STUDIES ON THE BIOLOGY AND COLOUR POLYMORPHISM OF TWO SIBLING SPECIES OF MARINE GASTROPOD (LITTORINA)

Thesis submitted in accordance with
the requirements of the University of
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"Shortly before sunset on a clear evening in summer, when foliage is fresh and full, the leaves of an oak are seen from certain directions to be aflame with orange light: the familiar tree becomes transmuted into a veritable burning bush - though only the elect have eyes to see it so. This reality may readily be proved by holding a flame-coloured nasturtium petal between the tree and the eye and comparing the tints thus brought together. But most of us make a mental reservation, based upon long years of experience, and we reject the evidence of the senses. Leaves, we know, are green in summer, not golden; and unless we are specially observant or specially trained, we refuse to accept the facts of colour as they are - we refuse to see that the distant firs are pale grey, or that the snow on the hills is blue where it lies in shade and warmly tinted where bathed in sunlight, that indeed it may be almost any tint except the white which we think it is. Experience has taken much of the colour and variety out of life, and we go on our way almost unconscious of the real appearance of the visual signs we have learned from earliest childhood to construe." (from Cott, 1940 - Adaptive Colouration in Animals, p. 2)

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ABSTRACT

An examination is made of the selective agents responsible for variation in shell morphology and shell colour in two sibling species of intertidal gastropod, *Littorina obtusata* and *L. mariae*. The species status of these two siblings is confirmed using the morphology of the shell, reproductive organs, and radula, as well as proteins, shell colour and intertidal zonation. One of the major differences between the species is in shell size, with *L. mariae* usually being much smaller than *L. obtusata* except on very exposed shores where the two siblings are similar in size.

Evidence from shell injuries in field collections in addition to predation experiments leads to the conclusion that many of these differences in shell size within and between *L. obtusata* and *L. mariae* can be attributed to the effects of predation by crabs, especially *Carcinus maenas*. An important component to this type of predation is that the selective pressures exerted by juvenile crabs as opposed to adults favours different strategies in shell size of the snails.

From an examination of stomach contents in some intertidal species of fish, it has been found that one species, *Blennius pholis*, is an important predator on *L. obtusata* and *L. mariae*. Only juvenile individuals of the two species have been found in the stomachs.

The colour polymorphism in the shells, involving four principle morphs - citrina, olivacea, light reticulata, and dark reticulata, occurs in both species with different proportions, olivacea being the most common in *L. obtusata* but the rarest in *L. mariae*. A small number of breeding experiments show that the polymorphism is genetically controlled; for the two common phenotypes in *L. mariae*, the data show dark reticulata to be completely dominant to citrina.

There are predictable changes in phenotype frequencies with exposure throughout the British Isles, the two species converging in frequencies on exposed shores and diverging in sheltered shores. It is shown that these changes reflect similar changes in the background on which each phenotype is cryptic. In *L. obtusata* the frequencies correlate with the species of alga on which it is found, while in *L. mariae*, the frequencies are correlated with the microdistribution of juvenile individuals within plants of the same species.

Underwater observations demonstrate that even the apparently conspicuous citrina are remarkably cryptic on the plants when the illumination is by light transmitted through the plant fronds. Examination of the colour of juvenile shells, which often differs from that of the adults, shows similar detailed crypsis, including a mimetic relationship between juvenile citrina and the white tubes of a polychaete *Spirorbis* sp. Predation experiments with *Blennius pholis* in the laboratory support strongly the suggestion that these cryptic relationships are the result of visual predation. It has been further possible to relate in both species differences in colour between the juvenile and adults and the developmental variability between shores to subtle differences in the background colour of the plants. The maintenance of this colour polymorphism is discussed with special reference to a multi-niche model.

The large amount of local variation within shores seen in *L. mariae*, involving differences in shell size and colour as well as other characters, is discussed in reference to possible

taxonomic subdivisions within this species. It is shown that this variation is adaptive owing to very localized differences in the habitat. No conclusions could be reached on the taxonomic status of the "dwarf" form within this species.

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SECTION I

GENERAL INTRODUCTION

Phenotypic variation within and between populations is widespread in many plants and animals. Many evolutionary interpretations of this variation have been made involving both selective and non-selective processes. One type of variation which lends itself to study is that of genetic polymorphism. This has been defined by Ford (1940) as "the occurrence together in the same locality of two or more discontinuous forms of a species in such proportions that the rarest of them cannot be maintained merely by recurrent mutation". In many species, the morphs are both numerous, strikingly different from one another, and highly variable in frequencies between localities.

Work by Cain and Sheppard (1950, 1954) and Sheppard,(1951) on the land snail Cepaea has provided a great deal of insight into variation within and between localities. These studies have demonstrated the role of visual selection acting on shell colour and banding, the frequencies of the different morphs often being correlated with the habitat on which they are most cryptic. In addition to selection for crypsis, there is some suggestion that apostatic selection (Clarke, 1962), as well as climatic selection (Cain and Currey, 1963; Arnold, 1968; Jones, 1973) can also influence the morph frequencies.

The intertidal prosobranch *L. obtusata* has long been known as exhibiting a similar variety of colour morphs to *Cepaea*, the proportions of which often change markedly from locality to locality apparently without relation to the habitat. This species is abundant and lives on intertidal algae throughout the North Atlantic coasts; it is very sedentary, rarely moving between plants, it has no pelagic larval phase, and lives for up to at least 2 years. In addition, the sexes are separate, unlike the hermaphrodites found in the pulmonates.

Recent work has shown that what was once thought to be the single species *L. obtusata*, is in fact 2 siblings *L. obtusata* and *L. mariae*, the two occurring on different levels on the shore but with a zone of overlap (Sacchi and Rastelli, 1966). I chose these two siblings for the present work since they were eminently suitable for a study parallel to that of *Cepaea nemoralis* and *Cepaea hortensis*. The main purpose was to determine primarily the reasons for the geographical variation in morph frequencies, and secondarily to gain some understanding of the causes for intrapopulation variation.

In this thesis, I shall firstly describe the survey that I carried out in order to assert whether the two siblings, *L. obtusata* and *L. mariae*, described by Sacchi and Rastelli in French localities, were also present in the British Isles. These sections also describe additional characters which can be used to separate the two species in females and juveniles. Following this, a description is given of the influence by some predators on the two species in relation to shell morphology and colour. In subsequent sections, I shall describe the colour polymorphism in its various aspects, that is to say, the genetics of the morphs, their distribution both on a geographical and microhabitat level, and their ontogeny. The complex nature of the changes in morph frequencies within and between these two species, which became apparent during this study, have enabled a fairly comprehensive understanding of several aspects of adaptation in some populations even to the fine structure of the environment.

As a result of my three years work, the suspicion began to arise that there may be two taxonomic sub-divisions within one of the siblings _L. mariae_. This matter is considered in the final results section and it is concluded that, although there is considerable variation within _L. mariae_, this variation can probably be entirely attributed to microhabitat differences which have been discussed throughout the body of this thesis. There follows a general discussion and synthesis.

SECTION II

GENERAL MATERIALS AND METHODS

1. Study Areas

The collections in this study of the flat winkle were obtained from the coasts of Ireland, Wales, and Southern England between 1970 and 1973 (fig 1). The sites were chosen so as to sample a wide range of shore exposures which would include ecologically diverse habitats.

The first collections were made in Anglesey, North Wales (fig 2), where, over short distances, large changes in exposure to wave action occur. From an examination of the samples from Anglesey, it became apparent that there were predictable changes in the winkle on shores of different exposure; a parallel set of sampling sites was required to determine whether the trends observed were applicable in other places.

The second area of survey was the Isles of Scilly which lie off the south-western coast of Cornwall (fig 3). These were chosen because an earlier trip had shown that the winkles were very different in shell colouration from those observed in Anglesey; these differences may be correlated with the extreme exposure present on these islands. The large islands are distributed as an atoll, each displaying a coastline beaten by the full swell of the Atlantic and an inner sheltered coastline protected from all but the more violent storms. Collections on each of the large islands were made to sample both types of shorelines. A more intensive sampling effort was made on White Island off St Martins, which provided a wide range of exposures over very short distances. On Bryher, one of the western islands, two collections were made at closely-spaced localities to provide an additional comparison of the effect of extremes in exposure on the flat winkles. Many of the Scillonian sites were resampled a year later to supplement small numbers and to determine the extent of stability in the populations from 1971 to 1972.

In July 1971, a small number of collections were made on the coasts of Wales, Somerset, Devon, and Cornwall to determine whether clinal variation would connect the very different winkles of Anglesey to those of the Isles of Scilly. The second purpose for the collections was to compare the flat winkle from the Bristol Channel with those from the Menai Straits in Anglesey. Both of these areas have strong currents and in the Menai Straits one of the colour morphs of L. obtusata had its highest frequency in the locality with the greatest current.

In August 1972, 19 collections were made in the Republic of Ireland predominantly on the western coast (fig 1). The choice of localities was again to provide collections of similar distance from one another and at the same time to provide as diverse a range of ecological conditions as possible. In the north-western part in County Sligo, 3 samples were obtained within 4 km of each other which spanned the complete range of exposure conditions as seen on the Isles of Scilly. This provided another comparison with Anglesey and the Isles of Scilly for observing the change in the winkles over short distances.

Three collections were obtained from a two day trip to Scotland in September 1972. On-shore winds at each locality resulted in very small samples being obtained as the intertidal region remained covered with water.

Figure 1

Figure 1

Localities sampled around the British Isles

1-27: Anglesey (see fig 2)
28: Llwyngwril
29 - Llanrhystyd
30 - Newport
31 - Dale Fort
32 - Dale Bay
33 - Port Eynon
34 - Barry
35 - Seawall
36 - Severn Bridge
37 - Weston-super-Mare
38 - Lynmouth
39 - Buck Mills
40 - Rock
41 - Looe
42 - Wembury
43a - Dartmouth Harbour
43b - Bodinnick Harbour
44 - Penzance Harbour
45-72 - Isles of Scilly (see fig 3)
73 - Dunfanaghy
74 - Curran's Fort
75 - Milkhaven
76 - Streedagh House Bay
77 - Staad Bay
78 - Barnatra
79 - Tiraun Point
80 - Leckanvy
81 - Little Killary Bay
83 - Black Head
84 - Liscannor
85 - Shot Head
86 - Adrigole Harbour
87 - Ballycotton
88 - Ballynagaul Bay
89 - Ballynagaul Point
90 - Bray Head
91 - Portrane
92 - Sea Scale
93 - Loch Awun
94 - Ballyluchan

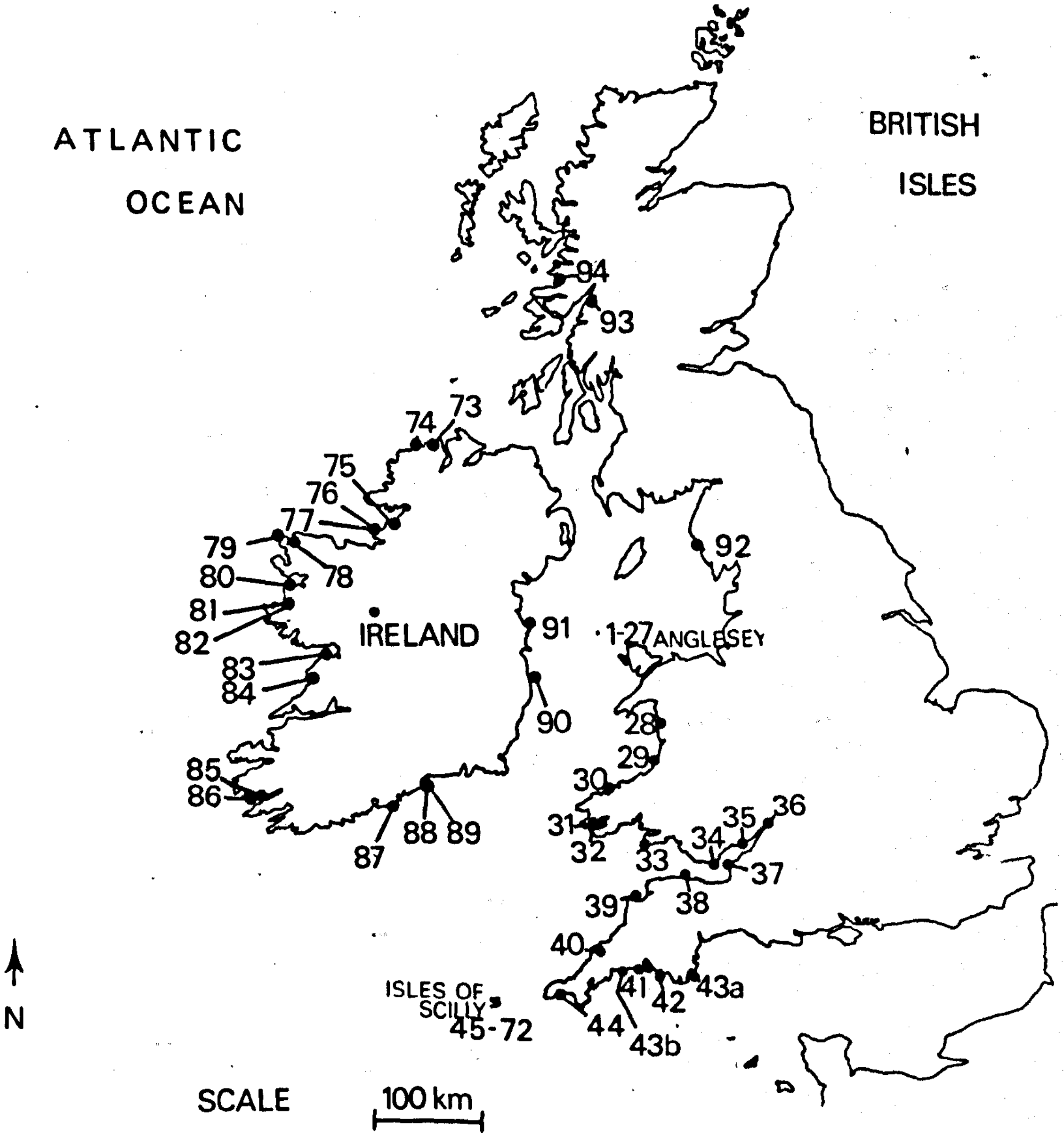
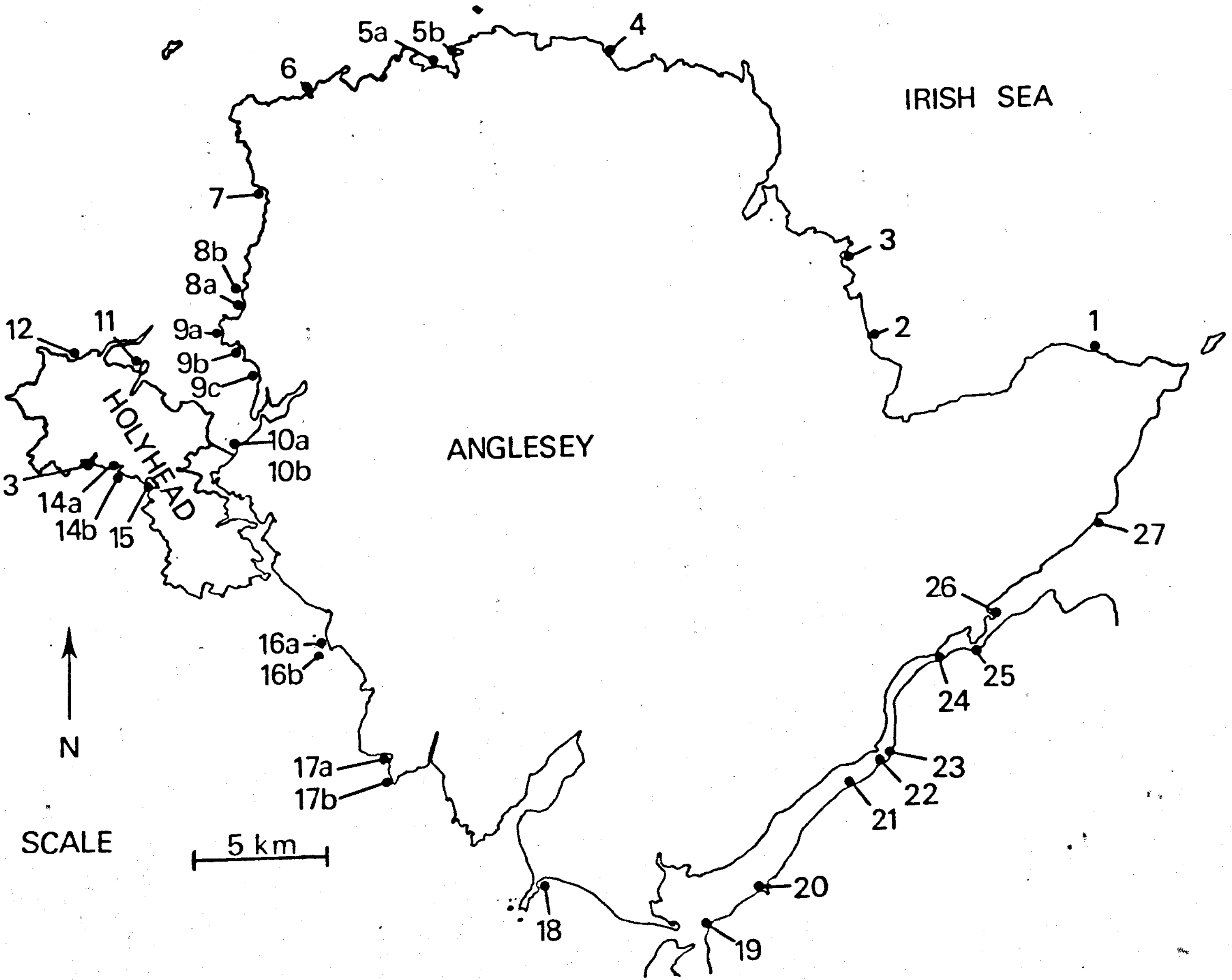

ATLANTIC
OCEAN
BRITISH
ISLES
94
93
73
74
75
76
77
79
78
80
81
82
IRELAND
91
·1-27
ANGLESEY
92
83
84
90
28
29
85
86
30
88
89
87
31
32
33
34
35
36
37
38
39
40
ISLES OF
SCILLY
45-72
41
42
43a
44
43b
N
SCALE
100 km

Figure 2

Figure 2

Localities sampled around Anglesey, North Wales

1 - Fedwfawr
2 - Benllech
3 - Moelfre
4 - Bull Bay
5a - Cemaes Bay
5b - Cemaes Head
6 - Hen Borth
7 - Porth Swtan
8a - Sandy Bay
8b - Sandy Point
9a - Penrhyn Point
9b - Penrhyn-mawr
9c - Penrhyn Bay
10a - Causeway
10b - Four Mile Bridge
11 - Holyhead Bay
12 - North Stack
13 - Porthdafarch
14a - Cliff Hotel Bay
14b - Cliff Hotel Point
15 - Trearddur Bay
16a - Rhosneigr Bay
16b - Rhosneigr Point
17a - Cwyfan Bay
17b - Cwyfan Point
18 - Llanddwyn
19 - Ysgubor Isaf
20 - Caernarvon
21 - Llanfair Hall
22 - Port Dinorwic Point
23 - Port Dinorwic Bay
24 - Train Bridge
25 - Menai Bridge
26 - Navy Station
27 - Beaumaris



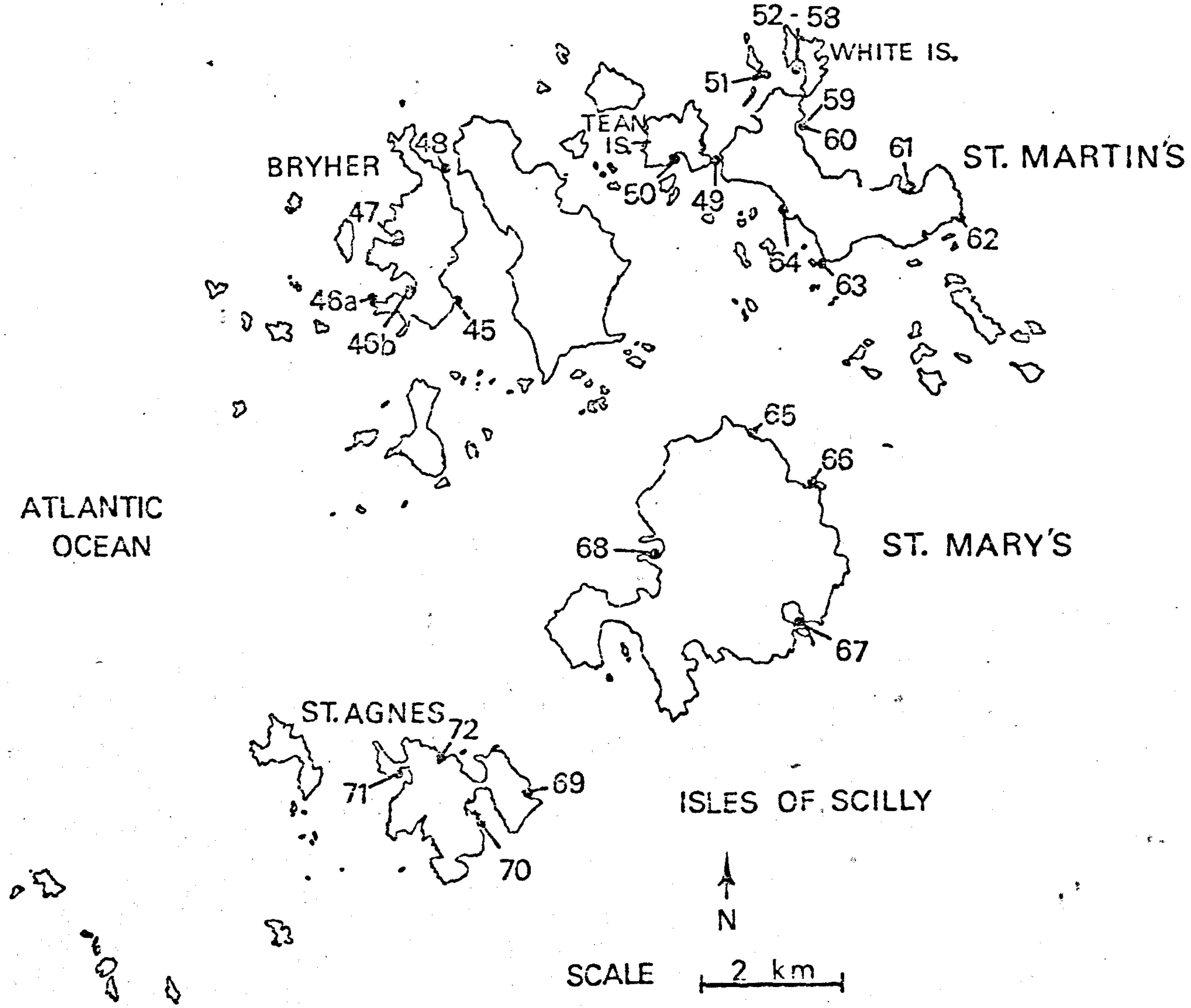
IRISH SEA
ANGLESEY
HOLYHEAD
1
2
3
4
5a
5b
6
7
8a
8b
9a
9b
9c
10a
10b
11
12
13
14a
14b
15
16a
16b
17a
17b
18
19
20
21
22
23
24
25
26
27
N
SCALE
5 km

Figure 3

Figure 3

Localities sampled around the Isles of Scilly

45 - Brow
46a - Heathy Point
46b - Heathy Bay
47 - Popplestone
48 - Hangmar
49 - Tean Sound
50 - Cow House
51 - Pernagie
52 - White No. 1
53 - White No, 2
54 - White No. 3
55 - White No. 4
56 - White No. 5
57 - White No. 6
58 - White No. 7
59 - Top Rock Point
60 - Top Rock Bay
61 - Bull's Porth
62 - St. Martin's Head
63 - Old Quay
64 - Lawrence Bay
65 - Innisidgen
66 - Toll's Island
67 - Porth Hellick
68 - Porthloo
69 - Dropnose
70 - Cove
71 - Perigli
72 - Killier


52 - 53
WHITE IS.
51
59
60
61
ST. MARTIN'S
TEAN IS.
48
BRYHER
50
49
47
64
62
63
46a
45
46b
65
66
ATLANTIC OCEAN
68
ST. MARY'S
67
ST. AGNES
72
69
71
ISLES OF SCILLY
70
N
SCALE
2 km

The intensive work on aspects of the biology of the two species of flat winkles, was carried out mainly at 2 sites in Anglesey, Sandy Bay and Porth Swtan. These were chosen as they exhibited high densities of the flat winkle, colour morphs in workable frequencies, little interference from human activity, and a general high diversity in other intertidal species, both in flora and fauna.

2. Estimation of the Habitat

At each of the localities in this study, a number of ecological parameters were estimated to allow a comparison of the winkles on shores with similar ecological conditions. The following parameters have been used:

(i) exposure
(ii) shore profile
(iii) substrate composition
(iv) % algal cover
(v) fucoid species present
(vi) abundance of *Ascophyllum*
(vii) abundance of flat winkle.

The means of estimation for each of the factors is discussed below.

(i) Exposure

As ecological conditions change from one locality to another, so the interactions between species and selective regimes acting on individuals can be expected to change. A difficulty which has occurred in work on littoral ecology has been an inability to assess objectively the ecological conditions at each locality. There have been various attempts to produce a repeatable and useful method of describing rocky shores. Descriptions of the physical attributes and a resulting exposure scale require a great deal of time and even then are not found to be easily repeatable. One of these methods is to determine the number of storms that hit a shore from greater than three miles at sea every 100 days (Moore, 1935). Another method is to determine the amount of swash above a predicted tide level on a series of days (see Lewis, 1964 for review). These methods are useful in their own right but require repeated visits over different times of the year and if the locality is far distant, the method is impractical.

From comprehensive surveys around British coasts a very similar pattern of community change can be observed with different amounts of wave action (Lewis, 1964; Stephenson and Stephenson, 1949). The reliability of these changes has led to a biological exposure scale which allows a rapid and accurate estimation of the amount of wave action (Ballantine, 1961). This is of particular use to a student of population variation, for whom, over a wide geographical area, the amount of time at each locality is limited. Its advantage is that other workers with some knowledge of species recognition will come to the same assessment of the exposure conditions. Although many anomalies exist within some shores, they do not generally blur the assessment of exposure.

Some of the general changes in the indicator species used in this study are as follows. In the very sheltered shores there is a luxuriant growth up and down the shore of the long-fronded knotted wrack, Ascophyllum nodosum. This is the predominant alga on these shores. Near its lower limit, occasional plants of the bladder wrack (Fucus vesiculosus) are found interspersed with Ascophyllum. The major zone below Ascophyllum is one dominated by the serrated wrack, F. serratus. This zone is not wide nor is it continuous as is that of Ascophyllum. The plants of F. serratus in sheltered localities are relatively large, reaching lengths of 1.5m. Below F. serratus there are the large plants of Laminaria saccharina, seldom seen as they are exposed only on spring tides. Above Ascophyllum, occurs a zone of the spiral wrack (F. spiralis), and above it, occasional plants of the channel wrack (Pelvetia canaliculata) near the upper shore. The black lichen Verrucaria maura found only in the splash zone is compressed to a narrow zone of about 1m above extreme high water (EHW). Barnacles are generally absent from these shores, partly because of the thick algal mat which prevents settlement of the larvae; occasionally on some algal free rocks, Balanus balanoides is found. Other species of both the flora and fauna which are restricted to very sheltered shores are given in Lewis (1964). The shore profiles in the sheltered shores are as a rule, gently sloping with a sand and mud substrate overlying rocks. Sediment is very prevalent on the plants, unlike these from more exposed shores. This is due not only to the amount of sediment present on the substrate but also to the stagnant water on the plants. An example of a sheltered shore with the characteristics given above is the causeway between Anglesey and Holy Island (Plate 1).

With increasing wave action there is a gradual increase in the numbers of F. vesiculosus with a concomitant reduction in the numbers and size of Ascophyllum. The F. serratus zone is wider and much more luxuriant than in the more sheltered conditions. It also shows a greater overlap with F. vesiculosus and Ascophyllum. Below F. serratus, L. saccharina is replaced by L. digitata. Above Ascophyllum and F. vesiculosus, F. spiralis is relatively less abundant than in sheltered conditions while Pelvetia becomes often more common. The black lichen (*Verrucaria*) is also much more abundant occupying a zone of up to 5m. This is due to the increased amount of splash that is present from wave action at high tide. Various patches of barnacles are present on the algal free rocks. The limpet *Patella vulgata*, which was absent on the sheltered shores, is now locally abundant on many of the rocks. The profile of these shores is variable, often showing a great deal of heterogeneity within one shore. The substrate is generally one of rock with various areas of sand as in the runoff channels. Little sediment is present either on the substrate or on the plants. These shores generally contain the highest diversity of both floral and faunal species. This applies also to the variation in colour forms of the flat winkles. An example of a shore with intermediate exposure is Sandy Bay on the west side of Anglesey (plate 2).

As one moves to shores of yet greater wave action, there is a progressive reduction in the numbers and size of the individuals of the major fucoids. The greater portion of the shore is covered with short and stocky individuals of *F. vesiculosus* and *F. serratus*. *Ascophyllum* which was the predominant species in sheltered conditions is now absent except for occasional short plants found in the most sheltered conditions, behind boulders and in crevices. *F. spiralis* is absent but *Pelvetia* maintains a wide zone near the top of the shore. The most striking characteristic of the exposed shores is the increase in height up the shore of the *Verrucaria*. This lichen which in sheltered shores occupied only a 1m zone above MHWS now occupies a zone up to 10 m in height. This species provides, then, a most useful indicator of the amount of wave action, as its upper limit is a function of the amount of splash present above the high tide mark. A number of changes occur also in the animal communities. Barnacles which were very uncommon in the more sheltered areas now became very common in the intertidal region. The limpet Patella aspera gradually replaces P. vulgata in abundance. The slope of such shores is as high as 60° owing to their proximity to the headlands. The substrate is generally rock with little observable sediment. An example of a shore with the conditions described is a locality near North Stack, Anglesey (plate 3).

With greater amounts of wave action the fucoids completely disappear. The Verrucaria zone increases in height and can be found up to 20m above the high water mark. With the absence of the fucoids, Littorina obtusata and L. mariae lack the food plant and are thus not found in these shores of increased exposure.

In addition to the indicator species described, the amount of fetch was used to supplement the biological exposure scale. This is the approximate distance that waves can travel before hitting the shores at various angles. It is generally this character that allows quite a reasonable initial estimation of exposure without one's being aware of the biotic components present.

A large number of biotic and abiotic parameters can be used to estimate exposure and allow some ability to compare shores. There will not always be agreement on the exact exposure of a given shore because emphasis will be placed on different variables. This difficulty in assessment does not blur the average consensus of what is a sheltered and what is an exposed shore. Lewis (1964) in his extensive and thorough review on exposure states "The most

Plate 1

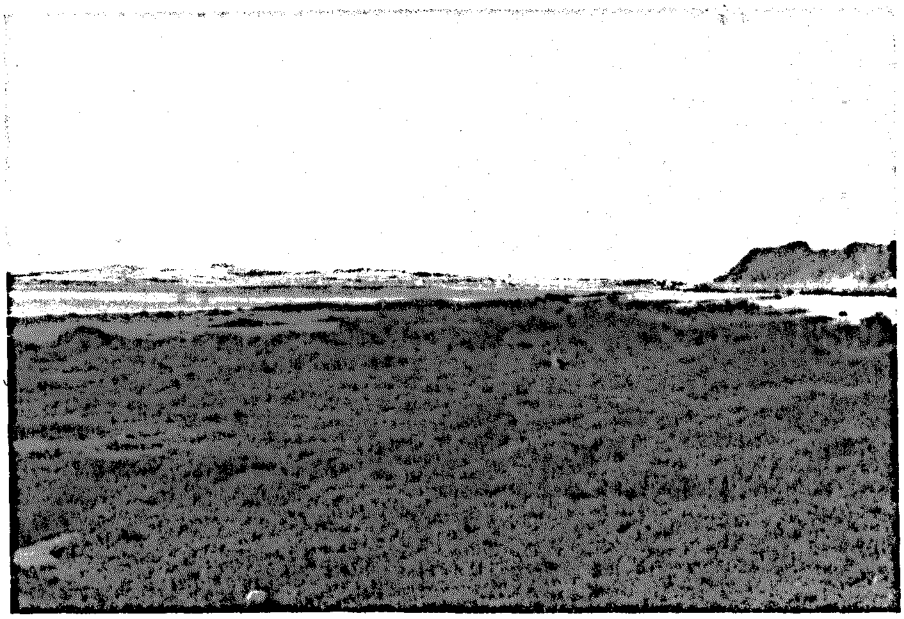


Plate 1

Example of sheltered locality at Causeway (No 10a) between Anglesey and Holy Island. Note predominance of Ascophyllum, shallow slope, smooth profile and mud substrate.

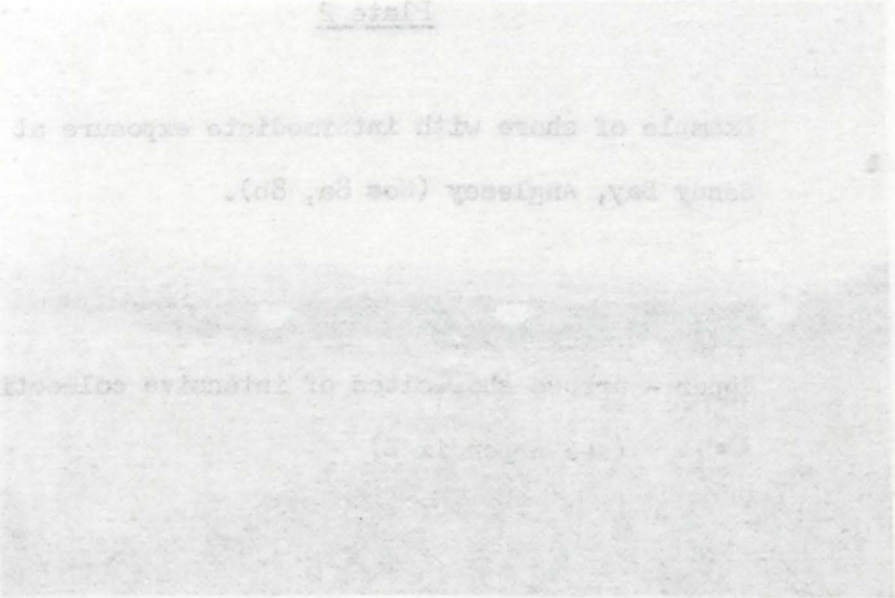


Plate 2

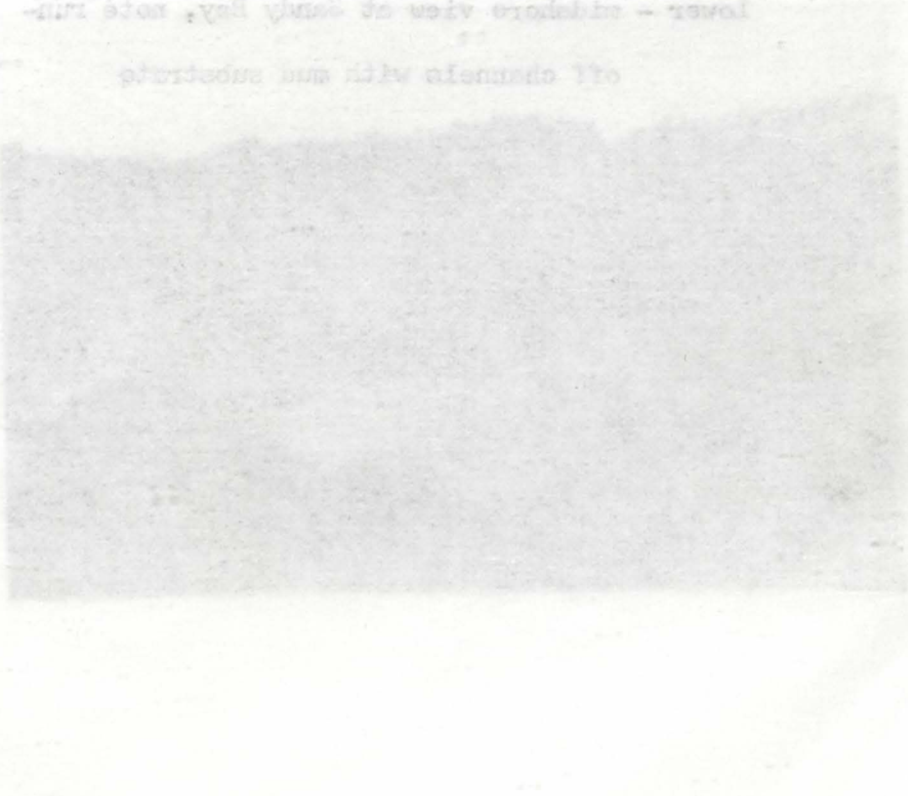


Plate 2

Example of shore with intermediate exposure at Sandy Bay, Anglesey (Nos 8a, 8b).

Upper - arrows show sites of intensive collecting (see Appendix E)

Lower - midshore view at Sandy Bay, note run-off channels with mud substrate

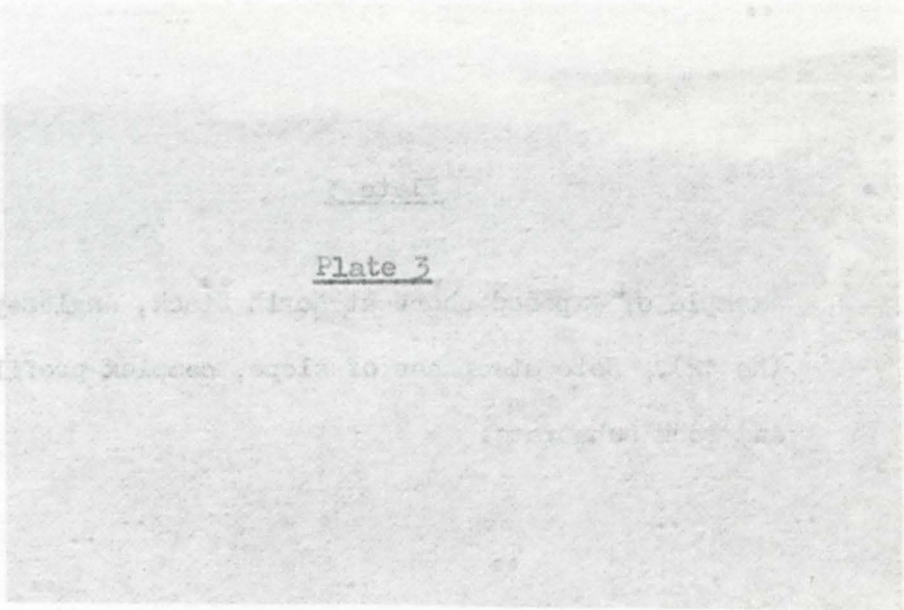


Plate 3

Plate 3

Example of exposed shore at North Stack, Anglesey (No 12). Note steepness of slope, complex profile, and rock substrate.

realistic course, therefore, appears to be to describe shore populations in the broad context of the major acknowledged trends, relying upon subjective estimates based on accumulated experience, and without due concern about strict comparisons which it will probably never be possible to make" (p 288).

(ii) Shore Profile

The amount of heterogeneity in a habitat may have an effect on the diversity of species and kinds of individuals found in the habitat. The shore profile is often a good indicator of habitat heterogeneity. With a smooth profile, there, is generally an absence of tidepools, crevices, boulders and micro-exposure variation. With a more complex profile many of these factors are now present which provide opportunities for other kinds of species. In shores of these kinds there is often a greater diversity of algal species because of the variety of substrates and micro-exposure differences. An index of 1-5 was used to estimate the extent of heterogeneity by the complexity of the shore profile. The least complex, i.e., the smoothest profiles were given the lowest value and the most complex the highest. It is also an index of the amount of heterogeneity in the exposure conditions within one shore and may influence the variation seen within one population of the winkle.

(iii) Substrate

If a Littorina is shaken from its plant during a storm it has to be able to return to the plant for feeding. Depending upon the nature of the substrate this is not always possible. If mud or fine sediment is present, the flat winkles upon crawling a few centimeters are soon suffocated by the fouling of the respiratory apparatus. This was very evident in all the localities that were sampled in the Bristol Channel. During collecting, any snail that was dislodged from plant to the substrate disappeared into 0.5m of mud. Very few individuals were seen in any of these sites so it appeared likely that the nature of the sediment did in some cases influence the population size of Littorina. The sediment also affects the visual properties of the algae and of the shell. In these localities, both the plants and the winkles were covered with a thin layer of silt which gave them a dark-grey appearance. In view of the possible influence of the substrate on the fucoids and, therefore, the winkles, each locality was recorded for the predominant substrate. In general, sheltered shores have a mixture of mud, sand, and rock substrates while the more exposed shores have predominantly rock with some areas of sand.

(iv) % Algal Cover

As the two species of flat winkle in this study are dependent upon the large fucoids for food and shelter, an index was established which allowed the ranking of shores according to the amount of algal cover that was present.

1 : less than 10% algal cover at collecting site
2 : 10%-30% algal cover at collecting site
3 : 30%-70% algal cover at collecting site
4 : 70%-90% algal cover at collecting site
5 : 90%-100% algal cover at collecting site

This index is not independent of the exposure index, since the amount of wave action is often inversely proportional to the amount of algae present. On some sheltered shores, however, the fucoids are very rare probably through silting or brackishness. This index is probably more useful biologically than the crude exposure scale.

(v) Fucoid Species Present

It is important to understand the variation and the distribution of the plants, since each species of fucoid is different in colour, texture and morphology. When a change in the species of the fucoid occurs it is likely that there will be some effect on the winkle. When all the fucoids were present there was a greater amount of heterogeneity in the habitat than when only one of the species was present. For this reason the presence or absence of the four major fucoid species were recorded. The species include *Fucus serratus*, *F. vesiculosus*, *F. spiralis*, and *Ascophyllum*.

(vi) Abundance of *Ascophyllum*

This species of alga dominates the midshore positions in sheltered conditions. It is the only large fucoid which is distinctly olive-green in appearance rather than olive-brown as the *Fucus* spp. The relative abundance was ranked on a 1-5 scale. Shores which had only occasional plants of *Ascophyllum*, relative to the other fucoids, were given the lowest value. Those shores in which all of the midshore position was occupied by *Ascophyllum* were ranked the highest.

1 : less than 10% relative occurrence
2 : 10%-30% relative occurrence
3 : 30%-70% relative occurrence
4 : 70%-90% relative occurrence
5 : 90%-100% relative occurrence

The actual relative occurrence was estimated in an area of 100 square meters at the collecting site.

(vii) Abundance of the flat winkle

Estimates of abundance in this study provide only a rough guide to the actual density of winkles. The actual collecting time for each sample was recorded and when the total number of individuals were counted, the number per unit time could be calculated. These values were then ranked on a 1-5 scale for direct comparison with estimates of exposure etc. Localities which were resampled on different days did not differ significantly in the two estimates of abundance. This applied both to shores with very sparse populations and those with large ones.

(viii) Other Factors

There are often local anomalies in a habitat, such as high densities of Carcinus maenas, proximity to seabird colonies, the amount of current, the presence of fresh-water streams etc., which could have some influence on winkles present at that locality. These influences could possibly be of greater importance than the exposure at that locality and mask relationships with the winkles to exposure conditions. When evident these local anomalies were noted in the field and will be discussed in reference to the variation observed in the winkles.

3. General Collecting Methods

The localities chosen encompass a wide range of ecological conditions varying from sheltered bays with luxuriant algal growth as in the Menai Straits to exposed headlands with less algae as in the Isles of Scilly. The main purpose for this wide geographical survey was to determine whether clinal variation existed in certain characters of the flat winkle and whether there were any predictable trends among ecologically similar shores.

The procedure for collecting was to initially choose an area on the shore which seemed representative of the shore as a whole. A single plant, randomly chosen in the area, was sampled for all the flat winkles. Following this, additional plants were chosen at least 3 meters from the first and from each other. In each case, the algal frond was violently rubbed against the underlying rock and collections made of individuals that had fallen on to the rock. The frond was then checked for any remaining individuals. It was hoped that this method would reduce a large portion of the sampling error in visual collecting (see below).

Where possible, two major subsamples were taken from each shore, including representatives of the flat winkle from the lower parts of the shore and those near the upper mid-shore positions. The fucoids are generally well zoned and provide an indication of the actual shore height (Lewis, 1964) so the lower sample was restricted to _Fucus serratus_ which occurs between the _Laminaria_ zone and the midshore _Ascophyllum_ zone. The midshore sample was taken from _Ascophyllum_, _F. vesiculosus_, and occasionally _F. spiralis_. In one locality which was sampled, the low shore collection was obtained by wading and diving because of neap tidal conditions. This manner of collecting was only used once as it seemed that there was too great a possibility that non-random collections would be made since only a small percentage of the winkles could be seen, these being the brighter coloured and larger shells.

The amount of time spent at each locality in the geographical survey was a function of the density of the _Littorina_. Where possible, a 250 ml container was filled with snails before moving on to the next sampling site. When the densities were low, this was not possible, so collections were continued until the tide rose. This was an especially common situation on the low shore sample as the submersion time of the lower positions occurred not only earlier but for a longer period of time than the positions further up the shore.

For the more intensive studies, carried out in Anglesey, a second method of collecting was used involving the use of a fine mesh net (30 meshes per inch), 1m in length and 0.7m in diameter. An algal frond was cut at the base, lifted into the net, and shaken violently. The majority of individuals except some of the juveniles less than 2mm in size became dislodged from the frond and fell to the bottom of the net. In some situations, juveniles were found in the air bladders of Ascophyllum and F. vesiculosus. These were not dislodged from the algal fronds during shaking and so individual bladders were examined and collections made of the winkles. The net method of collecting eliminated all visual bias that may have been present in the first method of collecting. The main purposes of the net collections were to examine the distribution of shell sizes within populations and to determine whether subtle changes occurred in morph frequencies over the year.

To determine whether a visual bias occurred with the first method of collecting, some localities were resampled in Anglesey using both the visual and the net collecting method. The results of these comparisons given in table 1 indicate that there is some visual bias of the order of a few percent, with the brighter shells being more common in the visual collections. There seems no reason for believing that this will not also be the case in the geographical survey where only visual collecting was used. As the amount of discrepancy between the two methods of collecting does not seem to be related to the frequencies of the morphs in the population it may be assumed that in all the localities examined, a relatively constant error of this magnitude in the same direction will probably occur.

Table 1: Comparison of visual and net collecting methods for estimating frequencies of light-coloured shells

N	Frequency of light-coloured shells	
	Visual	Net
171	71%	70%
254	39%	38%
46	18%	16%
74	13%	10%

The samples were preserved in 10% formalin in seawater at each collecting site. Those individuals required for experiments were placed in moist algae, returned to the laboratory at Liverpool, and kept in circulating seawater at 12°C. Those required for behavioural observations were not kept longer than 4 days in the laboratory after which they were returned to the field and new individuals collected.

In this study, a number of additional collections have been made, mainly in Anglesey, for work on the biology of the flat winkle. The methods and purposes of sampling for each of these will be discussed in the appropriate sections.

4. Examination of Samples

The following observations were made on each of the adult shells in the samples from the geographical survey.

1: shell colour

2: maximum shell diameter

3: sex of individual

4: morphology of the penis

Maximum shell diameter (appendix B) was measured to the nearest 0.1mm with a vernier calipers. The adult shells were recognized by the presence of a thickened lip which is a sign that shell growth is complete and maximum diameter attained. In addition to the length, some comparisons were made on the relative weights. For these purposes, the body was removed from the shell after boiling; after this the shell was placed in a drying oven at 30°C for 24 hours for removal of the moisture. The shell was then remeasured for maximum diameter and weighed to the nearest 0.001mg. Shells showing any evidence of injury during the lifetime were excluded because any fragment missing from the main body whorl contributed to a significant reduction in weight.

The sex was determined by the presence or the absence of the penis which itself was characterized as resembling the type of L. obtusata, L. mariae or intermediate (see Section III, 2, iii). To gain some indication of the variability in length of the didal tubule in L. mariae, the penis was removed at the base, and measurements made to the nearest 0.1mm on the tubule and the total penis length.

In 8 localities from each of the geographical areas, an examination was made of the radula morphology. Where the two species were disimilar in shell size, comparisons were made between the species using both males and females. Among populations where difficulty was encountered with separating females into L. obtusata or L. mariae, because of a similarity in shell size, only males were used initially for radula comparisons. When these proved to be discrete (see Section III, 2, iv) the females were then examined. To provide a comparison with the individuals from the field, the radula was examined from 4 individuals of each species that had been raised from eggs in the laboratory. For preparation, radulae were removed from the buccal sac and placed in a 0.1M KOH solution and heated gently for 15 minutes to remove ligaments and muscles. They were then taken through the following series of solutions for staining and clearing:

1 : 100% Glacial Acetic Acid
2 : 0.8g fuchsin stain in 5ml 20% C_3H_7OH
3 : 100% Glacial Acetic Acid
4 : 2 parts Glacial Acetic Acid: 1 part Methyl Salicylate
5 : 1 part Glacial Acetic Acid: 1 part Methyl Salicylate
6 : 1 part Glacial Acetic Acid: 2 parts Methyl Salicylate
7 : 100% Methyl Salicylate

The cleared radulae were then mounted in a DePeX solution on glass slides. They were viewed under various powers and sketched with the aid of a camera lucida. Four radula from each species were taken from the 0.1m KOH solution and prepared for viewing under a scanning electron microscope.

The examination of these characters provided a general picture of the occurrence and variation in *L. obtusata* and *L. mariae*. During the course of the study a number of additional characters have been examined: of these, the methods of sampling, scoring techniques, and procedure will be discussed in detail in the various sections of the thesis.

SECTION III

SPECIES CHARACTERISTICS

1. Introduction

It was a bimodality of adult shell size which led Sacchi (1964) to suspect that there were two genetical groups living sympatrically. When comparing a number of localities, he found two length modes on the majority of shores. This observation had been made as early as 1915 by Dautzenburg and Fischer who considered the smaller form to be only a dwarf variety. Colman (1932) was also aware of the two forms but considered the differences to be one of age. Sacchi and Rastelli (1966) using additional characteristics besides shell size were able to split the highly variable Littorina obtusata into two species - a larger obtusata, and a smaller mariae.

Some of the other major characteristics used to separate the two siblings were in the morphology of the penis (fig 6). In L. obtusata the organ is very muscular and stout with more than 20 adhesive glands. In L. mariae the organ is relatively long and slender with less than 12 adhesive glands. The major difference lies in the length of the tubule which extends beyond the glands. In L. obtusata this tubule constitutes less than 10% of the total length of the penis but in L. mariae it accounts for 50% of the entire length.

Other differences between the siblings noted by Sacchi and Rastelli were in the shell shape, the amount of sexual dimorphism in shell size, the sex ratio, colour morph frequencies, micro-distribution, and abundance on shores of different exposure.

A difficulty that Sacchi (1964) had encountered was the separation of the females of the two siblings where the adult sizes overlapped. This was also true for the separation of the juveniles which show very little development of the reproductive organs.

One of the purposes of this study was to determine whether the two siblings occurred around the British Isles and if so to determine the nature of variation of shell and colour in each of the two groups. I tried first to see whether a bimodality existed in adult shell size and whether the anatomical differences noticed by Sacchi and Rastelli would correlate with the two size groups. When this turned out to be the case, additional characters were searched for to allow an accurate identification of the individuals in the field and laboratory. When this became possible I was then able to examine both inter- and intrapopulation variation in both siblings.

2. Results

(i) Shell size

All the localities in this study in which adequate sampling was possible, including those from Wales, southern England, the Isles of Scilly, and Ireland yielded the expected bimodality in adult shell sizes. In all individuals of the two size classes, there are also the characteristic differences in the morphology of the penis; this therefore supports the observations of Sacchi and Rastelli (1966).

L. obtusata is always larger than L. mariae within the same locality throughout the study area (appendix 2,& plates 4, 5 and 6).

Plates 4, 5 and 6

Shells of L. obtusata and L. mariae. The following three photographs show representatives of the two species from three shores varying in exposure. The shells (approximately natural size and colour) were chosen to indicate the average adult size and colour variation in each species from these shores. In each photograph, upper shells are L. obtusata while lower shells L. mariae. Descriptions of the colour phenotypes are given in Section III, 2, ix.

Plate 4

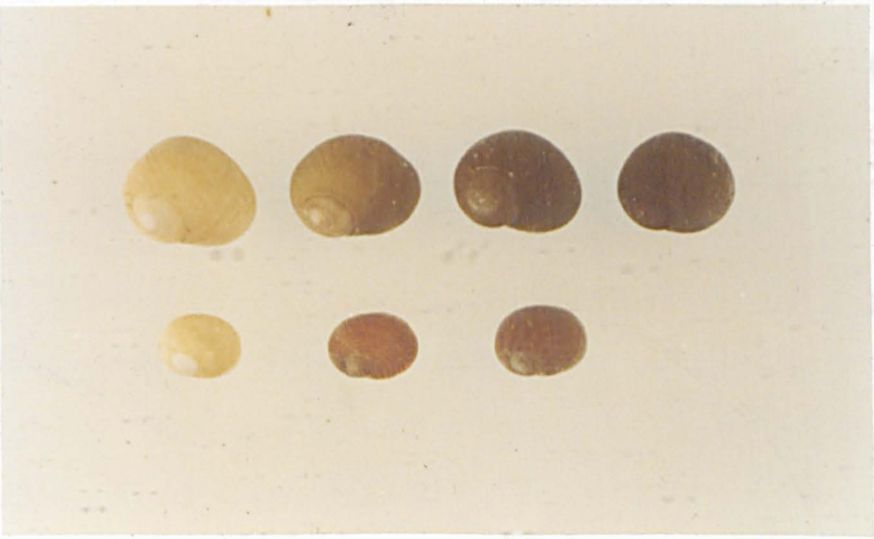
L. obtusata and L. mariae from Menai Bridge, a very sheltered locality in Anglesey. Note differences in size and colour variation.

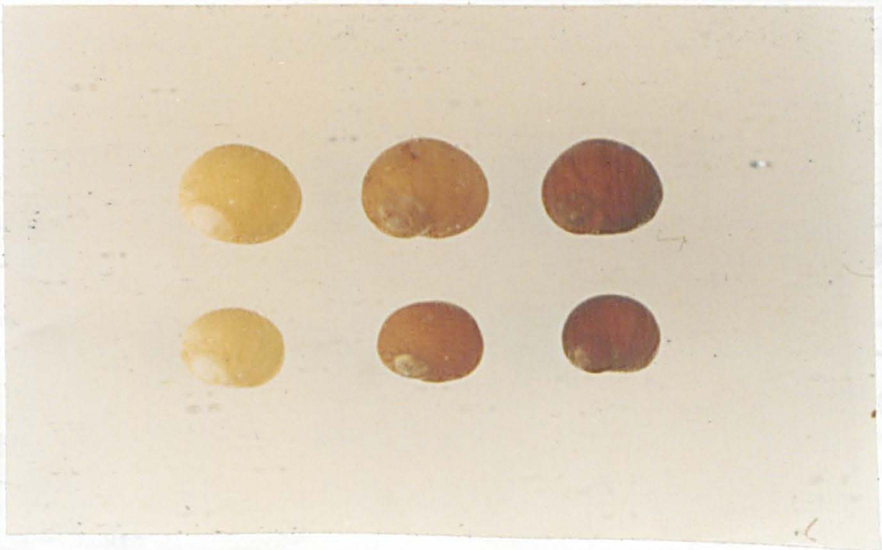
Plate 5

L. obtusata and L. mariae from Trearddur Bay, a semi-exposed locality in Anglesey.

Plate 6

L. obtusata and L. mariae from Heathy Point, a very exposed locality on the Isles of Scilly. Note reduction in size (from Plate 4) of L. obtusata and increase in size of L. mariae. Note also similarity in colour variation.





In the four geographical regions there is a similar pattern of shell size and variation in each of the species (table 2).

Table 2: Mean adult shell size for males of *L. mariae* and *L. obtusata*.

Area	*L. mariae*				*L. obtusata*			
	number of localities	$\bar{x}$ (mm)	s	range of $\bar{x}$	number of localities	$\bar{x}$ (mm)	s	range of $\bar{x}$
Wales	37	9.40	1.71	6.3-12.0	43	15.86	0.79	14.1-17.3
Southern England	6	9.91	2.39	7.4-12.5	9	15.93	0.64	15.2-17.1
Isles of Scilly	22	10.04	2.19	5.1-13.5	29	16.10	0.68	14.3-17.3
Ireland	15	10.03	2.00	5.7-12.8	22	16.65	1.05	13.0-17.7

Of the two species, *L. mariae* is more variable than *L. obtusata*. A variance-ratio test calculated between the two species in the four geographical areas shows that in all cases the difference in the variation is significant (table 3).

Table 3: Variance-ratio test of adult shell sizes between *L. mariae* and *L. obtusata*.

Area	*L. mariae* s^2	*L. obtusata* s^2	F ratio	P
Wales	2.92	0.62	4.71	<0.005
South England	5.71	0.41	13.92	<0.005
Isles of Scilly	4.80	0.46	10.44	<0.005
Ireland	4.00	1.10	3.63	<0.005

The range in variation of L. mariae, from 5.1mm to13.5mm can take place over very short distances. In Ireland (fig 1) the smallest (5.7mm) and largest (12.8mm) mean shell size are found at Milkhaven and Streedagh House Bay, which are separated by only 7km. This is a similar pattern to that observed in the Isles of Scilly (fig 3) at Heathy Bay and Heathy Point. These two localities are separated by 200m and show a change in adult shell size from 6.0mm to 12.0mm, respectively. These differences in adult shell size in L. mariae show a relationship to changes in size of L. obtusata. In this species, the largest sizes are often found in localities where L. mariae is the smallest. For example, at Milkhaven in Ireland, where the smallest L. mariae are found, the largest mean size (17.7mm) of L. obtusata is found. These differences are shown in calculations of the correlation coefficient for each sample where the two species occur (table 4).

Table 4: Correlation coefficients for adult shell sizes between L. mariae and L. obtusata.

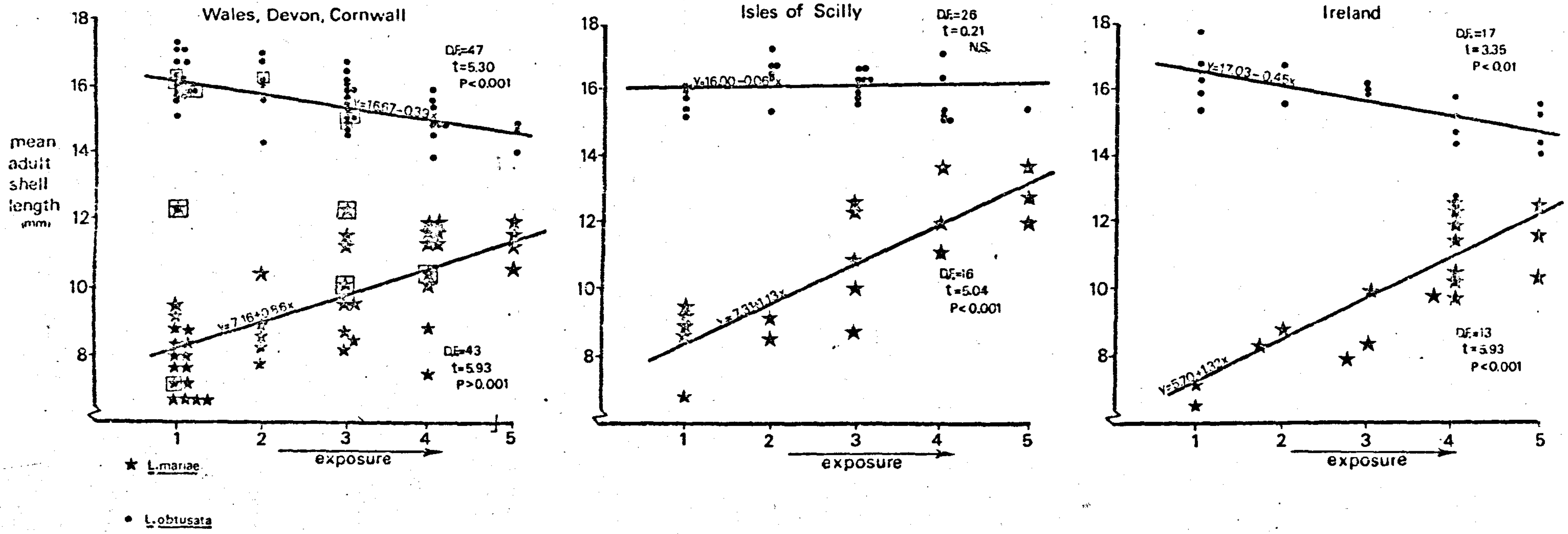
Area	r	d.f.	P
Wales	-0.56	35	< 0.001
Southern England	-0.24	4	N.S.
Isles of Scilly	-0.08	20	N.S.
Ireland	-0.53	13	< 0.05

It can be seen that in Wales and Ireland there is a significant negative correlation between the adult shell sizes of L. mariae and L. obtusata. Southern England, and the Isles of Scilly both show the same trend, although without a significant correlation.

Figure 4

Figure 4

Relation between exposure and shell size. Regression analysis weighted according to sample size and based upon mean adult length in males. t-values give comparisons between observed and zero slopes. Mean sizes in square symbols show localities from southern England including those from Devon, Cornwall, and Somerset. Raw data for both males and females given in Appendix B.


Wales, Devon, Cornwall
Isles of Scilly
Ireland
mean adult shell length (mm)
18
16
14
12
10
8
1
2
3
4
5
exposure
Y=16.67-0.33x
D.F.=47
t=5.30
P<0.001
Y=7.16+0.86x
D.F.=43
t=5.93
P>0.001
Y=16.00-0.06x
D.F.=26
t=0.21
N.S.
Y=7.31+1.13x
D.F.=16
t=5.04
P<0.001
Y=17.03-0.45x
D.F.=17
t=3.35
P<0.01
Y=5.70+1.32x
D.F.=13
t=5.93
P<0.001
★ L.mariae
● L.obtusata

The shell size in the males of both species shows a predictable change according to the amount of exposure on a shore. In L. mariae, there is a significant increase in adult shell size from sheltered to exposed conditions in all the geographical areas (fig 4). L. obtusata, always the larger of the two species, shows the opposite pattern in Wales, South England, and Ireland, while in the Isles of Scilly, no change is present. In other words, on sheltered shores the two species are very dissimilar in size but show a convergence on shores of greater exposure. L. mariae shows the greatest amount of change between the species.

These changes in shell size in both species appear to be a function of at least two factors - the amount of wave action, and the amount of predation by crabs. These are discussed in detail in subsequent sections (see Section IV, 5).

(iii) Shell Shape

Although the shells of the two species are similar in most respects, they can be distinguished by subtle differences in the thickness of the lip relative to the diameter of the aperture. In L. mariae this is generally thicker than in L. obtusata (plate 7). This character often allows the majority of adults to be separated, both males and females.

In some localities there are differences in the weight of the shell per unit length. It can be seen from the data collected at three localities that L. mariae is relatively heavier than L. obtusata for an equivalent shell size (fig 5). The amount of this difference is reduced when comparing the species from the region of overlap on the shore (see fig 14 for vertical distribution).

Although shells size and lip thickness are useful taxonomic characters for the adults, they provide little help in separating

Plate 7

Plate 7

Comparisons between shell thickness and shell shape in L. obtusata and L. mariae.

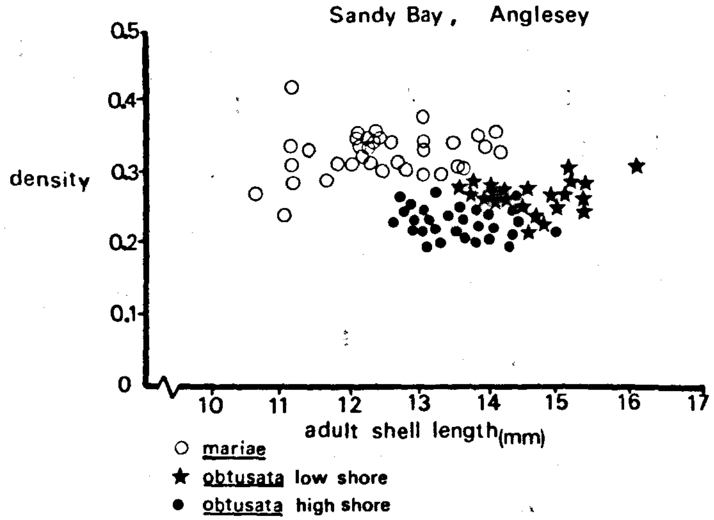
Upper - Lip thickness in adult shells of L. obtusata (top) and L. mariae (bottom) from Porth Swtan, Anglesey. Note proportionately thicker lip and smaller aperture in L. mariae. Shells about $1\frac{1}{2}$ times natural size.

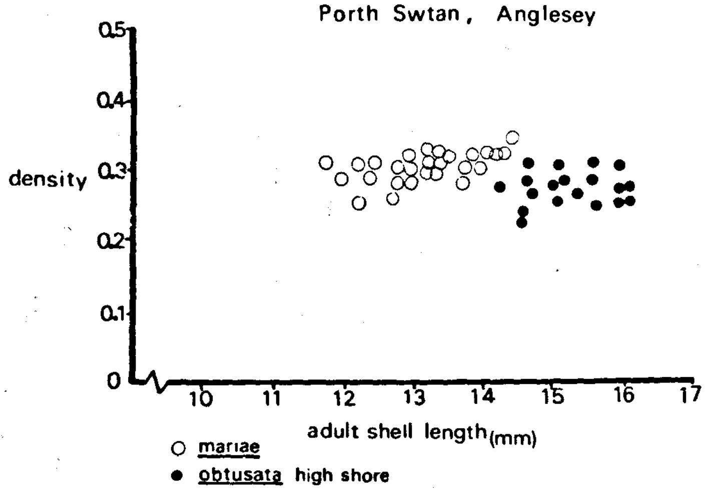
lower - juvenile shells of L. obtusata (top) and L. mariae (bottom) from Menai Bridge, Anglesey. Note that in L. obtusata the angle between the outer tip and columellar lip (shown by arrow) is much more angular than in L. mariae. Scale in mm.

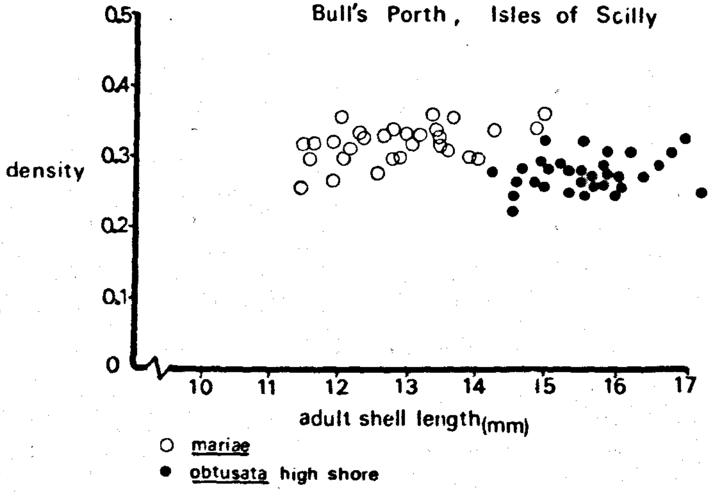
Figure 5

Figure 5

Shell density in L. obtusata and L. mariae. Values show estimates of density – $\frac{\text{dry shell weight in mg}}{(\text{shell diameter})^3}$ for individual adult males; one locality (Sandy Bay) shows L. obtusata males collected from the low-shore or region of overlap with L. mariae (see fig 14).


Sandy Bay, Anglesey
density
0.5
0.4
0.3
0.2
0.1
0
10
11
12
13
14
15
16
17
adult shell length(mm)
○ mariae
★ obtusata low shore
● obtusata high shore


Porth Swtan, Anglesey
density
0.5
0.4
0.3
0.2
0.1
0
10
11
12
13
14
15
16
17
adult shell length(mm)
○ mariae
● obtusata high shore


Bull's Porth, Isles of Scilly
density
0.5
0.4
0.3
0.2
0.1
0
10
11
12
13
14
15
16
17
adult shell length(mm)
○ mariae
● obtusata high shore

the juveniles. In these, shell size and lip thickness are generally very similar in both species. One difference is present in the shape of the shell in the area of connection between the inner and outer lip. In L. obtusata, this area is very angular compared with L. mariae (plate 7).

Another difference in the juveniles between the species is in the sculpturing on the periostracum. In both species, there are a series of minute ridges which run perpendicular to the lip. These are not readily visible by the naked eye, but can easily be seen with a 10X handlens. In L. obtusata the ridges are approximately uniform in height or with one high ridge followed by one low ridge; in L. mariae there is a sequence of one high ridge followed by two to four low ridges repeated over the shell. These can be seen in the scanning electron microscope pictures (plate 8). These differences are most strongly expressed in localities where the two species are very dissimilar in adult shell size. In localities where the species are similar in adult size, the differences in the ridges are less pronounced but still distinguishable in most cases.

(ii) Penis Morphology

Throughout the study area, anatomical examination of adult males showed the presence of either one of the two types of penis (fig 5) described by Sacchi and Rastelli (1966). Using these characters, there is no overlap and thus confusion in identifying the males of the two species. No truly intermediate types were found in this study.

Some variation occurs in the morphology of the penis, most noticeably in L. mariae. In this species, the distal tubule can constitute anywhere from 10-50% of the overall length of the penis. Sacchi and Rastelli (1966) have suggested that the differences in morphology of the reproductive organs between the species may act as

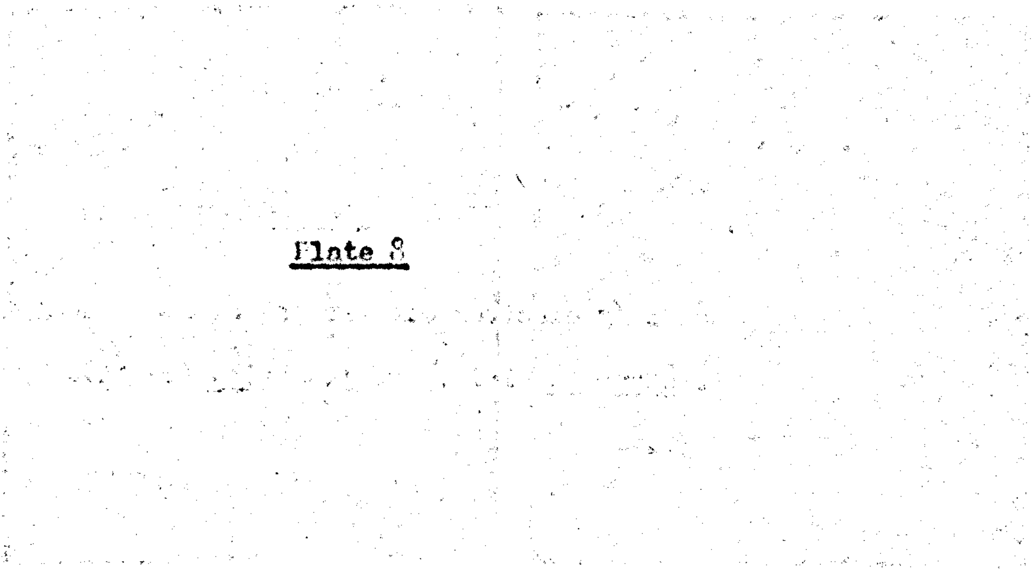


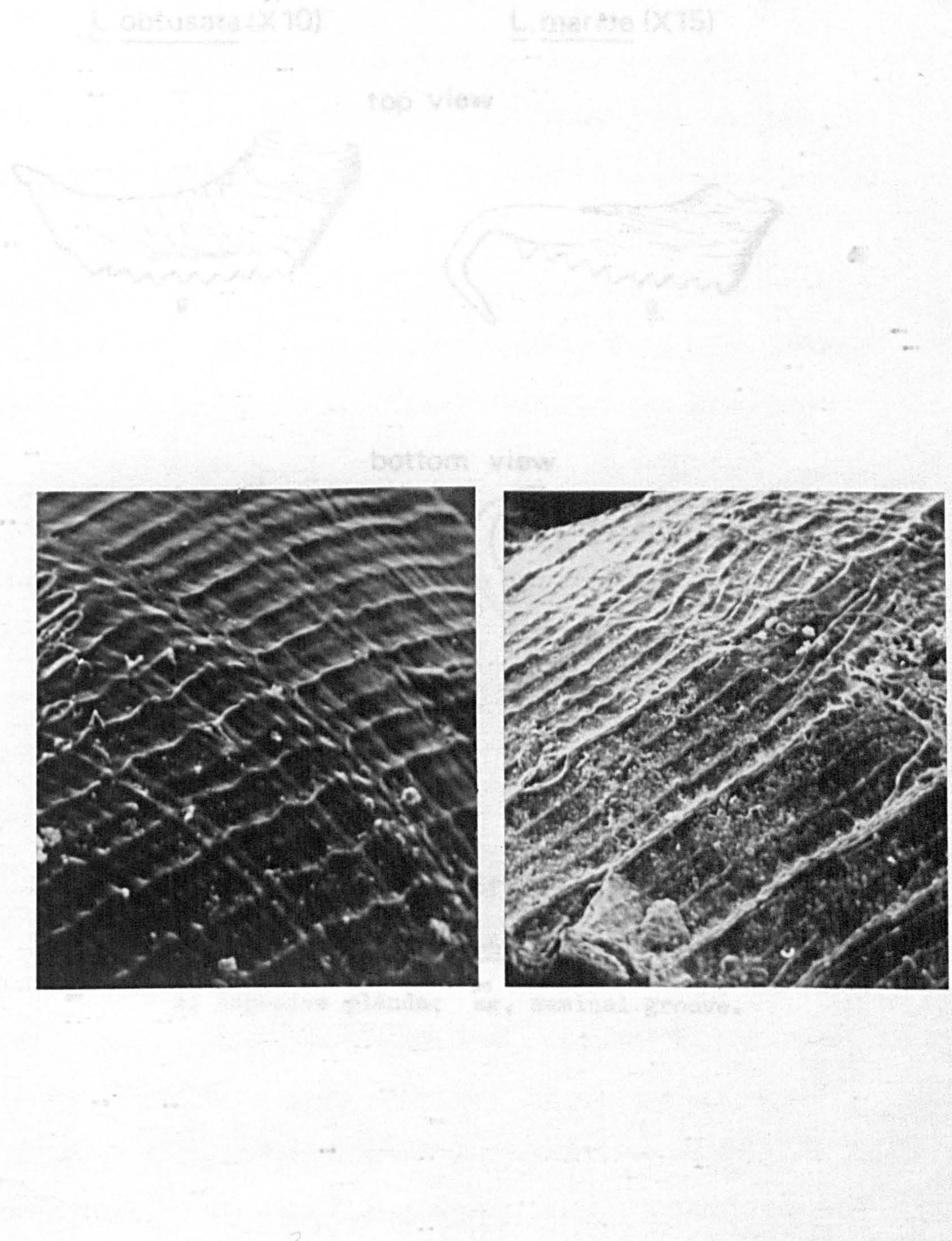
Plate 8

Plate 8

Scanning electron micrographs of shell sculpturing.

L. obtusata (left) and L. mariae (right). Scale

X 30.



s, adhesive glands; sg, seminal groove.

2

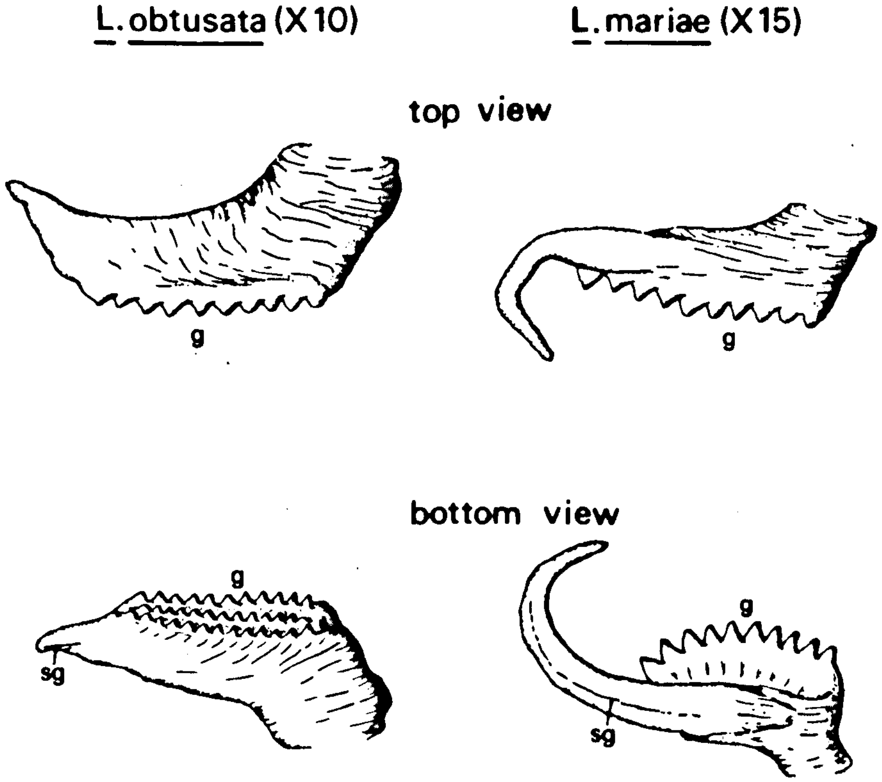


Fig. 6. Diagramatic sketches of dorsal and ventral views of penis in *L. obtusata* and *L. mariae*. g, adhesive glands; sg, seminal groove.

isolating mechanism. It is also possible that the relatively long tubule in L. mariae is an adaptation allowing the male to copulate successfully, since in this species there is a marked sexual dimorphism in shell size. The female is much larger than the male, so it is conceivable that some difficulty could be encountered during copulation if the male had only a short distal tubule.

These two suggestions can be tested by comparing the relative length of the tubule with amount of difference in shell size between L. mariae and L. obtusata and with the amount of sexual dimorphism in shell size in a large number of localities. If isolating mechanisms are involved, then one might expect that as the two species become more similar in adult shell size, the relative length of the distal tubule should increase. If the sexual dimorphism is involved, then one might expect that the relative length of the tubule should decrease in localities where there is a greater similarity in the sizes of the two sexes.

A comparison involving 33 localities shows that Sacchi and Rastelli (1966) may well be right in suggesting that the difference in the morphology of the penis between the species acts as isolating mechanisms (fig 7). There is a significant increase in the relative length of the tubule in L. mariae when the two species are very similar in shell size. When the two species are very dissimilar in shell size, there is probably little chance for hybridization owing to the differences in shell size alone. The suggestion of a relationship with the sexual dimorphism is less likely to be true; in localities where the two sexes are very similar in size, the relative length of the terminal filament is highest, the opposite of what would be predicted (fig 8).

This study confirms Sacchi and Rastelli's (1966) observations

Figures 7 & 8

Variation of relative tubule length in penis in L. mariae. Each point on these figures represents the mean relative tubule length $(\frac{\text{tubule length}}{\text{total penis length}})$ estimated from about 10-20 males in each locality. Regression analysis weighted according to sample size.

Figure 7

Comparison between tubule length and the amount of difference in adult shell size between *L. mariae* and *L. obtusata*, C.D shell length between species is

$$\frac{\bar{x}_{L.O} - \bar{x}_{L.M}}{S_{L.O} + S_{L.M}}$$

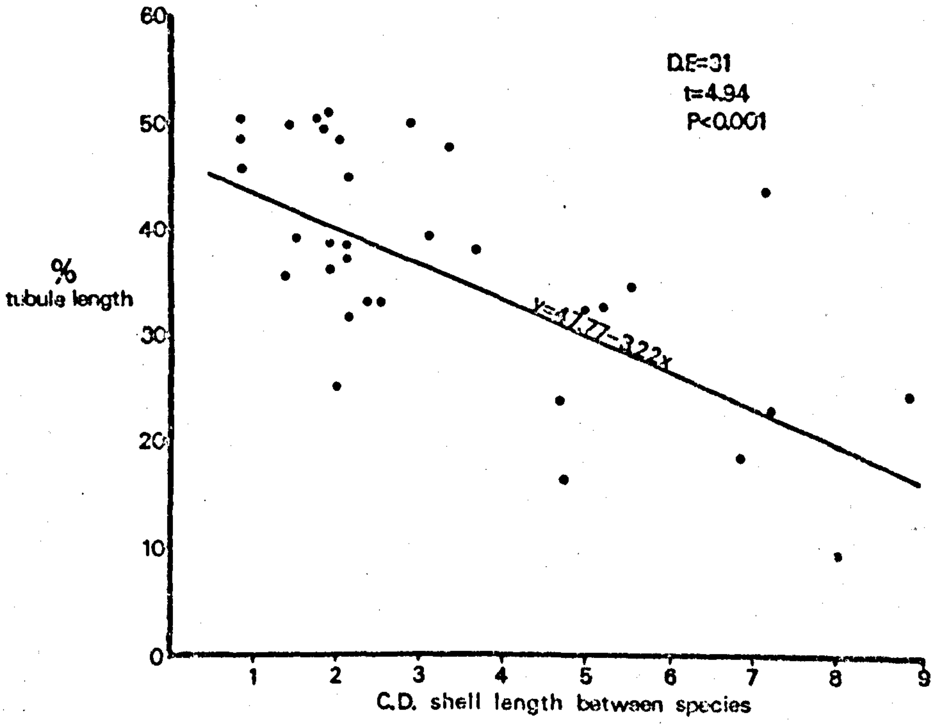
where $\bar{x}$ is the mean shell size in males of the two species and S the standard deviation. (from appendix B).

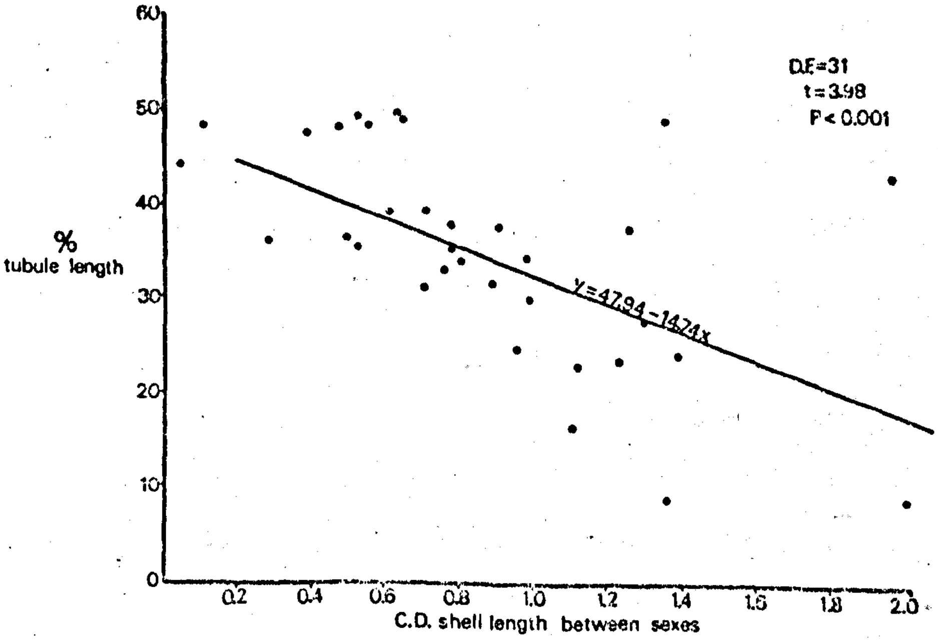
Figure 8

Comparison between tubule length and the amount of difference in mean adult shell size between females and males (see section III, 2.vi) in L. mariae. C.D. shell length between sexes is

$$\frac{\bar{x}_f - \bar{x}_m}{S_f + S_m}$$

where $\bar{x}$ is the mean shell size of females and males respectively and S the standard deviation (from appendix B).


60
50
40
30
20
10
0
%
tubule length
D.F.=31
t=4.94
P<0.001
Y=47.77-3.22x
1
2
3
4
5
6
7
8
9
C.D. shell length between species

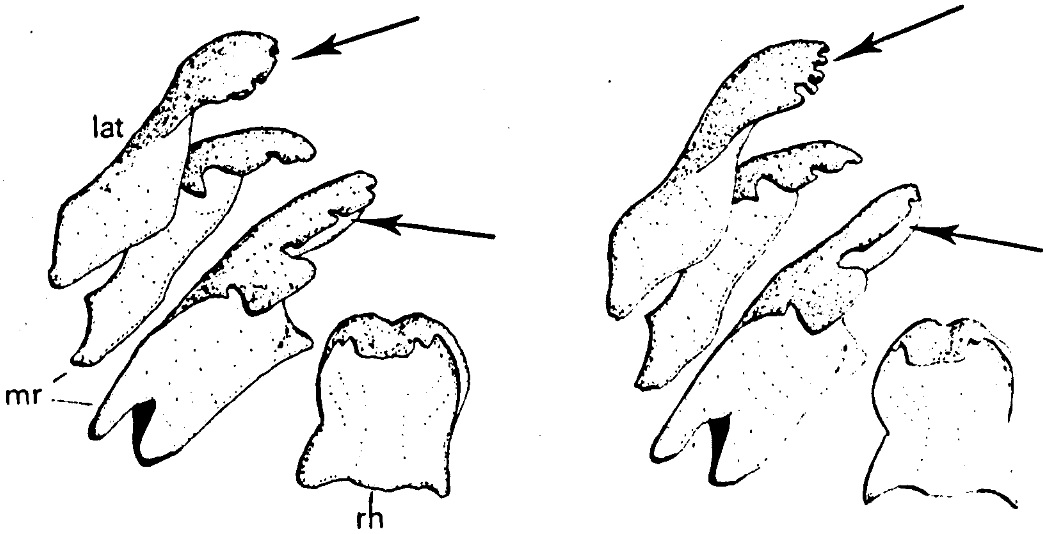

60
50
40
30
20
10
0
%
tubule length
D.F.=31
t=3.98
P<0.001
Y=47.94-14.74x
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
C.D. shell length between sexes

on the usefulness of the penis in separating the males between *L. obtusata* and *L. mariae*. Some difficulty is encountered with the females especially when the two species are similar in shell size and shape. In order to identify these individuals, it is necessary to examine other characters; for this reason, a study was made of the morphology of the radula.

(iv) Radula Morphology

Owing to the usefulness of the radula in the taxonomy of Gastropods, I thought that an examination of this character might provide an additional means of separating the two siblings when other characters were not discrete. The results of this examination show that there are distinct differences between the radula of the two siblings which allow a separation of the species. One of the differences lies in the shape of the cusps on the lateral tooth row. In *L. mariae* they are very lobate in appearance while in *L. obtusata* they are angular and smaller (fig 9). This difference allows separation of about 60% of the individuals in a sample. The end of the radula, which is used for rasping, is often worn resulting in a blurring of the distinction between the species. However examination of the middle positions or those near the point of formation, which are always unworn, show the same lobate and angular cusps that are evident in unworn distal positions of the radula.

The inner marginal teeth provide a more useful character for separation of the species. In *L. mariae* there is generally one large cusp visible while in *L. obtusata* two or three are present (fig 9, plate 9). There is about 5% overlap between the species in this character. The examination of the individuals reared in the laboratory showed the same interspecific differences. These individuals had been raised on the same food plant under similar light



L. obtusata

(X 275)

L. mariae

(X275)

Fig. 9. Camera lucida drawings of radulae teeth in L. obtusata and L. mariae. lat, lateral; mr, marginal; rh, rhachidian. Arrows show major differences between species.

Plate 9

[illegible]

[illegible]

[illegible]

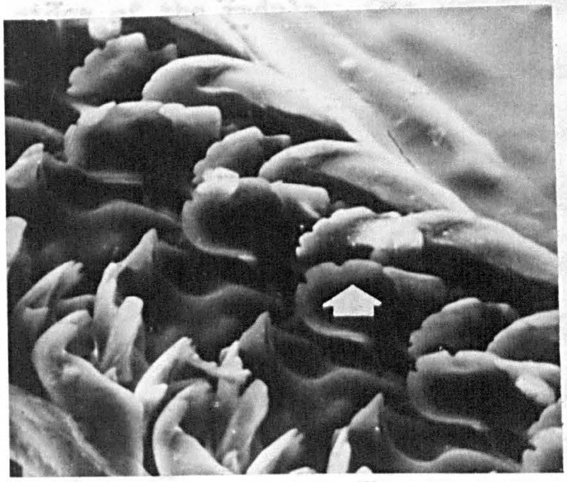
[illegible]

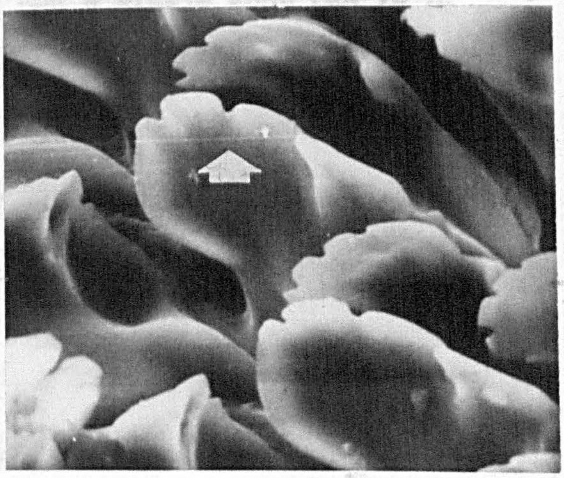
Plate 9

Scanning electron micrographs of radulae. Arrows show major difference between species. L. obtusata, upper two; L. mariae, lower two, scale - left X 150, right X 300.

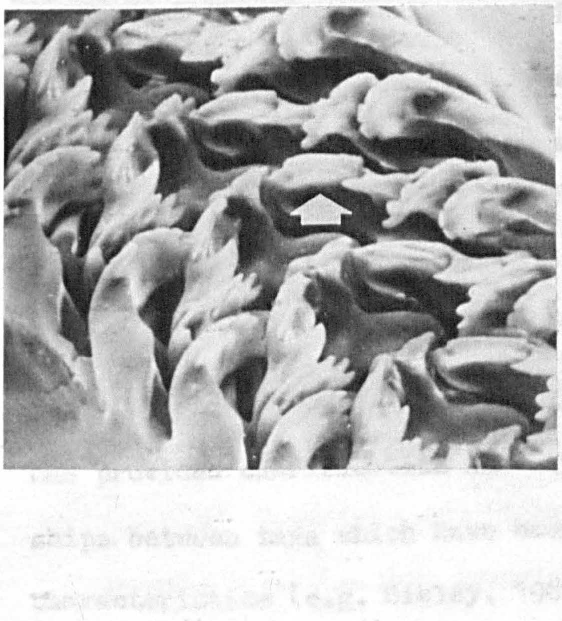
and temperature conditions.

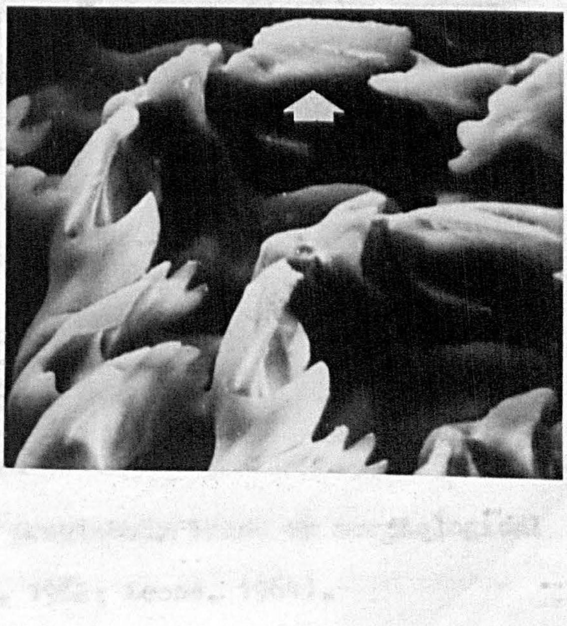
In L. obtusata some asymmetry has been seen between the two sides of the radula.





In summary, the differences in the morphology of the radula provide additional evidence for the specific status of the two siblings. Using this character and those of the shell morphology and sculpturing, one is able to separate reliably almost all but a few of the very smallest individuals (5mm) into either L. *obtusata* or L. *mariae* in the samples that have been taken in this study. All of the differences observed between the species to this point have involved morphological characters. In the early parts of this study,





this problem

ships between taxa which have been previously drawn on morphological characteristics (e.g. Sibley, 1957, 1962; Leone, 1964).

and temperature conditions.

In L. obtusata some asymmetry has been seen between the two sides of the radula. One individual had a single cusp (as in L. mariae) on the teeth in the left inner marginal row but on the right side, it had 3 cusps. This was consistent over the entire length of the radula. Another individual had single and triple cusps on one side. It is not known whether this is an inherited trait or whether it is a phenotypic response to some environmental stimulus. In these two individuals there were no indications in the morphology of the shell that would suggest hybridization.

In summary, the differences in the morphology of the radula provide additional evidence for the specific status of the two siblings. Using this character and those of the shell morphology and sculpturing, one is able to separate reliably almost all but a few of the very smallest individuals (<2mm) into either L. obtusata or L. mariae in the samples that have been taken in this study. All of the differences observed between the species to this point have involved morphological characters, In the early parts of this study, it was conceivable that these differences were produced by environmental differences. For this reason a study was undertaken to determine whether there were biochemical differences between the species.

(v) Biochemical Differences

(a) Introduction

Electrophoresis has recently become widely used for the comparison of biochemical characteristics within and between species. It has provided clarification and often posed new problems on the relationships between taxa which have been previously based on morphological characteristics (e.g. Sibley, 1960, 1962; Leone, 1964).

One advantage of protein characters lies in their developmental proximity to the actual genotype. Some sibling species are morphologically similar in most respects but show very discrete differences at the proteins level (Ayala _et al_., 1970). In some situations new siblings have been detected by these differences where before only one species was recognised (Manwell and Baker, 1963; Manwell _et al_., 1967).

The recognition of hybrids between sibling species is often very difficult using only morphological characters. Some of these difficulties are overcome by electrophoretic analysis. This technique has been successfully used in molluscs (Schwabl and Murray, 1970), fish (Abramoff et al., 1968; Hagen, 1967), amphibians (Coates, 1967; Fox _et al_., 1961) and reptiles (Crenshaw, 1965; Dessauer _et al_., 1962).

Disc-electrophoresis has been used in this study on _Littorina_ to determine whether there were differences in the proteins between the species and if so whether any hybridization was evident. The presence of overlap in some shell characters might not allow an accurate recognition of possible hybrids; I felt, owing to the success of previous workers, that if hybrids occurred they would be detectable from this method.

Some electrophoretic work has been done on the genus _Littorina_ (Cheng, 1964; Wium-Anderson, 1970; Snyder and Gooch, 1973); these works have not included _L. obtusata_ and _L. mariae_, but _L. littorea_, _L. saxatilis_, and _L. striata_. There were differences in the proteins of each of these species.

(b) _Materials and Methods_

Animals used for this investigation were collected from Menai Bridge where the two species are very dissimilar in size, and at

North Swtan where there is a greater similarity in shell size. The two species were both collected from F. serratus at the same position on the shore. They were returned to the laboratory and kept in sea-water for three days before the enzyme extracts were taken. Four individuals of L. obtusata and L. mariae that were reared from eggs in the laboratory were also used for providing a comparison with the field populations.

For preparation of the enzyme extract, the shell of the animal was broken and the hepatopancreas removed. The extract was weighed and then diluted with distilled water to a ratio of 0.1ml H_2O to 20mg. The hepatopancreas was homegenized with a glass rod until all large particles were broken; this solution was centrifuged for 7 minutes at 3000 rpm.

For separation of the proteins from the supernatant, polyacrylamide gel columns were used with a vertical disc-electrophoresis apparatus (Sargent, 1969). The buffer and gel solutions are the same as those described by Davis (1964). One minor modification that has been used is in the preparation of the extract. Rather than polymerizing the sample in a large-pore gel, it was mixed with 2 drops of 40% sucrose solution and then layered on to the upper gel. This method, combined with a low initial amperage (2mA per tube), produced a good stacking of proteins without the need to polymerize. After 5 minutes, the amperage was increased to 5mA per tube and this current continued until the end of the run. The front which was marked by means of Bromophenol Blue was allowed to migrate for a distance of 45mm measured from the top of the small-pore gel column. To avoid heating of the gels because of the current, all runs were made in a refrigerator at 3°C. When the run was complete, the tubes were removed from the apparatus and the gels taken out by rimming with a fine needle. These were placed directly into a staining solution.

Staining was done either for esterases or general proteins in the following solutions:

Esterases: 18mg α naphthyl acetate (dissolved in
2ml acetone)
80mg Fast Blue R R
100ml TRIS-Glycine buffer, pH 8.5(3g TRIS,
10g glycine, 500ml H_2O)
time 30 minutes

General Protein: 1g naphthalene black 10B
100ml 7% glacial acetic acid
time - 60 minutes

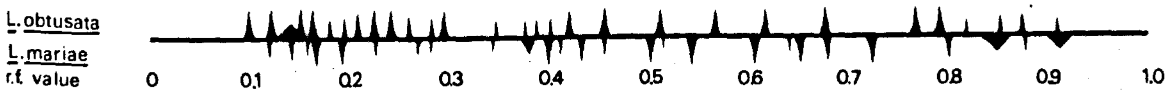
Destaining of the naphthalene was accomplished by washing the gels for 3 days in 7% glacial acetic. After this time, the gels were stored in individual tubes in a 10% glacial acetic acid solution.

For the scoring of the different bands on the gel, it was necessary to calculate the r.f. value for each owing to slight differences in the distance travelled by the tracking dye, (43-47mm). The r.f. value is the ratio of the distance travelled by a protein band to the total length of the gel (as measured from the Bromophenol Blue marker).

(c) Results

A number of differences exist between L. obtusata and L. mariae in both the esterases and the general proteins. The esterases provide the least useful of the proteins for taxonomic purposes owing to the occurrence of each of the major bands at the same position in both species (plate 10). When r.f. values are calculated for all identifiable bands in each individual, and plotted on a horizontal r.f. axis, a number of minor differences in the migration rate are evident between the species (fig 10). For example, when comparing the positions of the most discrete and deeply stained bands occurring in the central portion of the gel it can be seen that in *L. obtusata* the bands occur in at least one of the following positions - 0.51, 0.57, 0.62, 0.67. However in *L. mariae* they occur at the following - 0.50, 0.54, 0.60, 0.65, 0.67 and 0.73. Although the bands appear equivalent on the gel, it is apparent from scoring a large number of individuals (*L. obtusata* N = 100; *L. mariae* N = 63) that subtle differences are present.

Fig 10: Comparison between positions of all recognizable bands in 100 individuals of *L. obtusata* and 63 individuals of *L. mariae* from Menai Bridge.



Staining for general proteins provides a more useful taxonomic character owing to discrete differences between the species in the migration of the major bands (plate 11). As with the esterases, the general proteins were divided into three classes according to their velocity of migration. In Class G_2, *L. obtusata* is characterized by a dark band at the 0.65 position while *L. mariae* has a dark band at 0.73. These differences were also present in individuals that had been reared in the laboratory.

Plate 10

Plate 10

Polyacrylamide gells stained with α naphthyl acetate for esterases. *L. obtusata*, 4 gels on left; *L. mariae*, 4 gels on right. All individuals collected from Menai Bridge.

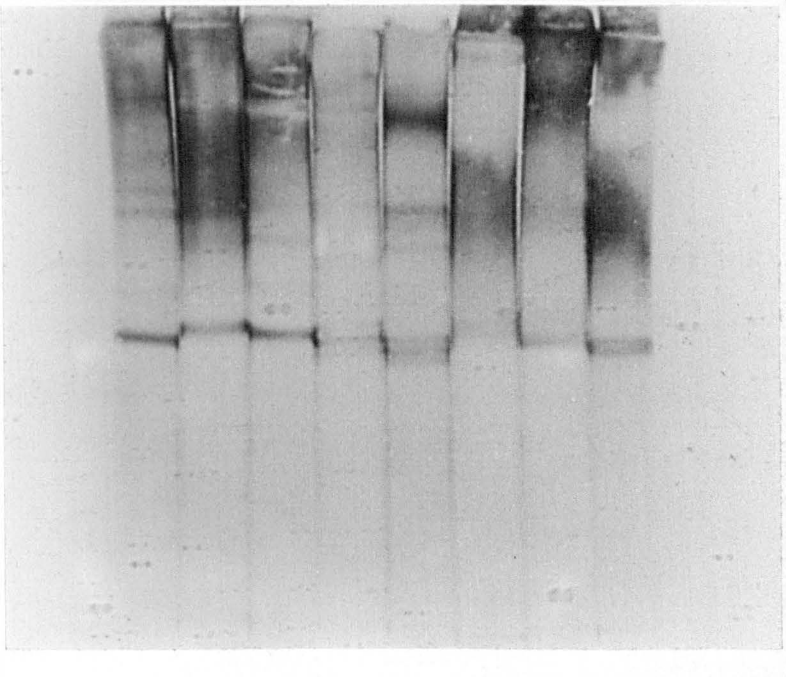
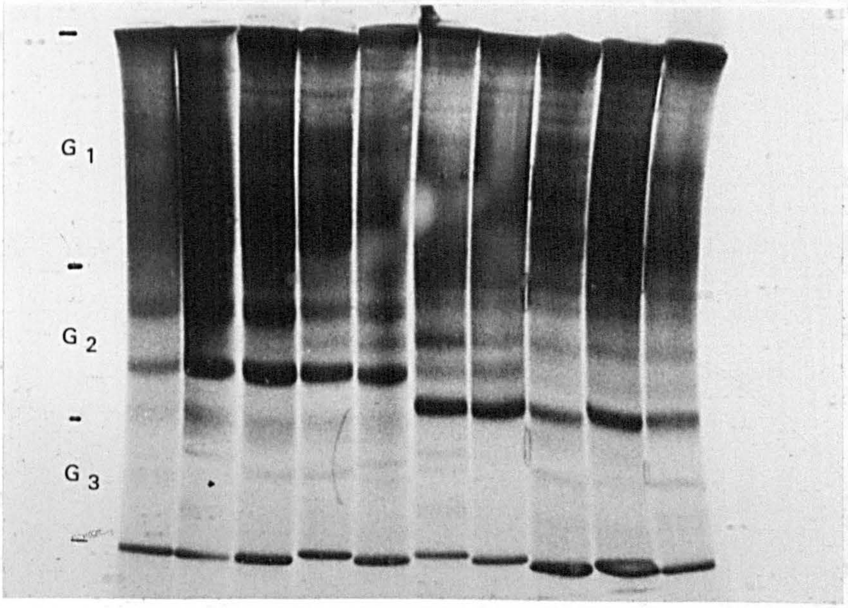


Plate 11

Plate 11

Polyacrylamide gels stained with napthalene 10B for general protein. L. obtusata, 5 gels on left; L. mariae, 5 gels on right. All individuals collected from Menai Bridge.


G 1
G 2
G 3

In Porth Swtan, where the two species are similar in some of the morphological characters, there was the same amount of discreteness in the biochemical characters that was present in the Menai Straits where the two species are very dissimilar morphologically. No indication of any hybridization was evident from the examination of protein bands from 100 individuals of each species at both localities. It would appear that *L. obtusata* and *L. mariae* can be treated as valid species at least in the British Isles.

The data presented this far on morphological and biochemical characters have allowed almost all individuals within any sample except the very smallest (2mm) to be recognized. It is therefore possible to compare other differences between the species such as the amount of shell dimorphism between the sexes, the geographical distribution of the species, and aspects of the ecology and distribution within the intertidal region.

(vi) Sexual Dimorphism in the Shell

(a) Introduction

Sacchi (1968) has noted that in both *L. mariae* and *L. obtusata* the females tend to be larger and more globular than the males. He found that the difference was much more pronounced in *L. mariae* over a large number of shores in Brittany. There was also a variation between the shores in the amount of difference between the sexes. For example, in calm but not estuarine conditions the two sexes of *L. obtusata* are very similar in size while in exposed areas, the males are relatively smaller than the females. Sacchi suggested that the males could be more affected by turbulence than the females. There have been no other suggestions to account for the sexual dimorphism within and between the species. The collections made around the British Isles in this study can be used for comparison with Sacchi's observations; since samples from a large number of localities are available, there is some opportunity for determining the significance of the dimorphism in the sexes.

(b) Methods

Estimates of the sexual dimorphism were made by calculating a coefficient of difference which is based upon the means and the variances of the two sexes. The equation (from Mayr, Linsley, and Usinger, p 146)

$$C.D. = \frac{\bar{x}_1 - \bar{x}_2}{s_1 + s_2}$$

where $\bar{x}_1$ and $\bar{x}_2$ are the mean shell sizes of the larger and smaller sex and s_1 and s_2 the standard deviation around each of the means.

(c) Results

The data presented here support Sacchi's observations that the female is generally larger than the male in both species (appendix B) and that this difference is greater in L. mariae (table 5).

Table 5: Comparison between species in the amount of sexual dimorphism in shell size.

Locality	Species	No. of localities	mean C.D.	t-test	P
Wales, south England	L. mariae	25	0.89	12.28	<0.001
	L. obtusata	37	0.19		
Isles of Scilly	L. mariae	17	0.84	4.58	<0.001
	L. obtusata	28	0.29		
Ireland	L. mariae	14	1.27	6.02	<0.001
	L. obtusata	19	0.30		

The amount of difference between the sexes is not constant between localities. L. mariae shows a reduction in the dimorphism in populations having a larger mean shell size in each of the three geographical areas (fig 11). A similar trend is evident in L. obtusata but in this species the differences are not significant. L. mariae shows a greater amount of change in dimorphism between localities compared with L. obtusata.

(d) Discussion

The difference in the sexual dimorphism is another character which separated the two species. The dimorphism is most pronounced in populations of L. mariae with a relatively small adult size. However, in localities where the mean adult shell size approaches that of L. obtusata there is a reduction in the dimorphism to a point where little difference in size exists between the sexes (the common situation in L. obtusata). In other words, as the two species converge in adult shell size, there is also a greater similarity in the sexual dimorphism of the two species.

Guiterman (1971) has suggested that the small adult size of L. obtusata on exposed shores is advantageous for hanging on in conditions of heavy wave action. If this is the case - that wave action favours an optimal shell size - then the convergence between the two species and the similarity in the sizes of the sexes in L. mariae on exposed shores would seem to be an advantageous response.

As argued in Section IV, 5, the general small size of the shells of L, mariae in sheltered localities is advantageous when considering the influence of predation by crabs. It is not clear why in these localities a marked sexual dimorphism exists. The relatively large sized females may have a number of advantages such as egg carrying capacity which offset the disadvantage from predation by crabs.

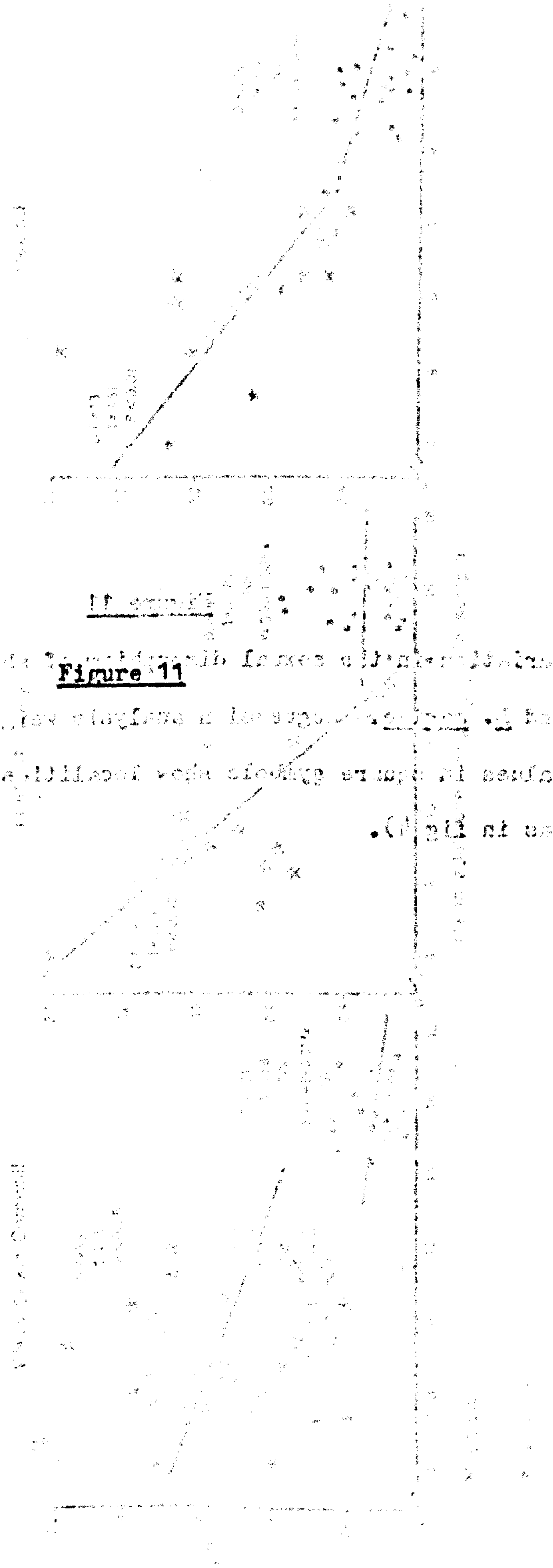
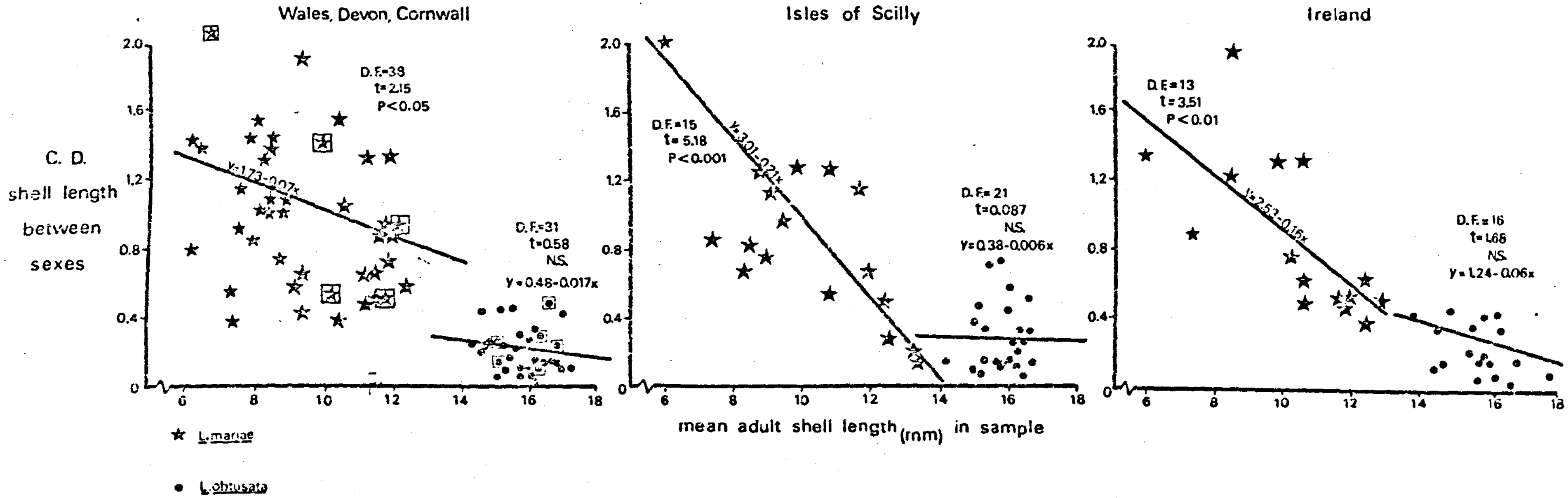


Figure 11

Figure 11

Variation in the sexual dimorphism of shell size in *L. obtusata* and *L. mariae*. Regression analysis weighted for sample size. Values in square symbols show localities from southern England (as in fig 4).


Wales, Devon, Cornwall
Isles of Scilly
Ireland
C. D.
shell length
between
sexes
2.0
1.6
1.2
0.8
0.4
0
6
8
10
12
14
16
18
D.F.=38
t=2.15
P<0.05
y=1.73-0.07x
D.F.=31
t=0.58
N.S.
y=0.48-0.017x
D.F.=15
t=5.18
P<0.001
y=3.01-0.21x
D.F.=21
t=0.087
N.S.
y=0.38-0.006x
D.F.=13
t=3.51
P<0.01
y=2.53-0.16x
D.F.=16
t=1.68
N.S.
y=1.24-0.06x
mean adult shell length (mm) in sample
★ L. mariae
● L. obtusata

(vii) Geographical Distribution

Sacchi (1969) has found L. obtusata and L. mariae on the majority of shores that he sampled, always appearing when the large intertidal fucoids were present. L. obtusata was more abundant on sheltered shores while L. mariae was more abundant on exposed shores. From these observations Sacchi felt that L. mariae was more tolerant to the effects of heavy wave action.

The results from collections made in my own study show that in both sheltered and exposed conditions a great deal of variation can occur in the abundance of both species of winkle. Generally speaking the trend is similar to that observed by Sacchi, at least in L. obtusata which is more common on average in sheltered conditions. L. mariae is less regular showing an increase with exposure in two of the areas and a decrease in the third (fig 12).

In L. obtusata, the amount of algal cover provides a better fit to the changes in abundance (fig 13). There is in each geographical area a significant relationship between an increase in the algal cover and an increase in the numbers of L. obtusata. No such relationship is present in L. mariae. This species is restricted to one of the intertidal fucoids, Fucus serratus. In sheltered shores where algal abundance is high, F. serratus is found only in a narrow band on the lower shore. With increasing exposure, there is a reduction in the overall amount of algae but an actual increase in the relative amount of F. serratus. On these shores, this species often constitutes the main fucoid in the general algal cover, but in these localities individual plants of all species are rare. As L. mariae is found mainly on this species, one would expect no correlation with general algal abundance.

There are some exposed shores with a very rich and luxuriant

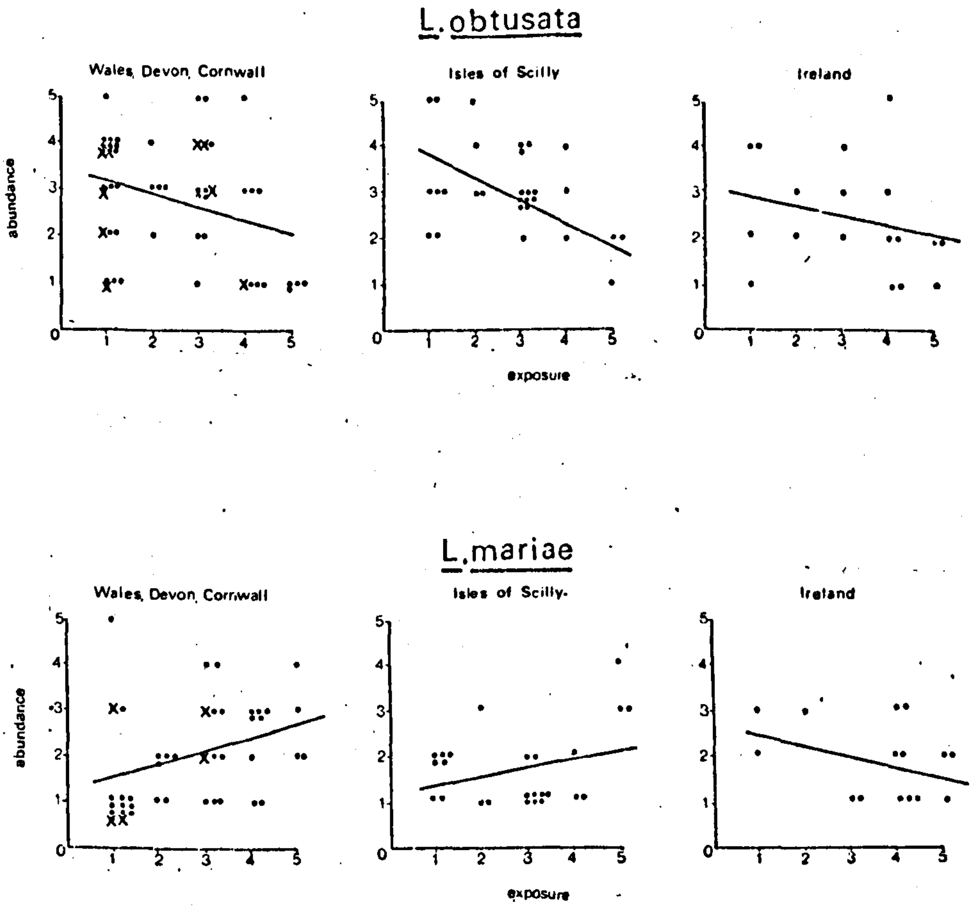
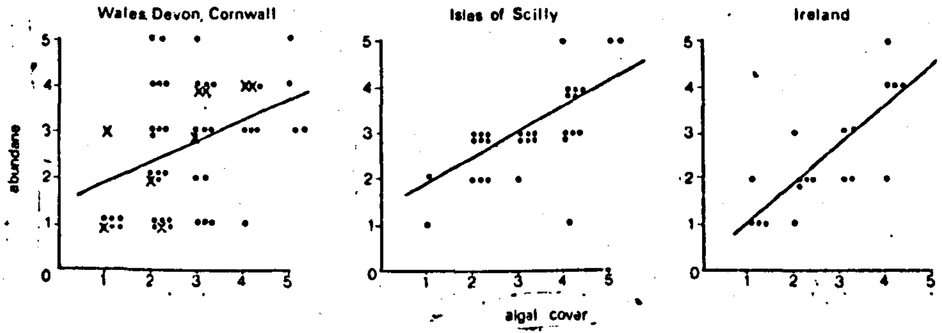


Fig. 12. Relation between exposure and abundance of winkles. Regression of y (abundance) on x (exposure) calculated for each area. Values with X show localities from southern England. Raw data shown in Appendix A.

L.obtusata



L. mariae

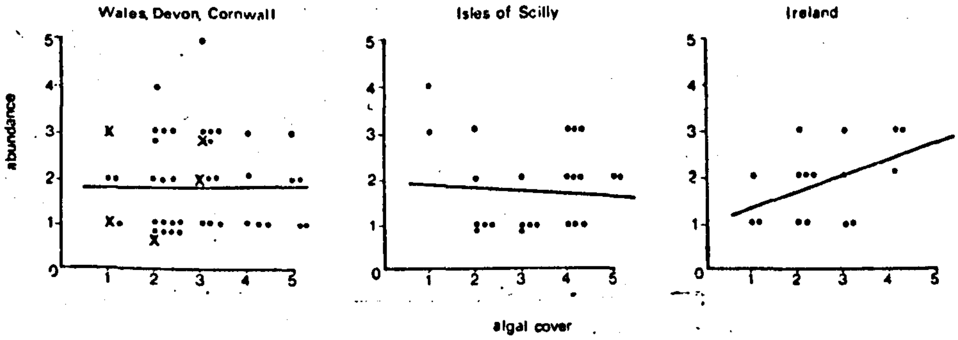


Fig. 13. Relation between algal cover and abundance of winkles. Calculations and symbols as in Fig. 12.

cover of F. serratus that lack both L. mariae and L. obtusata even though these localities are continuous with shores where the Littorina are very abundant. These situations have been found in all the major geographical areas which were visited in this study. It would seem therefore that there are factors influencing the abundance of Littorina other than the amount of food plant present. One characteristic of these shores is the smoothness and uniformity of the rock platform underlying the fucoids. There seem to be very few microenvironmental irregularities such as boulders and crevices which might allow the Littorina to find shelter during heavy wave action. It is possible that on these shores the snails are physically incapable of maintaining a population owing to the impact of the algae on the flat and hard surface.

(viii) Vertical Distribution

L. mariae and L. obtusata have different vertical distributions in the intertidal region. The former occurs from just above the main Laminaria zone, up to the mid-shore, while the latter is found from slightly below mid-shore, just overlapping with L. mariae up to the MHWM which is at the top of the F. spiralis zone (fig 14). These observations made in this study agree with those made by Sacchi (1969).

The distribution of L. mariae is closely concordant with the distribution of F. serratus on the majority of shores. In sheltered localities this species of alga is limited to a narrow zone above Laminaria except in moist conditions, as in run off channels and tidepools, where it can be found much higher on the shore. Near the upper edge of the zone it is often found underlying plants of Ascophyllum. In each of these situations, L. mariae can be found on F. serratus and only infrequently on Ascophyllum even though the latter species may be adjacent to or overlying F. serratus. On shores of greater exposure, where the upper edges of the *F. serratus* zone are higher on the shore, the distribution of *L. mariae* follows this species. When *F. vesiculosus* is abundant among the *F. serratus*, *L. mariae* is often found on the species, though not as commonly as on *F. serratus*. On some of the exposed shores, crevices often cut high into the shore, in which flora are more representative of the lower shore areas. In these conditions, *L. mariae* can be found with the same abundance as on the lower shore.

The distribution of *L. obtusata* up and down the shore is generally wider than that of *L. mariae*. On sheltered shores, it can be found on all the fucoids including the lower shore *F. serratus*, the mid-shore *F. vesiculosus* and *Ascophyllum*, and the upper shore *F. spiralis*. Where it overlaps with *L. mariae* on the *F. serratus*, individuals of both species can be found adjacent to each other on the same plant. However, *L. obtusata* is not common in this area as it reaches its greatest abundance further up the shore near the upper edge of the *Ascophyllum* zone. On shores of greater exposure the distribution of *L. obtusata* is more restricted than in sheltered shores. The lower edge of its range is displaced upwards, although it is still found on all the species of algae, most abundantly on *F. vesiculosus* and *F. spiralis*. Since *L. mariae* is displaced upwards relatively more than *L. obtusata* there is consequently a greater amount of overlap on these shores than in sheltered ones. There is however some separation on these exposed shores because *L. obtusata*, in the region of overlap with *L. mariae*, is most commonly found on fucoids in microhabitats which offer the greatest shelter from the action of waves such as those behind boulders etc.

The differences in the amount of overlap between the species on sheltered and exposed shores is even more expressed when the tide is

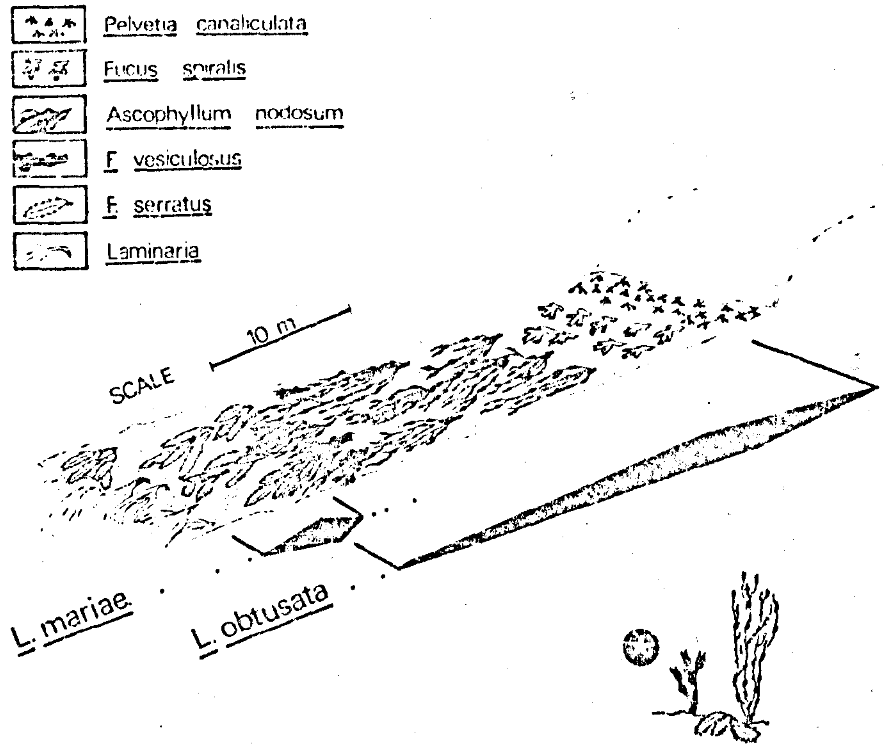
Figure 14

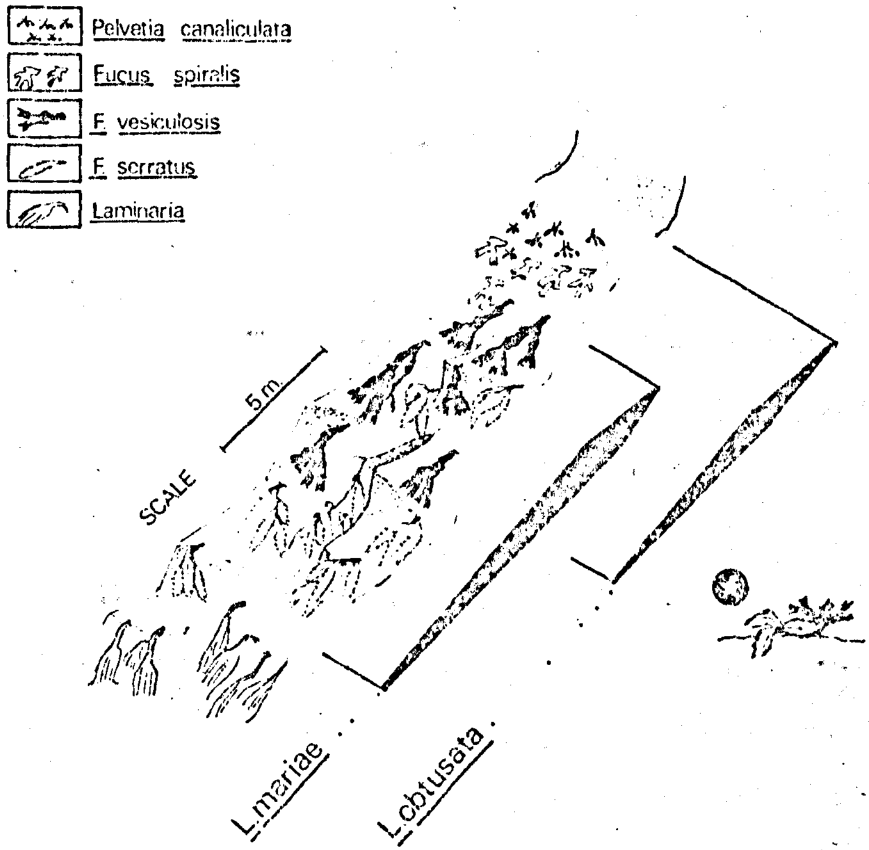
Figure 14

Typical zonation on sheltered and exposed shores. Small figures on right show spatial realtionships of algae at high water in region of overlap between L. obtusata and L. mariae.

Upper - sheltered shore

Lower - exposed shore. Note the absence of Ascophylum and replacement by F. vesiculosus.


Pelvetia canaliculata
Fucus spiralis
Ascophyllum nodosum
F. vesiculosus
F. serratus
Laminaria
SCALE
10 m
L. mariae
L. obtusata


Pelvetia canaliculata
Fucus spiralis
F. vesiculosis
F. serratus
Laminaria
SCALE
5 m
L.mariae
L.obtusata

in. Observations with the use of SCUBA in relatively sheltered shores have shown that plants of Ascophyllum and F. vesiculosus often 3m in length, stand vertical in the water because of the presence of airbladders. F. serratus on the other hand, lacking any form of airbladder, lies relatively flat on the bottom (fig 14). Therefore, what is observed to be a region of overlap on the shore during low tide, is in conditions of high water very good habitat separation. On exposed shores, the amount of spatial overlap is similar at low and high tide, because of the lack of Ascophyllum and the occurrence of the bladderless form of F. vesiculosus. Therefore the plants on which L. obtusata are found (mainly F. vesiculosus) lie in the same manner as F. serratus on which L. mariae occurs. The large amount of spatial overlap on these shores would seem to represent a real overlap in the habitat in both low and high tide positions unlike the situation in sheltered shores where the overlap occurs mainly when the tide is out.

(ix) Colour Polymorphism

(a) Introduction

Variation in the shell colour of the L. obtusata complex around the European coasts has been described by numerous authors in the last 60 years (e.g. Dautzenberg and Fisher, 1915; Barkman, 1956; Sacchi, 1961.). Generally speaking the colours occur as discrete phenotypes or morphs and have accordingly been given varietal names; the most common forms are citrina, olivacea, and reticulata (plates 4, 5 and 6). The expression of each of these phenotypes appears to be inherited (see Breeding Experiments, Section VI, 2).

It has been noted by Barkman (1956) that in the L. obtusata complex the shells of juvenile individuals often become darker with increasing growth of the shell. For example, some of the juvenile classified as citrina became olivacea in the adults while some of the juvenile olivacea become adult reticulata. Since the major purpose of this entire study was to compare and discuss the differences in morph frequencies between populations, it was necessary to work with adult shells in which the growth was complete or in other words, where no further change occurred in the basic shell colour. Therefore, unless otherwise mentioned, all discussion of morph frequency in this study are based upon adult shells.

In this section, methods of scoring and description of the morphs are given with a very generalized comparison of the differences between _L. obtusata_ and _L. mariae_. The detailed examination of the polymorphism is discussed in sections VI, VII and VIII.

(b) Description of Morphs and General Comparison between Species

Citrina:- This morph is usually lemon-yellow in appearance, it is found abundantly throughout the study area, more commonly in _L. mariae_ than in _L. obtusata_. A small proportion of the individuals within any sample can be either almost white (var. ablbescens) or yellowish-orange (var. aurantia). To provide some indication of the variation within the morph, three broad, continuous categories have been scored - white, yellow and orange.

Olivacea:- This olive-coloured shell is the most common morph in _L. obtusata_ and the rarest in _L. mariae_. From scoring some of the preliminary samples, it was apparent that there were minor variations in the shade, ranging from a light-olive through to a dark-olive. Accordingly, the morph was scored into 3 shades - light, intermediate and dark.

Reticulata:- In simplest terms, this common morph can be described as a pattern of dark intersecting lines on a somewhat lighter ground colour (usually orange, olive, or brown, but occasionally yellow, or red-brown). In this study, I have mainly followed Sacchi's (1961) method of scoring in recognizing two general phenotypic classes of the morph - light and dark. These two classes are often discrete and can therefore be treated as distinct morphs themselves. The difference between the two is owing to changes in the ground colour. In light reticulata, it is orange or occasionally yellow, this latter colour occurring only in L. mariae on some sheltered shores. In dark reticulata, the ground colours are more variable ranging from olive-green to olive-brown, through brown to red-brown. The olive ground colour is most prevalent in L. obtusata while brown is most common in L. mariae. All of these dark reticulata, irrespective of ground colour are phenotypically very dark in appearance relative to citrina or light reticulata.

On many shells, the ground colour is a very dark shade of brown in which the dark intersecting lines are scarcely visible. These shells do in fact grade into a completely dark brown (almost black) shell, known to some workers as the variety fusca. Light reticulata are intermediate in darkness between citrina and dark reticulata, roughly equivalent to intermediate shades of olivacea. The actual colour of the intersecting lines on these shells is lighter than those on dark reticulata; this is probably owing to the infusion of the ground colour into the dark brown lines seen on darker shells, such as those with an olive or brown ground colour.

Uncommon Shell Colours:- Some varieties listed by Dautzenberg and Fisher (1915) occurring at low frequencies are rubens (vermillion), inversicolour (two brown bands on an orange shell), zonata (two orange bands on a brown shell), alternata (one orange band on a brown shell). Only one of these varieties has been seen in this study (var. inversicolour) and has been scored accordingly. It is very uncommon in both L. obtusata and L. mariae.

Discreteness of the Morphs:- Although there is generally a a discontinuity in shell colours between the morphs, there are some localities where overlap occurs. In L. mariae from sheltered shores, citrina grades into light reticulata the latter usually being far less common than the former. In L. obtusata on these shores, citrina often grades into olivacea. In both species, there is not a complete blurring of the morphs but one of a bimodality with intermediates occurring up to a frequency of about 20% of the least common phenotype. In exposed conditions, there is no overlap between these morphs. Light and dark reticulata are usually discontinuous from each other, but in the Isles of Scilly and some localities in Ireland this is not the case for here the two phenotypes often merge continuously with each other showing no obvious bimodality. In these cases, scoring into two classes was done using reference shells from Anglesey where light and dark reticulata are discrete.

Although some uncommon shell colours such as albescens, aurantia, and fusca have been named as separate varieties by Dautzenberg and Fischer (1915), I have found that these grade continuously with one or other of the major morphs. Since in this study I have concentrated on a purely phenotypic classification using 4 broad classes of shell colour each of these uncommon varieties have been scored separately, but within one of the 4 major colour morphs - citrina, olivacea, light reticulata, dark reticulata. Shores in which uncommon varieties become locally abundant shall be mentioned in the text.

3. Conclusions

The data presented here on morphological, biochemical, and ecological characters in L. obtusata and L. mariae provide additional support for the validity of the two siblings as first recognized by Sacchi and Rastelli (1966). With the additional taxonomic characters presented in this study it is now possible to identify to species, not only the females but also the juveniles except for those less than 2mm (which themselves may be identified by colour in some cases, see Section IXI). This is of essential importance in ecological and genetical studies which involve comparisons between the two siblings.

L. obtusata and *L. mariae* are widespread, occurring on most rocky shores with fucoids in the British Isles and Ireland. Although it is often very difficult to make an accurate determination, there seems to be little if any indication of hybridization between the siblings. This conclusion has been reached in this study from an examination of both the morphology of the penis and the general protein characters. A low frequency of hybridization is still possible but larger samples are required to verify or reject the possibility.

Evidence began accumulating in the final stages of this study which indicated that *L. mariae* may itself be composed of two groups with morphological and ecological differences but with a complete gradation in all characters. For the greater part of the following discussions *L. mariae* is treated as a single heterogeneous group. More intensive work on this matter should clarify the present problem.

SECTION IV

SHELL INJURIES IN LITTORINA

1. Introduction

It is necessary to understand, as much as possible, the biology of a species in order to gain some clues to the factors or selective agents that might act on the populations. In this section, data is presented on the influence of crabs as predators on the flat winkles.

In all the collections made in this study, some individuals from each sample had evidence of injuries sustained during shell growth. In the juveniles, when injuries occurred at the lip, which resulted in pieces missing from the shell, the broken areas were replaced during subsequent growth. The replacement of the broken areas leaves a scar and subsequent evidence of the breakage (fig 15). It is possible then to detect some indication of the individual's history by examining the shell injuries sustained during growth.

The shell injuries may be sustained in a variety of ways. While observing the crab *Carcinus maenas* in the laboratory feeding on large *obtusata*, I noticed that many of the snails survived the attacks but had a large portion of their outer whorl missing. During an attack, the crab holds the shell with one claw and breaks successive pieces of the outer whorl with the other claw. This process is generally continued around the outer whorl until the body of the snail is exposed and then eaten. Often when the crab encountered the thicker parts of the whorl, further breakage was not possible. In these cases the snail was dropped and others attacked. In some situations, the snail was dropped during the initial handling phases and not picked up again by the crab. In

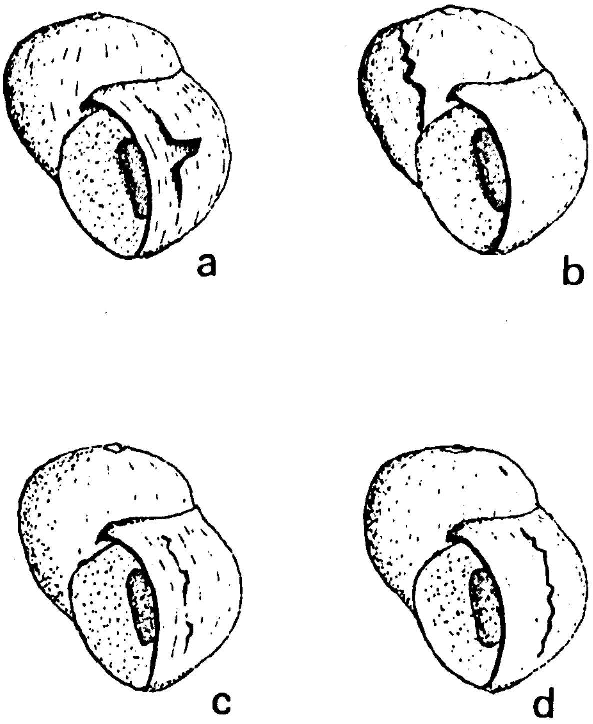


Fig. 15. Typical injuries on adult shells. a, major injury having occurred when winkle was approaching adult size; b, major injury at ½ adult size; c & d, minor injuries.

both of these situations, the snails eventually crawled back to the algae without apparent injury to the soft parts within. When the snails, that showed shell breakage, were removed and kept in holding tanks for 2 months, the missing parts of the shell were replaced leaving a scar very similar to that observed from individuals in the field. Of 238 attacks by the crabs in the laboratory, 124 (52.1%) were unsuccessful, although each resulted in some breakage to the shell. It is not unreasonable to expect that some of the snails attacked in the field might exhibit evidence of these unsuccessful attacks. It is known that the crabs Carcinus and Portunus prey extensively on the dogwhelk (Thais) in the field and often leave evidence of the injuries described above (Ebling et al., 1964).

Avian predators may account for some of the observed shell injuries (Moore, 1938). The oystercatcher Haematopus ostralegus is capable of breaking gastropod shells with its powerful beak (Dewar, 1913). Gulls (Larus spp.) also can be expected to cause some shell injuries as they are known to be predators on Littorina (Pettitt, 1971). The importance of these species as predators on L. obtusata and L. mariae is not known. At various times in three years of field observations on a large number of shores a watch was kept but no examples of predation by any species of bird on the two species of flat winkle have been recorded. It is likely, however, that many cases of predation have been overlooked. Estimates of the intensity of predation by birds are quite unreliable if the observer is himself obvious to shy species.

Shell injuries may also occur in the field from the actions of storms. The frequent injuries I have observed in L. saxatilis in some localities may be due to these. This species which is often found on small rocks may suffer injuries to the shell when the rocks are rolled against each other during storms. Even with the species found on the algal fronds (*L. obtusata* and *L. mariae*), injury may result from the lashing of the fronds against the underlying rocks. If this manner of injury is a common one, one expects that the highest injury rate should be found on shores with a great deal of wave action especially when there is a large number of small rocks and pebbles. In *L. obtusata* and *L. mariae* this is not the case because the samples with the highest frequency of injuries are found in the very sheltered localities with a sand or mud substrate (see Section IV 1, 3, (i)). Crabs are in fact much more abundant on these shores than on the more exposed ones (Ebling *et al.*, 1964).

Although all of the factors listed may contribute to the frequency of injured shells, it seems that the wide distribution of *Carcinus* on many of the shores sampled, and the similarity in shell breakage by crabs in the laboratory to the injuries found in the field, provide some reason to believe that the proportion of shell injuries in each sample does reflect in part the intensity of predation on *L. obtusata* and *L. mariae*. This suggestion has also been made by Crothers (1968) in his work on the biology of the shore crab, *Carcinus maenas*. By considering only the major injuries (fig 15a, b), I felt that some estimate of the influence of crab predators could be obtained.

In this study comparisons are made of the frequency of injuries with the following geographical areas, species, shore heights, colour phenotypes, and adult shell size. The results of these comparisons led to a series of predation experiments in the laboratory to determine the potential influence of crabs as selective agents on the shell morphology of the flat winkle.

2. Materials and Methods

All adult or near adult specimens collected in the geographical survey were scored for major injuries. In order to gain some understanding of variation up and down the shore a series of collections were made up and down the shore at 5 localities in Anglesey.

Comparisons were also made between shell colours and frequencies of injuries. As the number of injured shells in a single sample is often small, the different morphs were combined into two major classes: light-coloured shells and dark-coloured shells. The former includes citrina and inversicolour while the latter includes olivacea, light reticulata, and dark reticulata. The lumping into two classes is not a good technique as it may mask many differences present between phenotypes within one class. However, the two groups that have been used, represent what to the human eye appear as relatively conspicuous and inconspicuous shells against different background colours on the algae. To supplement small sample sizes, and give some indication of stability, 5 samples were made of L. obtusata between 1970 and 1972 at one locality (Menai Bridge) where

(1) the overall injury rate was high and

(2) both light- and dark-coloured shells were similar in abundance.

For the relationship with shell size, the total frequency of injuries was compared to the mean adult size of the males (from appendix B) in each population. The mean size, using both males and females was not used owing to the presence of a sexual dimorphism, the females being slightly larger than the males.

Any variation of the sex-ratio between the samples would therefore influence the mean shell size.

3. Results from Field Collections

(i) Geographical Comparison

Considering the four geographical areas (Wales, South England, Isles of Scilly, Ireland) there are very few differences between means and ranges of the frequencies of injured shells in both *L. obtusata* and *L. mariae* (table 6, see appendix C for raw data). When the two species are compared in each area with a paired t-test, there are no significant differences between the means.

Table 6: Geographical variation in the frequencies of injuries

AREA	SPECIES	NO. OF LOCALITIES	X	S	RANGE
Wales	*L. obtusata*	31	9.5	9.9	0 - 44
	L. mariae	25	9.6	9.7	0 - 44
South England	*L. obtusata*	9	11.8	13.9	0 - 37
	L. mariae	6	13.2	16.0	0 - 34
Isles of Scilly	*L. obtusata*	28	12.0	8.4	0 - 30
	L. mariae	18	13.4	9.1	0 - 30
Ireland	*L. obtusata*	17	12.8	10.5	2 - 47
	L. mariae	12	7.7	7.4	0 - 23

In Wales and South England, the highest frequencies of injuries in both species occur in sheltered bays and harbours, all of which have a mud or sand substrate and very little wave action (figs16 and 17). It is unlikely that the shell injuries in these localities are due to physical aspects of the habitat such as wave action on rocks. On the Isles of Scilly there is no such relationship with sheltered conditions. In L. obtusata, the injury rate is relatively constant from sheltered to exposed conditions except for 3 localities of intermediate exposure on St. Agnes (Cove, Pereglis, Killier, see fig 3) which have elevated frequencies. Samples of L. mariae from these localities were not obtained owing to conditions of neap tide at the time of collections; no comparisons are therefore possible. In the remaining localities in the Isles of Scilly, the injury rate of L. mariae is relatively high in intermediate exposure except for one very sheltered and one very exposed shore where it is also high. In Ireland, the sample of L. obtusata and L. mariae with elevated frequencies of injuries occur mainly in conditions of intermediate exposure, similar to the pattern observed in the Isles of Scilly.

It cannot be asserted that the changes in frequencies of injuries between localities are representative of different amounts of predation by crabs. It would first be necessary to have information on the density of crabs and their feeding behaviour in each locality to determine whether the number of injuries among living winkles reflects actual changes in the level of predation. The only observations of this kind that have been made by myself were in noting, during the geographical survey, when crabs were extremely abundant. When the field notes were reviewed, there were 7 localities in the British Isles where this occurred. They were the following: Cemaes Bay (Anglesey), Four Mile Bridge (Anglesey), Train Bridge (Anglesey),

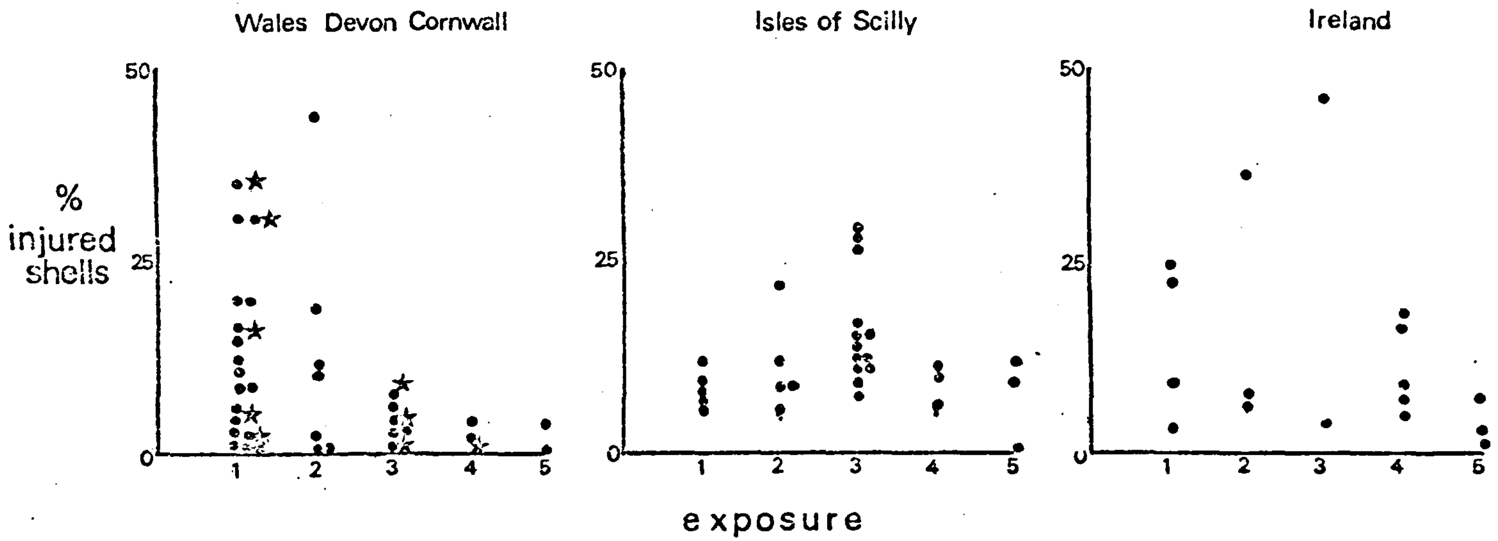


Fig. 16. Relation between exposure and per cent injured shells in L. obtusata. Values marked with star are localities from southern England. Raw data presented in Appendix C - table Ci.

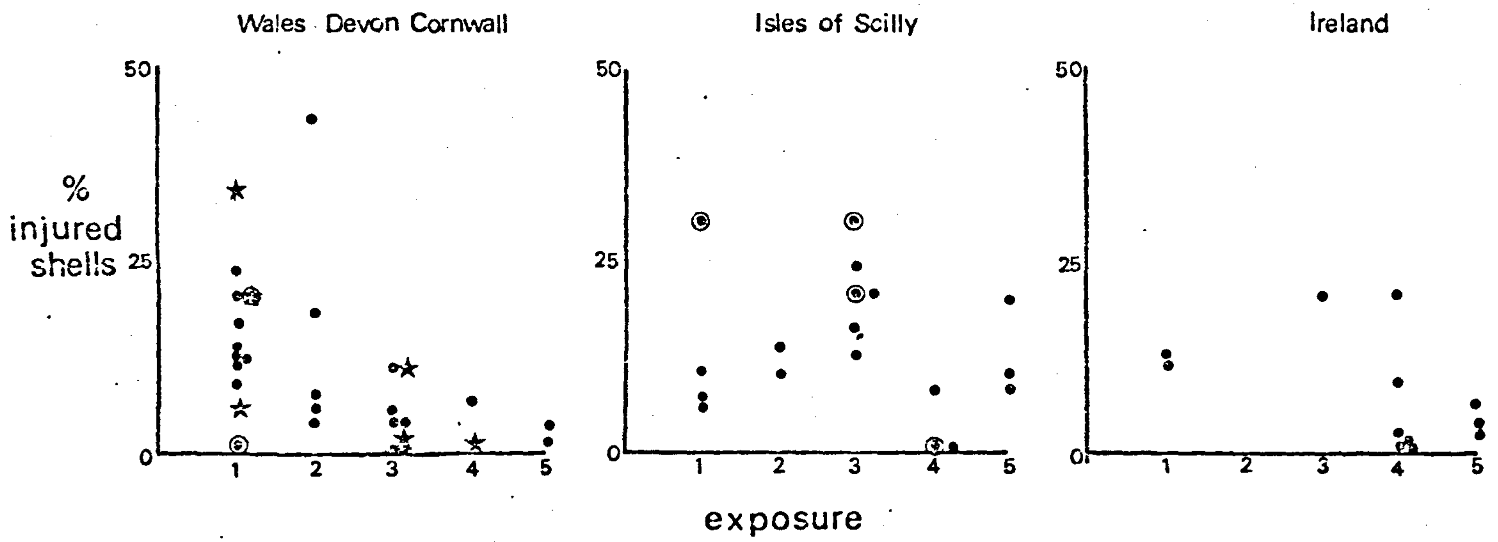


Fig. 17. Relation between exposure and per cent injured shells in L. mariae. Values marked with star are those localities from southern England while those marked with circle show samples with less than 10 individuals. Raw data in Appendix C - table Cii.

Newport (South Wales), Bodinnick (Cornwall), Porthloo (Isles of Scilly), Liscannor (Ireland). Examination of the shell injuries in these localities shows that in each case except one (Porthloo) the frequency of injuries were the highest observed in each of the geographical areas. This suggests very strongly that the number of injuries observed in at least some of the localities may be a function of the level of predation by crabs.

(ii) Variation within shores

The major observation that can be made from the collections at 5 localities in Anglesey is that in all cases there is a reduction in the number of injuries as the observer moves up the shore (fig 13,&table 7). Comparison of the injury rate between L. mariae and L. obtusata, irrespective of their position on the shore, demonstrates that in each of the 5 localities, L. mariae has more injuries than L. obtusata, although only in two (Porth Swtan, Sandy Bay) are the differences significant. These results are not unexpected in view of the vertical distribution of the two species, L. mariae being found most abundantly in the lower shore positions (see fig 14). When the two species are compared from the region of overlap on the shore, the frequency of injuries is not significantly different. There is, in fact, at Porth Swtan a slight excess of injuries on L. obtusata over L. mariae on both collecting dates in the region of overlap. At Sandy Bay, the same slight excess of injuries occurs at only one of the two collecting dates (table 7c). of the 3 remaining localities, (2 show a similar excess of injuries in L. obtusata in the region of overlap (Penrhynmawr, Cemaes Bay, table 7e and 7g), while the third (Causeway, table 7f), has a deficiency.

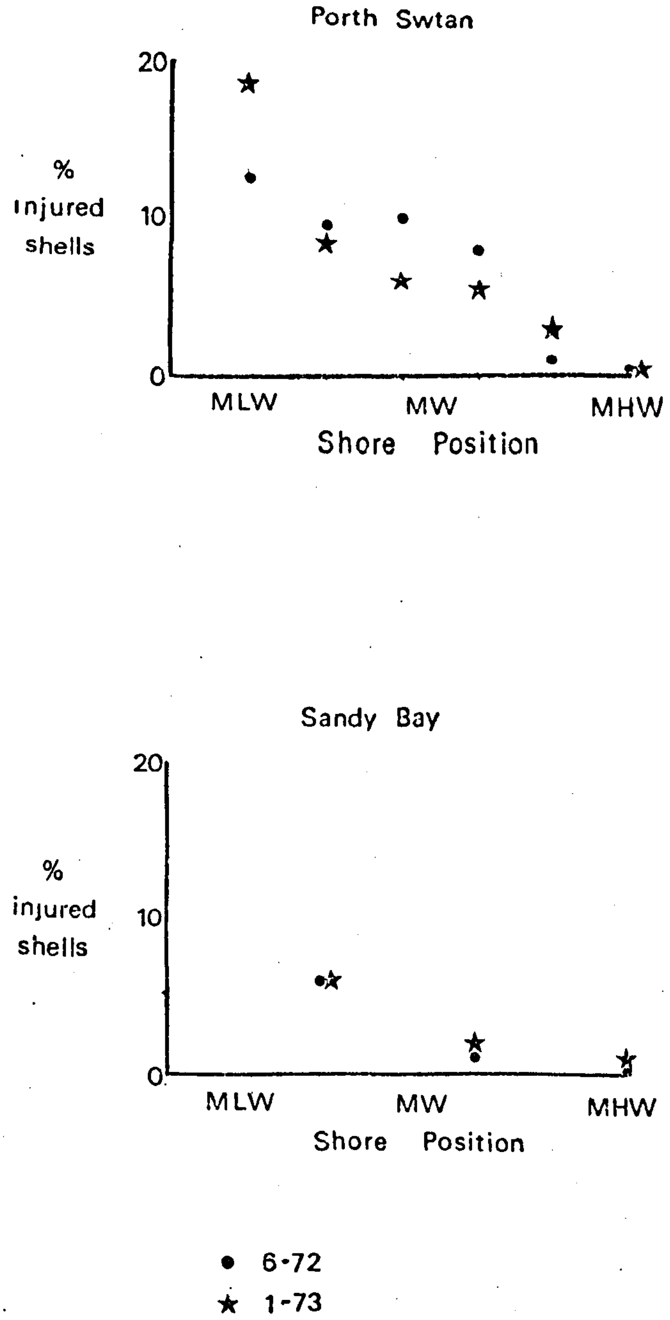


Fig. 18. Relation between shore height and per cent injured shells in two localities in Anglesey. Numbers for L. obtusata and L. mariae pooled in these two graphs (see Table 7a, b, c & d for actual numbers and comparison between species).

Table 7: Comparison of adult shell injuries in *L. mariae* and *L. obtusata* with different shore heights. Tests used are 2 x 2 chi-squared with Yates Correction (1 d.f.) and Fisher's Exact Probability.

Table 7a: Shell injuries from Porth Swtan in June, 1972.

Locality	Date	Shore Position	Species			
			L. mariae		*L. obtusata*	
			inj.	not inj.	inj.	not inj.
Porth Swtan	6/72	*F. spiralis*	0	0	1	230
		Ascophyllum	0	3	1	195
		F. vesiculosus	9	109	2	21
		F. serratus	10	96	4	26
		F. serratus	6	74	3	15
		Laminaria	6	39	0	0
		Total	31	321	11	487

χ^2 test between species (total injuries)

	inj.	not inj.
L. mariae	31	321
L. obtusata	11	487

$\chi^2 = 17.74$
$P < 0.001$

χ^2 test between species on *F. vesiculosus* and *F. serratus* (major region of overlap)

	inj.	not inj.
L. mariae	25	279
L. obtusata	9	62

$\chi^2 = 0.89$
N.S.

χ^2 test between upper and lower shore positions of *L. mariae*

	inj.	not inj.
upper shore	19	205
lower shore	12	113

$\chi^2 = 0.24$
N.S.

EXACT test between upper and lower shore positions of *L. obtusata*

	inj.	not inj.
upper shore	4	446
lower shore	7	41

EXACT P = .00001

Table 7b: Shell injuries from Porth Swtan in January, 1973

Locality	Date	Shore Position	Species			
			L. mariae		*L. obtusata*	
			inj.	not inj.	inj.	not inj.
Porth Swtan	1/73	*F. spiralis*	0	0	0	157
		Ascophyllum	0	7	5	150
		F. vesiculosus	7	64	1	67
		F. serratus	5	111	3	10
		F. serratus	8	89	0	0
		Laminaria	11	47	0	0
		Total	31	318	9	384

χ^2 test between species (total injuries)

	inj.	not inj.
L. mariae	31	318
L. obtusata	9	384

$\chi^2 = 14.49$
$P<0.001$

EXACT test between species on *F. vesiculosus* and *F. serratus*

	inj.	not inj.
L. mariae	12	175
L. obtusata	4	77

EXACT P = 0.78

χ^2 test between upper and lower shore positions of *L. mariae*

	inj.	not inj.
upper shore	7	71
lower shore	24	247

$\chi^2 = 0.00$
N.S.

EXACT test between upper and lower shore positions of *L. obtusata*

	inj.	not inj.
upper shore	6	347
lower shore	3	10

EXACT P = 0.003

Table 7c: Shell injuries from Sandy Bay in June, 1972

Locality	Date	Shore Position	Species			
			L. mariae		*L. obtusata*	
			inj.	not inj.	inj.	not inj.
Sandy Bay	6/72	*F. spiralis*	0	0	4	791
		F. vesiculosus	0	0	3	363
		F. serratus	21	375	12	170
		Total	21	375	19	1324

χ^2 test between species (total injuries)

	inj.	not inj.
L. mariae	21	375
L. obtusata	19	1324

$\chi^2 = 18.9$
$P < 0.001$

χ^2 test between species on *F. serratus*

	inj.	not inj.
L. mariae	21	375
L. obtusata	12	170

$\chi^2 = 0.18$
N.S.

EXACT test between upper and lower shore positions in *L. obtusata*

	inj.	not inj.
upper shore	7	1154
lower shore	12	170

EXACT $P = 0.0000005$

Table 7d: Shell injuries from Sandy Bay in January, 1973

Locality	Date	Shore Position	Species			
			L. mariae		*L. obtusata*	
			inj.	not inj.	inj.	not inj.
Sandy Bay	1/73	*F. spiralis*	0	0	5	435
		F. vesiculosus	0	0	4	217
		F. serratus	46	606	7	140
		Total	46	606	16	792

χ^2 test between species (total injuries)

	inj.	not inj.
L. mariae	46	606
L. obtusata	16	792

$\chi^2 = 10.81$
$P < 0.01$

χ^2 test between species on *F. serratus*

	inj.	not inj.
L. mariae	46	606
L. obtusata	7	140

$\chi^2 = 0.69$
N.S.

EXACT test between upper and lower shore position in *L. obtusata*

	inj.	not inj.
upper shore	9	652
lower shore	7	149

EXACT P = 0.02

Table 7e: Shell injuries from Penrhynmawr

Locality	Date	Shore Position	Species			
			L. mariae		*L. obtusata*	
			inj.	not inj.	inj.	not inj.
Penrhynmawr	6/72	*Ascophyllum*	0	0	2	493
		F. serratus	6	139	7	71
		Total	6	139	9	564

EXACT test between species (total injuries)

	inj.	not inj.
L. mariae	6	139
L. obtusata	9	564

EXACT P = 0.09

EXACT test between species on *F. serratus*

	inj.	not inj.
L. mariae	6	139
L. obtusata	7	71

EXACT P = 0.09

EXACT test between upper and lower shore positions of *L. obtusata*

	inj.	not inj.
upper shore	2	493
lower shore	7	71

EXACT P = .00002

Table 7f: Shell injuries from Causeway

Locality	Date	Shore Position	Species			
			L. mariae		*L. obtusata*	
			inj.	not inj.	inj.	not inj.
Causeway	6/72	*Ascophyllum*	0	0	12	162
		F. serratus	5	21	12	92
		Total	5	21	24	254

EXACT test between species (total injuries)

	inj.	not inj.
L. mariae	5	21
L. obtusata	24	254

EXACT P = 0.09

EXACT test between species on *F. serratus*

	inj.	not inj.
L. mariae	5	21
L. obtusata	12	92

EXACT P = 0.33

χ^2 test between upper and lower shore positions of *L. obtusata*

	inj.	not inj.
upper shore	12	162
lower shore	12	92

$\chi^2 = 1.54$
N.S.

Table 7g: Shell injuries from Cemaes Bay

Locality	Date	Shore Position	Species			
			L. mariae		*L. obtusata*	
			inj.	not inj.	inj.	not inj.
Cemaes Bay	6/72	*Ascophyllum*	0	0	82	110
		F. serratus	77	90	25	25
		Total	77	90	107	135

χ^2 test between species (total injuries)

	inj.	not inj.
L. mariae	77	90
L. obtusata	107	135

$\chi^2 = 0.077$
N.S.

χ^2 test between species on *F. serratus*

	inj.	not inj.
L. mariae	77	90
L. obtusata	25	25

$\chi^2 = 0.01$
N.S.

χ^2 test between upper and lower shore positions of *L. obtusata*

	inj.	not inj.
upper shore	82	110
lower shore	25	25

$\chi^2 = 0.59$
N.S.

The amount of difference between the injury rate of the two species at each locality irrespective of position shows some relationship with the amount of exposure at that locality. Of the 5 localities just discussed, the 3 which showed no significant difference between the species (tables 7e, 7f and 7g) were all in very sheltered conditions, while the remaining 2 localities which showed a significant excess of injuries in L. mariae were both in conditions of intermediate exposure (tables 7a, 7b, 7c and 7d). These differences can be accounted for in terms of the amount of algal cover up and down the shore; this influences the height to which Carcinus are generally found on most shores. Although my own observations (made predominantly in summer) are anecdotal, it seemed that when the fucoids were continuous from the lower to the upper regions of the shore, Carcinus was similar in abundance at all levels. When the fucoids were only sparsely present, Carcinus was more abundant on the lower levels and very rare on the upper regions. This distribution also applied to Cancer which is generally found lower on the shore than Carcinus.

These distributions are not unexpected owing to the problems of desiccation and susceptibility to predation by birds which crabs encounter on the upper levels of the shore when no fucoids are present for cover. It is in the sheltered conditions that one often finds a continuous cover of fucoids and a similarity in the injuries between the species while in more exposed conditions one finds a reduction in the fucoid cover and an excess of injuries in L. mariae. The distribution of the crabs as they relate to the frequency of shell injuries in the two species requires a great deal more investigation before the observed relationships can be fully understood.

During the final two months of this study on the siblings, it was in some cases possible to divide L. *mariae* itself into two groups ("dwarf" and "normal"), both living on the same shore, and make comparisons between the injuries. Three localities from Anglesey were chosen where the two forms were relatively discrete in morphology. The major morphological difference between the two is the size of the adult shell, the "dwarf" having a mean size of 8mm and the "normal" a size of 11mm.

The results of the comparison in shell injuries between the two is given in table 8. In 2 of the 3 localities the "dwarf" has significantly more injuries than the "normal" while in the 3rd locality there is a similar excess which is, however, not significant.

These two groups are found on F. *serratus* at the same level on the shore suggesting that the actual intertidal position is not the only factor for consideration as was initially noted for the frequency of injuries between L. *obtusata* and L. *mariae*.

Table 8: Number of injuries in small-shelled "dwarf" and large-shelled "normal" forms of L. *mariae*. Collections of these forms made within 5 meter radius.

Locality	Date	Group	Shell Injuries	
			inj.	not inj.
Sandy Bay	4/73	"normal"	78	385
		"dwarf"	75	227
			$\chi^2 = 6.79$	$P < 0.01$
Hen Borth	4/73	"normal"	12	68
		"dwarf"	23	53
			$\chi^2 = 4.38$	$P < 0.05$
Cliff Hotel	4/73	"normal"	64	132
		"dwarf"	51	96
			$\chi^2 = 0.78$	N.S.

As these two groups are found at the same level on the shore, the difference in the numbers of injuries is not likely to be due to the relative abundance of the crabs. The differences may reflect the crabs' success in predating the small-shelled "dwarf" compared to the larger "normal" (see Section on predation experiments). However differences in the microdistribution of the two groups in the field suggest that the "dwarf" and "normal" individuals are subjected to different kinds of predation by crabs.

"Dwarf" individuals are generally found on F. serratus, occurring in run-off channels, from the upper tidal positions. In these channels, the substrate is often sand and silt, and the individual F. serratus plants are sparse occurring as isolated clumps. "Normal" individuals of L. mariae occur also on F. serratus but on those plants which are present on rocky substrates with little silt. These two microhabitats occur within 1m meter of each other. Although only a few observations have been made on the crabs in these two positions, they indicate that there is an interaction between microhabitats (silty vs. rocky) and the average size of crabs present. In the silty area, occupied by the "dwarfs", many small-sized crabs were seen while in the rocky area, occupied by the "normals", both small and large crabs were seen.

These anecdotal observations on the size of the crabs show that one should not consider only the amount of predation judged by the number of shell injuries. Following the predation experiments (see Section IV, 4) it will be argued that many of the morphological differences between L. obtusata and L. mariae and those within the latter species are attributable to predation by crabs having different sizes. Before this however, the relationship between shell injuries and shell colour is discussed.

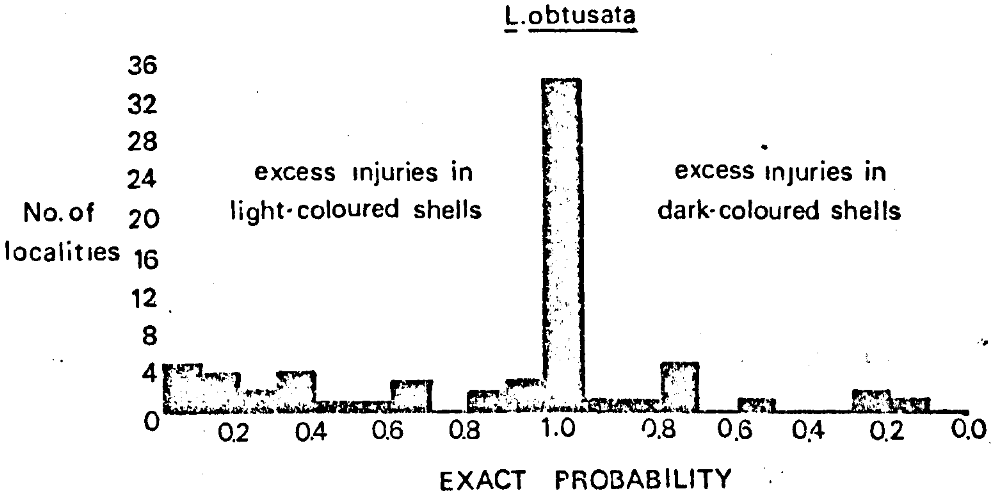
(iii) Relationship between shell colour and injuries

(a) Introduction

The record of shell injuries on living individuals allows a comparison to be made between the susceptibility of shells differing in colour. It thus becomes possible to study differences in the life histories between the morphs. Such studies could provide some understanding of the way in which the colour polymorphism is maintained. Unlike the examination of broken shells of *Cepaea* around thrush anvils, which has provided *direct* evidence of selective predation (Sheppard, 1951), the examination of living individuals with injured shells provides evidence only of some forms of *attempted* predation. Such an examination does not give the effective predation and thus has nothing to say about real selective differences which may exist between the morphs. However, if the assumption is made that the ability to escape after an attack is independent of shell colour, one can assume that any differences observed in the frequency of injuries between morphs is a direct indication of the selective response of the predator to the different colour morphs. It must be remembered that this comparison provides some information on only one of many possible species of predators, and not necessarily the most important one. Despite these difficulties in interpretation, the comparison of morphs legitimately provides some additional information on the complex life-history of each individual.

(b) *L. obtusata*

Of the 70 localities where comparisons were possible between the injuries of the two colour groups, only 2 showed significant differences ($P < 0.05$) between the groups. With this number of comparisons, one expects 5% or 3 localities to show significance at this level by chance. However the two localities (Porth Cwtan, no. 7; Looe, no. 41) both show an excess of injuries in the light-coloured shells. When one examines the probability calculations of the remaining localities, there is a general tendency for the light-coloured shells considered locality by locality to have an excess of injuries also. This can be seen in a probability histogram (fig 19) in which the horizontal axis is composed of a probability scales from 0-1-0. The estimate from each sample is plotted on the axis according to whether there is an excess or deficiency of injuries in the light-coloured shells. The left hand probability scale represents those localities with an excess of injuries in the light-coloured shells and the right hand scale those localities with a corresponding deficiency. This method of comparing localities is intended to provide only a visual presentation of the probability distributions. It can be seen that of the 70 localities presented, 25 show an excess of light-coloured shell injuries, 34 show equal frequencies of injuries, and 11 show a deficiency of injuries in light-coloured shells. Considering those samples with probabilities greater than 0.5 but less than 1.0 the number of localities on the left and right hand scales are similar with 9 showing an excess and 8 a deficiency of injuries in the light coloured shells. If one considers the lower probabilities of 0.5 or less, there are 16 localities with an excess and only 3 with a deficiency. Calculation of a goodness-of-fit test for the difference in the numbers of these localities gives a χ^2 value of 7.5 ($P < 0.01$). If these comparisons are meaningful, there is then reason to suspect that the light-



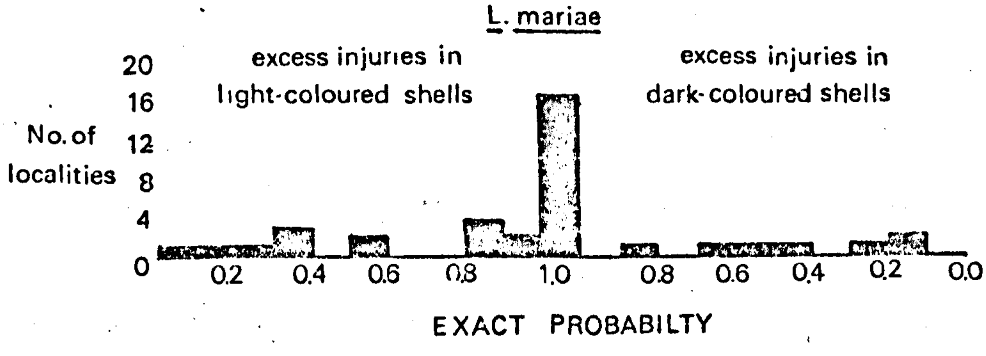


Fig. 19. Relation between shell injuries and shell colour. Exact probabilities calculated for each locality from data in Appendix C. Histograms show number of localities having a given probability on the horizontal axis. Localities grouped into probabilities of 0.1 units.

coloured or apparently conspicuous shells are on average, considered locality by locality, slightly more heavily predated by crabs than the dark shells. When all localities are pooled and the totals taken (appendix C) there are however no significant differences between the two colour classes.

The two samples from April and May (1972) both show a deficiency in light-coloured shell injuries; out of a total of 5 samples, these deficiencies would be expected by chance. However, one characteristic of these two samples, which separates them from all others in the shell injury analysis, is that they were composed entirely of juvenile shells (about ½ adult size) while all other samples had only adult or near adult sized individuals. More extensive studies of this kind may provide evidence for differences in selective coefficients of the shell colour among the different age classes.

Table 9: Occurrence of injuries in light- and dark-coloured shells of *L. obtusata* at Menai Bridge

Date	Light Shells		Dark Shells		χ^2	P
	inj.	not inj.	inj.	not inj.		
Oct. 1970	133	237	127	334	6.5	0.01-0.025
May 1971	55	55	168	207	0.73	0.25-0.50
July 1971	49	127	80	256	0.79	0.25-0.50
April 1972	7	35	26	96	0.18	0.50-0.75
May 1972	5	158	16	297	0.63	0.25-0.50
Total	249	612	417	1190		

Sum of $\chi^2_{[5]} \doteq 8.83$ 0.10-0.25

Pooled $\chi^2_{[1]} = 2.36$ 0.10-0.25

Heterogeneity $\chi^2_{[4]} = 6.47$ 0.10-0.25

In summary, the frequencies of injuries between light and dark-coloured shells are similar over most of the study area. However, in the 19 localities where comparisons of the shell colours gave probabilities less than 0.5, sixteen of these showed an excess of injuries to the light-coloured shells. The localities in which the largest samples were obtained (North Swtan, no. 7; Menai Bridge, no. 25; Looe, no. 41) all had significant ($P < 0.05$) differences between the shell colours with the light-coloured shells showing an excess of injuries. More extensive studies on this subject are required to determine whether these differences reflect real changes in the selective coefficients of the shell colours and if these differences have a relationship to the age or size of the shells.

(c) L. mariae

In this species, of the 38 localities where comparisons are possible between the two shell colours, only 1 (st. Martin's Head), has significant differences at the 0.05 probability level. One expects this by chance but it is interesting to note that the light-coloured shells are again represented in a higher frequency than expected (appendix C).

In the distribution of the probabilities (fig 19), 14 localities have an excess of light shell injuries, 17 have no difference, and 7 have a deficiency. The number of localities with an excess and a deficiency are not significantly different ($\chi^2 = 1.5$; $P = 0.1$-0.25). Considering the probabilities less than 0.5, there are 6 localities with an excess of light shells injuries and 4 with a deficiency. It would seem therefore that from the geographical survey, L. mariae does not show the same pattern as L. obtusata, in which the light shells were slightly more injured in a significantly greater number of localities. When considering all the localities together for L. mariae, there is, unexpectedly, a significant excess of injuries in the light-coloured shells ($\chi^2 = 17.0$; $P < 0.001$). These results reflect the fact that most of the large samples in this species, or in other words those which contribute most to the totals, have been collected from sheltered localities. In these, not only are the frequencies of injuries high in a sample but also the frequency of light-coloured shells is high (see Appendix C). These two factors therefore lead to an apparent excess of light-coloured shell injuries when lumping all the localities. The differences observed are, therefore, not meaningful for comparing light and dark shells within a population.

At Porth Swtan and Sandy Bay, each where 3 samples were collected between July 1971 and March 1972 there is in each case an excess of light-coloured shell injuries, though only 2 approach formal significance (table 10). The heterogeneity chi-square is not significant allowing each of the 3 samples to be pooled. When this is done, there is, at Porth Swtan, a significant difference ($P < 0.05$) in the numbers of injuries between the light-coloured and dark-coloured shells with the former being more common than the latter. At Sandy Bay, the differences are not significant ($P = 0.10-0.25$).

(d) Discussion

If the greater number of injuries on the light-coloured shells in some localities for both L. obtusata and L. mariae does reflect a greater amount of predation by crabs, there must be some compensating advantage to preserve these colour phenotypes in the population. When one compares numbers of injuries between the

Table 10: Occurrence of injuries in light- and dark-coloured shells in *L. mariae* at Porth Swtan and Sandy Bay.

Locality	Date	Light Shells		Dark Shells		χ^2	P
		inj.	not inj.	inj.	not inj.		
Porth Swtan	6/72	9	45	22	276	3.81	0.05–0.10
	1/73	7	50	24	268	0.53	0.25–0.50
	3/72	12	49	57	374	1.34	0.25–0.50
	Totals	28	144	103	918		
Sandy Bay	6/72	8	92	13	283	1.29	0.25–0.50
	1/72	25	286	21	320	0.61	0.25–0.50
	3/72	22	70	56	315	3.49	0.05–0.10
	Totals	55	448	90	918		

<u>Porth Swtan</u>	P
sum of $\chi^2_{[3]} = 5.68$	0.10–0.25
Pooled $\chi^2_{[1]} = 5.16$	0.01–0.025
Heterogeneity $\chi^2_{[2]} = 0.52$	0.90–0.95

<u>Sandy Bay</u>	
sum of $\chi^2_{[3]} = 5.39$	0.10–0.25
Pooled $\chi^2_{[1]} = 1.33$	0.10–0.25
Heterogeneity $\chi^2_{[2]} = 4.06$	0.10–0.25

two classes of shell colour within each species with the frequencies of each class in the population there does not appear to be a relationship. In L. obtusata the three localities which showed a significant excess of injuries in the light shells were Porth Swtan, Menai Bridge, and Looe. The frequencies of the light coloured shells in the population are 1.6%, 40% and 27% respectively. The mean frequency of the light shells throughout the study area is 12.9%. In L. mariae the only two localities with probability levels less than 0.10 were Porth Swtan and St. Martin's Head, with light shell frequencies of 10% and 6% respectively. The mean frequency in this species of the light-coloured shells is 47%. If the light-coloured shells are being maintained by frequency-dependent selection one would expect that the relative number of injuries would be reduced when there is a low frequency of light shells in the population. From the results observed in this comparison, this does not seem to be the case. It would be necessary however to consider the injuries on the very small or juvenile stages of the different colour classes. This may show that there is an advantage at this stage if low frequencies of the phenotype are present in the population.

(iv) Adult shell size and injuries

(a) Introduction

Variation in shell size of marine molluscs has been widely studied by numerous workers. Some of the species include Littorina picta (Struhsaker, 1967), L. saxatilis (James, 1968; Fischer-Piette et al. 1968), L. neritoides (Natale and Sacchi, 1964), L. scutulata and L. planaxis (North, 1954), Thais lapillus (Kitching et al., 1966; Largen, 1971; Crothers, 1973) and

Zoila friendi (Wilson and Summers, 1966). Various explanations have been offered to account for the geographical variation in shell size and shape. These include linkage, pleiotropy, non-adaptive variation, stunted and optimal growth, light conditions, historical effects, desiccation, wave action and predators. In the majority of cases evidence to support these explanations rests mainly on inferences from correlation. Evidence to be presented in this study demonstrates that the amount of predation by crabs, as deduced from the number of shell injuries, may be of great importance in influencing the shell size and shape of intertidal gastropods.

(b) L. obtusata

In L. obtusata, there are significant positive correlations between the frequencies of all injuries and the mean adult shell size in a sample (fig 20). In the area of Wales and southern England and in Ireland calculation of a t-test for the slope of the regression line gives probabilities of less than 0.001 and 0.05 respectively. On the Isles of Scilly, no trend is evident (P = 0.30-0.40). Sacchi (1969) has interpreted the large size of L. obtusata in some localities as an indication of optimal growth conditions. It would seem however that the relationship observed with shell injuries provides another variable in interpreting the significance of shell size.

In each of the geographical areas there are differences in the mean shell size between localities (14.5-17.0mm) without any observed changes in the frequencies of injuries. The rule, however, is that when the number of injuries are high, i.e. above 10%. then large shells are usually found.

These observations from the geographical survey have a

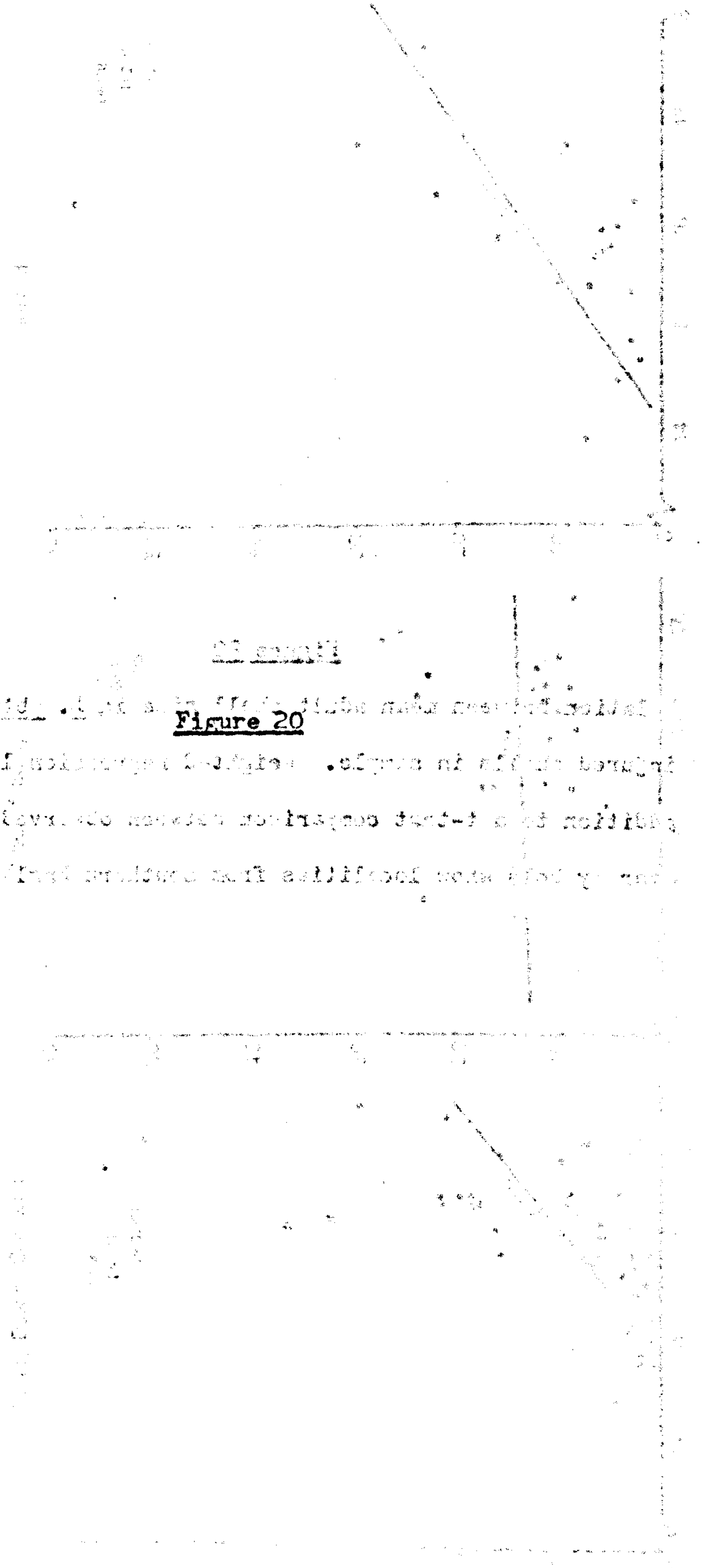
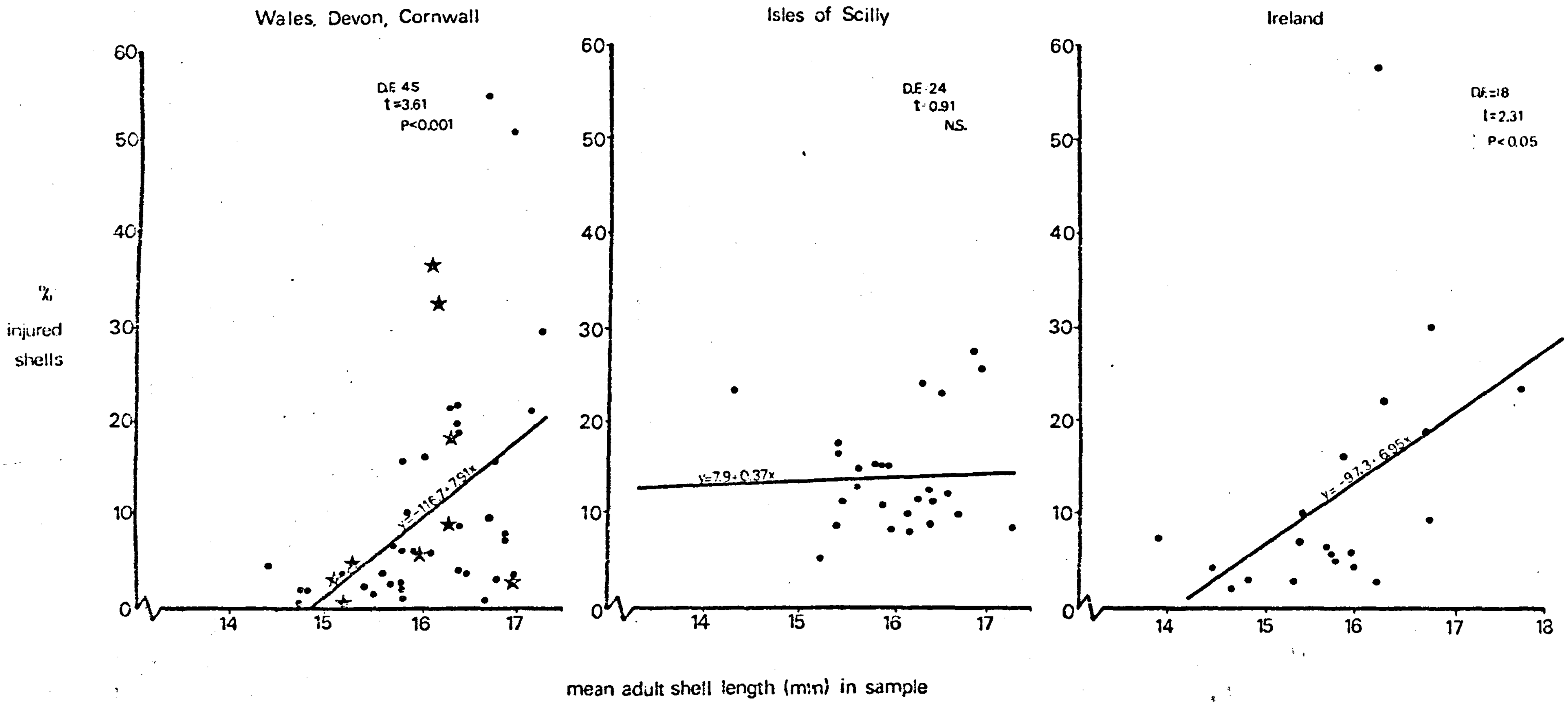


Figure 20

Figure 20

Relation between mean adult shell size in L. obtusata and percent injured shells in sample. Weighted regression lines are shown in addition to a t-test comparison between observed and zero slopes. Star symbols show localities from southern England.

L. obtusata


Wales, Devon, Cornwall
Isles of Scilly
Ireland
%
injured
shells
60
50
40
30
20
10
0
14
15
16
17
18
D.F. 45
t=3.61
P<0.001
y=-116.7+7.91x
D.F. 24
t=0.91
N.S.
y=7.9+0.37x
D.F.=18
t=2.31
P<0.05
y=-97.3+6.95x
mean adult shell length (mm) in sample

similar pattern to what is observed within shores (table 11). In L. obtusata there is an increase in the mean adult shell size from the upper to the lower positions on the shore. Over this vertical range, there is also a corresponding increase in the frequencies of injured shells (table 7). In one of the localities (Cemaes Bay, 7g) where the overall number of injuries is very high in both positions on the shore, not only is the mean adult shell size in the locality as a whole very large, but also there is little difference between the shell size in the upper and lower positions. It appears, therefore, that in both comparisons (between shores and within shores) there is a tendency for populations of L. obtusata that have a high level of predation by crabs (judged from the frequency of shell injuries) to have large adult shell sizes.

Table 11: Comparison of adult shell size on upper and lower shore positions with the frequency of injuries

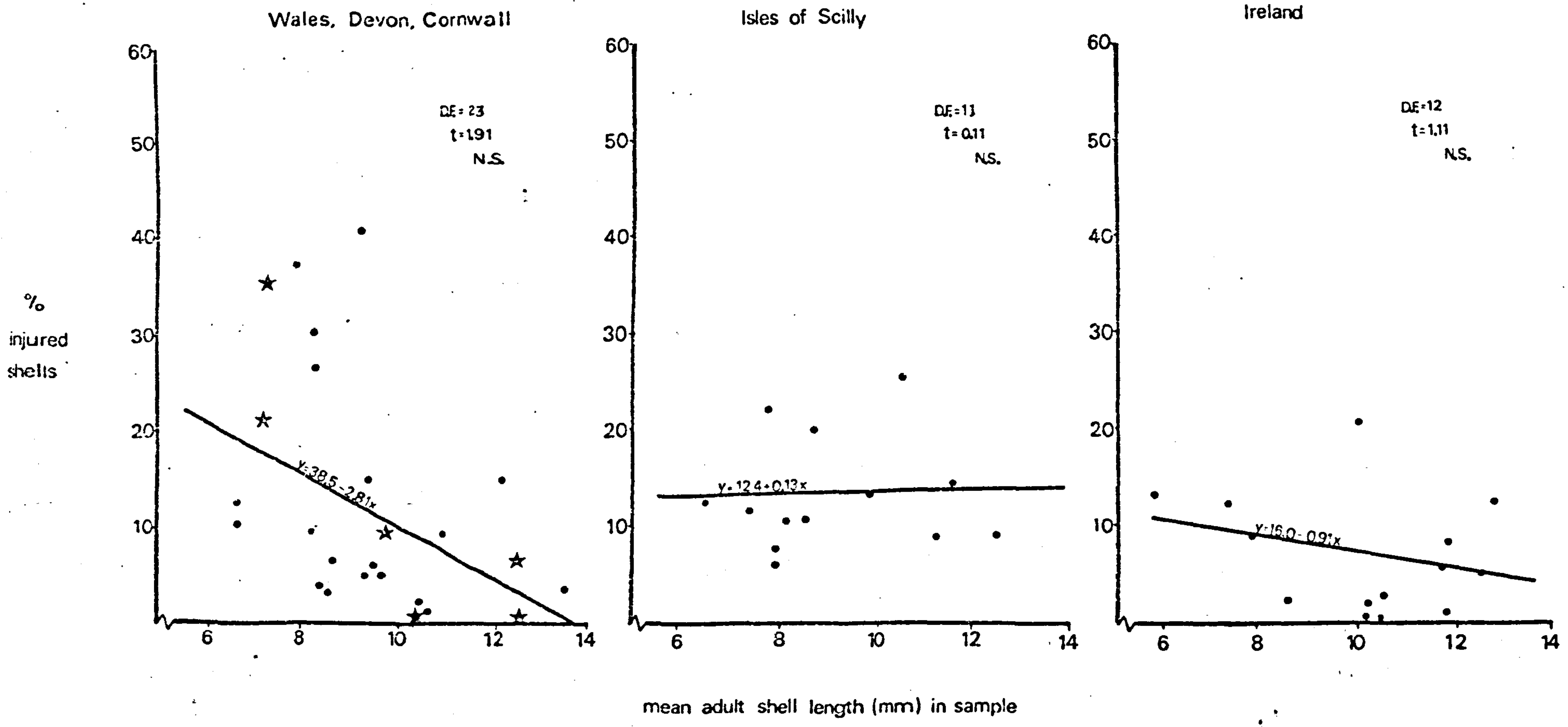
Locality	Tidal Height	N	Mean Adult Shell size (mm)	S.D.	% injuries
Porth Swtan	upper shore	231	15.1	0.45	0.4
	lower shore	48	16.6	0.53	14.6
Sandy Bay	upper shore	200	13.3	0.51	0.5
	lower shore	182	16.0	0.87	6.5
Hen Borth	upper shore	117	14.6	0.69	0.0
	lower shore	69	16.1	0.72	8.0
Cemaes Bay	upper shore	30	16.6	0.83	42.7
	lower shore	30	16.9	1.18	49.5

Figure 21

Figure 21

Relation between mean adult shell size in *L. mariae* and percent injured shells. Weighted regression and t-test shown (as in fig 20). Star symbols show localities from southern England.

L. mariae


Wales, Devon, Cornwall
Isles of Scilly
Ireland
%
injured
shells
60
50
40
30
20
10
6
8
10
12
14
D.F.=23
t=1.91
N.S.
y=38.5-2.81x
D.F.=11
t=0.11
N.S.
y=12.4+0.13x
D.F.=12
t=1.11
N.S.
y=16.0-0.91x
mean adult shell length (mm) in sample

(c) L. *mariae*

In this species there is only a slight relationship between size and injuries. Rather than showing a positive relationship, as observed in L. *obtusata*, there is a negative relationship such that high frequencies of injuries are associated with a decrease in the mean adult size (fig 21). The slope of the regression lines is not significant in each of the three geographical areas.

In L. *mariae* comparisons similar to those made in L. *obtusata* within shores are not possible owing to the narrow vertical range that this species occupies. However the occurrence together at the same level on the shore of the small shelled "dwarf" and the large shelled "normal" permit the demonstration of the same relationship between injuries and shell size as was observed in the geographical survey. That is, high injury rates were associated with decreased shell size, the opposite of L. *obtusata*. It has already been shown (table 8) that the "dwarf" has in two of three localities significantly more injuries than the "normal" on the same shore. The mean shell size of the "dwarf" is from 7.5-9.2mm in the three localities while the "normal" varies from 11.5-12.6mm. There is again, as in the geographical survey, a tendency for populations of L. *mariae* that have a large number of injured shells to have very small sized individuals, exactly the opposite to that observed in L. *obtusata*.

4. Predation Experiments

(i) Introduction

The correlations observed in this study between mean shell size and the frequency of injuries does not itself imply any causal relationship between the two. There may be many other factors influencing the size such as selection by wave action, optimal level of egg production, desiccation, isolating mechanisms etc. However the close relationship observed in *L. obtusata* and to a lesser extent in *L. mariae* between the shell size and frequency of injuries suggests that the amount of predation by crabs may be an important selective force on the mean shell size in the population. If this is the case then it would be reasonable to expect the two species to show a response in the same direction. This does not occur, for *L. obtusata* becomes larger and *L. mariae* smaller in localities where the frequency of injuries is high. To test some of the relationships which have been observed, a series of predation experiments were set up to determine whether individuals of different shell sizes would have differential abilities to withstand predation by crabs. The secondary purpose of the experiments was to determine whether the crabs would prey preferentially on either the light- or dark-coloured shells.

(ii) Materials and Methods

Collections of *Carcinus maenus* and *Cancer pagurus* were obtained from the shore at Sandy Bay, Anglesey. The crabs were placed in moist seaweed and returned to the laboratory where they were kept in circulating seawater at 12°C for a period of 3 days without feeding before the commencement of the experiments. Individuals of *Carcinus* were divided into 3 length categories (1.5-2.5cm; 4.0-6.0cm; 9.0-11.0cm) and placed in separate 20 litre glass tanks. For each length category, two replicate tanks were used each containing 3 individuals of the same length class. With *Cancer*, only 2 length classes were available (4.0-6.0cm; 14.0-18.0cm). Three individuals of the smaller length class were placed in one 20 litre glass tank, while of the larger length class, 3 individuals were placed in a 200 litre fibre glass tank. In all the tanks the water was circulated through a glass wool filter and kept at 10-13°C with cooling units.

The Littorina were obtained also from Sandy Bay, Anglesey, returned to the laboratory and kept in holding tanks for the experiments. The following 5 groups of Littorina were used in multiple-group predation experiments:

L. mariae ("dwarf") adults from lower shore
L. mariae ("normal") adults from lower shore
L. obtusata adults from lower shore
L. obtusata adults from upper shore
L. obtusata juveniles from mid-shore

These were also used in the two-group experiments with the addition of another class-juvenile "normal" mariae.

Five individuals from each group were placed in the bottom of each tank with the crabs. To stop the snails from crawling off the bottom, sand was placed around the periphery of each tank. Guiterman (1971) had found this method to be an effective way of keeping the snails within reach of the crabs. As soon as the snails had been added the crabs generally began to feed. The animals were then left undisturbed for 12 hours after which time the remaining snails were removed and scored. This cycle was repeated for 3 days with the 1.5-2.5cm crabs and for 7 days with the larger size classes. To differentiate the lower and upper shore adult L. obtusata, a small inconspicuous file mark was made on each individual on the apex in opposite directions relative to the position of the aperture. These experiments led to a further set which involvdd comparing only 2 rather than 5 groups in each experimental tank. In some of the comparisons a possibility existed that the observed differential rate of attack could be due to a chemical released by the Littorina which cause irritation to the crab. To overcome this, the bodies of the Littorina were removed by boiling and replaced by a small piece of beef liver which was forced into the shell.

In none of these experiments did Cancer predate any individuals, although they were seen doing so in field observations. For the discussion of the experiments, this species has not been included.

(iii) Results

(a) Multiple-Choice Experiments

The summarized data involving the comparison of the 5 groups at one time are presented in table 12. The major observation that can be made from these experiments is that the crabs are more successful at predating some of the groups and that the success is a function of the size of the crab.

Carcinus of the smallest length classes (Tanks 1, & 2) were successful in predating only the juvenile obtusata. During observations on the feeding behaviour, the small crabs were seen handling the other groups as well, but were unsuccessful in either cracking or breaking the shells. The "dwarf" mariae are of the same shell diameter as the juvenile obtusata (6-8mm) but differed in having a relatively thick lip. The crabs attacking the dwarf mariae were seen to be unable to crack the lip but readily did so with the thin-lipped obtusata. When the juveniles were removed from the tanks, and the small crabs were

Table 12: Results of successful predation by *Carcinus* on different groups of *Littorina*.

Size of Predator	Tank		*Littorina* Group*				
			ma (dw)	ma (nor)	ob (juv)	ob (low)	ob (up)
1.5-2.5cm	1	added	20	20	20	20	20
		eaten	0	0	9(45.0)	0	0
	2	added	20	20	20	20	20
		eaten	0	0	8(40.0)	0	0
4.0-6.0cm	3	added	75	75	75	75	75
		eaten	26(34.7)	0	47(62.7)	0	0
	4	added	75	75	75	75	75
		eaten	21(28.0)	2(2.7)	45(60.0)	0	1(1.3)
9.0-11.0cm	5	added	75	75	75	75	75
		eaten	67(89.3)	30(40.0)	55(73.3)	2(2.7)	55(73.3)
	6	added	75	75	75	75	75
		eaten	62(82.7)	40(53.3)	67(89.3)	1(1.3)	40(53.3)

* ma (dw) - *L. mariae* ("dwarf") adult, size range 6-8mm

ma (nor) - *L. mariae* ("normal") adult, size range 10-13mm

ob (juv) - *L. obtusata* juveniles from shore, size range 6-8mm

ob (low) - *L. obtusata* adults from lower shore, size range 16-17mm

ob (up) - *L. obtusata* adults from upper shore, size range 13-15mm

( ) - bracketed numbers are percentages

offered only the other groups, there was a great deal of attempted predation but in no cases were the attempts successful.

The intermediate sized crabs (Tank 3, 4) preyed predominantly on juvenile *obtusata* and to a lesser extent on "dwarf" *mariae*. In Tank 3, 64.4% of the predated shells were juvenile *obtusata* and 35.6% "dwarf" *mariae*. In Tank 4, the frequencies of the two were 65.2% and 30.4% respectively; the remainder were 2.7% normal *mariae* and 1.5% upper shore *obtusata*. In neither of the two tanks were any of the lower shore adult *obtusata* successfully predated. The different groups can be ranked according to their decreased susceptibility to predation by crabs. They are as follows: juvenile *obtusata* 64.8%, "dwarf" *mariae* 33.5%, "normal" *mariae* 1.0%, upper shore *obtusata* 0.8%, lower shore *obtusata* 0.0%.

The crabs of the largest sizes (Tank 5, 6) also preyed predominantly on the juvenile *obtusata* and "dwarf" *mariae*. However, whereas the three remaining groups were relatively immune from predation by the smaller crabs, two of the groups ("normal" *mariae* and upper shore *obtusata*) now constitute relatively large numbers of predated individuals. The mean frequencies of each group in the predated shells are: dwarf *mariae* 30.8%. juvenile *obtusata* 29.1%, upper shore *obtusata* 22.7%, normal *mariae* 16.8%, lower shore *obtusata* 0.8%. The larger crabs seem to have a much wider choice available than smaller individuals (table 13).

If the mean frequencies are calculated for the entire set of 6 tanks including the 3 size classes of *Carcinus* (table 13), the different groups of *Littorina* can be listed in order of susceptibility to predation:

juvenile *obtusata*	61.3%
adult *mariae* ("dwarf")	21.3%
adult *obtusata* from upper shore	7.8%
adult *mariae* ("normal")	6.1%
adult *obtusata* from lower shore	0.1%

These results show that the most susceptible shells are those of small size (6-8mm) and thin lip, followed by those of small size (6-8mm) and thick lip, followed by two groups of a larger size (10-15mm) and a thick lip, and lastly those of largest size (16-17mm) and thickest lip.

Table 13: Frequencies of individuals predated (from table 12)

Size of predator	Tank	Total added	% eaten	Freq. of predated shells				
				ma(dw)	ma(nor)	ob(juv)	ob(low)	ob(up)
1.5-2.5cm	1	100	9.0	0	0	100.0	0	0
	2	100	8.0	0	0	100.0	0	0
4.0-6.0cm	3	375	19.5	35.6	0	64.4	0	0
	4	375	13.4	30.4	2.9	65.2	0	1.5
9.0-11.0cm	5	375	55.7	32.0	14.4	26.3	1.0	26.3
	6	375	56.0	29.5	19.1	31.9	0.5	19.1
Mean				21.3	6.1	61.3	0.1	7.8

There were no significant differences in the proportions of light or dark shells taken by *Carcinus*. It is known that this species of crab does have some colour vision (von Buddenbrock and Frieduch, 1933 from Waterman, 1961) and should be presumed to be capable of differentiating between cryptic and conspicuous shells. However the glass substrate in the experimental tanks bears no resemblance to the fucoid substrate that the *Littorina* are normally found on in the field. In the glass tanks neither the light nor the dark shells can be classified as being more or less conspicuous. These experiments therefore provide no information on whether the predator exerts a selective force on the frequency of shell colour.

(b) Two-Group Experiments

The purpose of the multiple-group experiments was to provide some indication of the ability of different sized crabs to predate on different groups of *Littorina*. The results demonstrated that when each size class of crab is presented with 5 snail groups, varying in shell size and thickness, there is a greater amount of successful predation on the smaller sized and thinner lipped shells in all cases. The smallest crabs predated only the small and thin-lipped shells, while the larger crabs took individuals of all the shell groups. The total number of successfully predated shells was therefore higher in the tanks with the larger crabs (table 13). Although these experiments provided some indication of the susceptibility to predation of the different shell groups, they do not provide information on any choice exerted. Therefore a series of experiments were performed with *Carcinus* to compare the following:

adult "dwarf" *mariae* (6-8mm) vs. juvenile *obtusata* (6-8mm)
adult "dwarf" *mariae* (6-8mm) vs. juvenile "normal" *mariae* (5-8mm)
adult "normal" *mariae* (10-13mm) vs. adult *obtusata* lower shore (16-17mm)

adult "normal" *mariae* (10-13mm) vs. adult *obtusata* upper shore (13-15mm)

adult *obtusata* lower shore (16-17mm) vs. adult *obtusata* upper shore (13-15mm)

Table 14: Successful predation by crabs on "dwarf" *mariae* and juvenile *obtusata* of equivalent sizes.

Size of crabs	"dwarf *mariae*	juvenile *obtusata*	χ^2 [1]
1.5-2.5cm	1	11	6.7
4.0-6.0cm	6	14	2.4
9.0-11.0cm	14	17	0.1

Table 15: Successful predation by crabs on "dwarf" *mariae* and juvenile "normal" *mariae* of equivalent sizes

Size of Crabs	"dwarf' *mariae*	"normal" *mariae* (juvenile)	χ^2 [1]
1.5-2.5cm	0	9	7.1
4.0-6.0cm	8	15	1.5
9.0-11.0cm	19	18	0.0

The results in tables 14 and 15 show that the smallest crabs predate successfully significantly more juvenile *obtusata* and and juvenile "normal" *mariae* than equivalent-sized adult "dwarf" *mariae*. The largest crabs show no significant different in the numbers of the two groups predated. The reason for the difference between the small and large crabs is due to the method of feeding in the two groups. The small crabs insert the claw into the aperture and the outer whorl is broken in successive stages from the lip. Thus the success of the attack is influenced by the shell thickness of the lip and outer shorl. In juvenile obtusata and juvenile "normal" mariae the lip and outer whorl are very thin compared to the adult "dwarf" mariae accounting for the low amount of predation on the latter by the small crabs. The larger crabs have two methods for breaking the shells. For shells with an aperture large enough for the claw to be inserted the method just described is often used. The second method, employed predominantly on the "dwarf" mariae, involves breaking the entire shell into two pieces. The crab accomplishes this by grasping the shell at the spire and columella base and then crushing it.

From these results, it follows that the relative advantage of the adult "dwarf" mariae over the equivalent-sized juvenile obtusata and juvenile "normal" mariae will be dependent upon the extent of predation by smaller-sized crabs. If this is frequent, then the early thickening of the lip, as observed in the "dwarf" would be selectively advantageous for reducing the amount of successful predation. In view of the anecdotal observations that have been made (see page 87) on the relationship between the distribution of small crabs and the seemingly concordant distribution of the "dwarf", it may be that the intensity of predation by small crabs could have been one of the selective pressures contributing to the distribution and perhaps the evolution of the "dwarf" mariae.

This advantage of the adult "dwarf" is present only when comparing this group with equivalent-sized juveniles. When the "dwarf" is compared to "normal" adults the advantage is lost (table 16). There is in fact a significantly greater number of the "dwarf" successfully predated over the "normal".

Table 16: Successful predation by crabs on adult "dwarf" and adult "normal" *L. mariae*.

Size of crabs	"dwarf" *mariae*	"normal" *mariae*	χ^2 [1]
1.5-2.5cm	0	0	-
4.0-6.0cm	8	0	6.12
9.0-11.0cm	20	8	4.32

These results are the opposite of what was expected from the distribution of injuries in the field which showed the "dwarf" to have significantly more shell injuries. It was felt that the "dwarf", with a very small aperture, would be at an advantage compared to the "normal" with a larger aperture, as crabs would be unable to insert their chelae into the opening in order to break off pieces of the outer whorl. However, as described above, the larger crabs have a second method of feeding which involves breaking the entire shell rather than the outer whorl. In view of these observations it will be important to determine the exact distribution of the different size classes of crabs throughout the year to see if any relationship exists with the distributional differences observed between the "dwarf" and "normal" *mariae*.

From the observations in the field on the increased number of shell injuries as one moves down the shore (which appeared to be a function of the number of crabs), the small thick shell of *mariae* might be advantageous over that of *obtusata* in reducing the amount of successful predation. In the following experiment, adult individuals of "normal" *mariae* and *obtusata* collected from the same point on the shore are different in their susceptibility to crab predation but it is the "normal" *mariae* which has the disadvantage (table 17).

Table 17: Successful predation by crabs on adult "normal" *mariae* and adult lower shore *obtusata*.

Size of crabs	"normal" *mariae*	*obtusata* (lower shore)	$\chi^2_{[1]}$
1.5-2.5cm	0	0	-
4.0-6.0cm	0	0	-
9.0-11.0cm	15	0	13.1

The *obtusata* from the region of overlap with *mariae* have a relatively large and thick shell which gives them the advantage at least at the adult stage. This advantage is not present when comparing the juveniles with the adult *mariae* (see table 12). In this case the thickening of the lip at about 12mm as observed in "normal" *mariae*, would be advantageous compared with *obtusata* which does not develop a thickened lip until 16mm.

The final comparison made was between *obtusata* adults from the lower and upper shore positions. The significant relationship observed in the geographical survey between increased injury rate in a sample and increased mean adult size, and the same relationship within a shore suggested that the large adult size may be advantageous in cases of high amounts of crab predation. The results given in table 18 demonstrate that adult obtusata from the lower positions are indeed less susceptible to predation than those from the upper shore.

Table 18: Successful predation by crabs on lower and upper shore adult *L. obtusata*

Size of crab	*obtusata* (lower shore)	*obtusata* (upper shore)	χ^2
1.5-2.5cm	0	0	-
4.0-6.0cm	0	0	-
9.0-11.0cm	17	0	15.1

These results are in accordance with the multiple-group experiments (table 12) and those by Suiterman (1971). The large thick shells found on the lower shore could be an adaptation against predation by crabs compared to the smaller and thinner shells found on the upper shore where little evidence of crab predation is present. Those from the upper shore show only a slight thickening of the lip after the juvenile stages unlike *L. obtusata* and *L. mariae* from the lower shore where the lip is proportionally thicker.

5. Discussion

The previous experiments have shown that there are three factors to be considered when interpreting the influence of crabs on the morphology of the *Littorina*; these are the size of the shell, thickness of the lip, and size of the crab. The interrelationship of these factors allow different strategies to be developed by the *Littorina* in conditions of predation by crabs. When considering both juvenile and adult shells undergoing predation by large crabs, the order of decreasing susceptibility is "dwarf" *mariae*, "normal" *mariae*, and *obtusata*, or in other words the smallest shells are most susceptible while the large shells least so. Small crabs are usually incapable of successfully preying on any of these adult shells. Therefore, if only large crabs are to be considered, one strategy possible for the *Littorina* would be to develop larger and thicker shells. Within *L. obtusata* this does seem to be the case not only between localities but within them. However one must also take into account the role of small crabs, which can successfully predate most shells without a thickened lip, in other words the juveniles. There would be, in these conditions, an advantage to an early development of the thickened lip. In reference to this character, the order of decreasing susceptibility to successful predation is *obtusata*, "normal" *mariae*, and "dwarf" *mariae*, the opposite order to that seen from predation by large crabs. Therefore, the overall advantage or disadvantage of each *Littorina* group will be a function of the proportion and abundance of each size-class of crabs.

Previous studies on gastropod shell morphology have emphasized the role of abiotic forces in accounting for geographical variation in the shell. In *L. obtusata*, Sacchi (1969) has suggested that the large adult size observed in sheltered shores is due to optimal growth conditions, the smaller size on exposed shores due to growth stunting and the influence of waves. These are similar to the conclusions that Barkman (1956) drew from his thorough study of the same species.

In *L. saxatilis* there is variation in the amount of sculpturing and shell size on shores of different exposure. Small adult shells are found on exposed shores and large shells on sheltered ones. These differences have been explained in terms of wave action in exposed conditions which select for a smaller shell, and in terms of optimal growth in sheltered conditions resulting in large shell sizes. Even within one shore there are differences in shell size. The individuals on the lower shore are relatively large while those from the upper shore are smaller (Heller pers. comm.). No explanations of these data have considered the effect of predation by crabs which in this case would seem to conform well with that observed in *L. obtusata*.

In a study of variation in shell size and sculpturing in *L. picta* in the Hawaiian Islands Struhsaker (1967) has found a pattern of variation comparable to that observed in *L. obtusata*, at least in respect of shell size. Within one shore, individuals found on the more exposed parts with low slope angles have shells which are relatively small and unsculptured. Those found on the more sheltered areas with high slope angles (i.e. the landward side of a boulder) have large sculptured shells. The small shells on the more exposed conditions are very resistant to the effects of wave action for large sculptured shells are rapidly washed off in these conditions. Fishcer-Piette and Gaillard (1961) have noted that in *L. saxatilis* from more exposed areas, the shells are more heavily sculptured compared with those in sheltered conditions, the opposite of what Struthsaker has noted in L. picta. With reference to the function of the sculpturing in L. picta and L. saxatilis, Struthsaker (1967) has stated the following: "This discrepancy may be explained if the selective factor of the environment is assumed not to be acting mainly on the sculpture form differences, but rather on other factors linked genetically with sculpture form, e.g. size, physiological resistance to wave force, submergence, desiccation, etc. The linkage between these factors may be opposite in the two species". In reference to the larger size of L. picta in sheltered conditions, she comments that the large size is disadvantageous where there is heavy wave action but does not suggest any advantage for the increased size. This study makes no reference to the role of predation as another possible selective agent.

There is variation in the shell size of L. neritoides in which the more exposed shores have smaller individuals and the sheltered shores larger ones (Natale and Sacchi, 1964). The same is true for L. planaxis which has been explained in terms of wave action in exposed areas and possible salinity differences in sheltered areas (North, 1954).

Variation in shell size of Zoila friendi and Cypraea tigris have been accounted for in terms of temperature differences between areas (Wilson and Summers, 1966; Schilder, 1962). In other cases, the amount of variation has been explained in terms of historical factors such as in the mud-living species Potamopyrgus (Winterbourne, 1972). In none of these cases have the influence of predators, such as crabs, been considered as selective agents.

Vermeij (1973) working with tropical littorinids, neritids, and limpets has found extensive variation in the shell size and sculpturing within and between species on particular shores. Using his own data in addition to the observations from other workers in more temperate areas he concludes that the variation in shell morphology is attributable to environmental selection for minimizing water loss from shells, selection for thermoregulatory control by changing the proportion of reflective and absorptive areas on the shells, and selection for reducing the effect of heavy wave action on shells. This study by Vermeij involved comparing morphological characteristics of each species to their distribution within each shore. Throughout this study there has been little consideration given to the role of biotic factors, such as predators, as selective agents in the different adaptive strategies of shell size.

In the dogwhelk, *Thais lapillus*, populations from exposed shores have relatively thin shells with large apertures while those from sheltered conditions tend to be thick, highly sculptured and have only a small aperture. In two excellent papers on this species, Ebling *et al* (1964) and Kitching *et al* (1966) have shown using both ecological and experimental data, that the amount of wave action in exposed areas and the amount of predation by crabs in sheltered areas is sufficient to account for the morphological variation in their study. They have shown experimentally that thin shells with a large aperture are more capable of clinging to the rock substrate than the thicker shells with smaller aperture in conditions of wave action. They have also shown that the crabs *Carcinus* and *Portunus* which are most abundant in sheltered areas are less capable of successfully preying upon thick shelled individuals with small apertures compared with the thin shelled individuals.

Crothers (1973) working with the same species found that in exposed conditions small shelled individuals were present while in sheltered areas larger individuals occurred. He concluded that wave action on exposed conditions would provide the best explanation for the small sized individuals. He suggested that crabs might have more difficulty in breaking the larger shells found in sheltered conditions but noted that the very large crabs were able to break all the shells. From this he felt that the large size in these conditions could not be satisfactorily explained when considering predation by crabs. In this study, Crothers did not consider the interrelationship between the amount of predation by different sizes of crabs on juvenile and adult individuals of the dogwhelk.

There seems to be general agreement that on exposed shores the reduction in size of the shell is related to the effects of wave action. These observations have been made with a wide variety of species and have involved many experimental demonstrations that very large shells are less capable of adhering to rock substrate during wave action. The larger shells, often more heavily ridged, in sheltered conditions have been explained in number of ways . Some of these that have already been cited are selective regimes pertaining to disiccation, thermoregulation, linkage effects, optimal growth, and temperature and predation by crabs.

A reasonable conclusion, that can be drawn from my own observations on L. obtusata and L. mariae is that the variation in shell size of both species are explicable in terms of wave action and predation by crabs. The convergence in size of the two species on exposed conditions with L. obtusata becoming smaller and L. mariae larger combined with the experimental work of Guiterman (1971), suggest very strongly that the amount of wave action is indeed the selective agent acting on shell size in these conditions. In sheltered areas, the very large L. obtusata and the very small L. mariae enjoy an advantage when predation by crabs is present. The two opposing strategies seem to be related to the amount of predation by crabs of different sizes. With very small crabs, an early maturation with thickening of the lip as observed in L. mariae provides an advantage over equivalent sized thin-lipped L. obtusata. When large crabs are considered, both L. mariae and small shelled L. obtusata are at a disadvantage when compared with the large shells of L. obtusata found in sheltered localities and the lower shore positions where the amount of predation by crabs is greatest. Each locality will differ to some degree in the abundance of each species of crab (i.e. Cancer, Carcinus, Portunus) and probably in the age classes present. All of these biotic variables should be considered for an exhaustive survey in attempting to account for variations in size and shape of shells.

SECTION V

ROLE OF FISH AS PREDATORS ON LITTORINA

1. Introduction

Pettitt (1971) in a review of the predators on Littorina lists 12 species of fish recorded as having Littorina in the stomachs. The subtidal species which move in with the tide to feed include the flounder, Pleuronectes flessus, the dab, P. limanda, the pollack, Pollachius virens, the whiting, Gadidus merlangus, the dragonet, Callionymus lyra, and some members of the wrasse family, Labridae. The intertidal species include two species of the blenny, Blennius pholis and Chirolophis galeritas, the rock goby, Gobius pagenellus, the sea scorpion, Acanthocottus bubalis and the rockling, Onus tricirratus. The importance of each of these species as predators on Littorina cannot be established from the literature as there are seldom seasonal records of numbers and proportions of Littorina in the stomach contents. Another factor is that the different species of Littorina have often not been separated and have therefore been treated as one group. This is due to the difficulty in identifying each of the species, especially in the juvenile stages; these appear to provide the majority of individuals predated.

In this study, collections of intertidal fish have been made at three shores in Anglesey from July 1972 to April 1973, in order to determine which intertidal species are preying on Littorina, the proportion and abundance of each species of Littorina taken, and also the length distribution and colour morph frequencies of the predated snails.

2. Materials and Methods

At Porth Swtan, Sandy Bay, and North Stack, small tidepools were sampled by initially removing the large algae from the pools and then adding a small amount of the water-soluble anaesthetic quinoldene. The anaesthetised fish present in the tidepool, often floating to the surface, were placed in a container containing 50% formalin. Those individuals not completely anaesthetised were kept in a quinoldene solution until no further movement occurred. These were then placed in the formalin solution. A ventral incision was made in the abdominal wall to facilitate preservation of the stomach contents. To gain some indication of possible seasonal differences in the feeding habits of the blenny, collections were made in July, October, December, February and April from 1972-73.

For examination of the stomach contents, the entire digestive tract was removed and flushed with running water. All gastropods present were recorded for species, shell length, and shell colour. The latter character was useful only in L. mariae as this was the only species to show development of the colour polymorphism at the shell sizes present in the stomachs.

3. Results of Stomach Analysis

(i) Species of Intertidal Fish

Of the six species of littoral fish collected, B. pholis was the only species which showed any evidence of predation on gastropods during the time of the study (Table 19). This species was the most common on all the shores sampled and is widespread and common on most of the British shores (Barret and Yonge, 1958). The abundance of gastropods in this species has also been noted by Quasim (1957) in his work on the biology of the species in Wales. He found that up to 54% of the blennics during certain times of the year have gastropods present in the stomachs.

The absence of gastropods in the other species of fish may be a local phenomenon restricted to the area where the collections were made. It is possible that if a greater number of shores had been collected, some indication of predation by these species would have been present. In the review by Pettitt (1971) *Gobius pagenellus* and *Cottus scorpius* have been recorded as taking gastropods.

Table 19: Abundance of gastropods in 6 species of intertidal fish.

Species	No. Stomachs Examined	Abundance of Gastropods
Gobius paganellus	18	absent
G. ruthensparri	23	absent
Blennius pholis	137	frequent
Cottus bubalis	24	absent
C. scorpius	24	absent
Onus mustellus	16	absent

(ii) *Species of Gastropods*

With familiarity of the genus *Littorina*, one is able to identify the different species in the stomach contents, including some of the very small juveniles down to a length of 1mm. In all three localities collected in this study, the blennies were found to have predated 9 of 11 species of gastropods found on each of the shores (table 20).

Table 20: Species of gastropod found in stomachs of *Blennius pholis*.

Species	abundance		
	absent	occasional	frequent
L. mariae			•
L. obtusata			•
L. saxatilis			•
L. neritoides		•	
L. littorea		•	
Lacuna vincta		•	
L. pallidula		•	
Thais lapillus		•	
Gibbula cineraria	•		
G. lineata	•		
Patella vulgata		•	

The occurrence of each species in the stomachs was related to the position at which the blenny was collected. Those from the lower shore areas, in the Fucus serratus zone, had chiefly L. mariae with occasional individuals of the chink shell (Lacuna spp.) the Limpet (Patella sp.), and the edible winkle (L. littorea). From the mid-shore areas, in the Ascophyllum zone, the chief gastropods were L. obtusata, some L. saxatilis and Thais lapillus. Blennies from the upper shore where fucoids were rare, had predominantly L. saxatilis with occasional L. neritoides. The relative abundance of each species in the stomachs closely approximates the abundance and distribution of each species on the shore.

In general, it would seem that the blenny is opportunistic in its choice of gastropods, taking those individuals most commonly found in the area, a conclusion also reached by Quasim. In one blenny, from Porth Swtan, collected from the lower shore, 20 individuals of the chink shell (Lacuna vincta) were found in the stomach. This species is normally found near the low-tide positions and often below the low-tide mark. Extensive collecting in the vicinity where the blenny was captured yielded no individuals of the chink shell, but abundant individuals of Littorina mariae and occasional Lacuna pallidula neither of which were present in the stomach. In view of the very restricted home range occupied by the blenny (Quasim, 1957) and the characteristic colouration of the chink shell, it is possible that a search image is capable of overiding the abundance of other prey species.

(iii) Shell Abundance

The examination of the stomach contents of 135 blennies has shown generally an absence or low number of snails in each stomach although occasionally with more than 20 individuals (figs 22, 23, and 24). These histograms are summarized in Table 21 which give the average number of individuals in each species that were predated. In Porth Swtan and Sandy Bay, L. saxatilis is relatively more common in the stomachs than L. mariae and L. obtusata, while at North Stack the opposite is true with L. saxatilis now being the least common.

Table 21: Mean number of Littorina per stomach (see figs 22, 23, and 24 for numbers of blennies and individual variability).

Locality	Date*	L. mariae	L. obtusata	L. saxatilis
Porth Swtan	July/72	2.0	0.5	4.1
	Oct/72	1.3	1.4	12.0
	April/73	0.6	0.3	1.9
Sandy Bay	July/72	0.8	0.0	4.2
	Oct/72	0.0	0.0	0.0
	April/73	0.4	2.3	4.5
North Stack	July/72	3.1	2.7	0.4
	Oct/72	-	-	-
	April/73	2.2	4.0	0.7

*collections were attempted on December/72 and February/73 in the localities but no blennies were captured or seen.

Many blennies had no snails present in the stomachs while others in each of the three localities had more than 20 individuals present. This would seem to indicate that the blenny is potentially capable of exerting a large influence on the Littorina at some times and must therefore be considered as one of the possible selective agents in the life history of the snails. The data presented on the numbers in the stomachs must certainly be underestimates since occasional shells so far digested as to be unreadable for size and species were not included in the counts. For example, the shells smaller than 1mm were most commonly seen in the stomach and only rarely at the end of the digestive tract in the intestine. In this latter position, those which were seen were very thin and broken The larger shells were similar in abundance in the stomach and intestine and did not show the same level of shell digestion in the intestine as the smaller individuals.

The differences noted between the numbers of each species consumed is partially a reflection of the microhabitat where the blennies were collected and does not seem to be due to any preference for one species or another. When each blenny was captured, notes were taken on the general position and abundance of different species of gastropods at the point of the capture. When the results of the stomach contents were compared to the field notes, it is evident that the relative abundance of each species in the stomachs is similar to what is observed in the field where the blenny was collected.

Observations made while SCUBA diving during high tide provide some information on the numbers of Littorina consumed in a given time period. Data on this subject were not obtainable from the stomach analysis. During one dive lasting 75 minutes, a blenny approximately 70mm in length consumed 14 juvenile L. mariae all on a single plant within 1 hour. After this period of one hour, the plant was cut at the base and returned to shore for counting the remaining snails present. These consisted of 64 juveniles and 17 adults of L. mariae plus 9 adult Lacuna pallidula. Therefore, within one hour, 18% of the juvenile snails on the plant were predated. The general paucity of F. serratus (where L. mariae is found) in the area of observation implied that if this intensity of predation was continued over a longer period of time the numbers of

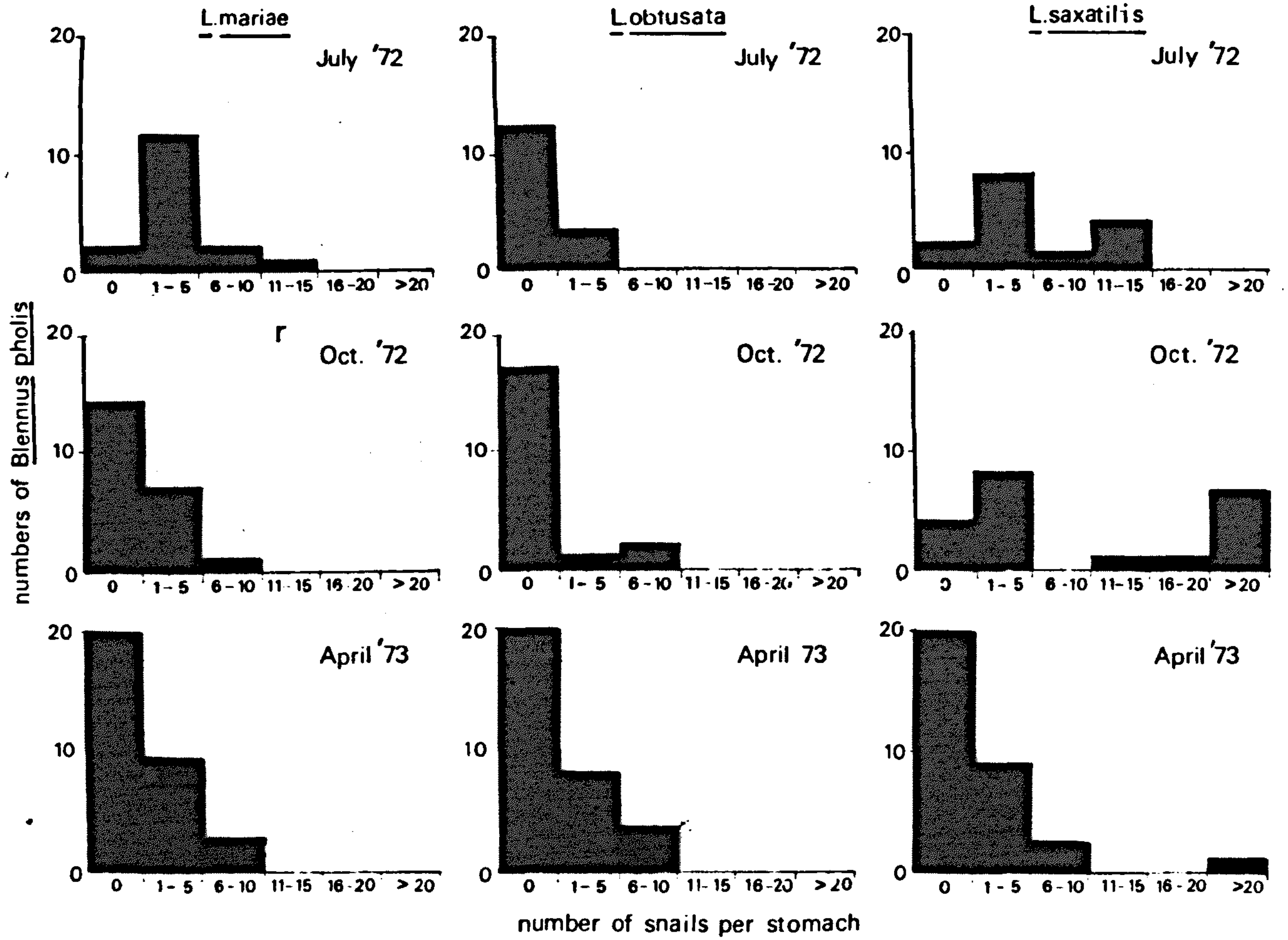


Fig. 22. Numbers of Littorina found in stomachs of Blennius pholis at Porth Swtan.

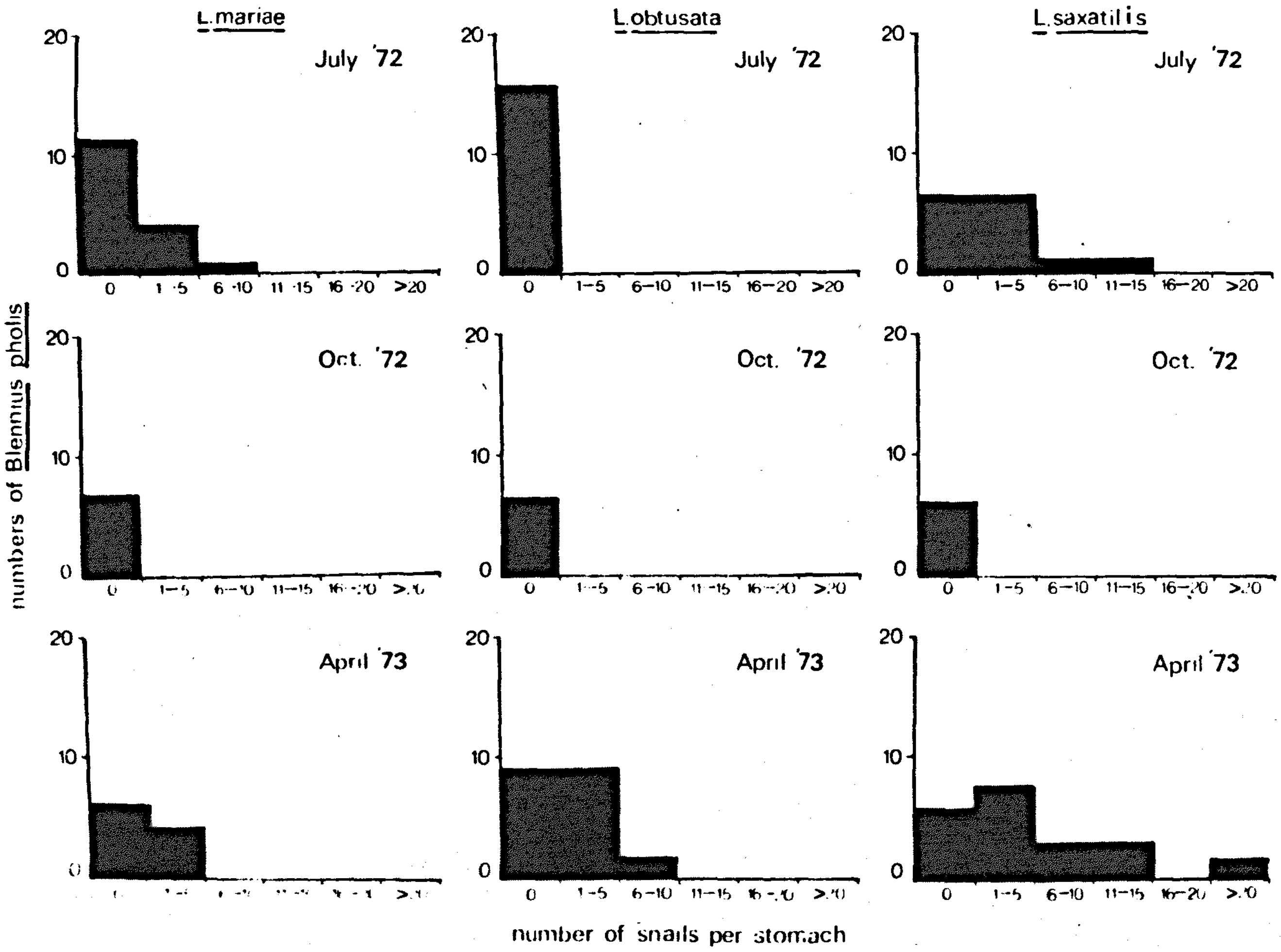


Fig. 23. Numbers of Littorina found in stomachs of B. pholis at Sandy Bay.

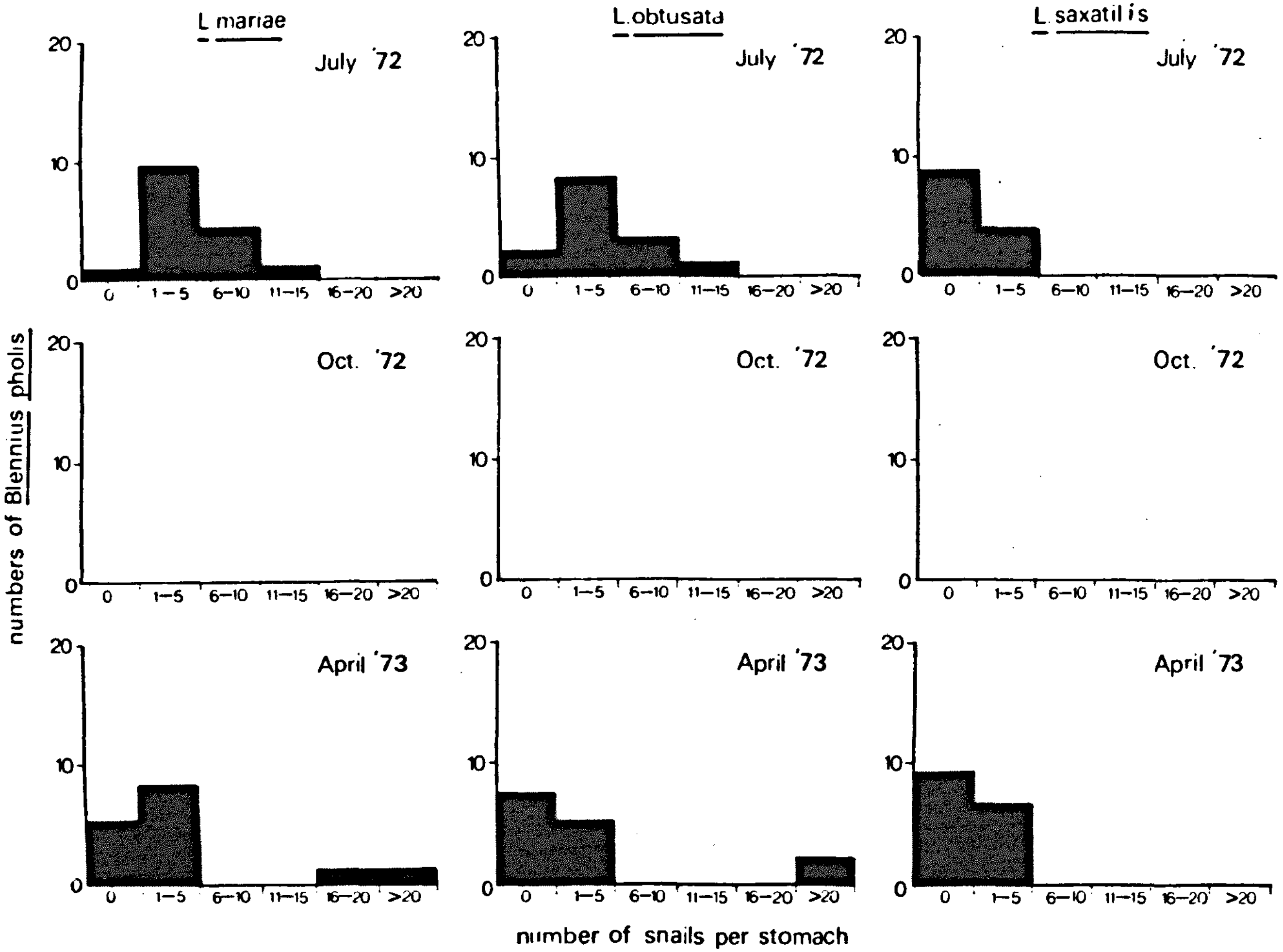


Fig 24. Numbers of *Littorina* found in stomachs of *B. pholis* at North Stack.

No blennies were captured in October.

the juvenile *L. mariae* in the area would be greatly depleted, unless there was constant recruitment. During a second dive of the same duration, a second blenny on the same shore was seen to feed on 9 juveniles of *L. obtusata* within one hour. These individuals constituted 6% of the total juveniles on the plant. The observations on the two blennies are of limited value but underline the need for a comprehensive ecological study on the interaction between the different species of *Littorina* and the predator *Blennius pholis*.

(iv) *Shell size*

The size of shells predated by the blennies range from 0.5mm-8.0mm in the three species, with the mode occurring at 1-3mm. The mean size of the predated shells shown in Table 22 demonstrate that the greatest amount of predation by the blenny occurs on the juvenile stages soon after hatching.

Table 22: Mean shell size of *Littorina* found in stomachs (see fig 25).

Locality	Date	*L. mariae*	*L. obtusata*	*L. saxatilis*
Porth Swtan	July/72	1.9	2.2	2.1
	Oct/72	1.9	1.9	2.2
	April/73	2.9	2.2	2.2
Sandy Bay	July/72	2.7	-	2.1
	Oct/72	2.3	1.8	1.9
	April/73	2.3	1.8	1.9
North Stack	July/72	2.1	2.0	2.4
	Oct/72	-	-	-
	April/73	3.1	1.7	2.9

Shells with diameters less than 1mm occur with relatively low frequency in the stomachs relative to the 1-2mm class (Fig 25). Because of the ease with which the smallest shells are crushed and digested by the blenny, it is highly probable that they are taken at a higher frequency than was actually recorded. The larger shells are not as readily broken or digested. This potential error will result in a higher observed mean size of the predated shells.

There is a positive correlation between the size of the blenny and the size of the snail eaten. Weighted linear regressions were calculated between the standard length of the blenny and the mean shell size for each species in the stomachs (figs 26, 27 and 28). It is apparent from these figures that in each of the species from the different localities, there is a general tendency for larger blennies to be consuming larger sized shells.

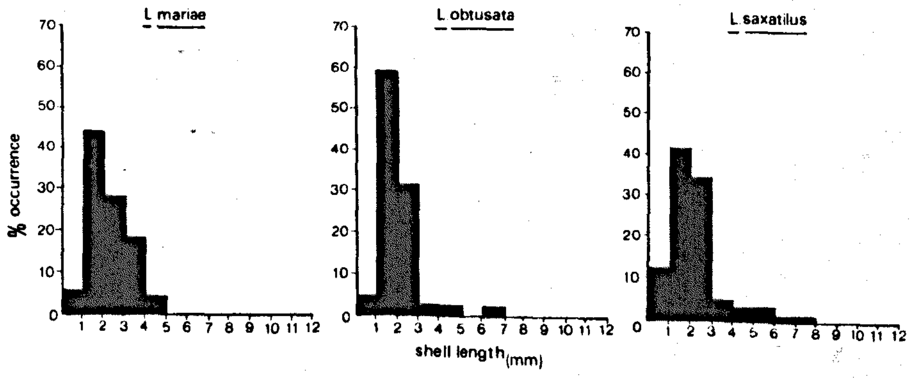
Observations on the feeding behaviour of the blenny in the laboratory indicated that snails less than 3mm were readily picked up and swallowed by all size-classes of blenny (40-100mm). However with shells greater than 5mm in diameter, the blenny readily took these into its mouth but always ejected them, presumably because the shell was too large to swallow. It seemed therefore that there was an upper limit in shell size that the blennies were successfully capable of predating.

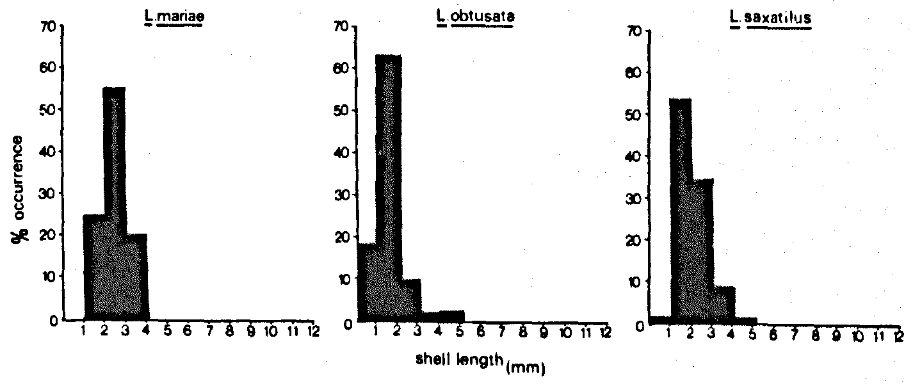
To determine whether this occurred in the field, the standard length of the blenny was compared to the largest shell found in the stomach. As the blennies and the predated shells in the three localities have a similar mean size and range they have been considered together. The results shown in fig 29 demonstrate that in

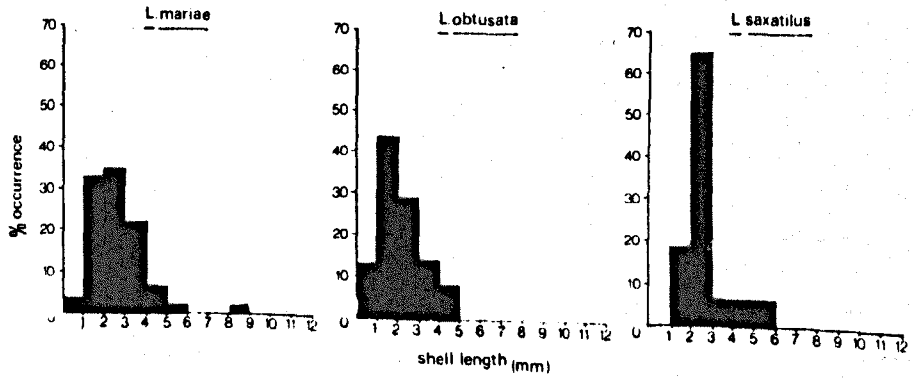
Figure 25

Figure 25

Distribution of shell sizes in stomachs of B. *pholis*. Top, Porth Swtan; centre, Sandy Bay; bottom, North Stack. See appendix E - fig E_1 which shows the distribution of shell sizes in the population for L. *obtusata* at Sandy Bay.


L. mariae
L. obtusata
L. saxatilus
% occurrence
0
10
20
30
40
50
60
70
1 2 3 4 5 6 7 8 9 10 11 12
shell length (mm)


L. mariae
L. obtusata
L. saxatilus
% occurrence
0
10
20
30
40
50
60
70
1 2 3 4 5 6 7 8 9 10 11 12
shell length (mm)


L. mariae
L. obtusata
L. saxatilus
% occurrence
0
10
20
30
40
50
60
70
1 2 3 4 5 6 7 8 9 10 11 12
shell length (mm)

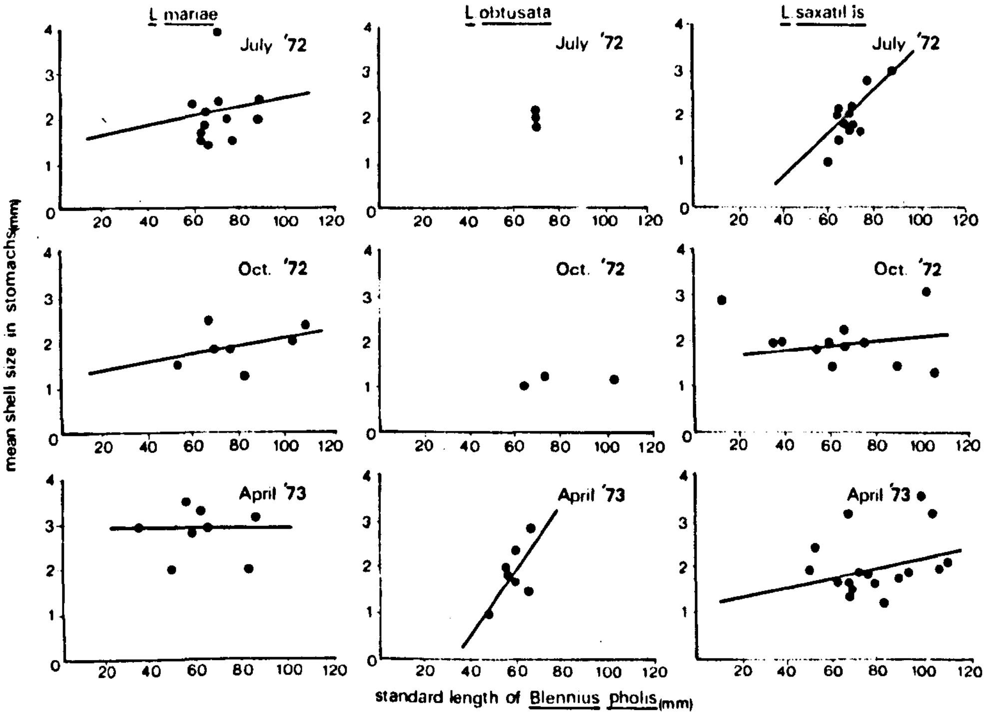


Fig 26. Relation between length of B. pholis and mean shell sizes in stomachs at Porth Swtan.

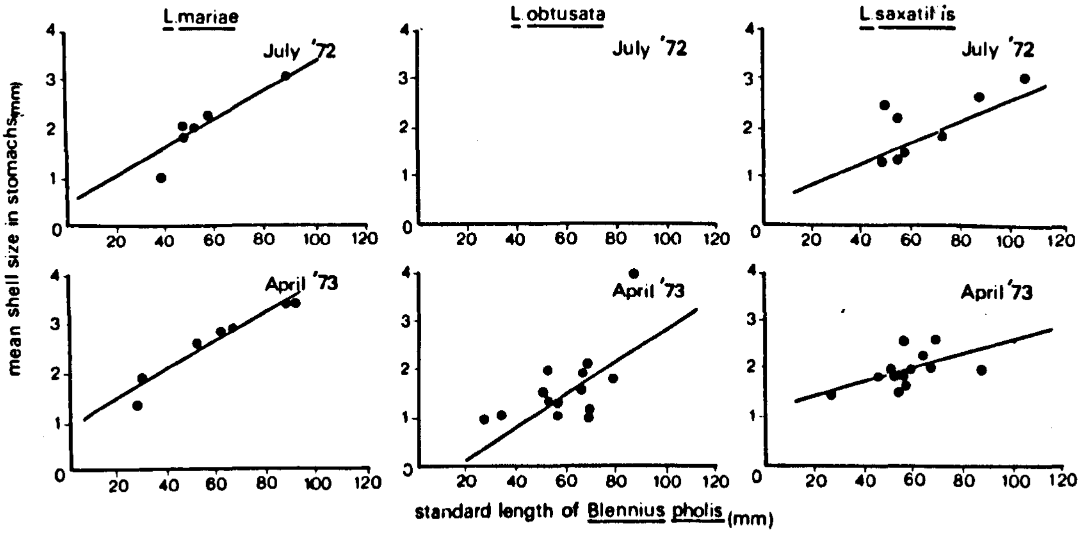


Fig 27. Relation between length of B. pholis and mean shell sizes in stomachs at Sandy Bay.

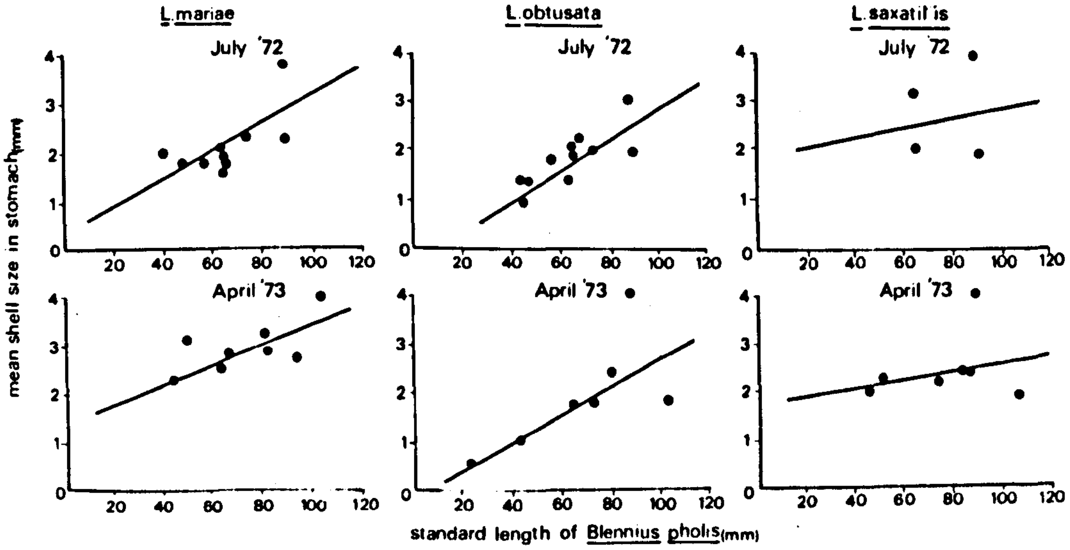


Fig 28. Relation between length of B. pholis and mean shell sizes in stomachs at North Stack.

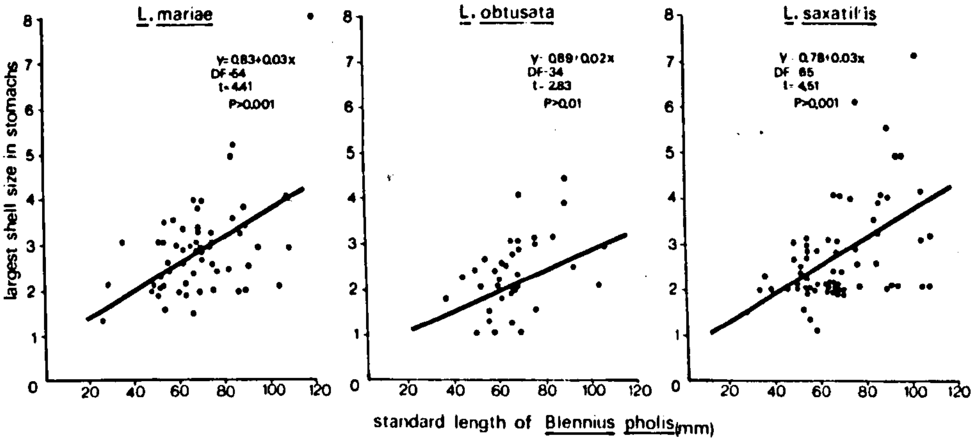


Fig. 29. Relation between length of B. pholis and size of largest shells in stomachs.

each of the three species there is a highly significant relationship between the length of the blenny and the largest snail consumed. The smallest blennies take relatively only small snails while the larger blennies take not only small shells but slightly larger ones. The largest shell taken was 8.0mm by a 120mm blenny, the largest individual captured. This is about the largest size of blenny that is found in the field.

It was argued (see section IV, 5) that the small adult shell size of L. mariae in sheltered conditions has an advantage when considering predation by adolescent crabs. It may be that a smaller adult size than observed would be favoured in these conditions. However there would come a point when the advantages to smallness would be lost owing to predation by blennies. It is interesting to note that the smallest adult shell-sizes of L. mariae (6mm) are at the upper edges of the range in sizes that the blenny is able to swallow. This possibly could account for the lower limit of adult shell size in this species, rather than explanations concerning egg-carrying capacity etc.

In summary, taking Quasim's observations which showed that the younger age classes of the blenny predate proportionately more gastropods than older individuals and the relationship observed above between blenny size and shell size, there is reason to believe that highest amount of predation on the Littorina by blennies occurs on the juvenile snails soon after hatching. The adults or the shells larger than 8-9mm appear to be immune from predation by this species of blenny.

(v) Shell colour

It is only in L. mariae that it is possible to score the different colour morphs at the shell sizes found in the stomachs, at which in L. obtusata and L. saxatilis, the shell colours form a continuous variable with many of the lighter coloured shells becoming darker with increased size.

In the 3 localities, the morph frequencies in the stomachs do not differ significantly from the frequencies observed at the same localities in the field (table 23). Calculation of the heterogeneity χ^2 for each locality gives values of 0.62 (4d.f.) for Porth Swtan, 0.00 (3d.f.) for Sandy Bay, and 5.93 (2d.f.) for North Stack; each of these are non-significant. When the data are pooled, there are still no significant differences between morph frequencies in the stomachs and those in the field from each locality ($\chi^2 = 1.88$, 0.94, 0.11 respectively). At Sandy Bay the differences observed between the July/72 and April/73 sample show a change in the numbers of each morph predated. This however, is a reflection of differences in the site where the blennies were collected. The collection of blennies in July was taken in run-off channels where the L. mariae were 95.6% citrina while those from April were collected from tidepools and rock platforms where citrina was only 16.1%. The results from the three localities then tend to indicate that if there is an overall tendency for the blennies to predate one morph more than the other the differences are very small; differences on the order of 5%, not detectable in these samples, could still be very important.

In the blennies, which had consumed more than 1 individual, it was in theory possible to determine where there was a searching image for one morph such that different blennies would have a higher number of the same morph in their stomach than would be separated from the population morph frequencies. When this was performed, no cases were found in which the proportions of the morphs differed significantly from the expected, as judged from the population morph frequency. However, these results are insufficient for deciding whether or not any search image is present owing to the heterogeneity in morph frequency within shores and the lack of information on morph frequencies at the exact location where each blenny was feeding.

Table 23: Numbers of different colour phenotypes of L. mariae in stomachs. Heterogeneity χ^2 (mentioned in text) calculated using 2 d.f. for samples with expected numbers less than 5.

Locality	Date		citrina	reticulata	P (EXACT)
Porth Swtan	July/72	stomachs population	8 14	25 79	0.29
	Oct/72	stomachs population	1 8	13 58	1.00
	April/73	stomachs population	2 8	16 51	1.00
Sandy Bay	July/72	stomachs population	8 43	0 2	1.00
	April/72	stomachs population	1 14	6 73	1.00
North Stack	July/72	stomachs population	8 7	32 41	0.58
	April/72	stomachs population	1 9	30 45	0.09

4. Conclusions

The results of the stomach analysis of the blenny has shown that this species could be an important influence on the life history of the winkles. In the remaining parts of this thesis, the colour variation in the shells of *L. obtusata* and *L. mariae* is considered in detail; in these sections the importance of the blenny as a visual predator will become evident.

SECTION VI

GENETICS OF COLOUR POLYMORPHISM

1. Introduction

The wide range of shell colours seen in many of the prosobranchs have not yet been studied genetically. In *Littorina* there have been various attempts to differentiate between genetic and environmental factors by comparing differences in salinity, illumination, wave action, and food types for the different shell colours (Linke, 1934; Barkman, 1956; Gaillard, 1965). All the evidence which has been collected, suggest that these factors play no significant role in the determination of shell colour. The reason for the lack of direct genetical data is probably due to the difficulties encountered in keeping the very small juveniles alive in the laboratory under highly artificial conditions.

In this study, a series of breeding experiments were carried out with both *L. obtusata* and *L. mariae* in an attempt to gain some understanding of the genetics of the polymorphism. One advantage of these two species over some of the other Littorinids is the absence of a pelagic larval phase; upon hatching, the minute snails (about 0.5 mm in size) remain on the fucoids during their subsequent development. In many of the crosseswhich were carried out, the progeny died soon after hatching. In others, however, some success was achieved in bringing individuals through to a size at which they could be reliably scored for some phenotypes. As this has apparently not been done successfully before, the techniques employed will be described in some detail.

The results that have been obtained provide demonstration that the different colour phenotypes are indeed genetically determined, although the exact mode of inheritance must await further breeding experiments.

2. Materials and Methods

For preliminary breeding experiments, collections of adult *L. obtusata* and *L. mariae* were obtained from Menai Bridge in Anglesey where the two species were very dissimilar morphologically and could be identified by shell size alone. As it was initially not possible to determine the sex of individuals, the snails were sorted only into colour groups and then placed in separate glass tanks with 3 species of algae-*Fucus serratus*, *F. vesiculosus*, and *Ascophyllum*. Seawater was added to the tanks after which they were placed in a cold room at 14°C with a 12 hour photoperiod.

According to Fretter and Graham, 1962 (p. 389), eggs of *L. obtusata* are normally laid within 2 hours of copulation. It seemed, however, that if females had been collected in the field just after a mating, the normal egg laying sequence would be interrupted owing to the transport and artificial conditions in the laboratory. For this reason all egg masses appearing within the first four days were discarded. After this time, algal fronds with single egg masses (i.e. from individual females) were removed and placed in separate 10l glass tanks with fresh sea water.

In view of the suspected sensitivity of eggs and young snails to contaminants in the seawater, all possible contact with metal or plastic was avoided. Aeration was accomplished through glass tubing rather than conventional airstones. Tanks with glass molds were used in preference to those with metal casing. Although it cannot be asserted, it is felt that the relative success of the breeding experiments were in part due to these precautions.

As the egg masses and young snails are found mainly on the algal fronds it is imperative that the algae be maintained in a healthy condition. Dr. E. Burrows (pers. comm.) has found that a mixture of soil extract and a salt solution in seawater is sufficient to culture *Laminaria* in the laboratory. This solution has also been used in this study for *Fucus* spp. and *Ascophyllum*. The preparation is as follows:

Soil Extract - 100 g of unfertilized soil added to 1 litre distilled water
- autoclave for 3 hours, filter, reautoclave, and refilter

Salt Solution - 2 gms sodium nitrate ($NaNO_3$)
- 0.4 gms di-sodium hydrogen orthophosphate dihydrate ($Na_2HPO_4 2H_2O$)
- 0.1 l distilled water.

25 ml of soil extract and 5 ml of salt solution were added to each litre of seawater in the tanks. Every week, the tanks were completely drained and the water discarded. The algae, with the snails, were left exposed to air for 4 hours to allow drying - as occurs twice daily in natural conditions. After this time, fresh seawater was added with the appropriate amounts of soil and salt solution. This procedure allowed plants of *F. serratus* to be kept for up to one year in the laboratory. With *F. vesiculosus*

and Ascophyllum, the plants still died within 3 weeks of being brought in from the field. As the egg masses of L. obtusata were laid only on these two species, most of these crosses were therefore lost.

In those eggs which survived, hatching took place anywhere from 4-8 weeks after the egg masses initially appeared. Some of the newly hatched individuals became dislodged from the fronds and fell to the bottom of the tank. These individuals would probably have been lost had it not been for a great deal of green algal growth on the sides and bottom of the tanks which provided food for the young snails. In many cases, these individuals crawled up the sides of the tank and beyond the water level. These were replaced on the algal fronds with a fine forceps, taking caution not to injure the fragile shells.

After having developed a basic technique from these preliminary crosses, a series of additional breeding experiments were undertaken. Most of the adults from these experiments were collected from Porth Swtan where a greater variety of phenotypes occurred compared with those from Menai Bridge. One of the restrictions in the preliminary crosses was the dependence on assortative colour matings as the sex was not determinable when the animal was alive. It was found, however, from watching the snails move in the field, that by gently lifting the shell and tilting it backwards, the sex could be determined from the presence or the absence of the penis. It was possible therefore to conduct matings between colour morphs which was not done during the first set of matings.

For both species, the sexes were separated and placed in tanks with 3 species of seaweed - F. serratus, F. vesiculosus and Ascophyllum. It was observed that egg masses appeared in the tanks for a period of 1 day after the females had been separated from the males, indicating that the egg laying sequence can be postponed for at least 24 hours after copulation. 7 days after no further egg masses appeared, the breeding crosses were begun. As single male-female pairs did not result in egg masses, 15 of each sex were placed together in each tank for the different crosses. When an egg mass appeared, it was placed in a separate tank with the corresponding algal frond. In two cases, individual females were seen laying the eggs. After completion, these females were removed and kept in separate tanks to determine whether additional egg masses appeared. Neither of them did, although it is possible that if they had been left undisturbed initially extra egg masses may have appeared.

All the egg masses from these crosses were treated in the same way as described for the preliminary crosses. Again, most of the egg masses laid on _F. vesiculosus_ and _Ascophyllum_ died owing to the inability to keep these two species of algae alive in the laboratory.

The possibility exists that some of the breeding crosses were contaminated with individuals present on algae which was brought in from the field. In each case that fresh algal fronds were required for the crosses, they were carefully visually inspected a number of times and all egg masses and snails removed. The algal fronds were placed in the tanks only when repeated inspection showed no more individuals present. Despite this method of repeated examination, it is possible that some small juveniles were overlooked. This is more likely to be the case in the _L. obtusata_ crosses, in which fronds of _Ascophyllum_ and _F. vesiculosus_ were used. These two species did not survive well in the laboratory and were consequently replaced with healthy plants from the field. Detection of small individuals on these plants is difficult since individuals often crawl into the air bladders (see page) and can be easily overlooked. This problem was not encountered with *F. serratus* since the fronds of this species are leaf-like on which even the smallest individuals can be seen. Since all the crosses of *L. mariae* were raised on *F. serratus*, it is likely that all of the progeny observed came from the egg masses laid in the laboratory.

After hatching, observations were made on the development of the shell colours to provide a comparison with individuals collected from the field. In some cases for *L. mariae*, all the individuals were removed from the one species of algae that they are normally found on (*F. serratus*) and individually placed on *Ascophyllum*, the common food plant of *L. obtusata*. This was to determine whether the species of algae would have an effect on the development of the shell colour. In each of the 5 crosses where this was performed, the new increments of shell had the same colour as the previous growth, suggesting therefore that food differences play no role in shell colour.

There are differences in size of the two species at which the different colour phenotypes can be reliably scored (see Section IX). In *L. mariae* at Porth Swtan the juveniles , soon after hatching are either white or dark brown corresponding to the phenotypes citrina and reticulata (Plate 26). Unfortunately, reticulata could not be scored separately for light and dark shades as is possible in the adults. In *L. obtusata*, more difficulty is encountered since most of the juveniles are very pale yellow right after hatching (Plate 25) and only begin to develop discontinuities at 3-6mm in size. All of the L. obtusata in the crosses had died before it could be asserted that no further change in shell colour would occur.

The different matings carried out for each species were the following:

	Number of crosses	
L. mariae	survived	died
citrina x citrina	5	2
citrina x light reticulata	2	0
citrina x dark reticulata	10	1
dark reticulata x dark reticulata	12	3
light reticulata x dark reticulata	0	2
L. obtusata		
citrina x citrina	5	6
citrina x olivacea	3	10
citrina x dark reticulata	0	4
olivacea x olivacea	1	6
olivacea x dark reticulata	0	4
dark reticulata x dark reticulata	1	3

L. obtusata has normally been treated before L. mariae in most of this thesis, but in this section L. mariae is considered first, since most of the breeding data comes from this species.

3. Results

(i) L. mariae

The results from these breeding experiments, given in Table 24 show that there is indeed a strong genetic component in the determination of shell colour. Many of the phenotypic ratios at North Swtan (No. 39-70) are explicable in terms of a genetical model of two alleles at a single locus with dark reticulata dominant to citrina.

dark reticulata x dark reticulata (No. 57-No. 70)

All these matings would be expected to produce progeny which were either all reticulata or reticulata and citrina with a 3:1 ratio. One of the 12 matings, No. 64, gave all reticulata, while the remainder gave reticulata and citrina usually with the former being much more common than the latter. Among these segregating crosses, there is significant heterogeneity ($\chi^2_{(11)}$ = 48.97; $P < 0.001$), the greatest contribution to the chi-squared coming from No. 66, which has an excess of citrina. If this cross is removed, the heterogeneity becomes non-significant ($\chi^2_{(10)} = 3.12$; $P > 0.9$). When these segregating crosses (excluding No. 66) are tested for departures from the 3:1 ratio, there are no significant differences from the expected in each cross considered separately. However, examination of the table shows a general trend for citrina to be less common than expected; when the data are pooled from these crosses, there is in fact, a slight but significant departure from an expected 3:1 ratio ($\chi^2_{(1)} = 6.54$; $P < 0.05$). The deficiency of citrina in

Table 24: Breeding data for *L. mariae* (single clutches)

Locality	Cross No.	Parents male	Parents female	Progeny citrina	Progeny olivacea	Progeny reticulata***
Menai Bridge*	36	cit	cit	92	0	9 (light)
	37	cit	cit	37	0	0
	41	cit	cit	41	0	0
Porth Swtan**	39	cit	cit	37	0	0
	40	cit	cit	12	0	18
	41	cit	lt ret	20	0	15
	42	cit	lt ret	26	0	28
	43	cit	dk ret	7	0	20
	44	cit	dk ret	3	0	28
	45	cit	dk ret	9	0	28
	47	cit	dk ret	8	0	11
	48	cit	dk ret	6	0	34
	49	dk ret	cit	0	0	5
	51	dk ret	cit	26	0	28
	54	dk ret	cit	6	0	19
	55	dk ret	cit	29	0	61
	56	dk ret	cit	3	0	10
	57	dk ret	dk ret	14	0	49
	58	dk ret	dk ret	6	0	35
	59	dk ret	dk ret	8	0	25
	61	dk ret	dk ret	5	0	16
	62	dk ret	dk ret	11	0	39
	63	dk ret	dk ret	14	0	55
	64	dk ret	dk ret	0	0	58
	65	dk ret	dk ret	13	0	58
	66	dk ret	dk ret	36	0	24
	67	dk ret	dk ret	10	0	40
	68	dk ret	dk ret	5	0	33
	70	dk ret	dk ret	3	0	11

* phenotype frequencies in population - citrina 98%, light reticulata 2

** phenotype frequencies in population - citrina 15%, light reticulata 5 ,
dark reticulata 80.

***-unscorable for light and dark unless specified.

- all reticulata from Porth Swtan much darker than those from Menai Bridge.

these crosses, in addition to the excess observed in No. 66, may simply be due to sperm storage. In view of the frequency of citrina and dark reticulata in the population, (15% and 80% respectively), it is not unexpected that one of the 12 crosses has a significant excess of citrina nor is it unexpected that the remaining crosses show a tendency for reticulata to be more common than expected assuming of course that matings occur at random in the field.

citrina x dark reticulata (No. 43-No. 56)

On the hypothesis of a single locus, the progeny of these crosses should be either cell reticulata or reticulata and citrina in a ratio of 1:1. One of crosses (No. 49), although with only 5 individuals present, does give all reticulata while the remaining 9 crosses segregate into the two phenotypes. Among these, there is a significant heterogeneity ($\chi^2_{(9)} = 19.60$; $P < 0.001$). 3 of the crosses (No. 47, No. 51 and No. 56) give the expected 1:1 ratio, or at least non-significant departures from it, while the other 6 crosses (No. 43, 44, 45, 48 and 54) give significant deviation with citrina always less frequent than expected, the same trend that was observed in the dark reticulata x dark reticulata crosses. If one is to assume that sperm storage from random matings has occurred, the deviations are in the expected direction with reticulata progeny more common than expected.

Another test of the single locus model is to compare the observed number of segregating crosses with the expected number. At Porth Swtan, the frequencies of dark reticulata and citrina are 80% and 15% respectively. On the hypothesis of a single locus, these would give allele frequencies of approximately

0.6 and 0.4 assuming dark reticulata to be dominant to citrina. Therefore, the proportion of total dark reticulata which will be heterozygous will be

$$\frac{2(0.6 \times 0.4)}{0.8} = 0.6$$

If this is the case, then one expects $(0.6^2)12$ or 4.3 of the 12 crosses involving dark reticulata x dark reticulata to segregate into the two phenotypes. The observed number segregating are 11; this is significantly more than the expected ($\chi^2_{(1)} = 16.3$; $P < 0.001$).

	seg	non seg
exp	4.3	7.7
obs	11	1

A similar comparison can be made with the 10 dark reticulata x citrina crosses. One expects 0.6 x 10 or 6 of these to segregate into the two phenotypes. The observed number is 9 which is again significantly more than that expected ($\chi^2_{(1)} = 4.0$; $P < 0.05$).

	seg	non seg
exp	6	4
obs	9	1

In both cases, therefore, there are significantly more segregating crosses than one would expect from the estimated frequency of heterozygotes on a single locus-two allele model. However, if one is to assume that sperm storage has occurred, as was mentioned earlier, then most of the crosses would be expected to segregate into the two phenotypes. For example, non segregating crosses are expected from the following matings:

	male		female
1	R/R	x	R/R
2	R/r	x	R/R
3	R/R	x	R/r
4	R/R	x	r/r
5	r/r	x	R/R

where R/- is dark reticulata and r/r citrina. If females in all of these matings, except the first and second, had mated once before and stored sperm from either R/r or r/r males, then in each case the progeny will segregate into the two phenotypes with ratios dependant upon the proportion of sperm used from each male. Some of the citrina that have been observed in the progeny (i.e. No. 43, 44, 45, 48, 54 and 55) may in fact be due to stored sperm from these matings. What has been calculated to be significant deficiencies of citrina from a 1:1 ratio in these crosses is then open to question.

citrina x citrina (No. 39, 40)

These two crosses gave progeny which were either all citrina, or citrina and reticulata, with the reticulata slightly more common. If citrina is recessive, then one of the crosses (No. 39) fits well with the expectation for a recessive x recessive cross. The occurrence of reticulata in the second cross (No. 40) does not conform to the hypothesis, unless of course the citrina female had stored sperm from a previous mating with a dark reticulata, not an unlikely assumption judged by the frequencies of the two in the population.

citrina x light reticulata (No. 41 and 42)

Both of these matings gave progeny with citrina and reticulata in a ratio of 1:1 since there are only two matings involving the light reticulata, little can be inferred from the data.

When considering all the 26 crosses from Porth Swtan, in 6 there are significant departures from the expected ratios according to the single locus model. If one can assume that sperm storage has occurred, then these departures from the expected values are in the direction that would be predicted if matings had occurred at random in the field. It is not necessary to introduce any other restrictions such as disassortative mating, sperm viability etc. The crucial question on which the single locus model depends is whether in fact sperm storage does occur. Cursory observations were made to determine whether this was the case by a dissection of 5 individuals from Porth Swtan. However, an examination of the genital duct yielded no sperm in these females. It is known that in the river snail <u>Viviparus</u>, sperm can be stored for up to 11 months in the albumen gland of the female (Ankel 1925, from Fretter and Graham 1962, p. 369). Whether, in fact, this occurs in <u>Littorina mariae</u> is not at present known and awaits further investigation.

An alternative genetical model involving two loci, rather than one, has been suggested by Dr. Deborah Charlesworth. This model accounts for most of the ratios in the progeny without invoking as much sperm storage as was necessary in the single locus model.

Assume that there are two loci each with two alleles: R/r and C/c where the dominant allele for both loci must be present for the expression of reticulata. The different genotypes with the corresponding phenotype will be the following:

R/-	C/-	reticulata
R/-	c/c	citrina
r/r	C/-	citrina
r/r	c/c	citrina

With the dark reticulata x dark reticulata matings, one expects not only ratios of 1:0 and 3:1 as observed in a single locus model, but also one of 9:7. This last named ratio provides a closer fit to cross No. 66 where there were more citrina than reticulata. However, the significant deviations observed from a 3:1 ratio, with citrina less common than expected, are not overcome with the two loci model.

In the dark reticulata x citrina matings, in addition to ratios of 1:0 and 1:1 observed with the single locus model, one expects also a ratio of 3:1. These three ratios are similar to what is observed. This would account for what appeared to be significant deficiencies of citrina from a 1:1 ratio.

The citrina x citrina matings rather than resulting only in citrina progeny in the single locus model, are expected to produce not only pure citrina with two loci but also citrina and reticulata in a ratio of 1:1. This latter ratio does correspond to cross No. 40.

With breeding results that have been obtained from Porth Swtan, no definite conclusions can be drawn on the type of genetical model involved. It would seem that 2 loci rather than a single one would offer a more parsimonious explanation for the observed crosses. However, until it has been demonstrated that sperm storage does not occur it seems just as reasonable to assume that at Porth Swtan, a single locus with at least two alleles are present with dark reticulata dominant to citrina.

Three matings were carried out with adults from Menai Bridge. At this locality, 98% of individuals are citrina while the remaining 2% are citrina with a very faint expression of the reticulata pattern. These latter individuals are not a discrete phenotype but show a continuous range from citrina to light reticulata. In two of the crosses (No. 37 and 41) there were only citrina produced, results which are expected of citrina are recessive. However in cross No. 36, although most of the individuals are citrina, a few show very faint expression of reticulations with the same continuous range as was seen in the field where the adults were collected. This seems unlikely to be due to sperm storage, since the frequency of light reticulata in the population is only 2%. What is more likely to be the case is that the wide range of expressions of reticulations are controlled at a number of separate loci from the yellow ground colour.

(ii) L. obtusata

The breeding data for L. obtusata shown in Table 25 provide little information on the mode of inheritance of shell colour. The majority of progeny had died before they could be reliably scored owing to the fact that in this species there is a continuum of shell colours until about 6mm in size and a tendency for light coloured shells to become darker with age. Thus individuals scored as citrina in the juvenile stages may change to olivacea with new increments of shell growth. Likewise juveniles scored as olivacea may become reticulata with increased growth. These factors combined with the possibilities of contamination and sperm storage allow only general inferences to be made.

At Menai Bridge the matings of citrina x citrina and citrina x olivacea each gave progeny which segregated for the two phenotypes. The single cross of olivacea x olivacea (No. 22), gave only olivacea although only 3 individuals had survived. It would seem from these crosses that citrina is more likely to be dominant than recessive to olivacea. However owing to the possibility of contamination and sperm storage, this may not be so.

In the 3 crosses from Porth Swtan, the two of citrina x citrina gave in one case (No. 31) citrina and olivacea and in the other (No. 29) citrina, olivacea and reticulata. The 4 individuals of reticulata in this cross may imply that the reticulations are controlled by a separate locus. This is certainly possible since the reticulata pattern is found on an olive ground colour in this species, similar to olivacea in appearance. If this is so, then the dark reticulata x dark reticulata cross (No. 34), can be interpreted as the reticulate pattern segregating into reticulate and non reticulate individuals. From the proportion which is not significantly different from a 3:1 ($\chi^2_{(1)} = 1.05$; P = > 0.5), it would seem

Table 25: Breeding data for L. obtusata (single clutches)

Locality	Cross No.	Parents male	Parents female	Progeny citrina	Progeny olivacea	Progeny reticulata
Menai Bridge*	2	cit	cit	12	12	0
	3	cit	cit	13	9	0
	11	cit	cit	2	9	0
	18	ol	cit	15	7	0
	20	ol	cit	25	13	0
	21	cit	ol	9	10	0
	22	ol	ol	0	3	0
Porth Swtan**	29	cit	cit	7	10	4
	31	cit	cit	12	12	0
	34	dk ret	dk ret	0	10	43

* morph frequencies in population - citrina 35%
- olivacea 65%

** morph frequencies in population - citrina 8%
- olivacea 40%
- light reticulata 15%
- dark reticulata 37%

that the reticulate pattern is dominant to its absence.

Until further breeding experiments have been carried out with this species, little if anything can be reliably interpreted from these crosses.

4. Discussion

From the limited amount of data that has been obtained from these breeding experiments it seems reasonably certain that the different colour phenotypes are genetically controlled. In L. mariae the two major phenotypes citrina and dark reticulata behave generally as allelomorphs (at a single locus) with dark reticulata dominant to citrina. It is also possible that two complimentary loci are involved, as those proposed by D. Charlesworth.

The reticulate pattern may be controlled at a separate locus from that of the shell colour, as was suggested in one of the crosses of L. obtusata. If this is the case then the dark brown ground colour of dark reticulata in L. mariae may be in actuality citrina with an infusion of the black reticulations. The results observed, therefore, may simply reflect segregation of the reticulate/non-reticulate character, on a citrina background, with reticulate dominant to non-reticulate. Since all combinations of ground colour and reticulations have been seen when surveying a large number of localities, it is quite likely that the two characters are separately controlled. A difficulty at present most evident in L. mariae, is determining what is the undiluted ground colour of dark reticulata. In this study, I have used Sacchi's classification (see Section III, 2, ix) which separates reticulata into two phenotypic categories, light and dark. It must be borne in mind during subsequent discussions on the polymorphism that although light or dark reticulata are phenotypically similar

from individual to individual or locality to locality, they probably constitute a large number of different genotypes.

SECTION VII

GEOGRAPHICAL VARIATION OF THE COLOUR POLYMORPHISM IN L. OBTUSATA AND L. MARIAE

1. Introduction

Barkman (1956) working with samples of the flat winkle from Holland, Norway, Scotland and Brittany found that the common colour morphs - citrina, olivacea, and reticulata (see Section III, 2, ix for description) vary considerably from shore to shore. When Sacchi and Rastelli (1966) split L. obtusata into two species, L. obtusata and L. mariae, they pointed out that the morph frequencies differed between the species at the same locality. Many of the changes in frequency that had been observed between localities by earlier workers such as Barkman, were in fact changes in the abundance of the two species.

With further work by Sacchi (1969) in Brittany, it was apparent that the highly variable morph frequencies in each species generally behaved in a predictable pattern on shores of different exposure. He found that on sheltered shores, olivacea is the most common morph in L. obtusata while citrina is the most common in L. mariae; on exposed shores, dark reticulata predominated in both species. To account for these changes, he suggested that selection was acting on pleiotropic characters rather than on shell colour itself. Similar conclusions were also reached by Gratton (1969) with regard to the variation in L. mariae from South Wales.

This section describes the geographical variation of the polymorphism in L. obtusata and L. mariae from the samples collected in Wales, South England and Ireland. The pattern of variation is similar to that observed by both Sacchi and Gratton. The results, however, suggest very strongly that in both species, visual selection for crypsis, rather than pleiotropy, is the predominant factor responsible for the various frequencies in each of the common colour phenotypes.

2. Results

(i) L. obtusata

Phenotype frequencies were found to vary not only between major geographical areas but also between adjacent shores. Repeated sampling at some of the shores over a period of 3 years, 1970-1973, has shown that the frequencies did not change during this time (see appendix D for raw data). Table 26 gives a summary of the data for each of the areas which have been sampled. The appropriate maps and graphs (figs 30-40) are collectively given on pp. 170-182.

(a) citrina

This is one of the most conspicuous and discrete of the phenotypes in L. obtusata. It occurs throughout the study area with a mean frequency of 13% and a range from 0-49. It has never been observed to be the most common phenotype in any single population.

On the coast of Anglesey, citrina is found with a frequency of 8% (range 0-40). The highest values occur in some of the very sheltered localities of the Menai Straits and on the exposed headlands at North Stack (Fig 30). There is some regularity in frequency changes of citrina within the Menai Straits where there is a change from 0% at Ysgubor Isaf on the southern end of the straits, to 40% at Train Bridge in the middle, after which there is a decline to 14% at Beaumaris on the northern end. Although all of these localities have been treated as being very sheltered, there are large differences in the velocity of the current between the localities. During the tidal change, a large volume of water moves through the straits, moving most rapidly in the narrowest parts of the channel which are

Table 26: Distribution of phenotype frequencies for L. obtusata among each of the major study areas (see appendix D for raw data)

Locality	citrina			olivacea			light reticulata			dark reticulata			No. of Localities
	$\bar{x}$	S.D.	range	$\bar{x}$	S.D.	range	$\bar{x}$	S.D.	range	$\bar{x}$	S.D.	range	
Anglesey	9.2	11.4	0-40	74.4	21.6	16-100	2.4	5.2	0-25	14.0	14.0	0-70	29
Rest of Wales	3.2	5.1	0-12	86.6	16.9	52-100	0	0	0	10.2	13.6	0-35	9
South England	11.1	11.1	0-24	49.8	41.6	1-100	0	0	0	39.1	33.4	0-75	9
Isles of Scilly	25.4	10.8	3-49	2.6	5.2	0-20	6.9	5.8	1-28	65.1	12.4	45-92	28
Ireland	9.4	11.6	0-32	56.8	32.5	10-100	0.6	1.3	0-4	33.2	23.1	0-70	19

found near Train Bridge. Here citrina reaches its highest frequencies. At either end of the straits, the channel is much wider and the current velocity correspondingly slower, at least in the intertidal region. In these localities citrina occurs in very low frequencies.

A series of collections made off South Wales provides a comparison to the observed correlation in the Menai Straits. The localities found in the narrow parts of the Bristol Channel, as at Severn Bridge, have a very rapid current compared to the wide channel at Barry. If citrina has an advantage in strong current, the frequency should be higher at Severn Bridge than at Barry. This is not the case, for in these localities, citrina is completely absent (fig 31).

In Southern England, citrina has an average frequency of 11% (range 0-24). The highest values occur at Looe, one of the more exposed localities, and at Bodinnick a very sheltered shore although with some current. A comparison of the frequency of citrina with the amount of exposure from Wales and Southern England shows that there is no significant correlation (fig 37).

On the Isles of Scilly, off the south-western coast of Cornwall (fig 32), citrina becomes more common than in any of the other geographical areas, reaching a mean frequency of 25% (Range 0-50). Unlike Wales and South England where citrina was only rarely abundant, the majority of localities on the Isles of Scilly have a relatively high citrina frequency. In a series of collections at White Island off St. Martins (fig 33), the highest frequency of citrina (48%) is present in the conditions most protected from waves, while the lowest frequency (34%) occurs in the exposed locality. Citrina is least common at Lawrence Bay (No. 64) and Old Quay (No. 63), two of the most sheltered localities that were sampled on the islands. There is, therefore, no general pattern of change with different exposures (fig 38).

In Ireland (fig 34), citrina occurs with a mean frequency of 9% (range 0-32), which is very similar to Wales. The highest frequency was found in the north-west of Ireland at Streedagh House Bay. When this shore was first visited the initial impression of the colours and general diversity of the intertidal region appeared very similar to shores in the Isles of Scilly where the frequency of citrina was also high. At Milkhaven, a very sheltered locality 4km from Streedagh House Bay, citrina is completely absent. When considering all the samples from Ireland, there is a significant increase in the frequency of citrina with increased exposure (fig 37).

Although citrina is generally lemon-yellow in colour, one occasionally finds shells which are very pale, almost white in appearance. These are absent in Wales, but occur with low frequencies (≃ 5%) in Ireland and Southern England. On the Isles of Scilly, they are more abundant comprising 20% of the total citrina population in some localities with intermediate exposure (e.g. Porth Hellick). Another variation of citrina is the occurrence of yellowish-orange shells. These are found throughout the study area, usually comprising about 10% of the total citrina population. There are two localities where the yellowish-orange shade is more common than the lemon-yellow shade. These are Dale Fort (South Wales) and Streedagh House Bay (Ireland), both of intermediate exposure.

(b) Olivacea

Of all the phenotypes in <u>L. obtusata</u>, olivacea is the most cryptic to the human eyes. It occurs in the study area with a mean frequency of 47% and a range from 0-100. In Anglesey its mean frequency is 74% (range 16-100) and it is the most common phenotype in most localities. The lowest and highest values are found at North Stack and Four Mile Bridge respectively. These two localities, only 7km apart, represent two extremes in the conditions of exposure for Anglesey, Four Mile Bridge being very sheltered and North Stack very exposed. This is a general pattern in Anglesey for olivacea to be very abundant in all localities except in the more exposed conditions.

In the rest of Wales, south of Anglesey, olivacea is still the most common morph in all of the localities, occurring with an average frequency of 86% (range 52-100). The lowest value is again found in the most exposed conditions, at Dale Fort.

In southern England, olivacea is relatively less common than in Wales ($\bar{x}$ = 49%; range 1-100). In the very sheltered localities such as Rock, Bodinnick, and Dartmouth, the frequencies are very high, as they are in sheltered and semi-exposed conditions in Wales. On shores of intermediate exposure (Lynmouth, Looe, Wernbury) there are relatively low frequencies of olivacea compared with shores of equivalent exposure in Wales. An unpaired t-test between the means in these localities (3 from South England and 12 from Wales) gives a significant value of 4.09 for 13 D.F. ($P < 0.002$).

When all the localities from Wales and southern England are considered, there is a significant decrease in the frequency of olivacea on shores of increasing exposure (fig 38).

While olivacea is the most common phenotype in Wales, on the Isles of Scilly off the south-west coast of Cornwall, it is the rarest occurring with a mean frequency of 3% (range 0-20). The highest values occur in the relatively sheltered localities on the inner sides of the islands at Lawrence Bay and Old Quay. The majority of other localities from these islands are in exposed conditions and show a very low frequency of olivacea.

In Ireland, olivacea is the most common phenotype on most shores ($\bar{x}$ = 57%; range 10-100). In the most northerly sheltered locality, Dunfanaghy, olivacea is present at 95% frequency. 30km to the west at Curran's Port a very exposed area, the frequency drops to 14%. A similar pattern is present in two localities further south. One of these, Milkhaven, is very sheltered and shows a frequency of 82%, while 4km away on the exposed shore at Staad Bay, the frequency drops to 36%. The remaining localities in Ireland show a similar pattern between frequency and exposure, (see fig 35). This can also be seen in Fig 38 which shows a significant reduction in frequency with increased exposure.

Within the olivacea there is variation in the expression of the olive colouration from an olive-yellow through to a dark olive-green. In Anglesey, the three categories (light, intermediate, dark) occur with frequencies of 4.9, 88.2, and 6.9% of total olivacea respectively, the intermediate being the most common shade in all of the localities. There is little variation in the light but the dark shows a slight increase on shores of greater exposure.

Between Anglesey and the Bristol Channel, the proportion of the dark-olivacea increases and becomes more common than the intermediate shade. At Severn Bridge in the Bristol Channel, it reaches 100% of the olivacea morph. The increase in the dark is not correlated with increased exposure. In the one locality where the intermediate is most common (Newport), the shore conditions are very sheltered.

In southern England (ol_1 - 1.3; ol_2 - 57.1; ol_3 - 41.6), there is also a high proportion of the dark shells although a great deal of variation occurs. On the more exposed shores where olivacea is abundant (Lynmouth, Buck Mills) the frequency of dark olivacea are 75% and 100% respectively. In the sheltered localities (Rock, Bodinnick, Dartmouth) the values drop to 7%, 40% and 25%. At Weston-super-Mare, in the Bristol Channel, the conditions are very sheltered yet the frequency of the dark olivacea is 87%. In the south part of Devon and Cornwall where olivacea is rare, those that are found are predominantly of the intermediate shade, as they are in Wales.

Although olivacea is very uncommon in the Isles of Scilly some variation does occur in the proportion of each shade. The mean frequencies for the light, intermediate and dark shades are 46.7%. 31.6% and 21.7% respectively. The major difference from the localities in Wales and south England is the relatively high frequency of the light shade. The majority of these samples contain less than 10 individuals of olivacea and are therefore subject to a greater sampling error, but the mean frequencies do suggest that olivacea is on average lighter than those from the mainland. It is not possible from these samples to determine whether there is a relationship to exposure. In general there would not seem to be, as the islands are generally more exposed than Wales. If there were a relationship with exposure the dark rather than the light should have predominated.

In Ireland, the general variation in the shades is similar to Anglesey with the frequencies being 15.6, 62.3 and 22.1% for the light, intermediate, and dark shades respectively. Where the dark shade shows an increase in frequency, the localities are relatively exposed, just as in Anglesey. It is never as common as the intermediate shade as was evident in the Bristol Channel.

Another aspect of the colouration of the olivacea is the existence of slight differences in hue. Most frequently olivacea is of an olive-green appearance but occasionally it is olive-brown or slightly red. In Wales and Ireland the usual colour is olive-green with occasional exposed shores having olive-brown individuals. The effect of the olive-brown compared to the olive-green is to give the shells a slightly darker appearance. In southern England, individuals from sheltered conditions are generally olive-green while on the more exposed shores, rather than being olive-brown, as in Wales they are reddish. This is most developed on the Isles of Scilly where the majority of individuals of olivacea are reddish.

To summarize, olivacea is the most common phenotype on the majority of shores in Wales, and shows a reduction in frequency on the more exposed conditions. In southern England it is common only in the very sheltered conditions just as in sheltered localities in Anglesey. On the Isles of Scilly, olivacea is now the least common phenotype in most localities, but again occurs with higher frequencies on shores with increased shelter. Ireland shows a similar pattern to Wales with olivacea being the most common phenotype in most localities, except the most exposed. Of the three shades of olivacea (light, intermediate, dark), the intermediate is the most common in Anglesey and Ireland. In the rest of Wales and southern England the dark form is most common except in some of the very sheltered conditions and when the morph is rare in the population. In these cases the intermediate shade predominates. On the Isles of Scilly, the light shade is most common on the majority of localities. With reference to the tone, olivacea is generally olive-green in Ireland and Wales except on exposed conditions when the colour is occasionally olive-brown. In the south of England and in the Isles of Scilly, olivacea appears slightly reddish.

(c) Dark Reticulata

As with olivacea, the dark reticulata is very cryptic on most of the intertidal fucoids. It is found throughout the study area with a mean frequency of 37% and a range from 0-92. The pattern of variation is the opposite to that of olivacea in that it is common when the latter is rare, owing to the relative constancy of frequencies in citrina and light reticulata.

In Anglesey, the average frequency is 14% (range 0-70), with the greatest abundance occurring on the western side of the island. The highest values observed are found on the exposed localities at Hen Borth and Porth Swtan. The phenotype is generally absent or rare in all sheltered conditions as for example in the Menai Straits.

The localities between Anglesey and the Bristol Channel have low frequencies similar to the Menai Straits. The most exposed locality in these collections occurs at Dale Fort where the highest abundance of dark reticulata is present (35%). In southern England the phenotype is relatively more common ($\bar{x} = 39\%$; range 0-75). The very sheltered localities (Rock, Bodinnick, Dartmouth) have very low frequencies which are similar to those associated with equivalent conditions in Wales. However on the more exposed sites dark reticulata becomes the most common phenotype. Including all the localities from Wales and south England, there is a significant ($P < 0.01$) increase in the frequency with increased exposure (fig 39).

On the Isles of Scilly the phenotype is very common in all localities ($\bar{x} = 65\%$; range 50-92). The higher frequencies are generally associated with conditions of greater exposure such as are found on the outer shores of the islands. The series of collections from White Island taken over a steep exposure gradient, shows a similar pattern of frequency change. The dark reticulata is least common in the sheltered bay and most common at the exposed site at Top Rock Point. Over all the localities from the islands, there is only a slight and non-significant (P = 0.10-0.20) increase in frequency with increased exposure (fig 38).

In Ireland ($\bar{x}$ = 33%; range 0-70), the pattern of variation is similar to that seen in Wales. Where olivacea is abundant in sheltered conditions, dark reticulata is rare. On the more exposed shores, olivacea decreases in frequency with a consequent increase in dark reticulata. These changes can also be seen over very short distances such as the Dunfanaghy-Curran Port collections where over a distance of 30km the frequency changes from zero to 60%. The same pattern occurs at Milkhaven (fig 35) over a distance of 750m. Here the dark reticulata change from 0-50% from sheltered to exposed conditions. Although there are predictable changes in frequency, the inclusion of all the samples from Ireland shows that there is only a slight and non-significant (P = 0.05-0.10) trend with changes in exposure (fig 39).

There is some variation in the ground colour of the dark reticulata. When olivacea is common, the reticulate pattern is found generally on an olive-green or olive-brown ground colour, the latter being more common on exposed shores. This is the usual situation in Wales, except for one locality (Llwyngwril) where of 7 dark reticulata found one had a red-brown ground colour. In southern England and on the Isles of Scilly this ground colour becomes more common and is often present on all the dark reticulata shells except on shores with very sheltered conditions, where the ground colour is olive-green or olive-brown. In Ireland, the red-brown ground colour occurs on some of the more exposed shores, most frequently in the northern locality of Curran's Port where it is present in 70% of the dark reticulata shells (fig 36).

In Ireland, the Isles of Scilly and some localities in southern England, the red-brown ground colour is present on both juvenile and adult shells, while in Wales and North Devon only adults have it. In the localities where the red-brown colour was absent from the juvenile, the adults showed different degrees of wearing on the shells. Those showing the least amount had the reticulate pattern on a dark-olive ground colour. In some cases parts of the shell were worn exposing a dark red colour under the dark olive. The most extreme cases of wearing resulted in the entire shell appearing a very dark red. When unworn dark reticulata and olivacea from these two sites were abraded against a rough surface, the outer colour was removed leaving the reddish colour underneath. It appears therefore that the red-brown colouration in these two localities is secondary and due to environmental factors. When dark reticulata and olivacea shells from Anglesey are abraded against the underlying rocks, the underlying colour is not red but olive-green or olive-brown, the same as the unworn colours. It is possible therefore that the secondary reddish colouration seen in Wales and north Devon has some ecological significance as it occurs on the edges of the range of the red-brown colouration seen in the Isles of Scilly and south Devon and south Cornwall, where both juvenile and adults show the expression.

In summary, the dark reticulata are relatively uncommon in Wales and Ireland except on the more exposed shores. In southern England and the Isles of Scilly, it becomes the most common phenotype, showing a further increase in the more exposed conditions.

The ground colour is basically olive-green or olive-brown, the latter being more common on shores of greater exposure. In southern England, the Isles of Scilly and in parts of Ireland, the ground colour is reddish-brown rather than olive.

(d) Light Reticulata

This phenotype is intermediate in appearance between the conspicuous citrina and the cryptic dark reticulata and olivacea. It is the least common in most areas, occurring with a mean frequency of 3.2% and a range of 0-28.

In Anglesey ($\bar{x}$ = 2.4%; range 0-25) it is present only when dark reticulata is abundant. The highest frequencies occur on the shores of intermediate exposure which in general have the greatest diversity of habitats. Between Anglesey and south England, the phenotype is not present in any of the localities sampled. On the Isles of Scilly, it becomes more common ($\bar{x}$ = 6.9%; range 1-28) and occurs on all of the shores. The highest frequencies are found at Lawrence Bay and Toll's Island, a sheltered and exposed shore respectively. At White Island (fig 33) there is little variation in frequency over a series of exposure gradients, as is also evident from fig 34 in which all the localities in the islands are compared.

In Ireland ($\bar{x}$ = 0.6%; range 0-4) the phenotype is absent on most shores except where dark reticulata are common. On these light reticulata occur at low frequencies which is similar to the pattern observed in Anglesey.

(e) Uncommon Phenotypes

Throughout most of the study area the rare phenotypes listed in Section III, 2, ix are absent or occur with frequencies less than 1%. One exception occurs at White Island on the Isles of Scilly (No. 52) where the banded phenotypes (inversicolour) reach 6% in frequency.

Figures 30-36

Phenotype frequencies of L. obtusata around the British Isles. For reference to locality names mentioned in text, see figs 1, 2 & 3. Raw data given in appendix D - table Di. In the following figures, some of the localities show more than one diagram; these represent additional samples taken at one year intervals.

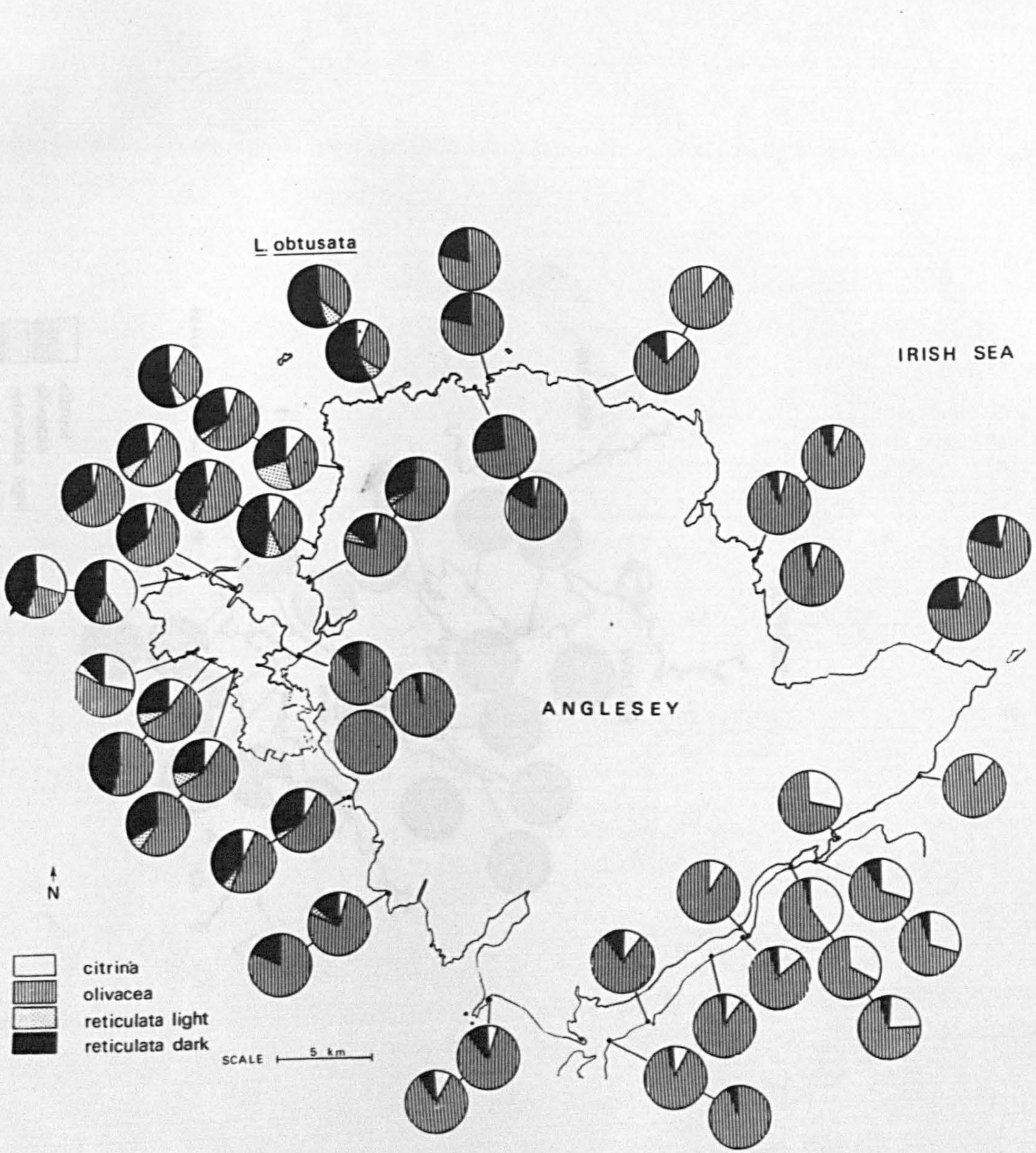


Fig. 30. Phenotype frequencies in L. obtusata around Anglesey, North Wales.

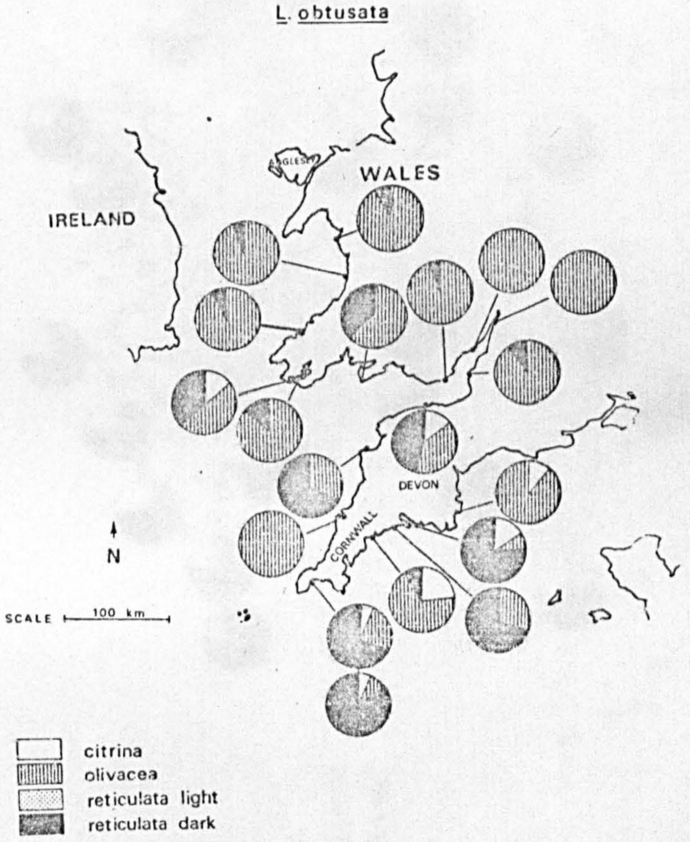


Fig. 31. Phenotype frequencies in L. obtusata between North Wales and southern England.

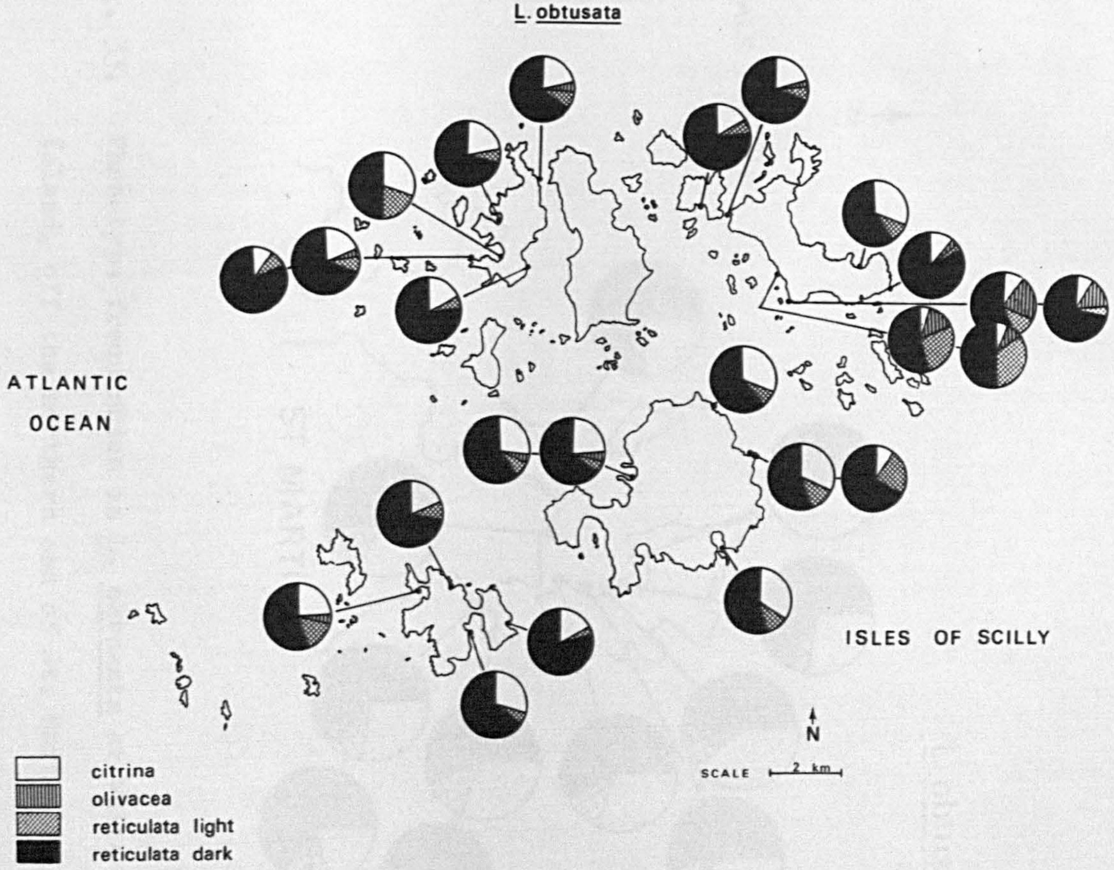


Fig. 32. Phenotype frequencies in L. obtusata around the Isles of Scilly.

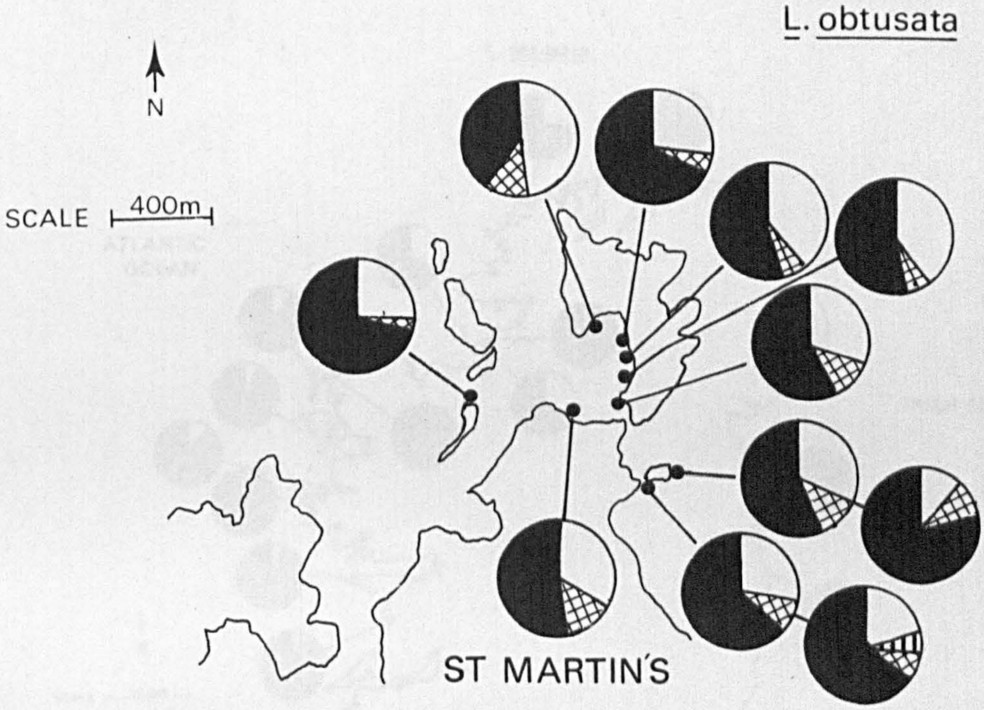


Fig. 33. Phenotype frequencies in L. obtusata at White Island, off the northern end of St. Martin's on the Isles of Scilly.

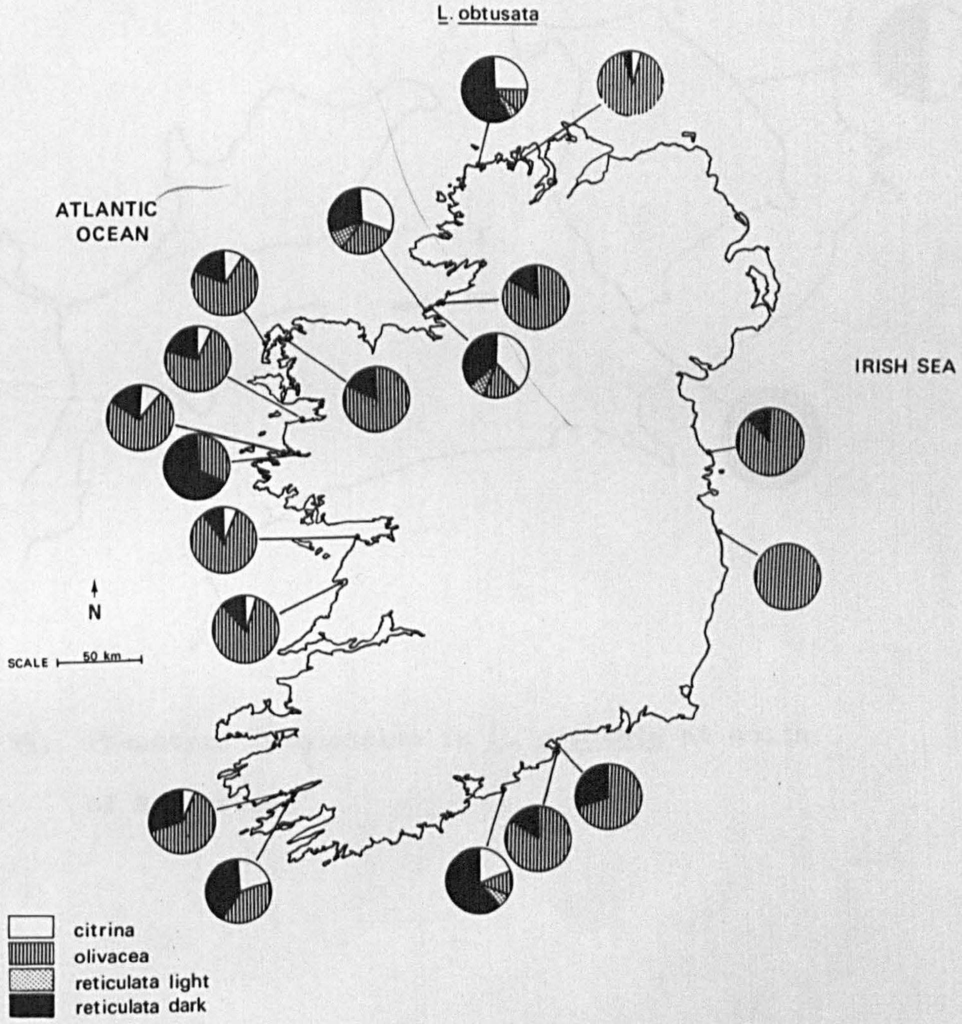


Fig 34. Phenotype frequencies in L. obtusata around Ireland.

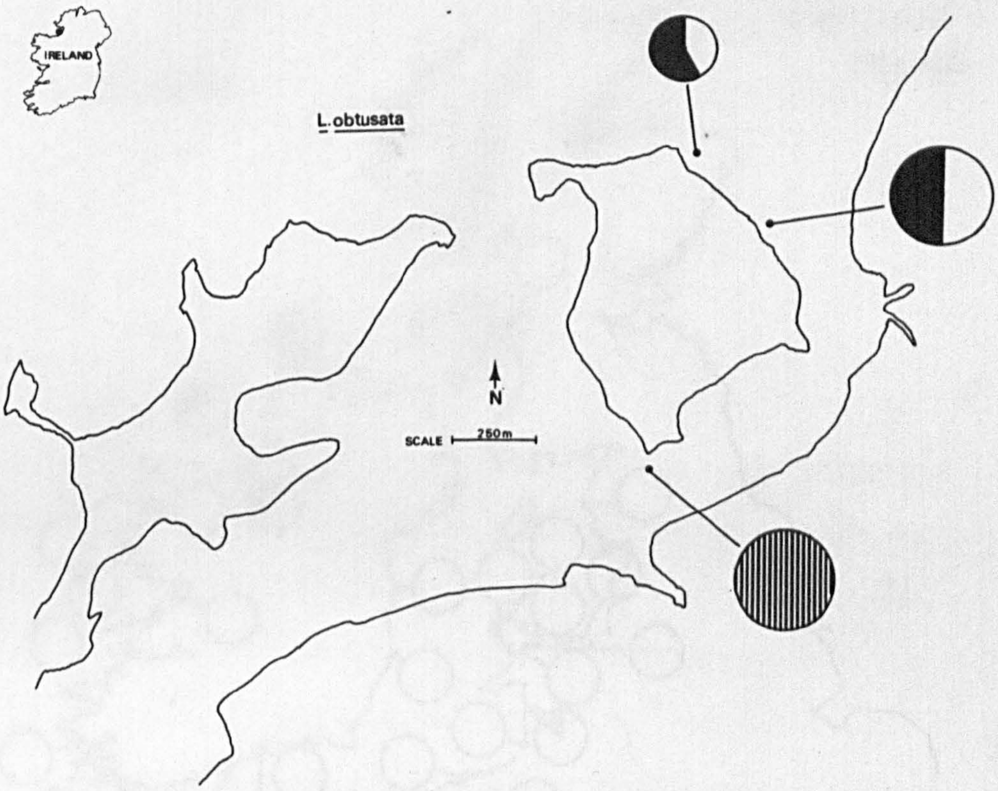


Fig 35. Phenotype frequencies in L. obtusata at mouth of Milkhaven.

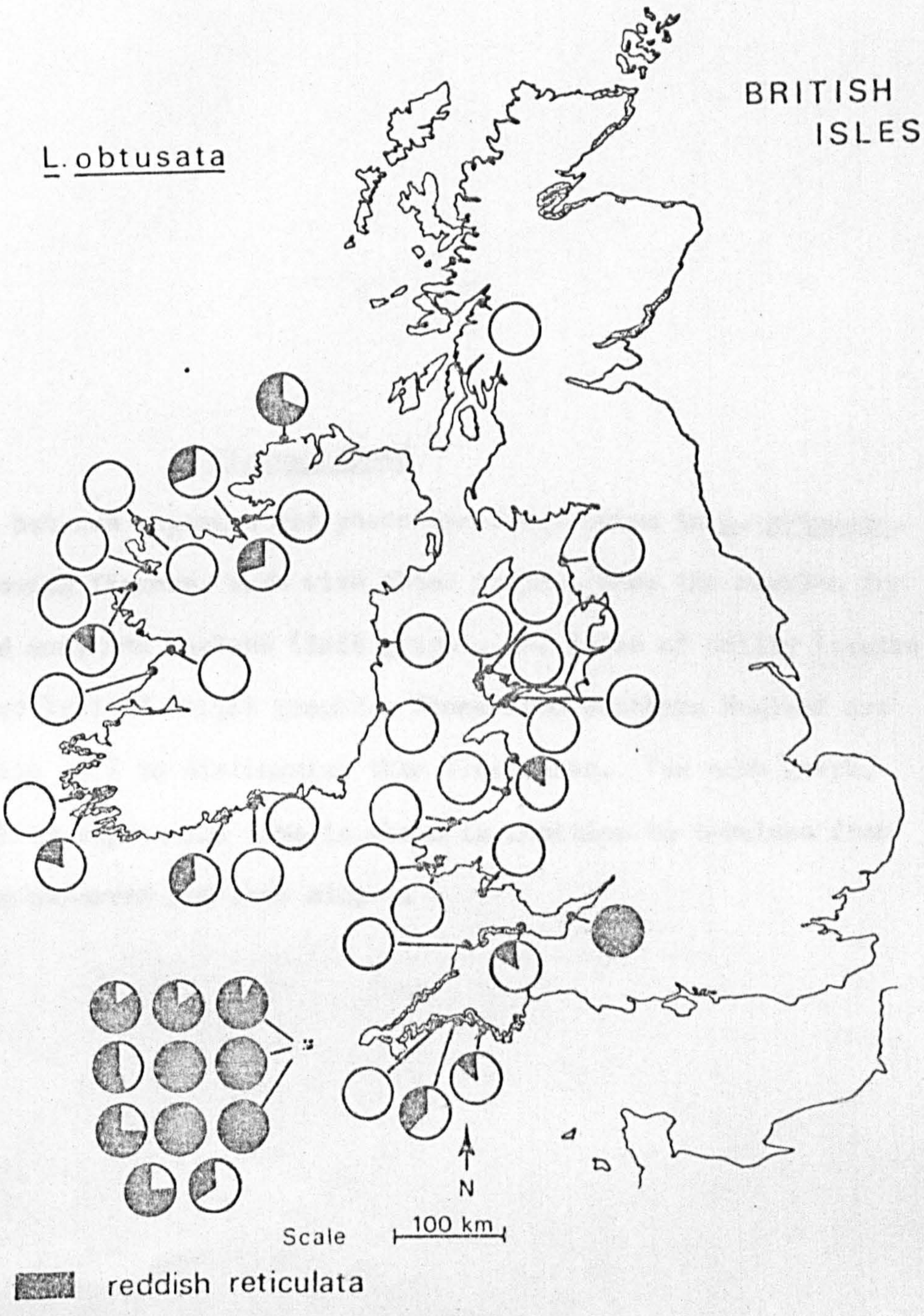


Fig 36. Occurrence of red-brown phenotypes in L. obtusata around the British Isles. Shaded portions of diagrams show proportions of the total dark reticulata in any sample having a red-brown colouration. Heavy set coastlines show approximate distribution of Devonian Red Sandstone (from Geological Map of British Isles, 1969). Isles of Scilly, off south-western tip of Cornwall lacking Red Sandstone.

Figures 37-40

Relation between exposure and phenotype frequencies in L. obtusata. The following figures, each with three graphs, show the results for Wales and southern England (left graph), the Isles of Scilly (centre graph) and Ireland (right graph). Those from southern England are marked with an X to distinguish them from Wales. For each graph, the weighted regression line is shown in addition to t-values from comparing observed and zero slopes.

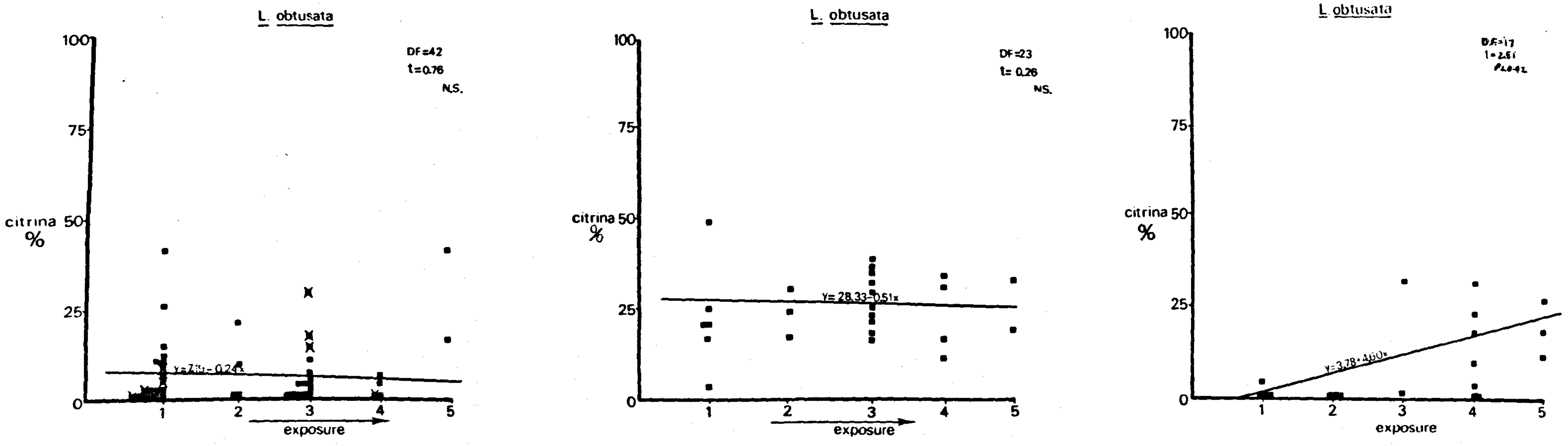


Fig 37. Relation between exposure and citrina. Wales and southern England (left), Isles of Scilly (centre), Ireland (right).

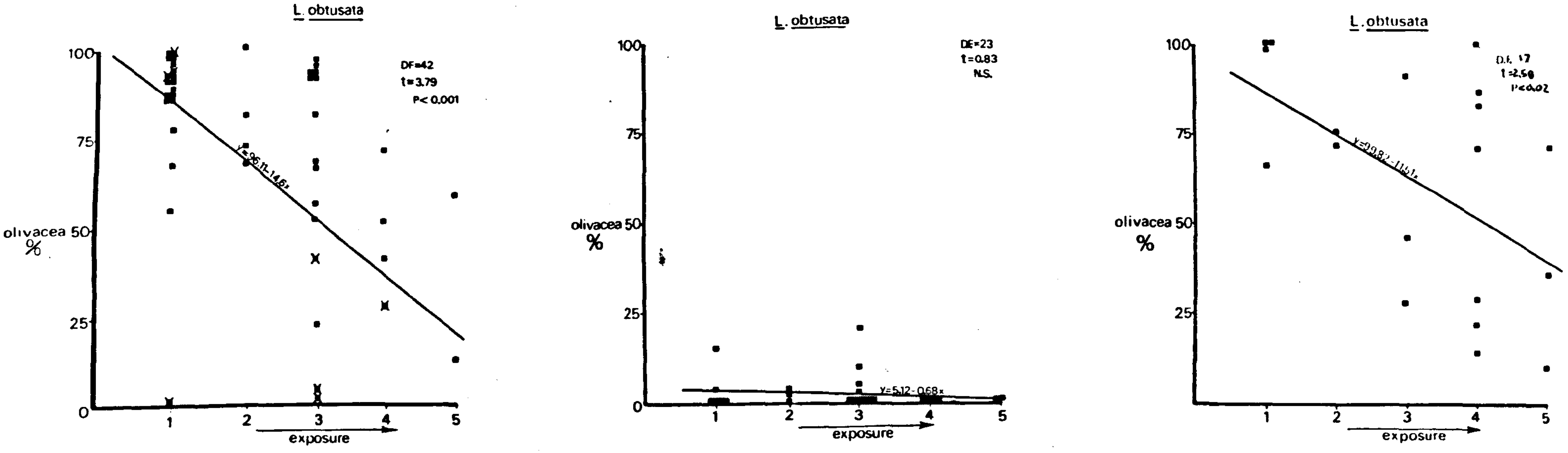


Fig 38. Relation between exposure and olivacea. Wales and southern England (left), Isles of Scilly (centre), Ireland (right).

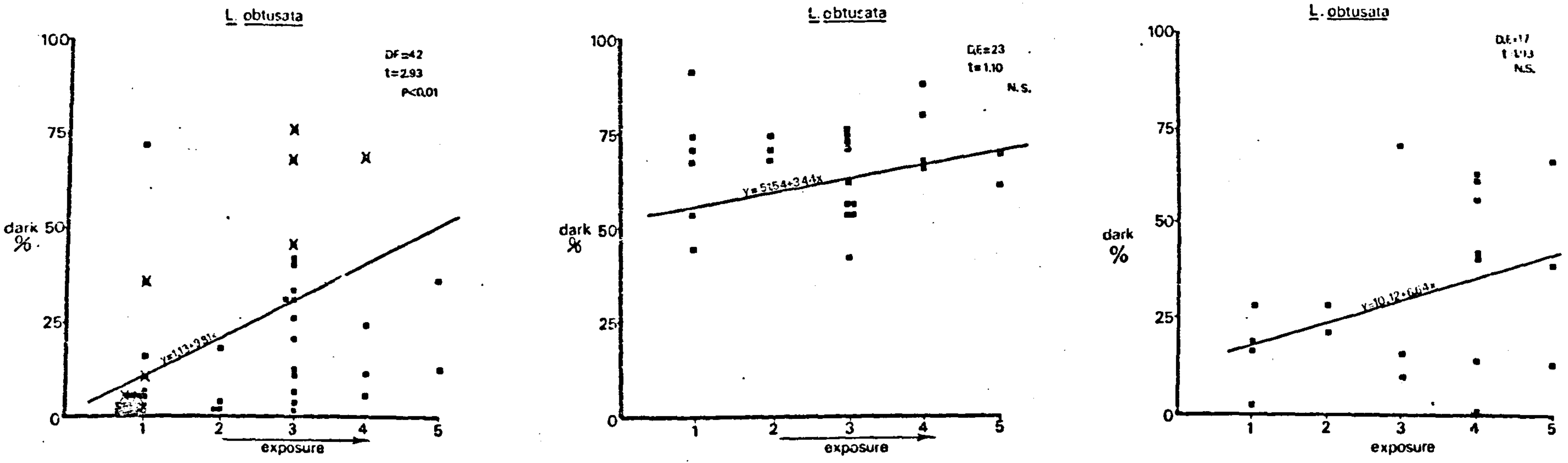


Fig 39. Relation between exposure and dark reticulata. Wales and southern England (left), Isles of Scilly, (centre), Ireland (right).

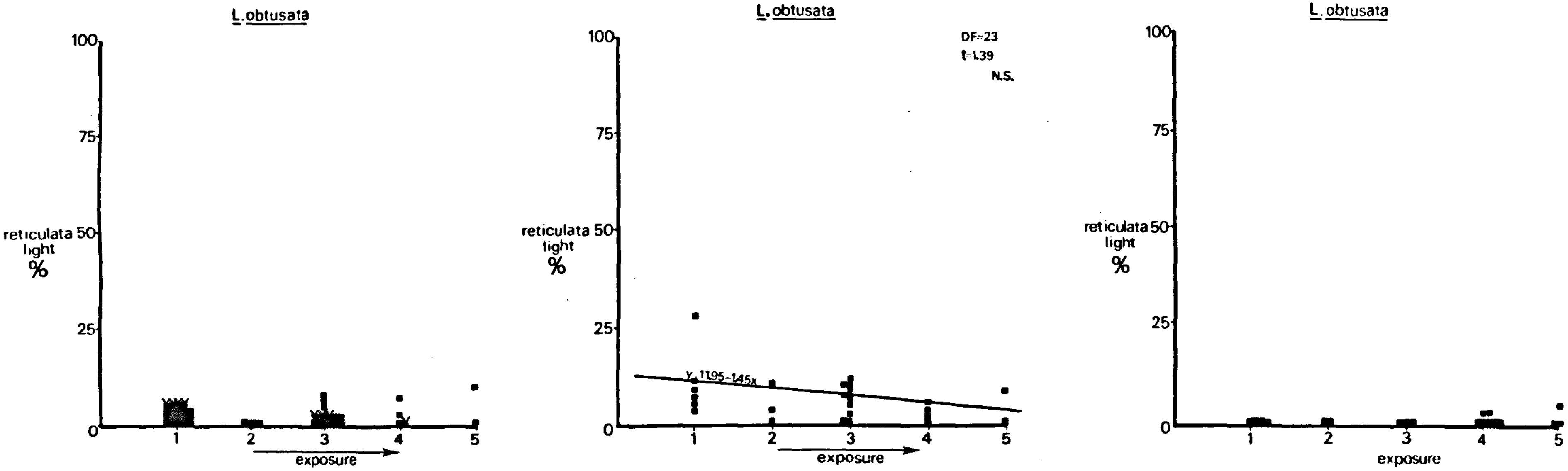


Fig 40. Relation between exposure and light reticulata. Wales and southern England (left), Isles of Scilly (centre), Ireland (right).

(ii) *L. mariae*

The general pattern of frequency distributions in each of the geographical areas is given in table 27. When these data are compared with those of *L. obtusata* (table 26), it is apparent that there are major differences in the abundance of each of the phenotypes and their range of variation. As with *L. obtusata*, the frequencies were approximately constant during 3 years at those localities where repeated sampling was performed (see appendix D for raw data). The relevant figures (figs 41-52) showing the geographical variation in frequencies are given on pp 192-204.

(a) *citrina*

This is the most common, and to the human eye, the most conspicuous of the phenotypes in *L. mariae* occurring with a mean frequency of 47% and a range of 0-100. It is easy to find throughout the study area but shows large changes in abundance between localities.

In Anglesey ($\bar{x}$ = 45%; range 1-100) the phenotype is very common in the sheltered Menai Straits where of the 9 localities it is present with greater than 95% frequency in all (fig 41). Five km to the north of the straits at Fedwfawr, a very exposed shore, the frequency is reduced to 1%. This pattern of change is also present within localities. Of 8 sites in Anglesey where two samples were obtained, one from the sheltered parts of the shore and the other from the more exposed, in every case the citrina were more abundant in the sheltered areas. A paired t-test between the frequency of

Table 27: Distribution of phenotype frequencies in L. *mariae* among the major study areas (see appendix D for raw data)

Area	citrina			olivacea			light reticulata			dark reticulata			No. of localities
	$\bar{x}$	S	range	$\bar{x}$	S	range	$\bar{x}$	S	range	$\bar{x}$	S	range	
Anglesey	44.6	36.5	1-100	0.2	1.2	0-6	19.1	22.3	0-70	36.1	25.4	0-98	36
Rest of Wales	66.0	29.7	50-100	0	0	0	10.2	7.9	0-15	23.8	29.2	0-67	5
South England	26.0	35.1	0-100	1.4	7.0	0-11	1.4	4.1	0-10	72.6	36.9	0-100	6
Isles of Scilly	43.1	28.7	0-100	2.9	7.5	0-22	15.5	16.3	0-54	38.5	36.3	0-100	18
Ireland	44.1	35.4	0-100	0.0	0	0	11.3	16.6	0-56	44.6	33.8	0-100	15

citrina in the two samples from each of the 8 sites gives a highly significant difference ($t = 4.17$; P 0.01).

Apart from Anglesey, the remaining localities from Wales (fig 42) show a similar pattern of variation. At the exposed shore at Dale Fort and the sheltered shore at Dale Bay, 5km apart, there is an increase in the citrina from 28% to 84% respectively. In southern England, citrina is common only at Bodinnick and Penzance, two of the sheltered localities. It is completcly absent from the most exposed shore (Buck Mills). One exception to the relationship of citrina with sheltered conditions occurs at Weston-super-Mare in the Bristol Channel. This is a very sheltered locality and has no citrina present. When considering all of the samples taken from Wales and southern England there is a significant increase (P 0.001) in the frequency of citrina on shores offering greater shelter (fig 38).

On the Isles of Scilly, (fig 43) citrina is also very common on the majority of shores ($\bar{x} = 43$; range 0-100). At Heathy Point and Heathy Bay, on the western side of Bryher, over a distance of 400m, there is a change in frequency from 16-87% from the exposed to the sheltered conditions. This magnitude of change can also be seen in the series of collections at White Island (fig 44) over a distance of 100m. From the very exposed conditions at site No. 7 to the sheltered conditions in the bay at site 6 there is an increase in the frequency of citrina from 11-90%. Comparison of all of the localities in the Isles of Scilly shows that, as in Wales and southern England, there is a significant (P 0.01) increase in the frequency of citrina with increased shelter (fig 48).

In Ireland the mean frequency of citrina is 44% with a range of 0-100 (fig 45). The pattern of variation is similar to that observed elsewhere in this study. The sheltered shores are characterized by a high frequency of citrina and the exposed shores by a low frequency. For example, at the exposed conditions at Curran's Port and the very sheltered conditions at Dunfanaghy, separated by 30km there is an increase in the frequency of citrina from 27 to 100%. At Staad Bay and Milkhaven, an exposed and a sheltered shore 4km apart, the frequency changes from 18 to 100%. The remaining localities in Ireland show similar patterns of variation on the shores of different exposure. This can be seen in fig 48 which shows a significant increase in frequency on shores with more sheltered conditions.

There is variation in the colour of citrina, as was also observed in L. obtusata. Most commonly the shells are lemon-yellow in colour but occasionally yellowish-white or yellowish-orange shells are found. In Ireland, Wales and south England the yellowish-white individuals comprise about 5% of the total citrina but on the Isles of Scilly the frequency now rises to about 25%. The yellowish-orange shade is more common than the yellowish-white shade occurring with frequencies (of total citrina) of about 10% in Wales, 2% in south England and Isles of Scilly, and about 20% in Ireland. The highest frequencies observed (62% and 63%) occur at Dale Fort, in south Wales and Streedagh House Bay in Ireland. These are the same two localities where the yellowish-orange shade was very common in L. obtusata.

In summary, citrina is generally the most common phenotype in L. mariae. In each of the major geographical areas sampled, it is relatively uncommon or absent in conditions of exposure but shows an increase in abundance, often reaching 100%, in sheltered conditions. These changes can occur over very short distances even within a single shore, where there are sharp differences in the amount of exposure.

(b) Olivacea

While this was the most common phenotype in L. obtusata it is the rarest in L. mariae occurring with a mean frequency of 0.5% and a range of 0-22 (fig 49). In Anglesey it is present in only 2 out of 28 localities (North Stack, Rhosneigr) with a frequency of 5.7% and 0.5% respectively. It is absent in the rest of Wales and southern England except for one sample from Bodinnick where it occurs with a frequency of 10.5%. On the Isles of Scilly it is slightly more common ($\bar{x} = 2.9\%$; range 0-22%) and is found in 4 out of 26 localities. The highest values occur in the sheltered conditions at Lawrence Bay. In Ireland it is absent from all the localities which were sampled.

(c) Dark Reticulata

In L. mariae this phenotype appears very cryptic to the human eye throughout the study area. It is abundant in all of the geographical areas occurring with a mean frequency of 36.0% and a range of 0-100. The pattern of variation is generally the inverse of that of citrina so that on shores where citrina is rare, dark reticulata is common (see fig 50).

In Anglesey ($\bar{x} = 36\%$ range 0-98) the phenotype is absent in the Menai Straits but becomes more common in the exposed conditions on the western side of the island. Samples from more sheltered and more exposed conditions at each of eight localities were compared; in each case the dark reticulata are more common in the exposed sample ($t = 9.87$; $P < 0.001$). The changes in frequency can occur over very short distances. At Sandy Bay, over a distance of 25m along the shore there is a change in frequency of dark reticulata from zero to 75%. At Rhosneigr, within 50m it changes from 5-96%. In both of these localities there are, over these distances, large differences in the amount of exposure with the dark reticulata predominating in the exposed conditions.

In the samples from Wales, apart from Anglesey, the phenotype is absent from the very sheltered locality at Dale Bay but occurs at a frequency of 70% 5km away at the more exposed Dale Fort. In southern England ($\bar{x}$ = 73%; range 0-100), one locality sampled in the Bristol Channel (Weston-super-mare), although very sheltered, shows it has only dark reticulata. This is the only locality in the geographical survey where the phenotype predominates on a very sheltered shore. The remaining two sheltered localities from southern England have low frequencies of the phenotype (15%, 0%). The two exposed localities have, as in Wales, high frequencies of dark reticulata (95%, 90%).

On the Isles of Scilly ($\bar{x}$ = 38%; range 0-100), the same relationship between frequency and exposure is evident (fig 43). At Heathy Bay and Heathy Point, a sheltered and a very exposed locality, there is a change in frequency from 3 to 76% over a distance of 400m. In the samples from White Island, over a distance of 100m, dark reticulata increase from zero to 88% from the sheltered to the exposed sample. Shores of intermediate exposure generally have intermediate frequencies.

In Ireland ($\bar{x}$ = 45%; range 0-100) a similar pattern occurs. In sheltered conditions the phenotype is rare or absent (i.e. Milkhaven) while in more exposed shores (i.e. Staad Bay) it is very common. In some of the very exposed shores, such as Tiraun Pt., Cloonagh, and Ballynagaul Pt., the dark reticulata is present at frequencies of only 35%, 27% and 25% respectively. These values are unusually low for the large amount of exposure at the 3 localities. They do not, however, destroy the relationship between an increase in exposure and an increase in dark reticulata (fig 50).

Although light and dark reticulata are generally phenotypically discrete, there are various situations where this is not the case. At Tiraun Point and Cloonagh in Ireland, the majority of "dark" reticulata are intermediate between the two phenotypes generally recognized as light and dark reticulata. Although variation of this nature was not seen in Wales, it is occassionally seen in the Isles of Scilly.

There are also differences in the ground colour of dark reticulata. In Wales, the colour is generally brown but in southern England, the Isles of Scilly and parts of Ireland, the ground colour is red-brown. Fig 47 gives the distribution which is very similar to that seen in L. obtusata (fig 36). In L. mariae the red-brown colour is always present in both juvenile and adult shells, unlike some individuals of L. obtusata from North Devon and Wales, where the red-brown was present only in adults which were well worn (p168).

In summary, dark reticulata are very common on most sheltered shores but increase in frequency, often up to 100%, with greater amounts of exposure. The differences in frequency are also present within shores where there are steep gradients in the exposure. Variation in the ground colour from the common brown to a red-brown occur in parts of the range very similar in distribution to that seen in L. obtusata.

(d) Light Reticulata

This phenotype is intermediate in overall darkness between citrina and dark reticulata. On many of the backgrounds that it is commonly found on, it is reasonably cryptic in appearance. Despite its crypsis, it is relatively uncommon in most localities, occurring throughout the study area with a mean frequency of 16%

and a range of 0-70. Unlike the other phenotypes in L. mariae it does not show a predictable change in frequency with exposure between the geographical area.

In Anglesey ($\bar{x}$ = 19%; range 0-70) the phenotype is very uncommon in the Menai Straits, which are very sheltered, although in the remaining localities it is often abundant in both sheltered and exposed conditions. In the 8 localities, where two samples were taken at each, a paired t-test shows that there is a significantly greater frequency of light reticulata in the sample from the more sheltered conditions ($t = 4.68$; $P < 0.01$). The most extreme change was noted at Rhosneigr where over a distance of 50m the frequency changes from 3-70% between the exposed and sheltered positions.

In Wales apart from Anglesey, the phenotype is present in only two of five localities (Dale Fort, Dale Bay) with frequencies of 16% and 5% respectively. It is absent from southern England except for one very sheltered site (Bodinnick) where it occurs in 11% of the specimens. Although there would seem to be a correlation between light reticulata and shelter, when all the localities from Wales and southern England are compared, there are no predictable changes with different levels of exposure (fig 51).

On the Isles of Scilly ($\bar{x}$ = 15%; range 0-52) the highest frequencies are observed at Lawrence Bay and Old Quay, two of the more sheltered shores. The two samples from Heathy Point and Heathy Bay separated by 400m do not show an increase in the more frequency of light reticulata in the more sheltered conditions and fail to follow the pattern which was present in Anglesey. In the samples from White Island the change in frequency with exposure is present, for in these collections the most exposed site (No7) lacks the phenotype while the samples from the bay (Nos. 5 and 6) have a frequency of 25%. When all the localities are compared from the islands there is a significant ($P < 0.02$) increase in frequency in conditions of greater shelter (fig 51).

In Ireland ($\bar{x} = 11\%$; range 0-56) the pattern is opposite to that seen in the Isles of Scilly. Rather than an increase in the more sheltered conditions there is a decrease (fig 51) though it is not significant ($P = 0.10-0.20$). The very exposed site at Cloonagh has the highest frequency observed. In this locality the light reticulata are slightly darker than in most situations and are only distinguishable from the dark reticulata by an arbitrary separation used for convenience of scoring. The pattern of frequency change of the light reticulata in Ireland is similar to that observed in L. obtusata for this phenotype. In this species, light reticulata was common only when dark reticulata was common.

(e) Uncommon Phenotypes

The only other phenotypes seen were occasional banded individuals (inversicolour). These occurred in only 2 localities in the Isles of Scilly and two localities in Ireland, the highest frequency observed being 7% (1 individual out of a total of 15) in the Isles of Scilly.

(iii) Comparison of Phenotype Frequencies Between L. obtusata and L. mariae

The two species, L. obtusata and L. mariae show very dissimilar patterns of abundance in the major colour phenotypes. In L. obtusata, the most common phenotype is olivacea followed by dark reticulata, citrina, and light reticulata, in order of decreasing abundance. In

Figures 41-47

Phenotype frequencies of L. mariae around the British Isles. Since this species varies a great deal in shell size, some attempt has been made to incorporate this into the following figures by showing localities with very small shelled adults (< 8mm) by a double circle diagram. All other localities, shown with single circle have larger sized adults. Raw data is given in appendix D - table Dii. Small circle diagrams show samples with less than 10 individuals.

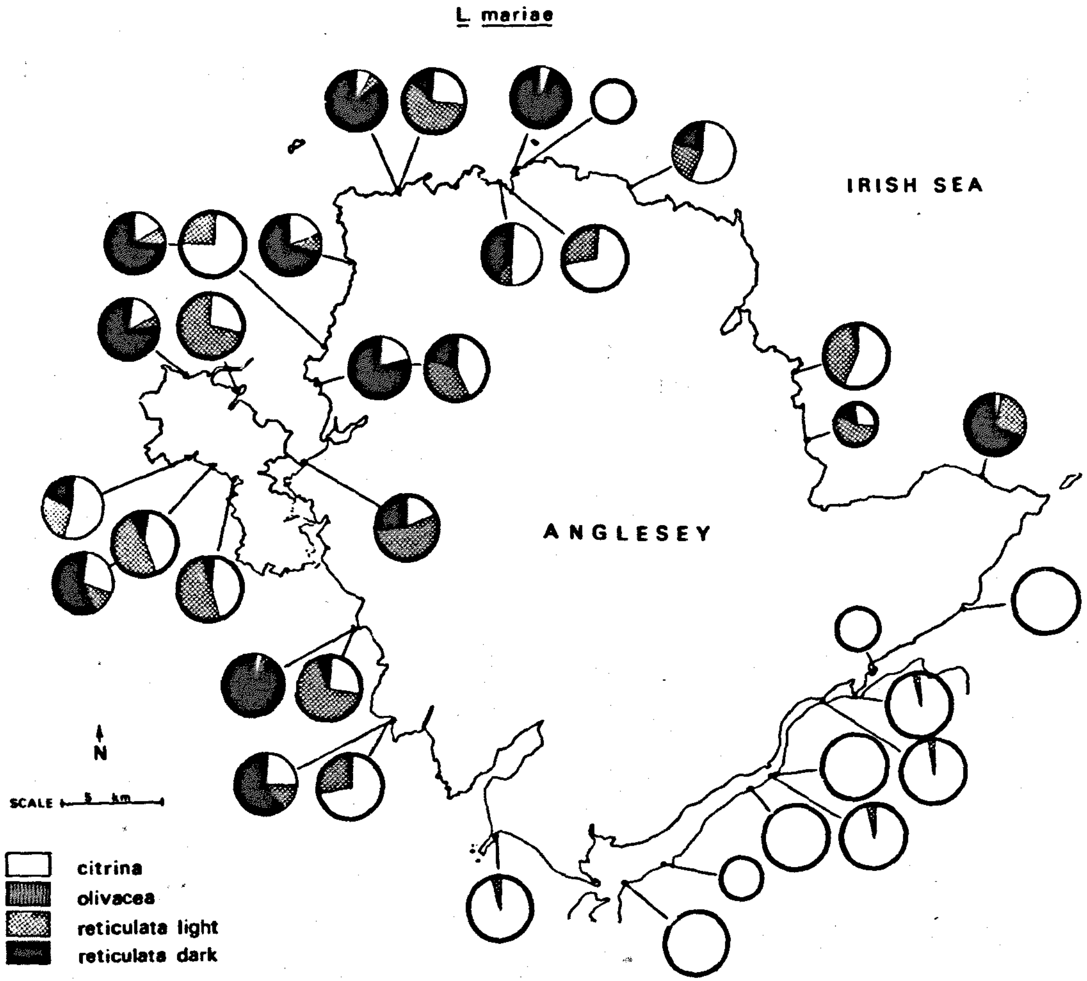


Fig. 41. Phenotype frequencies of L. mariae around Anglesey. The two diagrams at some localities show samples collected from sheltered (silt) and exposed (rock) conditions at the same locality. Since the sheltered conditions always had adults less than 8 mm (see Section X) they are marked with the double-circle. These two diagrams correspond to the "dwarf" and "normal" forms of L. mariae.

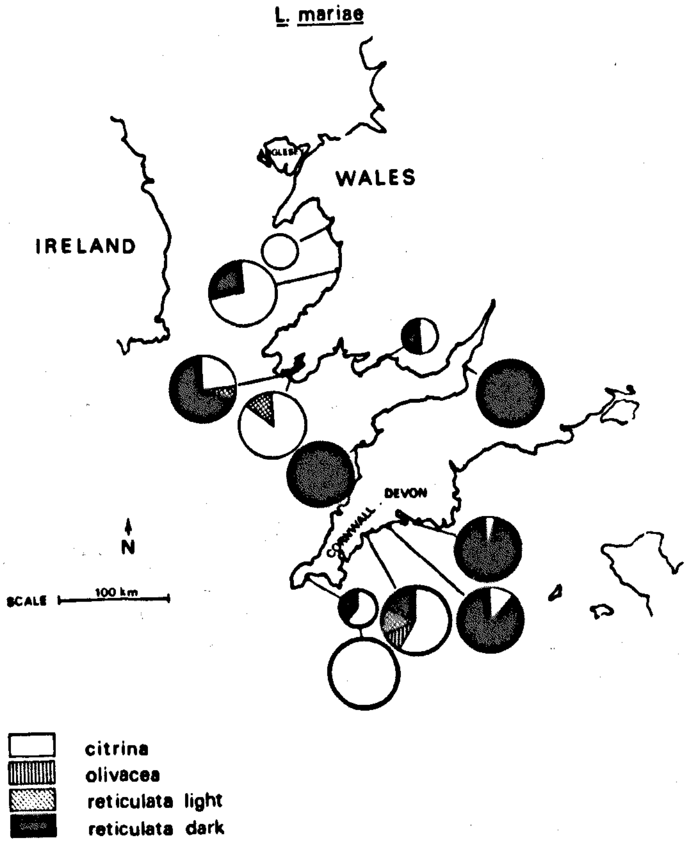


Fig. 42. Phenotype frequencies in L. mariae from Wales and south England.

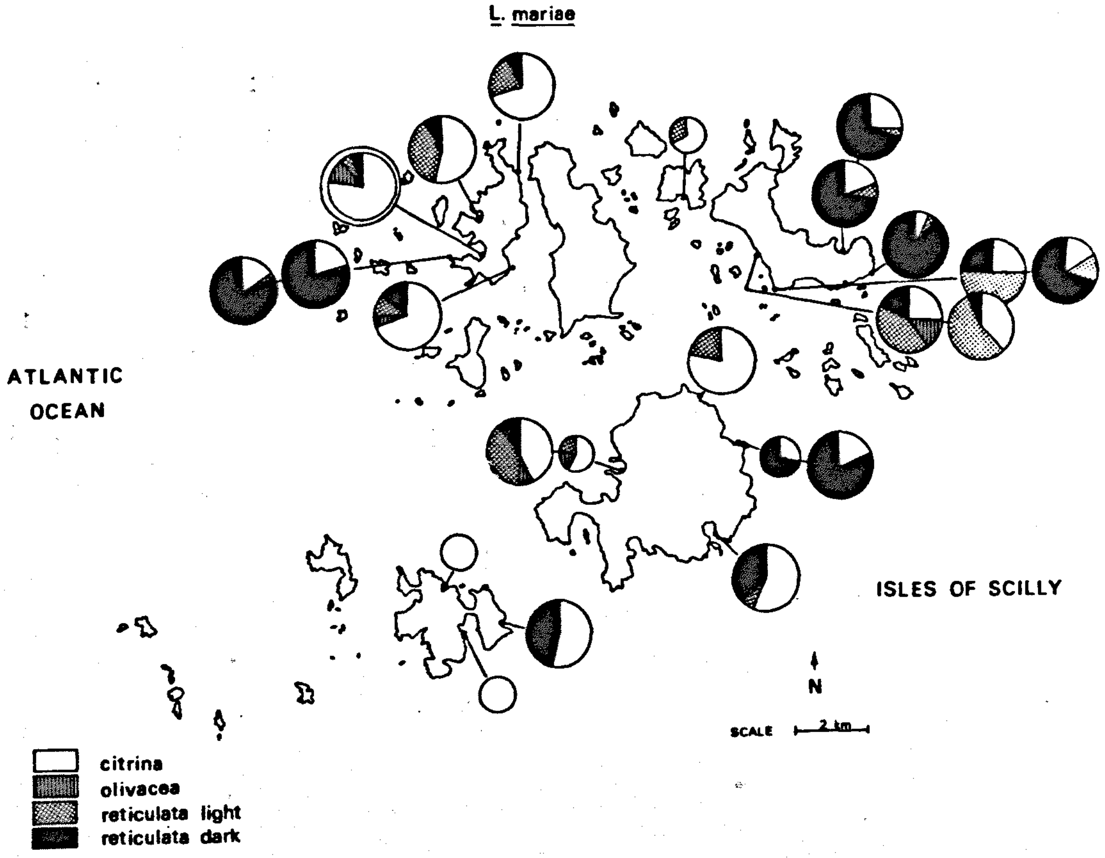


Fig. 43. Phenotype frequencies in L. mariae from the Isles of Scilly. The presence of a second pie diagram at some localities are from samples collected 1 year following the first collection.

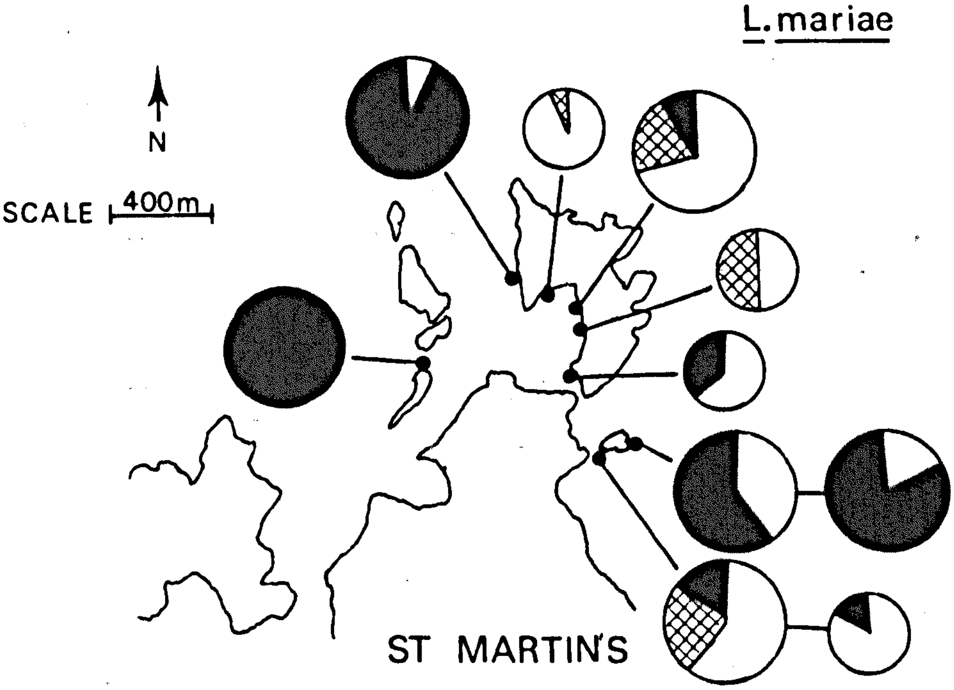


Fig. 44. Phenotype frequencies in L. mariae at White Island. Second sample at some localities collected 1 year after first.

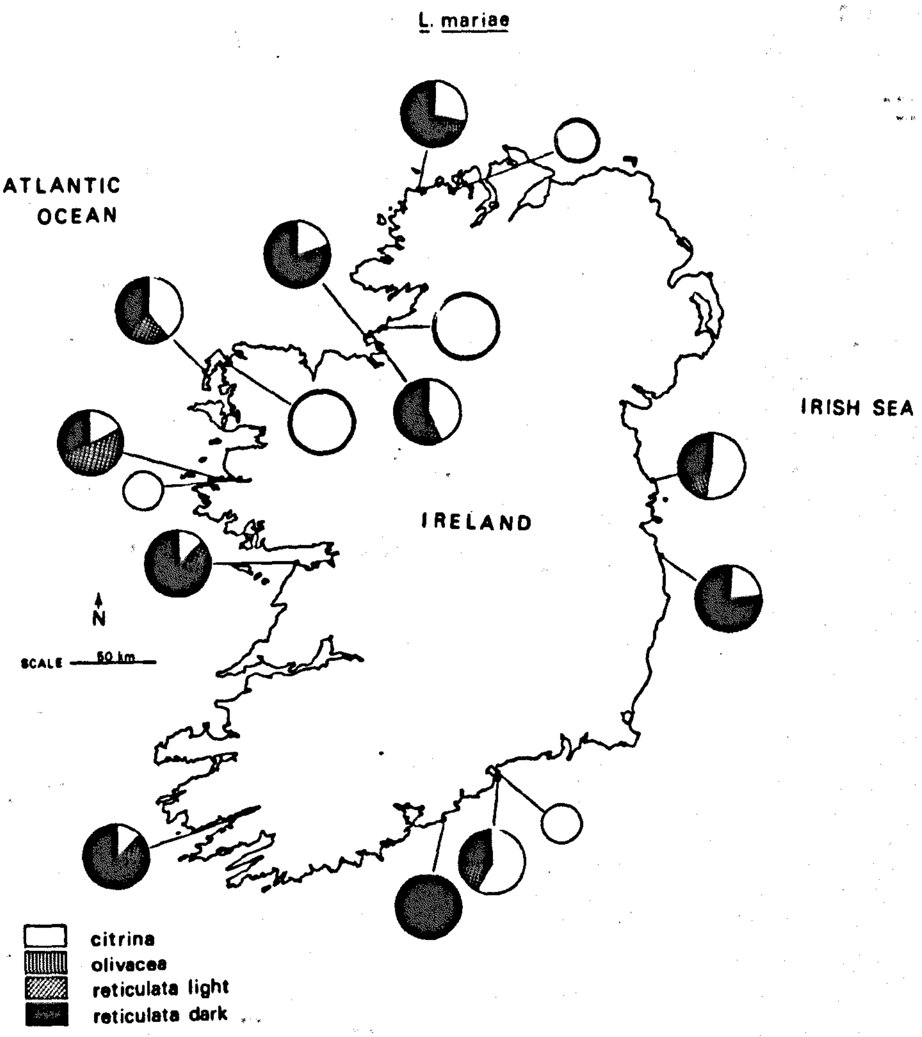


Fig 45. Phenotype frequencies in L. mariae around Ireland.

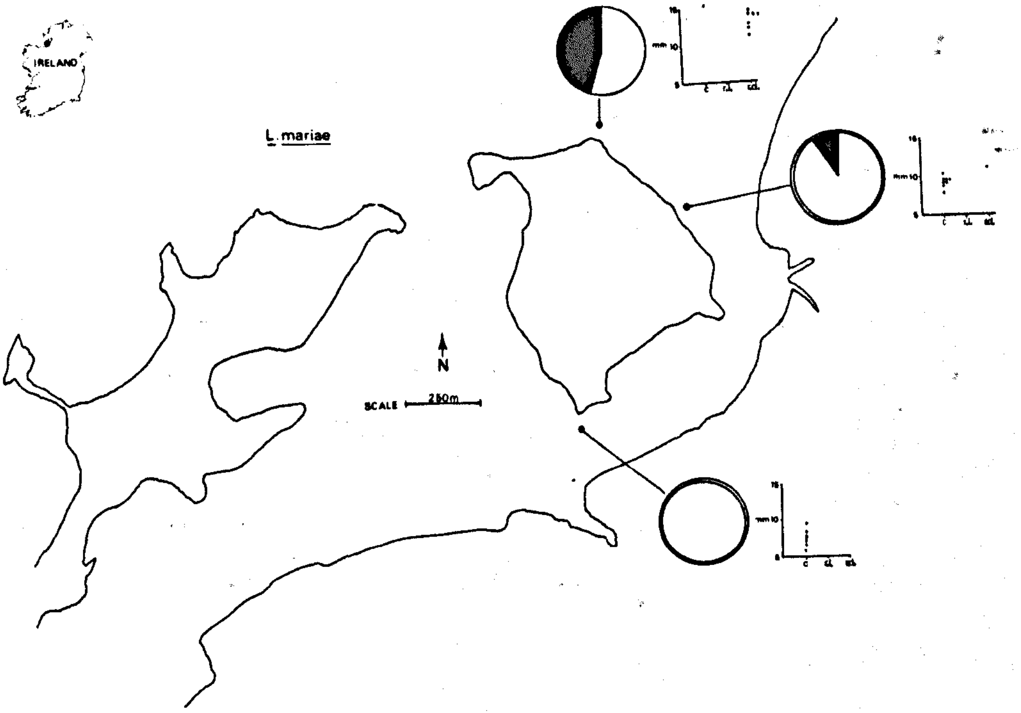


Fig 46. Phenotype frequencies in L. mariae at mouth of Milkhaven. Graph to right of each diagram shows size distribution in adult males of citrina (c) and dark reticulata (r.d).

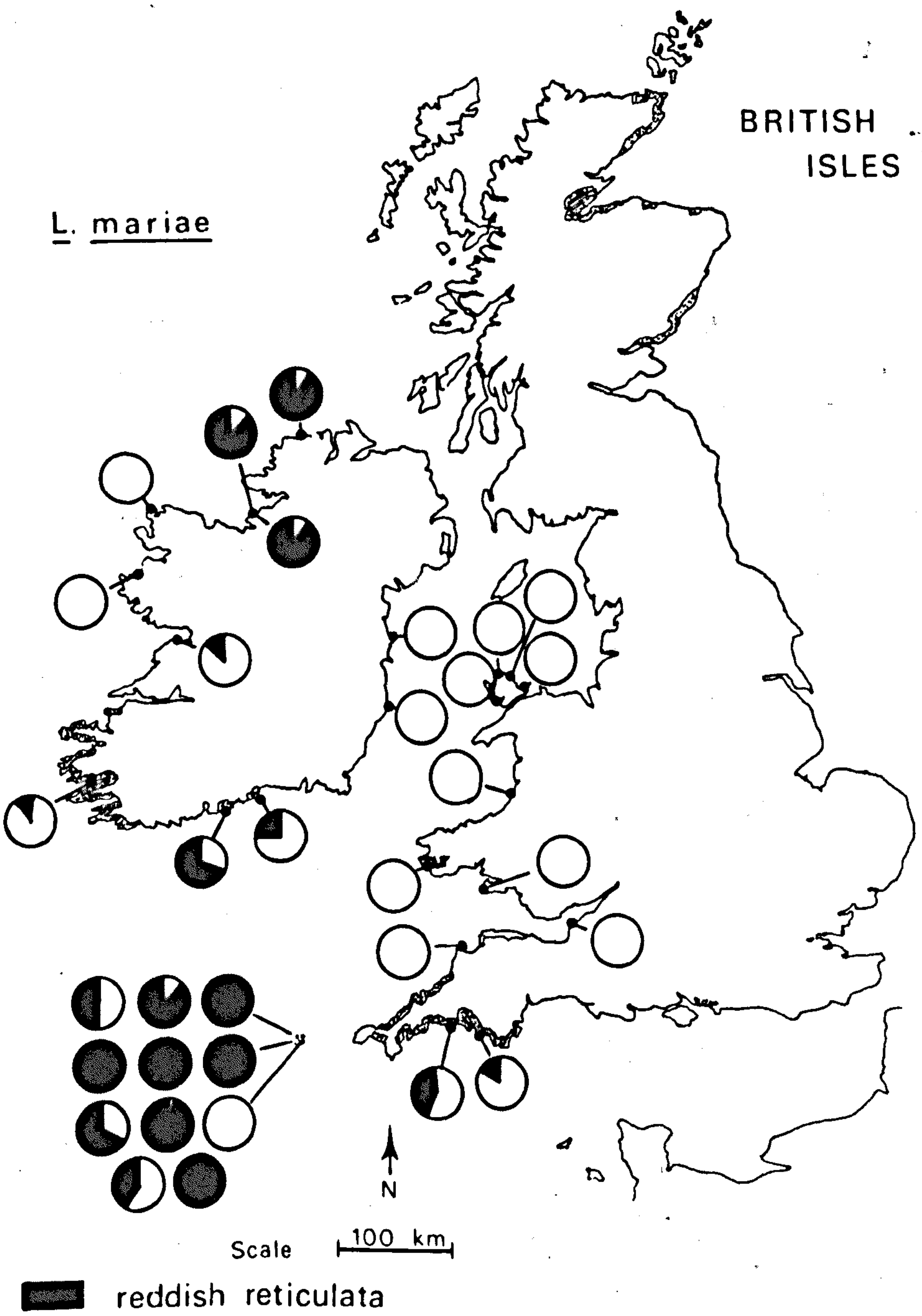


Fig 47. Occurrence of red-brown phenotype in L. mariae around the British Isles (for details see fig 36).

Figures 48-51

Relation between exposure and phenotype frequencies in L. mariae. For each graph, the weighted regression line is shown in addition to t-values from comparing observed and zero slopes. To provide some indication of the mean shell size at the different localities L. mariae is divided into three broad groups - dwarf, intermediate and normal, with lengths of 6-8mm, 8-11mm and 11-13mm respectively. These are discussed in detail in Section X.

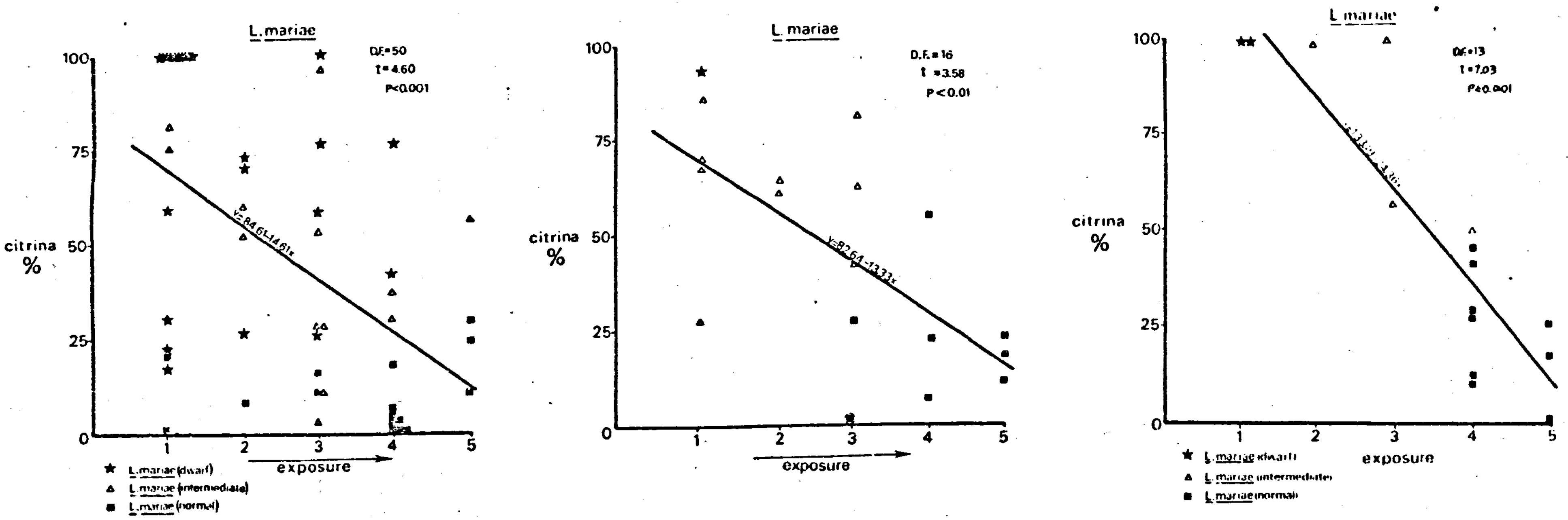


Fig 48. Relation between exposure and citrina. Wales and southern England (left), Isles of Scilly (centre), Ireland (right).

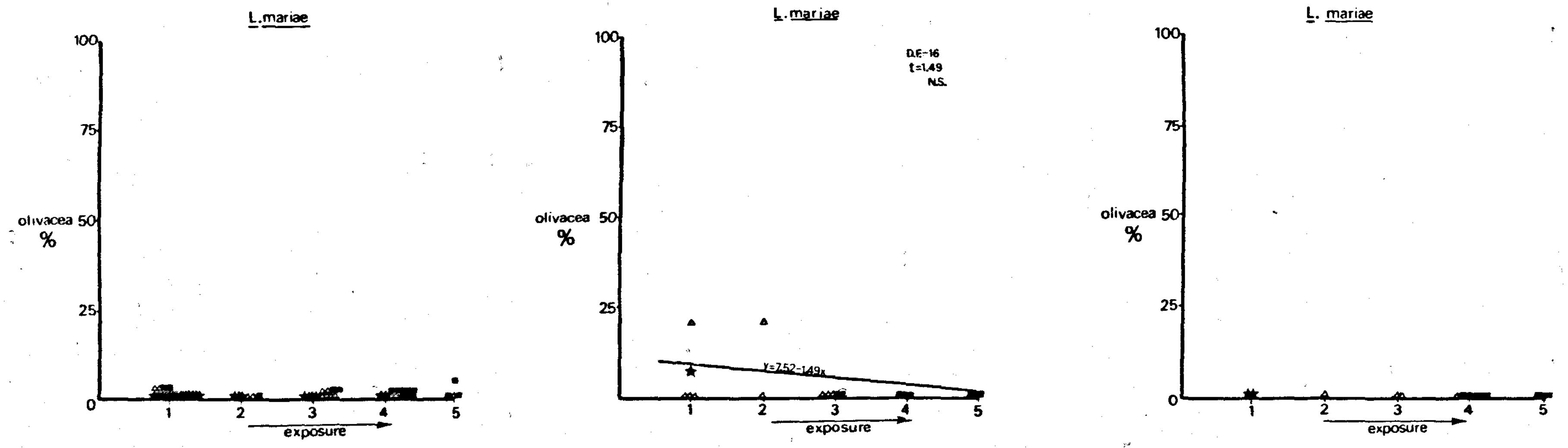


Fig 49. Relation between exposure and olivacea. Wales and South England (left), Isles of Scilly (centre), Ireland (right). Dwarf (star), intermediate (open triangle), normal (solid square).

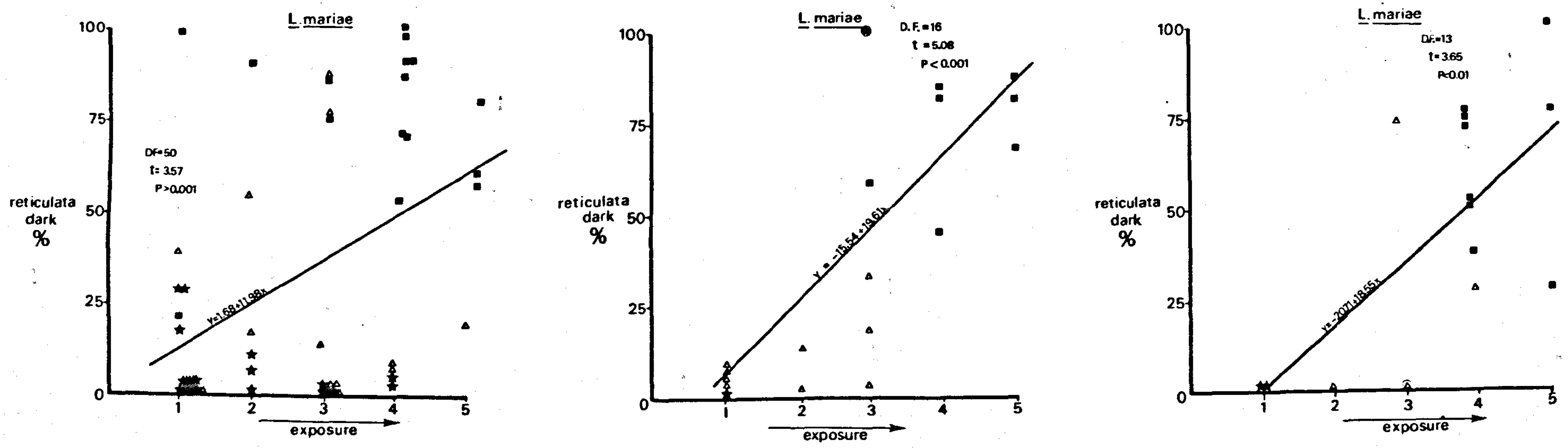


Fig 50. Relation between exposure and dark reticulata. Wales and southern England (left), Isles of Scilly (centre), Ireland (right). Dwarf (star), intermediate (open triangle), normal (solid square).

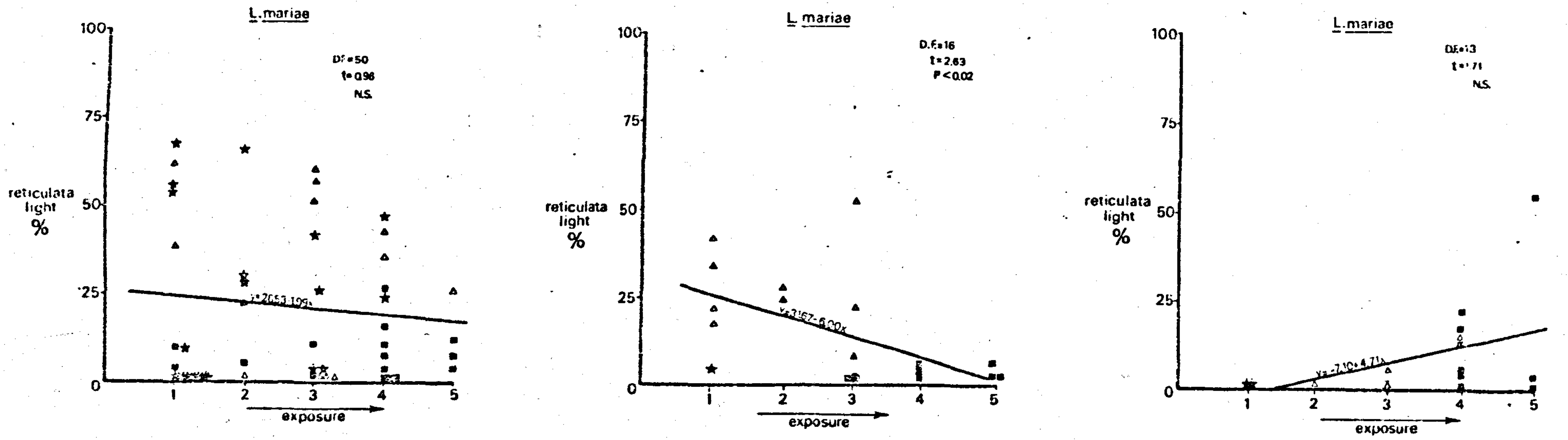


Fig 51. Relation between exposure and light reticulata. Wales and southern England (left), Isles of Scilly (centre), Ireland (right). Dwarf (star), intermediate (open triangle), normal (solid).

L. mariae the sequence is citrina, dark reticulata, light reticulata, and olivacea. Therefore, the phenotype which is most common in L. obtusata is the rarest in L. mariae.

Of the two species, L. mariae is much more variable within and between shores. While changes in frequency of 80% over a distance of 100m are not uncommon in this species, this magnitude of change has never been observed in L. obtusata.

In L. mariae, throughout the study area between and within shores, citrina is relatively uncommon on exposed conditions but becomes the predominant phenotype in sheltered conditions often reaching 100%. In L. obtusata there is no such change in the abundance of citrina. In Wales and southern England the phenotype in this species is relatively uncommon on most shores but occasionally reaches frequencies of 40% as in the sheltered Menai Straits, and the exposed headland at North Stack. On the Isles of Scilly, which are on average more exposed than the mainland, citrina is more abundant than in Wales and southern England and occurs frequently in all conditions of exposure. In Ireland, the phenotype is less common than in the Isles of Scilly and shows a significant increase in frequency with greater exposure. There is, therefore, in L. obtusata a general pattern of frequency change in citrina opposite to that of L. mariae.

In L. obtusata, olivacea is the most common phenotype in the majority of localities in Wales and Ireland and shows a significant increase in frequency on shores of increased shelter often composing all the individuals in the population. Over these localities the phenotype is rare or absent in L. mariae. On the Isles of Scilly, olivacea becomes the rarest of the major phenotypes in L. obtusata but again shows higher frequencies in the more sheltered localities.

Although also uncommon in L. mariae, olivacea reaches its highest frequencies on the Isles of Scilly in the same localities where it is most common in L. obtusata, that is in the more sheltered conditions.

In both species, dark reticulata shows an increase in frequency on shores of greater exposure throughout the study area. Even over small distances with an exposure gradient this change is evident, although it is much more pronounced in L. mariae than in L. obtusata. On these shores there is also a greater similarity in the ground colour of the shells. In the more sheltered conditions the ground colour of L. obtusata is olive-green but with greater exposure it is more often olive-brown. The olive-brown is phenotypically very similar to the brown ground colour of L. mariae which shows little change between sheltered and exposed conditions. Also, within the phenotype olivacea, there is often an increase in darkness on more exposed conditions. These olivacea are phenotypically very similar to the dark reticulata of L. mariae. Minor variations in the colour of the dark reticulata such as the red-brown phenotype show a very similar distribution in the two species. This ground colour is very abundant on the exposed shores from southern England, the Isles of Scilly and some localities in Ireland.

Light reticulata is uncommon in both species but is somewhat more frequent in L. mariae. In this species, the phenotype shows a slight increase in abundance in conditions of shelter in Wales and the Isles of Scilly but in Ireland the reverse is the case. In L. obtusata the phenotype is uncommon in most localities except in the Isles of Scilly. Here it reaches its highest frequency in the same sheltered locality (Lawrence Bay) where the phenotype is most frequent in *L. mariae*. In general however there is little relationship in frequency between the two species.

It is possible to obtain a quantitative value for the similarity or dissimilarity of the phenotype frequencies between the species for each locality. This value was estimated by the modulus:

$$\text{C.D.}_{\text{colour}} = \frac{\left| x_{a1} - x_{a2} \right| + \left| x_{b1} - x_{b2} \right| + \ldots\ldots \left| x_{i1} - x_{i2} \right|}{n}$$

where x is the frequency; a, b... the phenotype: 1, 2 the species: and n the number of phenotypes. This index is essentially the summed values of the differences between each phenotype of both species divided by the number of phenotypic classes used. The index is similar to one used by Cain and Harrison (1958) for estimating taxonomic affinities.

When the index from each locality is plotted against the amount of exposure, it can be seen that in each of the three geographical areas the two species are very dissimilar in sheltered conditions but on shores of greater exposure there is a significant trend for a greater similarity in the major phenotypic classes (fig 52). The index used here has not included the variation in colour within the phenotype olivacea of *L. obtusata* which on exposed shores becomes not only phenotypically darker but also more brown; both of these characters give the olivacea a greater resemblance to the dark reticulata of *L. mariae* which is common in the exposed conditions. The index has not included the greater similarity of the ground colour in dark reticulata on exposed shores. If all these characters were to be considered in the index, the trend towards similarity on exposed shores would be even more striking.

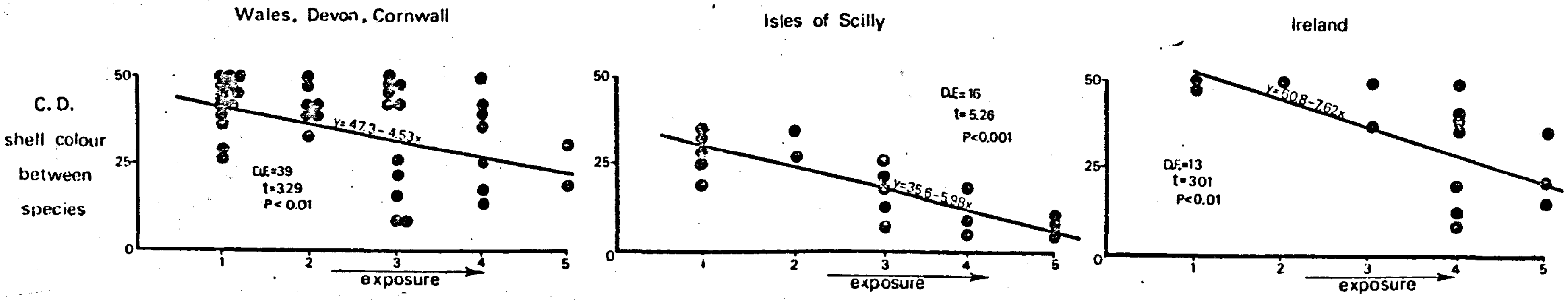


Fig 52. Relation between exposure and the Coefficient of Difference Index for phenotype frequencies between species (see text). Note that with greater exposure, L. obtusata and L. mariae become more similar in their phenotype frequencies.

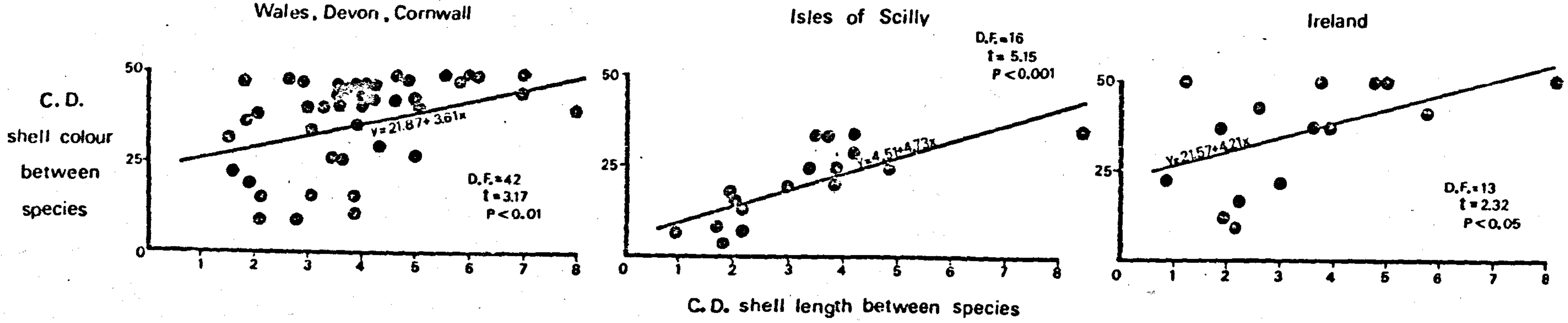


Fig 53. Comparison between Coefficient of Difference indices for shell size and shell colour (see text). Note that as the two species become more dissimilar in shell size, they also become more dissimilar in the frequencies of the colour phenotypes. C.D. index for shell length calculated from equation given in fig 7.

These changes have a parallel when considering the variation in shell size of the two species. In very sheltered conditions, the average adult shell size in L. *mariae* is about 7mm while in L. *obtusata* it is about 16mm. On very exposed shores adult shells are about 13mm and 14.5mm for the two species respectively (see fig 4). Therefore they tend to diverge in shelter and converge in exposure. It is possible to compare these changes with those from the colour frequencies. When this is done, there is in each of the geographical areas a significant positive relationship (fig 53). Where the two species are most different in adult shell size, they are most different in the phenotype frequencies. Where they are most similar in size, they are also most similar in the phenotype frequencies.

These observations are of some interest as they show a pattern of change opposite to that observed by Clarke (1962) on *Cepaea nemoralis* and C. *hortensis*. In these two species he has found an inverse relationship in the frequency of the light coloured shells between the two species where they overlap spatially. In L. *obtusata* and L. *mariae* the greatest amount of spatial overlap occurs on exposed shores (fig 19); on these, the two species are most similar in their phenotype frequencies.

3. Discussion

In both species the highly cryptic phenotypes show predictable changes in frequency with differences in exposure. In L. *obtusata*, olivacea predominates in shelter while dark reticulata become common in exposed conditions. These correlations have also been noted by Barkman (1956) and Sacchi (1961, 1969). Both of these authors felt that the dark reticulata, though cryptic, had a linked character which gave the phenotype a relative advantage for adhesion to the substrate during heavy wave action. In _L. mariae_ on exposed shores there is also a predominance of dark reticulata; in sheltered conditions, rather than an increase in olivacea there is a predominance of the apparently conspicuous citrina. Sacchi (1969) and Gratton (1969) felt that these differences were also explicable if the dark reticulata could adhere better in wave action and if the conspicuous citrina had pleiotropic characters which provided an advantage in sheltered conditions to overcome the supposed greater susceptibility to visual predators. Light reticulata, which are uncommon in _L. obtusata_ and locally abundant in _L. mariae_, show in some cases an an inverse and in other cases a direct relationship with changes in exposure. Sacchi had found this phenotype in _L. mariae_ to be more common in sheltered conditions and because of the light colouration proposed a similar explanation with that put forward for citrina. In the remainder of this thesis, I will attempt to show that the hypothesis of linked characters is not necessary to account for the variation and abundance either of the cryptic or of the apparently conspicuous phenotypes, but rather that these differences are generally explicable in terms of a selective regime favouring crypsis in response to subtle changes in the background colouration.

The main abiotic factor which differs between sheltered and exposed shores is the amount of wave action. This directly or indirectly affects a large number of biotic parameters on each shore (see Section II, 2). The main food plant and background of _L. obtusata_ on sheltered shores is _Ascophyllum_ and to a much smaller extent, _Fucus serratus_ and _F. spiralis_. The colour of _Ascophyllum_ is basically olive-green with some variation occurring on the orange fruiting bodies and in young plants which are often an olive-yellow (plate 12). The phenotype olivacea is very cryptic on all the species of algae and is the only one cryptic on Ascophyllum. There is in fact, a general positive relationship between the frequency of this phenotype and the abundance of Ascophyllum in Wales, southern England and Ireland (fig 54). However, on the Isles of Scilly, over the same range of Ascophyllum abundance, olivacea are very uncommon on the majority of shores, and where found they are slightly reddish in appearance. Apart from these islands, therefore, the presence of Ascophyllum is generally associated with an abundance of olivacea, the most cryptic phenotype on the olive-green algae.

On exposed shores, there is an upward extension of F. serratus and a replacement of Ascophyllum by F. vesiculosus. On these two species which here constitute the major background for L. obtusata, olivacea is still very cryptic, especially on F. vesiculosus. Various authors (Walton, 1915; Pettitt, 1973) as well as myself have noticed the striking similarity in appearance between the airbladders of F. vesiculosus and the olivacea phenotype. Despite the crypsis, there is, on these shores, a reduction in frequency of olivacea and a corresponding increase in dark reticulata, a change which Sacchi interpreted as selection on a non-visual character linked or associated with dark reticulata. However, examination of the algal colour, shows that F. serratus and F. vesiculosus are olive-brown on the leaf and dark-brown on or near the stem (plates 13 and 14), rather than being olive-green as in Ascophyllum. On the dark brown background, which often comprises more than 50% of the plant, dark reticulata are to the human eye much more cryptic than olivacea.

If it is true that the abundance of either dark reticulata and olivacea in L. obtusata is owing to their crypsis on the different

[illegible]

Plate 12

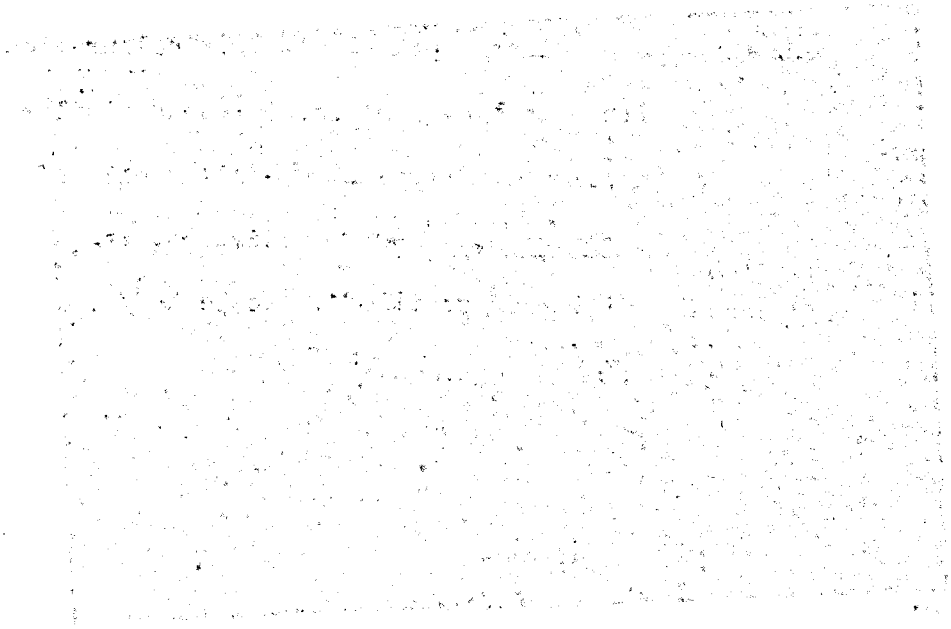


Plate 12

Variation in colour of Ascophyllum at Cuaseway (No 10a)

Upper - Note change from olive-yellow to olive green with the smaller fronds being lighter in colour. Scale X $^1/10$.

Lower - Note uniform dark olive-green colouration with both small and large fronds similar in colour. Arrows show adult shells of L. obtusata, phenotype olivacea, in undisturbed positions. Scale X $^1/6$.

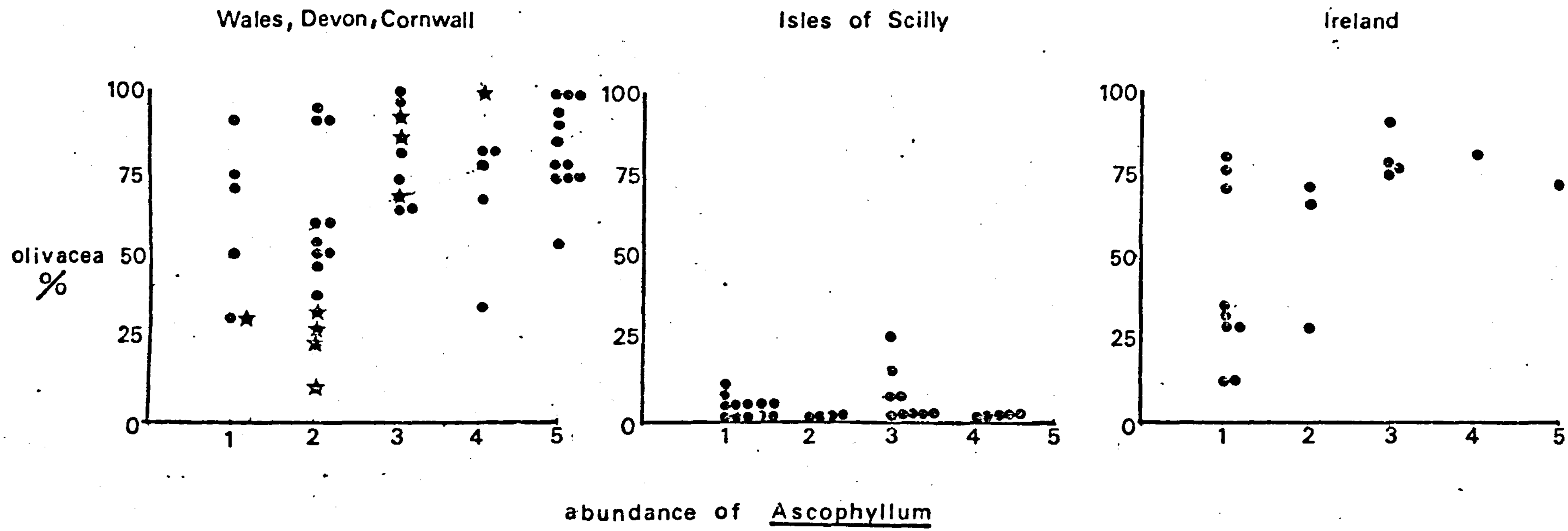


Fig. 54. Relation between abundance of Ascophyllum and frequency of olivacea in L. obtusata. Star symbols show localities from southern England.

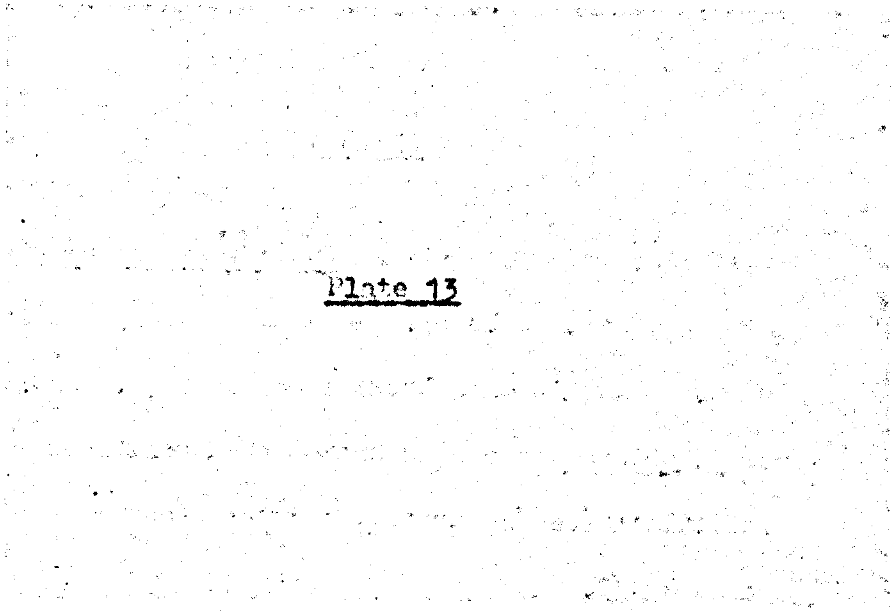


Plate 13

Plate 13

Variation in colour within Fucus vesiculosus plants at Sandy Bay, Anglesey. Note that a large segment of the plant is dark brown in appearance. Individuals of L. obtusata shown in undisturbed positions: Dark reticulata (centre arrow), olivacea (outer arrows). Scale X 1/3.

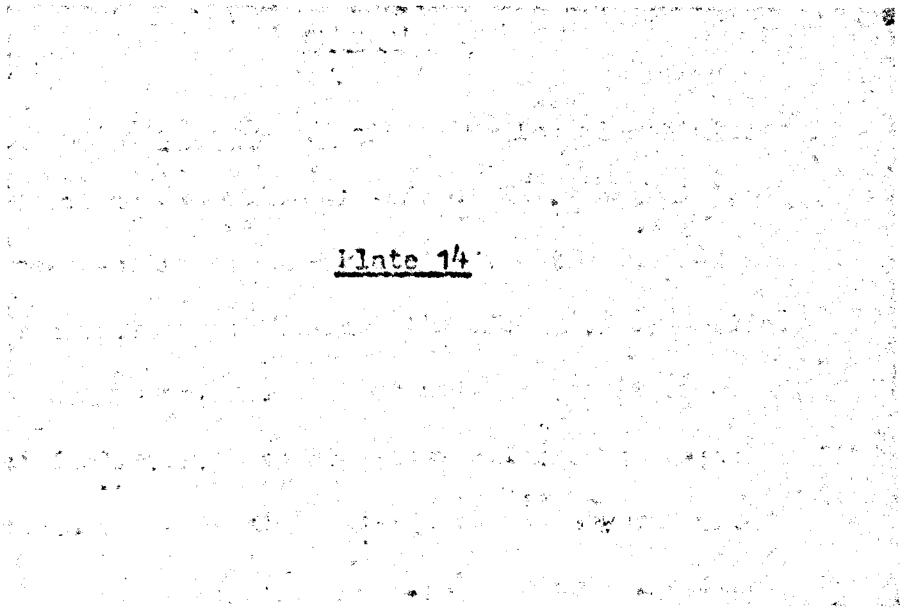


Plate 14

Plate 14

Variation in colour within F. serratus at Sandy Bay, Anglesey. Note that the stem and branches are dark-brown while the leaf areas are olive-green to olive-brown. Adult citrina and dark reticulata (top arrows) placed on stem to show conspicuousness of citrina and excellent crypsis of dark reticulata. Lower arrow shows dark reticulata in undisturbed position. Scale X 1/4.

species of algae, then one might expect to find some relationship within a locality between dark reticulata and Fucus spp and between olivacea and Ascophyllum. Although data of this sort are available from only a single locality (Sandy Bay, Anglesey), this does indeed seem to be the case but only when comparing Ascophyllum with F. serratus (see tables E (ii) and E (iii) in appendix E). Dark reticulata are significantly more common on F. serratus than on Ascophyllum (paired t-test, $t = 5.31$, d.f. $= 6$; $P < 0.01$) while olivacea show the opposite pattern (paired t-test, $t = 5.63$, d.f. $=$ 6; $P < 0.001$). On the other hand, when Ascophyllum is compared with F. vesiculosus, there is no significant difference in its phenotype frequencies although there is a similar trend to that observed with the Ascophyllum - F. serratus comparison (see table E (ii) in appendix E). Again there are no significant differences between the phenotype frequencies when Ascophyllum is compared with F. spiralis but in this case, the trend is in the opposite direction.

Since the main food plants of L. obtusata and L. mariae on exposed shores are F. serratus and F. vesiculosus, the observation made above on within shore distribution provide some support for interpreting higher frequencies of dark reticulata as visual selection for crypsis on these shores.

On surveying the samples, it appears that in L. obtusata there are a number of small colour differences in certain phenotypes between those from sheltered shores and those from exposed shores which correlate with similar differences in the colour of the background. For example, on sheltered shores olivacea is olive-green and very similar to the colour of Ascophyllum while on exposed shores it is olive-brown, very like the colour of the fronds of F. serratus and F. vesiculosus which predominate on these shores. There are corresponding changes in the colour of dark reticulata. This phenotype is uncommon on sheltered shores, and in such areas, it is a dark olive-green. It is very common on exposed shores where it lives on Fucus spp, and here it is a dark olive-brown. It is interesting to note that in L. mariae, the dark reticulata show no such change in colour with exposure of the shores. In this species the phenotype is generally dark brown which is very similar in appearance to the phenotype of L. obtusata on exposed shores. It would seem therefore, that when the two species are both found on Fucus spp, not only is there a greater similarity in frequency (see fig 52) but also a greater similarity in appearance of the dark reticulata.

Since the abundance of dark reticulata in L. obtusata is closely related to the abundance of backgrounds on which it is cryptic, there seems to be no reason to resort to pleiotropic characters as was done by Sacchi (1969) and Gratton (1969) to explain the high frequencies of this phenotype on exposed shores.

The distribution of the red-brown phenotype in both L. obtusata and L. mariae is strikingly similar (figs 36 and 47); this suggests a relationship to a common ecological factor. The red-brown colouration is usually found in both species on the same exposed shores of southern England, the Isles of Scilly and parts of Ireland. If it is a response crypsis, then the most obvious factor which might be important is the colouration of the fucoids. But in the areas where the red-brown phenotype is common, the fucoids do not appear to be different from the usual olive-brown colouration seen in Anglesey. A biotic variable which showed a similarity in distribution to the red-brwon shells was the presence of the topshell Monodonta lineata. This specisa, according to Barret and Yonge (1958) is found in south-western England between Anglesey and Dorset. Although this may be the case, the species was only noticed (due to their abundance) in Cornwall, the Isles of Scilly and the northern parts of Ireland. The *Monodonta* probably have little effect on *L. obtusata* and *L. mariae* but the similarity in distribution to the red-brown shells does indicate that at least one other species of gastropod becomes common for some reason in the same shores where the red-brown phenotype is abundant.

Guiterman (1971) has observed in Anglesey that during storms *L. obtusata* crawl from the fucoids to the underlying rocks and crevices where they seek protection from the action of waves. If this is a general behaviour on exposed shores, and visual predation occurs at this time, then rock colour may be an important factor.

The major sedimentary rock type which has a red colouration is Devonian Old Red Sandstone. The major outcrops on the shores of Great Britain occur in north Somerset, south Devon, and Cornwall. In Ireland the outcrops occur predominantly in the south and to a lesser extent in the northern part (figs 36 and 47). It can be seen that there is a general relationship between the distribution of the red sandstone and red-brown phenotype. A similar relationship has also been noted by Dr. J. Heller (pers. comm.) working with *L. saxatilis* in Pembrokeshire, Wales. Although this relationship is suggestive of crypsis, the highest frequencies of the red-brown phenotype occur in the Isles of Scilly where the red sandstone is absent. On these islands the major rock type is granite, which is occasionally pink but seldom red as is the Devonian Sandstone.

The general rock colour will be of little importance to the *Littorina* if most surfaces are covered with encrusting algae. Some of the encrusting forms are the pink *Lithophyllum*, the medium red

Rhodochorton, and the dark red Hildenbrandia. The relative abundance of these in the study area is not known. The only observations that have been made were that in southern England and especially in the Isles of Scilly each locality was very rich in variety of colour compared with the exposed shores of Wales, owing in part to the abundance of the red seaweeds, Rhodophyceae. However, during the time of collections, L. obtusata and L. mariae were not found on any of the red algae. It is possible that the colour of the olive-brown fucoids, could be influenced by reflected or transmitted light from adjacent red algal fronds when the tide is in. This will be considered in more detail in one of the following sections (see page).

Light reticulata in L. obtusata and L. mariae show differing patterns of variation with exposure. In L. obtusata, it is relatively uncommon in most localities from Wales, southern England and Ireland except in situations where the dark reticulata is abundant, that is, in exposed shores. On the Isles of Scilly, the light reticulata are more frequent on shores with more sheltered conditions. On most shores, therefore, this phenotype is associated with Fucus spp. rather than Ascophyllum. In L. mariae, which is generally restricted to F. serratus, the phenotype is more common than in L. obtusata. In Wales, southern England and the Isles of Scilly, it is more abundant on sheltered shores while in Ireland the reverse occurs. On one exposed locality in Ireland (Tiraun Point) where the light reticulata are common, the phenotype is very cryptic on the F. serratus. On these shores, the plants were very leathery and brownish-orange in appearance (similar to light reticulata) rather than the more common olive-brown. However in other localities in Ireland, Wales, and the

Isles of Scilly where light reticulata are common, the F. serratus are the typical olive-brown. There is some variation of colour on the plant such as the brownish-orange fruiting bodies which the light reticulata are very well camouflaged. The phenotype did not, however, seem more abundant on the fruiting bodies during collections. Although this background colour may provide some advantage to the light reticulata, it does not account for the change in frequency between shores, for the brownish-orange fruiting bodies are present on F. serratus in all localities. The reason for the general association of the phenotype in both L. obtusata and L. mariae with the Fucus spp. rather than with Ascophyllum is obscure, but it may reflect crypsis on some parts of the plant. Some evidence for this is presented in Section VIIIon micro-distributional differences between the phenotypes.

The most conspicuous of the various phenotypes in both species is citrina. It is relatively less common in L. obtusata and shows a correlation in frequency with shores of greater exposure or in some cases sheltered shores with the presence of current. In L. mariae it is uncommon in exposed conditions but increases in frequency (often to 100%) in sheltered conditions, the opposite to that of L. obtusata. Gratton (1969) has suggested that the colour of shells in sheltered conditions in L. mariae would be of little importance because of the increased turbidity of the water which would affect the vision of the predator. Sacchi (1969) has noticed that citrina and light reticulata are found more on the outside of the algae clump than the cryptic olivacea and dark reticulata, which spend more time in the interior of the algal clump. This would then result in a greater amount of predation by shore-birds on the shells at the surface of the algae. In order to account for the preservation of the citrina and light reticulata in the population, Sacchi felt that there must be associated characters with the two phenotypes which gave an advantage.

The analysis so far presented does not provide any clear explanation on what the countervailing advantages of the citrina phenotype may be. The suggestions offered by Sacchi (1969) and by Gratton (1969) seem unlikely in view of the very predictable pattern of frequency change with changes in the amount of exposure throughout the entire British Isles, not only between shores but also within them. Results to be presented in the following section on microdistribution show that the apparent conspicuousness and abundance of citrina are explicable in terms of selection for crypsis. Following these observations the nature and significance of the colour polymorphism in the two species will be discussed in detail.

SECTION VIII

MICRODISTRIBUTION STUDIES

1. Introduction

Evidence presented in the previous section has shown some correlation within a population of phenotype frequencies with certain species of algae in the direction expected if selection for crypsis is occurring. However, the colour of each phenotype in *L. obtusata* and *L. mariae* has a counterpart at least somewhere *within* each of the fucoids; furthermore, the whole colour range shown by each of the fucoids is usually matched by the colour range of the snails. This heterogeneity in background colour on single plants of the fucoids may alter the crypsis and therefore selective value of the different shell-colour phenotypes depending on their position on the plant. If precise selection for crypsis occurs, the microdistribution of the different shell phenotypes may show some close correspondence to the distribution of background colour, at least at some time during the snails' life-history.

A series of observations were carried out in the field by scoring the exact position of the winkles on each plant. It was evident at the onset of these observations that very little information would be obtained from *L. obtusata* owing to their tendency to drop from the fronds with the slightest disturbance of the algae. This behaviour is not present in *L. mariae* for even with a great deal of algal agitation, the snails remain attached to the frond. In view of this, I decided to restrict the observations to *L. mariae*.

In this section, I shall initially describe some of the morphological and colour variation in *F. serratus*; following this, comparisons are made of the microdistributions between citrina and dark reticulata and between juveniles and adults. The results of these observations are then discussed in respect to within shore and between shore variation in phenotype frequencies.

2. Morphological Variation in F. serratus

There is a close relationship between the colour of each plant and its relative distance from the holdfast. In the majority of cases, the stem and base of the leaf are dark-brown or black. This colour becomes progressively lighter through to an olive-yellow near the leaf tip. The largest difference is between the stem and the leaf, and stated simply, these two parts supply the two general background colours (dark-brown; olive-brown to olive-yellow) present on F. serratus.

After a large number of shores had been visited in this study, it became evident that F. serratus varied morphologically a great deal between and within shores. In sheltered conditions, the plants were very large, consisting mainly of a broad-leafed frond with a very small, thin stem; in exposed conditions, the plants were smaller in size, with narrow leaves and a very large, thick stem (fig 55; plate 15).

To provide an estimate of the relative amount of stem and leaf, measurements were made on a number of plants at 9 shores varying from the sheltered Menai Straits to the very exposed Sandy Point. Three vertical transects were set up in the F. serratus zone each from the Laminaria zone up to the lower Ascophyllum or F. vesiculosus zone. Individual plants were taken at 2 meter intervals along each transect. All the fronds on each plant (about 20) were measured firstly for total length, using the base of the holdfast as the origin, and secondly for stem length (from holdfast to base of leaf).

The results of these observations show a significant reduction ($P < 0.001$) in total length of the plants from shelter to exposure (fig 56) with a corresponding change in the proportion of the stem (fig 57). It is evident that in the Menai Straits, the stem in most cases constitutes only 10% of the total frond length. In 12% of the fronds, it reaches about 35% of the entire length. On the most exposed shore, Sandy Point, at least 50% of the total frond length is composed of stem. In 16% of the cases the entire frond is stem. As poorly anchored plants are readily torn away during heavy wave action the advantage of the longer and thicker stem in these conditions is evident.

There is a great deal of variation in morphology of F. serratus within a shore of intermediate exposure which generally shows the same pattern with exposure conditions as is seen between shores. The plants occurring in runoff channels are very similar to those seen in sheltered localities such as the Menai Straits. The individual plants are large and have only a small weak stem, which is generally anchored to a small rock under the silt. Individuals from the same shore, but on a rockplatform (usually more exposed conditions), are shorter with a proportionately larger and stronger stem. There are also differences between plants of varying ages; smaller individuals, presumably younger, have mainly leaf and little stem, while older individuals have proportionately less leaf and more stem.

Plate 15

Morphological variation in F. serratus between sheltered and exposed conditions.

Upper - F. serratus from sheltered conditions at Sandy Bay, Anglesey. Note general shape and colour. Scale X $\frac{1}{4}$.

Lower - F. serratus from exposed conditions at Sandy Bay. Scale X $\frac{1}{3}$.
Arrows show dark reticulata of L. mariae in undisturbed positions (bottom snail is juvenile). Note how the white body of the snail is the visible (see Appendix II for description of variation in the body colour.

Plate 15

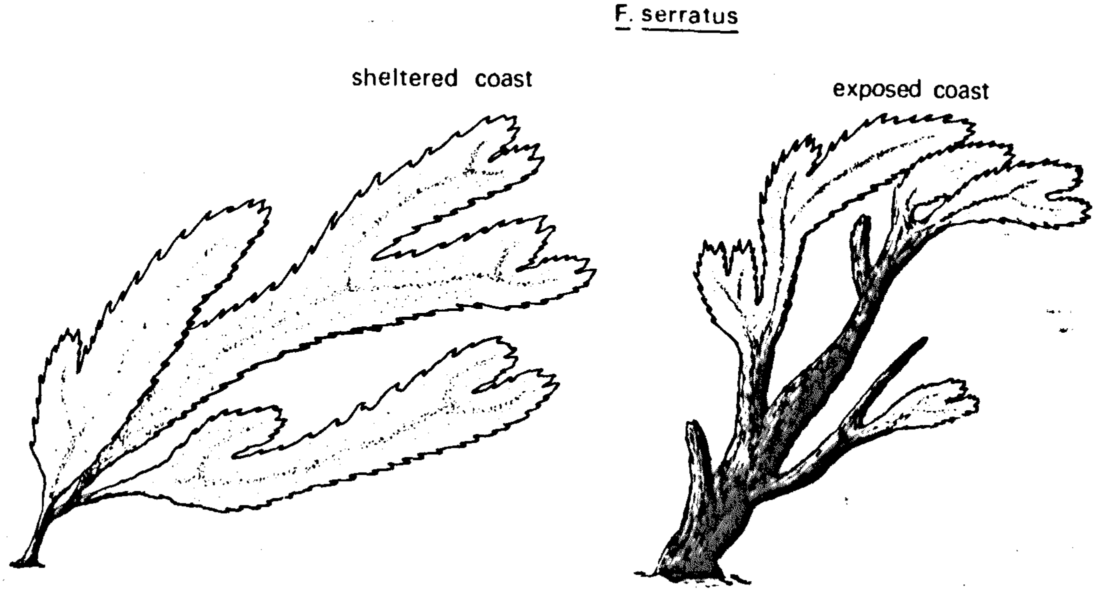


Fig. 55. Generalized sketch of F. serratus from sheltered and exposed shores.

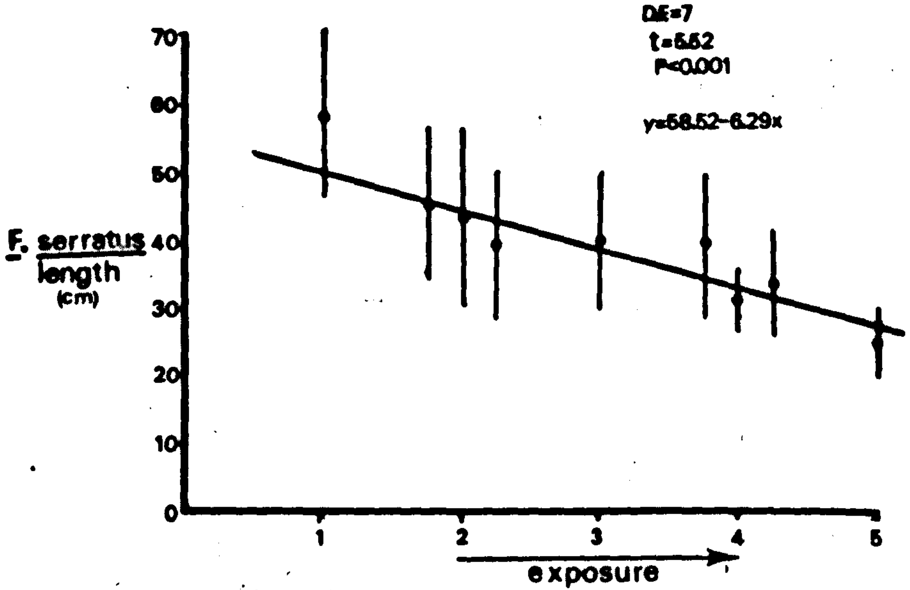
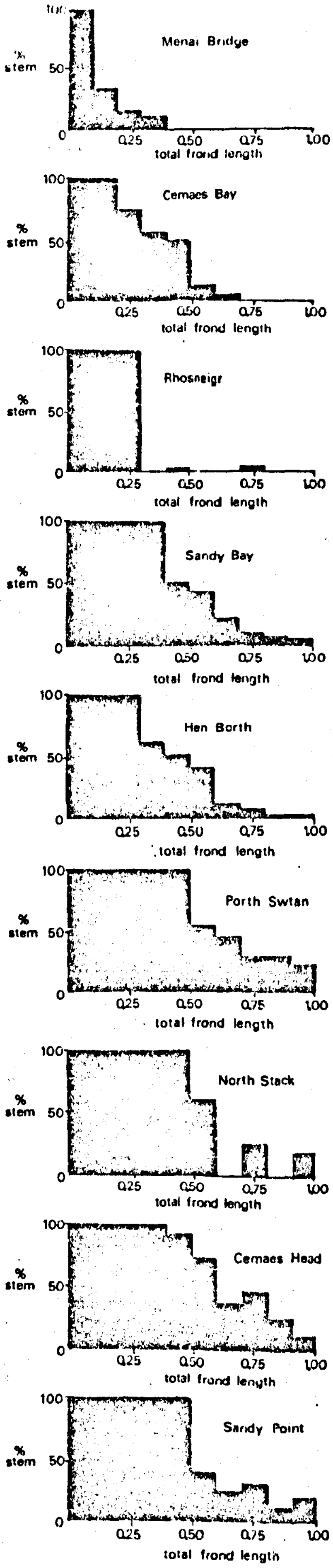


Fig. 56. Comparison between exposure and length of F. serratus.

Figure 57

Figure 57

The relative length of stem in *F. serratus* on shores varying in exposure. Localities arranged from most sheltered (top) to most exposed (bottom). Note that the amount of shading on each histogram reflects the relative amount of stem.


Menai Bridge
Cemaes Bay
Rhosneigr
Sandy Bay
Hen Borth
Porth Swtan
North Stack
Cemaes Head
Sandy Point
% stem
100
50
0
0.25
0.50
0.75
1.00
total frond length

It will be shown in the remaining parts of this section that these differences in the amount of stem and leaf provide an explanation of the general frequencies for citrina and dark reticulata in L. mariae.

3. Comparisons between citrina and dark reticulata

The greater part of this work was carried out in two localities in Anglesey (Porth Swtan, Sandy Bay) where L. mariae was abundant and had colour phenotypes in workable frequencies. Some additional data were collected from 7 other localities which differed in phenotype frequencies and in exposure.

The following observations on position were recorded for each adult snail:

a) plant substrate - stem, leaf

b) distance of snail from base of plant measured along axis of frond: and total length of frond (measured to nearest 0.5cm with 1m rule).

c) background colour (only at Porth Swtan)

   1 - dark-brown or black as on stem or leaf-stalk

   2 - olive-brown as on leaf

   3 - olive-yellow to orange as on leaf tip or fruiting bodies.

The plant substrate (a) provides a general comparison with background colour while the relative distance to the tip of the frond (b) provides a more exact comparison with changes in background along the length of the frond. At Porth Swtan, it was further possible to obtain a subjective grading of the background colour (c) at the exact position where the snail was found, irrespective of the general position occupied. These estimates take into account the minor variations in background colour. For example, the leaves of young plants are generally lighter in appearance than those from adult plants: the light-coloured fruiting bodies are developed in some plants and not in others: some leaves are very dark in appearance, owing in part to heavy grazing which removed the lamina or leaf leaving a dark mid-rib. This grading of the colour was made by an assistant who in each case was unaware of the shell colour.

To interpret the micro-distribution of the phenotypes it was necessary to gain some indication of the movement by individuals on the plant. During all of the microdistribution studies (made at low tide in daylight) no large-scale movements along the length of the fronds were observed. Most of the animals showed no movement; when it did occur, it was restricted to small areas on a single leaf or stem with occassional individuals crossing over to adjacent fronds at the same level. Diving observations at high water showed similar amounts of movement to that observed at low water. During darkness, both at low and high water, most of the animals were moving, though again in only small areas on the plant. It would seem that these winkles are remarkably sedentary in their habits.

At Porth Swtan, the comparison of citrina and reticulata demonstrates that the two phenotypes are not randomly distributed with respect to background colour. Although there are no significant differences from each of the 5 sampling dates, there is a consistent trend for the reticulata to be more commonly associated with a dark-brown background (table 28). As the heterogeneity χ^2 is non-significant (P = 0.50-0.90), the 5 samples can be pooled; this yields a significant χ^2 of 9.23 ($P < 0.01$).

Table 28: Comparisons between citrina and reticulata of L. mariae on different background colours of F. serratus at Porth Swtan. All snails recorded were adults.

Date	Phenotype	Background colour			$^{2}_{(2)}$	P
		1 (dark)	2	3 (light)		
12/4/72	citrina	11	10	12	2.88	0.25-0.50
	reticulata	43	48	26		
17/4/72	citrina	12	12	12	1.13	0.50-0.90
	reticulata	50	60	37		
24/4/72	citrina	29	24	19	2.73	0.25-0.50
	reticulata	84	101	46		
28/4/72	citrina	12	17	11	4.99	0.05-0.10
	reticulata	74	60	24		
2/5/72	citrina	11	9	10	2.20	0.25-0.50
	reticulata	40	50	26		

Total $\chi^2_{(10)}$ = 13.93 0.50-0.90

Pooled $\chi^2_{(2)}$ = 9.23 0.005-0.01

Heterogeneity $\chi^2_{(8)}$ = 4.70 0.50-0.90

Reticulata in these comparisons includes both the light and dark phenotypes, the former comprising only 9% of the individuals. When these two phenotypes are compared with respect to the background colour, there is a significant excess of the light reticulata on the lighter parts of the plant (table 29), or in other words, on the leaf.

Table 29: Comparison between light reticulata and dark reticulata and between light reticulata and citrina on different background colours (pooled data from table 28).

Phenotype	Background Colour 1 (dark)	 2	 3 (light)	$\chi^2_{(2)}$	P
light reticulata dark reticulata	18 273	32 287	22 137	7.38	< 0.025
light reticulata citrina	18 75	32 72	22 64	3.37	0.10-0.50

Comparison of the light reticulata with citrina shows no significant difference in the association with background colour.

The data recorded for the positions of L. mariae on F. serratus show that the majority of individuals are found near the end of the frond, generally on the leaf. Relatively few (11%) are found on the stem of the plant. Among these, however, the reticulata are relatively more common than citrina but not significantly so (table 30). When the position of the two phenotypes are compared with respect to the overall length of the frond, the reticulata are relatively more common than citrina near the base of the plant and citrina is relatively more common near the tip of the frond (table 31). These differences are again not significant ($P = 0.010-0.25$). Owing to these observations made initially at Porth Swtan, a series of other localities were sampled to determine whether similar trends occurred elsewhere.

In the 4 additional localities, there were, as in Porth Swtan, no significant differences between the distribution of citrina and reticulata (tables 30 and 31); but in each locality, although only a small proportion are found on the stem, of these, the reticulata were relatively more common than citrina as in Porth Swtan.

Table 30: Relationship between colour phenotypes of adult L. mariae and stem-leaf position on F. serratus. Shores arranged from most sheltered to most exposed.

Locality	Phenotype	Position of F. serratus		$\chi^{2}(1)$	P
		stem	leaf		
Rhosneigr	citrina reticulata	1 4	41 64	-	0.65 (EXACT)
Sandy Bay	citrina reticulata	6 15	125 192	0.06	0.50-0.90
Hen Borth	citrina reticulata	1 12	19 95	-	0.65 (EXACT)
Porth Swtan	citrina reticulata	20 84	204 666	0.71	0.25-0.50
Sandy Pt.	citrina reticulata	2 18	14 52	-	0.34 (EXACT)

The data presented so far have included only adult snails. In 4 of the 5 localities, it is also possible with a small amount of data, to compare the stem-leaf positions of citrina and dark reticulata in the juvenile shells (table 32). In each of the 6 comparisons there are again no significant differences between the phenotypes although in each case, the trend is in the same

Table 31: Relationship between colour phenotypes of adult *L. mariae* and position on *F. serratus*. In some localities, the lower positions have been pooled to provide cells with expected values greater than 5.

Locality	Phenotype	Position on *F. serratus**				χ^2	P
		0-0.49	0.50-0.69	0.70-0.89	0.90-1.0		
Rhosneigr	citrina	0	5	16	21	$1.13_{(2)}$	0.50-0.90
	reticulata	3	10	26	29		
Sandy Bay	citrina	6	12	60	64	$2.00_{(3)}$	0.50-0.90
	reticulata	9	26	76	96		
Hen Borth	citrina	0	2	6	12	$2.69_{(1)}$	0.10-0.25
	reticulata	7	18	39	43		
Porth Swtan	citrina	7	19	88	110	$5.40_{(3)}$	0.10-0.25
	reticulata	26	78	343	303		
Sandy Pt.	citrina	0	4	7	5	$0.19_{(1)}$	0.50-0.90
	reticulata	4	8	32	26		

*0.0 (stem) - 1.0 (leaf)

Table 32: Relationship between colour phenotypes of juvenile *L. mariae* on stem-leaf position of *F. serratus*.

Locality	Shell size	Phenotype	Stem	Leaf	$\chi^2_{(1)}$	P
Sandy Bay	juvenile	citrina	6	22	1.49	0.10-0.50
		reticulata	24	41		
	intermediate	citrina	4	37	0.22	0.50-0.90
		reticulata	11	64		
Porth Swtan	juvenile	citrina	7	9	0.32	0.50-0.90
		reticulata	21	16		
Cemaes Head	intermediate	citrina	6	9	0.97	0.25-0.50
		reticulata	42	36		
Sandy Pt.	juvenile	citrina	12	6	0.19	0.50-0.90
		reticulata	70	23		
	intermediate	citrina	12	4	0.25	0.50-0.90
		reticulata	39	7		

direction as in the adults, that is, an association between dark reticulata and stem and between citrina and leaf.

There are indications, therefore, of a slight but consistent trend for the two phenotypes in both adults and juveniles to be unequally distributed on the plant. The stem and base of the plant are dark-brown in colour on which dark reticulata are highly cryptic while citrina highly conspicuous (plate 14). The slight tendency for dark reticulata to be relatively more common than citrina on this part of the plant is not unexpected if visual predators prey upon the least cryptic shells. A second possibility, not necessarily contradictory from the first, is that of microhabitat preferences for each of the phenotypes.

With the data that have been presented up to this point, it has not been possible to account for either the existence or the variation of citrina (except in so far as it is the inverse of dark reticulata). If microdistributional differences were responsible for the polymorphism, it would seem that a reticulata phenotype which varied continuously from a dark through to a light brown would be the best strategy for crypsis owing to the fact that the plant varies in this manner. There appears at first sight to be no counterpart in the background colouration which would provide citrina with camouflage. It seemed plausible initially that the discrete nature of the phenotype and its low frequencies on exposed shores could be attributable to apostatic selection by visual predators such as the blenny. This type of selective regime could also account for the increase in frequency on sheltered shores, often up to 100%, if the second species L. obtusata, was taken into account; on these shores this species is composed predominantly of the dark and very cryptic phenotype, olivacea.

Clarke (1962) has suggested that this form of selection might account for the inverse relationship between the frequencies of yellow shells in Cepaea nemoralis and effectively unbanded shells in C. hortensis where the two species occur together. He has argued that if two similar species have a common predator, and if one has a high frequency of a certain morph, then owing to a search image by the predator, selection would favour a different morph in the second species, one which was dissimilar in appearance to that in the first.

There are other explanations which could be invoked to account for the occurrence of citrina involving selective regimes which do not act directly on the shell colour but rather on non-visual associated characters, the conclusions reached by Sacchi and Gratton working on L. mariae. Goodhart (1963), working with C. nemoralis, has suggested that for populations living in a stable environment, selection will build up a genetic background with each major gene resulting in a "co-adapted" complex. When individuals of the population migrate to a new habitat the visible manifestations of the major genes, such as colour, may be themselves disadvantageous or neutral but since these characters are tightly linked with a large number of other advantageous characters, the colour phenotype will be retained in the population. In this way, adjacent populations may show completely different patterns of phenotype frequencies over ecologically similar conditions. Ford (1964) has argued that selection may be favouring genes controlling a character without acting directly on the character itself. This explanation was presented to account for sharp changes in frequency of what appears to be insignificant spots on the underwing of the meadow-brown butterfly (Maniola jurtina). It would be possible to argue that in L. mariae, the bright colouration of citrina and its high frequencies in sheltered areas are a reflection of hidden advantageous characters important for survival in these conditions.

However, in each locality where citrina is present, there is a great deal of variation in the expression of the colour. The majority of individuals are very brilliant while others are very dull and apparently more cryptic. If the genes responsible for the expression of citrina are associated with hidden advantages, and the bright colouration is disadvantageous to some extent owing to the presence of visual predators, it would seem therefore that in all populations, there is an opportunity for combining the advantageous hidden characters to the citrina which are duller in appearance. It would seem unlikely that linkage groups would be so tight that they could not be modified by the action of natural selection. In fact, the significance of the brilliant colouration in citrina is not owing to hidden advantages but owing to the colour itself as the following observations will demonstrate.

During the final phases of this study, I had begun underwater observations with SCUBA for comparing microdistributions at low and high water. At this time I was able to observe one of the predators, Blennius pholis, in its natural habitat. In the zone of L. mariae blennies were seen under or within Fucus serratus plants preying upon the juvenile snails. When under the plant, they would swim out from the stem, and while still underneath the plant, stop and look upward searching for Littorina on the fronds above them. When a snail was seen, they would swim upwards, pluck it from the frond and swallow it. From my vantage point on the side of the plant, the reticulata appeared very cryptic while citrina stood out. However, when I moved to the base of the plant, and was now viewing the snails from the same position as the blenny, that is from below the fronds, the significance of the apparently conspicuous citrina colouration became clear.

Although the leaf of F. serratus is olive-brown when viewed with reflected light, the most common situation from a human standpoint, when viewed from below with transmitted light, the view seen by the blenny, the leaf appears a brilliant yellow (plates 16 and 17). Citrina, when seen against this background, are extremely well camouflaged while dark reticulata stand out as a conspicuous black shell. If citrina was in the least way duller in appearance, the very exact crypsis would be lost. Near the base of the leaf, less light is transmitted owing to the increased thickness of the frond at this point. In this position both citrina and dark reticulata stand out while light reticulata blend in. From the observations on the blenny, it would seem that there are three major shell colours which provide maximum crypsis: a dark-brown shell on the stem of the plant, a light brown shell at the base of the leaf, and a bright yellow shell on the leaf proper. The phenotypes, dark reticulata, light reticulata and citrina correspond to these colours.

Experiments were set up in the laboratory to determine whether the blenny would demonstrate a preference for a certain phenotype depending upon the position of the snail on the plant and the manner of illumination of the frond. In the procedure, a F. serratus plant was placed in each of three glass tanks illuminated from above by a flourescent light with two 60w tubes. One blenny was then put in each tank and allowed one day for habituation to the artificial conditions. Individuals of citrina and dark reticulata of the same size were presented in pairs to the blennies in the following 4

Plate 16

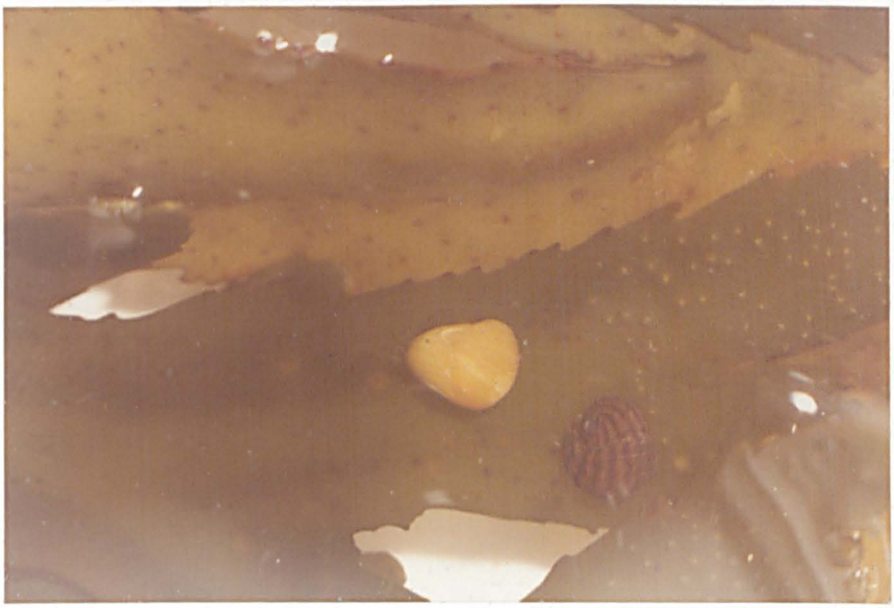
Plate 16

Effect of reflected and transmitted light on colour of F. serratus im relation to crypsis of citrina and dark reticulata (light source - double 6W fluorescent tubes).

Upper - major light source from above fronds (reflected light) with small amounts from below (transmitted light).

Lower - major light source from below shining through fronds (transmitted light).

Note. The shells in the upper and lower plate are the same individuals and show natural colouration (see note in Plate 17).



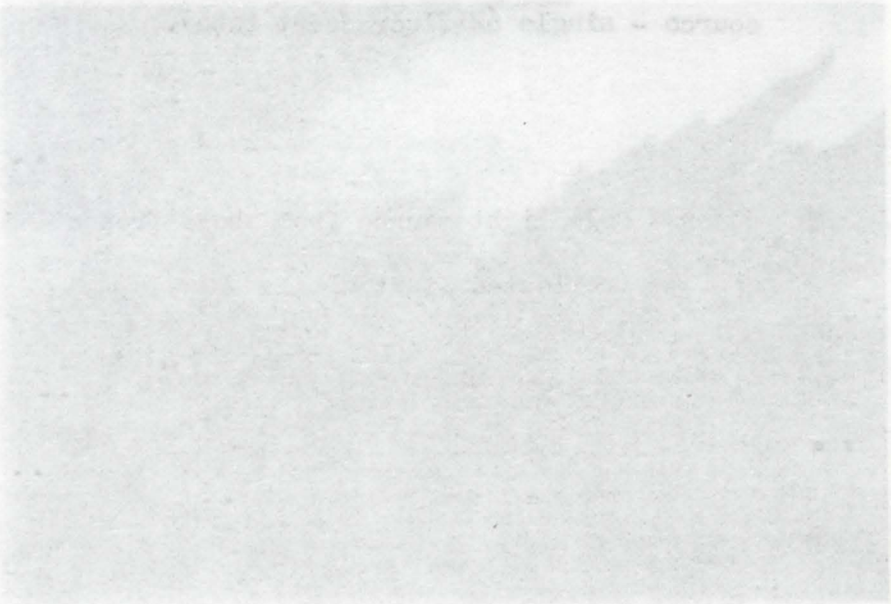


Plate 17

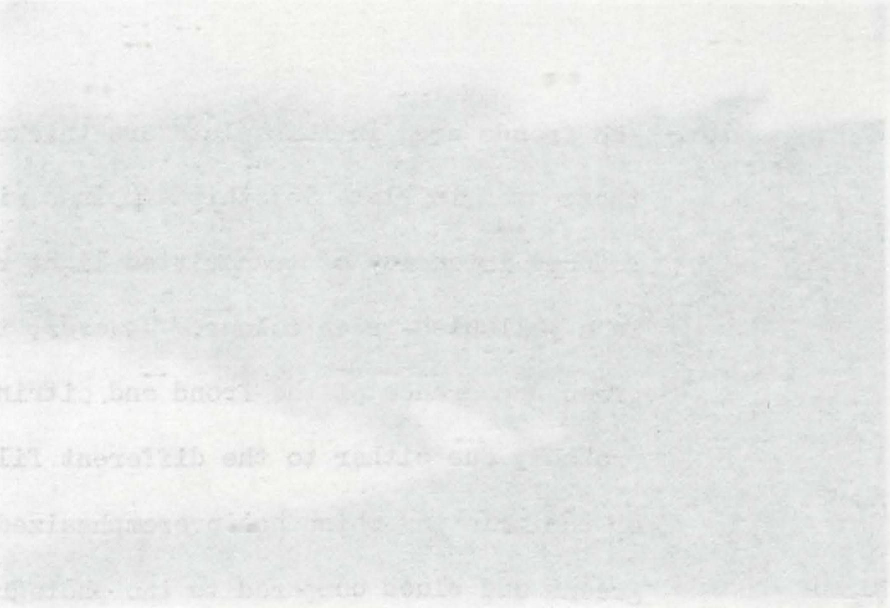


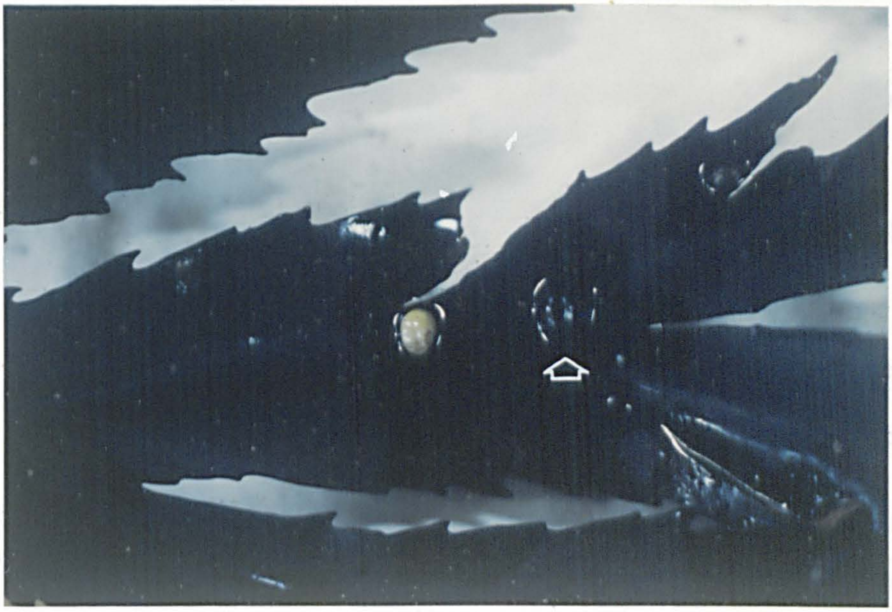
Plate 17

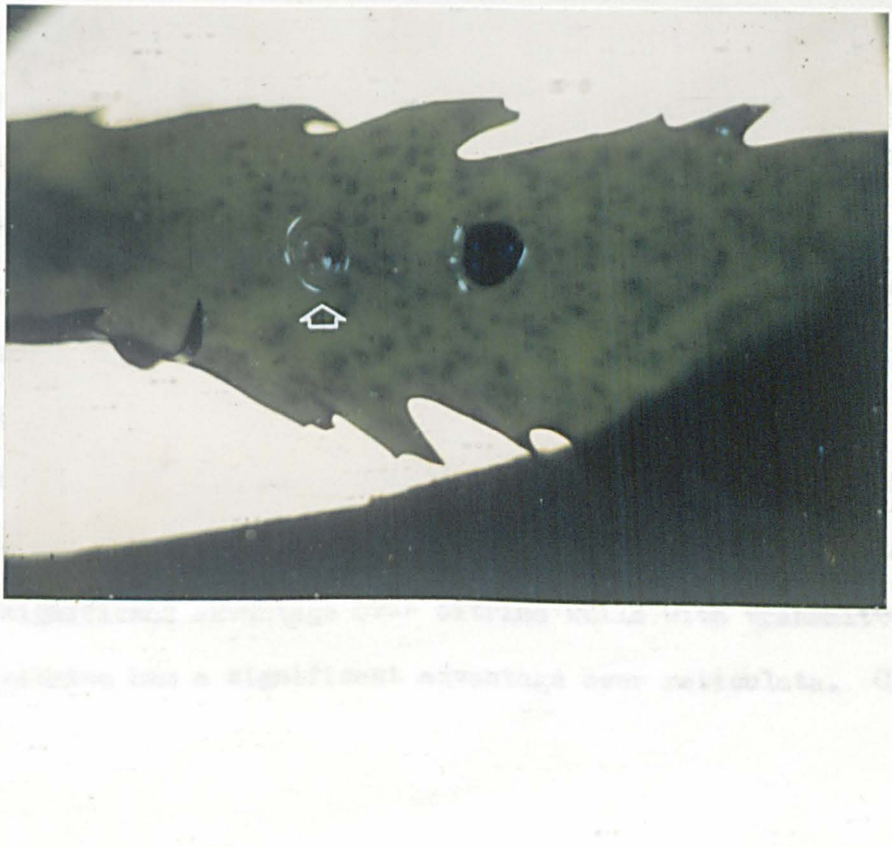
As in Plate 16 except for reduced intensity (light source - single 6W fluorescent tube).

Upper - sole light source from above fronds (reflected light)

Lower - major light source from below (transmitted light)

Note. The fronds used in this plate are thicker than those used in Plate 16; this combined with the reduced intensity of transmitted light results in a yellowish-green colour. However, the very green appearance of the frond and citrina are probably due either to the different film used or the printing which has overemphasized the greens and blues compared to the photographs in Plate 16.





conditions:

1: on a F. serratus leaf at the bottom of the tank so that the blenny would approach the snails from above. Illumination of the frond was only reflected by light.

2: on a F. serratus leaf which was held near the surface so as to allow the blenny to approach the frond from underneath. The illumination of the leaf was by transmitted light, as seen by the blenny.

3: on a F. serratus stem which was placed at the bottom of the tank allowing the blenny to approach from above.

4: on a F. serratus stem which was held near the surface again to allow the blenny to approach from underneath. Since the stem is opaque to light it appears much the same colour as in No. 3.

The citrina and dark reticulata were placed 4cm from each other in each case. Usually within 30 seconds of presenting the snails the blenny approached the frond and took one or both of the phenotypes (plate 18). For the purpose of this experiment the first snail to be taken was recorded. On each of 20 days in succession the blennies were presented with the 4 substrates in alternating sequences. Fifteen minutes were allowed between each trial. The general sequence was to present the substrate which would make the phenotype taken on the previous trial the most cryptic.

The results of these experiments presented in table 33 show that on an F. serratus leaf with reflected light, dark reticulata has a significant advantage over citrina while with transmitted light citrina has a significant advantage over reticulata. On

Plate 18

Plate 18

View of Blennius pholis in relation to hunting positions. Photographs show also citrina and dark reticulata of L. mariae on F. serratus frond illuminated with transmitted light (double 6W fluorescent tube).

Upper - position of blenny at rest.

Lower - blenny preying upon dark reticulata.

Following the photograph, a second blenny (blurred) took citrina.

Note. Although these photographs have been taken in the laboratory through the side of a glass tank, the general positions of the blenny and overall colouration are very similar to what was seen while diving (see text).

Table 33: Results of predation experiments comparing citrina and dark-reticulata on *F. serratus*.

Substrate	Tank	1st Taken		$\chi^2_{(1)}$	P
		citrina	reticulata		
F. serratus leaf with reflected light	1	13	7	1.8	0.10-0.25
	2	15	5	5.0	0.025-0.05
	3	12	8	0.8	0.25-0.50
	Total	40	20		
		Summed $\chi^2_{(3)}$ =		7.6	0.05-0.10
		Pooled $\chi^2_{(1)}$ =		6.8	0.005-0.01
		Heterogeneity $\chi^2_{(2)}$ =		0.8	0.25-0.50
F. serratus leaf with transmitted light	1	3	17	9.8	< 0.005
	2	8	12	0.8	0.25-0.50
	3	4	16	7.2	0.025-0.01
	Total	15	45		
		Summed $\chi^2_{(3)}$ =		17.8	< 0.005
		Pooled $\chi^2_{(1)}$ =		15.0	< 0.005
		Heterogeneity $\chi^2_{(2)}$ =		2.8	0.05-0.10
F. serratus stem at bottom of tank	1	14	6	3.2	0.05-0.10
	2	18	2	12.8	< 0.005
	3	17	3	9.8	< 0.005
	Total	49	11		
		Summed $\chi^2_{(3)}$ =		25.8	< 0.005
		Pooled $\chi^2_{(1)}$ =		24.2	< 0.005
		Heterogeneity $\chi^2_{(2)}$ =		1.6	0.25-0.50
F. serratus stem at surface	1	14	6	3.2	0.05-0.10
	2	15	5	5.0	0.025-0.05
	3	11	9	0.2	0.50-0.90
	Total	40	20		
		Summed $\chi^2_{(3)}$ =		8.4	< 0.005
		Pooled $\chi^2_{(1)}$ =		6.7	0.01-0.05
		Heterogeneity $\chi^2_{(2)}$ =		1.7	0.25-0.50

the stem, reticulata has an advantage in both positions of the stem. This is to be expected since, as mentioned, no light passes through the stem when it is held at the surface of the tank.

Many more experiments are required for understanding the effect of illumination with reflected and transmitted light on the degree of crypsis obtained by the various phenotypes. Comparisons must be made between dull and bright citrina and between the different shades of reticulata depending upon the thickness and illumination of the frond. The small number of experiments which have been performed in this study have shown that what has been considered a highly conspicuous phenotype is in fact highly cryptic; thus associated characters and hidden advantages would not appear to be necessary to account for the presence of this phenotype.

3. Comparisons between Juveniles and Adults

The observations made on the cryptic nature of citrina and light and dark reticulata and their microdistribution provide a partial explanation for the occurrence of these phenotypes within populations. The next question is whether they can account for the change in frequency of these phenotypes between shores. It will be recalled that in L. mariae citrina predominates in shelter while dark reticulata predominates in exposure. Light reticulata is usually less common then either of these phenotypes and does not show a predictable trend with exposure.

The observations on the morphology of F. serratus within and between shores may provide some explanation for the increase in frequency of dark reticulata from sheltered to exposed shores. If the snails were randomly distributed on the plant, then the abundance of dark reticulata that has been observed on exposed shores could simply be a reflection of the relatively large stem in these conditions (see fig 55). However, even if this were the case one would not expect more than 50% dark reticulata rather than the observed 90% as the stem seldom constitutes more than 50% of the entire plant. A more important criticism that can be made is one concerning the assumption of random distribution on the plant. It is quite evident that even on exposed shores, the majority of adult L. mariae are found on the leaf of the plant, the opposite of what would have been predicted if the high frequency of dark reticulata was owing to an association with the stem (tables 30 and 31).

When this work on the microdistribution was being carried out, I was also analysing the stomach contents of the blenny (see Section V). This had showed that there was no evidence of predation on adult L. mariae, but that there was extensive predation on juveniles less than 6mm. As the blenny appeared to be one of the major predators on L. mariae, it seemed possible that the juveniles were the unit of selection in the colour polymorphism, and that, if this were true, their microdistribution of the juveniles on the plants would be of more importance than that of the adults. Owing to this, I returned to the majority of localities where the measurements were made on F. serratus, and remeasured the microdistribution of juvenile and intermediate size classes.

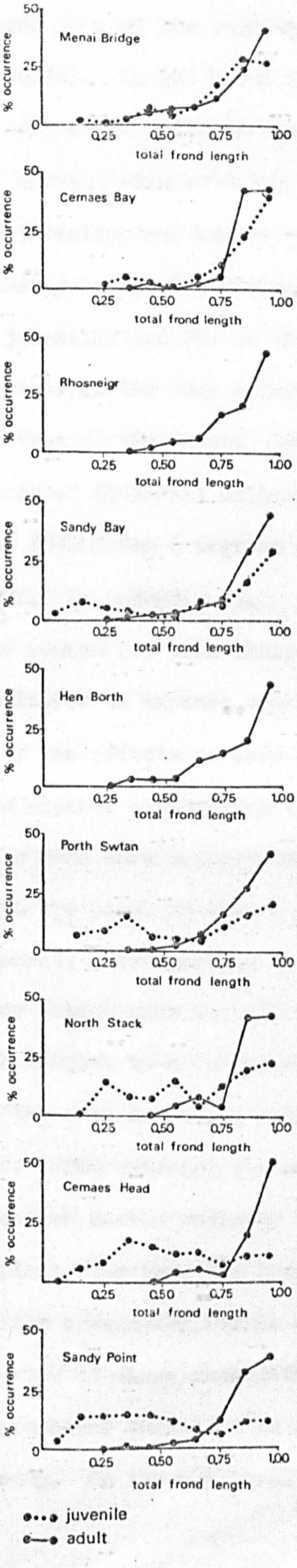
The results of these measurements are presented in appendix F and summarized in fig 58. This figure shows the distribution of the juveniles (dotted line) and adults (solid line) in the series of localities ranked from sheltered to most exposed. The series of graphs on the left hand side represent the frequencies of occurrence at different points from the base to the tip of the plant. It is apparent that in the most sheltered locality (Menai Straits) the juveniles and adults do not differ in their relative distribution, both being found near the leaf tip. However, in the localities of increased exposure, while the adults show little change in the distribution, the juveniles become relatively more common near the base of the plant. In the most exposed shore (Sandy Point), there is almost a complete separation between the distributions of the juveniles and adults, the former being very abundant near the base of the plant (see table F ii in appendix F for χ^2 analysis).

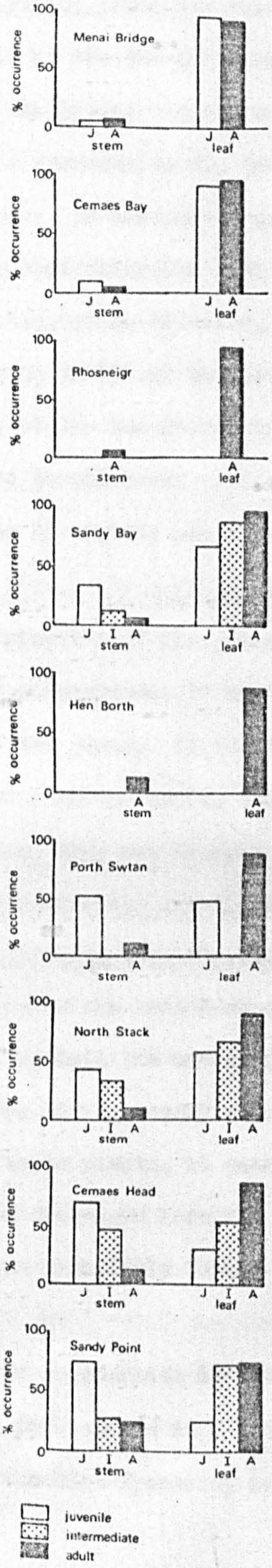
Another way of comparing the distributions is to consider the proportion found on the stem and the leaf. This takes into account the variation in F. serratus between shores. This is shown on the

Figure 58

Figure 58

Distributions of juvenile and adult *L. mariae* on *F. serratus* from a number of shores varying in exposure. Localities arranged from most sheltered (top) to most exposed (bottom). Left column shows frequencies of juveniles and adults found at different positions on the frond, values near 1.0 representing the leaf tip. The right column shows frequencies of juveniles, adults, and in some cases intermediate shell sizes on the stem and the leaf. See appendix F for raw data.


Menai Bridge
Cemaes Bay
Rhosneigr
Sandy Bay
Hen Borth
Porth Swtan
North Stack
Cemaes Head
Sandy Point
% occurrence
0
25
50
0,25
0,50
0,75
1,00
total frond length
juvenile
adult


Menai Bridge
Cemaes Bay
Rhosneigr
Sandy Bay
Hen Borth
Porth Swtan
North Stack
Cemaes Head
Sandy Point
% occurrence
0
50
100
J
I
A
stem
leaf
juvenile
intermediate
adult

right hand side of the page (juveniles-plain; intermediates-dotted; adults-solid). In the Menai Straits 96% of the juveniles and 94% of the adults are found on the leaf of the plant. In the more exposed shores, such as Porth Swtan, the frequencies are 50% and 92% for juveniles and adults respectively. On the most exposed shore (Sandy Point) the distribution is more divergent with 25% of the juveniles and 75% of the adults located on the leaf. There is, therefore, on the more exposed shores up to 75% of the juveniles on the stems of the plant. Calculation of the heterogeneity between the shores of different exposure for the juvenile and adults gives a χ^2 of 309.03 for 6 degrees of freedom ($P < 0.001$; see also table F iii in appendix F).

The reason for this change in distribution of the juveniles from sheltered to exposed shores is not understood. It may be caused by the effects of wave action on the plant. If the juveniles are less capable of adhering to the weed than the adults (this seemed to be the case when collections were made) they may distribute themselves on the plant in such a way as to reduce the likelihood of dislodgement. Observations made on F. serratus when the tide was rising or ebbing show that in exposed shores the leaf part of the plant is subject to a "whiplash" effect against the underlying rocks. The stem and base, being far more rigid, remain relatively immobile. From watching the motion of these plants, it seemed that the juveniles snails would be less often dislodged from the stem of the plant than from the leaf which was constantly lashed against the underlying rocks.

In view of these observations, it is appropriate to reconsider the geographical variation in phenotype frequencies in 7 localities in Anglesey. On the sheltered shores, the high frequency of citrina can be attributed to the distribution of the juveniles and adults which in these conditions are predominantly on the leaf of the plant. However, this would only be true if all the snails which were predated were on the underside of the frond. Since this is where the yellow shells appear cryptic when viewed with transmitted light. This is not true, for the blennies were often seen within the F. serratus clump taking snails on the fronds below them, a situation which should favour if not dark reticulata, at least light reticulata since illumination is now with reflected light. The reason for the high frequencies of citrina was again revealed from diving observation.

When approaching F. serratus from above, the visible fronds do indeed appear an olive-brown, a background colour on which both light and dark reticulata are quite cryptic. However when light passes through these upper fronds, all those on the interior of the clump are illuminated with yellow light on which the citrina are better camouflaged than both light and dark reticulata. The transmission of the yellow light was most evident in fronds with very thin broad leaves which occur mainly in sheltered coasts. It would seem, therefore, that the high frequency of citrina in these conditions relates to its cryptic nature on the majority of positions on the plant.

On shores with greater exposure, citrina decreases in frequency with a corresponding increase in dark reticulata. The reduction in citrina would be expected owing to the tendency for fewer individuals to be found on the leaf and greater numbers on the stem. The frequency of dark reticulata does in fact correlate with the relative numbers of individuals which are found on the stem of the plant (fig 59). These individuals are not the adults as were initially expected but the juveniles. This strongly suggests that the major advantage of dark reticulata is owing to its very cryptic nature on the stem and that the adult frequencies in a population reflect principally the abundance of the juvenile age classes on this part of the plant. There are some suggestions that this crypis may be yet further enhanced by the orientation of the snail, which is usually towards the base of the plant. The dark lines of the reticulate pattern appear to act as disruptive colouration breaking up the outline of the shell when the snail is orientated along the longitudinal axis of the stem (see appendix G).

Fig. 51. Proportion of juveniles on stem in relation to frequency of dark reticulata in the population. From appendices G & F.

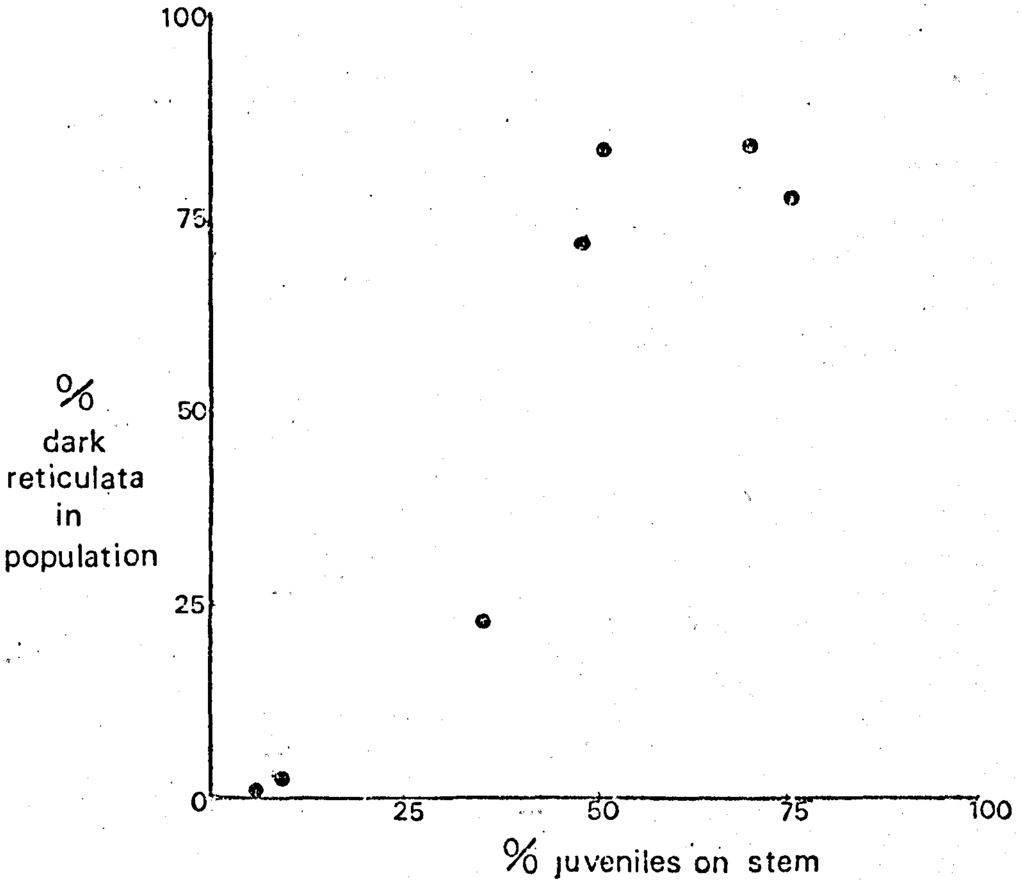


Fig. 59. Proportion of juveniles on stem in relation to proportion of dark reticulata in the population. From Appendices C & F.

4. General Discussion of Colour Polymorphism

A number of important factors have emerged from the studies on microdistribution in L. mariae; these include the demonstration of changes in the distribution of juveniles on the plant from sheltered to exposed shores, and the cryptic appearance of each of the phenotypes depending upon their position on the plant and the manner of illumination of the frond. These observations provided also an understanding of the variation in phenotype frequencies in some localities in Anglesey. It is possible, therefore, to re-examine the changes in phenotype frequencies that were observed in samples collected in the geographical survey around the British Isles (see section VII).

Throughout the study area, in Wales, south England, the Isles of Scilly and Ireland, there was a general correlation between shelter and high frequencies of citrina and between exposed conditions and high frequencies of dark reticulata. This is what would be expected if the observations at the 7 localities in Anglesey are of general relevance. One notable exception occurred in southern England at Weston-super-Mare; this locality is very sheltered and has, instead of a high frequency of citrina, a 100% frequency of dark reticulata. However, examination of the field notes for this locality showed that the algal fronds were covered with sediment, characteristic of many parts of the Bristol Channel. It would seem, therefore, that if little or no light could pass through the fronds, citrina would not be expected to occur.

Light reticulata was found mainly in sheltered and semi-exposed shores in Wales, south England and the Isles of Scilly. This phenotype is cryptic, when viewed with transmitted light through thicker parts of the leaf such as the areas near the stem-leaf connection. Although no information is available from the majority of these shores, it is possible that the frequencies with which the phenotype are found might be in proportion to the areas of the intermediate substrate. In Ireland, the phenotype is very uncommon except in one very exposed locality where it reaches a frequency of 50%. One would have expected dark reticulata to predominate in these conditions if all or most of the juveniles were on the stem of the plant. There was one exceptional difference in the Fucus serratus at this locality and this was that the fronds were very leathery and almost orange in appearance viewed with reflected light. Light is only partially transmitted through leathery or thick fronds and so, there would be a large part of the plants at Cloonagh which would appear intermediate in colouration between the yellow on the leaf and the dark brown on the stem. This may provide some indication for the relative abundance of light reticulata in this locality.

The striking similarity between the colouration of dark reticulata and the colouration of the stem observed in Anglesey raises some questions concerning the occurrence of the red-brown colouration of dark reticulata seen mainly in the Isles of Scilly. It was argued (see p. 220) that the occurrence of

Devonian Red Sandstone and encrusting red algae might provide an explanation for the red-brown shells if the individuals were found off the algae on these substrates. A difficulty with this hypothesis was that individuals were never found in these positions at least during the time of the collections. If the juvenile individuals are found predominantly on the stem as they are in Anglesey in exposed conditions, and rarely on the underlying substrate, the correlation with the red-brown shells and the reddish substrate is not readily understood. However, if consideration is given to the type of illumination of the plant, it is evident that the colouration of the stem will be influenced not only by its own inherent properties but also by the colour of the light which illuminates it. The light which reflects off the surface of red sandstone, pink granite, or red encrusting algae, might well result in a reddish appearance to the normally dark brown stem of *F. serratus*, giving the red-brown shells a well camouflaged appearance. Unfortunately, underwater observations were not made in these localities and so no support can be given for the suggestion.

Another factor to emerge from the microdistribution studies in *L. mariae* is one concerning the scarcity of the phenotype olivacea in this species. It will be recalled that in *L. obtusata*, olivacea is usually very common. On *F. serratus*, to which *L. mariae* is principally restricted, this phenotype appears quite cryptic when it occurs on the exterior fronds which appear olive-brown when viewed from above. However, on portions of the plant such as the stem, it is not nearly as cryptic as dark reticulata nor is it as cryptic as citrina when viewed from below the fronds (Plate 19). When a comparison is made with light reticulata, olivacea again does not appear as cryptic in conditions of transmitted light through the relatively thick parts of the leaf but is relatively more cryptic with reflected light. Therefore, one would not expect olivacea to be common in L. mariae, but assuming that morph frequencies are principally determined by the proportion of backgrounds on which each is cryptic, olivacea should occur with at least low frequencies. This is the case for two localities on the Isles of Scilly, but in general the phenotype is absent. It is remarkable that another species of gastropod, Lacuna pallidula, which is also found on the fronds of F. serratus, is usually green in the adults; shells of this species are slightly smaller and much less common than those of L. mariae, but very much resemble the phenotype olivacea found in L. obtusata. It is possible that there may be some interaction between L. mariae and Lacuna via the predator.

The cryptic nature of citrina that was initially observed in L. mariae also provides some understanding of the variation in frequency of this phenotype in L. obtusata (see section VII, 2, a). It has been noted that citrina is relatively more common on the Isles of Scilly, which are, overall, more exposed than other parts of the study area. In Wales, south England, and Ireland the phenotype is most abundant in some of the sheltered shores with a great deal of current and in most of the exposed shores where it reaches frequencies up to 40%. This pattern

Plate 19

Plate 19

Comparison between citrina and olivacea in L. obtusata on leaf of F. serratus with both transmitted and reflected light. Photograph taken in the field under natural lighting conditions.

of change in *L. obtusata* is tending to the opposite pattern to that seen in *L. mariae*, which shows the highest frequencies of citrina on sheltered shores, with or without the presence of current. At first sight, these changes in *L. obtusata* could be attributable to apostatic selection operating between the species. However there are reasons to suggest that the change may be owing to selection for crypsis. In very sheltered shores, *L. obtusata* is found predominantly on *Ascophyllum* on which the phenotype olivacea is very well camouflaged. Except for occasional fruiting bodies and light coloured juvenile plants, there are very few background colours in this species on which citrina are cryptic. The fronds are opaque and therefore do not transmit light. On more exposed shores, *Ascophyllum* is replaced by *F. vesiculosus* which is very similar to *F. serratus* in that the coloration is basically olive-brown. A more important similarity is the occurrence of the thin leaf which provides the same bright yellow background when viewed with transmitted light. When citrina is placed against these fronds, the phenotype is very cryptic just as with *F. serratus*. Owing to the narrowness of the leaf and to the opaque air bladders, there is only a limited number of positions through which light is transmitted. This may eventually account for the upper limit to the frequencies of citrina observed on exposed shores in *L. obtusata*. It has not been possible to account for the high frequencies of citrina in sheltered areas where rapid current is present since in such situations the species is found predominantly on *Ascophyllum* rather than the thin-leafed *F. vesiculosus* and *F. serratus*.

In the comparison between the phenotype frequencies in the species (Figs. 52 & 53), it was shown that on sheltered shores where the two species were most dissimilar in shell size and also show the greatest spatial separation, the phenotype frequencies were very dissimilar. In more exposed shores, where there was a convergence in size and also a greater overlap on the shore, the two species were very similar in their phenotype frequencies. It seems that these changes in frequencies can be interpreted mainly in terms of selection for crypsis owing, in L. mariae to differences in the distribution of juveniles on F. serratus from sheltered to exposed shores, and in L. obtusata to the occurrence of different algal species from shelter to exposure.

Although a great deal more information is required, especially on the distribution of juvenile L. obtusata, the data which have been presented on L. mariae allow two generalizations.

(1) Each of the phenotypes are very cryptic when considering the backgrounds as seen from the hunting positions of the blenny, that is, with both reflected and transmitted light.

(2) The phenotype frequencies in different populations are probably due to selection by visual predators, acting directly on shell colour, the frequencies of which are principally determined by the relative proportion of background colours on which the juvenile snails are found.

The understanding that was gained from this work on micro-distribution stimulated other observations on the actual development of shell colours in the very young post-hatching individuals. These are presented in the next section.

SECTION IX

THE ONTOGENY OF SHELL COLOUR

1. Introduction

It has been noted by authors working with colour polymorphisms in molluscs that young individuals often vary continuously in colour and so are difficult to score. In addition to this is the tendency for light-coloured shells to darken with age (see p. 60). Barkman (1956) working with L. obtusata, has noted that juveniles scored as citrina often become adult olivacea and that juvenile olivacea occasionally become adult dark reticulata. Sacchi (1964) working on the same species, but in a different area, had no difficulty in scoring juveniles reliably.

During the course of this study, it became apparent, from field collections and laboratory-reared animals, that juveniles of L. mariae and L. obtusata differed from each other in the shell size at which the colour phenotypes became discrete. It was first observed at Porth Swtan that in L. mariae, individuals less than 2mm were as discrete in colour as the adults (10-14mm). L. obtusata, in the same locality, cannot be scored reliably until 5-6mm. Up to this size, colour varies continously, the animals generally becoming darker with age. Individuals of both species that were bred and raised in the laboratory (Section VI) show the same differences that were observed in the field. The high level of predation on juveniles by a visual predatory, the blenny, implies that the discreteness of the phenotypes in L. mariae or the lack of it in L. obtusata may have some significance in the juveniles. Further observations and collections of juveniles were made at Porth Swtan and other localities to find out if there were ecological reasons which could account for these differences between the species, and if so, how these differences would affect the understanding of the polymorphism.

2. L. mariae

At Porth Swtan, the shells of L. mariae are usually yellow (citrina) or dark brown (dark reticulata). Examination of individuals varying in size from 1 to 13mm (adult size) shows that all the size classes of dark reticulata are the same colour, suggesting that colour changes do not occur during growth. In adult shells this is also evident, for in these the colour of the early whorls of the shell is the same as the later whorls (plate 20a) There are, however, changes in the shell colour of citrina during growth. The shells of the juveniles are white until a size of 3-4mm at which size the entire lip begins to lay down a yellow pigment and continues to do so through the remaining growth phases until adult size is reached. The overall impression given by an adult animal is of a yellow shell, although with a white tip on the apex indicative of the shell colour in the early stages of growth (plate 20).

Because of predation by the blennies on the juveniles, it would appear that the white colouration of young citrina would be at a disadvantage to the yellow as they are no longer cryptic against the leaf of the plants (plate 21) . However, when collecting juveniles less than 4mm, many of the white shells were initially overlooked because of their similarity in appearance to a white tube-dwelling polychaete, Spirorbis sp. which is found cemented to the fronds.

The polychaete is often very common on the plants (plate 22) and it seemed possible that some protection may be afforded to the white snails owing to this resemblance, assuming that Spirorbis itself was less subject to predation than the snails. Observations on the blenny in the laboratory suggested that this was indeed the case.

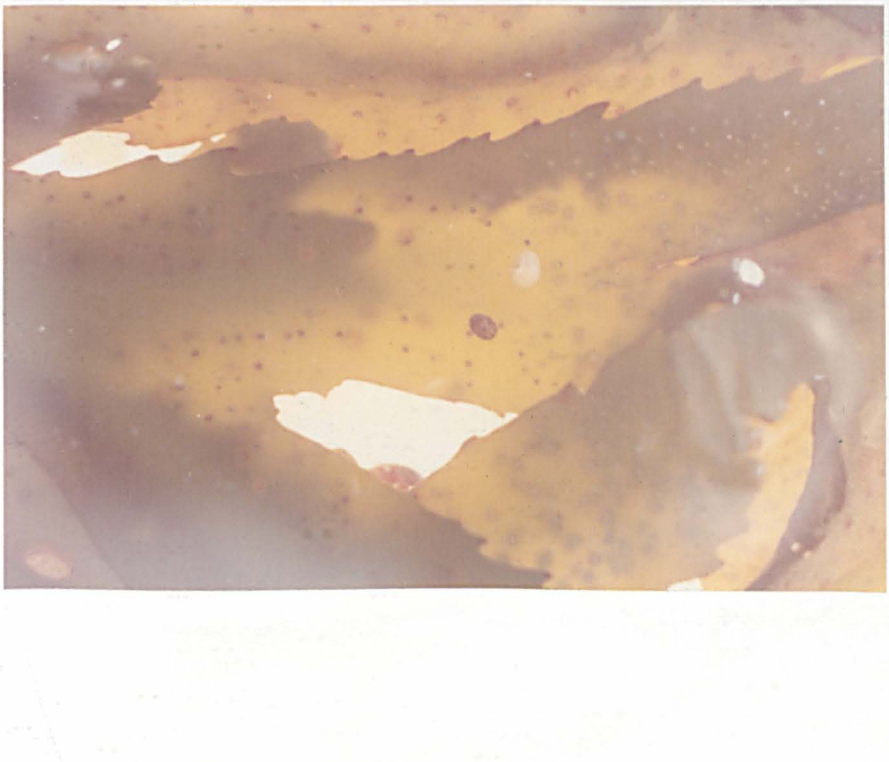
Plates 20 and 21

Plate 20

Colour of apex in adult shells in L. mariae. Note white tip in yellow and orange shells (citrina) and its absence in dark reticulata.

Plate 21

Juveniles of citrina and dark reticulata in L. mariae on F. serratus leaf. Note white colouration of citrina; this causes the white tip seen above in Plate 20. Illumination of frond with predominantly transmitted light. Compare with Plates 16, 17 and 18.



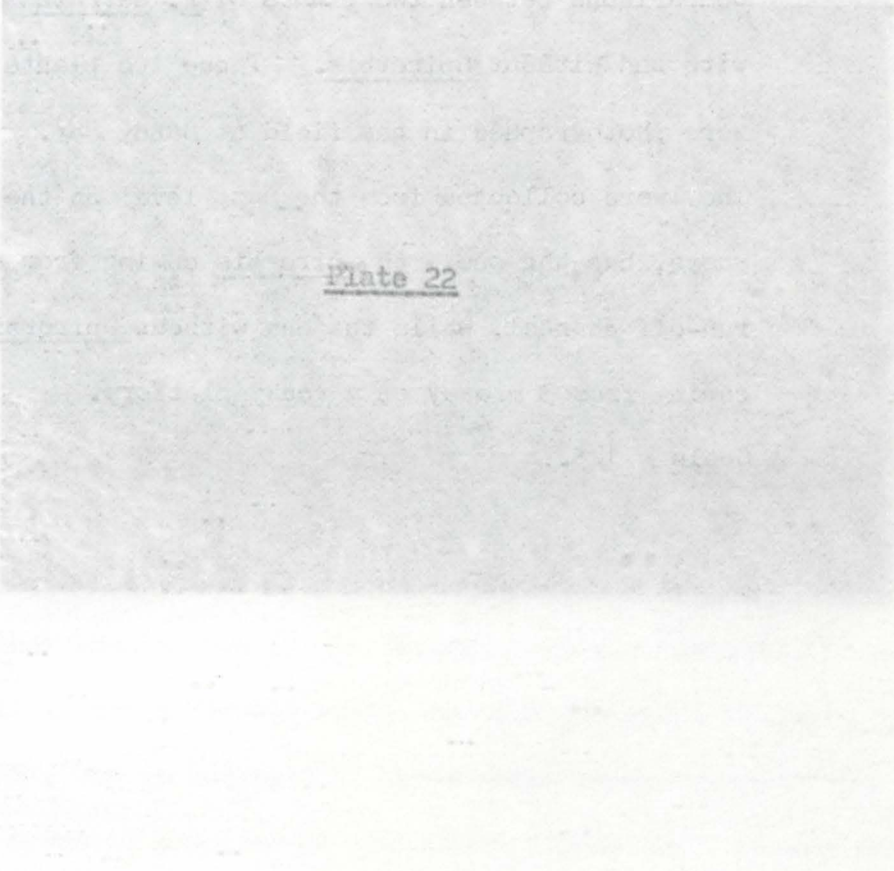


Plate 22

Plate 22

Comparisons between two plants of *F. serratus* with and without *Spirorbis*. These two plants were photographed in the field at Sandy Bay. They were collected from the same level on the shore, but the one with *Spirorbis* coming from a run-off channel, while the one without *Spirorbis* coming from 3 m away on a rocky platform. Scale X 1/5.

When a plant of F. serratus with abundant Spirorbis was presented to blennies that had not been fed for three days, the fish searched the plant but did not eat any of the Spirorbis, although occasional attempts were made to pluck the cemented tubes from the plants. When snails were placed on this same plant and offered to the blennies, they ate most of them without hesitation. Similar patterns of behaviour have been seen in the field during diving observations. But examination of stomachs from field-caught individuals demonstrates that some predation does occur on polychaete tubes. Quasim (1957), in his study on the food of Blennius pholis has found up to 28% of the individuals have some evidence of chaetae, tubes or opercular lids in their stomachs. These were predominantly from serpulid rather than spirorbid worms. By comparison, 37% of the blennies had eaten gastropods. Quasim did not measure the relative abundance of each food type within stomachs; it was not possible, therefore, to establish the amount of predation on the different foods. From my own observations on the blenny, it would seem that Spirorbis is eaten less often than snails, and there would then be an advantage to the snail which resembled it.

Closer examination of the juvenile citrina demonstrated that the shell is not uniformly white, as with juveniles of Lacuna pallidula (plate 25), but is composed of three white bands between which there are two areas of translucent grey shell (plate 27). During the earliest stages of growth the upper border of the outer whorl, which is translucent and thus grey in appearance. The lower edge of the white band is bordered by another area of translucent shell which is itself bordered by a second white band. The lower part of the whorl, which is not visible unless the shell is removed from the surface of the plant, is composed of a much broader area of translucent shell than is present on the visible upper parts of the whorl. There is a third, very narrow, white band at the lower edge of the translucent area. This band is not as intensely white as the upper two bands. When the shell is placed on a frond, the translucent areas appear dark because of the brown colouration of the underlying plant and of the dark hepatopancreas. No such darkening is evident on the white bands because of their opaqueness. The overall impression gained from viewing these white-banded shells on the plant is of a white-spiral which is remarkably similar to the tube of *Spirorbis*. A series of photographs (plates 23-28) showing a number of different shells among *Spirorbis* tubes, attempts to demonstrate the apparent significance of the white-banded shells.

There are a number of further observations which support the suggestion of mimetic resemblance. One of these is the similarity in size between the tubes of *Spirorbis* and the white-banded shells. Measurements of the diameter of 100 tubes at Porth Swtan gave a mean size of 2.6mm with a maximum of 3.5mm. This maximum size of the tubes corresponds to the size at which the white-banded shells begin to develop the yellow pigmentation in the shell, that is at 3-4mm. As snails grow beyond the size of the largest *Spirorbis*, the likelihood of confusion of the two is probably reduced and the advantage to the white-banded shells lost.

There is a similarity in distribution on the shore between *Spirorbis* and white-banded shells. *Spirorbis* is usually restricted to *F. serratus* and is not found on the algal species higher up the shore. The white-banded shells occur only in *L. mariae* which is itself usually restricted to *F. serratus*; *L. obtusata*, which is

Plates 23 - 28

Examples of different colour phenotypes in juvenile snails sitting among Spirorbis tubes on F. serratus. All the following photographs have been taken in the field under sunlight with illumination from above the fronds. Since Spirorbis tubes are usually found near the base of the leaf, which is relatively thicker than the distal parts of the leaf, very little light is transmitted through this area when the source of illumination is from behind the frond. For standardization of the background colour, an opaque white card has been used. All snails were collected from Porth Swtan, Anglesey, except for one on Plate 24 which came from Menai Bridge.

Scale 2½ times natural size.

Plate 23

Dark reticulata of L. mariae

Plate 24

Yellow citrina of L. mariae from Menai Bridge. Citrina juveniles in this locality lack white spiral shell. Compare with Plate 27.

Plate 25

Pure white shell of juvenile Lacuna pallidula showing more crypsis than yellow shells (Plate 25) although less crypsis than white-banded shells (Plates 27, 28, & 29).

Plate 26

White-banded shell of L. mariae (top left); opaline shells of L. obtusata (top centre and top right; bottom (black arrow) dark reticulata shell of L. mariae.

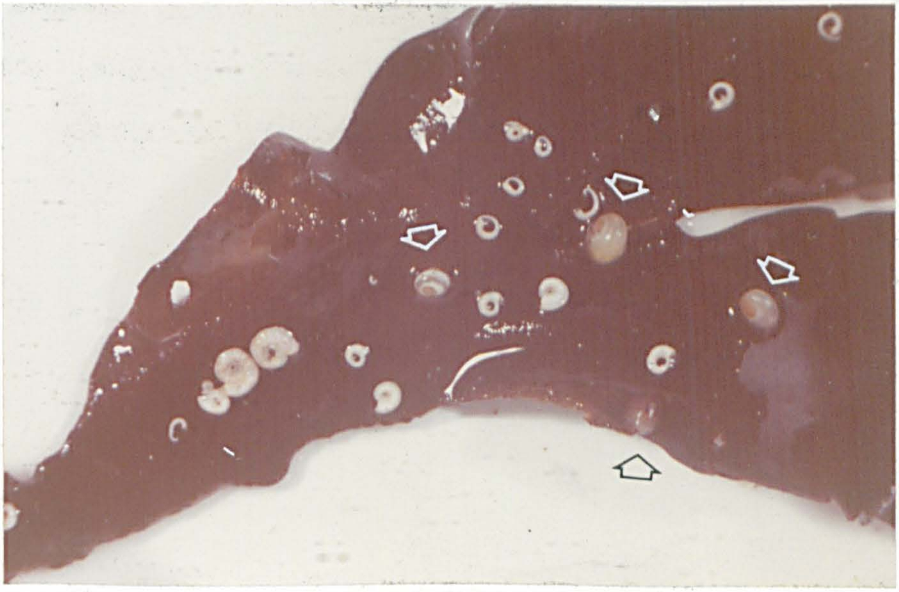


Plate 27

White-banded shell of L. mariae (left) and opaline shell of L. obtusata (right). Note, in L. mariae how the dark colour of the F. serratus can be seen through the translucent area between the opaque white bands.

Plate 28

White-banded shell of L. mariae amid Spirorbis.

found on algal species higher up the shore, shows no development of the white-banded shells (plates 26 and 27).

At Porth Swtan, Spirorbis is most abundant on the stem and leaf-base of F. serratus, and is only rarely found on the leaf proper. As has been discussed in the previous section, 50% of the juvenile L. mariae are also found near the base of the plant while the adults usually occur on the leaf tip. It would seem, therefore, that even within plants, there is a similarity in distribution between Spirorbis and white-banded shells.

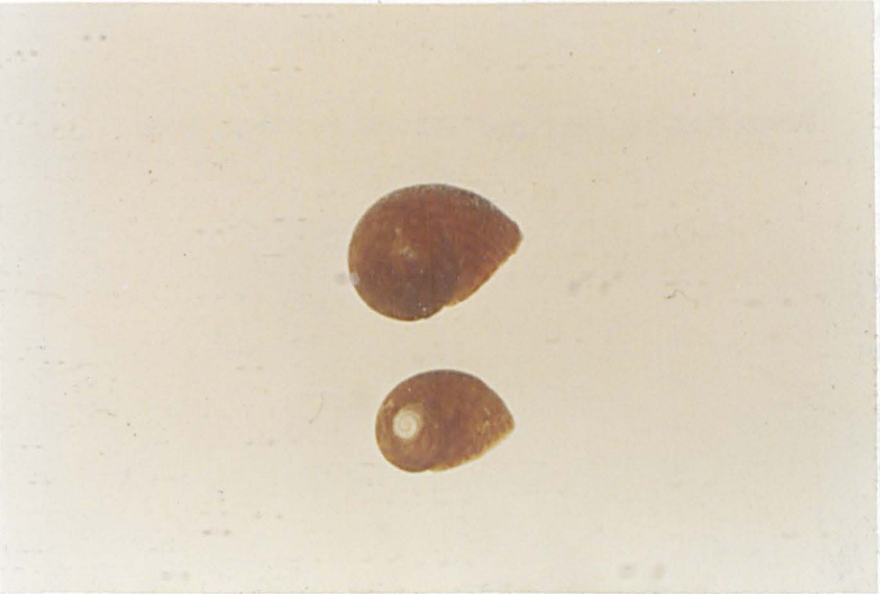
For comparisons with these observations made at Porth Swtan, a number of other localities in Anglesey were visited. One factor which was noted at these localities but not at Porth Swtan was that Spirorbis was not uniformly distributed on all the plants in the F. serratus zone. Plants which occur on rocky platforms have only occasional individuals of Spirorbis present, and in some cases lack them entirely. In these situations, the white-banded shells were found only in juvenile citrina and not in the juvenile dark reticulata. However, in run-off channels or in those areas underlain by pebbles, sand, or silt, Spirorbis is very common (up to 10per sq. cm) and in these areas the white-banded shell is seen also in juveniles of dark reticulata. In adult shells this can be seen as a white-spiral tip (plate 29).

Changes in the abundance of Spirorbis were seen in a series of collections made at Penrhyn from an exposed and rocky point to a sheltered and silty bay. On the point, Spirorbis were very uncommon but increased in abundance in the bay where they often completely covered most of each plant. The white-banded shells were present in all the citrina in both areas, but in dark reticulata, they occurred in only 1 out of 25 shells (4%) in the exposed shore where

Plate 29

Plate 29

Variation in the apex colour of adult dark reticulata in L. mariae. Both snails collected from Penrhyn-mawr.



Spirorbis was uncommon and in 19 out of 22 shells (86.4%) in the bay where Spirorbis was very common (fig 64). Similar correlations were also found at Rhosneigr (fig 66) and Hen Borth (fig 68). If it is true that the white-banded shells are indeed mimics, then the occurrence of this form in the normally very cryptic dark reticulata (plate 27) implies that the advantage of mimicry is greater than that of crypsis when the model, that is Spirorbis, is very abundant.

Another factor which supports the mimetic hypothesis is that one does not find white-banded shells when Spirorbis is absent from the locality, such as at the very exposed conditions at North Stack; here the juveniles are either a pale yellow or one of the various shades of reticulata. However, the converse of this is not true, that is, one occasionally finds Spirorbis moderately abundant in some localities, as for example in Menai Bridge and yet the juvenile citrina are pale yellow rather than white-banded. These exceptions suggest that some other factor is operating in these localities. However, it will be recalled from figure 58 in Section VIII that in very sheltered areas, as in the Menai Straits, greater than 90% of the juveniles are found on or near the leaf tip. In these positions Spirorbis is seldom found, being restricted more to the base of the plant. The advantage, therefore of retaining the yellow pigmentation in the juveniles is evident since on the leaf this phenotype is very cryptic as was shown in plates 16 and 17 (Section VIII).

Some of the differences between populations can be seen in diagrams which show the relationship between shell size and colour (fig 60). These localities were chosen to show the major variation in developmental pathways of L. mariae. At Penrhyn-mawr, it was possible to divide L. mariae into "dwarf" and "normal" forms according to sculpturing and shell size. It can be seen that there are major differences in the development of the polymorphism. In the "dwarf" form shells up to a size of 0.8mm are opaline (translucent shell overlying a pale body) after which all individuals develop the white-banded shells. This phenotype persists until a shell size of about 3.5mm, when the outer lip begins to change colour. On some of the shells the entire lip, rather than three bands, becomes an opaque white. The expression of this colour continues through to the adult stage. More commonly there is the expression of a yellow pigment on the lip, which persists to give the common citrina. About half of the shells develop an orange colouration on the lip; a few of these remain orange, but almost all develop the reticulata pattern giving the phenotype light reticulata. One other pathway that the white-banded shells take is the development of the dark reticulata.

In the "normal"form of L. mariae at this locality the major difference from the "dwarf" form occurs in the time of development of both light and dark reticulata. These two phenotypes do not go through the white-banded phase, as in the "dwarf" form, but develop independently from the opaline phase. The major phenotypes (citrina and reticulata) can therefore be distinguished at a very early stage. This pattern is the same as was initially described at Porth Swtan.

In the sample from Menai Bridge, the juveniles completely lack the white-banded phenotypes. From the opaline phase at hatching, the shells gradually become pale yellow and in almost all individuals a more intense yellow develops by a size of 3mm,which persists until adult size is reached, resulting in the typical citrina. Some of the pale yellow juveniles become orange, and in some cases a reticulata pattern is developed on an orange or yellow ground colour.

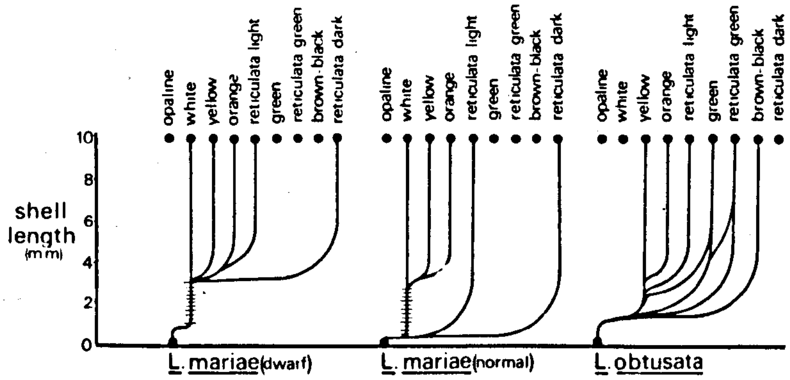
Figure 60

Figure 60

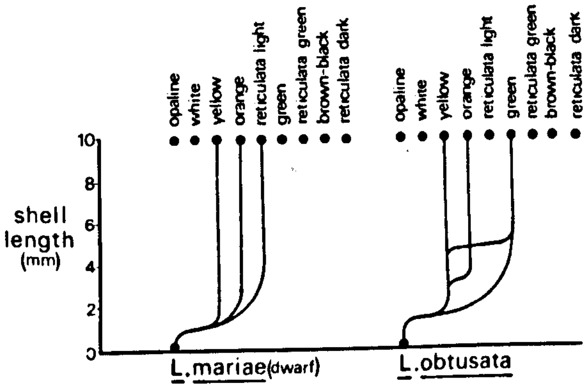
Developmental variation in shell colour within and between localities.

a.	Penrhyn mawr	Note the occurrence of the white-banded shell (hatched) in both "dwarf" and "normal" mariae but not in obtusata. One difference between "dwarf" and "normal" shells are in the development of light and dark reticulata; in the "dwarf" these phenotypes develop after the white-banded phase, while in the "normal" development occurs directly from the opaline phase.
b.	Menai Bridge	Note the absence of the white-banded phase in mariae.
c.	Severn Bridge	Note the relative short duration of the opaline phase compared to the other localities. No L. mariae were found in this locality.

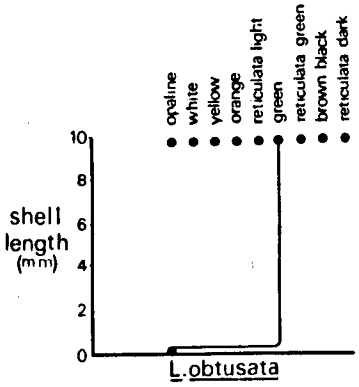
Penrhyn-mawr



Menai Bridge



Severn Bridge



In each of the localities that have been discussed there is a great deal of variation in the developmental pathways of each of the phenotypes. The diagram that has been presented to show the relationship of shell size to colour is a simplification, as it shows only the most common situation on each shore. It is possible that when these and other localities are examined in more detail a continuum in all the developmental pathways will be found. It is apparent, from the observations in this study, that there is developmental variability not only in the size at which phenotypic discontinuities occur but also in the pathways by which the different phenotypes develop. In L. *mariae* many of these differences appear to have ecological meanings in view of the microdistribution of juveniles and adults and their relationship to the presence or absence of the white tubes of *Spirorbis*.

To test some of these observations some simple predation experiments were carried out for determining whether the white-banded shells of juvenile citrina and juvenile dark reticulata were at an advantage compared to yellow shells of similar size on fronds where *Spirorbis* was either present or absent. Four blennies, of between 60 and 70mm in length, were collected from Porth Swtan and placed in separate glass tanks with circulating seawater at 12-15°C. White-banded and dark reticulata juveniles were obtained from this locality while yellow shells of equivalent size were obtained from Menai Bridge. The tanks were illuminated from above with a double filament fluorescent light. The procedure in the experiment was to put 3 snails on a frond, place it at the bottom of the tank and record the first snail taken. Two kinds of frond were used, one entirely lacking *Spirorbis* and the other one having densities of about 4 tubes per square cm (i.e. plate 25). Four tests per blenny were carried out each day over a period of 24 days.

The results of these experiments are given in Table 34. They show that the yellow shell is at a disadvantage to both white-banded and dark reticulata shells when Spirorbis is either present or absent. As there is no apparent heterogeneity in the results from each fish, the data have been pooled to provide a comparison between the fronds with and without Spirorbis. It can be seen that there are significant differences between the two ($P < 0.01$). This suggests that although the white-banded shells are at an advantage on both fronds compared to the yellow shells, this advantage is accentuated when Spirorbis is present.

Table 34: Results of predation experiments comparing white-banded, yellow, and dark reticulata shells on F. serratus with and without Spirorbis. Data shows first animal taken in each test.

Tank No.	frond with Spirorbis			frond lacking Spirorbis		
	white banded	yellow citrina	dark reticulata	white banded	yellow citrina	dark reticulata
1	2	15	7	6	13	5
2	4	13	7	7	10	7
3	1	21	2	3	16	5
4	2	20	2	6	14	4
Totals	9	69	18	22	53	21

$\chi^2_{(2)}$ between totals = 7.43 P = 0.01-0.025

It is interesting to note that there were relatively few white-banded shells taken even when Spirorbis was absent from the plants. The shells appear to be as highly conspicuous as citrina and yet are taken with about the same frequency as the highly cryptic dark reticulata. However the blennies in this experiment were collected from a locality where most plants had a moderate number of Spirorbis present. So it is possible that they are still confusing a single white-banded shell on a frond with a Spirorbis tube. It would be necessary to repeat these experiments with blennies collected from a Spirorbis - free habitat or with those raised in the laboratory.

There are many other experiments necessary to verify this suggestion of a mimetic resemblance between white-banded shells and the tubes of Spirorbis. One of these would involve comparing translucent or opaline shells with those having the white bands present. However, considering the predation experiments that were done and the remarkable similarity of the field distribution, it is reasonable to suspect that the white-banded shells are capable of gaining some advantage by their resemblance to Spirorbis. If this is the case, then the delayed development of the yellow pigment in citrina and a replacement with white bands is advantageous owing to their mimetic resemblance to Spirorbis; in the same way, the expression of the yellow pigment in larger shells is advantageous owing to the cryptic nature of this phenotype on the leaf. The juveniles of dark reticulata are also very cryptic on or near the base of the plant. The occasional occurrence of the white-banded shells in this phenotype suggests that the resemblance to Spirorbis provides a greater advantage than resemblance to the stem when Spirorbis is very abundant.

3. L. obtusata

This species does not show as much phenotypic discontinuity between the different colours as L. mariae, either in juvenile or in adult shells. This, together with the tendency to change colour during growth, makes scoring of juveniles into discrete phenotypes very difficult in many localities.

At Porth Swtan and Penrhyn-mawr the newly hatched shells are opaline in colour (plates 26 and 27,&fig 60). The shells themselves are translucent but they enclose a pale body with a slightly pigmented hepatopancreas. The opaline shells persist until about 2mm at which size the lip begins to lay down one of a number of colours. One of these is yellow, which eventually produces the common citrina. However, some of these yellow shells at 3-4mm change to orange, occasionally with reticulations, while others change from yellow to green. In each case this secondary colour persists until adult size is reached. The reticulations on the orange produces the light reticulata phenotype while the green colouration gives the common olivacea phenotype. The olivacea phenotype most commonly develops from the opaline phase. On this pathway, the green shells up to 6mm may develop a reticulate pattern. Another pathway from the opaline phase leads to a brown-black shell. In all of the pathways at these two localities there is a great deal of variation in size at which the colours express themselves. At Menai Bridge, there are fewwphenotypes, although the broad development of each is similar to those at Penrhyn-mawr. One minor difference is that the green shells (olivacea) can develop later from the yellow shells, up to size of 5mm. This results in the adult olivacea appearing much lighter in colour than the equivalent phenotype from Penrhyn-mawr. Two generalizations are possible at these localities which have been discussed: (1) there is little change in colour on any of the shells past 6-7mm and (2) up to about 5mm there is a continuum between all colours.

L. obtusata differs from *L. mariae* in lacking the white-banded shells in the juvenile stages and in having continuous variation in colour on the juvenile shells. These differences are to some extent correlated with differences in the habitat of the two species. *L. mariae* is restricted to *Fucus serratus*; this species of alga consists of a discrete stem often with *Spirorbis*, and a broad leaf (plate 14). There are two major background colours which correspond to the two most common shell colours. One of these is the dark-brown stem where the dark reticulata are very cryptic, while the other is the bright yellow coloured leaf when illuminated from behind where the citrina is very cryptic. On exposed shores *L. obtusata* is found mainly on *F. vesiculosus*, which usually lacks *Spirorbis*; the broad pattern of morphology in this species is similar to *F. serratus*. There is a dark brown stem and a light coloured leaf. However, owing to the narrowness of the leaf and the presence of paired vesicles, there is only a small area where transmitted light would provide citrina with a cryptic advantage. There is also a large amount of intermediate area between leaf and stem which is not present in *F. serratus*. This often composes 50% of the entire length of the plant. The colour is dark-brown when viewed from above, but a dark yellow-green when viewed with transmitted light. Another character of *F. vesiculosus* is that when covered with water the plant stands vertically owing to the airbladders; *F. serratus* on the other hand lies relatively flat on the bottom. This affects the illumination and thus the background colour on which the snails are found. All of these characters in *F. vesiculosus* contribute to what appears to be a continuous range in background colours on the plant.

Although no measurements of the distribution of juvenile *L. obtusata* have been made, they have been seen on all the different parts of the plant. It is possible that the continuous variation in shell colours, up to a size of 6mm, may be generated by selection by visual predators in the context of a continuum of background colours. All the shell colours that have been seen have an equivalent colour at least somewhere on the plant. What would be of importance to know, is why there is a development of discontinuity in shell colours on snails greater than 6mm in size. The understanding of this may come from microdistributional studies which in *L. mariae* have shown the discreteness in shell colour to be related to a discreteness in the background colour. It may be that *L. obtusata* greater than 6mm assort themselves on specific parts of the plant which have discontinuous background colours. Unfortunately, no data are available on this subject.

In sheltered shores, as at Menai Bridge, *L. obtusata* is found predominantly on *Ascophyllum*. This species of alga does not have the stem-leaf morphology that is shown by *F. serratus* and *F. vesiculosus* but is rather composed of long narrow leathery fronds with large air bladders. Most of the plant is olive-green in colour, but younger fronds and fruiting bodies are often tinged with orange or yellow. This species also stands vertically in the water, but because of the opaqueness of the fronds, the manner of illumination does not appear to affect the background colours on the plant very much. In this species there is no development of the dark brown colour that is characteristic of the stem in *Fucus* spp. At Menai Bridge, the shells of both juvenile and adult *L. obtusata* are seldom dark-brown, a shell colour which was very common on shores with *F. vesiculosus*. The juveniles up to 6mm in size show a continuous range in colour, including opaline, yellow, orange and green, and are on average lighter in colour than the adults. When observing the distribution of these individuals on the *Ascophyllum*, they were not restricted to the light-coloured fronds or to the yellow or orange fruiting bodies but were found on all parts of the plant, just as were the adults. The most important factor is proportions of each on different positions; this, however, is not known.

The citrina and olivacea shells of *L. obtusata* at Menai Bridge are often very difficult to distinguish in both the juveniles and adult stages. This was not the case in more exposed localities such as Porth Swtan and North Stack where *L. obtusata* is found mainly on *F. vesiculosus* and to a lesser extent on *F. serratus*. In these two localities, citrina and olivacea were quite discrete in most shells greater than 5mm. In other words, when *L. obtusata* is found on *Fucus* spp. it is similar to *L. mariae* in having very clear differences between citrina and the darker shells. The differences in the degree of discreteness that have been found, depending upon an association with *Ascophyllum* or *Fucus*, may relate to the illumination of these plants. The only yellow colouration that is present on *Ascophyllum* is on younger fronds and fruiting bodies. These vary continuously in colour from the basic olive-green colouration on the rest of the plant. All the fronds are relatively thick and therefore opaque to light. In *Fucus* the occurrence of the leaf results in a relatively large and discrete area where citrina is highly cryptic when viewed with transmitted light. The variation in the degree of discontinuity between citrina and the dark coloured shells in *L. obtusata* in these situations, may be, as in L. mariae, owing to the amount of discontinuity in the background colour.

In the localities which have been discussed, as the newly hatched shells of L. obtusata (0.5mm) do not develop any pigmentation until a size of about 1.5-2.5mm. This seemed to imply that the individuals were incapable of producing the pigments until a certain shell size had been reached, perhaps owing to biochemical limitations. There is, however, one locality sampled (Severn Bridge) in which even the newly hatched juveniles were dark green in appearance (fig 60). This colour persisted through to the larger shell sizes. There was no pathway through the yellow shell stage which has been found in the other localities. At Severn Bridge the only species of alga seen was Ascophyllum. Unlike those at Menai Bridge, each plant was very dark in appearance, partly because of the sediment which settles on the plants. It may be, therefore, that even the opaline phase in the newly hatched shells or the absence of it at Severn Bridge, has some advantage.

Some relevant observations were made at Sandy Bay in Anglesey. In this locality, examination of the stomachs of blennies (caught in the Ascophyllum zone) showed that all the individuals of L. obtusata that had been eaten were less than 6mm in size. However when one tries to find the snails of this size on a frond of Ascophyllum, they are only about one third as common as the larger sizes. The reason for this became evident when it was noticed that many of the small individuals which had been previously overlooked were present within the large air bladders and could only be seen when the air bladder was broken open. In most of these air bladders there was a small hole of about 1 to 3mm in diameter through which the snails must have gained entry. Often, shell sizes of 4mm were found in the bladders which had an entry hole of only 1mm. This implies that the snails must have crawled into the bladder when they were about 1mm in size and spent all the time feeding inside the bladder, protected not only from dessication and wave action, but also from predators.

An estimate was obtained of the proportion of individuals on the plant found in the air bladders. The shell sizes of all the individuals that could be found on the outside of the frond and in the bladders were measured. This was done for 5 Ascophyllum plants which were widely separated over the shore. The results of this comparison (table 35) show that 62% of the 0.5-5mm class are found within the air bladders. This is in comparison to 11% and 0% for the 5-10mm class and 10-15 class respectively. No information is available on other localities for comparison with these observations.

Table 35: Numbers of individuals of L. obtusata found on frond and within air bladders of Ascophyllum at Sandy Bay, Anglesey (see appendix E, fig E for distribution of shell sizes in population).

Shell Size	Position	
	frond	air bladder
0.5-5mm	45	75
5-10mm	94	12
10-15mm	133	0

This behaviour of the juvenile snails introduces another variable when attempting to understand the reasons for the absence of pigmentation in shells up to 2.5mm in size. It is not known whether the distribution observed is due to a behavioural preference of the juvenile snails or to the elimination by predators of many of the individuals on the outside of the frond. If it is due to a behavioural preference, then it is reasonable to suggest that there has been selection for a delay in the time at which the colours develop until the individuals are exposed to a variable background colour. One could also argue that there has been no selection for an expression of colour until the individuals leave the air bladders and crawl to the surface of the plant.

It is apparent that many factors must be considered when interpreting the development and variation of shell colour in L. obtusata The small number of observations that have been made to account for continuous and discontinuous colour phenotypes in this species are based on a very incomplete understanding of the complex life history in juveniles and adults. Between each population of L. obtusata there is not only variation in the overall phenotype frequencies, but also in the amount of discreteness between the phenotypes, the time at which they develop, and the pathway which is followed in development. Some of these differences are correlated with changes in the background colour over what appear, at first sight, to be similar ecological conditions. For the differences which are not at present understood, it is possible to suggest the action of hidden advantages or of random fluctuation permitted by very weak selection. These are however, of little value at the present owing to the superficial understanding of the life history in this species.

SECTION X

EVIDENCE FOR THE POSSIBLE OCCURRENCE OF TWO SIBLING GROUPS WITHIN L. MARIAE

1. Introduction

In the final stage of this study, the suspicion began to arise that the two sibling species L. obtusata and L. mariae, initially proposed by Sacchi and Rastelli (1966), may be actually three sibling species. The amount of morphological and colour variation within one of the siblings, L. mariae, greatly exceeded that found in the other sibling L. obtusata. It had been noted at many localities that the exposed part of the shore gave samples of L. mariae with large adult shell sizes (12mm) and high frequencies of dark reticulata while the sheltered areas gave L. mariae with smaller adult shell sizes (9mm) and high frequencies of citrina. Since this change followed the same pattern that was present between localities which were exposed and sheltered, it was felt that this was an example of precise local adaptation within shores. There seemed however to be a number of additional characters which also changed over very short distances. To determine whether this character variation was of taxonomic importance, further studies were carried out at a few localities in Anglesey.

2. Results

(i) Sandy Bay

One of the first indications that led to the suggestion of a taxonomic subdivision was the examination of adult shell sizes from the 8 collections made at Sandy Bay between November 1971 and August 1973 (appendix B - table ii). It is apparent at this locality that there is considerable variation in size of adult shells in collections made at the same position on the shore. When for each of these samples size is compared to colour phenotype frequencies (appendix E table Ev) there is a positive correlation between the samples with the small adult size and high frequencies of citrina. When the adult males of citrina and dark reticulata are separately plotted for shell size it is apparent that most of the citrina are smaller than the dark reticulata (fig 61). In some of the larger samples there is in fact a bimodality in adult size. The examination of the females (fig 62), shows again a similar trend as in the males, although in this case with greater overlap.

When the extremes of the small-shelled and large-shelled adults are compared, there are a number of additional characters other than size and colour which differ. One of these is in the sculpturing of the shell, a character which was initially discussed (p.35) in reference to the differences between L. obtusata and L. mariae. In the small-shelled adults and the majority of juvenile citrina L. mariae, there is a repeated sequence in ridge sizes across the outer whorl. A high ridge is generally followed by 2-4 low ridges, most commonly 3, all equal distance apart from each other. In the large-shelled adults and almost all the juvenile dark reticulata, the difference between the high and low ridges is much less pronounced and with a highly variable sequence of low-high ridges. Another difference is that in the small-shelled adults (mainly citrina), the longitudinal ridges are continuous along the shell, while in the large-shelled adults they are broken each 0.3-1.5mm leaving slight gaps along each ridge, not in corresponding positions across the shell.

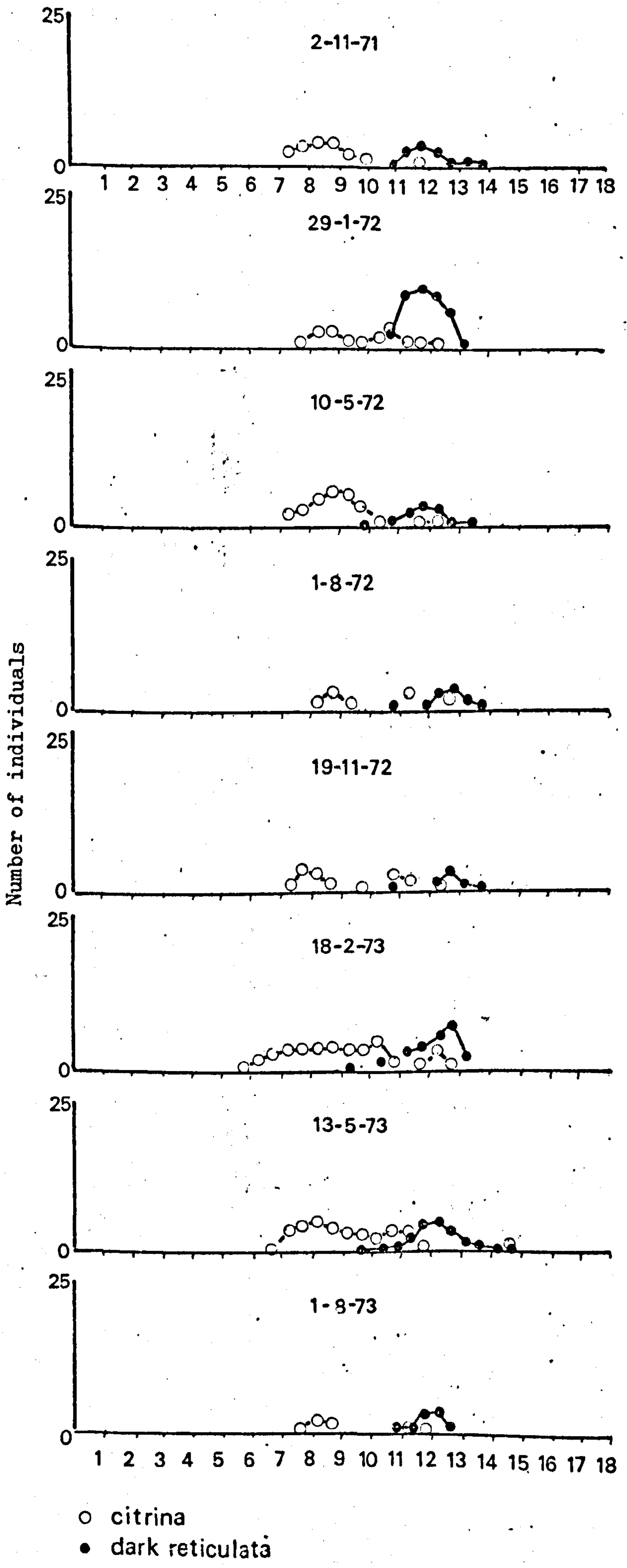
There are differences in the anatomy of the penis, for in the small-shelled adults the relative length of the distal tubule (see fig 6) is less than in the large-shelled adults although only slightly.

Figures 61 & 62

Distribution of adult shell sizes in citrina and dark reticulata at Sandy Bay, Anglesey. Corresponding phenotype frequencies given in Appendix E table E (v).

Figure 61

Male size distribution of adult shells.


Number of individuals
25
0
2-11-71
29-1-72
10-5-72
1-8-72
19-11-72
18-2-73
13-5-73
1-8-73
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
○ citrina
● dark reticulata

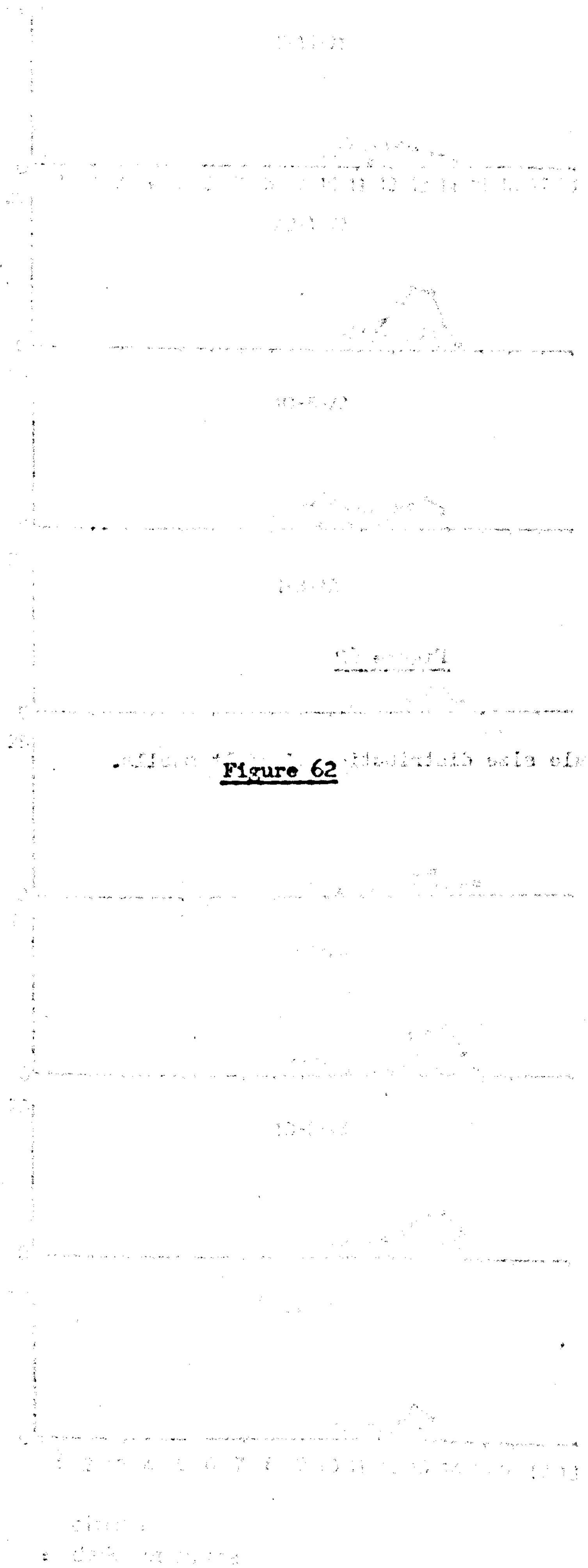
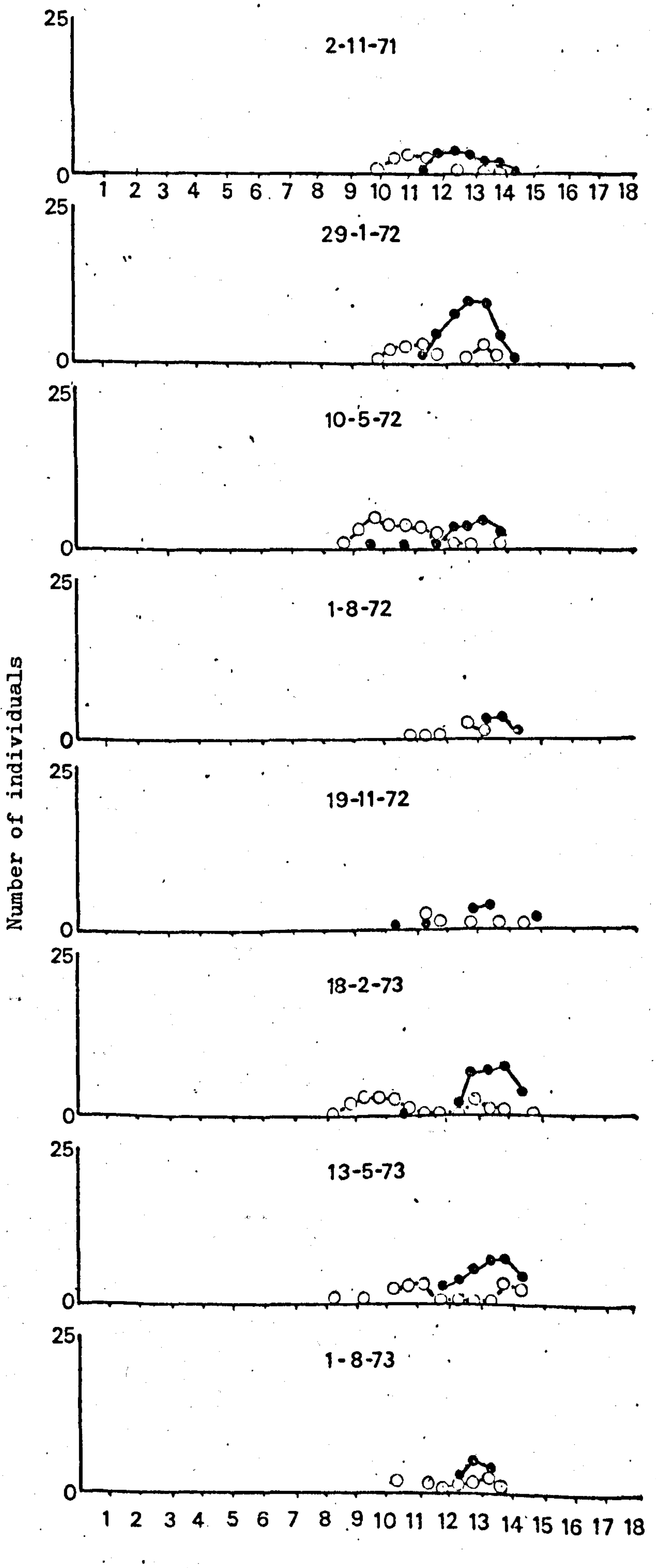


Figure 62

Figure 62

Female size distribution of adult shells.



○ citrina
● dark reticulata

This appears to be a function of adult size since individuals intermediate in size between small and large-shelled individuals have an intermediate length of the distal tubule.

Differences are present, not only in phenotype frequencies but also in minor variations in shell colour. The small-shelled adult citrina are often pale yellow, almost white, while in the large-shelled adults, the citrina are usually lemon-yellow. The dark reticulata, although very uncommon in the small form, often have a white-banded apex (see plate 29). The apex in larger form only occasionally has this character, normally being dark-brown as the rest of the shell. There are subtle differences in the ground colour of the light reticulata phenotype. In the small-shelled adults, the colour is generally a fawn-green while in the larger form it is usually orange. There is a corresponding difference in the lip colour, which in the small form is lightly pink and in the large form, white or violet.

The small- and large-shelled individuals are very predictable in where they are found on the shore. In all the runoff channels where a great deal of sediment is present, collections yield mainly small-shelled adults with high frequencies of citrina. On the rock platforms 3 meters away, the collections are composed mainly of large-shelled adults with high frequencies of dark reticulata. The <u>Fucus</u> <u>serratus</u> in each of these positions are very different in morphology. Those from the runoff channels are characterized by a short, thin stem and long broad fronds. Those from the rock platform have larger and thicker stems and shorter fronds. This is the same pattern observable between sheltered and exposed shores but in this case within a shore.

In all the differences that have been cited between the small-shelled and large-shelled adults, there is considerable overlap. However, within the samples shown in figs 61 and 62, in the sculpturing are very discrete when the smallest adult citrina and the largest dark reticulata are compared. On adults which are intermediate in size, there is intermediacy in the sculpturing and penis.

A series of collections were made on a horizontal transect at Sandy Bay to provide some indication of the amount of discreteness or intermediacy of the different characters which have been described. The transect was chosen so as to pass from rock platforms to low lying run-off channels with a sand and small pebble substrate (fig 63). The individuals were scored for shell size, sculpturing, colour phenotype, presence or absence of white tip to dark reticulata and ground colour of light reticulata. The results from each sample are presented in two ways: (1) a scatter diagram showing adult male shell sizes for each colour phenotype and (2) pie diagrams showing the phenotype frequencies of small-shelled (less than 10mm) and large-shelled forms (greater than 10mm). Some individuals had a shell size of slightly less than 10, nevertheless had all other characters resembling the larger-shelled individuals; these were accordingly placed in this group. In the same way, individuals slightly greater than 10mm, but with characters more like the small-shelled form (such as sculpturing) were placed in the smaller-shelled groups. The two pie diagrams, therefore, reflect principally the phenotype frequencies of smaller and larger shells. For convenience in discussion these will henceforth be referred to as "dwarf" and "normal" forms.

The results from these collections are given in fig 63. What is initially evident is the absence of dark reticulata in the dwarf form except for 2 individuals from site No. 8. These two individuals were included in the dwarf because of their similarity in sculpturing and penis morphology. From the scatter diagrams it can be seen that in each sample, citrina is on average smaller than light reticulata which is on average smaller than dark reticulata. The mean size of each of the phenotypes are 7.5mm, 9.0mm and 12.1mm respectively. Although the majority of small individuals are citrina, the large individuals can be either dark reticulata or citrina. Light reticulata cover the range from small to large but are on average intermediate in size. When all the samples are considered together, citrina shows a bimodality in shell size, one mode occurring at 7mm and the other at 12mm. Light and dark reticulata are unimodal at 9 and 12mm respectively. In all of the citrina except for those above 10mm, the sculpturing was characteristic of the dwarf while those shells above 10mm had the normal sculpture. The occasional dark reticulata less than 10mm were intermediate in sculpturing while those greater than 10mm were normal. Most of the light reticulata regardless of size had intermediate sculpturing. However, the two smallest individuals of this phenotype were most like the dwarf.

The white tip to the dark reticulata shell is not common in this locality and occurs in only 3 of 141 shells. All of these with the white tip had intermediate sculpturing. In size, these individuals were on average larger than the most of the citrina but were smaller than most of the dark reticulata. In these situations it is not clear as to whether the individuals are dwarf or normal.

The dwarf is most common in the samples from the low lying silty area. Comparing samples No. 5, 6 and 7, it can be seen that in No. 5 where a rock substrate is present, most of the shells in the sample are the normal dark reticulata. In No. 6, an intermediate area, the range in size of the dark reticulata increases and the dwarf citrina become more common. This is more expressed in No. 7 where the majority of individuals are now the dwarf. In this sample, individuals from 8-11mm are intermediate in sculpturing between the dwarf and normal. They were included in the normal because of the relatively long distal tubule of the penis. The F. serratus plants from No. 5 have a narrow leaf with a relatively large thick stem and only occasionally Spirorbis. Those from No. 7 had a broad leaf and small stem which was often densely covered with Spirorbis. The site in between (No. 6) had intermediate characters. These same trends apply to all the other sites at this locality. For example, from sites Nos. 2-4, or from Nos. 8-9.

The very predictive nature of many of these character changes between the dwarf and normal, depending on the habitat sampled, and the occurrence of multiple-character intermediates in the intermediate habitats suggests a number of possible explanations; these range from very local adaptation within a species to sibling species with no hybridization but convergence. In an attempt to provide some insight into these possibilities, a number of other localities in Anglesey were extensively sampled in a similar way to Sandy Bay.

(ii) Penrhyn

Just south of Sandy Bay, a series of collections were made connecting a rocky exposed headland to a muddy sheltered bay. Previous collections had shown that the exposed locality had the characteristic large-shelled individuals with a high frequency of dark reticulata while the most sheltered part of the bay had very small shelled individuals with predominantly light reticulata. The purpose of the additional collections was to determine if the dwarf and normal would grade into each other or change in the proportion of each. The results are presented in fig 64 which shows the most exposed site (No. 1) on the upper northernmost position of the figure, and the most sheltered on the lower right. The most exposed site has individuals typical of the normal 11-14mm in size, high frequencies of dark reticulata, irregular sculpturing, and relatively long distal tubule. The smallest dark reticulata however, has a white tip to the shell, similar to the dwarf but has an intermediate penis and intermediate sculpturing. Of the 5 citrina present, 2 were outside the size range of 96% of the range of dark reticulata. These however had sculpturing like the normal. The next more southernly collection (No. 2) was made in a runoff channel on a shore which was otherwise very exposed. In this sample all the adult individuals were either citrina or light reticulata with a mean size of 7.6 and 8.0mm respectively. The sculpturing of all these individuals were characteristically of the dwarf. There were some dark reticulata in this sample but they were all thin-lipped, each having the sculpturing of the normal.

In the next southern sample (No. 3) which also came from a runoff channel, there is little change from the previous sample except for the presence of some large-shelled light reticulata and one dark reticulata with a white tip each having intermediate sculpturing. The citrina and smaller shelled light reticulata were dwarf in all characters. In the remaining samples that were taken into the bay, small-shelled individuals predominate but with high frequencies of the dark reticulata. These dark reticulata were intermediate in sculpturing while the citrina of the same size had the common dwarf sculpture. Once again however, the largest dark reticulata approximated to the sculpture in the normal form. With all the characters that were used to separate the dwarf and normal there is a gradation from one to the other in each sample from Penrhyn, even within a single colour phenotype. One fact that does emerge from the examination of these samples is that citrina of less than 8mm were always dwarf in all characters. The light and dark reticulata less than 8mm were occasionally dwarf in sculpturing but more often than not intermediate. Most of these have been included in the dwarf because they were on average more similar in size to this form than the normal.

One factor of interest is the occurrence of the white tip to the reticulata shells. It increases from 2% on the headland to 94% in the bay. On the exposed shores it occurs only on the small shelled reticulata but on the sheltered shores it occurs on both small and large-shelled individuals, although more common in the former.

There is a close relationship of the dwarf and normal to the substrate, similar to that observed in Sandy Bay. On the headland (No. 1), when a rocky substrate was present, the normal form predominates. When sand and silt was present, as in the runoff channels (Nos. 2 and 3), the dwarf is most frequent. In the sheltered bay (Nos. 5 to 9), the majority of the substrate is sandy with occasional small rock platforms, as in No. 9. Each of these substrates had the different kinds of F. serratus described in Sandy Bay except for one difference. The plants in the bay were all covered with some sediment which gave them a much darker appearance than those of Sandy Bay, a situation which may account for the low frequencies of citrina (p.253).

(iii) Cliff Hotel (west of Treardour Bay), Rhosneigr, Cemaes Bay, Hen Borth.

The results from these localities are presented in figs 65 to 68. All of them show the same pattern that was evident at Sandy Bay and Penrhyn. Samples from the most exposed parts of the shores on a rock substrate are characterised by a high frequency of dark reticulata, large adult shell size, irregular sculpturing, relatively long distal tubule, and a low incidence of a white tip to the shells of reticulata. On sites from greater shelter or those with a sand substrate irrespective of exposure, the mean size of citrina decreases while dark reticulata remain about the same. The light reticulata are generally intermediate in size. The sculpturing of the smallest adults of citrina show the regular sequence of one high ridge followed by 3 low ridges. Larger individuals of citrina or the smaller ones of light and dark reticulata have an intermediate type of sculpture between the dwarf and normal. Those individuals of reticulata which are most like the dwarf in sculpturing generally have a white tip to the shell, as in Penrhyn. The amount of variation in all characters is greatest in the samples which were made on substrates that lay between rock platforms and expanses of sand, or in other words intermediate. Those collected from F. serratus on expanses of sand generally have characters of the dwarf in all the colour phenotypes. Even in these situations, however, the reticulata are on average larger in shell size than the citrina.

(iv) Other Localities

Further collections were made at Menai Bridge, Moelfre, Porth Swtan and North Stack all in Anglesey. The two sheltered localities, Menai Bridge and Moelfre, had only populations of L. mariae with all the characters of the dwarf. The exposed localities, Porth Swtan and North Stack, had only populations of L. mariae with all individuals having characters similar to the normal. All of these localities had some variation in the kinds of substrate present, although in general they were either very sandy and sheltered or very rocky and exposed. It is not surprising therefore that these localities did not show variation in shell size, colour frequencies and sculpturing between different positions on the shore.

The majority of samples from Wales, southern England, the Isles of Scilly, and Ireland that were collected from the geographical survey were re-examined to determine whether the individuals in a sample were mixtures of dwarf and normal forms. The soft parts of these individuals had been removed previously so that it was only possible to look at adult size, colour, and sculpturing. The main criterion used to determine whether mixtures were present was differences in the adult shell size of the different phenotypes.

It appears that in all but two localities the adult shell size was the same in all the phenotypes within a sample. There was also, except in the same two localities, no differences in the sculpturing between the different shell sizes or colour phenotypes.

The localities which did show differences were on the Isles of Scilly at Top Rock Point and Top Rock Bay (Nos. 59 and 60), two localities separated by about 20 meters. When these were re-examined, the citrina was on average smaller than dark reticulata and had sculpturing very similar to the dwarf seen in Anglesey. The larger shelled citrina and the dark reticulata had sculpturing very similar to the normal. Individuals of intermediate size as in Anglesey, were intermediate in sculpturing. The citrina in these samples were bimodal (7mm and 11mm) in length while the reticulata were unimodal (11mm) in length.

When an examination is made of the sculpturing in the remaining samples from the geographical survey, the sample from each locality is again apparently homogeneous, that is to say, all the individuals in a sample have about the same pattern of sculpturing. For example, in all the very sheltered localities from Wales, southern England, the Isles of Scilly, and Ireland (figs 41-51), L. mariae are very similar to the "dwarf" seen in Anglesey. The sculpturing is typically one large ridge followed by 3 small ridges in sequence. On all of the shores of intermediate exposure, which outnumber the very sheltered and very exposed, the sculpturing is intermediate. On the very exposed shores, throughout the study area, there is typically "normal" sculpturing, with the reduced height difference between low and high ridges and with irregular sequences.

3. Discussion

It is clear from the data presented that the variation in *L. mariae* greatly exceeds that which I found in my original samples in most of the localities in both *L. mariae* and *L. obtusata*. In view of these findings, it is necessary to consider whether *L. mariae* is composed of two taxonomic groups within some localities or whether the variation observed is very local adaptation within a single species.

In all the characters of the dwarf and normal forms, there is a gradation between the two. It is true that citrina in some cases shows a bimodality involving a large number of characters. More often however, there is a continuum with a single mode, the two ends of the continuum having characters of the dwarf and normal forms respectively. When one compares discrete substrates, such as silt and rock, the mode is shifted towards the dwarf in the former case. Mixtures of the two habitat yield intermediate forms. If the two forms are considered as taxonomic groups, it is necessary to invoke either a large amount of hybridization in the intermediate areas or convergence between the two forms.

Either of these situations could be possible. The variation may represent an extremely complex polymorphic situation, this would require a linkage group between colour phenotype and the following characters - habitat preference, mean adult shell size, shell sculpturing, penis morphology, colouration of the juvenile snails (white or black among dark reticulata), and the ability to express these character complexes in any number of intermediate states, in all combinations according to the intermediacy of the habitat. In view of the general studies that have been carried out on polymorphic species, this would be an exceptional but not impossible situation. In view of the sedentary nature of these winkles, it is also possible that the variation observed is due to precise local adaptation to microhabitat differences over very short distances.

Whatever the actual situation is, the variation in all characters is predictable in its occurrence, to some extent understandable in relation to differences in the habit. Thus the frequencies of citrina and dark reticulata would be predicted if crypsis were responsible in view of the variation in the morphology of F. serratus between areas of silt, rock, and intermediate substrates (Section VIII, 3 & 4). There is some evidence that an early development of a thickened lip which is observed in the dwarf is advantageous during predation by juvenile crabs, while the normal form are at an advantage with larger crabs (Section IV, p. 113). The presence or absence of the white apex on dark reticulata seems to track the abundance of a white tube-dwelling polychaete on the F. serratus (Section IX, 2). The significance of the variation in the sculpturing is not understood at the present time although further work may indicate the function of this character, and provide some understanding of its variation.

In summary, with the present data, it cannot be asserted whether the variation in L. mariae is a reflection of a taxonomic subdivision. It is clear that the variation is predictive and that of the characters which differ between adjacent sites on a shore, some understanding has been achieved on their adaptive significance. In general, it appears that the amount of variation within a population of L. mariae reflects principally the biotic and abiotic heterogeneity in the habitat. In view of the microdistributional differences that have been observed between citrina and reticulata, and the differences in the juvenile and adult positions within a shore (Section VIII) it will be important to understand whether these differences represent intraspecific or interspecific variation, but only further work will clarify this. The variation of L. mariae within shores was detectable only from intensive sampling of rock and silt substrates; it is possible that this variation, undetected in the geographical survey where collections were restricted to a small part of the shore (p. 20), may be actually widespread throughout the British Isles.

Figures 63 - 68

Variation in phenotype frequencies and adult shell size at a number of localities in Anglesey. In the following figures, the double circle diagram shows the phenotype frequencies of individuals with "dwarf" characters while the single circle diagram shows those with "normal" characters. Graph beside each sample shows adult size of all individuals in sample in relation to colour phenotype.

Key: Plain - citrina (c.); cross-hatched - light reticulata (r.l.); black- dark reticulata (r.d.); ● - reticulata with white apex; X - light reticulata with fawn-green colour; r - rock substrate; r.s. - rock-silt; s - silt substrate.

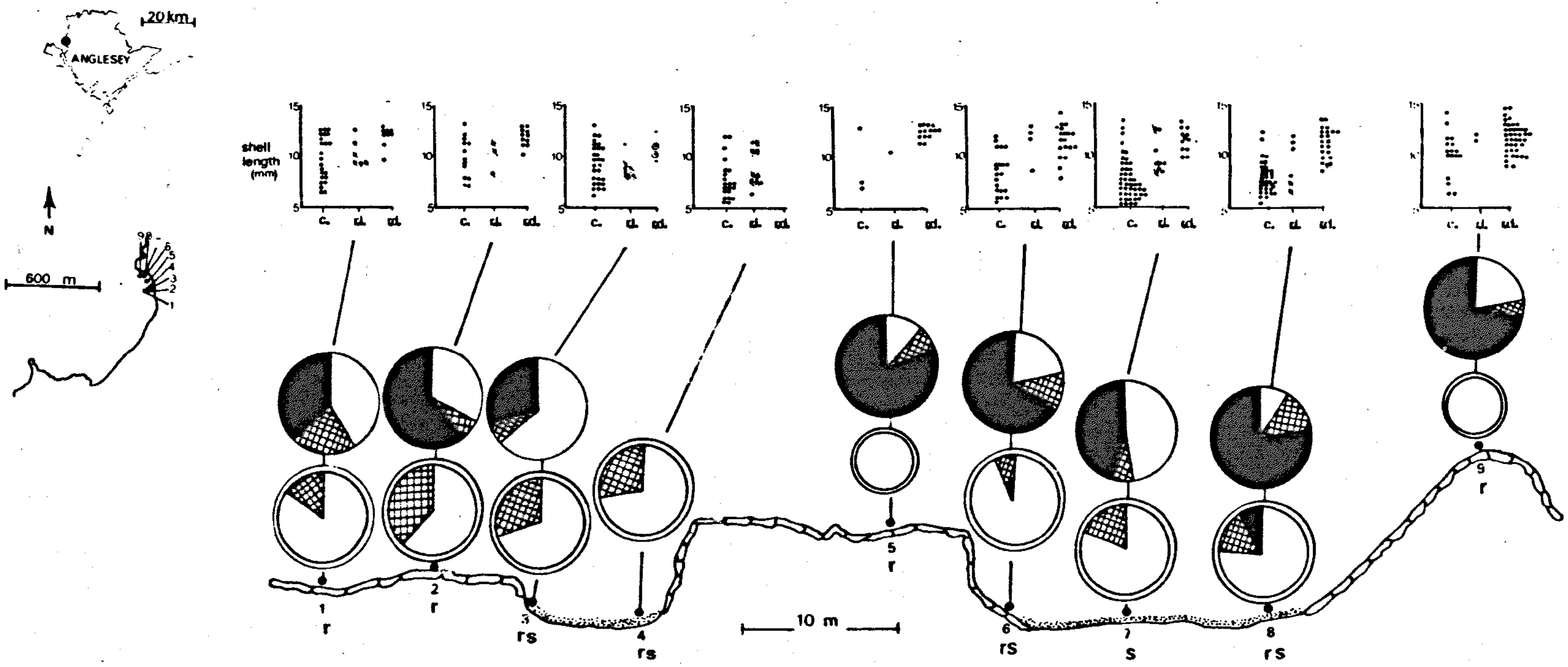


Fig. 63. Horizontal transect at Sandy Bay.

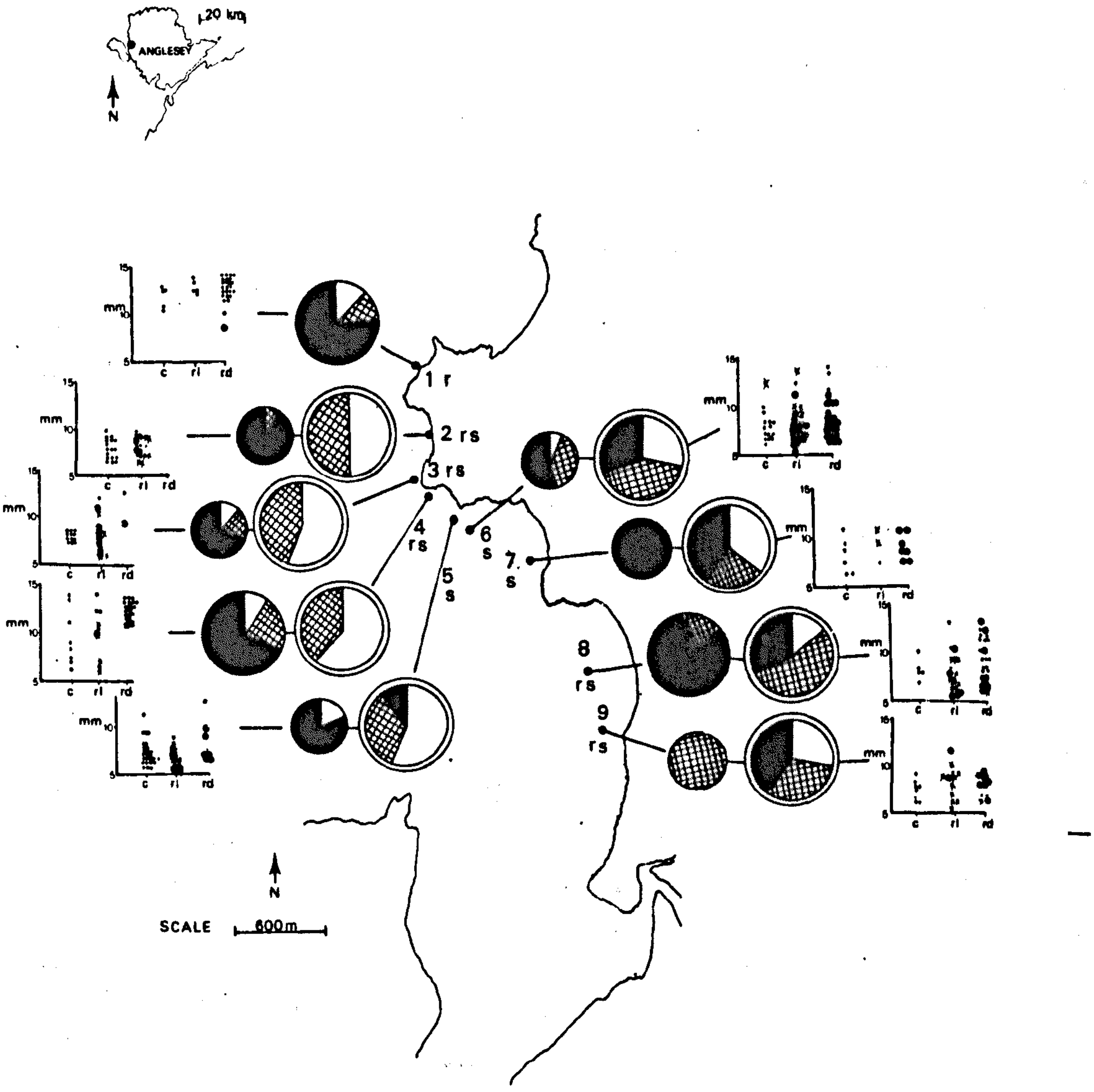


Fig. 64. Variation at Penrhyn.

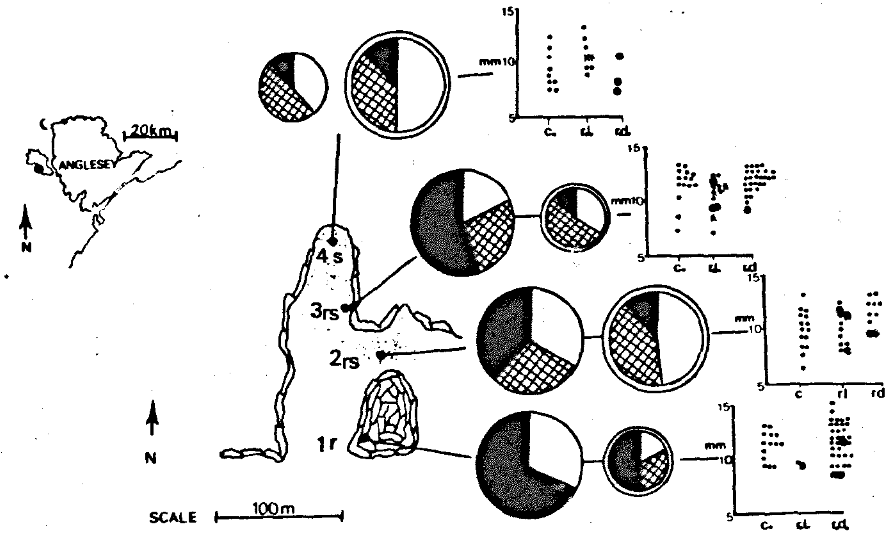


Fig. 65. Variation at Cliff Hotel.

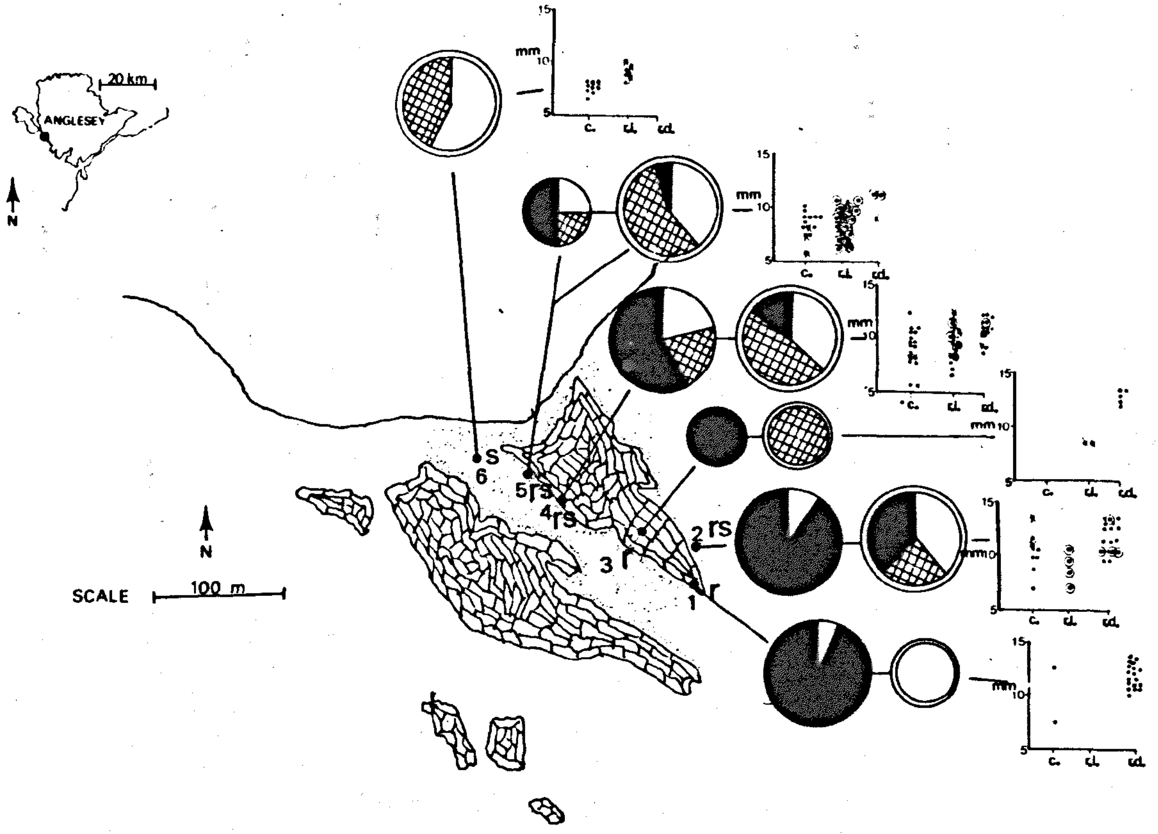


Fig. 66. Variation at Rhosneigr.

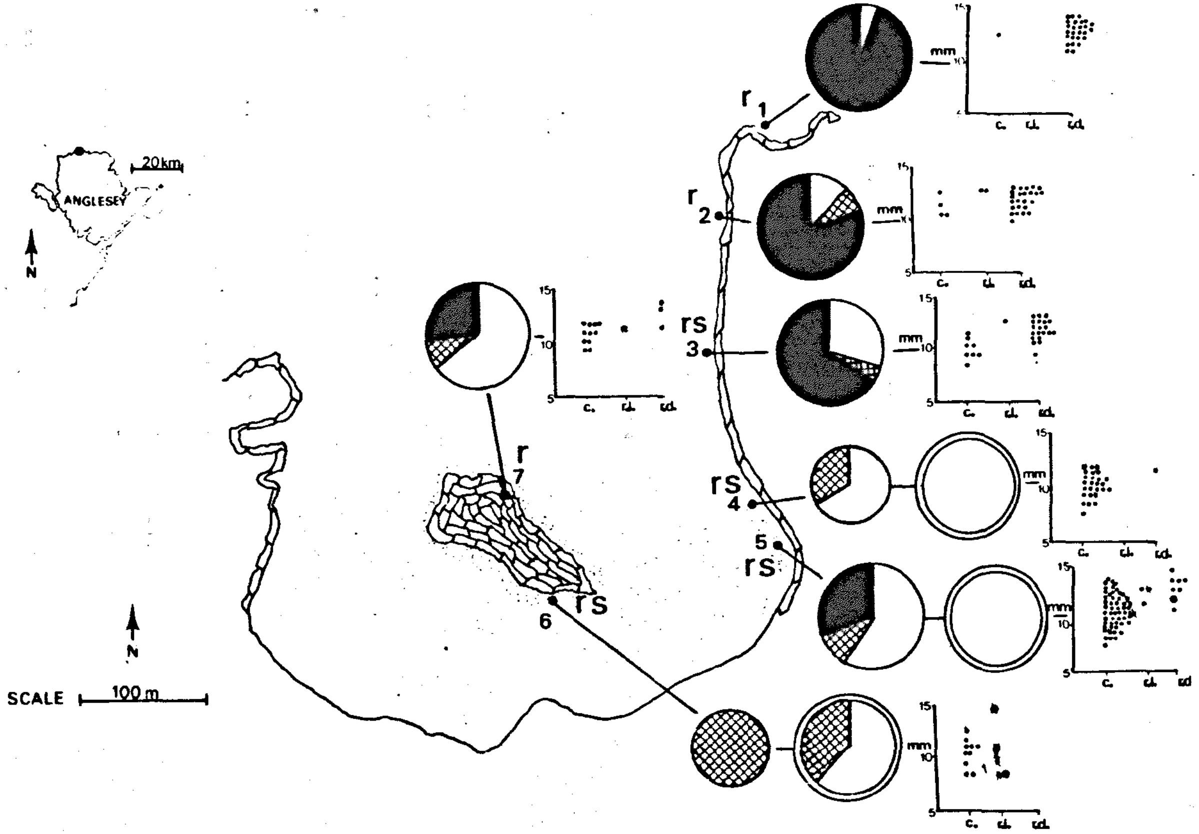


Fig. 67. Variation at Cemaes Bay.

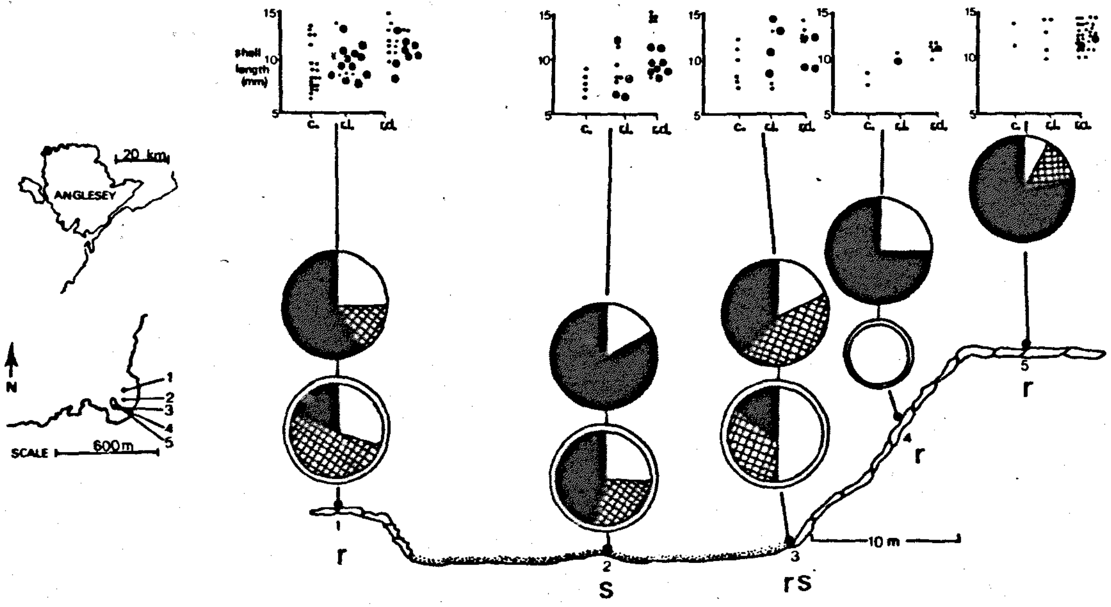


Fig. 68. Horizontal transect at Hen Borth.

GENERAL CONCLUSIONS AND DISCUSSION

This study has attempted to examine only a few of the many interrelationships between species and their habitats. Of these, it has been possible to relate a large amount of the variation in shell morphology and shell colour within and between species to the effects of predation and background.

The results presented in Section IV have suggested that the variation in adult shell size between localities and between species can be interpreted as the result of selection by wave action on exposed shores and by crabs in sheltered shores. One of the interesting features to emerge from the predation experiments was that predation by juvenile Carcinus as opposed to adult ones would lead, in shell sizes, to different strategies. The early thickening of the lip and thus small adult size seen in L. mariae seems to be at an advantage when considering predation by juvenile crabs while the large adult size in L. obtusata at an advantage when considering predation by adult crabs. If as different strategies as these are possible between sympatric species, it would be of interest to determine whether within a species the variation around the mean adult size (see Appendix E - fig E) is itself being selected for. Work as this may provide some test of the proposals by Van Valen (1965) who considered the relationship between continuous morphological variation and niche-width within a population.

Examination of the stomach contents in Blennius pholis (Section V) demonstrated considerable predation on Littorina spp. What was to become an essential factor for interpreting shell colour, especially in L. mariae (see Section VIII, 4), was that the predation by blennies was predominantly on the juveniles with very little on the adults.

The variation in phenotype frequencies of the different shell colours showed predictable changes with exposure (section VII). In each of the species these changes correlated well with the backgrounds on which each phenotype was cryptic. In *L. obtusata*, this was due mainly to differences in the proportion of the various algal species on which this species of winkle is found (Section VII, 3), while in *L. mariae*, it was due to changes in the distribution of juvenile snails on single plants of the same species of alga from shelter to exposure (Section VIII, 4). Even within plants there appears to be a correlation with background and phenotype. All of these observations suggested the role of visual predators, such as the blenny, as primarily responsible for the different phenotype frequencies between and within shores.

What has been demonstrated by these relationships between phenotype and background is the significance of colour *variation*. It has not accounted for the significance of the polymorphism, that is, the occurrence of discrete forms in a population. The ecological reasons for discreteness have not been extensively considered in many polymorphic studies. One notable exception is the study of mimicry (see Sheppard, 1969) in which the intermediate forms would be presumably at a disadvantage to both the mimics and non-mimics. Another is the work by Giesel (1970) on the polymorphism in the limpet. His study suggests that the discontinuous nature of the morphs relates to the discontinuous nature of the background. In other studies, such as those of *Cepaea*, where it has not been possible to relate some of the morphs to specific backgrounds within a population, apostatic selection has been used to account for the discreteness of morphs (Clarke, 1962). In this species, however, Cain, King and Sheppard (1960) have remarked that the three common colour morphs may in actuality correspond to three common backgrounds within a habitat, the intermediates of which appear relatively uncommon.

The underwater observations on the hunting positions of the blenny (p. 237) provided the clues as to the nature of phenotypic discreteness in L. mariae. They have shown that the colour of F. serratus seen by transmitted light is a brilliant yellow on which the apparently conspicuous citrina shells are remarkably cryptic. The discontinuity in the colour, between citrina and the other phenotypes seems primarily due to the discontinuity in the background. Since intermediate backgrounds were relatively uncommon, it is reasonable to assume that the predators have selected out intermediate phenotypes. This would seem to conform well with the work of Mather (1955) in which he suggested that if two separate modes on a continuum are favoured, disruptive selection will result in either isolation or polymorphism, the latter being controlled by super-genes.

The observations made on the ontogeny of shell colour (Section IX) have shown that the occurrence of discontinuities in the juvenile stages is also relatable to ecological factors. Thus in L. mariae, the discrete nature of dark reticulata and citrina at the post-hatching stage relates to the shells being either cryptic against the stem, or cryptic among the white tubes of Spirorbis, a relationship suggested to the mimetic. Intermediate individuals would again be at a disadvantage since they would be neither cryptic nor mimetic. In L. obtusata, the shell colours are continuous in the juveniles on what appears as a continuous background colour. Variation in the amount of discontinuity in the adult shells between localities has in some cases been related to discrete background, although in general with this species, the reasons for discrete phenotypes are not well understood.

Ford and Huxley (1927) were the first to examine developmental rates of characters in an evolutionary context. Although their own work was mainly on the eye colour of *Gammerus*, they cite various examples of African mammals in which the amount of darkening in the coat colour varies according to the age of the individual and the locality from which it was found. They point out that this developmental variability is itself probably responding to selection since the different rates are correlated with certain ecological conditions. Wanscher (1971) has considered the developmental genetics of shell colour in some molluscs showing how different colours and patterns occurring during growth can be interpreted as certain genes being regulated for activity. As his interests were mainly the "effects of genic time factors" during growth he has not alluded to the ecological significance of the variation in developmental rates.

In *L. mariae* it would appear that selection has favoured a delay in the expression of the yellow pigment when *Spirorbis* is abundant as well as the development of opaque white band on the translucent shell. When *Spirorbis* is very abundant, the expression of dark reticulata is also delayed, the juveniles appearing identical to the white-banded shells of juvenile citrina (see p. 268). When *Spirorbis* is absent, the yellow pigment of citrina appears much earlier. In at least one situation (p. 270), where the juveniles are found predominantly on the leaf (on which citrina is cryptic), the yellow pigmentation is present soon after hatching. It would seem therefore that the time of expression of each phenotype is responding to selection from predation by a visual predator such as the blenny. This type of examination of shell colour provides an opportunity for more detailed work. If it is true that the greatest elimination by predators on intertidal gastropods is on the juveniles, it would be profitable to attempt a complete life-history examination of the colour variation and the changes with time.

What has become evident from the study of the shell colour in at least L. mariae is that there seems to be extensive crypsis at all life stages. Yet the question arises, important in the understanding of every polymorphism, how this colour variation is maintained. Heterozygote advantage would provide sufficient conditions for the maintenance. It has not, however, been possible to identify heterozygotes (see Section VI) and thus no information can be given on their relative abundance or fitness. Clarke (1962) has suggested that frequency-dependent selection is capable of maintenance when the fitness is inversely proportional to the frequency of a genotype. In L. obtusata and L. mariae this certainly may be occurring although the small amount of data on shell injuries do not suggest this. It was shown (p. 95) that light-coloured shells, which are often more heavily injured than dark-coloured shells within a locality, do not show a reduction in the relative number of injuries when they are rare in the population. This would however require further investigation. Overall, the phenotype frequencies in both species correlate very well with the proportion of backgrounds on which each is cryptic not only between but within localities. From the abundance of particular types of background within localities and even within plants this would seem to fit the requirements of a "multi-niche" polymorphism.

Levene (1953) has been the first to examine this mathematically.

He considered the case of two niches which were each inhabited by two morphs, the relative fitness of each being a function of the niche it was in. New zygotes settle at random between the niches and then remain in their respective position with no migration between the niches. Selective elimination takes place and the proportion of the total population which make up the next breeding individuals is constant from each niche. Therefore, regardless of the selective differences between the morphs in each niche, some density dependence must be involved in order to make population contribution from each niche constant. Levene has found that a stable equilibrium could be maintained with these conditions. Other workers such as Levins and MacArthur (1966), Prout (1968) and Maynard Smith (1970) have also considered this model and expanded it to some extent, for example, using habitat selection. If migration is allowed between the niches, the maintenance is still possible although larger selective differences are required between the morphs in each niche.

From observation on blennies in the laboratory and in the field while diving, it would seem that the fitness of each phenotype on the plant is a function of two separate factors 1) its crypsis and 2) the density of snails on the plant where it is found. Various authors such as Tinbergen (1960) and Clarke (1962) have discussed the role of prey density and it would seem to be a widespread phenomena that when a particular form of prey becomes rare, it is less subject to predation than when it is common. The blennies certainly did spend much more time feeding on parts of the plant where the snails were abundant irrespective of the amount of crypsis or conspicuousness of the shells present. If one takes into account these two components of fitness then the stability of frequencies can be interpreted in the following way.

Consider a simplified situation in L. mariae where the plant is composed of a stem on which dark reticulata are cryptic and a leaf on which citrina are cryptic. Assume that the juveniles of the two phenotypes distribute themselves at random between the stem and the leaf (as in Levene's model) and that the blennies, being a rational predator, preys upon that which it sees, i.e. the conspicuous shells. Thus they would reduce the numbers of dark reticulata on the leaf and the numbers of citrina on the stem. If all the conspicuous shells are consumed, then the stem will be composed of entirely dark reticulata, and the leaf entirely citrina. Up to this stage the situation described does not differ from Levene's model. However, if it can be assumed that the blennies will continue to predate the snails, a likely assumption, then the success of predation will be a function of the density in each microhabitat. For example, if the blenny initially begins to search the stem, he may find a dark reticulata and develop a search image for this cryptic phenotype. With further predation this will lower the density on the stem and thus the frequency of dark reticulata relative to the citrina found on the leaf. It is conceivable that if the search image is very good, all the dark reticulata will be eliminated from the stem. What is more likely, however, is that when the density on the stem becomes very low and individuals hard to find, then it is an advantage for the blenny to search where the density is higher, that is, in this particular situation, on the leaf. This seems reasonable in that one would expect selection to favour a hunting strategy which minimizes the search time, all things being equal. Therefore, if citrina are now predated, the same factors should be operating to lower the density on the leaf to an approximate level to that on the stem; this would then reduce the frequency of citrina. If the density on the leaf drops below that on the stem, which would mean a corresponding reduction of citrina relative to dark reticulata, then the strategy of the blenny should be to search on the stem where the snails are more common. This type of alternating predation could go on until all the snails are consumed. Again, what is more likely is that when the snails become rare, the blenny will move to another plant. It is apparent that this type of predation will in theory lead to a stable equilibrium in which the fitnesses of dark reticulata and citrina approach equality when their densities in the two microhabitats become equal.

It is interesting to note that the frequencies of the two phenotypes after the selective predation will be the same as the frequencies after the density-dependent predation only when the two microhabitats are in equal abundance. That is to say, when there is random distribution on the plant, there will be equal numbers on the stem as there is on the leaf. After the conspicuous shells are eliminated, the densities of individuals on the two microhabitats will be the same, as well as the frequencies of dark reticulata and citrina. The density-dependent predation which follows, as discussed above, should stabilize the frequency at 50% for each phenotype. However, if the ratio of the two microhabitats is not equal, which is more realistic, then the two phenotypes contribute different proportions of conspicuous shells in the two habitats. For example, consider a plant where the stem and leaf constitute 10% and 90% respectively of the area and the frequencies of dark reticulata and citrina are 20% and 80% respectively. If these individuals now breed, and the juveniles distribute themselves at random, 90% of the dark reticulata will be found on the leaf, on which they are very conspicuous; yet only 10% of the citrina will be found on the stem, where this phenotype is very conspicuous. If as described before, the blenny eliminates all of the conspicuous shells, it will have consumed 90% of the dark reticulata population and only 10% of the citrina. The frequency of dark reticulata is in fact reduced to 2.7% after this elimination. If this type of predation were to be continued in subsequent generations, the dark reticulata would be eliminated from the population even though there is 10% of the habitat which provide it with an advantage. However, when one considers the role of density-dependent predation, the frequency of dark reticulata should increase. The density of snails on the leaf is now 4 times as great as on the stem as a result of elimination of the conspicuous shells, and therefore, the hunting strategy of the blenny should then tend to reduce the density on the leaf, and thus the frequency of citrina. If this is reduced to an equivalent density to that on the stem, the frequencies of the two phenotypes will be 10% and 90% for dark reticulata and citrina, the same as the proportions of backgrounds on which each is cryptic. Regardless of the initial frequencies of the phenotypes, in the population the end result of density-dependent predation as described will be to return the phenotypes to a frequency which approximates the frequencies of backgrounds. The effect of migration between the niches is either to stabilize or to destabilize depending upon the reasons for the movement. For example, if the stem and leaf are equally preferable by the snails for basic requirements such as food, then it is reasonable to assume that the amount of migration from one to the other is a function of the densities in the microhabitats. In the example described above, the density on the leaf was 4 times that on the stem after the conspicuous shells had been eliminated. The probability of citrina moving on to the stem should be greater than that for dark reticulata moving on to the leaf. If this is the case then these individuals would probably be eliminated owing to their conspicuousness. This would then tend to accelerate the approach to equilibrium. If however, the migration is not a function of density but of random movement, the proportion of dark reticulata moving onto the leaf would be greater than the proportion of citrina moving onto the stem. This would then tend to destabilize the equilibrium.

The simplified situation described above is basically Levene's model viewed in an ecological context. However, the actual relationships between the phenotypes and their background in these winkles is far more complex. For example, one does not find only dark reticulata on the stem and only citrina on the leaf at any stage from juvenile to adult, although the trend is in the expected direction within each age class (see p. 235). There are two reasons why such a relationship is not expected. One of these is that dark reticulata is also cryptic on the leaf when viewed with reflected light while citrina on the stem are themselves protectively coloured owing to their resemblance to Spirorbis. How this will affect the maintenance is not at present known, although it would certainly not tend to destabilize the equilibrium since migration from the stem to the leaf or from the leaf to the stem would not reduce the crypsis and thus fitness of each of the phenotypes. There are evidently many factors to be considered when attempting to account for the maintenance of this colour polymorphism.

One fact to emerge from the stomach analysis of the blennies was that the phenotype frequencies in the stomachs did not differ significantly from those in the population (Section V, 5). There are different ways of interpreting these data. One would be to assume that the blenny does not distinguish among the different colour phenotypes and thus exerts no visual selection on the population. This would seem unlikely owing to the excellent crypsis of each phenotype somewhere on the plant (see Plate 16 & 17) in addition to the predation experiments (pp. 243& 274), which showed that the blenny does consume initially the most conspicuous shells. If the blenny preys only on conspicuous shells in the field, then the similarity in the phenotype frequencies in the stomachs could be explained only if the proportion of each phenotype on its "wrong" background is similar in all cases, an exceptional though not unreasonable possibility. It is difficult to account for the data if predation is entirely density-dependent as described above in the model. This would lead in theory in this model to an individual blenny having at one time a large excess of one phenotype and at another time, an excess of the other. However the examination of individuals did not bear this out for there were no cases which had significant differences between the stomach and the population (p. 135). If the blennies were able to detect very subtle differences in density between the microhabitats, then the frequencies of the phenotypes taken would be similar to those in the population. Predation of this sort would then be only indirectly selective. It is interesting to note that if most of the shells found in the stomachs were indeed taken because of their conspicuousness then the majority of predation has been selective without any overall change in the phenotype frequencies in the population as a whole, at least at equilibrium frequencies. If this is true, and of relevance to other polymorphisms, it leads to the conclusion that the calculations of selective coefficients among different phenotypes based on changes in frequency at the population level may be greatly underestimating the actual selective differences between phenotypes.

It is difficult to believe that with continued selection against conspicuous shells, a strong microhabitat preference would not eventually develop in both citrina and dark reticulata. Any tendency for citrina to remain on the leaf, at least among individuals past the white-banded phase, should give an advantage over those which move randomly and in some cases on to the stem. With dark reticulata this will not be true for this phenotype is cryptic not only on the stem but on the leaf with reflected light. On the leaf, the greatest crypsis is achieved by the dark from near the leaf base or on any of the thick fronds which are more opaque to light compared with the thin leaf near the frond tip. The data on the microdistribution between citrina and dark reticulata (p. 231) showed a slight correlation in the expected direction but this could be due to predation rather than a habitat preference. It would be profitable if further studies are carried out on this species by marking individuals of different size classes and observing individual movement on plants throughout stages of the life history. In addition, studies of this kind may provide some information on the amount of predation which is selective, the fitness of each phenotype on each of the backgrounds, and how the polymorphism is maintained.

The results of this thesis have, to some extent, contributed to the understanding of variation in <u>L. obtusata</u> and <u>L. mariae</u>. It would seem that these two species are eminently suitable for a number of further studies and may demonstrate principles important to other species in interpreting ecological factors responsible for variation in colour within populations.

APPENDIX A

DESCRIPTION OF SAMPLING LOCALITIES

The table which follows includes the estimates at each locality of exposure, algal cover, Ascophyllum abundance, boulder abundance, and predominant substrate (rock, sand or mud). In addition to these, the abundance of L. obtusata and L. mariae have been included. In each of the estimates, except substrate, a scale of 1 to 5 designates conditions in order of increasing occurrence.

Station Number	Locality	Exposure (1-5)	Algal Cover (1-5)	*Ascophyllum* abundance (1-5)	Boulder abundance (1-5)	Predominant Substrate			Snail Abundance	
						rock	sand	mud	*obtusata*	*mariae*
1	Fedwfawr	4	2	1	4	+			1	1
2	Benllech	3	2	1	2		+		1	1
3	Moelfre	3	3	2	2	+	+		3	2
4	Bull Bay	2	4	5	3	+		+	1	1
5a	Cemaes Bay	2	5	5	1		+		4	2
5b	Cemaes Hd.	4	2	1	4	+			1	3
6	Hen Borth	3	5	4	2	+	+		5	3
7	Porth Swtan	4	2	2	3	+			5	2
8a	Sandy Bay	3	3	2	2	+	+		5	3
8b	Sandy Pt.	4	2	1	4	+			3	3
9a	Penrhyn Pt.	4	4	2	2	+			3	3
9b	Penrhyn-mawr	1	3	5	2		+	+	1	1
9c	Penrhyn Bay	1	3	4	1		+	+	4	1
10a	Causeway	1	4	5	1			+	4	1
10b	Four Mile Bridge	1	3	5	1			+	4	-
11	Holyhead Bay	1	5	5	2	+		+	3	1
12	North Stack	5	1	1	1	+			1	2
13	Porthdafarch	5	1	1	4	+	+		1	3
14a	Cliff Hotel	4	2	2	3	+	+		3	3
14b	Cliff Hotel Pt.	5	2	1	1	+			1	4
15	Treaddur Bay	3	3	2	2	+	+		3	2
16a	Rhosneigr Bay	2	3	2	3	+	+		3	2
16b	Rhosneigr Pt.	4	3	1	1	+			1	3
17a	Cywfan Bay	2	5	4	3	+			3	1
17b	Cywfan Pt.	5	1	1	1	+			1	2
18	Llanddwyn	3	2	3	1		+		4	2
19	Ysgubor Isaf	1	2	5	1			+	4	1
20	Caernarvon	1	2	5	1		+	+	4	1
21	Llanfair Hall	1	2	5	1		+	+	5	-
22	Pt. Dinorwic Pt.	1	4	5	4	+		+	3	1

Station Number	Locality	Exposure (1-5)	Algal Cover (1-5)	<u>Ascophyllum</u> abundance (1-5)	Boulder abundance (1-5)	Predominate Substrate rock	sand	mud	Snail abundance <u>obtusata</u>	<u>mariae</u>
23	Pt. Dinorwic Bay	1	2	3	1			+	2	1
24	Train Bridge	1	3	3	3		+	+	3	5
25	Menai Bridge	1	2	3	1	+	+	+	3	3
26	Navy Station	2	4	4	3	+		+	3	2
27	Beaumaris	1	3	4	2	+	+	+	4	1
28	Llwyngwril	3	2	2	1	+	+		3	1
29	Llanrhystyd	3	2	2	3	+	+		2	1
30	Newport	1	3	3	2	+	+	+	4	-
31	Dale Fort	3	2	2	4	+			2	4
32	Dale Bay	1	3	4	1		+	+	2	1
33	Port Eynon	3	2	2	2	+	+		3	1
34	Barry	2	2	3	2	+		+	2	-
35	Seawall	1	1	4	1			+	1	-
36	Severn Bridge	1	1	4	2			+	1	-
37	Weston-Super-Mare	1	2	3	3			+	1	1
38	Lynmouth	3	3	2	3	+	+		4	-
39	Buck Mills	4	1	1	3	+	+		1	1
40	Rock	1	4	4	1		+		4	-
41	Looe	3	3	2	4	+	+		4	3
42	Wembury	3	3	2	4	+	+		3	2
43a	Dartmouth	1	4	3	1			+	4	-
43b	Bodinnick	1	1	3	1			+	3	3
44	Penzance	1	2	2	1		+	+	2	1
45	Brow	1	5	3	3	+	+	+	5	2
46a	Heathy Bay	1	4	3	1		+	+	3	2
46b	Heathy Pt.	5	2	1	3	+			2	3
47	Popplestone	1	5	4	1		+		5	2
48	Hangman	3	2	1	2	+	+		3	1
49	Tean Sd.	2	4	3	4	+	+		3	-
50	Cow House	1	2	4	3	+	+		2	2
51	Pernagie	3	3	1	4	+	+		3	1

Station Number	Locality	Exposure (1-5)	Algal Cover (1-5)	*Ascophyllum* abundance (1-5)	Boulder abundance (1-5)	Predominate Substrate			Snail Abundance	
						rock	sand	mud	*obtusata*	*mariae*
52	White 1	3	3	1	4	+			2	-
53	White 2	3	4	1	4	+			4	2
54	White 3	3	4	1	4	+	+		4	-
55	White 4	2	4	2	3		+		3	1
56	White 5	1	4	4	2		+		3	2
57	White 6	1	4	4	1		+		1	-
58	White 7	5	1	1	5	+			1	3
59	Top Rock Pt.	3	4	2	4	+			4	2
60	Top Rock Bay	2	4	4	2	+	+		4	3
61	Bull's Porth	5	1	1	4	+			2	4
62	Head	4	3	2	2	+			3	2
63	Old Quay	3	3	3	4	+	+		3	1
64	Lawrence Bay	1	2	3	1		+		3	1
65	Innisidgen	3	3	3	4	+	+		3	1
66	Tolls Is.	4	2	1	4	+			2	1
67	Hellick	3	3	1	3	+	+		3	1
68	Porthloo	2	4	3	2	+	+		5	1
69	Dropnose	4	4	1	4	+	+		4	1
70	Cove	3	2	1	3	+	+		3	1
71	Perigli	3	2	1	2	+	+		3	1
72	Killier	3	2	2	2		+		3	1
73	Dunfanaghy	1	2	3	1		+	+	1	-
74	Curran's Port	4	2	1	3	+	+		2	3
75	Milkhaven	1	4	3	1		+		4	2
76	Stredagh House Bay	4	4	2	1		+		5	3
77	Staad Bay	5	2	1	1	+			2	2
78	Barnatra	1	4	4	1		+		4	3
79	Tiraun Pt.	4	2	1	3	+	+		3	1
80	Leckanvy	2	3	3	2		+	+	3	-
81	Killary Bay	3	3	2	2		+		4	1
82	Cloonagh	5	1	1	2	+			1	1
83	Black Head	4	2	1	1	+			2	2

Station Number	Locality	Exposure (1-5)	Algal Cover (1-5)	*Ascophyllum* abundance (1-5)	Boulder abundance (1-5)	Predominate Substrate			Snail Abundance	
						rock	sand	mud	*obtusata*	*mariae*
84	Liscannor	3	3	3	1		+		2	-
85	Shot Head	4	1	1	3	+			1	1
86	Adrigole Harbour	1	4	5	1		+	+	2	-
87	Ballycotton	5	1	1	3	+			2	2
88	Ballynagaul Bay	2	3	2	3	+	+	+	2	3
89	Ballynagaul Pt.	4	2	1	3	+			2	1
90	Bray	4	1	1	1	+			1	2
91	Portrane	3	3	2	2		+	+	3	1
92	Seascale	3	2	3	4	+	+		3	-
93	Loch Awun	2	2	3	1	+	+		3	-
94	Ballyluchan	3	4	4	1	+	+	+	3	-

APPENDIX B

MEAN ADULT SHELL SIZES OF L. OBTUSATA AND L. MARIAE

The maximum diameter of the shell (see fig below) was measured to the nearest 0.01 mm using a vernier calipers. In some localities, additional samples were obtained to provide estimates of stability in shell size through the season and between years. The samples of *L. obtusata* (table B (i)) occasionally include two estimates of shell size, one from the bottom of the shore at the region of overlap with *L. mariae* and the other from the top of the shore. Most of the localities, however, were sampled in the middle or upper shore positions. Collections of *L. mariae* (table B (ii)) in some localities include comparisons between rock substrates and adjacent silt substrates.

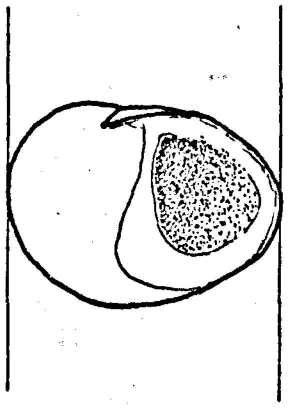


Table B (i): Distribution of adult shell sizes in *L. obtusata*

No.	Locality	Date		Males			Females		
				N	$\bar{X}$	S	N	$\bar{X}$	S
1	Fedwfawr	11/70		20	15.2	0.71	26	15.3	0.63
		6/71		10	15.4	0.64	11	15.4	0.47
2	Benllech	2/71		50	14.7	1.55	45	15.2	1.22
3	Moelfre	2/71		20	15.7	0.93	26	16.1	0.95
		3/73		20	15.9	1.17	20	15.9	1.20
4	Bull Bay	2/71		9	16.3	1.14	9	16.1	0.71
		5/72		8	16.1	1.07	9	16.2	0.84
5a	Cemaes Bay	3/71	(L)*	30	16.9	1.10	30	17.2	1.05
			(H)**	30	16.6	0.83	30	16.5	0.63
		3/73	(L)	40	17.0	0.67	40	17.1	0.61
			(H)	20	16.6	0.71	20	16.5	0.84
5b	Cemaes Hd.	3/73		12	15.4	0.97	14	15.4	1.21
6	Hen Borth	3/71		109	15.1	0.89	127	15.2	0.83
		3/73		30	15.2	0.61	30	15.3	0.87
7	Porth Swtan	2/71	(L)	30	15.6	0.51	30	16.1	0.20
			(H)	30	14.6	0.81	30	15.0	0.79
		3/73	(L)	10	15.9	0.63	10	15.9	0.87
			(H)	30	14.3	0.27	30	14.9	0.31
8a	Sandy Bay	11/71	(L)	10	16.3	0.74	10	16.3	0.78
			(I)***	53	13.9	0.71	66	14.0	0.88
			(H)	125	13.1	0.59	157	13.3	0.64
		1/72	(L)	29	15.4	0.47	25	15.9	0.71
			(I)	32	13.8	0.47	33	14.1	0.66
			(H)	67	13.0	0.58	81	13.2	0.83
		5/72	(L)	36	15.4	0.76	23	15.3	0.74
			(I)	56	13.8	0.63	84	14.0	0.71
			(H)	74	13.0	0.59	57	13.3	0.48
		8/72	(L)	10	15.7	0.45	10	15.7	0.52
			(I)	6	13.9	0.63	4	14.0	0.39
			(H)	99	13.1	0.56	67	13.4	0.42
		11/72	(L)	42	15.8	0.37	51	15.9	0.73
			(I)	50	13.8	0.62	74	13.9	0.55
			(H)	42	13.1	0.45	51	13.8	0.72
		2/73	(L)	52	15.5	0.39	57	15.5	0.47
			(I)	62	13.5	0.57	64	13.9	0.51
			(H)	59	13.1	0.41	45	13.4	0.68
		5/73	(L)	49	15.8	0.70	35	15.9	0.49
			(I)	22	14.0	0.69	17	14.0	0.73
			(H)	43	12.9	0.40	40	13.1	0.48
		8/73	(L)	17	16.1	0.40	21	16.0	0.49
			(I)	18	14.1	0.69	24	14.4	0.96
			(H)	73	13.2	0.78	94	13.4	0.85
8b	Sandy Pt.	5/72		12	14.1	0.87	12	14.4	0.71
9a	Penrhyn Pt.	3/73		15	16.1	0.51	15	16.2	0.43

*,**,*** - see end of table

Table B (i): *L. obtusata* contd.

No.	Locality	Date		Males			Females		
				N	$\overline{X}$	S	N	$\overline{X}$	S
9b	Penrhyn mawr	3/71	(L)	7	15.2	1.61	8	15.9	1.05
			(H)	24	15.4	0.77	26	15.4	0.55
10a	Causeway	4/71		20	15.8	0.63	20	15.9	0.71
10b	Four Mile Bridge	4/73		20	17.1	0.51	20	17.3	0.47
11	Holyhead Bay	4/71	(L)	31	16.5	0.83	30	16.7	0.77
			(H)	24	16.2	0.71	27	17.0	0.63
12	North Stack	5/71		20	14.4	1.10	20	15.1	1.45
		6/72		16	14.4	0.87	14	15.2	0.93
13	Porthdafarch	5/71		8	14.7	1.30	9	15.1	0.63
14a	Cliff Hotel	5/71	(L)	18	15.5	0.83	19	15.8	0.95
			(H)	31	14.9	0.89	37	15.2	0.63
15	Trearddur Bay	5/71		50	16.1	0.71	50	16.5	0.64
16a	Rhosneigr Bay	5/71		30	15.8	1.14	30	15.7	1.09
16b	Rhosneigr Pt.	5/71		20	15.1	0.64	12	15.2	0.78
17a	Cywfan Bay	5/71		50	16.5	0.89	41	16.5	0.55
		4/73		15	16.9	0.65	15	16.9	0.47
18	Llanddwyn	5/71		104	14.4	2.12	150	15.3	1.34
		9/72		50	14.6	1.91	50	15.3	1.21
19	Ysgubor Isaf	5/71	(L)	30	15.8	0.63	30	16.2	0.63
			(H)	30	15.8	0.83	30	16.1	0.87
20	Caernarvon	5/71		30	15.9	0.61	30	16.1	0.69
21	Llanfair Hall	5/71		30	15.9	0.78	30	16.3	0.82
22	Port Dinorwic Pt.	5/71		30	16.3	0.70	30	16.4	0.69
23	Port Dinorwic Bay	5/71		30	16.3	0.77	30	16.5	0.63
24	Train Bridge	5/71		30	16.3	0.93	30	16.4	0.71
		5/72		20	16.2	0.77	20	16.4	0.84
		4/73		30	16.2	0.81	30	16.3	0.97
25	Menai Bridge	5/71	(L)	28	16.4	0.71	30	17.0	0.77
			(H)	27	16.8	0.95	27	17.0	0.83
		4/72	(L)	30	16.2	0.59	30	16.9	0.83
			(H)	30	16.5	0.81	30	16.8	0.89
26	Navy Station	5/71		15	16.1	0.73	15	16.2	0.64
27	Beaumaris	5/71	(L)	30	15.7	0.77	30	15.9	0.45
			(H)	30	15.7	0.69	32	15.7	0.55
28	Llwyngwril	7/71		17	16.1	0.58	23	16.2	0.79
29	Llanrhystyd	7/71		24	16.8	0.67	18	17.0	0.66
30	Newport	7/71		18	17.3	0.80	19	17.3	0.62

Table B (i): L. obtusata contd.

No.	Locality	Date		Males			Females		
				N	$\bar{X}$	S	N	$\bar{X}$	S
31	Dale Fort	7/71		20	15.6	0.41	23	16.1	0.66
32	Dale Bay	7/71		21	15.7	0.69	32	17.4	0.70
33	Port Eynon	7/71		21	16.9	0.65	30	17.4	0.58
34	Barry	7/71		16	17.0	0.45	19	17.0	0.62
35	Seawall	7/71		1	15.9	-	-	-	-
36	Severn Bridge	7/71		1	14.7	-	-	-	-
37	Weston-Super-Mare	7/71		27	16.0	0.70	21	16.0	0.72
38	Lynmouth	7/71		34	16.4	1.02	37	16.4	0.56
39	Buck Mills	7/71		10	15.2	0.45	11	15.7	0.67
40	Rock	7/71		14	17.1	0.71	11	17.7	0.71
41	Looe	7/71		27	15.3	0.53	21	15.6	0.60
42	Wembury	7/71		25	15.2	0.52	35	15.4	0.55
43a	Dartmouth	7/71		23	16.4	0.63	32	16.8	0.70
43b	Bodinnick	5/72		11	15.9	0.70	14	16.9	1.24
44	Penzance	4/71		13	15.9	0.68	22	16.2	1.06
		5/72		10	15.7	0.83	12	16.1	1.19
45	Brow	4/71		26	15.9	0.45	26	16.7	0.77
46a	Heathy Pt.	4/71		12	15.3	0.77	15	15.6	0.94
		5/72		10	15.1	0.96	10	15.2	0.73
46b	Heathy Bay	5/72		6	15.8	0.90	4	16.4	0.91
47	Popplestone	4/71		30	16.0	0.77	30	16.2	0.63
48	Hangman	4/71		31	16.2	0.77	30	16.4	0.55
49	Tean Sd.	4/71		19	15.4	1.22	21	16.0	0.77
50	Cow House	4/71		15	14.3	1.61	18	14.6	0.55
51	Pernagie	4/71		30	15.6	0.71	30	16.5	0.55
52	White No1	4/71		30	17.0	0.75	30	17.1	0.81
53	White No 2	4/71		30	17.3	0.89	30	17.2	0.71
54	White No 3	4/71		28	16.7	0.83	30	17.1	0.71
55	White No 4	4/71	(L)	19	16.8	0.63	22	17.3	0.71
			(H)	30	16.5	0.83	30	16.9	0.71
56	White No 5	4/71	(L)	17	16.1	0.77	18	17.0	0.77
			(H)	10	15.9	1.30	7	16.7	0.63
57	White No 6	4/71		13	15.5	0.45	14	16.4	0.77
58	White No 7	4/71		1	15.3	-	2	16.1	0.68

Table B (i): *L. obtusata* contd.

No.	Locality	Date		Males			Females		
				N	$\bar{X}$	S	N	$\bar{X}$	S
59	Top Rock Pt.	4/71		24	16.4	0.71	25	16.7	0.71
		5/72		11	16.2	0.77	11	16.5	0.64
60	Top Rock Bay	4/71		17	16.4	0.55	11	16.9	1.00
		5/72		30	16.4	0.84	30	16.8	0.94
61	Bull's Porth	4/71		15	15.4	0.89	15	15.5	1.09
62	St. Martin's Hd.	4/71		34	15.2	0.71	45	15.7	0.63
63	Old Quay	4/71		30	16.3	1.05	30	16.6	1.00
		5/72		10	16.0	0.64	10	16.2	0.43
64	Lawrence Bay	4/71	(L)	30	15.8	0.95	28	16.0	0.55
			(H)	30	16.3	0.71	30	16.6	0.63
		5/72		14	16.0	0.87	14	16.5	0.73
65	Innisidgen	4/71	(L)	30	15.8	0.95	28	16.0	0.55
			(H)	30	16.3	0.71	30	16.6	0.63
66	Toll's Island	4/71		30	16.4	0.89	30	16.7	0.55
		5/72		15	16.2	0.54	15	16.1	0.96
67	Hellick	4/71	(L)	40	15.9	0.71	30	16.1	0.83
			(H)	30	16.5	0.83	30	17.1	0.77
68	Porthloo	4/71		30	16.6	0.89	30	16.7	0.71
		5/72		30	16.7	1.10	30	16.8	0.94
69	Dropnose	4/71		30	17.1	0.77	30	16.9	0.71
70	Cove	4/71		30	16.9	0.63	30	17.1	0.86
71	Periglis	4/71		29	16.7	0.95	30	17.5	0.74
72	Killier	4/71		35	16.3	0.83	29	16.5	0.71
73	Dunfanaghy	8/72		25	16.8	0.69	27	16.8	0.84
74	Curran's Port	8/72		23	14.6	0.92	20	14.9	0.79
75	Milkhaven	8/72		25	17.7	0.79	28	17.5	0.68
76	Streedagh House Bay	8/72		58	16.0	0.65	78	16.2	0.75
77	Stand Bay	8/72		20	14.5	1.41	16	14.8	0.82
78	Barnatra	8/72		29	15.9	0.55	29	16.2	0.82
79	Tiraun Pt.	8/72		16	13.8	0.64	29	14.5	0.91
80	Leckanvy	8/72		26	15.3	0.64	12	15.8	0.85
81	Little Killary Bay	8/72		21	15.7	0.61	22	15.9	0.49
82	Cloonagh	8/72		21	15.7	0.70	25	15.8	0.72
83	Black Head	8/72		29	15.4	0.96	26	16.0	0.63
84	Liscannor	8/72		11	16.1	0.78	15	16.9	0.98
85	Shot Head	8/72		35	14.8	0.58	28	15.3	0.49
86	Adrigole Harbour	8/72		19	16.3	0.83	20	17.0	1.29

Table B (i): L. *obtusata* contd.

No.	Locality	Date	Males			Females		
			N	$\bar{X}$	S	N	$\bar{X}$	S
87	Ballycotton	8/72	40	15.4	0.77	35	15.8	0.78
88	Ballynagaul Bay	8/72	8	16.8	0.38	7	16.9	0.57
89	Ballynagaul Pt.	8/72	11	15.8	1.12	15	16.6	0.78
90	Bray	8/72	4	13.0	0.90	2	14.0	1.10
91	Portrane	8/72	28	16.2	0.71	17	16.4	0.91
92	Seascale	9/72	14	16.7	0.75	3	16.3	1.17
93	Loch Awun	9/72	12	15.9	0.87	7	16.2	0.91
94	Ballyluchan	9/72	15	16.0	0.25	10	16.5	0.47

* low shore

** high shore

*** intermediate or middle shore

Table B (ii): Distribution of adult shell sizes in *L. mariae*.

No.	Locality	Date	Males			Females		
			N	$\bar{X}$	S	N	$\bar{X}$	S
1	Fedwfawr	11/70	26	11.4	0.65	29	12.1	0.79
2	Benllech	2/71	3	9.5	0.30	3	12.2	1.12
3	Moelfre	2/71	26	8.4	0.83	34	10.0	0.63
4	Bull Bay	2/71	20	8.5	1.04	28	11.1	0.83
5a	Cemaes Bay	3/71	6	9.1	1.01	10	11.3	1.10
		3/73	19	8.5	0.87	24	10.4	1.03
5b	Cemaes Hd.	3/73	41	11.5	0.96	38	12.5	1.08
6	Hen Borth	3/71	64	10.5	1.30	67	12.3	1.26
		3/73 (s)*	16	8.2	0.77	8	10.3	1.61
		3/73 (r)**	25	11.2	0.87	25	12.5	1.07
7	Porth Swtan	2/71	30	12.0	0.61	30	13.7	0.68
		11/72	15	11.9	0.57	15	13.4	0.76
8a	Sandy Bay	11/71	45	9.6	1.61	41	11.0	1.93
		1/72	61	11.7	1.58	62	12.9	1.91
		5/72	51	9.7	1.98	47	11.8	2.10
		8/72	12	12.0	0.57	10	12.2	0.73
		11/72	17	11.5	0.41	15	12.8	1.31
		2/73	69	11.1	1.73	57	11.9	1.90
		5/73	50	11.7	0.83	50	12.8	1.07
		8/73	20	11.7	0.44	23	12.5	0.91
8b	Sandy Pt.	5/72	30	11.6	0.63	30	12.9	0.84
9a	Penrhyn Pt.	4/73 (s)	12	7.5	1.31	8	9.7	0.71
		(r)	11	11.4	0.98	13	13.0	0.95
9b	Penrhyn-mawr	3/71	21	8.7	0.52	25	9.9	1.09
10	Causeway	6/72	23	8.0	1.41	20	10.2	1.31
11	Holyhead Bay	4/71	3	6.3	0.89	2	7.0	0.89
12	North Stack	5/71	24	11.5	0.55	27	12.4	0.55
		9/71	31	11.4	0.61	30	12.5	0.48
		4/72	26	11.5	0.59	32	12.5	0.60
		4/73	36	11.5	0.47	38	12.5	0.41
13	Porthdafarch	5/71	30	11.4	0.83	16	12.6	1.00
14a	Cliff Hotel	5/71	40	10.4	1.41	53	11.9	1.26
		4/73 (s)	25	8.4	0.61	14	10.9	1.31
		(r)	15	11.8	0.83	15	12.6	0.77
14b	Cliff Hotel Pt.	4/73 (r)	15	11.9	1.07	15	13.1	0.64
15	Trearddur Bay	5/71	46	9.5	1.18	36	10.9	1.14
		6/72	19	9.4	0.87	21	11.0	1.23
16a	Rhosneigr Bay	5/71	42	7.6	0.83	54	8.3	1.38
		4/73 (s)	20	7.6	0.75	20	9.5	1.40
16b	Rhosneigr Pt.	4/73 (r)	12	10.6	0.81	12	12.4	0.86

* silt substrate ** rock substrate

Table B (ii): *L. mariae* contd.

No.	Locality	Date	Males			Females		
			N	$\bar{X}$	S	N	$\bar{X}$	S
17a	Cwyfan Bay	5/71	36	8.8	0.92	37	10.9	0.99
		4/73 (s)	11	7.6	0.92	12	10.0	1.73
17b	Cwyfan Pt.	4/73 (r)	8	10.6	0.46	15	12.0	0.51
18	Llanddwyn	5/71	75	8.4	0.95	54	10.3	1.09
		9/72	15	8.3	0.66	15	10.4	0.91
19	Ysgubor Isaf	5/71	4	8.0	0.70	3	10.0	0.70
		6/72	4	8.1	0.61	2	10.0	0.10
20	Caernarvon	5/71	3	7.6	1.31	3	8.9	1.21
21	Llanfair Hall	5/71	8	8.3	1.20	5	9.0	0.83
22	Port Dinorwic Pt.	5/71	30	6.5	0.63	30	8.6	0.89
23	Port Dinorwic Bay	5/71	10	6.4	0.65	10	8.7	1.03
24	Train Bridge	5/71	30	8.9	0.74	30	10.9	0.71
		5/72	30	8.9	0.56	30	10.8	0.76
		4/73	30	9.0	0.66	30	10.9	0.82
25	Menai Bridge	5/71	22	9.0	1.18	12	11.5	1.14
		4/72	30	9.1	1.11	30	11.7	0.98
26	Navy Station	5/71	2	8.4	0.30	-	-	-
27	Beaumaris	5/71	5	7.2	1.43	-	-	-
29	Llanrhystyd	7/71	23	8.4	0.71	25	10.1	0.93
31	Dale Fort	7/71	24	11.4	0.90	32	12.4	0.90
32	Dale Bay	7/71	7	9.4	1.04	6	10.6	1.08
37	Weston-Super-Mare	7/71	7	12.3	0.80	9	13.5	0.67
39	Buck Mills	7/71	14	10.3	0.80	10	11.0	0.56
41	Looe	7/71	30	10.0	0.60	30	11.9	0.95
42	Wembury	7/71	28	12.5	0.74	23	13.3	0.62
43	Bodinnick	5/72	9	7.4	0.99	7	10.9	0.57
44	Penzance	4/71	-	-	-	3	9.5	0.85
		5/72	12	6.8	0.73	7	8.3	1.21
45	Brow	4/71	30	9.0	1.61	30	10.9	0.89
46a	Heathy Pt.	4/71	19	12.6	0.64	12	13.0	0.86
		5/72	15	12.3	0.75	10	12.9	0.71
46b	Heathy Bay	5/72	8	6.0	0.23	12	9.2	1.05
47	Popplestone	4/71	30	9.5	1.09	30	11.4	0.89
50	Cow House	4/71	2	5.1	0.0	2	10.4	0.63
51	Pernagie	4/71	1	12.2	-	4	13.8	1.14
55	White No 4	4/71	1	10.4	-	3	12.8	1.0

Table B (ii): *L. mariae* contd.

No.	Locality	Date	Males			Females		
			N	$\overline{X}$	S	N	$\overline{X}$	S
56	White No 5	4/71	6	9.2	0.89	7	11.6	1.26
58	White No 7	4/71	22	11.8	0.77	24	13.4	0.63
59	Top Rock Pt.	4/71	30	12.5	1.14	31	13.5	0.95
		5/72	14	12.3	1.30	15	13.5	0.87
60	Top Rock Bay	7/71	30	8.4	1.73	30	10.6	0.89
		5/72	3	7.9	0.59	5	10.0	0.95
61	Bull's Porth	4/71	15	13.5	1.26	15	13.5	1.63
		5/72	14	13.5	0.94	13	13.5	0.71
62	St. Martin's Head	4/71	26	12.1	1.05	19	13.4	0.63
63	Old Quay	4/71	12	8.6	0.83	12	10.0	0.89
		5/72	9	8.5	0.78	10	10.1	0.52
64	Lawrence Bay	4/71	5	9.0	1.09	8	9.9	0.63
		5/72	4	8.9	0.84	5	10.1	0.89
65	Innisidgen	4/71	6	9.8	0.63	5	11.5	0.71
66	Toll's Island	4/71	1	12.0	-	3	12.2	0.63
		5/72	7	13.5	0.78	9	13.7	1.23
67	Hellick	4/71	25	10.9	1.70	18	12.3	1.05
68	Porthloo	4/71	17	9.1	0.73	23	11.4	1.17
		5/72	13	9.2	0.88	14	11.4	1.21
69	Dropnose	4/71	5	10.9	1.30	8	13.2	1.05
70	Cove	4/71	1	12.4	-	1	12.4	-
72	Killier	4/71	2	8.2	0.63	-	-	-
74	Curran Port	8/72	48	11.7	0.76	43	12.5	0.76
75	Milkhaven	8/72	29	5.7	0.56	24	7.9	1.0
76	Streedagh House Bay	8/72	28	12.8	0.94	35	13.8	0.90
77	Staad Bay	8/72	40	12.5	0.93	42	13.2	1.13
78	Barnatra	8/72	10	7.4	1.15	13	8.8	0.63
79	Tiraun Pt.	8/72	11	10.0	0.86	7	11.3	0.78
81	Little Killary Bay	8/72	1	8.5	-	1	10.4	-
82	Cloonagh	8/72	8	10.3	1.13	9	11.6	0.94
83	Black Head	8/72	26	11.9	0.94	16	13.0	0.96
85	Shot Head	8/72	41	10.4	0.93	39	11.8	0.98
87	Ballycotton	8/72	13	11.8	0.99	9	12.7	0.99
88	Ballynagaul Bay	8/72	10	8.8	1.02	8	11.4	1.29
89	Ballynagaul Pt.	8/72	10	8.4	0.97	9	10.6	0.84
90	Bray	8/72	23	10.5	0.92	26	11.6	1.26
91	Portrane	8/72	6	10.0	0.91	5	11.8	0.54

APPENDIX C

SHELL INJURIES IN L. OBTUSATA AND L. MARIAE

The following tables show the distribution of injuries among light-coloured and dark-coloured shells and the percentage of total injuries in the sample. Light-coloured shells include all variations of citrina while dark-coloured shells include olivacea, light and dark reticulata, red-brown, and banded individuals. Comparisons between the two classes are made using Fisher's Exact Probability test (two-tailed).

Table C(i) Distribution of shell injuries and comparison between colour phenotypes in <u>L. obtusata</u>

No.	Locality	Light Shells		Dark Shells		Total inj.	P (Fisher's Exact)
		inj.	not inj.	inj.	not inj.	%	
2	Benllech	0	3	2	80	2.4	1.00
3	Moelfre	0	4	2	96	2.0	1.00
4	Bull Bay	1	5	6	51	11.1	1.00
5a	Cemaes Bay	-	-	12	19	44.0	-
7	Porth Swtan	2	4	7	371	2.8	0.02
8a	Sandy Bay	0	11	1	96	0.9	1.00
9b	Penrhynmawr	0	1	8	279	2.8	1.00
10a	Causeway	-	-	7	98	6.7	-
10b	Four Mile Bridge	-	-	31	72	30.1	-
11	Holyhead Bay	0	3	7	209	3.2	-
12	North Stack	-	-	1	24	4.0	-
13	Porth da farch	0	3	0	14	0.0	-
15	Trearddur Bay	0	1	3	136	2.1	1.00
16a	Rhosneigr Bay	0	2	1	102	1.0	1.00
17	Cywfan Bay	0	1	22	170	11.4	1.00
18	Llanddwyn Is.	1	11	11	240	4.6	0.87
19	Ysgubor Isaf	1	3	22	231	8.6	0.63
20	Caernarvon	4	9	20	172	11.7	0.07
21	Llanfair Hall	4	10	30	165	16.3	0.35
22	Port Dinorwic Pt.	3	16	22	88	19.4	0.73
24	Train Bridge	20	29	29	55	35.1	0.15
26	Navy Station	4	34	22	74	19.4	0.15
27	Beaumaris	3	11	19	107	15.7	0.23
28	Llwyngwril	0	1	9	154	5.5	1.00
29	Llanrhystyd	-	-	3	100	2.9	-
30	Newport	1	1	45	102	30.9	1.00

Table C(i) *L. obtusata* contd.

No.	Locality	Light Shells		Dark Shells		Total inj.	P (Fisher's Exact)
		inj.	not inj.	inj.	not inj.	%	
31	Dale Fort	1	23	7	166	4.1	1.00
32	Dale Bay	0	4	1	217	6.5	1.00
33	Port Eynon	-	-	18	213	7.8	-
34	Barry	-	-	2	63	3.1	-
35	Seawall	-	-	4	44	8.3	-
36	Severn Bridge	-	-	0	40	0.0	-
37	Weston super Mare	-	-	5	83	5.7	-
38	Lynmouth	3	31	16	186	8.1	1.00
39	Buck Mills	-	-	1	21	4.5	-
40	Rock	-	-	1	39	2.5	-
41	Looe	20	238	21	602	4.7	0.01
42	Wembury	0	9	0	43	0.0	-
43a	Dartmouth	1	6	12	67	15.1	1.00
43b	Bodinnick	4	8	11	18	36.6	1.00
44	Penzance	2	3	26	56	32.2	0.41
45	Brow	3	24	18	126	12.3	1.00
46a	Heathy Pt.	1	5	4	45	9.1	1.00
47	Popplestone	6	30	10	132	8.9	0.15
48	Hangman	2	34	9	59	10.6	0.85
49	Tean Sd.	1	40	6	103	4.7	0.76
50	Cow House	3	4	5	23	22.9	0.36
51	Pernagie	5	27	14	89	14.1	0.97
52	White No.1	9	31	20	130	15.3	1.00
53	White No.2	3	85	17	193	6.7	0.21
54	White No.3	8	42	8	87	8.5	0.36
55	White No.4	7	86	8	158	5.8	0.52

Table C(i) L. obtusata contd.

No.	Locality	Light Shells inj.	Light Shells not inj.	Dark Shells inj.	Dark Shells not inj.	Total inj. %	P (Fisher's Exact)
56	White No.5	2	20	4	60	7.0	0.96
57	White No.6	1	15	1	16	6.1	1.00
58	White No.7	-	-	0	3	0.0	-
59	Top Rock Pt.	5	35	11	102	10.5	1.00
60	Top Rock Bay	6	43	7	101	8.3	0.36
61	Bull's Porth	0	8	3	13	12.5	0.55
62	St. Martin's Head	1	10	4	76	5.5	0.96
63	Old Quay	2	19	12	82	12.2	1.00
64	Lawrence Bay	1	4	10	128	7.7	0.67
65	Innisidgen	14	76	23	123	15.7	1.00
66	Toll's Is.	5	35	9	80	10.9	0.89
67	Porth Hellick	17	80	16	142	12.9	0.13
68	Porthloo	8	29	10	97	12.5	0.10
69	Dropnore	7	38	27	265	10.1	0.29
70	Cove	15	45	39	102	26.9	0.79
71	Periglis	9	24	29	76	27.5	1.00
72	Killier	4	21	35	84	27.1	0.25
73	Dunfanaghy	0	1	6	58	9.2	1.00
74	Curran's Port	0	21	1	38	1.7	1.00
75	Milkhaven	-	-	24	76	24.0	-
76	Streedagh House Bay	1	42	5	90	4.3	0.78
77	Staad Bay	1	11	1	32	4.4	0.93
78	Barnatra	-	-	3	83	3.5	-
79	Tiraun Pt.	0	6	5	54	7.7	1.00
80	Leckanvy	0	1	4	68	5.5	1.00

Table C(i) *L. obtusata* contd.

No.	Locality	Light Shells		Dark Shells		Total inj.	P (Fisher's Exact)
		inj.	not inj.	inj.	not inj.	%	
81	Little Killary Bay	-	-	5	68	6.8	
82	Cloonagh	0	7	9	59	12.0	1.00
83	Black Head	0	2	5	46	9.4	1.00
84	Liscannon	5	5	10	12	46.9	1.00
85	Shot Head	2	16	1	74	3.2	0.19
86	Adrigole Harbour	0	2	11	35	22.9	1.00
87	Ballycotton	4	17	5	97	7.3	0.09
88	Ballynagaul Pt.	-	-	8	39	17.0	-
89	Ballynagaul Bay	-	-	6	10	37.5	-
90	Bray	-	-	1	5	16.7	-
91	Portrane	-	-	2	64	3.0	-
92	Sea Scale	-	-	13	26	33.3	-
93	Loch Awun	0	1	6	27	17.7	1.00
94	Ballyluchan	-	-	5	26	16.1	-

Table C(ii) Distribution of shell injuries and comparison between colour phenotypes in *L. mariae*

No.	Locality	Light Shells		Dark Shells		Total inj.	P (Fisher's Exact)
		inj.	not inj.	inj.	not inj.	%	
3	Moelfre	6	204	3	47	5.6	1.00
4	Bull Bay	3	41	0	32	5.5	0.38
5a	Cemaes Bay	11	14	-	-	44.0	-
7	Porth Swtan	0	3	2	21	7.7	1.00
8a	Sandy Bay	2	9	7	31	18.4	1.00
9b	Penrhynmawr	1	12	-	-	7.7	-
12	North Stack	0	22	1	118	0.7	1.00
13	Porth da farch	3	97	1	79	2.2	0.73
15	Trearddur Bay	7	42	6	51	12.3	0.77
16a	Rhosneigr Bay	4	37	3	109	4.6	0.17
17	Cywfan Bay	2	23	0	9	5.7	1.00
18	Llanddwyn Is.	5	115	0	3	4.1	1.00
19	Ysgubor Isaf	1	4	-	-	20.0	-
20	Caernarvon	0	7	-	-	0.0	-
21	Llanfair Hall	3	10	-	-	23.0	-
22	Port Dinorwic Pt.	13	107	0	4	10.5	1.0
24	Train Bridge	12	81	-	-	12.9	-
26	Navy Station	1	6	-	-	14.3	-
27	Beaumaris	1	13	-	-	7.1	-
28	Llwyngwril	0	4	-	-	0.0	-
29	Llanrhystyd	1	46	0	16	1.6	1.00
31	Dale Fort	4	51	4	135	4.6	0.32
32	Dale Bay	3	13	0	3	15.8	1.00
37	Weston super Mare	-	-	1	18	5.3	-
39	Buck Mills	-	-	-	35	0.0	-

Table C(ii) L. mariae contd.

No.	Locality	Light Shells		Dark Shells		Total inj.	P (Fisher's Exact)
		inj.	not inj.	inj.	not inj.	%	
41	Looe	2	12	9	86	10.1	0.85
42	Wembury	0	2	1	79	1.2	1.00
43b	Bodinnick	11	21	-	-	34.4	-
44	Penzance	1	4	-	-	20.0	-
45	Brow	7	61	1	32	7.2	0.38
46a	Heathy Pt.	1	9	3	39	12.5	1.00
47	Popplestone	5	58	6	38	10.3	0.41
48	Hangman	2	6	1	1	30.0	1.00
50	Cow House	3	4	0	3	30.0	0.58
51	Pernagie	-	-	1	4	20.0	-
58	White No.7	1	4	8	32	20.0	1.00
59	Top Rock Point	7	25	4	40	14.5	0.21
60	Top Rock Bay	9	95	11	51	12.0	0.13
61	Bull's Porth	4	34	9	107	8.4	0.80
62	St. Martin's Hd.	2	1	2	41	8.7	0.03
63	Old Quay	0	8	7	14	24.1	0.14
64	Lawrence Bay	1	3	0	11	6.7	0.53
65	Innisidgen	3	13	1	3	20.0	1.00
66	Tolls Island	0	3	0	4	0.0	-
67	Porth Hellick	2	25	4	13	13.6	0.28
68	Porthloo	0	6	1	3	10.0	1.00
69	Dropnore	0	9	0	7	0.0	1.00
74	Curran's Port	1	37	8	108	5.8	0.60

Table C(ii) *L. mariae* contd.

No.	Locality	Light Shells		Dark Shells		Total inj.	P (Fisher's Exact)
		inj.	not inj.	inj.	not inj.	%	
75	Milkhaven	16	110	-	-	12.7	-
76	Streedagh House Bay	3	24	2	30	8.5	0.83
77	Staad Bay	0	13	5	66	6.0	0.84
78	Barnatra	4	28	-	-	12.5	-
79	Tiraun Pt.	0	21	0	27	0.0	-
82	Cloonagh	0	7	1	32	2.5	1.00
83	Black Head	0	5	1	38	2.3	1.00
85	Shot Head	0	12	0	96	0.0	-
87	Ballycotton	-	-	2	42	4.5	-
89	Ballynagaul Pt.	0	21	1	40	1.6	1.00
90	Bray	1	19	1	69	22.2	0.79
91	Portrane	4	15	3	10	21.9	1.00

APPENDIX D

DISTRIBUTION OF COLOUR PHENOTYPES IN L. OBTUSATA AND L. MARIAE

In all the samples, the following phenotypes have been scored:

citrina -	c1	white or pale yellow
	c2	yellow
	c3	orange (include aurantia)
olivacea -	ol1	olive-yellow
	ol2	olive-green
	ol3	dark olive
reticulata -	r1	light reticulata
	r2	dark reticulata (includes olive-green, olive-brown, and black individuals)
	r2'	dark reticulata (red-brown)
banded -	inversicolour	

In the majority of localities, adult individuals constitute the greater part of the samples. The remaining individuals that were scored are slightly sub-adult, very similar in size to the adults but lacking the thickened lip.

Table D (i): Distribution of phenotypes in *L. obtusata* around the British Isles.

No.	Locality	Date	citrina			olivacea			reticulata			banded	Totals
			c_1	c_2	c_3	ol_1	ol_2	ol_3	r_1	r_2	r_2'		
1	Fedwfawr	11/70	-	3	-	-	34	-	-	11	-	-	48
		6/71	-	1	-	-	17	-	-	5	-	-	23
2	Benllech	2/71	-	10	3	28	263	14	-	4	-	-	322
3	Moelfre	2/71	-	4	-	11	70	13	-	2	-	-	100
		3/73	-	7	-	91	14	-	-	4	-	-	120
4	Bull Bay	2/71	-	6	-	4	41	9	-	7	-	-	67
		5/72	-	2	-	-	15	2	-	-	-	-	19
5a	Cemaes Bay	3/71	-	-	-	6	201	58	-	80	-	-	345
		3/73	-	1	-	4	105	30	-	21	-	-	161
5b	Cemaes Hd.	3/73	-	-	-	-	23	5	-	7	-	-	35
6	Hen Borth	3/71	-	10	3	-	57	-	20	140	-	-	230
		3/73	-	5	-	-	48	-	8	99	-	-	160
7	Porth Swtan	2/71	-	21	3	5	138	-	23	167	-	-	357
		6/71	-	4	-	-	25	-	1	29	-	-	59
		4/72	-	6	1	-	40	1	3	43	-	-	99
		11/72	-	1	-	-	11	-	-	14	-	-	26
		3/73	1	7	-	-	39	-	4	52	-	-	103
8a	Sandy Bay	see	Appendix E										
8b	Sandy Pt.	5/72	-	-	-	2	8	1	1	14	-	-	26
		3/73	-	-	-	-	7	-	-	12	-	-	19
9a	Penrhyn Pt.	4/73	-	-	-	-	12	-	1	24	-	-	37
9b	Penrhyn-mawr	3/71	-	1	-	26	188	4	6	64	-	-	289
		4/73	-	-	-	4	58	-	1	33	-	-	96

Table D (i): L. obtusata contd.

No.	Locality	Date	citrina			olivacea			reticulata			banded	Totals
			c_1	c_2	c_3	ol_1	ol_2	ol_3	r_1	r_2	r_2'		
9c	Penrhyn Bay	4/73	-	-	-	2	35	5	-	10	-	-	52
10a	Causeway	4/71	-	1	-	6	200	6	-	16	-	-	229
		4/73	-	-	-	-	53	-	-	1	-	-	54
10b	Four Mile Bridge	4/73	-	-	-	-	71	-	-	-	-	-	71
11	Holyhead Bay	4/71	-	3	-	2	91	33	-	90	-	-	219
		6/72	-	1	-	-	50	12	-	34	-	-	97
12	North Stack	5/71	-	21	3	-	6	2	-	27	-	-	59
		4/72	-	9	-	-	8	-	-	15	-	-	32
13	Porthdafarch	5/71	-	3	-	-	7	3	2	5	-	-	20
14a	Cliff Hotel	5/71	-	2	-	10	153	-	8	59	-	-	232
		4/73	-	-	-	1	14	-	-	13	-	-	28
14b	Cliff Hotel Pt.	4/73	-	-	-	-	29	-	-	18	-	-	47
15	Trearddur Bay	5/71	-	1	-	18	52	2	10	45	-	-	128
		6/72	-	-	-	3	9	-	1	7	-	-	20
16a	Rhosneigr Bay	5/71	-	2	-	5	55	8	-	26	-	-	96
16b	Rhosneigr Pt.	5/71	-	6	-	3	71	8	4	62	-	-	154
17a	Cywfan Bay	5/71	-	1	-	12	142	-	2	35	-	-	192
		4/73	-	-	-	3	21	-	-	5	-	-	29
18	Llanddwyn	5/71	-	12	-	18	203	8	-	20	-	-	261
		9/72	-	15	-	15	191	10	-	12	-	-	243
19	Ysgubor Isaf	5/71	-	4	-	12	220	4	1	16	-	-	257
20	Caernarvon	5/71	-	13	-	16	158	5	-	13	-	-	205

Table D (i): L. obtusata contd.

No.	Locality	Date	citrina			olivacea			reticulata			banded	Totals
			c_1	c_2	c_3	ol_1	ol_2	ol_3	r_1	r_2	r_2'		
21	Llanfair Hall	5/71	-	14	-	10	168	13	-	4	-	-	209
		9/72	-	9	-	6	141	10	-	3	-	-	169
22	Port Dinorwic Bay	5/71	-	19	-	-	109	5	-	-	-	-	133
23	Port Dinorwic Bay	5/71	-	7	-	-	53	6	-	-	-	-	66
24	Train Bridge	5/71	-	34	-	3	40	1	-	3	-	-	81
		5/72	-	19	-	-	38	-	-	-	-	-	57
		4/73	-	19	-	-	56	-	-	2	-	-	77
25	Menai Bridge	5/71	-	46	2	11	113	5	-	11	-	-	190
		4/72	-	38	-	6	84	4	-	3	-	-	135
		4/73	-	12	1	-	40	1	-	-	-	-	54
26	Navy Station	5/71	-	34	4	4	86	6	-	-	-	-	134
27	Beaumaris	5/71	-	28	5	5	221	3	-	1	-	-	225
28	Llwyngwril	7/71	-	1	-	-	140	18	1	5	1	-	166
29	Llanrhystyd	7/71	-	-	-	-	20	75	-	8	-	-	103
30	Newport	7/71	-	2	-	2	124	16	-	5	-	-	149
31	Dale Fort	7/71	-	10	14	-	35	68	-	72	-	-	199
32	Dale Bay	7/71	-	4	-	-	54	139	-	25	-	-	222
33	Port Eynon	7/71	-	-	-	2	42	110	-	77	-	-	231
34	Barry	7/71	-	-	-	-	13	47	-	3	-	-	63
35	Seawall	7/71	-	-	-	-	-	48	-	-	-	-	48
36	Severn Bridge	7/71	-	-	-	-	30	10	-	-	-	-	40

Table D (i): *L. obtusata* contd.

No.	Locality	Date	citrina			olivacea			reticulata			banded	Totals
			c_1	c_2	c_3	ol_1	ol_2	ol_3	r_1	r_2	r_2'		
37	Weston-Super-Mare	7/71	-	-	-	-	11	70	-	-	7	-	88
38	Lynmouth	7/71	1	26	7	1	22	70	-	90	17	-	234
39	Buck Mills	7/71	-	-	-	-	-	7	-	15	-	-	22
40	Rock	7/71	-	-	-	-	37	3	-	-	-	-	40
41	Looe	7/71	5	194	59	-	10	-	-	400	208	5	881
42	Wembury	7/71	-	8	1	-	3	-	-	37	3	-	52
43a	Dartmouth	7/71	-	6	1	6	52	20	1	-	-	-	86
43b	Bodinnick	5/72	-	12	1	1	25	10	-	5	-	-	54
44	Penzance	4/71	1	3	1	-	-	-	19	63	-	-	87
		5/72	-	1	-	-	2	-	-	21	-	-	24
45	Brow	4/71	-	21	6	-	-	-	8	16	101	-	152
46a	Heathy Pt.	4/71	-	4	-	-	1	-	3	6	40	-	54
		5/72	-	4	-	-	-	-	1	1	29	-	35
46b	Heathy Bay	5/72	-	3	1	-	-	-	-	3	6	-	13
47	Popplestone	4/71	-	30	6	-	-	2	11	50	79	-	181
48	Hangman	4/71	1	31	4	1	-	-	7	26	68	1	139
49	Tean Sd.	4/71	-	39	2	6	-	-	-	25	78	-	150
50	Cow House	4/71	1	5	1	1	-	-	4	11	11	-	34
51	Pernagie	4/71	4	22	6	-	-	2	2	6	93	-	135
52	White No 1	4/71	-	60	10	-	-	-	2	68	65	11	216
53	White No 2	4/71	1	75	12	-	-	-	27	83	94	6	298

Table D (i): L. obtusata contd.

No.	Locality	Date	citrina			olivacea			reticulata			banded	Totals
			c_1	c_2	c_3	ol_1	ol_2	ol_3	r_1	r_2	r_2'		
54	White No 3	4/71	4	38	9	2	-	-	12	19	59	4	147
55	White No 4	4/71	2	78	13	-	-	-	27	33	104	2	259
56	White No 5	4/71	1	19	2	-	-	-	4	27	38	1	92
57	White No 6	4/71	-	15	1	-	-	-	3	2	12	-	33
58	White No 7	4/71	-	1	-	-	-	-	-	-	2	-	3
59	Top Rock Pt.	4/71	8	43	7	1	-	-	21	32	58	1	171
		5/72	-	2	-	-	2	-	-	5	15	-	24
60	Top Rock Bay	4/71	5	36	8	-	-	-	12	10	84	1	156
		5/72	1	27	-	-	4	-	6	15	73	-	126
61	Bull's Porth	4/71	-	8	-	-	-	-	1	4	11	-	24
62	St. Martin's Head	4/71	1	8	2	-	2	-	2	-	76	-	91
63	Old Quay	4/71	1	8	1	10	9	3	8	3	61	-	104
		5/72	-	2	-	-	3	-	1	-	17	-	23
64	Lawrence Bay	4/71	-	5	-	11	11	-	41	21	55	-	144
		5/72	-	2	-	2	-	-	10	11	3	-	28
65	Innisidgen	4/71	9	69	12	6	1	6	31	47	46	1	234
66	Toll's Island	4/71	8	28	6	-	-	-	7	11	71	-	131
		5/72	-	4	-	-	-	-	-	10	30	-	44
67	Hellick	4/71	19	65	13	-	-	-	20	91	54	-	262
68	Porthloo	4/71	-	25	12	2	3	-	6	7	89	-	144
		5/72	-	14	5	-	2	-	3	2	48	-	74
69	Dropnose	4/71	1	39	5	-	-	-	9	66	148	2	270

Table D (i): <u>L. obtusata</u> contd.

No.	Locality	Date	citrina			olivacea			reticulata			banded	Totals
			c_1	c_2	c_3	ol_1	ol_2	ol_3	r_1	r_2	r_2'		
70	Cove	4/71	2	52	6	-	-	-	5	86	44	2	197
71	Periglis	4/71	1	30	2	1	4	-	16	35	49	-	138
72	Killier	4/71	-	24	1	-	16	-	-	33	81	-	160
73	Dunfanaghy	8/72	-	1	-	6	45	12	-	-	-	-	64
74	Curran's Port	8/72	-	13	-	5	3	-	2	24	53	-	100
75	Milkhaven	8/72	-	-	-	5	47	15	-	33	-	-	100
76	Streedagh House Bay	8/72	1	20	22	6	16	6	4	7	43	2	127
77	Stead Bay	8/72	-	9	3	1	10	1	4	14	6	-	48
78	Barnatra	8/72	-	-	-	10	46	17	-	13	-	-	86
79	Tiraun Pt.	8/72	1	5	-	5	30	15	-	-	5	-	61
80	Leckanvy	8/72	-	1	-	15	31	6	-	7	9	-	69
81	Little Killary Bay	8/72	-	-	-	-	10	9	-	54	-	-	73
82	Cloonagh	8/72	-	7	-	9	40	7	-	11	1	-	75
83	Black Head	8/72	-	2	-	9	35	2	1	5	-	-	54
84	Liscannor	8/72	-	10	-	3	9	4	-	6	-	-	32
85	Shot Head	8/72	-	18	-	4	21	6	1	33	9	-	92
86	Adrigole Harbour	8/72	-	2	-	5	14	10	-	1	15	-	47
87	Ballycotton	8/72	-	20	1	3	6	3	6	49	35	-	123
88	Ballynagaul Bay	8/72	-	-	-	1	9	2	-	5	-	-	17

Table D (i): *L. obtusata* contd.

No.	Locality	Date	citrina			olivacea			reticulata			banded	Totals
			c_1	c_2	c_3	ol_1	ol_2	ol_3	r_1	r_2	r_2'		
89	Ballynagaul Pt.	8/72	-	-	-	4	22	15	-	6	-	-	47
90	Bray	8/72	-	-	-	-	4	1	-	1	-	-	6
91	Portrane	8/72	-	-	-	-	50	10	-	8	-	-	68
92	Seascale	9/72	-	-	-	2	25	4	-	5	-	-	36
93	Loch Awun	9/72	-	1	-	8	16	8	-	11	-	-	44
94	Ballyluchan	9/72	-	-	-	-	23	8	-	-	-	-	31

Table D(ii): Distribution of phenotypes in *L. mariae*

No.	Locality	Date	citrina			olivacea			reticulata			banded	Totals
			c_1	c_2	c_3	ol_1	ol_2	ol_3	r_1	r_2	r_2'		
1	Fedwfawr	11/70	-	1	-	-	-	-	20	55	-	-	76
		6/71	-	-	-	-	-	-	3	14	-	-	17
2	Benllech	2/71	-	2	-	-	-	-	4	1	-	-	7
3	Moelfre	2/71	-	110	-	-	-	-	78	1	-	-	189
4	Bull Bay	2/71	-	44	-	-	-	-	16	13	-	-	73
		5/72	-	15	-	-	-	-	4	1	-	-	20
5c	Cemaes Bay	3/71	-	12	-	-	-	-	4	-	-	-	16
		3/73	-	45	-	-	-	-	18	-	-	-	63
5b	Cemaes Hd.	3/73	-	7	-	-	-	-	-	96	-	-	103
6	Hen Borth	3/71	12	7	6	-	-	-	97	10	-	-	132
		3/73(s)*	5	5	3	-	-	-	29	5	-	-	47
		(r)**	-	6	-	-	-	-	1	51	-	-	58
7	Porth Swtan	2/71	1	24	-	-	-	-	13	90	-	-	128
		6/71	3	21	-	-	-	-	13	130	-	-	167
		4/72	-	4	-	-	-	-	1	26	-	-	31
		11/72	-	6	-	-	-	-	-	35	-	-	41
		3/73	-	6	-	-	-	-	-	31	-	-	37
8a	Sandy Bay	11/71	5	30	1	-	-	-	10	40	-	-	86
	(see also Appendix E)	1/72	4	24	1	-	-	-	3	44	-	-	76
		5/72	7	48	1	-	-	-	18	31	-	-	105
		8/72	1	7	-	-	-	-	2	12	-	-	22
		11/72	2	10	-	-	-	-	4	16	-	-	32
		2/73	7	40	2	-	-	-	22	54	-	-	125
		5/73	10	65	2	-	-	-	26	101	-	-	204
		8/73	5	56	3	-	-	-	17	123	-	-	204

* silt substrate
** rock substrate

Table D(ii): *L. mariae* contd.

No.	Locality	Date	citrina			olivacea			reticulata			banded	Totals
			c_1	c_2	c_3	ol_1	ol_2	ol_3	r_1	r_2	r_2'		
8b	Sandy Pt.	5/72	-	3	-	-	-	-	-	75	-	-	78
		3/73	-	1	-	-	-	-	-	49	-	-	50
9a	Penrhyn Pt.	4/73(s)	9	8	3	-	-	-	6	-	-	-	26
		(r)	-	1	-	-	-	-	3	34	-	-	38
9b	Penrhyn mawr	3/71	5	33	-	-	-	-	42	6	-	-	86
		4/73	2	32	-	-	-	-	34	18	-	-	86
9c	Penrhyn Bay	4/73	-	13	-	-	-	-	41	21	-	-	75
10a	Causeway	4/71	-	2	-	-	-	-	6	3	-	-	11
11	Holyhead Bay	4/71	-	1	-	-	-	-	7	-	-	-	8
		6/72	-	13	-	-	-	-	30	-	-	-	43
12	North Stack	5/71	-	16	6	1	7	-	8	101	-	-	139
		9/71	1	8	-	-	-	-	3	52	-	-	64
		4/72	-	5	-	-	-	-	3	49	-	-	57
		4/73	-	11	-	-	-	-	10	53	-	-	74
13	Porth da farch	5/71	-	61	39	-	-	-	49	35	-	-	184
14a	Cliff Hotel	5/71	-	71	-	-	-	-	76	15	-	-	162
		4/73(s)	1	11	-	-	-	-	16	3	-	-	31
		(r)	-	15	-	-	-	-	8	27	-	-	50
14b	Cliff Hotel Pt.	4/73(r)	-	15	1	-	-	-	3	32	-	-	51
15	Trearddur Bay	5/71	-	45	4	-	-	-	53	4	-	-	106
		6/72	-	7	1	-	-	-	23	-	-	-	31
16a	Rhosneigr Bay	5/71	1	33	7	-	1	-	101	9	-	-	151
		4/73(s)	-	22	-	-	-	-	49	5	-	-	76
16b	Rhosneigr Pt.	4/73(r)	-	1	-	-	-	-	1	27	-	-	29

Table D(ii): *L. mariae* contd.

No.	Locality	Date	citrina			olivacea			reticulata			banded	Totals
			c_1	c_2	c_3	ol_1	ol_2	ol_3	r_1	r_2	r_2'		
17a	Cywfan Bay	5/71	2	64	8	-	-	-	29	1	-	-	104
		4/73(s)	-	20	-	-	-	-	15	-	-	-	35
17b	Cywfan Pt.	4/73(r)	-	12	-	-	-	-	7	29	-	-	48
18	Llanddwyn	5/71	-	100	20	-	-	-	2	-	-	-	122
		9/72	-	35	6	-	-	-	1	-	-	-	42
19	Ysgubor Isaf	5/71	-	10	-	-	-	-	-	-	-	-	10
		6/72	-	16	-	-	-	-	-	-	-	-	16
20	Caernarvon	5/71	-	7	-	-	-	-	-	-	-	-	7
21	Llanfair Hall	5/71	-	24	-	-	-	-	-	-	-	-	24
22	Port Dinorwic Pt.	5/71	-	72	48	-	-	-	4	-	-	-	124
23	Port Dinorwic Bay	5/71	-	16	7	-	-	-	-	-	-	-	23
24	Train Bridge	5/71	-	88	20	-	-	-	2	-	-	-	110
		5/72	-	147	30	-	-	-	3	-	-	-	180
		4/73	-	54	12	-	-	-	-	-	-	-	66
25	Menai Bridge	5/71	-	89	18	-	-	-	3	-	-	-	110
		4/72	-	120	4	-	-	-	7	-	-	-	131
26	Navy Station	5/71	-	7	-	-	-	-	-	-	-	-	7
27	Beaumaris	5/71	-	13	-	-	-	-	-	-	-	-	13
28	Llwyngwril	7/71	-	4	-	-	-	-	-	-	-	-	4
29	Llanrhystyd	7/71	-	30	17	-	-	-	-	16	-	-	63
31	Dale Fort	7/71	-	20	35	-	-	-	9	131	-	-	195
32	Dale Bay	7/71	-	15	1	-	-	-	3	-	-	-	19
33	Port Eynon	7/71	-	1	-	-	-	-	-	1	-	-	2
37	Weston super Mare	7/71	-	-	-	-	-	-	-	19	-	-	19
39	Buck Mills	7/71	-	-	-	-	-	-	-	35	-	-	35
41	Looe	7/71	-	14	-	-	-	-	-	55	40	-	109
42	Wembury	7/71	-	2	-	-	-	-	-	67	13	-	82

Table D(ii): *L. mariae* contd.

No.	Locality	Date	citrina			olivacea			reticulata			banded	Totals
			c_1	c_2	c_3	ol_1	ol_2	ol_2	r_1	r_2	r_2'		
43b	Bodinnick	5/72	1	10	1	2	-	-	2	3	-	-	19
44	Penzance	4/71	-	3	2	-	-	-	-	3	-	-	8
		5/72	-	23	1	-	-	-	-	-	-	-	24
45	Brow	4/71	8	60	-	2	-	2	16	4	4	3	99
46a	Heathy Pt.	4/71	2	7	1	-	-	-	1	2	39	-	52
		5/72	-	6	-	-	-	-	-	-	21	-	27
46b	Heathy Bay	5/72	-	35	-	3	-	-	1	-	-	-	39
47	Popplestone	4/71	-	63	-	1	1	-	34	-	-	-	99
48	Hangman	4/71	5	3	-	-	-	-	2	-	-	-	10
50	Cow House	4/71	2	5	-	-	-	-	3	-	-	-	10
51	Pernagie	4/71	-	-	-	-	-	-	-	-	5	-	5
53	White No.2	4/71	-	5	-	-	-	-	-	-	3	-	8
55	White No.4	4/71	-	3	-	-	-	-	3	-	-	-	6
56	White No.5	4/71	5	7	-	-	-	-	2	-	-	1	15
57	White No.6	4/71	4	5	-	-	-	-	1	-	-	-	10
58	White No.7	4/71	-	5	-	-	-	-	-	-	40	-	45
59	Top Rock Pt.	4/71	10	22	-	-	-	-	-	3	41	-	76
		5/72	1	4	-	-	-	-	-	-	30	-	35
60	Top Rock Bay	4/71	20	84	-	-	-	-	32	6	13	-	155
		5/72	-	7	-	-	-	-	-	-	2	-	9
61	Bull's Porth	4/71	3	34	-	-	-	1	8	3	104	-	153
		5/72	-	6	-	-	-	-	1	2	19	-	28
62	St. Martin's Hd.	4/71	-	3	-	-	-	-	2	9	30	-	44

Table D(ii): *L. mariae* contd.

No.	Locality	Date	citrina			olivacea			reticulata			banded	Totals
			c_1	c_2	c_3	ol_1	ol_2	ol_3	r_1	r_2	r_2'		
63	Old Quay	4/71	3	5	-	-	-	-	16	-	-	-	24
		5/72	-	4	-	-	-	-	4	-	14	-	22
64	Lawrence Bay	4/71	-	4	-	1	2	-	6	2	-	-	15
		5/72	2	1	1	-	2	-	5	-	-	-	11
65	Innisidgen	4/71	9	7	-	-	-	-	4	-	-	-	20
66	Toll's Island	4/71	-	2	-	-	-	-	-	-	8	-	10
		5/72	-	1	-	-	-	-	1	-	10	-	12
67	Hellick	4/71	13	14	-	-	-	-	3	8	6	-	44
68	Porthloo	4/71	1	5	-	2	-	-	2	-	-	-	10
		5/72	2	12	-	1	-	1	10	2	1	-	29
69	Dropnore	4/71	3	6	-	-	-	-	-	-	7	-	16
70	Cove	4/71	2	-	-	-	-	-	-	-	-	-	2
72	Killier	4/71	-	2	-	-	-	-	-	-	-	-	2
74	Curran's Port	8/72	2	31	5	-	-	-	9	4	95	-	146
75	Milkhaven	8/72	3	123	8	-	-	-	-	-	-	-	134
76	Streedagh House Bay	8/72	1	9	17	-	-	-	3	4	25	-	59
77	Staad Bay	8/72	-	8	5	-	-	-	3	2	64	2	84
78	Barnatra	8/72	2	30	-	-	-	-	-	-	-	-	32
79	Tiraun Pt.	8/72	2	19	-	-	-	-	10	17	-	-	48
81	Little Killary Bay	8/72	3	6	-	-	-	-	-	-	-	-	9
82	Cloonagh	8/72	-	3	4	-	-	-	24	9	-	-	40
83	Black Head	8/72	1	3	3	-	-	-	6	31	3	-	47
85	Shot Head	8/72	1	10	1	-	-	-	13	73	4	6	108

Table D(ii): Distribution of phenotypes in *L. mariae*

No.	Locality	Date	citrina			olivacea			reticulata			banded	Totals
			c_1	c_2	c_3	ol_1	ol_2	ol_3	r_1	r_2	r_2'		
87	Ballycotton	8/72	-	-	-	-	-	-	-	8	18	-	26
88	Ballynagaul Bay	8/72	-	1	3	-	-	-	-	-	-	-	4
89	Ballynagaul Pt.	8/72	2	19	-	-	-	-	11	8	2	-	42
90	Bray	8/72	-	12	8	-	1	-	-	69	-	-	90
91	Portrane	8/72	1	15	3	1	-	-	2	7	2	-	31

APPENDIX E

RESULTS FROM INTENSIVE SAMPLING AT SANDY BAY, ANGLESEY BETWEEN NOVEMBER 1971 AND AUGUST 1973.

Collections at this locality were made at 3 month intervals over a period of 2 years. In each case, a sample was obtained from the upper, middle and lower portions on the shore providing a comparison between individuals inhabiting different species of algae, namely F. spiralis and F. vesiculosus, Ascophyllum, and F. serratus (see Plate 2 for sampling positions). As all of these samples have been made with the use of a net (see Section II, 2), no visual bias will be present which could affect the observed phenotype frequencies of the more conspicuous shells.

In the following series of tables, raw numbers and frequencies of the different phenotypes are shown in relation to algal species for juvenile* and adult L. obtusata in addition to length distributions of juveniles and adults (Fig. E). Table E (iv) gives the frequencies of the phenotypes in L. obtusata and L. mariae in a transect taken from the top to the bottom of the shore in April 1972. Table E (v) shows the frequencies of phenotypes in adult L. mariae over the two years (Appendix D table (ii) shows raw data).

*Since the juveniles cannot be reliably scored on sizes less than 4 mm, only individuals greater than this size have been used.

Table E (i): Results for *L. obtusata*

Date	Position	Age	Sex	citrina	olivacea	reticulata light	reticulata dark
2/11/71	*F. spiralis*	Adult	male	5	60	4	56
			female	8	70	8	71
		Juvenile	male	1	22	3	24
			female	3	30	2	50
			?*	5	21	1	39
	Ascophyllum	Adult	male	3	19	3	28
			female	3	21	5	37
		Juvenile	male	1	13	2	23
			female	1	18	1	23
			?	4	55	6	65
	F. serratus	Adult	male	-	2	1	7
			female	-	3	-	7
		Juvenile	male	1	4	1	5
			female	1	7	-	12
			?	2	11	1	34
29/1/72	*F. spiralis*	Adult	male	4	31	1	31
			female	4	39	2	36
		Juvenile	male	5	33	1	28
			female	3	58	-	46
			?	5	22	3	36

* individuals less than 4mm in size

Table E (i): *L. obtusata* contd.

Date	Position	Age	Sex	citrina	olivacea	reticulata light	reticulata dark
	Ascophyllum	Adult	male	-	24	-	8
			female	-	20	-	13
		Juvenile	male	2	8	1	11
			female	4	16	2	17
			?	3	97	7	65
	F. serratus	Adult	male	1	11	-	17
			female	3	10	-	12
		Juvenile	male	3	7	-	4
			female	1	6	-	9
10/5/72	*F. spiralis*	Adult	male	2	52	-	20
			female	2	32	-	23
		Juvenile	male	2	43	4	36
			female	6	60	4	43
	Ascophyllum	Adult	male	4	25	2	25
			female	2	42	4	36
		Juvenile	male	-	32	1	35
			female	6	58	12	48
			?	6	15	2	43
	F. serratus	Adult	male	2	9	-	25
			female	2	8	1	12
		Juvenile	male	2	4	-	5
			female	5	6	-	13
			?	8	-	3	2

Table E (i): <u>L. obtusata</u> contd.

Date	Position	Age	Sex	citrina	olivacea	reticulata light	reticulata dark
1/8/72	<u>F. spiralis</u>	Adult	male	7	55	4	33
			female	6	35	2	24
		Juvenile	male	1	30	10	25
			female	5	34	11	19
			?	37	48	3	29
	<u>Ascophyllum</u>	Adult	male	1	2	-	3
			female	-	3	-	1
		Juvenile	male	1	4	-	3
			female	-	7	2	10
			?	12	29	3	35
	<u>F. serratus</u>	Adult	male	-	2	-	8
			female	-	2	-	8
		Juvenile	male	1	2	-	3
			female	-	2	-	4
			?	3	3	2	3
19/11/72	<u>F. spiralis</u>	Adult	male	4	30	-	18
			female	3	26	1	21
		Juvenile	male	2	13	-	13
			female	5	26	2	18
			?	20	78	8	66
	<u>Ascophyllum</u>	Adult	male	4	26	1	19
			female	5	33	2	34

Table E (i): *L. obtusata* contd.

Date	Position	Age	Sex	citrina	olivacea	reticulata light	reticulata dark
		Juvenile	male	-	4	-	16
			female	1	23	3	36
			?	12	88	3	62
	F. serratus	Adult	male	1	13	1	26
			female	1	10	-	19
		Juvenile	male	2	15	-	7
			female	-	5	1	8
			?	4	7	-	11
17/2/73	*F. spiralis*	Adult	male	2	29	4	17
			female	2	35	3	17
		Juvenile	male	-	20	2	21
			female	-	33	2	21
			?	26	86	1	81
	Ascophyllum	Adult	male	1	40	-	21
			female	6	35	4	19
		Juvenile	male	2	24	3	25
			female	2	34	1	29
			?	19	93	13	115
	F. serratus	Adult	male	2	31	5	21
			female	3	18	1	24
		Juvenile	male	-	8	1	10
			female	1	13	1	17
			?	4	7	1	20

Table E (i): *L. obtusata* contd.

Date	Position	Age	Sex	citrina	olivacea	reticulata light	reticulata dark
13/5/73	*F. spiralis*	Adult	male	2	24	4	13
			female	2	23	6	9
		Juvenile	male	0	16	2	10
			female	0	20	0	14
			?	-	9	-	15
	Ascophyllum	Adult	male	2	12	1	7
			female	-	10	3	4
		Juvenile	male	1	14	2	12
			female	-	24	-	22
			?	-	35	-	33
	F. serratus	Adult	male	-	10	3	28
			female	-	12	3	20
		Juvenile	male	-	9	-	6
			female	-	5	1	7
			?	-	7	-	5
1/8/73	*F. spiralis*	Adult	male	6	35	5	27
			female	5	60	3	26
		Juvenile	male	2	34	6	33
			female	4	49	2	45
			?	16	50	6	47

Table E (i): L. obtusata contd.

Date	Position	Age	Sex	citrina	olivacea	reticulata light	reticulata dark
1/8/73	Ascophyllum	Adult	male	1	10	1	6
			female	-	10	-	14
		Juvenile	male	-	10	-	13
			female	1	13	1	14
			?	2	29	2	13
	F. serratus	Adult	male	-	7	-	10
			female	1	9	-	11
		Juvenile	male	-	3	-	7
			female	-	5	-	6
			?	-	3	-	4

Table E (ii): Phenotype frequencies in adult *L. obtusata* between November 1971 and August 1973.

Date	Shore Position	N	citrina	olivacea	reticulata light	reticulata dark
19/11/72	*F. spiralis*	282	4.8	46.1	4.3	44.8
	Ascophyllum	119	5.0	33.6	6.7	54.7
	F. serratus	20	0.0	25.0	5.0	70.0
29/1/72	*F. spiralis*	144	5.6	48.6	2.1	46.7
	Ascophyllum	65	0.0	67.7	0.0	32.3
	F. serratus	54	7.4	38.9	0.0	53.7
10/5/72	*F. spiralis*	131	3.1	64.1	0.0	32.8
	Ascophyllum	140	4.3	47.9	4.3	43.5
	F. serratus	59	6.8	28.8	1.7	62.7
1/8/72	*F. spiralis*	166	7.8	54.2	3.6	34.4
	Ascophyllum	10	10.0	50.0	0.0	40.0
	F. serratus	20	0.0	20.0	0.0	80.0
19/11/72	*F. spiralis*	103	6.8	54.4	1.0	37.8
	Ascophyllum	124	7.3	47.6	2.9	42.7
	F. serratus	71	1.4	32.3	1.4	64.9
17/2/73	*F. spiralis*	109	3.7	58.7	6.4	31.2
	Ascophyllum	126	5.6	59.5	3.2	31.7
	F. serratus	105	4.8	46.7	5.7	42.8
13/5/73	*F. spiralis*	83	4.8	56.6	12.0	26.6
	Ascophyllum	39	5.1	56.4	10.3	28.2
	F. serratus	84	0.0	35.7	7.1	57.2
1/8/73	*F. spiralis*	167	6.6	56.9	4.8	31.7
	Ascophyllum	42	2.4	47.6	2.4	47.6
	F. serratus	38	2.6	42.1	0.0	55.3

Table E (iii): Phenotype frequencies in juvenile *L. obtusata* between November 1971 and August 1973.

Date	Shore Position	N	citrina	olivacea	reticulata light	reticulata dark
2/11/71	*F. spiralis*	135	3.0	38.5	3.7	54.8
	Ascophyllum	82	2.4	37.8	3.7	56.1
	F. serratus	31	6.5	35.5	3.2	54.8
29/1/72	*F. spiralis*	173	4.6	52.0	0.6	42.8
	Ascophyllum	61	9.8	39.3	4.9	45.9
	F. serratus	30	13.3	43.3	0.0	43.3
10/5/72	*F. spiralis*	198	4.0	52.0	4.0	40.0
	Ascophyllum	192	3.1	46.9	6.8	43.2
	F. serratus	35	20.0	28.6	0.0	51.4
1/8/72	*F. spiralis*	135	4.4	47.4	15.6	32.6
	Ascophyllum	27	3.7	40.7	7.4	48.2
	F. serratus	12	8.3	33.3	0.0	58.4
19/11/72	*F. spiralis*	79	8.9	49.4	2.5	39.2
	Ascophyllum	83	1.2	32.5	3.6	62.7
	F. serratus	28	7.1	35.7	3.6	53.6
17/2/73	*F. spiralis*	99	0.0	53.5	4.0	42.5
	Ascophyllum	120	3.3	48.3	3.3	45.1
	F. serratus	51	2.0	41.1	3.9	53.0
13/5/73	*F. spiralis*	62	0.0	58.1	3.2	38.7
	Ascophyllum	75	1.3	50.7	2.7	45.3
	F. serratus	28	0.0	50.0	3.6	46.4
1/8/73	*Ascophyllum*	52	1.9	44.2	1.9	52.0
	F. spiralis	175	3.4	47.4	4.7	44.5
	F. serratus	21	0.0	38.1	0.0	61.9

Table E (iv): Phenotype frequencies in adult *L. obtusata* and *L. mariae* on transect from upper to lower shore positions at Sandy Bay on 4/72.

Littorina spp	Species of algae	Shore Position	N	citrina	olivacea	reticulata
L. obtusata	*F. spiralis*	a(HWM)	587	8.2	50.8	41.0
		b	183	6.0	49.7	44.3
		c	361	8.8	49.5	41.7
	F. vesiculosus	d	289	4.5	49.5	46.0
		e	224	3.6	52.7	43.7
		f	460	5.2	44.2	50.6
		g	159	4.8	48.5	46.7
		h	84	8.4	43.4	48.2
		i	122	9.8	33.6	56.6
	Ascophyllum	j	272	9.0	67.9	23.1
		k	248	6.5	53.8	39.7
		l	323	4.9	50.6	44.5
		m	274	4.0	49.8	46.2
		n	534	5.0	47.8	47.2
		o	243	9.9	48.6	41.5
	F. serratus	p	233	1.7	43.8	54.5
		q	305	5.3	43.3	51.4
		r	194	5.4	45.4	49.1
		s	370	5.4	37.8	56.8
		t	145	4.1	40.0	55.9
		u(LWM)	130	3.9	42.3	53.8
L. mariae	*F. serratus*	p	249	40.2	-	59.8
		q	152	46.1	-	53.9
		r	158	48.7	-	51.3
		s	193	47.4	-	52.6
		t	92	45.0	-	55.0
		u(LWM)	117	44.3	-	55.7

Figure E

Figure E

Distribution of shell sizes in *L. obtusata* on *F. spiralis.* juveniles (small dots); adults (large dots).

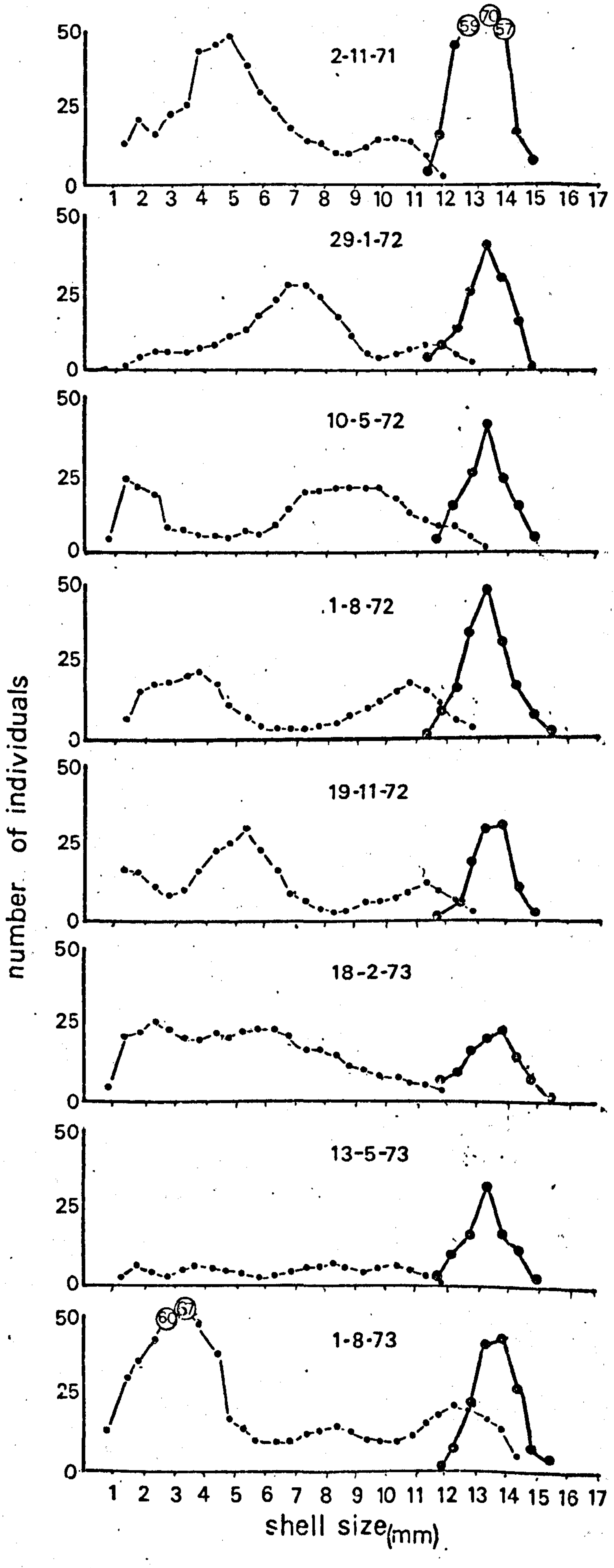

50
25
0
2-11-71
59
70
57
29-1-72
10-5-72
1-8-72
19-11-72
18-2-73
13-5-73
1-8-73
60
57
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
number of individuals
shell size(mm)

Table E (v) Phenotype frequencies in adult L. *mariae* between November 1971 and August 1973.

Date	N	citrina	reticulata light	reticulata dark
2/11/71	86	41.9	11.6	46.5
29/1/72	76	25.0	15.8	59.2
10/5/72	105	53.3	17.1	29.6
1/8/72	22	36.4	9.1	54.5
19/11/72	32	37.5	12.5	50.0
17/2/73	125	39.5	17.6	42.9
13/5/73	204	37.7	11.8	50.5
1/8/73	204	32.4	8.3	59.3

APPENDIX F

Results for microdistribution studies in *L. mariae*

The following tables (Fi & Fii) show the raw data for the position measurements on *F. serratus* of juveniles and adults and the chi-squared analysis between the size classes. Table Fiii gives the stem-leaf comparisons of the size classes. This data has been summarized in Fig. 58.

Table F(i) Relationship of shell size and phenotype in *L. mariae* to microdistribution on *F. serratus*

Locality	Size of Shell in mm.	Pheno-type	Position on *F. serratus**										
			0-0.09	0.10-0.19	0.20-0.29	0.30-0.39	0.40-0.49	0.50-0.59	0.60-0.69	0.70-0.79	0.80-0.89	0.90-1.0	Total
Menai Bridge	adult	cit.	0	0	3	3	7	9	12	15	34	56	139
	(5-10)	ret.	-	-	-	-	-	-	-	-	-	-	0
	Juv.**	cit.	0	3	1	2	13	8	15	27	47	47	163
	(1-5)	ret.	-	-	-	-	-	-	-	-	-	-	0
Cemaes Bay	adult	cit.	1	0	0	1	0	0	1	2	9	10	24
	(5-10)	ret.	0	0	0	0	0	0	0	0	2	1	3
	Juv.	cit.	4	0	1	4	2	1	3	6	13	23	57
	(1-5)	ret.	0	0	0	0	0	0	0	0	1	1	2
Rhos-neigr	adult	cit.	0	0	0	0	0	2	3	8	8	21	42
	(5-10)	ret.	0	0	0	1	2	5	5	11	15	29	68
Sandy Bay	adult	cit.	0	0	0	2	4	6	6	13	47	64	142
	(10-15)	ret.	0	0	1	3	5	7	19	22	54	96	207
	Juv.	cit.	0	0	2	1	3	2	1	10	7	15	41
	(5-10)	ret.	0	1	1	3	4	2	5	11	24	24	75
	Juv.	cit.	0	1	1	0	1	1	3	2	7	12	28
	(1-5)	ret.	3	10	6	4	2	2	6	5	10	17	65
Hen Borth	adult	cit.	0	0	0	0	0	1	1	3	3	12	20
	(10-15)	ret.	0	0	1	3	3	3	15	18	21	43	107
Porth Swtan	adult	cit.	0	1	1	2	3	5	14	43	45	110	224
	(10-15)	ret.	0	5	5	6	10	24	54	118	225	303	750
	Juv.	cit.	0	0	1	3	1	2	1	2	3	3	16
	(1-5)	ret.	0	4	4	6	2	1	2	4	6	8	37

Table F(i) contd.

Locality	Size of Shell in mm.	Pheno-type	Position on *F. serratus**										
			0-0.09	0.10-0.19	0.20-0.29	0.30-0.39	0.40-0.49	0.50-0.59	0.60-0.69	0.70-0.79	0.80-0.89	0.90-1.0	Total
North Stack	adult	cit.	0	0	0	0	0	0	0	0	1	1	2
	(10-15)	ret.	0	0	0	0	1	2	0	1	8	8	20
	Juv.	cit.	-	-	-	-	-	-	-	-	-	-	0
	(5-10)	ret.	0	0	1	1	1	0	1	3	7	7	21
	Juv.	cit.	-	-	-	-	-	-	-	-	-	-	0
	(1-5)	ret.	0	0	6	3	3	7	2	2	8	9	40
Cemaes Hd.	adult	cit.	0	0	1	0	0	0	0	0	0	4	5
	(10-15)	ret.	0	1	1	1	0	5	7	3	20	48	86
	Juv.	cit.	0	1	0	0	1	0	0	2	4	7	15
	(5-10)	ret.	0	1	2	1	10	9	13	9	15	18	78
	Juv.	cit.	0	0	1	1	1	1	1	0	2	1	8
	(1-5)	ret.	0	2	9	21	17	11	13	11	12	12	108
Sandy Pt.	adult	cit.	0	0	0	0	0	0	4	2	5	5	16
	(10-15)	ret.	0	1	2	1	0	3	5	10	22	26	70
	Juv.	cit.	0	0	1	2	0	0	5	4	2	2	16
	(5-10)	ret.	0	2	2	2	3	3	1	6	13	14	46
	Juv.	cit.	0	2	1	2	4	0	2	2	3	2	18
	(1-5)	ret.	5	13	15	15	12	13	5	5	9	11	103

* Values near 0 represent the dark-brown stem while those near 1 are the olive-brown or olive-yellow leaf.

** Juv. - Juvenile

Table F(ii) χ^2 analysis of relation between shell size in *L. mariae* and microdistribution on *F. serratus* (from Table F(i). In localities where two chi-squared values are given, the first is between adults and intermediates while the second is between adults and juveniles.

Locality	Shell Size	Position on *F. serratus*				χ^2	P
		0-0.49	0.50-0.69	0.70-0.89	0.90-1.0		
Menai Bridge	Adults	13	21	49	56	5.21 (3)	0.10 - 0.25
	juveniles	19	23	74	47		
Cemaes Bay	Adults	2	1	13	11	0.05 (1)	0.25 - 0.50
	juveniles	11	4	20	24		
Sandy Bay	Adults	15	38	136	160	14.27 (3)	< 0.001
	intermediate	15	10	52	39	57.75 (3)	< 0.001
	juveniles	28	12	24	29		
Porth Swtan	Adults	33	97	431	413	51.15 (3)	< 0.001
	juveniles	11	6	15	11		

Table F(ii) *L. mariae* contd.

Locality	Shell Size	Position on *F. serratus*				χ^2	P
		0-0.49	0.50-0.69	0.70-0.89	0.90-1.0		
North Stack	Adults	1	2	10	9	0.04 (1)	0.50 - 0.90
	intermediate	3	1	10	7	1.53 (1)	0.25 - 0.50
	juveniles	12	9	10	9		
Cemaes Hd.	Adults	4	12	23	52	20.51 (3)	< 0.001
	intermediate	16	22	30	25	59.23 (3)	< 0.001
	juveniles	42	26	25	13		
Sandy Pt.	Adults	4	12	39	31	8.61 (3)	0.025 - 0.05
	intermediate	12	9	25	16	62.10 (3)	< 0.001
	juveniles	59	20	19	13		

Table F(iii) χ^2 or Fisher's Exact test analysis of stem/leaf position between juvenile and adult *L. mariae*. Two chi-squared values at some localities show adult vs intermediate and adult vs juvenile respectively.

Locality	Shell Size	POSITION		$\chi^2_{(1)}$	P
		stem	leaf		
Menai Bridge	Adults	3	136	-	0.52 (EXACT)
	juveniles	6	163		
Cemaes Bay	Adults	1	26	-	1.00 (EXACT)
	juveniles	4	55		
Sandy Bay	Adults	23	326	4.09	0.025 - 0.05
	intermediate	15	99	49.64	<0.001
	juveniles	32	61		
Porth Swtan	Adults	118	856	59.34	<0.001
	juveniles	27	26		
North Stack	Adults	2	20	2.49	0.10 - 0.25
	intermediate	7	14	5.96	0.01 - 0.025
	juveniles	17	23		
Cemaes Hd.	Adults	11	80	26.20	<0.001
	intermediate	44	48	64.68	<0.001
	juveniles	80	36		
Sandy Pt.	Adults	17	69	0.02	0.50 - 0.90
	intermediate	14	58	60.38	<0.001
	juveniles	85	26		

APPENDIX G

Observations on the orientation of *L. mariae* in relation to camouflage.

During the studies on the microdistribution between phenotypes, I initially felt that the direction which a snail was pointed might provide some indication of what part of the plant the individual would eventually move to. If citrina, found on the stem, were orientated towards the leaf and dark reticulata, on the leaf, orientated towards the stem, this might suggest some behavioral preference for the different habitats. Many of the individuals, regardless of their orientation, did not move at all during the time of observations (up to 3 hours at low tide and 1 hour at high tide while diving). Those individuals , however, which did move often did so with a circular path or in a straight path backwards and forwards. From both of these observations, it was clear that the orientation could not be used as an indication of potential position.

The observations which were made are, however, useful because they show that in resting position, both phenotypes orientate towards the base of the plant along the axis of the frond. The resting orientation was confirmed during diving observation. The reason for this orientation towards the base is probably due to the influence of current. Guiterman (1971) has shown that there is least hydrodynamic force on shells of L. obtusata which are pointed in the direction of an oncoming current. On the plants of F. serratus, current flow is generally from the holdfast to the leaf tip so it is not unexpected that individuals orientate themselves towards the base along the frond axis.

Although dark reticulata is a very cryptic phenotype on or near the base of the plant, the crypsis is enhanced when the individual is orientated with the longitudinal axis of the frond. The main reason for this is that the reticulate pattern on this phenotype is often a series of dark lines which run perpendicular to the outer lip (Plate 16); when the shell is orientated along the axis of the frond, this pattern acts to break up the outline of the shell. This would offer an explanation of why the shells, especially the juveniles, are not uniformly dark brown. If individuals were randomly orientated then one might expect a uniform colour but since in resting position they are found mainly pointed along the frond axis, probably in response to current, the actual "reticulate" pattern may be itself at an advantage.

There is a great deal of variation between localities in the expression and pattern of the reticulata phenotype. These minor variations do not appear to affect the basic visual properties of the shell and therefore might be considered "inconsequential noise" owing to developmental processes or in the genetic background. It is equally possible, however, that these minor variations reflect subtle differences in the camouflage owing to behavioural and habitat differences. Studies on the orientation may provide some understanding of these phenomena.

APPENDIX H

Observations on a polymorphism in the body colour of *L. mariae* and *L. obtusata*.

There is a polymorphism in the body colour in L. mariae and L. obtusata in which the head, tentacles and dorsal part of the foot can be either pale yellow or black. When the animal is stationary these characters cannot be seen owing to the thick nature of the shell; it was for this naive reason that very little attention was paid to the occurrence and variation of the body pigmentation in the course of the study. In view of the observations which were made while diving it became apparent that about 30% of the individuals were in fact moving on the algal fronds at which time the colouration of the body was visible and could potentially, therefore, affect the crypsis of each shell colour on different backgrounds. Although very little quantitative data was collected, it is possible to make some generalizations concerning the body colour.

In broadest terms, there is a positive correlation between light coloured shells (i.e. citrina & light reticulata) and unpigmented body and between dark coloured shells (olivacea, dark reticulata) and a pigmented body. There would appear to be a very strong linkage between citrina and an absence of pigmentation, for of approximately 7000 individuals of this phenotype examined, only one individual had a black body. Among the dark coloured shells, no such relationship is evident, for approximately 50% of these individuals can have a pale body.

It is highly likely that the body colour is an inherited trait and not merely a phenotypic one in response to some environmental factor. In the breeding experiments which were done for shell colour (section VI), it was observed that all the citrina progeny, from matings involving citrina x citrina, citrina x dark reticulata, and dark reticulata x dark reticulata had pale yellow bodies. The reticulata, on the other hand, were either pale yellow, intermediate or dark in colour. In the crosses involving dark reticulata x citrina (dark x pale body) about $\frac{1}{2}$ of the reticulata were pale and the other $\frac{1}{2}$ pigmented. In two crosses of dark reticulata x dark reticulata, both having dark bodies, the progeny were predominantly dark bodied. Unfortunately all of these crosses were lost in a fire before they could be properly scored for body colour. It was possible, therefore, to gain only a general impression of how the colouration was assorting among the different matings. The data do not provide any information of dominance relationships but they do suggest that the presence or absence of pigmentation is an inherited character.

If one regards the body colour as a response to selection for crypsis, the basic relationship of shell and body colour can be partially understood. It was first observed in the laboratory that when citrina and dark reticulata were placed on a stem of _F. serratus_, not only were citrina far more conspicuous but also their movement was much more evident than that of dark reticulata. This was because the black tentacles of dark reticulata could not be readily seen on the dark-brown background while the pale yellow tentacles of citrina accentuated the already conspicuous shell colour. The same was true for a dark reticulata which had a pale yellow rather than black body. When citrina was placed on the leaf of the plant and viewed with transmitted light it was quite evident that the pale yellow body was distinctly advantageous for camouflage when the animal was moving. Among dark reticulata placed in this position, the presence of the pigmented body accentuates the conspicuousness when the animal is moving. However, when these are compared to the same phenotype with a pale body, the movement in these latter individuals is as difficult to detect as in citrina. The question arises, therefore, of what is the significance of the variation in body colour among the dark reticulata. If a predator such as the blenny is able to detect the tentacle movement, then one might expect some relationship of body colour to the background. To determine if this was the case a small number of observations were made at 4 localities in Anglesey, where dark reticulata were abundant and showed the two morphs of body colour. The procedure was to provide a comparison of the stem and leaf positions of the two morphs.

The results of these observations, shown in the following table demonstrate that in 2 of the 4 localities, the individuals with the black bodies are significantly more common on the stem of the plant. In the other two localities, one shows a similar trend, while the other shows no difference. Therefore, at least in two cases, the pattern of distribution is the same as would be expected if selection for crypsis has occurred. If these cursory observations are of any relevance to other populations or for themselves, there is potentially a number of further studies which could be undertaken. The major limitations to the small amount of data presented here are that the observations were made only at one point in time, and only on adult individuals. Also, nothing is known of the geographical variation and only little of the inheritance. Future studies may elucidate the genetic interaction and ultimately the nature of the variation.

Table H. Relationship between body colour in _L. mariae_ and plant position.

Locality	Species	Body colour	Position stem	 leaf	$\chi^2_{(1)}$
Sandy Bay	_mariae_	light	20	47	10.45
		dark	13	4	
Hen Borth	_mariae_	light	17	42	2.74
		dark	23	27	
Porth Swtan	_mariae_	light	28	62	13.51
		dark	34	19	
Sandy Pt.	_mariae_	light	6	14	0.00
		dark	11	27	

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