FACTORS AFFECTING GENE-FREQUENCIES IN SOME POPULATIONS OF *CEPAEA*

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Thesis submitted for the degree of Doctor of Philosophy in the University of Oxford.

"From my experience in collecting the snails, I should not be able to deny a certain apparent tendency to mimicry, if it were asserted; but I should hardly dare assert it. In one locality, quite protected from the light, there seemed to be a preponderance of darker shells and fused bands; among the honeysuckle undergrowth full-banded varieties were more abundant and not easy to see; in more than one locality among yellow leaves, the yellow bandless variety, or 00300, was common. It may have been imagination, but the idea of mimicry suggested itself".

HOWE, J.L. 1898: American Naturalist.

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ABSTRACT OF THESIS

This thesis describes a number of studies which are concerned with the genetics of natural populations of the polymorphic land snails of the genus Cepaea Held.

Chapter 1 reports the results of breeding experiments with Cepaea hortensis (Müll.) and Cepaea nemoralis (L.). The mode of inheritance of some of the principal characters has been determined. In C.hortensis the dominance of pink to yellow shell colour is confirmed, and dark yellow is shown to be dominant to light yellow. The dominance of unbanded to banded is confirmed. This locus is probably linked to shell colour. Full pigmentation, partial pigmentation and absence of pigmentation of the bands form an allelic series with a dominance hierarchy decreasing in that order. Fusion of bands is probably multifactorially controlled. Brown shell colour is associated with infertility.

In C.nemoralis it is shown that the gene for absence of pigmentation of the bands may prevent the expression of genes controlling colour of the shell. Reddish body colour is dominant to gray.

Chapter 2 describes a survey of populations of C. nemoralis on sand dunes at Berrow in Somerset. The distribution of phenotypes at the present day is compared with that found in 1926 by Captain C.Diver and Professor A.E.Boycott. Clines

in phenotype-frequency have persisted from 1926 to the present, but there have been consistent and significant changes in the proportions of two of the principal forms. The proportion of dark browns has nearly halved, and the proportion of 00300 has doubled since 1926. The changes are interpreted as the results of natural selection.

In Chapter 3 the data for the frequencies of dark brown and 00300 in the Berrow collections have been used to calculate the selective value of these forms between 1926 and 1959-60. Assuming a generation time of three years, the dark brown phenotype has been at an overall disadvantage of 6.2% per generation, while the 00300 phenotype has enjoyed an overall selective advantage of 5.2%. Heterogeneity among the estimates of the selection coefficient of 00300 suggests that there are differences in the selective value of this phenotype in different parts of the area studied.

In Chapter 4 the influence of the mating system on the effective population size of C.nemoralis is discussed. C.nemoralis is hermaphrodite with obligatory cross-fertilisation. Sperm from several matings may be stored for more than a year. Breeding experiments show that the mean number of matings contributing to each brood is approximately two. Multiple mating and sperm storage serve to minimise the effects of population fluctuations and of increased variance of brood-size, which might otherwise reduce the effective size of populations.

Chapter 5 is an analysis of the structure of a population of C.nemoralis at Wittenham Clumps, Berks. The standard deviation of dispersal per generation is estimated as 15 metres, and the area of the "neighbourhood" as 1180 sq. metres. Population density declined during the three years' study from about 2 per sq. metre to 0.25 per sq. metre. Predation in the colony is at a maximum in May and June. Differential selection by visual predators has been demonstrated. Thrushes tend to select more of the pink and brown snails; mammals select more yellow snails.

Chapter 6 describes the populations of C.nemoralis inhabiting the Isles of Scilly. Although the populations of different islands are geographically isolated, they are, with few exceptions, phenotypically homogeneous. In comparison with populations from the Oxford region they appear to fall largely within a single habitat group. Strong evidence of natural selection in at least one population is provided by continuing disturbances of the Hardy-Weinberg equilibrium.

PREFACE

With the exception of Chapters 2 and 3, each part of this thesis is a separate study and is complete in itself. The work in Chapters 2 and 3 was carried out in collaboration with Mr.B.C.Clarke, and the division has been introduced to clarify our separate responsibilities. Although we have co-operated at every stage of the work, Mr. Clarke is primarily responsible for the form of Chapter 2. An earlier version was included as Chapter VI in a thesis entitled "Some factors affecting shell colour polymorphism in Cepaea" submitted by him for the degree of Doctor of Philosophy in the University of Oxford. On the other hand, Chapter 3 is entirely my own work.

I have included acknowledgements at the end of each chapter, but I should like to express my gratitude especially to Dr.A.J.Cain and Dr.P.M.Sheppard for their constant help and encouragement. I should also like to thank Professor Sir Alister Hardy, F.R.S., and Dr.E.B.Ford, F.R.S., for the facilities which they have provided in the Department of Zoology, Oxford, where this work was carried out during the tenure of a National Science Predoctoral Fellowship.

INTRODUCTION

Land snails of the genus Cepaea Held provide abundant material for the study of the genetics of natural populations. The remarkable polymorphisms of these animals embrace a wide variety of phenotypes, and the relative frequencies of the different forms vary widely from population to population.

Two species of the genus are found in the British Isles, Cepaea nemoralis (L.) and Cepaea hortensis (Müll.). Superficially the two are very similar in appearance, exhibiting a wide range of parallel variations (Taylor, 1914). Since in exceptional cases viable hybrids may be produced (Lang, 1908), the species are very closely related; but in the wild they behave as good biological species. They frequently occur in mixed populations with no indication of introgression.

The search for an explanation of the mechanism by which the frequencies of forms are controlled has produced a number of detailed studies of both species. The differences of interpretation of these studies is as remarkable as the polymorphism itself. Diver (1940) has suggested that the different proportions of the forms result from the random fluctuations of gene frequencies, Lamotte (1951) has postulated very high mutation rates, Schilder and Schilder (1957) have emphasized the importance of gene flow, and Cain and Sheppard (1950, 1954) have concluded that the controlling factor is natural selection. More

recently the importance of natural selection has been accepted by most workers although there is still disagreement about the relative power of different components (Lamotte, 1959). There are even some authors who still regard all variation in Cepaea as random (Van Valen, 1960).

The studies described in this thesis were begun with the object of investigating the forces affecting gene-frequencies in populations of Cepaea. All the field work was carried out with C.nemoralis. Since I am indebted to other workers for the indispensable knowledge of the genetics of C.nemoralis, I have attempted to repay the debt by providing information on the genetics of C.hortensis. The results of the breeding program are described in Chapter 1.

In collaboration with Mr. B.C.Clarke, I have studied the populations of C.nemoralis on the dunes at Berrow, Somerset. Through the kindness of Captain C.Diver, who generously allowed us to use his records, we were able to compare the frequencies of forms in these populations after an interval of 33 years. These studies, which have enabled us to make estimates of the magnitude of natural selection, are described in Chapters 2 and 3.

The mating system of C.nemoralis is discussed in Chapter 4. I have attempted to determine the extent to which multiple mating and sperm storage influence the effective population size and counteract the consequences of reduced mobility.

Chapter 5 reports a detailed study of a colony of C.

nemoralis at Wittenham Clumps, Berks. I have estimated population parameters by capture-recapture methods. The principal differences between this and other studies of Cepaea populations were that:

1. Animals were individually marked.

2. Animals were not displaced when marked.

3. The colony was observed at frequent intervals over a relatively long period.

Finally, Chapter 6 describes the populations of C.nemoralis in the Isles of Scilly. Since the populations of different islands are geographically isolated from one another, they provide an opportunity of discovering whether populations of Cepaea diverge in isolation.

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CHAPTER 1. THE INHERITANCE OF SOME CHARACTERS IN *CEPAEA*.

Chapter 1

THE INHERITANCE OF SOME CHARACTERS IN CEPAEA

1. INTRODUCTION

In recent years the remarkable polymorphism of snails of the genus Cepaea Held has continued to attract considerable attention. The variation from place to place in the proportions of the different phenotypes has proved to be useful material for the study of evolution in natural populations (Cain and Sheppard, 1950, 1954; Clarke, 1960; Lamotte, 1951, 1959; Schnetter, 1951; Sheppard, 1951). The foundation of such population studies must always be a knowledge of the mode of inheritance of the characters of the polymorphism.

Since most of the field work has been carried out with Cepaea nemoralis (L.), it is natural that relatively more is known of the genetics of this species (Lang, 1908, 1911, 1912; Lamotte, 1951, 1954; Cain and Sheppard, 1957; Cain, King and Sheppard, 1960). Recently, however, Lamotte (1959) and Clarke (1960) have discussed the relative distribution of phenotypes in mixed populations of Cepaea nemoralis (L.) and Cepaea hortensis (Müller). Their work has served to point out that our knowledge of the genetics controlling the polymorphism is much more meagre in C.hortensis than in C.nemoralis.

2. PREVIOUS STUDIES

Cepaea hortensis has the distinction of having provided one of the first demonstrations that the laws of Mendelian inheritance apply to animals as well as to plants (but see Bateson and Saunders,

1902). Lang (1904) had already begun his pioneering researches on Cepaea at the time of the rediscovery of Mendel's experiments, and he pointed out that the inheritance of banding in C.hortensis could be explained in a similar manner.

Lang was not the first to undertake the breeding of C.hortensis. There are indications of the mode of inheritance of banding in the work of Seibert (1876), who found that banded parents produced only banded offspring, and of Brockmeier (1888), who obtained a banded individual from the offspring of an unbanded snail. However, it was Lang who first understood the Mendelian nature of the inheritance. In his first paper on the breeding of hortensis (1904) he established the important points (1) that Cepaea cannot self-fertilize and (2) that sperm from several matings can be stored for long periods without losing its visibility. By means of both backcross and F_2 generations, Lang showed that the unbanded, 00000, condition is dominant to the five-banded, 12345, condition. In only one individual (a 12045) did the banded offspring over 7 mm in diameter fail to develop all five bands. Lang suggested that pink is dominant to yellow, on the evidence of a mating of a pink with a yellow that produced all pink offspring. His matings also demonstrated the heritability of the tendency to fusion of bands.

In a later paper (1906), Lang showed that in C.hortensis the five-banded pattern may be recessive to 10345. His mating of two animals of the pattern 12045 suggests that this form is not determined by a single Mendelian gene.

More recently Boettger (1950) has stated that the hyalozonate

condition (var. arenicola MacGillivray) is recessive to normal pigmentation of the bands, but unfortunately he does not give any data. He also claims that the series of band formulae (12345), (123)(45), (12)3(45), (12)345 and 12345 form an allelic series with each form dominant to all those which follow it. Such an explanation is inconsistent with the data of Lang (1904) (and see below).

3. METHODS

Breeding stocks were either collected from wild populations or taken from the offspring of matings in progress. In most cases the animals were isolated well before the completion of the shell. They were therefore unfertilized prior to the intended mating. A few adult animals from the wild have been used (see Table 1) and these may have produced some young from previous matings (Lang, 1904; Murray, this thesis). Where possible the offspring of the virgin parent have been separated from those of the animal collected as an adult.

Each breeding pair was placed in an 8-inch flower pot half filled with earth and covered with a sheet of glass. The animals were fed on lettuce and occasionally on carrot. Algae growing on the walls of the pot served to supplement the diet. Chalk was always available. Eggs laid in the pots were usually allowed to hatch there. In 1961, however, many of the clutches were removed to rearing boxes before hatching.

The young snails were reared in plastic boxes. Damp paper in the bottom served to maintain a moist atmosphere. The snails were fed on lettuce, oatmeal and powdered chalk.

The scoring of individual characters is discussed in the appropriate sections below. Each brood was scored when the animals in the brood had reached a size at which the character in question could be distinguished. Consequently the size at scoring differed from brood to brood.

4. RESULTS

The parents and offspring of 29 matings of Cepaea hortensis are shown in Table 1. In addition, Table 2 shows five matings of Cepaea nemoralis with the resulting offspring. The hortensis matings provide information on the inheritance of shell colour, banding, pigmentation of bands and fusion of bands. The nemoralis matings demonstrate an epistatic effect of the hyalozonate condition on shell colour and produce some information on the inheritance of body colour and of an unusual band-formula.

5. MATINGS WITH CEPAEA HORTENSIS

a. Shell Colour:

Successful matings have been achieved with three of the principal shell colours: dark yellow, pale yellow and pink. The dark yellow shells are a vivid yellow (var.lutea Picard). If the periostracum is removed, the underlying calcareous layers are seen to be distinctly yellow. In the pale yellow shells (vars.alba Picard and flavovirens Picard), any yellow colour which may be present is restricted entirely to the periostracum. The distinction is therefore made on the ground colour of the calcareous material. The pink shells of matings 37, 38

Table 1. Cepaea hortensis: a list of matings and their progeny.

Key to provenance of parental stocks.

AW = Avebury, Wilts.

BO = Begbroke, Oxon.

BS = Berrow, Somerset.

BW = Burderop Down, Wilts.

CB = Cothill, Berks.

FB = Faringdon, Berks.

IB = Ickford, Bucks.

IS = Isles of Scilly.

SW = Silbury Hill, Wilts.

WB = Wittenham Clumps, Berks.

YW = Yatesbury, Wilts.

Key to phenotypes.

Colour:

P = Pink.

DY = Dark yellow

PY = Pale yellow.

Y = Yellow, depth uncertain.

Banding:

00000 = unbanded

00300 = midbanded.

12345 = five-banded (all five bands present in adults unless otherwise stated).

Fusions not scored.

Other symbols:

ad = adult when collected, possibly not virgin.

ar = var. *arenicola*, bands unpigmented.

lu = var. *lurida*, bands partially pigmented.

TABLE 1

MATING NUMBER	PROVENANCE	PARENTS	PROGENY		REMARKS
1	FB	DY 00000	DY 00000	12	
	FB	DY 00000	Y 12345	8	
3	FB	DY 00000	DY 00000	26	
	FB	DY 00000	PY 00000	11	
			Y 12345	11	
			Y 12345ar	1	
6	FB	PY 00000	PY 00000	43	
	FB	PY 00000	Y 12345ar	14	
10	FB	PY 00000	PY 00000	11	
	FB	PY 00000			
11	FB	DY 00000	DY 00000	2	
	FB	PY 00000	PY 00000	6	
			Y 12345	5	
12	FB	DY 00000	DY 00000	12	
	FB	PY 00000	PY 00000	8	
			Y 12345ar	2	
13	FB	DY 00000	DY 00000	6	
	FB	PY 00000	Y 12345	7	
14	FB	DY 00000	PY 00000	1	
	FB	PY 00000	Y 12345	1	
15	FB	DY 00000	DY 00000	63	
	FB	PY 00000			
16	FB	Y 12345ar	Y 12345ar	7	12045-1
	FB	Y 12345ar			
17	FB	Y 12345	Y 12345	5	
	FB	Y 12345ar	Y 12345ar	3	
18	FB	Y 12345	Y 12345	9	
	FB	Y 12345ar	Y 12345ar	7	
20	FB	Y 12345	Y 12345	72	
	FB	Y 12345ar			

Table 1 (contd.)

MATING NUMBER	PROVENANCE	PARENTS	PROGENY		REMARKS
28	FB	Y 12345	Y 12345	15	
	SW	Y 12345lu			
29	AW	adP 12345	P 12345	10	Prog.of either parent
	FB	Y 12345	Y 12345	4	
			P 12345	15	Prog. of Y 12345
			Y 12345	12	
30	AW	adP 12345	P 12345	6	Prog. of P12345
	FB	Y 12345	Y 12345	4	
			P 12345	13	Prog.of Y 12345
			Y 12345	17	
34	FB	Y 12345	Y 12345	77	
	FB	Y 12345	Y 12345ar	23	
35	IB	adBY 00000	DY 00000	12	Prog.of either parent
	FB	Y 12345ar	Y 12345	7	
			DY 00000	4	Prog.of Y 12345
			Y 12345	2	
37	CB	P 00000	P 00000	1	
	FB	Y 10345	Y 12345	2	
38	CB	P 00000	P 00000	4	
	FB	Y 12345	Y 12345	2	
39	CB	P 00000	P 00000	19	
	YW	P 00000	DY 00000	4	
42	Prog. 20	Y 12345	Y 12345	43	
	Prog. 28	Y 12345	Y 12345lu	15	
45	Prog. 15	DY 00000	DY 00000	38	
	FB	PY 00000	PY 00000	23	
46	Prog. 29	P 12345	P 00000	20	
	Prog. 3	DY 00000	DY 00000	22	
50	Prog. 28	Y 12345	Y 12345	5	
	Prog. 28	Y 12345	Y 12345lu	2	

Table 1 (contd.)

MATING NUMBER	PROVENANCE	PARENTS	PROGENY		REMARKS
51	Prog. 28	Y 12345	Y 12345	7	
	Prog. 16	Y 12345ar	Y 123451u	12	
52	Prog. 20	Y 12345	Y 12345	14	
	Prog. 16	Y 12045ar	Y 12345ar	12	
53	Prog. 15	DY 00000	DY 00000	11	
	Prog. 6	FY 00000	FY 00000	21	
			Y 12345	5	
54	Prog. 15	DY 00000	DY 00000	2	
	Prog. 6	FY 00000	FY 00000	4	
			Y 12345ar	2	

TABLE 2

MATING NUMBER	PROVENANCE	PARENTS	PROGENY		REMARKS
31	WB	Y 12345	P 00300	66	
	BO	w 00300hz	P 12345	54	
43	IS	adP 12300	P 00300	31	
			P 12345	37	
47	Prog. 31	P 00300	Y 00300	8	Probably prog. of Y 12345 only
	BS	adY 12345hz	Y 12345	11	
48	Prog. 31	P 00300	w 00300hz	1	
	Prog. 31	P 00300			
59	BW	adY 12345gb	Y 12345rb	26	
			Y 12345gb	39	

Table 2. Cepaea nemoralis: a list of matings and their progeny.
Key as for Table 1 with additions.

gb = gray body.
rb = reddish body.
hz = hyalozonate.
w = colourless shell.

and 39 are clear, rosy pinks (var. incarnata Picard), but the banded pinks of matings 29, 30 and 46 have a decidedly dusky cast.

Among the initial matings, numbers 1 to 15 demonstrate that the yellow colours segregate, with dark yellow dominant to light yellow. Ignoring the banded offspring for the moment, the pale yellow/pale yellow matings (6 and 10) produce only pale offspring, while dark yellow/dark yellow (1 and 3) and dark yellow/pale yellow (11-15) produce either all dark yellows (1, 13 and 15) or both dark and pale yellows (3, 11 and 12). An exception is number 14, where the single unbanded offspring is pale. Three test crosses (45, 53 and 54) of the dark yellow F_1 offspring of mating 15 segregate for dark and light yellow.

Unfortunately I have been unable to distinguish light and dark yellows among the banded offspring. The effect of banding seems to be to lighten the ground colour of the shell in all cases. Occasionally a flush of yellow can be seen in the calcareous layer below the periostracum, but its appearance is not constant enough to be scored with confidence.

It is therefore not possible directly to observe linkage between the yellow colours and banding. By analogy with C.nemoralis, however, it is to be expected that the loci for colour and banding are linked; and there is some indirect evidence in this case. If the loci are unlinked, then in mating 53 (and to a lesser extent in mating 54) there is a deficiency of dark yellows which is just short of significance ($\chi^2_{(1)} = 3.125$). If in this case the loci for colour and banding are completely linked in repulsion in the dark yellow

parent then the deficiency is negligible (P between 30 and 50%). One other mating is suggestive. In number 3, the expectation with random assortment is 9 dark yellow unbandeds to 3 pale yellow unbandeds to 4 bandeds. With complete linkage in coupling in both parents, no pale yellow unbandeds are expected. Either with linkage in repulsion in both parents or with linkage in coupling in one and repulsion in the other, the expectation is 2 dark yellow unbandeds to 1 pale yellow unbanded to 1 banded. The agreement of the observed proportions with both the 9:3:4 and the 2:1:1 ratios is nearly perfect (P between 80 and 90% for both); but of the two, the 2:1:1 ratio is a slightly closer fit ($\chi^2_{(2)}$ = 0.234 against 0.398). Therefore there is some evidence to suggest linkage between yellow and banding.

Matings 29, 30, 37, 38, 39 and 46 demonstrate that pink and yellow segregate, and number 39 shows that pink is dominant. (The pink parent of mating 46 may be the progeny of the pink parent of mating 29. Since the latter was collected as an adult, the genotype of its offspring cannot be determined with absolute certainty.) These matings confirm the conclusions of Lang (1904).

Matings 37 and 38 suggest linkage between colour and banding, but more offspring are needed before this hypothesis can be considered proved.

Seven matings were begun to investigate the inheritance of brown shells (var. olivacea Taylor). Unfortunately, these have completely failed to produce offspring.

b. Banding:

Since the dominance of the unbanded to the five-banded type in C.hortensis was clearly demonstrated by Lang (1904), no special effort was made to investigate this point. Lang's results are abundantly confirmed, however, in many of the present matings (e.g. 1, 3 and 46). Only one variant of the full five-banded condition has appeared among the offspring, a 12045 in mating 16. Lang (1904) records a similar single occurrence in a brood of 43, and he has presented evidence (1906) which indicates that the expression of the 12045 phenotype is not dependent on a single gene.

c. Pigmentation of Bands:

The inheritance of two modifications of the normal chocolate-brown bands has been investigated. In the first (var. arenicola MacGillivray) the bands are devoid of all pigmentation. In the second (var. lurida Moq.) the pigment is dilute, and the bands are of a reddish-brown colour, intermediate between those of arenicola and of the normal type.

Matings 16, 20, 34 and 52 show conclusively that completely unpigmented bands are recessive to pigmented bands. Partial pigmentation of the bands is also controlled by a single gene and is recessive to full pigmentation (matings 28 and 50).

Two matings suggest that the three grades of band pigmentation may be controlled by an allelic series of three genes. Number 42 is a cross of two normally banded animals, one heterozygous for the gene for dilute pigmentation and one heterozygous for the gene for absence

of pigmentation. The offspring are 3:1 normal and dilute. Again, mating 51 shows a 1:1 segregation of animals with normal and dilute pigmentation resulting from a cross of a dilute/normal heterozygote with a homozygote for absence of pigment.

There is, of course, the alternative explanation that both of the animals carrying the gene for pigmentlessness also carried genes for dilute pigmentation. The parent in number 42 would have to be a heterozygote and that in number 51 a homozygote for the dilute condition. In the latter case it would also be necessary for both parents in mating 16 at least to be heterozygous for this gene. These contingencies seem to be rather unlikely, particularly as the gene for dilute pigmentation is not common in the populations from which these stocks were taken. The four parents of matings 16 and 20, three of which would have had to carry the gene, were taken from two collections of 371 (103 banded) and 137 (88 banded) neither of which contained a single specimen with dilute pigmentation of the bands.

I have discussed the point in some detail since a case of complementation with similar phenotypes has been found in C.nemoralis (Dr.A.J.Cain, personal communication). An animal with pigmentless bands mated with one with dilute pigmentation produced offspring with dark, fully pigmented bands. If the hortensis genes do indeed form an allelic series, then this would be the first case in which the genetic systems controlling shell polymorphism in the two species have been found to differ. On the other hand it would seem, by analogy

with Drosophila, altogether reasonable for there to be genes with similar effects at more than one locus in each species. In this case we may expect to find at other loci in each species other genes to reestablish the genetic homology of the two species.

d. Fusions:

Some information on the inheritance of coalescence of bands is provided by these matings. Mating 20 is the most useful example. Although neither parent shows any tendency towards fusion, several of the offspring have fused bands.

In order to score the condition of fusion at the same stage in development of each shell, the band-formulae were scored at the point where the shells reached $4\frac{1}{2}$ whorls. No shell was scored which had not completed $4\frac{1}{2}$ whorls. The use of this convention reduces the variation which would be introduced by differences in the time of lip formation, and avoids the scoring of fusions which take effect just before the lip.

The scores for mating 20 are:

12345 - 27

123(45) - 1

12(34)5 - 2

1(2345) - 1

They indicate that the inheritance of fusions does not depend on the segregation of an allelic series of major genes. Together with the results of Lang (1904), they suggest that fusion of bands is

multifactorially controlled in C.hortensis, as it is in C.nemoralis (Cain, King and Sheppard, 1960).

6. MATINGS WITH CEPAEA NEMORALIS

a. Band Pigmentation and Shell Colour:

Mating 31 demonstrates an epistatic effect on shell colour by a gene producing pigmentless or hyalozonate bands. The hyalozonate parent is phenotypically cream-coloured because of its yellowish periostracum, but the underlying calcareous layer is quite white. The offspring of a mating of this animal with a yellow, normally-banded animal are without exception pink, indicating that the cream-coloured animal was genetically a homozygous pink. Mating 47, a backcross using an adult hyalozonate, produced no offspring attributable to the progeny of number 31; but the single offspring of number 48, an F_2, again showed the colourless shell with hyalozonate banding.

The epistasis is interesting because animals are known with pigmentless bands on a pink ground colour (var. lateritia Dumont and Mortillet). An effect of this kind might well explain some, though not all, of the associations between pigmentless bands and colour of shell noted by Cain and Sheppard (1954).

b. 12300:

"Mating" 43 records the offspring of an animal of unusual band-formula. It was collected as an adult so that the other parent (or parents) of the progeny is unknown. It is not yet known whether the type 12300 is heritable, but if it is, then it is likely to be recessive to 12345 and 00300.

c. Body Colour:

The offspring of "mating" 59 are also derived from a single animal taken as an adult from the wild. This brood shows a 1:1 segregation of animals with the normal gray bodies and with the less common reddish bodies. No other reddish offspring were found in 34 other families obtained from as many parents from the same colony. Since this mating is apparently a backcross and since the gene for reddish body is uncommon, it is almost certain that this gene is dominant to that for gray body. Both colours vary in intensity of pigmentation. The lightest gray animals are almost white.

Cain and Sheppard (1952) have discussed the inheritance of body shade in nemoralis. They conclude that body shade is heritable and is probably multifactorially controlled. They recognized the occurrence of different body colours but scored them all against neutral shades. Therefore the occurrence of genes segregating for body colour is compatible with their results.

7. GENE NOMENCLATURE

As knowledge of the genetic basis of the polymorphism in Cepaea has increased, it has become necessary to develop a consistent system of nomenclature which is capable of incorporating new discoveries without changes in existing symbols. Cain and Currey (in preparation) have suggested such a system for Cepaea nemoralis. Each locus is represented by a symbol, and each allele at that locus is indicated by an appropriate superscript.

The polymorphisms of Cepaea nemoralis and C.hortensis parallel

one another to a remarkable degree. Although the frequency of similar forms in the two species is often very different, almost all of the forms are common to both species. Indeed in crosses between the species, the genes behave as if they were homologous, and dominance relationships are undisturbed (Lang, 1904, 1908).

It seems to be desirable, therefore, to adopt for C.hortensis the nomenclature proposed for C.memoralis. The genes which have been investigated for C.hortensis can be symbolised in the following manner.

a. Ground colour of the shell: G

G^{P} = Pink

G^{DY} = Dark Yellow

G^{PY} = Light Yellow

The dominance decreases in the order listed.

b. Banding: B

B^{O} = Unbanded

B^{B} = Banded

Unbanded is dominant to banded.

c. Pigmentation of bands and lip together: P

P^{B} = Dark brown or black bands and lip

P^{L} = Partial pigmentation of bands and lip (var. lurida Moq.)

P^{T} = Transparent or hyalozonate bands and white lip (var. arenicola MacGillivray)

The dominance decreases in the order listed. P^{L} has not been

described for C.nemoralis; it is not homologous with orange bands (Cain, King and Sheppard, 1960). In nemoralis, the hyalozonate condition affects the colour of the bands and the lip, both of which are normally dark. Another (non-allelic) gene (Dr. A.J.Cain, personal communication) produces a white lip only. Since what is probably the homologue of the latter gene (Lang, 1904, 1908) is the "normal" allele in hortensis, the P locus produces a visible effect only on the pigmentation of the bands. In hortensis with dark lips, the P alleles may be expected to affect the colour of both lip and bands.

For C.nemoralis a new symbol is proposed:

a. Dermal pigmentation, or colour of the body and foot: D

D^R = Reddish body colour

D^G = Gray body colour

Reddish body is dominant to gray body.

8. CONSISTENCY OF RESULTS

The segregations for colour, banding and pigmentation of bands have been tested for agreement with the expected Mendelian ratios and for homogeneity among broods. Mather (1951) has described a convenient method for a joint comparison of backcross and F_2 segregations. In general, the results are satisfactory.

The segregations for pigmentation of bands show no significant departures from expectation and no heterogeneity among the broods. The overall agreement is good.

Among the matings segregating for banded and unbanded, there is more variation. One of the F_2 segregations, number 13, differs significantly from a 3:1 ratio ($\chi^2_{(1)} = 5.77$). The overall agreement, however, is good (P lies between 70 and 80%), and the heterogeneity among broods fails to reach the 5% level of significance.

There are two groups of segregations for colour to be tested. Those for pink and yellow show no significant departures from expectation and no heterogeneity among broods. The dark yellow/light yellow segregations also show no significant departures and no heterogeneity, but the deviations are considerably greater. If only the backcrosses are considered, then there is heterogeneity at the 5% level of significance. It is unlikely that the heterogeneity is due to differential viability, since the deviations are in different directions and the overall agreement is good. The most likely explanation is that linkage with banding has prevented the complete scoring of some of the broods (see above).

There is, therefore, no evidence of differential fertility or viability of different phenotypes in those matings which produced offspring. Of course, the tests of heterogeneity do not exclude the possibility of viability differences in the matings tested. Differences which would be of considerable importance in determining the proportions of phenotypes in wild populations could pass undetected in broods having the numbers of offspring so far obtained. It will be necessary to raise more offspring before the question can be satisfactorily answered.

One class of matings was particularly unsuccessful. At the same time that matings 1 to 15 were begun with young yellow animals, 7 matings were also established in which at least one member was a young brown animal. Nine of the 15 matings of yellows produced young, but not a single mating with browns produced any young at all. The deficiency of successful matings with browns is significant at the 5% level (Armsen, 1955). If reduced fertility is responsible for this deficiency, then it could be a contributing factor to the lowering of the frequency of browns in natural populations and to the difference of response of the two species of Cepaea to the action of natural selection, Clarke, 1960).

SUMMARY

1. In Cepaea hortensis:

a. The dominance of pink to yellow shell colour is confirmed.

b. Dark yellow shell colour is dominant to light yellow.

c. The dominance of unbanded to banded is confirmed. This locus is probably linked to that for shell colour.

d. Full pigmentation, partial pigmentation and absence of pigmentation of the bands form an allelic series with a dominance hierarchy decreasing in that order.

e. Fusion of bands is probably multifactorially controlled.

f. Brown shell colour is associated with infertility.

2. In Cepaea nemoralis:

a. In some cases, hyalozonate banding suppresses the expression of the genes for ground colour of the shell.

b. Reddish body colour is dominant to gray.

3. A system of gene nomenclature is proposed in accordance with that of Cain and Currey.

4. The agreement of the segregations with expected Mendelian ratios is satisfactory.

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CHAPTER 2. CHANGES OF GENE-FREQUENCY IN *CEPAEA NEMORALIS* (L.).

Chapter 2

CHANGES OF GENE-FREQUENCY IN CEPAEA NEMORALIS (L.)

With Bryan Clarke

1. INTRODUCTION

It has not often been possible to observe the spread or decline of individual genes in natural populations. Seasonal fluctuations in the proportions of various inherited characters have been observed by Timoféef-Ressovsky (1940) in Adalia, by Gershenson (1945) in Cricetus, and by Dobzhansky (1943, 1948), Dubinin and Tiniakov (1945, 1946), and Carson and Stalker (1949) in Drosophila. Changes in the frequency of genotypes over longer periods have been reported by Fisher and Ford (1947) and Sheppard (1951a, 1956) in Panaxia, by Kettlewell (1958) in Biston, by Komai (1956) in Harmonia, by Dobzhansky (1958) in Drosophila, and by Schnetter (1951) and Goodhart (1956, 1958) in Cepaea. In most of these cases the action of natural selection has been inferred, but only in the studies on Panaxia has it been possible to obtain a precise estimate of the magnitude of the selective forces acting upon an individual Mendelian population. Allison (1954) has also made such an estimate in his studies of human sickle-cell polymorphism, but it was calculated for a static population.

In 1926, Captain C.Diver and the late Professor A.E. Boycott made very detailed and extensive collections from a

large population (or series of contiguous populations) of the polymorphic land snail Cepaea nemoralis (L.) on the sand dunes at Berrow, in Somerset. Captain Diver has very kindly allowed us to make use of their data, which have never before been published. This has given us the unique opportunity of studying in detail the changes that have taken place in a natural population during a period of about eleven generations.

2. DESCRIPTION OF THE SAMPLING AREA

The sand dunes at Berrow, Somerset, run for three miles south from the village of Brean towards Burnham-on-Sea. The dunes border directly on mud flats that stretch about two miles out to sea at low tide. At Brean, and for about a mile and a half south of it the dunes are covered by caravan sites, holiday camps and bungalows.

The collections described in this paper were taken in an area between Berrow Church (National Grid Reference S.T. 293524) and a point about two thousand yards north of it. The width of the dunes (east to west) varies from about three hundred yards in the northern part of this area to about six hundred yards in the south. In the southern part of the area, the dips between the sandhills are occupied by the greens and fairways of the Burnham Golf Course.

The dunes can be divided into a number of components. There is a sea ridge of mobile or semi-mobile dunes which are quite low, rarely exceeding fifteen feet in height. Behind

this, to the east, there is a long dip or series of slacks. To the east of the dip there is the main ridge of more stable dunes, some of which are sixty feet or more in height. Beyond the main ridge are more slacks bordering the coast road. On the other side of the road are houses and low-lying fields.

In the southern part of the area, the slacks east and west of the main ridge are occupied by the short cultivated turf of the golf course.

Boley (1944) has described the vegetation of the area. The sea ridge is mainly moving sand, partly covered by marram grass (Ammophila arenaria (L.) . In some places, however, there are patches of short turf and clumps of Iris foetidissima L.

The main ridge is largely covered by short turf, with some marram, and a scattering of other plants including Senecio, Ranunculus, Trifolium, Lotus, Rubus, Cynoglossum, Hedera, Oenothera, Taraxacum, and clumps of Iris foetidissima. Large areas of the main ridge have become overgrown with dense patches of sea-buckthorn (Hippophaë rhamnoides L.). Some of the hollows between the dunes have standing water for a part of the year. Rushes (Juncus sp.) and horsetails (Equisetum sp.) occur within them.

Cepaea nemoralis is common almost everywhere on the main ridge except in the dense patches of sea-buckthorn. The snails are particularly numerous in and around clumps of Iris. A single

clump, two or three feet in diameter, may contain as many as a hundred snails.

Boycott and Diver reported that the sea-ridge was completely bare of C.nemoralis. We have found, however, that there are a number of flourishing colonies, particularly in the more stable parts. They may have become established since 1926.

In addition to C.nemoralis, the snail fauna of the dunes includes Helix aspersa Müll. which is extremely common, and Helicella virgata (Da Costa), Helicella caperata (Montagu), and Cochlicella acuta (Müll.) which are sparsely distributed. Cepaea hortensis (Müll.) occurs along the edge of the coast road but does not extend on to the dunes.

The existence of many broken shells shows that both C.nemoralis and H.aspersa are frequently attacked by birds and small mammals. Rabbits seem to be common throughout the area, and song-thrushes (Turdus ericetorum Turton) are often seen, particularly in the patches of buckthorn.

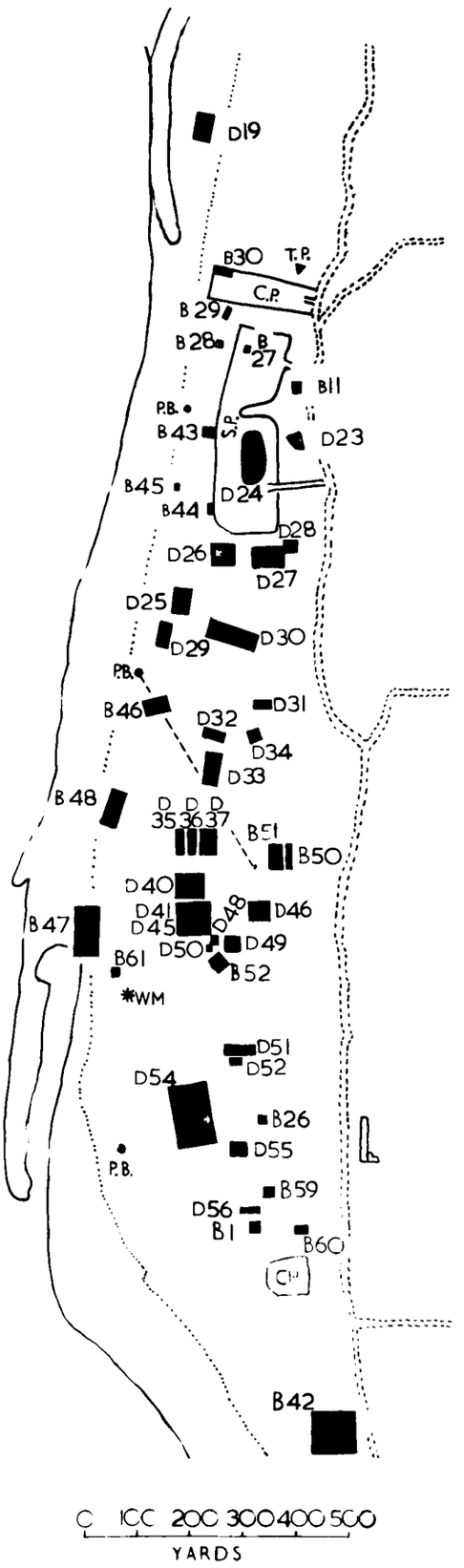
3. METHODS

(i) Mapping.

Figure 1 is a sketch map of the area drawn to scale from the Ordnance Survey 25 in. map. The collecting places are marked in black and are also drawn to scale. Those bearing the prefix D are localities previously sampled by Boycott and Diver. Those prefixed B are new localities sampled only by us.

Boycott and Diver recorded their samples with careful

Figure 1. Sketch map of the sand dunes at Berrow, Somerset. The solid black patches represent collecting areas. They are drawn to scale. The prefix D indicates a locality sampled by Boycott and Diver. The prefix B indicates a locality sampled by us alone. Double dotted lines represent roads. The continuous line shows the approximate high water mark. CH = churchyard; C.P. = car park; P.B. = pill box; S.P. = sand-pit; T.P. = trig.point; WM = windmill.


D19
B30
T.P.
C.P.
B 29
B 28
B 27
B11
P.B.
B 43
S.P.
D23
B45
D24
B44
D28
D26
D27
D25
D29
D30
P.B.
B46
D32
D31
D34
D33
B 48
D D D
35 36 37
B51
B50
D40
D41
D45
D48
D46
B47
D50
D49
B61
B52
*WM
D51
D52
D54
B26
D55
P.B.
B59
D56
B1
B60
CH
B42
C 100 200 300 400 500
YARDS

descriptions of the places from which they were taken and also marked them upon a 6 in. Ordnance Survey Map. We have established the position of our samples in a similar manner, and in addition, we have pinpointed them by taking bearings on fixed points with a prismatic compass. In the course of this work it became apparent that the Ordnance Survey map was inaccurate. This disturbing fact made it necessary for us completely to resurvey the whole area. When we had made our map, we found that part of the area had also been resurveyed by the Somerset County Council. There were negligible differences between the two maps.

It has now been possible to establish with a reasonable degree of accuracy, the position of most of Boycott and Diver's collections. We believe that in most cases the error is not greater than fifteen yards.

(ii) Sampling.

Random samples of C.nemoralis were collected during 1959 and 1960 from forty localities, twenty of which had previously been sampled by Boycott and Diver. Eighteen localities studied by the earlier workers were not sampled by us, either because they had become overgrown with buckthorn, or because snails were otherwise scarce. During sampling each collecting area was searched carefully to avoid taking too high a proportion of snails that were obvious to the eye.

(iii) Scoring of Samples.

The snails were scored for maturity, condition, colour and banding according to the criteria given by Cain and Sheppard

(1950, 1954) and Clarke (1960). There is one way in which these collections differ remarkably from those described by Cain and Sheppard. A number of the samples contain shells that are very pale yellowish-brown in colour, usually with a darker brown apex. They are quite distinct from the normal dark brown forms of nemoralis, which also occur at Berrow, and they have been recorded as a separate class (Pale Brown).

Each of us (JM and BC) scored the samples independently, and then compared our results. The differences were slight. There were none in the scoring of 00000, 00300 or dark browns. In a few samples, however, we differed in distinguishing between some of the pinks and pale browns. In one sample (B30) the difference of scoring amounted to 6% of the total number of snails scored, in four samples (B1, D26, B26, B27) it was about 3.5%, in one (D48) about 2.5%, and in two (B29, D31) about 1.5%. Because of these differences, pale browns have not been analysed in detail.

In only two samples (B29, B47) were there any differences in the scoring of yellow shells (0.5% and 2.5% respectively). In B29 the difference was due to one shell, which was doubtfully yellow or pale brown. In B47 two shells were doubtfully yellow or pink.

There were considerable differences in the scoring of 10345 (as distinct from 12345). These were due to the occurrence, in many shells, of very faint traces of the second band on the

upper whorls when it was absent on the lower ones. The frequencies of 10345 have not been analysed in detail.

4. RESULTS

Table 1 records the phenotypes of the 12,658 live adult snails collected at Berrow (7030 by Boycott and Diver, 5628 by us). Details of the phenotypes of young snails, and of dead shells, will be reported in a later publication. In Table 1, the collecting localities are arranged in geographical order, from north to south. Successive samples from the same locality are arranged in chronological order. The classification of phenotypes (vertical columns) is straightforward except for the columns marked 00345*. The figures in these columns refer not only to 00345 but also to 00045 and 00005. In the table, pale and interrupted bands are not distinguished from the normal full bands.

At a number of localities, our samples differ significantly from those taken by Boycott and Diver. An overall comparison of the two sets of samples shows that these differences are too great to be the result of random errors in sampling ($p < 0.001$, for both colour and banding). At eight localities (B29, B28, B43, D24, B44, B45, D52 and D54) we ourselves have taken more than one sample. The sum of χ^2 for differences between our successive samples is not significant for colour ($\chi^2_{(10)} = 5.09$, $p > 0.8$), but is significant for banding ($\chi^2_{(10)} = 19.24$, $p < 0.05$). The latter high value of χ^2 is almost entirely due to one locality (B45), which

Table 1. The composition of samples of *Cepaea nemoralis* collected at Berrow, Somerset.

Table 1

Locality	Yellows					Pinks					Pale Browns					Dark Browns			Unusual Banding Types	Total	Comments	
	00000	00300	00345*	10345	12345	00000	00300	00345*	10345	12345	00000	00300	00345*	10345	12345	Banded	Unbanded	Fusions in 12345				
D19	1	4	5	1	19	1	5	7	1	40	-	4	6	1	42	-	5	X	-	142	AEB & CD	22.9.26
	-	7	9	-	29	-	4	18	3	40	-	4	4	3	10	-	-	26	1-PB12045	132	BC & JM	3.6.59
B30	-	4	3	-	21	-	2	4	2	20	-	4	1	1	14	-	6	25	-	82	BC & JM	8.7.59
B29	1	18	4	3	114	2	8	4	-	85	-	2	3	-	32	-	13	83	1-P12045	290	BC & JM	8.7.59
	-	4	3	-	34	-	2	-	-	27	-	-	-	-	6	-	4	40	-	80	BC & JM	9.8.60
B28	-	3	3	-	58	-	1	3	3	39	-	-	2	-	22	-	1	49	4-Y02345 2-P02345 1-P12045 1-PB02345	143	BC & JM	8.7.59
	-	-	-	-	28	-	1	4	1	17	-	-	-	-	5	-	-	20	-	56	BC & JM	9.8.60
B27	-	5	1	2	39	-	6	-	-	30	-	-	1	1	5	-	1	17	1-PB02345 2-P02345	94	BC & JM	8.7.59
B11	-	-	-	-	10	-	1	-	-	2	-	-	-	-	-	-	-	2	-	13	BC & JM	6.7.59
D23	-	3	4	1	45	4	2	2	1	36	-	1	-	-	4	-	-	X	-	103	AEB & CD	20.9.26
	1	9	1	2	51	-	9	2	-	34	-	-	-	-	1	-	-	10	-	110	BC & JM	7.7.59
B43	-	2	1	1	50	-	6	-	4	27	-	-	-	-	-	-	1	11	-	92	BC & JM	10.7.59
	-	8	6	6	141	1	3	4	6	100	-	-	-	-	3	-	-	35	3-Y12045 2-P12045	283	BC & JM	9.8.60
D24	-	2	5	8	63	-	2	2	5	61	-	-	-	-	-	-	-	X	1-Y12045	150	AEB & CD	20.9.26
	-	7	5	3	30	2	2	-	-	27	-	-	-	-	-	-	-	6	1-P12045	76	BC & JM	7.7.59
	-	1	2	-	14	-	3	-	1	10	-	-	-	-	1	-	-	8	-	32	BC & JM	8.6.60
B44	-	-	1	-	23	-	1	1	-	23	-	-	-	-	-	-	-	8	-	49	BC & JM	10.7.59
	-	3	4	-	59	-	2	2	-	42	-	-	-	-	-	-	-	17	1-Y12045 2-P12045	115	BC & JM	9.8.60

Table 1 (contd.)

Locality	Yellows					Pinks					Pale Browns					Dark Browns		Fusions in 12345	Unusual Banding Types	Total	Comments
	00000	00300	00345*	10345	12345	00000	00300	00345*	10345	12345	00000	00300	00345*	10345	12345	Banded	Unbanded				
B45	-	26	4	8	123	-	8	2	6	67	-	-	-	-	1	-	-	20	1-Y12045 2-Y02345 1-P12045 1-P02345	250	BC & JM 10.7.59
	-	1	-	-	62	1	1	1	2	39	-	-	-	-	2	-	-	32	-	109	B.C. & J.M. 9.8.60
D28	-	2	1	6	220	-	-	2	2	148	-	-	-	-	1	-	-	X	2-Y12045 1-Y02345 2-P12045	387	AEB & CD 20.9.26
D27	-	11	4	6	279	1	7	15	6	219	-	-	-	-	2	-	-	X	4-Y12045 2-P12045	556	AEB & CD 20.9.26
D26	-	7	6	6	239	-	6	3	2	101	-	-	-	-	-	-	-	X	1-P12045	371	AEB & CD 20.9.26
	-	10	4	8	89	-	4	9	6	86	-	-	-	-	-	-	-	25	(1-Y12045) (1-P12045) (2-P02345)	220	B.C. & J.M. 4.6.59
D25	-	3	6	1	147	-	4	2	2	98	-	-	-	-	1	-	1	X	1-P12045	266	AEB & CD 20.9.26
	-	5	2	7	80	-	4	2	3	47	-	-	-	-	1	-	-	23	1-Y02345	152	BC & JM 4.6.59
D29	-	5	-	-	28	-	2	-	-	12	-	-	-	-	-	-	1	X	-	48	AEB & CD 20.9.26
	-	22	3	7	207	-	4	6	6	80	-	-	-	-	-	-	-	67	2-Y12045 5-Y02345	342	BC & JM 7.7.59
D30	-	11	2	3	153	-	-	-	-	57	-	-	-	-	-	-	2	X	2-Y12045	230	AEB & CD 24.9.26
	-	22	6	7	124	-	8	4	2	40	-	-	-	-	-	-	-	39	1-Y10045 1-Y12045 4-Y02345 1-P02345	220	BC & JM 4.6.59
D31	-	3	-	-	17	-	-	-	1	2	-	-	-	-	-	-	-	X	-	23	AEB & CD 20.9.26
	-	17	-	-	30	-	4	-	1	7	-	-	-	-	-	-	-	8	-	59	BC & JM 9.7.59
B46	-	5	-	-	31	-	1	-	-	17	-	-	-	-	-	-	-	8	-	54	BC & JM 10.7.59
D32	-	25	1	7	329	-	4	-	-	23	-	-	-	-	-	-	-	X	-	389	AEB & CD 23.9.26
	-	4	-	1	15	-	-	-	-	-	-	-	-	-	-	-	-	5	1-Y02345	22	BC & JM 4.6.59
D34	-	5	-	7	148	-	-	-	-	11	-	-	-	-	-	-	-	X	-	171	AEB & CD 23.9.26
	-	6	-	2	38	-	-	-	-	1	-	-	-	-	-	-	1	7	1-Y02345	49	BC & JM 4.6.59

Table 1 (contd.)

Locality	Yellows					Pinks					Pale Browns					Dark Browns		Fusions	Unusual Banding Types	Totals	Comments
	00000	00300	00345*	10345	12345	00000	00300	00345*	10345	12345	00000	00300	00345*	10345	12345	Banded	Unbanded	i12345			
D33	-	10	-	3	294	-	1	-	-	5	-	-	-	-	-	-	2	X	1-Y12045	316	AEB & CD 23.9.26
	-	7	-	3	149	-	-	-	-	5	-	-	-	-	-	-	-	37	2-Y02345	166	BC & JM 4.6.59
B48	-	3	-	-	15	-	-	-	-	-	-	-	-	-	-	-	-	2	-	18	BC & JM 10.7.59.
D35	-	-	1	6	188	-	2	-	-	10	-	-	-	-	-	-	-	X	-	207	AEB & CD 23.9.26
	-	2	1	-	11	-	1	-	-	4	-	-	-	-	-	-	-	2	-	19	BC & JM 4.6.59
D36	-	8	-	8	152	-	1	-	2	11	-	-	-	-	2	-	2	X	1-Y12045	187	AEB & CD 23.9.26
	-	4	1	4	34	-	-	1	-	7	-	-	-	-	-	-	-	4	-	51	BC & JM 4.6.59
D37	-	17	10	15	373	-	2	1	1	27	-	1	-	-	8	-	7	X	-	462	AEB & CD 23.9.26
B50	-	12	-	-	83	-	-	-	-	2	-	-	-	-	-	-	-	55	-	97	BC & JM 9.8.60
B51	-	8	-	-	65	-	-	-	-	3	-	-	-	-	-	-	-	31	-	76	BC & JM 14.9.60
D40	-	9	1	6	125	-	1	-	3	27	-	-	-	-	-	-	5	X	1-Yalbino 00345 2-Y02345	180	AEB & CD 20.9.26
	-	8	2	2	69	-	5	-	1	13	-	-	-	-	-	-	3	10	2-Y12045 1-Y02345	106	JM & BC 8.7.59
D46	-	18	-	9	113	-	1	-	3	17	-	-	-	-	-	1	6	X	-	168	AEB & CD 24.9.26
	-	13	-	1	42	-	-	-	-	1	-	-	-	-	-	-	1	10	1-Y02345	59	BC & JM 8.7.59
D41	-	69	9	18	374	-	14	4	3	66	-	-	-	-	-	10	22	X	3-Y02345 1-Y12045	593	AEB & CD 24.9.26
D45	-	8	2	2	26	-	-	-	-	5	-	-	-	-	-	3	1	X	-	47	AEB & CD 24.9.26
D48	1	49	19	25	265	-	11	-	1	31	-	-	1	1	2	18	45	X	-	469	AEB & CD 24.9.26
	-	18	2	2	49	-	1	-	1	4	-	-	-	-	-	-	9	9	3-Y02345	89	BC & JM 8.7.59
D49	-	6	-	11	40	-	2	-	-	5	-	-	-	-	-	5	5	X	-	74	AEB & CD 24.9.26
B47	1	6	3	6	33	-	3	-	-	7	-	-	-	-	-	8	6	9	-	73	BC & JM 10.7.59
D50	-	13	4	14	65	-	1	1	-	12	-	-	-	-	-	4	18	X	1-Y12300 5-Y02345	133 145	AEB & CD 24.9.26
	-	21	7	12	71	-	1	-	-	7	-	-	-	-	-	-	20	11	1-P02345		BC & JM 8.7.59

Table 1 (contd.)

Locality	Yellows					Pinks					Pale Browns					Dark Browns			Unusual Banding Types	Totals	Comments
	00000	00300	00345*	10345	12345	00000	00300	00345*	10345	12345	00000	00300	00345*	10345	12345	Banded	Unbanded	Fusions in 12345			
B52	-	-	1	-	4	-	2	-	-	-	-	-	-	-	-	-	-	2	1-Yalbino 12345	8	BC & JM 15.9.60
B61	-	5	2	-	18	-	1	-	-	3	-	-	-	-	-	3	4	3	-	36	BC & JM 16.9.60
D51	-	34	7	14	144	-	2	-	2	6	-	-	-	-	2	24	21	X	-	256	AEB & CD 24.9.26
D52	-	29	35	24	206	-	4	3	4	19	-	-	-	-	5	28	67	X	1-Y10045 1-Y12045 1-P02345	427	AEB & CD 29.9.26
	1	29	13	12	148	-	-	1	1	7	-	-	-	-	-	-	28	24	1-Y10045 2-Y02345 1-P02345	244	BC & JM 8.9.59
	-	8	4	7	45	-	-	-	-	1	-	-	-	-	-	3	5	7	1-P12045	74	BC & JM 8.6.60
	-	3	1	2	24	-	1	-	-	2	-	-	-	-	-	1	4	6	1-Y12045	39	BC & JM 8.8.60
D54	1	36	1	24	133	-	5	-	3	23	-	-	-	-	-	-	18	X	1-Y10045 3-Y02345 1-P02345	249	AEB & CD 22.9.26
	-	38	5	15	53	-	4	-	-	6	-	-	-	-	1	-	1	13	(1-Yalbino 12345 4-Y02345)	128	BC & JM 8.7.59
	2	80	6	43	196	-	14	1	9	18	-	-	-	-	-	5	3	26	-	377	BC & JM 8.8.60
	-	20	-	5	40	-	3	-	1	4	-	-	-	-	-	-	2	8	-	75	BC & JM 14.9.60
B26	-	21	1	1	50	-	2	-	-	6	-	-	-	-	-	-	11	3	1-Yalbino 12345 1-Y12045	24	BC & JM 8.7.59
D55	-	43	1	46	138	-	3	1	5	11	-	-	-	-	-	-	55	X	1-Yalbino 10345 2-Yalbino 12345	311	AEB & CD 20.9.26
B59	-	7	-	4	62	-	-	-	-	1	-	-	-	-	8	-	5	9	-	87	BC & JM 16.9.60

Table 1 (contd.)

Locality	Yellows					Pinks					Pale Browns					Dark Browns		Fusions in 12345	Unusual Banding Types	Totals	Comments
	00000	00300	00345*	10345	12345	00000	00300	00345*	10345	12345	00000	00300	00345*	10345	12345	Banded	Unbanded				
D56	-	19	-	2	70	-	-	-	1	4	-	-	-	-	5	1	13	X	-	115	AEB & CD 20.9.26
	-	40	-	7	205	-	5	-	1	14	-	2	-	-	9	2	16	48	1-Yalbino 12345 1-Y12045 1-P12045	304	BC & JM 8.8.60
B1	-	4	-	1	50	-	1	-	-	10	-	-	-	-	-	1	7	14	-	74	BC & JM 3.6.59
B60	-	-	-	1	46	-	-	-	-	4	-	-	-	-	2	1	1	11	-	55	BC & JM 16.9.60
B42	-	4	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	2	1-P02345	10	BC & JM 10.7.59
Grand Totals	9	999	235	458	7461	12	216	112	108	2123	0	18	18	7	197	118	449		118	12658	

contributes one degree of freedom and 10.12 to the value of the χ^2. It also makes the largest contribution (1.53 with one degree of freedom) to the heterogeneity χ^2 for colour.

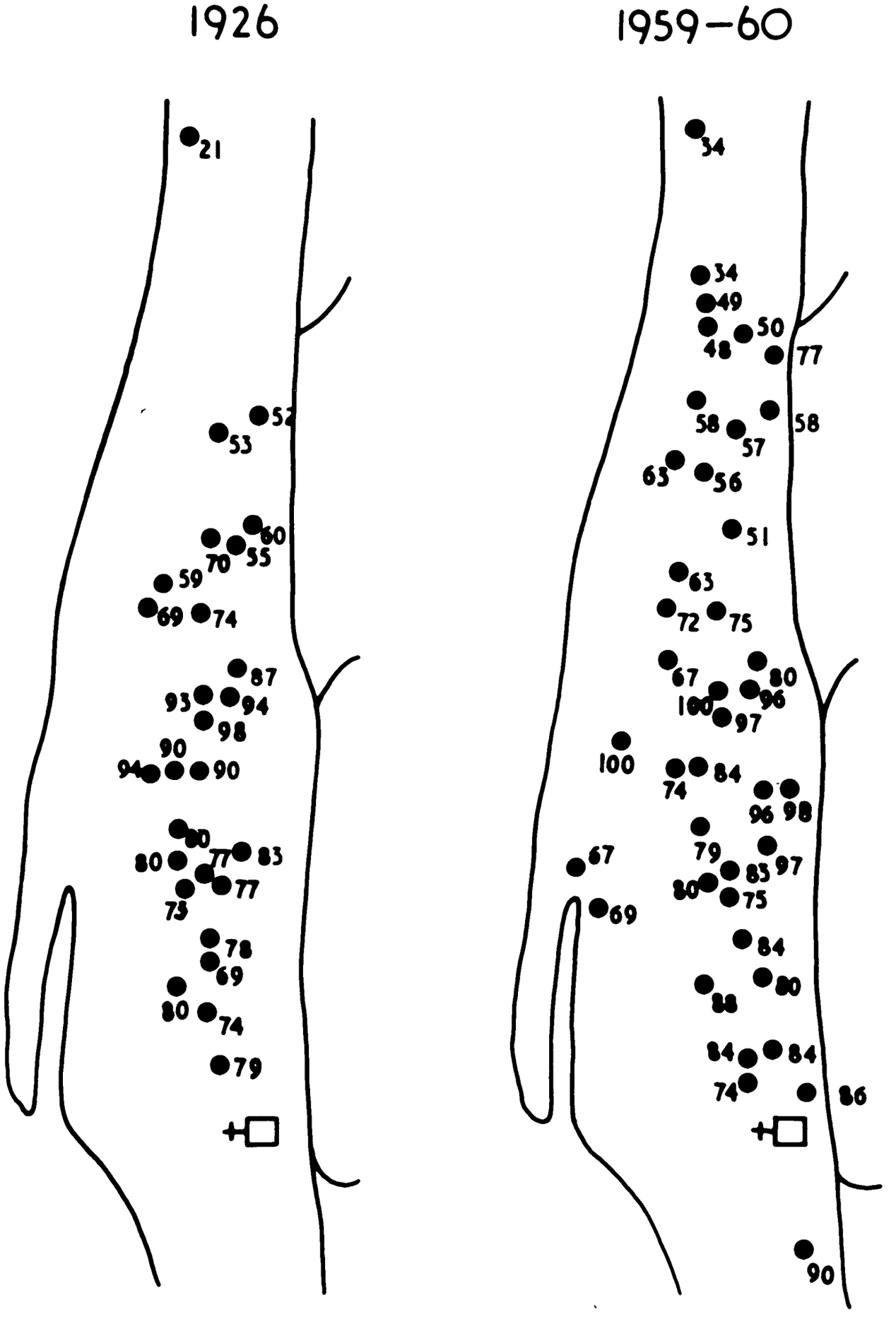
B45 is not one of the localities sampled by Boycott and Diver, and its position was not accurately determined at the time of the first collection. It seems likely that the second collection was not taken from exactly the same place as the first. If B45 is excluded, there is no evidence of heterogeneity between successive samples, either in colour or banding.

Boycott and Diver did not score fusions of bands but we have recorded them. Once again, our samples from B45 are significantly different ($p < 0.001$), but if this locality is excluded there is no further evidence of heterogeneity.

Figures 2-6 record the distributions of various phenotypes in the samples collected at Berrow. On each figure, the left-hand diagram shows the percentages in samples collected by Boycott and Diver, and the right-hand diagram shows the percentages in our samples. All the frequencies are rounded off to the nearest percent. except those less than one percent.

Figure 2 shows the percentages of yellow shells. In both series of samples there is evidence of a cline in these percentages from north to south. The north part of the area shows lower frequencies (20-60%) of yellows. In the centre of the area there is a region where the frequencies exceed 90%. South of this the frequencies are again slightly lower.

Figure 2. Cepaea nemoralis at Berrow, Somerset. The percentages of yellow shells at various localities in 1926 (diagram on the left) and in 1959-60 (diagram on the right). There are obvious morph-ratio clines at both times. In this figure, and the following ones, the proportions are recorded to the nearest percent., except when they are less than one percent.


1926
1959–60
21
52
53
60
55
70
59
69
74
87
93
94
98
90
94
90
80
80
77
83
77
73
78
69
80
74
79
34
34
49
50
48
77
58
58
57
65
56
51
63
72
75
67
80
100
96
97
100
84
74
96
98
79
67
83
97
80
75
69
84
80
88
84
84
74
86
90

% YELLOW

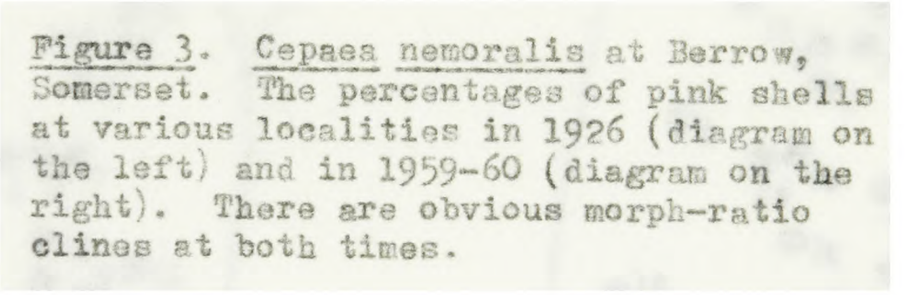
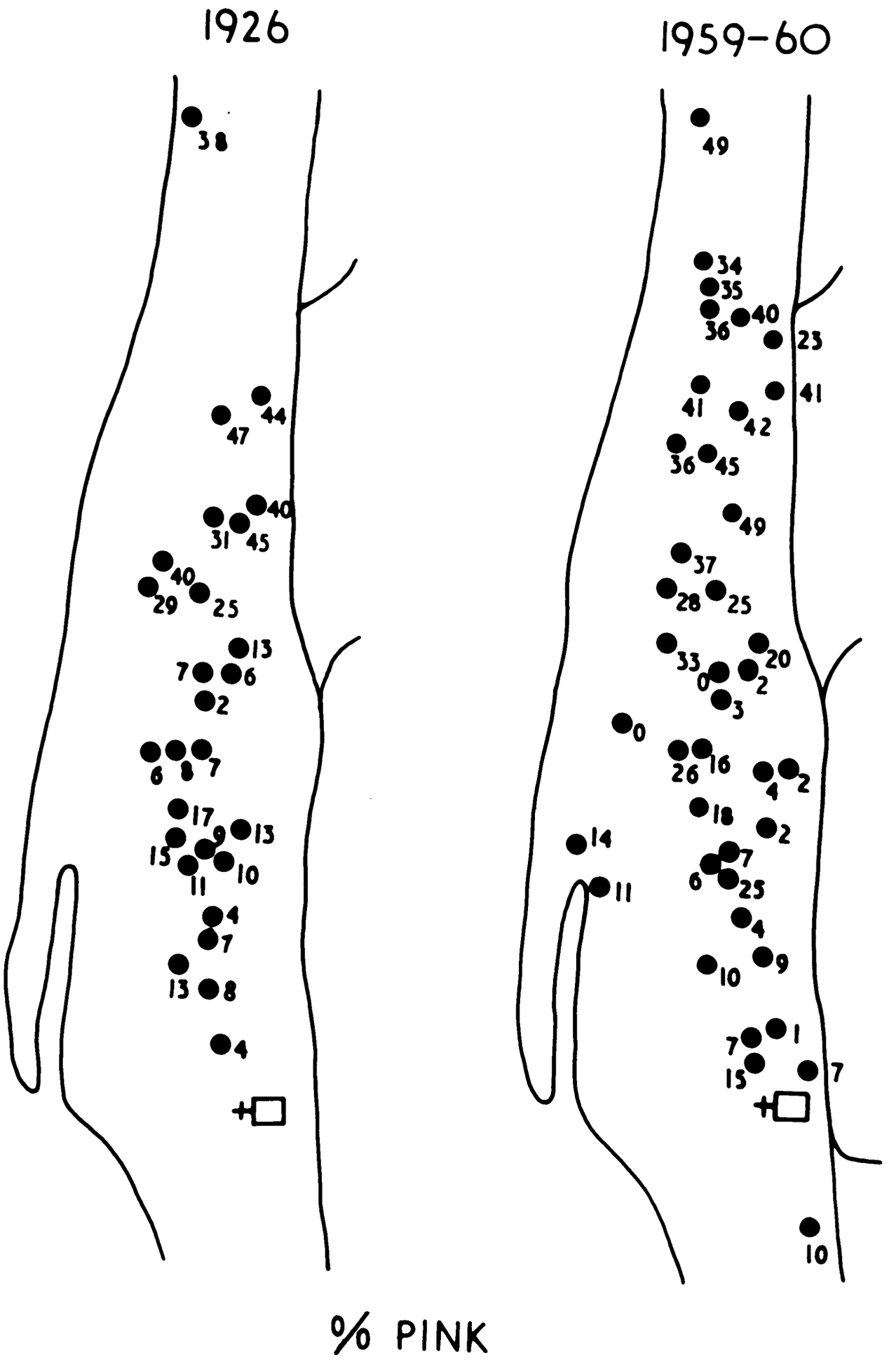


Figure 3. Cepaea nemoralis at Berrow, Somerset. The percentages of pink shells at various localities in 1926 (diagram on the left) and in 1959-60 (diagram on the right). There are obvious morph-ratio clines at both times.


1926
38
44
47
40
31
45
40
29
25
13
7
6
2
6
8
7
17
9
13
15
10
11
4
7
13
8
4
1959-60
49
34
35
40
36
23
41
41
42
36
45
49
37
28
25
33
20
0
2
3
0
26
16
4
2
18
2
14
7
6
25
11
4
9
10
1
7
15
7
10
% PINK

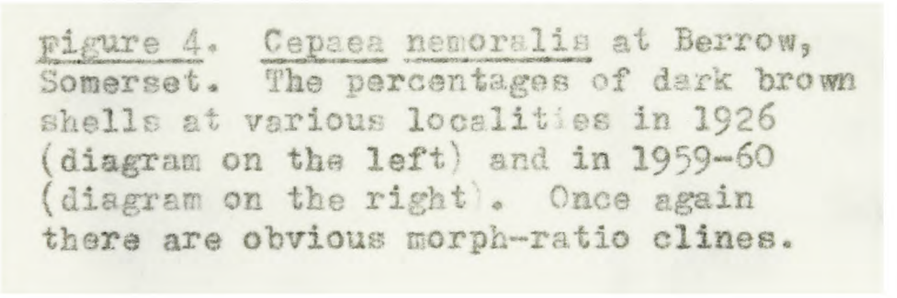
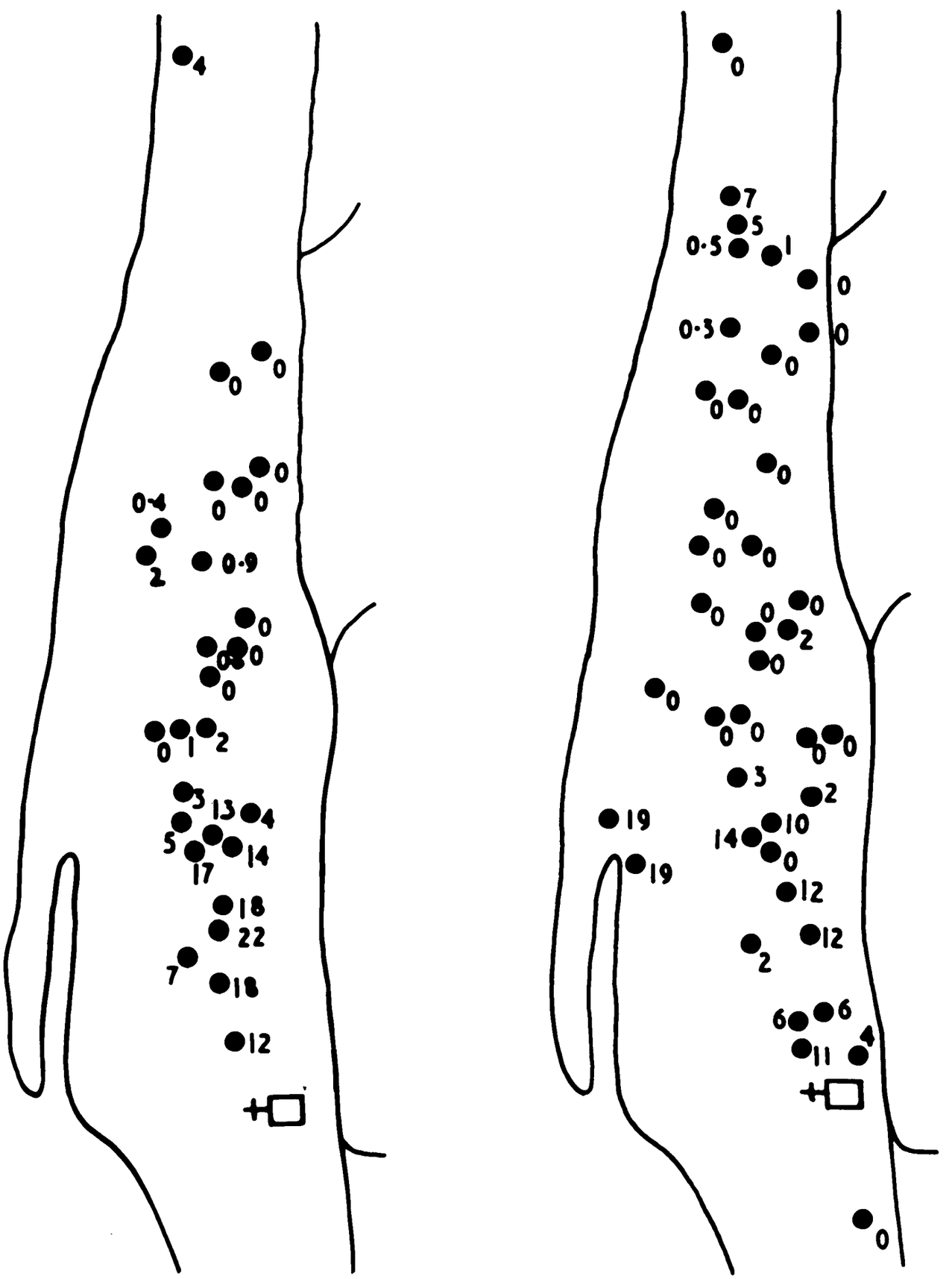
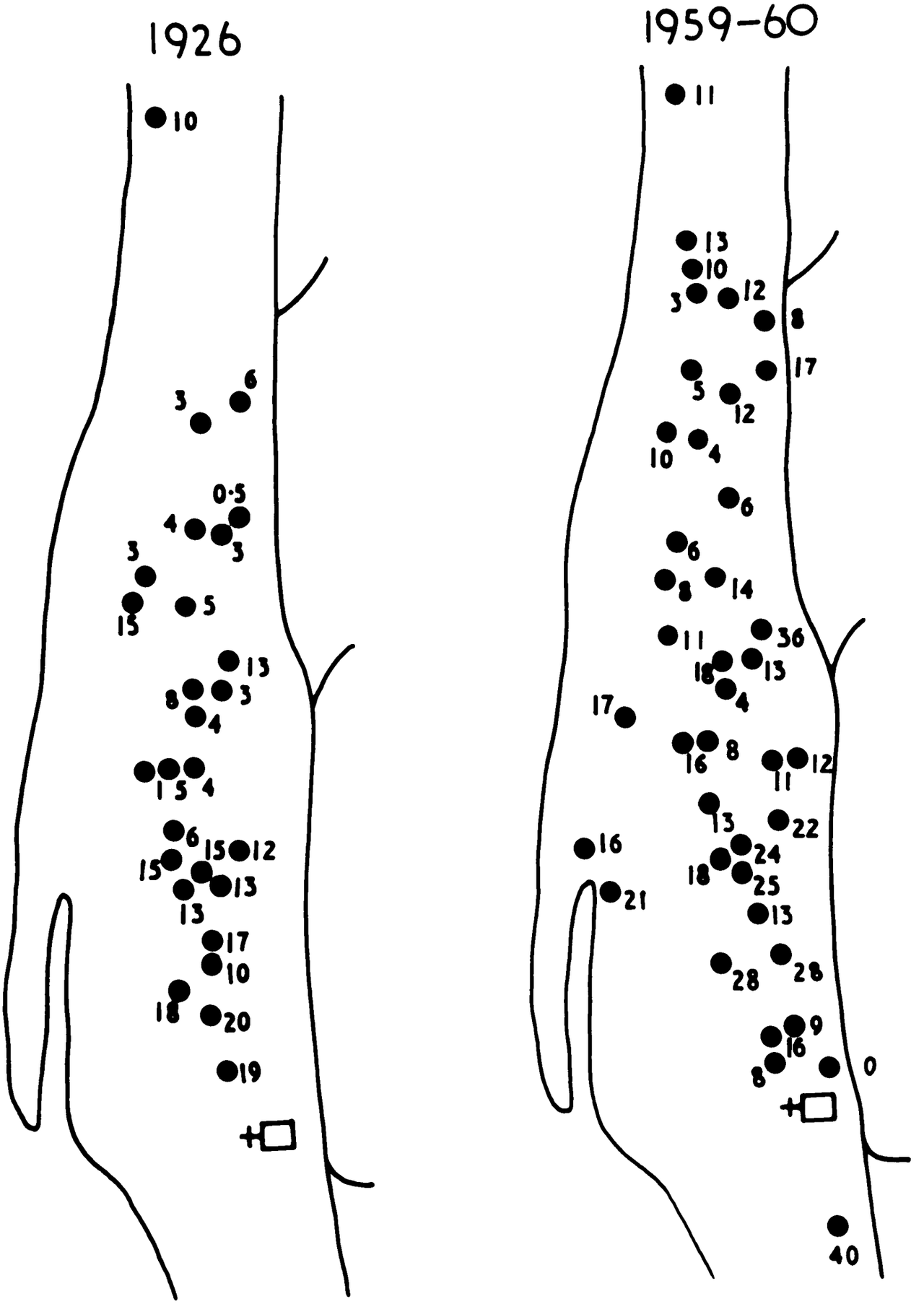


figure 4. Cepaea nemoralis at Berrow, Somerset. The percentages of dark brown shells at various localities in 1926 (diagram on the left) and in 1959-60 (diagram on the right). Once again there are obvious morph-ratio clines.


1926
1959-60
4
0
0
0
0
0
0
0
0
0·4
0
0
2
0·9
0
0
0
0
0
0
1
2
3
13
4
5
14
17
18
22
7
18
12
7
5
0·5
1
0
0·3
0
0
0
0
0
0
0
0
0
0
0
2
0
0
0
0
0
0
3
2
19
10
14
0
19
12
12
2
6
6
4
11
0

% BROWN

Figure 5. *Cepaea nemoralis* at Berrow, Somerset. The proportions of single-banded (00300) shells at various localities in 1926 (diagram on the left) and in 1959-60 (diagram on the right). The proportion of 00300 for each locality is calculated as a percentage of the total number of banded yellows, pinks and pale browns in the sample (see text).


1926
10
3
6
0·5
4
3
3
5
15
13
8
3
4
15
4
6
15
12
15
13
13
17
10
18
20
19
1959–60
11
13
10
3
12
8
17
5
12
10
4
6
6
8
14
11
36
18
13
4
17
16
8
12
11
13
22
16
24
18
25
21
13
28
28
9
16
8
0
40

% 00300

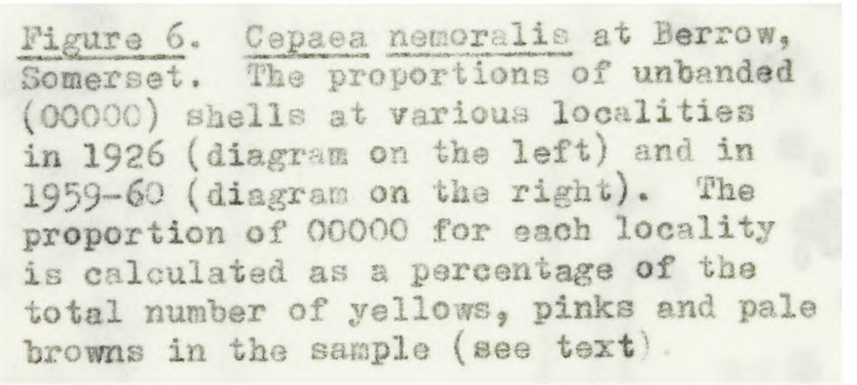


Figure 6. Cepaea nemoralis at Berrow, Somerset. The proportions of unbanded (00000) shells at various localities in 1926 (diagram on the left) and in 1959-60 (diagram on the right). The proportion of 00000 for each locality is calculated as a percentage of the total number of yellows, pinks and pale browns in the sample (see text).

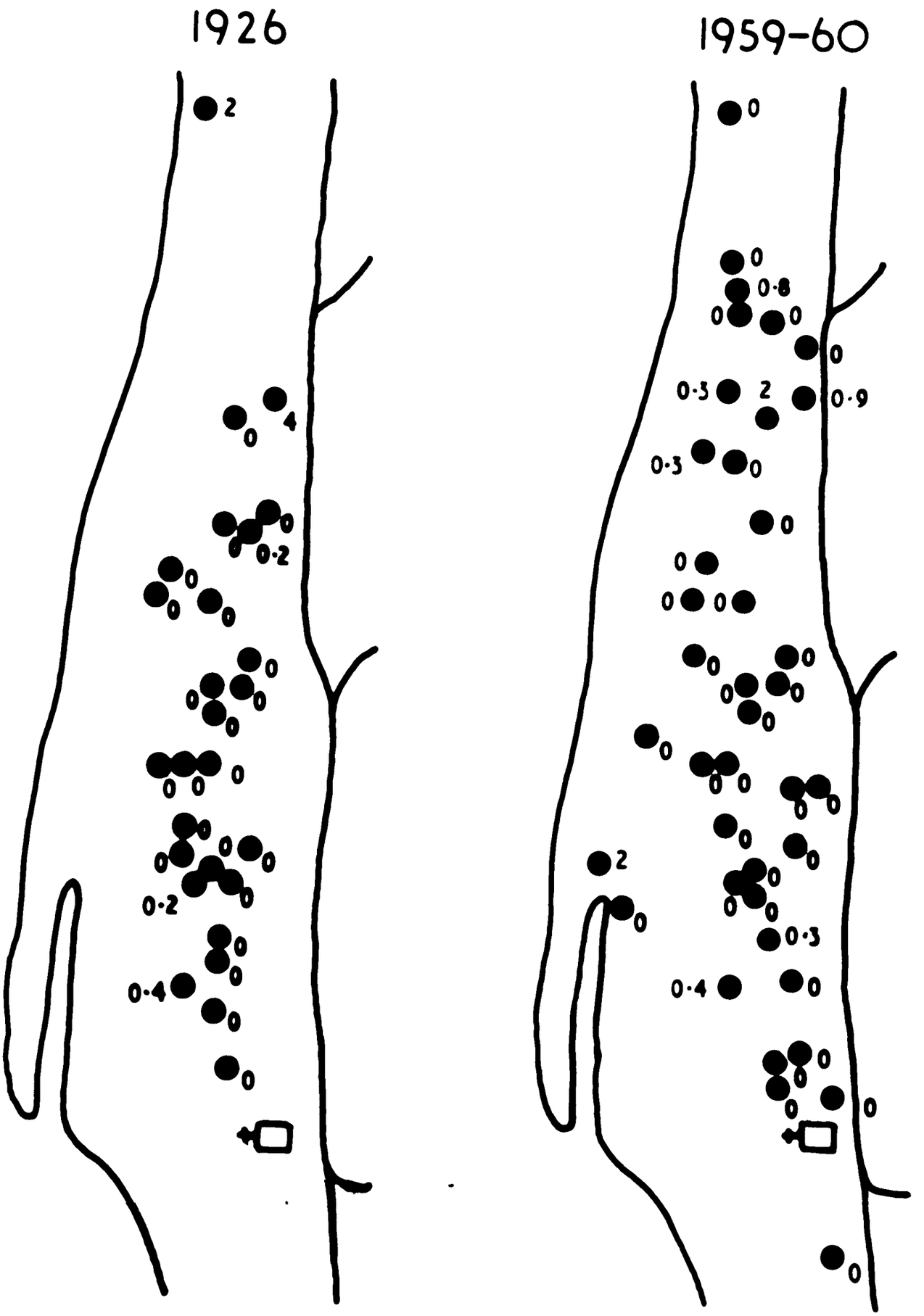

1926
1959-60
% 00000

Figure 3 shows the percentages of pink shells, which may be subject to errors due to confusion with pale browns (see above). It seems clear, however, that there are morph-ratio clines which are opposite in sense to those displayed by the frequencies of yellows.

Figure 4 shows the percentages of brown shells (dark browns). Once again there is evidence of morph-ratio clines. In this case there are higher frequencies in the southern part of the area which fall off to zero in the north. There is also evidence of an overall decrease in the frequency of dark browns between 1926 and 1960 (see below).

Figure 5 shows the distribution of the single-banded form (00300). Because the genes for dark brown shell colour and for the unbanded condition (00000) are both known to be at least partially epistatic to the gene for 00300 (Lamotte, 1954; Cain and Sheppard, 1957; Cain, King and Sheppard, 1960) it was considered best to express the frequency of this form at any locality as a percentage of the total number of banded yellows, pinks and pale browns. The figure shows that there is a slight increase in the frequency of 00300 from north to south. There is also evidence of an overall increase in the proportions of 00300 between 1926 and 1960 (see below).

Figure 6 gives the distribution of unbanded shells (00000). Because the gene for dark brown is partially epistatic to all forms of banding, the frequency of unbanded shells at any locality has

been expressed as a percentage of the total number of yellows, pinks and pale browns. It can be seen that this form is rare throughout the area, but that there are two regions where it seems to be commoner than elsewhere. These regions roughly correspond with areas where the frequency of brown shells is relatively high (see Figure 4).

5. DISCUSSION

Figures 4 and 5 suggest that the frequencies of 00300 and dark brown have changed, over the whole area, between 1926 and 1960. Twenty localities were sampled at both times. Of these, eighteen show an increase in the proportion of 00300, and two show a decrease. This can hardly be accidental ($p < 0.01$). The changes are shown graphically on Figure 7. The hatched columns indicate the frequency of 00300 in 1926, the solid black columns give its frequency in 1959-60. It can be seen that at many localities the changes are large. If all the twenty collections taken in 1926 are added together, the overall percentage of 00300 is found to be 7.3%. The overall percentage for 1959-60 is 14.7%. If each locality is given equal weight, regardless of the size of the sample taken from it, then the average frequency of 00300 for 1926 is 8.4% and for 1959-60 it is 15.1%. In general, the proportion of 00300 seems to have approximately doubled during a period of thirty-three years.

Dark browns have been recorded from fourteen out of the twenty localities. Of these, twelve show a decrease in the

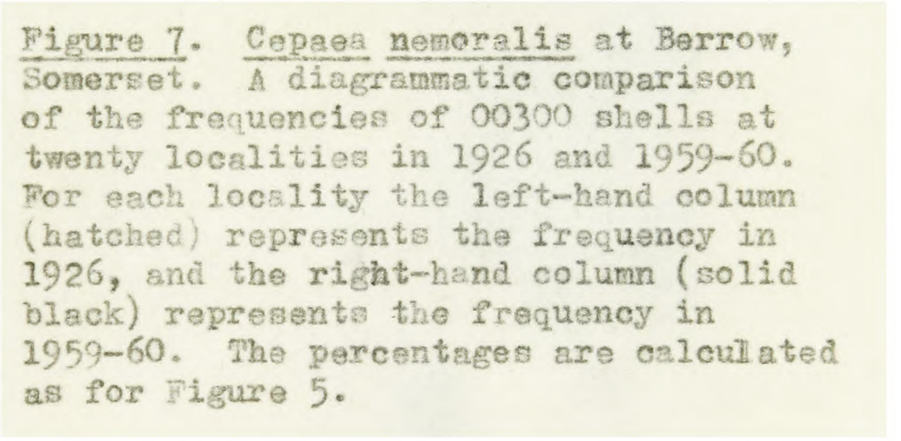


Figure 7. Cepaea nemoralis at Berrow, Somerset. A diagrammatic comparison of the frequencies of 00300 shells at twenty localities in 1926 and 1959-60. For each locality the left-hand column (hatched) represents the frequency in 1926, and the right-hand column (solid black) represents the frequency in 1959-60. The percentages are calculated as for Figure 5.

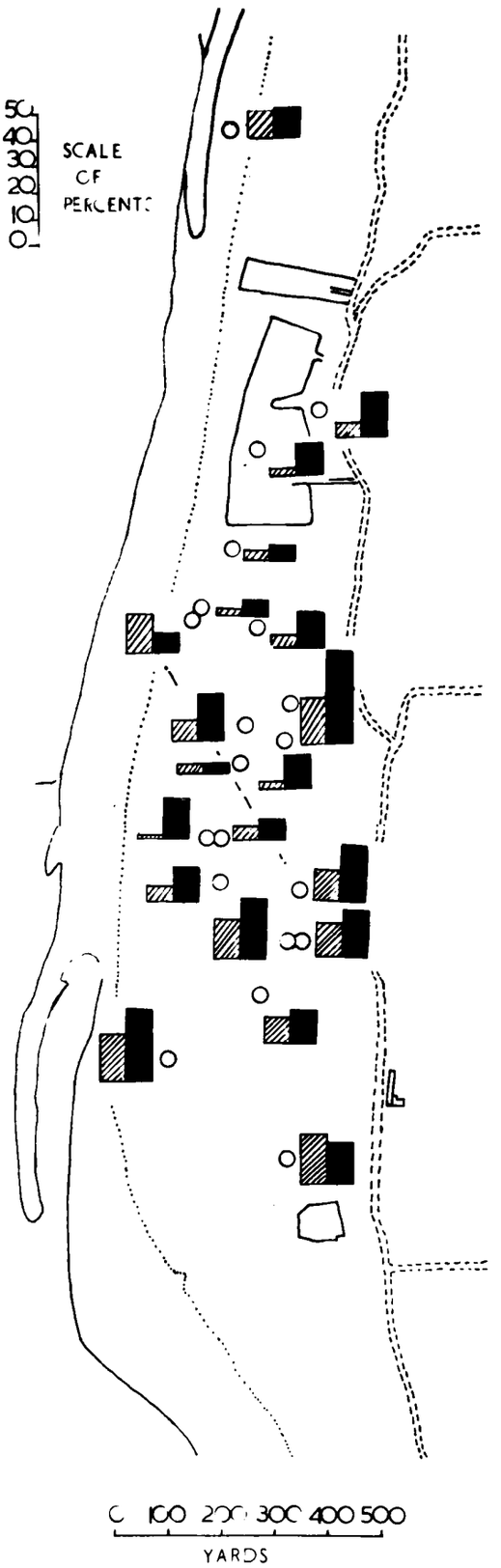
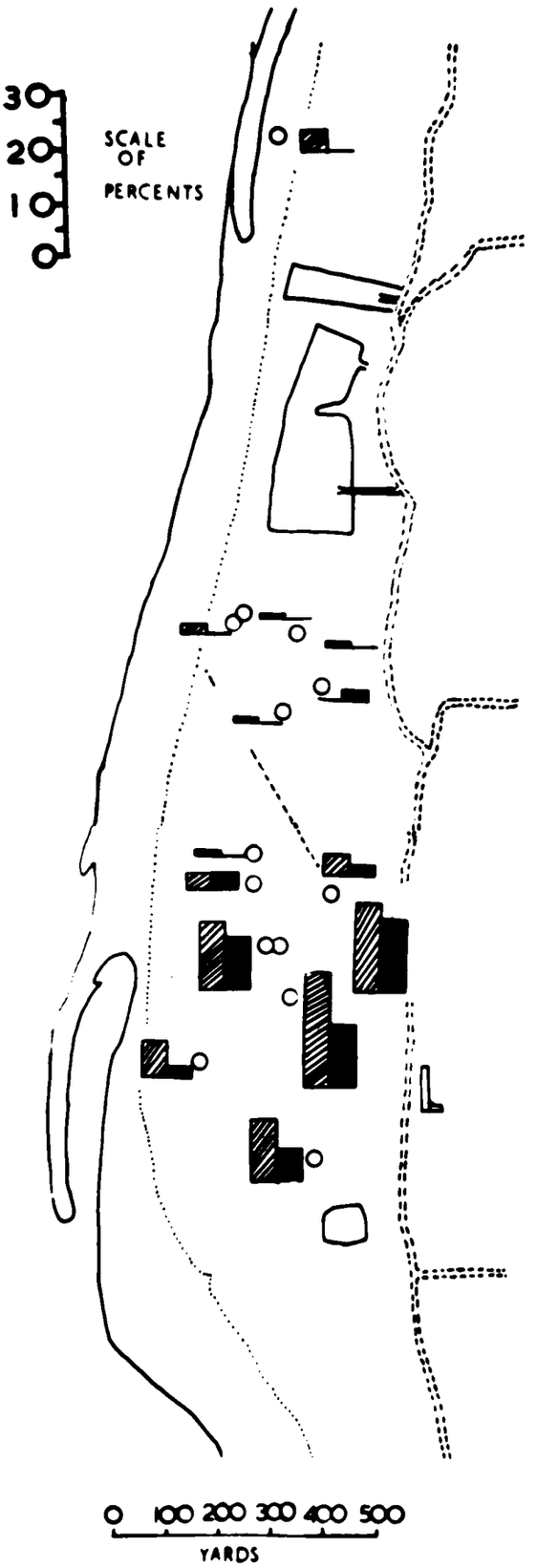

SCALE
OF
PERCENT
0 10 20 30 40 50
0 100 200 300 400 500
YARDS

Figure 8. *Cepaea nemoralis* at Berrow, Somerset. A diagrammatic comparison of the frequencies of dark brown shells at fourteen localities in 1926 and 1959-60. For each locality the left hand column (hatched) represents the frequency in 1926, and the right hand column (solid black) represents the frequency in 1959-60.


30
20
10
0
SCALE OF PERCENTS
0 100 200 300 400 500
YARDS

proportion of such forms, one shows no change, and one shows an increase (which is due to the appearance of a single dark brown snail in our collection). Once again, this can hardly be fortuitous ($p < 0.02$). Figure 8 shows the changes graphically; the hatched columns representing the frequency in 1926, and the black columns giving the frequency in 1959-60. The overall percentage of dark browns in the fourteen samples taken in 1926 is 5.5%. The overall percentage for 1959-60 is 3.2%. The average percentage for 1926 is 6.2%, and for 1959-60 is 3.9%. In general, the proportion of dark browns seems to have nearly halved.

The inheritance of the 00300 and dark brown varieties is known. Both are controlled by single dominant genes, which are inherited in a simple Mendelian fashion (Lamotte, 1954; Cain and Sheppard, 1957; Cain, King and Sheppard, 1960). They are not linked. Both phenotypes are easily scored, and their development and expression are apparently unaffected by the environment. The observed changes of phenotype-frequency must therefore represent changes of genotype-frequency. Such changes immediately suggest the action of natural selection, but there are other possibilities that must be considered.

Since the differences between 1926 and 1959-60 are statistically highly significant ($p < 0.001$), they can hardly be the result of random sampling errors. Mistakes in locating Boycott and Diver's collections, however, could give rise to significant differences (as apparently happened among our samples

at locality B45), but they would not be expected to produce differences that were consistent in direction.

Disparities between the methods of sampling used by the earlier workers and those used by us might conceivably be responsible for consistent and significant differences in phenotype-frequency. This is unlikely to be true for several reasons:-

(i) Boycott and Diver took great care to avoid bias in sampling, as we did also.

(ii) Previous experience has shown that samples collected from the same place by different workers (over a short period of time) do not differ significantly from each other (Clarke, 1960).

(iii) At most localities the collecting was done either on short turf, where all phenotypes are quite obvious, or else in clumps of Iris, where many of the snails were detected by touch.

(iv) At most localities the differences were large (see above).

(v) Samples from thrush-stones and collections of dead shells, many of which were bleached, differed only slightly from samples of live snails taken from the same locality (Clarke and Murray, in preparation).

Sedlmair (1956) has reported that various phenotypes of Cepaea do not behave identically under particular environmental conditions. This state of affairs could give rise to the observed differences only if the environmental conditions during

1959 and 1960 consistently favoured patterns of behaviour that were different from those obtaining in 1926. Since our samples were taken on several occasions spread over two years, and under diverse conditions of weather, temperature and humidity, this explanation seems most unlikely. Points (i) to (v) above also argue against it.

The possibility remains that the differences were the result of random genetic drift (Wright, 1940, 1955), rather than natural selection It can be discounted. Random drift would be expected to have its most obvious effects in small isolated populations, but it would not be expected to give rise, in a series of such populations, to differences that were consistent in direction. The snails in the collecting area cannot be regarded as a single panmictic Mendelian population because of the heavy morph-ratio clines. Lamotte (1951) has calculated that the panmictic unit in Cepaea is not more than a hundred yards in diameter. Our own unpublished observations on movements of C.nemoralis at Berrow support Lamotte's conclusions. Even where no physical barriers exist, the average annual movement of individual snails seems to be less than twenty yards.

The number of snails at Berrow is very large indeed. Boycott and Diver collected 7030 live adults, and we collected 5628. The collecting places occupy less than one fifteenth of the total area surveyed. It is very unlikely that our samples ever constituted

more than 50% of the populations sampled. If only a quarter of the total area were habitable to snails, these figures would give an estimated total population of about 42,000 adult individuals. It is very probably a gross underestimate.

It cannot be argued that the observed differences between 1926 and 1959-60 are due to a catastrophic decline in the numbers of Cepaea followed by repopulation, since the broad pattern of morph-ratio clines has persisted virtually unchanged. It seems necessary to conclude that the changes are results of the action of natural selection. It may be profitable to consider the nature of the selective forces that have brought them about.

Two sorts of natural selection are known to influence the distribution of phenotypes in populations of Cepaea. The work of Cain and Sheppard (1950, 1954), Sheppard (1951b) and Clarke (1960, 1962) has indicated that visual selection by predators, particularly thrushes, is important in determining the proportions of various phenotypes. These studies have also suggested that non-visual selective forces are operating. Boettger (1954), Sedlmair (1956) and Lamotte (1959) have reported differences in survival between various phenotypes of Cepaea under adverse conditions of temperature and humidity. Either or both of these mechanisms may be responsible for the changes in phenotype frequency at Berrow, but the ultimate cause must be some systematic change in the environment.

The most obvious environmental changes that have taken place

since 1926 are first, the overgrowth of sea-buckthorn and second, the general stabilization of the dunes. The first change has certainly affected the distribution and abundance of snails, but may not have directly influenced the overall frequencies of phenotypes, since snails become scarce in the areas of buckthorn. The spread of these shrubs, however, may have allowed an increase in the number of song-thrushes, to which they afford shelter and protection, and consequently may have intensified the action of visual selection upon the snails.

The second change, less clear than the first, has involved the replacement of marram by other grasses and herbs. It is most obvious on parts of the sea ridge, where it seems to have produced a greener and more uniform background. The phenotypes favoured by visual selection against the more green and uniform backgrounds are yellow and effectively unbanded shells (Cain and Sheppard, 1950, 1954). The latter category includes all shells that have the upper two bands missing (e.g. 00300, 00345, 00045 as well as 00000). Thus the decline of browns and the increase of 00300 may both be due, at least in part, to this change. We have direct evidence of visual selection against the dark brown phenotype at Berrow (Clarke and Murray, in preparation).

An interpretation in terms of visual selection is supported by two further observations. Fifteen of the twenty localities show the 00345* phenotype, and in ten of them the proportion of this form has increased since 1926. Again, if the frequency of

yellow shells is considered apart from the frequency of dark browns (in other words, if the proportion of yellows is calculated as a percentage of the total number of yellows, pinks and pale browns) it is found that fourteen out of the twenty localities show an increase of this form.

A number of observations suggest, as might be expected, that visual selection by predators is not the only kind of natural selection acting to influence the proportions of phenotypes at Berrow. The morph-ratio clines recorded on Figures 2-5 cannot easily be correlated with any evident differences in background. The southern part of the area, for instance, where dark browns are most common, is not obviously darker than the northern part.

The clines might conceivably be due to an incomplete mixture of several accidentally distinct populations. If this were so, they would be expected to break down over a period of time. The speed of the breakdown would depend upon theaverage mobility of the snails. There is, however, no obvious sign of a "levelling-out" of phenotype-frequencies since 1926. Such a tendency to uniformity should give rise to negative correlations, from place to place, between the frequencies of individual varieties and the changes of frequency that have taken place. No such negative correlations have been found, in spite of the fact that the proportions of some phenotypes change drastically over very short distances (a hundred yards or less, see Figures 2-6). We hope to examine this problem in greater detail when we know more about

the movements of individual snails.

Another observation suggests the action of non-visual selection. If the frequency of yellow shells is expressed as a percentage of the total number of yellows, pinks and pale browns, it is independent of the frequency of dark browns. The relation between the two proportions can then be examined by normal statistical methods. If visual selection alone were operating, we would expect a negative correlation between them, since yellow shells would be at a disadvantage against backgrounds where dark browns were favoured (and vice-versa). In fact, there is a slight positive correlation (not significant) between them.

In a forthcoming paper we shall consider what magnitude of selective forces would be necessary to bring about the changes observed at Berrow (Clarke and Murray, this thesis).

6. CONCLUSIONS

We have shown that the proportions of at least two phenotypes (00300 and dark brown) have changed drastically and significantly between 1926 and the present day (1959-60), and that these changes are almost certainly the result of natural selection. There is evidence that both visual and non-visual selection have been acting simultaneously. From the data presented in this paper it should be possible to estimate the magnitude of these natural selective forces.

This study provides further evidence against the hypothesis

that the distribution of genotypes in Cepaea is largely determined by random genetic drift (Lamotte, 1951, 1959; Van Valen, 1961).

7. SUMMARY

1. In 1926, Captain C.Diver and the late Professor A.E.Boycott collected random samples from populations of the polymorphic land snail Cepaea nemoralis (L.) on sand dunes in Somerset

2. The area was revisited during 1959 and 1960, and further random samples were taken from the localities studied by the earlier workers.

3. A comparison of the two series of samples showed consistent and significant differences, particularly in the frequencies of two phenotypes (single-banded shells and dark brown shells).

4. The overall proportion of single-banded shells has doubled since 1926. The overall proportion of dark brown shells has very nearly halved.

5. Possible causes of the differences are discussed, and it is concluded that they are the results of natural selection. It is likely that both visual and non-visual selection have been acting simultaneously.

6. The data presented in this paper allow an estimate of the magnitude of natural selective forces acting on individual Mendelian populations.

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CHAPTER 3. CHANGES OF GENE-FREQUENCY IN *CEPAEA NEMORALIS* (L.). II. THE ESTIMATION OF SELECTIVE VALUES.

Chapter 3

CHANGES OF GENE-FREQUENCY IN CEPAEA NEMORALIS (L.)

II. THE ESTIMATION OF SELECTIVE VALUES

With Bryan Clarke

1. INTRODUCTION

In a recent paper (Clarke and Murray, in press), we have described the present distribution of phenotypes of the land snail Cepaea nemoralis (L.) on sand dunes at Berrow in Somerset. The careful records of Captain C.Diver and the late Professor A.E. Boycott allowed us to compare the phenotype-frequencies found in 1959-60 with those in 1926. The results were striking. While the stability of steep morph-ratio clines has been maintained over the intervening period, at least two phenotypes show systematic changes of frequency. Thus although the highest proportions of dark brown shells are found in the same places as before, there has been a consistent overall decline in the frequency of this phenotype throughout the area sampled. Similarly there has been a general increase in the proportion of single-banded (00300) shells.

We have presented reasons for attributing these changes to the action of natural selection (Clarke and Murray, in press). Because of the precision of the historical records, the evidence for genetic continuity provided by the morph-ratio clines, and

the consistency of the changes in phenotype-frequencies, we consider this to be a unique opportunity for obtaining quantitative estimates of the forces of natural selection.

Both of the genes ($\underline{G}^{B}$ for dark brown and $\underline{M}^{3}$ for 00300: Cain and Currey, in preparation) are inherited as Mendelian dominants. Their phenotypic expressions seem to be unaffected by direct environmental influences, and segregation ratios are approximately normal (Lamotte, 1951; Cain and Sheppard, 1957; Cain, King and Sheppard, 1960).

2. THE FITNESS OF THE DARK BROWN PHENOTYPE

(i) Changes in the Frequency of Dark Brown

Twenty areas on the Berrow dunes were sampled both in 1926 and in 1959-60. Six of the resulting pairs of samples contain no dark browns and yield no information on changes of gene-frequency. The remaining fourteen pairs are listed in Table 1 with the percentage of dark brown shells in each sample, the total numbers of shells, and the frequencies of the $\underline{G}^{B}$ gene calculated from the Hardy-Weinberg ratio. Of the fourteen comparisons, twelve show a decrease in the frequency of the gene, and one shows no change. The increase in the fourteenth case is the result of a single brown individual in our sample of 49 snails from a locality where Boycott and Diver found none in 171.

In seven of the pairs of samples only one member of the pair contains any dark brown. For the purpose of computation, the

1	2	3	4	5	6	7	8	9	10	11	12
No.	$\%_o$	% assumed	$\%_n$	N_o	N_n	p_o	p_n	q_o	q_n	S	$V_{(S)}$
D19	3.5	0.76	0	142	132	0.018	0.004	0.982	0.996	0.1390	0.00958
D25	0.4	0.66	0	266	152	0.002	0.003	0.998	0.997	-0.0370	0.01696
D29	2.1	0.29	0	48	342	0.011	0.001	0.989	0.999	0.2194	0.02014
D30	0.9	0.45	0	230	220	0.005	0.002	0.995	0.998	0.0838	0.01308
D34	0	0.58	2.0	171	49	0.003	0.010	0.997	0.990	-0.1109	0.01686
D33	0.6	0.60	0	316	166	0.003	0.003	0.997	0.997	0	0.01277
D36	1.1	1.96	0	187	51	0.006	0.010	0.994	0.990	-0.0472	0.01212
D40	2.8	-	2.8	180	106	0.014	0.014	0.986	0.986	0	0.00462
D46	4.2	-	1.7	168	59	0.021	0.009	0.979	0.991	0.0791	0.00920
D48	13.4	-	10.1	469	89	0.069	0.052	0.931	0.948	0.0290	0.00120
D50	16.5	-	13.8	133	145	0.086	0.072	0.914	0.928	0.0190	0.00096
D52	22.2	-	11.5	427	357	0.118	0.059	0.882	0.941	0.0745	0.00035
D54	7.2	-	1.9	249	580	0.037	0.010	0.963	0.990	0.1234	0.00122
D56	12.2	-	5.9	115	304	0.063	0.030	0.937	0.970	0.0734	0.00117

TABLE 1.

Selection coefficients of the dark brown phenotype at Berrow in Somerset.

$\%$ = phenotype frequencies
N = sample sizes
p and q = gene frequencies
S = selection coefficients
$V_{(S)}$ = variances of S

Those columns referring to 1926 bear the subscript $_o$; those referring to 1959-60, the subscript $_n$. For those samples in which no dark browns were found, column 3 lists a frequency based on the assumption that the sample contained 0.999 dark brown individuals.

other member was assumed to include 0.999 dark brown individuals. Column 3 of Table 1 gives the assumed percentages of dark browns for those samples where the percentage was actually zero.

(ii) Coefficients of Selection

Let G^B represent the gene for dark brown, and G^X all other members of the allelic series controlling ground colour of the shell. According to the Hardy-Weinberg ratio the frequency of the three genotypes will be:

Genotype	$G^B G^B$	$G^B G^X$	$G^X G^X$
Frequency	p^2	$2pq$	q^2

If S represents the selective disadvantage of the dark brown phenotype, then the fitness of the genotypes can be written as:

Genotype	$G^B G^B$	$G^B G^X$	$G^X G^X$
Fitness	1-S	1-S	1

The change of gene-frequency as a result of a single generation of selection is (Falconer, 1960):

$$\Delta q = \frac{Sq^2 (1-q)}{1-S (1-q^2)}$$

By treating this expression as a differential equation and integrating, it is possible to express the selection coefficient S in terms of the initial and final gene-frequencies q_o and q_n and the number of generations t involved (Lush, Bulmer, personal communication§):

$$S = \frac{\log_e \left[\frac{q_n(1-q_o)}{q_o(1-q_n)}\right] - \frac{1}{q_n} + \frac{1}{q_o}}{t + \log_e \left(\frac{q_n}{q_o}\right) - \frac{1}{q_n} + \frac{1}{q_o}}$$

This expression allows us to calculate the variance of the coefficient of selection with respect to the two sample sizes N_o and N_n such that:

$$V(s) = \frac{\left(\frac{1}{q_n} + \frac{1}{1-q_n} + \frac{1}{q_n^2}\right)^2 \frac{q_n(1-q_n)}{2N_n} + \left(\frac{1}{q_o} + \frac{1}{1-q_o} + \frac{1}{q_o^2}\right)^2 \frac{q_o(1-q_o)}{2N_o}}{\left[t + \log_e \left(\frac{q_n}{q_o}\right) - \frac{1}{q_n} + \frac{1}{q_o}\right]^2}$$

The results of these calculations for the fourteen pairs of samples, using a generation time of three years (see below) are shown in columns 11 and 12 of Table 1.

The various estimates of the selection coefficient can be combined into a weighted mean value:

$$\bar{s} = \frac{\sum \frac{s_i}{V(s_i)}}{\sum \frac{1}{V(s_i)}}$$

together with its variance:

$$V(\bar{s}) = \frac{1}{\sum \frac{1}{V(s_i)}}$$

Thus by combining the estimates, the mean selective coefficient S is found to be 0.0619 ± 0.0118 (S.E.).

3. THE FITNESS OF THE SINGLE-BANDED PHENOTYPE

(i) Changes in the Frequency of 00300

When considering the changes that have taken place in the frequency of the single-banded type at Berrow, all twenty comparable pairs of samples may be used. The percentages of 00300 shells, the sample sizes, and the Hardy-Weinberg gene-frequencies are listed in Table 2. Since the gene for the unbanded condition is epistatic to that for 00300 and since there is also an interaction of some kind between the genes for dark brown and for banding, the percentages of 00300 have been calculated only from the banded yellow, pink and pale brown shells in each sample (Murray and Clarke, in press). Hence the sample sizes are slightly smaller than the corresponding ones in Table 1.

Of the twenty comparisons, eighteen show an increase and two a decrease in the frequency of 00300.

(ii) Coefficients of Selection

If $\underline{M}^{3}$ represents the gene for 00300 and $\underline{M}^{-}$ the unmodified banded condition, then the frequencies of the genotypes and their fitness can be represented as:

Genotype	$\underline{M}^{3}\underline{M}^{3}$	$\underline{M}^{3}\underline{M}^{-}$	$\underline{M}^{-}\underline{M}^{-}$
Frequency	p^2	$2pq$	q^2
Fitness	1	1	1-S

1	2	3	4	5	6	7	8	9	10	11
No.	$\%_o$	$\%_n$	N_o	N_n	p_o	p_n	q_o	q_n	s	$V_{(s)}$
D19	9.6	11.4	135	132	0.049	0.059	0.951	0.941	0.0186	0.00132
D23	6.1	16.5	99	109	0.031	0.086	0.969	0.914	0.0949	0.00172
D24	2.7	12.3	150	106	0.014	0.064	0.986	0.936	0.1299	0.00216
D26	3.5	6.4	371	220	0.018	0.033	0.982	0.967	0.0549	0.00115
D25	2.6	5.9	265	152	0.013	0.030	0.987	0.970	0.0736	0.00193
D29	14.9	7.6	47	342	0.078	0.039	0.922	0.961	-0.0755	0.00204
D30	4.8	13.6	228	220	0.024	0.070	0.976	0.930	0.0969	0.00095
D31	13.0	35.6	23	59	0.067	0.198	0.933	0.802	0.1167	0.00330
D32	7.5	18.2	389	22	0.038	0.096	0.962	0.904	0.0885	0.00252
D34	2.9	12.5	171	48	0.015	0.065	0.985	0.935	0.1262	0.00257
D33	3.5	4.2	314	166	0.018	0.021	0.982	0.979	0.0144	0.00198
D35	1.0	15.8	207	19	0.005	0.082	0.995	0.918	0.2147	0.00476
D36	4.9	7.8	185	51	0.025	0.040	0.975	0.960	0.0437	0.00299
D40	5.7	12.6	175	103	0.029	0.065	0.971	0.935	0.0749	0.00143
D46	11.8	22.4	161	58	0.061	0.119	0.939	0.881	0.0687	0.00123
D48	14.8	23.8	405	80	0.077	0.127	0.923	0.873	0.0538	0.00071
D50	12.6	17.6	111	125	0.065	0.092	0.935	0.908	0.0360	0.00111
D52	9.9	13.0	332	315	0.051	0.067	0.949	0.933	0.0273	0.00050
D54	17.8	28.0	230	567	0.093	0.151	0.907	0.849	0.0545	0.00031
D56	18.8	16.4	101	286	0.099	0.086	0.901	0.914	-0.0157	0.00081

TABLE 2

Selection coefficients for the single-banded (00300) phenotype at Berrow in Somerset. Explanation as in Table 1.

In order to consider selection as acting always <u>against</u> the decreasing genotype, we can use a modification of the expressions given above for the dark brown condition. It would, of course, be possible to use the former expressions, but then selection would be expressed relative to the dominant gene and would be opposite in sign to that relative to the recessive. The relation of the two methods of expressing the selective coefficient is:

$$S_{(\text{dominant})} = \frac{-S_{(\text{recessive})}}{1-S_{(\text{recessive})}}$$

Expressed as selection <u>against the recessive</u> the change in the frequency of $\underline{M}$ becomes:

$$\Delta q = \frac{Sq^2(1-q)}{1 - Sq^2}$$

and:

$$S = \frac{\log_e \left[\frac{q_o(1-q_n)}{q_n(1-q_o)}\right] + \frac{1}{q_n} - \frac{1}{q_o}}{t + \log_e \frac{1-q_n}{1-q_o}}$$

and:

$$V_{(S)} = \frac{\left(\frac{1}{q_n} + \frac{1}{1-q_n} + \frac{1}{q_n^2}\right)^2 \frac{q_n(1-q_n)}{2N_n} + \left(\frac{1}{q_o} + \frac{1}{1-q_o} + \frac{1}{q_o^2}\right)^2 \frac{q_o(1-q_o)}{2N_o}}{\left[t + \log_e \left(\frac{1-q_n}{1-q_o}\right)\right]^2}$$

The resulting coefficients and their variances are shown in columns 10 and 11 of Table 2. Combining the estimates of S we find that:

$$\bar{S} = 0.0521 \pm 0.0076 \text{ (S.E.)}$$

4. DISCUSSION

In order to calculate general estimates of the forces of natural selection, we have found it necessary to make a number of assumptions. We have estimated gene-frequencies by assuming that the Hardy-Weinberg law provides an adequate description of the distribution of gene-frequencies in our samples. The Hardy-Weinberg law, of course, applies to large, randomly-mating populations in the absence of mutation, migration and selection. It is unlikely that mutation and migration will have a serious effect on the gene-frequency distribution. Lamotte (1951) has presented evidence against assortative mating in C.nemoralis, and our sampling areas are small enough to be considered as panmictic units (Lamotte, 1951). However, since we are attempting to measure natural selection, we must determine its effect on our gene-frequency estimates.

Barring unlikely contingencies such as meiotic drive, which seems to be ruled out by good segregation ratios from breeding experiments (see above), the Hardy-Weinberg ratio will be re-established with the union of gametes in each generation. Let us consider the case in which the fitness of the three genotypes is represented by:

p^2	$2pq$	q^2
1-S	1-S	1

The initial frequency of the recessive homozygote will be q^2. After selection is complete, which will be the extreme case for the adult population, the proportion of recessives in the population is:

$$\frac{q^2}{1-S(1-q^2)}$$

In order to determine how much difference to the estimation of S might be caused by equating this quantity with q^2, we have recalculated the selection coefficient of dark brown for a typical example (D 56) on the assumption that the Hardy-Weinberg ratio had been disturbed by a selective force of 6%. Although there is a 2-4% difference in the estimated gene frequencies, the resulting effect on the value of S is negligible. Instead of 0.0734, it becomes 0.0735. Thus a moderate amount of natural selection will have little effect on the determination of the size of the selection coefficient, despite small errors in the estimation of gene frequency. It would be possible by iterative calculations to apply a correction to S, but the gain in accuracy is hardly worth while.

We have made two assumptions about the nature of the selection coefficients themselves. First, the fitness of the dominant homozygote and the heterozygote have been assumed to be equal. This is probably not true since the fitness of the heterozygote in a polymorphic system is likely to be greater than that of either

homozygote. However, in the cases of both of the dominants which we have considered, the gene-frequencies, and therefore the percentages of dominant homozygotes, are low. This is particularly true of the dark browns, where homozygotes in excess of 1% are expected in only one sample out of twenty-eight. Hence the selection coefficients of the dominants can be considered to represent most nearly those of the heterozygotes.

Second, in order to arrive at an estimate of the mean selective coefficient in each case, we have assumed the value of S to be the same in each set of samples. It is possible to test the homogeneity of the estimates by calculating χ^2 with K-1 degrees of freedom, where K is the number of individual estimates.

$$\chi^2_{(K-1)} = \sum \frac{(S_i - \bar{S})^2}{V_{(S_i)}}$$

In the case of dark brown, $\chi^2_{(13)} = 12.87$; and the probability lies between 30 and 50%. There is thus no evidence of heterogeneity among the estimates of S for dark brown, despite the fact that in half of them an assumed value has been used for either the initial or final gene frequency. A word of caution is necessary, however, since this good agreement should be interpreted partly as a reflection of the rather large variances of the individual estimates. This is particularly true of those based on assumed frequencies. On the other hand if we consider only those seven pairs of samples where no assumptions were necessary, the mean value of S is only

slightly altered. Instead of 0.0619 it becomes 0.638. The change is accompanied by an increase in the standard error of $\bar{S}$ from 0.0118 to 0.0122.

In the case of 00300, where individual variances are smaller, $\chi^2(19) = 33.03$. Since this figure is significant at the 5% level there is evidence of real differences in the value of S among the twenty samples, a not altogether surprising conclusion considering the wide range of frequencies over the area sampled. We cannot therefore place much confidence in the standard error of $\bar{S}$ for 00300.

An examination of the individual contributions to the value of χ^2 for 00300 shows that one sample (D29) contributes almost a quarter of the total. Since there is no independent reason for excluding this value, it is not legitimate to remove it from the calculations. However it is interesting that without this extreme value, χ^2 is no longer significant, and $\bar{S}$ is increased from 0.0521 to 0.0559.

The model that we have used implies that selection takes place by steps in a series of discrete generations. In a long-lived animal such as C.nemoralis the process is continuous since there is no break between generations. This particular problem has been discussed by Haldane (1926). He has concluded that the model for selection based on overlapping generations is negligibly different from that based on discrete generations.

Finally, we must consider the generation time in C.nemoralis. For an animal with discrete generations, this is simply the elapsed

time between two equivalent stages in successive generations. With overlapping generations, the important time is that at which each individual has made its mean contribution to the next generation. Hence generation time should be measured from zygote formation to the mid-point in the reproductive life of the average individual. Even when maintained in optimum conditions where feeding and growth can continue throughout the winter, C.nemoralis rarely becomes adult within one year. It seems likely that the average individual begins to breed in its second autumn or third spring, when it is eighteen months to two years old. If we consider that adult snails are eliminated from the population at a rate of about 50% per year, then the generation time should be about three years.

Since these calculations are necessarily rather rough, we have indicated in Figure 1 the relation between generation time and the selection coefficient. Sample D56 (dark brown) has again been used. The selection coefficient is plotted against generation time in years. Over the range which we have investigated the relationship is very nearly linear. A reduction of the generation time from three to two years would reduce the value of the selection coefficient in this case from 7.3% to 4.9%.

5. CONCLUSIONS

We have estimated the magnitude of the selective forces necessary to bring about observed changes in the proportions of two phenotypes in populations of Cepaea nemoralis at Berrow in Somerset.

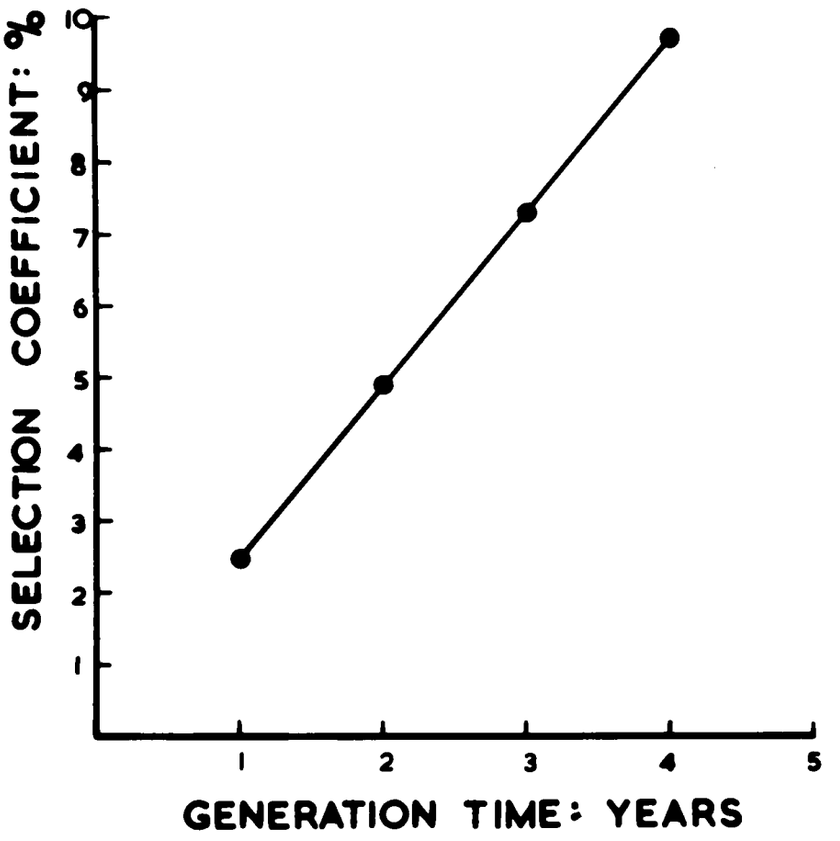


Figure 1. The relation of generation time to the magnitude of the selection coefficient in sample D 56 (dark brown).

Over the period 1926 to 1959-60, the dark brown phenotype has been at an average disadvantage of about 6.2%, while the 00300 phenotype has been at an average advantage of about 5.2% per generation. These figures are based on a generation time of three years. In the unlikely event that the generation time in C.nemoralis is as low as two years, then these estimates would be reduced to 3.8% and 2.8% respectively.

There is no evidence of variation from place to place in the value of the selection coefficient of dark brown. On the other hand, it is likely that there are real differences in the magnitude of the selection coefficient of 00300 at different localities. The heterogeneity among the estimates of the selection coefficient of 00300 is of importance for two reasons. First, it explains the large differences in the frequency of the single-banded type within the area. Second, it shows that the selective value is not associated with a single obvious feature of the habitat. It is likely that each value is a result of the combined action of many different forces.

We have no information on fluctuations in the magnitude of the selection coefficients during the intervening period between the collection of the two sets of samples. However, any fluctuations in the direction and intensity of the selection would necessarily increase the intensity of selection for a part of the time. Hence our estimates are minimum values for the magnitude of selection.

Finally, with natural selection of this intensity, there can be little scope for the action of dispersive processes in the

determination of gene-frequencies.

6. SUMMARY

1. Assuming a generation time of three years, the dark brown phenotype in populations of Cepaea nemoralis at Berrow in Somerset has been at an overall disadvantage of 6.2% per generation over the period 1926 to 1959-60.

2. During the same period, the single-banded (00300) phenotype has enjoyed an overall selective advantage (expressed as the disadvantage of the normally banded phenotype) of 5.2% per generation.

3. With this level of selection, negligible errors are introduced by calculating gene-frequencies from the Hardy-Weinberg ratio.

4. No heterogeneity is demonstrable in the individual estimates of the selection coefficient of dark brown. Heterogeneity among the estimates of the selection coefficient of 00300 suggests real differences in the value of S in different parts of the area studied.

5. Over the range investigated, a direct linear relationship obtains between lengths of generation time and magnitude of the selection coefficient.

6. Natural selection of this intensity precludes the action of dispersive processes in the determination of gene-frequencies.

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CHAPTER 4. MULTIPLE MATING AND EFFECTIVE POPULATION SIZE IN *CEPAEA NEMORALIS*.

Chapter 4

MULTIPLE MATING AND EFFECTIVE POPULATION SIZE IN CEPAEA NEMORALIS

1. INTRODUCTION

One of the outstanding characteristics of populations of the land snail, Cepaea nemoralis (L.), is the ubiquity of the polymorphism of colour and banding pattern. Monomorphic colonies have been found, but they are exceedingly rare. For example, Lamotte (1951) cites only two such cases out of a thousand colonies studied in France. They were samples of 300 from Gergovie (Puy-de-Dôme) and of 100 from Chagny (Saône-et-Loire) made up entirely of yellow unbanded individuals. Even these populations may be polymorphic, since in samples of this size, the dominant gene for absence of bands could conceal a low frequency of its allele for banding. Moreover, the gene for absence of bands is epistatic to that for mid-banded, which is normally an important element of the polymorphism.

The rarity of monomorphic colonies is noteworthy since variations in the proportions of the different phenotypes have been attributed by some authors (e.g. Diver, 1940, and Lamotte, 1951, 1959) to the action of random genetic drift. If such a dispersive process is responsible for the observed frequencies, then in isolated colonies fixation is to be expected. The rarity of fixation suggests that the effects of genetic drift are being reduced in most populations of Cepaea.

In order for genetic drift to play a significant role in the alteration of gene frequencies, the effective population size must be relatively small. The effective size may, of course, be very different from the actual number of animals in the population. It is usually smaller but in certain cases can be larger than the number of breeding individuals.

The mating system of Cepaea nemoralis has a profound influence on the effective size of its populations. These snails are hermaphrodite, they mate more than once, and they store sperm for long periods. Each individual may, in a single brood, produce offspring from several matings. I have attempted to discover the number of productive matings per individual in order to investigate the effects of such a mating system on the decay of variability in populations of Cepaea.

2. METHODS

The average number of matings from which each animal is producing young can be estimated from a comparison of the frequency of certain forms in a population with the proportion of those forms appearing in the progeny of selected individuals from that population. In a random-mating population where each brood results from a single mating, among broods produced by animals of the recessive phenotype, the proportion of broods containing individuals showing a dominant character will be the same as the proportion of that phenotype in the population. If each brood results from two or more matings, then the number of broods containing individuals showing the dominant

character will be proportionally increased.

The colony chosen for this study is located at the foot of Burderop Down near Wroughton, Wiltshire (grid reference SU 158768). An area 50 yards east-west by 10 yards north-south was sampled. It lies between the Ridgeway (here, at the foot of the downs) on the south and a hedge on the north. The vegetation consists of long grass, nettles and umbellifers.

Three pairs of genes suitable for this study are found in the colony. All produce clearcut phenotypes and segregate in a Mendelian manner. Their genetics have been reviewed by Lamotte (1951) and Cain and Sheppard (1954). Additional data are given by Cain and Sheppard (1957) and Cain, King and Sheppard (1960). Briefly, the universal recessive is the yellow five-banded, 12345, type. Dominant to it are the genes for pink, mid-banded or 00300 and unbanded or 00000. The loci for 12345/00000 and yellow/pink are strongly linked, and 00000 is epistatic to 00300; but these complications do not affect the present study.

This particular colony was chosen because all three dominant genes are found with low frequencies, decreasing in a series: pink > 00000 > 00300. It was hoped that one of the three might be represented in about half the resulting broods. At this frequency, the estimate of the number of matings has the greatest precision. In the event, pink proved to be the most useful type. Samples were collected on four occasions, three in June 1960 and one in May 1961.

Fifty yellow unbanded snails from the first sample were isolated in individual breeding containers. These are flower pots half filled with earth and covered with sheets of glass. The animals were fed on lettuce and occasionally on carrot. Lumps of chalk were always available. The algae growing on the side of the pots provided an additional source of food. Eggs were allowed to hatch in the breeding pots, but the young snails were transferred to plastic boxes for rearing on a diet of lettuce, oatmeal and powdered chalk.

During the summer of 1960, 35 snails produced young, one died and 14 others failed to reproduce. This failure cannot be considered as evidence that these snails had not been previously fertilized. Seven of the 14 survived the winter, and one produced a brood of 16 young in September of 1961. This represents a storage of sperm for at least fifteen months since the last occasion on which this animal could have mated. It confirms reports by Lang (1904, 1911) of sperm storage for more than a year.

3. RESULTS

Of the 1630 young removed from the breeding pots, 1419 lived to an age when they could be scored for colour and banding pattern. The scores and the number of broods containing each type are shown in Table 1. Two of the possible phenotypes, the double dominants for colour and banding pattern, are not represented in the offspring. It is possible that some of the yellow unbanded individuals also

NUMBER	±12345	YELLOW 00300	00000	±12345	PINK 00300	00000	TOTAL SCORED	TOTAL HATCHED
1.	PRODUCED IN 1961		ONLY					
2.	17			3			20	21
3.	18	8					26	33
4.	15		1				16	18
5.	47						47	56
7.	38			12			50	59
8.	43						43	49
9.	37						37	39
10.	32		2	5			39	41
12.	38						38	40
13.	41						41	45
14.	20						20	23
16.	26			16			42	48
17.	33			13			46	49
18.	12			17			29	32
19.	16			1			17	29
21.	21			22			43	47
22.	29			26			55	56
23.	31			25			56	87
25.	2						2	2
26.	30		10				40	55
30.	17			23			40	40
31.	66						66	69
32.	43			36			79	86
33.	48			4			52	55
34.	35						35	40
35.	48			4			52	53
36.	24			24			48	50
38.	43						43	50
39.	30						30	39
40.	55						55	57
41.	30			22			52	54
42.	51						51	58
44.	63						63	77
45.	11			9			20	41
46.	26						26	32
TOTAL	1136	8	13	262			1419	1630
% of TOTAL	80.0	0.6	0.9	18.5			MORTALITY = 12.9%	
No. BROODS	35	1	3	17				

TABLE 1. Offspring of homozygous recessive animals taken as adults from a wild population of Cepaea nemoralis.

carry the gene for 00300. but this is unlikely since the gene is unlinked to the other banding locus and would be assorted at random between the unbanded and five-banded offspring of the broods containing unbanded young.

It is immediately obvious from Table 1 that a number of the broods show drastic departures from good Mendelian ratios. Since one parent in each case was a homozygous recessive, each brood should be a backcross producing either all dominants, all recessives or a 1:1 segregation of each. In fact seven of the broods show significant differences from a 1:1 ratio with respect to pink, while all of those which are segregating for 00000 and 00300 show similar disturbances. Brood 10 shows departures from the 1:1 ratio in two segregations, probably as a result of linkage in the "male" parent, with the genes for pink and unbanded in repulsion. There is thus evidence for multiple matings in ten broods. Departures from Mendelian ratios demonstrate multiple matings, but good ratios do not necessarily represent single matings. Obviously, multiple matings with animals of the same genotype will produce results which are indistinguishable from the broods of single matings. Also a mating with a homozygous dominant followed by one with a homozygous recessive will simulate a single mating with a heterozygous animal. Therefore it is important to look at the distribution of dominants in the offspring. The proportion of broods containing dominants is very different from the proportions of those dominants found in the parent colony.

Table 2 gives the proportion of dominants in the samples from the parent colony. The samples were compared for homogeneity

	% Pink	%00000	%00300	N
Sample 1	20.4	4.6	3.7	108
Sample 2	23.8	13.6	0.7	147
Sample 3	10.4	7.5	2.8	106
Sample 4	16.7	11.1	0.0	18
TOTAL	18.7	9.2	2.1	379
TOTAL of 1, 2 & 4	22.0	9.9	1.8	273

TABLE 2. The composition of samples of Cepaea nemoralis from the colony from which breeding stocks were obtained.

with respect to colour in two ways. Samples 2, 3 and 4 were each separated into four parts, from the northeast, northwest, southeast and southwest quarters of the area respectively. When the score of each quarter is summed for all three samples there are no significant differences among the quarters ($P > 0.20$). However when the samples from different dates are compared, there is a suggestion that sample 3 differs from the others in having too few pinks (P between 0.05 and 0.02). Therefore comparisons with the breeding results have been made both with and without sample 3.

Figure 1 shows the expected increase in the proportion of broods with some pink offspring as the number of matings per individual increases. If $\bar{P}$ and $1-\bar{P}$ represent the proportions of the pink and yellow phenotypes within the colony and $\bar{B}$ the percentage of broods containing pink offspring, then with one mating:

$$\bar{B} = \bar{P}$$

With two matings: $$\bar{B}_2 = \bar{P} + \bar{P}(1-\bar{P})$$

and similarly: $$\bar{B}_3 = \bar{P} + \bar{P}(1-\bar{P}) + \bar{P}(1-\bar{P})^2$$

$$\bar{B}_n = \bar{P} + \bar{P}(1-\bar{P}) + \bar{P}(1-\bar{P})^2 \ldots\ldots \bar{P}(1-\bar{P})^{n-1}$$

In Figure 1 the solid curve represents the expected increase in the proportion of broods containing pinks, based on the total of all the samples (% pink = 18.7). If sample 3 is omitted, then the increase is represented by the broken curve.

The horizontal solid line shows the proportion of broods which contain at least one pink individual among the offspring. Each brood

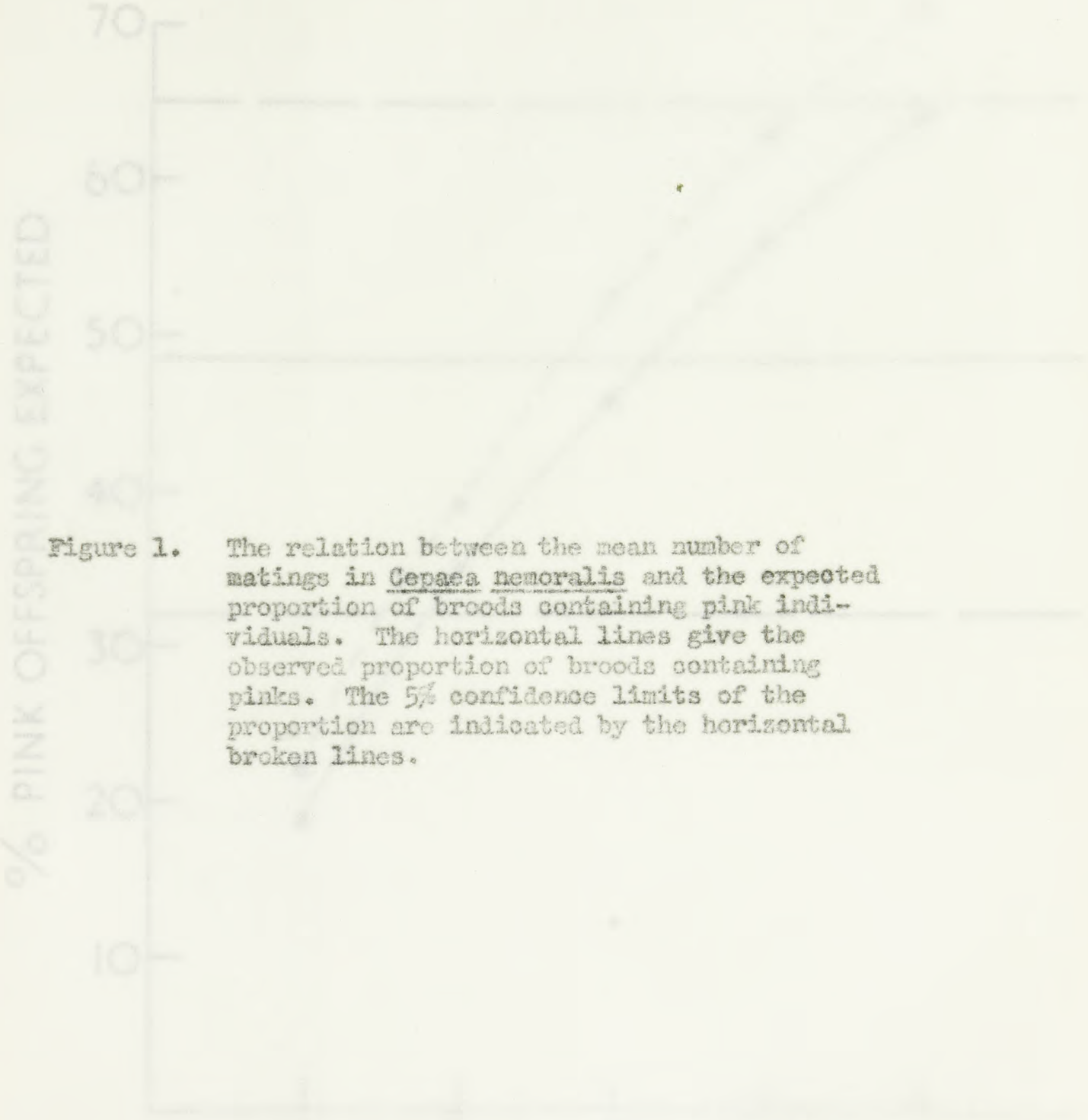
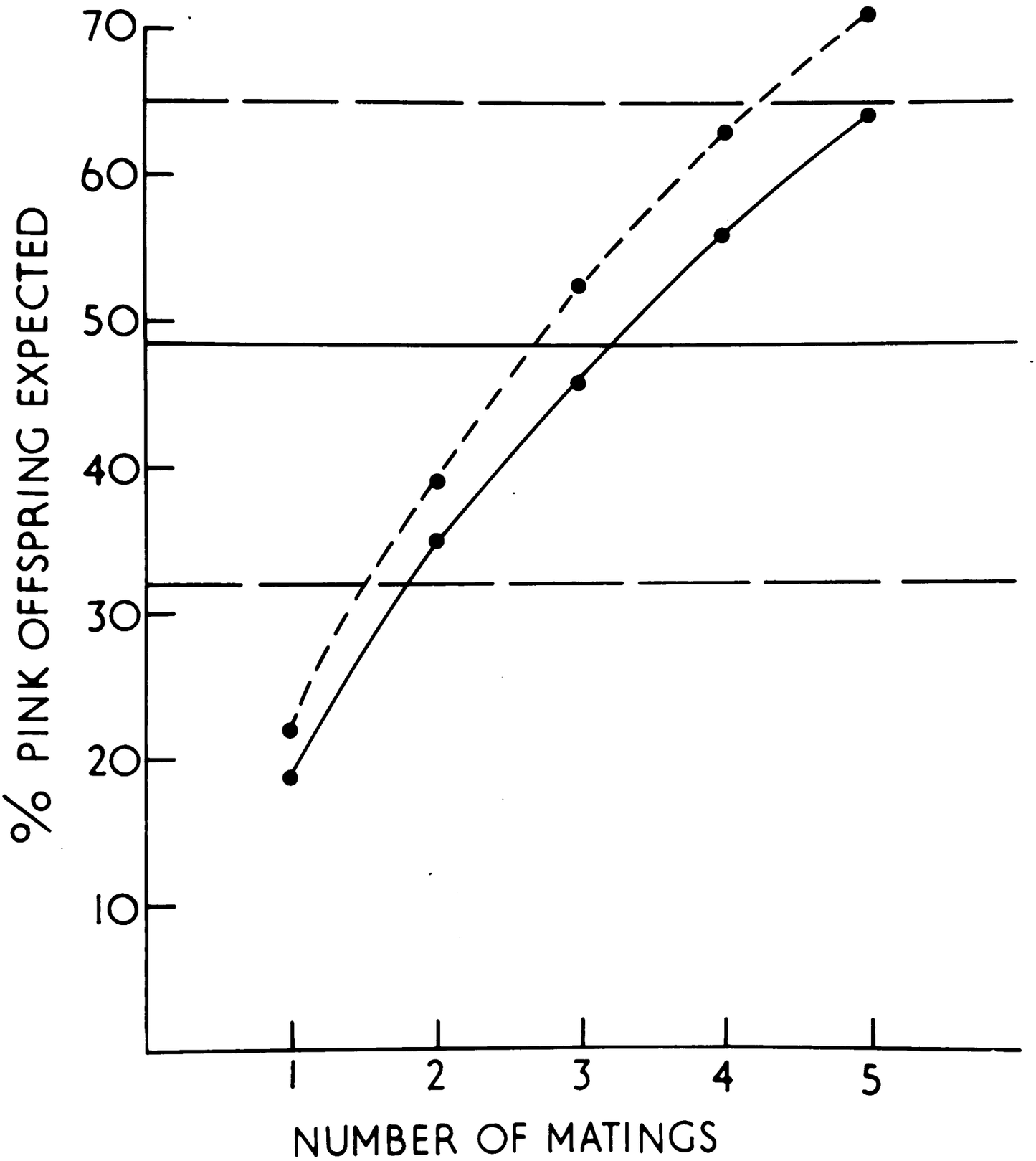


Figure 1. The relation between the mean number of matings in Cepaea nemoralis and the expected proportion of broods containing pink individuals. The horizontal lines give the observed proportion of broods containing pinks. The 5% confidence limits of the proportion are indicated by the horizontal broken lines.


70
60
50
40
30
20
10
% PINK OFFSPRING EXPECTED
1
2
3
4
5
NUMBER OF MATINGS

is counted here as a single observation. For this reason the 5% confidence limits, represented by the horizontal broken lines, are rather wide.

An attempt was made to use more of the available information by considering the proportion of pink to yellow offspring within each brood. Although a maximum likelihood method was developed by Dr. M.G.Bulmer for estimating the average number of matings per individual from the proportions within broods, it became apparent during the analysis that the assumptions of the method were probably not justified. It is very doubtful, for example, whether two matings separated by long periods of time would contribute equally to the subsequent offspring of a particular individual. If sperm from more recent matings fertilize a higher proportion of the eggs in a clutch than sperm which have been stored for some time, then an estimate of multiple mating based on the proportion of types within that brood would be highly misleading.

This objection does not apply to the estimate based on the proportion of broods containing dominants. Matings in the distant past, from which no sperm remain viable, will of course be ignored; but they are of no particular interest to the present study. The important thing to measure is the number of matings which are capable of contributing contemporaneously to the offspring of an individual. Therefore, I shall consider each brood as a single observation.

From Figure 1 it is possible to obtain an estimate of the average number of productive matings per individual in C. nemoralis. The average

for the observed broods is between two and five matings per individual, with about three as the most likely value. If population sample 3 is excluded, then the estimates are reduced by about one third of a mating. Since it is to be expected that the number of matings per individual will be distributed as a Poisson series, and since the zero term of the distribution cannot be determined (see above), then the mean of the Poisson series will be slightly smaller. If $\bar{X}$ is the mean of the truncated distribution, and m is the Poisson mean, then

$$\bar{X} = \frac{m}{1 - e^{-m}}$$

If $\bar{X}$ is 3, then the mean of the Poisson distribution is 2.8.

The average number of matings can be estimated by a second method. It is a useful check since it is independent both of the proportion of matings containing pinks, from which the first estimate is derived, and of the proportions within the broods. The broods are divided into those which conform to good Mendelian ratios and those which do not. The two groups seem to be distinct. If the number of matings follows a Poisson distribution, then the proportion of good Mendelian ratios is related to the mean of the distribution. For a truncated Poisson distribution, if each animal has mated r times, then the probability that all r matings are with individuals of the same genotype (and therefore that the offspring

will conform to a Mendelian ratio) is (Bulmer, personal communication):

$$\frac{e^{-m}}{1-e^{-m}}\left(\frac{m^r}{r!}\right)\left(D^r + H^r + R^r\right)$$

where D, H and R are the proportions of dominant homozygotes, heterozygotes and recessive homozygotes in the population. The sum of all r terms for the whole distribution is then:

$$\frac{e^{-m}}{1-e^{-m}}\left[(e^{mD}-1) + (e^{mH}-1) + (e^{mR}-1)\right]$$

Among the 35 broods, 28 or 80% show good Mendelian ratios for the pink/yellow segregation. Equating this percentage with the summation above and substituting the values of D, H and R (0.01, 0.18 and 0.81) derived from the samples from the colony, the value of m is found to be 1.3, corresponding to a mean of 1.8 for the truncated distribution.

This value is considerably smaller than that derived from the proportion of broods containing pinks. There are several factors which may contribute to the discrepancy. The most important of these is probably the sampling variance of the estimates both of the proportion of broods containing pinks and of the proportion of good Mendelian ratios. The overlap in the confidence limits for these two proportions would allow values of m from 1.4 to 2.3.

Another factor could be the omission, from the sum of the probabilities of like matings, of one class of matings capable of producing good Mendelian ratios. A double mating with equal contributions from a dominant homozygote and a recessive homozygote would

produce a 1:1 ratio simulating a single mating. There are also triple matings which might produce such a result; although as the number of matings rises, the possibility of unequal contributions and therefore of a detectable disturbance increases. These types of matings were excluded from the summation because a) dominant homozygotes are rare (1%) in the population, b) the contributions of different matings may or may not be equal and c) the summation becomes much more difficult if they are included. However if one double mating of the D + R type is included among the "good" ratios, then the value of m should be raised from 1.3 to 1.5.

Another source of error may be the occurrence of multiple matings of the type H + R in which the contribution of the R mating is reduced. Of the ten good Mendelian ratios for pink/yellow, six show a deficiency of pinks and only three a deficiency of yellows. This is striking since the overall proportion of pinks is in excess (see below). Although the numbers are small, it suggests that some of the excess yellows could result from small contributions from R matings in the relatively distant past.

It seems that the estimate based on Mendelian ratios is subject to a greater variety of errors than the estimate based on brood-proportions. It is likely, therefore, that the true mean value for the number of matings is closer to 2.8 than to 1.3. However since the null hypothesis is that each animal produces young from only one mating at a time, a conservative estimate of the mean value of m is two matings.

4. DIFFERENTIAL FERTILITY

The overall phenotypic proportions in the offspring of the sample should bear a definite relationship to the proportions in the population from which the sample was drawn. Since in this case only the offspring of recessives have been reared, the proportion of individuals of dominant phenotype among the offspring will be:

$$\frac{pq}{q^2 + pq} = p$$

Therefore the expected overall proportion of any dominant is equal to the gene frequency of that dominant in the parent population.

From Tables 1 and 2 it can be seen that the pink offspring make up 18.5% of the total instead of the expected 10%. There are at least two possible explanations for the discrepancy. The first is that the gene frequency of pink in the population is indeed 18.5% and that the population samples are significantly deficient in pinks compared with the parent population. The only evidence in favour of this hypothesis is the slight heterogeneity of the successive samples (see above). On the other hand, the most extreme sample does not approach the proportions demanded by a gene-frequency of 18.5%.

An alternative explanation for the excess of pink young is differential fertility of pink and yellow parents. The broods containing pink offspring tend to be slightly larger than those which do not. Omitting the very small brood, number 25, the mean brood size of all-yellow broods is 39.8, while that of broods containing some pinks is 43.5. It might be argued that the brood size of the all-

yellow broods is biased, since small broods would be expected to lack pinks through sampling errors more often than large broods. However if only the broods which contain pinks are compared, the mean of the eight broods with the highest proportion of pinks is 46.3 compared with a mean of 39.8 for the eight with the lowest proportions.

The effect is not very great, for the regression of brood size on the proportion of pink within the brood (in angular measurement) is not significantly different from zero ($t_{(15)} = 0.82$). There may be other factors contributing to the discrepancy, but it is interesting that there is an indication of differential fertility in yellow and pink individuals, particularly as the difference is being expressed by means of the contributions of the "male" parent.

The disturbance of the overall pink/yellow ratio in the offspring is another reason for using the proportion of broods containing pinks, rather than the proportions within broods, for the estimates of multiple matings.

5. DISCUSSION

The concept of effective population size has been discussed by Wright in a series of pioneering papers (e.g. 1931, 1938, 1939, 1940). The effective size of a population is "the number of individuals that would give rise to the sampling variance or the rate of inbreeding appropriate to the conditions under consideration, if they bred in the manner of the idealized population" (Falconer, 1960). Usually the effective population size is smaller than the number of breeding individuals, but in special circumstances it may actually be larger.

The effective population size N_e is not equal to the actual population size N (1) when reproduction is bisexual, (2) when the numbers of the sexes are unequal, (3) when the population fluctuates in numbers, or (4) when family size is non-random. The breeding structure of populations of Cepaea nemoralis has a direct effect on the relation of N_e to N in all of these situations.

In Cepaea, cross-fertilization is obligatory. Since the probability of self-fertilisation in the ideal population is equal to that of any other possible mating, then obligatory cross-fertilization results in a slight decrease in the rate of inbreeding and hence an increase in effective population size (Falconer 1960):

$$N_e = N + \frac{1}{2} \quad \text{(approx.)}$$

Unless the population size is very small it is unlikely that this change will be of any importance.

Of considerably greater significance to the effective size of populations of Cepaea is the fact that these snails are hermaphrodite. In populations of bisexual animals, an inequality of the sexes will reduce the effective population size to twice the harmonic mean of the numbers of males and females (Wright 1938):

$$N_e = \frac{4N_{\delta}\, N_{\varphi}}{N_{\delta} + N_{\varphi}}$$

In the extreme case of a very few individuals of one sex and unlimited numbers of the other, the effective size is approximately four times

the number of the least numerous sex. With hermaphroditic animals, however, the equality of the sexes is automatically adjusted so that there is no reduction in population size.

If the population fluctuates in size from time to time, then the effective size will be closer to the minimum than to the maximum value. For n successive population sizes, the effective population size is the harmonic mean of the numbers at each time (Wright 1938):

$$N_e = \frac{n}{\sum_{i=1}^{n} \frac{1}{N_i}}$$

It is in the case of short term fluctuations that the system of multiple matings and sperm storage in Cepaea will have its greatest effect. If each animal produces offspring from an average of two matings and is capable of sperm storage for more than a year, then the effects of a severe reduction in population size will be mitigated. Without this system, the maximum number of diploid sets of genes that can be present when the population is at its smallest is 2N, provided that each animal has been previously fertilized. In a bisexual population the maximum is 1.5N.

In the case of Cepaea, each animal carries an average of 3N diploid sets of genes, twice the maximum of a bisexual population and 1.5 times the maximum of a hermaphrodite population without storage. Thus if a "crash" of population numbers occurs in Cepaea followed by a swift recovery, the effective population size will be the harmonic mean of the population sizes before and after the disaster and at least 1.5 times the population size at the time of minimum size.

$$N_e = \frac{3}{\frac{1}{N_{max}} + \frac{1}{1.5N_{min}} + \frac{1}{N_{max}}}$$

In this way the mating system provides a reservoir of variability during temporary depletions in numbers.

Finally, the effective population size is affected by the distribution of family size. In the ideal population of bisexual animals, where the population size is stable, the mean number of gametes contributed by each individual is 2. Since the contributions vary according to a Poisson distribution, the variance of gamete numbers is also 2. If the variance is larger or smaller than 2, then the effective population size is not the same as the actual population size. Wright (1938, 1940) shows the relation to be:

$$N_e = \frac{4N - 2}{2 + \sigma_k^2}$$

where σ_k^2 is the variance of the number of gametes contributed by each parent. When σ_k^2 = the mean $m_k = 2$, then N_e is almost the same as N. In the special case when each animal provides exactly two gametes, the variance term becomes zero and the effective population size is very nearly 2N. When the variance is larger than the mean, then the effective population size is less than the actual population size.

Crow and Morton (1955) have shown that if the survival of young is at random, the variance of brood size tends to approach

the mean as the numbers of surviving offspring decrease. Laboratory populations of Drosophila appear to conform to the assumption of random survival, yielding estimates of N_e of from 0.35 to 0.90N. However if each brood tends to survive or perish as a unit then the variance tends to increase with decreasing numbers of survivors. For example, if the variance of family size among the offspring of the 35 individuals recorded here for Cepaea nemoralis is adjusted to a mean of 1 (for a population of hermaphrodites), it becomes 1.12 if survival is assumed to be at random and 52.20 if survival is by broods (for the method of adjustment, see Crow and Morton, 1955). In the absence of modifying conditions, the latter value would represent an effective population size of only 4% of the actual population size!

There is no direct evidence for the validity of one model of survival rather than the other in natural conditions. It seems likely that in Cepaea a change takes place in early life. A clutch of eggs may survive or perish as a unit; but once an individual has hatched, its survival, if not random, is unlikely to be highly correlated with the fate of other members of the same brood.

The breeding structure of the Cepaea population operates in two ways to limit the restriction of effective population size by an increased brood variance. First, the offspring of each individual are divided into a number of broods produced over a period of months or even years. It is highly unlikely that the fates of members of different broods will be correlated with each other. Second, each brood is composed of contributions from one "female" parent and an

average of two "male" parents. The result of such a brood composition is to minimize the effects of any tendency for unit-survival of groups of offspring from single parents. Consequently there will be a reduction of the variance of the gametic contributions of individuals.

In general, therefore, the mating system of Cepaea nemoralis minimizes the effects of the several factors that reduce the effective size of populations. Since processes having a dispersive action on gene frequencies depend on a restriction of population size (I exclude changes in the magnitude and direction of the systematic forces (Wright, 1949, 1955), which are not random), then populations of Cepaea nemoralis are buffered against random changes in gene frequency. It seems likely that in Cepaea, and probably in other species of snails as well, the system of multiple mating and sperm storage has been evolved to protect organisms of rather limited mobility from impoverishment of the gene pool. Even in the extreme case of a population founded by a single fertilized individual, the gene pool will consist on the average of six haploid sets of genes. The homeostatic action of the mating system, and the magnitude of the effects of natural selection (Clarke and Murray, this thesis), can be invoked to explain the rarity of monomorphic colonies in this highly variable species.

SUMMARY.

1. The land snail Cepaea nemoralis (L.) is a hermaphrodite with obligatory cross-fertilization. The breeding system of its populations includes both multiple mating and sperm storage.

2. The mean number of matings contributing to each brood is conservatively estimated to be not less than two.

3. There is some evidence of differential fertility between yellow and pink "male" parents.

4. Multiple mating and sperm storage serve to minimize the effects of population fluctuations and of increased variance of brood-size, which might otherwise reduce the effective size of populations.

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CHAPTER 5. MOVEMENT AND MORTALITY IN A COLONY OF *CEPAEA NEMORALIS*.

Chapter 5

MOVEMENT AND MORTALITY IN A COLONY OF CEPAEA NEMORALIS

1. INTRODUCTION

One of the powerful tools for the study of the structure of animal populations is the analysis of multiple capture data. The work of Lincoln (1930) on waterfowl, of Jackson (1939 and later papers) on the tsetse fly, and Dowdeswell, Fisher and Ford (1940) on the butterfly Polyommatus icarus are classics in this field. More recently Leslie and Chitty (1951), Leslie (1952), Moran (1952) and Darroch (1958, 1959) have discussed various methods of grouping the observations and of obtaining estimates of the variance of population sizes and mortality. Leslie, Chitty and Chitty (1953) and Moran (1952) have pointed out some of the problems, both biological and statistical, which much be faced in applying capture-recapture techniques.

Marking studies of several kinds have been undertaken with Cepaea nemoralis (L.) by a number of workers. Sheppard (1951) used a marked population to demonstrate seasonal changes in the selective value of different phenotypes in this snail, but the marks served mainly to identify the area from which the animal had been taken by a thrush. Lamotte (1951) used marked animals to obtain rough estimates of population size by means of the "Lincoln Index". He also studied the movements of marked animals, but in every case, large numbers of animals were collected, marked,

and released at a single spot. As Lamotte points out, this local overcrowding is likely to cause an immediate dispersal of the marked individuals. Schnetter (1951) has made a similar study which shows extreme dispersal.

The present sampling program was designed to minimize the disturbance to the wild population. Some of the advantages of using large samples were sacrificed in order to study intensively a small area in which each snail is individually marked and returned without displacement. A long sampling chain has secured unusually high recapture rates which permit the division of the data for the investigation of differences in death rates both among sub-groups and at different times. From the recorded movements of animals in the area it is possible to deduce the size of the "neighbourhood" (Wright, 1946) and hence to estimate effective population size.

2. THE COLONY

a. General:

The colony chosen for this study is located at Wittenham Clumps, Berkshire. The Sinodun Hills, on which the clumps are situated, are small outliers of the Lower Chalk, cut off from the main mass of the Berkshire Downs by the intervening Greensand (Arkell, 1947). In the early Iron Age a hill fort was built on one of them, and it is in the ditch of this fort that *Cepaea nemoralis* thrives.

An area 10 by 4 metres was staked out in 2-metre squares

on the inner bank of the ditch on its northwest side. The plot extends from the bottom of the ditch almost to the top of the bank. Footpaths one to two metres wide bound the plot at the top and bottom. The area beyond the top path is under cultivation. Paths 1 metre wide were made to bound the plot on either side. Figure 1 is a general view of the sampling area from the outer bank of the ditch, showing the squares into which the area was divided.

Within the area itself, conditions were disturbed as little as possible. Care was taken always to stand in the same places. This procedure restricted the damage to the vegetation to the area of two footprints at the bottom and in the centre of each 2-metre square. These spots were rapidly reduced to bare, hard-packed earth.

Some changes in the habitat were unavoidable. The most noticeable of these is shown in Figures 2, 3 and 4. Figure 2 was taken on 19 March, 1960. It shows the bank in its winter condition. The sampling area can be seen outlined by the surrounding paths. Figure 3 was taken two months later on 4 May, 1960. The spring vegetation is well grown, and there is little difference between the sampling plot and the surrounding area. In Figure 4, however, the progressive change that takes place through the summer months has become apparent. The photograph was taken on 28 July, 1960. It shows that the sampling area has lost much of the long, dry grass which is seen elsewhere. The dry stems tend to break off during sampling. Consequently, the vegetation of

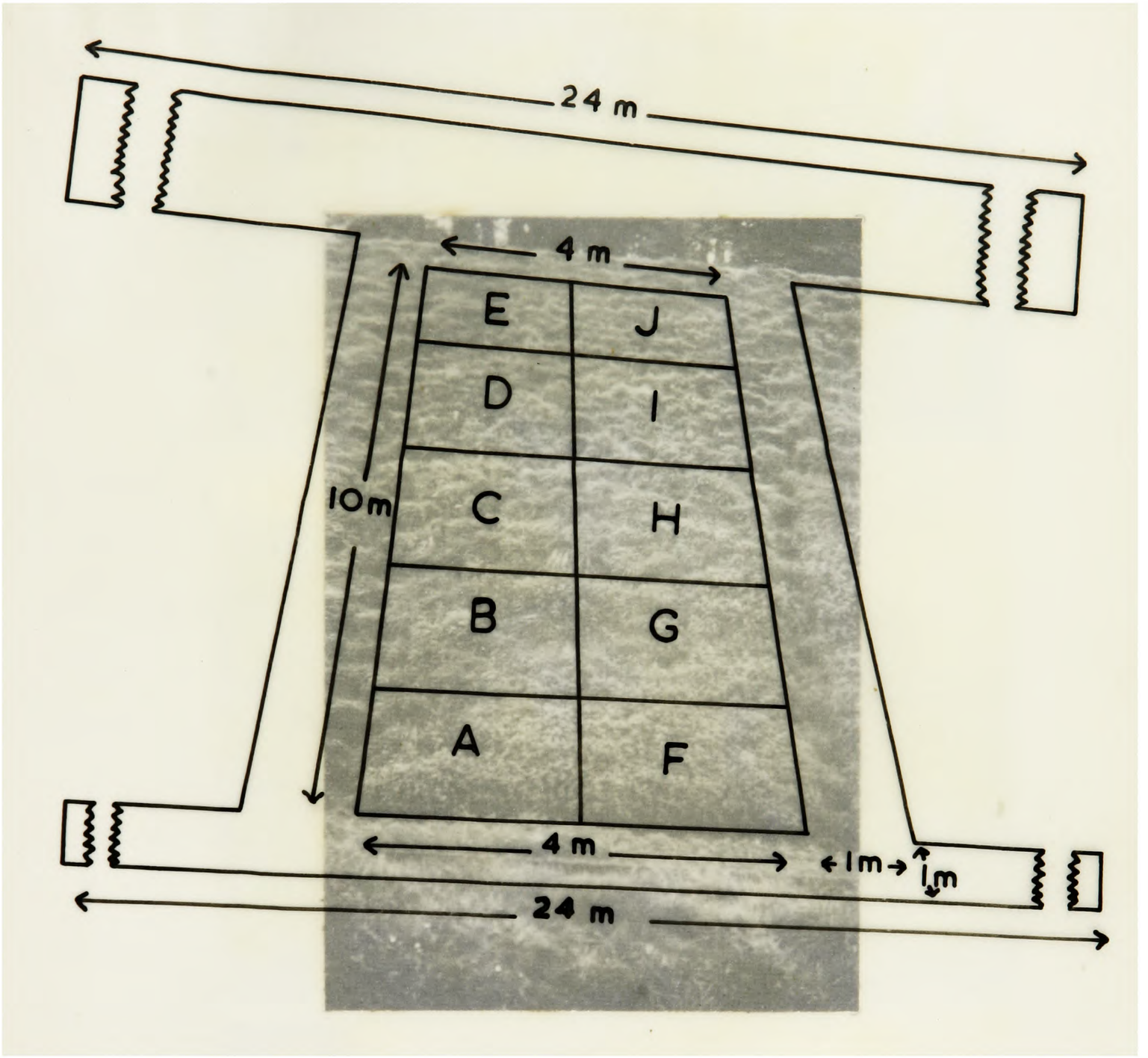

24 m
4 m
E
J
D
I
C
H
B
G
A
F
10 m
4 m
1 m
1 m
24 m



Figure 1. The sampling area at Wittenham Clumps, Berkshire.

Figure 2. Winter (19 March, 1960)

Figure 3. Spring (4 May, 1960)

Figure 4. Summer (28 July, 1960)

General views of the sampling area at Wittenham Clumps, Berkshire, at different seasons.







the sampling area tends to be shorter and rather greener than the surrounding parts.

Another change that is less easy to illustrate is a reduction in the amount of matted vegetation around the roots of the plants. Again, this is the result of continued searching for animals, and is unavoidable.

b. Vegetation:

Figure 1 shows the principal features of the vegetation of the sampling area. The bottom third, rather askew, is a thick clump of stinging nettles (Urtica dioica L.). The nettles give way in the middle section to long grasses and a variety of broad-leaved plants. Toward the top of the bank the vegetation becomes shorter and more even. Table 1 lists the more important components of the plant community.

c. Other Molluscs:

The ditch at Wittenham Clumps is inhabited by a wide variety of gastropods. Table 2 lists the species which have been found. The relative abundance of the shelled species is shown in Table 3. Each spring all shells which could be found within the sampling area were collected. With some reservations, the shells collected in the springs of 1960 and 1961, on which Table 3 is based, can be considered as the production of 1959 and 1960. The principal bias will be in favour of the larger species. Since no attempt was made to sift the soil and dead vegetation many individuals of the smaller species almost certainly have been overlooked. The second source of

Plant	Distribution
Common grasses:	
Dactylis glomerata L.	General
Agropyron repens (L.).	General
Arrhenatherum elatius (L.).	General
Phleum bertolonii DC.	Top half
Common broad-leaved plants:	
Ranunculus sp.	Top half
Trifolium campestre Schreb.	Top half
Lotus corniculatus L.	Top half
Lathyrus pratensis L.	Top half
Pastinaca sativa L.	Centre
Urtica dioica L.	Dominant in A, F and G
Primula veris L.	Top half
Lysimachia nummularia L.	Top half
Myosotis arvensis (L.).	Centre
Lamium purpureum L.	Bottom half
Lamium album L.	Centre
Plantago media L.	Top half
Plantago lanceolata L.	Top half
Campanula glomerata L.	Top half
Galium verum L.	Top half
Galium aparine L.	General
Scabiosa columbaria L.	Top half
Bellis perennis L.	Top half
Achillea millefolium L.	Top half
Cirsium vulgare (Savi).	Bottom half
Cirsium palustre (L.).	Bottom half
Cirsium acaule (L.).	Bottom half
Centaurea scabiosa L.	General
Centaurea nigra L.	General
Leontodon hispidus L.	E and J
Sonchus oleraceus L.	General
Hieracium sp.	General

TABLE 1. Common plants of the sampling area at Wittenham Clumps, Berkshire.

Family Pomatiidae

Pomatias elegans (Müller). [a]

Family Cochlicopidae.

Cochlicopa lubrica (Müller).

Family Enidae.

Ena obscura (Müller)

Family Helicidae.

Arianta arbustorum (L.).

Cepaea nemoralis (L.).

Helix aspersa Müller. [b]

Hygromia striolata (C. Pfeiffer).

Hygromia hispida (L.).

Monacha cantiana (Montagu).

Helicella caperata (Montagu).

Helicella virgata (da Costa).

Helicella itala (L.).

Family Arionidae.

Arion intermedius Normand.

Arion circumscriptus Johnston.

Arion hortensis Férussac.

Arion ater (L.).

Family Zonitidae.

Oxychilus draparnaldi (Beck).

Retinella nitidula (Draparnaud).

Family Vitrinidae.

Vitrina pellucida (Müller). [c]

Family Limacidae.

Agriolimax reticulatus (Müller).

TABLE 2.

Mollusca from the sampling area at Wittenham Clumps, Berkshir

a. No live animals found.

b. A single shell fragment.

c. A single living animal.

Blocks	Pomatias elegans	Arianta arbustorum	Hygromia sp.	Monacha cantiana	Helicella caperata	Helicella virgata	Oxychilus draparnaldi	Retinella nitidula	Other species	Total numbers
A/F	2.0	0.2	71.3	9.8	1.8	4.5	1.3	8.5	0.7	449
B/G	2.0	0.7	65.7	11.4	2.0	7.6	2.8	7.6	0.2	458
C/H	0.5	-	52.3	21.3	3.8	8.7	1.3	11.5	0.6	390
D/I	-	-	47.5	26.2	5.4	10.4	1.0	8.9	0.5	202
E/J	-	-	42.7	27.1	2.1	7.3	3.1	17.7	-	96
Total	1.3	0.3	60.3	16.2	2.8	7.3	1.8	9.6	0.5	1595

TABLE 3. Percentages of empty shells removed from the sampling area at Wittenham Clumps, Berkshire, in the springs of 1960 and 1961.

error is that some of the shells are probably older than the year before collection and were overlooked the year previously or were newly uncovered after being buried for a time. On the whole, however, the collections support the qualitative impressions of the relative abundance of species.

An interesting point is shown by Table 3. Monacha cantiana and Hygromia striolata and hispida are inversely related in abundance. With the transition from the relatively more moist and shaded lower slope to the exposed and dry upper slope, the proportion of Hygromia falls and of Monacha rises. Although these animals are almost invariably found in association, this inverse relationship may point to some form of competition between them.

3. SAMPLING METHODS.

Sampling of the Wittenham Clumps population was carried out from 20 May, 1959 to 3 August, 1961. With the exception of the winter months, sampling took place at least once in almost every weekly period. The intervals between samples varied slightly, since an effort was made to choose the most favourable day in each period, i.e. a day during or immediately following rainfall. However, an attempt was also made to prevent the distortion of the pattern of weekly sampling. During periods of drought, the population was sampled just after daybreak in order to find animals which might be tempted to activity by the dew.

On each occasion the sampling area was searched methodically,

from square A to square E and then from F to J. All empty Cepaea shells were removed, but with the exception of two damaged animals, no live Cepaea were ever taken from the area. As each animal was found, its exact position was recorded, and if it were unmarked, it was given a distinctive mark. It was then replaced immediately in its former position.

A dentist's drill held either in a pin vice or in a hand drill was used to mark the snails with small holes through the lip. Holes were drilled in two rows, each row with five holes corresponding to the five positions where bands may occur. These ten positions made possible a simple code for identifying individual animals. Figure 5 illustrates a five-banded snail, number 37. The mark is permanent and easily read, even after the hole has been repaired from within. An additional advantage is that the marks are usually left intact on predated shells, so that for many animals it is possible to specify the time of death (see below). The chief disadvantage of the method is that it cannot be applied to young snails.

After the sampling area had been checked, the surrounding areas were searched for predated shells. The overlay on Figure 1 shows, distorted by perspective, the standard areas for these collections. They include the sampling area itself, the 1-metre wide paths around the area, the top and bottom paths for 10 metres to the right and left of the area, and an equivalent length of the edge of the ploughed field just beyond the top path. In



Figure 5. A five-banded example of Cepaea nemoralis. The lip has been marked with small holes for individual identification. This mark is no. 37.

addition, other nearby areas were searched from time to time, but the resulting collections were kept separate from those from the standard areas.

4. MOVEMENT AND THE "NEIGHBOURHOOD".

For each pair of successive captures, the elapsed time and the displacement between captures has been recorded. If an animal was captured at times a, b and c, for example, measurements were made for the periods a to b and b to c. No record was included for the period a to c as this observation is partially dependent on the other two. The mean displacements between successive captures separated by one to five weeks are shown in table 4.

The interpretation of these observations presents some difficulties. First, there is evidence that the contributions of sub-groups of observations are not wholly equivalent. If only captures which were made after an interval of one week (see below) are considered, there are differences between phenotypes and between observations made at different times of the year.

In table 5 the data for intervals of one week are separated into the yellow and pink classes. The brown class has not been included since very few observations are available for this group. The mean distance moved between successive observations is greater for the yellow class. The means of the two distributions are significantly different from one another ($P < 0.01$).

In table 6 the data are divided into observations made

Weeks Between Samples	Mean Displacement in Metres	Number of Observations
1	1.40	161
2	1.75	74
3	1.69	55
4	1.73	40
5	1.66	22

TABLE 4. Mean displacement of Cepaea nemoralis between successive captures separated by differing lengths of time.

Displacement in Metres	Pink	Yellow
0.1 - 0.5	22	9
0.6 - 1.0	23	11
1.1 - 1.5	22	13
1.6 - 2.0	11	7
2.1 - 2.5	6	10
2.6 - 3.0	5	3
3.1 - 3.5	3	3
3.6 - 4.0	1	3
4.1 - 4.5	0	1
4.6 - 5.0	0	1
Number	93	61
Mean Displacement	1.24	1.69

TABLE 5. Weekly displacement in Cepaea nemoralis. A comparison of observations for the pink and yellow classes.

Displacement in Metres	April - May	All Other Months
0.1 - 0.5	25	6
0.6 - 1.0	25	12
1.1 - 1.5	17	21
1.6 - 2.0	10	9
2.1 - 2.5	5	11
2.6 - 3.0	1	7
3.1 - 3.5	3	3
3.6 - 4.0	1	3
4.1 - 4.5	1	0
4.6 - 5.0	1	0
Numbers	89	72
Mean Displacement	1.18	1.68

TABLE 6. Weekly displacement in Cepaea nemoralis. A comparison of observations for April and May with those for the remainder of the year.

at the beginning of seasonal activity (April and May) and during the remainder of the year. The difference in mean weekly displacement for the two periods is highly significant ($P < 0.001$). It is particularly noteworthy that displacement in the early season, when activity is greatest in the population, should be significantly smaller than later in the year when fewer animals are in evidence. This seasonal fluctuation of activity is shown in figure 6, which gives the monthly average numbers of animals per sample. Since each sample represents an equivalent sampling effort, the comparison of means provides a rough estimate of relative activity, although the numbers taken at any given time will also reflect changes in population density.

The importance of this inverse relationship between activity and mean displacement is that it demonstrates that dispersal is not the direct result of the normal activity cycle of the animal. It seems, rather, that dispersal is the result of overcrowding (Lamotte, 1951; Schnetter, 1951) or of a search for an optimal environment.

An alternative explanation of the smaller mean displacement in April and May must be considered briefly. Since it appears that there are differences among the phenotypes in mean displacement, it is conceivable that differential behaviour of phenotypes could give rise to the seasonal differences. Schnetter and Sedlmair (1953) and Sedlmair (1956) have indeed reported behavioural differences of phenotypes in Cepaea with respect to humidity and temperature under experimental conditions. However, if the distribution of

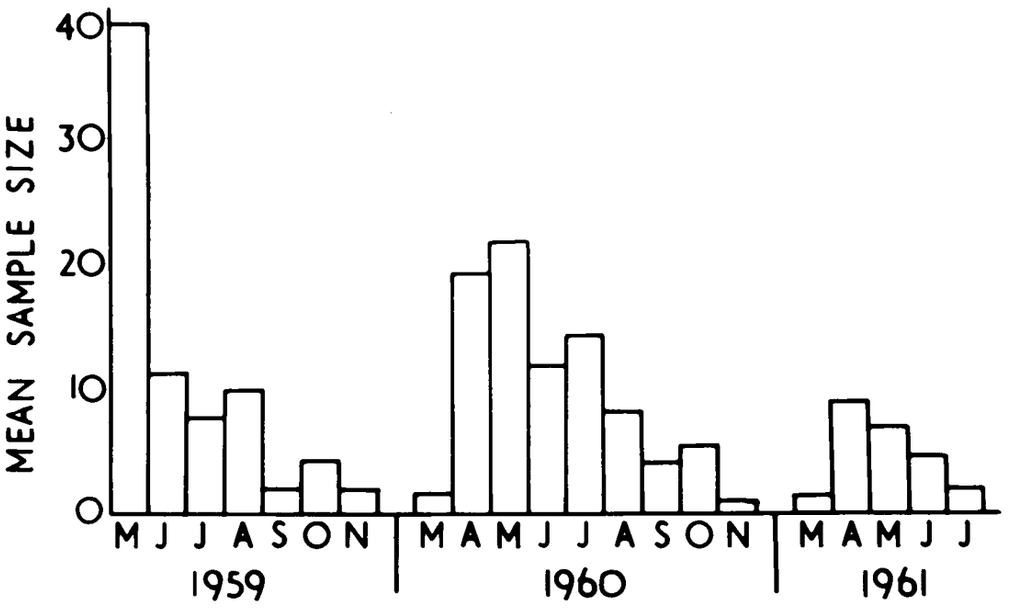


Figure 6. Seasonal fluctuation of activity in Cepaea nemoralis at Wittenham Clumps. Mean sample size is shown for each month in which sampling took place.

the yellow and the pink classes in the observations for April and May and for the rest of the season is tested, the agreement is very good indeed ($\chi^2_{(1)} = 0.065$; $P > 0.80$). Therefore there is strong evidence that differential behaviour of different phenotypes is not responsible for the seasonal differences in movement.

A second interpretational difficulty is that the observed mean displacements are large compared with the size of the sampling area. From table 4 it can be seen that observations separated by more than two weeks show no increase in mean displacement. It is likely that there are two factors contributing to this effect. The first is, of course, that with the small sampling area, very large displacements will not be recorded. Hence there is a limit above which further increase in mean displacement cannot be detected. The other is that while short-term displacement is large, this displacement does not necessarily represent dispersal, which may take place at a slower rate. While the first factor will inevitably begin to operate at some time, the evidence that maximum activity does not increase the observed displacement (see above) suggests that the second may also be important.

Fortunately there is independent evidence from predated shells which helps to clarify the situation. The sampling area is bounded on its upper side by a stony, cultivated field. The stones along the edge of the field are frequently used by thrushes (Turdus ericetorum Turton) as anvils for breaking snail shells. Predated shells are found scattered along the stone border, indicating that the thrushes do not select a single stone for use

over long periods. Observations by Sheppard (1951) and especially by Morris (1954) confirm that snails are not carried for long distances and that thrushes tend to use the nearest convenient anvil.

As stated above, all predated shells were collected from the border of this field for a distance of ten metres on either side of the sampling area. In order that no marked shells should be overlooked, as soon as a single marked shell was found in the first pair of ten-metre strips, the search was extended to two further ten-metre strips from that date onwards. When a marked shell appeared in the second pair, a third was added. In this way the strip under observation always ended with a ten-metre length at either end in which no marked shells had ever been found. The shells collected from the newly-added strips were not included with the collections from the "standard" areas (see above). The result of this method of collection is that the distribution of predated shells is unlikely to be affected by the loss of extreme observations. At the end of the sampling period, a 64-metre length of the border was being searched, although only one marked shell (at 15 m) appeared at a distance of more than 10 metres from the sampling area.

It is possible to compare, therefore, estimates of dispersal based on the displacement of live individuals per week and on the displacement of animals from the time of the last recorded capture to death on a thrush anvil. In order to determine the size of the "neighbourhood" (Wright, 1946) and the effective size of the population,

the measurements of displacement must be related to the standard deviation of the dispersal of animals per generation. In chapter 3 (and see below) generation time has been estimated at approximately three years. Since there is little activity during the winter from November to March, the yearly dispersal of Cepaea should be related to an active season of about 35 weeks.

In the first instance, each observation of displacement during a single week has been treated as a measure of dispersal. Considering each observation to be a deviation from a mean of zero, then the standard deviation of the weekly dispersal is:

$$\sigma = \sqrt{\frac{\Sigma d^2}{n-1}} = 1.703 \text{ metres}$$

Extrapolation of this figure for three seasons produces a rough estimate of dispersal per generation:

$$\sigma = 1.70 \sqrt{105} = 17.3 \text{ metres}$$

It is interesting that an estimate by Lamotte (1951) for the colony at Coquerel (Somme, France) is remarkably similar (16.3 metres), although the sampling conditions were very different.

As a check on the rather rough estimate of dispersal, the 24 shells found on thrush stones along the edge of the field were scored for elapsed time and displacement since each was last captured. The regression of displacement on the square root of elapsed time is positive, although not significantly different from zero ($t_{(22)} = 1.30$; P between 0.2 and 0.3). The

equation of the regression line is:

$$y = 1.60 + 0.658x$$

The facts that the regression is not significant and that the constant is large compared with the coefficient of x suggest again that the short-term movements of the animals are large compared with the long-term dispersal.

It is necessary to extrapolate the regression line in order to estimate dispersal over a three-year period. For a 35-week season the mean dispersal is 8.3 metres. This value represents, however, the projection of a two-dimensional dispersal onto a line. Hence the mean dispersion in two dimensions is:

$$\sqrt{2 \times (8.3)^2} = 11.7 \text{ metres}$$

This figure, of course, should be compared not with the standard deviation of the dispersion but with the extrapolated mean of the individual measurements of dispersion, i.e. with:

$$1.4 \sqrt{105} = 14.3 \text{ metres}$$

Considering the approximations involved, these two estimates are in remarkably good agreement.

It is noteworthy that the estimate based on predated shells is lower than that from weekly displacement. It indicates again that dispersal is less than might be expected from observations of short-term displacement.

From these considerations, it seems that the standard deviation of the dispersal per generation in the Wittenham Clumps colony is slightly less than 17 metres, approximately 15 metres.

Wright (1946) has shown that the area of the panmictic unit or neighbourhood in a continuous population is:

$$\pi(2\sigma)^2$$

where σ is the standard deviation of the dispersal per generation. This represents the area of a circle with a radius of twice the standard deviation. In the Wittenham colony, the width of the habitable area is less than 4 σ or 60 metres, and therefore the full extent of the theoretical area (2827 sq. metres) cannot be attained. The neighbourhood is reduced by the area of the two segments of a circle of 2 σ radius that extend into unfavourable habitats. Each of these has an area:

$$\frac{4\pi\sigma^2\theta}{360} - 2\sigma^2 \sin\theta = 824 \text{ sq. metres}$$

Subtracting twice this area from the theoretical area gives an estimate of 1180 sq. metres for the approximate size of the neighbourhood in the Wittenham Clumps colony.

5. POPULATION SIZE.

The size of the population inhabiting the sampling area at Wittenham has been estimated by the method described by Dowdeswell, Fisher and Ford (1940) and in greater detail by Ford (1953). Dowdeswell and Durran (personal communication) have prepared a program for carrying out the necessary calculations on the Mercury electronic computer. Figure 7 shows the mean weekly population sizes per month for the three seasons under consideration. Each season's captures have been treated

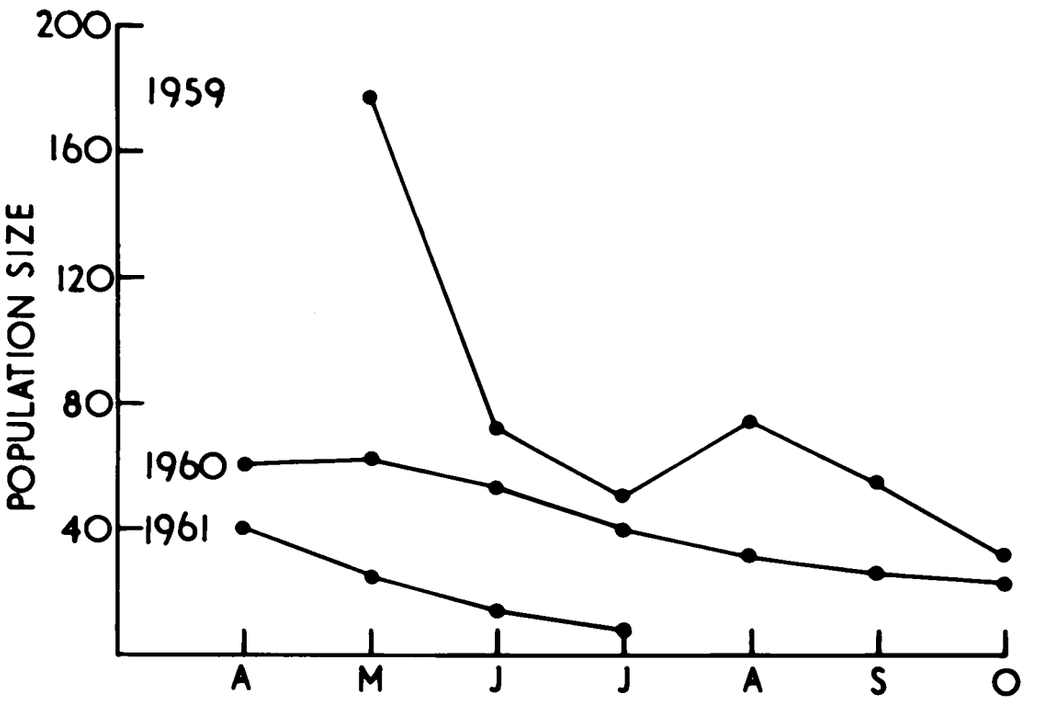


Figure 7. Mean weekly population size for each month for which estimates are available.

separately. It is immediately obvious that the population at Wittenham has decreased drastically during the period of the observations.

A portion of the downward trend in each season is attributable to the effects of dilution by migration. Since the weekly movement of the animals is large compared with the size of the sampling area, the immediate effect of this movement is to exchange marked animals for unmarked ones. An overestimate of population size within the sampling area during the early part of any sampling period is the result. The effect is progressively reduced as the length of the sampling chain increases. In any case, it is unlikely that the estimates are seriously affected by dilution. The number of catches frequently equals 50% of the estimated population size, and on one occasion reached 84% fairly early in the season (11-13 May, 1960: Captures = 47: Estimate = 58).

In addition, the comparison of sizes in the same month in successive years demonstrates that the principal component in the downward trend is the real decrease in numbers of animals. Searches of nearby areas indicate that this reduction is not confined to the sampling area alone, but that it has occurred throughout the entire population at Wittenham Clumps.

During the early season of 1959, the population of the 40 sq. metres of the sampling area can hardly have been less than 80 animals. This density of 2 per sq. metre represents an effective population size of 2360 for the neighbourhood, which is a "large"

population from the point of view of gene-frequency dispersion (Wright, 1946; Falconer, 1960). By the middle of the 1960 season, the density had dropped by 50%. In 1961 the population size fell even further until the density reached the low value of 0.5 to 0.25 per sq. metre. Large samples during this period numbered 15 on 11 April and 8 on 12 June. A density of 0.25 per sq. metre represents a population size for the neighbourhood of only 295, which is approaching the size of a "small" population.

In the absence of other disturbing factors, random fluctuations in gene-frequencies may be expected in neighbourhood populations of an effective size of 200 or less (Wright, 1946). In the Wittenham population, however, such random drift is unlikely to be important for a number of reasons. First, the effective population size will be the harmonic mean of successive population sizes (Wright, 1938). In addition, the mating system of Cepaea provides an extra homeostatic device that increases by 50% the minimum population size during short-term fluctuations (Chapter 4).

Since all these considerations apply to populations in the absence of selection, by far the most important factor in the determination of gene-frequencies is natural selection (see below). Although all the components of selection cannot be assessed, it is possible to observe relative differences in the survival value of phenotypes.

6. MORTALITY.

Differential mortality may be detected by two different methods. A direct measure of some of the differences is possible from observations on dead and predated shells, while the analysis of multiple captures produces estimates of survival of living animals.

a. Differential Predation:

On each sampling occasion, all of the dead and predated shells from the "standard" areas (see above) in the Wittenham colony were collected. The shells could be separated into three groups: those predated by thrushes (*Turdus ericetorum* Turton), those predated by small mammals (rabbits and/or rodents) and those which were unbroken. Morris (1954) has described the different patterns of breakage characteristic of thrush and mammal predation. Doubtful cases were assigned to one class or the other according to whether or not they were found in the vicinity of a thrush anvil. The 427 shells collected during the three seasons from the standard areas at Wittenham are classified in table 7 by phenotype and by the cause of death where this can be determined.

In figures 8 and 9 the monthly totals of predated shells are shown in the form of histograms. Thrush-predated shells are indicated by the hatched portions of each column, mammal-predated shells by the white portion. The scores for November through February represent the equal distribution of the predated shells collected at the end of February.

	Pink ±12345	Pink 00000	Yellow ±12345	Yellow 00000	Brown 00000	Total
Thrush-predated	150	16	78	6	9	259
Mammal-predated	57	9	43	9	1	119
Shell unbroken	20	3	20	3	3	49
Total	227	28	141	18	13	427

TABLE 7. Empty shells of Cepaea nemoralis taken from 1959 to 1961. All banded shells are scored as ±12345.

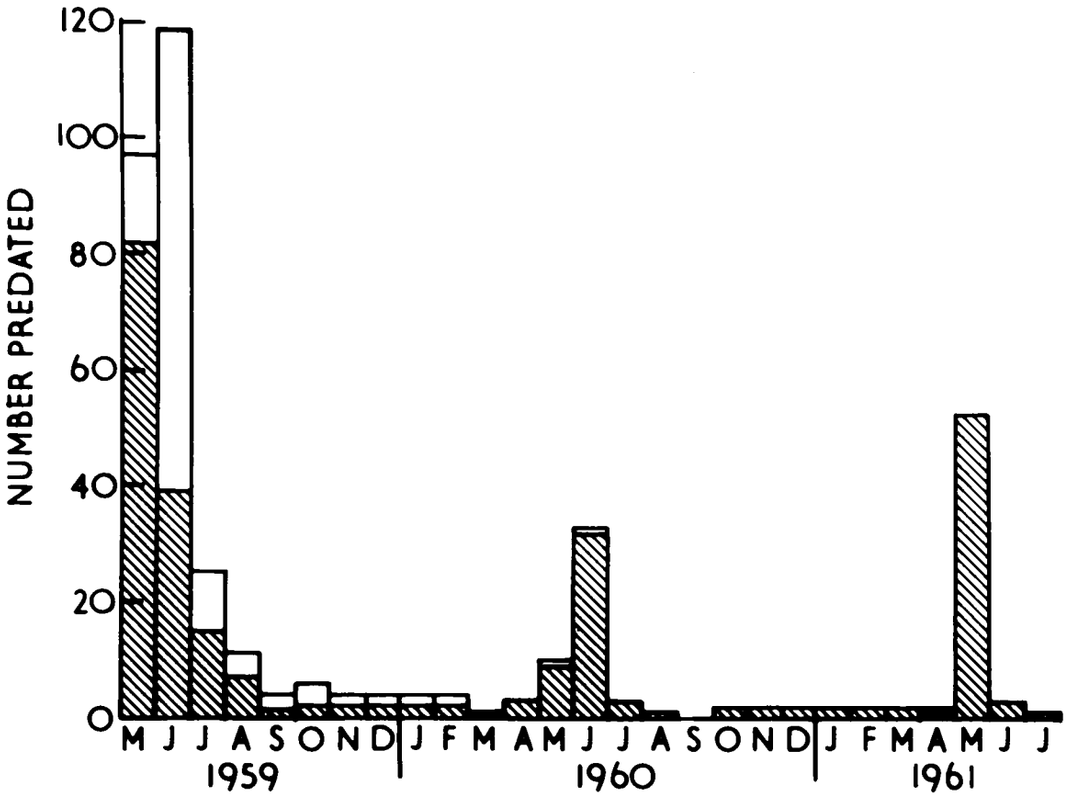


Figure 8. Monthly totals of all predated shells collected from the "standard" areas at Wittenham Clumps. The hatched portion represents the thrush-predated shells, the plain portion mammal-predated shells. The scores for November through February represent the equal distribution of the predated shells collected at the end of February.

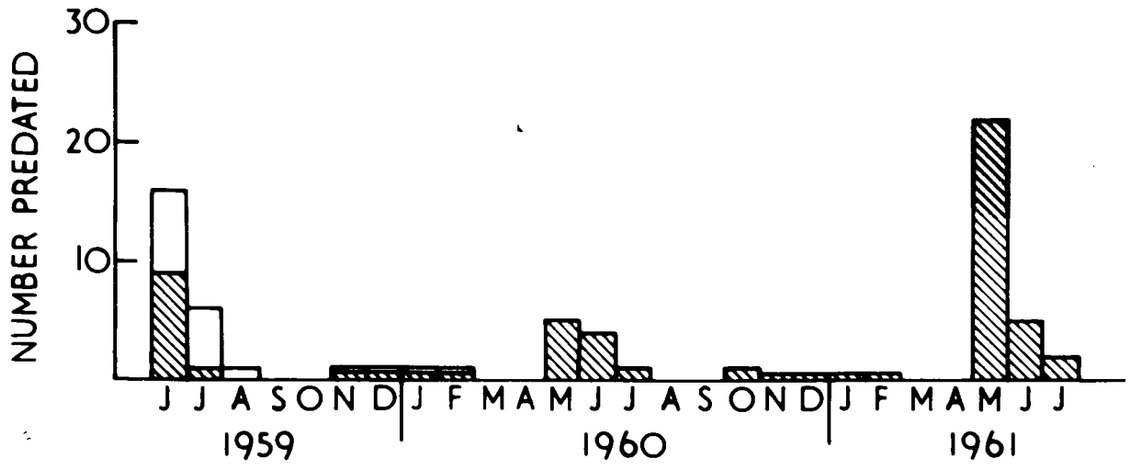


Figure 9. Monthly totals of marked predated shells both from the "standard" areas and elsewhere. Conventions as for figure 8.

The variation in the intensity of predation is remarkable. In each year there was a period of very heavy predation in May and/or June with very little during the remainder of the year. This pattern differs in two important respects from cycles of predation in other areas. First, Lamotte (1951) has found that in many colonies of C.nemoralis in France, the maximum intensity of predation, particularly by thrushes, occurs during the winter. The situation at Wittenham is quite different, with very few animals being taken in winter. It seems likely that Lamotte's failure to find evidence of visual predation in many colonies reflects a difference between England and France in the yearly cycle of predation.

Dr. P.M.Sheppard (personal communication) has found that in another colony in the Oxford area (Wytham Woods) the intensity of thrush predation depends on the availability of other foods. In periods of drought, thrushes turn to Cepaea as a reserve food supply. The situation in the Wittenham colony seems to be different. Figure 10 shows the monthly rainfall reported by the Meteorological Office in Abingdon, Berks, for the periods of the Wittenham observations. The record drought of August and September 1959 was marked by a decline in intensity of predation at Wittenham. In the relatively dry months of April 1960 and March/ 1961 predation was negligible. Only in the month of May 1961 was an unusually dry month accompanied by an increase in the intensity of predation. It seems likely that the key to the intensity of predation at Wittenham is to be found in the breeding activity of the thrushes, which is at

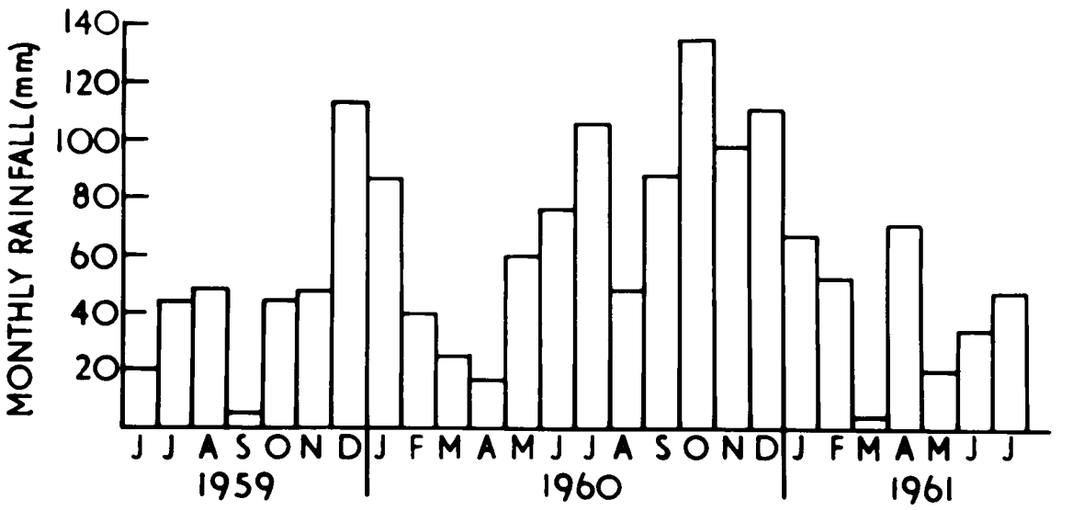


Figure 10. Monthly rainfall for Abingdon, Berks.
By courtesy of the Meteorological Office.

its height in May and June (Witherby et al., 1938). At this season the thrushes' demands on the available food supply will be at a maximum.

A remarkable feature of figures 8 and 9 is the change in the proportion of thrush- and mammal-predated shells in successive months. The figures immediately suggested to me that a subjective change had occurred in the criteria by which the shells were scored. The whole of the material was therefore scored for a second time with almost identical results. I have no satisfactory explanation for the decline in the intensity of predation by mammals. It is possible that an outbreak of myxomatosis among the rabbit population could be a contributing factor, but a single rabbit carcass near the sampling area is the only suggestive fact.

Cain (1953) has suggested that since rabbits and many small rodents are colour-blind, the basis of discrimination in visual predation by these animals will be tone and not colour. It is to be expected, therefore, that the result of selection by mammals will be different from that of selection by thrushes, which are able to distinguish colours. In the predominantly green habitat at Wittenham, the yellow shells should be least conspicuous to the thrushes but most conspicuous to mammals selecting by tone (Cain, 1953).

If the distribution of yellows and non-yellows (pinks and browns) in the two groups of predated shells is tested, there is a significant excess of yellows among the shells predated by mammals

($\chi^2 = 4.016$; $P < 0.05$)

Predator	Yellow	Non-yellow	
Thrush	84	175	259
Mammal	52	67	119
	136	242	378

This difference is not attributable to changes from year to year in the proportion of yellow shells in the thrush-predated group, since the collections of thrush-predated shells for the three years show no heterogeneity ($\chi^2_{(2)} = 2.736$; $P > 0.20$).

Lamotte (1951) has argued that the failure to detect differences in the composition of predated and non-predated dead shells is evidence that visual predation is unimportant in many of the colonies which he has studied. It is interesting that in the Wittenham colony, where the components of visual predation are demonstrably different, there is no detectable difference between the total of all predated shells and the unbroken dead shells ($\chi^2 = 1.785$; $P > 0.10$).

	Yellow	Non-yellow	
Predated shells	136	242	378
Unbroken dead shells	23	26	49
	159	268	427

The balance of two components of selection is not at all equivalent to selective neutrality of the genes in question.

Another method of investigating the effects of selective

predation is to compare the proportions of phenotypes in the marked population with the proportions of marked dead shells. Table 8 shows the number of animals of each phenotype marked in each year, and table 9 the recoveries of marked dead shells. Part of the latter were collected from the "standard" areas and were included among the collections discussed above, but part were found elsewhere. Unfortunately it is only in the group of thrush-predated shells that the number of shells is sufficient for useful comparisons to be made.

If the proportions of yellow and non-yellow shells in the marked, thrush-predated shells and in the remainder of the marked animals are compared, the difference is not significant ($\chi^2 = 1.288$; $P > 0.20$). However, if only those animals marked in 1959 (which have been exposed to the hazards of predation for the longest time) are considered, then the thrush-predated group contains a significant excess of pink and brown shells ($\chi^2 = 4.912$; $P < 0.05$).

	Yellow	Non-yellow	
Thrush-predated shells	5	21	26
Other marked shells	37	43	80
	42	64	106

By either of the two methods of comparison which have been considered, there are no detectable differences in the proportions of the different band-formulae among any of the sub-groups.

	Pink ±12345	Pink 00000	Yellow ±12345	Yellow 00000	Brown 00000	Total
1959	52	9	37	5	3	106
1960	43	4	35	5	5	92
1961	19	3	6	2	0	30
Total	114	16	78	12	8	228

TABLE 8. Numbers of snails of each phenotype marked in the Wittenham Clumps colony.

	Pink ±12345	Pink 00000	Yellow ±12345	Yellow 00000	Brown 00000	Total
Thrush-predated	35	5	19	2	3	64
Mammal-predated	7	1	5	2	0	15
Shell unbroken	2	1	6	0	0	9
Total	44	7	30	4	3	88

TABLE 9. Recoveries after death of marked snails in the Wittenham Clumps colony.

b. Differential Survival:

It is possible to estimate the survival of Cepaea nemoralis in the Wittenham colony by two independent methods, using dead shells and live captures respectively.

For 72 of the 88 animals recorded in table 9, the time of death is known. It is therefore possible to determine exactly the survival rate of this group for each period. The survival is expressed as a percentage of those animals which were alive at the beginning of the month, which survived until the end of the month. Deaths during the winter were assumed to be distributed evenly over the period from November through February.

Figure 11 shows the survival of this group during the three years. It demonstrates again that the rate of survival fluctuates violently from time to time. There is, of course, a direct relationship between the survival rate of this group and the intensity of predation illustrated in figure 9.

The survival rates for the Wittenham population have also been calculated by the method described by Ford (1953, and see above). The program by Dowdeswell and Durran provides the standard error of the rate of survival (see also Leslie and Chitty, 1951). Table 10 shows the mean weekly survival rate of all phenotypes in the three successive years. The values for the three years indicate that the overall rate of survival has been fairly constant at about 90% per week over the period of the observations.

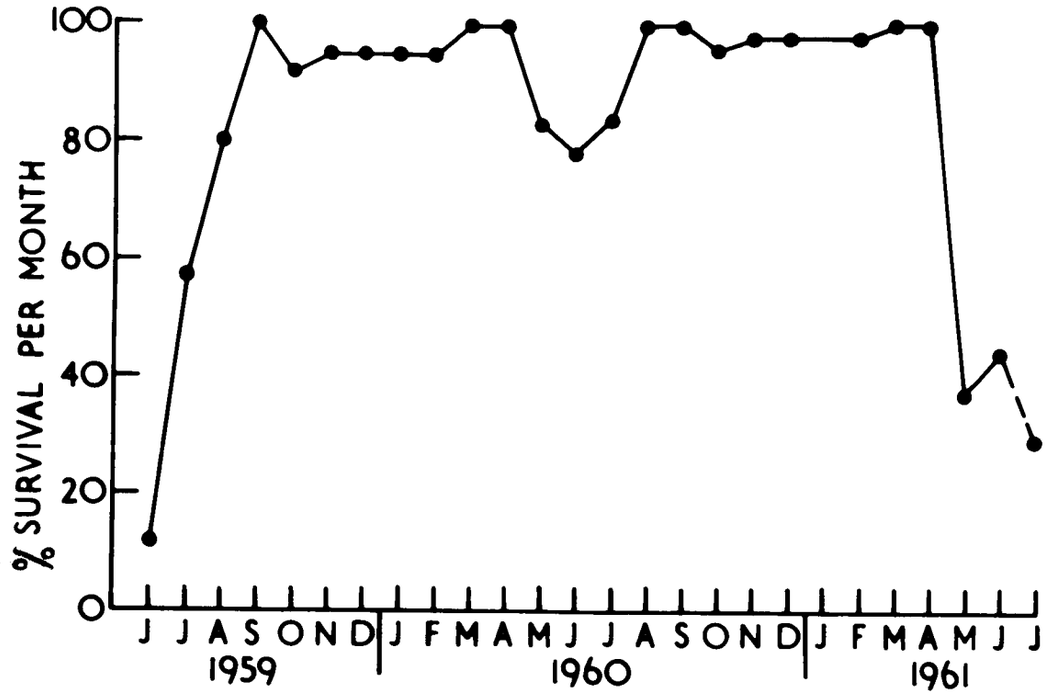


Figure 11. Monthly survival rate of marked snails for which the dates of death are known.

	Weekly Survival	5% Limits	Sampling Period
1959	.87	± .044	May-October
1960	.91	± .013	March-October
1961	.87	± .047	March-July

TABLE 10. Estimates of survival of Cepaea nemoralis at Wittenham Clumps, Berks.: Mean weekly survival of all phenotypes in each year.

	Weekly Survival	5% Limits
March-April	.88	± .138
May-June	.79	± .077
July-August	.95	± .106
September-October	.89	± .333

TABLE 11. Estimates of survival of Cepaea nemoralis at Wittenham Clumps, Berks.: Mean weekly survival of all phenotypes for different periods in 1960.

	1959	1960	1961
Pink ±12345	.89 ± .060	.90 ± .017	.81 ± .087
Pink 00000	.81 ± .151	.79 ± .143	.95 ± .068
Yellow ±12345	.86 ± .092	.94 ± .025	.82 ± .112
Yellow 00000	—	.77 ± .091	—
Brown 00000	—	.91 ± .079	—

TABLE 12. Estimates of survival of Cepaea nemoralis at Wittenham Clumps, Berks.: Mean weekly survival of phenotypes in each year.

In table 11 the data for 1960 have been divided into bi-monthly periods in order to demonstrate the fluctuations in survival rate, which are masked in the overall calculations. A comparison of table 11 with figures 8, 9 and 11 makes it clear that the survival rate follows closely the observed intensity of predation. Although the confidence limits of the estimates are rather wide, the heavy predation in May and June is reflected in the low survival rate for that period.

The differences in the survival rates of different phenotypes, shown in table 12, are more difficult to interpret. The results for 1959 suggest that a single estimate for overall survival of all the phenotypes is an adequate description. On the other hand there seem to be real differences between phenotypes both in 1960 and 1961 which are opposite in direction in the two years. It is unfortunate that the data are insufficient for the investigation of differences among phenotypes at different times of the year.

7. CONCLUSIONS.

The population of *Cepaea nemoralis* which inhabits the ditch of the Iron Age fort at Wittenham Clumps is not a single random-mating group. The size of the neighbourhood is determined in one dimension by the dispersal of the animals. In the other, the neighbourhood is limited by the width of the habitable area. From the standard deviation of dispersal per generation, 15 metres, the size of the neighbourhood is estimated to be 1180 sq. metres

Compared with a theoretical circular area of 2827 sq. metres. During the period of the observations the density of the population has fallen from approximately 2 animals per sq. metre to 0.25 per sq. metre.

It is clear from this study that there are differences in the survival value of different phenotypes in the Wittenham colony. Although a net effect of visual selection cannot be demonstrated, an analysis of the components of the selection shows that the apparently neutral predation consists of opposing forces of sufficient power to be detected in relatively small samples. Since visual predation is but one of many types of selective forces that act on populations of Cepaea, there will be little opportunity for the operation of processes that take place "in the absence of selection".

8. SUMMARY

1. A portion of the colony of Cepaea nemoralis (L.) at Wittenham Clumps, Berkshire, has been studied for three seasons by means of the method of multiple captures. Each animal was given an individual mark and returned immediately to its former position.

2. The standard deviation of dispersal per generation is estimated as 15 metres. The panmictic area or neighbourhood in the Wittenham Clumps colony is estimated as 1180 sq. metres.

3. Predation in this colony is at a maximum during May and June.

4. Predators exert differential selective pressures on the phenotypes. Thrushes take a higher proportion of pink and brown snails. Mammals take a higher proportion of yellows.

5. These results support the hypothesis that the proportions of the phenotypes in Cepaea nemoralis are controlled by natural selection, and that visual predation is an important component of this selection.

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CHAPTER 6. *CEPAEA NEMORALIS* (L.) IN THE ISLES OF SCILLY.

Chapter 6

CEPAEA NEMORALIS (L.) IN THE ISLES OF SCILLY

1. INTRODUCTION

The polymorphic land snail Cepaea nemoralis (L.) is characterised by extreme variation in the frequency of its different forms. Significant differences in the proportions of the colour and banding varieties may occur with a separation of only a few yards. In a continuous cline, frequencies may change by as much as 40% in 500 yards (Clarke and Murray, this thesis).

To account for this remarkable variability a number of processes have been invoked. Random differentiation by genetic drift was suggested by Diver (1940) and has been championed by Lamotte, who also found that his model depended upon an exceedingly high mutation rate (Lamotte, 1951, 1959). Schilder and Schilder (1957) have sought an explanation in terms of the spread of genes from centres of high frequency, but they do not offer reasons for the initial frequencies. In contradistinction to these essentially random processes, Cain and Sheppard (1950, 1954) have found evidence of the control of frequencies by natural selection through the agency of visual predation. They have emphasized, however, that visual selection must be balanced by some form of non-visual or physiological selection in order for the polymorphism to persist.

Because of this disagreement over the control of the polymorphism, the populations of Cepaea in the Isles of Scilly are of

considerable interest. The populations are isolated from each other and from the mainland. Geology, climate, and in many cases habitats are similar. Hence the different expectations of the various theories may be examined in a series of closed populations.

The Isles of Scilly form a compact archipelago about thirty miles from the British mainland southwest of Land's End. There are about 140 islands ranging in size from St Mary's, over two miles in diameter, down to isolated rocks uncovered only at low tide. The whole group represents a single intrusive granite mass which has been subsequently eroded and partially submerged. The slaty rocks which originally overlay the granite have been entirely removed except for a small area on White Island. With the exception of a few minor deposits such as old beaches, alluvium, and glacial drift, the principal features of the islands' geology are the granite and the blown sand derived from it. The subsidence of the area has almost certainly taken place very recently. It is indeed possible that some of the major separations have occurred in historic times as there is evidence of human habitation of the fourth century A.D. now covered by high tides (Barrow, 1906).

2. METHODS

A. Collecting.

In the summer of 1960 collections were taken from six of the principal islands of the group: St Martin's, St Mary's, Tresco, Tean, White Island, and Great Ganilly. St Helen's and Great Innisvouls were also visited, but no snails were found. Fifteen

areas on the six islands yielded samples of thirty or more individuals: ten of the samples exceeded a hundred. All of the samples consisted of empty or broken shells except that from White Island Middle Bar.

In the following summer further collections were taken on St Martin's, St Mary's, Tresco and Great Ganilly. Five other islands, St Agnes, Gugh, Annet, Samson and Bryher, provided collections for the first time. All of the major islands have now been visited and all but St Helen's are represented by at least one sample. Thirty-five samples collected in 1961 consisted of at least thirty individuals and twelve exceeded a hundred. Again most of the collections were of empty or broken shells with the exception of one sample from St Martin's Top Rock Valley Bar, which contained only live animals, and one from Annet East where live and dead were lumped in order to make up a sample of the requisite size.

B. Scoring.

The shells were scored for condition, maturity, colour and banding pattern. Since most of them were broken, a convention was adopted to avoid duplication in scoring. Only shells with more than half the lip intact were scored, except in a few cases where almost the entire shell was preserved and where no other fragment could possibly be the missing part. A few smaller fragments have also been scored in order to record the presence of

rare types in areas where they would otherwise appear to be absent. The maximum error introduced by this bias in favour of rarities occurs in the 1962 sample from Great Ganilly, where the inclusion of a yellow five-banded fragment raises the percentage of yellow shells from 2.5 to 4.4%. Because the shells of young snails are difficult to score, their scores have not been included in the analysis.

The colour forms of C.nemoralis in the Scilly populations are quite clear-cut. The brown phenotype has not been found, and the yellow and pink classes are distinct except in the two samples from Gugh. The four "yellows" in the Gugh Carn sample are almost devoid of pigment, while some of the unbanded individuals in the Dropnose Porth sample are of an intermediate orange-pink colour.

The banding patterns were scored according to the system described by Cain and Sheppard (1950). Banded shells with a reduced band formula could be separated without difficulty into two groups. One of these includes the formula 00300 and modifications of it with one or two additional, poorly defined bands. The other consists of modifications of the formula 12345, chiefly 00345. All banded shells are therefore classed as either 00300 or as 12345. The percentage of effectively unbanded shells has, however, been calculated independently of this grouping and includes all shells with bands one and two absent. The full scores and the samples are deposited in the Oxford University Museum

zoological collections.

The scoring of fusions is complicated in these samples by the incompleteness of many of the shells. I have followed the Cain and Sheppard (1950) convention of scoring fusions at a point on the final whorl 90° from the lip. Broken shells were regarded as having fused bands if a missing portion might have shown a fusion. Consequently the score for fusions is a maximum. It is expressed as a percentage of the fully five-banded class.

3. RESULTS

The composition of the fifty samples collected in 1960 and 1961 is given in Tables 1 and 2. The distribution of the forms is very different from that on the mainland.

Cain and Sheppard (1950, 1954) have devised a method of displaying the phenotypic characteristics of populations of *Cepaea nemoralis* on a scatter-diagram. When the percentage of yellow shells is plotted against the percentage of effectively unbanded shells (i.e. with bands 1 and 2 missing), samples from similar habitats in the same region tend to be grouped together. Figure 1 is a diagram of this type for the Oxford area (Cain and Currey, in preparation).

In contrast to this diversity, Figure 2 shows the distribution found in the Isles of Scilly. With a few exceptions, the samples form a compact group comparable with single habitat groups

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	PINK		YELLOW											CHROMOSOME FREQUENCIES			
LOCALITY	00000	00300	±12345	00000	00300	±12345	TOTAL	EFFECTIVELY UNBANDED	% YELLOW	% 00000	% 00300	% EFFECTIVELY UNBANDED	% FUSIONS	p (P0)	q (P5)	r (Y0)	s (Y5)
ST.MARTIN'S																	
Lower Town Camp	29	82	40	-	40	14	205	151	26.3	14.1	69.3	73.7	29.6	.074	.413	0	.513
Middle Town Depression	41	12	15	-	17	6	91	70	25.3	45.1	58.0	76.9	33.3	.258	.239	0	.503
Middle Town Down 2	133	25	14	3	14	21	210	175	18.1	64.8	52.7	83.3	17.1	.390	.185	.016	.409
Top Rock Valley	10	48	47	-	15	5	125	73	16.0	8.0	54.8	58.4	53.8	.041	.559	0	.400
ST.MARY'S																	
Garrison 1	38	53	53	19	10	13	186	119	22.6	30.6	48.8	64.0	34.8	.044	.481	.123	.352
TRESCO																	
Main	7	33	28	2	1	-	71	48	4.2	12.7	54.8	67.6	*	-.021	.816	.087	.118
Farm	15	27	48	9	5	8	112	66	19.6	21.4	36.4	58.9	*	.011	.546	.102	.341
TEAN																	
1.	55	55	51	-	9	11	181	148	11.0	30.4	50.8	81.8	50.0	.166	.502	0	.332
2.	27	47	21	-	2	3	100	88	5.0	27.0	67.1	88.0	55.6	.146	.630	0	.224
3.	31	21	19	16	10	14	111	85	36.0	42.3	48.4	76.6	54.2	.106	.294	.135	.465
4.	50	9	21	15	1	5	101	83	20.8	64.4	27.8	82.2	31.3	.190	.354	.213	.243
7.	10	1	53	9	-	27	100	20	36.0	19.0	1.2	20.0	96.3	.020	.380	.080	.520
GREAT GANILLY																	
Total	47	1	32	-	-	-	80	48	0	58.8	3.0	60.0	*	.357	.643	0	0
WHITE ISLAND																	
South	1	6	39	-	-	10	56	7	17.9	1.8	10.9	12.5	44.9	.009	.568	0	.423
Middle Bar(live)	-	1	16	-	1	12	30	2	43.3	0	6.7	6.7	64.3	0	.342	0	.658

TABLE 1. The composition of samples of *Cepaea nemoralis* collected in 1960 from the Isles of Scilly.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
	PINK			YELLOW										CHROMOSOME FREQUENCIES			
LOCALITY	00000	00300	±12345	00000	00300	±12345	Total	EFFECTIVELY UNBANDED	% YELLOW	% 00000	% 00300	% EFFECTIVELY UNBANDED	% FUSIONS	p (P0)	q (P5)	r (Y0)	s (Y5)
ST.MARTIN'S																	
Lower Town Camp	8	41	8	-	23	4	84	72	32.1	9.5	84.2	85.7	16.7	.049	.384	0	.567
Middle Town Depression	23	8	13	1	11	12	68	43	35.3	35.3	43.2	63.2	16.0	.183	.223	.013	.581
Middle Town Down 1	33	2	5	4	7	9	60	46	33.3	61.7	39.1	76.7	14.3	.319	.103	.061	.517
" " " 2	27	1	4	4	1	7	44	33	27.3	70.5	15.4	75.0	27.3	.360	.117	.096	.427
" " " 3	25	14	11	2	11	13	76	52	34.2	35.5	51.0	68.4	4.2	.174	.241	.023	.562
" " " 4	21	17	17	-	7	11	73	45	24.7	28.8	46.2	61.6	25.9	.156	.347	0	.497
" " " 5	2	21	29	-	12	6	70	33	25.7	2.9	48.5	47.1	28.6	.015	.478	0	.507
" " " 6	-	15	13	-	5	3	36	19	22.2	0	55.6	52.8	18.8	0	.529	0	.471
" " " 7	1	70	36	-	18	9	134	88	20.1	0.7	66.2	65.7	17.8	.004	.548	0	.448
Turfy Hill East	5	4	8	5	4	11	37	18	54.1	27.0	29.6	48.6	5.6	.047	.218	.099	.636
Top Rock Valley Bar	2	16	27	-	5	6	56	23	19.6	3.6	38.9	41.1	87.9	.018	.539	0	.443
Top Rock Valley Inland	12	24	48	1	4	6	95	40	11.6	13.7	34.1	42.1	16.7	.054	.605	.017	.324
Top Rock Valley Bar (Live)	1	16	15	-	10	8	50	27	36.0	2.0	53.1	54.0	86.4	.010	.390	0	.600
ST. MARY'S																	
Garrison 1	41	34	35	10	4	6	130	89	15.4	39.2	48.1	68.5	26.8	.105	.503	.115	.277
" 2	27	26	37	5	3	7	105	61	14.3	30.5	39.7	58.1	20.5	.096	.526	.070	.308
" Shore	17	8	13	6	4	3	51	34	25.5	45.1	42.9	66.7	12.5	.124	.371	.135	.370
Airport	2	2	5	59	16	29	113	72	92.0	54.0	34.6	63.7	38.2	-.006	.047	.328	.631
Bant's Carn	30	55	44	-	9	9	147	93	12.2	20.4	54.7	63.3	11.3	.108	.543	0	.349
TRESCO																	
Main	16	93	72	7	3	4	195	130	7.2	11.8	55.8	66.7	12.7	-.017	.749	.078	.190
East End	31	164	72	4	10	4	285	209	6.3	12.3	69.6	73.3	20.5	.034	.715	.030	.221
North Field	32	14	22	11	3	3	85	63	20.0	50.6	40.5	74.1	22.7	.115	.438	.181	.266
South Field	32	15	33	18	-	4	102	76	21.6	49.0	28.8	74.5	19.2	.019	.517	.267	.197
Farm	15	18	41	7	4	16	101	54	26.7	21.8	27.8	53.5	44.7	.044	.439	.072	.445
GREAT GANILLY																	
Total	23	4	16	-	1	1	45	28	4.4	51.1	22.7	62.2	35.3	.301	.489	0	.210
ANNET																	
East (Part live)	11	8	4	8	3	-	34	30	32.4	55.9	73.3	88.2	75.0	.065	.367	.271	.297
ST. AGNES																	
Monolith	3	20	12	3	13	1	52	36	32.7	11.5	71.7	69.2	46.2	.007	.421	.053	.519
St. Warna West	1	23	11	3	3	3	44	29	20.5	9.1	65.0	65.9	21.4	-.036	.584	.083	.369
GUGH																	
Carn	22	128	10	-	4	-	164	154	2.4	13.4	93.0	93.9	50.0	.070	.775	0	.155
Dropnose Porth	34	22	2	7	-	-	65	63	10.8	63.1	91.7	96.9	0	.064	.607	.329	0
SAMSON																	
Houses	10	3	18	4	-	1	36	21	13.9	38.9	13.6	58.3	40.0	.012	.615	.206	.167
Puffin	17	4	25	14	1	7	68	37	32.4	45.6	13.5	54.4	74.2	.037	.394	.225	.344
North	28	22	34	9	5	5	103	65	18.4	35.9	40.9	63.1	39.5	.081	.490	.118	.311
BRYHER																	
Heathy Hill	-	11	21	-	2	1	35	16	8.6	0	37.1	45.7	15.8	0	.707	0	.293
Additional Samples																	
TRESCO																	
Main 1957 (McWhirter)	10	44	28	3	2	1	88	63	6.8	14.8	61.3	71.6	20.8	0	.739	.077	.184
Main 1961b (Creed)	6	28	25	2	-	1	60	41	5.0	13.3	50.0	68.3	15.8	-.025	.801	.094	.130
East End 1961b (Creed)	14	86	34	3	7	-	144	111	6.9	11.8	73.2	77.1	22.6	.017	.718	.044	.221

TABLE 2. The composition of samples of Cepaea nemoralis collected in 1961, with an additional sample taken in 1957, from the Isles of Scilly.

in Figure 1. They are remarkably pink and effectively unbanded, a composition characteristic of woodlands in the Oxford region.

Most of these samples were, indeed, collected from rather similar habitats. In Scilly, most of the populations live on rough pasture land more or less overgrown with gorse, brambles, bracken, ragwort, and even in some cases heather. The acid conditions inland are reflected in the relative thinness of the shells (see below).

Two of the samples which are separated from the main group in Figure 2 (White Island Middle Bar and Tean 7) belong to a rather special habitat class. They represent populations living among stones just above the seaside shingle beaches where the vegetation consists largely of grass and thrift (Armeria maritima). The samples are heavily banded with high percentages of fusions. A third (White Island South) is made up of scattered shells from a less precisely defined seaside area on White Island. It agrees with the other two in its high percentage of bandedness, but the degree of fusion is not remarkable.

The 1960 sample from Top Rock Valley was also collected from a seaside area with a fringe of large stones by the shore. After the collection was taken, it became apparent that the population from this stone border was different from that farther inland. Consequently in 1961 the area was divided into two parts, Top Rock Valley Bar (the stone border) and T.R.V. Inland (beginning thirty

Figure 1. A scatter-diagram showing the relation between percentage of yellow shells and percentage of effectively unbanded shells for different habitats in the Oxford area (after Cain and Currey, in preparation).

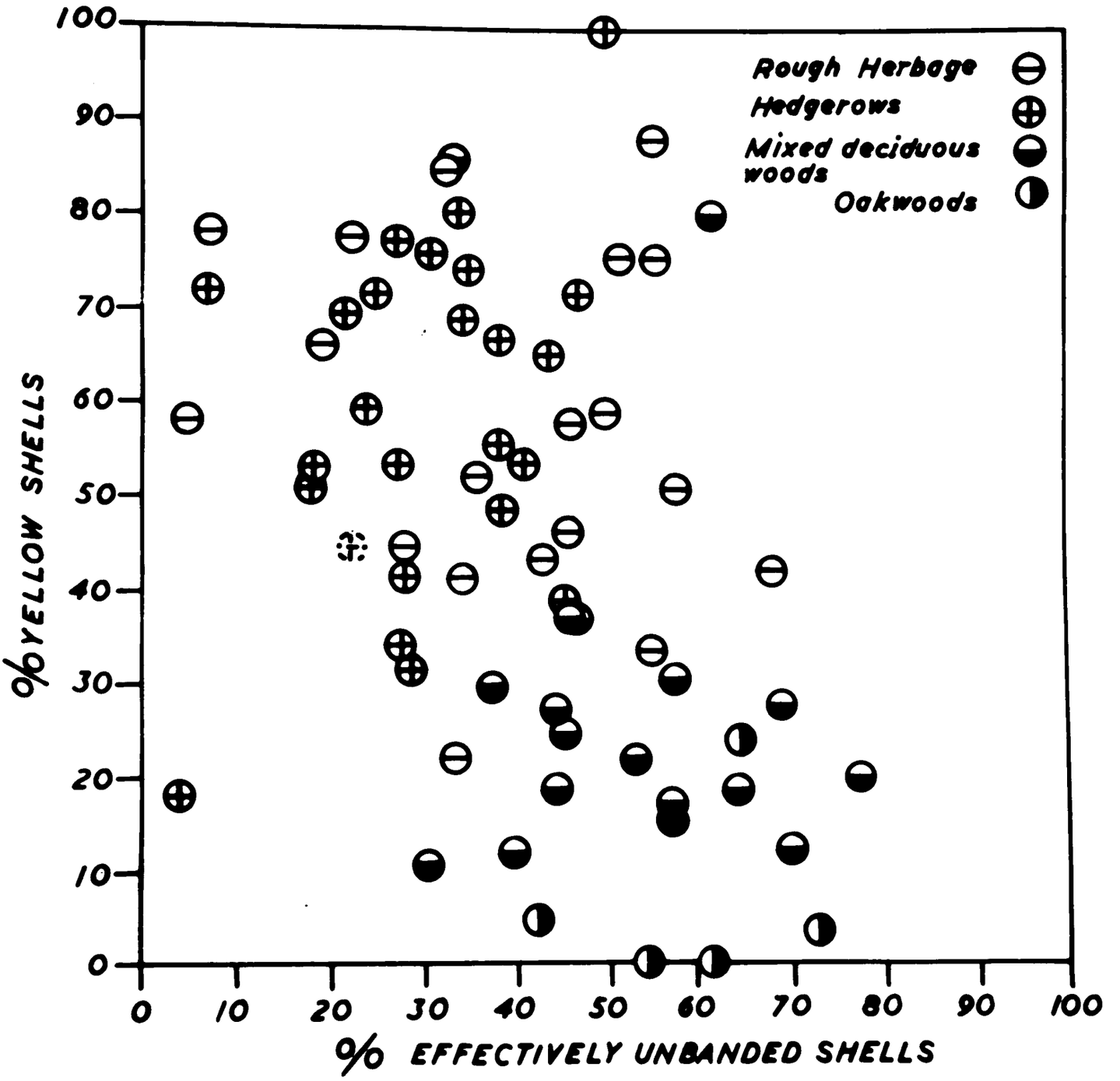
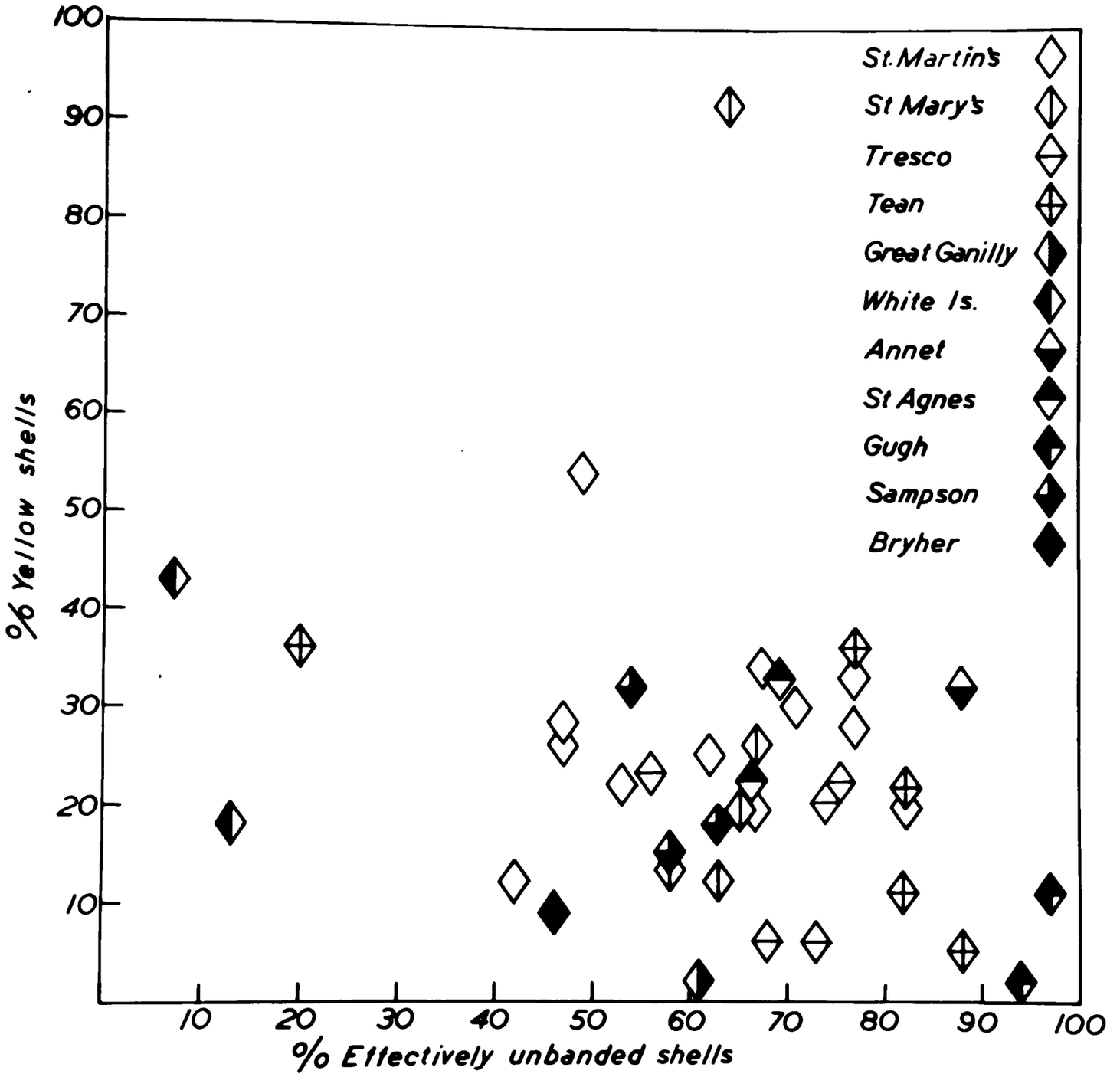

Rough Herbage
Hedgerows
Mixed deciduous woods
Oakwoods
% YELLOW SHELLS
% EFFECTIVELY UNBANDED SHELLS
0
10
20
30
40
50
60
70
80
90
100

Figure 2. A scatter-diagram showing the relation between percentage of yellow shells and percentage of effectively unbanded shells in the Isles of Scilly.


100
90
80
70
60
50
40
30
20
10
% Yellow shells
10
20
30
40
50
60
70
80
90
100
% Effectively unbanded shells
St. Martin's
St Mary's
Tresco
Tean
Great Ganilly
White Is.
Annet
St Agnes
Gugh
Sampson
Bryher

yards from the bar and extending another thirty inland). The vegetation of the bar consisted largely of grass and thrift, and inland, of rabbit-grazed pasture with brambles and bracken. Curiously enough, the percentage of effectively unbandeds was almost identical in the two samples (41 and 42%) and neither approached the aberrant 1960 samples. However, the percentage of fusion differed strikingly, 88% at T.R.V. Bar and 17% at T.R.V. Inland compared with 54% in the combined 1960 sample.

One other sample, Annet East, was taken from a similar seaside bar in 1961. The percentage of effectively unbanded shells is unusually high (88%), but among the few five-bandeds, three out of four show some degree of fusion.

Among the thirty-five 1961 samples, there are two that are rather different from the rest. Turfy Hill East contains about 20% more yellow shells than one might expect, but it is one of the smallest samples recorded (37). St Mary's Airport on the other hand is a large sample (113) and is very different from any other collection from Scilly. The percentage of yellow shells (92%) is more than double that of any other sample except Turfy Hill East and White Island Middle Bar. Moreover more than half of the shells are unbanded yellows, a type which is often rare and is completely absent in a third of the samples. The habitat does not show any particularly outstanding features. The sample was taken from near the edge of tall sea cliffs where the

vegetation consisted chiefly of grass and thrift, with bracken and yarrow in patches.

4. CONSISTENCY OF SAMPLES

A number of localities have been sampled on more than one occasion. These multiple samples throw light on two problems inherent in this study.

First, do the collections of predated and empty shells reflect the true proportions of different forms within the living populations? In only one locality have I been able to collect enough of both living and dead snails to make a reliable comparison. Top Rock Valley Bar produced 56 empty shells on 16 July, 1961, and 50 live snails four days later. The samples contain too few unbanded shells for a comparison of this form, but there are no significant differences between the two samples with respect to colour (P between 0.10 and 0.05) and bandedness (00300/12345: P between 0.30 and 0.20).

Second, are the populations stable enough for samples taken in successive years to be comparable? Eight localities can be tested for agreement between 1960 and 1961. Oneof these is Top Rock Valley (see above). Not unexpectedly, the lumped Bar and Inland samples of 1961 differ significantly from the 1960 sample with respect to the distribution of banding patterns (00000/00300/12345: $P < 0.02$). Because the 1960 Top Rock Valley sample was thought to be heterogeneous beforehand, it may be excluded from consideration.

	Colour	Banding
χ^2	6.765	15.613
Degrees of Freedom	5	12
Probability	>0.20	>0.20

TABLE 3. A comparison of successive samples from the same localities for homogeneity of colour and banding pattern.

The other seven localities may be considered together. Lower Town Camp, Middle Town Depression, Middle Town Down 2, St Mary's Garrison 1, and Tresco Farm have been compared with respect to colour (Pink/Yellow) and three banding patterns (00000/00300/12345). Because of small numbers in the yellow class, only the three banding patterns can be compared in the Tresco Main samples. For similar reasons only a banded/unbanded comparison is possible for Great Ganilly.

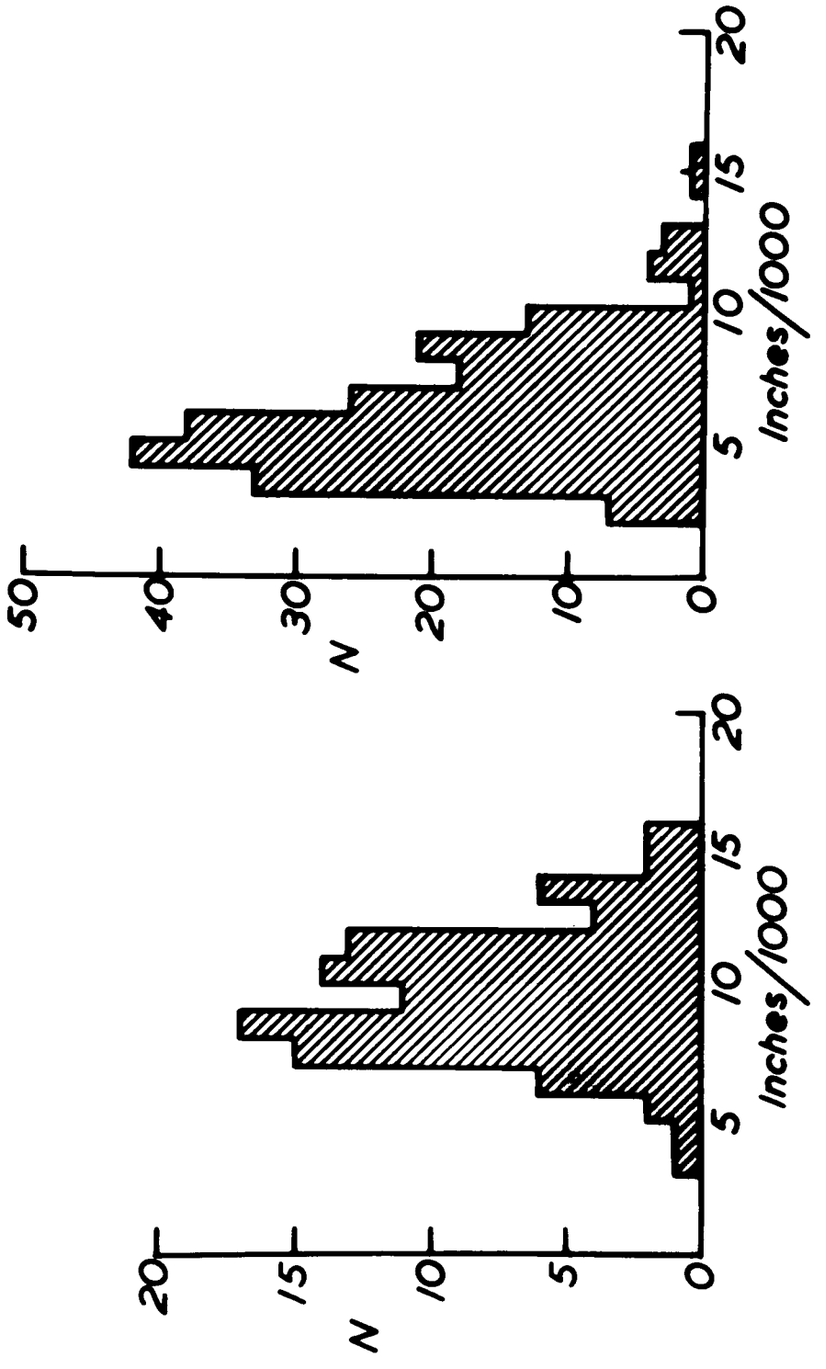
Among the twelve individual comparisons, the value of χ^2 reaches the 5% significance level in one case (Lower Town Camp for banding), but the over-all summation of χ^2 shows that there is no significant heterogeneity between samples of different years. Table 3 gives the values of χ^2 and the corresponding probabilities for the two classes.

5. SHELL THICKNESS

The Cepaea populations in the Scilly Isles vary considerably in strength of shell. Shells from seaside populations are heavy and strongly built while those from inland, where conditions are more acid, tend to be thin and diaphanous. In order to demonstrate the differences, two populations were compared: Tean 7 from a rocky bar on the north shore of Tean and Middle Town Down (1960) from the hill above Middle Town almost in the centre of St Martin's and approximately one quarter mile from the nearest shore.

Weights could not be compared since many of the shells

Figure 3. A comparison of shell thickness between a seaside population (Team 7) and an inland population (Middle Town Down, 1960).


50
40
30
20
10
0
N
0
5
10
15
20
Inches/1000
20
15
10
5
0
N
0
5
10
15
20
Inches/1000

were broken. Consequently thickness was measured with a micrometer at a standard position; the site of band three, five mm from the lip. The distribution of measurements for the two populations is shown in Figure 3. Shells from Tean 7 have a mean thickness of 9.15 thousandths of an inch with a standard deviation of 2.397. Those from Middle Town Down on the other hand have a mean of 5.56 thousandths and a standard deviation of 2.353. The means of these two samples are significantly different with $P < 0.001$. There is no evidence of a difference in the distribution of thicknesses among the colour and banding classes within the populations.

Oldham (1928) has studied the effect of calcium abundance on shell size and weight in Arianta arbustorum (L.). The snails were fed a standard diet of cabbage and oatmeal, with half of each brood receiving an addition of chalk. The shells of those given chalk were only slightly larger than but more than 3½ times as heavy as the controls. A similar effect might be expected in Cepaea. Although there is a remote possibility that differences in the age structure of the populations might account for the differences in shell thickness, it is almost beyond doubt that the thicker shells of the seaside populations reflect the amount of calcium carbonate available to them from shells washed up from the sea, a source denied to the inland populations.

6. CHROMOSOME FREQUENCIES

Perhaps the most remarkable features of the Scilly populations

are the considerable disturbances that occur in the joint distribution of the colour and banding classes. The association of pink/yellow with banded/unbanded was tested for all samples in which the expectation in each class is five or more. Among eight such samples taken in 1960, five show a significant departure from random assortment. Six of twelve samples collected in 1961 show a similar disturbance. In several of the collections the difference is spectacular, for example the 1960 Middle Town Down sample:

	Pink	Yellow	
Unbanded	133	3	136
Banded	39	35	74
	172	38	210

$\chi^2 = 62.7382$

P far less than 0.001

Considering the magnitude of the disturbances it is interesting that not all of the deviations are in the same direction. The most striking differences are those in which there is an excess of pink unbanded and yellow banded shells, but in two collections these classes are significantly deficient.

The expression of the pink/yellow and banded/unbanded phenotypes is under the control of two pairs of allelic genes with the genes for pink and unbanded dominant (e.g. Cain and Sheppard

1957). The interpretation of the departures from random assortment depends to a large extent on the strength of linkage between these two loci determining colour and banding. If recombination occurs at even a modest frequency then such a marked disturbance must be maintained by a powerful selective force. Unfortunately the cross-over value for these loci has not been determined. Apart from a case reported by Fisher and Diver (1934) in which multiple fertilization cannot be excluded, only two instances of crossing over have been reported. Since the two animals in which crossing over occurred are offspring of a single mating, the resulting cross-over value is not generally applicable (Cain et al., 1960).

The importance of linkage in maintaining the observed associations is supported by a comparison of the distribution with respect to colour of two unlinked loci controlling banding patterns. The gene for 00300 is dominant to that for 12345 and belongs to a different linkage group from the banded/unbanded and pink/yellow loci. When the association of 00300/12345 and pink/yellow was tested as before, none of fifteen samples showed a significant departure from random assortment. While this by no means excludes the action of natural selection in these cases, since selection must be very powerful indeed to produce a significant association between unlinked genes, it seems that the linkage groups for colour and banding are behaving as super-genes in which crossing over is a rather rare event.

On this hypothesis chromosome frequencies have been calculated as for four alleles. These can be derived directly from the expansion of the polynomial $(p + q + r + s)^2$, or more conveniently from a modification of Wellisch and Thomsen's (1930) formulae for the gene frequencies of the ABO blood groups.

$$p = 1 - (q + r + s)$$

$$q = \sqrt{\overline{Y5} + \overline{P5}} - \sqrt{\overline{Y5}}$$

$$r = \sqrt{\overline{Y5} + \overline{Y0}} - \sqrt{\overline{Y5}}$$

$$s = \sqrt{\overline{Y5}}$$

$\overline{Y5}$, $\overline{Y0}$ and $\overline{P5}$ here represent the frequencies of the yellow banded, yellow unbanded, and pink banded phenotypes respectively. The chromosome frequencies are given in columns 15 to 18 of Tables 1 and 2.

7. DISCUSSION

Two points of interest emerge from the gene frequency tables. First, there is a considerable diversity among the samples. The chromosome carrying genes for pink and banded, for example, is present at frequencies varying from 10 to 82%, even if the aberrant St Mary's Airport sample (5%) is excluded. Intermediate frequencies are evenly represented. This variability is in strong contrast to the phenotypic similarity displayed in Figure 2. Since the modification of this genetic diversity has been accomplished by several genetically different but visually equivalent means (the increase of the frequencies of the 00000, 00300, and 00345 banding patterns),

there is strong evidence that phenotypic stability is being maintained by natural selection acting on the visual characters of the shell.

Further evidence of the operation of natural selection is provided by the samples from St Warna West, St Mary's Airport, and Tresco Main. Calculation of chromosome frequencies from the observed phenotype frequencies results in negative frequencies for the chromosome carrying the genes for pink and unbanded. These obviously impossible answers reflect disturbances of the Hardy-Weinberg equilibrium.

Tresco Main is the most thoroughly studied of the areas showing "negative" frequencies. Four samples from this locality have been taken, the two additional ones being collected in 1957 by K.G.McWhirter and in August 1961 by E.R.Creed. Although the individual samples contain too few yellow shells for a valid comparison of their homogeneity with respect to colour, their excellent agreement in banding patterns (for 00000/00300/12345, P lies between 0.90 and 0.95) leaves little doubt of their comparability. When the joint distribution of colour and banding in the combined samples is tested, the probability of random association between pink/yellow and banded/unbanded is far less than 0.001. In this case however there is evidence of a disturbance that cannot be accounted for by this association alone. Three of the four samples show a negative frequency for the chromosome

carrying the pink and unbanded genes and the fourth frequency is zero. These figures indicate that even in the complete absence of this chromosome, the pink unbanded class is smaller than would be expected from the joint occurrence of the chromosomes carrying the pink and banded and the yellow and unbanded genes. Therefore selection must be acting either against the pink unbanded or against the yellow banded class. Furthermore, since the successive samples indicate that the genetic constitution of the population is relatively stable, a balancing selective force is probably operating. In a polymorphic system such as this, the balance could well be maintained by the advantage of heterozygous combinations.

The importance of the C.nemoralis populations of the Isles of Scilly to the general problem of the control of the frequency of the polymorphic forms in this species stems from the similarity of the climatic and vegetational features of the islands together with their complete isolation. If the frequencies of the several forms were under the "control" of random processes such as mutation or genetic drift then with complete geographical isolation, divergence might be expected to occur on the separate islands. Such is not the case. Figures 1 and 2 demonstrate that most of the Scilly samples form a compact group comparable with a single habitat group from the Oxford area. Moreover the differences within islands are as large as or larger than the differences between islands (cf. Tean 1960 and St Mary's 1961).

We must conclude therefore that a systematic process, natural selection, is responsible for determining the phenotypic composition of the Scilly populations. The genetic composition of the samples leads to the further conclusion that this selection is acting on the visual characteristics of the shells, since the phenotypic similarity is imposed on an underlying chromosomal variability. In addition to this indirect evidence of the effect of selection, there is direct evidence that a continuing systematic force is operating in the case of the Tresco Main population to produce a disturbance of the random assortment of the super-genes or chromosomes. It seems highly unlikely, therefore, in the presence of such selective forces that random processes can play a significant role in the control of the polymorphism in these populations.

8. SUMMARY

1. Populations of Cepaea nemoralis from the Isles of Scilly were sampled in 1960 and 1961. The collections were scored for colour and band-formula.

2. With few exceptions, the Scilly populations are phenotypically homogeneous. In comparison with populations from the Oxford region they appear to fall largely within a single habitat group.

3. Calculation of chromosome frequencies shows an underlying genetic diversity, suggesting that the phenotypic similarity is

the result of the action of natural selection on the visual characteristics of the shells.

4. Strong evidence of natural selection in at least one population is provided by continuing disturbances of the Hardy-Weinberg equilibrium.

5. The thickness of shells is related to the availability of calcium carbonate in the form of sea shells.

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