulatus* in the field has helped elucidate the seasonal activity of adults at a site in Britain. The general pattern of activity lasts from late August to May with an obligatory period of inactivity between May and August. There were two peaks in activity with a marked peak in March-April and a more diffuse and reduced peak between September and November. There was, however, significant variation in activity from year to year at this site. This general pattern of activity conforms with the geographic variation in activity shown in *D. reticulatus* across it's range (Syzmanski 1987a). The absence of a winter diapause is probably a reflection of the mild winter conditions experienced and the slightly longer than expected summer diapause is perhaps a reflection of the high temperatures and low humidities (for a tick) that can be experienced in the summer months on sand dunes.

The most similar activity pattern to that at Morfa Harlech is found in S.E. France (Gilot *et al.* 1973, Martinod and Gilot 1991). The similarity to the French populations may suggest a

common origin, but they are more likely to reflect responses to the prevailing climatic conditions. This is supported by the fact that different activity patterns were generated at Morfa Harlech in different vegetation communities.

It was very interesting to discover that the ticks exhibited a plastic behavioural response (variation in seasonal activity) within a local area. The ticks at the dune sites (ungrazed and grazed) appeared to be responding to the macro-temperature, whereas those at the marsh site appeared to be responding to the photoperiod. A microclimatic effect of vegetation temperature and humidity on activity was found at the grazed site and vegetation temperature had an effect at the marsh site. Such variation in behaviour within a population is likely to reflect individual responses to microenvironmental cues.

Further work is needed to determine which micro-environmental cues the ticks are responding to. Data needs to be collected within the ticks habitats on variables such as temperature, RH, solar radiation, soil moisture content and water table depth. Continuous measurements could be made by use of a data-logger in situ. The activity of ticks placed in tubes on site could be measured at regular intervals, this data can supplement that collected by blanket dragging. In conjunction with the field study, laboratory experiments in a controlled environment could be carried out. Variables such as photoperiod, temperature and humidity could be controlled and the resulting questing behaviour of the adults monitored using time-lapse video. An attempt was made to conduct such experiments during this study but insufficient data were collected, primarily due to a lack of ticks.

The adults have been shown to persist in a range of habitats within a local area. Quantitative analysis of the vegetation shows that there are clear-cut divisions in the communities found at the dune (ungrazed and grazed sites) and the marsh sites. Sub-communities within the dune and marsh site were also identified. Having identified these different tick habitats further work could be conducted to determine the survival and development of all stages of tick within these habitats. Such experiments will, of course, require a successful laboratory culture of ticks to provide adequate numbers.

The vegetation communities are quite different to those found in other studies such as

swampy mixed wood (Nosek 1972), grasslands and forests (Gilot *et al.* 1974), xeric scrub habitats (Immler 1973) and dense woods (mixed and deciduous), peat bogs and in meadows (near bushes and tress) (Szymanski 1986). It is possible that the populations on the Atlantic coast of France occur in similar dune communities (Gilot pers.comm.). However, as mentioned, Daniel *et al.* (1986) assume that the nature of the vegetation is not in itself important in accounting for the distribution of *D. reticulatus* and Immler (1973) believed that this tick could survive apparently unfavourable habitats by persisting in suitable microclimates, he states that "The lowest stratum of the vegetation is of primary importance for the existence of this species." It was of note that, as in other studies, a seasonal sex ratio variation was observed in this tick. Males tended to appear earlier than females in the autumn, but females predominated for much of the year and were numerically dominant. No wholly adequate explanation has been offered to account for this, but it is possibly the result of the XX-XO sex determining mechanism found in other species of *Dermacentor*. The survival of a large number of cohorts of ticks reared through several generations could be monitored in the laboratory to investigate this phenomenon further.

The assessment of the on-host phase of adults and immatures was not a great success. Though we have positive proof of adults feeding on cattle at Harlech and evidence that *D. reticulatus* and *I. ricinus* occur on the same host individual which may be of some significance (see later) , the data is very limited . Somewhat surprisingly the local rabbit population did not appear to play a role in maintaining the tick population. Ideally the cattle hosts could be inspected every 2-4 weeks throughout the year, but particularly during the period of adult activity off-host. Morfa Harlech is not an ideal site for regular cattle inspection given the large area from which the cattle would have to be driven. Also, unless the farmer has a particular interest in ticks (or diseases thereof) it is likely to cost a fairly large sum of money to enable regular inspection over several seasons.

Monitoring of the immature stage was more productive once a suitable site (marsh site)

had been found. From the data collected it appears that the larval activity is over by mid-July and that nymphal activity peaks during July. Larvae were recorded on bank voles (*Clethrionomys glareolus*) and common shrews (*Sorex araneus*), nymphs were recorded on *C. glareolus*, *S. araneus*, wood mice (*Apodemus sylvaticus*) and pygmy shrews (*Sorex minutus*). *C. glareolus* was the most important host species at this site. Differences in the tick burdens of the different age-classes and sexes were observed in this species. Further study is required to determine the seasonal activity of the immature phase. Sampling should occur bi-weekly between May and September and at monthly intervals for the rest of the year in case the activity is prolonged as result of the mild winter climate. Laboratory studies would help elucidate host-preferences and the effects of host resistance on mortality. Clearly this is a major undertaking in itself.

The seasonal activity patterns of *D. reticulatus* and *I. ricinus* were clearly different at the sites studied in Wales. *I. ricinus* had a bi-modal pattern with a spring/ early summer and autumn peak, and it was active during the spring/summer when *D. reticulatus* had entered the summer diapause. In the winter months when *D.reticulatus* was active, *I. ricinus* was inactive. The investigation into the cold-hardiness of the two species (as measured by the supercooling points) revealed that the SCP's in *I. ricinus* were generally lower than in *D. reticulatus* but there was so much variation that there was no significant differences with mean SCP values in the range -6 to -8°C for *D. reticulatus* and -4 to -12°C for *I. ricinus*. Both species have SCP's which would enable them to withstand moderate sub-zero temperatures and their SCP values are comparable to those of the Antarctic tick *I. uriae* (Lee and Baust 1987). There were no obvious differences in the SCP's of lowland and upland populations of *I. ricinus*. The SCP did not give an indication of how *D. reticulatus* is able to remain active during the winter months when *I. ricinus* is inactive. Further work is required to investigate the seasonal variation in the SCP and accumulation of cryoprotectants in both species. Experiments should also be conducted to determine the activity threshold temperature in *D. reticulatus* and the threshold temperature for active water uptake in both species.

The CEH value of *D. reticulatus* as determined in this study was very similar to the CEH value known for *I. ricinus* (Lees 1946), thus the CEH value did not give an indication of whether *D. reticulatus* is able to withstand greater conditions of water stress than *I. ricinus* and is, therefore, able to exist in drier environments than *I. ricinus*. Further experiments to investigate the whole-body permeability, water mass, capability of water-vapour uptake and survivorship in a range of humidity and temperature conditions are required to reveal differences in the physiological adaptations of both species which may contribute to the observed differences in their distributions and habitat preferences.

It is unfortunate that the experiment to test the competitive interaction hypothesis was not completed through reasons beyond my control. This study would have assessed the effects of host resistance to *I. ricinus* on the fecundity of female *D. reticulatus* in order to see whether such an interaction was significant enough to suppress the productivity of *D. reticulatus* to such a degree that it could be excluded from habitats favourable to *I. ricinus*. A pair of twin sheep with no previous exposure to ticks were to have been used. One individual was to have been exposed to *D. reticulatus* and one individual exposed to a combined infestation of *D. reticulatus* and *I. ricinus*. The fecundity was to have been measured over several challenges. In southern Africa, an introduced species *Boophilus microplus* has replaced in many areas the indigenous species *B. decoloratus* and this has occurred through interspecific competition acting via host resistance (Norval and Short 1984). The feeding on cattle by *B. microplus* enhances the cattle's resistance to *B. decoloratus* and it was also shown that *B. microplus* feeds more successfully on cattle than *B. decoloratus*. Such interspecific competition between *D. reticulatus* and *I. ricinus* might account for the restricted distribution of *D. reticulatus* in Britain, where it might persist in habitats unfavourable to *I. ricinus*. Further to this study, the suitability of different cattle breeds might be investigated. Welsh black cattle (descended from an ancient breed of forest cattle) appear to be suitable and presumably a different breed (Devon red cattle?) plays a similar role in S.W.England. Other breeds may prove unsuitable and, therefore,

contribute to limiting the distribution of *D. reticulatus* in Britain.

The field and laboratory experiments proposed in this chapter are required to determine how *D. reticulatus* has attained it's current distribution and to give a comprehensive overview of the life-history of this species in Britain.

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APPENDIX I

THE EFFECTS OF HOST RESISTANCE ON FEEDING SUCCESS AND FECUNDITY IN *DERMACENTOR RETICULATUS*

INTRODUCTION

The initial aim of this study was to investigate the possibility of there being interspecific competition between *D. reticulatus* and *Ixodes ricinus*. As we've seen, *D. reticulatus* has a restricted distribution in Britain, whereas *I. ricinus* is the commonest and most widespread species in Britain. It is possible that interspecific competition, acting through the host's immune response, assists in restricting the distribution of *D. reticulatus*. The intention was to compare the effects of the immune response on the feeding success and fecundity of *D. reticulatus* i) on a host sequentially challenged by *D. reticulatus* and ii) on a host (twin) sequentially challenged by *D. reticulatus* and *I. ricinus* together. In some areas of southern Africa (Norval and Short 1984) interspecific competition was shown between *Boophilus decoloratus* and *Boophilus microplus* and was thought to account for the exclusion of the endemic *B. decoloratus* by the introduced *B. microplus* in these areas. Cross-resistance was first shown between larvae of *Dermacentor andersoni* and *Dermacentor variabilis* (Trager 1939). Significant cross-resistance has also been shown between *Amblyomma americanum* and *Rhipicephalus sangineus* (Brown and Askenase 1981) and guinea-pigs sensitised to *A. americanum* were resistant to *D. variabilis* but not *D. andersoni* (Wikel 1982). There is also evidence of cross-reactivity of the antigens for *D. reticulatus* and *I. ricinus* (Martinod *et al.* 1985).

Unfortunately, for a number of reasons this experiment was not completed. However, enough data was collected to investigate the effect of host resistance on *D. reticulatus* alone. The immune system of the host is known to be stimulated by the ticks through the salivary antigens and tick-derived products (Trager 1939, Allen 1973, Roberts 1968). Immunity as defined by Willadsen (1980) denotes " any immunologically mediated response that is disadvantageous to the parasite". Frequently this immunity is only partial as a proportion of parasites complete their life-cycle, in which case resistance is the more

accurate term. The effects of immunity range from simple rejection of the parasite with little apparent damage, to interference with feeding, extension of the engorgement period, reduction in engorged weights, inhibition of egg laying and a decrease in the viability of the eggs, to the death of the parasite on the host. A number of studies have investigated the effects of host resistance on the feeding success of ticks, for example, *D. variabilis* feeding on guinea-pigs (Trager 1939), *D. andersoni* on guinea-pigs (Allen 1973), *Haemaphysalis longicornis* on cattle (Sutherst *et al.* 1979), *Haemaphysalis punctata* on sheep (Alani and Herbert 1987), *I. ricinus* on rabbits (Bowessidjaou *et al.* 1977) and *Rhipicephalus appendiculatus* on rabbits and cattle (Branagan 1974). There have as yet been no such studies on *D. reticulatus*.

MATERIALS AND METHODS

One of a pair of twin Dorset sheep was used as the experimental host in this study. The ticks were maintained on the sheep enclosed in a cotton sleeve attached to a shaved area on the right or left flank. A new sleeve was attached to a new area with each infestation. For each infestation the host was challenged with 10 male and 10 female *D. reticulatus*. The following parameters were measured at each infestation:

1) Engorgement period - number of days each female tick was engorged

2) The percentage of ticks successfully engorging

3) Engorgement weight of each female tick

4) The percentage of these ticks producing eggs

5) The weight of the egg mass produced by each tick

6) The number of eggs produced (derived from 5)

7) The egg conversion factor (ecf) as a measure of the efficiency of each tick in converting the bloodmeal into eggs, the ecf= egg mass weight/ engorged weight.

8) The hatchability of the eggs produced, expressed as the percentage of eggs hatching into larvae.

A total of 8 infestations were carried out which was assumed to be sufficient to induce an immune response. Each new challenge was conducted immediately after the ticks from the previous infestation had fed to repletion. The egg number (6) was calculated by measuring the weight of a series of egg batches consisting of 100 eggs each to determine the value of the weight of an individual egg which was calculated as being $= 0.042 \pm 0.007$ mg

RESULTS

The results are shown in tables A1.1 and A1.2 and figs. A1.1-A1.3. Tables A1.1 and A1.2 show that there is a significant negative correlation between the engorgement period and the number of infestations and the most marked decrease in the engorgement period occurred at the 7^{th} infestation. There was no significant change in the percentage of ticks successfully engorging at each infestation. The engorged weight of females decreased significantly with increasing number of infestations (fig. A1.1 and table A1.2) and the most pronounced effect occurs at the 7^{th} infestation. However, the percentage of engorged ticks that then produced eggs did not alter significantly with increasing number of infestations (tables A1.1, A1.2). The egg mass weight also shows a significant decrease with increasing infestation with the most marked reduction occurring at the 7^{th} infestation (fig. A1.2, table A1.2). Clearly correlated with this the number of eggs produced per individual decreases with increasing number of infestations. The egg conversion (ecf) (fig. A1.3), a measure of the efficiency of the ticks in converting the bloodmeal into eggs, also decreases with a clear reduction at the 7^{th} infestation. Of the eggs laid, the percentage of these eggs that hatch (hatchability) does not alter significantly with the number of infestations, though there is a marked reduction at the 8^{th} infestation.

Table A1.1: The effects of sequential infestation of a sheep host on the feeding success and fecundity of *D. reticulatus*.

Infestation	Engorgement period (days)	% ticks engorging	Engorged ticks producing eggs (%)	No. eggs	Hatchability
1	12.1± 0.6	100	100	4794± 388	98.1± 1.5
2	11.3± 0.5	80	100	3745± 327	95.5± 2.9
3	14.3± 1.3	60	100	3493± 539	92.3± 1.8
4	9.3± 0.2	70	100	3537± 258	84.5± 14.7
5	10.0± 0.6	100	100	3701± 286	91.4± 2.1
6	12.0± 0.8	80	71	2749± 677	92.6± 0.5
7	8.5± 0.3	40	100	1455± 472	97.2± 0.6
8	9.0± 0.6	40	67	1187± 192	18.2± 13.8

Table A1.2: Values and significance of the Spearman's rank correlation (r_s) between the number of infestations and parameters of the tick feeding success and fecundity.

Parameter	r_s	N	significance
Engorgement period	-0.424	56	p<0.002
% ticks engorged	-0.618	8	p>0.05
♀ Engorged weight	-0.294	56	p<0.05
% ticks producing eggs	-0.655	8	p>0.05
Egg mass weight	-0.475	43	p<0.002
Egg number	-0.528	43	p<0.002
Egg conversion factor	-0.355	43	p=0.02
Hatchability	-0.279	32	p>0.01

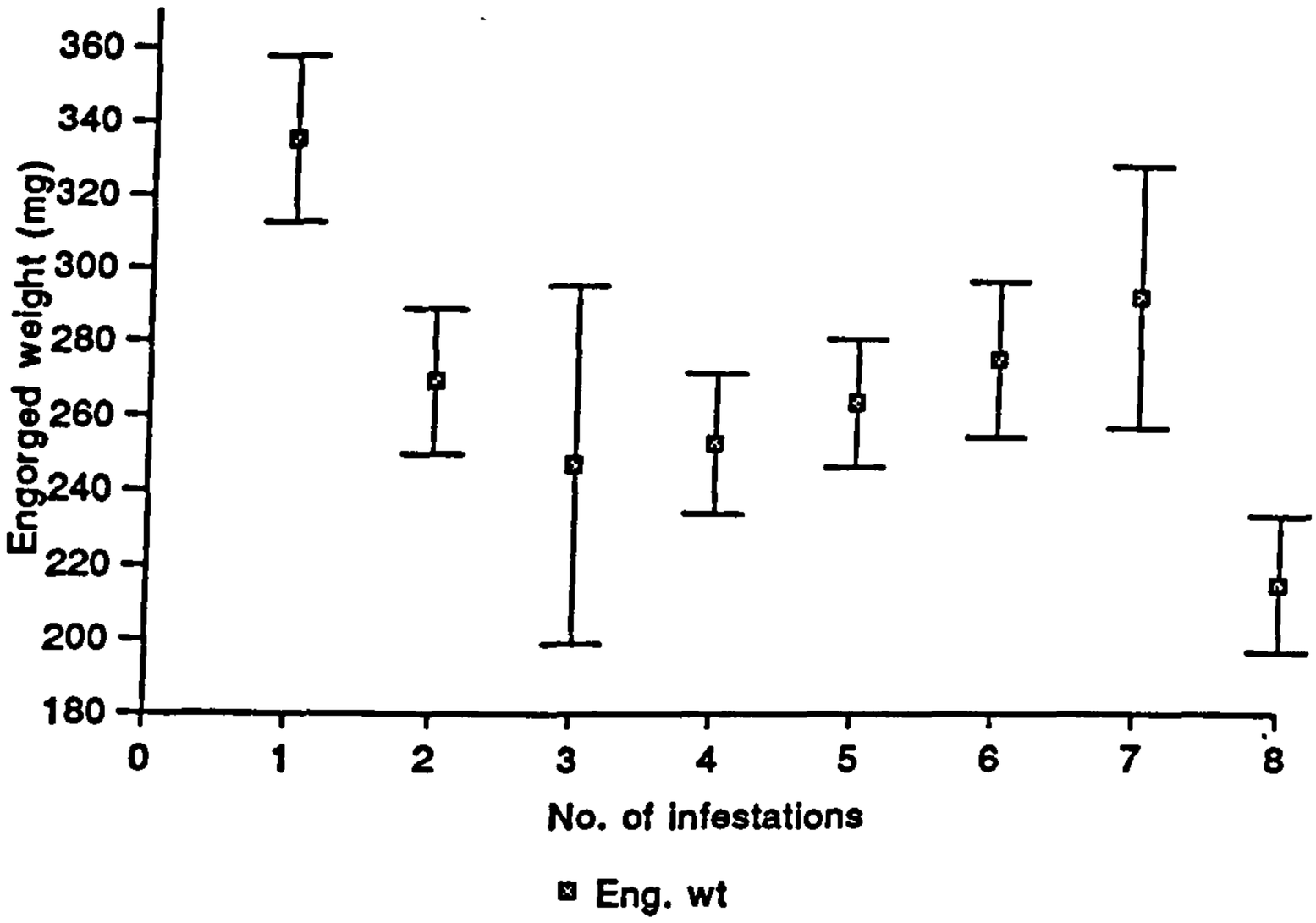

Fig. A1.1: The change in the mean engorged weight (Eng. wt) (± S.E.) of female *D. reticulatus* with increasing number of infestations.

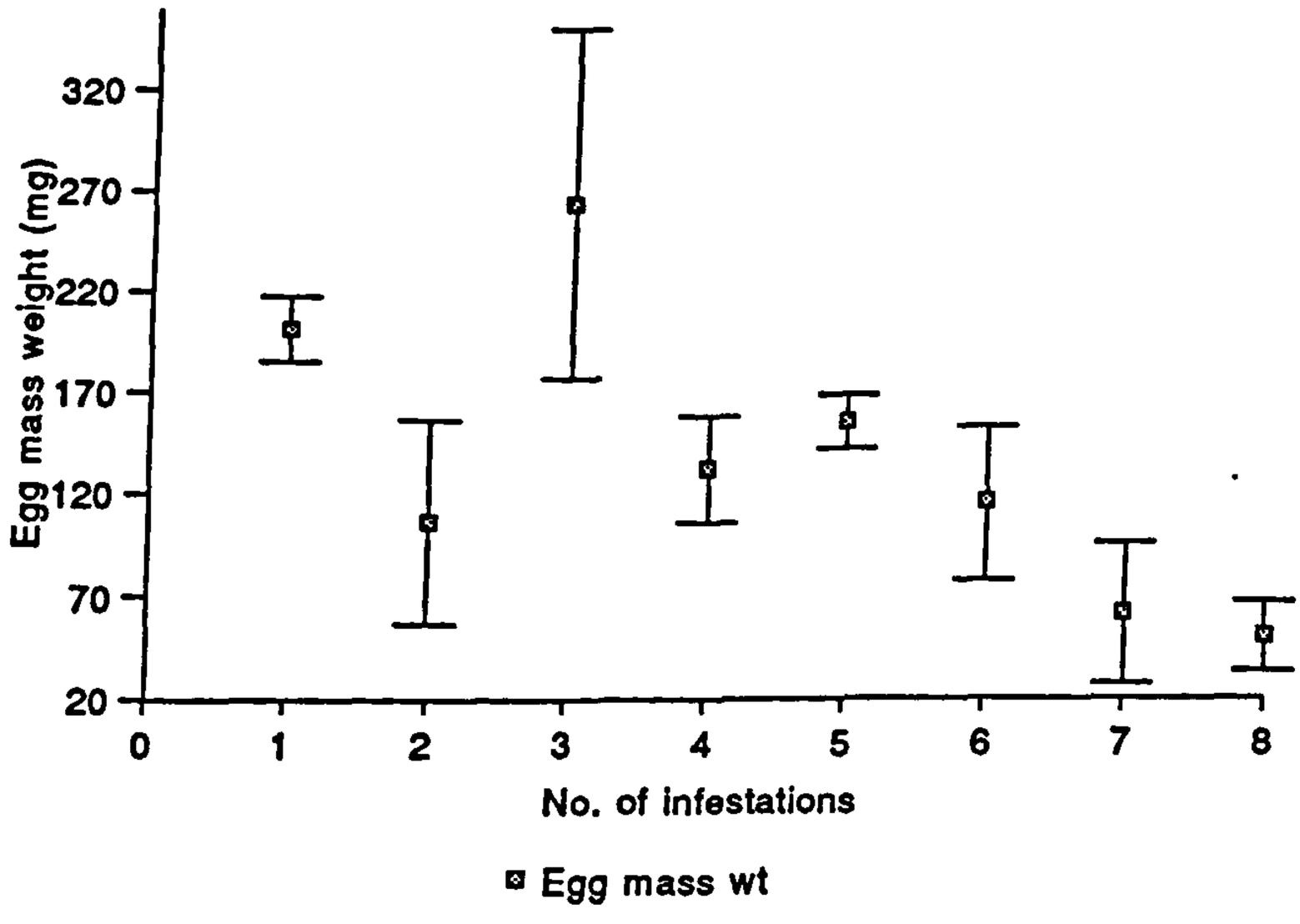

Fig. A1.2: The change in the mean egg mass weight (egg mass wt.) (± S.E.) with number of infestations.

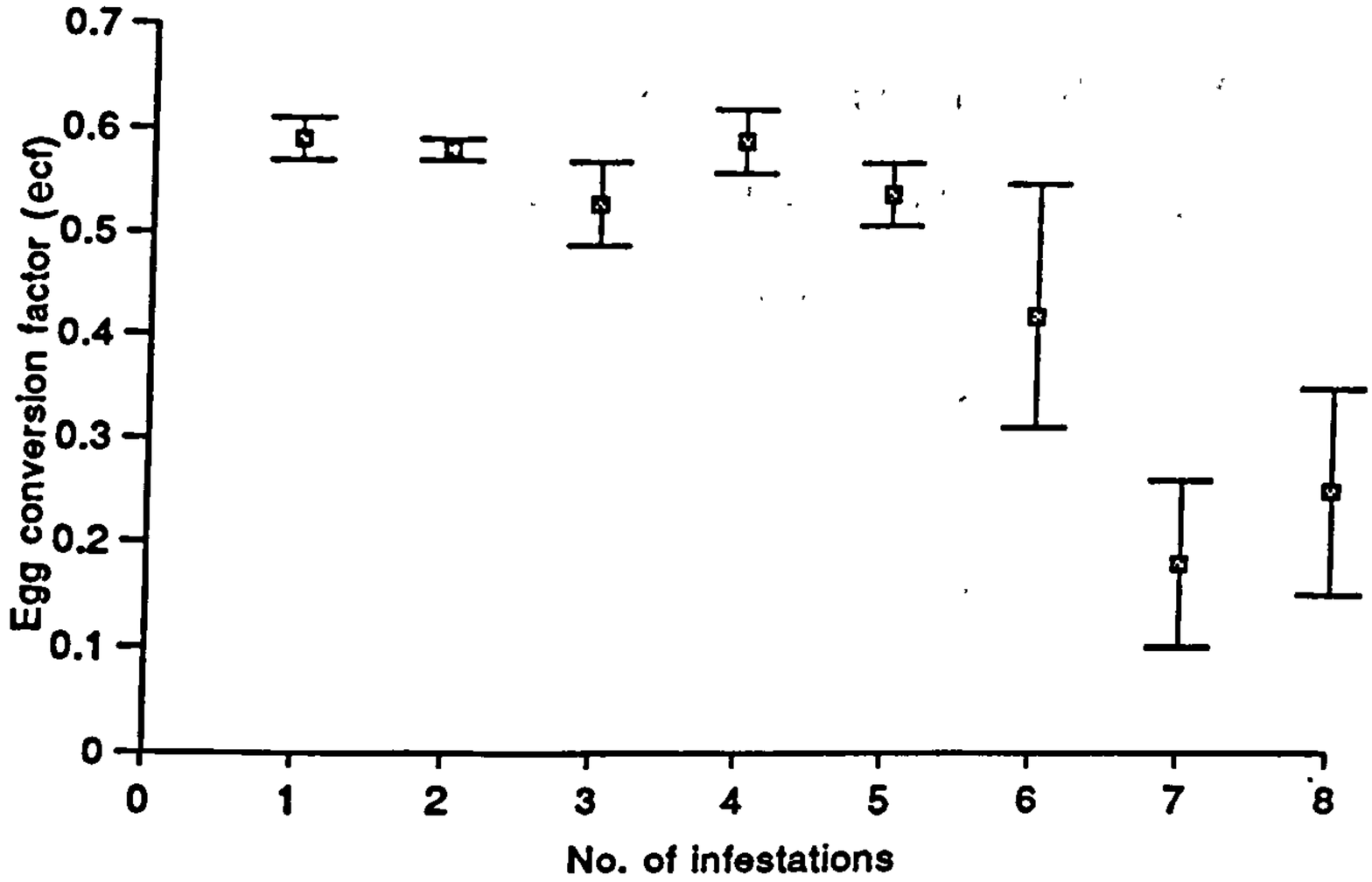

Fig. A1.3: The change in the egg conversion factor (ecf) with number of infestations.

In summary, there appears to have been an immune response initiated at the 7th infestation leading to a reduction in the engorgement period, engorged weight, egg mass weight and egg conversion factor which has led to a reduction in the fecundity of *D. reticulatus*. However, the percentage of ticks engorging and the hatchability of eggs produced did not alter significantly. It is also of note that there was a significant positive correlation between the engorged weight of ticks and the egg mass weight produced (combining the data from all infestations, $r_s = 0.552$, $p < 0.002$). This linear relationship between engorged weight and egg mass weight has also been found in *I. ricinus* (Honzáková *et al.* 1975, Gray 1981).

DISCUSSION

The results show that the sequential feeding of *D. reticulatus* on a host sheep initiates an immune response in the host which affected the feeding and reproductive performance of the ticks. This immune response manifested itself in reducing the engorgement period, engorgement weight, egg mass weight and thus, egg number and the egg conversion factor of ticks. The immune response had no effect on either the percentage of ticks engorging or the hatchability of the eggs produced. The immune response becomes apparent at the 7th infestation. The overall effect of this response will be to produce smaller engorged weights of females and smaller egg masses. Bearing in mind that these results represent the immune response initiated in a single host animal, the results are compared to those of other studies.

Considering first those parameters which showed a significant negative correlation with increasing number of infestations. The engorgement period showed a decrease in this study, whereas in most other studies, the engorgement period increased as the immune response was expressed (Willadsen 1980). This was the case with *Boophilus microplus* feeding on cattle (Hewetson 1971), *Hyalomma anatolicum excavatum* and *Rhipicephalus*

sangineus on cattle (Kohler *et al.* 1967) and *I. ricinus* on rabbits (Bowessidjaou *et al.* 1977). However, Fujisaki (1978) found that the engorgement was unaltered in *Haemaphysalis longicornis* feeding on rabbits. The prolonged feeding period is thought to be due to interference in the feeding process by the immune response of the host making it more difficult for the tick to obtain a sufficient bloodmeal. The shortening of the feeding period may be due to the unsuitability of the bloodmeal so that the ticks disengaged earlier to avoid damage from the host's immune system.

The percentage of ticks successfully engorging did not significantly alter in this study, whereas in other studies this parameter has decreased as the number of infestations increased. For example, in *Amblyomma americanum* feeding on cattle (Strother *et al.*1974), *D. variabilis* feeding on guinea-pigs (Trager 1939), *Haemaphysalis punctata* on sheep (Alani and Herbert 1987) and *Hyalomma anatolicum excavatum* and *R. sangineus* (Kohler *et al.* 1967). However, this value did not alter in *Haemaphysalis longicornis* (Fujisaki 1978).

In this study the engorged weight of females decreased with increasing number of infestations and this trend was also found in *B. microplus* (Hewetson 1971, Wagland 1978) *D. variabilis* feeding on guinea-pigs (Allen 1973), *Haemaphysalis leporispalustris* feeding on rabbits, *H. longicornis* on cattle (Sutherst *et al.* 1979) and on rabbits (Fujisaki 1978), *Ixodes holocyclus* on cattle (Doube and Kemp 1975) and *Rhipicephalus appendiculatus* on cattle (Branagan 1974).

The percentage of engorged ticks that produced eggs did not alter significantly, however, in other studies the percentage of ticks engorging decreased with repeated infestations e.g. in *H. punctata* (Alani and Herbert 1987) and *I. ricinus* (Bowessidjaou *et al.* 1977).

The egg masses produced and the number of eggs produced showed a significant decrease with increasing number of infestations as was found in *B. microplus* (Hewetson 1971), *H. punctata* (Alani and Herbert 1987) and *I. ricinus* (Bowessidjaou *et al.* 1977).

The egg conversion factor showed a significant decrease as the immune response was initiated, indicating that the ticks became less efficient at converting the bloodmeal into

eggs. The correlation between the ecf and engorged weight of ticks ($r_s = 0.026$, $p > 0.1$, N=42) indicates that the size of the tick has no effect on the efficiency to convert the bloodmeal to eggs, though this may be confounded by the increasing immune response of the host. Using a different index of efficiency, Gray (1981) found that smaller *I. ricinus* were more efficient at converting the bloodmeal into eggs than larger ticks, but Bennett (1974) found that smaller *B. microplus* were less efficient at producing eggs than larger ticks.

The viability of the eggs, as shown by the percentage hatching of the eggs did not significantly alter in this study, although there was a marked decrease at the last infestation. The viability was significantly reduced in *B. microplus* (Hewetson 1971), *H. punctata* (Alani and herbert 1987), *I. ricinus* (Bowessidjaou *et al.* 1977) and this is generally the case in ticks (Willadsen 1980). However, the viability was unaltered in *H.longicornis* (Fujisaki 1978).

There is evidence for at least two types of response in the studies so far (Willadsen 1980), physical removal of the parasite probably induced by the irritation of tick attachment and the actual inhibition of feeding. In this study the assumption is that the reduced engorgement weights, reduced egg laying and viability are the effects of the host's immune response, but it is also possible that these effects are the result of poor nutrition (Willadsen 1980). The best evidence of a direct host response was in *I. ricinus* (Bowessidjaou *et al.* 1977), although female ticks engorging on rabbits were of low weight, these weights were insufficient to account for the observed reduction in egg laying, suggesting that there had been a toxic effect.The immune response initiated by *D. reticulatus* shows many similarities with other species of tick, but there are some differences. As mentioned, *H. longicornis* feeding on rabbits initiated a different response to that found in other species of tick in that although the engorged weights of females were reduced, the number of ticks engorging, length of engorgement period and hatching rates were all unaltered (Fujisaki 1987). In *D. reticulatus* the immune response was not apparent until the 7^{th} infestation,

whereas it manifested itself much earlier in other species. For example, in *B. microplus* the immune response appeared after 8 days from the initial infestation of a naive animal (cattle) (Roberts 1968). Similarly, a single infestation of *H. longicornis* produced resistance in host rabbits (Fujisaki 1978). Guinea-pigs acquired resistance after the 2nd and 3rd infestation (Allen 1973). The immune response of *I.ricinus* was initiated after four infestations on rabbits (Bowessidjaou *et al.* 1977) and also after the 4th infestation of *H. punctata* on sheep (Alani and Herbert 1987).

The type of reaction elicited by the tick will depend on the nature of the immunogen (Askenase *et al.* 1982), the host species (Loomis 1977), the immune capabilities of the exposed host (Wikel 1982) and the history of prior exposure (Askenase *et al.* 1982). Different host species and breeds may vary in their responses to different species of tick and much less is known about the immune responses of sheep than of cattle, rabbits and guinea-pigs (Alani and Herbert 1987). In this study there has clearly been an immune response initiated in the experimental host by *D. reticulatus*, and this response appears to be induced more slowly than in other species of tick. However, only one experimental animal has been used so that no firm conclusions can be drawn, except to state that an immune response appears to have been elicited and that this response reduces the fecundity of *D. reticulatus*.

Further work requires the use of larger samples of experimental animals, perhaps investigating the immune responses in different host species such as Welsh black cattle, sheep, rabbits and dogs and to investigate the effects of host immunity on the immature instars. A further investigation into the immunological aspects of and the persistence of this immunity will be essential for the development of an integrated control programme against *D. reticulatus*

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APPENDIX II

TRIAL USE OF DELTAMETHRIN POUR-ON FOR THE CONTROL OF *IXODES RICINUS*

INTRODUCTION

The following experiment was conducted to gain experience of the control of ticks in the field. Nineteen yearling cattle grazing on an 80 acre rough pasture at Hafotty farm, Llandonna, Anglesey were used in the trial. The farm has a history of Redwater fever. The study consisted of two trials, the initial trial commencing on the 6th May 1988 and the second trial on the 2nd June 1988. In the initial trial the group was divided at random into:

Group 1 - 14 animals treated with 10 ml Deltamethrin solution applied along their backs.

Group 2 - 5 untreated animals to act as controls.

In the second trial the fourteen previously treated cattle in group 1 was divide into two groups of seven animals:

Group 1a - 7 animals treated as before.

Group 1b - 7 animals treated with 5 ml Deltamethrin along their backs plus a total of 5 ml sprayed on their axillae.

Group 2 - 5 untreated animals (same individuals as before)

The animals in group 1b were treated under the axillae as a highly significant number of ticks had been found there during the first trial.

In both trials the cattle grazed on the same rough pasture and were not separated into their respective groups. The cattle were examined and weighed after one week, two weeks and four weeks for each of the trials. The number of adult, nymphal and larval ticks on each animal was recorded during each inspection. The tick burdens (mean number of ticks per animal) was calculated for each group.

RESULTS

In the first trial no significant difference was found between the tick burdens of the treated and control groups (fig A2.1a,b), though there was a slightly higher tick burden in the control group. The tick burden showed a marked decline during the first week of the trial and this followed a similar trend in rainfall. There was a significant correlation between the tick burdens of the treated animals and total weekly rainfall (one-way ANOVA, $p=0.009$), but not between the tick burdens on the control animals and rainfall ($p=0.066$).

In the second trial, all three groups show no significant difference in their tick burdens (fig A2.2a,b), though the control group do have a slightly higher tick burden. again there was a decrease in tick numbers during the first week of the trial followed by a slow increase in numbers. This followed a similar trend in rainfall, though the correlation was not significant for any of the groups (Group 1a $p=0.903$, Group 1b $p=0.822$, Group 2 $p=0.749$).

The two groups of cattle (treated and control) showed no significant difference in live weight gain (fig. A2.3). This was not unexpected given the similarity of the tick burdens of the treated and control animals. One animal in the treated group of the first trial contracted redwater fever during the trial but was successfully treated.

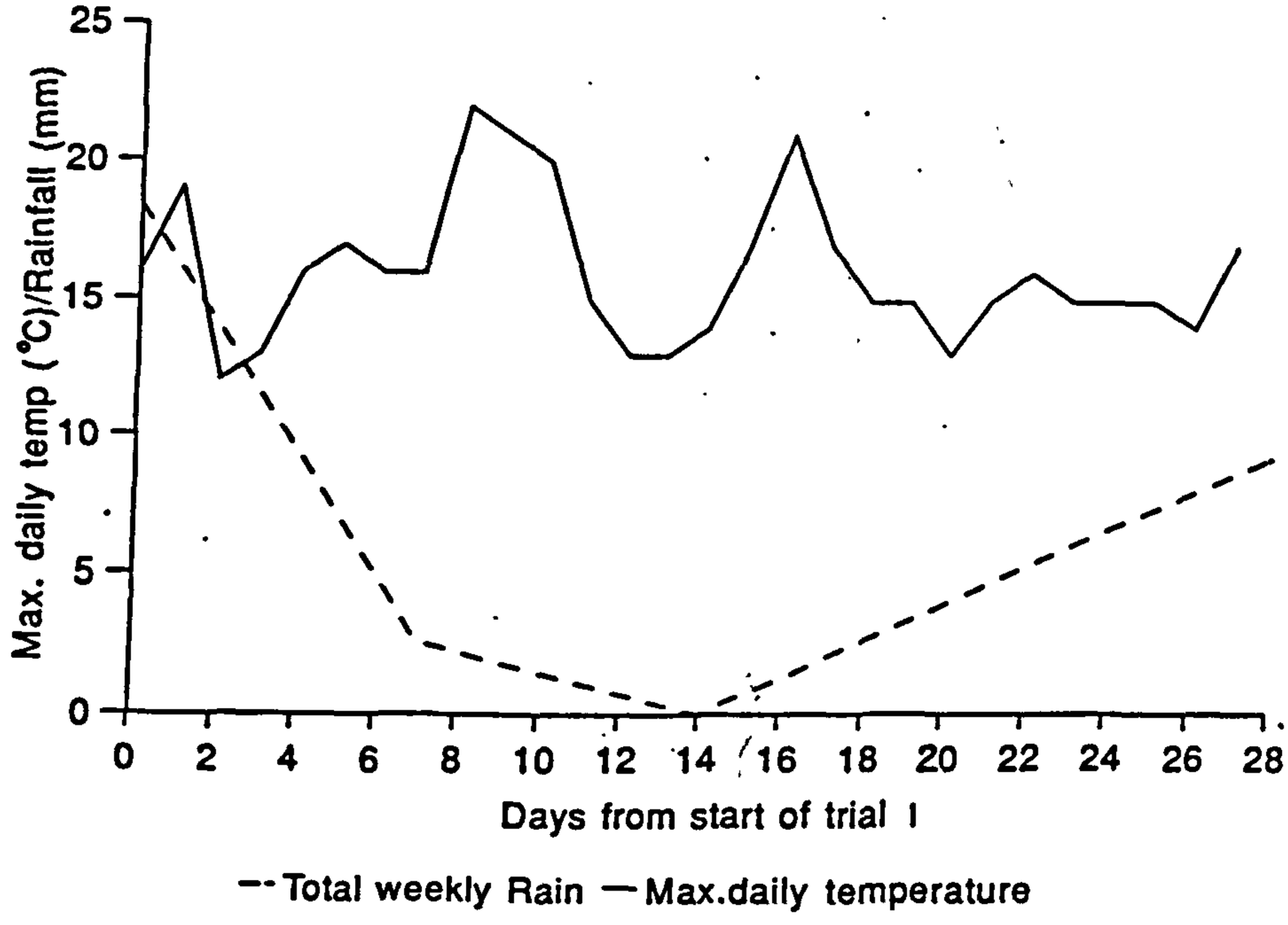

Fig. A2.1a: Maximum daily temperature and total weekly rainfall during trial 1.

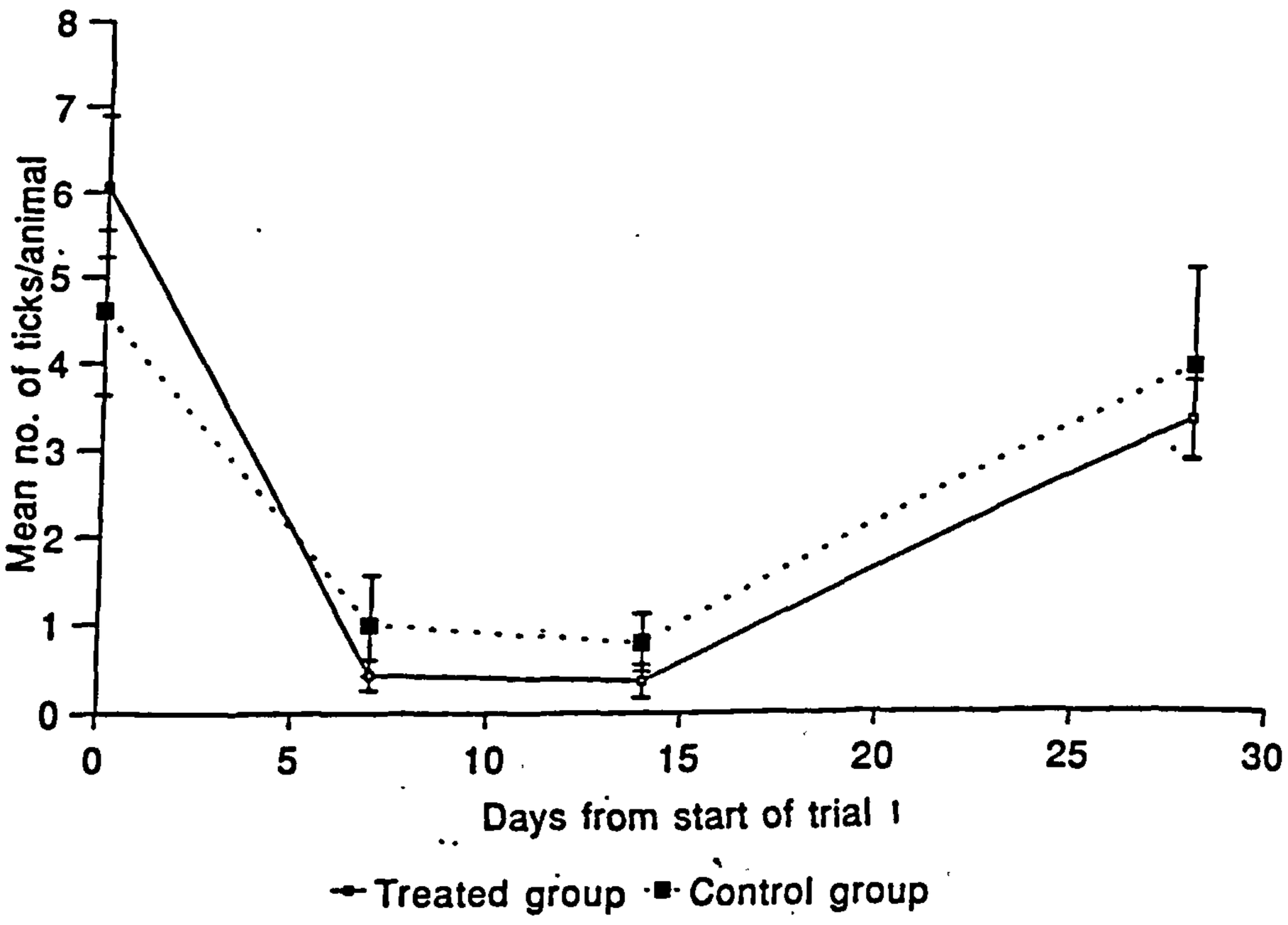

Fig. A2.1b: Mean tick burdens (± S.E.) of treated and control animals, trial 1.

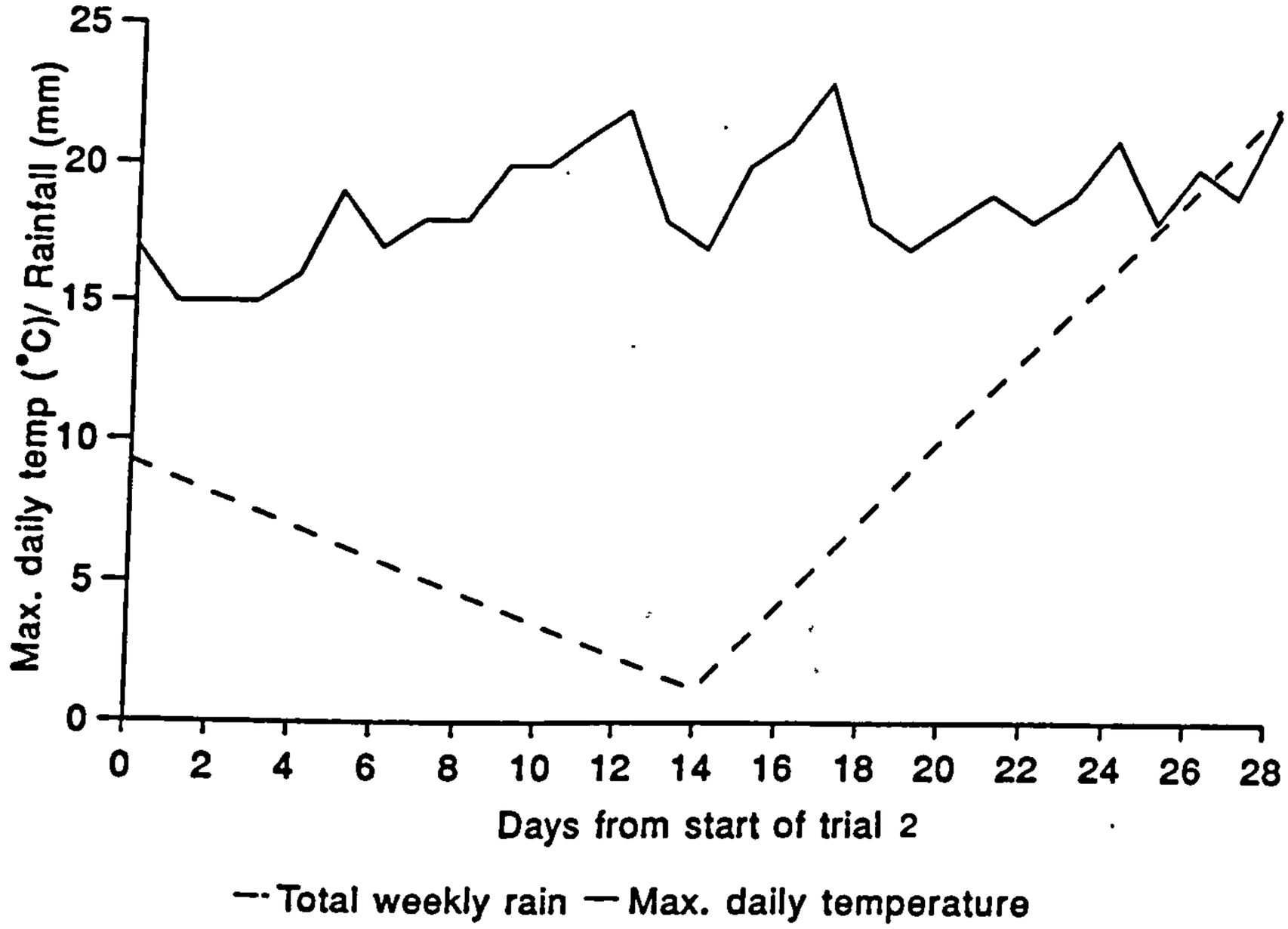

Fig. A2.2a: Maximum daily temperature and total weekly rainfall during trial 2.

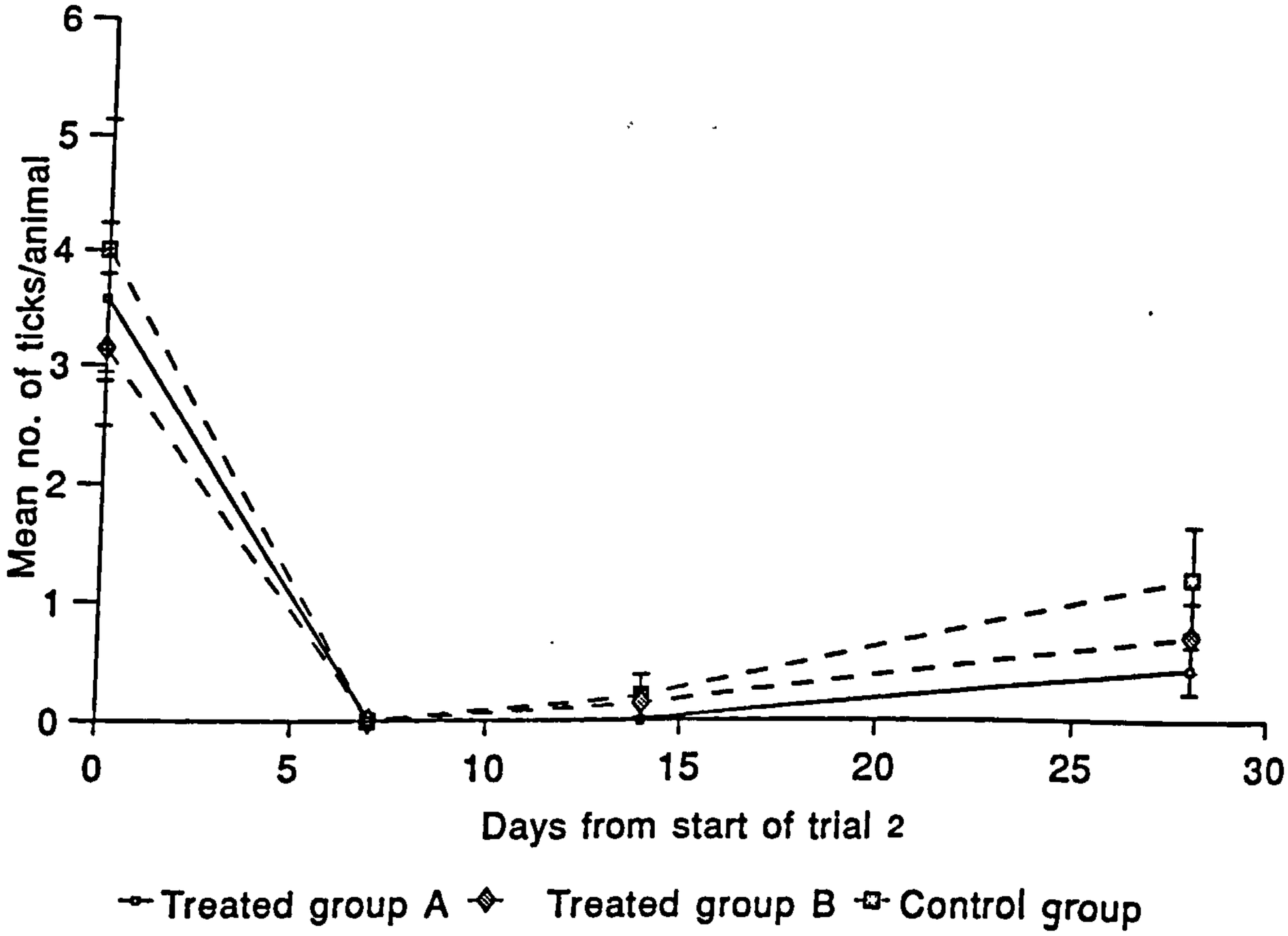

Fig. A2.2b: Mean tick burdens (± S.E.) of treated and control animals, trial 2.

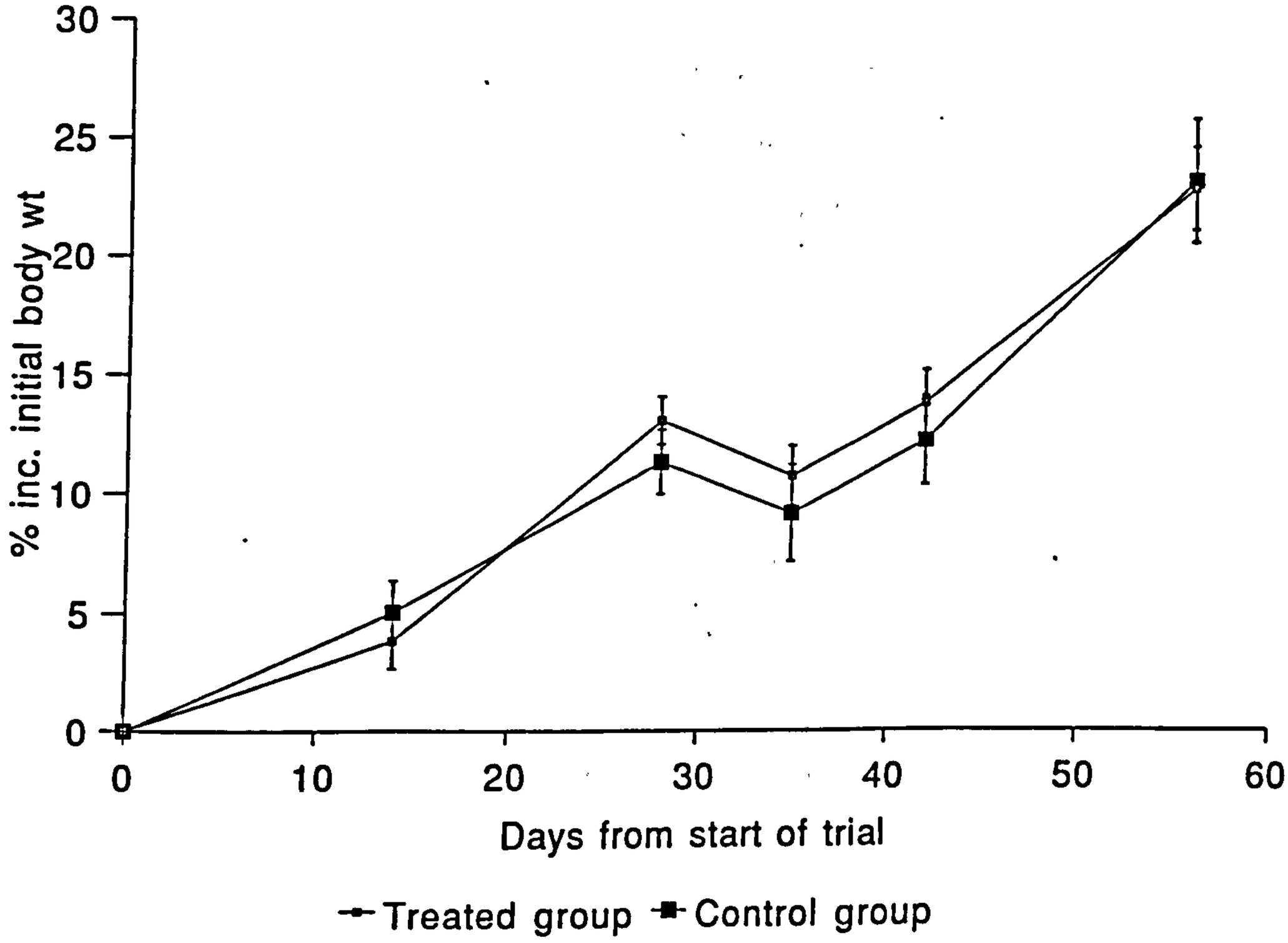

Fig. A2.3: Mean weight gain (± S.E.) of treated and control animals in both trials.

DISCUSSION

Though these results show no conclusive evidence that the deltamethrin pour-on formulation had any significant effect on the tick burdens of cattle, such pour-on treatments have been shown to significantly control ticks on cattle (Taylor and Elliot 1987). The problems with this trial were two-fold:

i) The control group were not separated from the treated animals so that cross-contamination (transference of acaricide through contact with treated animals) will have occurred so that the control animals were in effect treated.

ii) The remarkably good spring in 1988 with a very low rainfall led to a truncation of spring activity in *Ixodes ricinus* and resulted in a lower level of activity over an extended period (pers. obs). This will have reduced tick burdens irrespective of treatment. This draws attention to how important it is to take into account the climatic conditions during trials and I would suggest in future that, where possible, complementary sampling of the trial pastures should be conducted to give an indication of the level of tick activity.

At this farm it was hoped to assess the degree of protection from redwater fever the treatment gave. One animal contracted the disease during the trial and ticks were present on cattle for most of the trial. Given that it requires only a single adult or nymph to transmit redwater fever (Donnelly and Peirce 1975) the treatment did not offer complete protection. However, it may have reduced the probability of cattle contracting the disease. The decision to treat depends on the size of the tick problem and the incidence of disease. If the farm has a large tick population and the cattle are regularly exposed to ticks then it is probably unwise to treat as cattle are more likely to acquire and retain an immunity with persistent exposure (Gray 1980). If the tick population is small and cattle are infrequently exposed to ticks, then their immunity could wane and cattle may therefore be more susceptible to developing the disease. In this case treatment with a pour-on formulation would be recommended.

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APPENDIX III

Recipes for electrophoresis stains:

Isocitrate dehydrogenase:

Isocitric acid	100 mg
$MgCl_2$	25 mg
NADP	8 mg
MTT	5 mg
PMS	8 mg
Tric HCl	25 ml
2% Agar	20 ml

Phosphoglucomutase:

Glucose-1-phosphate	80 mg
Glucose-6-phosphate dehydogenase	15μl
NADP	8 mg
$MgCl_2$	25 mg
MTT	5 mg
PMS	8 mg
Tris HCl	25 ml
2% Agar	25 ml