Studies on the biology of the butterflies anthocharis cardamines (L) and pieis napi (L), in relation to speciation in Pierinae

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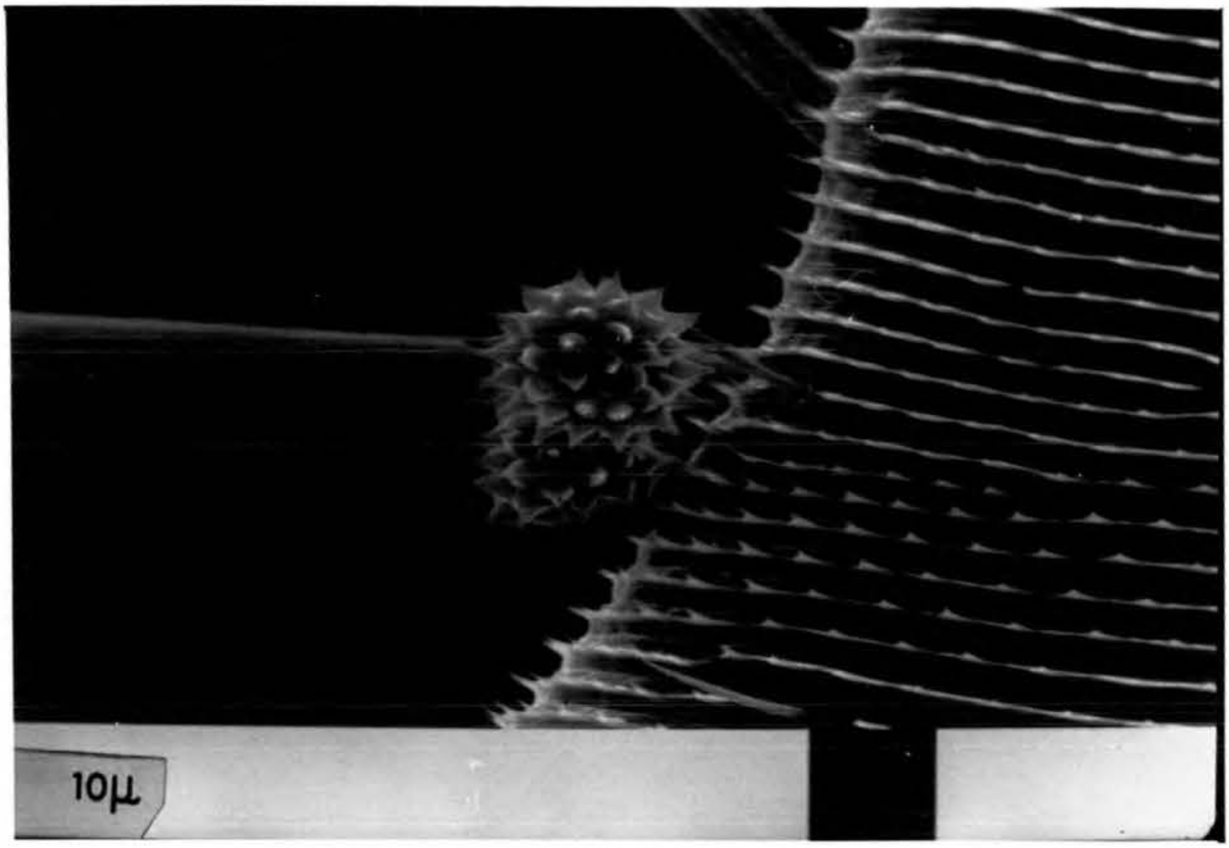
Steven Peter Courtney

Being a Thesis offered in candidature for
The Degree of Doctor of Philosophy at
The University of Durham, 1980.

Proboscis of *Anthocharis cardamines* with *Bellis perennis* pollen. (S.E.M.)

Proboscis of *Anthocharis cardamines* with *Alliaria petiolata* pollen grain.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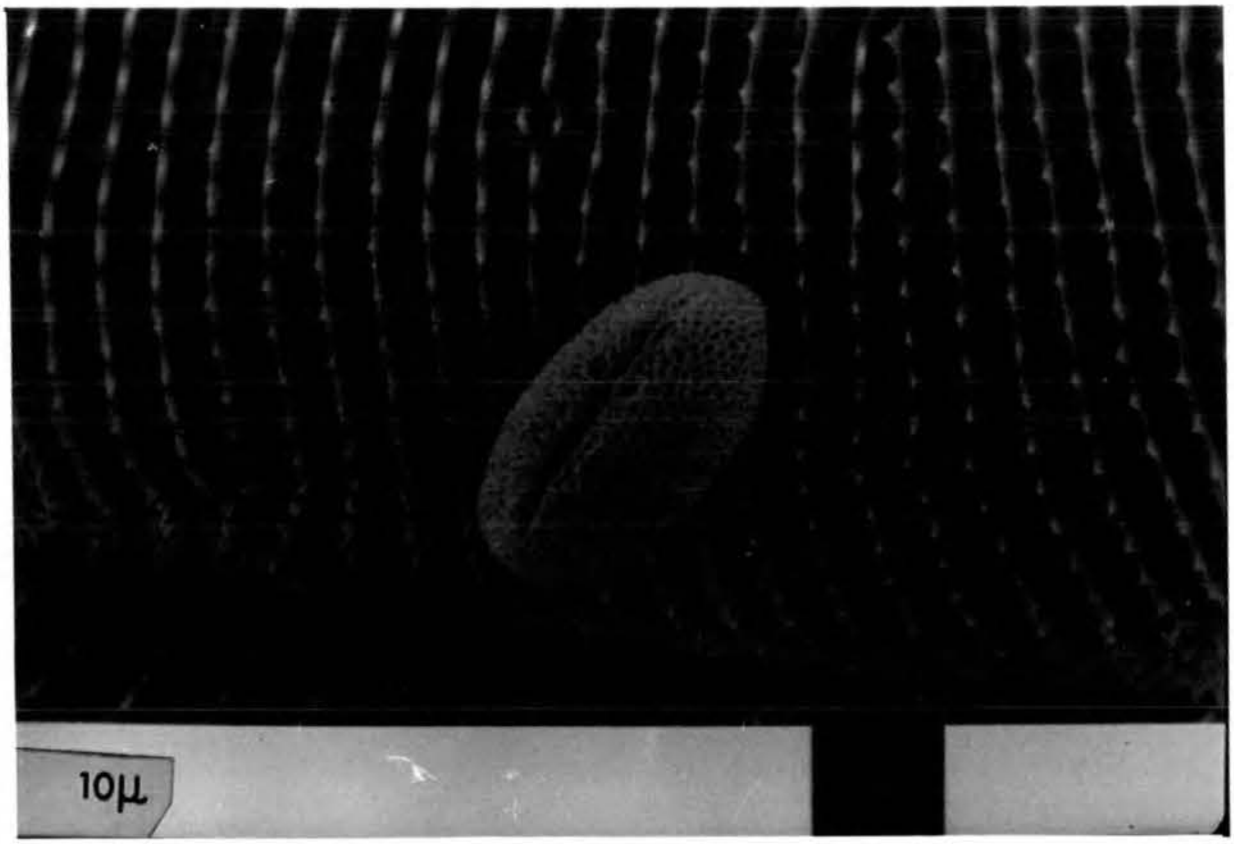
10µ

Today, this insect, and the world I breathe,
Now that my symbols have outelbowed space,
Time at the city spectacles, and half
The dear, daft time I take to nudge the sentence,
In trust and tale have I divided sense,
Slapped down the guillotine, the blood-red double
of head and tail made witness to this
Murder of Eden and green genesis.

The insect certain is the plague of fables.

Dylan Thomas

Abstract

The evolutionary biology of Pierinae is described in three separate studies. In Part One, the population biology of the Orange Tip butterfly (*Anthocharis cardamines*) is described. Colonies of this species are localised in riverbank habitats in Co. Durham. Using mark-recapture studies, the population size and movements of individual males were assessed. Studies of individual pre-adult survival indicated that food-plant-related mortality and parasitisation were important causes of death. However a key-factor analysis for one population showed that failure of adult females to lay all their eggs was the most important factor influencing population size.

The adaptiveness of oviposition behaviour was examined by following individual females and by mapping the distribution of eggs upon foodplants. It was shown that many aspects of oviposition choice were best interpreted as chance outcomes of searching behaviour. The observed differences in larval survival on different foodplants, and the constraints placed by time shortage on oviposition were used to construct a simple optimality model. It was shown that oligophagy, the typical condition of *A.cardamines* populations, leads to increased individual fitness when compared to monophagy. The effect of this stabilising selection on future speciation in Pierinae is discussed.

The concept of effective population size is developed for A.cardamines, and it is shown that allelic variation at one locus conforms to the predictions of sampling theory in small populations.

Part two describes the mating behaviour of several Pierinae, and demonstrates that reproductive isolation in this group is not a result of male behaviour, but of female discrimination of male characters. The agreement of observed behaviour with the predictions of sexual selection is noted, and the likely effects of sexual selection in past and future speciation is discussed.

Part three describes the amount of structural gene change that has occurred during the evolution of Pierinae. It is shown that little genetic differentiation at such loci occurs prior to or during speciation. Rather, differentiation of species appears to occur at other loci, including those involved in local adaptation, as was described for montane and lowland populations of the Green-Veined White butterfly (Artogeia napi) in Co. Durham.

The importance of these results to the wider field of speciation studies is discussed.

Acknowledgements

This project was inspired by the work of Sydney Bowden, to whom I owe a special debt. His freely-given help and advice, particularly in obtaining stocks of animals, has aided me immeasureably. Others who have collected specimens are C. Aquilina, L. Gall, C. Oliver, M. Pyornila, A. M. Shapiro and my mother, Una Courtney.

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Finally, I wish to acknowledge the guidance given over the last four years by my supervisor, Lewis Davies. The lessons I have learnt from him extend far beyond the narrow confines of this research. I offer him affectionate and warmest thanks.

CONTENTS

APPENDICES

1. Introduction

The Pierinae butterflies provide an unrivalled opportunity for evolutionary studies, in that the group is both generally distributed and well-studied. The economic importance of several Holarctic species has meant that attention has been focussed on aspects of their biology only infrequently examined for other Lepidoptera. Thus it is that the pathology (David, 1978), parasitology (Sato, 1976, 1979) and other aspects of their ecology, such as seasonal movements (Baker, 1968; Jones, 1977), oviposition behaviour (Behan and Schoonhoven, 1978) and relationships to foodplants (Rothschild, 1975; Feeny, 1975) are relatively well known. In addition the species are often easy to rear (David and Gardiner, 1961 a b) which leads to their frequent use as laboratory animals. Hence the comparative physiology and biochemistry (Turunen, 1977) have also been studied. Of particular importance is the fact that some species have been the subject of intense neurophysiological experimentation (Chun and Schoonhoven, 1973; Kolb, 1978).

In common with most butterflies, the taxonomy of Pierinae is extremely well known. Some genetic work has been done (e.g. Shapiro, 1971, 1975; Thomson, 1946; Bowden, 1956, 1972) but evolutionary studies have tended to concentrate on particular species or subspecies problems. In particular the well-known *Artogeia napi/ bryoniae* group of taxa have captured much attention (Muller and Kautz, 1939; Bowden, 1972). It was the opportunities provided by this species group which initially stimulated my interest in the Pierinae as a whole.

The Pierinae butterflies, then, were ideal subjects for studies on adaptation and speciation. In particular the cross-breeding experiments of Lorkovic (1958) and Bowden (1972) and others allow a biological species concept to be used within the group with a degree of accuracy rarely found elsewhere.

Originally it was intended to work with *Artogeia napi* L. in all aspects of the work described below. However this rapidly proved impossible; in particular it was soon established that the species presents insuperable problems to studies of population ecology, since it is often extremely mobile (as are *A.rapae* and *P.brassicae*). In addition it was found that the ecology of *A.napi* was in any case being studied elsewhere (by E. Lees, A. M. Shapiro, F. Chew, C. Wiklund), and it was felt prudent to diversify effort into other species for comparative purposes.

Chapter 2 describes the population biology of the

Orange Tip butterfly, Anthocharis cardamines. This species was chosen for ecological work since it seems, uniquely among British Pierinae, to live in discrete colonies, at least in the Northern part of its range. Further, the species offers wide opportunity for studies on behavioural and ecological adaptations as seen in a member of the tribe Euchloini. This tribe has been rather poorly investigated as compared to the well-known Pierini of the genera Pieris, Artogeia, Pontia and Aporia. The results gained from the Orange Tip, together with some incidental observations made on some other Palaearctic Euchloini in North Africa, will be useful for comparison with these Pierini. The relationship of the Orange Tip to its cruciferous food-plants was chosen for particular attention, since there is good reason to believe that hostplant related evolution is of major importance in diversification and speciation in butterflies and other insects (Ehrlich and Raven, 1965; Zwolfer, 1974).

When I started this work, no detailed ecological studies on the Orange Tip, or indeed any Euchloini member, had been completed. However, midway through 1978, contact was made with C. Wiklund of Stockholm University, who has been engaged on a 5 year ecological study of A.cardamines. Much that is reported in Chapter 2 agrees with the conclusions of his work (Wiklund and Ahrberg, 1978);

however, all material reported here was derived quite independently.

The study of reproductive isolation and of the origin of isolating mechanisms is central to the study of speciation. Chapter 3 describes the mating behaviour of animals of this sub-family, and demonstrates the mechanisms responsible for reproductive isolation among the three Pierinae commonest at Durham (A.cardamines, A.rapae and A.napi). The likely effects of selection acting on the behaviours described are examined, and the importance of such selection to future speciation discussed.

Chapter 4 describes an investigation into the amount and nature of genetic change during both local adaptation and speciation. Local populations of the Green-Veined White (A.napi) were examined using electrophoretic methods, as were populations at increasing levels of divergence, in order that the amount of gene change during speciation might be assessed. At the inception of this study, relatively few investigations of this sort were available for other organisms (e.g. Avise et al, 1975; Ayala, 1975) but since that time other studies have been published, usually agreeing with the initial findings of little gene change during speciation. One feature that distinguishes the present study is that the taxonomy of the Pierinae is well known, thus avoiding complications caused when taxonomic status is unsure (e.g. Brittnacher et al (1978) studied Speyeria butterflies of uncertain levels of divergence).

The research reported here thus forms studies of several different aspects of the evolutionary biology of Pierinae, which are briefly discussed in relation to speciation of that group in the general discussion.

Species names used frequently in the text

The classification of Pierinae is discussed in App. 14.

Pierinae

Artogeia napi L. Green Veined White
Artogeia bryoniae O.
Artogeia virginiensis Edw.
Artogeia ergane Gyr.
Artogeia melete Men.
Artogeia krueperi Stgr.
Artogeia mannii Myr.
Artogeia rapae L. Small White
Pieris brassicae L. Large White
Pieris cheiranthi Hbnr.
Pontia daplidice L. Bath White
Pontia chloridice Hbnr (= Pontia beckerii Edw)
Synchloe callidice Hbnr (= P.occidentalis Rkt and
P.protodice Bois Lact.)
Aporia crataegi L. Black-veined White

Euchloe ausonia Hbnr.
Euchloe ausonides Bois.
Euchloe tagis Hbnr.
Euchloe belemia Espc.
Euchloe olympia Edw.
Elphinstonia charlonia Donzel
Anthocharis cardamines L. Orange Tip
Anthocharis sara Bois.
Anthocharis damone Bois.
Anthocharis gruneri Herch-Seffr.
Anthocharis belia L.
Anthocharis genutia F.
Anthocharis lanceolata Bois.
Zegris eupheme Esp.

Papilionidae

Papilio machaon L. Swallow Tail

Pieridae

Leptidea sinapis	L.	Wood White

Danaidae

Danauus plexippus	L.	Monarch

Nymphalidae

Limenitis camilla	L.	White Admiral
Inachis io	L.	Peacock
Vanessa cardui	L.	Painted Lady
Aglais urticae	L.	Small Tortoiseshell
Euphydryas editha		
Euphydryas aurinia	Rtmg.	Marsh frittilary

Satyridae

Eumenis semele	L.	Grayling
Maniola jurtina	L.	Meadow Brown
Coenonympha tullia	M/r.	Large Heath
Coenonympha pamphilus	L.	Small Heath
Pararge aegeria	L.	Speckled Wood
Lasiommata megera	L.	Wall

Lycaenidae

Thecla betulae	L.	Brown Hairstreak
Quercusia quercus	L.	Purple Hairstreak
Strymonidia pruni	L.	Black Hairstreak
Lycaena phlaeas	L.	Small Copper
Lycaena dispar	Haw.	Large Copper
Heodes virgaureae	L.	Scarce Copper
Maculinea arion	L.	Large Blue
Aricia agestis	Sff.M/r.	Brown Argus
Polyommatus icarus	Rtmbg.	Common Blue
Carterocephalus palaemon.	P/s.	Chequred skipper
Thymelicus lineola	Och.	Essex Skipper

Thymelicus sylvestris	Poda.	Small Skipper
Ochlodes venatus	Brm. Grey	Large Skipper

<u>Cruciferae</u>

Nasturtium officinale	R.Br.	Water Cress
Rorippa sylvestris	L.	Creeping Yellow Cress
Barbarea vulgaris	R.Br.	Yellow Rocket, Winter Cress
Arabis hirsuta	L.	Hairy Rock Cress
Turritis glabra	L.	Glabrous Tower-Cress
Cardamine amara	L.	Large Bitter Cress
Cardamine pratensis	L.	Cuckoo Flower, Lady's Smock
Cardamine hirsuta	L.	Hairy Bitter Cress
Cochlearia officinalis	L.	Common Scurvy Grass
Hesperis matronalis	L.	Dame's Violet
Alliaria petiolata	Bieb.	Garlic Mustard
Brassica oleracea	L.	Wild Cabbage
Brassica rapa	L.	Turnip
Sinapis arvensis	L.	Charlock
Hirschfeldia incana	L.	Hoary Mustard
Capsella bursa-pastoris	L.	Sheperd's-Purse
Thlaspi arvense	L.	Field Penny-Cress
Thlaspi alpestre	L.	Alpine Penny-Cress
Isatis tinctoria	L.	Woad

2. The population biology of the Orange-Tip, *Anthocharis cardamines* Linn

"For the young pointer can no more know that he points to aid his master, than the white butterfly knows why she lays her eggs on the leaf of the cabbage"

Darwin, The Origin of
Species, 1st Edn.

The Population Biology of the Orange-Tip, *Anthocharis cardamines Linn*

i. Introduction

a. Introduction

> "P.D. alis integerrimis rotundatis albis: primoribus media fulvis, posticis subtus viridi nebulosis"
>
> Linnaeus. Syst. Nat. ed.x.p.468

The Orange Tip butterfly is a familiar member of the British fauna, well known to both entomologists and more casual observers. It is an attractive species, popular with the public, and associated by many with the sights and sounds of the English spring. The first British writer to note the species was Sir Thomas de Mayerne of the court of Charles I, who in 1634, published his '*Theatrum insectorum*' which describes the species. Later writers such as John Ray (in his *Historia Insectorum*, 1710) continued to note its presence in Britain. (Ford, 1945, gives a summary of such writers). The species has probably been present since 10,000 B.P. It is unlikely that the species was able to survive the oscillations in Temperature of the Allerød Interstadial, although one of its foodplants is recorded from Godwin's Zone III. Dennis (1978) has argued convincingly that the animal would not survive at the low temperatures of this period and that the species could not have survived in Britain prior to the beginning of the Interglacial proper. This is contrary to the earlier suggestions of Ford (1945) and Beirne (1947). The distinctness of *A.cardamines* in Britain and Ireland has been used in these arguments to suggest an early colonisation of those islands. In addition to pigmentational differences between these and the nominate race, British *A.cardamines* has a karyotype of n=30 (Bigger, 1978). Throughout the rest of its range, which extends across Europe and Asia to Japan, n=31. The animal is widely distributed and common throughout this region, but has a slightly northern distribution, being absent from South Spain, North Africa and the Atlantic Islands. Related species occur in southerly areas of Asia and Europe(belia L., damone Bdvl., gruneri Hch.-Sfr). In North America, A.sara Bdvl. represents the species group.

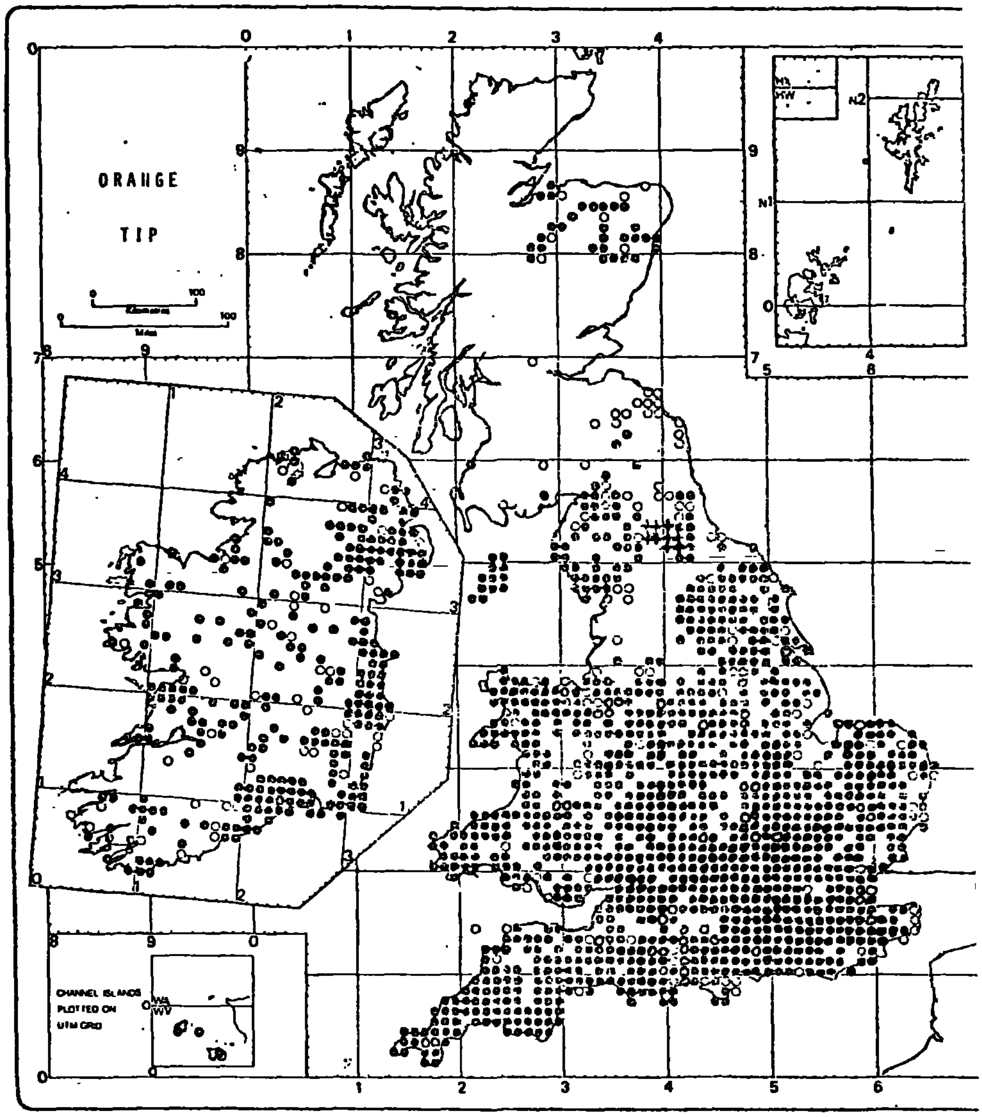
Fig. 1 shows the results of the Biol. Rec. Centre for the Orange Tip. Within the British Isles the species has a disjunct distribution, being generally common in the South, the Isle of Man and in Ireland, but becoming less common in more hilly areas. A gap occurs in its present distribution with a northerly limit in the Borders country followed by a reappearance in the North East of Scotland. This is a relict distribution caused by the contraction of a former, continuous range. As recently as the last century, the Orange Tip was common in many Scottish counties, which it is only now recolonising. The contraction in range mirrors that found in other butterfly species, e.g. Pararge aegeria (Downes, 1948). The history of the Orange Tip in Scotland is discussed by Smith (1949) and Long (1979).

b. Habitats

In the south of Britain, the Orange Tip is a butterfly of riversides, glades, meadows and lanes and may be found generally wherever its cruciferous foodplants abound. However in the Northern part of its range, a marked restriction in habitat occurs, and the animal is found only along riverbanks

Fig. 1. The distribution of *Anthocharis cardamines* in the British Isles.

Open and Closed circles represent past and present records of *A.cardamines* held by the Biological Records Centre, Monks Wood. Crosses indicate new records obtained in the present study.


ORANGE
TIP
0 100
Kilometres
0 100
Miles
CHANNEL ISLANDS
PLOTTED ON
UTM GRID
WA
WV

which may also be the foci for dispersal. For instance in Aberdeen and Kincardine-shires, Palmer and Young (1977) mapped the distribution of the species and found that it coincided with river valleys. This is also the case in the North of England - during the present study the species was only rarely found away from the main rivers of the area (exceptionally at the bog at Cassop Vale). This restriction in the habitats of the butterfly parallels a similar reduction in the major foodplant Alliaria petiolata, which is a common riverside plant in Co. Durham, but which occurs in only 4% of hedgerows, surveyed by Bailey (1979). This contrasts with Worcestershire where the plant is much more widespread, being found in 60% of hedges. A.petiolata is found in only 8% of Co. Durham roadside verges (Anon, 1979).

In Sweden, Wiklund and Ahrberg (1978) have noted that the habitats chosen by the two sexes are different, the males being localised in adult feeding areas, whilst the females are more wide ranging in their search for larval foodplants. This is also the case in North England and in Scotland, where Morison (1965) remarked on the tendency of males to remain in one part of the habitat of the species. Other species of butterfly show sexual differences in movement patterns and habitat selection, e.g. Aglais urticae (Baker, 1972).

c. Foodplants

A.cardamines is oligophagous as a larval feeder, being associated with several related species of plants, in the Family Cruciferae. This is typical of Pierinae as a whole, although very few species use such a large number of foodplants.

Artogeia napi (the Green-Veined White) and *Euchloe ausonia* display similarly wide ranges of foodplants used, but most Pierinae are more restricted, e.g. *Artogeia rapae* and *Pieris brassicae* are associated mostly with cultivated Brassicas; *A.belia* is restricted to *Biscutella spp.* *Zegris eupheme* to *Hirschfeldia incana*; *A.virginiensis*, a sibling species of *A.napi* feeds only upon *Dentaria spp.* It is not known whether oligophagy is the ancestral condition in *Anthocharis* as it is in the *A.napi*-group.

Like all other Euchloini studied, the Orange Tip uses the flowers and seedpods of its foodplants. In attacking only the reproductive parts of its host, it may be said to act as a predator. Only rarely do Euchloini attack other parts of the plant, usually in conditions of crowding or food shortage (n.b. *Euchloe hyantis* is an exception (Opler, 1974)). This contrasts with the behaviour of most Pierini, where the leaves are the food (the animal being a parasite in attacking non-essential plant parts). This seed/leaf difference in life-style is consistent worldwide in the two tribes of the Pierinae and may form the major ecological difference allowing coexistence at least in the Holarctic. (The currently accepted classificatory groups of the Sub-Family are given in a later section - App.14). Thus it is relatively common to find a single plant supporting both *A.cardamines* larvae and those of *A.napi* or *A.rapae*.

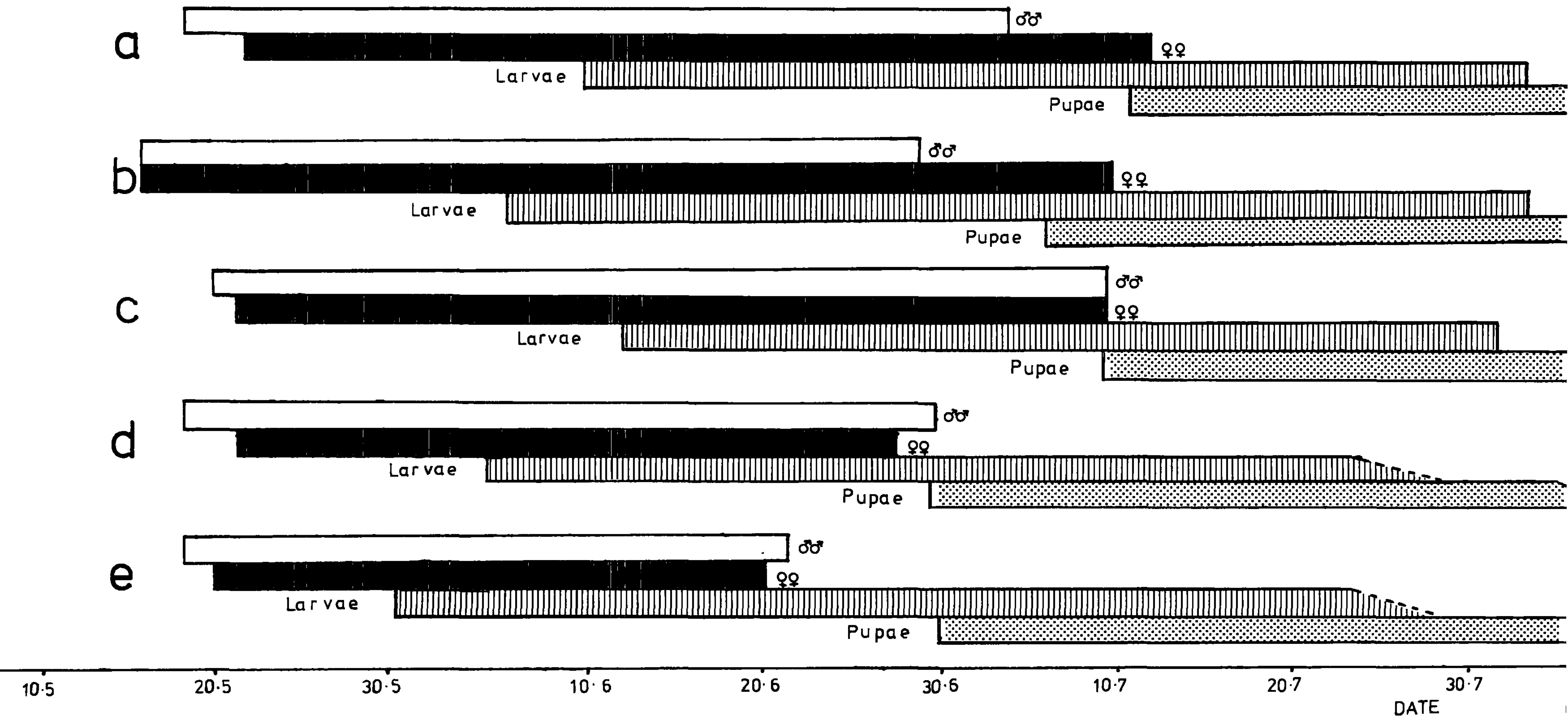
The Orange Tip is univoltine throughout its range, although occasional reports of second broods have been recorded (see Williams, 1915). These are probably only retarded 1st brood specimens. Neave (1914) and Williams (1915) give several instances of pupae eclosing in the summer, or even autumn, instead of the previous spring. The latest flight observed in the present study was in mid-August. Univoltinism appears to be prevalent in the Euchloini, although some, e.g. Euchloe ausonia are multiple-brooded. Pierini, however, are generally multivoltine - the single brooded A.virginiensis being an exception. In this case univoltinism is a derived characteristic (Shapiro, 1971) and appears adaptive in synchronising the butterflies emergence with the growth of its vernal foodplant. There is little evidence for asserting the adaptiveness of univoltinism in Euchloini, such as Anthocharis, although certain facts are persuasive. The cue for host-finding appears to be the sight of the flower (section 2 vii), whilst the food, the reproductive growth of the plant, is usually vernal. This contrasts with the Pierini where hostplant location is, initially, a visual response to vegetative parts (Ilse, 1926; Jones, 1977) which in many crucifer species grow for extended periods. For instance, in Co. Durham, A.petiolata flowering and seedset is vernal, but there is a resurgence of leaf growth later, which coincides with the second brood of A.napi. The local differences in voltinism patterns of A.napi are discussed in section 4.i.

Occasionally diapausing pupae of A.cardamines are found which lie over two winters, emerging as adults in the spring two years after feeding as larvae (Mitchell, 1896). This was also recorded naturally in one specimen of the present study. In addition pupae were kept in differing conditions of light and temperature, in order to find those conditions which would break diapause. Only those pupae subjected to prolonged cold periods eclosed, those kept at room temperature remaining as apparently healthy pupae. It is thus possible to both delay and hasten emergences - producing a flight in mid-winter if wished (emergence is generally 3-4 weeks after removal from the refrigerator).

Fig. 2 presents the phenology of the Orange Tip at Durham. Eclosion takes place from mid-May onwards, continuing until the end of June. (Wailes (1857) gives May and June as typical for the species in Durham and Northumberland). Males generally appear approximately a week before the females (although in very late springs the emergence may be synchronous). This is typical of most butterfly species. Scott (1978) and Wiklund and Fagerstrom (1977) have recently produced models of this situation, based on premises of male competition for matings with females. Both models argue that selection between males for the scarce resource, matings with females, will lead to protandry, since the number of possible matings to the individual male is thus maximized.

Oviposition occurs during June and early July; the larvae feed up during July and August and most have pupated by the second week of the latter month. Minor differences in timing occur at the other sites in Co. Durham but the above description appears generally applicable to the whole of the North of England. Occasional emergences as early as March

Fig. 2 Phenology of A.cardamines at Durham 1977-79 (a,b,c) and at Ljusterö (d) and Ingarö (e), Sweden 1976 (after Wiklund and Ahrberg, 1978)



may be dismissed as aberrations. However, there exists a clear difference in adult flight times between Co. Durham and other areas. For the South of England most observers regard the Orange Tip as a species of late April and May, rather than June. For instance Williams (1915) gives as 'unusual' dates such as June 16th and 17th - the period of peak egglaying at Durham. Similarly Chapman (1888) gives dates of July 12th-19th for pupation, when many Durham larvae are in their early instars. The nominate race in Sweden also flies earlier (Fig. 2). Further north in Britain, the relict Scottish population, for instance at Abernethy, flies even later than at Durham - June being typical.

d. Adult

The adult butterfly has a wing expanse of some 40 mm, Williams (1915) giving a range of 30-52 mm (the largest specimens being of the Continental, nominate race), the female being larger by some 5-6 mm. The ground colour is white and is produced by pterins, as in other Pieridae. In the male the outer half of the wing has bright orange scales, giving the species its name. On the upperside, the apex is black, as is the discoidal spot; the wing bases are dusted with black scales and the fringe of the wing is chequered with black and white. In the female both the apical marking and the discoidal spot are larger; sometimes the latter may have a white spot within it, reminiscent of other Euchloini. On the underside the apex is white with black and yellow scales, giving a mottled green appearance. This is also found on the hindwings which have intricate 'green' markings, covering most of the undersurface. This yellowish-green colour is typical of most Euchloini and some Pierini (e.g. *Pontia daplidice*) and is an 'impressionist' effect, yellow and black scales together giving the appearance of green (Wigglesworth, 1928).

Two characters of variable expression are the tinting of yellow on the male underside near the costal margin of the forewing and the colour of the female upperside which is often ochreous. Both these characters are common in Ireland and have been used to describe the Irish race (*hibernica*) as distinct from the British (*britannica*). These characters are also found in Manx and Welsh specimens, which has led to these animals being placed in the Irish subspecies. Both characters are also found in Durham specimens. Other variation of the Orange Tip has been but little studied, the work of Williams (1915, 1957) being exceptional, though occasional captures of spectacular gynandromorphs and of yellow-tipped specimens have been recorded. During the present study a full gynandromorph was captured at Durham in June, 1977; additionally, a specimen of ab.*salmonea* (in which the apical orange of the male is replaced by salmon-pink) was bred from a pupa taken at Witton Park in 1978.

The colouration of the Orange Tip has caused much comment. The underside mottling was cited by Wallace (1878) as a fine example of camouflage:'The little orange tip butterfly often rests in the evening on the green and white flowerheads of an umbelliferous plant, the wild chervil (*Anthriscus sylvestris*) and that when observed in this position the beautiful green and white mottling of the undersurface completely assimilates with the flowerheads and renders the creature very difficult to be seen'. Similarly Kettlewell (1958) argued that the mottling had evolved through natural selection via predation. He cited spiders as potential selective agents, but most would agree that birds are more likely predators; the camouflage seems most suited to deceive the vertebrate eye. (It should be noted that at rest the wings are folded so that the orange forewing is completely hidden by the hindwings). Some authors have suggested that the camouflage of the Orange Tip is adapted to particular roosting sites - the difficulty of detecting the animal upon *Anthriscus sylvestris* L. has been often noted. However, the animal is equally well protected on other plants such as Bracken (*Pteridium aquilinum* L.) (Lucas, 1912) and *A.petiolata*. The mottling more likely acts as a general disruptive pattern, equally effective in many roosting sites. The orange colouration of the male has also been the subject of comment, Darwin and Wallace arguing variously for sexual selection and aposematism as factors in its evolution; this topic is discussed briefly in section 3 iv.

The plants mentioned above as roosting sites are typical for the species. The flowerheads of other umbellifers and crucifers are also used for nocturnal roosting; however the animal only flies in direct sunshine, and when forced into inactivity by lengthy cloudy periods, it will settle onto any available surface or inflorescence. During such periods of weather with intermittent sunny spells, the butterfly basks with wings open, using sunshine to raise its body temperature (Clench, 1972) to a level suitable for activity (as in Heodes virgaureae (Douwes, 1976)). This basking posture is well-known throughout the Pierinae. The species is associated with particular types of flowering plants in the area around Durham. (Appendix 1). Notably, the Orange Tip uses, as nectar sources, common flowers of the Caryophyllaceae. Especially noteworthy is the importance of larval foodplants as nectar sources for the adults. This may indicate some behavioural interaction amongst oviposition and feeding stimulants, or that the animal is adapted visually to certain wavelengths of light. It may be significant that many of the nectar plants of the Orange Tip are coloured white or blue to human eyes - many of these plants additionally fluoresce in the UV range. Similarly coloured plants are recorded for Swedish A.cardamines. The movements, mating and oviposition behaviour of adults are described in later sections (2.iii, 3.ii, 2.vii).

e. Egg

The eggs are laid singly upon the flowerheads of Cruciferae during June and July. They are typically placed at the base of pedicels, more rarely upon the bloom or stem. They are of the typical Pierid form, being approximately 1 mm in height, ribbed with a variable number of longitudinal ridges. The colour when laid is usually pure white, turning pinkish-orange within 24 hours, and subsequently to a deep orange-red, which is very easy to detect against the background of the yellow or white inflorescence. Due to the colour changes of the egg, it is possible to age an egg during the first three days of its life, although caution must be used. When oviposition is inhibited by bad weather, the proximal egg within the female, being fertilised, may develop the red colour (confirmed by dissection). Other Euchloini also show egg colour changes, e.g. the several species discussed by Opler (1966).

Immediately prior to hatching, the egg becomes a dark brown in colour. On hatching, the larva normally eats the transparent egg case, but rarely to completion. This proved of great value in following and confirming the hatching of eggs.

The duration of the egg stage is variable, 7 to 10 days from oviposition to hatching being typical. As recorded by Williams (1915) during warm weather, development is quicker than in cold spells.

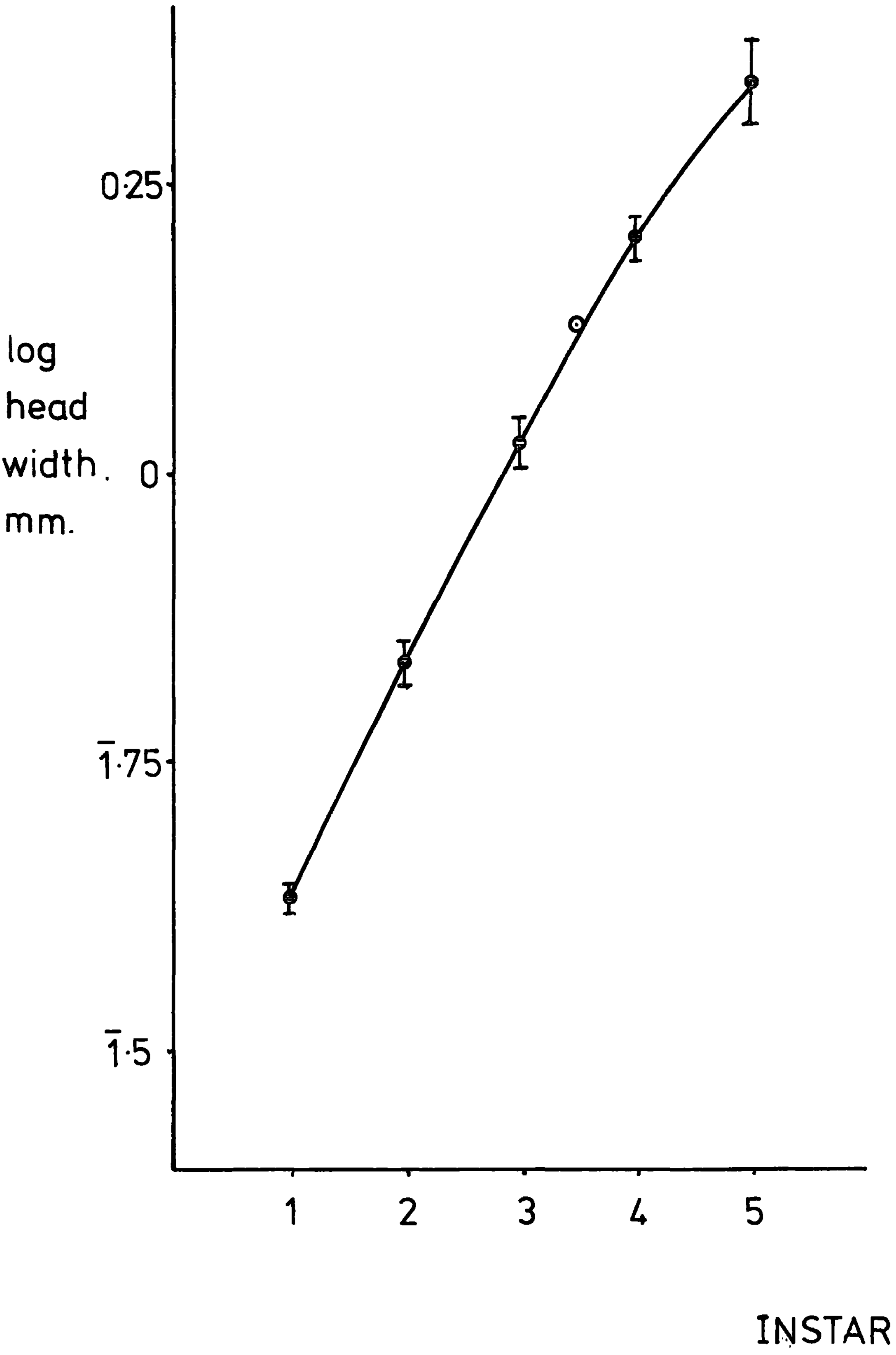
f. Larva

The duration of each larval instar is similarly variable; Chapman (1888) records a total duration from hatching to pupation of 16 to 24 days. At Durham, longer larval periods were normal, with some larvae developing over up to 36 days. There are five instars in all, as confirmed by head capsule width measurements (Fig. 3).

Upon hatching the larva is approximately 2 mm in length and is pale in colour. The body is covered with black spots and black hairs which are forked at the tip. The head is black. The animal feeds up rapidly, and in the

Fig. 3 Head capsule widths of *A.cardamines* larvae ($\bar{x} \pm 2$s.e.)

⊙ records one animal intermediate between instars 3 & 4.



second instar develops a green body and head. Later instars also develop a broad lateral stripe of white, which extends the full length of the larva and encloses the spiracles. Feeding takes place at both day and night. At first the larva feeds upon the flowerbuds and open flowers of its foodplant, and at this stage it may be difficult to detect. Later stages feed upon the developing seedpods.

> "At first it (the larva) requires but little food, and the pods, growing with great rapidity, fully keep pace with its requirements and furnish an abundant supply. It is curious to observe with what pertinacity the caterpillars in confinement select the pods and neglect the leaves of these plants"
>
> Newman (1869).

Occasionally the larva may in fact descend the plant and feed upon the leaves, or actually leave the plant altogether. In particular, when feeding on very small crucifers this may be necessary. Leaving depleted foodplants to search for alternative hosts has also been found for other species - e.g. Melitaea harrisii Sedr. (DeThier, 1959) Euphydryas editha (White, 1974). However, in most circumstances the larva completes development upon a single plant or even a single flowerhead of the largest crucifers. This happy circumstance makes the survival of individual larvae easy to follow. Similarly, the cast skin from each ecdysis provides clues as to larval survival and development.

When fully grown the larva measures some 35 mm, exceptionally 40 mm, and at this point enters a wandering phase, descending the foodplant and searching out a pupation site. Occasionally larvae of a distinct blueish colour occur; specimens of this type occurred at Wolsingham in 1979.

Originally this was described as a separate species 'alberti' (Albert, 1894; cited in Williams above) but is now clearly an occasional form of A.cardamines proper.

The larvae are easy to rear in suitable containers; During the present study this was routinely done in petri dishes. One egg or larva was reared in each dish (to avoid cannibalism) and was provided with foodplant material - typically a sprig of A.petiolata. Foodplant material was kept fresh by a small piece of damp cotton wool, and was changed when it dried out or was consumed. Dishes were periodically cleaned of frass. Immediately prior to pupation larvae were transferred to bigger containers.

The bifurcate hairs of the young larvae often hold droplets of clear liquid; Ford (1945) and others record that these are used in an association with ants. During the present study ants were frequently found ascending the stems of crucifers, especially A.petiolata, and were seen to feed on nectar. The species included Formica and Myrmica spp, but no instance was found of any association between A.cardamines and these species. (Baker found that survival of A.cardamines is better on plants which also bear ants - pers. comm.)

Another habit often associated with a mutualism with ants is cannibalism, for which A.cardamines larvae are notorious amongst entomologists. Williams (1915) and Ford (1945) both record that the habit is restricted to young larvae, although some antagonism (not leading to killing) is also found in later instars, and pupae may be killed if left in the same container as large larvae. Cannibalism is discussed more fully in section 2.iv.

The habit of wandering away from the foodplant makes the pupa almost impossible to find in the field. Frohawk (1915) and T. Dunn (pers.comm.) have each found a single example upon A.petiolata, but both Frohawk and Williams were of the opinion that the species 'but seldom pupates upon its foodplant'. In captivity pupation will take place upon any thick stemmed plant, the larva spinning a thick pad of silk to which it is attached anally. The larva also spins a girdle of silk which supports the animal around the middle, the ventral part being free of support. Pupation therefore takes place head upwards, and occurs over a period of 24 hours. The pupa is from 15 to 25 mm in length, pointed at either end, and considerably harder than most Pierinae pupae. When first formed it is green, but this gradually fades to a drab buff. Like other Pierinae species, there is some variation in the colouration, occasional specimens maintaining their green hue. There may also be environmental influences upon pupal colour (evidence summarised in Williams, 1915). Immediately prior to eclosion, the pupa 'colours up', the developing wings of the adult showing through the pupal skin, now very thin. In this way it is easy to sex pupae before emergence, and to keep the sexes separate.

g. Aims of the study

The central problem addressed in this investigation was the relationship of adaptations to individual fitness, that is, do 'adaptations' lead to maximal numbers of offspring in the next or succeeding generations, and have such adaptations arisen by natural selection? Section 2.iii describes the movement patterns of one population and the censussing of adult numbers, which have bearing on population genetics and dynamics (section 2.viii and 2.vi). Female fitness was investigated by way of oviposition behaviour (section 2.vii) and the survival of eggs (section 2.iv). This led to the study of the relations between the animal and its foodplants (section 2.v) and to the production of a k-factor analysis for one population, in order that individual adaptations might be viewed against a proper background of fitness within a population. The results of these analyses are discussed in relation to the known local history and variation of <u>A.cardamines</u>. (Male fitness is discussed briefly in Chapter 3). Section 2.ix then draws together these various investigations to consider the evolution of life-history characters, their evolutionary 'stability' and their likely effects upon future evolution, diversification and speciation in the Pierinae.

Fig. 4. The location of _A.cardamines_ populations (overlay) in the vicinity of Durham.

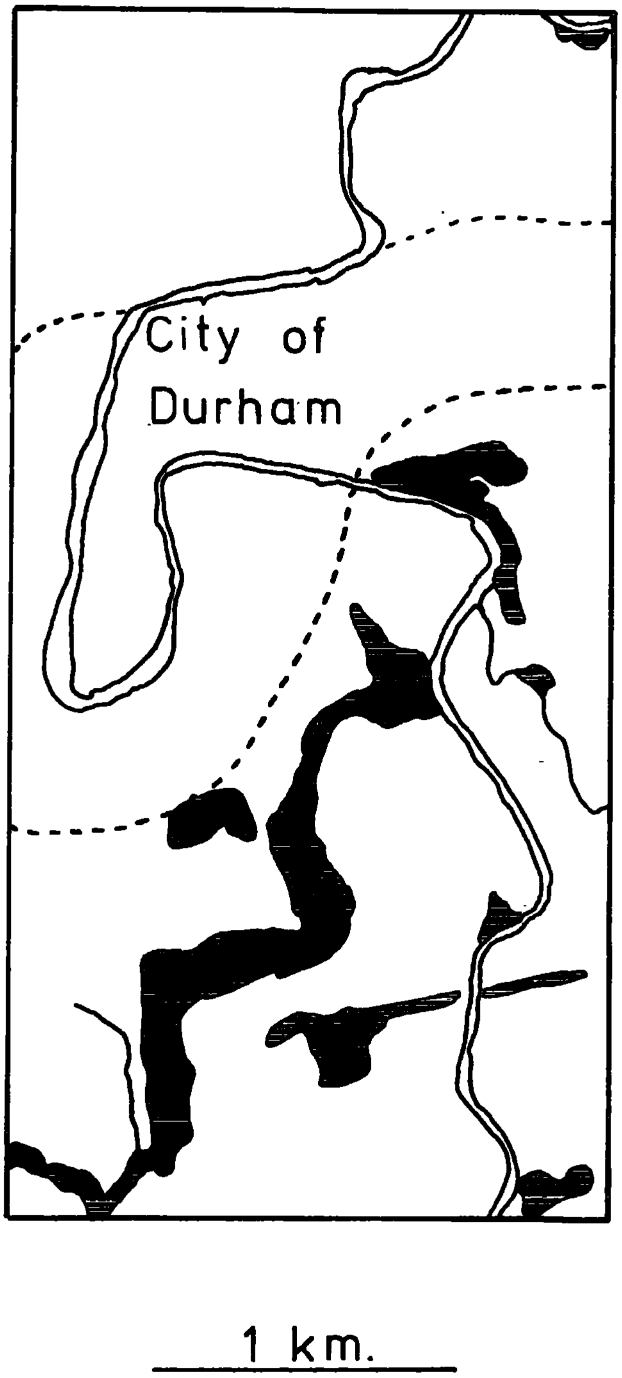
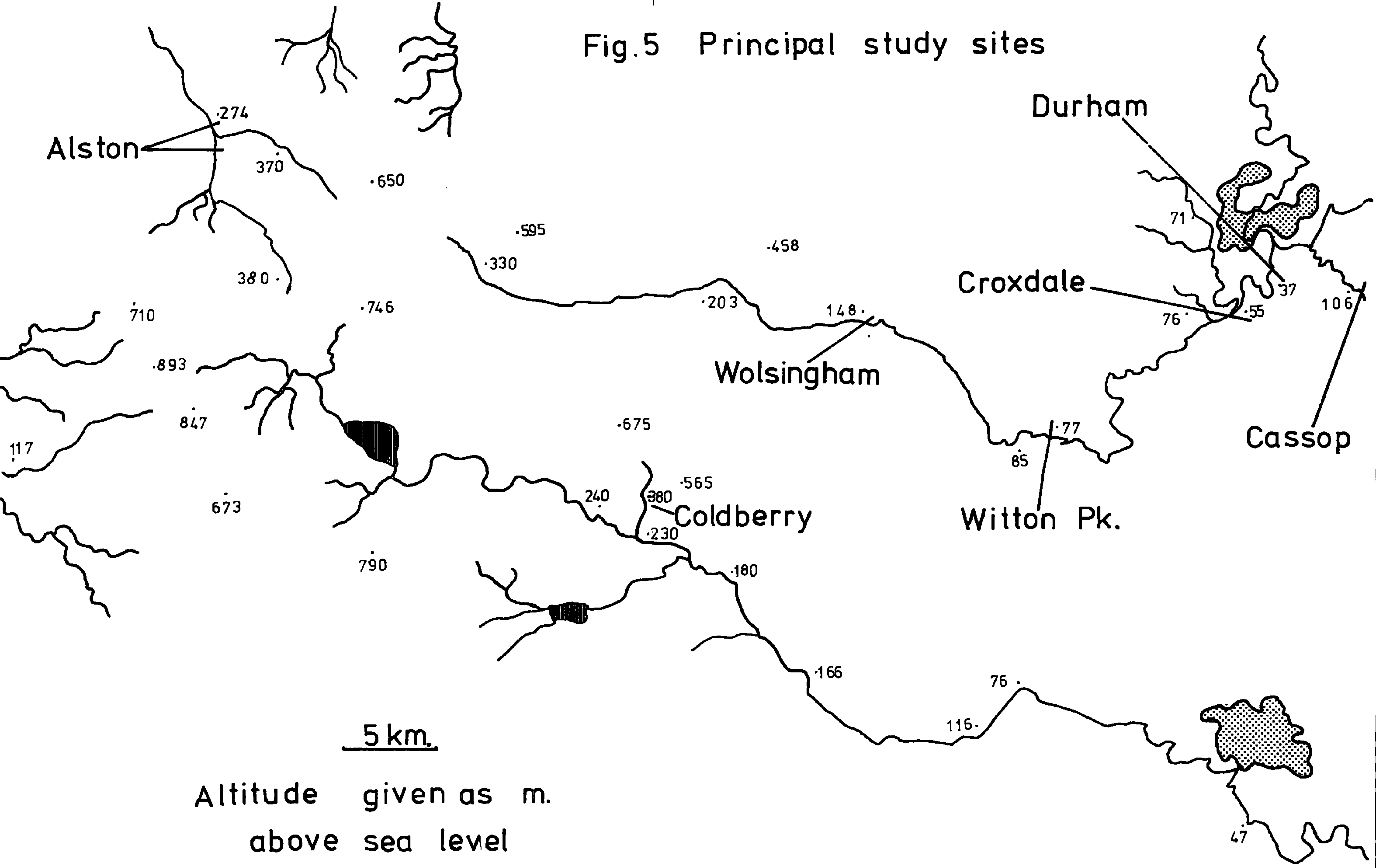

City of
Durham
1 km.

Fig.5 Principal study sites



Altitude given as m. above sea level

ii. Study Sites

The population chosen for most intense study was that occupying the banks of the river Wear upstream of Durham City. Fig. 4 maps the area. This population is easily accessible throughout its extent, with firm boundaries placed by Durham City which is unsuitable habitat for both A.cardamines and its foodplants. The banks of the river Wear provide ideal conditions for the growth of several crucifer species, particularly A.petiolata, Barbarea vulgaris, Brassica rapa, Hesperis matronalis and Sisymbrium officinale, in a band of low disturbed vegetation some 10-20 m. wide. Capsella bursa-pastoris is more generally distributed over the whole area and is not restricted to the streamsides as are the above species. Cardamine pratensis occurs in nearby meadows. The riverbanks are subject to heavy flooding in some winters and silt deposition may have important effects in preventing growth of crucifers. The weather at Durham City is summarised in the Annual reports of the Durham Observatory and Manley (1941) has summarised the weather over the period 1880-1940. Other populations exist nearby to the one chosen for study - at Shincliffe Hall (400 m. upstream/South) and at the Sands, Durham (4 km. downstream/1.5 km. N.N.E.) with similar arrays of crucifers. A more recently derived population is that at Houghall, in meadows some 750 m. E. of the Durham population. This population, associated with C.pratensis, is believed to have been started in 1975 or 1976. Colonisation also took place at the Zoology Field station, probably in 1977; again C.pratensis was the larval foodplant.

Similar conditions to those at the main Durham site were found upstream at Croxdale (4 km. S.W. Grid reference NZ 250380) and Witton Park (15 km. S.W., NZ 174309) (Fig. 5) where adult populations were sampled and larval survival followed. Flooding becomes progressively less important upstream, although silt deposition does occur at Witton Park; here it may serve to keep areas free of herbage and open for crucifer growth. Further upstream at Wolsingham (18 km. W.S.W. of Durham; NZ 065375) inundation in winter is important in maintaining the flooded areas which support large stands of Cruciferae, particularly C.pratensis and C.amara. In addition the rocky riversides support A.petiolata and B.vulgaris with a very rich meadow flora adjacent.

These four areas were the sites for most research. Additional observations were also made at various localities in the upper reaches of the Wear, Tees and Tyne valleys. In particular, fortuitous finds, at Coldberry in 1978, of a small population outside the normal habitats of the species, and at Alston in 1977 of a large uniform stand of H.matronalis, allowed specific questions to be addressed. These areas at high levels in the Pennines, are quite different to those at lower altitudes; there are few suitable nectar plants and normal roosting sites are absent, except in the valley bottoms. In addition to all these riverside sites, some observations were made at Cassop Vale.

This site, which lies in the Wear catchment area, some 8 km. S.E. of Durham, supports a wide range of plants associated with magnesium limestone, and also includes a large marshy area with <u>C.pratensis</u>.

iii. Movements and Population sizes of adults

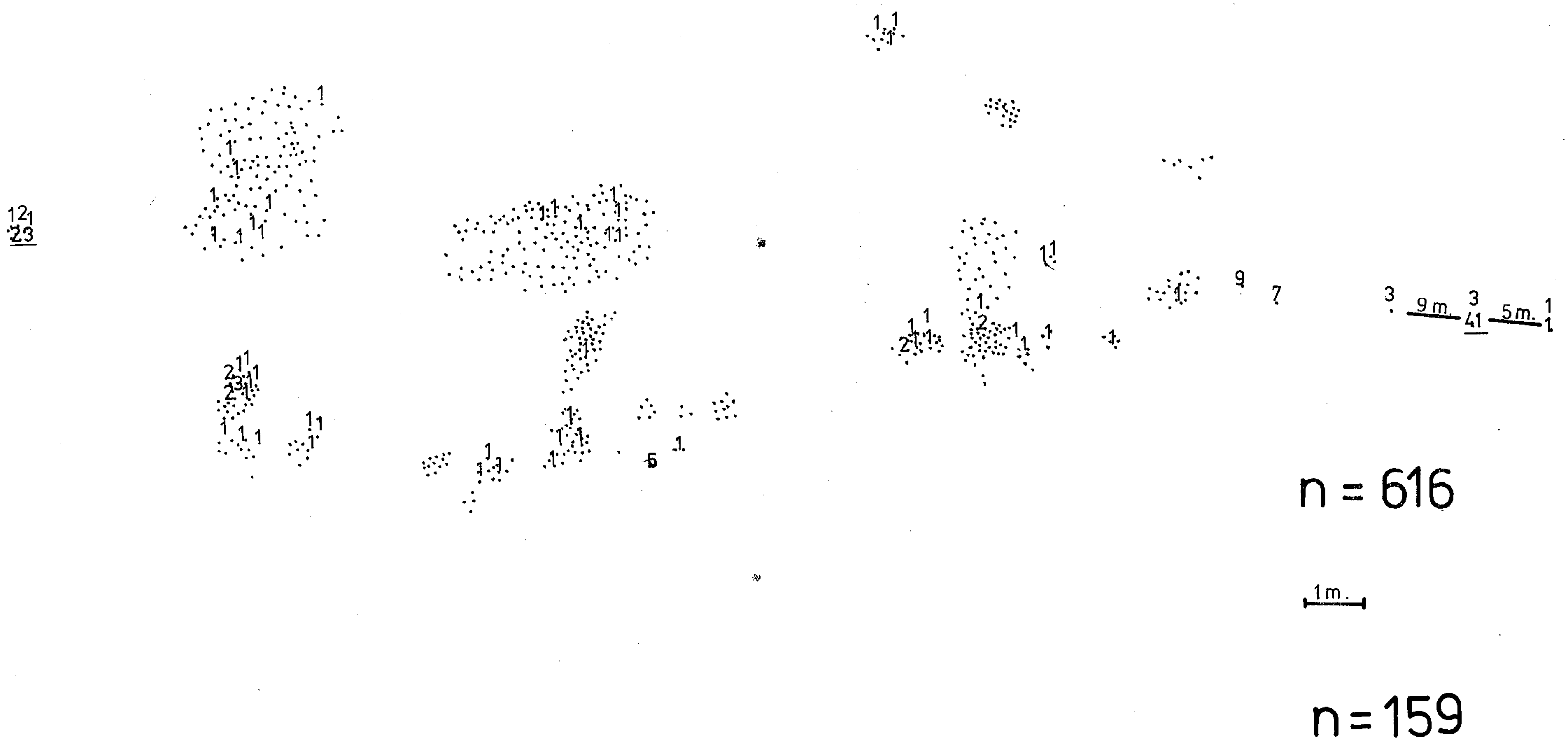
a. Introduction

The movement patterns of a species may have great influence upon the evolution of characters. Immigration may swamp local differentiation or facilitate the spread of advantageous traits. Alternatively the fragmentation of demes with little movement between them may lead to random effects on gene frequency, and to local extinctions. Lepidoptera, as relatively large, mobile insects, have been investigated repeatedly by numerous observers. Several species of Pierinae have been found to be long-distance migrants. *Pontia daplidice*, *Pieris brassicae* and *Artogeia rapae* are familiar immigrants to Britain, *P.brassicae* maintaining only precarious populations in the Durham area, periodically subject to incursions from the south. Other species may restrict their movements to much smaller distances. Scott (1975) studied 13 species of Nearctic butterfly using mark-recapture methods, and found every intermediate state between long distance migrant and purely sedentary species. Previous studies on Euchloini suggest that they may be animals of small movements. Thus Evans (1955) for *Anthocaris reakirtii* (= *Anthocharis sara*), Scott (1975) for *E.ausonides* and incidental observations made on *A.cardamines* by Morrison (1965) all suggest that small movements are typical for these species (males).

In the present study, it was decided to investigate movements within the Durham population, which, as noted above, is of easy access throughout. The methods in use were those of Scott. In essence these consist of catching and individually marking butterflies, making recaptures on following days in the same area. The location of every capture is carefully marked on a large scale map, carried in the field, together with any incidental observations (such as weather). Afterwards, on return to the laboratory, the recapture events were recorded on similar maps, one for each individual animal, allowing a picture to emerge of that animals movements within the study area. Examples of such results are shown in Fig. 6.

Mark-recapture methods may also be used for population sizes, thus providing useful information in both ecological and genetic studies, as pioneered by Fisher and Ford (1947) for *Panaxia dominula* L., *Euphydryas aurinia* and other Lepidoptera. Numerous studies have now taken place, and several different marking schemes exist. The date-specific marks used by Fisher and Ford are usually applied as small spots of enamel paint. However this has several disadvantages, as do individual marking schemes using small paint dots, e.g. Frazer (1960), and are only appropriate when very large numbers of animals are being marked, e.g. Watt et al (1977). In the present study it was decided to apply instead a large individual number to each butterfly caught, using felt tip pens. This marking scheme has the advantages of being rapid, quick drying and relatively undisturbing to the animal, in that an undersurface is used. Other schemes use upper surfaces so that predation risks are not increased (the spots being invisible when the insect is at rest). A red individual number placed in the orange

Fig. 36 The distribution of <u>H. matronalis</u> plants at Alston 27·6·79 (from field sketch). Each point signifies one plant. Overlay – <u>A. cardamines</u> eggs on plants



apical marking of the male and a black number in the same position on the female were invisible when the animals were at rest. Additionally the mark is sufficiently distinct to be read at a distance without catching the animal if so wished (in practice this was not attempted).

One matter for concern in any recapture scheme is the problem caused if handling increases emigration; this is especially so with butterflies, which are sensitive to disturbance. The methods employed by others to overcome this include chilling and shading the insects into a passive state before release. In the present study it was felt sufficient to place the marked animal deep within a clump of grass or other herbage, and to fold the grasses back over the animal, preventing it from flying out. A brief watch was then kept until the animal emerged crawling from the grass, when it would fly away, apparently unalarmed, and immediately engage in courtship, feeding or other 'normal' behaviour. Individual animals were frequently recaptured many times within a single day, suggesting strongly that handling had not caused emigration in these cases.

b. Patterns of movement

In capturing animals for the above studies, it rapidly became apparent that females were captured very infrequently when compared to males and that female recapture events were very scarce indeed.

Scarcity of females has been noted by Church (1957) and Bowden (1957) for A.napi. In A.cardamines as in A.napi there is a 1;1 sex ratio at emergence, and samples captured which depart from this ratio probably represent a biased sample. There is no evidence for female survival being shorter than male (in the lab the converse is true) but there is abundant evidence for other bias* 1-females are less conspicous than males (since females fly more slowly through an area) within the habitat and also show different habitat selection to males, frequently moving away from the riverbanks. For both these reasons it was decided to augment recapture studies with direct observations on the movements of both species.

Of 26 females followed individually, all eventually left the area of study at least once, moving away on a course perpendicular to the river. Nine of these animals had also been seen to enter in this way and 13 of the 26 were followed until they re-entered the area (the other 13 were lost to sight almost immediately, normally on the opposite side of the river to the observer. They may have subsequently returned to the habitat). Of 25 males followed individually none left the area of the riverbanks, and very few casual observations of males were made outside this area. (Total length of observation time - males 245 minutes, females 105 minutes). When within the habitat both sexes frequently showed directionality of flight (as noted for many species, including Acardamines, by Baker (1969)). This behaviour has important effects on oviposition patterns and

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*It is possible to confuse the female in flight with other white Pieridae. However, this is not a possible source of error here, since all white Pieridae were being caught for other purposes.

additional results on movement patterns are discussed in section 2.vii.

Scott (1975) has produced several methods for analysing the results of mapping individual animal movement. These include:-

di = minimum distance between successive captures.

R = Range = distance between two most distant captures.

D = Sum of all di.

V = D/Time between first and last captures = 'overall speed'.

The results of these methods when applied to A.cardamines males are presented in Table 1. There are insufficient data for analysis of females to be worthwhile. Also shown are Scott's results for Euchloe ausonides for comparative purposes. It will be seen that the values for A.cardamines males are small - particularly the ranges and speeds of animals in 1977 and 1978.*1 This agrees well with observed behaviour. Males are constantly in flight up and down the riverbanks and are a typical 'patrolling' species (sensu Scott, 1974).*2 This small degree of movement also agrees well with the results of Evans (1954, 1955) on Nearctic Anthocharis males and the observations of Morison (1965).

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*1 The difference between 1979 and previous years was very noticeable in the field. Other aspects of population biology, particularly floral choice (for nectar), changed dramatically.

*2 That is, their mate-location behaviour appears to be based on making rapid transits of suitable habitat, searching for females from the air. This contrasts with 'perching' species which adopt a small area and remain there, often on a flower or the ground, flying up and making inspections of insects entering their field of vision. Most Pieridae are patrollers. Examples of perchers are Aglais urticae (Baker, 1972) and Pararge aegeria (Davies, 1978).

Table 1

Results of analyses using methods of Scott (1975). A.cardamines ♂♂

	1977	1978	1979	E.ausonides ♂♂ (From Scott, 1975)
No. Animals Marked	127	168	145	97
No. Animals Recaptured	62	91	84	54
No. Recaptures	108	150	172	110
Mean R (m)	211.5	184.2	483.5	329.0
Mean D (m)	247.0	253.5	751.4	463.0
Mean V (m/day)	97.8	88.7	150.1	139.0

> "From long observation of the males it seemed that each male confined its flight to a rather restricted area, though it flew rapidly, particularly when aided by the wind".

The results disagree, however, with the suggestion of Baker (1972) that A.cardamines is migratory and, moreover, uses sun orientation in such movements. In the North of England few males are ever found away from river courses, whilst Owen (1977) records only 22 A.cardamines in 10,730 butterflies caught in his suburban garden. Fig. 7 shows the distribution of ranges recorded for marked animals at Durham. The mean recorded range, with one outside the area at Shincliffe Hall (500 m distant) is 302 m. The maximum possible range within the area studied is 1,800 m. There is little evidence for Baker's contention, at least in the population here studied.

The difference in habitat selection of the two sexes has been recorded by Wiklund and Ahrberg (1978) who suggest that it arises from the two sexes seeking different resources (virgin/receptive females and oviposition sites) which are distributed differentially. This agrees well with the situation at Durham, where some small patches of Cruciferae, particularly C.bursa-pastoris, occur away from the river. In the case of males searching for females, patrolling a section of riverbank would certainly produce more contacts with females than ranging the whole area searched by females, since the overall density would be small but the females eventually return to the riverbanks. (In effect the resource, females, is distributed patchily over the total area, and males are maximising their chances

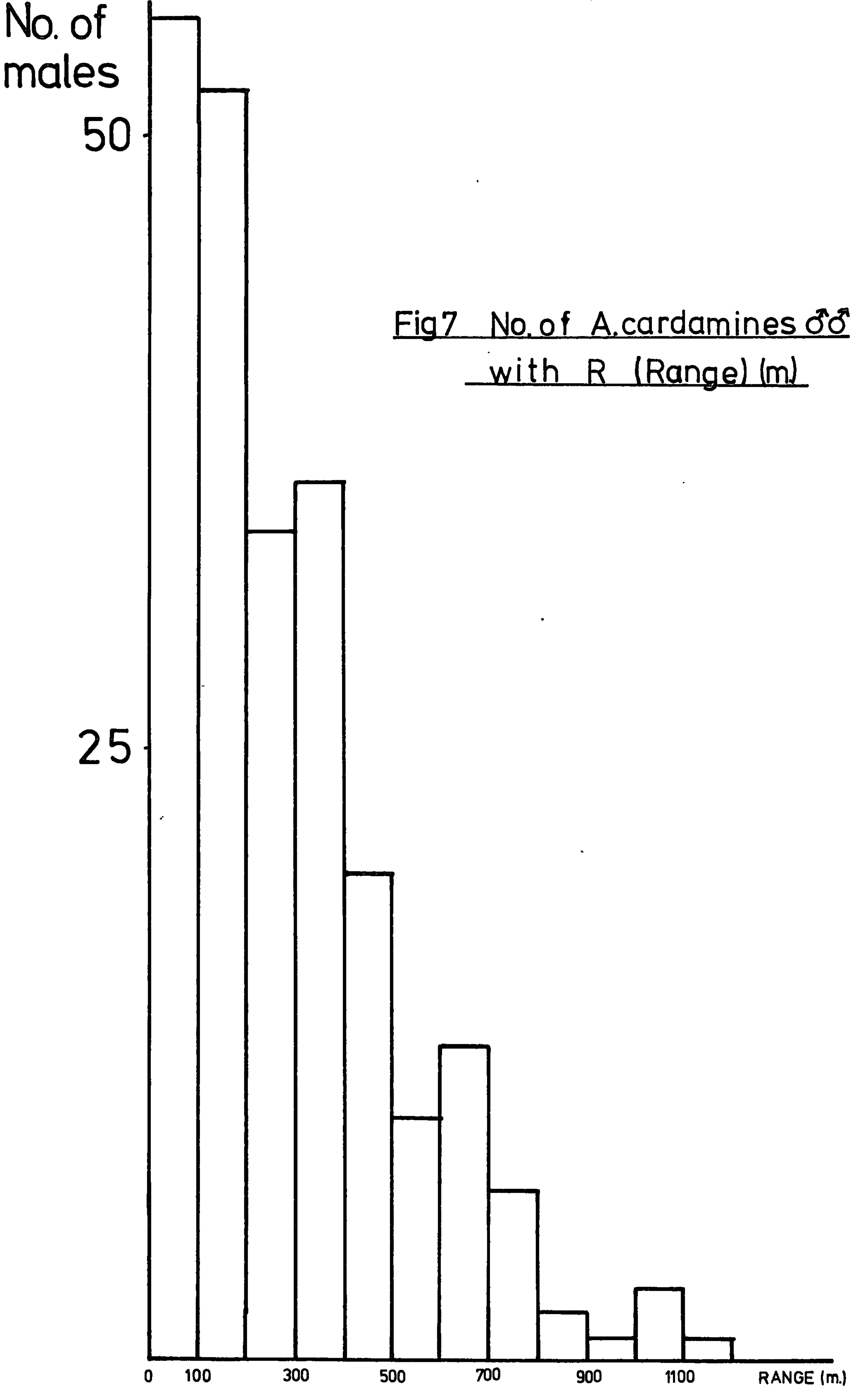

Fig 7 No. of A.cardamines ♂♂ with R (Range) (m)

of utilising the resource by recognising the optimal area). Patrolling a small section of riverbank may also be advantageous to males in allowing them to adopt a 'familiar area' where the animal is aware of the distribution of nectar sources, roosting sites, etc. On the other hand the evolution of a small home range, as appears to be the case in male A.cardamines, would not be expected where the distribution of resources is unequal amongst patches (and the animal unable to gauge a patch's productiveness), since a larger area would always produce some contacts and in a mixed population of 'large movers' and 'small movers', the latter strategists would be gradually eradicated by stochastic variations in success (the mean number of matings for both strategies would be the same, but more nulls would occur for 'small movers'). It may well be that the small movement recorded here is not an evolved characteristic per se, but stems from the tendency of males to stay near good sources of nectar.

c. Population size

The accurate determination of population size is a subject to which considerable attention has been given in ecology, with several alternative methods of calculation from recapture data, being available. Of these the methods of Manley and Parr (1968) and Jolly (1965) are the most widely used, and have the advantages of not assuming constant death rates, giving daily estimates of mortality and other population parameters. These most powerful methods are, however, not appropriate when population

Table 2

Daily Survival Rate (Ø) and Population Size N of *A.cardamines* populations

		Ø	N♂	Est. N♂+♀
A.cardamines	1977	0.799	173	346
	1978	0.884	298	596
	1979	0.866	243	486

Table 3

Daily survival rate (Ø) in other studies

	Ø	Reference
Parnassius phoebus	0.842	Scott, 1973.
Euchloe ausonides	0.835	" "
Maniola jurtina	0.75-0.93	Dowdeswell et al, 1949.
Pararge megera	0.789	Parr et al, 1968.
Coenonympha tullia	0.70	Turner, 1963.
Polyadryus arachue	0.794	Scott, 1973
Lycaena arota	0.763-0.790	" "
L.Xanthoides	0.893-0.932	" "
Polyommatus icarus	0.744	" "
Polyommatus icarus	0.83	Dowdeswell et al, 1940.

size is small, as here. In these circumstances the Fisher-Ford method is most appropriate. This method assumes both age-independence of mortality, and a constant survival rate, although it is robust against small variations in either of these. The method (outlined in Appendix 2) uses date-specific marks, and produces estimates of daily population size, gain and loss.

Table 2 gives the results when applied to the Durham data. The method does not distinguish between the effects of emigration and death or between immigration and emergence. Very little evidence exists for migration between populations. Of 83 animals (47 in 1977, 36 in 1979) taken at the Shincliffe Hall population (which lies only some 500 m upstream from the Durham site) only one was marked. Similarly none of the 40 animals examined at the Durham Sands population in 1979 were marked. Additionally no eggs were found on crucifers within the city and in other areas away from the populations studied - arguing strongly against female emigration from these populations. Thus mortality is ascribed to be the main cause of adult loss and immigration is not believed to be diluting the population to any significant extent.

d. <u>Adult survival</u>

The mean survival of adult males is given in Table 2. There is evidence that female survival exceeds this, the animal using her eggs as an emergency energy source. The range of mean male survival for the three years agrees well with that found for other species of Holarctic butterflies. These include species such as C.tullia, which is colonial, where there is little chance of emigration, and whose mean survival is less than found here. Tropical butterflies typically survive for much longer periods (Gilbert and Singer, 1975).

The causes of adult mortality are largely unknown, as is typical for butterflies. One animal was found to have been killed by a motor vehicle (in 1977). Known causes of death include bird predation (Knight, 1972) and one case of 37 adults in captivity predated by a carnivorous beetle (Floersheim, 1906). Spiders may take a few animals, but the major cause of morta-lity is probably senescence. The observed life expectancies are not very much smaller than those expected in the lab. One cause that has operated on the Durham population in the past, is deliberate predation by humans - entomologists and children. Collecting ceased in 1976. In sampling for electrophoretic and other studies, animals were either taken from those reared as pupae, from nearby populations (e.g. Shincliffe Hall) or from adults flying at the end of the emergence.

iv. Survival of the immature stages

a. Introduction

The varying patterns of survival at differing stages in the life history of an organism are believed to have important effects upon the evolution of adaptive characteristics. MacArthur and Wilson (1967), Wilbur et al (1974) and others have for instance discussed the importance of selective regimes associated with reproductive effort and survivorship patterns. One aspect of the present study was to describe the population background to fitness by following the survival of eggs, larvae and pupae of the Orange Tip, in order to gain insights into individual fitness and adaptations. Additionally, although many authors have reported investigations into the survival of immature Lepidoptera and have constructed life tables representing these patterns, relatively few have recorded the details of intra-population variability in survival. It was decided therefore to investigate and to identify states associated with differing survival of A.cardamines young stages (these results are reported in later sections). The present section discusses the sources of mortality.

Studies on the survival of Lepidoptera larvae, etc., generally consist of either repeated censusing at intervals during development (e.g. Pyornila, 1976) or the following of cohorts of animals, often individually marked. The latter approach has been particularly common in studies of butterfly species (e.g. Lycaena phlaeas (Dempster, 1971)) where, often, overall densities within the habitat are low, and the larvae remain in a relatively small area. The Orange Tip is an ideal subject for the latter type of study in that host specificity restricts movements of individuals, and often the larva may complete its development upon a single seedhead of a large crucifer such as Apetiolata. The survival of animals was followed at a number of sites during the three years of this study. Early in the season, before female emergence and oviposition, study areas were visited and an area of habitat was selected for mapping.[*1] This took place by exhaustively searching for Cruciferae, and entering details of their location, species and 'size' on a sheet of paper to produce a diagram of every crucifer within a length of riverbank or other habitat. 'Size' of crucifer was indexed by the number of flowerheads - that is the number of potential oviposition sites. A copy of this diagram was carried on every subsequent visit and amendments were made as necessary (e.g. by the emergence of new plants or the budding of new flowerheads).

In this way a map of part of a particular habitat was built up. As soon as female emergence commenced, the study sites were re-visited and every crucifer inflorescence examined for the presence of A.cardamines eggs. This species

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[*1] As far as possible the same areas were chosen from year to year. However, extensive subsidence of riverbanks at Durham caused the loss of two of four mapped areas in 1977-8 and the extensive modification of a third in 1978-9. Similarly, at Croxdale in 1978, logging and clearance destroyed part of the study area whilst actually under investigation. To compensate for these events, additional patches were chosen where necessary.

of butterfly provides an opportunity not found in any other yet studied, that of finding every egg within an area.[*1] The red stage of the egg which may last up to a week is readily detectable and, since every oviposition site is examined, there is no danger of missing a single ovum (care must be paid not to miss the earlier white stage however). The eggs were recorded on photocopies of the crucifer map and the visits were repeated as often as possible, at least every six days, so that all subsequent ovipositions were recorded and complete maps of the distributions of eggs over crucifers were possible. In addition, on every visit all crucifers known to have previously borne an egg or larva were examined. If the animal was still present, it was recorded together with its size or instar (based on colour, length and headcapsule width) and any details of its behaviour (for instance, large larvae moving down to feed on the leaves). If, however, the animal was no longer present and no clue as to its survival (or death) such as egg case or exuviae, was found on the plant or underneath, every surrounding crucifer was searched. If the animal was still undiscovered (as was almost invariably the case) then it was marked down as a 'disappearance'. On subsequent visits the crucifer was examined again; occasionally the animal had

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[*1] Other studies on butterfly cohorts use one of two methods for detecting eggs; either (1) searching within quadrat for any visible egg or larva - this is usually unproductive at low densities (e.g. Thecla pruni (Thomas, 1974), or (2) following individual females and marking oviposition sites. This is very laborious and may lead to considerable bias if individual females differ in hostplant choice.

reappeared (usually when first instar larvae were feeding within inflorescences). If the animal was not subsequently found then it was recorded as dying, and was deemed to have done so in the same stage as when last recorded. This procedure which follows that of Dempster (1971a) leads to a slight bias in the data obtained, since it is possible that between visits an animal may develop to a further stage before dying. This is almost certainly the case in several instances of both egg and first instar death of the present study. However, provided that visits are frequent relative to the development of the animal (as here) this inaccuracy does not become a major problem.[*1] Other information noted was the presence of potential predators (ants, sawflies, spiders) or competitors (Molluscs and other Lepidoptera) together with comments on the condition of the foodplant (e.g. dry and dessicated, dying or infected by fungal hyphae). In this way details were obtained of the survival of individuals both within particular circumstances and within the population. The survivorship data from the sites at Durham in 1977-79 and from Witton, Wolsingham and Croxdale in 1978-79, are summed in Fig. 8 and Appendix 3. This represents the survival of 1,798 eggs. The survival of animals at different sites and in different conditions are discussed in later sections.

[*1] Some animals were killed by humans - trampling, picking, etc. In handling such data the procedure followed in constructing life tables was to include the animal as surviving its penultimate stage, but not as entering the stage of its death.

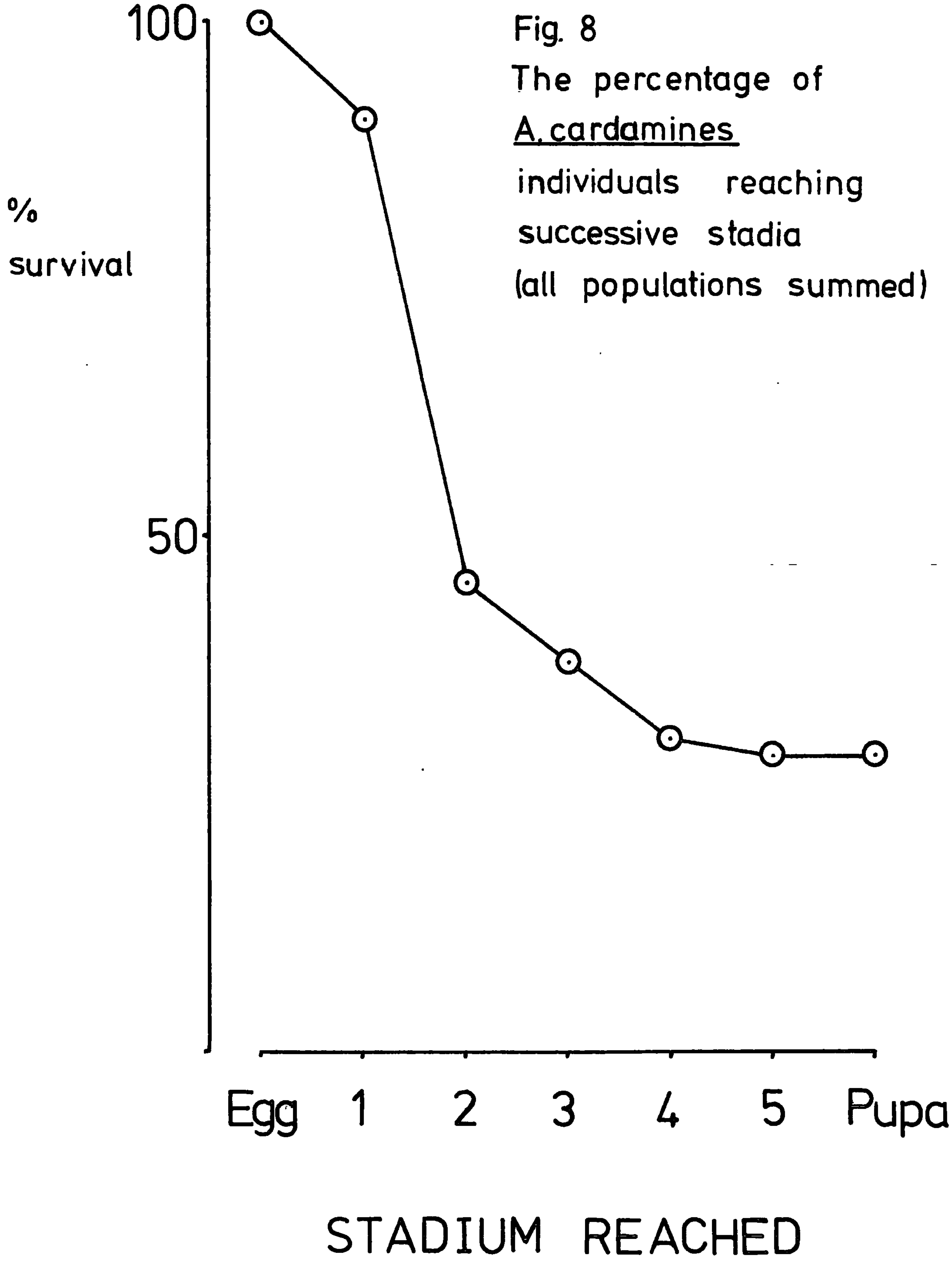


Fig. 8
The percentage of
<u>A. cardamines</u>
individuals reaching
successive stadia
(all populations summed)

The general form of the survival curve (Fig. 8) is similar to that recorded for other Palaearctic species of butterfly. In *Papilio machaon* (Dempster et al, 1976), *A.rapae* (Richards, 1940; Hasni, 1977) *L.phlaeas* (Dempster, 1971a) and in the moth *Tyria jacobeaea* L. (Dempster, 1971b) few eggs die, but this is followed by heavy mortality in the early instars. In all species mortality is less in later instars, but it should be noted that both for these species and the Orange Tip, parasitism is not included.

b. Egg mortality

Of 1,798 eggs, 173 are recorded as dying before reaching the first instar. As noted above, this is certainly an over-estimate. The causes of mortality are given in Table 4.

Table 4:

Source of mortality	Number killed
Dessication	16
Philaenus sp	2
Failed to hatch	1
Trichogramma sp	2
Cannibalism	15
Cannibalism?	85
Unknown	46
Drowning	6
	173

A frequent cause of death was 'dessication'. These were eggs that were laid, coloured up and showed every sign of developing normally but were later found to have collapsed internally. These were in every way similar to eggs which sometimes died in the lab. in conditions of heat stress. Richards (1940) recorded that temperatures above 30°C caused dessication of many *A.rapae* eggs, and it may be significant that in A.cardamines dessication appeared to occur only towards the end of the oviposition period. One egg was recorded as failing and was presumed infertile, or imperfectly developed. Low infertility is typical of butterflies, e.g. A.rapae (Harcourt, 1966).

Two eggs were seen to blacken up in the manner symptomatic of Trichogramma parasitisation (the parasites escaped). A low level of egg parasitisation is usual for most butterfly species, although some Lycaenids, with overwintering egg stages, may suffer greater losses - e.g. Plebejus icariodes Bds. (Downey, 1962) and Quercusia quercus L. (Thomas, 1976). Two eggs were, subsequent to laying, enclosed in the secretion of spittle-bugs. Neither developed beyond the pale red stage. Eggs lost to cannibalism are distinctive in being partially eaten, whilst the category of cannibalism? covers those eggs which disappeared whilst on the same flowerhead as other eggs or larvae. This includes cases of probable cannibalism, but some of such losses were not so incurred, and should properly be included in the 'unknown' category. These were eggs which disappeared without trace, with no larvae nearby. Possible causes for this mortality include invertebrate predation and the dislodging of eggs. In one instance an egg was laid on the growing tip of S.officinale and soon fell off (this was not on a study site and is not included above). Dislodging occurs in Thecla pruni and may occur in some A.rapae populations due to rainfall (Dempster, 1967). However, in other A.rapae populations rainfall is believed to have only small effects (Harcourt, 1966), (These studies were, however, carried out in climatic conditions quite unlike those in England) whilst in Lycaena dispar (Duffey, 1968) and T.pruni, earwigs may take many eggs. In A.cardamines, rainfall is held to be unimportant. At Witton Park on the 6th of June, 1979, 13 eggs were mapped immediately prior to a torrential thunderstorm. All were still present after the storm had passed.

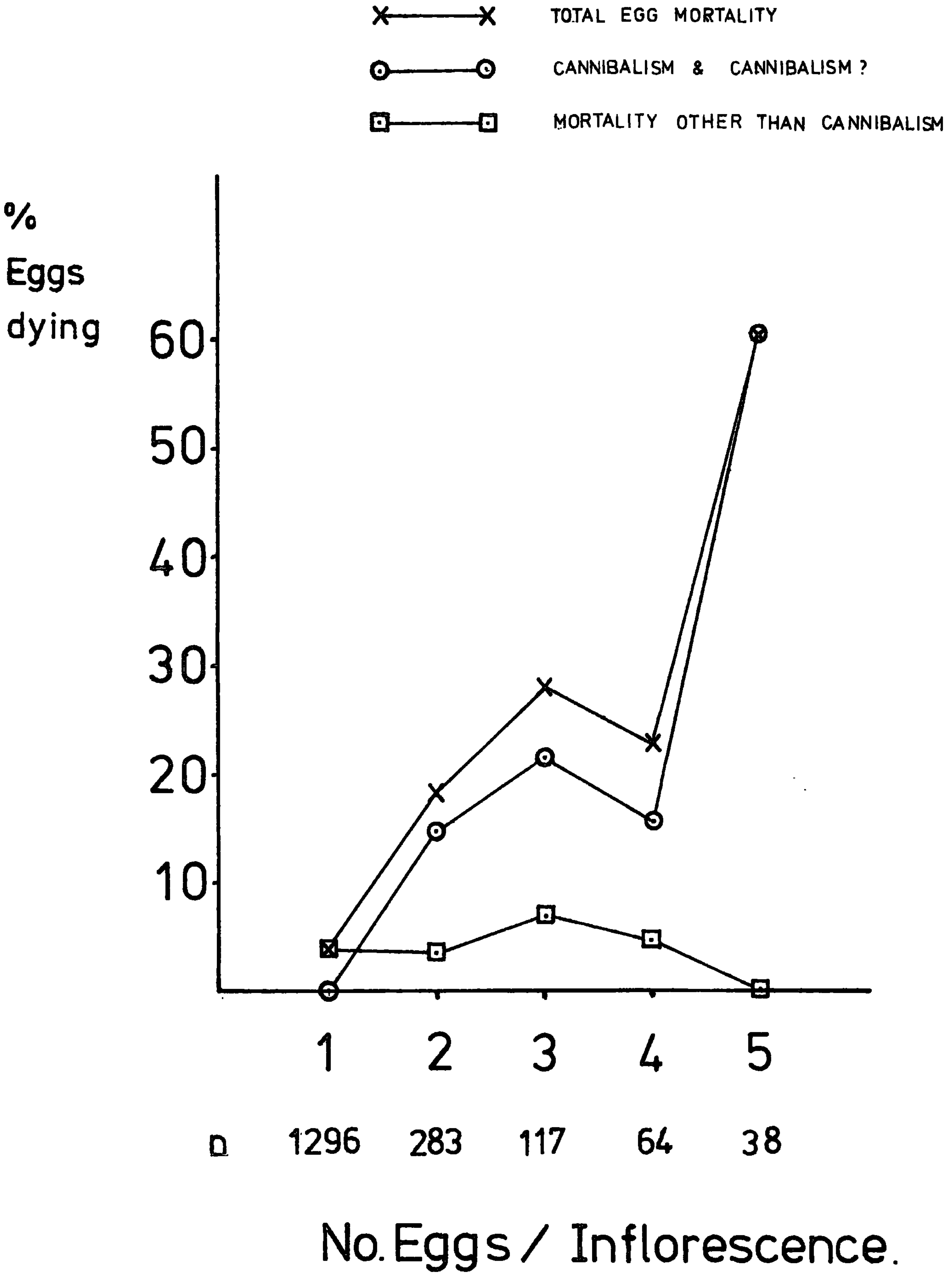
Williams (1915) records one egg killed by having another laid upon it.

Cannibalism is discussed more fully below.

c. Cannibalism

One of the most noted facts concerning A.cardamines is the cannibalism of the early instars. In the populations studied, cannibalism was occasionally noted, particularly when several eggs were laid upon a single flowerhead (Fig. 9). Only cannibalism of eggs was recorded, although in the lab. larval cannibalism is known. It seems likely that cannibalism of larvae in the field is relatively rare, where an aggressive larva would be able to overcome only a quiescent opponent (e.g. when preparing to moult). Egg cannibalism is also frequent in A.rapae but not in other Pierini. An interesting comment on this behaviour is recorded by Gardiner (1974); In discussing P.brassicae he records how the first larva hatching from a batch of eggs eats not only its own eggshell but also the top of those nearby, thus causing a synchronous emergence from the egg

Fig. 9 The effect of egg density upon egg mortality



of this gregarious species. This is strikingly similar to the situation in A.rapae and A.cardamines except that in these cases nearby eggs are destroyed. The young larva eats through the side of its own shell and immediately devours most of the empty case. Should an unhatched egg be in the vicinity, that also is devoured. It seems likely that the differing behaviour of newly hatched P.brassicae, A.rapae and A.cardamines larvae arose from the same ancestral response - the use of the egg case as food by the newly hatched larva. It may well be, therefore, that some instances of 'cannibalism' (perhaps the majority of those in A.rapae) result from 'mistaken identity'. However, once this circumstance has arisen, it may be the precursor for further evolution and the evolution of cannibalism proper, where this is of individual advantage (for instance when larvae are subject to competition for food resources).

It is normally stated that cannibalism is a habit restricted to the early instars (Williams, 1915) (Ford, 1945). In the present study, antagonism was noted occasionally in larvae of later instars, where two animals on the same food-plant would appear to spar with rapid head movements. This sort of conflict might even lead to an animal leaving the foodplant, but must be rare in the field. Inadvertently leaving pupae in a container with larvae led to the discovery that they may also be cannibalised, although this was seldom the case and highly unlikely to occur in the wild. Nevertheless, the behaviour is of interest since it suggests that cannibalism might be linked to an olfactory response.

Cannibalism in A.cardamines usually entails only the killing of an opponent, and actual consumption of the conspecific appears only partial. Is the behaviour spiteful? (sensu Hamilton, 1970). That is, has the behaviour evolved under individual advantage or by inclusive fitness? A 'spiteful' gene hypothetically comes to dominate a population not by increasing the success of individuals carrying it, but by decreasing the proportion of non-cannibals. Several conditions are favourable to the possible evolution of spite in A.cardamines. The over-dispersion of eggs by females, and particularly the habit of laying one egg per visit means that competition between siblings will be less frequent than expected from a random distribution. Since most competitors will not be closely related, antagonistic larvae will not be selected against by kin selection.

The fragmented nature of demes of A.cardamines in northern Britain, and the small effective population sizes also favour random genetic events (section 2.viii) and the rapid fixation of favourable traits. This arises due to the initial advantage of a spiteful gene in a large population being small, since spiteful individuals are rare and few non-spiteful individuals killed. In small populations, each individual contributes a greater proportion of the next generation; thus spiteful genes will come to dominate the population faster, and may be helped by random fluctuations in initial gene frequencies. It is perhaps significant that A.mannii, a sibling-species of the 'accidental' cannibal A.rapae (Powell, 1908), and an Australasian Eurema sp. (Jones, pers.comm.) are both highly cannibalistic and highly localised butterflies.

However cannibalism may have evolved under 'normal' individual selection, if it favours individuals which as larvae might otherwise have succumbed to food shortage due to depletion by other individuals. The killing of pupae would then become understandable as an 'accidental' outcome of olfactory stimuli which normally release killing of eggs or larvae. Thus it is not necessary to invoke 'spite' to explain A.cardamines cannibalism. 'Spite' remains an as yet unconfirmed principle.

d. Larval mortality

Most mortality of immature A.cardamines occurs in the larval stages and is particularly heavy in the early instars. This pattern is common to many other species of Lepidoptera and is normally ascribed to losses of small vulnerable larvae to invertebrate predators; for instance in studies of A.rapae by Richards (1940), Dempster (1967) and Harcourt (1966). Rainfall may also have an effect by drowning or dislodging small larvae of some species. In the present study little evidence was found for the operation of any of these factors. Only one instance was seen of predation - in 1978 at Durham when a larva of the DunBar Moth (Cosmia trapezina L.) was seen to kill a third instar larva. Spiders, ants and sawflies were all common upon the foodplants, but no evidence was ever found of interactions with these groups. (Spider attacks leave distinctively damaged larvae in A.rapae and L.phlaeas). Wiklund and Ahrberg (1978) record only two instances of invertebrate predation - the loss of late instar larvae to predatory bugs. Similarly, periods of heavy rainfall were not associated with increased larval death.

There is evidence to suggest that the larval foodplants might be responsible for substantial mortality. Upon hatching, the tiny larva is faced with penetrating the defences, mechanical and biochemical, of the crucifer. In fact, a great many fail to establish themselves. Comparisons of larval survival upon different foodplants (Section 2.v) indicate significant and consistent differences at this point, strongly suggesting that the foodplants themselves are strongly influencing larval survival. Support for this deduction comes from recent studies on A.rapae (Jones and Ives, in press) and on the chrysomelid beetle Phyllotreta nemorum (Nielsen, 1978) which attack the leaves and seedpods of crucifers respectively. In both species the success of the larva upon hatching, in penetrating at once the plants defences, was critical to larval survival. Similarly, in Papilio machaon, in laboratory culture (where predators are excluded) larvae dying on unsuitable foodplants did so predominately in the first instar (Wiklund, 1975). An identical view has independently been reached for Swedish populations of A.cardamines where, again, initial larval penetration is important, survival thereafter being relatively unaffected by the foodplant (Wiklund and Ahrberg, 1978).

One source of larval mortality also related to the foodplant was 'drowning'. On several occasions at Durham in 1977 and at Croxdale in 1977 and 1978, crucifers collapsed under their own weight and, being situated on the banks of streams or rivers, submerged their seedheads and any caterpillars attached. Larvae were not killed directly by immersion, and on rescue usually survived (Lycaena dispar larvae can withstand long periods of winter floods). However, when submerged,larvae ceased moving and feeding and must normally have died rapidly. In all, 38 eggs and larvae were lost in this way.

Relatively few larvae were lost in the last two instars (36; 7·3% of those entering the 4th instar). In A.rapae (Dempster, 1967; Baker, 1970) and A.napi (Lees and Archer, 1974) this loss was demonstrated to be due to bird predation.[*1] The following species of potential bird predators occurred at the study sites.

Grasshopper Warbler	Locustella naevita
Sedge Warbler	Acrocephalus schoenobaenus
Garden Warbler	Sylvia borin
Whitethroat	S.communis
Lesser Whitethroat	S.curruca
Willow warbler	Phylloscopus trochilis
Dunnock	Prunella modularis
Robin	Erithacus rubecula

[*1] Bird predation may also be important in other species - for instance in Ladoga camilla it has been suggested to act as the key factor determining population sizes and changes (Pollard, 1979).

All these insectiverous birds could be predators but in fact only one of them was seen to search in the micro-habitat which included A.petiolata and A.cardamines - The Sedge Warbler. This species is known to be a major predator of P.machaon larvae (Dempster et al, 1976) and it is significant that the only site where late instar mortality was high (Durham A in 1977. 46% loss) was also the only site included in a Sedge Warbler territory. After 1977 abundance of these birds fell, frustrating efforts to link predation by this species with A.cardamines loss. An experiment was conducted akin to those of Baker, and of Lees and Archer where larvae were reared in captivity until the 4th instar and then placed upon foodplants in the field. Half of these were protected from bird but not invertebrate predation by a bag of netting. The other half (20 in each group) were left unprotected. Survival was better in the unprotected group (19 survived 6 days) than in the protected group (17 survived) and the experiment was not repeated.

A priori one would not expect heavy bird predation upon A.cardamines larvae - the larvae are at low densities compared to the semi-natural populations studied by Baker, and the artificial ones of Lees and Archer. Birds are only likely to invest time searching for such prey at a relatively high density or in their normal habitat. The major bird predators on suburban A.rapae are House Sparrows (Passer domesticus) and Starlings (Sturnus vulgaris) (pers. ob.) which do not hunt in riverside areas.

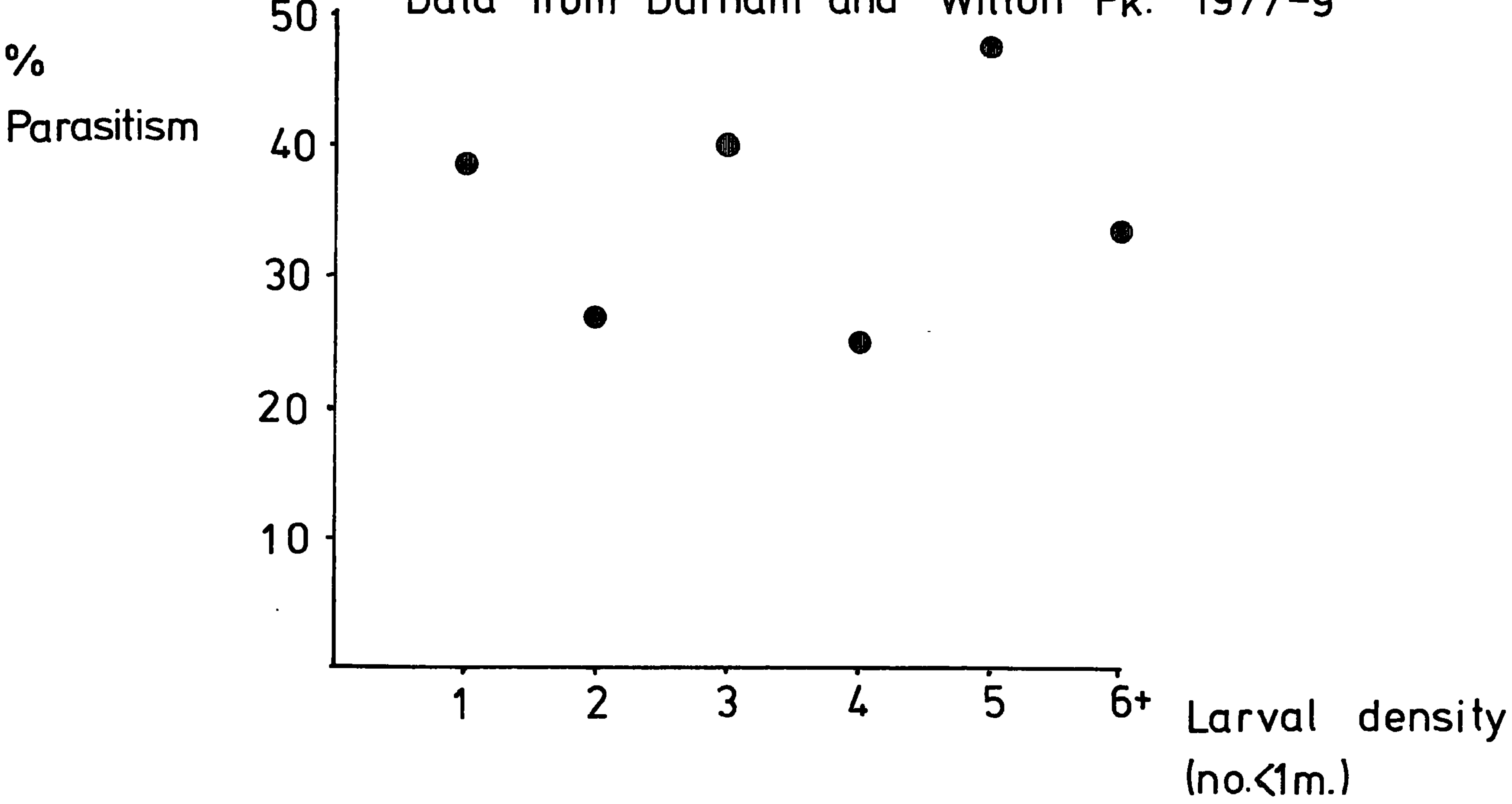
A major source of mortality in mature larvae is parasitisation. Only one species of parasite was reared in the present study, the Tachinid Phryxe vulgaris. This species lays its eggs directly onto the host and attacks from the 3rd instar onwards in A.cardamines. This species of Tachinid also attacks other butterflies - e.g. L.phlaeas, (Dempster, 1971a), L.dispar (Duffey, 1968), Lasiommata magera L. (Richards, 1940), Aglais urticae (Pyornila, 1976) and A.rapae - and is known from many other Lepidoptera as well. It attacks only the late instars in A.rapae (Richards, 1940; Harcourt, 1966) and Aporia crataegi L. (Stellwag, 1924). The parasite develops within the larval tissue and usually emerges after pupation, although occasionally the maggot may emerge from a prepupa (in this case the maggot is usually small and dies). The parasite emerges either within days of the pupation of its host or lies over until the following spring, emerging soon after the removal of pupae from the refrigerator. The parasite therefore does not seem to spend its first brood in A.cardamines and is either bi- or tri-voltine. The incidence of parasitisation at the study sites is given in Table 5.

Table 5

Locality and Date		N	Overall larval density, larvae/ m of habitat	Overall % parasitisation
Durham	1977	76	2.68	38.2
	1978	29	1.35	44.8
	1979	46	1.99	43.5
Croxdale	1978	27	0.91	11.1
	1979	26	1.92	15.4
Witton Park	1978	24	1.72	16.7
	1979	53	2.94	41.5
Wolsingham	1978	42	1.15	4.8
	1979	79	3.03	7.6
Barnard Castle	1978	5	-	20
Egglestone	1978	8	-	0
Alston	1977	12	-	0
	1978	9	-	0
	1979	12	-	0

It may be seen that loss to *Phryxe* parasitisation was consistently heavy at Durham, but varied in intensity at other sites. This may reflect habitat differences between sites, the presence of alternative hosts, the recent origin of some *A.cardamines* populations or other factors. Wiklund and Ahrberg (1978) note that parasitisation is low on plants of marshy habitats - as is the case at Wolsingham. However, variation in loss may also reflect broader habitat preference of the parasite, perhaps linked to the presence of alternative hosts (as in the parasites of *Coleophora alticolella* - Randall in prep). The absence of parasitisation in upland sites may reflect this (the sites also support large numbers of *A.napi*, a species which is very little parasitised, Lees, pers comm.) Alternatively it may reflect the fact that these populations are recently derived - the parasite may not have had sufficient time to find these new colonies. However, this is not a plausible explanation for variation among the main sites. Similarly there is no evidence to suggest that the differing parasitisation rates are a result of overall larval densities, although it is known that *Phryxe vulgaris* responds to increasing densities of *A.rapae* by increasing searching effort and attack rate in some populations (Harcourt, 1966) but not others (Richards, 1940). Nor was any evidence found for the direct effect of larval aggregation or density upon parasitisation (Fig. 10). Other Tachinids do show increased attack rate with increasing host density, e.g. *N.anoena* on *Panolis flammea* (Gurjanova, 1977).

Fig. 10 The effect of <u>A.cardamines</u> larval density upon parasitism by <u>Phryxe vulgaris</u>.
(Density is taken as the number of larvae within 1m. of the 2nd or later instars)
Data from Durham and Witton Pk. 1977-9



The present study records P.vulgaris as the only parasite of A.cardamines. This was also the case in Berkshire in 1979 (Waage, pers comm). In the past another Tachinid has been identified as the sole parasite of some British populations - Exorista vulgaris, another polyphagous species (Williams 1915). In the summers of 1905 and 1906, this species caused particularly heavy losses. Similarly, in A.sara, Tachinids are the major parasites. These situations contrast markedly with that of A.cardamines in Sweden, where no Tachinid parasites were observed but instead the Braconid Apanteles saltator, which may cause very heavy losses. Pyornila (1976) has argued that P.vulgaris is inferior in competition with Braconids when attacking the host A.urticae and is excluded. Phryxe vulgaris is also inferior to Apanteles spp. in A.rapae (Richards, 1940). In A.rapae, Phryxe and Apanteles spp seem to attack different populations (Harcourt, 1966; Parker, 1970). Again, around Durham, suburban populations of A.rapae and P.brassicae are attacked by Braconids, populations near riverbanks only by Tachinids. It is apparent that inter-relations between parasites, hosts and habitats are exceedingly complex.*1

Additional late instar death was known to have been caused by disease. The most frequent affliction was bacterial attack, the symptoms of which are a distinctive blackening of the larva, first as a small spot on the skin

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*1 Audcent (1942) in his review of the hosts of parasitic Diptera records only Phryxe vulgaris from A.cardamines. In their similar review of the hosts of parasitic Hymenoptera, Morley and Rait-Smith (1933) do not have any record for A.cardamines.

but later all over, and an increasing sluggishness of the animals.[*1] The bacterium enters on the ingested food material and attacks through the gut wall (Atwa and Abdel-Rahman, 1974) and may be important in controlling outbreaks of the pests A.rapae and Thymelicus sylvestris (Arthur and Angus, 1965). Granulosis virus also attacks Pierids and may cause substantial mortality, particularly when the animal is under stress (David et al, 1972). Viral attack was suspected in several epidemics in the laboratory, but no field evidence for its attacking A.cardamines was found. Like parasitoids, granulosis viruses are only moderately specific, and strains from one Pierid species will infect another (Payne, pers. comm).

In 1979 siliquae and stems of A.petiolata at Croxdale and, to a lesser extent, Witton Park, were infected by hyphae of a white oomycete fungus.[*2] The fungus caused rapid deterioration and drying of the siliquae and some 4th and 5th instar larvae disappeared. These may merely have left the foodplant in search of better quality food; however, at least one was killed by the fungal hyphae growing through from its midgut. Other larvae ate infected siliquae without ill effect. A.rapae is resistent to some entomophilous fungae (Puttler et al, 1976).

3 larvae failed to pupate properly.

Larval survival was not related to density (Appendix 4).

[*1] Diagnosed by C. Payne of the Glasshouse Crops Research Institute.

[*2] Identified by G. Banbury of the Department of Botany, Durham.

e. Pupal mortality

Deaths of pupae in the laboratory were generally caused by disease or parasitism from the larval stage. However, 5 were lost because of problems of eclosion and others died from being kept in improper conditions of high humidity. The former losses are probably due to natural malfunctions and most studies show a few such losses.

Pupal mortality in the field was less easy to examine. In the autumns of 1977 and 1978, batches of pupae were put out on the riverbanks of the Wear. These were animals which had been allowed to pupate on the stems of A.petiolata, which were then fixed to other vegetation in the field. Unfortunately, in both years all such pupae were lost (20 in 1977-78, 17 in 1978-79). Survival was followed until inundation by the rising river water in November/December, but thereafter none was found, despite careful mapping and marking of the plants - prior to inundation, only one pupa is known to have disappeared - in 1978 when the whole A.petiolata plant disappeared, suggestive of human interference. Following these failures, and in order to look for pupal parasitoids, a further 23 pupae were put out in August, 1979. All were retrieved in early October - none were parasitised and all bar one emerged successfully.

Little success was thus gained in estimating or identifying the causes of pupal mortality. In other species of Pierid, pupal losses occur due to parasitoids (Hasni, 1977) and to predation, especially by birds.

f. Interspecific competition

Competitive interactions with other species are important in the evolution of niche occupation. Well documented studies on 'competitive release' (e.g. Lack, 1971) have shown that species are often prevented from exploiting the full and potential range of habitats, food items, etc., by the presence of another species which excludes it from those resources. In particular, competition between closely related species has been felt to be important in such situations - this view was expressed by Darwin in the 'origin of Species' and was particularly favoured by Elton (1946). If one favours an 'ecological' species concept then this process of competition and evolution amongst very closely related groups might be considered an integral part of the speciation process.

During fieldwork on the mapped sites and elsewhere, note was taken of other species feeding upon Cruciferae. These included the crucifer specialist *Plutella maculipennis* (the Diamond-back moth) at low-levels in 1977, with *Cepaea sp.* and *Limax sp.* at Croxdale in 1979. These three species were found exclusively upon the leaves of *A.petiolata* and were not observed to interact with *A.cardamines* at all. Various sawfly larvae and many spittlebugs (*Philaenus spumarius* L.) occupied a number of crucifer inflorescences, but evidence was not found of direct antagonistic competition apart from the two eggs destroyed by spittle (q.v.); rather *A.cardamines* females appeared to avoid laying eggs on those inflorescences (invariably the terminal ones) occupied by a competitor. This was 'interference' competition rather than 'exploitation' (*sensu* Miller, 1967).

Three other species of insect were frequently found upon crucifers. *A.napi* occurred generally upon *A.petiolata*, *B.vulgaris*, *H.matronalis* and *C.amara*, *A.rapae* upon *A.petiolata*, *B.vulgaris* and *B.rapa*, whilst a chrysomelid beetle (*Phyllotreta cruciferae* Goez.) caused heavy defoliation of *C.amara* at Wolsingham in all three years. These species ordinarily did not interact with *A.cardamines*. However, when *A.cardamines* larvae have consumed all the siliquae of some plants (*C.amara* and *B.vulgaris*) or the ageing of the foodplant causes siliquae to be unattractive (*A.petiolata*, *Barbarea vulgaris*, *H.matronalis* but not *B.rapa*) the larvae may attack the plant stem or leaves (particularly of *A.petiolata*). In these circumstances the leaf-feeders may be competitors of the Orange Tip. Thus at Wolsingham in 1979, three *A.cardamines* larvae ate out the siliquae of *C.amara*, descended the plant, found the leaves already consumed by *A.napi* (others moved down on plants grazed by chrysomelids but this was rarely complete) and were forced to move to new plants where they completed development. Similarly at Wolsingham in 1979, a single plant of *B.vulgaris* supported 38 *A.cardamines* larvae, 3 *A.napi* and 7 *A.rapae* larvae. Two *A.cardamines* larvae moved down to feed on the leaves, whilst two *A.napi* and three *A.rapae* moved up from the diminished leaves onto the seeds. Again in 1978 an *A.rapae* larva moved up onto the seeds of a *B.vulgaris* plant where two *A.cardamines* larvae were feeding. These were the only observed cases of competition; more frequently both A.cardamines and the Artogeia species were able to complete development without interacting. (It was not possible, however, to study Artogeia populations rigorously).

In order to investigate the behavioural response of larvae to interspecific contacts, 3rd instar larvae of A.cardamines were placed in individual containers in which a 1st or 2nd instar larva of A.napi had also been placed (15 trials in all). Also in each container were placed one leaf and one siliqua of A.petiolata. In this way the preferred food of either species was present, but placed in such a way that encounters were frequent. Young larvae of A.napi were chosen so as to give an advantage to the larger cannibalistic A.cardamines, which were at a size where they might have moved down onto A.petiolata leaves in the field. Interest was centred on the possibility of aggressive encounters between species but none was seen. Each species grazed solely on its normal food and encounters were uneventful. From these experiments and field observations it is apparent that competition between these two species is of the 'exploitation' rather than 'interference' type and is not antagonistic.

Competitive interactions between Pierid species are not limited to the larval stage - if competition for larval food has been a force in the species' evolutionary pasts, then one might expect ovipositing females to lay their eggs in such a way as to minimise such competition. One possibility is that females may respond to the eggs of other species with avoidance behaviour (e.g. Pieris brassicae (Rothschild and

Schoonhoven, 1977)) or by distributing their eggs differentially amongst potential hosts. To test these ideas the distributions of A.cardamines and A.napi eggs were compared at one site (Alston) where the large uniform stand of H.matronalis eliminated variations in plant species, age, etc., which contribute to variation in egg loads. The results are presented in Table 6.

Table 6

Co-occurrence of A.cardamines and A.napi upon plants of H.matronalis at Alston

	27.6.77	22.6.78	2.7.79
No. of plants	616	672	510
No. of plants with Ac eggs	68	72	45
No. of plants with An eggs or larvae	53	24	27
Expected* plants with both species	5.85	2.57	2.38
Observed plants with both species	18	14	19
χ^2_c	5.38	6.65	11.66
	$p<0.05$	$p<0.01$	$p<0.001$

*From combined probabilities of both species, e.g. in 1977, $= \frac{68 \times 53}{616}$

If the two species were distributing their eggs differently, by whatever means, one would expect the observed co-occurrences of both species to be less than that predicted from their independent abundances. In all cases there was a significant excess of such events over that predicted. A.cardamines and A.napi eggs are strongly and positively associated with each other. This arises because of the strong edge - effects in both species (section 2.vii).

One additional observation was that A.cardamines females may sometimes lay their eggs on leaves and stems when there are already other eggs present. Similarly A.cardamines larvae eat out seedheads most rapidly when there are several larvae present; thus situations of intra-specific competition are those in which inter-specific effects are most likely to occur.

Owen (1959) has argued from the observation that British populations of A.cardamines and A.napi use different parts of the same plants to the view that competition between these species has resulted in exclusive niches. This view is made untenable, however, by two sets of facts: firstly, that both species show the behaviour normal to their respective tribes, i.e. that leaf or siliqua feeding is ancestral to the present situation; secondly that the respective habits of the two species are maintained in isolation from the other. However, occasional competitive interactions between species may serve to re-inforce niche selection. Similar situations occur in other species pairs in the Pierinae, e.g. P.protodice and Euchloe hyantis in California (Shapiro, 1975); and P.brassicae and Zegris eupheme, pers.obs. in North Africa. In other Lepidoptera, the situation of Papilio indra (Rkt) and P.rudkini (Cusk.) is strikingly similar to the present case; again competition only occurs between species in conditions of food shortage (Emmel and Emmel, 1969).[*1]

[*1] Grosvenor (1912) records A.napi in the drought year of 1911 attacking A.petiolata seeds.

One further area of interaction between species is the support by Pierinae populations of mutual diseases and parasites. It is known that granulosis viruses are sufficiently general to attack several Pierini. Similarly the Braconid, Apanteles glomeratus is a common parasite of Pierids, and in Japan parasites reared from A.rapae will infect A.napi, although A.melete is immune to its attacks (Sato, 1976). Again in Germany, A.glomeratus populations which attack the pest Pierinid Aporia crataegi are obliged to undergo alternate generations in P.brassicae (Wilbert, 1957). Rothschild (1975) gives a list of many shared parasites of A.rapae and P.brassicae. It is not apparent, however, to what extent the bacterial and parasite populations attacking A.cardamines were maintained upon other Pierinae. No bacterial loss was found in the few A.rapae and A.napi taken in. However, in transplanted larvae three A.rapae (of 33) but no A.napi (of 21) were found to be parasitised at Durham in June-July 1979. The parasite was Phryxe vulgaris, which was also found in one of two A.rapae from Croxdale, but in no other samples. This parasite is also known to attack A.urticae , but was not found when rearing large numbers of this species from Shincliffe and elsewhere in 1978 (other Tachinids and a Braconid killed over 50% of A.urticae). A full investigation of interactions of A.cardamines and P.vulgaris would require detailed population studies of all the parasite's hosts.

v. Relationships of A.cardamines and its foodplants

a. Introduction

The fact that most phytophagous insects are restricted to a narrow range of acceptable foodplant species is hypothesised to be the result of coevolution between the insect and plant species (Brues, 1924). This 'coevolutionary' hypothesis advanced for butterflies by Ehrlich and Raven (1965), has been the subject of much research and the presence of 'allelochemical' substances (sensu Whittaker and Feeny, 1971) has been identified in many plant groups. Thus tannins, cardiac glycosides, alkaloids, cyanogens and other toxic or distasteful substances have been linked in many studies with deterrence or inhibition of feeding in herbivores (Lundgren, 1975; Lukefahr and Martin, 1966; Atsatt and O'Donald, 1976). The activity of insects as grazers is thus held to be the 'raison d'etre', the evolutionary pressure leading to the production of secondary plant substances (Fraenkel, 1959). The counter-adaptations of insects to these deterrents have also been elucidated, e.g. detoxifying enzymes (Krieger et al, 1971) or life-history characteristics (Dixon, 1976).*1 The adoption of monophagic strategies is believed to be the necessary corollary of such specialisations, and has led to further adaptations associated with such a mode of life, particularly in host finding and selection. Frequently it has been found that erstwhile deterrent substances are used by specialist herbivores as location-cues and feeding stimulants. Abundant research has linked

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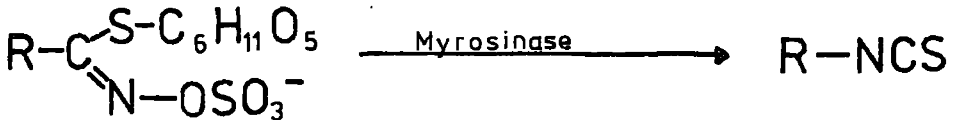
*1 In some instances herbivores have also been shown to sequester such allelochemicals for their own defence - against predation (Rothschild, 1972) or parasitoids (Smith, 1978). Plant defences are not limited to chemical deterrence and may include physical characters (Feeny, 1975) and visual mimicry, etc. (e.g. plants mimicing butterfly eggs - Gilbert, 1975).

monophagic strategies by insects with the presence of allelochemicals (reviewed by Chapman, 1974) and plant-feeding is currently regarded as an evolutionary 'hurdle' which relatively few insect groups have overcome (Southwood, 1973). There are, however, some indications that allelochemicals may not greatly influence the actual number of insects species feeding upon a particular plant species (Lawton, 1978).

Ehrlich and Raven (1965) showed that particular families of butterflies were restricted in their hostplants. The butterflies of the Pierinae are restricted to Cruciferae and Capparidaceae (and a few other isolated instances on other Families), with Holarctic representatives feeding mostly upon the Cruciferae, a plant group that supports a large fauna from many insect groups. All these insects tend to be crucifer specialists. The primary chemical defence of the Cruciferae is known to be the presence of glucosinolates or mustard oils.[*1] The general form of glucosinolates and their derivatives are given below.



$$R{-}C\overset{\displaystyle S{-}C_6H_{11}O_5}{\underset{\displaystyle N{-}OSO_3^-}{}} \xrightarrow{\text{Myrosinase}} R{-}NCS$$

The R-group may be represented by many different groups. More than 70 such glucosinolates are known. The presence of mustard oil glucosides has been shown to have a deterrent effect upon the feeding of non-crucifer feeding insects (Erikson and Feeny, 1974) but in most crucifer specialists these chemicals are feeding stimulants which identify the

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[*1] Some Crucifers have evolved other defences as well as mustard oils, e.g. *Erysimum* has cardenolides, *Iberis* has curcubitains (Nielsen, 1978).

plant as suitable food. Thus animals as diverse as a sawfly (Sehgal and Vjagir, 1977), a fly (Mukherji and Harcourt, 1970), various beetles and weevils (e.g. Free and Williams, 1978), an aphid (Moon, 1967), and aphid parasites (Hymenoptera - Read et al, 1970), all use the presence of mustard oil glucosides as cues for feeding, ovipositing, etc. Indeed the close relation between the biology of Pieris butterflies and that of their hostplants is one of the best worked paradigms of the coevolutionary approach, with research on the group starting with that of Verschaffelt (1910). (Both adults and larvae of Pieris spp are sensitive to glucosinolates).

Different crucifer species have very different glucosinolate profiles, but the effects of differing glucosinolates upon crucivores has been little studied,*1 although studies by Blau et al (1978) suggest that variations in glucosinolate concentration in food has little effect upon the growth of Pierini. Other dietary factors such as nitrogen may be more important (Slansky and Feeny, 1977).

One possible way in which closely related species may avoid competition is specialisation upon differing foodplants. Hicks and Tahvanainen (1974) described the host-plant specificity of cruciferous Chrysomelidae, and showed that little overlap occurred between species. Similarly Ohsaki (1979) showed that some sympatric Japanese populations of

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*1 This forms the long term research aim of F. Chew's group.

Pierini species seemed to select different Cruciferae (albeit with some overlap). Stride and Straatman (1962) have emphasised the importance of colonisation of novel foodplants to possible race formation in Papilionids. Downey and Dunn (1964) and Goodpasture (1974) have observed competitive exclusion and competitive 'release' between related species of Lycaenid on shared plant species. Similarly the recent formation of Pieris cheiranthi from P.brassicae (Kudrna, 1973) is closely linked to the adoption of a new foodplant - Tropaoleum. Again A.virginiensis, derived from A.napi, appears to be facultatively monophagous upon Dentaria spp. and has evolved life history characteristics enabling it to specialise solely on that genus (Hovanitz, 1963). Z.eupheme and other Euchloini seem to be more obligatorily monophagous.

It is thus apparent that speciation and foodplant exploitation may be closely linked. Observations such as those on A.virginiensis and P.cheiranthi, and the studies of Singer (1971) who showed local differences in hostplant selection in Euphydryas editha, all suggest that differences amongst foodplants may be important selective factors. The maintenance of oligophagy[*2] in many Pierinae is thus of

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[*1] This forms the long term research aim of F. Chew's group.

[*2] Wiklund (1974) has distinguished two forms of oligophagy - one in which an animal species may attack all or many related plant species in a habitat ('Polyphagic type') and another form in which the animal throughout its range attacks many species, but which attacks only one or a few in any particular area ('Monophagic' type). Most Pierini are examples of the first type, E.editha of the second.

interest since it seems not only that oligophagy may be adaptive in these species, but also that this situation is dynamic.

In oligophagous Pierini different hostplants are known to differ in suitability for larval growth (Takata, 1961; Dowdeswell and Willcox, 1961; Chew, 1974, 1975) and Chew (1977ab) has discussed several factors which might explain the persistence of the oligophagous habit in North American Pierinae, notably habitat heterogeneity and foodplant unpredictability (but also historical events).

The studies on larval survival and development outlined below were carried out to investigate the maintenance of oligophagy in A.cardamines. By demonstrating the fitness of eggs laid upon differing foodplant species it was hoped to illustrate the relative advantages of different strategies of foodplant exploitation, and to demonstrate the circumstances in which foodplant specialisation might occur. Section 2.v details the effects of the foodplants upon A.cardamines; Section 2.vii relates these results to observed female oviposition and section 2.ix discusses the evolution of foodplant exploitation strategies.

b. Foodplant characteristics and larval survival

Figs. 11 to 17 record the survival of A.cardamines larvae upon differing crucifer species at particular sites and times. Survival appears to vary greatly with foodplant species. Two methods are used for these comparisons - overall larval survival and an Index of survival (Table 7). The index of survival is calculated as the mean number of stadia which are survived. Thus an animal which dies in the 3rd instar

Figs. 11-17. The survival of *A.cardamines* upon different hostplants.

Fig. 11. Durham, 1978.
12. Durham, 1979.
13. Croxdale.
14. Witton Park, 1978.
15. Witton Park, 1979.
16. Wolsingham, 1978.
17. Wolsingham, 1979.

Fig 11 The survival of *A.cardamines* upon different foodplants

Durham 1978

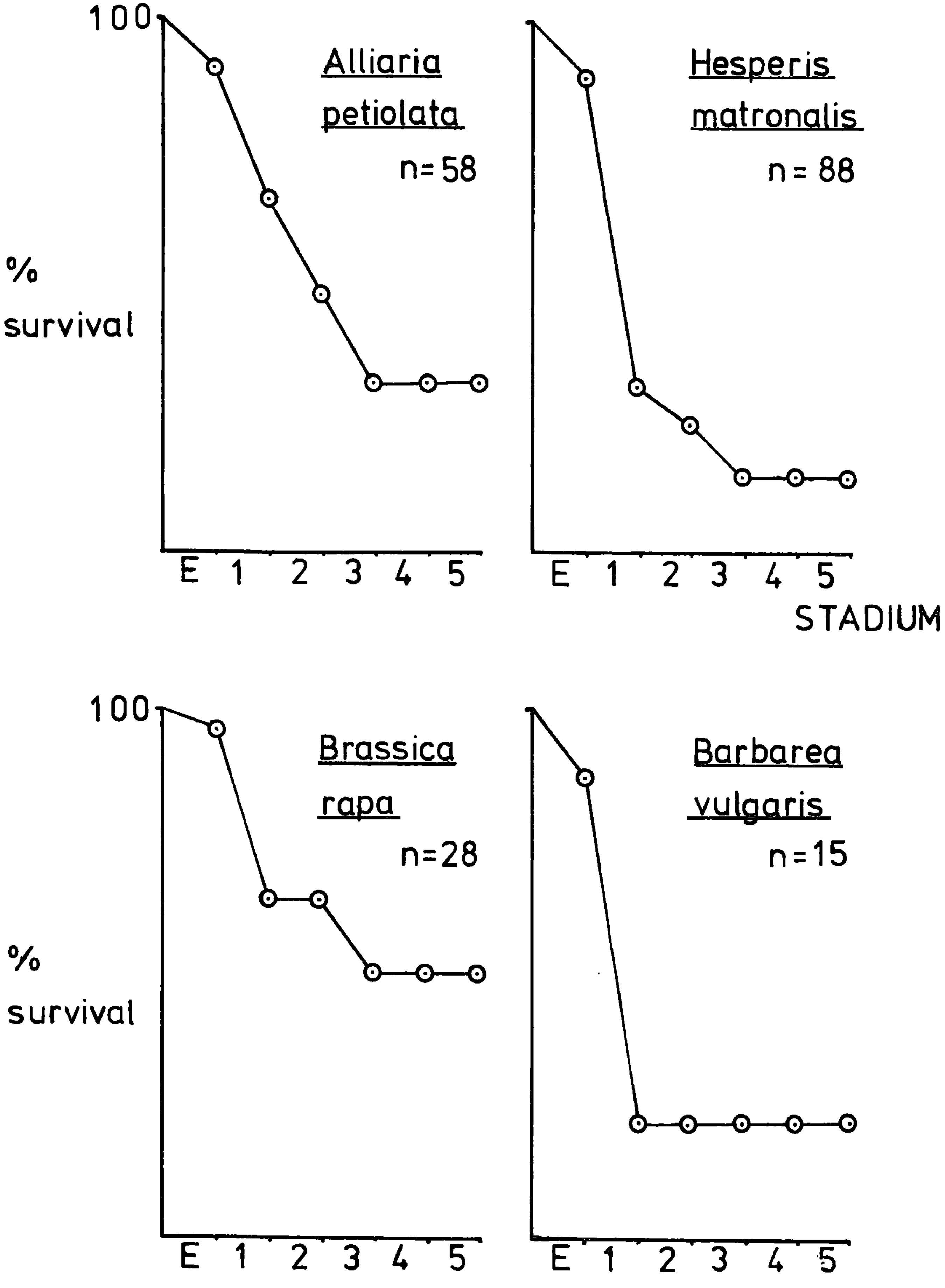

Fig 12 Durham 1979

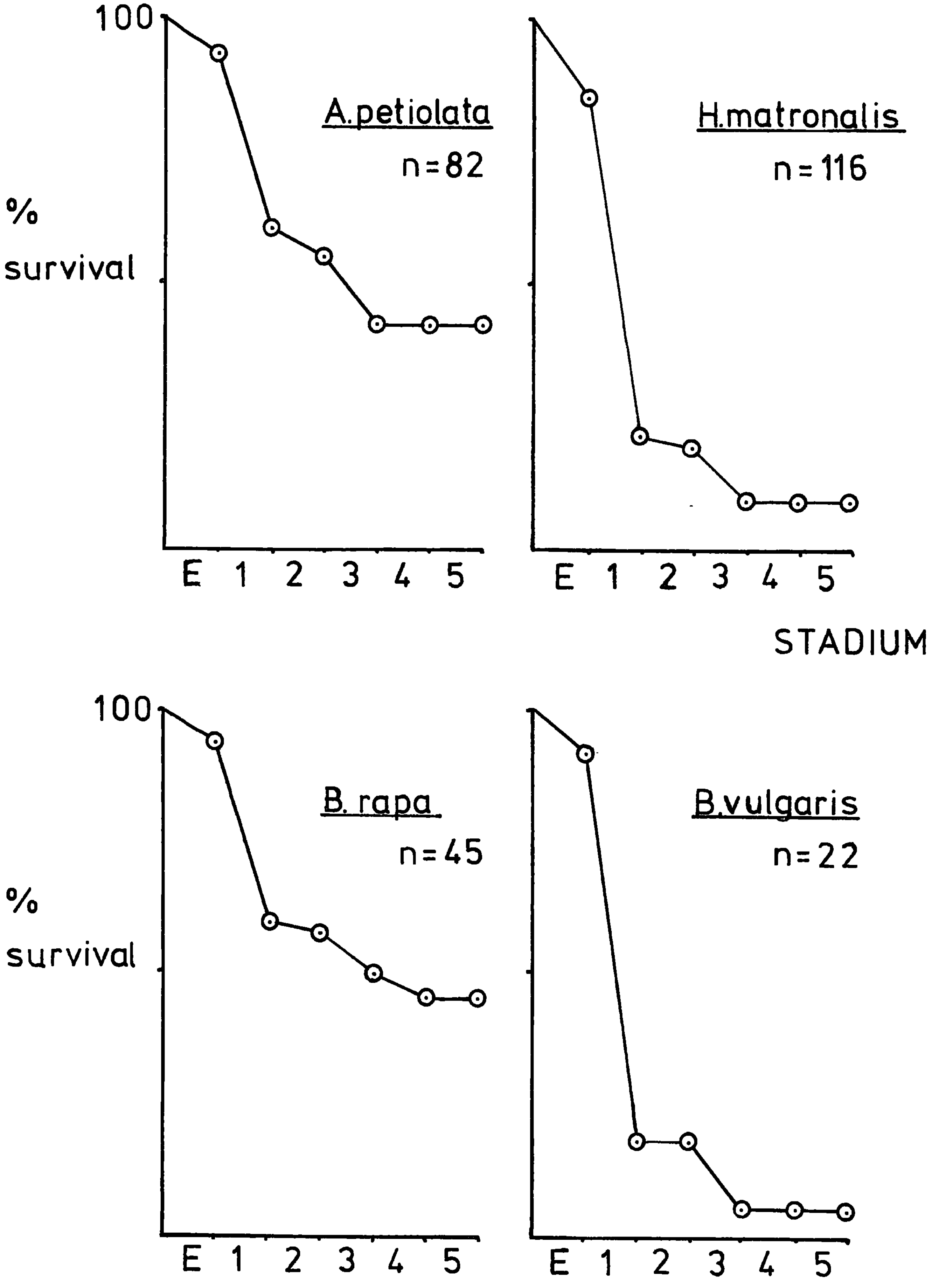

Fig 13 Croxdale

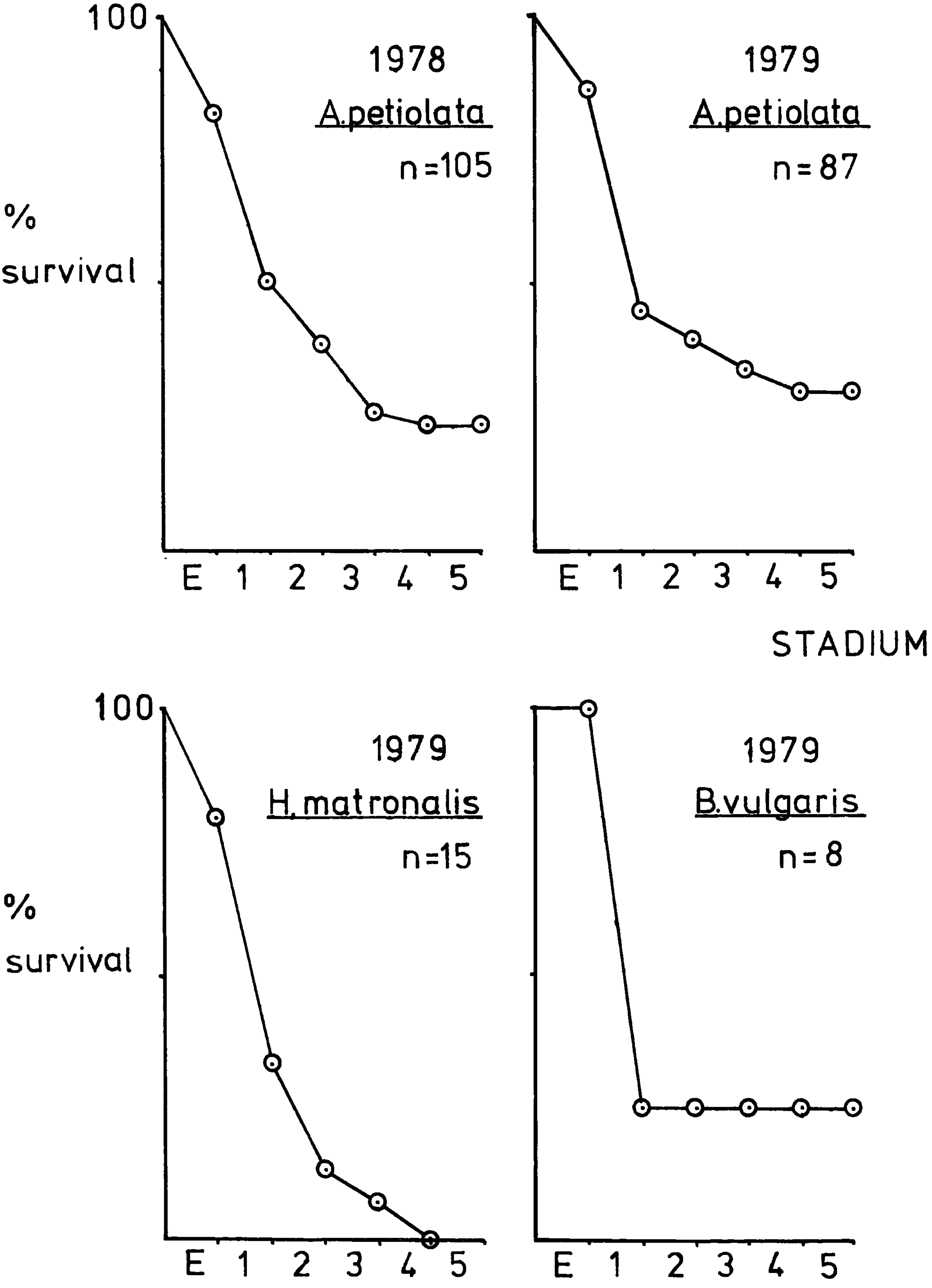

Fig 14 Witton Pk. 1978

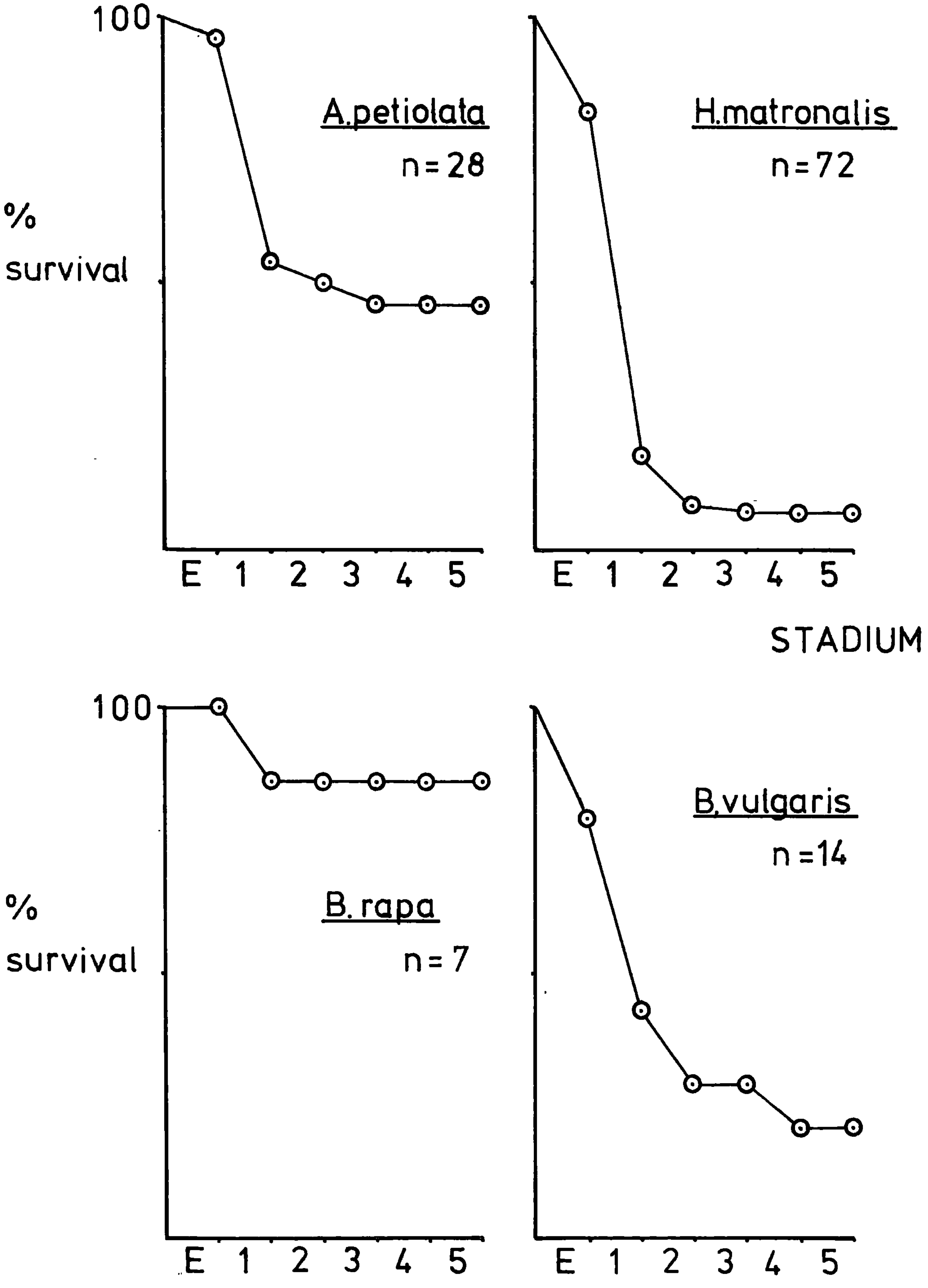

Fig 15 Witton Pk. 1979

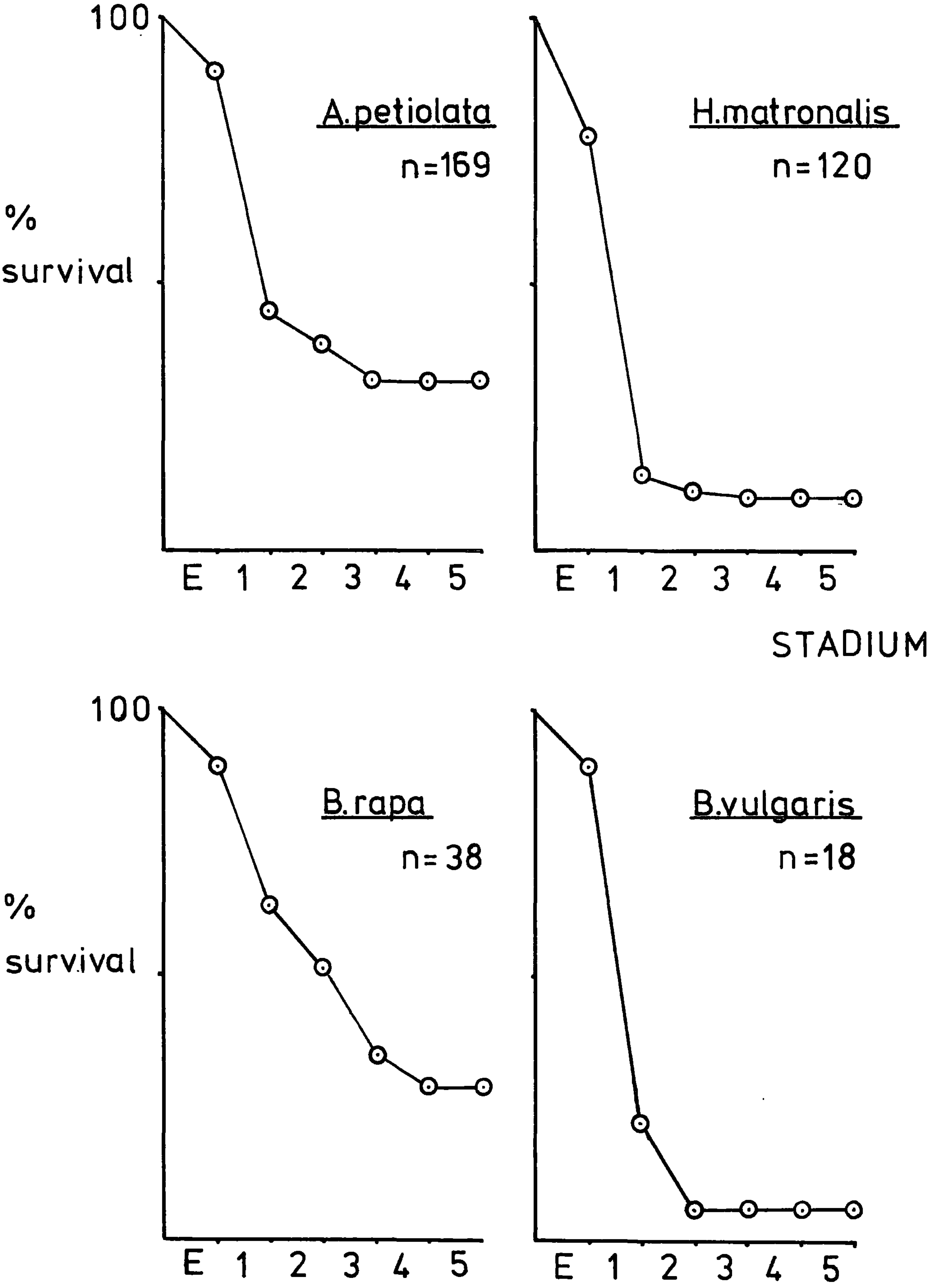

Fig 16 Wolsingham 1978

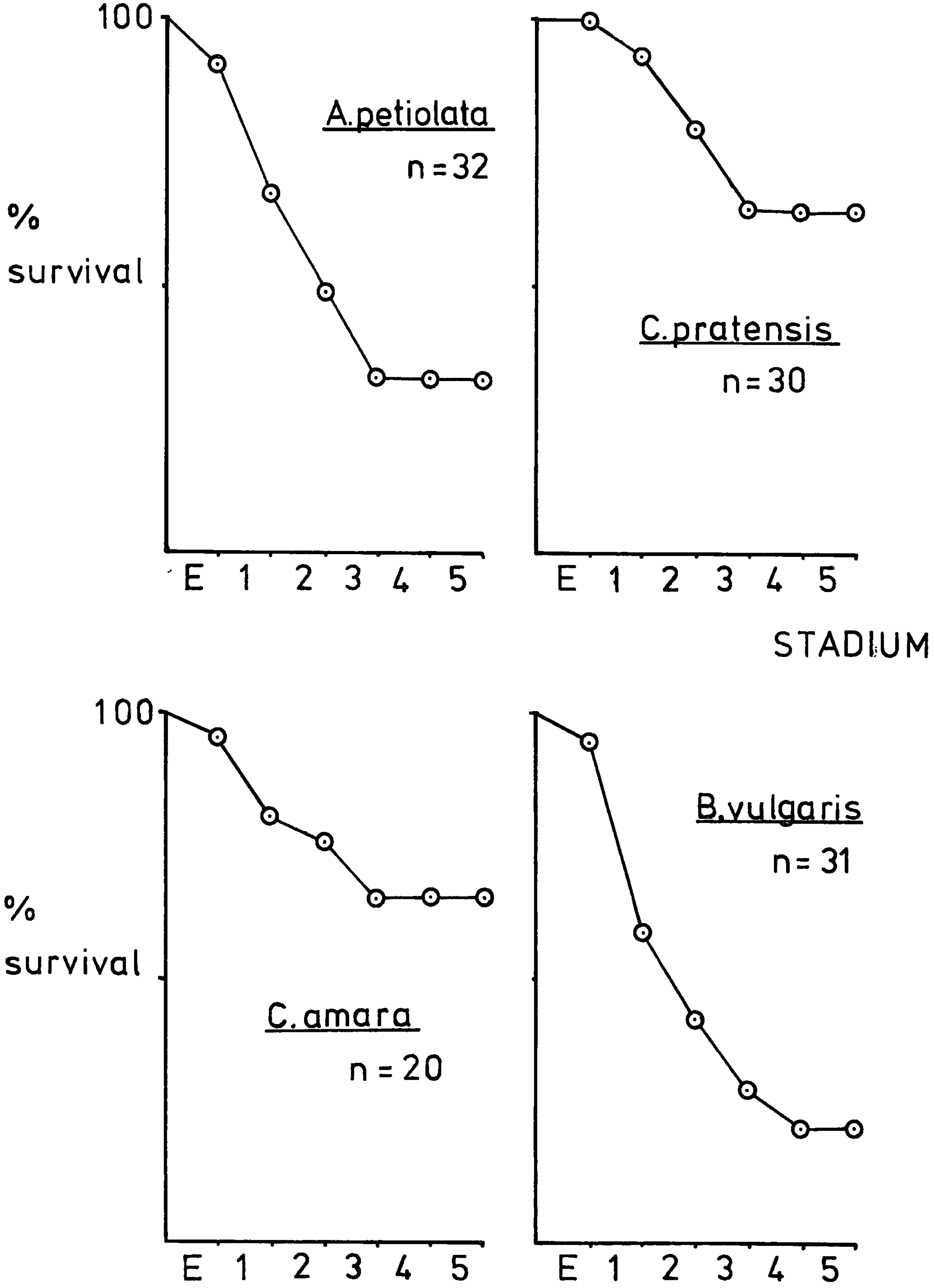

Fig 17 Wolsingham 1979

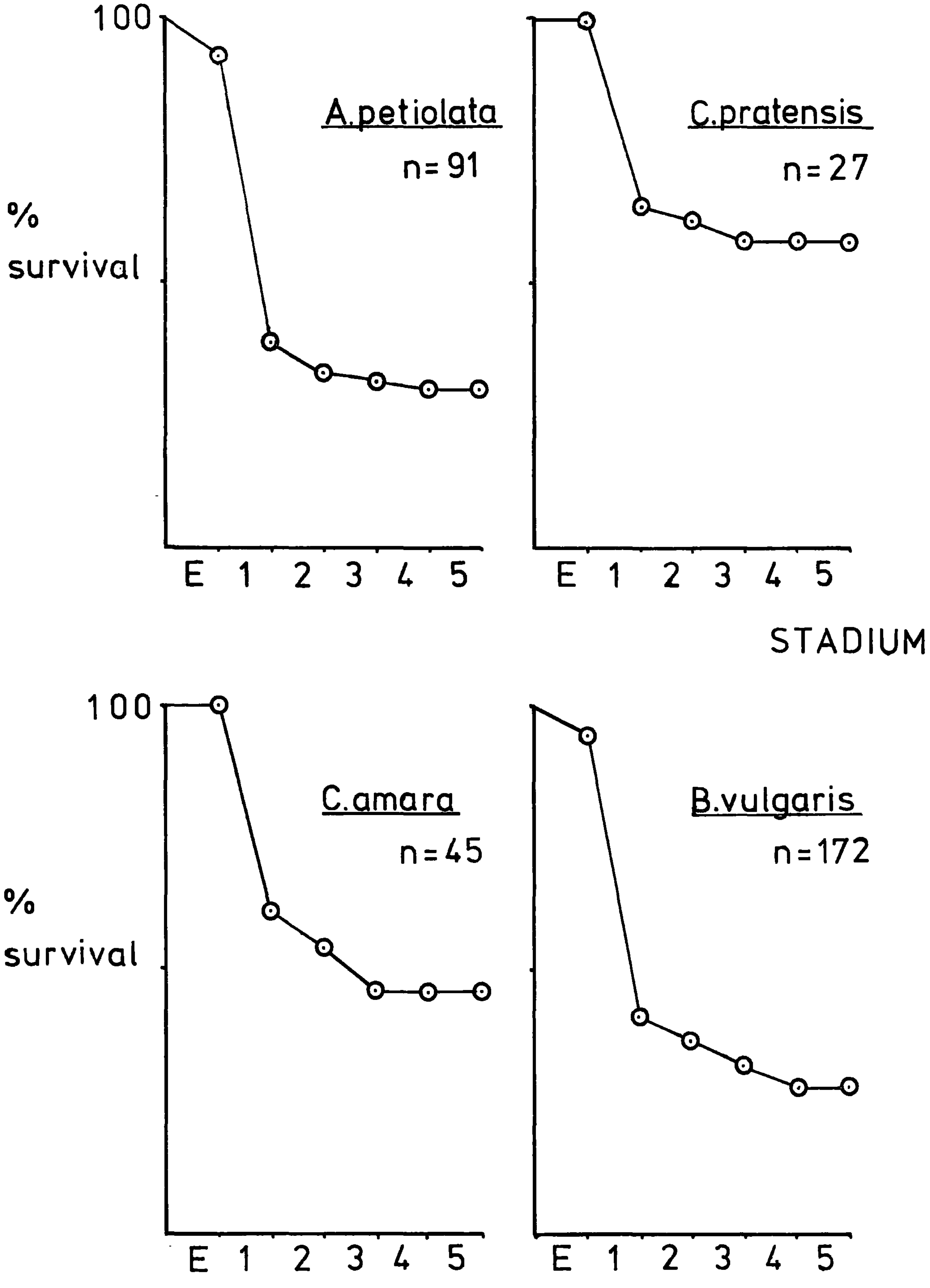

Table 7

The Index of survival of populations of A.cardamines upon different hostplants, with standard error in brackets

		Ap	Hm	Bv	Br	Cp	Ca	Rs
Durham, 1978		2.96 (.24)	1.84 (.20)	1.93 (.60)	3.75 (.45)			
	n	(54)	(88)	(14)	(28)			
1979		3.30 (.27)	1.53 (.15)	1.55 (.28)	3.44 (.37)			
	n	(79)	(116)	(22)	(43)			
Croxdale, 1978		2.39 (.22)						
	n	(98)						
1979		2.67 (.25)	1.33 (.29)	2.25 (.82)				
	n	(87)	(15)	(8)				
Witton Pk, 1978		3.30 (.48)	1.28 (.17)	2.21 (.61)	5.29 (.71)			
	n	(27)	(72)	(14)	(7)			
1979		2.51 (.19)	1.26 (.13)	1.33 (.29)	2.45 (.48)			
	n	(146)	(103)	(18)	(22)			
Wolsingham 1978		3.12 (.45)		2.68 (.37)		4.59 (.37)	4.45 (.51)	3.5
	n	(32)		(31)		(27)	(20)	(2)
1979		2.51 (.23)		2.61 (.17)		4.0 (.47)	3.48 (.38)	6.0
	n	(90)		(172)		(26)	(40)	(12)

Ap = A.petiolata
Hm = H.matronalis
Bv = B.vulgaris
Brr= B.rapa
Cp = C.pratensis
Ca = C.amara
Rs = Rorippa sylvestis

Table 8

Significance of χ^2 tests among animals entering and leaving stadia

Among localities and years

	STADIUM				
	ε	1	2	3	4
A.petiolata	n.s	p 0.001[*1]	n.s	p 0.05[*2]	-
H.matronalis	n.s	n.s	n.s	p 0.01[*2]	-
B.vulgaris	n.s	n.s	n.s	-	-
B.rapa	n.s	n.s	-	n.s	-
Among Foodplants species (all localities summed)	p 0.001[*3]	p 0.001[*4]	p 0.05[*5]	p 0.01[*5]	p 0.01[*6]

[*1] Heterogeneity caused by heavy 1st instar mortality at Wolsingham in 1979 - on Senescing A.petiolata?

[*2] Heterogeneity caused by consistently heavy 3rd instar mortality at Durham - bird predation?

[*3] Heavy egg mortality on H.matronalis - (cannibalism)

[*4] Very heavy mortality on H.matronalis and B.vulgaris

[*5] Heterogeneity from H.matronalis

[*6] Heterogeneity from many 4th instar larvae dying on B.vulgaris

Table 9

The establishment of A.cardamines in the laboratory. Animals taken as eggs from inflorescences of A.petiolata and placed upon crucifer material in the laboratory. Food maintained in a constant state of freshness

Ne = Number 'established' = no. reaching 3rd instar

Crucifer material	N	Ne
A.petiolata immature siliquae	40	34
ageing siliquae	40	13
flowers	20	13
leaves	20	2
H.matronalis siliquae	40	15
leaves	10	3

counts 3, whilst one dying as an egg counts zero. The index is particularly useful when overall survival is low. The two measures show broad agreement; there are few differences between populations at different sites and times suggesting strongly that the observed survival differences are due solely to the effects of the foodplants, and that variations, in time and space, of the major factors causing mortality, did not occur.

In order to identify the stadium or stadia at which these host-specific differences occurred, comparisons of those animals entering and leaving each stadium were made (summarised in Table 8). It is seen that significant differences occur among foodplants at every stadium. The difference in egg mortality is a density effect; other mortalities reflect the varying suitabilities of host plants.

Mortality particularly in the early instars is hostplant related. This could be due to some interaction of foodplant species with an agent of mortality such as invertebrate predation. However, rearing experiments in the laboratory indicate that such is not the case (Table 9). This suggests strongly that larval death is caused more directly by foodplant characteristics. It may be that A.cardamines larvae respond differentially to the different chemical defences of the various crucifers. Chew (1975) has shown that for some Pierinae certain plant species are toxic. In particular she has associated Barbarea vulgaris with toxicity to Anthocharis genutia in New England, although Shapiro records this plant as a major foodplant of A.sara in California (pers comm). In other crucifer feeding groups, B.vulgaris supports an independent 'niche' (Hicks and Tahvanainen, 1974) suggesting that it is distinct in some way. There is little evidence, however, for glucosinolates being responsible for larval death (Chew (1974) has presented glucosinolate profiles for several crucifer species). The presence of other plant substances such as cyanogenics is as yet undocumented. Similarly the different nutritive qualities of the foodplants for A.cardamines remain unknown.

One aspect of a host plants array of defences that has received relatively little attention is the presentation of mechanical problems to grazing. The observed pattern of larval survival in A.cardamines would appear to support the view that such defences are important. It is observed that once penetration has been achieved, larvae appear to survive well regardless of plant species.*1 (In the field it was frequently observed that one larva managed to survive on a plant of, e.g. H.matronalis, on which many other larvae died - suggesting that intra-population variability in foodplant quality is not a plausible explanation of this observation). There is a 'hurdle' which must be overcome in the early instars. To test the idea that differences in plant structure might be important in determining larval survival, various rearing experiments were carried out in the laboratory (also in Table 9). It was found that the age and dryness of a siliqua affected the initial penetration of larvae feeding upon A.petiolata and H.matronalis, and also that establishment varied with plant parts.

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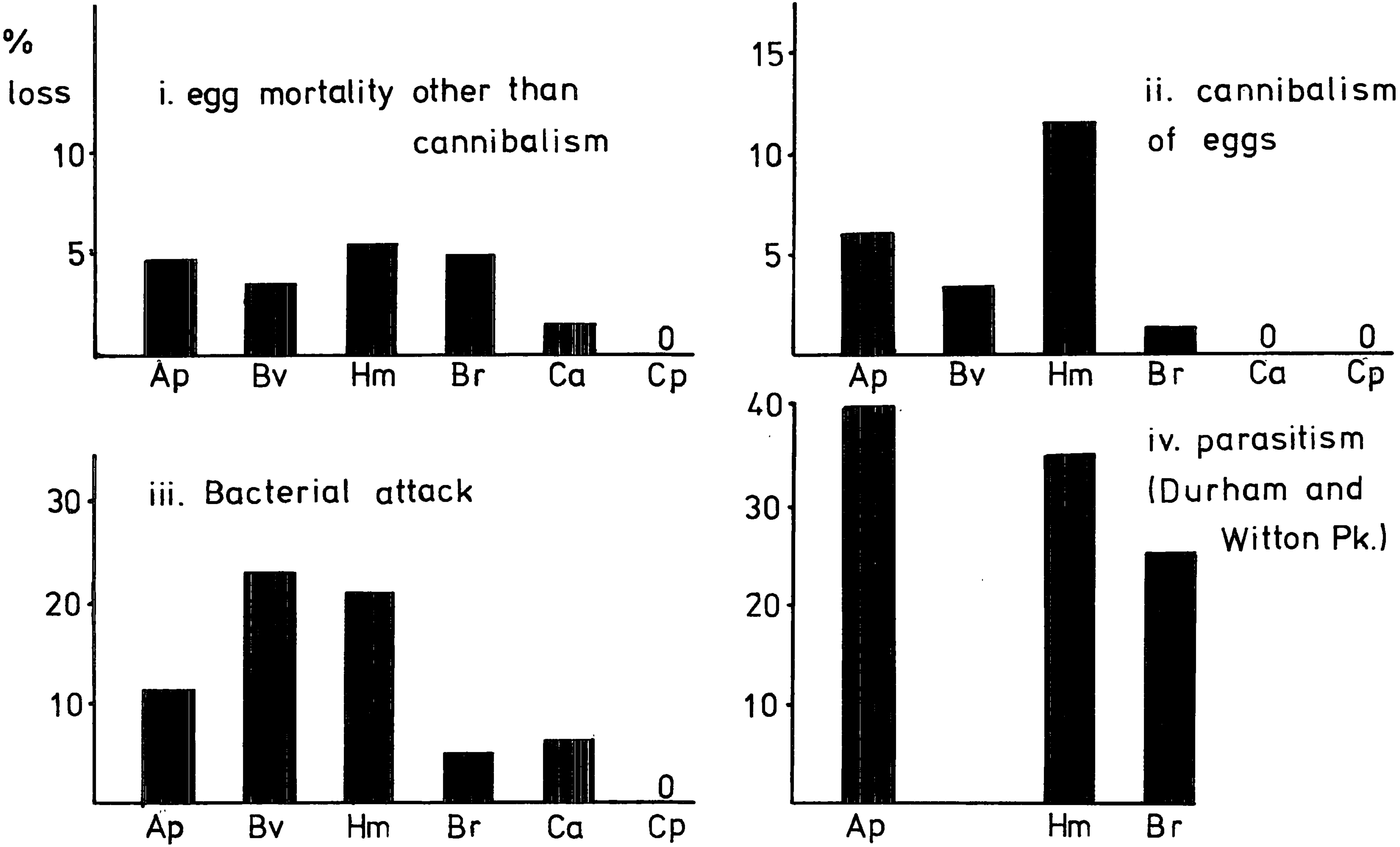
*1 This could also indicate the induction of e.g. detoxifying enzymes - this has been demonstrated by F. Chew (pers comm).

Feeny (1975) has demonstrated that the efficiency of A.rapae feeding is influenced by the proportion of dry matter in its diet, whilst Nielsen (1977), who has recorded a similar pattern of survival to A.cardamines in the Chrysomelid Phyllotreta nemorum (which is killed by several foodplants, including H.matronalis) attributes heavy 1st instar mortality to the presence of a wax layer on the siliquae. This waxy layer is particularly noticeable on the siliquae of H.matronalis. The view that physical properties of the foodplants of A.cardamines have important effects upon larval survival has been reached independently by Wiklund and Ahrberg for Swedish A.cardamines.

c. Interaction of foodplant species with other mortality factors

The various species of Crucifer differ greatly in growth form which results in females responding differently to the species (section 2.vii). It is to be expected that other factors may also interact with plant characters such as form and habitat type, and that these may influence larval survival upon the differing plants. For instance, Wiklund and Ahrberg (1978) noted (non-significant) differences in predation, parasitisation, dessication and drowning associated with different plants. Figure 18 records the differences shown in certain mortality factors between foodplant species. It is shown that cannibalism and bacterial death may be foodplant related but that there is little evidence for different foodplants being refugia from parasite attack (as noted for A.rapae by Pimentel (1961)). The concentration of cannibalism

Fig. 18. Mortality factors acting upon <u>A.cardamines</u> on different plant species.


%
loss
i. egg mortality other than cannibalism
10
5
0
Ap
Bv
Hm
Br
Ca
Cp
ii. cannibalism of eggs
15
10
5
0
0
Ap
Bv
Hm
Br
Ca
Cp
iii. Bacterial attack
30
20
10
0
Ap
Bv
Hm
Br
Ca
Cp
iv. parasitism (Durham and Witton Pk.)
40
30
20
10
Ap
Hm
Br

upon H.matronalis is a density effect caused by A.cardamines eggs being concentrated upon that species (section 2.vii). More interesting is the observation that bacterial attack may be associated with particular plant species. It is already known that viral attack of Pierinae is enhanced upon unsuitable food (David, 1978). It appears that 'stress' conditions lower the ability of larvae to withstand infection. In this context it is significant that bacterial attack is prevalent upon larvae reared on B.vulgaris.

d. The effect of foodplants upon larval development

The foodplant may influence other aspects of fitness beyond mere survival. It is known that A.napi grown on various Cruciferae in standardised laboratory conditions show differences in both development time to pupation and in the final pupal weight obtained (These are often linked, e.g. Wiklund, 1973, but not in A.cardamines). These may be themselves related to survival, for instance in a short growing season, or where increased development time leads to increased exposure to parasitoids. In A.cardamines these differences may also have other effects. Pupal size is related to both imaginal wingspan (influencing adult survival and activity) and to the number of eggs contained in females at emergence, as in A.rapae (Rahman, 1968). (Figs. 19 and 20). The pupal weights of A.cardamines reared in the field are given in Table 10. Anovar (one way - interaction is too high for two-way) shows no significant differences among sites. Significant differences are observed, however, on summing sites and comparing foodplants.

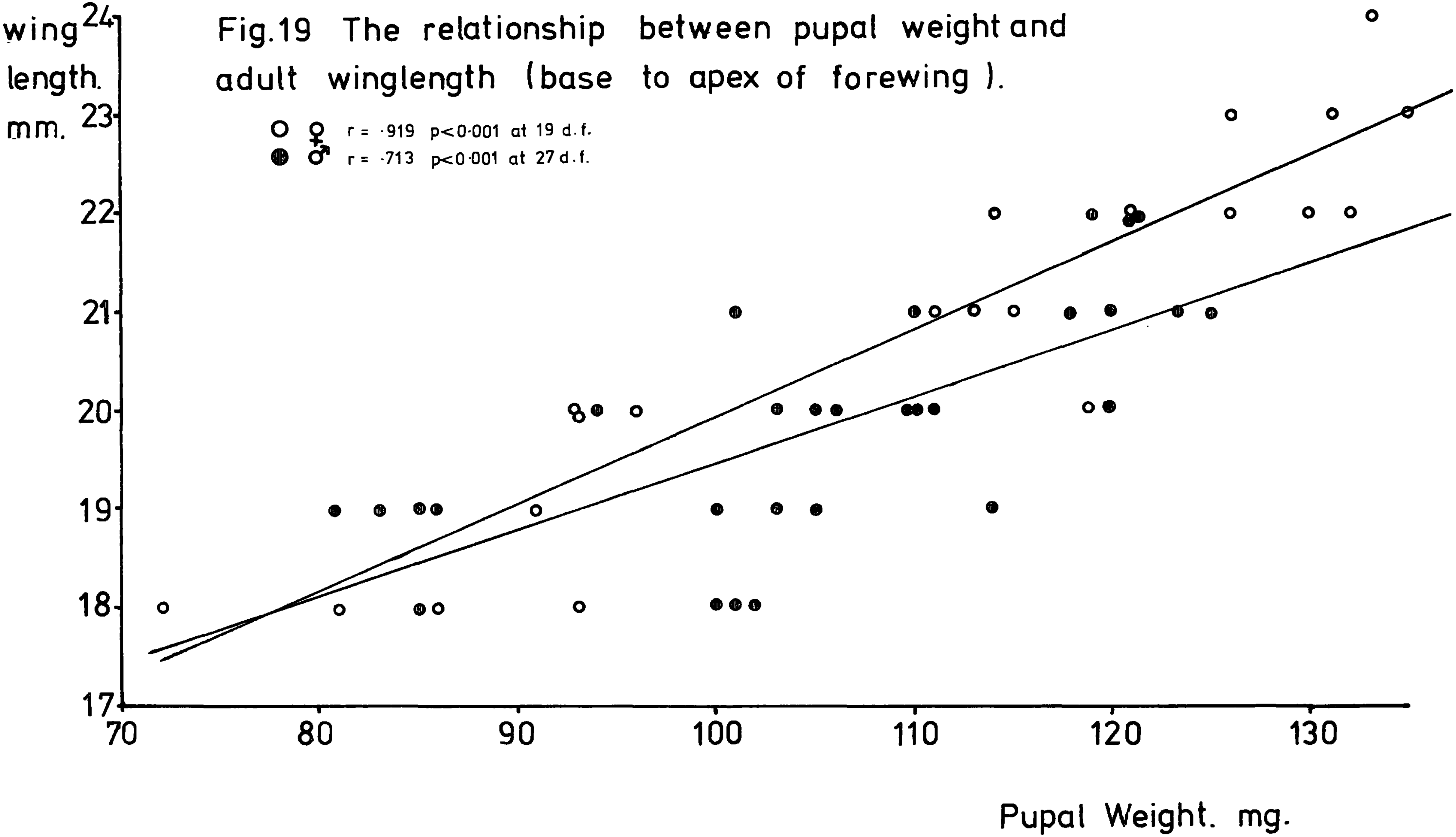


Fig.19 The relationship between pupal weight and adult winglength (base to apex of forewing).

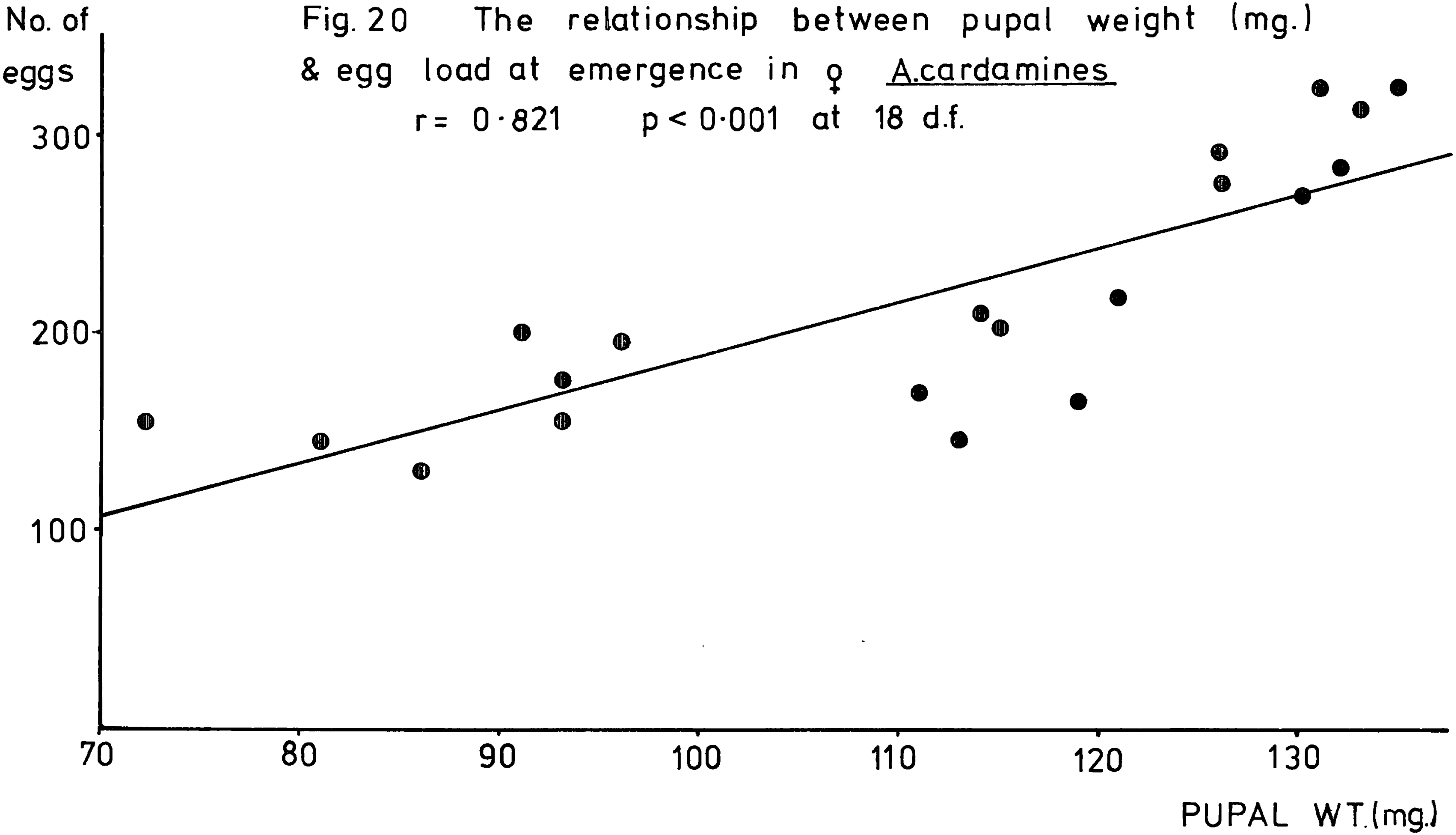


Fig. 20 The relationship between pupal weight (mg.) & egg load at emergence in ♀ A.cardamines

$r = 0 \cdot 821 \quad p < 0 \cdot 001$ at 18 d.f.

Table 10

Foodplant effects upon pupal weight

Foodplant species	$\bar{x}$ pupal wt (mg) + S.E.		n
A.petiolata	111.03	(1.78)	115
B.rapa	103.25	(2.90)	20
H.matronalis	99.11	(4.00)	27
B.vulgaris	93.68	(2.48)	41
C.pratensis	93.38	(6.06)	13
C.amara	82.56	(4.53)	13

ANOVAR (1 way) for all sites - amongst foodplants

Source	SS	DF	MS	F	
Foodplants	20869	5	4173.80	17.27	$p<0.001$
Residual	53908	223	241.74		
Total	74777	228			

ANOVAR (1 way) for pupae reared on *A.petiolata* - amongst sampling sites and times

Source	SS	DF	MS	F	
Sites	3613	7	516.14	1.58	n.s.
Residual	35090	107	327.10		
Total	38703	114			

The differences are consistent in the two years of study.

In studying the effect of foodplants upon development time, two approaches are possible - the study of larval growth under rigourously controlled conditions in the laboratory is useful in elucidating some aspects of foodplant differences (e.g. Chew, 1974). However, such studies are laborious, especially in intractable species such as A.cardamines, and may also ignore the effects in the field of, e.g. plant form upon micro-climate. In the present study it was preferred to follow the development of larvae in the field, thus gaining information on the general effects of the plant, but not on which aspect is responsible for observed differences. Total development time, from oviposition to pupation was recorded. The exact date of oviposition was not always known, although it could usually be inferred from the colour of the egg. In uncertain cases the last date possible was assumed. On reaching the 5th instar, the larva was brought into the laboratory until it pupated; again this is likely to produce a slight underestimate of development time. The results of these observations are recorded in Table 11. It is seen that significant differences occur in the development times of larvae on different foodplants, with H.matronalis and B.vulgaris once again proving the least suitable hosts with the longest development times. However, caution must be applied to these data, since Anovar (1 way) among sites for larvae upon A.petiolata is significant - indicating that development time may vary - with year in this case.

Table 11

Development times of larvae on differing crucifers (all sites summed) Days (+S.E.)

Ap	Hm	Bv	Br	Cp	Ca	Rs
33.34 (0.35)	36.38 (.84)	36.14 (.36)	32.43 (.70)	31.68 (.59)	32.60 (.58)	31.9 (.23)

---

1 way ANOVAR development time upon plant species

*excluding R.S.

Source	SS	df	M.S	F	
Foodplant	1351	5	270.2	4.07	$0.01 > p > 0.001$
Residual	23763	357	66.6		
Total	25114	362			

---

1 way ANOVAR development time upon A.Petiolata at different sites

Source	SS	df	M.S	F	
Site	530	7	75.7	6.78	$p < 0.001$
Residual	1853	166	11.16		
Total	2383	173			

Table 12

Hierarchies of suitability of crucifers for the support of A.cardamines larvae

	Survival (up to 5th)	Development time	Pupal weight	Bacterial attack
1	R.sylvestris	C.pratensis	(R.sylvestris)*	C.pratensis
2	C.pratensis	R.sylvestris	A.petiolata	B.rapa
3	C.amara	B.rapa	B.rapa	C.amara
4	B.rapa	C.amara	H.matronalis	A.petiolata
5	A.petiolata	A.petiolata	B.vulgaris	H.matronalis
6	B.vulgaris	B.vulgaris	C.pratensis	B.vulgaris
7	H.matronalis	H.matronalis	C.amara	R.sylvestris

High ranks to high survival, short development time, heavy pupae, low bacterial loss.

* only one animal of 14 survived to pupation.

e. Summary of plant effects

It is seen that three different criteria of hostplant suitability give remarkably similar hierarchies of suitability (Table 12). Eggs laid upon a foodplant species giving poor larval survival are likely to develop into small pupae, and to spend longer than the average in doing so. Moreover adult fitness of such pupae is likely to be impaired. It is possible to synthesise all these factors into a tentative index of foodplant suitability. Thus if

A = % eggs surviving to pupation (including deaths due to bacteria but not parasitisation)

$\text{Index} = \frac{A}{2}\left(\frac{A}{2} \times N\right)$ where N is the mean number of eggs/female (calculated from mean weight and graph 20)/maximum value for egg load.

The Index assumes that pupal weight affects the fitness of females only, not males, and also that winter death affects pupae independently of their larval hostplant. It indicates the magnitude of fitness differences of eggs on different hostplants. Table 13 records the results of calculating the index.

Table 13

	Durham		Witton		Wolsingham	
	78	79	78	79	78	79
A.petiolata	23.72	31.50	33.63	23.06	23.63	23.25
B.rapa	40.22	34.34	64.41	21.80		
H.matronalis	9.36	5.62	4.49	5.92		
B.vulgaris	13.18	3.01	11.84	3.61	13.69	16.39
C.pratensis					49.92	43.79
C.amara					47.23	31.34

f. Variation in plant abundance

Another foodplant related factor which may profoundly influence strategies of exploitation is the predictability of the resource (i.e. the Crucifer spp) in time and space. Most Cruciferae are annuals or biennials; those species which are perennials are often of early successional status. Wiklund and Ahrberg used this fact to argue that the Crucifer species must vary greatly in abundance from year to year and place to place, and that the polyphagic strategy of *A.cardamines* was in part a response to this. Cruciferae in Sweden do vary in abundance, sometimes quite dramatically (C. Wiklund, pers comm). Equally important may be variations in plant abundance over space. Chew (1975) felt this to be of major importance in the maintenance of polyphagy in *A.napi.*

The records of hostplant abundance at Durham, the maps of foodplants used for larval sites, and incidental observations on foodplants, allow the variation in crucifer abundance at sites in the North East to be investigated. The principal hostplants used in all populations studied were:

Locality	Foodplants	Locality	Foodplants
1. Durham Sands	Ap Hm Bv	16. Finchale	Ap
2. Durham	Ap Hm Bv Br	17. Darlington	Ap
3. Shincliffe Hall	Ap Hm Bv Br	18. Winston	Ap Bv Ca
4. Field Station	Cp	19. Ovington	Ap
5. Cassop Vale	Cp	20. Barnard Castle	Ap
6. Bear Park	Cp	21. Egglestone	Ap Hm Cp
7. Croxdale	Cp Hm Ap Bv	22. Middleton	Ap
8. Witton Park	Ap Hm Bv Br	23. Dent Bank	Ap Cp
9. Witton-le-Wear	Ap Hm Bv Br	24. Coldberry	Cp
10. Wolsingham A	Ap Bv Ca Cp	25. Alston A	Hm
11. " B	Ap Cp	26. " B	Ca Cp Ta No
12. Stanhope	Ap	27. Tynehead	Ca Cp
13. Wearhead	Ap Cp	28. Nenthead	Cp
14. Brasside	Cp	29. Langwathby	Cp No
15. Brancepeth Castle	Cp		

Ap *A.petiolata* Hm *H.matronalis* Bv *Barbarea vulgaris*
Ca *C.amara* Cp *C.pratensis* Ta *Thlapsi alpestre*
Br *Brassica rapa* No *Nasturtium officinale*

Fig. 21 The hostplants of *A.cardamines* populations

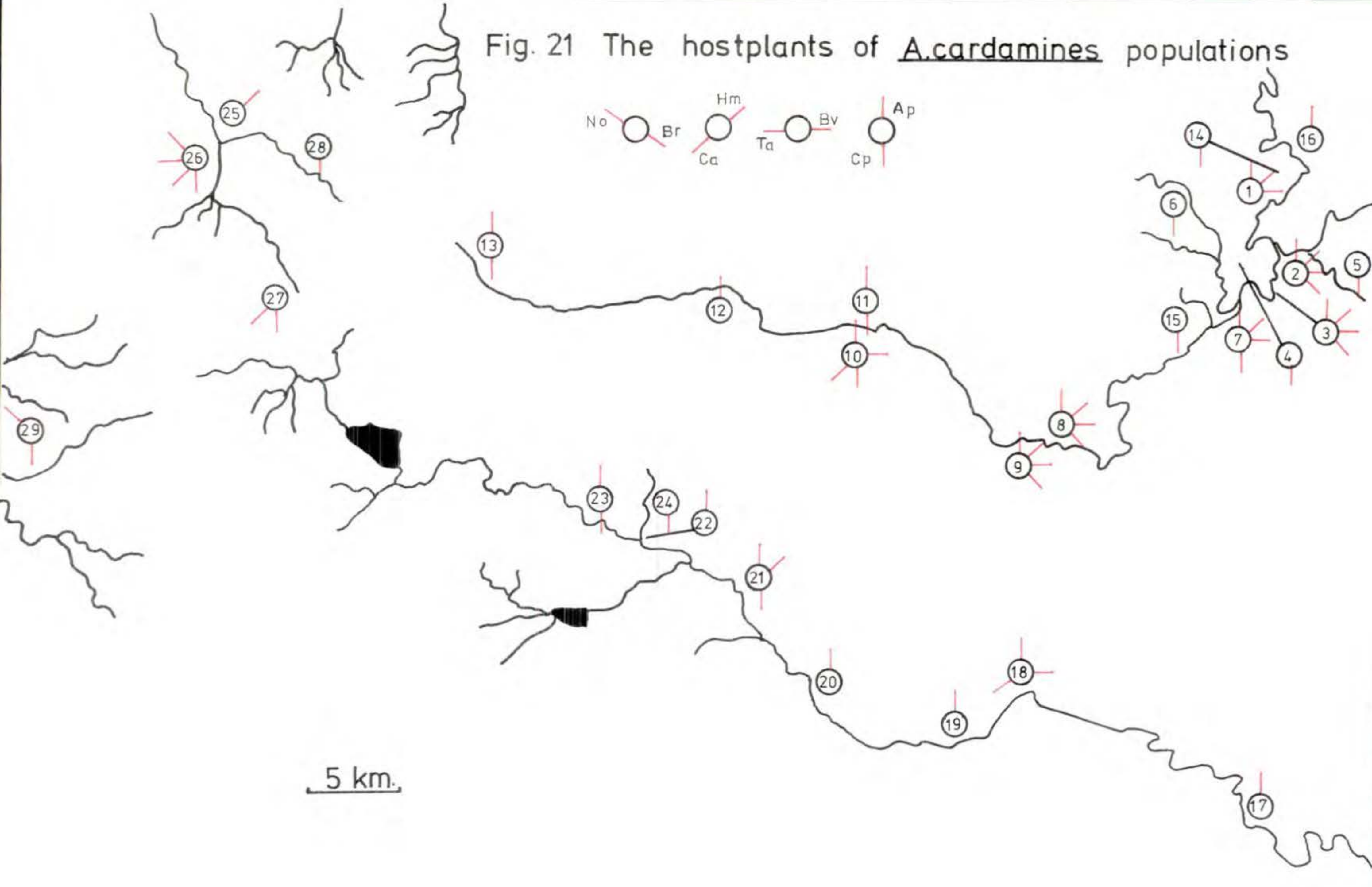


Table 14

Abundance of hostplants of A.cardamines at Durham (Total counts) and other sites (mapped section only)

		A.p	Hm	Bv	Br	Cbp	So	Ca	Cp
*Durham	1977	4350	195	235	410	430	45	-	-
	1978	10320	230	225	185	85	55	-	-
	1979	3100	115	75	85	50	80	-	-
Croxdale	1978	33	2	3	-	-	-	-	0
	1979	18	5	8	-	-	-	-	35
*Witton Park	1978	84	25	3	4	-	-	-	-
	1979	453	32	4	38	-	-	-	-
Wolsingham	1978	101	10	5	-	-	-	15	23
	1979	38	0	2	-	-	-	41	82

* + additional plants of *Cardamine hirsuta*, *Sinapis arvensis*

Counts for sites include only those areas counted in both years. Counts were made to the nearest 10 (*A.petiolata*) or 5 (other species) at Durham.

Fig. 21 maps these sites. It can be seen that sites separated by very little distance may have very different foodplants.

Table 14 records the abundance of hostplants at Durham and on the mapped sites from other localities. It may be seen that considerable variation occurred in crucifer abundance. For instance, one area ploughed in spring, 1977, was left fallow in subsequent years and large growths of Capsella bursa-pastoris were out-competed. Similarly the wet winters and springs of '78 and '79 encouraged crucifer growth, but caused extensive flood damage so that there was a great decline in crucifer abundance there.

g. The effect of the animal upon the plants

The grazing of A.cardamines larvae upon crucifer seed-heads is often devastating, and forms a ready clue to the animals presence. However, the total destruction of seeds may be small. Approximately 33% of the seed of an A.petiolata plant is lost in supporting one A.cardamines larva to maturity, most of the damage occurring as a result of the feeding of the last larval instar. Grazing of earlier instars is less damaging, and there is evidence that Cruciferae can compensate fully for such loss (Williams and Free, 1979). Thus it is unlikely that damage caused in or before the 3rd instar is permanent. The overall calculated loss of A.petiolata to A.cardamines at Durham is given in Table 15.

Table 15

Year	Number of *A.p.* plants	Est. no. of *A.cardamines* larvae (4th instar)	% seed loss of population.
1977	4,350	1,598	12.2
1978	10,320	754	2.4
1979	3,100	763	8.2

These figures match with those of Wiklund and Ahrberg, where up to c. 12% of seedset was lost by some plant species.

The nectar-feeding and pollinator activity of the population of *A.cardamines* at Durham has been investigated (Appendix 1) by means of pollen analysis and direct observations. Despite the strong association of *A.cardamines* adults with larval foodplants, the temptation to advance some co-evolutionary hypothesis must be avoided, since many Cruciferae are able to self-pollinate. *A.petiolata* for instance always shows full seed-set regardless of insect pollination. Nevertheless these studies demonstrate that the effect of *A.cardamines* upon Cruciferae is not entirely negative: the constancy observed may for instance greatly influence outcrossing in these species of plants.

vi. Population dynamics of the Durham Population

a. Introduction

Different factors affecting the survival of animals within the populations under study are outlined in the preceeding and following sections. All these factors at least potentially affect the fitness of eggs laid by an ovipositing female, and may act as selective agents. However before any statement is made linking such putative selective agents with observed 'adaptations', it is highly desirable to have some method of comparing the relative importance of different mortality factors, particularly if the selective influences of different factors are opposed. One such method is key-factor analysis. This approach, pioneered by Morris (1959) entails the repeated censusing of a population at successive intervals in order that the relative contribution of each mortality factor to total generation mortality can be assessed. The approach has proved particularly useful in demonstrating the dynamics of population determinants, and in resolving the debate over factors 'controlling' population numbers. (Varley et al, 1973). The method does not provide information on the importance of very long-term selective factors such as the extinction of populations, and ideally should be continued over many generations in order to provide information on the evolution of strategies; it does, however, give insights into actual selective pressures at a particular time.

For the method to be applied, it is essential that censussing should be as accurate and frequent as possible.

The procedure for censusing adults has been described. For egg and larval censussing, the mapped distributions were used, The incidence of eggs upon foodplants, and the survivaal of larvae upon each foodplant species was combined with the actual abundance of these species at Durham to give the overall number of animals surviving to each stage. The mortality at each stage is then expressed as the k or 'killing value', that is the difference between the logarithms of population size before and after mortality. In the present study it was necessary to estimate two such k-values, since no censussing was possible between late instar death, and the emergence of adults the following spring. However, $k_5 + k_6$, the death caused by parasites emerging before winter and by bacteria, can be estimated from the populations in the laboratory, the remaining residual mortality K_9 and K_{10} being what is left between the observed mortality k_2 - 8 and the known total generation mortality K. This residual mortality represents all death of pupae overwintering until emergence the next year, and it includes in it any loss to predators, parasites or weather factors. Most population studies include such a 'residual' factor, and in the present study this is limited to a fairly discrete part of the life-history. In other studies, for instance that on the Winter Moth (Operophtera brumata) (Varley and Gradwell, 1968), 'winter death' covered all mortality from adult emergence to counts of prepupal larvae severely limiting the inferences that may be made from the studies.

Merely to include all calculated pupal death as 'winter death' is to lose valuable information. The percentage of pupae entering the winter which are parasitised is known from the pupae brought into the laboratory. However, some of these would ordinarily fall to the other causes of death which make up pupal mortality. Following the guidelines of Varley et al (1973) only the first mortality factor to act must be considered as the cause of death, so it becomes necessary to estimate the loss of parasitised pupae to 'winter death'. This calculation and that of the actual loss caused as k_9 'winter death' and k_{10}, parasitisation, follows the following form.

$$N_i \xrightarrow{\text{winter death.parasitisation}} N_T$$

$$\xrightarrow[k_9]{} N_i + 1 \xrightarrow[k_{10}]{}$$

Where N_i = Popln. no. before winter.

N_T = Popln. no. after K_9 and k_{10}

$N_1 - N_T = T$ (Total death) (known)

q (Overall parasitisation rate) is known.

$$\therefore \quad k_9 + k_{10} = T$$

$$k_{10} = \frac{N_i - k_9}{Ni} \times q$$

$$\therefore \quad k_9 + \left\{ \frac{N_i - k_9}{N_i} \times q \right\} = T$$

Solve by Trial and Error.

e.g. If 100 larvae enter winter, and 20 eclose and 20 of those entering are estimated as parasitised

$$\text{THEN} \quad K_9 + \left\{ \frac{100 - k_9}{100} \times 20 \right\} = 80$$

$$\text{TRY} \quad k_9 = 70$$

$$\left\{ \frac{100 - 70}{100} \times 20 \right\} + 70 = 76 \neq 80$$

TRY $k_9 = 75$

$$\frac{100 - 75}{100} \times 20 + 75 = 80$$

$\therefore\ k_9 = 75$

$k_{10} = 80 - 75 = 5$

(In the above examples k-values are not expressed as logarithms, for the sake of simplicity).

The calculation of one k-value in particular is difficult - that of k_2 egg shortfall. This is the difference (as a logarithm) between the maximum number of eggs that might be laid under ideal conditions (where each female achieves maximum survival and lays all her eggs) and that number which are actually observed to be laid in the population. This k-value covers all loss to the population where the female lays less than the maximum number of eggs, including emigration, early adult death and inhibition of egg-laying by poor weather. It has proved difficult to assess accurately the effects of female movements. At least some females visit areas outside the normal study area at Durham (App. 1) and there may be some migration between populations. Any immigration will lead to an under-estimate of egg shortfall, but an over-estimate of female fecundity within the population, whilst emigration will lead to an underestimate of fecundity. Emigration will almost certainly exceed immigration, and egg shortfall is probably reasonably accurately determined. Female fecundity is probably under-estimated, but leaving the riverbanks is akin to death from the females' point of view. Only rarely (e.g. the colonisation of the Zoology Field Station or the 'founder event' at Coldberry) will females succeed in finding crucifers away from the river-banks which is the main habitat for Cruciferae in County Durham. Thus estimates of total female fecundity based on female fecundity within the population studied should not be greatly inaccurate.

The accurate calculation of egg shortfall has received surprisingly little attention from population workers: usually they are content to make dissections to establish the mean number of eggs carried by females (e.g. Varley and Gradwell, 1968; Banerjee, 1979) rather than the actual number laid under ideal conditions. Accurate estimation of the number of the youngest eggs in female ovaries is rarely possible (this group is in the majority in newly emerged females). However, in A.cardamines it is necessary to use such dissections, since the marked inhibition of egg-laying under laboratory conditions precludes any attempt to make direct observations on egg production. The mean eggload at emergence of 14 females, was 208 eggs. (This is probably an underestimate of the mean in the population).

For A.rapae, Gossard and Jones (1977) described egg production under ideal conditions of adult survival and nutrition, etc. They showed that females of that species may lay up to 300 eggs during adult life. Dissections of A.rapae females were made by Baker (1968) who gave a mean eggload per female of c.450 eggs (the much larger females of P.brassicae had from 625 to 800 eggs). A $\bar{x}$ egg production of c. $\frac{300}{450}$ x 208 = 139 seems reasonable on these results.

However other evidence exists to suggest that a maximum egg production figure of c.150 eggs per female should be used. The reasoning for this is: if a female produces and lays up to 30 eggs a day (except on the day of emergence) and lives approximately 6 days (as seems reasonable from male survival) then the number of eggs produced under ideal conditions by a female of average survival is 150. An estimate of k_2 based on a maximum production per female of 150 eggs should therefore seriously underestimate egg shortfall in total, and that caused by ♀ death, but should provide a good estimate of the remaining components of egg shortfall, i.e. emigration and oviposition inhibition. It should be noted that a k_2 based on 150, 300 or even 100 eggs as the maximum, does not alter the finding that egg shortfall is the mortality factor with the biggest k-value in all three years of study. A mean egg production of 150 is assumed.

Human effects on survival were not included in calculating k-values, since the sites on which survival was followed were atypically open and subject to disturbance. Thus egg and larval k-values are slightly under-estimated. Nor are animals removed for sampling included. Sampling of adults took place at Shincliffe Hall in 1977 and 1979. In 1978 pupae from the previous year were used to give adults. Retaining such pupae (from larvae followed as individuals) will inflate 'mortality' due to 'winter death'. In all three years winter death will be overestimated by c. 2 to 4%.

b. Results of k-factor analysis

The results of k-factor analysis are given in Table 16.

Tab 16

K FACTOR ANALYSIS FOR MORTALITY IN THE DURHAM POPULATION OF *A. CARDAMINES*

			Values of k		
STAGE	k	Cause of death	1977	1978	1979
ADULT	k_1	Sex ratio	(0.301)	(0.301)	(0.301)
	k_2	Egg shortfall	0.822	1.228	1.202
EGG	k_3	Cannibalism etc.	0.002	0.040	0.037
1ST & 2ND INSTARS	k_4	Foodplant-related	0.224	0.305	0.294
3RD INSTAR	k_5		0.047	0.149	0.096
4TH & 5TH INSTARS	k_6	Predation ?	0.046	0.001	0.004
PUPA	k_7	Parasitisation	0.081	0.137	0.196
	k_8	Bacteria	0.021	0.070	0
	k_9	Winter death*	0.250	-0.088	
	k_{10}	Parasitisation*	0.145	0.039	
	K	$(k_2 - 10)$	1.638	1.881	

* Calculated from residual mortality

Ideally, to identify any factor as the 'key' factor determining the direction and degree of population change, one should demonstrate a correlation between individual component k-values and overall generation mortality K. This is not possible on the limited data available. However, the analysis does identify the major contributing factors to population mortality, which allow some relative values to be attached to putative selective agents (section 2.vii). In all three years of study, egg shortfall is seen to be of paramount importance. Of secondary importance are mortality factors acting on the immature stages: foodplant related death, parasitisation (k_7 and k_{10}) and winter death, in that order of importance. Similar patterns of mortality, with egg shortfall as the most important contributing factor, are also found in the butterflies Thecla pruni and T.betulae (Thomas, 1974), L.dispar (Duffey, 1968) and in other Lepidoptera, e.g. Andraca bipunctata (Banerjee, 1979). It is also implied in other population studies which miss out adult factors, e.g. A.rapae (Dempster, 1967). Similarly in the Winter Moth, 'winter death', which is the key factor for that species, covers all mortality from female emergence until pre-pupal death. A major component of 'winter death' in this species may in fact be failure of females to lay full egg-loads.

c. Egg shortfall and weather

Most butterflies are inactive in periods of poor weather (e.g. Douwes, 1976). This is usually a response to lack of sunshine: most butterflies are heliothermic, showing pigmentational and behavioural adaptations so as to raise their body temperatures (via basking postures, etc.) (Clench, 1972). Not surprisingly, oviposition, dependent upon flight activity, appears to be concentrated upon sunny days (Biever et al, 1972; Rahman, 1969; Gossard and Jones, 1977) and in sunny parts of the habitats (Chew, 1977a) in most species.

It has been shown above that shortfall of egg production is the major source of mortality in the Durham population of A.cardamines, with a mean egg production per female (estimated) of 22.6, 8.9, 9.4 eggs in the three years of study.[*1] Casual observation in 1977 showed that bad weather might be contributing greatly to this shortfall, since on rainy, cold days (e.g. the period June 10th to 19th, 1977, when only 10.1 hours of sunshine were recorded at Durham) very few eggs were found to have been laid.[*2] To demonstrate the relations between daily sunshine, flight activity and oviposition, observations were made in 1978 and 1979 on the daily deposition of eggs. Mapped lengths of riverbanks (not those visited for larval survival) were visited during the evening of every day, after oviposition had finished, and every crucifer inflorescence was examined for the presence of newly laid A.cardamines eggs. In order to reduce the variance

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[*1] The number of eggs laid per ♀, and egg shortfall in the population may also be affected by foodplant availability and abundance, and the values given here may also reflect the increasing difficulty of finding hosts when they are rare.

[*2] Bad weather might influence shortfall in other ways than prevention of oviposition, e.g. by decreasing emigration or increasing mortality.

in the observation of oviposition rates, the following procedures were used:

1. Counts were restricted to the middle part of the flight period when most females had emerged.
2. Counts were also restricted to that period when A.petiolata plants were young. Observations ceased when inflorescences began to approach age class 3 (see later section).
3. Only inflorescences of A.petiolata were examined. Plants of other crucifer species were uprooted and removed from the population.
4. As each egg was found, it was removed from the plant, together with that part of the inflorescence on which it stood. This was usually a single pedicel.

These procedures thus avoided variance caused by variations in female numbers, foodplant ageing, species effects and oviposition deterrents.

Occasionally eggs of a light orange colour were found - these could have been eggs which had developed the orange colour in the female before oviposition or eggs which had been overlooked whilst in the white stage on the previous day. The latter was assumed to be the case, and such eggs (2 in 1978 and 9 in 1979) were added to the total for the day previous to the day of finding. The totals for each day were then compared to the daily sunshine record at Durham.

The results of these observations are given in Figs. 22 to 24. Fig. 22 shows the daily record for the two sites of 1979. These stretches of riverbank, although separated by some 300 m. show close agreement in daily fluctuations of egg deposition, suggesting that effects are general to the whole area. Figs. 23 and 24 show the relationship between

Fig. 22 Daily record of egg deposition on two lengths of riverbank DURHAM JUNE 1979

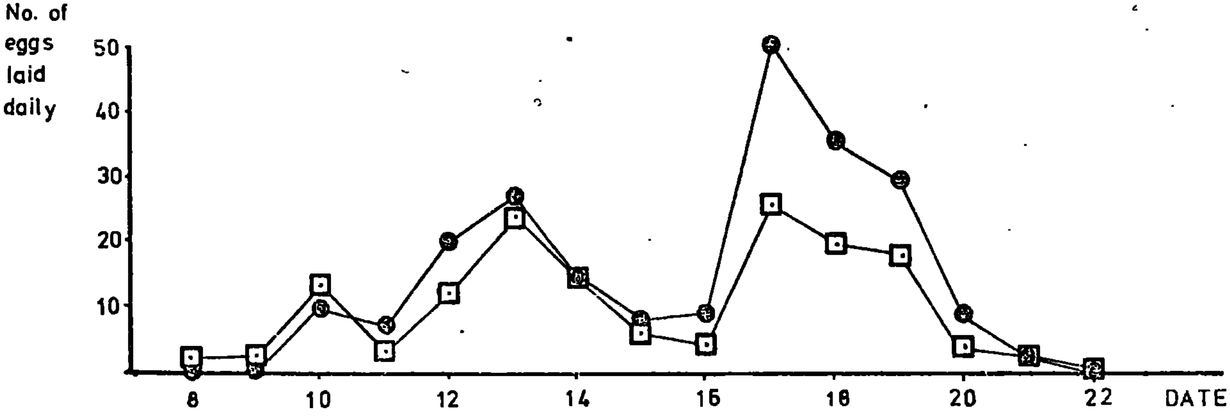


Fig.s 23 & 24 The relationship between egg deposition and daily sunshine in 1978 & 1979

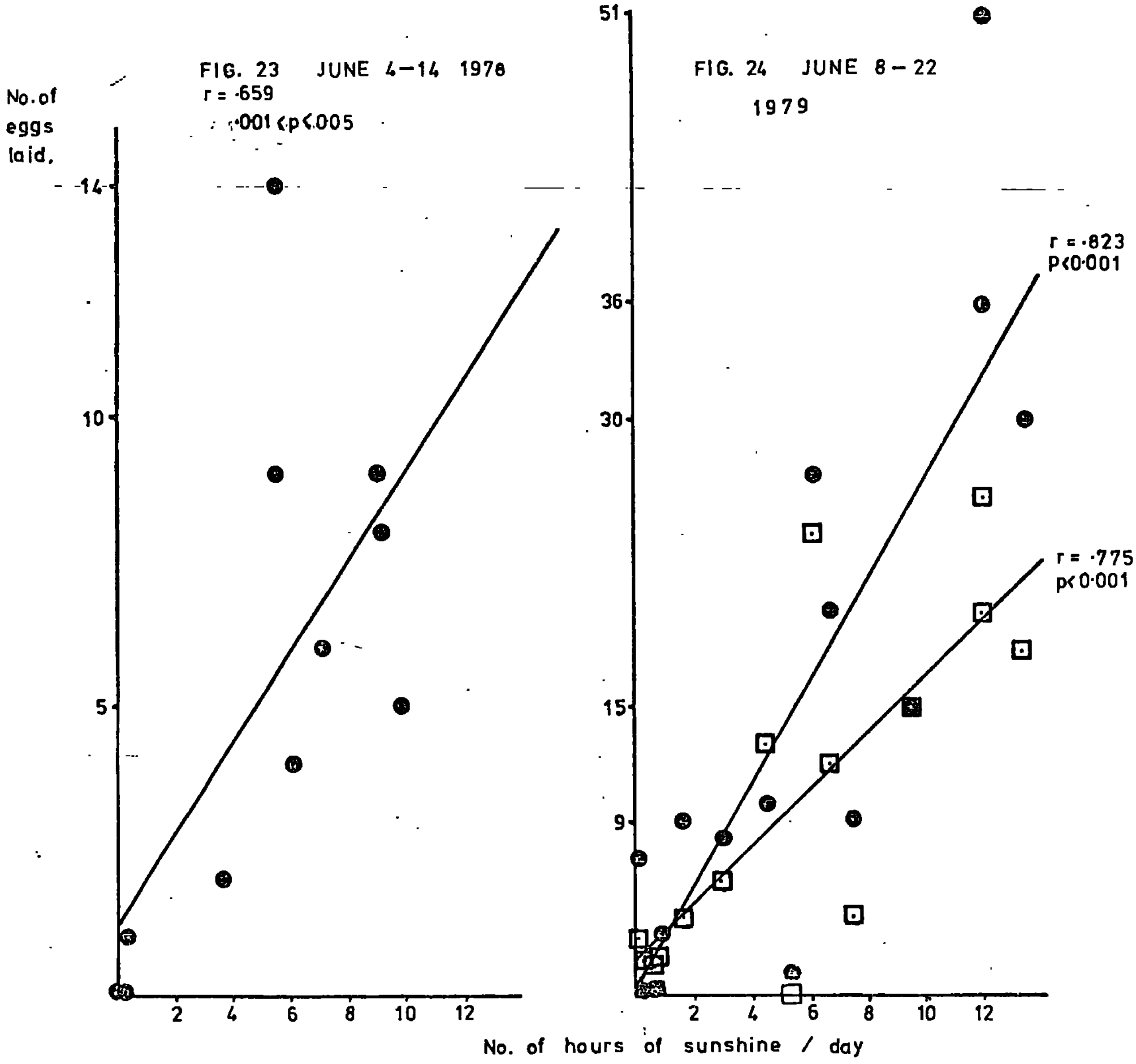
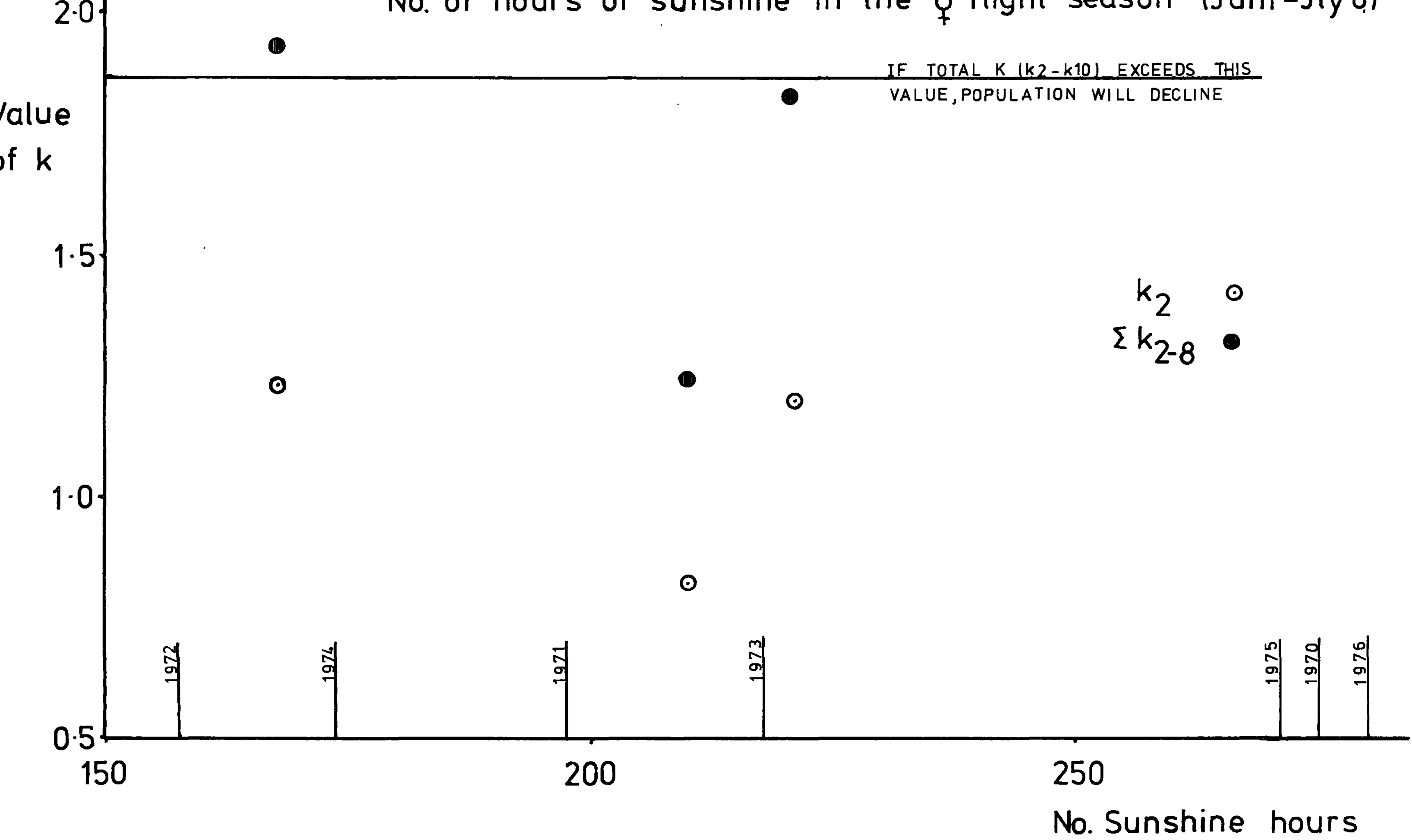


FIG. 23 JUNE 4–14 1978

FIG. 24 JUNE 8 – 22 1979

Fig. 25 Relationship between k_2 (egg shortfall), Σk_{2-8} and the No. of hours of sunshine in the ♀ flight season (Jun1–Jly 6)



recorded sunshine and oviposition. In all cases there is a marked increase in oviposition in sunny weather, and there appears to be a simple relationship between amount of sunshine, that is time available for female activity, and the number of eggs laid. However, caution must be used in applying these results, since it is known that in A.rapae, females may compensate for flightless days by increasing oviposition on the next favourable day. Up to 75% eggs may thus be held over to the next day (Jones, 1975).

If inhibition of egg-laying by bad weather is important, then it is to be expected that both k_2, egg shortfall and total mortality K at the Durham population should be related to the number of hours available for activity during the flight season. Fig. 25 shows that this prediction is not contradicted by the Durham results, but that it cannot be said to be confirmed on the limited data available. Nevertheless Fig. 25 suggests that the overall trend in A.cardamines numbers in the Durham area should be upwards in the 1970's, as was the case.

vii. The Adaptiveness of oviposition behaviour

a. Introduction

The oviposition behaviour of Pieridae has been much studied, by Ilse (1926, 1937), Terofal (1965), Schoonhoven and Behan (1971) and Traynier (1979) amongst others. Most of these authors have emphasised that there appears to be a chain of cues (sensu Kennedy 1965) where, by a sequence of responses to different stimuli, the female is led to lay her eggs in a suitable position. The chemoreceptive responses and apparatus of Pierini have in particular received much attention since they represent the hypothetical result of coevolution between the chemical defences of Cruciferae and the butterfly species (e.g. Verschaffelt, 1910). However, rather less attention has been focussed on other aspects of host-finding although colour vision (Ilse, 1937; Rothschild and Schoonhoven, 1977) and movement patterns of adults (Jones, 1977) are known to markedly affect the distribution of eggs upon plants. The view of Kennedy (1965) remains true to this day:

> "while the final links in such reaction chains..... are comparatively easy to see and to analyse, the earlier links, by which the plant is found and contacted, are not so. They constitute, I believe, the most backward part of the whole subject (host plant selection)......"

In the present study it was decided to investigate the oviposition behaviour of female A.cardamines, including the effect of both host location and host selection by the female upon the distribution of eggs in the field. The observations obtained were then used to explore the 'adaptiveness' of such behaviour.

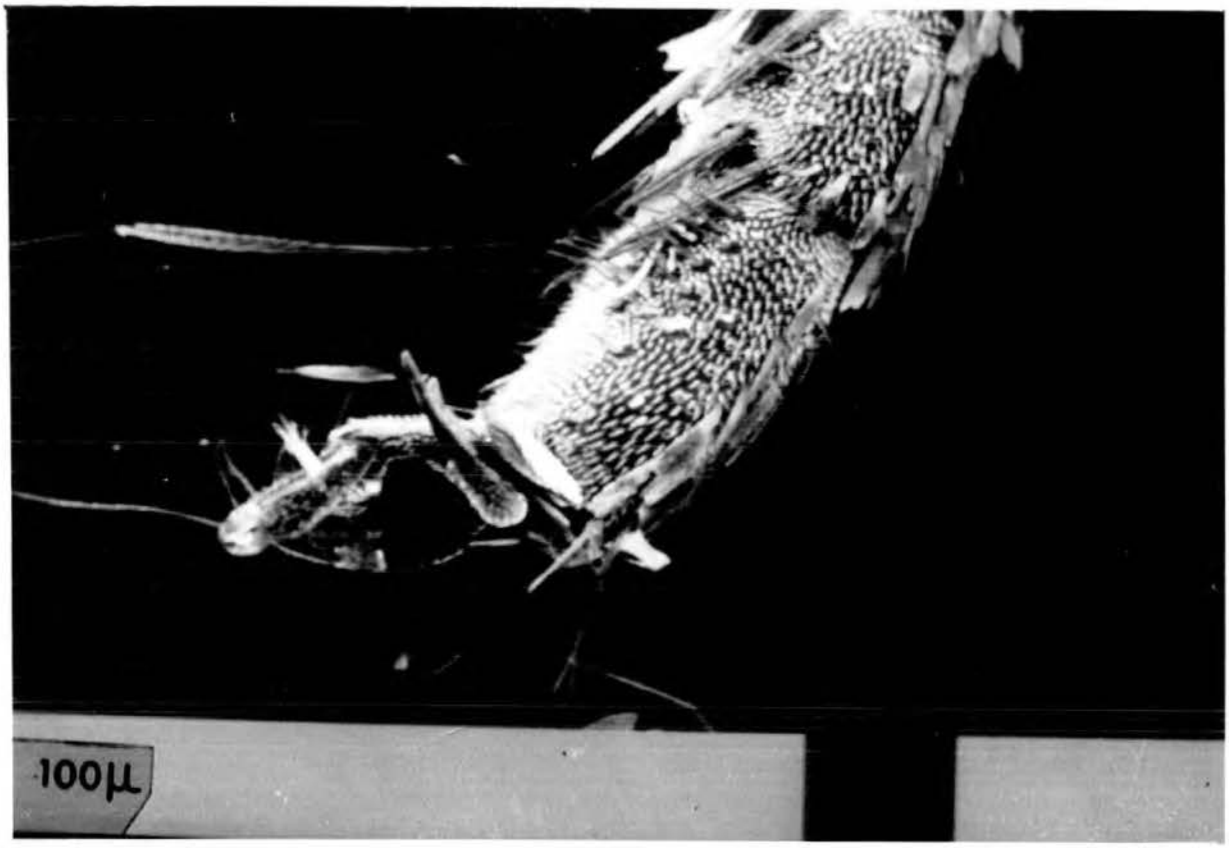
When searching for hostplants the female A.cardamines flies low above vegetation in a characteristic mode of flight, with high wing beat amplitude, typical of most egg-laying Pierinae females. During such flight she repeatedly investigates any object of the approximate size and colour of a crucifer inflorescence, including not only flowers but other (stationary) butterflies, pieces of paper, etc. Such flight is usually slow - interspersed with it occur flights of greater speed and distance.

Contacts between an investigating female and objects were very transitory and rarely involved the female settling if the object was not a crucifer or a nectar source. On contacting some crucifer individuals, females landed upon the inflorescence and after a second or so bent their abdomens around under the flowerhead; usually an egg was then deposited upon a flowerbud, a pedicel or the stem of the plant. No settling was observed without the following of either feeding or abdomen-bending, but abdomen-bending did not inevitably lead to oviposition. In these circumstances the females remained on the flowerheads for a long time (up to 30 seconds), and subsequently were often seen to perform similar behaviour, without ever laying an egg - perhaps indicating that they had no more mature eggs available for deposition that day.

These casual observations indicate that host location is initially visual and that thereafter the female possesses means for the identification of Cruciferae which are contact-dependent. Similar sequences of host location and identification are known from other Lepidoptera, where a non-specific visual

Fig. 26. Fore-Tarsus of A.cardamines female. (S.E.M.)

Fig. 27. Detail of Fig. 26. showing chemosensory hairs above tarsal claws.


100µ


40µ

cue is followed by a specific odour or contact stimulus (e.g. Yamamoto et al, 1969; Saxena and Goyal, 1978; Rausher, 1978). In Pierini adults, the contact stimulus is the presence of mustard oil glucosides. The sense organs sensitive to these chemicals are the tarsal hairs concentrated upon the forelegs of females (Fox, 1966). Chun and Schoonhoven (1973) have demonstrated the sensitivity of these chemoreceptive organs to a range of mustard oil glucosides.[*1] The mechanism appears to act by the abrasion of plant material by tarsal claws, leading to the release of volatile glucosides which then stimulate the tarsal hairs. Using Scanning Electron Microscopy, it was possible to demonstrate similar hairs on the tarsi of A.cardamines (Figs. 26 and 27); again they concentrated upon the fore and mid legs of females. It seems reasonable to assume that the tarsal hairs fulfil a similar function in Euchloini to that found in Pierini. The efficiency of host selection by A.cardamines is demonstrated by the fact that only a single instance was noted during this study of an egg being laid upon a non-crucifer. At Langwathby in 1978 an egg was found deposited upon a blade of grass - which was entangled with the flowerhead of a C.pratensis plant.

[*1] These receptors are also involved in sensitivity to sugars, and stimulation of the diffusely distributed hairs leads to proboscis extension. Sensitivity to glucosides is restricted to the clumps of hairs.

Two mechanisms of host selection are thus sufficient to explain host finding in A.cardamines - an initial visual response to an inflorescence, followed by a response by tarsal sensillae to glucosides. However, other responses are not excluded. In P.brassicae, although chemoreception is not suspected in host location (as opposed to host selection), there exist antennal receptors sensitive to flower odours and mustard oil glucosides. Examination of A.cardamines antennae reveal complex structures, with four or more different types of sensilla - it is therefore possible that the visual response of the females might be triggered or enhanced by volatile plant odours (including flower scents[*1]) although there was no evidence of this in the field. In other species, A.rapae respond to cabbage behind glass, and Traynier (1979) is of the opinion that olfaction is of little importance in host location. Finally, it should be noted that other plant chemicals or physical properties may be involved in, e.g. rejection of unsuitable oviposition sites (Wiklund and Ahrberg record instances of female A.cardamines rejecting crucifers which bent under their weight).

b. Methods and Observations

The methods involved in investigating oviposition behaviour were various. Some observations were made on captive females, but the majority of data was collected in the field, from observed egg distributions, and by following

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[*1] As shown for some species by Tinbergen (1951).

females and noting their success in host location and choice. These observations were augmented by transplanting and rearing experiments.

Following individual females was of key importance in this study. For this it was necessary to follow one animal for as long a period as possible, to observe her behaviour, including patterns of movement, and to record the characteristics of the plants, especially Cruciferae, which she encountered. This work was originally planned for 1978, but delayed by bad weather until the following year. Two or more observers were necessary, each equipped with binoculars and tape-recorder. One observer noted behaviour of the female, whilst the other noted the density, age, size and species of any crucifer inspected (these data were then compared to the population of plants as a whole). In all, 22 females were followed during the summer of 1979 (13 during the middle part of the flight season when A.petiolata was the major foodplant available, and was also in good condition - age classes 1 and 2. 9 during the latter part of the season, when a substantial part of the A.petiolata population was aged). The sequences of female behaviour were thus recorded, and the 'early phase' results are shown in Fig. 28. From these results, it is seen that certain activities were associated with others (Table 17). For instance, oviposition was frequently preceded or followed by 'long distance' flights of over 20 m. However, this was not always the case, sometimes oviposition was followed by feeding upon the same flower; this agrees with the obser-

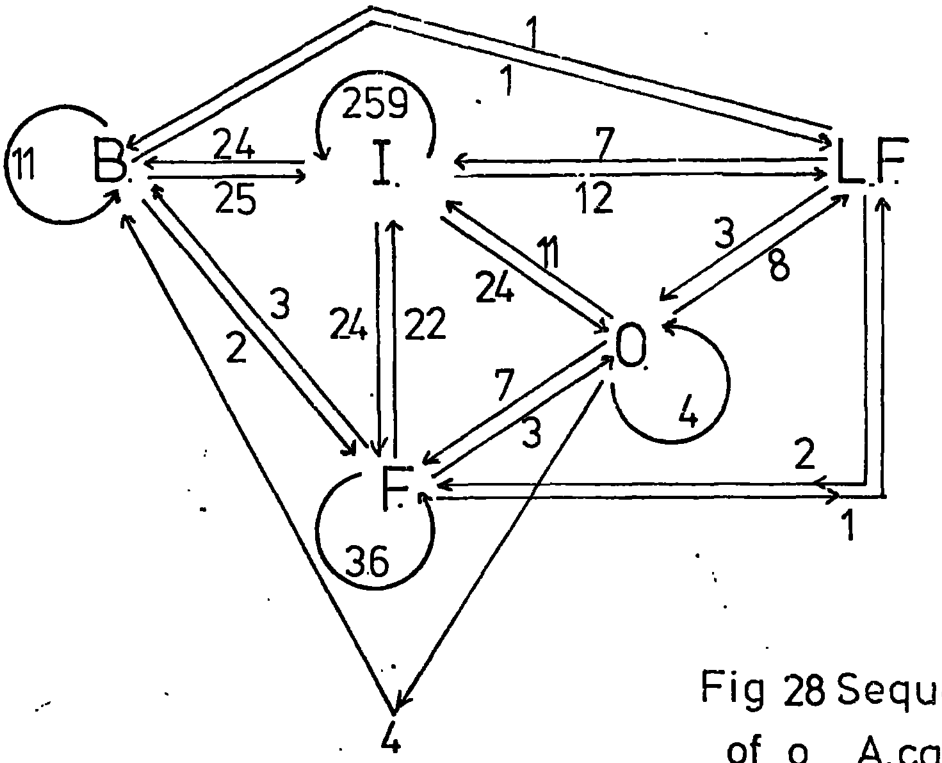

Fig 28 Sequences of ♀ A.cardamines Behaviour

	Exp	Obs		Exp	Obs
O→I	22·8	11	I→O	24·1	24
O	2·4	4	O	2·4	4
B	3·0	4	B	2·7	0
F	4·3	7	F	3·9	3
LF	1·5	8	LF	0·9	3
I→F	43·2	24	I→B	30·5	24
O	4·3	7	O	3·0	4
B	4·9	2	B	3·5	11
F	6·9	36	F	4·9	3
LF	1·6	2	LF	1·2	0

Tab17 Association of behaviours in Fig28

KEY

F	FEED
B	BASK
O	OVIPOSIT
I	INSPECT (without F,B,O)
LF	FLIGHT > 20m.

vations of Floersheim (1906) and contrasts with the view of Wiklund and Ahrberg, that "After depositing the egg she (the female A.cardamines) immediately flies off".

The aim of this study then was to outline foodplant finding and choice, and to show to what extent these behaviours might be viewed as 'adaptations', i.e. to what extent they contribute to fitness in the field, and to what extent they might have evolved by selection against alternative behaviour. A simple example of this approach is the following section on shaded foodplants. Observations at the Durham site indicated that shaded plants of A.petiolata and H.matronalis received few, if any, eggs. (Table 18). A similar observation was made for A.napi by Chew (1974) who noted that shaded plants of Cardamine cordifolia, despite being of an optimal species for larval growth, received no eggs.

Table 18

A.cardamines eggs upon shaded and unshaded plants at Durham, 1977 (June 4-7)

Plant species	Shaded		Unshaded	
	No. Plants	No. Eggs	No. Plants	No. Eggs
H.matronalis*1	36	3	123	45
A.petiolata*2	83	3	601	141

χ^2 (with Yates correction)

*1 5.45 $P < 0.05$

*2 11.83 $P < 0.001$

Two explanations of this behaviour are offered. Firstly that it may be adaptive, females avoiding laying eggs on shaded plants, because survival of immature stages is poor there. (This might be the case if shaded plants were e.g. a more favoured environment for predators, or were predominantly of a toxic species). No evidence was found for this suggestion. Thirteen larvae transferred at the 2nd instar to shaded H.matronalis plants at Durham in 1978, all completed development and left the plant. (Wiklund and Ahrberg record that shaded plants live longer than unshaded ones.). The alternative suggestion is that the observed egg distributions may reflect the area of activity of the females, since it is to be expected that females will restrict their activity to areas of direct sunshine, where body temperature will be kept high. This is the case in A.cardamines where individuals of either sex only entered shade to escape from interactions with other butterflies, and left shade immediately afterwards. Thus restriction of oviposition to plants in sunny areas, appears not to be an adaptation to avoid poor survival on other plants (but will maximize female activity). The alternative strategy of searching all areas, would evolve only when females found more plants, and laid more eggs, by doing so. Rather than being an adaptation per se, avoidance of shaded areas is likely to be a side-effect of other aspects of searching behaviour. This rather trivial example, of rejection of an alternative hypothesis, illustrates the approach to be followed in the subsequent sections.[*1]

c. Foodplant age

Investigations were carried out into the effect of foodplant age upon oviposition. Plants of A.petiolata were placed in 3 age-groups as follows:

Age-class 1. Less than 25% of seedpods mature.
Age-class 2. More than 25% of seedpods mature.
Age-class 3. Flowerhead senescing, seedpods hardening.

The number of A.cardamines eggs recorded on plants of each age-class was then recorded at dates when all three classes were common (Fig. 29). In the field,plants of age-class 3 receive significantly less eggs than those of the other two classes. This might reasonably be taken as adaptive behaviour by the female, since one intuitively feels that her fitness is maximized by laying eggs only on plants of good food quality; moreover, evidence exists to support this view. Establishment of young larvae is better on flowers and young siliquae of A.petiolata than an old siliquae (q.v. Table 9).[*2] Again, old larvae are more likely to leave the seedheads of old plants, moving down onto the less preferred leaves or even abandoning the plant altogether (Table 19).[*3]

[*1] Rausher (1979) has recently described the adult behaviour and larval survival of three tropical species in relation to shade. Shaded plants support better larval survival, but are not chosen by two of the three species. Rausher argues that this could be either a strategy (in time and space) or caused by differences in the rate of foodplant discovery amongst habitats.

[*2] Old siliquae are sometimes found on A.petiolata plants of Age-class 2, generally low down.

[*3] Again, in the case of several eggs being laid together, the first laid is the most likely to survive cannibalism.

Fig 29 The percentage of *A.petiolata* plants of different ages with *A.cardamines* eggs

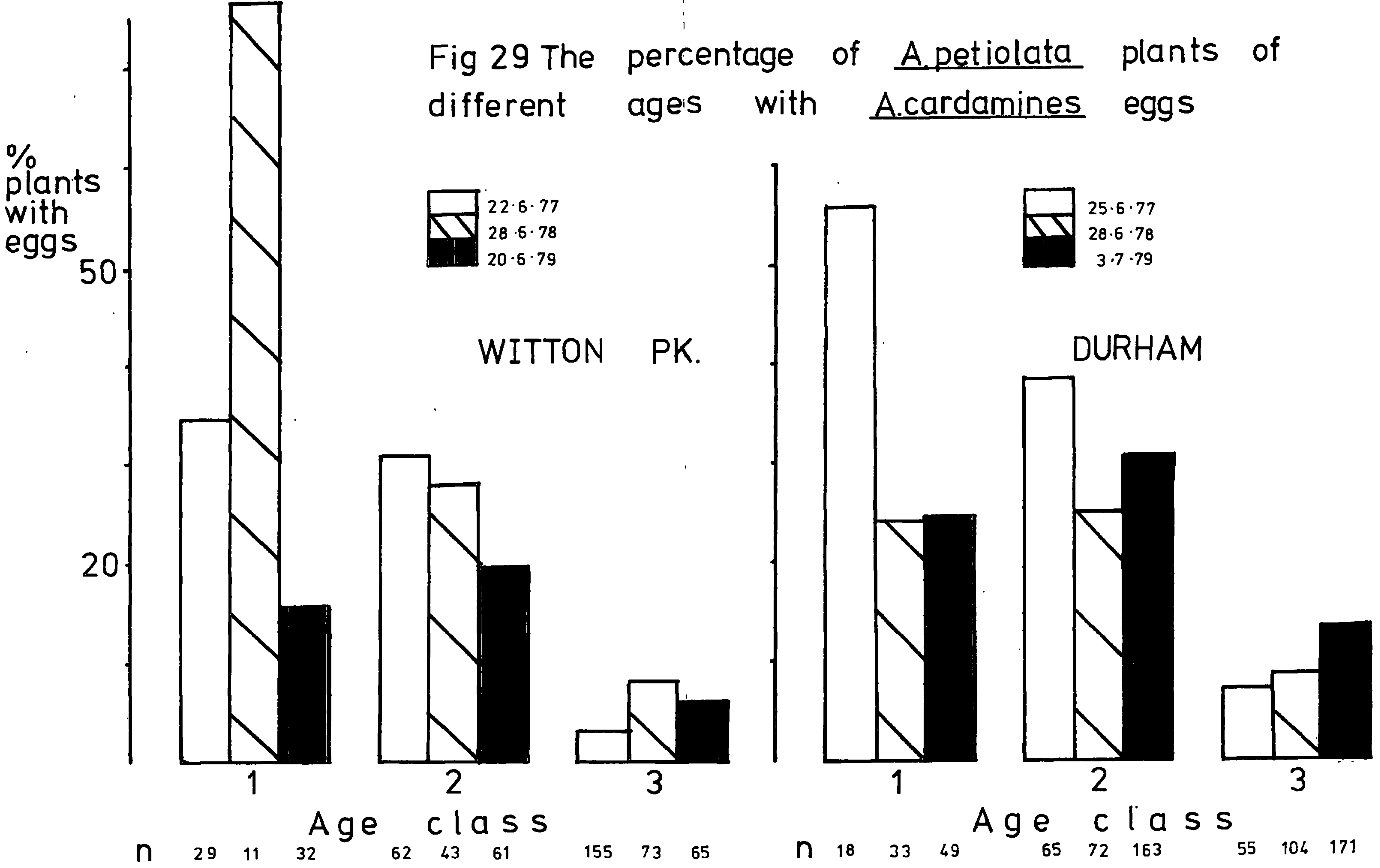

Table 19

Larval movement due to foodplant age (on sites 1978-9) (no. of larvae).

(3, 4, 5 instars)

Larval Responses	*A.petiolata* plants recorded as dry or senescing	Other *A.petiolata* plants
Remain on seeds	24	138
Transfer to leaves	12	1
Leave plant	12	2

1 2nd instar larva transferred from a senescing *A.petiolata* plant, but chose another and died.

Movement was also seen from depleted *B.vulgaris*, *C.amara* and *C.pratensis* and from the seedheads of *C.bursa-pastoris* and *S.officinale*, which offer little food.

Hence any female which specialises in laying her eggs on only young foodplants might be hypothesised to leave more surviving offspring than a female who is less specific in her choice, i.e. the behaviour is an 'adaptation' to avoid unsuitable plants. Similar situations are known to occur in the crucifer specialists Myzus persicae (Hodgson, 1978), Euchloe spp. (Opler, 1974) and A.rapae (Jones and Ives, in press), where young stages are concentrated on plants which maximize survival.[*1] Similarly Watanabe (1979) and Schweitzer (1979) have completed detailed studies on the effects of foodplant age upon the growth and survival of species of Lepidoptera; both studies support the view that early growth is optimal for larval growth, and that such 'foliage is an ephameral resource'. Wiklund and Ahrberg (1978) are of the opinion that in Swedish populations:

> "Females of A.cardamines only oviposit on young inflorescences which may be regarded as an adaptation to avoid that the host plants will have time to mature and cast their seeds before the larva has had time to complete its development."

However, an alternative hypothesis is possible; the size of the visual cue, the white florets of the inflorescence, is very much less on old flowers than young ones. If the size of the visual cue presented to the female is important in determining whether she finds that hostplant, then one would expect the same distribution of eggs as from the above hypothesis. To distinguish between these alternative

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[*1] Jonasson (1977) has described how female frit flies (Oscinella frit L) lay eggs on young shoots of oats, because larvae are unable to penetrate into old shoots.

Table 20

Responses of A.cardamines females to A.petiolata of different ages

(following observations 2nd part July 1979)

	Age Class			
	1	2	3	T
No. in A.petiolata sample*	49	163	171	383
% in A.petiolata popln.	12.8	42.6	44.6	
No. with A.cardamines eggs	12	50	23	85
No. of Inspections*	15	63	28	106
% of Inspections	14.2	59.4	26.4	
No. of ovipositions	3	8	2	13

$^{*}\chi$ 3 x 2 = 59.4

at 2 df $p < 0.001$

hypotheses, the age-classes of plants encountered by females were compared to the population of A.petiolata as a whole. If females encountered old plants in proportion to their abundance in the population, then one can reject the second hypothesis - that is, females would be seen to be refusing to lay their eggs upon old plants. However, if they did not encounter old plants (and given that they show investigative behaviour to any encountered object) then that is evidence supporting the second hypothesis. The results of these observations are given in Table 20; it is seen that females encounter significantly less plants of Age-class 3 than expected. The second hypothesis is thus supported; however, one can not yet reject the first hypothesis. One method of doing so would be to demonstrate that having encountered a crucifer inflorescence, the female is equally likely to lay on it, regardless of its age. Table 20 shows the limited data that are available on this point. The difference between acceptance of plants of differing ages is non-significant, but this is not sufficient to accept the null hypothesis of no difference between plants of different ages, on such a small sample. Wiklund and Ahrberg record that females were:

> "on several occasions observed to refuse to deposit eggs on aged and characteristically elongated inflorescences ... after being initially attracted by the bright colours of the petals."

More systematic data on this point are needed. It is clear in A.rapae (Jones, 1977) and E.hyantis (Opler, 1974) that female rejection of older plants does occur. Moreover, in

Nearctic Euchloe spp. (Opler, 1974) and in Z.eupheme (per. ob) plants which present the largest visual cue are avoided, and eggs are laid solely upon unopened flowerbuds which have a very small visual cue. In these species of Euchloinid hypothesis 2 is untenable, but in A.cardamines the evidence suggests that visual searching by the female, and the small size of the visual cue presented by old flowers, is responsible for most, if not all, of the observed age effects.[*1]

It appears that females of A.cardamines, rather than avoiding unsuitable plants, succeed in finding only favourable ones. Thus a female's fitness is greater than in the hypothetical alternative case (finding and using all plants), but this state is not shown to have arisen by selection against the alternative strategy. Rather it appears that this adaptation, which is seen to increase fitness (when compared to the alternative), arises from an incidental effect unrelated to direct selection.[*2]

d. Foodplant size

In studies of a number of butterfly species, hostplants of different size receive different numbers of eggs. Thus in

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[*1] A similar composite solution has been favoured by Jones and Ives (in press) who record that A.rapae females have a greater inclination to land on large, as opposed to small, cabbage plants (visual response?), but lay more eggs upon young as opposed to old plants. These two responses lead to most eggs being laid upon middle-sized, middle-aged cabbages.

[*2] There yet remains the possibility that receptiveness of the female to the visual cue is itself an adaptation against old foodplants. This appears unlikely, and is an ad hoc defence which should not be adopted in preference to the more successful hypothesis. (Popper, 1957).

Papilio xuthus (Tsubaki, 1979), T.pruni (Thomas, 1974), L.Dispar (Duffey, 1968), A.rapae (Jones and Ives, in press), plant size appears to influence whether eggs are laid.[*1] In Euchloinids, several Nearctic species are recorded as choosing plants more than 5 inches in height (Opler, 1974), whilst Siche (1912) noted that A.cardamines laid eggs on C.bursa-pastoris specimens greater than 1 foot in height.

In the present study, the effect of foodplant size was investigated in a number of different ways. The mapped egg distributions allow the distribution over foodplants of different size to be calculated. However, these data are collected throughout the field season, and if plants of different sizes age at different rates, or if plant density influences size, then bias will occur. One additional problem is that large foodplants, being rare, will contribute to variance disproportionately (as in T.pruni (Thomas, 1974)). For these reasons it was decided to sample a large stretch of riverbank at Durham, and to examine every A.petiolata inflorescence, during a short period of time, before any A.petiolata plants had senesced. Efforts were made to sample only in the centre of areas of A.petiolata, and areas which were shaded were excluded. Efforts were made, with limited success, to include numbers of plants of the largest sizes.

Size of an A.petiolata plant was taken to be the number of inflorescences in bud or flower; this corresponds to the number of oviposition sites available to the female A.cardamines. No attempt was made to record height or

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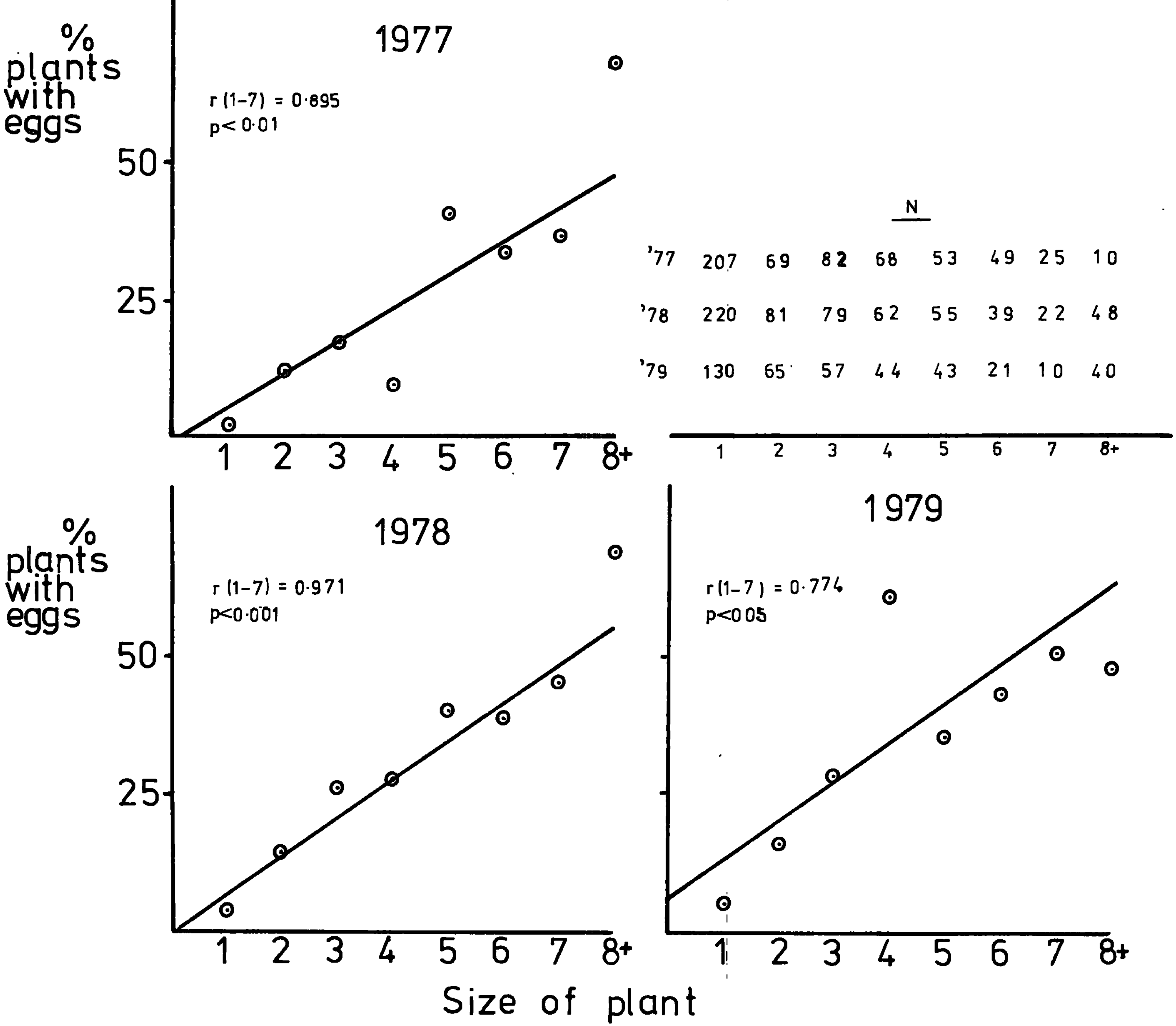
[*1] This does not seem to occur in A.napi macdunnoughi - data of F. Chew.

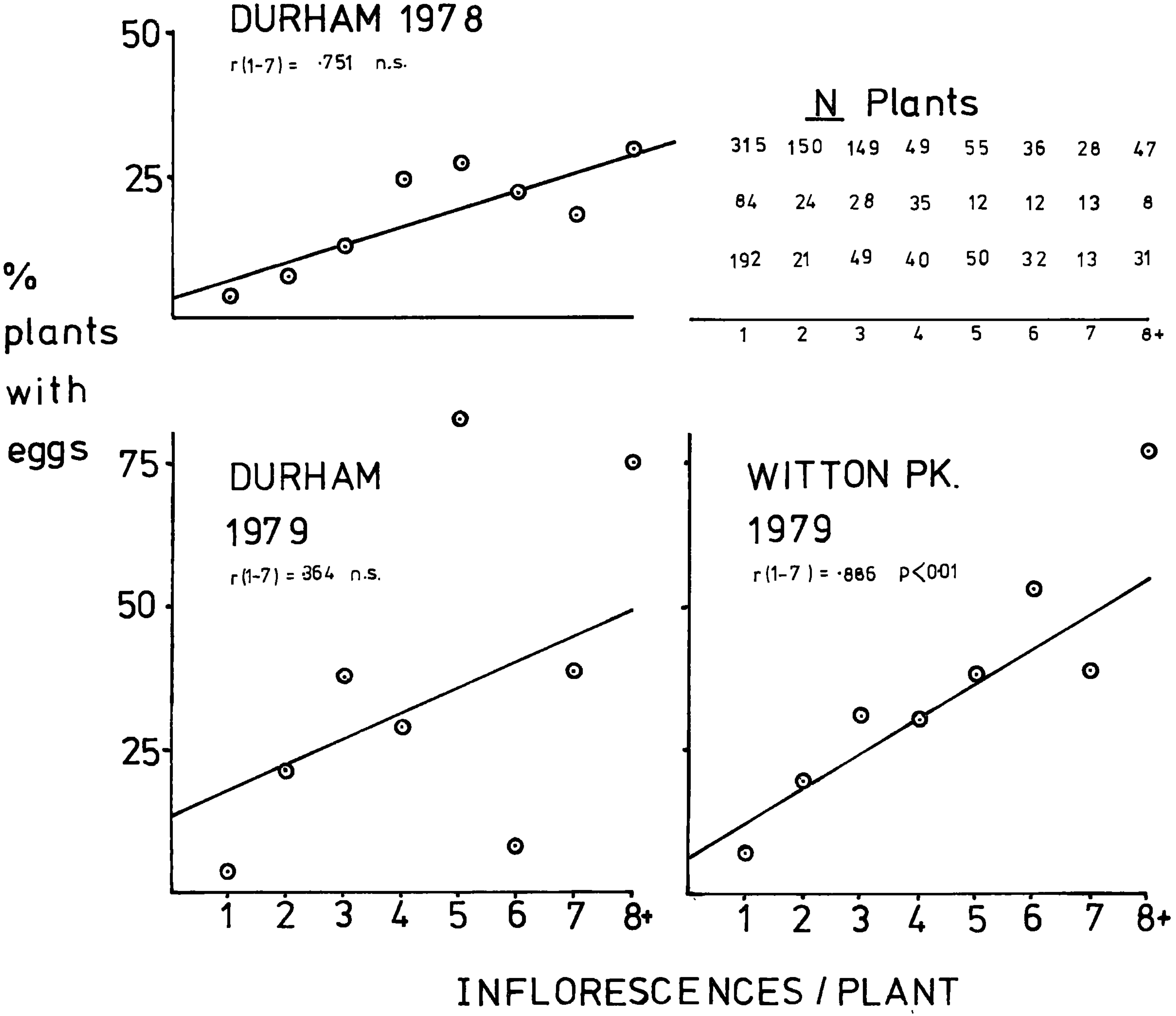


N

	1	2	3	4	5	6	7	8+
'77	207	69	82	68	53	49	25	10
'78	220	81	79	62	55	39	22	48
'79	130	65	57	44	43	21	10	40

Fig 30
Percentage of *A. petiolata* plants in each size class with *A. cardamines* eggs

DATES
4&5 · 6 · 77
16 · 6 · 78
19 · 6 · 79



N Plants

1	2	3	4	5	6	7	8+	
315	150	149	49	55	36	28	47	D. '78
84	24	28	35	12	12	13	8	D. '79
192	21	49	40	50	32	13	31	W. '79

Fig. 31 % of A.petiolata plants with A.cardamines eggs (on sites)

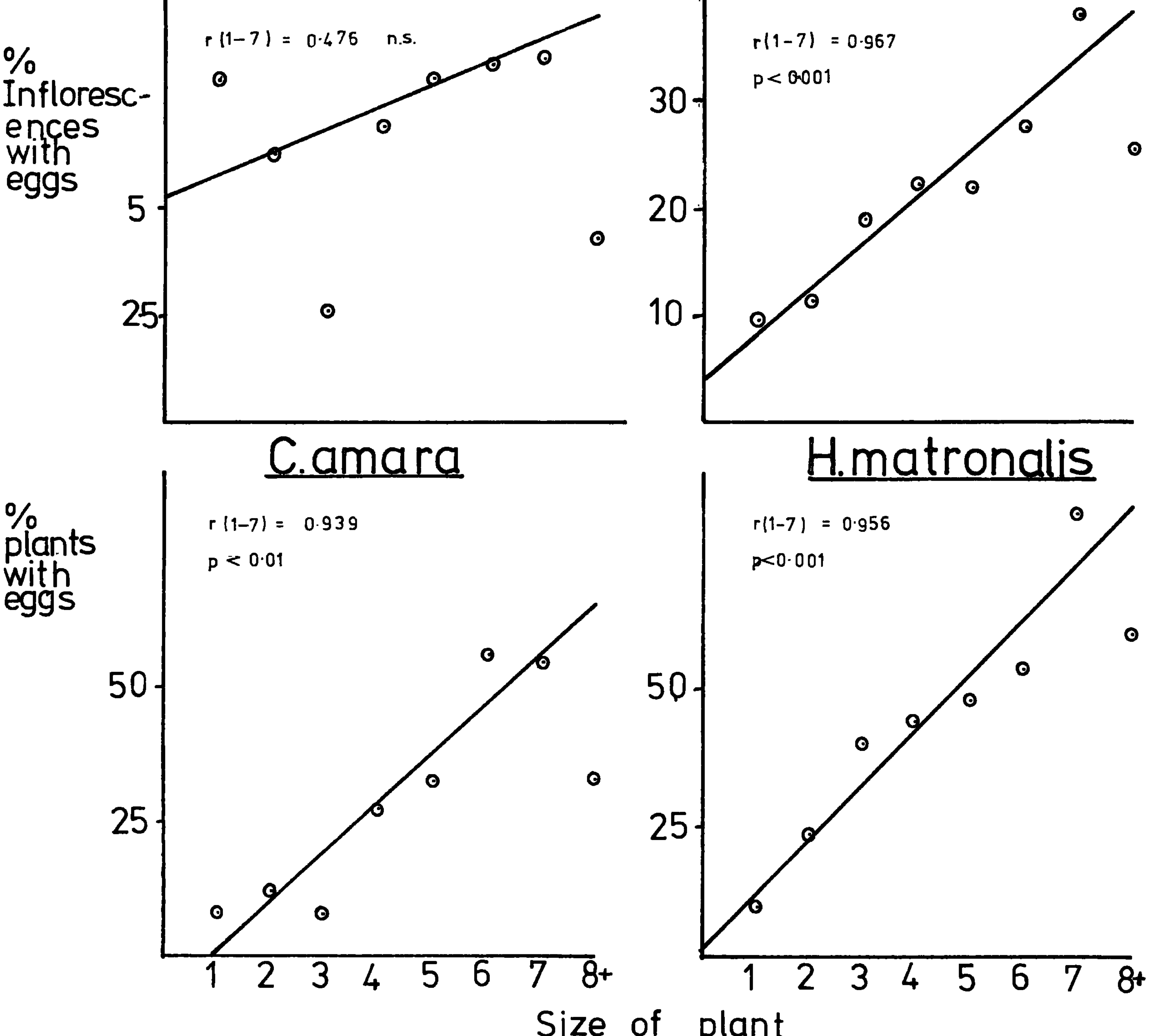


Fig 32

Percentage of plants and inflorescences of C.amara and H.matronalis with A.cardamines eggs

C.a. Wolsingham 9·6·79

H.m. Durham 10·6·78

N

Ca	124	16	13	18	15	7	9	6
Hm	156	17	30	9	21	13	6	10
	1	2	3	4	5	6	7	8+

width differences among plants of differing inflorescence number; all three characters appeared closely related. However some variation in height did occur, and this undoubtedly contributed to variance.

Fig. 30 records the results of these large scale searches, whilst Fig. 31 records the distribution of eggs upon A.petiolata of different sizes upon the mapped sites. Fig. 32 records two counts made for H.matronalis and C.amara. In all cases more eggs are seen to occur on larger foodplants. Several hypotheses are plausible explanations of this.
Hypothesis 1: That there is an interaction with another factor - efforts were made to counter this in sampling. (The interactions between age, size and density are discussed below).
Hypothesis 2: That the difference may reflect adaptive behaviour, that is the selection by the female of the most suitable plant for larval survival. Large plants might for instance resist dessication better, and provide more and better food than smaller plants. Such an adaptive hypothesis is favoured by Thomas (unpublished) for Maculinea arion, where predation is very much less upon larger plants.
Hypothesis 3: That the ovipositing female may perceive large plants more easily than small ones. This appears to be the case in A.rapae (Jones and Ives, in press). Curio (1977) has noted the general phenomenon of predators, whether using olfaction or vision, encountering and thus taking more large prey.
Hypothesis 4: That the differences may reflect nothing more than more eggs being laid because more sites are available

Table 21

Survival of A.cardamines on A.petiolata plants of differing sizes

Size of Ap	No. A.c.	% reaching pupae	Survival Index	(± S.E.)
1	46	32.6	2.98	(.34)
2	42	45.2	3.60	(.35)
3	51	25.5	2.64	(.29)
4	54	35.2	2.82	(.30)
5	69	26.1	2.53	(.26)
6	33	24.2	2.30	(.39)
7	23	43.4	3.43	(.54)
8+	163	36.8	2.95	(.19)

Data from all sites 1978 and 1979 (except Wolsingham in 1979 - a-typical survival data q.v.)

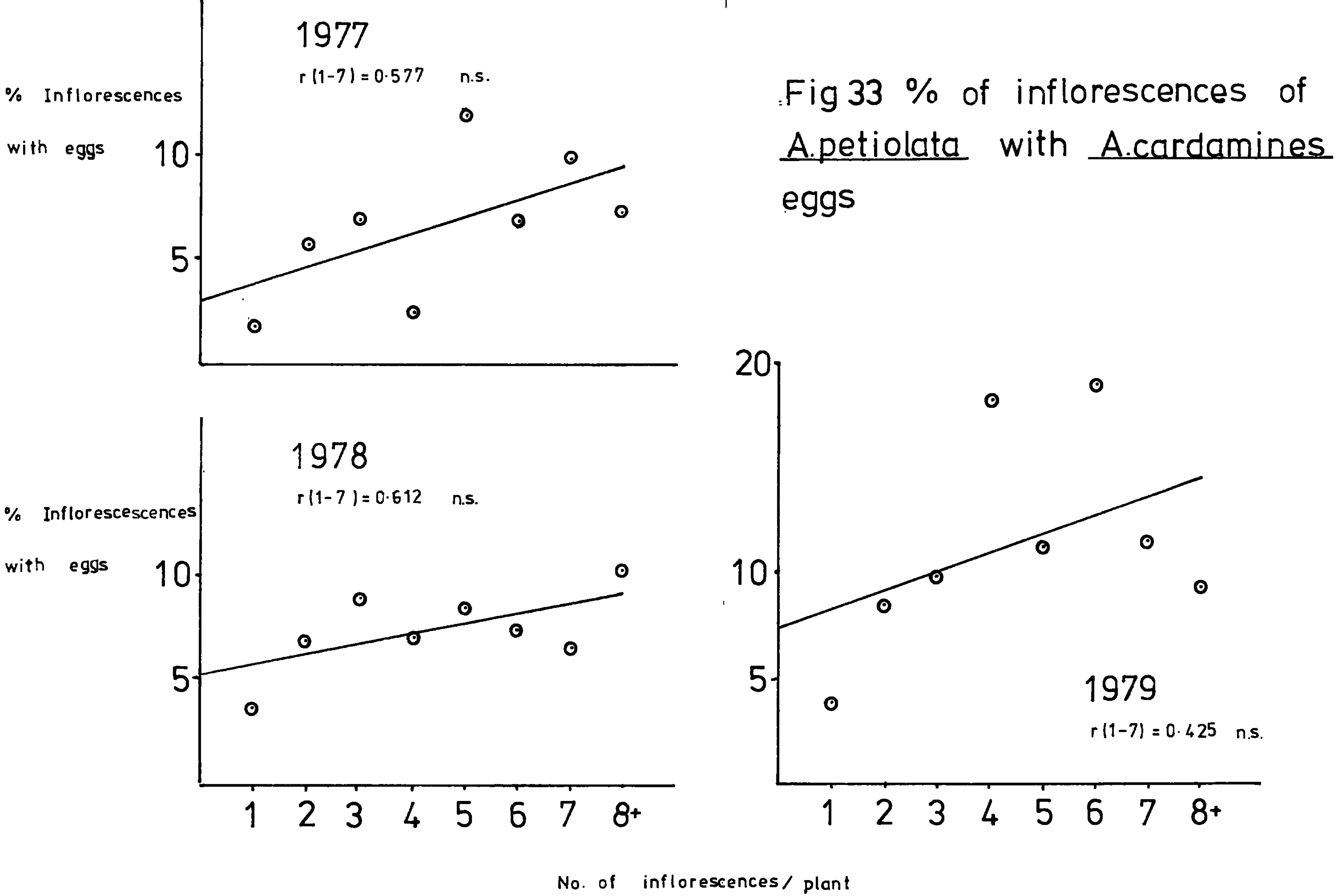


Fig 33 % of inflorescences of *A.petiolata* with *A.cardamines* eggs

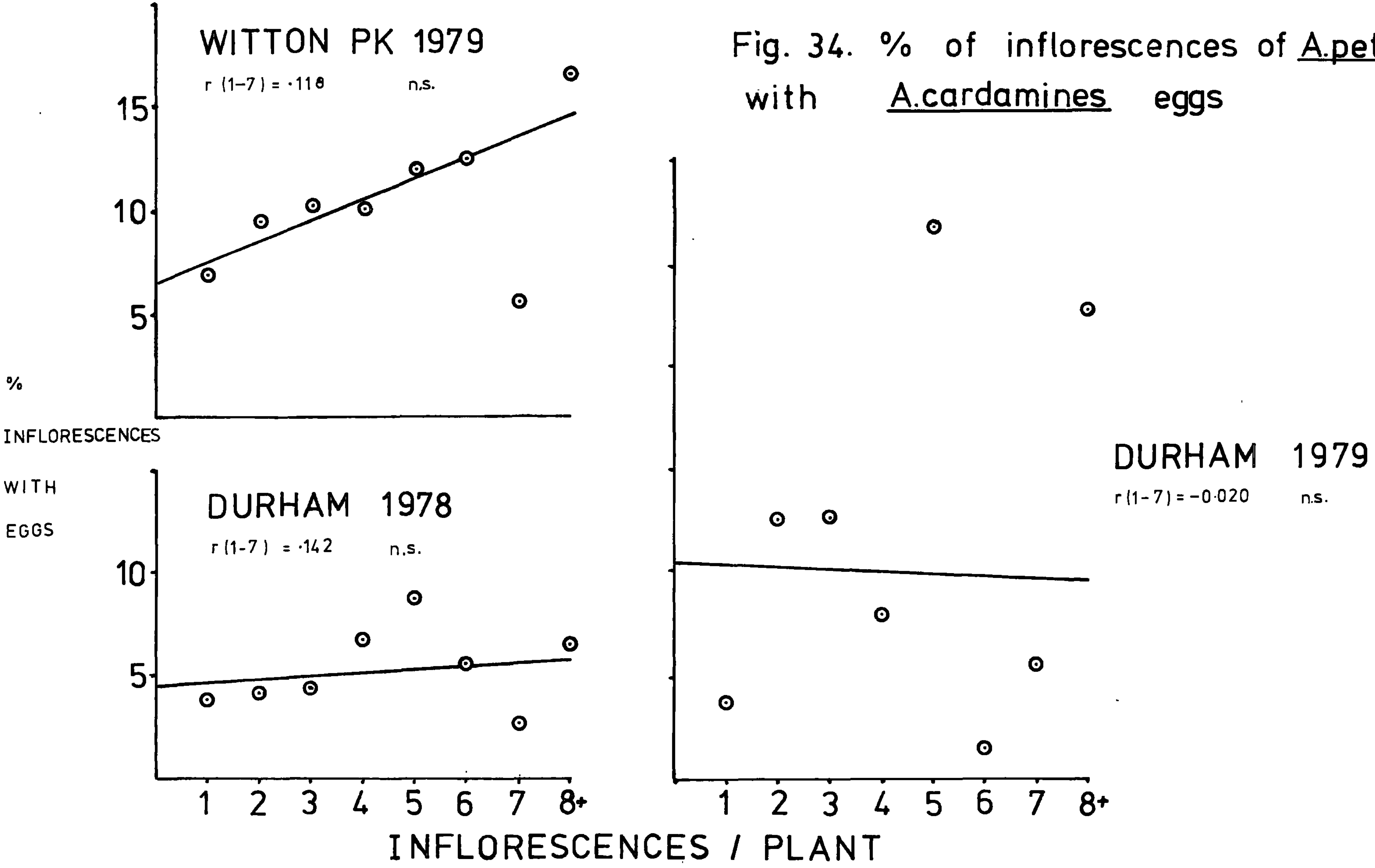


Fig. 34. % of inflorescences of *A.petiolata* with *A.cardamines* eggs

for them. Assuming hypothesis 1 to be incorrect, one may examine hypothesis 2 by comparing the survival of eggs laid upon plants of different sizes. There is no relationship between foodplant size and A.cardamines survival (Table 21), and this hypothesis may be rejected. To examine the last two hypotheses, the results of Figs. 30 and 31 are re-drawn as Figs. 33 and 34, which represent the percentage of oviposition sites which are utilised in each size class (corrected for double ovipositions). If size effects reflect simply the number of oviposition sites available, then one would expect no relationship to be shown in these figures, and the graphs to describe a line parallel to the X axis. This appears to be the case, thus suggesting that size effects are largely a result of oviposition site number. However, one notable feature of these figures is the under-representation of the very smallest foodplants, which receive fewer eggs than the others (this occurs in 5 of the 6 figures). Plants with one or two flowerheads are not receiving as many eggs as larger plants.

Hypothesis 3 suggests an explanation. If the visual cue to the female is partly dependent upon size, then one would predict distributions of the same shape as in Figs. 31 and 32. Appendix 5 presents this reasoning, and produces a simple model which appears to agree well with observed female behaviour. (The data for H.matronalis (Fig. 32) do, however, show a relationship between oviposition site number, and the % of such sites used. This may not be in opposition to applying hypotheses 3 and 4 to the A.petiolata data,

Tab 22

Female encounters with *A. petiolata* in different size classes compared to the population as a whole

Size	No Encounters (E)	No in Population (P)	E as % T_E	P as % T_P	E/P
1	62	179	21.3	34.6	0.346
2	28	90	9.6	17.4	0.311
3	57	70	19.6	13.5	0.814
4	38	53	13.1	10.3	0.717
5	63	57	21.6	11.0	1.105
6	16	27	4.7	5.2	0.592
7	7	15	2.4	2.9	0.466
8+	19	25	6.5	4.8	0.760
	$T_E = 291$	$T_P = 517$			

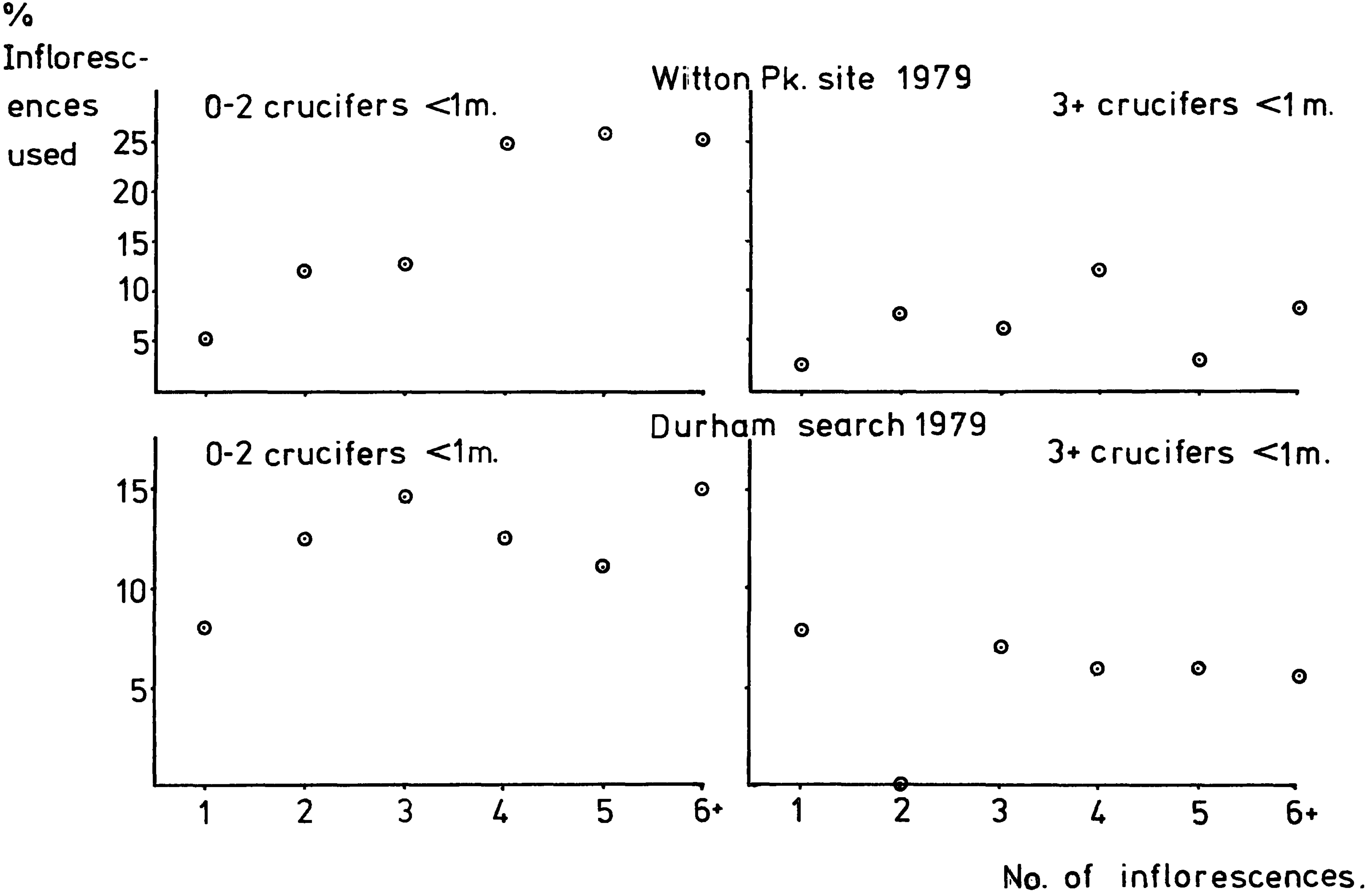
χ^2 2 x 8 TABLE

= 46.43

AT 7 d.f. $p < 0.001$

Fig. 35. The percentage of inflorescences on *A.petiolata* plants of different sizes, used as oviposition sites by *A.cardamines* at low and high plant densities (0-2 v 3+ crucifers within 1 m.)

The data presented are from the site mapped for larval survival at Witton Park in 1979, and the large scale search at Durham in 1979.


%
Infloresc-
ences
used
Witton Pk. site 1979
0-2 crucifers <1m.
3+ crucifers <1 m.
25
20
15
10
5
Durham search 1979
0-2 crucifers <1m.
3+ crucifers <1m.
15
10
5
1
2
3
4
5
6+
1
2
3
4
5
6+
No. of inflorescences.

since H.matronalis has very large and closely bunched flowerheads. Increasing the size of the bunch may increase the area over which the plant is visible. Saxena and Khuttar (1977) have shown that the visual angle subtended at the ommatidia is important in Lepidopteran orientation, rather than the size of distance of the perceived object).

One deduction that follows from hypothesis 3 is that the plants actually encountered by a female (as opposed to those in the population as a whole) should be distributed in size in the same way as the egg distributions - that is, the hypothesis predicts that females are not able to perceive small plants as efficiently as they do large ones. The results of the following observations (first period) are used to answer this prediction, in Table 22. It is seen that females encounter small plants significantly less than expected from the observed distribution of plants in the population as a whole.

A second deduction of this hypothesis is that, if plants growing closely together are perceived as a single unit by females, then small plants growing intermingled with other plants should receive a greater proportion of eggs than similar plants growing singly. The asymptote-like curve of Figs 33 and 34 should thus be maintained when isolated plants are examined, but a straight line parallel to the X axis should be found when groups of closely-packed A.petiolata plants are examined. This re-analysis was (Fig. 35) undertaken for two data-collections which were sufficiently large. Again the prediction is confirmed. This also shows the source of some of the variance in Figs. 33 and 34.

The composite final hypothesis is thus that larger foodplants receive more eggs because they have more available oviposition sites; however, female searching behaviour also influences egg distributions, particularly when the visual cue is small.

e. Density and edge effects

Casual field observations during 1977 revealed that individual crucifers sometimes received extra-ordinary numbers of A.cardamines eggs. Thus, in 1977 at Egglestone, one H.matronalis plant bore 68 eggs upon 10 flowerheads. Similar observations elsewhere indicated that there was much variance in egg loads upon plants and suggested that outlying plants in particular received heavy loads. Examination of mapped distributions confirmed this suggestion. At all sites and in all years, plants on the edge of a clump are more likely to be attacked by A.cardamines than those in the centre. This is most strikingly visible in the mapped distributions for the plot of H.matronalis at Alston. This fortuitous find of 1977 is important in that the stand of crucifers is uniform in species, size and flowering time; thus it is not subject to the sources of variance found in other sites. Fig. 36 illustrates the distribution of eggs and plants at Alston in 1977, whilst Fig. 37 presents the data from this site for all three years as density plots. Eggs are strongly concentrated upon outlying edge plants, although some are present on central plants as well.

The 'edge effect' has been noted repeatedly for insects particularly A.rapae (Kobayashi, 1957; Ashby, 1974;

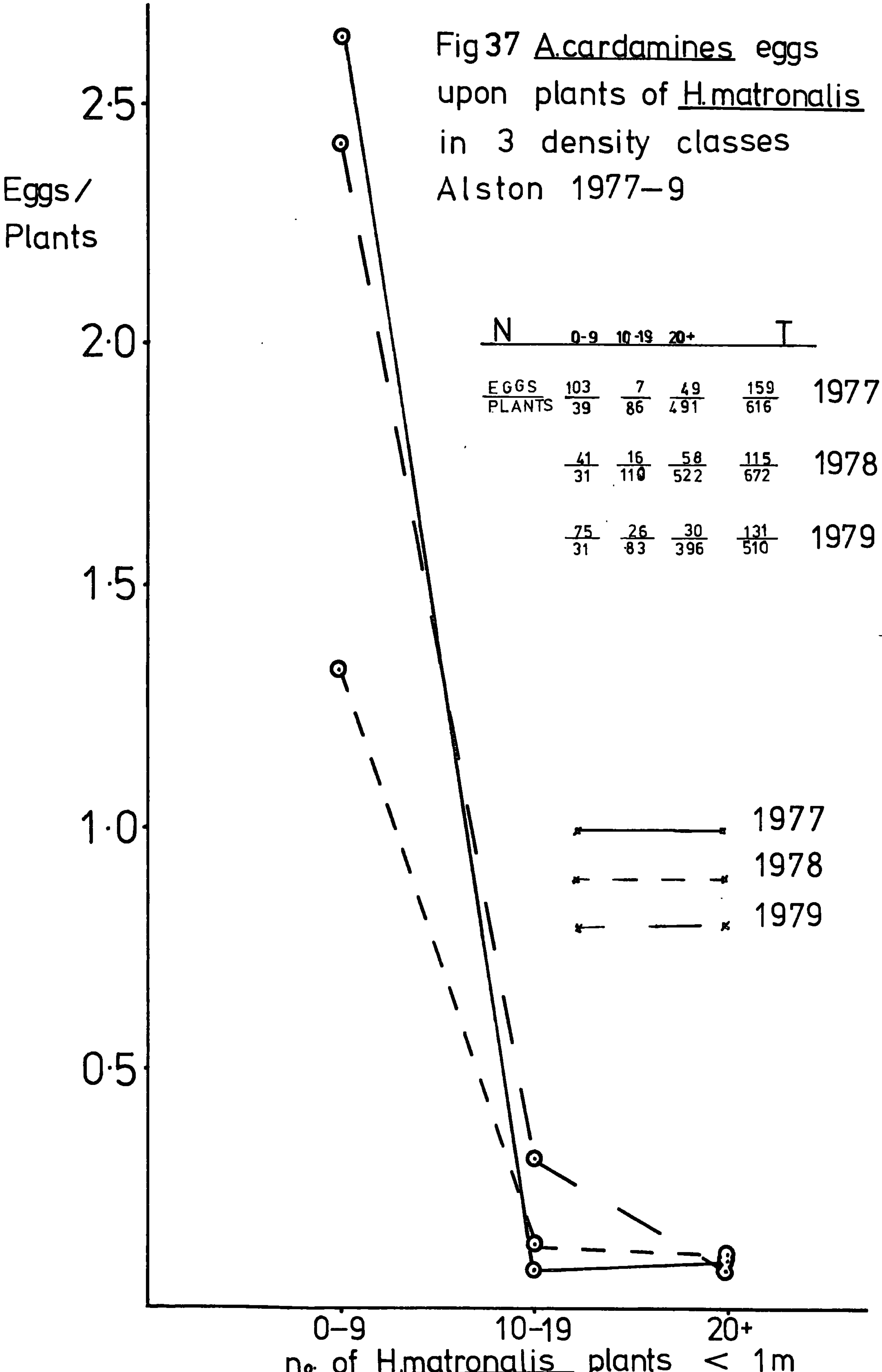


Fig 37 *A.cardamines* eggs upon plants of *H.matronalis* in 3 density classes Alston 1977–9

N	0-9	10-19	20+	T	
EGGS/PLANTS	103/39	7/86	49/491	159/616	1977
	41/31	16/119	58/522	115/672	1978
	75/31	26/83	30/396	131/510	1979

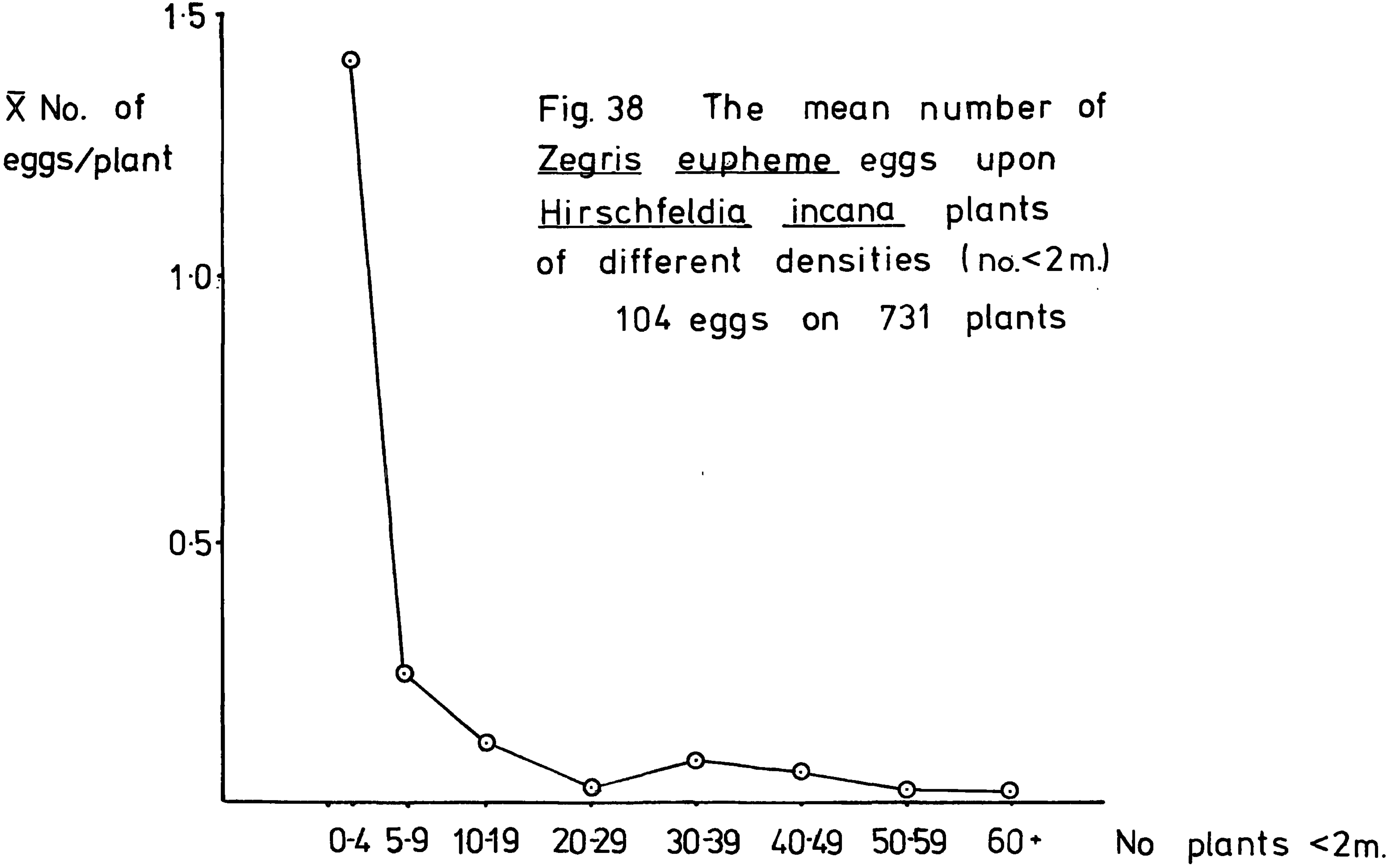


Fig. 38 The mean number of *Zegris eupheme* eggs upon *Hirschfeldia incana* plants of different densities (no.<2m.)
104 eggs on 731 plants

Cromartie, 1975; Jones, 1977) but also other butterflies, e.g. *Danaus chrysippus* (Edmunds, 1976), *Cupido minimus* (Gedge, 1897) and other Crucifer specialists, e.g. beetles, weevils and midges (Free and Williams, 1978, 1979). In *Pontia protodice* and *P.occidentalis*, edge effects cause interspecific competition although foodplant resources are not limiting to either species (Shapiro, 1975). A similar effect was observed here with *A.napi*, and in North Africa for *Euchloe belemia* and *E.ausonia*. *Zegris eupheme* again shows a very strong edge/density effect, in a population studied at Ifrane, Morocco. (Fig. 38).

Two hypotheses have been suggested to account for edge effects in Pierinae.

Hypothesis 1 - That the effect is adaptive, and arises from female choice for those areas where larval survival is best. As advanced by Shapiro (1975) this argument relies upon the fact that parasites of crucifer-specialists (e.g. the Braconids *Diaertiella rapae* (Read et al, 1970) and *Apanteles glomeratus* (Sato, 1979)) find their prey by searching for plant odours. Thus parasites are more likely to search areas of high plant density, and hence larvae placed as eggs upon isolated plants will survive better.

Hypothesis 2 - That the effect is of uncertain adaptive value and arises from female searching patterns. This was advanced for *A.rapae* by Jones (1977). It may arise in two manners:

2a - by females showing directionality of flight with a tendency to turn at clump edges (as in *A.rapae*).

2b - by females responding to the nearest crucifer at points along her flight path.

Other hypotheses which might explain edge effects are:

3 - that females are more responsive to plants after flying long distances. This might either be by laying more eggs or a lowering of any selectivity on the females part.

4 - that females are responding to differences in crucifer quality, caused by density effects on the crucifers themselves. This might be either:

4a - an adaptive response to those plants giving good larval survival (if, e.g. less crowded plants grew bigger, and bigger plants gave better survival)

4b - low density plants being more apparent to the searching females, by virtue of, e.g. bigger size.

In considering these hypotheses it is necessary to distinguish in some way between edge and density effects.. It is impossible to say which of these is the observed pattern of egg distribution, since edge plants must always be those at lowest density relative to the total patch. However, it is possible to determine the predicted effects of the different hypotheses. Thus hypothesis 1 predicts a density effect, where female A.cardamines should ideally lay eggs on crucifers with few other crucifers within that distance which is the perceptive range of the proposed parasite.

Hypotheses 2a and 3 predict edge effects, whilst 2b predicts a density effect dependent upon the perceptual range of the butterfly. Hypotheses 4a and 4b both predict density effects caused by interference or other interactions among plants.

Tab. 23. Survival of A.cardamines upon A.petiolata plants at different densities

No. crucifers 1< m	% larvae reaching 5th Instar	Survival Index (+ SE)	% Parasitisation*
0	38.4	3.07 (.26)	38.5
1	38.1	3.46 (.32)	50.0
2	27.8	2.56 (.29)	45.5
3	27.2	2.65 (.27)	42.8
4	22.6	2.48 (.28)	40.0
5	6.3	2.03 (.36)	33.3
6	32.4	2.79 (.46)	57.1
7	42.9	3.35 (.65)	0
8	40.0	2.96 (.52)	33.3
9+	31.7	2.90 (.29)	0

*Data from Witton and Durham only

In attempting to resolve which of these hypotheses is or are responsible for egg deposition patterns, it is important to note that a clumped distribution of eggs, which is the observed pattern, is disadvantageous to the population and the egg-laying females. Not only does this increase any competition for food both intra - and inter-specifically, it leads to cannibalism, and to significant loss of offspring.[*1] Hence any satisfactory explanation of the observed patterns must account for the stability and maintenance of the effect, despite selective pressure against it. It should also ideally apply to other species than A.cardamines. The generality of edge/density effects suggests that a general solution of the problem for all **Pierinae** is appropriate.

Hypotheses 1 and 4a hold that the effect is the result of adaptive behaviour where females choose optimal oviposition sites. There is no evidence to support this from the present study. Parasitisation and larval survival up to pupation (survival index q.v.) are not related to plant density (1 m is taken as an appropriate distance for both parasite discrimination and crucifer competition) (Table 23). It could be argued that the data on parasitisation reflect actual success at avoiding parasitisation rather than being a proper test of potential parasite behaviour. For this

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[*1] A clumped distribution will also lead to increased susceptibility to certain parasites and predators. As a strategy against any random source of mortality such as erosion, it is likewise a poor choice.

reason larvae of A.cardamines were transferred in summer of 1979 to plants of differing densities (larval density was carefully manipulated to be no more than 1 in an area of 1 m. around the plant chosen). On plants with no neighbours within a metre, 10 of 20 larvae were found to be parasitised, whilst on plants with more than 20 neighbours within a metre, 8 of 18 larvae were parasitised.*1 Since the behaviour underlying the edge effect cannot be maintained as a strategy leading to exploitation under hypotheses 1 or 4a in the absence of selection for it, and since such selection is not demonstrated in any of three years of study, both hypotheses may be rejected. Ashby (1974) gives data showing that mortality is higher on peripheral plants in A.rapae.

Hypotheses 4a and 4b hold that plants at low density are different in quality to those at high density. Such situations are well documented among many plant species, e.g. Sinapis alba and Lepidium sativum (Bazzaz and Harper, 1976). In wild parsnip the effect of density upon plant quality and through this upon animal grazing has been studied (Thompson and Price, 1977) and it was shown in this situation that the parsnip webworm (Depressaria pastinacella) optimises the quality of plants attacked, by selecting plants of low density. In A.cardamines similar situations could be envisaged, where for instance

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*1 larvae transferred as 3rd instar, collected as 5th. On A.petiolata plants 2 larvae were lost to other causes.

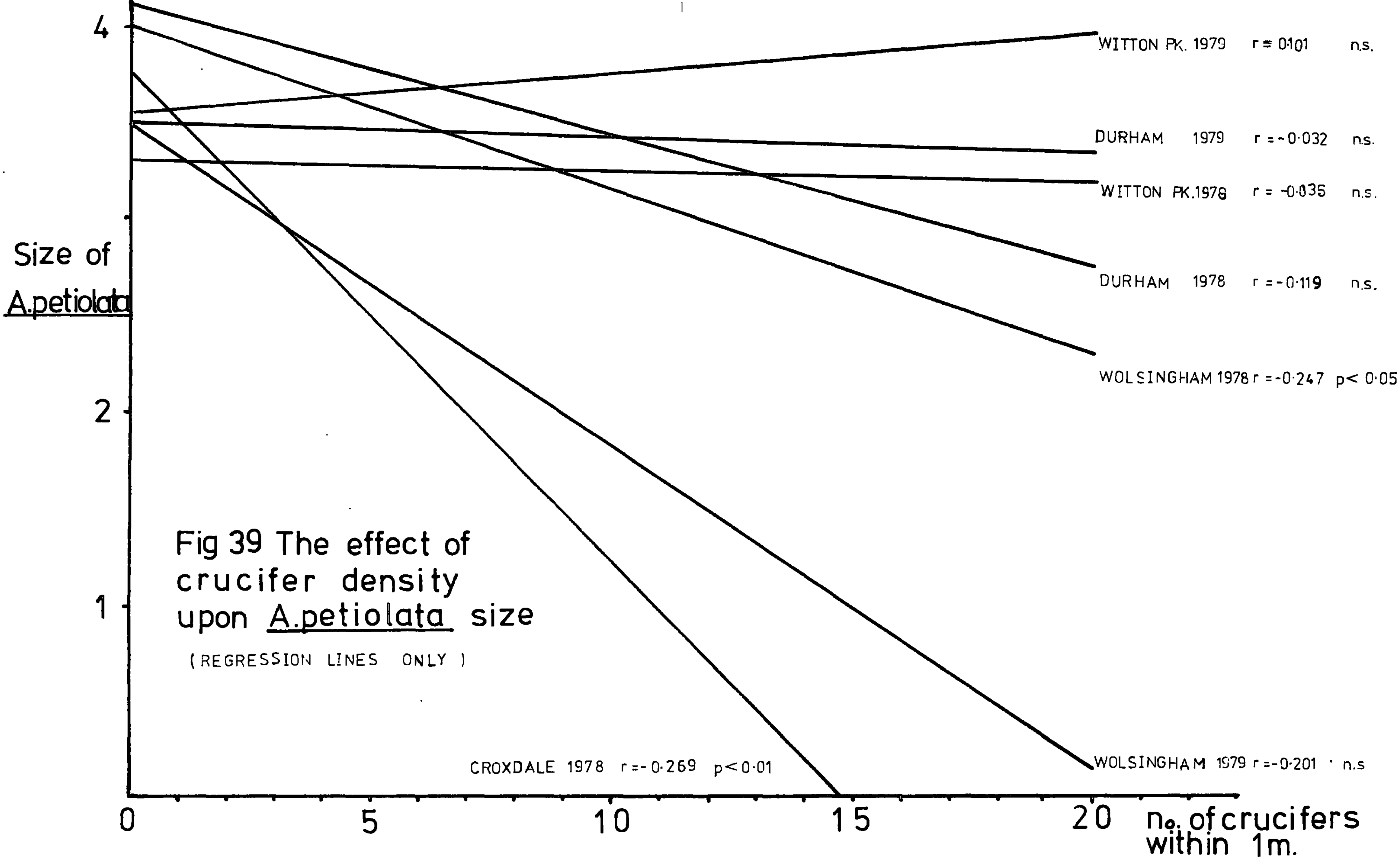

Fig 39 The effect of crucifer density upon *A.petiolata* size

(REGRESSION LINES ONLY)

low density plants were bigger and therefore had more oviposition sites. The mapped distributions allow the effect of density upon flowerhead size in A.petiolata to be investigated. (Fig. 39). It can be seen that density and size of A.petiolata are significantly related (but not at Durham or Witton) in some instances, although correlation values are very low, and much variance occurs. It may be that some **competition** (for soil moisture?) occurs sporadically in crucifer populations, and that this might have effects upon size. It is neither sufficiently strong nor frequent to be anything more than a sporadic and partial contributor to the edge/density effect.[*1] There remains the possibility that plants at different densities might senesce at different rates, thus affecting the time over which oviposition sites are available. Marked cohorts of A.petiolata plants at differing densities were followed in 1979 (20 in each group). Plants with no conspecifics within a metre reached age class 3 an average in 44.3 days from first flowering, which is not significantly different from those plants with 1 or more than 20 neighbours ($\bar{x}$ = 41.2, 45.3 respectively).

The remaining hypotheses - 2a, 2b and 3 - require information upon female behaviour in the field. This is particularly difficult to collect and liable to be subject

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[*1] In C.amara populations, e.g. at Wolsingham, size of inflorescence is positively associated with density, since plants are concentrated in the areas of best growth. Nevertheless an edge-effect of the distribution of A.cardamines eggs is found.

to considerable variation, due to individual differences in female behaviour. Some information is available, however, from the following of females (q.v) where the behaviour of females when approaching clumps of crucifers was noted, as was the number of neighbouring (within a metre) crucifers of each crucifer inspected. If hypothesis 2a <u>alone</u> is responsible for egg deposition patterns, then one would expect the female under observation to visit plants irrespective of the density of neighbours, to fly in straight lines, and to return at the edges of patches. The females observed (first period only) did fly in straight lines but turns were not associated with clump edges (Table 24).

<u>Table 24</u>

<u>Turning behaviour of searching females (1st part)</u>

No. of 'edges' found by ♀♀	39
No. of turns at 'edges'	10
No. of turns in 'centres'	24

On hypothesis 2b, females should respond to the nearest crucifer at points along her flight path. If in a 10 metre flight she searches for a foodplant every meter, then plants have a chance of receiving an egg directly inverse to the number of neighbouring crucifers, as below:

Table 25

Female encounters with A.petiolata plants at differing densities, compared to the total population

No. of other A.petiolata plants < 1m	No. Inspected I	No. in population (P)	I as % N_I	P as % N_P
0	87	45	29.9	8.7
1	79	64	27.1	12.4
2	27	64	9.3	12.4
3	30	70	10.3	13.5
4	26	62	8.9	12.0
5 - 8	39	97	13.4	18.8
9 - 16	1	75	0.3	14.5
16+	2	40	0.7	7.7
	$N_I = 291$	$N_P = 517$		

χ^2 2 x 8 = 147.1

at 7 d.f. $p < 0.001$ ($p = 2 \times 10^{-9}$)

Such behaviour would appear to be mal-adaptive in that many suitable foodplants are passed over. It does, however, resemble the observed behaviour of females, and might be envisaged to evolve as an adaptation against laying a second egg in the vicinity of the first (App. 6), or as a strategy against situations of low predictability of foodplants. From hypothesis 2b it may be predicted that females should encounter plants at low density less frequently than expected from the population of plants as a whole. (This is also expected from the rejected 4b). As is shown by the observations on female encounters (Table 25) this prediction is corroborated.

Hypothesis 2b would also be supported if oviposition was immediately followed by a dispersive move. Fig. 31 (q.v.) suggests that this may occur, as does the converse, oviposition following dispersion, which supports hypothesis 3. Other evidence exists for female behaviour being modifiable by searching success (see Appendix 6) and it is to be expected that in situations where a female carries many unlaid mature eggs, that she will be less specific in her choice of oviposition site than otherwise. This has been clearly shown in <u>A.rapae</u> females by Jones (1977).

It has not proved possible to demonstrate that any one factor is clearly responsible for producing the observed effects. However, it has been possible to reject certain hypotheses (1, 2a, 4a, 4b) as acting alone and to show that two at least are acting (2b, 3). These latter two are doubly interesting in that one predicts a density effect, the other an edge effect, strongly suggesting that no single solution will ever be available.

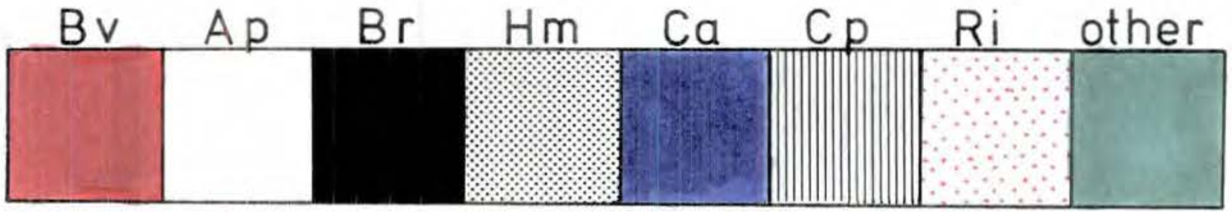
The compendium final hypothesis is then that female movement patterns and searching behaviour are responsible for producing observed egg distributions. This produces a clumped distribution, with resulting competition and death (and may lead to adaptations to counter these - see App. 6). However, the stability of such behaviour against such selection might be explainable as a generalised adaptation to overall low density of suitable oviposition sites. Wiklund has watched a single female A.cardamines fly for over two hours without once encountering a crucifer. The movement patterns and oviposition behaviour of A.rapae females are known to vary geographically in populations of very recent origin (Jones, 1977) strongly suggesting that such behaviours are both genetically determined and variable. The persistence of observed movement patterns in Durham A.cardamines remains an unresolved problem. However, one success of the final hypothesis is that, unlike the others, it gives a plausible account for 'edge effects' being general to the Pierinae, since it suggests a general adaptation to low foodplant (crucifer) density.

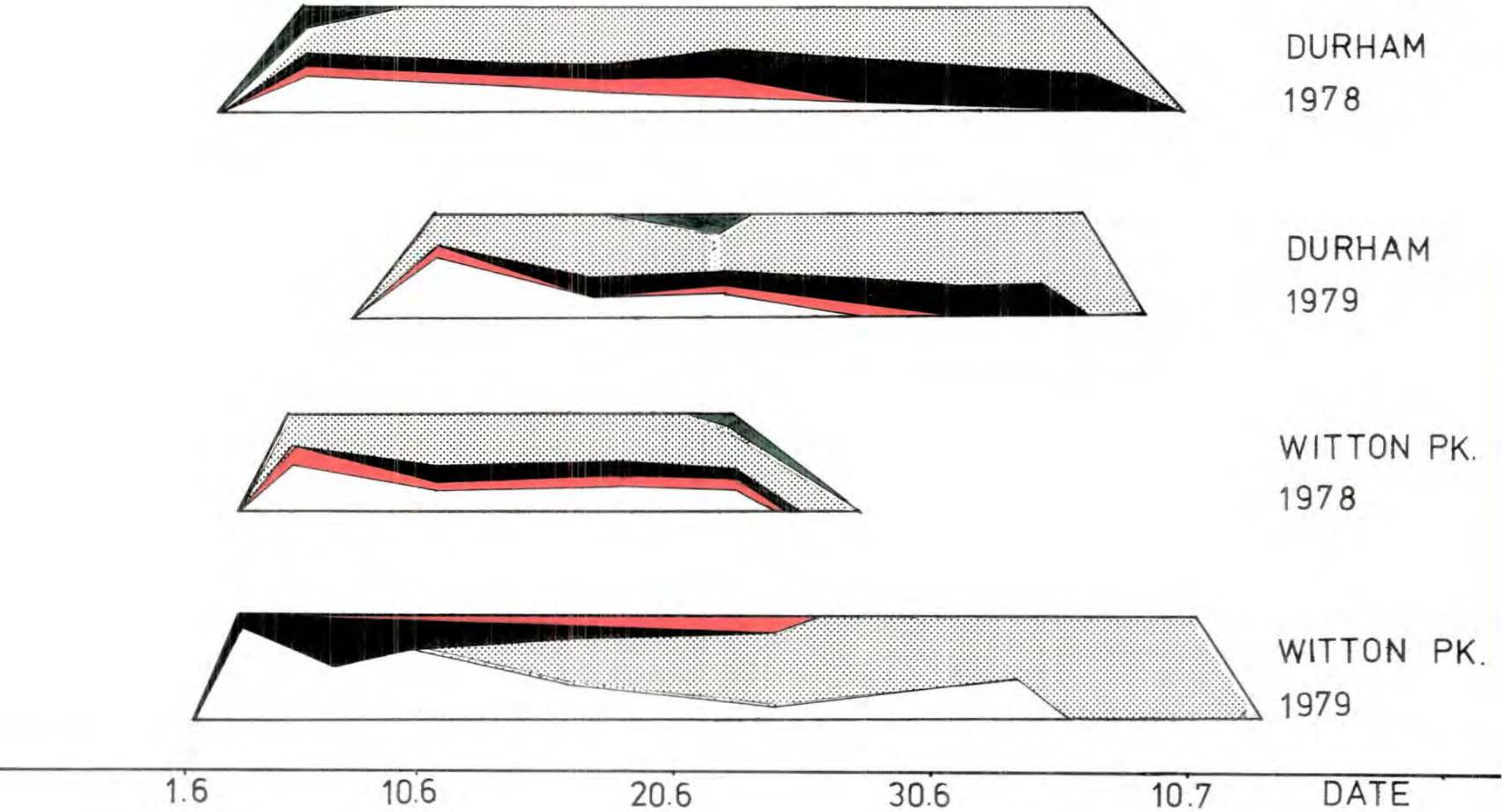
> "Different patches of plants may receive very different numbers of eggs, not because they differ in quality, but as a chance consequence of the female's behaviour"
>
> R. Jones, 1977.

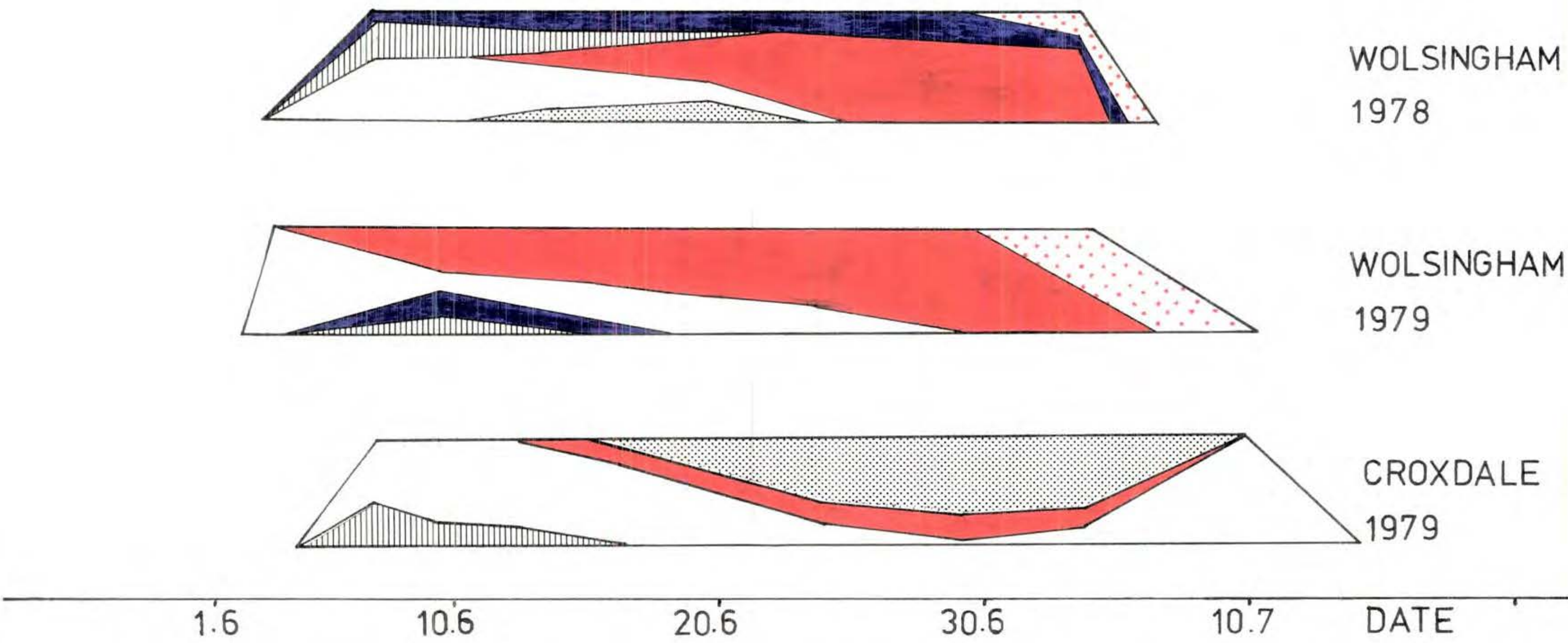
f. The distribution of A.cardamines eggs upon foodplants of different species

The effect of different foodplant characteristics upon

Fig. 40. The changes in host plant species used by *A.cardamines* during the flight season. The figures record the relative proportion of eggs laid upon different species between visits.




DURHAM
1978
DURHAM
1979
WITTON PK.
1978
WITTON PK.
1979
1.6
10.6
20.6
30.6
10.7
DATE


WOLSINGHAM
1978
WOLSINGHAM
1979
CROXDALE
1979
1.6
10.6
20.6
30.6
10.7
DATE

the oviposition behaviour of A.cardamines females has been detailed above. The influence of these upon the choice of different foodplant species as oviposition sites will now be considered.

Foodplant ageing is of great importance in determining which species are chosen at different points during the oviposition period of A.cardamines. Fig. 40 records the changes in foodplant choice (as % eggs laid between visits) upon the 4 main study sites in 1978 and 1979. It is clear that A.petiolata, C.pratensis and to a lesser extent C.amara senesce rapidly and are unavailable as oviposition sites late in the emergence, resulting in the selection of other plant species. The majority of eggs laid on the sub-optimal plant species are laid late in the season.*1 The data from all these sites support the similar observation of Wiklund and Ahrberg that in Sweden 'no single crucifer could serve as the only host plant for A.cardamines'.

Fig. 41 shows the observed deposition of eggs upon different foodplant species as egg:plant ratios. It is seen that these vary greatly with foodplant species, and that certain species appear to be chosen more frequently by ovipositing females. H. matronalis in particular bears heavy egg loads. This could indicate that the size of the visual cue, the inflorescence (which is far greater in this species than any other) is influencing choice between foodplant

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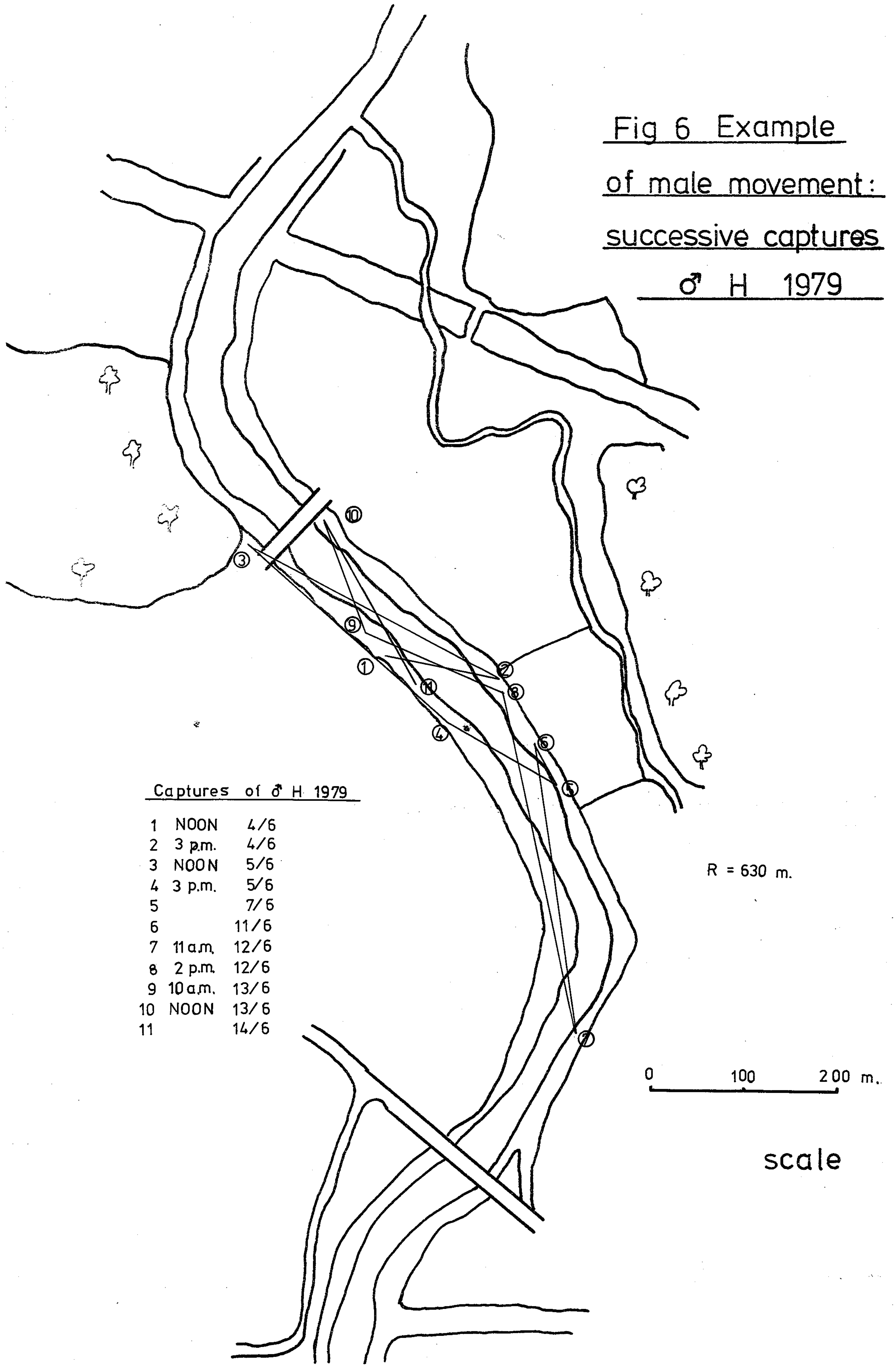
*1 Sisymbrium officinale in particular is a late flowering species.

Fig 6 Example of male movement: successive captures ♂ H 1979



Captures of ♂ H 1979

1	NOON	4/6
2	3 p.m.	4/6
3	NOON	5/6
4	3 p.m.	5/6
5		7/6
6		11/6
7	11 a.m.	12/6
8	2 p.m.	12/6
9	10 a.m.	13/6
10	NOON	13/6
11		14/6

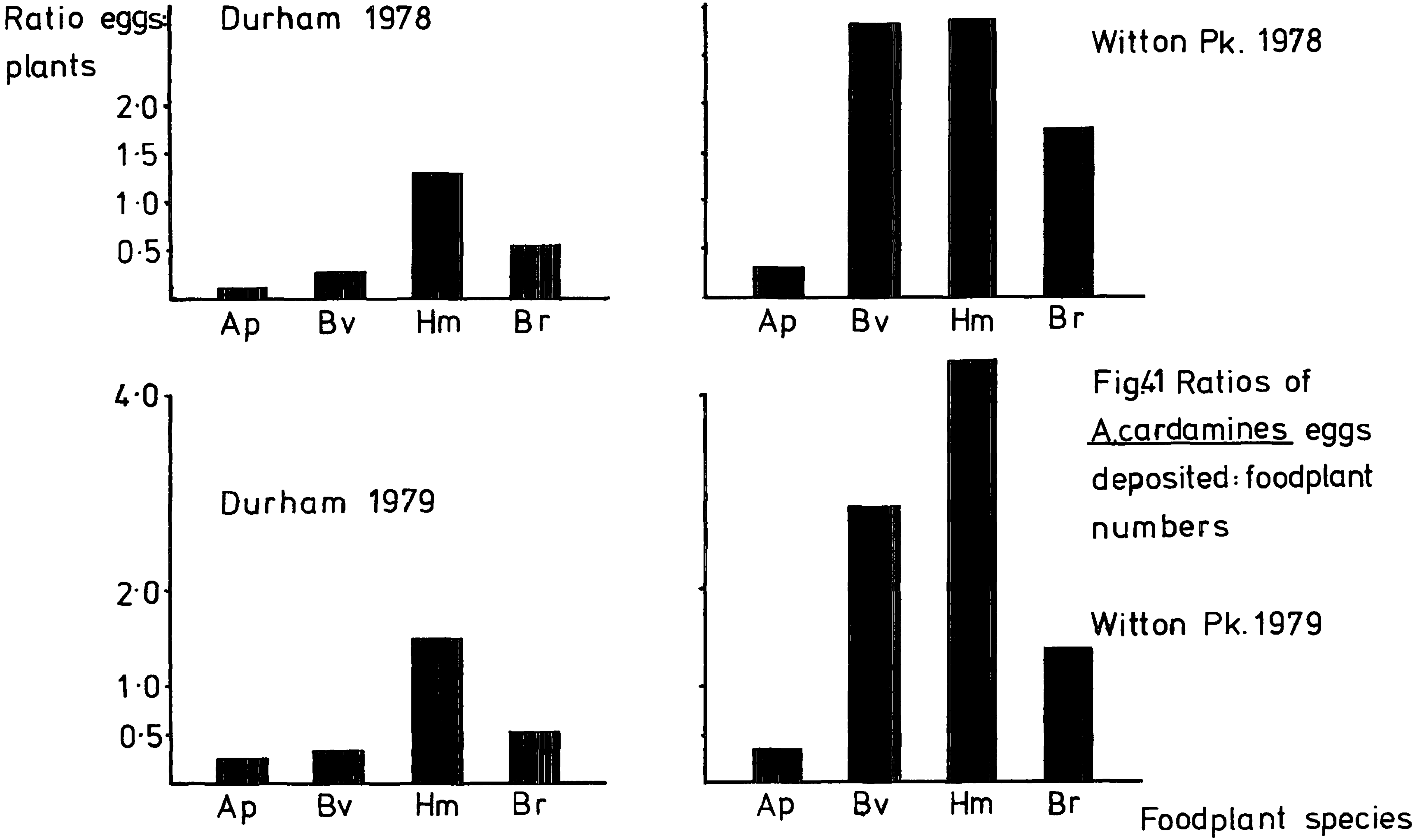


Fig.41 Ratios of <u>A.cardamines</u> eggs deposited: foodplant numbers

species, as it has been shown to do within the species. However, since these data are summed from the whole flight period they also reflect other differences between foodplants (the flowering period of H.matronalis is very long). In order to properly test the hypothesis that foodplant inflorescence size influences foodplant species choice, two distributions of eggs were detailed, upon single days and at sites where all foodplant species present were in full bloom together. Sampling was carefully limited to a small area to avoid density effects, and the egg-load and inflorescence characteristics of the foodplants were noted. These results are shown in Table 26. It is seen that considerable variation in egg-loads were found at these sites, and that, as predicted, this variation parallelled that in the size of the visual cue.*1

The effect of foodplant clumping upon the eggloads on different foodplant species was noted by Wiklund and Ahrberg, who showed that isolated plants of rare species receive more eggs than clumped, common ones. This may have effects in the present study - particularly it may lead to oviposition on the scattered plants of B.vulgaris - but it is not held to have any major effect, since the habit of almost every crucifer species examined was clumped.

g. The adaptiveness of foodplant species choice

It is apparent that a large percentage of the A.cardamines eggs, in any of the populations studied, were laid upon sub-

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*1 All the plant species examined are known to support larval growth, with the exception of Cochlearia officinalis, Scurvy grass, whose effects on larval growth are unknown. C.hirsuta supports A.cardamines growth in the laboratory, although Bowden (pers comm) regards it as unsuitable for A.napi.

Table 26

Foodplant Flower Characteristics and Eggloads - Wolsingham (9.6.79), Alston (26.6.79)

					Wolsingham		Alston	
Foodplant Species	Size of flowers (Cm^2) A	Modal No. of Flowers/ Flowerhead, B	Mean No. Flowerheads/ Plant C	Product AxBxC.	No. of Plants	No. of Eggs	No. of Plants	No. of Eggs
Cardamine amara	$\frac{3}{4} \times \frac{1}{2} \times 4$	4*	2.4	14.4	216	39	100	21
Cardamine pratensis	$\frac{3}{4} \times \frac{3}{4} \times 4$	8*	1.3*	23.4	148	25	100	23
Cardamine hirsuta	$\frac{1}{4} \times \frac{1}{4} \times 4$	4*	1.1*	1.1	100	0	100	1
Barbarea vulgaris	$\frac{1}{4} \times \frac{1}{4} \times 4$	32	9.8	78.4	8	57	-	-
Cochlearia officinalis	$\frac{1}{4} \times \frac{1}{4} \times 4$	11*	2.3*	6.3	43	0	100	3
Nasturtium officinalis	$\frac{1}{2} \times \frac{1}{4} \times 4$	8	1.2	4.8	-	-	20	7
Arabis hirsuta	$\frac{1}{4} \times \frac{1}{4} \times 4$	4	13	13.0	-	-	1	1
Thlaspi alpestre	$\frac{1}{8} \times \frac{1}{4} \times 4$	8	1.2	1.2	-	-	100	0

* from Wolsingham sample.

optimal foodplant species. In view of the heavy pre-adult mortality associated with these species (especially H.matronalis and B.vulgaris) such behaviour by ovipositing females appears maladaptive, particularly when one considers the large number of optimal foodplants which go unused at every site.

Several alternative hypotheses offer themselves as explanations of such behaviour, and these will now be considered in turn.

Hypothesis 1 - That females are unable to differentiate amongst crucifer species. As a pre-requisite for the evolution of selectivity amongst foodplants, it is necessary that ovipositing females are able to discriminate between such species. As pointed out by Stanton (1979) in work on chemotactile stimuli and oviposition in Colias spp, this discriminating limit to adaptation is not often considered in studies of foodplant choice, and yet may be of great evolutionary importance. The extent to which Pierinae females in vivo can distinguish amongst crucifers is not clear. Some species, such as Erysimum, which contain chemical deterrents other than mustard oils, are avoided, but it is not apparent to what extent the different glucosinolates are distinguishable. However, there is evidence that other crucifer specialists are able to distinguish among glucosinolates (Nair et al, 1973; Hicks, 1974; Sehgal and Vjagir, 1977; Nielsen, 1978), and it seems reasonable to assume that discrimination by A.cardamines females amongst crucifers could evolve if selection favoured it.*

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*Chew (1977) suggests that Nearctic A.napi females are beginning to recognise and avoid Thlaspi arvense.

Hypothesis 2 - That insufficient time has elapsed for the evolution of discrimination. H.matronalis, one of the least favourable foodplants, is an introduced species, now extensive in the wild, which has been present in the British Isles and Sweden for a relatively short time. It is a plausible hypothesis that insufficient time has elapsed for the evolution of foodplant discrimination in those areas. However, this hypothesis may be rejected on two counts. The Orange Tip has been recorded as using both H.matronalis and B.vulgaris over many generations in Britain. Newman (1869) records both species of crucifer as among the 4 major foodplants of the butterfly (and C.pratensis as an occasional host) and frequent notes in the entomological literature since that time have recorded the continuing use of these two hosts. Thus well over a hundred generations have passed in Britain on these foodplants, and despite the apparent strong differences in larval survival, no avoidance behaviour has evolved. The second piece of evidence, which allows one to conclusively reject this hypothesis, is that in Central Europe, where H.matronalis is native, the plant is still used by A.cardamines as a host (C.Hill, pers. comm.) In these areas the two species must have been in proximity for thousands of generations. Jones (1977) has shown how foodplant searching behaviour in A.rapae has changed in populations derived from a common stock in the 19th century.

Hypothesis 3 - That the apparent lack of selectivity is a strategy in time and space. Several authors are of the opinion that oligophagy, as practised by Pierinae, is a response to the unpredictability and instability of larval foodplants and the mortality factors associated with them. Thus A.rapae (Takata, 1961); A.napi (Chew, 1977) and A.cardamines (Wiklund and Ahrberg, 1978) have all been felt to adopt a 'polyphagous' type of oligophagy in order to overcome fluctuations in the availability of foodplants in either space or time. Chew emphasised variations of foodplant species over space as important in A.napi. This would not appear a satisfactory explanation in the populations of A.cardamines studied here, however. In almost every population encountered, at least one favourable foodplant (A.petiolata or C.pratensis) was present in large numbers (q.v. Fig. 21). Wiklund and Ahrberg have emphasised that unpredictability of foodplant associated mortality might lead to selection against specialisation. However, in the case of B.vulgaris it is apparent that mortality associated with this foodplant is predictable - mortality is consistently high in Anthocharis spp. fed on this foodplant - not just in County Durham, but in Sweden (Wiklund pers. comm) and the U.S.A. (Chew, 1977). In the long term evolution against use of such a consistently poor foodplant should have occurred.

As shown very convincingly by Wiklund and Ahrberg, oligophagy in A.cardamines is favoured by a complex of different foodplant related factors, which are hypothesised to have resulted in a strategy of foodplant exploitation; that is the ovipositing females adopt a wide range of host-plants as oviposition sites, in order to minimise the risks associated with choice of particular foodplants. In particular the variation in time of foodplant availability and foodplant-related mortality factors are suggested as the 'unpredictable' selective pressure which has led to the evolution of the strategy of oligophagy, including the use of foodplants of low suitability.[*1] However problems, both theoretical and practical, arise with the suggestion of such a hypothesis here. In order to test, or at least provide evidence supporting the hypothesis, a major research effort over many years, into the variability of mortality factors and foodplant availability, is necessary; this has been the aim of C. Wiklund's 5 year project on A.cardamines. Moreover, although evidence may be adduced on the longterm advantage to a genetic lineage of avoiding specialisation, this may not represent the actual interplay of selective forces within a population. For, if specialisation on say a particularly suitable foodplant species is of short-term advantage, then it is to be expected that specialisation will come to dominate the population, even if this is a long term disadvantage. Despite the fluctuations in foodplant availability over time and space (q.v.), these would appear to be the sort of conditions found in Co. Durham, where A.petiolata is in large fairly stable populations, widespread in space. A major disadvantage of hypothesising in favour of such strategies is that they are not evolutionarily stable (see Appendix 15) and may be used to explain away even results exactly opposite to those predicted (e.g. Rausher, 1979).

[*1] Wiklund and Ahrberg also note that oligophagy may be directly advantageous within a season due to the ageing and replacement of foodplant species - q.v.

The data collected in the present study might be variously argued as for or against the maintenance of oligophagy in A.cardamines. Foodplant populations are seen to vary, sometimes catastrophically; foodplant species are spread in time allowing a longer oviposition period of females. However, many of the foodplant related mortality factors appear relatively predictable. The 'strategy' hypothesis, as formulated thus far, is not favoured. What is needed is an hypothesis which shows how oligophagy may be directly and consistently adaptive (and thus evolutionarily stable).

Hypothesis 4 - Egg shortfall as a selective factor. The major mortality factor in the population of A.cardamines at Durham is egg shortfall, with only c.10% of eggs typically being laid in the population. In comparison to this, the mortality associated with foodplant species is small, as are all other mortality factors. Thus it is easily seen that selection for overcoming egg shortfall will be greater in intensity than other putative selection. In circumstances where such selective factors are opposed, it is to be expected that selection via egg shortfall, that is for increased fecundity, will be stronger. For instance, it is easy to envisage that selection for foodplant specificity will be opposed and may be over-ridden by selection for fecundity in those circumstances where increasing specificity of foodplant choice leads to decreasing egg production and consequent decreased fitness. In section 2.ix, a simple model of this situation is developed and typical values of foodplant related mortality applied. It is apparent that the predictions of the model are robust against variations in such mortality. To put this in terms of the individual female, which is subject to the vagaries of the weather and the difficulties of hostplant finding, it will be better to lay eggs in sub-optimal sites rather than to have those eggs remain unlaid. As a prediction from this hypothesis, it is to be expected that females will show increasing willingness to lay (even on sites of signalled low fitness, such as may be the case when other eggs are present - see App. 6) with increasing numbers of eggs to lay - for instance after flying a long distance, following bad weather, or in the first hours of daily oviposition. As egg load decreased one would predict that females would become less responsive. All these suggestions seem to be supported by casual observation on A.cardamines; rather better support comes from the studies of Jones (1977) who showed that A.rapae females were less responsive to cabbages as the egg-load carried was depleted.

Oviposition site selectivity, therefore, whether of foodplant species, or of plants of different density, size and 'quality' within a species, is not expected to occur when in opposition to selection for increased success of egg deposition. This agrees with the finding of Wiklund and Ahrberg that "when following ovipositing females it was obvious that they were prone to oviposit on any crucifer encountered".

viii. Local variation in A.cardamines

a. Introduction

County Durham is, at present, near the edge of the range of A.cardamines. During the 1880's there was a contraction from a formerly more extensive range, which led to populations in South Scotland becoming extinct, and to the species becoming less common in Durham, Cumbria and Northumberland (Long, 1979). The species probably rallied c.1930-40 and has slowly increased since that time. However, during the late 1960's and the 1970's, the species suddenly became far more frequently seen, and during the very favourable years of 1975 and 1976 expanded still further (T. Dunn, pers. comm.), including into upland areas at the heads of The Dales. Similar expansion was recorded in Norway (Haage, 1977) and increased numbers were reported for Southern England (Brown, 1970).

The causes of contraction and expansion remain obscure - Long favours winter weather effects. From the results of section 2.vi, it can be suggested that favourable summer weather may have led to the species' resurgence, as is hypothesised for other butterfly species. Whatever the cause(s), contraction of populations, local extinctions, colonisation events and rapid population growth may have led to important genetic effects. Founder effects and sampling effects on small populations may lead to random fluctuation and fixation of genes. Other aspects of population biology may also cause sampling effects on gene frequency, particularly neighbourhood size (Wright, 1940;

There is some evidence that different populations of A.cardamines studied here may begin to emerge earlier than others, and that this response is under genetic control. However, evidence is scanty, and considerable overlap occurs.

Kimura and Crow, 1963).

In the populations of A.cardamines studied here, neighbourhood size is probably very small, since males are observed to move very short distances only (section 2.iii). Female movement patterns will oppose this somewhat, but it is unlikely that extensive migration of females between populations occurs, particularly between populations in different river valleys. One dispersal/founder event was however seen when, in 1978, 37 eggs of A.cardamines were found on flowers of Cardamine pratensis at Coldberry (see Fig. 21). These eggs were all at the same stage of development and in close proximity to one another, and there can be little doubt that they were laid by a single female, dispersing from the small Middleton population. All bar 1 egg is known to have hatched, but only two larvae are known to have pupated, the remaining larvae disappearing during development (11 in the 1st, 12 in the 2nd, 5 in the 3rd, 4 in the 4th and 2 in the 5th instars. Disappearing larvae of late instars had eaten down their hostplant completely and presumably left in search of new food. Careful search revealed no larvae on surrounding C.pratensis). No adults or eggs were observed at Coldberry in 1979.

The Coldberry area is probably outside the tenable range of A.cardamines, although some moorland populations at comparable altitudes are known to have existed. (Fearnehough, 1953) recorded A.cardamines eggs on C.pratensis in Derbyshire moorland in 1952, but this population was eradicated by late blizzards in 1955). However, had more larvae survived at Coldberry, then a population may indeed have thrived, as a result of a founding event by a single female.

In other species of butterflies (e.g. Watt et al, 1977) effective population size is normally regarded as influenced only by absolute population size and neighbourhood effects. However, other influences may be more important than dispersal. Female monogamy, as shown by Begon (1978) in his studies on *Drosophila* spp., may lead to an inbreeding effect (Labine, 1960), particularly if most females are mated close to their emergence site, as seems likely in *A.cardamines*. Rather more importantly, strong variance in family size of different animals will lead to a very marked reduction in 'sampling effective population size'. In *A.cardamines*, very great variance in female success is suggested. Very few eggs are laid per female, due to inhibition of egglaying by bad weather (Section 2.vi). Additionally, changing foodplant availability and quality means that eggs laid at the end of the emergence period are placed upon ageing plants, or on species giving poor larval survival. Thus females emerging late will encounter only plants giving poor larval survival. The ratio of the fitness of early and late females is probably in excess of 4:1 at Durham.

Thus in populations of c.400-600 individuals, as at Durham, effective population size (Ne) will be small (c.100-150) since

$$Ne = \frac{N\,\bar{k}}{1 + \sigma^{2}\,k/\bar{k}}$$

where $\bar{k}$ and $\sigma^2_{k/\bar{k}}$ are the mean and variance of offspring number per parent, and assuming a ratio of 1:5 in k : $\sigma^2_{k/\bar{k}}$. This seems a reasonable and **conservative** estimate - several individual females are known to have laid more eggs in a single day than the population mean for the whole season.* Begon (1977) obtained a variance to mean (offspring/parent) ratio of 14.78 for a Drosophila subobscura population.

The size of the Durham population is probably typical of many other Co. Durham A.cardamines populations. A very few (Witton Park/Witton-le-Wear, Wolsingham) may be bigger, whilst many of the recently derived populations are probably smaller. The net effects of family size variation, inbreeding effects, neighbourhood effects, founder events and population declines and expansions lead one to expect that effective population sizes in most County Durham A.cardamines populations will be c.150, with some populations being even smaller. Such values are well within the range where one might expect random fluctuations and fixations of gene frequency. The present study was carried out in order to see if this prediction was confirmed.

b. Genetic variation

Electrophoretic analysis was carried out upon animals removed from populations (figured in Fig. 21). Assay procedures are given in App. 9 . The following loci were examined - GOT-1, αGPDH, XDH, G6PDH. Additionally F6DP was examined in 1979. Of these loci only GOT-1 and

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*Two females in 1978 laid 32 and 23 eggs respectively. A female in 1979 laid 18 eggs.

Tab. 27

Allelic frequencies at GOT-1 and F6DP for A.cardamines populations

Alleles are designated relative to the mobility of the commonest allele in A.napi, which is arbitrarily designated as allele 100.

Allelic frequency is expressed as the proportion of the sample taken.

		GOT - 1				F6DP
		1977	1978	1979		1979
	n		44		n	
Durham	p.25		.07		p. 33	
	p.29		-		p. 75	
	p.35		.84		p. 78	
	p.92		.09			
	p.120		-			
Shincliffe		66		42		42
		.06		.05		.29
		.02		-		.71
		.79		.83		-
		.12		.10		
		.02		.02		
Cassop		40				
		.08				
		-				
		.85				
		.08				
		-				
Witton Park		48	56	46		46
		.04	.04	-		.13
		-	-	-		.85
		.92	.86	.96		.02
		.04	.10	.04		
		-	-	-		
Wolsingham		42	72	64		64
		.05	.08	.06		.13
		-	.01	.03		.87
		.90	.79	.83		-
		.05	.08	.06		
		-	.04	.02		
Wearhead			16	28		28
			-	-		.07
			-	-		.93
			1.00	1.00		-
			-	-		
			-	-		
Alston		22	28	42		42
		-	-	-		.05
		-	-	-		.95
		1.00	1.00	1.00		-
		-	-	-		
		-	-	-		

F6DP showed any allelic variation (one animal from Durham, 1978, had a variant G6PDH allele). Esterase loci showed pronounced variability in both band positions and intensity. However, these proved intractable, since loci appeared to be overlapping upon gels, and under complex genetic control.

The allelic frequencies at GOT-1 for the years 1977-79 and at F6DP for 1979 are given in Table 27. It is seen that populations in the upper reaches of river valleys, at Wearhead and Alston, have allelic frequencies at GOT-1 different to populations from lower down the valleys. The two populations are fixed (or nearly so) for the allele -100, which is the common allele of other populations. This strongly suggests that such populations have been subject to genetic drift. However, since there remains the possibility that selection in the upland areas favours the 100 allele, the search for other polymorphic loci was maintained. F16DP was found to show variation, but in contrast to GOT-1 does not show fixation of the common allele in upland areas. Rather there appears to be a gradual decrease in the frequency of rare alleles with increasing altitude. These results are, again, consistent with both genetic drift and selection, and must be treated carefully in view of the small samples taken. (There is a real risk of damaging populations by removing more individuals).

At present there is some evidence that 'genetic drift', that is random fluctuations in gene frequency, may be occurring in these populations due to small effective population sizes and founder events. A larger sample of individuals, from a wider range of populations, is needed to support this contention.

ix. Discussion

The fragmentation of populations into small isolated demes has been repeatedly shown to have major influences on evolutionary change. Ehrlich (1965) emphasised the extremely fragmentary nature of the demes of the butterfly Euphydryas editha in one area, and suggested that evolution could be very rapid in such demes. Singer (1971) showed local adaptation of these populations to hostplants.

Local genetic events and adaptation may proceed rapidly in such isolates, and co-evolutionary changes, for instance, may arise much more swiftly than in large panmictic populations (Slatkin and Wilson, 1979). It is therefore to be expected that such local adaptation be an important contributing agent in the evolution of a species group. In Pierinae, local races are commonly found, as exemplified by the A.napi complex. Within the tribe Euchloini, examples of local races are the forms of Euchloe olympia (Wagner, 1977) and E.tagis (Sarlet, 1975). In the E.tagis complex local adaptation has reached the point where one isolate is currently designated a good species - E.pechi Stdgr. of the Djebel Aures region of Algeria. Local selective regimes within populations are thus implicated in speciation in that group.

Local and geographic differences in foodplant tolerance in the A.napi complex have been described by Bowden (1970) who showed that despite being attractive to females of Nearctic A.napi and A.virginiensis, Alliaria petiolata is toxic to the larvae of such taxa. The foodplant tolerances of American and British A.napi populations are different. Similar changes are known in the P.cheiranthi isolate from P.brassicae, and for the populations of E.editha studied by Singer (1971), where different populations are known to specialise on single, different plant species. Similar changes are also recorded for many other insects: Hsiao (1978) gives an example of geographic adaptation to otherwise sub-optimal hosts.

In A.cardamines some evidence of local adaptation has been found (Section 2.viii) where time of emergence may vary between populations, possibly to synchronise flight with foodplant availability. However, other aspects of foodplant-related behaviour appeared poorly suited to optimal use of resources, despite the localised and small demes (Sections 2.iii, vi). Despite wide variation in larval survival and fitness on different foodplants (Section 2.v), A.cardamines, both in Co. Durham and Sweden, is completely oligophagous and appears to utilise any available crucifer (a list of foodplants is given in Appendix 7). The non-specificity of A.cardamines, and of other Pierinae*, in the face of apparent strong selection requires some explanation. The gametic wastage caused by

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*Artogeia napi, A.melete, Pontia daplidice, P.sisymbrii, Synchloe callidice, Euchloe ausonia/ausonides, E.tagis, E.olympia, Authocharis genutia, A.lanceolata, A.sara are other widely oligophagous species. Only Zegris eupheme and the habitat specialists Artogeia ergane, A.bryoniae, A.krueperi and A.virginiensis appear to be monophagous. (Although Z.eupheme is recorded as monophagous, it undoubtedly uses other Cruciferae species apart from Hirschfeldia incana, such as Isatis tinctoria (pers. ob.)).

poor larval survival on toxic plants should lead to rapid counter-evolution, as noted for other butterfly species (e.g. Straatman, 1962).

As noted by Stanton (1979) in constructing explanations concerning hostplant-ranges of insects, it is important to recognise the physiological limits to discrimination. If A.cardamines females are unable to distinguish different Cruciferae, it is unsurprising that eggs are laid upon all available species. However, other crucifer-feeding insects are able to distinguish different crucifer species (e.g. Sehgal and Vjagir, 1977) and it seems reasonable to assume that Pierinae females can do so (Section 2.vii).

Adaptive explanations of host specificty or generality have previously emphasised the dynamics of ecosystems in time and space. Thus Singer (1971) believes abundance and predictability of foodplants allows the local specialisation of E.editha populations. Smiley (1978) has recently outlined a possible scheme for the evolution of monophagy from the oligophagous condition in Heliconids, based on progressive specialisation on abundant plant resources. By contrast, Takata (1961), Wiklund (1974a,b, 1975), Chew (1975, 1977a,b) and Wiklund and Ahrberg (1978) have emphasised the importance of foodplant unpredictability in time and space to the maintenance of oligophagy. However, a number of problems arise with the adoption of the hypothesis that oligophagy is a strategy to combat an essentially coarse-grained (sensu Levins, 1968) environment, where different populations, and different generations of a population may experience different selection regimes associated with foodplants. Firstly, several aspects of foodplant availability are predictable. As noted in earlier sections all A.cardamines populations in the Durham area are associated with at least one 'suitable' crucifer species (which appear, therefore, relatively predictable in both time and space). Similarly certain foodplants give consistently and predictably poor survival, not merely in Co. Durham, but worldwide, notably Barbarea vulgaris. Again, as noted in section 2.vii, the use of this foodplant cannot be explained as due to insufficient time having elapsed for the evolution of avoidance (as Chew (1977,b) has suggested for the use of Thlaspi arvense by Artogeia napi in the U.S.A.) From these considerations of predictability of foodplant effects, one would expect some counter-evolution on the part of the butterfly populations, and polymorphism for oviposition strategies, with some females laying on all crucifers, some only on the most suitable. This is not found.

Secondly, as a consequence of counter-evolution it is to be expected that local differences should exist between populations, particularly in such small isolates as the Co. Durham A.cardamines populations, in the manner of the E.editha populations studied by Singer. Oligophagy would not be 'evolutionarily stable' in all populations against invasion by the monophagous habit - for instance in populations with just one 'good' and one 'bad' foodplant. Wiklund and Ahrberg (1978) described a situation of unpredictability of foodplant resources, which should favour both local differentiation and polymorphism for exploitation strategies. A pure 'polyphagic' strategy may require group-selection scenarios in these conditions.

Thirdly, as noted by Jones and Ives (in press), the lack of selectivity between different foodplant species, by egglaying Pierinae, is suprising in view of the fact that such females show considerable choice within a food-plant species (avoiding, e.g. old plants). Jones and Ives point out that the lack of 'inter-specific' as opposed to 'intra-specific' selectivity may be due to consistent selection <u>within</u> species (e.g. old plants always give poor larval survival) but the inconsistency of selection between species. Selection against use of particular plant species depends upon which other species are present in the community. 'Intra-specific' selection they feel to be more predictable than 'inter-specific' selection.

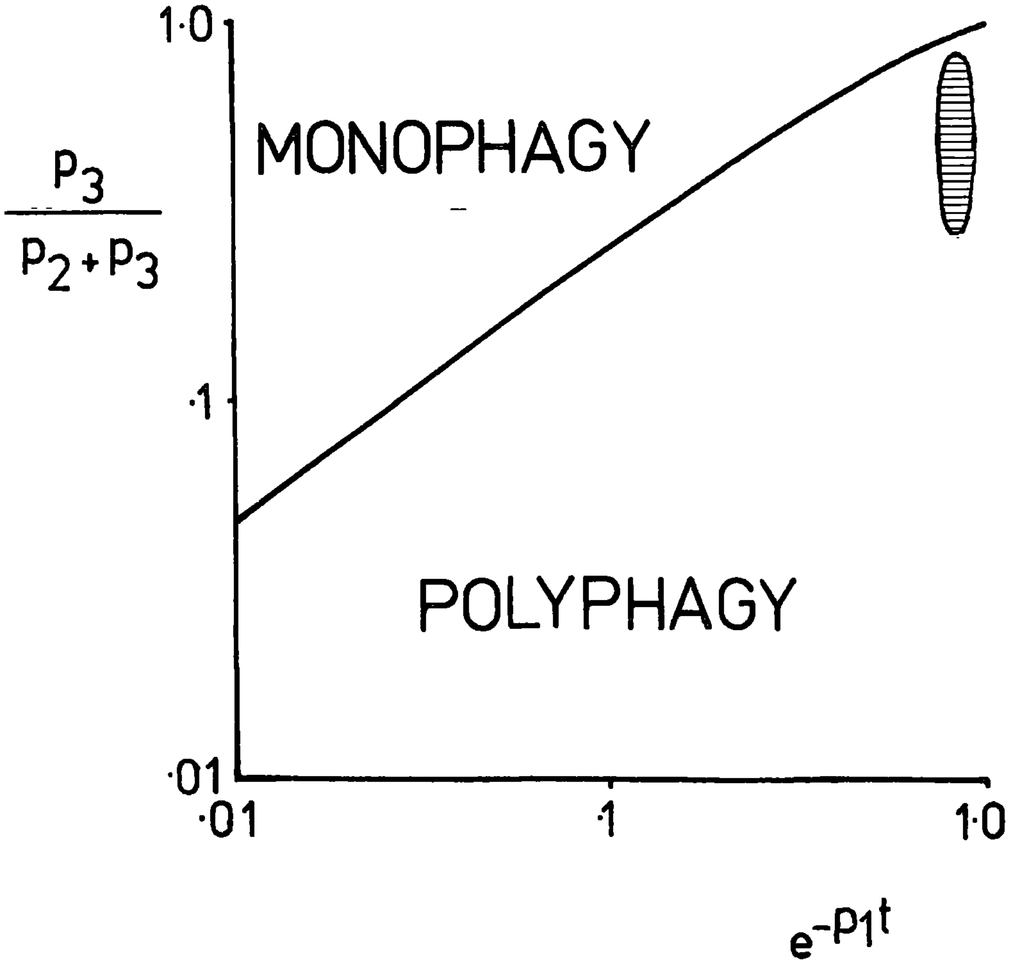
Related to this consideration is the change in female selectivity which is believed to underlie the 'edge effect' (Section 2.vii) and the occasional 'escalation' of contests between an ovipositing female and eggs (App. 6). If predictability of larval survival is the sole factor influencing oviposition choice, these effects would not be expected.

From all these considerations it was felt necessary to seek other factors which might be influencing the strategies followed by females. In Section 2.vi, it was shown that the major mortality factor in the Durham population was egg shortfall. In Section 2.vii egg shortfall was invoked as a possible selective factor to explain non-specificity of female oviposition choice. The basic premise of this suggestion is that a female 'attempts' to maximize her reproductive output, i.e. that natural selection acts on female oviposition by selection for the maximum number of offspring in succeeding generations. Thus a balance is held to be struck between strategies which lead to good larval survival (e.g. specialisation on 'good' plants) and those which lead to many eggs being laid (e.g. lack of specialisation). A situation akin to this was modelled by Levins and MacArthur (1969), who produced the figure shown here as Fig. 42. The model describes the optinal strategy, in terms of surviving offspring, for females in different conditions of $e^{-P_1 t}$ (The probability of failure to find foodplants on which to lay eggs) and $\frac{P_3}{P_2 + P_3}$ (The percentage of alternative host-plants which are unsuitable as food). Their model predicts therefore that if 50% of eggs are unlaid, the population of females should only be polyphagous if 30% or more of the extended diet items are suitable. The approximate conditions for <u>A.cardamines</u> at Durham are also indicated in Fig. 42. It is seen that the model predicts the maintenance of 'polyphagy'.

However, the model of Levins and MacArthur makes several assumptions which may be unreasonable in this study. Firstly, it assumes that females are unable to distinguish foodplants as good or bad, except for the very best foodplant.

Fig. 42 The model of Levins and MacArthur showing the predicted optimal strategy under different conditions of probability of not finding hostplant (e^{-p_1t}) and the proportion of alternative hosts that are suitable ($\frac{p_3}{p_2+p_3}$), Taken here as equating with the relative suitability of alternative food. The shaded area represents approximate conditions for A.cardamines at Durham, where c.90% of eggs are unlaid.

For derivation of model, see Levins and MacArthur (1969).



Evidence suggests rather that some crucifer-specialising herbivores can distinguish different crucifers including B.vulgaris (Hicks, 1974). Secondly, the model specifically assumes that the herbivore must be either monophagous or polyphagous, that is that the animal must use either one or all available food items. Thirdly, it assumes that monophagy is ancestral, polyphagy the derived state. In Pierinae, at least, the converse is implied. Fourthly, it assumes that foodplants are either suitable or unsuitable as food, with no intermediate cases. Additionally it has the disadvantage of not specifically taking account of differences in abundance, but has the advantage of not considering fluctuations in suitability (as Wiklund and Ahrberg (1978) suggest for the foodplants of A.cardamines).

A different model has therefore been constructed for the specific case of a herbivore, such as A.cardamines, which has several possible foodplants of different suitability, and which may adopt several different strategies of exploitation. The model presupposes that there is an array of N foodplants with N strategies of exploiting them (where each successive strategy adds the next most favourable foodplant to the array used by the herbivore. Initially the model assumes equal abundance of different foodplants).

t_j = searching time/plant for each strategy.

Thus t_1 is the searching time to find a single suitable plant under strategy 1, that is using only the most favourable foodplant

$$t_1 = 2t_2 = 3t_3 = Nt_N$$

T = total time available (expressed in t_N units).

X = maximum number of eggs to be laid.

ε_j = Number of eggs laid by each strategy (j)

$$= \left| \frac{T}{x\,t_j} \right| X$$

where $\left| \frac{T}{x\,t_j} \right|$ represents the relationship between total available time and the time necessary to lay all eggs using strategy j. The term may range from 0 to 1 (when total available time exceeds that necessary for egglaying, the term is held at its maximum of 1, any excess being ignored).

F_j = fitness of strategy j

$$= \sum_{i}^{n} \frac{\left| \frac{T}{x\,t_j} \right| X}{N} f_i$$

where f_i is the 'fitness' of eggs reared on the i^{th} foodplant, relative to the most favourable foodplant, taking values from 0 to 1.

Thus $f_1 = 1$.

As a simple example

Assume two foodplants, equally abundant, one giving 100% survival, the other 50%

$N = 2$

$t_1 = 2t_2$ $\qquad$ $f_1 = 1.0,\ f_2 = 0.5$

let $X = 100$ and $T = 150t_2$

$$\varepsilon_1 = \left| \frac{150}{100 \times 2} \right| 100 \qquad \varepsilon_2 = \left| \frac{150}{100} \right| 100$$

$$= 75 \qquad\qquad\qquad = 100$$

$$F_1 = \frac{75}{1} \times 1.0 \qquad F_2 = \left(\frac{100}{2} \times 1\right) + \left(\frac{100}{2} \times 0.5\right)$$

$$= 75 \qquad\qquad = 75$$

Thus each strategy leaves equal numbers of offspring in succeeding generations.

Elaborating the model slightly to include four hostplants of varying suitability.

$N = 4 \qquad t_1 = 4t_4 \qquad X = 100$

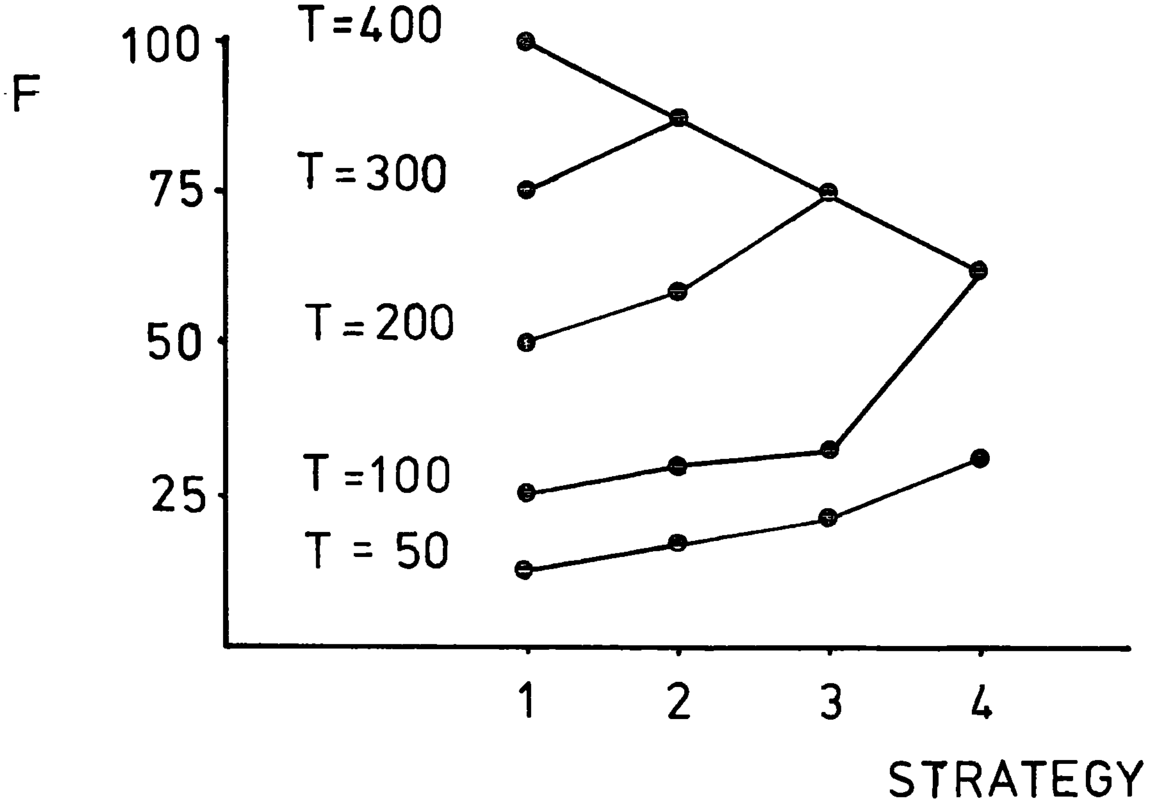
$f_1 = 1; \quad f_2 = 0.75; \quad f_3 = 0.5; \quad f_4 = 0.25$

T varying

gives F_j for the four possible strategies (using one, two, three or four hostplants) as in Fig. 43. It is seen that only when sufficient time is available to lay all or most eggs do specialist strategies lead to most surviving offspring. When time available is short, relative to that needed for maximum egg laying, strategy 4 (usage of all 4 plants) gives the maximal number of offspring.

To make this model applicable to the Durham population of A.cardamines, the crucifer array available is designated thus. Plant 1 is Brassica rapa, which gives the best larval survival (and fecundity in the next generation), Plant 2 is Alliaria petiolata, which from Table 13 is seen to have a 'suitability index' with a mean value of 0.66 of that of B.rapa. Barbarea vulgaris and Hesperis matronalis are designated plants 3 and 4 with mean suitability (based on values of suitability index, relative to B.rapa) of 0.28 and 0.18 respectively. Thus

Fig. 43 Relative fitness (F) of different strategies of foodplant exploitation with differring available searching Times (T).



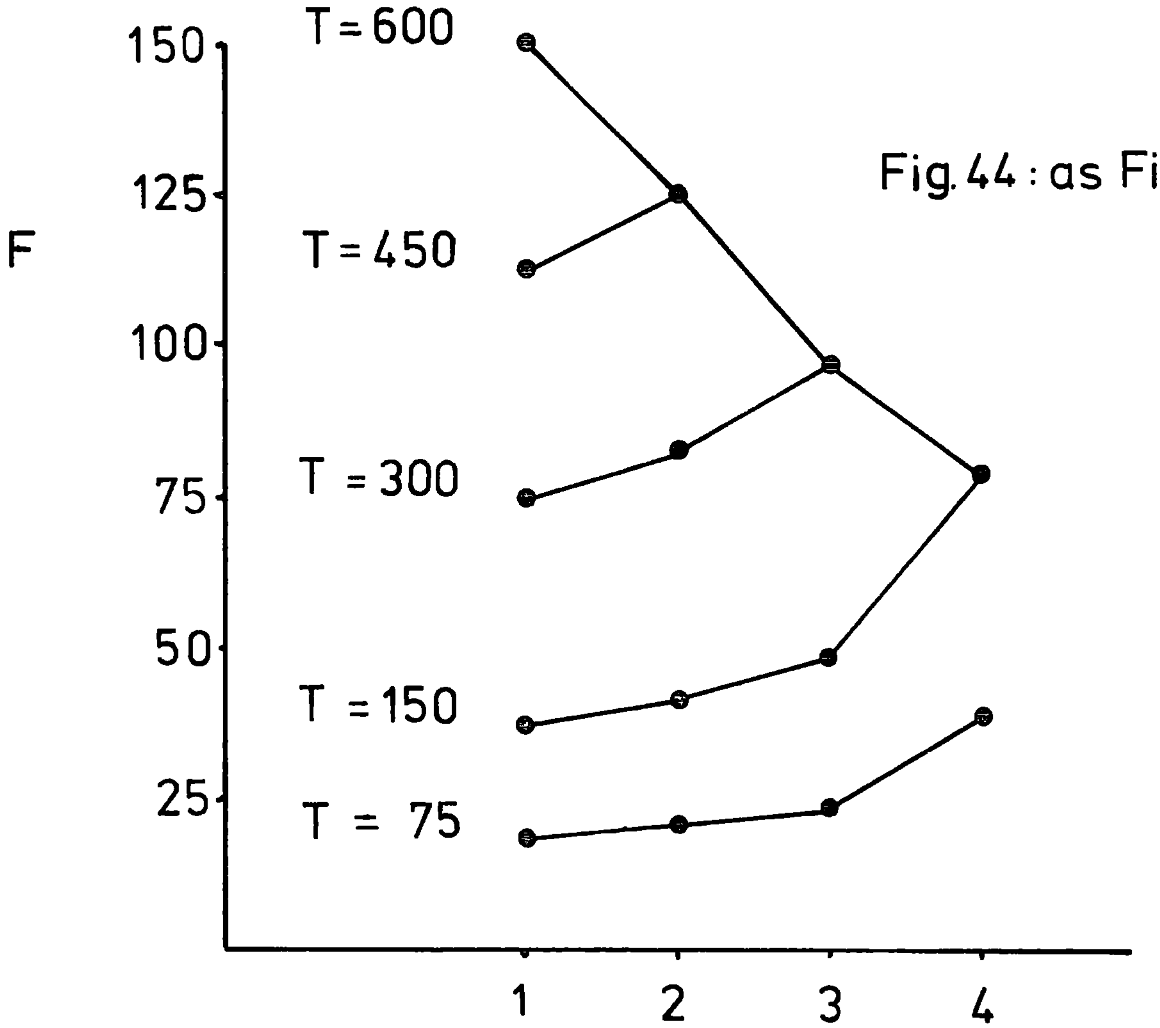


Fig. 44 : as Fig. 43

$N = 4 \quad t_1 = 4t_4$

$f_1 = 1, \quad f_2 = 0.66, \quad f_3 = 0.28, \quad f_4 = 0.18$

T = varying

X = 150, the value assumed for mean fecundity under optimal field conditions (q.v. Section 2.v). The results of this model (again assuming equal foodplant availability) are given in Fig. 44. Again, foodplant specialisation is shown to be advantageous only when much time is available.

To take account of differences in foodplant abundance and 'availability', the model must be further modified. No correction is needed for differences in crucifer sizes, but the different foodplants are not equally apparent to female A.cardamines (Section 2.vii) and it is invalid to assume that crucifer abundance directly measures availability. To overcome this, the number of plants of each of the four crucifers at Durham (from Table 14) was multiplied by the mean eggload per plant of that species (Fig. 41), relative to that of A.petiolata, to give the 'available' individuals of each species.

Table 28

The number of 'available crucifers' (n_i) at Durham in three years of study.

	1977	1978	1979
B.rapa	1722	777	357
A.petiolata	4350	10320	3100
B.vulgaris	588	563	188
H.matronalis	2106	2484	1173
TOTAL: (Z)	8766	14144	4818

$\bar{x}$ fecundity = T = 22.6 8.9 9.4

Then if t_4 for 1977 is 1

$$t_3 = \frac{8766}{6660}, \quad t_2 = \frac{8766}{6072}, \quad t_1 = \frac{8766}{1722}$$

$$= 1.32 \qquad = 1.44 \qquad = 5.09$$

Similarly for 1978,

$t_4 = 1$, $t_3 = 1.21$, $t_2 = 1.27$, $t_1 = 18.20$

and 1979,

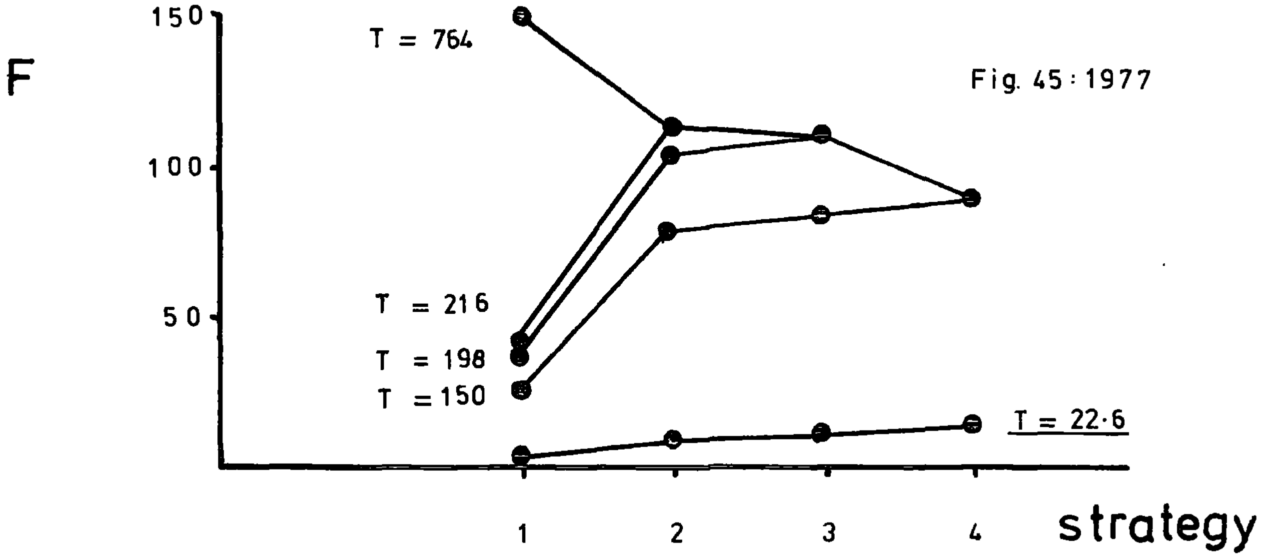
$t_4 = 1$, $t_3 = 1.32$, $t_2 = 1.39$, $t_1 = 13.50$

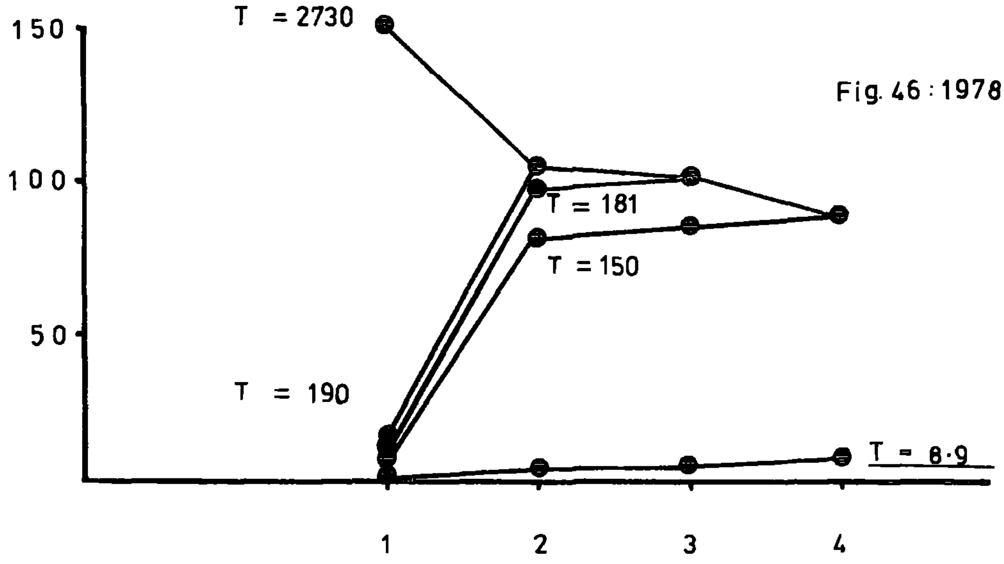
Calculating $\mathcal{E}_j$ as before, $Fj = e_i f_i$

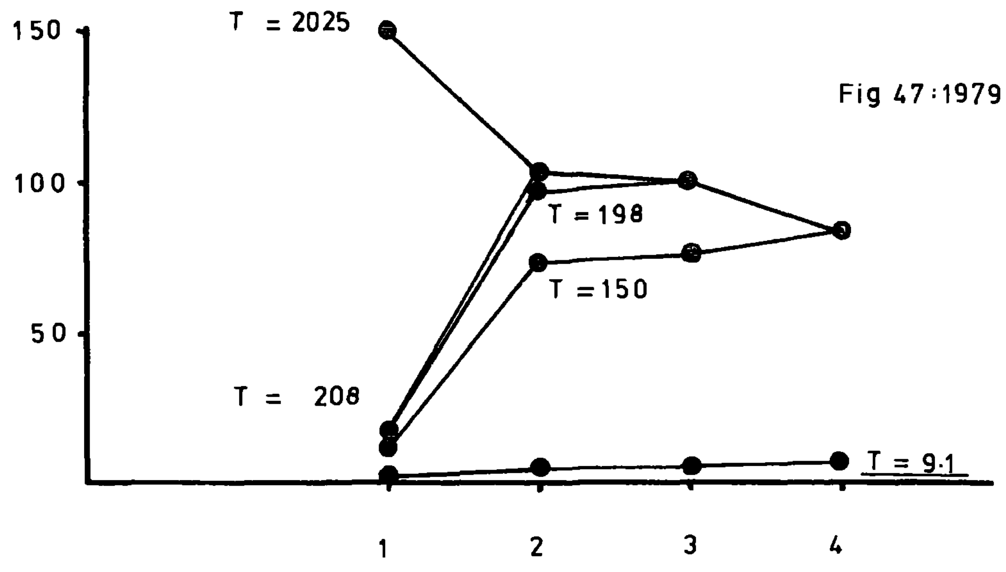
Where e_i = amount of $\mathcal{E}_j$ laid on plant$_i$

$$= \frac{\mathcal{E}_j \, n_i}{z}$$

Figs. 45 to 47. Fitness of different foodplant exploitation strategies where model parameters, of foodplant suitability and abundance, and of time available for egg-laying, are set by actual data from Durham.







The resulting models based upon the actual data on foodplant availability, suitability and successful host-locating (= fecundity) for the three years of study at Durham, are given in Figs. 45 to 47. For all three years the models predict that females laying eggs on all available foodplant species would leave the most offspring.

The above models of oviposition behaviour should be treated with caution, and ideally should be tested on populations other than those used to derive the models. A major shortcoming of the approach is that it assumes fixity of female responses, which does not seem justified in view of observed egg-deposition patterns. It seems likely that the decision whether or not to oviposit on an encountered crucifer is influenced by the physiological state of the individual butterfly. It is to be expected that females will be more prone to refuse encountered crucifers when few eggs remain to be laid - as shown for A.rapae by Jones (1977).

However, the models do indicate solutions to some problems concerning foodplant exploitation strategies. Firstly, even though some foodplants (e.g. Barbarea vulgaris) may be consistently, and predictably, bad food for larvae, more offspring will result from laying eggs on such species, if the alternative strategy is to have the eggs unlaid. Secondly, by demonstrating that 'polyphagy' may be of direct individual advantage within a population, it has become unnecessary to invoke arguments based upon future and past unpredictability of the foodplants. (This is not to say that unpredictability may not have some influence). 'Polyphagy' in these models is evolutionarily stable against invasion by 'monophagic' strategists except in the most optimal conditions for foodplant finding.

The above models, therefore, predict that phytophagous herbivores such as A.cardamines and other Pierinae should only specialise on foodplants when conditions are extremely favourable to hostplant finding. Ecological factors are thus opposing the evolution of 'monophagic' strategies. As Zwölfer (1974) has pointed out, a major influence on the speciation and subsequent co-existence of phytophagous insects appears to be the division of food resources amongst species: Petersen (1966) has shown that sibling groups of Pieridae and Nymphalidae tend to have different hostplants, whilst Armstrong (1945) described the differentiation of host-races of Lepidoptera species without geographic separation. Similar division of host-plants among sibling species of insects are known for other groups, e.g. Drosophilidae (Pipkin et al, 1966). Thus speciation itself is likely to be opposed by the 'enforced polyphagy' described above.

A related model to those above was produced by MacArthur and Pianka (1966), who showed that in the case of a predator foraging for patchily distributed prey, the optimal strategy (to minimise search time, when pursuit time is small) is to take any food items encountered, and to specialise on patch types. That is to be not a food-type specialist, but a habitat-type specialist. Such strategies appear common in foraging birds and mammals. The model also predicts that competition between related species of foraging animals should lead to little differentiation of food niches but rather differential habitat selection. Shorrocks (1975) has corroborated this prediction in British communities of Drosophila spp.

In very few cases have butterfly species within a community been held to divide food resources amongst themselves on the basis of foodplant species. Goodpasture (1974) describes an exceptional situation where species of Plebejus appear to have mutually exclusive diets. More commonly temporal separation (Shapiro, 1975 c) or spatial segregation by habitant selection occurs. Thus in the Satyrid gensus Lethe (Shapiro and Carde, 1970) and in Heliconidae (Benson, 1978; Young, 1978) and in three European (Petersen, 1954) and three Japanese (Ohsaki, 1979) Pierini, different species have similar host plant ranges, but are segregated by habitat selection.

The limiting factor to species-packing in Pierinae will therefore not be prey types (plant species) but habitat types. The monophagous Artogeia virginiensis, a member of the A.napi complex, is a habitat specialist rather than a hostplant specialist, and will use any available crucifer, although only Dentaria spp are normally available. Similarly the montane A.bryoniae is particularly associated with Biscutella spp. but will use other crucifers when these are available (Varga, 1965). Thus ecological selective factors will oppose species packing within habitats, and hence speciation.

In the long term it is to be expected that repeated ovipositions on sub-optimal plants will cause the evolution of larval tolerance towards such plants. The evolution of such tolerance may even be easier to encompass physiologically than changes in adult behaviour. However, as noted by Wiklund and Ahrberg (1978) the effect of A.cardamines on its individual hostplant species undoubtedly exceeds the effect of any one species upon A.cardamines. It is therefore to be expected, especially in view of the sometimes devastating predation of seeds by A.cardamines (and other species (e.g. White, 1971)) that hostplants should evolve both qualitative and quantitative defences to butterfly predation.

In Cruciferae, it is known that variations in concentration of the primary group of defence chemicals are genetic (Josefsson, 1970; Mullin and Saharrabundhe, 1977). The evolutionary response of the plant species might be increased production of deterrent glucosinolates, or decreased production of glucosinolates which act as stimuli to ovipositing females. Mitchell (1977) has described differential predation of Pieris brassicae upon morphs of Brassica oleracea differing in glucosinolate content. It is therefore to be expected that herbivores will profoundly affect the genetic constitution of crucifer populations.

Herbivores may also be expected to exert considerable influence on Cruciferae community structure within habitats. O'Dowd and Williamson (1979) have shown the theoretical conditions for mutual associations to arise between members of a community predated by a herbivore. McNaughton (1978) has shown an example of such conditions, where palatable grasses of the Serengeti plain suffer less grazing when growing in association with unpalatable species. Thus herbivore grazing may promote species diversity within a habitat.

X. The effect of human activity upon A.cardamines

There are no animal species which are completely unaffected by human activities. The relatively recent industrial and agricultural development of much of the Holarctic has had far-reaching effects upon the distribution, survival, genetic composition, etc., of animal populations. In very few animals are these effects known in any detail. In butterflies, the decline of many populations over the last two centuries is usually attributed to the destruction of habitats (Brewer, 1965; Pyle, 1977), although pesticides may have also been important recently. Collecting by entomologists has also exacerbated the scarcity of some insects (e.g. Lycaena dispar and Maculinea arion). In some chalk grassland Lycaenidae, the resurgence of grass growth following the extermination of rabbits by myxamotosis, is believed to be the cause of decline in some populations (Frazer, 1960, 1961).

In virtually no study has the exact cause of population decline been elucidated. However, it is important, if future management programmes are to have any chance of success, to gather as much information as possible about butterfly species, and the effects of man upon them.

The populations of A.cardamines which I have studied suffer considerable mortality due to human activity. Some adults are taken by entomologists (including the author) and one is known to have been killed by a motor vehicle. Probably such increase in adult mortality is unimportant, although it should be noted that the adult stage is the least numerous in the life-cycle, and killing or collecting a few adults can have a potentially disproportionate effect on populations.

Eggs and larvae, on the other hand, suffer considerable losses in riverbank populations, particularly when close to a town. Thus at Durham, Barnard Castle, Darlington and Witton Park, very heavy recreational use of the riverbanks lead to egg and larval death. This was primarily due to anglers trampling undergrowth and to the picking of A.cardamines bearing flowers. The losses of A.cardamines on sites to human activity are recorded in Table 29. (This undoubtedly over-estimates mortality somewhat, since sites chosen for study are automatically those of easy access to anglers and children).

Trampling undoubtedly is the most important of the two causes of death. Trampling also greatly decreases floral diversity, e.g. at Darlington (Holmes, 1978). There appears little that can be done about this, although some control of human activity may be necessary. At Durham, and along the Tees banks from Darlington to Barnard Castle, very extensive trampling has caused erosion of the riverbanks, as noted by Holmes. This exacerbates normal bank erosion, and cause serious problems. Fig. 48 shows a bank slip caused by river action, and Fig. 49, the total destruction of vegetation (foreground) caused by trampling in the area at Durham. (Some disturbance may, however, maintain open areas for crucifer growth).

The picking of flowers is hard to discourage.

Table 29

The losses of *A.cardamines* on study sites to human activity

		Animals lost to:		
		Flower picking	Trampling	% marked population lost to humans
Durham	1977	4 eggs		2.0
	78	11 eggs, 7 larvae	1 egg, 10 larvae	15.3
	79	6 eggs	3 eggs, 2 larvae	4.0
Croxdale	1978	0	10 larvae	9.3
	79	0	0	0
Witton Park	1978	2 eggs	1 larvae	2.5
	79	24 eggs, 21 larvae	13 eggs, 31 larvae	19.0
Wolsingham	1978	9 eggs	3 eggs	9.4
	79	0	*42 eggs, 6 larvae	12.2
			$\bar{x}$	8.2

* caused by cattle being driven over study site.

Fig. 48. Natural bank slip and subsidence at Maiden Castle Bridge. Durham, 1979.

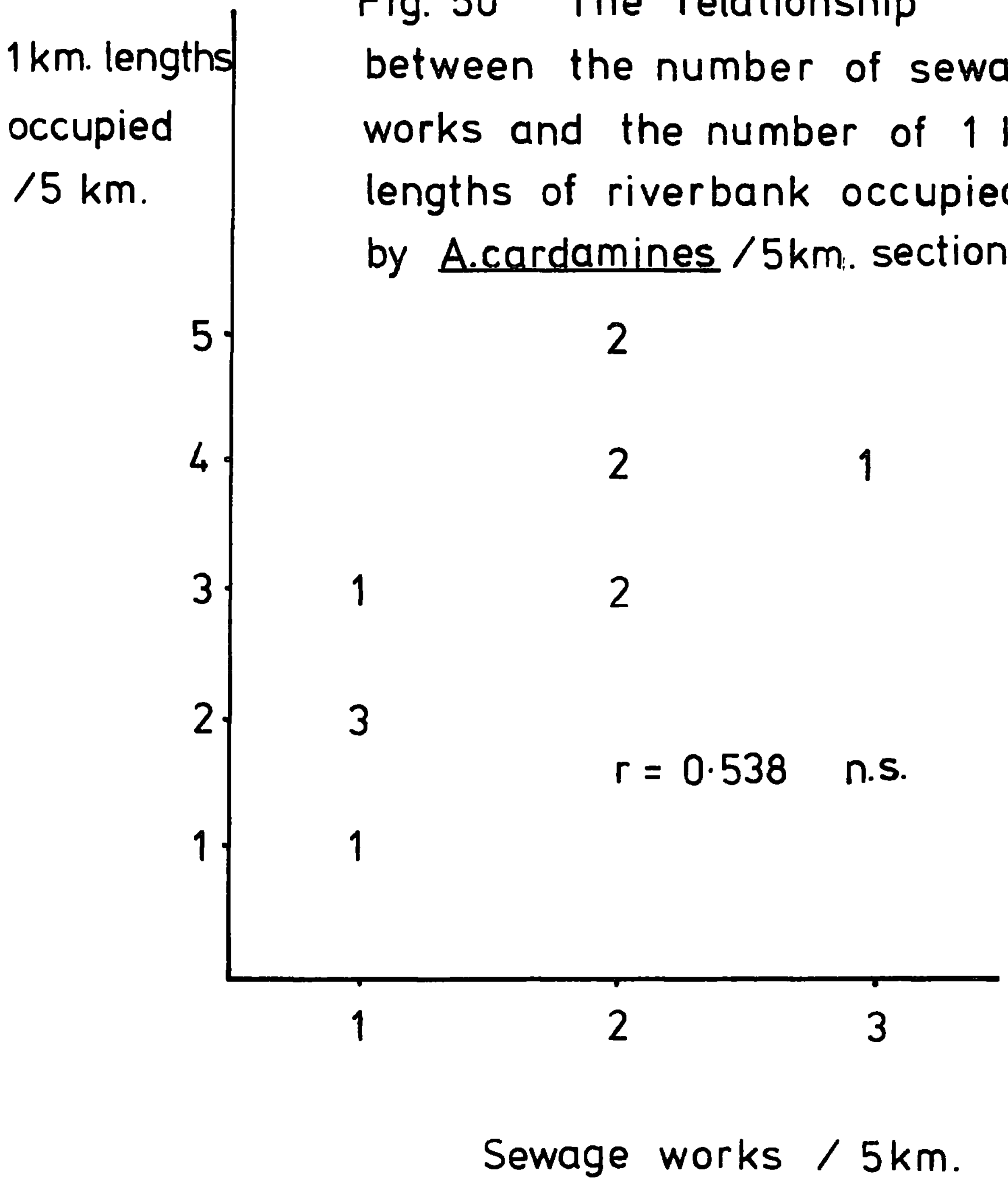
Fig. 49. Severe erosion and trampling caused by humans. Durham, 1979.

Unfortunately, some of the largest and most colourful blooms of riverbanks are Cruciferae - which leads to their being selected by both ovipositing A.cardamines and flower-picking humans. H.matronalis which receives the heaviest egg-loads, is also heavily selected for picking. B.rapa and B.vulgaris are rather less frequently plucked, and the strong-smelling A.petiolata almost never so.

The direct destruction of A.cardamines by man is small but significant in these populations. Indirect human effects are rather more difficult to assess. Brown (1970) attributed the increase in A.cardamines in the Bristol area following 1968 as due to increasing restraint in the use of weedkillers on roadside verges (a major habitat of Southern English A.cardamines). The role of pesticides in the mortality of A.cardamines is unsure, but likely to be concentrated upon dispersed populations associated with 'weedy' crucifers.

Habitat destruction is easy to see, but its importance is hard to assess. In species such as A.cardamines, however, which exist in small populations (around Durham), habitat destruction may lead to increasing isolation of demes, with consequent increasing risks of local extinction (as in M.arion). The relationship between one aspect of urban development and the distribution of A.cardamines is shown in Fig. 50, where 5 km lengths of the rivers Wear (from Chester-le-Street to Wolsingham) and Tees (Darlington to Barnard Castle) were examined. The number of 1 km lengths within each section known to be occupied by A.cardamines

Fig. 50 The relationship between the number of sewage works and the number of 1 km. lengths of riverbank occupied by <u>A.cardamines</u> /5km. section



were compared to the number of sewage works shown on Ordinance Survey maps.

It is seen that habitat selection by sewage engineers and A.cardamines is similar, in that the abundance of the butterfly and sewage works are related. This arises from both using non-wooded, non-steep riverbanks for their activities. Sewage works hardly pose a threat to A.cardamines, but the relationship does indicate the form habitat exploitation and destruction is likely to take - gradual, ever-increasing encroachment.

On the credit side, human activities may favour the creation of other habitats suitable for A.cardamines. Owen (1978) recorded A.cardamines larvae feeding on garden Arabis albida Stev. and stated that A.cardamines was therefore safe from decline due to herbicides, pesticides and habitat destruction. This facile view is not supported by this study - other garden crucifers such as Lunaria annua and Hesperis matronalis do not give good larval survival. It seems extremely unlikely that A.cardamines will ever become a truly urban species, as some other butterflies have done (Singer and Gilbert, 1978).

More important to A.cardamines will be the provision of weedy hostplants as a result of agricultural practices. Capsella bursa-pastoris flourished in one area at Durham which was fallow in 1977, but this land was not ploughed in subsequent years and grass took over. Elsewhere, particularly in the south of England, with vagrant A.cardamines populations, weedy crucifers such as Sinapis arvensis will be important.

It is likely that *A.cardamines* is maintained *above* the levels it might ordinarily reach by agriculture, as are *A.rapae* and *P.brassicae*.

The likely importance of different human-related factors to *A.cardamines* populations in northern and southern England are summarised:

1. Herbicides)
) More important in south, especially roadside areas.
2. Pesticides)
3. Entomologists. Concentrated in southern England.
4. Anglers (trampling). Important only in Northern riverside populations.
5. Gardens. Concentrated in south.
6. Agriculture (weeds). Concentrated in south.
7. Habitat destruction. Probably only important in small populations in the North.

Man has also markedly affected the ecology of *A.cardamines* by the introduction of non-native crucifers - in the present instance *Herperis matronalis* causes significantly high larval mortality. Other introduced crucifers include *Brassica canpestris* (wild cabbage) and *Isatis tinctoria*. It is pleasant to speculate that ancient British cosmetic practices may have indirectly favoured the colourful Orange-Tip via its foodplant, Woad.

CHAPTER 3

Mating behaviour and Reproductive Isolation in Pierinae

> "Ursula was watching the butterflies of which there were dozens near the water .... suddenly snapping out of nothingness into a jewel life ...... two white ones wrestling in the low air; there was a halo around them; ah, when they came tumbling near they were Orange-Tips and it was the orange that made the halo. Ursula rose and drifted away, unconscious like the butterflies"
>
> D. H. Lawrence, "Women in Love".

3. Mating behaviour and Reproductive Isolation in Pierinae

i. Introduction

With the adoption of the biological species concept in the middle of this century, zoologists such as Dobzhansky (1937) and Mayr (1942) emphasised the importance to speciation of reproductive isolation amongst taxa. The maintenance of genetic integrity, by the presence of barriers to inter-taxon breeding, became the only criterion for judging specific status. Despite many difficulties in application, the concept is now almost universally accepted, and has stimulated much work into the nature of isolating mechanisms, i.e. those characters which allow co-existence and integrity of sympatric gene pools (populations). Dobzhansky divided isolating mechanisms into two major classes - pre- and post-zygotic - depending upon whether isolation between taxa depends on mechanisms prior to, or after mating. Post-zygotic isolation may be important in some groups of organisms, but has received relatively little attention.*1

In animals, particularly where parental investment in gametes is high, pre-zygotic mechanisms are prevalent. Mechanical isolating mechanisms, which prevent inter-specific copulation, are known (e.g. Paulson, 1974) but rare. Similarly, what Dobzhansky and Mayr termed

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*1 The studies of Bowden (1972 a,b) and Oliver (1972 a,b, 1977) amongst Lepidoptera are exceptions.

'ecological' mechanisms may occur, although the demonstration of sympatric species differing in habitat selection, phenology, etc., can rarely be attributed to the result of reproductive isolation per se. Abundant research has, however, demonstrated in many groups ethological isolating mechanisms, based on species-specific courtship and mating patterns. This fact may be attributable to the relative ease of evolution of isolating mechanisms which avoid gametic waste by changes in courtship behaviour, in comparison with the relatively major genomic changes which may underlie remodelling genitalia, habitat shifts, etc.

Ethological work on courtship behaviour in butterflies began with the studies of Tinbergen et al (1942) who studied the releasers of courtship in Eumenis semele L. The Pierinae have received considerable attention, and studies of various Pierini species have been published. Thus the studies of Petersen and Tenow (1955) on Artogeia napi, Obara (1964 et seq) on A.rapae and Chovet (1977) on Pieris brassicae have all demonstrated fundamentally similar mating behaviour in these three species. Although Petersen and Tenow, and Lorković (1968) investigated mating behaviour with particular reference to reproductive isolation, studies on sympatric populations of closely related Pierinae species were lacking until the publication of work by Suzuki et al (1977) on four Japanese Pierini. The present study, started prior to the publication of that work, investigated the mating behaviour and reproductive isolating mechanisms of the 3 Pierinae that are normally commonest at Durham - A.napi, A.rapae and Anthocharis cardamines.* As conflicts were found between differing accounts of Pierinae mating behaviour, both between sources in the literature, and between these and results of the present study, it was felt necessary to make detailed study of disputed points. From the final description of mating behaviour and ethological isolation in sympatric populations, the likely evolutionary consequences and stability of such behaviour, particularly in relation to future speciation, will be discussed.

Butterfly courtship studies have generally emphasised the importance of chains of stimuli and responses in leading both sexes to successful copulation. The importance of visual cues in the initial attraction of males to females is stressed by Guillaumin (1977) in his review of the roles of differing stimuli. Thus in Eumenis semele L. (Tinbergen et al, 1942), Hypolimnas missipus L (Stride, 1958), Argynnis paphia L (Magnus, 1958 a), Heodes virgaureae L (Douwes, 1975 a) and other species, the importance of visual cues in this initial phase of the encounters of the two sexes has been demonstrated by means of models and other visual stimuli. As noted by Guillaumin, this non-specific attraction is generally followed by recognition phases and courtship proper, before copulation is effected. In Holarctic

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*P.brassicae fluctuates greatly in abundance here, and was rare in 1977 and 1978.

Pierinae, with the exception of Synchloe spp and Elphinstonia charlonia,* this general pattern is followed. Thus a visual response of the male, specific only to insects of approximate Pierinae size and colour, is followed by recognition of the female, courtship, acceptance (or rejection) of the male by the female, and finally copulation. A close similarity is found between all species in following this scheme.

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*Neither Synchloe nor Elphinstonia have androconia on male wings. In Synchloe callidice there appears to be a female-produced sex pheromone, as in moths.

ii. Male behaviour

Male Pierinae are patrollers (sensu Scott, 1974), that is they search for females by flying constantly within a habitat, investigating objects that are likely to be conspecific females. This behaviour is contrasted to that of 'perching' species, where sedentary males fly out at passing insects. Curio (1977) has shown that in a model of a predator searching for moving prey, the predator gains little advantage from increasing speed of movement. Instead, when the critical factor determining encounter rate is prey density, predators will maximize the number of contacts with prey by limiting their activity to areas of greatest prey density. In the present case, it is to be expected that males should concentrate their activity where females are most abundant - this is borne out for A.napi, A.rapae (Scott, 1975) and particularly the population of A.cardamines at Durham.* This may also lead to other aggregative behaviour such as 'hill-topping', noted for Pontia occidentalis (Sheppard, 1966).

Whilst patrolling, male Pierinae fly to and investigate

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*The relative advantages of perching and patrolling may depend upon the degree of aggregation or predictability of females, which may itself be dependent upon larval foodplant growth forms and abundance (Scott, 1975 b). In animals other than butterflies, male searching strategies akin to perching and patrolling may both exist within a single population, and may be pursued by different males - for instance the bee Centris pallida (Alcock et al, 1977).

many objects, including flowers, pieces of paper, other butterflies and other insects, such as bees. Some of these investigations may be aimed at potential nectar sources, but the majority investigate insects which may be potential mates. Guillaumin (1977) has distinguished this phase of behaviour as 'le vol d'approche' and has emphasised the lack of specificty in the male searching image. In Pieris brassicae, under caged conditions, "The sexually active male does not always immediately distinguish a female from a male" (David and Guardiner, 1961 b). Around Durham, frequent encounters were seen between all 4 common Pierinae members, with occasional interactions with other insects such as Aglais urticae, Maniola jurtina, various syrphids and bees. In Morocco, Zegris eupheme, a very large fast-flying Euchloinid, was seen to investigate insects of approximately the same colour as a Z.eupheme female - viz. Goneptery x rhamni L., Colias croceus Gffy., A.rapae, P.brassicae and other Z.eupheme males. Their lack of specificity in the initial approach is typically found in other butterflies, such as Hypolimnas missippus (where, as noted by Stride (1958) males will attempt to mate with other males), Heodes virgaureae (Douwes, 1975), Papilio xuthus L. (Hidaka and Kamashita, 1978) and Cartercephalus palaemon Pls. (Somerville, 1978).*

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*Non-specificity in male courtship is typical of many other groups of insects: In Odonata (Moore, 1952), in Rhagoletis spp (Bush, 1972), Drosophilidae (Speith, 1972), various Hymenoptera (Alcock et al, 1978) and Periplaneta spp (Simon and Barth, 1977) similar responses are seen.

Contacts between an investigating male and any insect other than a conspecific female are normally transitory. However, on some occasions, 'spiralling' occurs, with two males flying around and about one another, gradually increasing in height. As noted by Chovet (1977) for P.brassicae, this behaviour is most likely to occur when several individuals are present. Chovet hypothesised that this occurs because of the super-stimulation presented by several males in motion. As shown for E.semele (Tinbergen, 1951), A.paphia (Magnus, 1958, a,b) and H.virgaureae (Douwes, 1975 b) moving stimuli are more attractive than stationary ones in eliciting such 'pursuit' behaviour.*

After contacting and investigating another insect, the Pierinae male flies off unless the insect proves to be a Pierinae female. In this case, the male's response depends upon the female's behaviour - pursuit should she attempt to fly away. More frequently, the female is already at rest or soon settles - in these cases males flutter about the head of the female. Normally the females response is a 'mate-refusal' posture, with wings sharply deflected down and abdomen up-raised. This posture has been erroneously

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*Baker (1972) for the butterflies Aglais urticae and Inachis io L., and Davies (1978) for Pararge aegeria, have described spiralling behaviour as territorial defence and, in the latter study, as a signal based on the asymmetry of the contest. As pointed out by Scott (1975) such 'territoriality' may in fact be typical 'perching' behaviour and investigative forays. "Interference between butterflies during mating behaviour arises from the generalised nature of the stimuli involved in visual communication".

interpreted by Abbott (1965) as a mate-acceptance posture, but throughout Pierini its role is rather one of mate refusal, since the deflected wings prevent males from settling, whilst the raised abdomen prevents copulation.

It was first noted as such by Marshall (1891) and is also found in some Euchloini. (The release of the mate-refusal posture is discussed more fully in the next section). Males continue fluttering around the female for some time. If she persists in maintaining the mate-refusal posture, then the male departs. However, if the female closes her wings then the male will eventually settle and initiate copulation.

In order to study male investigative behaviour, experiments were carried out using 'fishing-pole' arrangements as in the studies of Tinbergen et al (1942). These involved the presentation to wild males of dead butterflies, or other models, suspended on a thin thread from a long pole. The reactions of wild males to these models was then noted. By manipulating the 'behaviour' of the models, it was possible to examine male responses to different stimuli.

The experimental design was to use pinned specimens of males and females of A.cardamines, A.rapae and A.napi, and to suspend these from a pole c.2 m in length which was held in the hand in habitats where males of these species were flying. Several different 'behaviours' were possible with the models; it was decided to employ three:

i. The model is maintained in the air whilst a wild male investigated

ii. On contact by the wild male, the model was dropped to the ground. For specimens set with wings open, the model is kept in its normal orientation, i.e. upper-surfaces uppermost.

iii. The model is dropped to the ground, but pulled over by means of the string to expose the under-surface of the wings.

Model behaviour ii mimics the mate-refusal posture of females, whilst behaviour iii mimics some features of the female closing wings. Whilst in flight, the models resembled quite closely normal butterflies, since the aerodynamic properties of the pinned specimens led them to 'fly' **head** foremost. Wild males appeared to investigate them freely and to behave in every way normally in response to the stimuli from these models.

Several interesting observations were made in the initial phases of this work. Models, that is specimens, which were completely set and dry had a very short life-span as useful tools. Antennae, legs and whole heads were lost rapidly, whilst wings became very tattered and were frequently knocked off specimens by males. It was apparent that the ebullient wild males were destroying the models by their investigations. This problem was overcome by using specimens kept frozen until use, which were more relaxed, and less brittle. A second observation was that it was important to use a standardised "response" by the model. If the model was moved away from the investigating male, preventing him from coming within c. 10 cm. of the model, the interaction could be prolonged almost indefinitely. Encounters between e.g. wild male _A.rapae_ and a male model _A.napi_ could be made to last a minute or more in this way, although once the male had been allowed to make contact, it would immediately fly away.

Prior to contact, the investigation could be prolonged, but not afterwards, unless the model was a female. The standardised response was to 'flutter' the model around only until a male reacted to it, when the model was held stationary. Other observations were made by initiating contacts using the model. Male butterflies invariably flew away from such investigations, even if the model was female. Female butterflies adopted the mate-refusal posture.

In all three years of study Orange Tip behaviour was studied in spring at Durham, whilst the other species were primarily studied during summer and autumn, at Witton Park and Durham City Allotments. The length of time spent by wild males investigating the various models was noted by means of a stop-watch, and any attempts at copulation were recorded.

Table 1 summarises the results of this study. It may be seen that males of all three Pierinae species investigate any model presented. However, males of A.cardamines distinguish females of their own species, and usually will only follow these down to the ground. Males of both Artogeia species are seen to spend much time fluttering around females of either species (and to a lesser extent A.cardamines females). Model behaviour iii, presenting underwing colouration, elicited most copulation attempts by these species, although some A.cardamines males attempted to copulate with model females with wings spread. Males of either Artogeia species are seen to attempt to copulate with females of the other species.

Table 1

The duration (d) of the responses to models ($\bar{x}$ in secs) (+ 2.S.E) and the number of copulation attempts (n_c)

A.cardamines males

Model	Wing Position	Model Behaviour	n	$\bar{x}d$	2 S.E	n_c
A.cardamines ♂	Open	i	56	1.4	.34	-
		ii	8	2.1	.74	-
		iii	-	-	-	-
A.cardamines ♀	Open	i	30	6.7	2.45	-
		ii	26	17.3	4.25	9
		iii	12	15.5	5.32	9
A.rapae ♂	Open	i	23	1.1	.12	-
		ii	4	2.0	0	-
		iii	-	-	-	-
A.rapae ♀	Open	i	21	1.2	.27	-
		ii	6	2.0	0	-
		iii	-	-	-	-
A.napi ♂	Open	i	25	1.1	.13	-
		ii	10	2.4	.51	-
		iii	-	-	-	-
A.napi ♀	Open	i	25	1.3	.27	-
		ii	18	2.2	.19	-
		iii	8	2.9	.25	-

Table 1 cont'd.

<u>A.rapae</u> males

Model	Wing Position	Model Behaviour	n	$\bar{x}d$	2 S.E	n_c
A.cardamines ♂	Open	i	30	1.7	.48	-
		ii	10	3.0	.26	-
		iii	-	-	-	-
A.cardamines ♀	Open	i	30	1.7	.42	-
		ii	8	4.0	.76	-
		iii	8	5.3	3.63	-
A.rapae ♂	Open	i	131	1.8	.14	-
		ii	21	2.6	.39	-
		iii	13	2.8	1.47	1
A.rapae ♀	Open	i	101	1.7	.13	-
		ii	84	4.7	.49	21
		iii	90	11.0	1.66	53
A.rapae ⚥	Closed	i	30	1.2	.13	-
A.napi ♂	Open	i	104	1.7	.12	-
		ii	40	2.3	.31	-
		iii	42	2.3	.13	-
A.napi ♀	Open	i	121	1.9	.77	-
		ii	84	4.5	.40	6
		iii	81	7.4	1.28	43

Table 1 cont'd.

<u>A.napi</u> males

Model	Wing Position	Model Behaviour	n	$\bar{x}_d$	2 S.E	n_c
A.cardamines ♂	Open	i	30	1.1	.09	-
		ii	10	2.0	0	-
		iii	-	-	-	-
A.cardamines ♀	Open	i	30	1.3	.26	-
		ii	17	4.8	1.85	-
		iii	10	2.4	.33	-
A.rapae ♂	Open	i	62	1.6	.31	-
		ii	20	2.3	.51	-
		iii	21	2.7	.73	-
A.rapae ♀	Open	i	60	1.8	.26	
		ii	64	5.2	.98	8
		iii	73	8.8	2.22	33
A.napi ♂	Open	i	88	1.6	.28	-
		ii	32	3.7	.81	-
		iii	22	2.3	.37	-
A.napi ♀	Open	i	64	1.8	.43	-
		ii	72	4.4	1.02	5
		iii	68	11.6	1.95	36
A.napi ♀	Closed	i	24	1.4	.47	-

These results are of interest for comparison with previous studies of Pierinae mating behaviour. Obara and Hidaka (1968) and Obara (1970) have demonstrated that male A.rapae are able to distinguish between the sexes as has been shown by Chovet (1977) for P.brassicae. In both species the basis of this sexual discrimination has been stated as differences in reflectance of Ultra-violet (U.V) light. Evidence in support of this is given by the fact that Pierinae butterflies will only mate in daylight or in artificial light with U.V. components added (Petersen and Tenow, 1955; David and Gardiner, 1961 b; Dusanssay and Delplanque, 1964). The importance of U.V. light in insect vision has been known since the work of Lutz (1933) who figured the U.V. absorbing wings of male P.brassicae. Further studies of Mazokhin-Porshniakov (1956) established the wide variation in reflectance and absorbance in butterflies wings, and showed sexual differences in such pattern. As shown by Hidaka and Okada (1970), sexual differences of U.V. absorbance in A.rapae, are produced by elipsoid particles packed into the gratings of scales. Abundance of these particles directly parallels U.V. absorbing capacity.

The structure of the Pierid eye also supports the view that the butterflies use U.V. vision extensively. Kolb (1977) studied the eye of P.brassicae, and showed that the corneal surface of each ommatidium was covered with minute nipples. As shown by Bernhard et al (1965), these nipples act as impedance converters which match the impedance of air to that of lens material. Nipples of different amplitude act optimally at different wavelengths of light. Low amplitude nipples (of c. 1700 Å), as in P.brassicae, transmit light of the U.V. range optimally.

Abundant evidence therefore suggests that U.V. reflectance and absorbance patterns in Pierini are the basis for sexual discrimination and attraction. The behaviour of males has been hypothesised to advertise sexual identity in A.rapae, where a stationary male, on being investigated by another male, 'flutters' his wings, thus illustrating his highly U.V. absorbent wing surfaces (Obara and Hidaka, 1964). The observed discrimination of male A.napi and A.rapae at Durham, where the sex, but not the species, of females is determined accurately by investigating males, might be explainable if female A.rapae and A.napi were alike in being U.V. reflective. However Bowden (1977) surveyed U.V. reflectance of A.napi, and showed that many of the taxa within that species group, including the populations in southern England, have females which are not significantly more reflective than males, although other taxa, particularly the upland A. bryoniae do have U.V. reflecting females.

Photographs were taken of males and females of all Pierinae species using U.V. illumination and filters as described by Bowden (1977) (Fig. 1). Females of A.napi from County Durham are seen to reflect more U.V. light than males. In particular 'upland' females with characteristic yellowish

Fig. 1. Ultra violet reflectance in Pierini butterflies.

A.napi ♂
Durham 1978
1st brood

A.napi ♀
Durham 1978
2nd brood

A.napi ♀
Langdon Common 1977

A.bryoniae ♀
ex Stocks
S.R. Bowden

A.rapae ♀
Durham 1979
2nd brood

P.brassicae ♂
Durham 1979
2nd brood

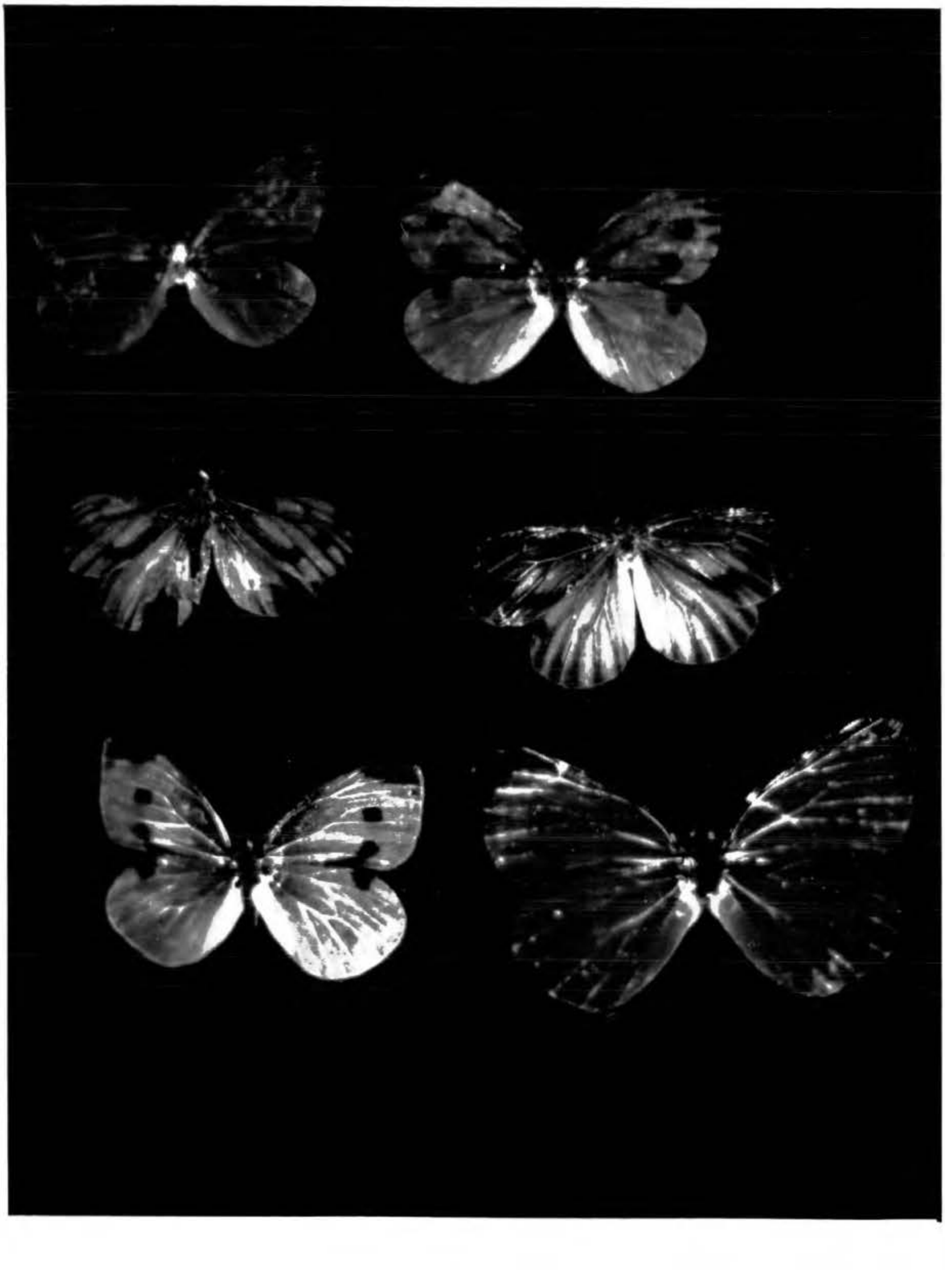


Fig. 2. Ultra violet reflectance in Euchloini and *Pontia*.

Anthocharis cardamines

♂ Durham, 1978 ♀

Zegris eupheme

♂ Ifrane, Morocco. 1978 ♀

Pontia daplidice

♂ Asni, Morocco. 1978 ♀

Euchloe ausonia ♂

Ifrane, 1978

Euchloe belemia ♀

Asni, 1978

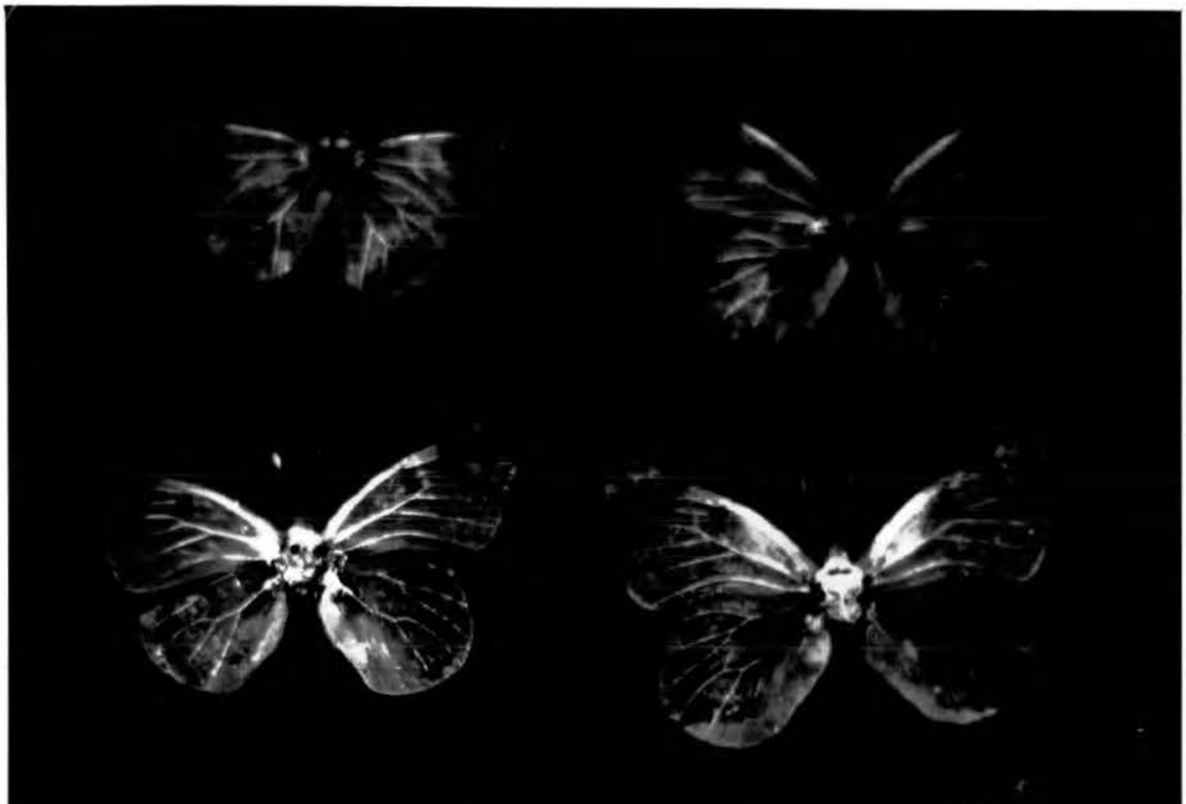
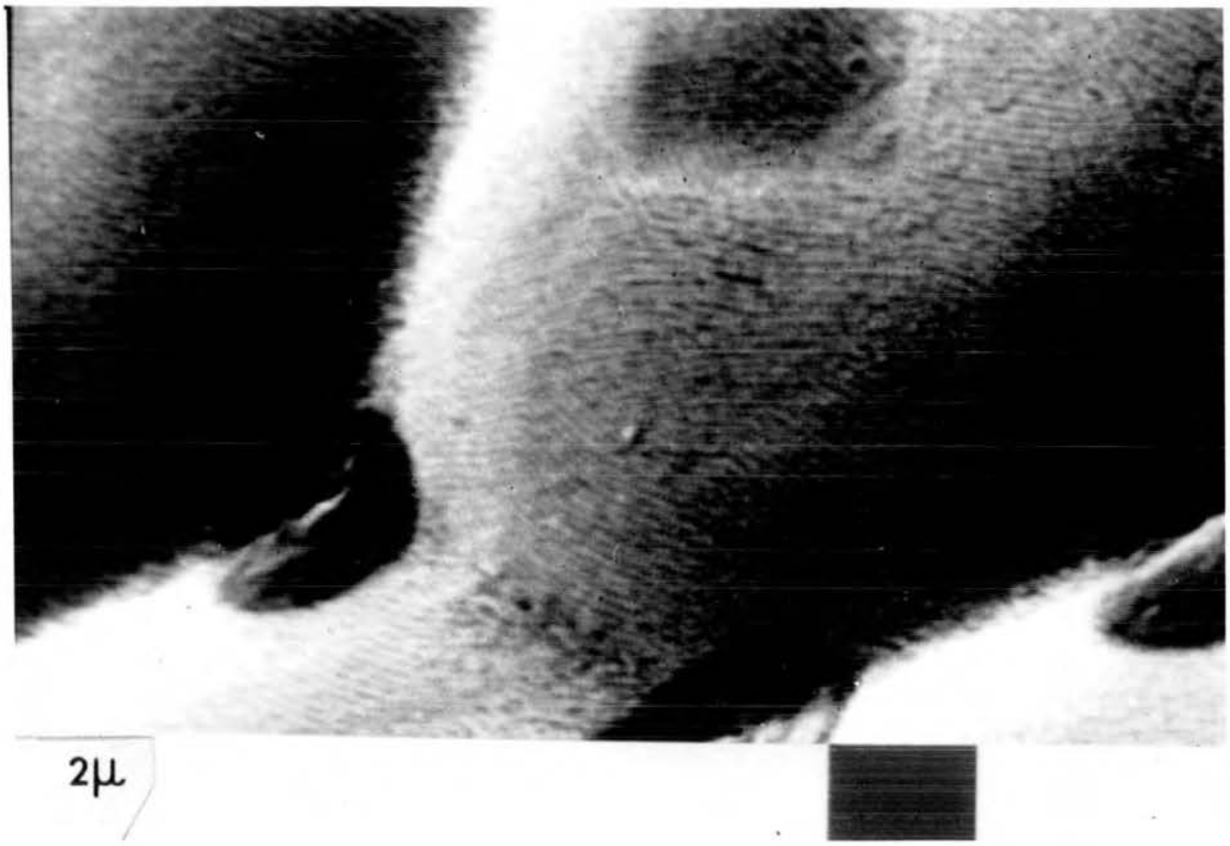
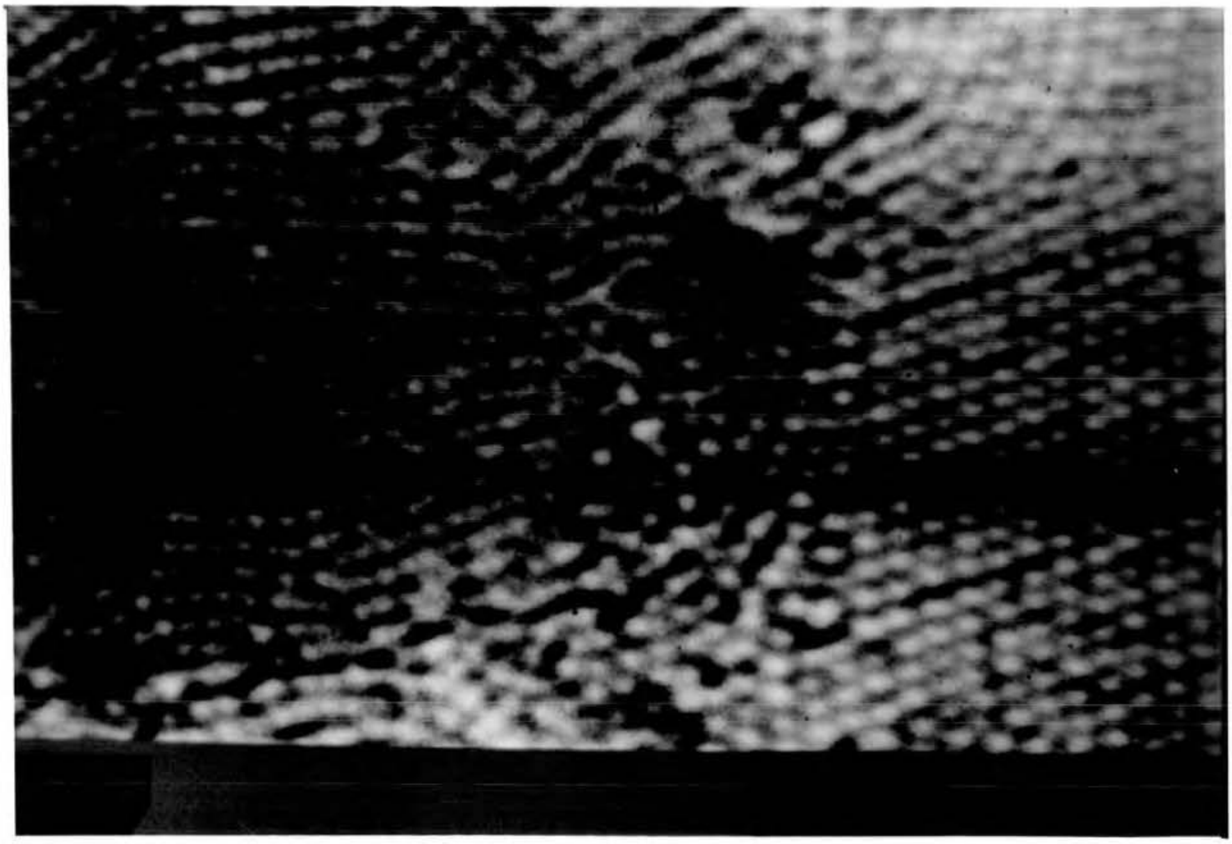


Fig. 3. Corneal surface of the ommatidia of _A.cardamines_ (S.E.M.) (rounded dark patches are of unknown origin, and appear pathological).

Fig. 4. Detail of Fig. 3., to show rows of low amplitude nipples covering the corneal surface.


2μ



upper-surfaces (see Chapter 4) reflect much U.V. light, which Bowden has shown for other A.napi (the two characters are only partly correlated).

A.cardamines females are readily distinguishable by all males. However, they do not reflect much U.V. light; rather, in U.V. illumination, female A.cardamines appear to glow a pale saffron colour. This may be the basis for sexual discrimination in that species. In other Euchloini, Scott (1973) has demonstrated sexual differences in U.V. reflectance in Euchloe ausonides. Fig. 2 shows that E.belemia and Zegris eupheme are sexually dimorphic in reflectance patterns, as is the Pierini member, Pontia daplidice.

As a final check on the idea that U.V. reflectance plays a major part in sexual identification, the surface of the cornea of the three species, A.rapae, A.napi and A.cardamines was examined for nipples of the sort described by Kolb (1977). In all three species, low amplitude nipples, which offer maximum transmission of light in the U.V. range, were found (e.g. A.cardamines, Figs. 3 and 4).

Model females of A.napi and A.rapae with wings closed were not attractive whilst in the air, but underside colouration was the stimulus for copulation attempts when on the ground. Obara (1970) found using grounded models only, that females with closed wings were more attractive to male A.rapae than females with open wings. Obara attributed the apparent superior stimulation caused by the undersurface of wings to under-sides having the optimal quantity of near-U.V. reflection. Obara's scheme for *A.rapae* mating held that virgin females, sitting on cabbage leaves, were the resource sought by males. His scheme therefore holds that only a single cue, underside colour, is necessary for copulation attempts by males. In fact most *A.rapae* females mate several times (see below) and most male:female encounters occur between flying insects. It is apparent that Obara's scheme must be modified. Rather than a single cue leading to copulation, several cues are perceived by the male in sequence. Firstly, an initial 'whiteness' which elicits approach. Secondly 'U.V. reflectance' which at close range signals sex and elicits pursuit and courtship. Thirdly the yellow underside colouration which, only after the other cues have acted, becomes stimulatory. This view of male responses was given unexpected support during attempts to photograph copulation attempts with dummies. On this occasion, it was found that a female model would lure a male down from the air, but that the male would 'lose interest' in the female on the ground, and would instead direct much attention to part of the camera equipment. Fig. 6 shows a male *A.rapae* attempting to copulate with the equipment, whilst ignoring a nearby female. Fig. 5 shows a 'normal' copulation attempt upon a female *A.rapae*. The equipment was a light-yellow in colour, and it is hypothesised that this yellow colour acted as a super-stimulus to the courting male. The

Fig. 5. Wild male A.rapae attempting to mate with dead female. The bent abdomen of the male, approaching the female from the side, is clearly visible. Also very noticeable is the pin and line used in manipulating the model.

Fig. 6. Male A.rapae attempting to copulate with yellow camera spool and ignoring female model.

equipment was totally ignored by patrolling males, but was always preferred to a model female once the male had been attracted down. Thus Obara's view of courtship being based on a single response of the male is replaced by the view that a catenary process occurs, the successful courtship of mates being dependent upon a chain of stimuli and responses. A similar conclusion has been reached for Heodes Virgaureae (Douwes, 1975) where copulation attempts are similarly released by underside colouration, and for Zizeeria mala L (Wago, 1978). In P.brassicae, Chovet (1970) has shown that the emission of a spermatophore by the male is a simple reflex reaction to tactile stimuli during copulation, which completes the reaction chain.

iii. Female responses and courtship

The above section has shown the responses of males to different stimuli and demonstrated that male discrimination does not underlie reproductive isolation in these Pierinae. However, interspecific matings between *A.napi* and *A.rapae* are rare.* The responses of females to male stimuli have, however, been rather less studied in butterflies than have male responses. Entomologists have traditionally felt that male pheromones, usually disseminated as androconial scales, are the stimuli leading females to successful courtship, as reviewed by Myers (1972). In relatively few studies has this been demonstrated. However, Taylor's work on reproductive isolation in *Colias* (Taylor 1970, 1973) and the elegant studies of Rutowski on *Eurema lisa* (1977 a,b), have demonstrated conclusively that male androconial scent is responsible for female acquiescence to mating in these Pierids.

In Pierini the androconia are large and distinctive and produce smells readily detectable to even the human nose. *A.napi* in particular smells strongly of lemons. Bergstrom and Lundgren (1973) and Hayashi et al (1978) have chemically characterised the scents of *A.rapae*, *A.napi*, *A.melete* and *P.brassicae*, which contain varying amounts of citral, geranial, neral and other substances in the different species. As noted by Bergstrom and Lundgren, androconial scent does not appear to have direct aphrodisiac function in Pierini, since hand pairing (i.e. tactile stimuli alone)

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*Hybrids of *A.rapae* and *A.napi* may be produced, albeit with difficult, in the laboratory by hand-pairing. The Hope Department Museum, Oxford, has a presumed hybrid which is wild-caught.

will lead to females copulating. Rather they argue that the scent acts to suppress the mate-refusal posture of females (q.v.)

The mate-refusal posture is one of several methods by which females respond to male investigations. If the female is already settled, she invariably adopts this posture. However, if she is contacted whilst in flight, she may alternatively settle and adopt the posture, drop in amongst herbage and escape attention, or fly upwards. In response to females flying upwards, males usually give chase and very lengthy spiralling may take place before a female drops to the ground or a male loses contact with her (usually the former event occurs). The behaviour of the male in relation to flying females was described by Suzuki et al (1977) as 'leading the female down' to the ground, and was noted as a courtship ritual. This was in total disagreement with my impressions, and in order to investigate the behaviour of males to flying females of A.rapae, slow motion cine film was taken during the autumn of 1979 to examine the precise nature of the interaction. The flight pattern was shown to be identical to that figured by Suzuki et al. That is, the male approached from below the female and flew into her from beneath, in a zig-zag flight path. Suzuki et al interpreted this as the male attempting to disseminate androconia by contact and leading the female to the ground. Close inspection of the film obtained suggested a different interpretation. It is seen that males often fly straight into the female, and lose considerable amounts of height as a result of the collision. Thus the zig-zag flight arises from males losing and regaining height. The female also loses height and is quite clearly battered by the male. It is suggested that although androconial scent transfer may be taking place, the male is attempting to force the female into alighting. The effect of male aggression will be discussed again below.

Obara (1964) and Obara and Nakagoshi (1974) have reported in detail the mate-refusal posture of *A.rapae* which has also been noted for other Pierini including *A.napi*, *A.canidia* L. and *A.melete* (Suzuki et al, 1977), *P.brassicae* (David and Gardiner, 1961 b), *Pontia daplidice* (pers ob) and the Euchloinid *A.cardamines*. Obara showed that in *A.rapae* only unmated females did not show the response and used this observation to suggest that *A.rapae* females were monogamous. However, as noted by Suzuki et al (1977), virgin females of *Artogeia* spp also sometimes adopt the posture. They therefore felt that

> "The following interpretation seems reasonable that the mate refusal posture of female *Pieris* (*Artogeia*) butterflies have been evolved firstly in favor of achieving copulation at their appropriate condition, or as a prenuptial posture by which they acquire adequate time to discriminate proper partners from others, but not for avoiding remating."

Different hypotheses have therefore been proposed for the evolution of the mate-refusal posture. Avoidance of multiple mating could be advantageous to females with limited time in which to lay their eggs. However, the lack of discrimination shown by males might equally lead to females adopting the behaviour, in order to avoid gametic loss due to inter-specific mating. In order to investigate the question of mating frequency in Pierinae, spermatophore counts were made upon female butterflies sampled for electrophoretic study, following the approach of Burns (1968). The number of spermatophores present in the bursa copulatrix of each female was noted. The bursa copulatrix and spermatophore of A.rapae are shown in Figs. 7 and 8.

It is known that the mate-refusal posture of A.rapae is 'released' or at least enhanced by the presence of a spermatophore within the bursa copulatrix. The elegant neurophysiological work of Obara et al (1975) demonstrated that stretch receptors around the bursa copulatrix are stimulated by the presence of a spermatophore. Injecting saline solution into the bursa copulatrix released mate-refusal behaviour. A virgin's bursa copulatrix does not stimulate the stretch receptors, nor do empty ones in which sperm is depleted. In other Lepidoptera an adequate sperm supply in the spermatheca is known to act as an inhibitor of receptivity to courtship, rather than the contents of the bursa copulatrix (Taylor, 1967). However, dissections on Co. Durham material confirmed the unimportance of the spermathecal store as a stimulus, since frequently animals with bulging spermathecae were found to have mated several

Fig. 7. Surface of bursa copulatrix of female A.rapae. The lamina dentata is clearly visible (S.E.M.)

Fig. 8. Spermatophore removed from the bursa copulatrix of a female A.rapae. This spermatophore is at approximately one half of the maximum distension (S.E.M.)


200μ

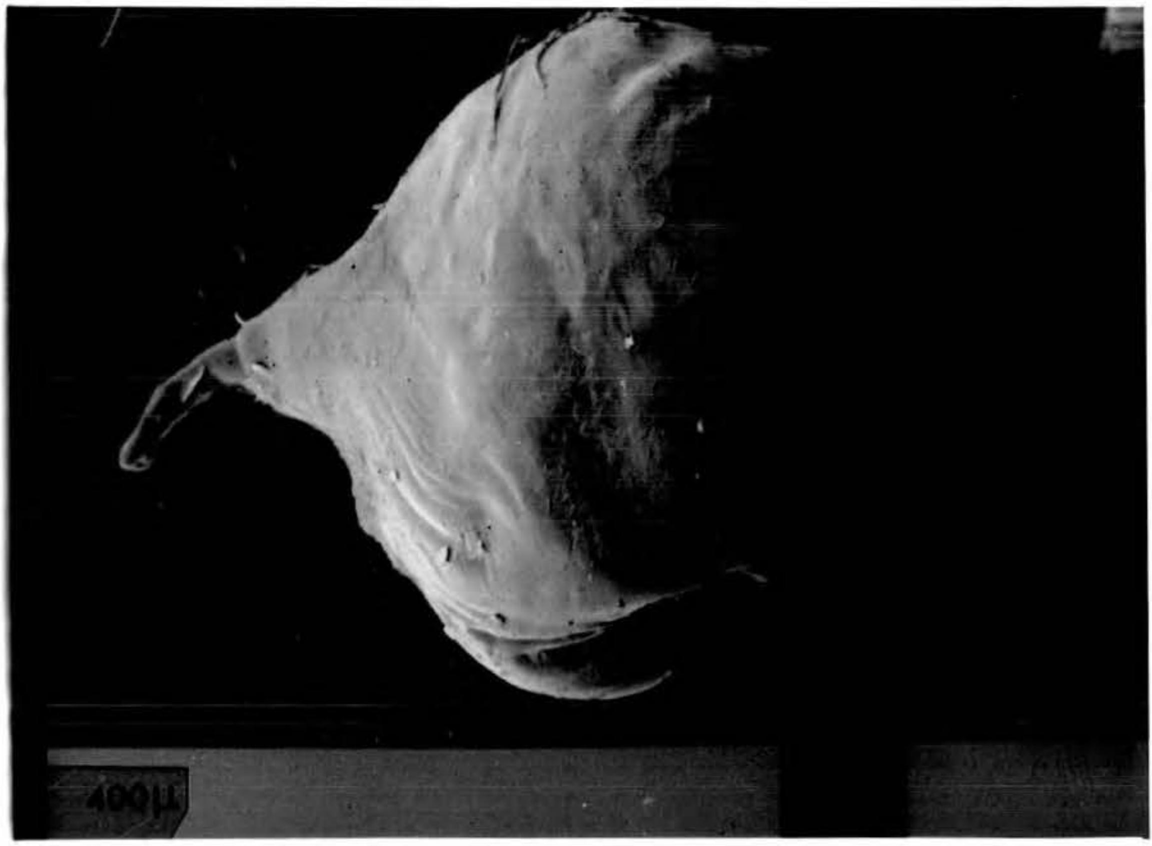
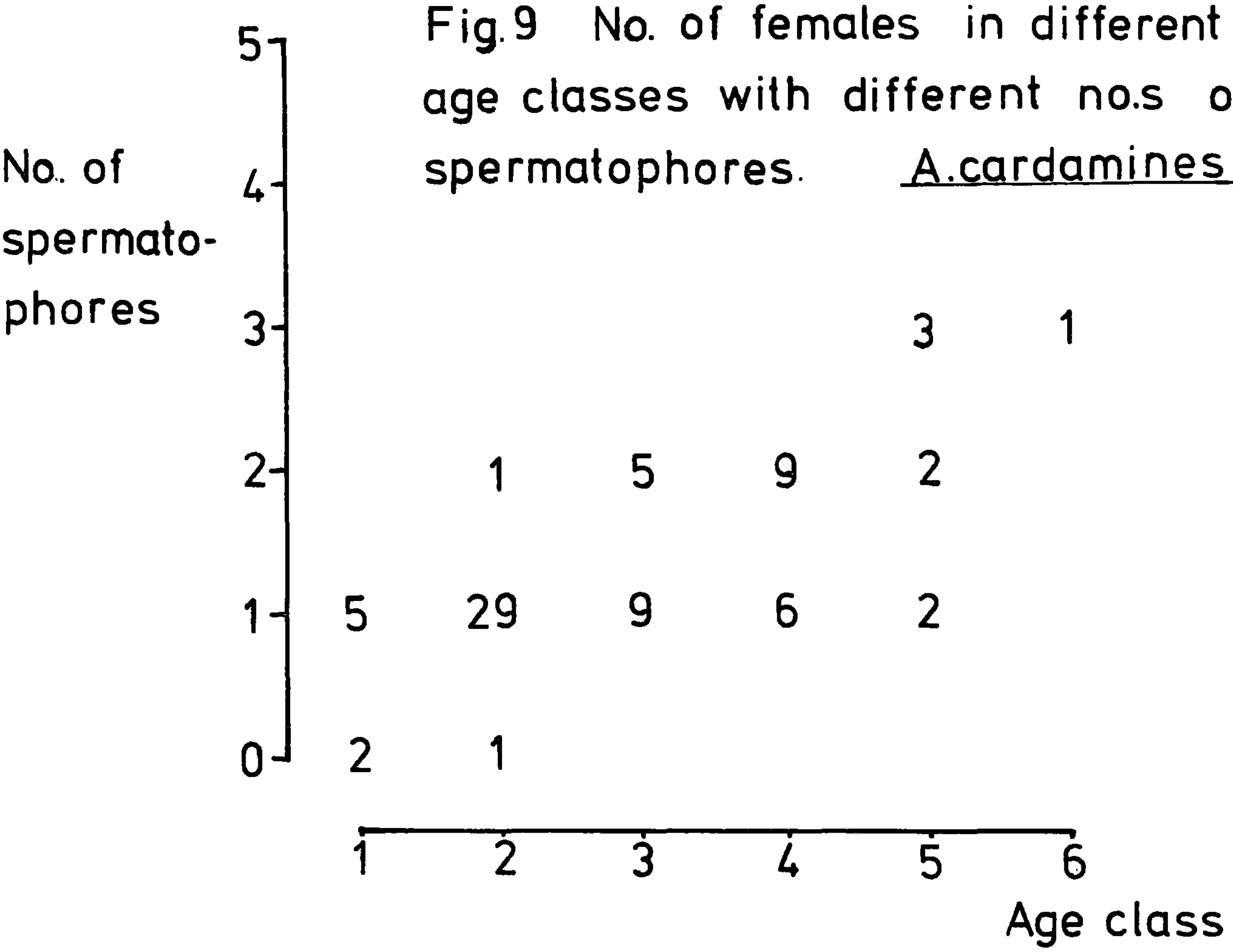

400μ

Fig. 9 No. of females in different age classes with different no.s of spermatophores. A. cardamines



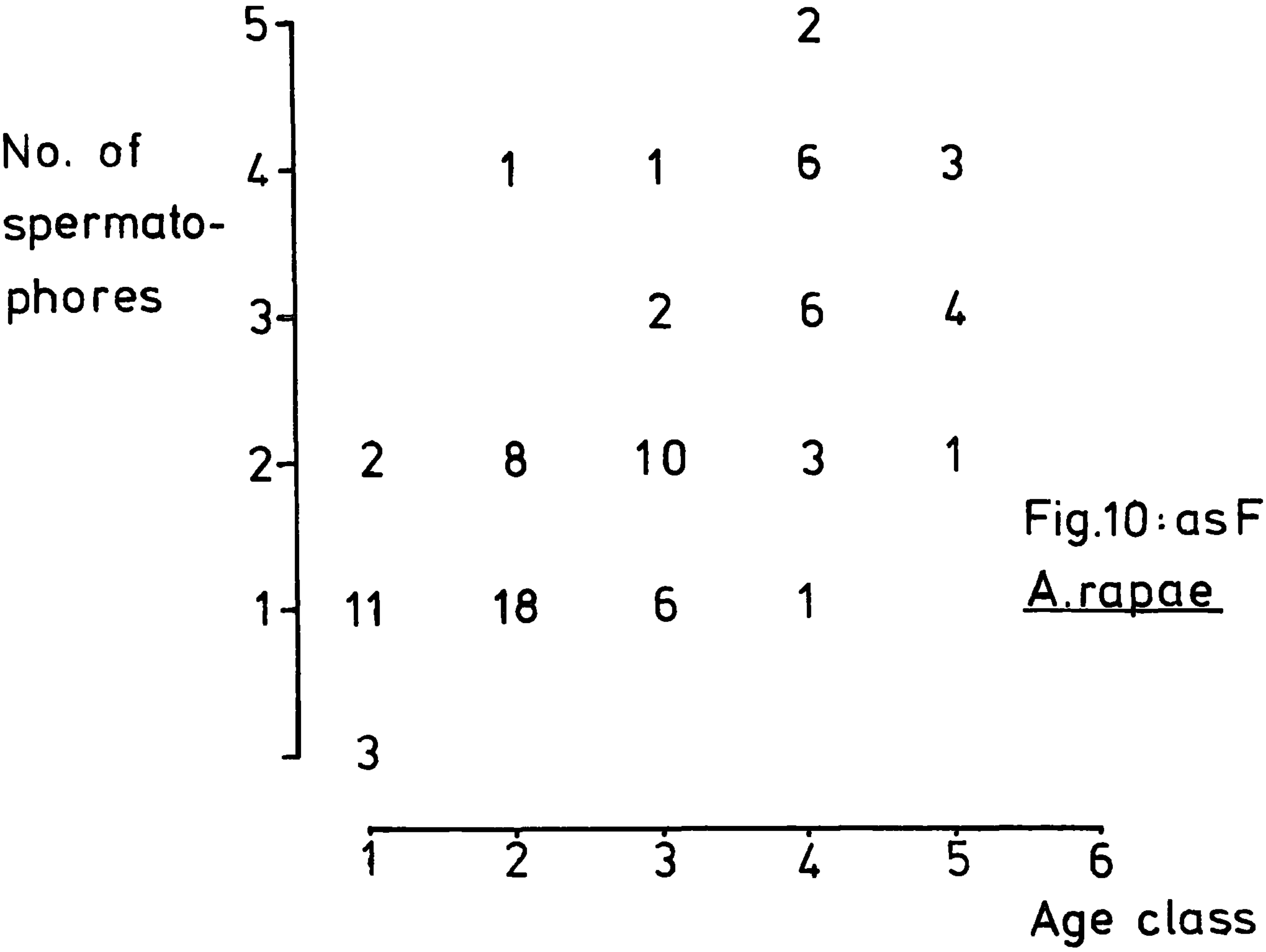


Fig. 10: as Fig. 9 A. rapae

times.

The animals dissected for spermatophore counts were additionally placed into age classes on the basis of the degree of wear on the wings (i.e. the number of scales lost, and the amount of damage to the wing edge). For A.napi, this has been demonstrated to be related to age by mark-recapture studies on one very large population in 1977 (Appendix 8). In A.cardamines and A.rapae no such relationship has been shown, and age classes of the three species should in no way be considered comparable.

Figs. 9 and 10 show the mating frequencies of all specimens of A.cardamines and A.rapae dissected. It is seen that A.cardamines tends towards female monogamy, as suggested by Wiklund and Ahrberg (1978), but that A.rapae females mate frequently and typically have more than one spermatophore in the bursa copulatrix. This species appears similar to P.brassicae, where in a culture of 20 females, 46 matings occurred in 10 days (David and Gardiner, 1961 b). As noted by David and Gardiner (1962 b), after 12 days from mating, female P.brassicae begin to lay infertile eggs. Similarly in the red boll worm, Diparopsis castanea Hmps. (Noctuidae) fertility of females declines with time since mating (Marks, 1976). The evidence therefore suggests that multiple mating in these species may be adaptive, by ensuring that all eggs laid by females are fertile. As noted by Parker (1970) and demonstrated in Drosophila melanogaster by Gromko and Pyle (1978), it is adaptive for the female to remate <u>before</u> the total exhaustion of sperm stored from the first mating.

Obara's suggestion that female <u>A.rapae</u> avoid remating is not confirmed. However, the mate-refusal posture may act to prevent remating except when the bursa copulatrix stretch receptors are unstimulated, i.e. when sperm supplies are dropping (but not exhausted). The relatively regular increase in mating frequency with age supports this view.

The response of the female to dwindling sperm supplies may thus be to remate. However, the stimulation of pressure receptors will not be digital, but rather analog - that is the receptors will not be either all stimulated or all not stimulated. --Situations of depletion will occur when receptors are partially stimulated. (Sugawara (1979) has recently shown that impulse frequency from bursal stretch receptors is related to bursal volume). What will be the females response in these cases? It was hypothesised that the mate-refusal posture of females in such states might be overcome by persistent male harassment. Since populations of <u>A.napi</u> differ widely in the density of animals, it was decided to compare females from differing populations and to see if increasing population density increased mating frequency. Such a relationship is already known for the moths <u>Plodia</u> <u>interpunctella</u> Hbn (Browes, 1975) and <u>Diparopsis</u> <u>castanea</u> (Marks, 1976) and for <u>Drosopophila</u> <u>melanogaster</u> (Gromko and Pyle, 1978). Harassment by males is known to change female behaviour in <u>Pontia</u>

protodice (Shapiro, 1970). The density of populations of *A.napi* was assessed directly by recording the length of time required to collect a sample of 25 adults (male and female). Populations were then grouped into three classes of density.

i. High - 25 animals caught in < 30 minutes.

ii. Intermediate - 25 animals caught in > 30 < 120 minutes.

iii. Low - 25 animals caught in > 120 minutes.

These classes were adopted because they offered clear cut divisions in the field, where *A.napi* is either abundant (i.e. within a large colony), common or occurs only as stragglers. The highest density recorded was at the **Langdon Common** Site (see Chapter 4) on 8th July, 1977, when 254 animals were caught by two observers in 45 minutes.

The results obtained from dissection of *A.napi* are given in Fig. 11. It is seen that mating frequency increases with age, as indexed by wing wear, as for *A.cardamines* and *A.rapae*. The results also indicate that more matings/female occur in populations at high density. This is made clear in Table 2, which shows the number of matings in old females (age classes 4, 5 and 6) from populations at different densities.

The role of male aggression in securing matings has been noted in other butterfly species, e.g. *Euphydryas editha* (Labine, 1966), *Heodes virgaureae* (Douwes, 1975 a), *Lycaeides argyrognomon* (Lundgren and Bergstrom, 1975), *Eurema lisa* (Rutowski, 1977a) and other species (Pliske, 1973). Physical battery is emphasised in some of these

FIG. 11 NUMBERS OF A.NAPI ♀♀ IN DIFFERENT AGE CLASSES WITH No.S OF SPERMATOPHORES. SAMPLES FROM POPULATIONS AT DIFFERENT DENSITIES (SEE TEXT)

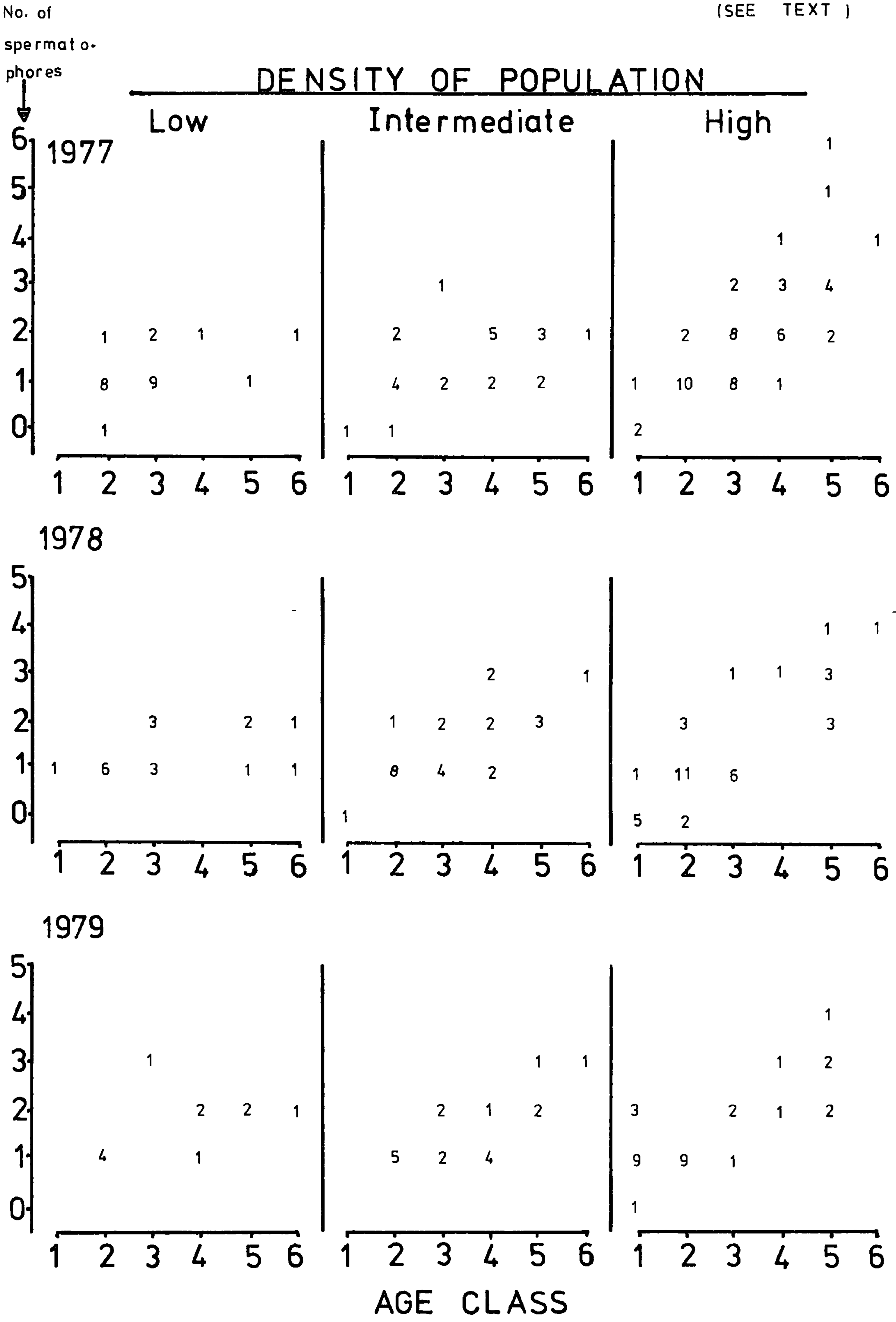


Table 2

The number of females in age categories 4, 5 and 6 with three or more, as opposed to two or less spermatophores. Females sampled from populations at different. All years summed

Number of Spermatophores	Density of Populations Low	Intermediate	High
0-2	14	27	15
3+	0	5	21

$\chi^2 = 22.68$

$p < 0.001$

(Expected Low 3+ = 4.44)

species and in Danauus plexippus (Rothschild, 1978) males may cause severe damage to unreceptive insects. In A.napi, persistence in trying to overcome the mate-refusal posture (as in L.argyrognomon) is prolonged but rarely causes severe damage. It involves continual battering of the female. Slow motion cine-photography confirms that full reflexion of the abdomen only occurs in response to actual contact. Females of A.napi and A.rapae will adopt the mate-refusal posture in response to any fast moving white object which comes near them, but will only show the full expression of the behaviour in response to contact (as shown by harassing females with white flowers).

Figs. 12 and 13 present the final scheme for mating behaviour in all three of the species studied, as flow diagrams for the behaviour of both sexes.

Figs. 12 and 13.

Flow Diagrams to illustrate male (Fig. 12) and female (Fig. 13) behaviour during courtship.

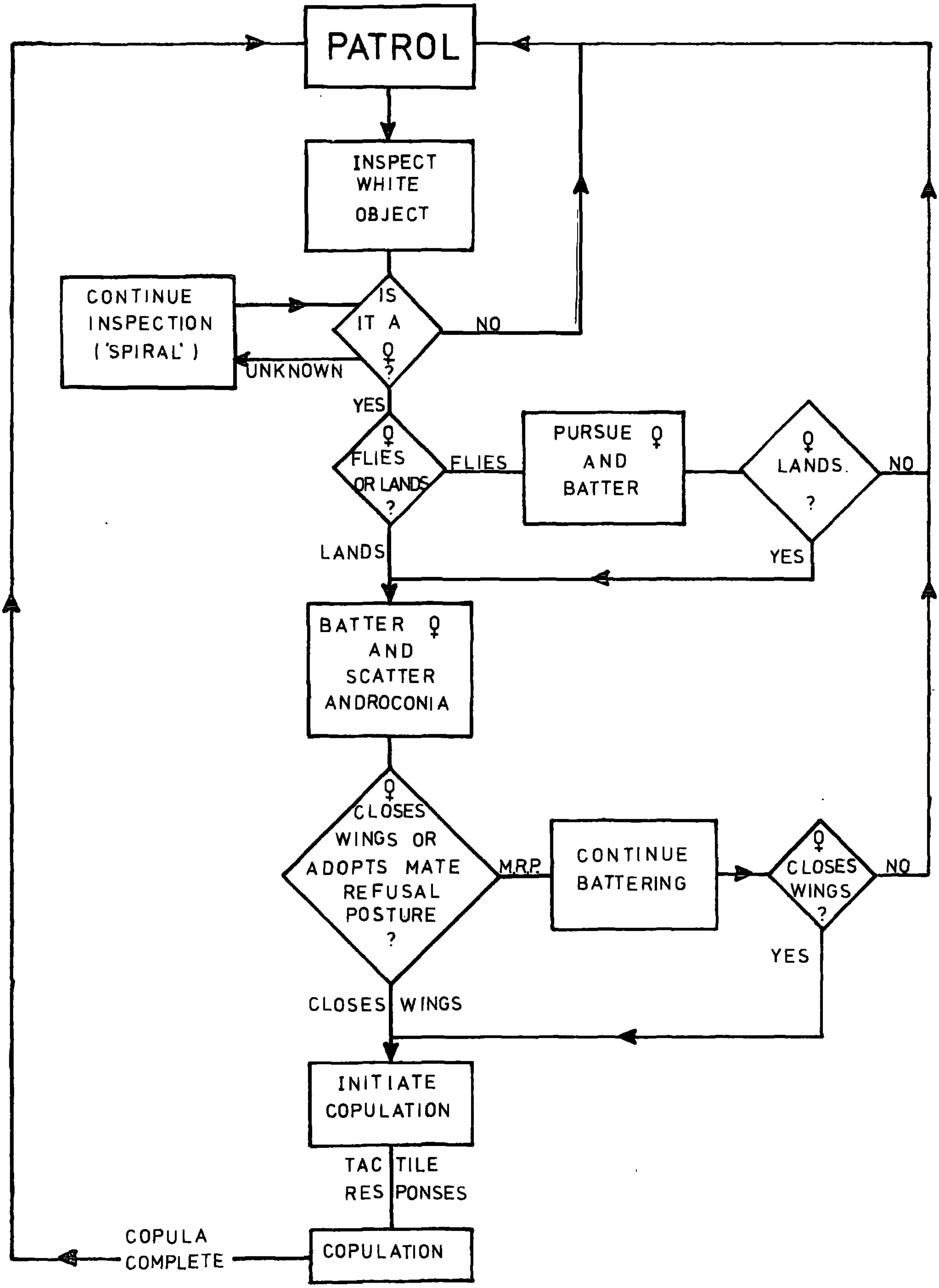

PATROL
INSPECT WHITE OBJECT
CONTINUE INSPECTION ('SPIRAL')
IS IT A ♀ ?
NO
UNKNOWN
YES
♀ FLIES OR LANDS ?
FLIES
PURSUE ♀ AND BATTER
♀ LANDS ?
NO
YES
LANDS
BATTER ♀ AND SCATTER ANDROCONIA
♀ CLOSES WINGS OR ADOPTS MATE REFUSAL POSTURE ?
M.R.P.
CONTINUE BATTERING
♀ CLOSES WINGS ?
NO
YES
CLOSES WINGS
INITIATE COPULATION
TACTILE RESPONSES
COPULATION
COPULA COMPLETE

FIG. 12: ♂

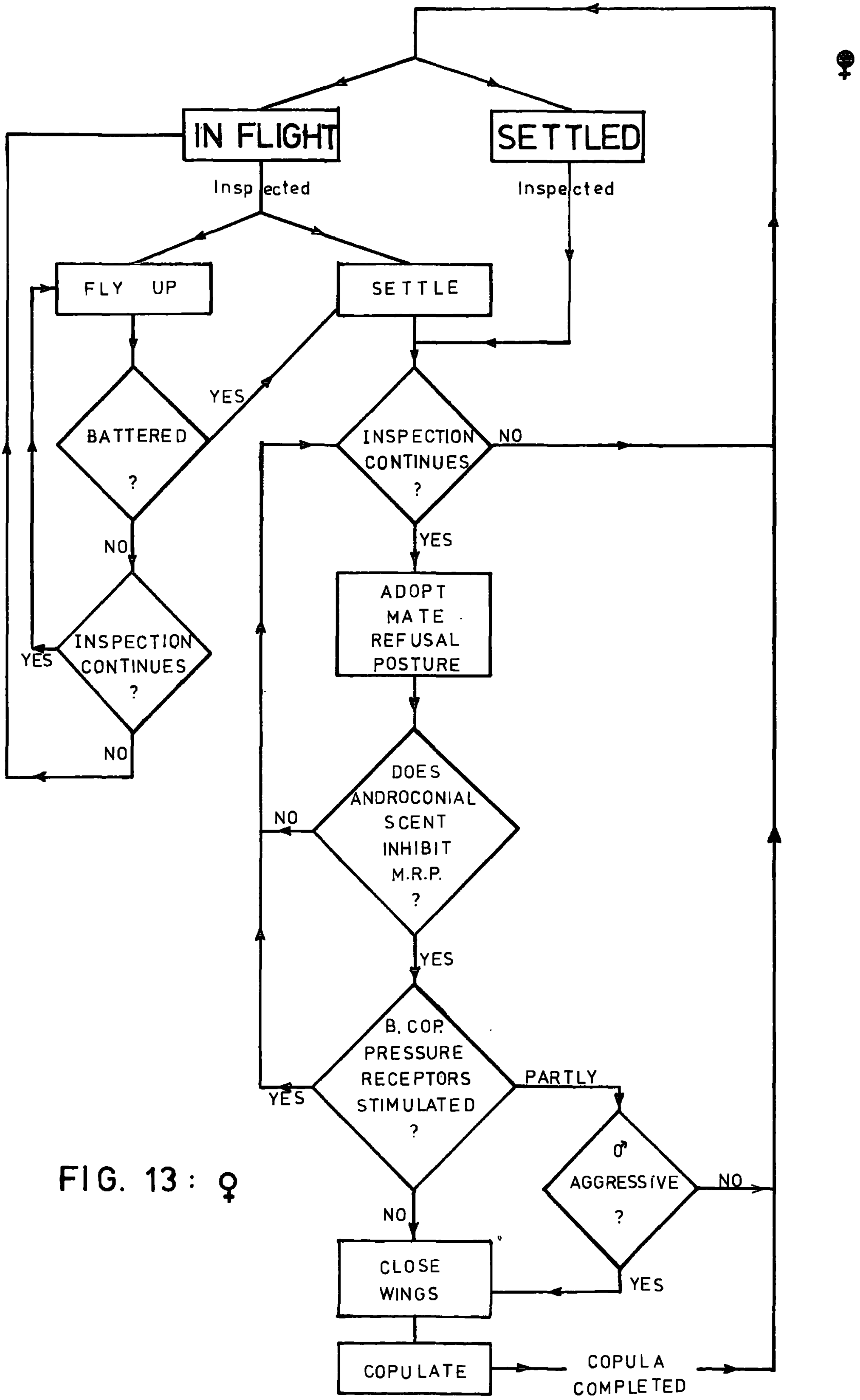

♀
IN FLIGHT
SETTLED
Inspected
Inspected
FLY UP
SETTLE
YES
BATTERED ?
NO
INSPECTION CONTINUES ?
YES
NO
INSPECTION CONTINUES ?
NO
YES
ADOPT MATE REFUSAL POSTURE
DOES ANDROCONIAL SCENT INHIBIT M.R.P. ?
NO
YES
B. COP. PRESSURE RECEPTORS STIMULATED ?
YES
PARTLY
♂ AGGRESSIVE ?
NO
YES
NO
CLOSE WINGS
COPULATE
COPULA COMPLETED

FIG. 13 : ♀

iv. Discussion

Students of the evolution of reproductive isolation have normally favoured one of two views. Dobzhansky (1938) and others have felt that the evolution of reproductive isolation, particularly ethological isolation, among sibling species was favoured by 'physiological disunity' of hybrids formed on secondary contact of derived populations. In these circumstances, selection will place a heavy premium on avoiding gametic wastage on infertile crosses, and reproductive isolation will evolve. This situation has been modelled by Crosby (1970).

Opposed to this 'adaptive' explanation of the origin of reproductive isolation is the view that impediments to gene-exchange between sibling taxa arise essentially by accident, and exist prior to secondary contact. The evidence for both theories was reviewed by Koref-Santribarez and Waddington (1958), who felt that most evidence favoured the latter theory. In some studies however (e.g. Wasserman et al, 1977) reproductive isolation is associated with sympatry, with geographic isolates showing little incipient isolation.

The mating behaviour of male and female Pierinae is hypothesised to have arisen under individual selection. In this section, the consistency of the observed patterns of mating behaviour with the predictions of sexual selection theory will be shown. It will then be shown how sexual selection may act to promote the formation of a reproductive isolating barrier (R.I.B.) prior to contact of populations, and evidence for this assertion will be presented. Finally, the role of sexual selection as both a 'creative' and a stabilising force in evolution will be discussed.

Several components of fitness may be influenced by mating encounters and some of these are summarised below as the advantages and disadvantages of mating to each sex of Pierinae once contact has been made.

Sex	Advantages	Disadvantages
Male	Increased number of offspring.	i. Risk of damage. ii. Time expenditure. iii. Investment in gametes.
Female	Increased fertility.	i. Risk of damage. ii. Time expenditure.

To males, time lost in mating will usually be unimportant, as will investment in sperm. In most species of Lepidoptera studied, for instance Plodia interpunctella (Brower, 1975) and Diparopsis castanea (Marks, 1976), male fertility does not decrease with repeated matings.* In these circumstances males should attempt to mate with any females encountered, as appears to be the case in Pierinae (and many other insects). Similarly, it is to be expected that males will not "risk" damage in encounters with other males, but should do so with females.

The fitness of a female is determined primarily by the number of eggs she succeeds in laying (Chapter 2). The increased fertility to be gained from repeated matings will

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*Rutowski (1979) has recently produced preliminary evidence for a decrease in male fertility following mating in Pontia.

thus be relatively unimportant (except when stores of sperm are depleted) compared to the loss of egglaying time copulation involves.* Thus it is expected that females should attempt to escape courtship where possible, and to give in to males only when the decrease in fitness due to time-loss is outweighed by increased fertility or the potentially catastrophic decrease in fitness caused by damage. Also, since a female's gametic investment is greater than a males, it is to be expected that she will maximize her fitness by avoiding infertile matings; thus the female sex should be the most active in reproductive isolation. Such is the case in Pierinae (preceding section) and most insects (e.g. Von Schilcher and Dow, 1977).

Such discrimination by females may lead to selection upon males, since females should only mate with males who have unambiguously demonstrated their suitability as mates (females should be 'coy' (Halliday, 1978)). One prediction from this is that females should mate preferentially with males giving optimal stimulation. For instance female Pierinae should mate with fresh males, since these have the greatest number of androconia (which old males have lost). 4 copulating pairs of <u>A.cardamines</u> were found during this study, together with 31 pairs of <u>A.napi</u> and 26 pairs of <u>A.rapae.</u> All males were in age-classes 1 (rarely) or 2.

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*Copula duration is temperature dependent (e.g. Petersen and Tenow, 1955) and may last several hours.

Suzuki et al (1977), working only with virgin females, described the behaviour of 4 Japanese Pierini species, and recorded 'species-specific' rituals in two species. In Artogeia melete, 'hovering' was noted where the males spent considerable time apparently disseminating androconia whilst hovering above the female without contact. In A.canidia 'caressing' was recorded, where the male flew in circles around the settled female, repeatedly making contact. Suzuki et al noted that these behavioural elements were not essential to mating in either species, and that frequently females accepted males without these stimuli. Neither element was noted in A.napi japonica or A.rapae crucivora. However in A.n.napi at Durham behaviour similar to both 'hovering' and 'caressing' is frequent, and is usually released when males encounter unreceptive females. Duration of female unreceptivity was much longer in A.canidia and A.melete than in A.rapae and A.napi in the study of Suzuki et al, and it seems reasonable to suggest that both 'species-specific' elements are in fact male responses to prolonged female scrutiny. These elements may indeed be almost fixed in the repertoire of the species concerned, but they are not species-specific rituals involved directly in reproductive isolation.

The mate-refusal posture of females, is hypothesised to have evolved within populations to prevent time loss in superfluous matings. It is probably also maintained in populations by such selection, although selection for avoiding infertile matings with other species may reinforce the behaviour, and favour its stability.* Selection for increasing the duration of female scrutiny may also be expected (and to be directional) in populations sympatric with other Pierinae. This may represent the situation studied by Suzuki et al.

Female scrutiny has also been invoked to explain the orange-apical patches of male A.cardamines. Darwin (1871) felt that the only satisfactory explanation of this colouration was that it had evolved via sexual selection for the stimulation of the female, in the same way as much colour and adornment in male birds. Wallace (1878) contradicted this view and held that the orange colouration was aposematic.

> "I am not aware that anyone has ever maintained that the brilliant colours of butterflies have 'commonly been acquired for the sake of protection', yet Mr. Darwin has himself referred to cases in which the brilliant colour is so placed as to serve for protection... The bright patch on the Orange-Tip butterflies, which Mr. Darwin denies are protective, may serve the same purpose."

However Wallace's explanation fails to demonstrate why only males should be aposematic. Both Darwin and Wallace were mistaken in believing, since female A.cardamines are white, that white colouration is the ancestral condition in the species. Many Euchloini females do have orange apices, however, e.g. A.belia, Zegris emphene and Colotis spp.

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*Inter-specific matings in Pierinae only take place when females are too fresh, with unexpanded wings, to adopt the mate-refusal posture (e.g. Shapiro, 1975 b). This is also the case in other Pieridae (Taylor, 1973).

It seems probable that female A.cardamines in fact show the derived state, the orange patches being ancestral. The 'advantage' to having white colouration may be participation in the oft-suggested mimetic complex surrounding Pieris spp. (App. 14). Female adoption of mimetic patterns with males retaining ancestral, unprotected patterns, is typical of tropical mimetic butterflies, and is most usually interpreted as due to sexual selection maintaining an otherwise disadvantageous character in the male (Turner, 1978).

Male persistence and unselectivity, female coyness and selectivity, and possibly some aspects of colouration, are all results typically found in other studies and regarded as the outcome of 'sexual selection'. This concept, introduced by Darwin 1871 in an attempt to explain the great diversity of animal colouration, was treated extensively by Huxley (1938) who distinguished two forms: 'female choice' (Inter-sexual or 'epigamic' selection) and 'male-contest' (Intra-sexual selection). Both authors, although realising the fundamental connection between natural and sexual selection, emphasised that sexual selection did not normally lead to adaptations promoting better individual survival in the environment. There is a pervading feeling in their writings that sexual selection is of small importance to evolution in the long term.

Indeed sexual selection may oppose adaptation to the non-sexual environment, for instance in birds of paradise.

The handicap principle of Zahavi (1975) emphasised this point, where male adornments were held to advertise superior ability to carry such handicaps. In criticising this theory, Halliday (1978) has emphasised that in the light of present evolutionary thinking, the distinction between natural and sexual selection becomes "trivial", since evolution is now seen to be concerned with gene replication rather than individual fitness.

The recent resurgence of interest in sexual selection, including detailed studies of the importance of such selection in the evolution of mating systems (e.g. Parker, 1970 a,b,c; Thornhill, 1978), has not replaced the underlying assumption of Darwin and Huxley - that natural selection may lead to 'evolutionary advancement' (e.g. new species) whilst sexual selection is of importance only within species. This appears to be the essence of Mayr's argument for maintaining the distinctness of the terms (Mayr, 1972). Such studies that have related sexual selection to speciation have concentrated upon selection for isolation during secondary contact of populations. Thus Trivers (1972) argued that sexual selection could lead to the exaggeration of species-specific characters, and thus reproductive isolation, in sibling taxa undergoing contact. Support for this view comes from many studies showing increased efficiency in reproductive isolation in sympatric as opposed to allopatric populations (e.g. Colias butterflies (Taylor, 1970), Continental

Drosophilidae (Wasseman et al, 1977; Templeton, 1979)).

Such a view clearly favours the 'adaptive' mode of speciation discussed above. However, Ringo (1977) suggested that sexual selection might be a potent force in promoting a R.I.B. prior to contact of taxa. Based on the earlier suggestions of Kessler (1962) and Spieth (1974), Ringo advanced the hypothesis that the extreme diversity of Hawaiian Drosophilidae was explainable by sexual selection acting on the very elaborate courtship rituals of these species to promote R.I.B.'s. Since many Hawaiian Drosophilidae exist in very small populations, Ringo felt that founder events and fluctuations in population size could have led to 'bottlenecking' and 'population flushes'. Ringo then suggested that as a consequence of such drastic population events, some courtship elements might be lost and other novel elements relatively easily introduced. The details of Ringo's hypothesis have met with criticism (Templeton, 1979) and tests are not yet forthcoming. His emphasis on loss of elements may be misguided (at least in the Drosophildae - see below) but his basic notion of the evolution of R.I.B.'s prior to secondary contact has stimulated further work.

Ohta (1978) used Ringo's scheme to establish a phylogeny of the Drosophila grimshawi species group. He hypothesised that in small populations, female discrimination should be relaxed, leading to the loss of courtship elements. He therefore argued that females of derived populations should respond to males with ancestral traits, but that the converse would not be true. He then constructed the phylogeny on the basis of observed courtship patterns in laboratory crosses of allopatric species. However, his work has met with criticism; Watanabe and Kawanishi (1979) re-analysed the data on the basis of the elaboration of male characters, rather than the loss of female discrimination, and produced an alternative phylogeny (almost the converse of that of Ohta) which agrees well with phylogenies based on other characters. From the arguments of both Ohta, and Watanabe and Kawanshi, it is possible to produce a scheme which predicts the presence and efficiency of R.I.B's on first contact of ancestral and derived populations (e.g. in the laboratory), and where the 'derived' population has either lost or gained new courtship elements. This is shown in Fig. 14.

This scheme predicts that loss of courtship elements will not inevitably or even normally lead to a R.I.B. 'Female simplification' will never produce a barrier to mating, whilst the loss of elements in both sexes may lead to barriers, but involves two 'loss' events. For constructing phylogeny on such a scheme, it will be more parsimonious to assume 'male elaboration' which normally will produce barriers to mating in one step. Moreover since male elaboration can be the result of directional selection (see below) it is not necessary to postulate

Fig. 14 Presence of ethological barriers to crossing on secondary contact of sibling taxa.

Courtship elements may be elaborated in males (by female choice) or lost in females, or in both sexes (founder events, etc.)

Contact	between	♂ elaboration	♀ loss	♀+♂ loss
Ancestral ♂	Derived ♀	Barrier	No Barrier	No Barrier[*1]
Derived ♂	Ancestral ♀	Barrier[*2]	No Barrier	Barrier

[*1] NO BARRIER unless ♂ requires stimulus from female before proceeding to next element (i.e. if courtship is a chain of stimuli and responses).

[*2] BARRIER if ♂ has changed an element. Introducing a new element will have uncertain results.

drastic reductions in population size, and the tenuous argument based on this may be relaxed. In the specific case of the Hawaiian Drosophilidae, loss of courtship elements appears unlikely in view of the extremely elaborate courtship rituals, which seem to be inherited en bloc (Templeton, 1979).

For sexual selection to operate as a contributing mechanism to speciation in any group, it is important that variation should exist in courtship-related characters, including behaviour. Moreover, as shown by Trivers (1972) directional change in such characters can result from female choice (over several generations) only when females are choosing 'relative' rather than 'absolute' characters. An absolute character is one which is either present or absent; an example is the orange apical patches of a male <u>A.cardamines</u>. (Variants are occasionally found of males without the orange colouration). A relative character is one which may be present in varying amounts, or in varying proportions. Only abrupt (and hence unlikely) changes in female choice can cause changes in an absolute male character.

An example of a relative male character is the androconial scent of Pierinae which is composed of many constituents. Hayashi et al (1978) showed that the difference between androconial scents of different species is not of which components are present, but of the relative proportions of each. In particular the androconial scents of A.rapae and of the sibling species A.napi and A.melete differ by relatively minor adjustments in the proportions of constitments.

As shown in the previous section, it is likely that differences in androconial scents alone are responsible for reproductive isolation, at least between A.rapae and A.napi. The important study of Lorkovič (1968) has established that such differences may exist in very recently derived populations of A.napi which are still isolated from one another. Lorković showed that females of A.napi napi refused to mate with males of A.(n.)balcana except when scent from A.n.napi males was also present. Hence populations which may only have become separated during the present interglacial have evolved an incipient isolating mechanism prior to secondary contact. Relatively minor adjustments in pheramone make-up may have led to speciation in other insects (e.g. Cardé et al, 1978).

Some evidence therefore exists for sexual selection having led directly to the formation of R.I.B.'s and hence to speciation.* The belated discovery, that the two processes may sometimes be related, is surprising and may

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*Carson and Bryant (1979) have recently shown that one sexual character, bristle number, differs in different derived populations of Drosophila sylvestris and forms an incipient R.I.B.

be a result of the long neglect of sexual selection and the relatively recent advance and application of the biological species concept, which for most groups of organisms remains an ideal. Only when speciation is conceived of solely in terms of reproductive isolation does sexual selection become apparent as a potential force in speciation. Neither reproductive isolation nor sexual selection is necessarily related to the environment, but both are intimately related to the evolution of mating systems. The widespread acceptance of the 'adaptive' modes for speciation (e.g. Bush, 1975) also tends to direct attention away from 'non-adaptive' forces such as sexual selection, which is moreover usually conceived of as having a stabilising influence.

The stabilising effect of sexual selection was discussed by Templeton, who recognised that this was a difficulty in arguments postulating changes in female preferences. Although rigorously uniform populations are not necessarily favoured by sexual selection (frequency-dependent scenarios are possible, for instance), such selection would tend to oppose novelty in males, unless it took the form of a super-stimulus. Novelty in females, as in female preferences would, however, be permitted, when all females are typically mated.

Templeton's solution to this objection to Ringo's hypothesis, was to fuse that notion with the founder-flush scenario, so that females in small, isolated populations would undergo selection for decreased discriminatory ability. A similar approach, more orthodoxically expressed, is to hold that species typically exist in demes, of varying size, which may undergo periods of isolation and reduction in size. In these circumstances 'conventions' of female choice could arise rapidly and become fixed in populations, with different conventions arising in differing geographical isolates. This is a mechanism whereby geographical speciation could take place rapidly, and in a non-adaptive mode.

In a majority of cases, sexual selection should lead to evolutionary stability. Since such selection is directional, it should typically maintain similar behaviours in related species. The close overall similarity in mating behaviour of most Pierinae supports this suggestion. Moreover, once the point of sympatry is reached, inter-specific effects should lead to the maintenance of similar isolating mechanisms, such as mate-avoiding behaviours. The widespread occurence of the mate-refusal posture again supports this suggestion in Pierinae.

CHAPTER 4

Gene change during the evolution of Pierinae

"There is a perfect graduation in variability, from butterflies of which hardly two can be found alike, to slight varieties, to well-marked races, to races which can hardly be distinguished from species, to true and good species."

Darwin, 1863.

i. Introduction

Probably few other animals are as widespread as Artogeia napi, the Green-veined white, or have as many named subspecies. Within its range, which covers the whole of the Holarctic, more than 34 local races have been named. Of these taxa, one pair in particular has received much attention from entomologists and systematisists, and has become known as the napi/bryoniae problem. Muller and Kautz (1939) published a monograph on the species pair, summarising a great deal of information from earlier workers. Muller and Kautz showed that in some areas the upland bryoniae form, characterised by heavy dark markings and yellow colouration of the female, and by univoltinism, was sufficiently distinct from the lowland, multivoltine, lightly marked napi form to be regarded as a separate species.

Far from resolving the issue of A.napi/bryoniae, the work of Muller and Kautz has stimulated much further research, and many authors have published studies relating to the status of the two forms (Petersen, 1952, 1955, 1958; Lorkovic, 1962, 1968; Moucha, 1962; Varga, 1967; Bowden, 1956, 1970, 1972; Warren, 1958, 1961). It is now apparent that in some areas (such as the Western Alps, and possibly the Carpathians and mountain ranges within the U.S.S.R.) populations of the two forms may be sympatric and distinct, whilst in other areas, integradation may occur, with all intermediate forms being found (e.g. Austria). Understanding of the situation is further complicated by the presence of northern subspecies of A.napi (e.g. A.n.adalwinda, A.n.bicolorata; and north British populations) which, occupying arctic or sub-arctic regions, resemble A.bryoniae but are more closely related to A.napi (Bowden, 1970). The precise nature of the relationship between A.bryoniae and A.napi is still unclear, and it is probably best to treat the two forms as sibling taxa approaching the status of species - i.e. as semi-species (Lorković, 1962).

Investigations centering on local differentiation of the A.napi species group has led to the biology of the species being extremely well studied. Thus karyological (Lorković, 1968), morphological (Warren, 1961; Petersen, 1947), phenological (Shapiro, 1975, Lees, 1970) and ecological studies (Dowdeswell and Willcox, 1965; Chew, 1975, 1977 a,b; Lees and Archer, 1974; Ohsaki, 1979) have described in detail particular aspects of biology. Studies have also been made of mating behaviour (Petersen and Tenow, 1954; Suzuki et al, 1978) and of reproductive isolation within the group (Lorković, 1968), with especially detailed investigations into hybrid viability (Bowden, 1970, 1972), to the extent that the biological species concept is more applicable within the A.napi group than perhaps any other taxon yet studied.*

One problem which besets any student of speciation is that of distinguishing differentiation which has occurred

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*Its application is, however, opposed by some (e.g. Warren, 1958).

prior to the evolution of reproductive isolation from that which has accumulated after speciation (e.g. Carson and Bryant, 1979). One method of circumventing this problem is to carry out studies of taxa at all levels of divergence, especially those approaching full specific status, but without complete reproductive isolation - as A.napi and A.bryoniae. Within Pierinae as a whole, the degree of divergence reached by populations is well known, and the Sub-Family therefore forms an ideal subject for the study of differentiation during speciation. It was decided, in this study, to investigate the amount of structural gene change during the evolution of Pierinae, using electrophoretic methods.

By the hard efforts of other researchers, cultures or population samples of numerous different Pierinae were obtained. It was also decided to investigate local differentiation of A.napi populations. In the North of England, A.napi exists in two forms - an upland, typically univoltine form (with many characters reminiscent of A.bryoniae or A.n.adalwinda) and a lowland bivoltine form. By investigating local variation at electrophoretically detectable loci, it was hoped to determine if selective influences, as manifested between lowland and upland A.napi in Co. Durham, might also be responsible for any observed differences between A.napi and A.bryoniae. Thus the degree of differentiation between taxa at all levels of divergence, from local populations to different tribes, was assessed, together with the likely contribution of structural gene change to speciation in Pierinae.

The use of electrophoretic methods in evolutionary studies has been a striking feature of the past two decades. Many of the controversies surrounding the techniques and results of the methods are not relevant here. For instance, for systematic use, it is not directly important for allelic variation at loci to be either subject or substantially neutral to selection. In general, studies of genetic differentiation based on electrophoretic detection of enzyme loci have produced similar results to studies based on cytology or morphological characters (e.g. Lakovaara et al, 1972; Johnson, 1975; Avise, 1975; Mickevich and Johnson, 1976; Bezy et al, 1977), although exceptions occur (e.g. Avise and Smith, 1974; Gorman and Kim, 1976). Electrophoretic studies have proved extremely useful in elucidating relationships in 'difficult' groups (e.g. Tilley et al, 1978) and have led to the discovery of new taxa (e.g. Ayala, 1973).

The degree to which taxa are distinguishable depends upon the variability of enzyme loci surveyed (Johnson and Mickevich, 1977). In groups which typically show high levels of polymorphism, such as Drosophilidae, genetic differentiation between species is high. In the D.Willistoni group, approximately 50% of loci differ between species. Ayala et al (1970) interpreted this result as evidence for major reorganisation of gene pools at speciation, in agreement with the earlier suggestions of Mayr (1942). In other groups where less variability is found, taxa are typically less distinguishable, for instance the species of the *Anopheles gambiae* complex (Mahon et al, 1976). Since estimates of genetic divergence will therefore vary from group to group, and also depend upon which loci are surveyed (Prakash, 1977), it is not permissible to compare directly the results of studies upon different groups. It is, however, important to include as many taxa and loci as possible in any particular study.

ii. Local populations under study

The Green-veined White is subject to pronounced local variation and differentiation within Britain. Heavy dark pigmentation, especially on the female upperside (typical of arctic-alpine forms of the species) is found in populations of upland areas of Wales and England, and is the typical form of Scotland and much of Ireland. Clinal variation in pigmentation characters, with northern/western/ upland populations being opposed to southern/eastern/ lowland ones, is concurrent with variation in female colouration (often ochreous in upland populations) and male androconial scale type (Thomson, 1970). Also allied with these changes, are differences between populations in migratory behaviour, foodplant utilisation, phenology and other aspects of the ecology of the species.

In Northern England, the lowland and upland forms exist in close proximity to one another (this is probably the situation in many other localities (e.g. Rosie, 1976). On the mass of the Pennines there exist many large populations of <u>A.napi</u> showing 'upland' characters, viz. univoltinism, heavy pigmentation, ochreous females, whilst in the lower reaches of the river valleys and plains to the East and West exist bivoltine populations without upland characters. Variation at electrophoretic loci was studied in these populations during the years 1977-9.

The location of study sites is shown in Fig. 1. Important aspects of each population are shown in Table 1.

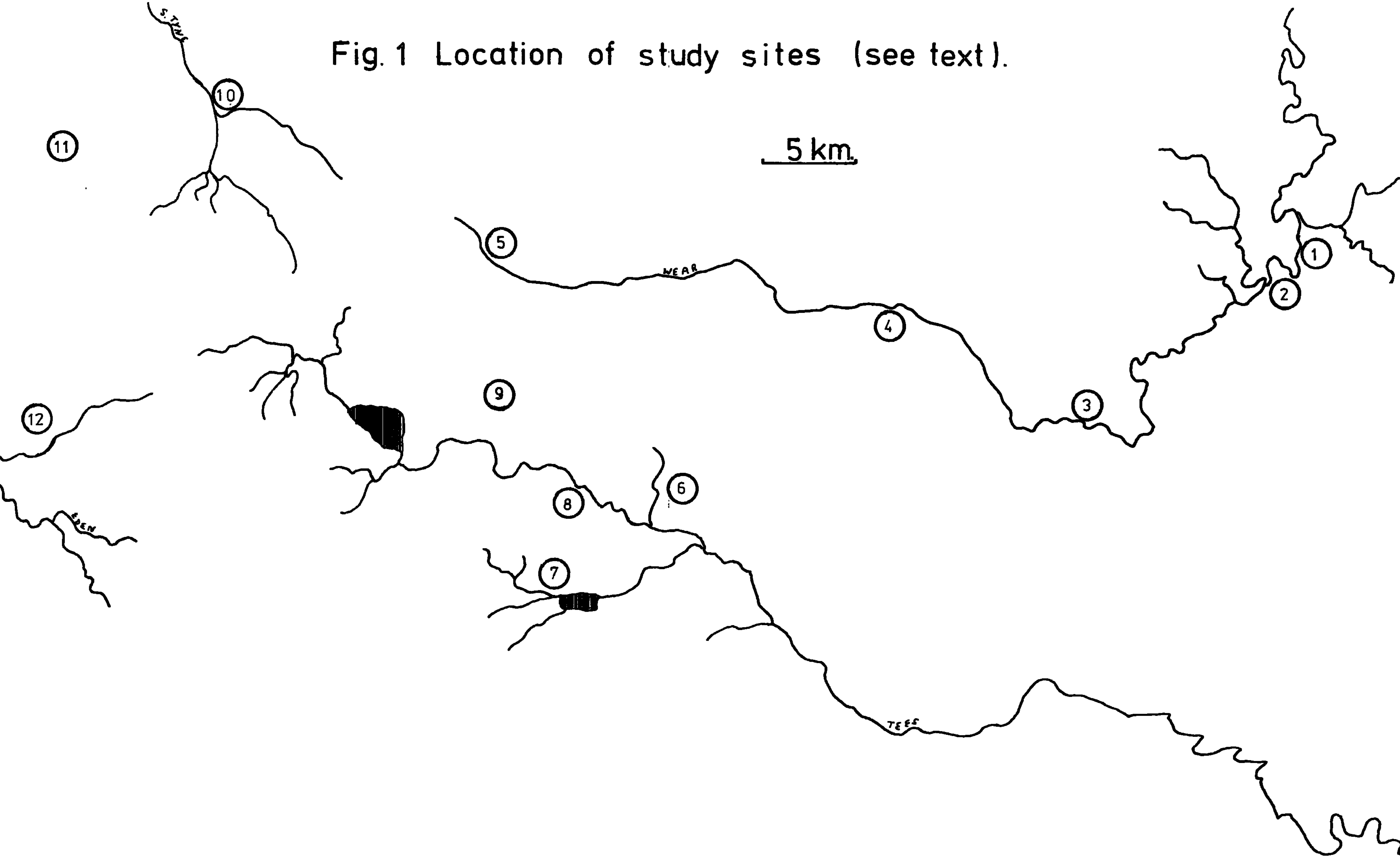

Fig. 1 Location of study sites (see text).

Table 1

Populations studied in the years 1977-9 with important features of biology

No. of Broods 1* at Hartside indicates that a few second brood specimens were taken in 1978, but not in other years.

Foodplants	Ap	Alliaria petiolata
	Br	Brassica rapa
	Hm	Hesperis matronalis
	Bv	Barbarea vulgaris
	Cp	Cardamine pratensis
	Ca	Cardamine amara
	No	Nasturtium officinale

Androconial Types	N	'*napi*' type
	M	'*meridionalis*' type
	A	'*adalwinda*' type

Locality No. (See Fig. 1)	Population	Altitude (m)	No. of Broods/ Yr.	Foodplants	% Females Ochreous	Predominant Androconial scale type
1	Durham	37	2	Ap Br Hm	0	NMA
2	Croxdale	55	2	Ap Hm Cp	0	NMA
3	Witton Park	78	2	Ap Br Hm	0	NMA
4	Wolsingham	148	2	Ap Bv Ca Cp	3	NMA
5	Wearhead	336	1	Ap Cp	25	A
6	Coldberry	380	1	Cp	43	A
7	Lune Valley	390	1	Cp	28	A
8	Holwick	262	1	Cp	10	A
9	Langdon Common	440	1	Cp	25-33	A
10	Alston	274	1	Hm Ca Cp	17	A
11	Hartside	571	1*	Cp	16	A
12	Langwathby	118	2	No Cp	0	NM

It is seen that upland populations of A.napi are typically univoltine, with a few second brood specimens (four females) being found at Hartside (Fig. 1. Locality 11) in 1978. Voltinism in A.napi is partly under genetic control, but whether an individual enters diapause or not is also influenced by photoperiod (Oliver, 1970; Shapiro, 1975 c, 1977). In upland areas of the Pennines, the distribution of obligate and facultatively univoltine A.napi populations is mosaic, with gene flow influencing local differentation (Lees, 1970; pers. comm). The population at Langdon Common is said by Lees to be obligately univoltine. In other populations, it is usually possible to produce second broods from apparently univoltine stocks by careful manipulation of photoperiod (e.g. P.n.microstriata in California (Shapiro, 1975 c)).

Univoltinism in upland forms of insects is usually held to be a response to a rigorous climatic environment, and a short developmental season which does not allow the support of second or subsequent broods. However, in the populations studied here, the flight of upland A.napi, although two weeks or more later than lowland forms (typically throughout June, as opposed to late May and early June) is not so late as to preclude a second brood (as at Hartside in 1978). Rather, as suggested by Shapiro (1975 c) univoltinism appears to be a derived state related to the restricted availability of vernal hostplants (as in A.virginiensis). C.pratensis, although present as a rosette later in the season, is most abundant and easily found (by humans) in Spring, flowering in upland areas being later than in lower river valleys, and synchronous with *A.napi* flight. The association of British univoltine *A.napi* with *C.pratensis* has been noted by others (e.g. Rosie, 1976; Lees, 1970), whilst Meeuse (1973) believed that the association of upland *A.bryoniae* with vernal *Biscutella* spp. was important in maintaining distinctness from lowland *A.napi*.

Shapiro (1976 a) noted that local adaptation of voltinism patterns in Pierini would be opposed and disrupted by gene flow from other areas. This is probably the situation at Hartside summit which, in spite of being at the highest altitude studied, produced a small second brood in 1978. The population is typically 'upland' in all other respects, but is situated at the summit of a steep escarpment and is subject to considerable invasion of animals from lowland areas. Numerous *P.brassicae* and *A.rapae* were captured at this site, all flying up-slope; elsewhere these species were almost never captured on moorland. The Hartside population is only 6-8 km. from suitable 'lowland' habitat, and it seems reasonable to suggest that it is subject to persistent immigration, thus altering the frequencies of genes present.

Also shown in Table 1 are the typical androconial scale types of *A.napi* populations surveyed. Much has been made of the use of androconial scale shapes as indications of phylogenetic origin (e.g. Warren, 1961;

Thomson, 1970), but the characters used are undoubtedly to a large part under environmental influence, with first and second brood specimens having different shaped androconia. The groupings used in Table 1 ('napi', 'meridionalis' and 'adalwinda') follow those of Warren (1968) who described the northern populations of British A.napi as a new British species A.adalwinda Thomsoni, later relegated to subspecific status. Recently it has been shown by Bowden that Irish A.napi also have the same scale types as Scottish and Northern English populations, and that therefore the original practice of referring to all such populations as A.n.brittanica should be retained (pers. comm). On the present evidence, it is clear that some scale types ('adalwinda', and 'narina' - present in some upland Co. Durham populations) are directly associated with altitude, and that the delineation of subspecies on such environmentally influenced characters is to be avoided, at least until the genetic basis (if any) of variation is known.

Pigmentation differences similarly exist between upland and lowland populations. Melanic markings of the upper-side of females are known to be at least partly under genetic control, and these vary with altitude. However, environmental effects may also be extremely important, as in underside melanic markings of both sexes, and great differences exist between the two broods of lowland populations. Again, these differences will not be discussed further.

One aspect of pigmentation known to be under genetic control is the ochreous ground colouration of some upland females (Lorković, 1962; Bowden, 1979). The strong association of this character with univoltine populations in Table 1 indicates some genetic differentiation, probably under selection (Arctic-alpine populations of the A.napi group typically have such females - e.g. A.bryoniae, A.n.adalwinda, A.n.hulda).

Populations were sampled during the flight period, and typically 25 adults were removed for electrophoretic study. This gives a large and sufficient sample for electrophoretic study (n = 50) without risking damage to the populations. However, the exact frequencies of rare alleles are not accurately measured by this sample size. Some of the populations studied in 1977 (a peak year following the good summers of 1975 and 1976) crashed in 1978 and 1979. Thus some samples in 1979 could not be taken due to population decline (Croxdale) or extinction (Holwick). Collecting in 1978 was greatly hampered by bad weather (some samples had to be taken by searching vegetation for quiescent adults) and sampling was not completed in that year (e.g. at Hartside).

In addition to the samples mentioned above, other collections were made. In the peak year of 1977, the very large population at Langdon Common was sub-sampled at the limits of the population (2 km NNE, 520 m altitude; 3 km S, 365 m altitude). In 1978 and 1979 no animals were seen at the Northern sub-sampling site. A small number of animals continued to be found at the southern site. Three samples of Southern English A.napi were also collected - at Redbourne, Herts (grid reference, TL100165) in August, 1977, by S. R. Bowden, and at Knodishall, Suffolk (grid reference TM 420720) in July-August, 1977, and 1978, by U. D. Courtney.

A sample of A.n.bicolorata was collected at Oulu, Finland, by M. Pyörnila in 1978. This univoltine subspecies was described by Petersen (1947) partly on the basis of females being of both ochreous and white types. The subspecies is typically more heavily marked than most Co. Durham specimens, but is analagous with these populations in being intermediate between 'lowland' A.napi napi and Arctic A.n.adalwinda.

iii. Local variation in electrophoretically detected genes

The Electrophoretic techniques used are described in Appendices 9 and 10. Few loci were found to show variation in natural populations and, of these, some were discarded for surveying populations, since there was reason to doubt that apparent phenotypic variation reflected true genotypic variation (App. 9). Of 24 loci which were suitable for the study of local variation, only 7 show appreciable degrees of polymorphism in A.napi or related taxa within Pierinae.* This low level of polymorphism contrasts with many other organisms which have been examined using similar techniques, although similar results to this study have been obtained in some organisms, where little (e.g. Brittnacher et al, 1978; Hedgecock et al, 1977) or no variation (Mashburn et al, 1978) was detected. Suggestions that degree of polymorphism may reflect underlying genetic strategies, for instance as adaptations to 'fine' or 'coarse-grained' environments, have been made (e.g. Valentine, 1975; Hedgecock et al, 1977), but such suggestions are hard to test. The overall similarity of related species, in degrees of polymorphism exhibited, regardless of differences in population sizes and ecology and in inter-population movement (present study; Brittnacher et al, 1978) suggests that such ideas are unfounded.

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*Several of the discarded loci, viz. PGM-1, PG1, SOD-1 and GOT-2 undoubtedly show genetic variation. Hence heterozygosities and degree of polymorphism is probably underestimated here.

It was decided to investigate local variation and differentiation between populations at 6 enzyme loci - Esterases 1 and 2 (EST-1, EST-2) Glutamate-Oxaloacetate Transaminase 1 (GOT-1), α-Glycerophophate Dehydrogenase 1 (α-GPDH-1), Glucose - 6 - Phosphate Dehydrogenase (G6PDH) and an enzyme which functions as an Aldehyde Dehydrogenase (AlDH) but which is highly active on hypoxanthine and related substrates (see App. 9). Of these loci EST-1 is highly polymorphic with 6 alleles detected in local populations. EST-2 and GOT-1 are commonly polymorphic, with most populations exhibiting low frequencies at a rare allele. α GPDH-1, G6PDH and AlDH are essentially monomorphic loci with only rare variants detected. All 6 loci were chosen for being easily and rapidly scored. Variation at the different classes of loci - highly polymorphic, weakly polymorphic and newly monomorphic, will therefore illustrate contrasting influences of selection and other factors on gene frequencies.

The frequencies within populations of alleles detected are given in App. 11, for all loci. The variation of allelic frequency at one locus, EST-1, is shown in Figs. 2 to 4, where gene frequencies over the range of populations studied in the years 1977-9 are given.

It is seen that allelic variation at EST-1 is not associated with other aspects of local variation. 'Upland' populations do not have gene frequencies substantially different to those of 'lowland' populations. This is

Figs. 2 to 4. Allelic Frequencies at EST-1 for the years 1977-9.

For names of sites see Fig. 1 and legend thereto.

a = 1st brood
b = 2nd " "

p. Allele

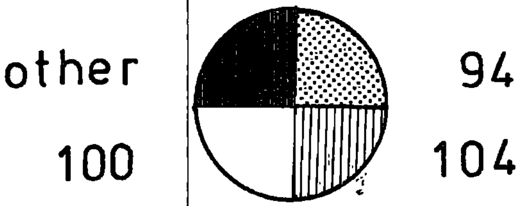
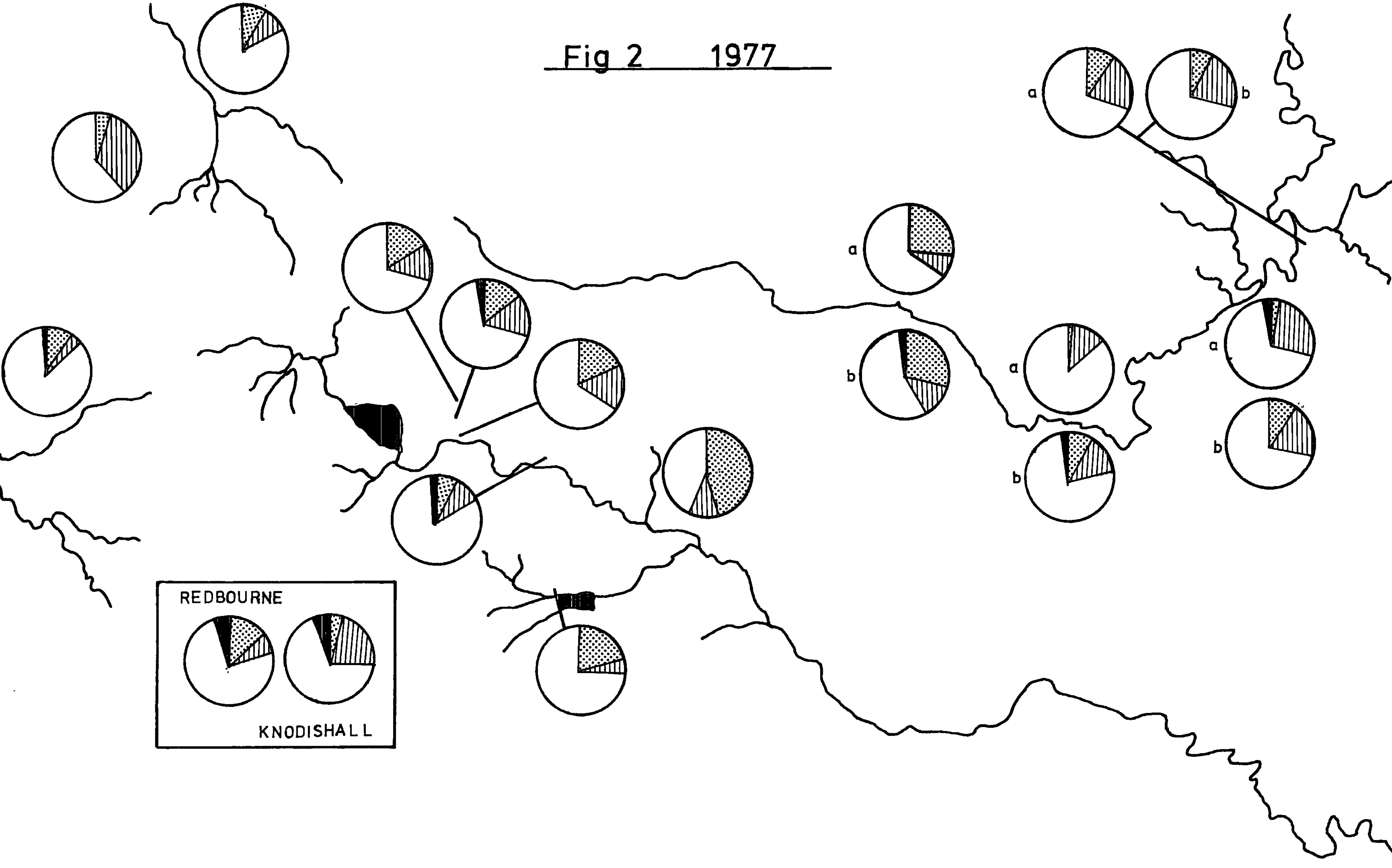


Fig 2 1977



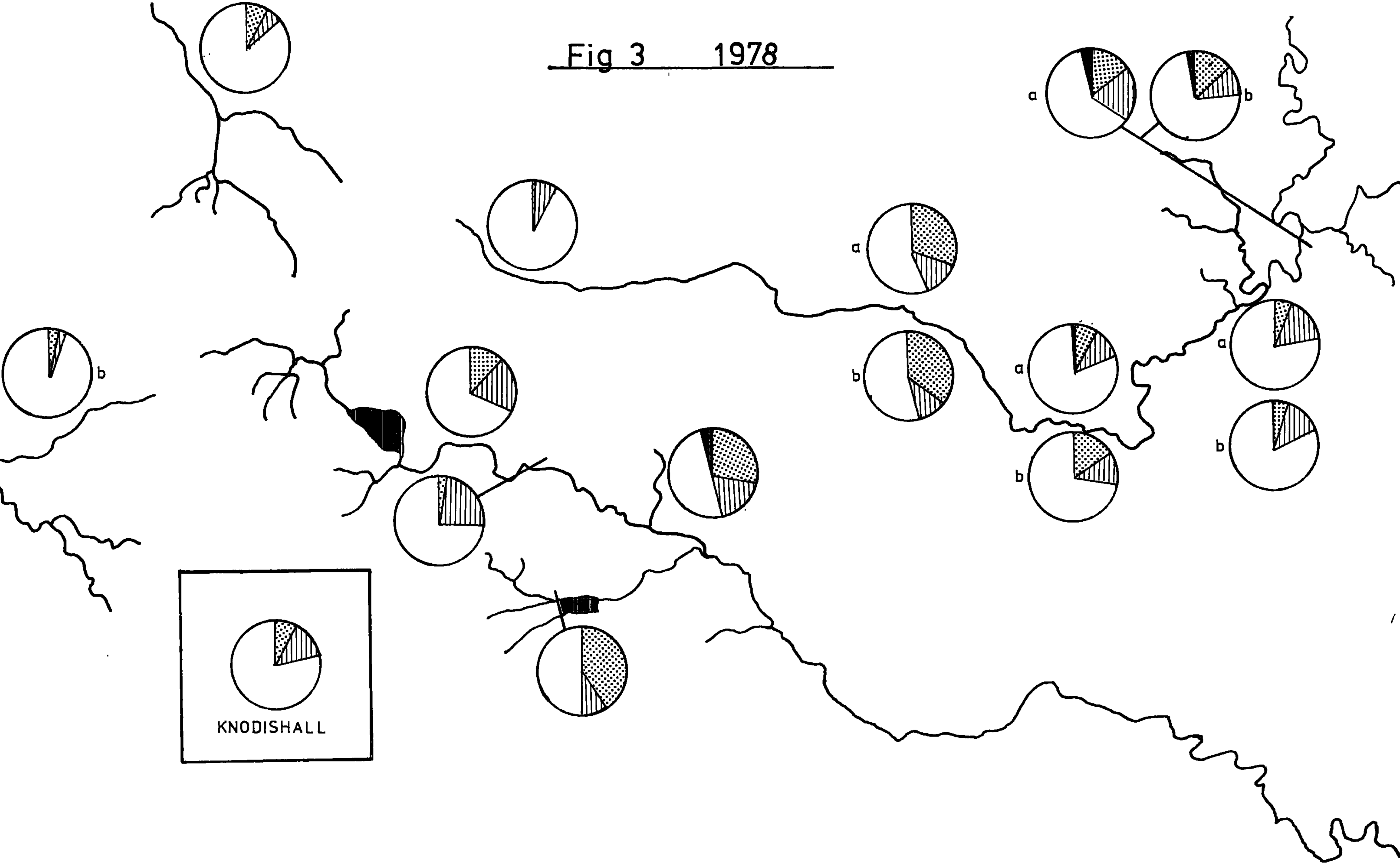
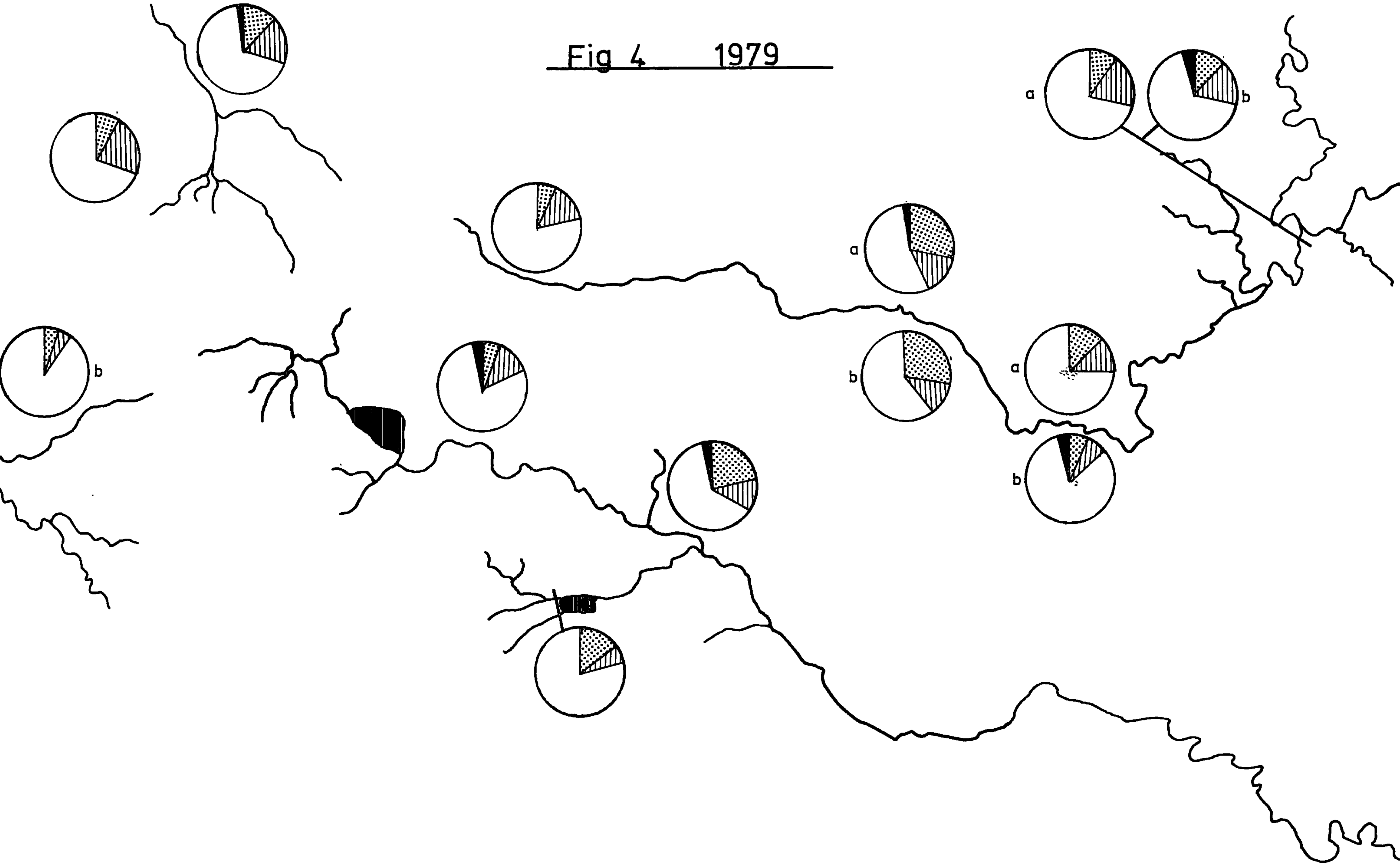


Fig 3 1978

Fig 4 1979



Figs. 5 and 6

The frequency of Est-1 alleles (94 - Fig. 5, 104 - Fig. 6) at study sites of different altitudes.

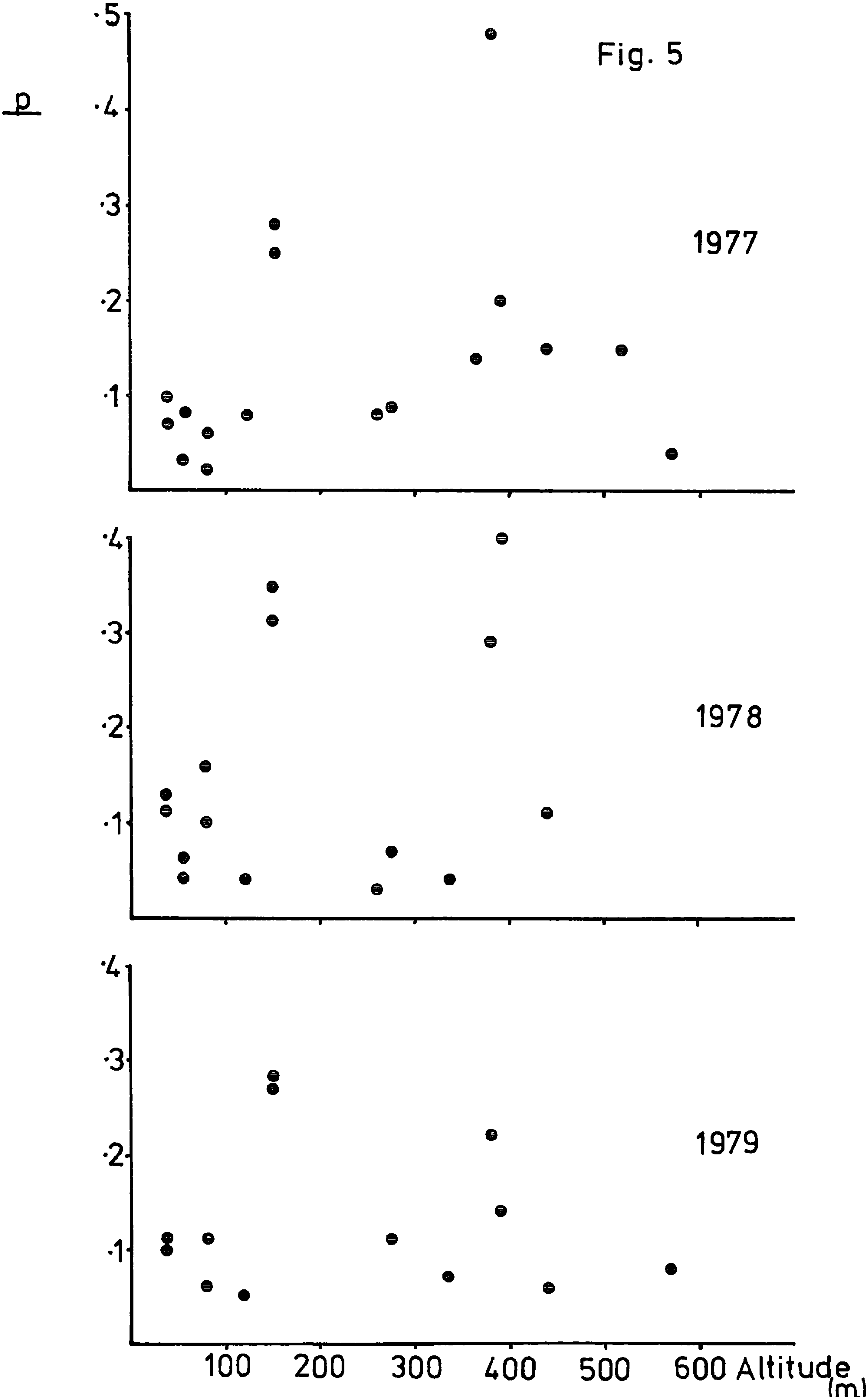
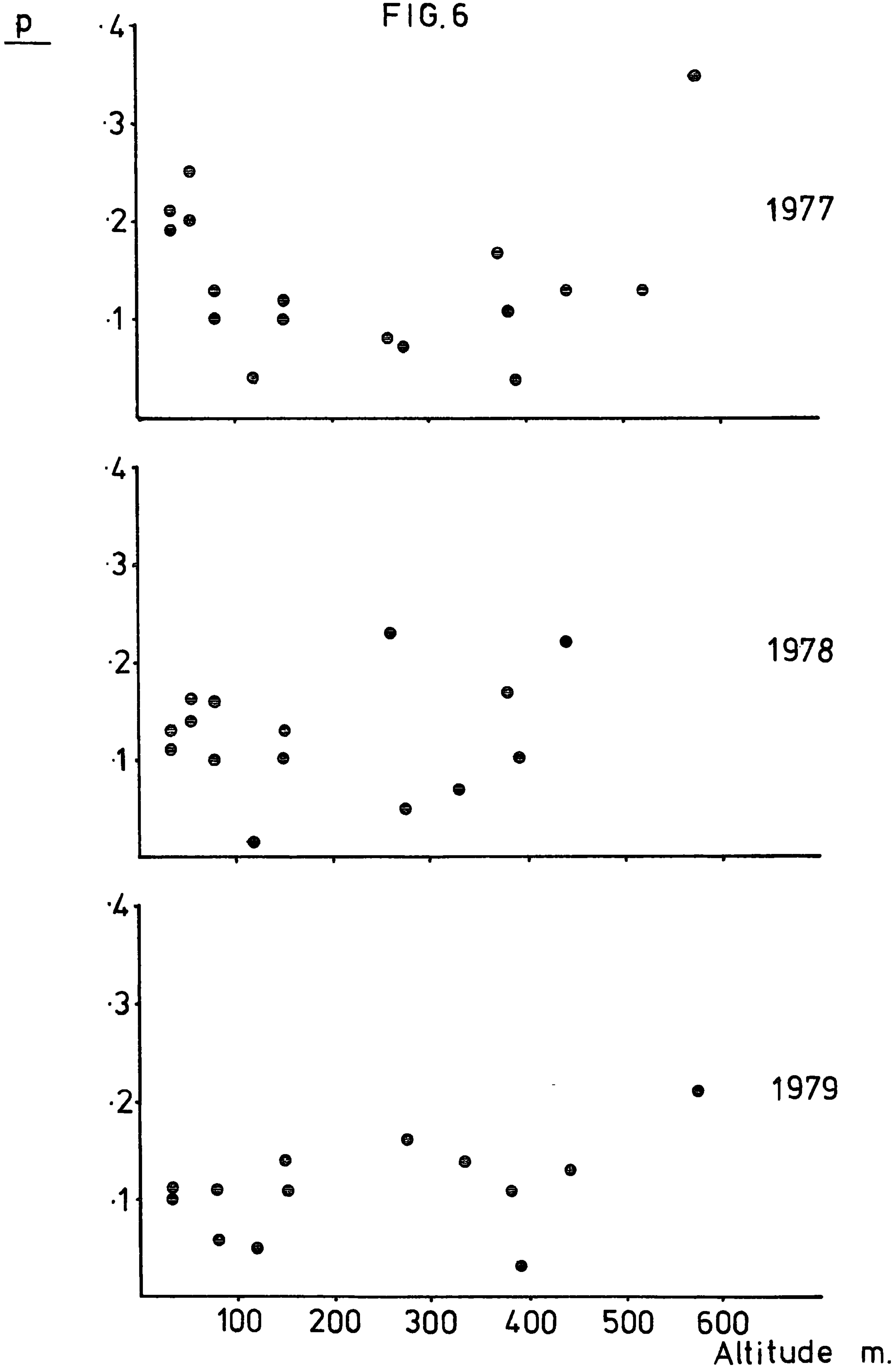


Fig. 5
p
·5
·4
·3
·2
·1
1977
·4
·3
·2
·1
1978
·4
·3
·2
·1
1979
100
200
300
400
500
600 Altitude (m)

FIG. 6



illustrated further in Figs. 5 and 6, where frequency of two alleles intermediate in abundance is shown to be unrelated to altitude of populations.

Variation at other loci also did not parallel obvious differences in population ecology (App. 11). EST-2 allelic variation appeared persistently at Wolsingham and most upland sites. Similarly GOT-1 variation was persistent at Durham and several upland sites. This pattern was repeated at the remaining loci, where such variants as were detected were not associated consistently with either upland or lowland populations. However, there was some indication that upland populations might be supporting greater levels of polymorphism. In particular the population at Langdon Common showed allelic variation at all loci surveyed, with the highest frequencies of rare alleles at GOT-1, α GPDH-1, G6PDH and AlDH. Other upland populations also showed frequent occurence of such alleles. Since differentiation at these loci is small even between species (αGPDH-1 and AlDH are stable across many species, with all Pierini members having the same common phenotype), it seems reasonable to postulate stabilising selection at these loci.* If this is correct, then it may be that low levels of heterozygosity are maintained under favourable conditions for populations, or when population size is very large. The association of

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*The alternative suggestion of low mutation rates is opposed by the fact that some variation is found.

rare variants at these 5 loci with upland populations, particularly in the years 1977 and 1978, then becomes understandable as a result of the relaxation of selectional constraints. During the warm summers of 1975 and 1976, A.napi undoubtedly prospered in many upland areas, such that population sizes in 1977 had reached very high levels (e.g. App. 8). Under these conditions of large population size and favourable climatic conditions, selection against rare variants may have been relaxed. Elsewhere, in lowland areas, such high levels were not reached and rare variants may have continued to be eradicated. The decline in the frequency of rare alleles in 1978 and 1979 supports this hypothesis, but it remains untested until further fluctuations in population size and gene frequency are monitored.

Variation at EST-1 shows some indications of perturbations during the course of this study, but, as with variation at the above loci, it is difficult to definitely associate fluctuations in allelic frequency with fluctuations in population size alone. Such variation as does appear between years, in Figs. 2 to 4, may as easily represent sampling error. Only continuing to monitor these populations over succeeding generations, particularly following the recovery from present low levels, will unambiguously demonstrate whether shifts in allelic frequency have occurred. Where sufficient samples have been taken to observe any stability of allelic frequencies (i.e. at lowland, bivoltine populations where 6 as opposed to 3 flight seasons have occurred*), it seems that allelic frequencies are stable. Moreover, populations separated by relatively small distances appear to maintain stable, different gene frequencies - populations with similar allelic frequencies have dissimilar populations interspersed between them (e.g. the sites at Durham and Witton Park are separated from similar populations at Langdon Common by dissimilar populations at Wolsingham).

This result is even more surprising when inter-population migration is considered. Lowland populations of A.napi are vagile, and may even be migratory in some instances (particularly in the South of England), whilst upland populations are typically colonial, and although extensive movements may occur in some upland areas, perhaps a majority of individuals remain within the colony. Thus lowland populations should be essentially panmictic, and there should be immigration of lowland individuals into upland areas, as was suggested by voltinism patterns (q.v). Similar conditions exist in the Alps, where A.bryoniae is typically colonial, and may exist in large colonies, and is contrasted to lowland A.n.napi which is migratory (Muller and Kautz, 1939) and may invade A.bryoniae habitats as a season progresses (Petersen, 1955).

Although extensive mark-recapture studies have not

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*This does not indicate 6 generations, since some caterpillars from first broods, particularly at Wolsingham, go into dispause. Thus first broods in any year consist of individuals which were laid as eggs in first brood of the preceding year and of individuals laid as eggs by the succeeding generation.

been made to determine long-distance dispersal, it does seem reasonable to assume migration between populations occurs (e.g. App. 1). However, striking differences in allelic frequency are maintained, e.g. at Wolsingham. Several explanations for this might be suggested. Firstly, it may be that random fluctuations in gene frequency have occurred in the past as a result of fluctuations in population numbers, and random genetic events (as is suggested for A.cardamines, Section 2.viii). Gene frequencies would then be maintained in the absence of strong immigration. However, the assumptions of this hypothesis do not seem reasonable. The population at Wolsingham is known to have been in good numbers for many years (T. Dunn, J. Richardson, pers. comm.), and extensive movements of individuals away from the main colony suggest that considerable migration is taking place between populations.

Secondly, differences in allelic frequencies at different sites may reflect some form of undetected selection, either at the EST-1 locus or at a closely-linked locus. In the absence of firm evidence in either direction, this hypothesis cannot be discussed further.

Thirdly, the observed differences between populations may reflect the presence of co-adapted gene complexes. Cain and Currey (1963) described 'area effects' in Cepaea spp., where colour-banding morphs showed sharp changes in frequency which could not be associated with any observable selective agent. Johnson (1976) extended this study and showed that allelic variation at a number of electrophoretic loci also showed sharp changes in frequencies at colour-banding boundaries. In the absence of any observable selection, the above authors concluded that different co-adapted genomes might have arisen which were equally viable, but which did not produce equally fit hybrids. In this case, different gene complexes would remain intact, with sharp boundaries in contact zones.

Essentially similar mechanisms have been postulated by White (1978), who argued that local chromosomal rearrangements, arising because of the protection afforded to local gene complexes, might be a major force in speciation. Vaurter and Brussard (1975), studying the variation of five electrophoretic loci in the butterfly *Plyciodes tharos* Drury, felt that

> "selective forces involved (in maintaining enzyme polymorphisms) are probably associated with the maintenance of co-adapted gene complexes, rather than direct interaction with the environment".

Again, like Bowden (1979), I find an 'area effect' hypothesis for the 'boundary phenomenon' in Cornish *Maniola jurtina* the only satisfactory explanation. The evolution of two separate co-adapted gene complexes, with extensive action of modifiers, would seem to explain both wing-spotting (Creed et al, 1970) and electrophoretic (Handford, 1973) discontinuities in the boundary region, with current populations being derived from two isolates in secondary contact. (Though they could even represent a sharpened cline (Endler, 1973)).

A major difficulty with postulating the coadaptation of gene complexes as an explanation for gene frequency differences, is that it is not an immediately testable hypothesis, given our present techniques for investigating gene interactions. Johnson (1976) recognised this:

> "An important difficulty in the controversy on the causes of area effects is that the major hypotheses are not mutually exclusive. Indeed, one would expect much synergism between isolation, environmental selection and co-adaptation."

Unequivocal demonstration of selective influences on EST-1 alleles is at present the only direct test of the hypotheses given for the explanation of allelic differences in A.napi populations. In the lack of such a demonstration, the circumstantial evidence points to an 'area-effect' type of mechanism. Allele 94 is common in only three populations (Wolsingham 4, Coldberry 6, Lune Valley 7) which are of very different ecologys and population sizes, but which are near to each other in space. Since A.napi in Britain probably derive from several different invasions during the present Interglacial (Bowden, 1970; Lees, 1970; Dennis, 1978) ample opportunity for the evolution and recont-act of different gene complexes has occurred.

The favoured hypothesis for the explanation of observed allelic variation at EST-1 is therefore that such variation reflects past isolation and recontact events, and does not reflect direct environmental selection. Major re-organization of the genome has not however taken place, with allelic frequencies remaining essentially similar. This last point is emphasised by the allelic frequency of the sample from

Oulu, Finland, shown in Table 2.

Table 2.

Allelic frequency at EST-1 for a population of A.napi sampled at Oulu, Finland

n	36
p92	.06
94	.08
100	.70
102	.06
104	.11

iv. Genetic change during evolution in Pierinae

A major assumption of the orthodox view of geographic speciation is that substantial genetic change must take place **prior** to recontact of isolates (e.g. Mayr, 1942, 1957). Although other modes of speciation may now be accepted as possible (e.g. sympatric, parapatric), the underlying assumption of the 'adaptive' nature of specific differences and of adaptive pathways to speciation remains unchallenged (e.g. Bush, 1975).

However, recently suggestions have been made that even in 'normal' geographic or allopatric speciation, the amount of gene change necessary for reproductive isolation to arise on recontact is small. Particularly the work of Avise (1975, 1977), Ayala (1975) and Ayala et al (1974) has suggested that little reorganisation of the genome takes place prior to or during speciation. In the present study it was decided to investigate how many genes encoding for electrophoretic loci had changed during the evolution of Pierinae, and to what extent such changes were associated with speciation events, particularly the A.napi/bryoniae semi-species but also other closely-related and (presumably) recently derived taxa such as Pieris cheiranthi and Pieris brassicae, and Euchloe ausonia and E.ausonides.

In all 21 or 22 loci were examined over 23 taxa or populations. (Numerous other loci were discarded for this work - see App. 12. In particular, Esterases were not used since homology of loci could not be reasonably assumed). This is a relatively large number of loci, compared to other electrophoretic studies of butterflies, which have tended to investigate allelic variation at only highly polymorphic loci, with 5-10 loci being typically examined. (Johnson, 1971, 1976; McKechnie et al, 1975; Jelnes, 1975 abc; Schrier et al, 1976; Watt, 1977; Eanes and Koehn, 1978; Cullenward et al, 1979). Only one study (Brittnacher et al, 1978) has reported electrophoretic results at a comparable number of loci (16), including at essentially monomorphic loci. This study, on the genus Speyeria (Nymphalidae) is also the only other study of genetic change during the evolution of a group of butterflies, and hence will be very useful for comparison with the present study.

The results of electrophoretic analysis are given in detail in App. 12, and are summarised here. In Tabs. 3 and 4 are shown the results of calculating the Indices of genetic Identity (I) and genetic Distance (D) proposed by Nei (1972). These Indices are computed as follows:

$$I_n = \frac{\Sigma x i y i}{\left(\Sigma x_i^2 \cdot \Sigma y_i^2 \right)^{\frac{1}{2}}}$$

$$D = - \ln I$$

where x_i and y_i are the frequencies in samples x and y of the ith allele. I is the mean of all I_n over all loci.

The values for I and D given undoubtedly underestimate genetic distance between the taxa studied, in that several of the more variable loci (and hence those most likely to be different between species) had to be discarded - in the case

Tabs. 3 and 4. **Genetic Identity (I) and Distance (D) of Nei (1972) calculated for all pairwise comparisons of taxa based upon 21 loci***

I lower left comparisons
D upper right comparisons

*Comparisons between Pn, Pr, Pb, Pd and Ac based on 22 loci (including GOT-1)

Pn	Artogeia napi (Co. Durham)
Pr	A.rapae (Co. Durham)
Pb	Pieris bassicae (Co. Durham)
Pc	P.cheiranthi
Pd	Pontia daplidice
Ac	Anthocharis cardamines
Ab	A.belia
As	A.sara
Ag	A.genutia
Ze	Zegris eupheme

Ea	Euchloe ansonia
Es	E.ansonides
Eb	E.belemia
Ec	Elphinstonia charlonia
Pnb	A.napi bicolorata
Pbry	A.bryoniae
Pm	A.melete
Pj	A.japonica
Pv	A.virginiensis
Pr2	A.rapae (Malta)
Pr3	A.rapae (Morocco)
PrC	A.rapae crucivora
Pb2	P.bassicae (Malta)

Table 3

	Pn	Pr	Pb	Pc	Pd	Ac	Ab	As	Ag	Ze	Ea	Es	Eb	Ec
Pn		.103	.098	.100	.538	1.017	1.055	.966	.965	.851	.926	.960	1.033	.960
Pr	.903		.150	.153	.527	.903	.928	.966	.964	.948	.810	.936	.907	.963
Pb	.907	.861		.006	.415	1.017	1.109	.974	.858	.856	.933	.936	1.026	.965
Pc	.905	.858	.994		.410	.986	1.025	.935	.826	.822	.931	.936	1.026	.989
Pd	.584	.591	.660	.663		.766	.783	.814	.818	.851	.789	.769	.840	.782
Ac	.362	.405	.360	.373	.465		.253	.279	.215	.422	.521	.482	.537	.563
Ab	.348	.395	.330	.359	.457	.777		.063	.322	.321	.454	.475	.479	.544
As	.380	.381	.378	.392	.443	.756	.939		.349	.337	.406	.424	.488	.565
Ag	.381	.381	.424	.438	.441	.807	.724	.706		.485	.471	.417	.460	.496
Ze	.427	.428	.428	.440	.427	.656	.725	.714	.616		.524	.476	.509	.562
Ea	.396	.445	.393	.393	.454	.594	.635	.666	.625	.592		.091	.061	.150
Es	.383	.392	.392	.392	.464	.618	.622	.654	.659	.621	**.913**		.089	.112
Eb	.356	.404	.359	.359	.432	.585	.619	.614	.631	.601	.940	.915		.080
Ec	.383	.382	.381	.380	.458	.570	.581	.568	.609	.570	8861	.894	.922	

	Pn	Pnb	P bry	Pm	Pj	Pv
Pn		.0008	.0007	.0007	.0495	.0335
Pnb	.9992		.0007	.0007	.0614	.0420
Pbry	.9993	.9920		0	.0488	.0337
Pm	.9993	.9920	1.0000		.0488	.0337
Pj	.9516	.9404	.9524	.9524		.0842
Pv	.9670	.9588	.9669	.9669	.9192	

	Pr_1	Pr_2	Pr_3	Pr_c
Pr1		.004	.002	.011
Pr2	.996		.002	.002
Pr_3	.998	.998		.003
Pr_c	.989	.998	.997	

	Pb	Pb_2	Pc
Pb		.001	.006
Pb2	.999		.005
Pc	.994	.995	

Table 4.

of Esterases, specific patterns were so different that it was impossible to say whether the same loci were being examined. However, the values for I and D given probably reflect actual differentiation at the loci concerned quite accurately. As Nei (1978) has shown, accurate calculations of genetic distance may be based on surprisingly small numbers of individuals if heterozygosity is low, as here. However, this is dependent upon assaying a large number of loci - as was also the case here.

Table 5 presents the mean values for I and D calculated for all pairwise comparisons of samples taken from populations at increasing degrees of divergence. In making such comparisons, taxa below the rank of congeneric (i.e. non-sibling) species were not used in comparisons of higher taxonomic rank, since this would lead to considerable bias - for instance use of the many taxa of the A.napi group, in say, inter-tribal comparisons would heavily bias the mean inter-tribal result towards the distance between A.napi and all others. Similarly P.cheiranthl and E.ausonides were not used for any comparisons other than at sibling species level, nor were P.bryoniae or P.n.bicolarata used at levels other semi-species and sub-species respectively.

The values of I and D given in Tabs. 3 and 4 show that little differentiation has taken place at the loci surveyed, throughout the evolution of Pierinae. Table 6 presents the results of similar comparisons on other species. It is

Table 5

Values of I and D (with standard errors) for comparisons amongst taxa at different stages of divergence.

Level of Divergence	No. of Comparisons	I	D
Geographic populations subspecies	8	0.9968 ± .0014	0.0029 ± .0012
Semi-species	1	0.9993	0.0007
Sibling species	8	0.958 ± .011	0.043 ± .011
Congeneric species	8	0.819 ± .033	0.206 ± .041
Sub Tribes	4	0.888 ± .016	0.120 ± .018
Contribal species	22	0.619 ± .009	0.482 ± .015
Tribes	32	0.403 ± .007	0.947 ± .011

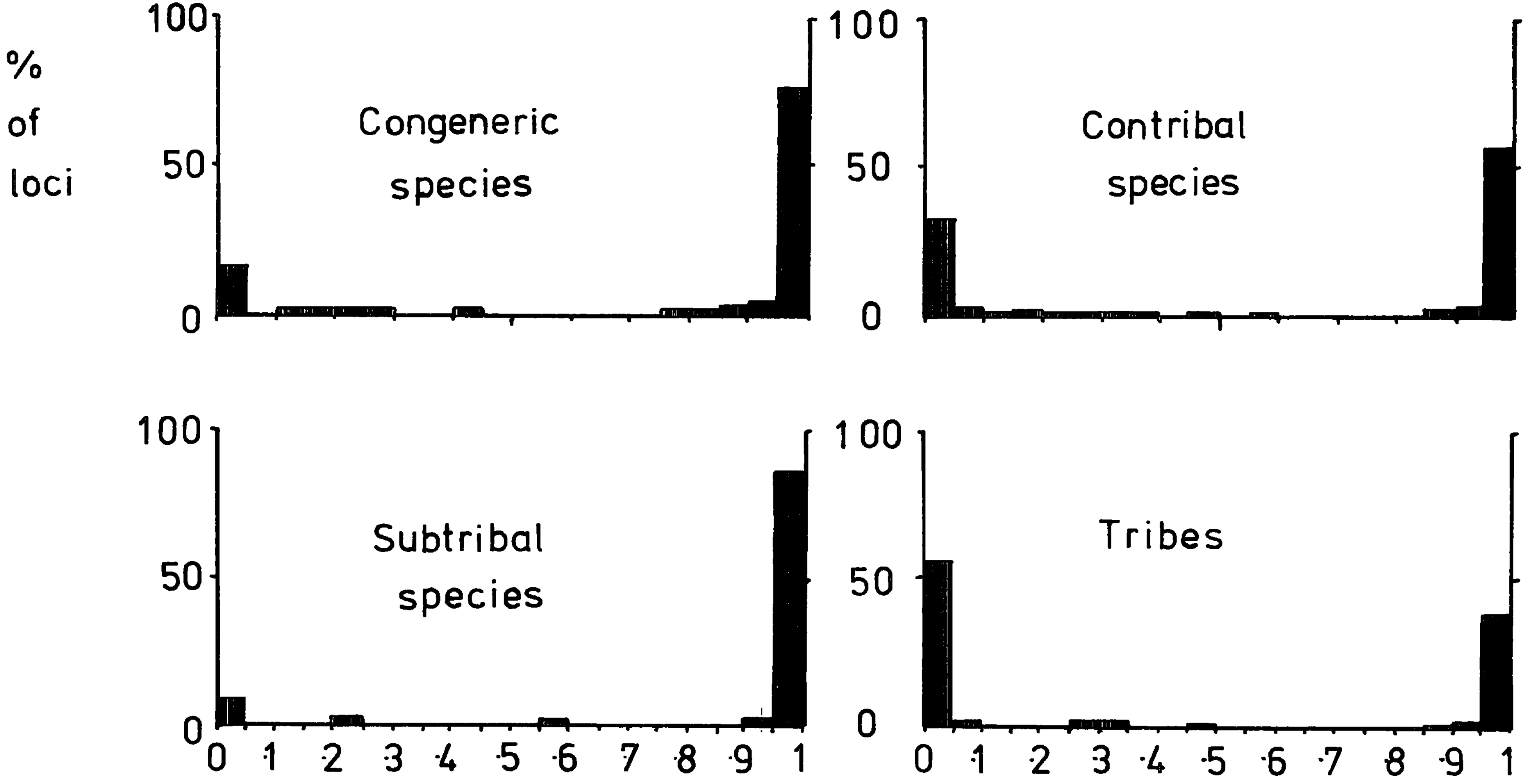
Table 6

Genetic Identity (I) between taxa of differing taxonomic levels

Taxa	I subspecies	I semi-species	I sibling-species	I congeners	Ref
Speyeria (butterflies)	0.977	-	-	0.833	Brittnacher et al, 1978.
Heliconius (")	0.963	-	-	0.688	Turner et al, 1979.
Drosophila willistoni	0.795	0.798	0.517	0.352	Ayala et al, 1974.
D.mulleri	0.878	-	0.788	-	Zouros, 1973.
Asterias (starfish)	-	0.672	-	-	Schopf and Murphy, 1973.
Taricha (salamanders)	0.836	-	-	0.745	Hedgecock and Ayala, 1974
Peromyscus (mice)	0.793	-	-	0.693	Avise et al, 1974.
" " "	0.949	0.837	0.945	0.716	Zimmerman et al, 1978.
Spermophilus (squirrels)	-	0.965	-	0.821	Cothran et al, 1977.
Salmo (trout)	-	-	0.975	-	Ryman et al, 1979.
Centrarchidae (fish)	0.843	-	-	0.544	Avise and Smith, 1977.

(I between genera = 0.297).

Fig. 7 Percentage of loci with values of I_n between taxa at different levels of divergence



seen that genetic differentiation in Pierinae is small compared to that of some other groups, particularly the highly polymorphic Drosophilidae. However, in the comparable study on Speyeria by Brittnacher et al (1979) relatively little differentiation between species was found, and electrophoretic techniques were not able to distinguish morphologically quite separate species. It may be that widely different rates of genetic change occur in different insect orders.

In Fig. 7 is shown the percentage of loci giving particular values for genetic Identity I. It is seen that relatively few intermediate loci occur - most are fixed either at similar alleles or at different ones. This is typically the shape of such histograms and Ayala and Gilpin (1974) have discussed the possible causes of such U-shaped figures, in relation to the neutralist/selectionist controversy concerning enzyme polymorphisms. However, the fixation of alleles may be an outcome of either neutral or selective processes, and histograms such as Fig. 7 may not safely be used on either side of the controversy.

In late 1978, Geiger published a short note concerning electrophoretic variation in A.rapae, A.napi, A.bryoniae and P.brassicae. The exact methodology used is obscure, and one enzyme reportedly surveyed is unknown in any other electrophoretic survey (Arginine kinase). Moreover a figure of electrophoretic mobility appears to have polarity reversed to that normally used (most enzymes are shown migrating to the cathode). Despite these reservations about methods used, Geiger's study is useful for comparison to the present study. He gives values for I between P.brassicae and A.napi and A.rapae of 0.48 and 0.41 respectively, and between A.napi and A.rapae of 0.68 These figures are much lower than those found here (Table 3), but probably represent nothing more than the fact that Geiger's survey was based on a substantially different and smaller set of enzyme loci, including several variable loci (SOD, PG1, PGM) which were discarded for use in the present study because accurate typing was not possible. In most other organisms studied PG1 and PGM are variable. Probably the correct level of divergence between A.napi, A.rapae and P.brassicae lies somewhat less than the values given in the present study. However a much larger survey of loci was undertaken here than by Geiger, and the present estimates of I and D are probably accurate. In only one case do the results of Geiger differ from the present ones - Geiger's figure shows that Malate Dehydrogerase - 2 shows small differences in migration distances between species. This was not found in the present study, despite extensive searching (App. 10). Rather MDH-2 was invariate across the SubFamily. Until the details of Geiger's technique become available, the truth of the situation cannot be ascertained.

Geiger's results are, however, useful in that he surveyed allelic frequencies in wild populations of *A.bryoniae* and found essentially similar frequencies to those in *A.napi*. He gives I for *A.napi* and *A.bryoniae* as 0.99. This is in agreement with the present study where I = 0.9993 (where, however, *A.bryoniae* was from a culture and not wild-caught - hence alleles are likely to be fixed and I over-estimated). However Geiger went on to interpret this close similarity as indicating that the two taxa could not be good species, since the degree of difference between them was less than between conspecific populations of other organisms studied (particularly *Drosophila* spp). Such an approach is *not* permissible; the genetic difference between *Pan* and *Homo* (I = .54) is less than between sibling species of *Drosophila* (King and Wilson, 1975), but no one would suggest that humans and chimpanzees are congeneric. Comparisons of genetic identity *within* groups may be useful as an indication of degree of difference, or more properly, time since isolation during which genetic differences may accumulate; however, they should not be used to define exact taxonomic status, particularly at the specific level - Ryman et al (1979) have for instance described sibling species of trout which are barely distinguishable at electrophoretic loci. The same was found for minnows of different genera by Avise et al (1975), whilst even in *Drosophila*, animals of the *D.pseudobscura* group may share all alleles but have sterile crosses (Prakash, 1972).

v. Discussion

The classical statement of geographic/allopatric speciation, as advocated by Mayr (1942), and discussed by Bush (1975) was stated succinctly by Remington (1958):

> "Speciation may be defined as the accumulation in a population of genetic differences from other populations until these in sum constitute a barrier of sterility or inviability sufficient to prevent swamping out of the differences, when or if the population becomes sympatric with closely related populations."

Central to this model of speciation is the concept of 'physiological disunity', where hybrids between isolates are inviable due to genetic disruptions of physiology. Although the model does not specifically call for large genetic change there is an implicit assumption that under this mode of speciation, 'physiological disunity' arises due to the gradual accumulation of many small genetic differences which act in concert to disrupt hybrid viability.

The widespread assumption of large genetic reorganisation prior to or during speciation was challenged by the work of Spencer (1940) and Hubby and Throckmorton (1968) on Drosophila, which showed that genetic differentiation between sibling species was less than that between corgeneric species - in contrast to the predictions of Mayr. Later studies by Ayala et al (1974) showed that semi-species and sibling species were not greatly more distinct than were subspecies, at electrophoretic loci.

As shown in this study, large-scale genetic differentiation is not associated with speciation; this result parallels that of other workers. Ayala et al (1974) felt that the genetic differentiation between subspecies was sufficient for speciation, and that additional genetic differentiation was not necessary during the evolution of reproductive isolation. However this study was on Drosophila, where the genetic differentiation of subspecies is large. More recently, studies by Avise and Ayala (1976) on minnows, and Tilley et al (1978) on salamanders, have provided evidence in support of theoretical studies by Avise and Ayala (1975) and Avise (1977), showing that speciation is not typically associated with major reorganisations of the genome (as detected by electrophoretic methods) and that most genetic differentiation takes place after speciation, perhaps partly as a result of gene flow being stopped - i.e. reproductive isolation may lead to an increased accumulation of differences.

The evidence for little differentiation during speciation thus rests on electrophoretic studies such as the present investigation on Pierinae. To what extent do electrophoretic methods detect genetic variation? Not all base substitutions cause charge changes, and thus do not affect protein migration distances. Similarly the recent discovery of an unsuspected wealth of hidden variation within allelic classes may call into question some estimates of genetic differentiation, although earlier fears on this point are now somewhat allayed. This topic is discussed at length in App. 10., which describes attempts to discover hidden variation in Pierini.

The currently accepted view on electrophoretic techniques (e.g. Throckmorton, 1977) is that they form an adequate sample of structural gene change during evolution. However as noted by Carson (1973, 1976) such genes may not be the loci determining 'important' changes, particularly in speciation. Using examples from the Hawaiian Drosophilidae, Carson showed that recently derived species, which may have I = 0.95, are not characterised by extensive modifications at isozyme loci; nor are point mutations or chromosomal inversions important in evolutionary change. Rather he felt (1973) that:

> "a drastic change in gene organization or regulation could occur without strongly affecting electrophoretically detected polymorphism."

The realisation that changes in gene action (rather than in the structure of proteins produced) might be important in evolution was first stated by Wilson (1975 a,b) who advanced the hypothesis that regulatory genes were controlling important morphological changes in the evolution of some vertebrates particularly primates. Compared to other vertebrates, primates have undergone marked morphological changes, but remain relatively close at electrophoretic loci (Cherry et al, 1978).

Models of gene regulation have typically been based upon studies of bacteria and other simple organisms. However, important studies by MacDonald et al (1977), MacDonald and Ayala (1978), Lewis and Gibson (1978) on the enzyme Alcohol Dehydrogenase (ADH) in Drosophila have clearly illustrated the potential importance of loci controlling activity of enzyme loci. MacDonald et al were able to isolate many different strains from natural populations all showing differences in the number of ADH molecules produced per individual. Moreover, their study also showed that many different loci all controlled activity of the ADH structural locus, and that the possible interactions amongst loci was thus very large.

MacDonald and Ayala (1978) recognised that 'structural' and 'regulatory' loci were not mutually exclusive classes, and that they did not include all loci within a genome. They offered the following working definition of regulatory loci as "those that control or modify the activity of other loci". Such regulatory loci may affect activity of genes (as ADH in Drosophila) or timing of gene action (as in developmental genes) or act as switch genes for large scale gene-blocs, or as combinations of these and other actions. Such evidence as exists suggests that the proportion of the genome given over to regulatory activity may be very large, and that very small base sequence differences may have large regulatory effects - Calos (1978) using recombinant DNA technology was able to sequence a gene 'promoting' a structural gene (lactose repressor - lac 1) and showed that a mutation causing a ten fold increase in expression was the result of a single base change in one position.

A number of authors have recently suggested that changes in regulatory genes may be fundamental in instances of speciation. Thus Richmond and Dobzhansky (1976) argued that ethological reproductive isolation among semi-species of Drosophila paulistorum, which was independent of allozymes and postmating isolatory mechanisms, might be due to regulatory differences. Kirkpatrick and Selander (1979) described sympatric sibling species of lake whitefish that appeared to be very recently derived and showed that:

> "speciation apparently has involved changes in genes regulating rate of maturation and time of spawning, rather than extensive modification of structural gene loci."

A similar situation was described by Ryman et al (1979) who showed that sympatric sibling species of trout differed in growth rates. Again Heinbach (1978) showed that two sympatric sibling species of Chironimidae differed in diel emergence time. Finally, on theoretical grounds, Endler (1977) has shown that coadaptive modifiers of the kind postulated for A.napi populations, may lead to speciation by modifiers leading to a deficiency in major gene heterozygote fitness.

Although these studies are plausible, they do usually lack exact mechanisms for gene action leading to speciation. Moreover there is a real danger of attributing every specific difference to 'regulatory loci'. Such, in fact, may be the case - it is indeed hard to imagine any pre-reproductive isolating mechanism which is not affected by developmental genes, for instance. The exact genetic mechanisms underlying both hybrid inviability and reproductive isolation should be carefully considered in each separate case.

Nevertheless the conceptual advance gained by the postulated importance of regulatory genes does allow the re-examination of two aspects of speciation theory. Firstly, the evolution of isolating mechanisms between taxa may depend upon genes affecting the timing of activity of animals. Gwadz (1970) has for instance shown that early breeding in an aberrant strain of Aedes atropalpus is under the control of a single locus affecting corpora allata hormone production. Alternatively, regulatory loci determining the activity of other genes, for instance the genes encoding for pheromone components in Pierinae, may affect reproductive isolation by varying the proportions of major constituents (Hayashi et al, 1978).

Secondly, the concept of 'physiological disunity' may be useful in examining hybrid inviability. It is likely that populations differing at 'structural' genes only will not form inviable hybrids, since such structural differences do not affect activity - heterozygotes at isozyme loci are just as fit as homozygous individuals. Nor is it likely that populations differing only at loci encoding amount of activity should form inviable hybrids. MacDonald and Ayala (1978) showed that high activity alleles tended to be dominant over low activity alleles of ADH (this has long been suspected to be the cause of most dominance). However populations differing in timing of gene activity might well be expected to form inviable hybrids, since basic developmental changes may well be de-synchronised in hybrids.

Oliver (1977, 1978, 1979), working on nymphalid butterflies of the genera Boloria and Phyciodes, observed that variance of mortality in inter-population and inter-specific crosses was high, with some broods being viable, others not. He attributed this variance as being the result of relatively few genes causing disruption of 'metabolic integration' ($\equiv$ physiological disunity). He was able to show that the cause of Phyciodes tharos x P.campestris hybrid failures was incorrect developmental interaction between egg cytoplasm and sperm nuclei, and that other developmental failures were probably due to the effects of juvenile hormone levels. He went on to consider other studies, particularly those on the A.napi group and felt that:

> "Incompatibility between P.napi and P.melete thus appears to be largely meiotic and structural in nature, whereas that between P.napi and P.bryoniae is basically regulatory".

This statement was based on the work of Bowden, particularly one paper (1953) describing the effect of hybridisation between A.napi and A.bryoniae upon emergence times of the two sexes. Typically males of either taxon emerge a few days prior to females, as is typical of most butterflies. However in hybrids, this pattern was disturbed.

If the male parent of broods was *A.napi* then F1 females emerged before males. However if the male parent was *A.bryoniae*, female hybrids emerged months after males. These results indicate that developmental genes were different in the two taxa, and that emergence time was probably under relatively simple genetic control, on the sex chromosones. Whether such perturbations of emergence times are important in preventing gene flow between *A.napi* and *A.bryoniae* is not clear. Other modifying genes, responsible for differences between *A.napi*, *A.bryoniae*, *A.virginiensis* and other taxa at the pigment encoding locus *sulphurea* are described by Bowden (1979).

A.bryoniae is unusual in that it appears to be a relatively ancient taxon which is present on different mountain ranges, and yet which is imperfectly isolated from its sister species, *A.napi*, in some populations. Petersen (1963) regarded these populations as resulting from the 'breakdown' of previously rigorous species isolating barriers. If this supposition is correct, it must indicate that the two taxa are very close genetically. The results of this study support this suggestion. However the 'breakdown' of isolating mechanisms requires some explanation. It may be that *A.napi* and *A.bryoniae* are distinct at only a very few regulatory loci which cause hybrid inviability in crosses. These genes appear in at least one case (Bowden, 1953) to be concerned with developmental programming. Additional support for this hypothesis comes from the fact that it is the univoltine populations of *A.bryoniae*, in close contact with bivoltine *A.napi*, which appear to have good reproductive isolation. However in the Eastern Alps at Modling, nr. Vienna, the bivoltine *A.bryoniae flavescens* occurs at lower altitudes than the nominate race of Switzerland, and it is this bivoltine race which interbreeds freely with *A.napi*. Thus degree of isolation appears to vary with voltinism patterns. It is suggested that the speciation of *A.bryoniae* from *A.napi*-like ancestors is based upon few genes encoding developmental programmes fitted to rigorous alpine environments. It is further suggested that this pattern of little structural gene change is typical of other speciation events in Pierinae (for instance the montane *A.ergane* and the woodland *A.virginiensis* both from *A.napi* ancestors, *P.cheiranthi* from *P.brassicae*, and the various Nearctic members of the *Euchloe ausonia* complex) which may however be based on relatively minor adjustments of physiology, as a result of 'regulatory' gene changes.

Chapter 5.

General Discussion.

The publication of books by Dobzhansky (1938) and Mayr (1942, 1963) constited an important event in the study of speciation. Both authors provided a wealth of theory and example in support of geographic differentiation and speciation, and the then lack of evidence supporting alternative modes of speciation (such as sympatric differentiation) must have led many to agree with Verne Grant:

> "Some readers may feel as I do that we are now close to having a satisfactory general theory of speciation."
>
> "The Origin of Adaptations", 1963.

However the "classical" allopatric model of speciation, based on selection in isolated populations leading to adaptive differentiation, is now supplemented by other models, e.g. 'founder-flush', 'area-effect', clinal and sympatric scenarios (terminology of White, 1978 a). Also important may be chromosomal events, particularly in some insects and mammals (e.g. Patton and Young, 1977; Bush et al, 1977; White, 1978 b).

An interesting assumption remains in much work on speciation, however. That is that speciation takes place as a result of adaptive phenomena. Thus Bush (1975) stated:

> "Speciation is ultimately an
> adaptive process that involves
> establishment of intrinsic
> barriers to gene flow."

White (1978 b) has similarly argued that even "non-adaptive" genetic events such as chromosomal rearrangements have importance in adaptation and become fixed in populations because of their protection of locally adapted gene complexes. On this view, chromosomal rearrangements become a second stage in 'area-effect' speciation, where local adaptation and evolution of gene complexes (as suggested for A.napi in Chapter 4) are the necessary first stage.

More explicitly 'adaptive' are the remaining currently accepted modes of speciation such as host-plant shifts (in the sympatric mode), sharpened clines, and when temporary relaxation of selection leads to populations inhabiting new 'adaptive peaks'. Adaptation is, however, most importantly an assumption of theory in the classical allopatric mode. This scenario holds that isolated populations adapt to their environments differently, and that these genetic differences are sufficient, on secondary contact, to keep gene pools separate, by mechanisms such as 'physiological disunity'.

It was particularly the latter assumptions concerning adaptation that lead Slobodkin and Rapoport (1974) to comment:

> "The assertion that speciation is a generally adaptive response regardless of environmental conditions seems to be untenable as a predictive theory, whatever value it might or might not have in retrospect."

The substance of Slobodkin and Rapoport's criticism is that of predictability*: _a priori_ there is no method of determining whether adaptation will favour speciation. Rather in Pierinae the converse appears to be true (Chapter 2). Although host plant specialisation appears to play an important role in speciation in Pierinae (and other phytophagous insects) most species of Pierinae are wide-ranging and are oligophogous upon Cruciferae. In Chapter 2 it was shown that the factors favouring oligophagy (shortage of oviposition time and possibly unpredictability of larval resources) were much stronger in _A.cardamines_ than factors favouring foodplant specialisation (differences in foodplant suitability for larval growth and survival). Hence specialisation and thus speciation in _A.cardamines_ is strongly opposed by such 'adaptation'. Similarly the widespread similarity of Pierinae in the form of mating behaviour (Chapter 3) is most simply interpreted as the result of

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*Predictability in evolutionary studies is discussed in App. 15.

strong directional selection maintaining ancestral behaviour. This will similarly oppose the formation of Reproductive Isolating Barriers and hence speciation.

Sexual selection need not, however, oppose speciation. The suggestion that sexual selection has played an important role in the evolution of Hawaiian Drosophilidae, and possibly in Pierinae and other insects, was also discussed in Chapter 3. Although this 'sexual-selection' hypothesis has been fused somewhat with the 'founder-flush' scenario (Templeton, 1979), there is an important distinction to be made. The 'founder-flush' idea hypothesises that a new adaptive peak is found as a consequence of small population size and the relaxation of normal selective constraints. The sexual-selection hypothesis similarly suggests a change to new characters (although not necessarily as a result of small population size) as a result of arbitrary changes in e.g. female choice, which will be largely unrelated to the environment. Hence in any organism existing in demes (as most species do), changes in sexually selected characters, and hence speciation, may arise in a way unrelated to the external environment.

In Chapter 4 it was shown that a 'genetic revolution', involving most of the genome, was not typically found to have occurred in speciation of Pierinae, as in other organisms. The notion that large parts of the genome underwent such reconstruction during speciation, was one of the tenets of the classical allopatric mode of speciation, which held that 'physiological disunity', the cause of the evolution of barriers to mating, was dependent upon the additive effects of many different loci, which had differentiated in isolates. However, as shown by Oliver (1979), in Phyciodes butterflies 'physiological disunity' may be the result of very few loci. Oliver emphasised the importance of developmental or regulatory genes, and suggested that asynchromy at these loci could be a major cause of e.g. hybrid inviability.

The possible importance of regulatory genes in speciation in the A.napi complex was discussed briefly in Chapter 4, where it was suggested that selective influences at such loci had led to differences between populations and hence to speciation. It may, however, be worth pointing to the possible plurality of influences at such loci.

In Chapter 2 it was shown that allelic variation at a structural locus, GOT-1, in A.cardamines appears to conform to the expectations of theories of genetic drift and sampling. The importance of such random effects to speciation was realised by Wright, who entitled one of his seminal works on effective population size: 'Breeding Structure of Populations in Relation to Speciation' (Wright, 1940). In particular random effects on gene frequency are to be expected when many loci affect the same aspect of a single phenotype. McDonald and Ayala (1978) showed for instance that many different loci affected the activity of the ADH enzyme locus in *Drosophila*. When, as in this case, such loci also happen to be effecting developmental or regulatory changes, the way is open for random fixation and differentiation at such loci, and hence, plausibly, to 'physiological disunity' via a non-adaptive route.

The allopatric model of speciation, once so monolithic, is now supplemented with alternative modes which students of speciation recognise may be equally important. In future investigations of the evolution of species, it may be useful to supplement 'adaptive' arguments with 'non-adaptive' mechanisms, and to recognise the plurality of factors affecting a species characteristics.

Appendix 1.

Floral Use and Pollen-Carrying by Anthocharis cardamines

Several studies have been reported on the flower-visiting behaviour of Holarctic butterflies. Watt et al (1974) and Wiklund (1977) showed that such species are typically generalist feeders and tend to concentrate their attention on the most abundant nectar-providing plant species. However, Watt et al also noted that floral colouration may be important in attracting insect pollinators, and that Colianae butterflies may visit primarily flowers of particular colours. Kay (1976) has shown that different coloured morphs of Raphanus raphanistrum L. differ in their attractiveness to A.rapae and other pollinators, with highly U.V. - reflective morphs being preferred. Miyukawa (1976) has investigated the flower-visiting behaviour of A.rapae in Japan, and has shown that the species prefer purple or blue model flowers to white, red or green ones, but shows little other preference. However, Scott (1975,b) stated that some preference amongst flowers occurred in a population of Euchloe ausonides which appeared to concentrate feeding upon the cruciferous larval host.

Many Cruciferae are self-incompatible; thus, C.pratensis, H.matronalis and many species of Brassica, Sinapis, etc., require cross-pollination by insects. However, other species such as A.petiolata, B.vulgaris and C.bursa-pastoris are able to self pollinate if outcrossing does not occur (Bateman, 1955; Free and Williams, 1977). Some butterflies are good pollinators and carry much pollen (McNeil, 1977), but others appear less good and may be 'parasitic' on the nectar-providing plants (Wiklund et al, 1979).

In order to examine adult nectar resources in the

Durham and Shincliffe populations of A.cardamines, the flowers visited by animals in the field were noted. These results, for 1977 and 1979, are given in Table (A.1.1). Also included are the results of identifying pollen carried by A.cardamines (1977) and other butterflies (1979) (expressed as the number of pollen grains of each plant species, and as the percentage of animals carrying such pollen).

Pollen analysis on Lepidoptera was pioneered by Mikkola (1971) in an effort to determine the origin of immigrant Lepidoptera, and has usually employed light microscopy (e.g. Wiklund et al, 1979). However, as shown by Turnoch et al (1978), Scanning Electron Microscopy (S.E.M.) is a much more accurate tool for finding and identifying pollen on specimens since it allows close examination of surface texture. This method was employed here, pollen being identified against a reference collection of plants growing in the study areas.

The results in Table (A.1.1) therefore allow one to see whether A.cardamines is a specialist or generalist forager, and whether it concentrates on larval host plants or plants of a particular colour; additionally the presence of any pollen from plants not found in the area strongly suggests that that animal has immigrated into the study areas. The other species of butterfly, which are all vagrant or migratory to a greater or lesser degree, are useful for comparisons.

It is seen that A.cardamines concentrates upon nectar sources in their order of abundance, and that blue and white

Table A.1.1.

The relative abundance of nectar providing plants, with the number of visits (i) by butterflies during observation, the number of pollen grains found (ii) and the percentage of butterflies examined with pollen of a particular species (iii).

Relative abundance assessed during early June. Feeding records for *A.urticae* in May, for other species in June.

Pollen analyses made for 1979 specimens, except for *A.cardamines* when 1977 specimens were used.

Table A.1.1 The relative abundance of nectar providing plants, with the number of visits (i) by butterflies during observation, the number of pollen grains found (ii) and the percentage of butterflies examined with pollen of a particular species (iii)

Plant Species or type	Ranked Abundance 1977	Ranked Abundance 1979	A.cardamines i_{77}	ii	iii	i_{79}	A.cardamines i_{77}	ii	iii	i_{79}	A.napi i	ii	iii
Alliaria petiolata (type)	1	2	65	205	68	29	23	176	77	22	104	65	64
Stellaria spp.	2	1	6	6	26	6	3	9	38		50	5	18
Eudymion non-scripta	3	5	18			2	3				4		
Silene spp.	4	4	12	26	42	14	5	41	54	7	6	4	6
Hesperis matronalis	5	9	19	458	53	20	2	248	62	1		37	12
Taraxacum officinale	6	3	8	4	11	8		4	15		16	54	41
Barbarea vulgaris	7	8	1	1	5			1	8				
Lathyrus pratensis	8	10	2			3							
	9	12	4										
Rubus idaeus	10	11	3										
Crataegus monogyna	11	6	1										
Lamium album	12	7	1										
Bellis perennis								3	15		3	2	12
Petasites hybridus													
Others				2	11	1		5	23		1		
Unknown					0			15	31			15	35
Total			140	700		83	36	502		30	184	165	
No. butterflies surveyed					19				13				17

Table A.1.1. cont'd.

Plant Species or Type	Ranked Abundance 1979	*A.rapae*			*A.brassicae*			*A.urticae*		
		i	ii	iii	i	ii	iii	i	ii	iii
Alliaria petiolata (type)	2	3	25	50		81	46	1	59	24
Stellaria spp.	1	1	4	10					2	10
Endymion non-sccipta	5				4					
Silene spp.	4		2	10		8	31			
Hesperis matronalis	9	1	106	30	1	168	54		12	15
Taraxacum officinale	3	10	76	90	4	132	100	18	196	91
Barbarea vulgaris	8		2	10						
Bellis perennis		2	29	30	2	5	23		1	5
Petasites hybridus								10	68	48
Others	6,7,10	1							1	5
Unknown			4	20		32	54		27	38
Total		24	248		10	426		29	366	
No. butterflies surveyed				10			13			21

flowers are particularly strongly represented. However, there also appears to be some association with Cruciferae. This is particularly apparent in some additional results from Wolsingham in 1977, given in Table (A.1.2), where there are many potential nectar sources, but adult feeding is concentrated upon Cruciferae.

Table A.1.2.

No. of observed visits of A.cardamines to flowers at Wolsingham, 1977

Plant Species	Males	Females
Geranium sylvaticum	14	18
Cardamine pratensis	28	13
Cardamine amara	20	-
Hesperis matronalis	7	-
Rubus idaeus	-	1
	69	32

Similarly, A.napi concentrates its feeding upon larval hosts at Durham, and at many other sites. Since only B.rapa and H.matronalis (of the Cruciferae at Durham) require out-crossing for seed set, it is unlikely that a mutualistic relationship has evolved between these and their herbivores. There may perhaps be some interaction of feeding and host-locating stimuli in A.napi and A.cardamines. In Thlaspi alpestre populations in Colorado, self-pollination accounts for c.70% of seed-set; the remaining pollination is effected by Phyllotreta abionica, a crucifer specialist, which is a major herbivore on the plant populations (Petersen, 1977). In the populations of A.cardamines studied by Wiklund and Ahrberg (1978) butterflies tended to feed upon blue or purple flowers, particularly Viola spp., but not from Cruciferae. However, elsewhere in Sweden, where A.petiolata is abundant, it is heavily used by A.cardamines (C. Wiklund, pers. comm.)

The results indicate that of 19 male and 13 female A.cardamines surveyed for pollen, only 4 females were seen to carry pollen from outside the immediate study area. This contrasts with 6 of 17 A.napi, 2 of 10 A.rapae, 7 of 13 P.brassicae and 8 of 21 A.urticae. This strongly suggests that the A.cardamines males surveyed were not immigrants, but that some females were visiting areas outside the study areas (i.e. immediately around the river-banks). Whether such females were truly migrating between populations or merely showing periodic incursions into the surrounding areas is not clear from these data. The 'unidentified' pollen carried on such females was of a uniform and large tricolpate form, with pitted surfaces. 'Unidentified' pollen on other species were of many different plants, and showed a great diversity of form.

Recently Wiklund et al (1979) have proposed that adult butterflies may be parasitic upon flowers, in the sense that nectar is removed from the flower by the long proboscis without transferring significant amounts of pollen. This hypothesis tends to oppose other opinions on the pollinator activity of butterflies (Levin and Bernbe, 1965; Wilson, 1969; Watt et al, 1974; Cruden and Hermann-Parker, 1979), but is given credence by the small loads of pollen carried by individuals of Leptidea sinapis L. in the study of Wiklund et al. These authors may have under-estimated the total numbers of pollen grains carried by butterflies, since their light-microscopic methods are not as efficient as S.E.M. In particular pollen carried within the facial cavity, behind the palps and proboscis, will not be shown by their methods. However, as pointed out by Wiklund (pers. comm), the significant portion of pollen carried by a butterfly is that which is easily transferred to another plant. It is not readily apparent how easily pollen carried within the facial cavity or upon the legs or even eyes (e.g. A.urticae) will be transferred to another flower. It seems reasonable to assume that pollen carried upon the proboscis is most easily transferred. The percentage of pollen carried upon different structures of the adult butterflies surveyed is given in Table (A.1.3).

It is seen that in all 3 Pierini, most pollen is carried in the facial cavity. In A.cardamines likewise, a majority of pollen is carried in the cavity, but occasional individuals had very large numbers of H.matronalis pollen (which appears to transfer readily to Lepidoptera probosces). A.urticae similarly carried a large proportion of pollen on the proboscis - in laboratory experiments, great numbers of grains were found to be present immediately after removal from a flower. Thus it appears that A.urticae, and to a lesser extent A.cardamines, would be relatively good pollinators, and transfer a significant amount of pollen, particularly over short distances (when consecutive visits

	A.c.		A.n.		A.r		P.b		A.u		
Pollen Type	Fc	P.	Fc	P.	Fc	P.	Fc	P.	Fc	P.	E
Alliaria petiolata type	82	18	88	12	84	16	100	0	33	64	3
Hesperis matronalis	63	37	100	0	95	5	95	5	-	100	-
Caryophyllacae	86	14	89	11	100	0	-	-	-	-	-
Taraxacum officinale	75	25	42	48	89	11	95	5	29	64	7
Petasites hybridus									46	35	19
Total Pollen	72	28	75	25	93	7	96	4	33	59	8

Table A.1.3. % of pollen in the Facial cavity (F.C), on the probocis (P) or eyes (E) of butterflies surveyed are most likely to be directed to conspecific flowers). The significance of pollen loads carried elsewhere than the proboscis, and the importance of butterflies as pollinators over long distances must await further study. Wiklund et al's interesting hypothesis must similarly await corroboration from other studies. The present data indicate that some butterflies may indeed be 'nectar-parasites', whilst others may be good pollinators.

Appendix 2.

Fisher-Ford Method of Mark-recapture analysis

The method uses date-specific marks, which are placed on every butterfly captured. The outline below follows that of Begon (1979).

The total number of marks caught on day i is given by:

$$m_i = \sum_j m_{ij}$$

where j is the day of marking. Each mark is i-j days old, and the total age of all marks is

$$\sum_j m_{ij} \, (i-j)$$

Summing this quantity for all days, the 'total days survived' by marks is:

$$\sum_i \sum_j m_{ij} \, (i-j)$$

= observed Total Days Survived (T.D.S)

The mean Age of all marks on day i is calculated thus:-

A_i = mean age on day i

M_i = Marks 'at risk' before day i sample

r_i = Marks from day i

At this time M_i marks have a total age of $A_i M_i$ whilst r_i marks have an age of 0. The mean age of all marks is therefore

$$\frac{A_i M_i + 0}{M_i + r_i}$$

Immediately before the i + 1 sample, the mean age is therefore:

$$A_i + 1 = \frac{A_i M_i}{M_i + r_i} + 1$$

The procedure is now to find, by trial and error, a daily survival rate, Ø, which in combination with the known number of marks captured and released, will give a value of $\sum_{1} A_i m_i$ equal to the observed T.D.S.

This is computed as follows.

Begin by constructing Trellis Diagram of marks and releases.

The data used are from Orange Tip males in 1977.

			Time of release of marks j															
Day	Captured	Released	1	2	3	4	5	7	9	10	14	15	16	17	23	26	35	36
i	n_i	r_i																
1	1	1																
2	0																	
3	1	1																
4	2	2	1															
5	2	2				1												
7	12	12																
9	13	13					1	5										
10	10	10				1	1	2	4									
14	14	14						2	2	2								
15	31	31						4	2	2	7							
16	39	39						2		1	5	18						
17	37	37						2	1	2	5	7	13					
23	27	27						1			1	2	2	6				
26	19	19										1	2	4	9			
35	5	5													1	3		
36	3	0														1	2	

From these data the days survived by marks in the samples are calculated - $\sum_{j} m_{ij}\,(i-j)$

			$\phi = 0.75$			$\phi = 0.8$		
Day i	m_i	$m_{ij}(i-j)$	M_i	A_i	$A_i m_i$	M_i	A_i	$A_i m_i$
1	0	-	0	0	0	0	0	0
2	0	-	0.75	1	0	0.8	1	0
3	0	-	0.53	2	0	0.64	2	0
4	1	3	1.14	1.69	1.71	1.31	1.78	1.8
5	1	1	2.36	1.61	1.61	2.65	1.70	1.7
7	0	0	2.45	2.87	0	2.98	2.97	0
9	6	14	8.13	2.49	14.9	9.58	2.60	15.6
10	8	17	15.85	1.96	15.7	18.07	2.10	16.8
14	6	32	8.18	5.20	31.2	11.50	5.49	32.9
15	15	61	16.63	1.91	28.7	20.40	3.48	52.2
16	26	52	35.73	1.67	43.4	41.11	2.38	61.9
17	30	84	56.04	1.78	53.4	64.09	2.22	66.6
23	12	91	16.56	7.08	85.0	26.50	7.41	88.9
26	16	97	18.38	5.69	91.0	23.30	6.67	106.7
35	4	39	2.10	11.79	47.1	5.68	11.67	50.7
36	3	12	6.08	4.06	12.2	9.34	7.16	21.5
		503			425.9			513.3
		= T.D.S.						

Calculate M_i by $M_{i+x} = \phi^{x}\,(M_i + r_i)$

A_{i+x} by $\dfrac{A_i\,M_i}{M_i + r_i} + x$

(Thus making allowances for days on which no captures were made)

Trying ϕ 0.75 first, $\sum A_i m_i < \text{T.D.S.}$ $\therefore$ TRY BIGGER ϕ

For ϕ of 0.8 $\sum A_i m_i = 513.3$.

From Regression line calculate true ϕ to give $\sum A_i m_i$ of 503

= 0.799.

To calculate Population size, first calculate the population size on each day N_i

When $N_i = \frac{(n_i + 1)}{(m_i + 1)} M_i$

The number entering the population is then

$$B_i = N_{i+1} - \phi N_i$$

Day i	Ni	ϕN_i	B
1		0.799	0.001
2	0.8	0.6	0.7
3	1.3	1.0	2.9
4	3.9	3.1	0.9
5	4.0	2.6	32.1
7	38.7	24.7	-5.5
9	19.2	15.3	6.8
10	22.1	9.0	13.6
14	24.6	19.7	21.1
15	40.8	32.6	28.3
16	60.9	48.7	29.9
17	78.6	20.5	36.6
23	57.1	29.1	-1.7
26	27.4	3.6	4.4
35	8.0	6.4	2.9
36	9.3	7.43	

In calculating Bi, the normal procedure is abandoned, and it is assumed that no animals enter on days when no captures occur. This is a reasonable assumption here, where adult butterflies will not fly in days of unsuitable weather.

$$\sum B_i = 173$$

Assume a 50:50 sex ratio.

Number of A.cardamines - 1977 = 346.

From the tables of Robson and Reigier (1964) re-calculated by Begon (1979) a population of 25-50 animals requires daily samples of 15, 20 or 30 animals for 0.5, 0.25 and 0.1 accuracy respectively. The indicated accuracy of the results, and population sizes, approaches 0.1 on most days.

Appendix 3

Survival data for various populations of A.cardamines, 1977-79

Data refer to site, year and the number of animals entering and leaving each stadium on different foodplants. Thus:-

	ε		1	
A_p	82	76	76	49
H_m	116	99	98	24

Indicates that 82 eggs upon A.petiolata gave 76 animals in the 1st instar and 49 in the 2nd instar. However, of 116 eggs on H.matronalis, 99 survived that stage, but one 1st instar larva was subsequently destroyed by human action, and only 98 are recorded as entering instar 1.

		Eggs		1		2		3		4		5	
Croxdale 1978	Ap	105	85	80	50	48	37	37	25	25	23	23	23
Croxdale 1979	Ap	87	76	76	39	39	35	35	30	30	26	26	26
	Hm	15	12	12	5	5	2	2	1	1	0		
	Bv	8	8	8	2	2	2	2	2	2	2	2	2
Witton Pk. 1978	Ap	28	27	27	15	14	13	13	12	12	12	12	12
	Hm	72	59	59	12	12	6	6	5	5	5	5	5
	Br	7	7	7	6	6	6	6	6	6	6	6	6
	Bv	14	11	11	6	6	4	4	4	4	3	3	3
Witton Pk. 1979	Ap	169	152	137	69	66	55	52	44	44	44	44	44
	Hm	120	92	75	14	14	11	11	10	10	10	10	10
	Br	38	34	24	17	11	9	9	6	6	5	5	5
	Bv	18	16	16	4	4	1	1	1	1	1	1	1
Wolsingham 1978	Ap	32	27	27	18	18	15	15	14	14	13	13	13
	Bv	31	29	29	18	18	13	13	9	9	7	7	7
	Cp	30	30	28	26	26	22	21	17	17	17	17	17
	Ca	20	19	19	16	16	15	15	13	13	13	13	13
	Ri	2	2	2	1	1	1	1	0				
Wolsingham 1979	Ap	91	85	85	35	35	30	29	27	27	26	26	26
	Bv	172	163	163	70	70	64	64	56	56	48	48	48
	Cp	27	27	26	17	17	16	16	15	15	15	15	15
	Ca	45	45	41	25	25	22	21	18	18	18	18	18
	Ri	-12 survive all stadia											
Durham 1977	all plants	198	197	197	117	97	98	98	81	81	74	74	73
Durham 1978	Ap	58	53	50	36	36	26	25	17	17	17	17	17
	Hm	88	78	78	27	27	21	21	12	12	12	12	12
	Br	28	27	27	18	18	18	18	14	14	14	14	14
	Bv	15	13	12	3	3	3	3	3	3	3	3	3
Durham 1979	Ap	82	76	76	49	49	45	44	34	34	33	33	33
	Hm	116	99	99	24	24	22	21	10	10	10	10	10
	Br	45	42	41	26	25	24	24	21	21	19	19	19
	Bv	22	20	20	4	4	4	4	1	1	1	1	1

Appendix 4.

The effect of larval density upon larval mortality

Data from cohorts described in App. 3.

Fig. A.4.1. The mortality of larvae at different numbers of larvae/inflorescence (all foodplant species).

Fig. A.4.2. The mortality of larvae at different instars upon A.petiolata, at different larval densities. (all sites summed). Density is taken as the number of larvae within 1 m.

FIG. A.4.1.

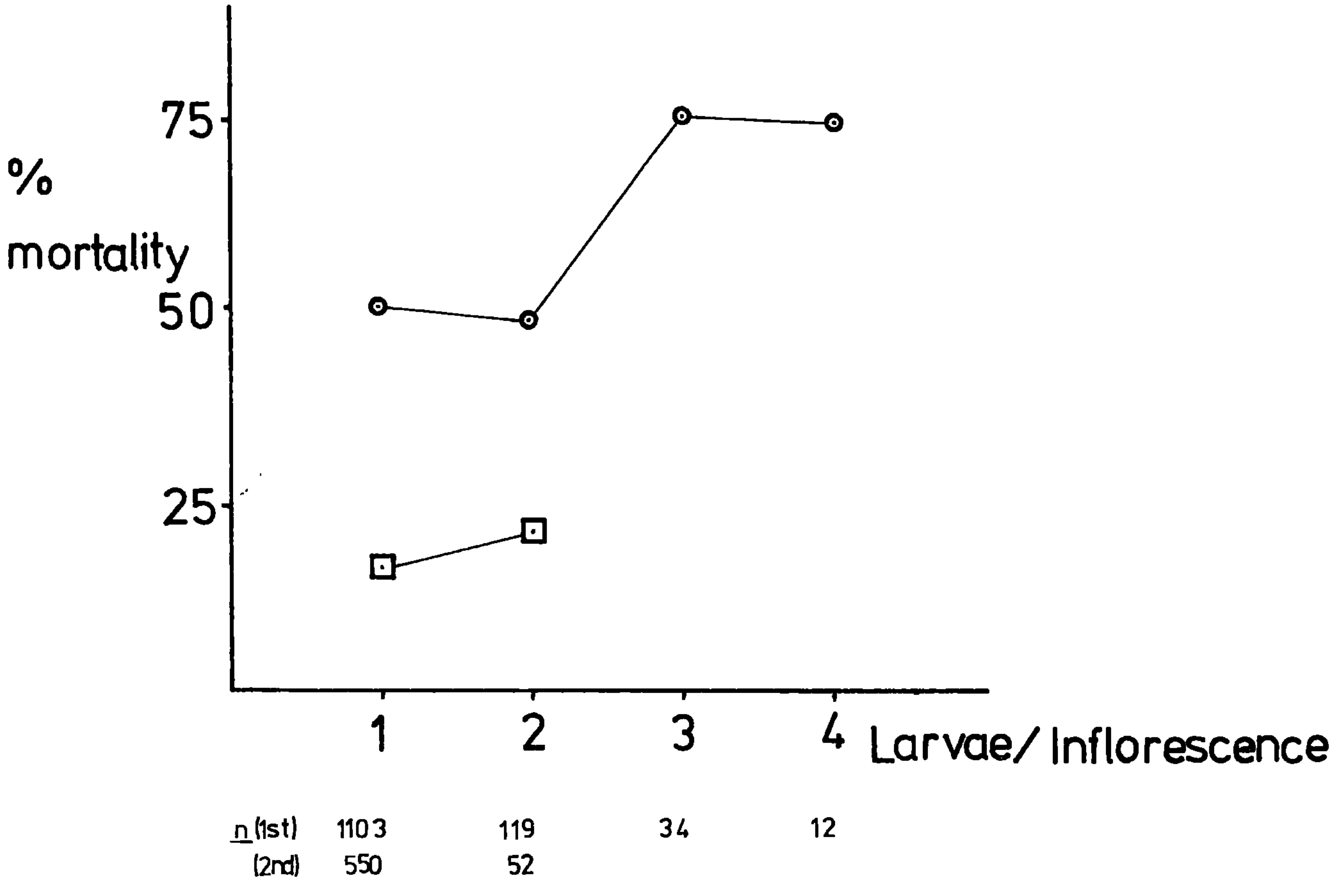
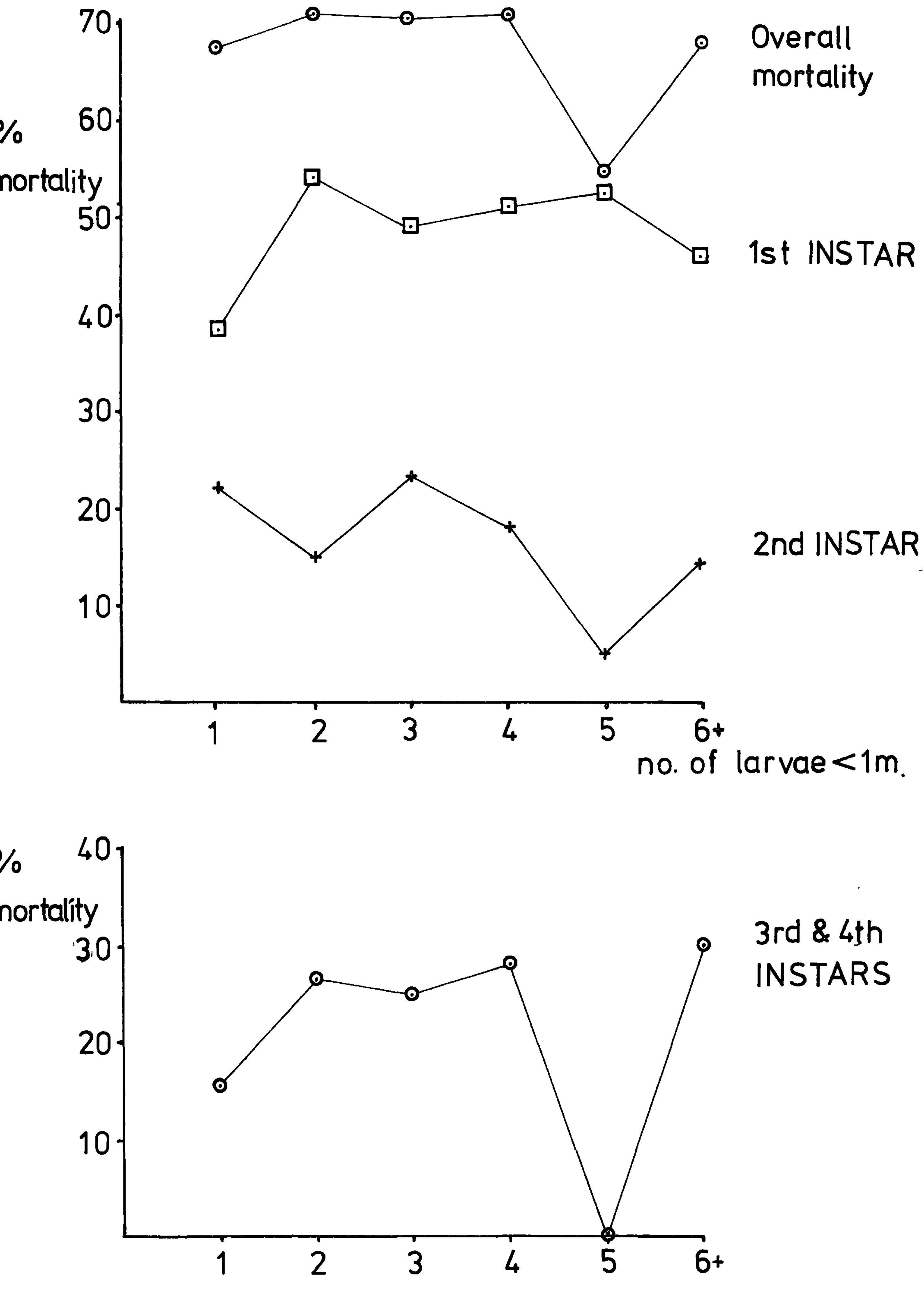

75
%
mortality
50
25
1
2
3
4
Larvae/ Inflorescence
n (1st) 1103 119 34 12
(2nd) 550 52

FIG. A.4.2.

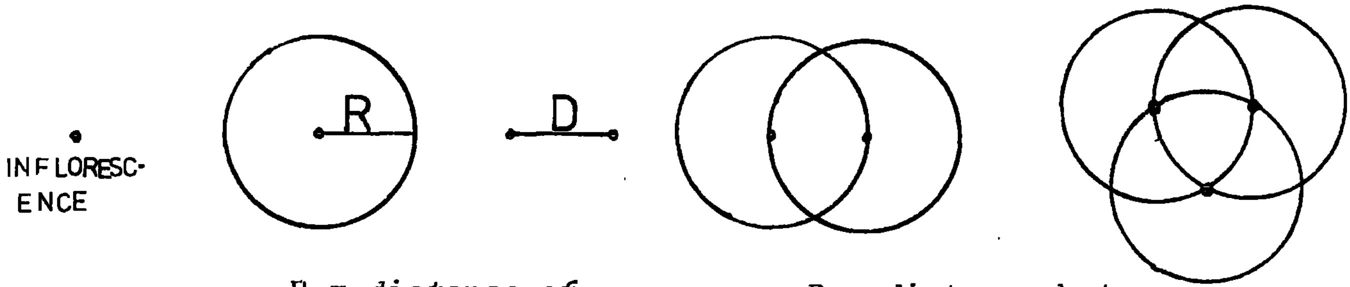

%
mortality
70
60
50
40
30
20
10
Overall mortality
1st INSTAR
2nd INSTAR
1
2
3
4
5
6+
no. of larvae<1m.
%
mortality
40
30
20
10
3rd & 4th INSTARS
1
2
3
4
5
6+

Appendix 5

Modelling size effects upon oviposition in A.cardamines

It has been suggested in Section 2.vii that plants of *A.petiolata* of size classes 1 and 2 receive fewer eggs than larger plants, because the visual cue attracting the animal to the plant is less. This section illustrates a simple model of searching behaviour of female *A.cardamines* for *A.petiolata* plants of different sizes, and compares this to the observed data.

Females are observed to orientate to the plant (from varying distances) and to fly towards and inspect inflorescences. Since larger plants, with several flowerheads, spread over a wide area, are visible over a larger area than smaller plants, it is to be expected that they would receive more visits and thus eggs from females. This reasoning is illustrated thus.



R = distance of ♀ A.C. visual response

D = distance between n and n + 1 inflorescences

However, the increase in area over which the plant is visible will not be linear with respect to increasing flowerhead numbers, since every additional inflorescence will 'overlap' to a greater degree with previous ones. The resultant graph of 'area over which plant is visible' and 'flowerhead number on plant' will be asymptotic.

Unfortunately a simple two-dimensional model will not suffice, since the female butterfly searches in three dimensions. In order to model this, a series of physical analogues were built. Apples were cut into hemispheres (since butterflies search from the side and from above, but not from below) and then into shapes illustrating varying degrees of overlap, as above. The weights of the resulting pieces were then obtained, and from these, assuming that weight is proportional to volume, the relative volumes of each increment in 'volume over which plant is visible'.

It should be noted that the model depends on two variables: D - the distance between successive <u>A.petiolata</u> inflorescences, and R - the visual range of female <u>A.cardamines</u>. Neither of these need be measured, since the model depends only on the <u>ratio</u> between these two. Increasing the visual range of the model animal will decrease the relative amount of each increment. Thus by comparing the model produced with the observed data on responses to, and egg loads on plants of different sexes, and given an average value for D, it will be possible to make predictions on the actual visual range of the female butterfly.

Fig. A5.1 represents the results of the apple models (mean of six trials) for ratios of R : D of 1:1 and 2:1, whilst Fig. A5.2 represents the data of **Figs.**33 and 34, and Table 22 (% eggs upon different sized plants and observed searching behaviour). All values are given as relative to that for plants with three flowerheads.

It is seen that egg load data agree reasonably well

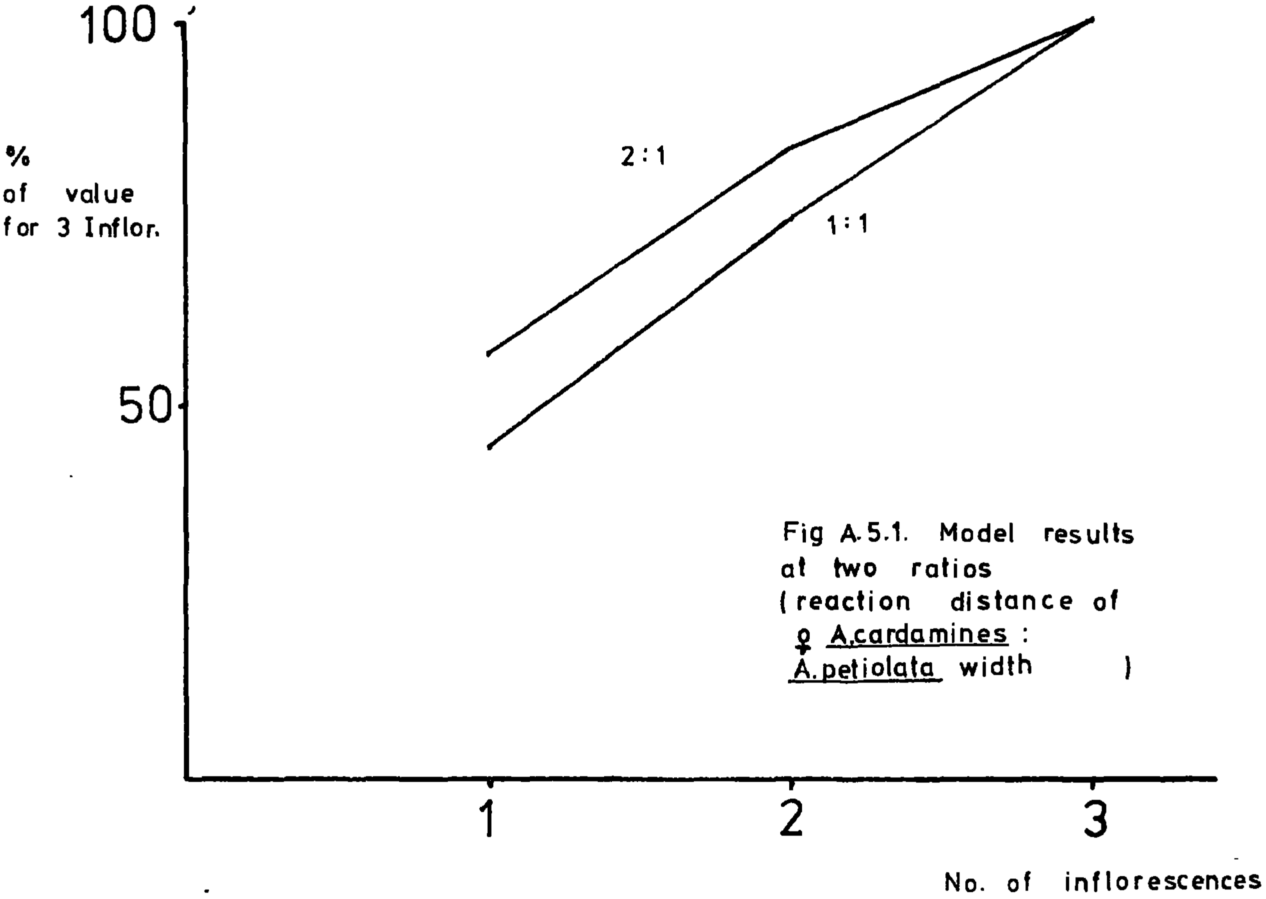


Fig A.5.1. Model results at two ratios (reaction distance of ♀ *A.cardamines* : *A.petiolata* width)

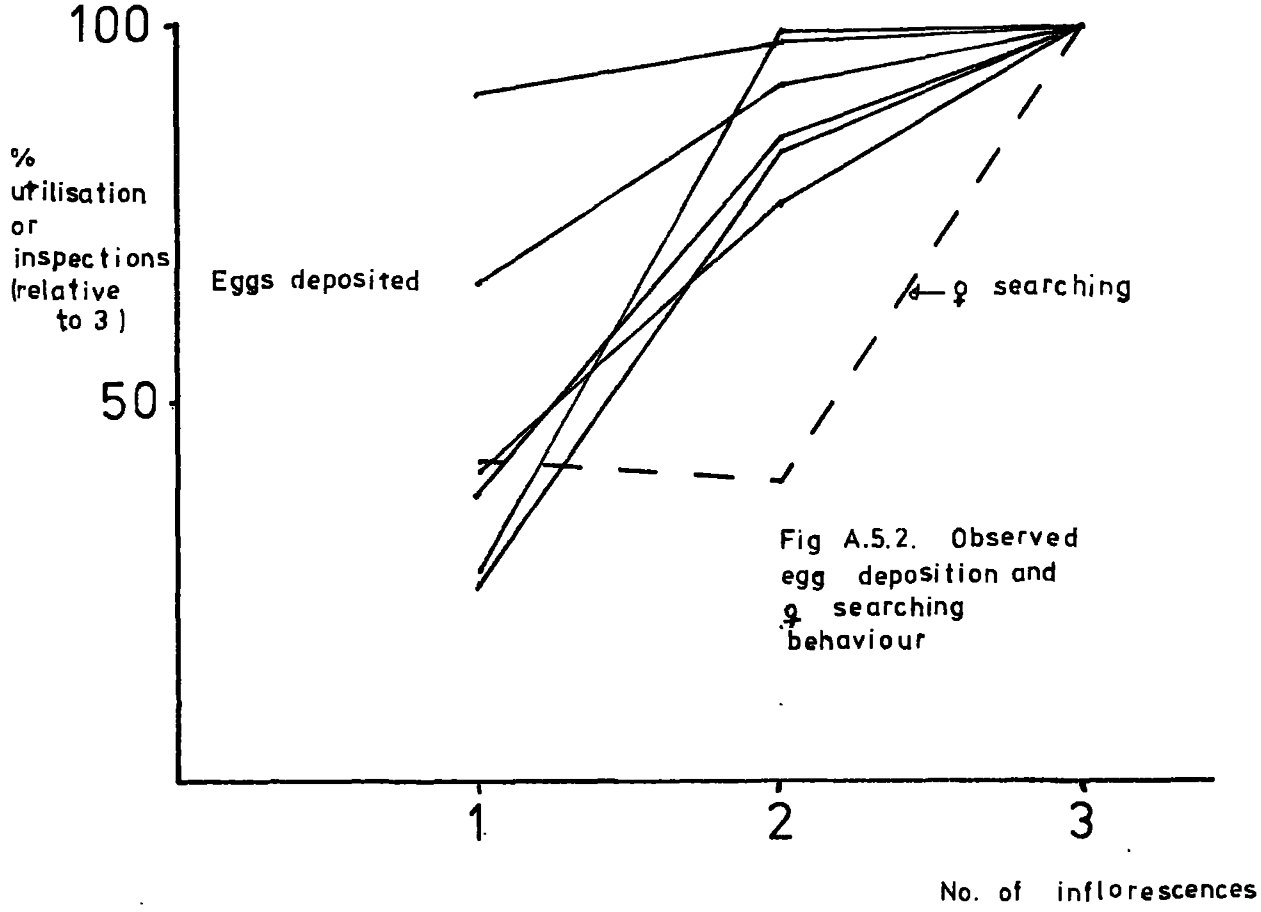


Fig A.5.2. Observed egg deposition and ♀ searching behaviour with the predicted distribution of the model, but that the limited data on female searching has an under-represented value for plants of two flowerheads.

The model produced thus far therefore indicates that female A.cardamines should have a mean reaction distance to A.petiolata inflorescences approximately equal to that of A.petiolata plant width. This appears to agree well with field observations, where reaction distances of ½ to 1 m. appeared common. This prediction could be adequately tested by filming female behaviour. The model may also be refined by the addition of further flowerheads, and by placing these in three dimensional space, rather than assuming that they are all in one plane, as here. (This assumption will produce an underestimate of predicted values for plants of 1 and 2 flowerheads).

Appendix 6

Oviposition deterrents in A.cardamines

> 'It is a wise instinct, if the expression may be pardoned, that prompts the female to lay only one egg on a plant, as in the case of such foodplants as Cardamine pratensis, one larva will devour the whole plant'
>
> H. B. Williams, 1915.

> 'On May 20th I noticed a female ovipositing on Capsella bursa-pastoris. The ova (sic) was deposited singly on the side of the clump of flower buds at the end of the stem. I afterwards examined the neighbouring Capsella plants, and found more eggs, all laid in a similar position and never more than one on each shoot'
>
> J. F. Bird, 1913.

The ova of Euchloini, as already noted, are unusual in being brightly coloured.[*1] This fact, when considered in conjunction with the strong cannibalistic tendencies of the larvae, suggested to me that the eggs' colour might act as a signal to approaching females that the inflorescence was already occupied. Initially this idea was suggested by the similar situation in Heliconius butterflies, where again cannibalism is common, and females refuse to lay eggs on already laden leaves. (This has led to the evolution of egg mimics (Gilbert, 1975). To this end, data were collected during 1977 on egg distributions, to see if eggs were distributed randomly upon flowerheads of foodplants. If females were avoiding competition between their own eggs and prior occupants, by avoidance behaviour, and refusing to oviposit near another egg, then one would expect the egg distribution over flowerheads to depart from the random, Poisson distribution. The preliminary results of 1977 supported this view (Table A.61)

[*1] The only Holarctic Pierini members to lay red eggs are Pontia spp, which are also unusual in laying eggs upon inflorescences.

Tab. A.6.1.

The distribution of A.cardamines eggs over A.petiolata inflorescences, 1977.

Number of Eggs/ Inflorescence	Durham		Croxdale	
	Obs	Exp	Obs	Exp
0	1554	1554.8	2	14.1
1	132	129.6	35	22.8
2	3	5.4	23	18.4
3	1	1.5	10	10.0
4+			1	5.4

χ^2 =	1.24	21.65
d.f. =	1	3
	n.s.	$p < 0.005$

The same conclusion was found to have been reached independently by a number of other researchers. Baker (unpublished) formulated similar ideas c.1968, and was able to show that females spent longer surveying egg-laden plants. He felt that the response was purely visual. The same view was reached in 1979 by J. Waage (pers. comm), and is implied in the comments of the entomologists above. Wiklund and Ahrberg (1978) were the first to publish such a conclusion. They also independently used comparisons with the Poisson distribution to test their hypothesis, and were able to show that "specimens of C.pratensis bearing a single egg were heavily over-represented, whereas specimens bearing two, three or more eggs were strongly under-represented, as were specimens bearing no egg at all."

However, comparisons with the Poisson distribution are not always a useful way of testing the hypothesis. Size, age and particularly the pronounced edge/density effects tended to produce a clumped distribution of eggs (especially in populations such as those at Alston). Thus it may not be valid to compare the actual distribution of eggs with the Poisson, since not all hostplants are equally available for oviposition. The study of Wiklund and Ahrberg was made on C.pratensis, a small and solitarily growing crucifer, which should not be subject to size or density effects. However, their comparisons were made with data collected throughout the flowering period of C.pratensis. Especially in a species with a very short individual flowering time such as C.pratensis, this procedure of summing data may cause departures from the

Poisson as an artefact, since once again flowers are of unequal availability - and may senesce after, e.g. receiving one egg. Summing several different Poisson distributions will lead to a distribution which departs significantly from the Poisson.

Nevertheless casual observation of female behaviour confirmed that a deterrent effect was taking place. Similar overdispersion has been noted for A.mannii (also cannibalistic (Powell, 1909)) A.rapae and P.brassicae (Rothschild and Schoonhaven, 1977), although this is contested by Traynier (1979) for A.rapae.[*1] The means by which ovipositing P.brassicae detect the egg batches is at least partly olfactory (Schoonhoven and Behan, 1978) although visual responses are also implied. In order to better test the hypothesis that ovipositing A.cardamines discriminate amongst egg-bearing and bare plants, some trials were made in 1978 using females in the laboratory. These females, in individual cages, were offered for a standard length of time (5 minutes), plants of A.petiolata which bore either a red (mature) A.cardamines egg, a white (freshly laid) egg or were unladen. It was predicted that if a visual response to the red egg was alone responsible for avoidance behaviour, then plants with such eggs would have less new eggs laid upon them than those plants either bare or with white eggs. Females were captured from the field and brought into the laboratory, and were allowed to lay at least one egg before beginning the experiment.

[*1] Avoidance of egg-bearing plants is also known for other butterflies, e.g. Battus spp (Ransher, 1979), moths, e.g. Hadema spp (Brantjes, 1976) and a weevil Callosobruchus maculatus (F) (Mitchell, 1975).

Only females which did lay eggs were used, and of 17 females brought in, only six readily laid eggs. The number of eggs laid by each female, and the randomized sequence of presentation are given in Fig. A.62. It is seen that both red and white eggs have deterrent effects upon oviposition, strongly suggesting that other responses, apart from or additional to a visual response to the red egg, are involved in avoidance behaviour.

One further interesting observation on eggs as deterrents was made. In the field, A.cardamines females consistently refuse to lay an egg upon an inflorescence already occupied. However, certain of the recorded egg distributions show extraordinary numbers of eggs upon plants. For instance, at Egglestone, in 1977, one H.matronalis plant received 68 eggs. Similarly at Alston, excessively clumped distributions were found. In these circumstances, sub-optimal areas of the plant were chosen as oviposition sites, such as stems and leaves, which were never recorded elsewhere. (See Williams, 1915, for another example). It is apparent that in circumstances of low foodplant availability, probably as a response to the female having flown a very long distance, that an Orange Tip female may choose to 'escalate' a contest that she would normally have settled by prior occupation. 'Escalation' is to be expected if the chance of finding an alternative site for her egg is lower than the chance of 'winning' the contest between her egg and the 'territory holder' (c.f. Maynard Smith and Parker, 1975)[*1]. The evolution of this strategy of avoiding competition and cannibalism, which may be stable across many Pierinae species (and which may have inter-specific effects, e.g. in N. Africa) would make a fascinating topic for further study.

[*1] The behaviour of females emigrating from oviposition sites except when these are at low density, may also be viewed as a strategy optimizer, where the 'costs and benefits' of emigration and oviposition are weighed (Parker and Stuart, 1976). Similar behaviour is known for Callosobruchus (Mitchell, 1975) and Coleoplora alticollela (Randall, pers. comm.)

Table A.6.2

The response of ovipositing females to egg-bearing plants (Nos. eggs laid)

	Egg Colour		
Female No.	White	Orange	No Egg
1	0	2	9
2	0	0	3
3	4	2	10
4	4	3	3
5	4	1	4
6	1	1	7
	13	9	36

Sequence

n n w n o n o w w o o n n w n o n o w w o o o w n n w o

Appendix 7

Hostplants of A.cardamines

The following species of Cruciferae have been noted as bearing A.cardamines eggs (but not necessarily supporting larval growth).

Nasturtium officinale
Rorippa sylvestris
Barbarea vulgaris
Cardaminopsis arenosa
Arabis hirsuta
A.gerrardi
A.albida
Turritis glabra
Cardamine amara
C.pratensis
C.hirsuta
Draba muralis
Cochlearia officinalis
Armoracia rusticana
Hesperis matronalis
Lunaria annua
L.biensis
Erysimum cheiranthoides
Arabidopsis thaliana
Sisymbrium officinale
Alliaria petiolata
Brassica oleracea
B.napus
B.rapa
B.nigra
Sinapis arvensis
S.alba
Capsella bursa-pastoris
Lepidium smithii
Thlaspi alpestre
T.arvensis
Isatis tinctoria
Raphanus raphanistrum
Reseda lutea (Resedaceae)

App. 8

Mark recapture studies on A.napi

Originally it had been intended to investigate population size and individual movement in A.napi in order to complement studies in population genetics (Chapter 4). It was rapidly found that very few recapture events occurred when marking a population at Durham, and this attempt was abandoned. However, unusual circumstances at Langdon Common, where in 1977 enormous numbers of A.napi were in flight in late June and early July, allowed some mark-recapture studies to be undertaken. Although individuals were seen dispersing from the main centre of the colony, and were quite common for miles around, most butterflies were concentrated on a flat area of some 200 metres square, which had an abundant growth of Cardamine pratensis in between grass tussocks. Marking took place over a period of six days in July, on four days of which captures were made. Effort was not equal on the four days of study: although 1½ man hours was put in to capturing animals on each day, butterflies were not equally active on each day due to weather conditions. After capture, individuals were brought to a holding cage and kept there until catching was completed. Then each individual was removed from the cage, sexed, a note taken of its wing condition and a number applied in red ink to the underside forewing (obscured at rest). The animal was then released at a central point. In all 563 individuals were marked and released (all damaged individuals were killed). Of the 503 individuals marked between the 7th and the 11th, 101 were recaptured at least once.

Notes were taken of the amount of wing damage and scale wear shown by each individual. Wing damage, although related to scale wear, also occurred on some very fresh specimens (these may have been attacked by birds). Scale wear was placed into 6 classes:

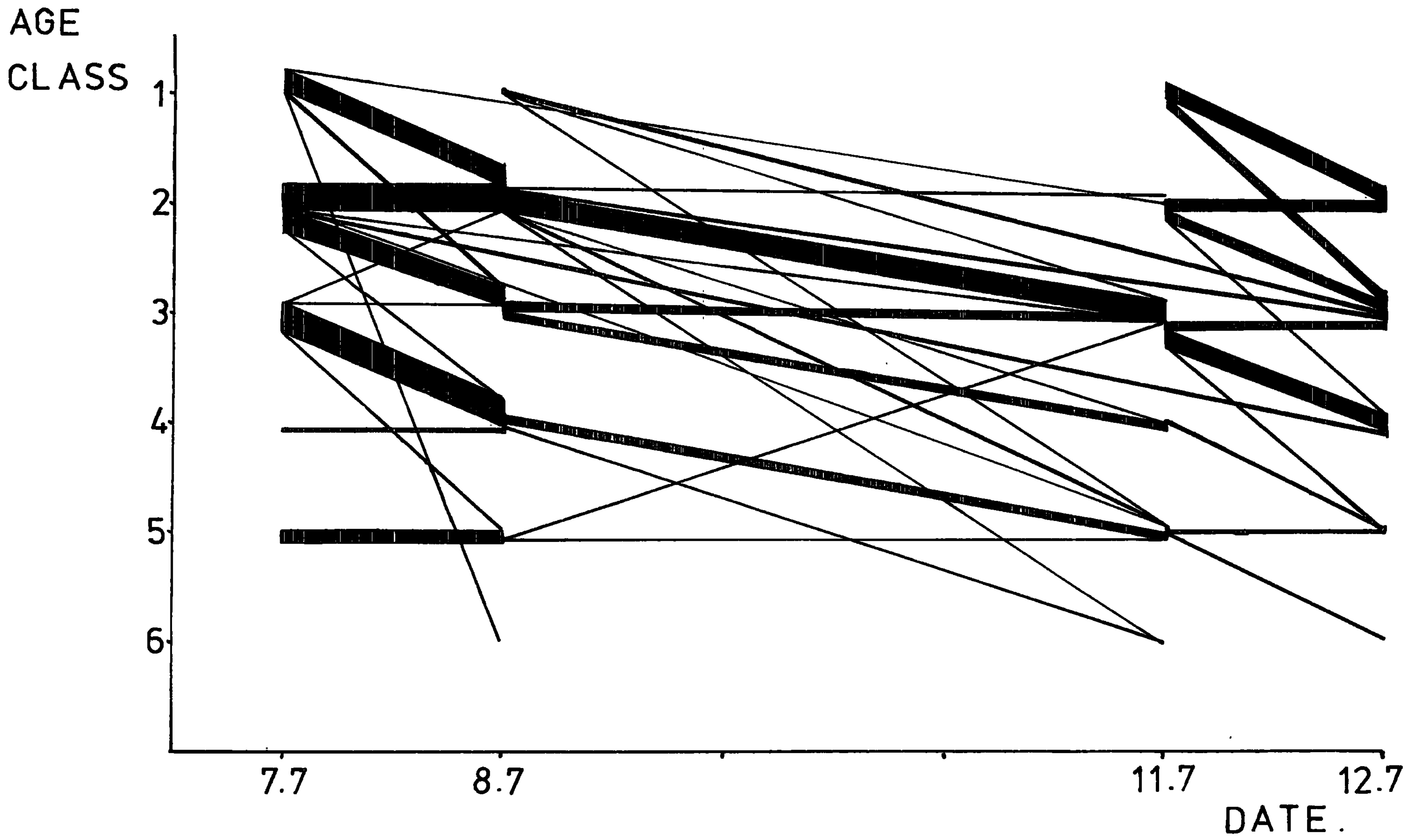
1.	'Very Fresh'	- Animal still moist from emergence.
2.	Fresh	- Few scales lost.
3.	Good	- Some scales lost.
4.	Average	- Wear noticeable.
5.	Worn	- Wear very noticeable.
6.	Very Worn	- Few scales remaining

The number of individuals captured on each date in each age class is given below:

Wear Class	Date 7/7	8/7	11/7	12/7	
1	25	36	33	18	
2	60	82	43	36	
3	31	68	35	25	
4	34	49	18	13	
5	26	15	15	4	
6	7	4	2	2	
	183	254	146	98	681

Individuals that were recaptured were seen to change wear class as shown in Fig. A.8.1., where each line signifies recaptures between dates moving from one age class to another (or staying in the same class). The width of the line signifies the number of individuals observed.

Fig. A.8.1. Changes in age class of individuals of *A.napi* during capture-recapture studies, Langdon Common, 1977. The width of each line is proportional to the number of individuals.


AGE
CLASS
1
2
3
4
5
6
7.7
8.7
11.7
12.7
DATE.

It is seen that only two individuals were classed in a lower wear class on subsequent captures, and that only rarely did an individual move rapidly from a low wear class to a high class. In general, scale wear increased gradually with time, and the age classes used therefore appear to be an appropriate index of individual age since emergence.

App. 9

Electrophoretic Techniques

The methods in use in this study were starch gel electrophoretic techniques based on the original methods of Smithies (1955), Hunter and Markert (1957) and Poulik (1957), as developed by Shaw and Prasad (1970) and as applied to butterflies by McKechnie et al (1975). The enzymes available for study in this way are soluble proteins, and are predominantly glycolytic enzymes (Chefurka (1958) reviews the differences between insect and vertebrate glucose metabolism).

Gels were made at a concentration of 13% starch, 32.5 g of starch/250 ml of gel buffer, cooked, and after degassing, were refrigerated until use. For assaying enzymes which used NAD or NADP as co-factors, 10 mg of the co-factor were added to the gel immediately prior to degassing, as this was found to sharpen bands markedly.

Animals were prepared for electrophoresis by removing abdomens, or rarely heads, and grinding these individually in a small volume of deionised water. (Initially grinding buffers were used containing co-factors, but these were not found to increase resolution). When volumes permitted, samples were then centrifuged to remove large cell debris; however frequently volumes were too small for this step. In such cases samples were absorbed through a layer of filter paper. Samples were absorbed onto pieces of filter paper (Whatman No. 1) 6 mm x 8 mm and loaded into slots in the gel.

Electrophoretic conditions followed those of McKechnie et al (1975). After initial experimentation with buffers, three buffer systems were used.

1. Discontinuous Tris-Citrate (Poulik) (DTC)

   Electrode buffer 0.3 M borate pH 8.2.

   (18.55 g Boric Acid, 2.4 g NaOH / L)

   Gel buffer 0.076 M tris - 0.005 M Citric acid pH 8.7

   (9.21 g tris, 1.05 g Citric acid / L)

2. Continvonstris Citrate (CTC)

   Electrode buffer 135 mM Tris, 45 m M citric

   (16.34 g Tris, 9.45 g Citric / L) pH 7.1

   Gel buffer 1:14 dilution of electrode buffer.

3. Tris-versene-Borate (TBE)

   Electrode buffer 0.5 M tris, 0.02 M EDTA,
   0.65 M boric acid, pH 8.0

   (60.6 g tris, 40.0 g boric acid, 6.0 g Na_2 EDTA / L)

   Gel buffer : 1:9 dilution of electrode buffer.

Electrophoresis was continued until front markers (bromophenol blue) or migration front (in DTC) had migrated 8 cm towards the anode. Gels were then removed and sliced horizontally, and the inner surface of each slice stained for enzyme activity.

The following staining procedures were used:

<u>Esterases</u> (EST) 3 ml 1% α-napthyl acetate (in acetone/
water $50^o/_{50}$)
100 mg Fast Blue RR
100 mg EDTA
in 100 ml buffer.

EST-1 and EST-2 were inhibited by eserine but not EDTA, suggesting that these loci were coding for cholinesterases. However strong inhibition of background esterases was effected by EDTA, making adult esterases more easily read. (Larval esterases are unaffected by eserine and are probably carboxylesterases (Clements, 1967)).

Lencine Amino Peptidases (LAP-1, 2, 3)

1 ml 0.1 M $MgCl_2$
10 mg leucyl B napthylamide HCL
in 100 ml buffer.
Incubate for 1 hour, then add 20 mg Black K.

Both EST and LAP staining procedures involve highly carcinogenic material.

Peptidases (PEP- 1, 2, 3)

Following the method of Lewis and Trulove (1969) In 50 ml of buffer were added 50 mg $MgCl_2$, 50 mg $MnCl_2$, 50 mg O-dianisidine (HCl_2), 1000 u Peroxidase and 10 mg purified *Crotalus adamanteus* (Diamondback Rattlesnake) venom, together with 80 mg of di- or tri- peptides. The gel slice was then incubated overnight.

A tripeptide (Glycylglycyl glycine) gave no Peptidase activity.

A dipeptide (Glycyl-L-Leucine) gave two peptidase loci (PEP - 1,2).

A second dipeptide (L-Valyl-L-Leucine) gave a third peptidase locus (PEP-3).

Glutamate - Oxaloacetate Transaminases (GOT- 1, 2)

To 100 ml buffer, add 0.5 mg Pyridoxal-5-Phosphate, 200 mg Aspartic acid, 100 mg Ketoglutaric Acid and 150 mg Fast Blue BB.

Peroxidase and Catalase (PER, CAT)

Soak gel slice at pH 6 for 45^{o} mins, at 4^{o} C. Then soak for 60 seconds in 1 g kI, 1 ml Acetic Acid in 50 ml of water.

Wash thoroughly in distilled water, then add 1 ml 3% H_2O_2 in 50 ml H_2O.

Peroxidase should stain as dark bands, Catalase as white bands on background. Catalase bands, poorly resolved, were visible on DTC gels, in approximately the same position as SOD bands.

NAD - Dependent Dehydrogenases

The same basic staining procedure was followed for several loci.

In 100 ml of buffer, 20 mg NAD, 20 mg Nitro Blue Tetrazolium and 3 mg Phenazine Metasulfate were added to, for:

i. Hydroxybutyrate Dehydrogenase (BDH)

10 mg Mg Cl_2, 500 mg NaCl and 250 mg B - hydroxybutyric acid.

ii. α Glycerophosphate Dehydrogenases (αGPDH - 1, 2)

10 mg $MgCl_2$ and 50 mg Na_2 α DL glycerophosphate

iii. Malate Dehydrogenases (MDH - 1, 2)

10 mg $MgCl_2$ and 250 mg Malic Acid.

iv. Aldehyde or Xanthine Dehydrogenases (AlDH/XDH)

20 ml Benzaldehyde or 25 mg Hypoxanthine

Xanthine dehydrogenase is a highly active enzyme in Pieridae (Lafont and Papillon, 1972), and varies greatly in activity during development, as might be expected in organisms where uric-acid synthesis is so important.

v. Staining for Alcohol and Octanol Dehydrogenases was not successful, although Helias (1977) has shown that these are active in larvae of P.brassicae.

NADP - Dependent Enzymes

These enzymes were stained using the following basic stain:

10 mg NADP, 20 mg NBT and 3 mg PMS in 100 ml of buffer, added to, for:

i. Glucose-6-Phosphate Behydrogenase (G6PDH)

100 mg Glucose-6-Phosphate.

ii. NADP - Dependent MDH or Malic Enzyme (ME)

100 mg Malic Acid, 10 mg $MgCl_2$.

iii. Isocitrate Dehydrogenase (IDH)

10 mg $MnCl_2$, 100 mg Isocitric Acid (Na salt).

iv. 6-Phosphogluconate Dehydrogenase (6PGDH)

10 mg Mg Cl_2, 100 mg Ba-6 Phosphogluonic Acid.

The following enzymes included coupling reactions to other enzymes:

Fumarase (FUM)

In 100 ml buffer, add 10 mg NBT, 3 mg PMS, 20 mg NAD, 100 mg Fumaric Acid and 300 u MDH.

Phosphoglucomutases (PGM)

In 100 ml buffer, add 12 mg NADP, 20 mg MT.T 3 mg PMS, 1 ml 0.1 M $MgCl_2$, 100 mg Glucose-1-Phosphate and 15 units G6PDH.

Phosphoglucose-isomerase (PG1)

In 100 ml buffer, add 10 mg NADP, 10 mg NBT, 3 mg PMS, 1 ml 0.1 M $MgCl_2$, 25 mg Fructose-6-Phosphate and 20 units G6PDH.

Hexokinases (HK-1, 2, 3)

In 100 ml buffer, add 10 mg NADP, 10 mg NBT, 3 mg PMS, 1 ml 0.1 M $MgCl_2$, 50 mg Glucose, 20 mg ATP and 20 units G6PDH.

Phosphomannose Isomerase (PM1)

Following the method of Nichols et al (1973) In 100 ml of buffer, add 20 mg Ba-D-mannose - 6-Phosphate, 10 mg NADP, 10 mg NBT, 3 mg PMS, 10 mg $MgCl_2$, 100 u PG1, 80 u G6PDH.

Adenylate kinase (AK)

To 100 ml buffer, add 0.5 ml 0.1 M $MgCl_2$, 20 1 8mM $MnCl_2$, 50 mg Glucose, 12 mg NADP, 3 mg PMS, 20 mg ADP, 20 mg M T.T., 50 u HK, 20 u G6PDH.

Aldolase (ALD)

To 100 ml buffer, add 250 mg Fructose-1 6-Diphosphate, 75 mg Arsenic Acid, 50 u Glyceraldehyde Dehydrogenase, 20 mg NAD, 3 mg PMS, 20 mg NBT.

Fructose-1-6-Diphosphatase (FDP)

To 100 ml buffer, add 125 mg Mg5O4, 20 mg Fructose-1-6-Diphosphate, 1 mg PMS, 10 mg MTT, 10 mg NADP, 20 u PG1, 20 u G6PDH.

Superoxide Dismutase (SOD)

Otherwise known as Indophenol Oxidase, or Tetrazolinm Oxidase, this enzyme appears as white bands against the blue backgrounds of many of the above stains.

The stain buffers in use (after initial trials) were:

1. 0.05 M Tris-HClpH 7.1
2. 0.076 M-Tris- citric pH 8.7

The stain buffers were used as follows:

Enzyme	Enzyme Commission No.	Stain buffer	Electrophoretic buffers
EST-1,2	3.1.1.8	2	DTC
LAP-1,2,3	3.4.11.1	2	TBE
PEP-1,2,3	3.4.13.11	1	TBE
GOT-1,2	2.6.1.1	1	DTC
BDH	1.1.1.30	2	DTC
α GPDH	1.1.1.8	2	DTC
MDH	1.1.1.37	2	DTC
AlDH	1.2.1.3/1.2.1.37	2	DTC
G6PDH	1.1.1.49	2	DTC
GPGDH	1.1.1.43	1	TBE
ME	1.1.1.40	2	DTC
FUM	4.2.1.2	1	DTC
HK	2.7.1.1.	1	CTC
PM1	5.3.1.8	1	CTC
AK	2.7.4.3	1	CTC
ALD	4.1.2.13	1	CTC
FDP	3.1.3.11	1	CTC

Criteria for accepting loci for survey work

Obviously, it is essential to have good activity at enzyme loci before they are suitable for electrophoretic survey work. BDH, IDH and αGPDH-2 were insufficiently active under the present procedures for accurate typing and were discarded. Similarly MDH-1 (which appeared to distinguish Euchloini from Pierini) and GOT-2 (which, running cathodally, appeared polymorphic) were insufficiently resolved for accurate typing. PGI was discarded since this enzyme-stain exhibited G6PDH activity under the procedure used. PGM and SOD (3 loci) exhibited good activity and good resolution but were also discarded because of difficulties in accurate typing. These two enzymes have different forms in the larvae (as have esterases) which are sometimes maintained in the adult. Especially in the case of PGM, which does not form hybrid bands (being a monomeric enzyme), there is no way to tell apart individuals heterozygous for adult PGM, and individuals homozygous for adult PGM but with some larval PGM remaining. Nair et al (1977) have described similar regulatory changes which may have caused over-estimation of heterozygosity and genetic distance. Similar problems exist with anodal SOD loci, where larval enzymes migrate similar distances to adult enzymes and cause complex over-lapping patterns. Cathodal SOD (SOD-3) gave easily typed bands (and appeared to distinguish between Artogeia and Pieris) but was not present in all samples surveyed.

Esterases were also not used for comparing taxa, despite their being easily resolved and scored. The reason for this is that evolutionary homology of different alleles and even loci cannot be assumed at esterases. Homology of loci is an assumption of comparative electrophoretic work, and appears justified at most enzymes, where for instance the number of loci encoding for a particular enzyme does not change. A second assumption is that alleles which migrate a similar distance have the same structure, and are genetically identical. However migration distance might be subject to convergence, where different genotypes give similar phenotypes. The net result of this second assumption will be a slight under-estimate of genetic Distance (one example may be the presence in A.napi and A.rapae of the phenotype G6PDH-105 which is the common allele of Anthocharis genutia), but it usually is disregarded as being negligible, and impossible to correct for. However when staining for esterases neither of the assumptions appears justified - most species have very distinct phenotypes, with varying numbers of bands. It appears likely that the number of esterase loci varies between different species of Pierinae, and in cases where migration distances are similar (for instance EST-1 in A.napi and some esterases in A.cardamines) the assumption of genetic identity does not appear justified.

App. 10

Electrophoretic heterogeneity of allelic classes

Coyne (1976) dismayed many workers in electrophoretic research by showing that genetic heterogeneity existed within previous electrophoretic classes of XDH in *Drosophila persimilis*. He demonstrated that allelic differences of migration distances were detectable at extremely small differences such as 0.4%. (For instance he distinguished alleles 98.4 and 98.0). By varying the concentration of acrylamide strength and buffers in the system he was using he increased the number of alleles detected at XDH from 5 to 23. He also indicated, by heat treatment, that further genetic variability might exist, in resistance to heat, although as Beckenbach and Prakash (1977) point out this could reflect variability at loci other than the XDH structural locus. If Coyne's finding had proven general, then many earlier findings using electrophoretic techniques would have been falsified, including estimates of genetic identity and distance. Some research supported this suggestion and enzymes of *Drosophila pseudobscura* (McDowell and Prakash, 1976) and *Colias* butterflies (Johnson, 1976, 1977) were shown to have 'hidden' variability. (However the results on *Colias* butterflies have been challenged by Cobbs and Prakash (1977) on methodological grounds). However other recent studies, for instance on HK of *Drosophila pseudobscura* and *D.persimilis* by Beckenbach and Prakash (1977) using many different conditions of electrophoresis have failed to find increased variability.

As Watterson and Anderson (1978) and Coyne et al (1978) point out, it appears that there are essentially two classes of enzyme variation - **nearly monomprphic** and highly polymorphic.

In the present study it was judged important to search for such 'hidden' variability, since the presence of such variability might invalidate the main conclusion of Chapter 4, that is that little gene change at electrophoretic loci has taken place.

To this end vertical slab acrylamide electrophoresis was undertaken, following the methods of Coyne (1976). Four buffer systems were used:

Tris-HCl	pH 9.0.
Tris-HCl	pH 7.0.
Tris-citric	pH 8.7.
Tris-citric	pH 7.1,

and two acrylamide strengths (5% and 7%) were used. Samples were prepared in the usual way, except that they were routinely centrifuged and loaded on the top of the gel using micropipettes.

Staining for enzymes was followed as in App. 9. The following enzymes were examined using the **eight** electrophoretic conditions outlined:

MDH-2, α-GPDH-1, AlDH, EST-1, EST-2

For esterases, A.napi specimens were examined.

For other loci, A.napi, A.rapae, A.cardamines and P.brassicae were compared

At MDH-2, α-GPDH-1, AlDH and EST-2 no additional variation was detected.

At EST-1, results indicated that allele 104 comprised two alleles (one being rare, but distinguishable by slower migration distances). Other alleles (94,100) showed no evidence of hidden variability.

Thus at essentially monomorphic, and at slightly polymorphic loci no evidence was gained of the presence of additional variation. At one highly polymorphic locus, an additional variant was found. Thus in Pierinae, which were studied here using predominantly monomorphic loci, the high genetic Identity found is probably _not_ due to cryptic variation being missed. Throckmorton (1977), reviewing _Drosophila_ systematics using electrophoretic techniques, felt that new data on cryptic variability had not altered the idea of minimal genetic change during speciation in that group, but that such new data did argue against electrophoretic results being used for phylogency or as molecular clocks. Finally, using the yet more accurate techniques of two dimensional electrophoresis on SDS-Acrylamide, Brown and Langley (1979) have investigated a large number of structural proteins in _D.melanogaster_. They found that only 6 of 54 loci were polymorphic, and suggest that earlier estimates of heterozygosity in Drosophila may be over-estimates.

App. 11

Gene frequencies in A.napi

The following pages give the allelic frequencies of EST-1, EST-2, GOT-1, α-GPDH-1, G6PDH and ALDH in the populations of A.napi studied. Frequencies are expressed as proportions of the sample.

Different alleles are noted and named under their mobility relative to the most common allele, which is arbitrarily assigned a migration distance of 100. Thus an enzyme coded by allele 94 migrates anodally 94% of the distance coded by allele 100, and has a frequency of p.94.

The first brood in bivoltine populations is designated by the letter a, the second brood by b. Univoltine populations have no letter attached.

ESTERASE - 1

Durham

	1977a	b	1978a	b	1979a	b
n	42	56	56	46	48	76
p94	.10	.07	.13	.11	.10	.11
100	.71	.71	.68	.78	.71	.67
102	-	-	.02	.02	-	-
104	.19	.21	.18	.09	.19	.17
107	-	-	-	-	-	.05

Croxdale

	1977a	b	1978a	b
n	32	40	32	28
p94	.03	.08	.06	.04
100	.69	.73	.78	.82
102	.03	-	-	-
104	.25	.20	.16	.14
107	-	-	-	-

Witton Park

	1977a	b	1978a	b	1979a	b
n	50	48	42	74	18	48
p94	.02	.06	.10	.16	.11	.06
100	.88	.79	.83	.73	.78	.83
102	-	-	-	-	-	.04
104	.10	.13	.07	.11	.11	.06
107	-	.02	-	-	-	-

Wolsingham

	1977a	b	1978a	b	1979a	b
n	40	50	54	48	66	72
p94	.25	.28	.31	.35	.27	.28
100	.65	.58	.56	.54	.58	.61
102	-	.02	-	-	.02	-
104	.10	.12	.13	.10	.14	.11
107	-	-	-	-	-	-

Holwick

	1977	1978	1979
n	48	30	0
p94	.08	.03	
100	.81	.73	
102	.02	-	
104	.08	.23	
107	-	-	

Coldberry

	1977	1978	1979
n	46	48	46
p94	.48	.29	.22
100	.41	.50	.65
102	-	.02	.02
104	.11	.17	.11
107	-	.02	-

Lune Valley

	1977	1978	1979
n	46	40	36
p94	.20	.40	.14
100	.76	.50	.83
102	-	-	-
104	.04	.10	.03
107	-	-	-

Alston

	1977	1978	1979
n	44	44	44
p94	.09	.07	.11
100	.84	.89	.70
102	-	-	.02
104	.07	.05	.16
107	-	-	-

	Langdon Common			St. John's Chapel	Langdon Beck
	1977	1978	1979	1977	1977
n	98	46	48	46	36
p94	.15	.11	.06	.15	.14
100	.69	.67	.78	.72	.69
102	.02	-	-	-	-
104	.13	.22	.13	.13	.17
107	-	-	-	-	-

p89 .04

Langwathby				
	1977b	1978b	1979a	1979b
n	48	54	16	58
p94	.08	.04	-	.05
100	.85	.94	1.00	.90
102	.01	-	-	-
104	.04	.02	-	.05
107	-	-	-	-

	Wearhead		Knodishall	
	1978	1979	1977b	1978b
n	56	28	34	36
p94	.02	.07	.03	.06
100	.91	.79	.72	.81
102	-	-	.06	-
104	.07	.14	.21	.14
107	-	-	-	-

	Hartside			Redbourne
	1977a	1978b	1979a	1977b
n	46	8	48	16
p94	.04	-	.08	.13
100	.61	1.00	.71	.75
102	-	-	-	-
104	.35	-	.21	.06
107	-	-	-	.06

ESTERASE - 2

SAMPLE SIZES AS ESTERASE 1

p100 = 1.00 in all samples except:

	Durham			Wolsingham			
	1977b	1978b	1979b	1977a	1977b	1978a	1978b
p96	.02	.04	.03	.03	.06	-	.06
100	.98	.94	.92	.98	.89	.96	.92
103	-	.02	.05	-	.04	.04	.02

	Wolsingham		Holwick	Coldberry	
	1979a	1979b	1978	1977	1979
p96	.06	.08	.03	.07	.04
100	.91	.92	.97	.93	.96
103	.03	-	-	-	-

	Lune Valley		Alston	Knodishall
	1978	1979	1978	1978
p96	.08	-	-	.06
100	.90	.97	.95	.92
103	.03	.03	.05	.03

	Langdon Common		Witton Park
	1977	1978	1979b
p96	.03	.02	.02
100	.96	.96	.96
103	.01	.02	.02

GOT - 1

Sample sizes as EST-1

p100 = 1.00 in all samples except:

Durham

	1977a	1977b	1978b	1979a	1979b
p35	.05	.05	.07	.02	.04
p100	.95	.95	.93	.98	.96

	Coldberry		Lune Valley	
	1977	1978	1977	1978
p35	.04	.04	.05	.05
100	.96	.96	.95	.95

	Langdon Common			St. John's Chapel
	1977	1978	1979	1977
p35	.09	.07	.04	.07
100	.91	.93	.96	.93

	Langwathby		Wearhead	
	1977b	1979b	1978	1979
p35	.02	.05	.04	.04
100	.98	.95	.96	.96

	Knodishall		Redbourne
	1977b	1978b	
p35	.06	.14	.13
100	.94	.86	.87

α GPDH-1: rare variants were present in the following frequencies:

Durham	1977b p102 = .04, 1978b p102 = .02
Witton	1978a p110 = .07, 1978b p102 = .01
Coldberry	1977 p102 = .02, 1978 p102 = .04, 1979 p102 = .02
Langdon Common	1977 p102 = .05, 1978 p102 = .04
Wearhead	1978 p102 = .01, p110 = .01
Knodishall	1977b p102 = .03

G6PDH : rare variants were present in the following frequencies:

Coldberry	1977 p105 = .02
Langdon Common	1977 p105 = .03
Knodishall	1977b p105 = .03

ALDH : rare variants were present in the following frequencies

Alston	1978 p103 = .02
Langdon Common	1978 p103 = .02

App. 12

Allelic frequencies at 22 loci in Pierinae

Allelic frequencies in 23 samples are presented, at loci discussed in App. 9. Alleles are assigned to classes based on their electrophoretic mobility relative to the most common allele in A.napi (samples of which were run as standards).

The following notation abbreviates sample species or populations.

P.n	Artogeia napi.	Co. Durham populations (summed)
P.nb	A.n.bicolarata	from Oulu, Finland, 1978. (3.7.78).
P.br	A.bryoniae	ex stocks of Bowden.
P.m	A.melete	" " " "
P.j	A.japonica	" " " "
P.v	A.virginiensis	ex stocks of C. G. Oliver (6) wild-sample North Bramford, NewHaven Co., CT., U.S.A. 28.4.79.
P.r	Artogeia rapae	Co. Durham
Pr(2)	A.rapae	Qormi, Malta 7/78.
Pr(3)	A.rapae	Asni, nr. Marrakesh, Morocco 5/78
Prc	A.rapae crucivora	Hokkaido, Japan 7/78
Pb	Pieris brassicae	Co. Durham
Pb(2)	P.brassicae	Qormi, Malta 7/78
P.c	Pieris cheiranthi	Tenerife, Canary Islands. 5/78.
Pd	Pontia daplidice	Asni, Morocco. 5/78.
A.c	Anthocharis cardamines	Co. Durham (summed)

A.b	*A.belia*	Ifrane, Middle Atlas, Morocco. 4/78.
A.s	*A.sara*	Gates Canyon, Vaca Hills, Solano Co., California. 4/79.
Ag	*A.genutia*	West Rock, NewHaven Co., Ct., U.S.A. 6/5/79.
Ze	*Zegris eupheme*	Ifrane, Middle Atlas, Morocco. 4/78.
Ea	*Euchloe ausonia*	Asni, Morocco 5/78.
Es	*Euchloe ausonides*	Rancho Cordova, Sacramento Co., California, 4/79.
Eb	*Euchloe belemia*	Asni, Morocco 5/78
Ec	*Euchloe charlonia*	Asni, Morocco 5/78.

AK	Pn	Pnb	Pbr	Pm	Pj	Pv	Pc	Pr(2)
n	42	-	16	20	8	12	34	16
p35	-	-	-	-	-	-	-	-
100	1.00		1.00	1.00	1.00	1.00	.94	1.00
110	-	-	-	-	-	-	-	-
120	-	-	-	-	-	-	-	-

Pr(3)	Prc	Pb	Pb(2)	Pc	Pd	Ac	Ab
16	6	30	16	10	22	22	14
-	-	-	-	-	1.00	1.00	1.00
1.00	1.00	1.00	1.00	1.00	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-

As	Ag	Ze	Ea	Es	Eb	Ec
6	12	**14**	26	14	24	10
1.00	.92	1.00	1.00	.79	1.00	1.00
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	.21	-	-
-	.08	-	-	-	-	-

ALDH	Pn	Pub	Pbr	Pm	Pj	**Pn**	Pr	Pr(2)
n	2260	22	16	20	8	18	96	32

Pr3	Prc	Pb	Pb(2)	Pc	Pd	Ac	Ab
24	6	84	28	10	32	656	20

As	Ag	Ze	Ea	Es	Eb	Ec
6	12	16	28	14	24	22

all have p100 = 1.00

except Pn p100 = .999 Pr p100 = .96
p103 = .001 p112 = .04

ALD

	Pa	Pab	Pbr	Pm	Pj	Pv	Pr	Pr(2)
n	42	-	16	20	8	12	34	16
p36	-		-	-	-	-	-	-
45	-		-	-	-	-	-	-
52	-		-	-	-	-	-	-
56	-		-	-	-	-	-	-
100	1.00		1.00	1.00	1.00	1.00	1.00	1.00
105	-		-	-	-	-	-	-

	Pr3	Prc	Pb	Pb2	Pc	Pd	Ac	Ab
	16	6	30	16	10	22	22	14
	-	-	-	-	-	-	-	1.00
	-	-	-	-	-	-	1.00	-
	-	-	-	-	-	1.00	-	-
	-	-	-	-	-	-	-	-
	1.00	1.00	1.00	1.00	.80	-	-	-
	-	-	-	-	.20	-	-	-

	As	Ag	Ze	Ea	Es	Eb	Ec
	6	12	14	26	14	24	8
	1.00	-	1.00	-	-	-	-
	-	-	-	-	-	-	-
	-	-	-	-	-	-	-
	-	1.00	-	1.00	1.00	1.00	1.00
	-	-	-	-	-	-	-
	-	-	-	-	-	-	-

FDP	Pn	Pnb	Pbr	Pm	Pj	Pv	Pr	Pr(2)
n	42	-	16	22	8	16	32	-
p10	-		-	-	-	-	-	-
33	-		-	-	-	-	-	-
75	-		-	-	-	-	-	-
78	-		-	-	-	-	-	-
80	-		-	-	-	-	-	-
100	.95		1.00	1.00	1.00	.94	.16	-
110	.05		-	-	-	.06	-	-
120	-		-	-	-		.84	1.00

FDP	Pr3	Prc	Pb	Pb2	Pc	Pd	Ac	Ab
n	16	6	24	16	10	22	222	14
p10	-	-	-	-	-	-	-	.86
33	-	-	-	-	-	-	.135	.07
75	-	-	-	-	-	-	.860	-
78	-	-	-	-	-	-	.005	.07
80	-	-	1.00	1.00	1.00	.95	-	-
100	.06	-	-	-	-	.05	-	-
110	-	-	-	-	-	-	-	-
120	.94	1.00	-	-	-	-	-	-

FDP	As	Ag	Ze	Ea	Es	Eb	Ec
n	6	12	14	26	14	24	8
p10	1.00	.08	1.00	.92	1.00	.92	1.00
33	-	.76	-	.04	-	-	-
75	-	.16	-	-	-	.04	-
78	-	-	-	-	-	.04	-
80	-	-	-	-	-	-	-
100	-	-	-	.04	-	-	-
110	-	-	-	-	-	-	-
120	-	-	-	-	-	-	-

FUM	Pn	Pnb	Pbr	Pm	Pj	Pv	Pr	Pr(2)
n	64	20	12	22	8	12	38	16
p20	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-
90	-	-	-	-	-	-	-	-
100	.97	1.00	1.00	1.00	1.00	1.00	1.00	1.00
108	.03	-	-	-	-	-	-	-
120	-	-	-	-	-	-	-	-
142	-	-	-	-	-	-	-	-

FUM	Pr(3)	Prc	Pb	Pb(2)	Pc	Pd	Ac	Ab
n	16	6	30	16	10	22	26	12
p20	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	1.00
90	-	-	.03	-	-	-	1.00	-
100	.94	1.00	.97	1.00	1.00	1.00	-	-
108	.06	-	-	-	-	-	-	-
120	-	-	-	-	-	-	-	-
142	-	-	-	-	-	-	-	-

FUM	As	Ag	Ze	Ea	Es	Eb	Ec
n	6	12	18	26	14	24	10
p20	-	-	-	.12	-	-	-
50	1.00	-	-	-	-	-	-
90	-	1.00	-	-	-	-	-
100	-	-	-	-	-	-	-
108	-	-	-	.88	.86	1.00	1.00
120	-	-	-	-	.14	-	-
142	-	-	1.00	-	-	-	-

G6PDH

	Pn	Pnb	Pbr	Pm	Pj	Pv	Pr	Pr(2)
n	2260	22	16	20	8	18	96	32
p45	-	-	-	-	-	-	-	-
48	-	-	-	-	-	-	-	-
72	-	-	-	-	-	-	-	-
91	-	-	-	-	-	-	.99	1.00
100	.998	.99	1.00	1.00	1.00	1.00	-	-
105	.002	.02	-	-	-	-	.01	-

	Pr(3)	Prc	Pb	Pb(2)	Pc	Pd	Ac	Ab
n	16	6	30	16	10	22	658	12
p45	-	-	-	-	-	-	.998	1.00
48	-	-	-	-	-	-	.002	-
72	-	-	-	-	-	1.00	-	-
91	1.00	1.00	-	-	-	-	-	--
100	-	-	1.00	1.00	1.00	-	-	-
105	-	-	-	-	-	-	-	-

	As	Ag	Ze	Ea	Es	Eb	Ec
n	6	12	20	26	14	24	16
p45	1.00	-	-	-	-	-	-
48	-	-	1.00	-	-	-	-
72	-	-	-	.96	1.00	.96	1.00
91	-	-	-	.04	-	-	-
100	-	-	-	-	-	.04	-
105	-	1.00	-	-	-	-	-

αGPDH

	Pn	Pnb	Pbr	Pm	Pj	Pv	Pr	Pr(2)
n	2260	24	16	20	8	18	122	32
p97	-	-	-	-	-	-	-	-
100	.991	1.00	1.00	1.00	1.00	1.00	.88	1.00
102	.007	-	-	-	-	-	.04	-
110	.002	-	-	-	-	-	.08	-

Pr(3)	Prc	Pb	Pb(2)	Pc	Pd	Ac	Ab
24	6	80	24	10	32	656	20
-	-	04	-	-	-	-	-
1.00	1.00	.91	1.00	1.00	1.00	1.00	1.00
-	-	.05	-	-	-	-	-
-	-	-	-	-	-	-	-

As	Ag	Ze	Ea	Es	Eb	Ec
6	12	18	28	14	24	16
-	-	-	-	-	-	-
1.00	1.00	1.00	-	-	-	-
-	-	-	-	-	-	-
-	-	-	1.00	1.00	1.00	1.00

GOT-1

	Pn	Pnb	Pbr	Pm	Pj	Pv	Pr	Pr(2)	Prc	Pb	Pc	Pd	Ac
n	2280	50	16	18	8	20	142	32	6	44	8	6	656
p25	-	-	-	-	-	-	-	-	-	-	-	-	0.043
29	-	-	-	-	-	-	-	-	-	-	-	-	0.006
35	-	-	-	-	-	-	-	-	-	-	-	-	0.878
76	.024	.06	-	-	-	-	.23	.19	-	-	-	-	-
92	-	-	-	-	-	-	-	-	-	-	-	-	0.064
100	.976	.94	1.00	1.00	1.00	.96	.77	.81	1.00	-	-	-	-
120	-	-	-	-	-	.04	-	-	-	-	-	-	0.009
132	-	-	-	-	-	-	-	-	-	1.00	1.00	1.00	-

HK-2

	Pn	Pnb	Pbr	Pm	Pj	Pv	Pr	Pr2
n	46	24	16	20	8	18	34	22
p20	-	-	-	-	-	-	-	-
68	-	-	-	-	-	-	-	-
76	-	-	-	-	-	-	-	-
82	-	-	-	-	-	-	-	-
90	-	.12	-	-	-	-	-	-
100	1.00	.88	1.00	1.00	1.00	1.00	.96	.95
118	-	-	-	-	-	-	.24	.05

Pr(3)	Prc	Pb	Pb(2)	Pc	Pd	Ac	Ab
20	6	34	18	10	34	42	18
-	-	-	-	-	1.00	.05	-
-	-	-	-	-	-	-	-
-	-	-	-	.20	-	.78	1.00
-	-	-	-	-	-	.17	-
-	-	-	-	-	-	-	-
.85	1.00	1.00	1.00	.80	-	-	-
.15	-	-	-	-	-	-	-

As	Ay	Ze	Ea	Es	Eb	Ea
6	12	18	28	12	28	14
-	-	-	-	-	-	-
-	-	-	.07	-	-	-
1.00	.83	1.00	.14	.17	.14	-
-	.17	-	-	-	-	-
-	-	-	-	.09	-	-
-	-	-	.79	.74	.86	1.00
-	-	-	-	-	-	-

HK-1 Both loci n's as above.

HK-3 Both loci p100 = 1.00 throughout.

LAP-1

	Pn	Pnb	Pbr	Pm	Pj	Pv	Pr	Pr(2)
n	48	22	16	20	8	16	48	28
p95	.02	-	-	-	-	-	.02	-
100	.98	1.00	1.00	1.00	1.00	1.00	.96	1.00
104	-	-	-	-	-	-	.02	-
108	-	-	-	-	-	-	-	-

	Pr(3)	Prc	Pb	Pb(2)	Pc	Pd	Ac	Ab
n	28	6	38	20	10	38	46	22
p95	-	-	-	-	-	-	.17	.36
100	1.00	1.00	1.00	1.00	1.00	1.00	.83	.18
104	-	-	-	-	-	-	-	-
108	-	-	-	-	-	-	-	.46

	As	Ag	Ze	Ea	Es	Eb	Ec
n	6	12	20	32	12	28	18
p95	-	-	.10	.21	-	-	-
100	-	1.00	.90	.15	1.00	.36	1.00
104	-	-	-	.58	-	.64	-
108	1.00	-	-	.06	-	-	-

LAP-2
(n's as
in LAP-1)

	Pn	Pnb	Pbr	Pm	Pj	Pv	Pr	Pr(2)
p90	-	-	-	-	-	-	-	-
95	-	-	-	-	-	-	-	-
100	1.00	1.00	1.00	1.00	1.00	.25	1.00	1.00
102	-	-	-	-	-	.75	-	-
105	-	-	-	-	-	-	-	-

Pr(3)	Prc	Pb	Pb(2)	Pc	Pd	Ac	Ab
-	-	-	-	-	-	-	-
-	-	-	-	-	.24	1.00	1.00
1.00	1.00	1.00	1.00	1.00	.76	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-

As	Ag	Ze	Ea	Es	Eb	Ec
-	-	1.00	-	-	-	-
1.00	.75	-	1.00	.83	1.00	1.00
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	.25	-	-	.17	-	-

LAP-3 n's as above. All have p100 = 1.00
except Pd p95 = .08
100 = .87
108 = .05

MDH-2

	Pn	Pnb	Pbr	Pm	Pj	Pv	Pr	Pr(2)
n	68	120	16	20	6	12	84	22

Pr(3)	Prc	Pb	Pb(2)	Pc	Pd	Ac	Ab
26	6	62	20	10	32	44	20

As	Ag	Ze	Ea	Es	Eb	Ec
6	12	20	28	12	28	12

all have p 100 = 1.00

ME-1	Pn	Pnb	Pbr	Pm	Pj	Pv	Pr	Pr(2)
n	40	22	14	22	10	16	64	20
p97	-	-	-	-	-	-	-	-
98	-	-	-	-	-	-	-	-
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

	Pr3	Prc	Pb	Pb(2)	Pc	Pd	Ac	Ab
	16	6	30	16	10	22	34	14
	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	1.00	1.00
	1.00	1.00	1.00	1.00	1.00	1.00	-	-

	As	Ag	Ze	Ea	Es	Eb	Ec
	6	12	14	26	16	28	12
	-	-	-	-	-	-	1.00
	1.00	1.00	1.00	1.00	1.00	1.00	-
	-	-	-	-	-	-	-

ME-2 n's as ME-1 All have p100 = 1.00

PEP-1

	Pn	Pnb	Pbr	Pm	Pj	Pv	Pr	Pr(2)
n	48	22	16	22	10	16	66	16
p100	1.00	1.00	1.00	1.00	1.00	1.00	.97	1.00
110	-	-	-	-	-	-	-	-
114	-	-	-	-	-	-	.03	-

	Pr(3)	Prc	Pb	Pb(2)	Pc	Pd	Ac	Ab
	20	6	32	16	10	28	44	16
	1.00	1.00	1.00	1.00	1.00	1.00	-	-
	-	-	-	-	-	-	1.00	1.00
	-	-	-	-	-	-	-	-

	As	Ag	Ze	Ea	Es	Eb	Ec
	6	12	18	28	14	28	12
	-	-	-	-	-	-	-
	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	-	-	-	-	-	-	-

PEP-2 n's as PEP-1

	Pn	Pnb	Pbr	Pm	Pj	Pv	Pr	Pc(2)
p32	-	-	-	-	-	-	-	-
72	-	-	-	-	-	-	-	-
78	-	-	-	-	-	-	-	-
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
116	-	-	-	-	-	-	-	-

	Pr(3)	Prc	Pb	Pb(2)	Pc	Pd	Ac	Ab
	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	1.00	-
	-	-	-	-	-	1.00	-	1.00
	1.00	1.00	1.00	1.00	1.00	-	-	-
	-	-	-	-	-	-	-	-

As	Ag	Ze	Ea	Es	Eb	Ec
-	-	-	-	-	.14	-
1.00	1.00	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	1.00	1.00	1.00	.86	1.00

PEP-3

	Pn	Pnb	Pbr	Pm	Pj	Pv	Pr	Pr(2)
n	46	22	16	22	10	16	42	16
p39	-	-	-	-	-	-	-	-
78	-	-	-	-	-	-	-	-
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

	Pr(3)	Prc	Pb	Pb(2)	Pc	Pd	Ac	Ab
n	20	6	26	16	10	28	40	16
p39	-	-	-	-	-	-	1.00	1.00
78	-	-	-	-	-	1.00	-	-
100	1.00	1.00	1.00	1.00	1.00	-	-	-

	As	Ag	Ze	Ea	Es	Eb	Ec
n	6	12	18	28	14	28	12
p39	1.00	1.00	1.00	1.00	1.00	1.00	1.00
78	-	-	-	-	-	-	-
100	-	-	-	-	-	-	-

PM1

	Pn	Pnb	Pbr	Pm	Pj	Pv	Pr	Pr(2)
n	42	-	16	20	8	12	34	16
p80	-		-	-	-	-	-	-
90	-		-	-	-	-	-	-
100	1.00		1.00	1.00	1.00	1.00	1.00	1.00
105	-		-	-	-	-	-	-

	Pr(3)	Prc	Pb	Pb(2)	Pc	Pd	Ac	Ab
n	16	6	30	16	10	22	22	14
p80	-	-	-	-	-	.36	-	-
90	-	-	-	-	-	.64	.09	-
100	1.00	1.00	1.00	1.00	1.00	-	-	-
105	-	-	-	-	-	-	.91	1.00

	As	Ag	Ze	Ea	Es	Eb	Ec
n	6	12	14	26	14	24	10
p80	-	-	-	-	-	-	-
90	-	-	-	.08	.28	.16	.20
100	-	-	1.00	.08	-	.08	-
105	1.00	1.00	-	.84	.72	.76	.80

6PGDH

	Pn	Pnb	Pbr	Pm	Pj	Pv	Pr	Pr(2)
n	48	22	16	22	10	16	66	16
p48	-	-	-	-	-	-	-	-
68	-	-	-	-	-	-	-	-
75	-	-	-	-	-	-	-	-
82	-	-	-	-	1.00	-	-	-
100	1.00	1.00	1.00	1.00	-	1.00	1.00	1.00
110	-	-	-	-	-	-	-	-
112	-	-	-	-	-	-	-	-

	Pr(3)	Prc	Pb	Pb(2)	Pc	Pd	Ac	Ab
n	20	6	32	16	10	28	44	16
p48	-	-	-	-	-	-	-	-
68	-	-	-	-	-	-	-	-
75	-	-	-	-	-	-	-	-
82	-	-	-	-	-	-	-	-
100	1.00	1.00	1.00	1.00	1.00	1.00	-	-
110	-	-	-	-	-	-	1.00	1.00
112	-	-	-	-	-	-	-	-

	As	Ag	Ze	Ea	Es	Eb	Ec
n	6	12	18	28	14	28	12
p48	-	1.00	-	-	-	-	-
68	-	-	-	-	-	1.00	1.00
75	-	-	-	-	.79	-	-
82	-	-	-	-	-	-	-
100	1.00	-	-	1.00	.21	-	-
110	-	-	.78	-	-	-	-
112	-	-	.22	-	-	-	-

App. 13.

The Genetics of EST-1 in A.napi

Allelic variation at EST-1 was confirmed by rearing families of A.napi. Pairings were achieved by mating virgin females reared from larvae; the male was killed after successful copulation, as was the female once eggs had been laid. Both adults were then subjected to electrophoretic analysis as were offspring on their emergence. The results of these experiments are given below.

Cross 1

Parental phenotypes	100/100 (♀)		100/104 (♂)
		x	
Fi phenotypes	100/100		100/104
Expected numbers	23.25		7.75
Observed numbers	25		6

Cross 2

Parental phenotypes	100/100 (♀)		100/104 (♂)
		x	
Fi phenotypes	100/100		100/104
Expected numbers	16.5		5.5
Observed numbers	18		4

Cross 3

Parental phenotypes	94/94 (♀)		100/94 (♂)
		x	
Fi phenotypes	94/94		100/94
Expected numbers	17.5		17.5
Observed numbers	20		15

Cross 4

Parental phenotypes	100/100 (♀)		100/100 (♂)
		x	
Fi phenotypes	100/100		
Expected Observed	28		

All 4 crosses were between animals homozygous at EST-2 and GOT-1, as were all offspring.

Population samples were in agreement with the predictions of Hardy-Weinberg distributions, with the exception of samples from Wolsingham in 1978 (1st brood) and 1979 (1st brood), at Hartside in 1977 and Durham in 1979 (1st brood). In all these cases heterozygotes were less abundant (n.s.) than predicted. This may have been the result of mixing within a sample animals derived from different populations (The Wahlund effect).

App. 14

Systematics and Classification of Pierinae

The White and Orange-Tip butterflies are a group which has been studied repeatedly by taxonomists and is the cause of much controversy yet. Some authors regard the two basic subdivisions, discussed in this thesis as the tribes Pierini and **Euch**loini, as worthy of sub-Familial rank, equal to the status of Rhodocerinae ('Yellows'), for instance Klots (1931) and Higgins (1975). However, the two groups are clearly closely related in some taxa (such as Pontia and Euchloe), as noted by Verity (1947), and intermediates occur (such as Colotis). It is preferable to retain the two tribes within a single Sub-Family, as in Ehrlich and Ehrlich (1961).

It is within tribal groupings, however, that most controversy exists. In this section I shall briefly discuss the current status of each taxon used in the text.

Within Pierini, there is great disparity in generic usage, Most American authors persist in using Pieris for a wide range of species including rapae, napi, virginiensis, beckerii (= chloridice), sisymbrium and protodice (= callidice). Most other authors have split this heterogeneous group into several genera. Thus Talbot (1932), Dixey (1932) and Mariani (1937) split the genus Pieris into 4, 2 and 3 genera respectively. Klots (1931) and Verity (1947) retained these subsidiary groups as subgenera, whilst Bernardi (1947) recognised some as genera (e.g. Pontia) but retained others within *Pieris*, although recognising that this genus remained heterogeneous. Most authors (e.g. Higgins and Riley, 1970) currently recognise three genera as follows:

Pieris	*Pontia*	*Synchloe*
brassicae	daplidice	callidice
rapae	chloridice	
mannii		
napi		
ergane		
virginiensis		
melete		
krueperi		
canidia		

However recently many authors have felt it desirable to recognise the difference between *P.brassicae* and other species members formerly in *Pieris*. Kudrna (1974) summarised the grounds for doing so, and emphasised the distinctness of *P.brassicae* from most related species, in androconial shape, male genitalia, chromosone number and other characters. He proposed the genus name *Artogeia* for other members of *Pieris*, in accordance with Verity's (1947) subgenera, and in agreement with that author's and with Klot's (1931) retention of *brassicae* as the type of *Pieris*. *Pieris* is accordingly now restricted to *brassicae*, *brassicoides* and *cheiranthi*, with *napi* as the designated type of *Artogeia*. This usage, favoured by Higgins (1975) and others, is retained throughout this thesis.

Within the genus Pieris, in the restricted sense currently accepted, there is an interesting example of speciation via geographical isolation. Upon the Canary Islands has evolved a distinctive isolate of P.brassicae - P.cheiranthi, which is unusual in feeding upon Tropaeoleum, as opposed to Cruciferae, and which is distinct from P.brassicae in androconial and genitalic shape, as well as by heavily increased pigmentation. On the Azores and Madeiran Islands occur superficially similar forms (azorensis and wollastoni) which are, however, little different from P.brassicae brassicae. Kudrna (1973) felt that all the differences of P.cheiranthi were sufficient to raise it to specific status, a procedure which has been followed here, but which has not yet found general approval. Certainly P.cheiranthi is very close to P.brassicae and might best be regarded as a sibling species.

Within Artogeia, several groupings of species are apparent. One, including A.rapae, A.mannii and A.canidia, are clearly related, although superficial resemblances may be deceptive. A.ergane is very alike in appearance to A.rapae, but is in fact derived from an A.napi like ancestor. In A.ergane, n = 26, as in many A.napi populations; A.ergane and A.napi also show many similarities of genitalia, and androconial structure and scent (Lorkovic, 1968, 1974). A.ergane is also very unusual in being monophagous as a larva, feeding only upon Aethionema in montane areas.

Its larvae are likewise distinct in being blue, as opposed to the typical apple-green of other Artogeia.

Other species closely allied to A.napi include A.virginiensis, a univoltine species monophagous upon Dentaria spp., which flies sympatrically with Nearctic A.napi and may form inter-species pairings (Chew, pers. comm.). Closely allied also is A.melete of Japan, which however have sterile Fi crosses to napi in the females (Bowden, 1975), with much chronosomal disturbance (Lorkovic, 1974). Extremely similar in appearance to A.melete is A.japonica, originally recognised as distinct from A.melete by Shirozu (1952). Until recently A.japonica has been regarded as a subspecies of A.napi, but Bowden has demonstrated that it is not in fact conspecific with A.n.napi, but will interbreed with A.n.nesis, also from Japan. He therefore raised A.japonica to specific rank, to include A.j.nesis. However, A.japonica may yet prove to be conspecific with another Far-Eastern taxon, A.n.dulcinea, which has priority (Bowden, pers. comm).

The diffentiation of other taxa within A.napi, particularly A.bryoniae, A.n.bicolorata and A.n.thomsoni are discussed in the text.

Within Euchloini, there has similarly been controversy over the specific status of some taxa, notably the several Nearctic members of the Euchloe ausonia complex (Opler, 1966). Klots (1930) completed a generic revision of the Tribe, and recognised the distinctness of Zegris, particularly its habit of pupating in a rudimentary cocoon. However he recognised its similarity to some *Euchloe*, particularly *E.olympia*. Dixey (1932) went further and placed *olympia* within *Zegris*. Whether *olympia* is in fact near to *Zegris* is debatable, since essentially superficial characters were used. Unfortunately, although material of *E.olympia* was obtained, it arrived too late for electrophoretic analysis which may have illuminated this point.

For a long period many authors used the generic name *Euchloe* indiscriminately to cover all species now within *Anthocharis* and *Euchloe*; Klots (1930) gives details. It is undoubtedly correct to regard them as separate genera as confirmed by the distant chromosomal identity inferred from the results of Lorkovic (1974). Within *Anthocharis*, Klots recognised two subgenera *Anthocharis* (to include *cardamines*, *sara*, *belia*, *cethura*, etc.) and *Falcapica* (to include *genutia*, *bieti*, *lanceolata* and *scolymus*).

Within *Euchloe*, Klots recognised two subgenera, *Euchloe* and *Elphinstonia*. Most species he referred to the former, to the latter only *tagis* and *charlonia*. Bernardi (1945) however removed *E.falloui* from *Euchloe* to *Elphinstonia* on the grounds of male genitalia similarities. These results are surprising in view of the close similarity between *E.ausonia* (subgenus *Euchloe*) and *E.tagis* and between *E.belemia* (also *Euchloe*) and *E.falloui*. It may be that genitalic characters are giving spurious groupings within *Euchloe*. Higgins and Riley (1970) retain

E.tagis (and the sibling species E.pechi) within the genus Euchloe, but regard E.charlonia as sufficiently distinct to warrant generic status. This procedure is followed here.

It is not a safe procedure to place over much confidence in electrophoretic results for taxonomic work. Although in general agreement with conventional taxonomy is good, exceptions occur. A particular drawback is that the characters provided by enzyme phenotypes would be ideal for cladistic approaches, except for the total lack of knowledge as to ancestral types. Thus only distantly related animals might be viewed as closely similar due to both possessing ancestral isozyme patterns. Nevertheless the results of Chapter 4 do bear close resemblence to the relationships outlined above. Pierini and Euchloini are readily distinct from one another, although some similarities do exist, notably between Euchloe spp. and P.daplidice, especially at G6PDH. This similarity could represent retention of ancestral types or convergence or both, or it could indicate that Pontia and Euchloe and perhaps closer than currently acknowledged - as both Verity (1947) and Higgins (1975) have felt.

Tab. 3 of Chapter 4 places P.brassicae closer to A.napi than that taxon is to A.rapae. This is undoubtedly partly an aberrant result - some of the discarded loci (e.g. SOD) linked the Artogeia species together. Nevertheless the small degree of difference between subtribal groups (Pieris V Artogeia spp; Euchloe spp. V. Elphinstonia) when compared with congeneric species, (D is *less* for subtribal groups. D = 0.120 as opposed to 0.206) may indicate that one if not both of these groups is over-split.

The relative distinctness of *E.ausonides* from *E.ausonia* may or may not indicate that the two taxa are specifically distinct (see discussion of Chapter 4 for the merits of using electrophoretic methods to assess specific status). This must await further study, particularly breeding experiments.

Finally, one suggestion that has been made repeatedly is that Pierini and perhaps Euchloini as well are involved in Mullerian mimetic complexes, and have evolved superficial resemblances for this purpose. This suggestion would certainly go a long way towards explaining the surprising convergence of some disparate taxa such as *A.rapae* and *A.ergane*, and explaining sexual dimorphism in some *Anthocharis* (Chapter 3)

Early work on the evolution of mimicry proved that birds attacked some betterflies, and that butterflies varied in their acceptability to such predators (e.g. Morton Jones, 1934; Carpenter, 1937). Marshall and Poulton (1902) recorded some African Pierinae as a posematic, and suggested that Holarctic representatives might equally be distasteful. This suggestion has been slow of acceptance. Colthrup (1913) for instance recorded Sparrows 'toying' with *A.rapae* (i.e. tasting and rejecting the butterflies) for 'sheer devilment', but felt "I should be very sorry to attribute the rejecting of A.rapae to distastefulness".

Shapiro (1974) discussed the incidence of beakmarks on some common butterflies and recorded a very high incidence of such marks in U.S.A. A.rapae populations. In the present study, some beakmarked individuals were found, including one A.rapae at Witton Park in 1978 which had evidently been 'tasted' and lost or released (the thorax of the insect was pierced). Other authors such as Marsh and Rothschild (1974), Aplin et al (1975) and Pettit et al (1977), have shown that Pieris and Artogeia may have biologically active chemicals, possibly sequestered from larval host plants, which may act as deterrents, but experimental proof has been lacking until the recent elegant work of Pough and Brower (1977) on the related Ascia monuste.

Some preliminary results were obtained in the present study using captive predators. It was found that hungry Dunnocks (Prunella modularis) and some starlings (Sturnus vulgaris) would take individuals of any Pierinae species offered (specimens from the deep freeze), although other starlings would greet P.brassicae especially with alarm calls. A few experiments were carried out using the methods of Pough and Brewer, withJackdaws (Corvus monedula) as the captive predator. These experiments involved offering the predator a choice between the hypothesised distasteful species and a known palatable butterfly species, after allowing the bird to become familiar with each butterfly type. By offering birds a choice, one overcomes problems caused by individual personality (as in starlings) and physiological states (e.g. hunger differences). If palatable prey are uneaten, then clearly the predator is "off-colour". The critical experimental measure is the ratio of unpalatable to palatable specimens taken.

Two Jackdaws were successfully trained in captivity. One was allowed to experience P.brassicae and Maniola jurtina (presumed palatable), the other experienced A.rapae and M.jurtina before commencing the experiment.

	Bird 1	Bird 2
No. M.jurtina offered	17	18
No. attacked	14	15
No. eaten	14	15
No. P.brassicae offered	-	18
No. attacked	-	10
No. eaten	-	0
No. A.rapae offered	17	-
No. attacked	14	-
No. eaten	12	

Bird 1 subsequently attacked but refused to eat 2 P.brassicae. Bird 2 attacked and ate 2 A.rapae and a female A.cardamines. From these results it is apparent that these Jackdaws did not find A.rapae distasteful. Nor did Bird 2 show significant evidence of learning within the short confines of this experiment, but continued to attack most white butterflies presented, despite the evident distastefulness of *P.brassicae*.

Clearly the hypothesis of Batesian or quasi-Batesian mimicry of *P.brassicae* by *A.rapae*, *A.napi* and *A.cardamines* is far from corroborated. Nevertheless the hypothesis does offer one explanation of the surprising superficial resemblence of *Artogeia*, *Pieris* and *Euchloe*, if white colouration is a signal to birds of distastefulness. That the white colouration of Pierini might be confusing as to identity is established - the bird-orientated Ernst Mayr felt (1942) that the 3 common species of *Pieris* were familiar examples of sibling-species - which they most certainly are not.

Appendix 15

Testability and Evolutionary Theory

In Chapter 5, Slobodkin and Rapoport's (1974) criticism of the 'adaptive' viewpoint in speciation studies was noted. A number of other authors have recently felt compelled to challenge the accepted methods of evolutionary science, as being tautologous, historical or leading to untestable hypotheses. Platnick (1978) for example, criticised the a posteriori methods of phylogenetic reconstruction as 'historical narratives', whilst Gould (1978) for similar reasons attacks sociobiologists for telling 'Just So Stories'. Peters (1976, 1978) used somewhat similar arguments to attack most current ecological theory as tautologous. These charges have prompted a number of defences of evolutionary theory in systematics (Downing, 1978) and ecology (Stebbins, 1977; Caplan, 1977; Castrodeza, 1977; Hairston, 1979). However with most authors addressing different problems confusion is now typical.

In this brief discussion I shall attempt to isolate those issues concerning natural selection and the methods of evolutionists which are major problems, by reference to the most influential (Popper) and effective (Lewontin) critics and defenders (Maynard Smith).

> "I have come to the conclusion that Darwinism is not a testable scientific theory, but a metaphysical research programme ....
> ...... Adaptation or fitness is <u>defined</u> by modern evolutionists as survival value, and can be measured by actual success in survival: there is hardly any possibility of testing a theory as feeble as this."
>
> K. Popper. 'Unended Quest', 1976.

These quotes are often used to suggest that Popper, the foremost advocate of deductive scientific method, regards evolutionary biology as unscientific. However, as shown below, he has in fact made progress to solving some of these problems, but is particularly hampered by his lack of familiarity with modern genetics (as shown by Ruse, 1977). Evolutionary Science to Popper is the 'New Synthesis' of Huxley (1942) (p. 170, 'Unended Quest').

By contrast, a prominent population geneticist, Lewontin, has also launched a number of attacks on evolutionary methods in some fields (Lewontin, 1977, 1977, 1978). His criticisms are particularly levelled at evolutionary ecologists who participate in the 'adaptationist programme', which Lewontin regards as intending to prove adaptation by repetitive example (as in the earlier field of ecological genetics).

The essentials of these criticisms are then that adaptation and natural selection are tautologous statements* and that the underlying principles and particular hypotheses of evolutionary studies are untestable. The problems of historical science, which Popper opposes, are not fundamentally at issue here, and have been discussed satisfactorily elsewhere (Olding, 1978), and I agree with Downing:

> "Discovery of the historical details of past selective pressures, though perhaps difficult or impossible in a given case, is both possible in principle and a logically separate problem anyway."

It is also important at this point to avoid the confusion caused by the Spencerian term 'survival of the fittest', which is undoubtedly a tautology. Although the term is not favoured by current evolutionists, some critics (e.g. Brady, 1979) have used it as a straw man to attack. In actuality, the fact that some _a posteriori_ arguments have been used in evolutionary studies does not mean this is always so.

Maynard Smith (1978) in his clearly stated defence of optimality arguments surrounding adaptation recognised that three classes of assumption were routinely made , concerning the heritability of, and constraints upon characters under consideration, together with their

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*Formally, tautologous statements are those from which no other statement is deducible. However, in common usage, tautology means 'saying the same thing twice', and it is in this sense that it has been most frequently used in relation to natural selection.

relationship to fitness.* The essential point of Maynard Smith's defence is that it is these ceteris paribus assumptions which are at test in optimality arguments rather than the general theorem of natural selection. Falsifying any particular adaptationist argument does not falsify natural selection itself. Similarly evolutionists routinely distinguish other evolutionary pathways than natural selection, such as preadaptation (as in the use of only young Alliaria petiolata inflorescences by A.cardamines), mutation, etc. There is no 'adaptationist programme' aimed at proving the generality of adaptation.

As shown by Brady (1979) the current debate has polarised opponents over two quite separate issues:

> "Critics have worried about the formulation of tests, defenders, about the formulation of the theory."
>
> Brady, 1979.

Considering first the theory of natural selection, Brady asked when, if ceteris paribus are usually tested, is the principle of natural selection itself under test? I contend that in fact it is never tested, and is in

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*Lewontin (1977) and Brandon (1978) make similar points.

fact untestable.[*1] As noted by Brady, a favourite ad hoc defence to the charge of tautology is to retort that mathematics is also tautologous. He quoted Haldane (1935) as stating:

> "The phrase 'survival of the fittest' is something of a tautology. So are most mathematical theories. There is no harm in saying the same truth in two ways."
>
> J. B. S. Haldane, 'Darwinism under Revision', 1935.

Brady suggests that Haldane here missed the important distinction between deductive (e.g. mathematical) truths and those aimed at causal explanation. In fact the phrase 'survival of the fittest' is at fault, since it implies such a causal approach. Haldane was, however, undoubtedly using it as a form of shorthand for a more precise genetic argument[*2] and was making the simple point that, at the genetic level, natural selection does approach mathematics (or better, arithmetics). Given two types of replicator, replicating at different rates, then at any point in the future the type which replicates more often will be more numerically abundant. This statement is not testable in

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[*1] Untestable, that is beyond the suggestion that environmental influences can affect gene frequencies.

[*2] This perhaps regrettable approach may have been fostered by the need for evolutionists at this time to emphasise the unity of evolutionary science, and to reconcile genetics with the natural selection of the naturalists.

the normal sense of the word, and no other statements are deducible from it (i.e. it is a formal tautology). Moreover it is not restricted to biological replicators (genes, chromosomes, genomes) but to all replicators in _all_ circumstances. Popper (1976) himself recognised this:

> "Darwinism becomes not merely applicable, but almost logically necessary in situations of self-reproducing variable bodies."

Natural selection then is not a testable theory*, but rather has at its base an arithmetic rule, which leaves the empirical description of factors affecting rate of replication to the observer.

As Brady points out, the most serious criticisms of evolutionary thought, although framed as attacks on theory, are really critical of methods - Maynard Smith showed that this typically meant the quality of _ceteris paribus_ assumptions. On this point Lewontin (1977) singled out the problem of assumptions regarding fitness - related characters, and showed that optimality studies often made great assumptions here. However, this is not always the case. In Section 2 ix is shown an optimality model concerning oviposition behaviour in _A.cardamines_

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*This view is not shared by some, e.g. Maynard Smith (1978).

which is constructed directly in terms of offspring number.

More importantly, Lewontin points out the tendency to explain away unpredicted results with *ad hoc* arguments. Wasserman (1978) and Gould (1978) make similar points, whilst Wilbur, in criticising the field of behavioural ecology, as exemplified by Krebs and Davies (1978) said:

> "The *modus operandi* of the behavioural ecologist, like that of the ethologists before, seems all too often to be - make field observations first, compare populations or species, reflect for a time and then construct a plausible explanation for the observations that is consistent with the latest evolutionary theory."
>
> Wilbur, 1979.

Certain procedures may alleviate these problems. Several authors have felt that such problems arise from an insufficient base of knowledge of the particular species before hypotheses and models are constructed (e.g. Lewontin, 1977). Similarly, Dayton (1973) discussing the disadvantages of simple models in ecology, felt that the only way to guard against making the 'right prediction for the wrong reasons' is to 'obtain a thorough understanding of the relevant natural history.' Again, Stanton (1979) has made the valid point that studies of foodplant exploitation in Pieridae usually ignore the limits to discrimination by ovipositing females. (In

Chapter 2 it was shown that such physiological limits may be very important in determining oviposition by A.cardamines).

These observations therefore suggest that a Baconian collation of data is necessary in evolutionary studies, where several plausible mechanisms for evolution, and typically several adaptive explanations are possible. Baconian methods are also implicit in the testing of such hypotheses. The concept of 'evolutionary stable strategy' (E.S.S.) makes this particularly clear; for a strategy to be considered stable, alternative strategies must be specified. Implicit in this statement then is the need for the method of 'strong inference' (Platt, 1964) and the setting up of many opposing hypotheses, as has typically been attempted throughout this thesis. This appears to be the consensus position also:

> "It is becoming the received view in the philosophy of science that hypotheses are not evaluated in isolation, but rather in comparison with rival hypotheses."
>
> Brandon, 1978.

Ecologists such as Holling (1965) and Wilson (1975) have also recognised the need for the method of strong inference to distinguish between hypotheses, and Holling in particular has emphasised that the only rigorous test of a hypothesis is one which distinguishes between it and its rivals.

Finally, it is worth emphasising that of all evolutionary arguments, those concerning strategies are most open to abuse and, used *a posteriori*, may even be used to explain away results entirely contrary to those predicted (e.g. Rausher, 1979). Given the fact that arguments concerning strategies also often unwittingly use group-selection scenarios (e.g. Levins, 1968) and the notion that oligophagy in *A.cardamines* is a result of foodplant unpredictability) it is apparent that such hypotheses are best considered in the light of competing hypotheses.

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