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

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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 (1950) 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 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 ST 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.

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 are damp. Rushes (*Juncus* sp.) and horsetails (*Equisetum* sp.) occur within them. The flora at Berrow has been described in detail by Boley (1944).

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. It seems likely that they 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 descriptions of the places from which they were taken, and also marked them upon a 6 in. Ordnance Survey

FIG. 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; W.M. = windmill.

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, presumably because some of the dunes have changed their positions since it was made. This disturbing fact made it necessary for us to completely 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. Eight 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 age (mature or immature), 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 Browns").

Each of us (J.M. and B.C.) scored the samples independently, and then compared our results. Apart from some simple errors of counting the differences were slight. There was 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 (B 30) the difference of scoring amounted to 6 per cent. of the total number of snails scored, in four samples (B 1, D 26, B 26, B 27) it was about 3.5 per cent., in one (D 48) about 2.5 per cent., and in two (B 29, D 31) about 1.5 per cent. Because of these differences, pinks and pale browns have been grouped together for detailed analysis.

In only two samples (B 29, B 47) were there any differences in the scoring of yellow shells (0.5 per cent. and 2.5 per cent. respectively). In B 29 the difference was due to one shell, which was doubtfully yellow or pale brown. In B 47 two shells were doubtfully yellow or pink, having been scorched in a brush fire.

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 (B 29, B 28, B 43, D 24, B 44, B 45, D 52 and D 54) we ourselves have taken more than one sample. The sum of χ^2 for differences between our successive samples is not significant for colour ($\chi^2_{10} = 5.09$, $p < 0.9$), 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 (B 45), which 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.

B 45 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 B 45 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 B 45 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 per cent.

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 per cent.) of yellows. In the centre of the area there is a region where the frequencies exceed 90 per cent. South of this the frequencies are again slightly lower.

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 p. 450).

TABLE 1
*The composition of samples of Cepaea nemoralis collected at Berrow.
 The 00345* column includes the numbers of 00045 and 00005 as well as 00345.*

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				
D 19	1	4	5	1	19	1	5	7	1	40	—	4	6	1	42	—	5	X	1-PB12045	142	AEB, CD
B 30	—	7	9	—	29	—	4	18	3	40	—	4	4	3	10	—	6	26	—	132	BC, JM
B 29	—	4	3	—	21	—	2	4	2	20	—	4	1	1	14	—	—	25	—	82	BC, JM
B 28	1	18	4	3	34	2	2	—	—	27	—	2	—	—	6	—	4	40	1-P12045	80	BC, JM
	—	3	3	—	58	—	1	4	3	85	—	—	3	—	32	—	13	83	4-Y02345	290	BC, JM
	—	—	—	—	—	—	8	3	—	39	—	—	2	—	22	—	1	49	2-P02345		BC, JM
	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1-P12045		BC, JM
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1-PB02345	143	BC, JM
B 27	—	—	—	—	28	—	1	4	1	17	—	—	—	—	5	—	—	20	—	56	BC, JM
B 11	—	5	1	2	39	—	6	—	—	30	—	1	1	1	5	—	1	17	1-PB02345	94	BC, JM
	—	—	—	—	10	—	1	—	—	2	—	—	—	—	—	—	—	2	2-P02345	13	BC, JM
D 23	—	3	4	1	45	4	2	2	1	36	—	1	—	—	4	—	—	X	—	103	AEB, CD
B 43	1	9	1	2	51	—	9	2	34	—	—	—	—	—	1	—	10	—	BC, JM	110	BC, JM
	—	2	1	1	50	—	6	—	4	27	—	—	—	—	—	—	11	—	BC, JM	92	BC, JM
	—	8	6	6	141	1	3	4	6	100	—	—	—	—	3	—	—	35	3-Y12045		BC, JM
D 24	—	2	5	8	63	—	2	2	5	61	—	—	—	—	—	—	—	X	2-P12045	283	BC, JM
	—	7	5	3	30	2	2	—	27	27	—	—	—	—	—	—	—	6	(1-Y12045)	150	AEB, CD
	—	1	1	—	14	—	3	—	1	10	—	—	—	—	1	—	—	8	1-P12045	76	BC, JM
B 44	—	—	1	—	23	—	1	1	23	8	—	—	—	—	—	—	—	8	—	32	BC, JM
	—	3	4	—	59	—	2	2	—	42	—	—	—	—	—	—	—	17	—	49	BC, JM
B 45	—	26	4	8	123	—	8	2	6	67	—	—	—	—	1	—	—	20	1-Y12045	250	BC, JM
	—	1	—	—	62	1	1	1	2	39	—	—	—	—	2	—	—	32	2-Y02345		BC, JM
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1-P12045	109	BC, JM
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1-P02345		BC, JM

D 28	.	—	2	1	6	220	—	—	—	2	148	—	—	—	1	—	—	X	2-Y12045 1-Y02345 2-P12045 4-Y12045 2-P12045 1-P12045 {1-Y12045 1-P12045} (2-P02345)	387	AEB, CD	20.9.26
D 27	.	—	11	4	6	279	1	7	15	6	219	—	—	—	2	—	—	X	2-P12045 4-Y12045 2-P12045 1-P12045	556	AEB, CD	20.9.26
D 26	.	—	—	7	6	239	—	6	3	2	101	—	—	—	—	—	—	X	1-P12045	371	AEB, CD	20.9.26
		—	—	10	4	8	89	—	4	9	86	—	—	—	—	—	—	25	{1-Y12045 1-P12045} (2-P02345)	220	BC, JM	4.6.59
D 25	.	—	—	3	6	1	147	—	4	2	98	—	—	—	1	—	1	X	1-P12045	266	AEB, CD	20.9.26
		—	—	5	2	7	86	—	4	2	47	—	—	—	1	—	23	BC, JM	152	BC, JM	4.6.59	
D 29	.	—	—	5	—	28	—	2	—	—	12	—	—	—	—	—	1	X	1-Y02345	48	AEB, CD	20.9.26
		—	—	22	3	7	207	—	4	6	80	—	—	—	—	—	—	67	—	342	BC, JM	7.7.59
D 30	.	—	—	11	2	3	153	—	8	—	57	—	—	—	—	—	2	X	2-Y12045	230	AEB, CD	24.9.26
		—	—	22	6	7	124	—	—	4	40	—	—	—	—	—	—	39	1-Y10045 1-Y12045	220	BC, JM	4.6.59
		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4-Y02345 1-P02345			
D 31	.	—	—	3	—	—	17	—	—	—	2	—	—	—	—	—	—	X	—	23	AEB, CD	20.9.26
B 46	.	—	—	17	—	—	30	—	4	—	7	—	—	—	—	—	—	8	—	59	BC, JM	9.7.59
		—	—	5	—	—	31	—	1	—	17	—	—	—	—	—	—	8	—	54	BC, JM	10.7.59
D 32	.	—	—	25	1	7	329	—	4	—	23	—	—	—	—	—	—	X	—	389	AEB, CD	23.9.26
		—	—	4	—	1	16	—	—	—	—	—	—	—	—	—	—	22	1-Y02345	22	BC, JM	4.6.59
D 34	.	—	—	5	—	7	148	—	—	—	11	—	—	—	—	—	—	5	—	171	AEB, CD	23.9.26
		—	—	6	—	2	38	—	—	—	1	—	—	—	—	—	1	X	1-Y02345	49	BC, JM	4.6.59
D 33	.	—	—	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
B 48	.	—	—	3	—	—	15	—	—	—	—	—	—	—	—	—	—	2	—	18	BC, JM	10.7.59
D 35	.	—	—	—	1	6	188	—	2	—	10	—	—	—	—	—	—	X	—	207	AEB, CD	23.9.26
		—	—	8	1	8	11	—	1	—	4	—	—	—	—	—	2	X	—	19	BC, JM	4.6.59
D 36	.	—	—	2	—	152	—	1	—	—	11	—	—	—	2	—	—	X	1-Y12045	187	AEB, CD	23.9.26
		—	—	4	1	4	34	—	—	1	7	—	—	—	—	—	4	—	—	51	BC, JM	4.6.59
D 37	.	—	—	17	10	15	373	—	2	1	27	—	1	—	8	—	7	X	—	402	AEB, CD	23.9.26
B 50	.	—	—	12	—	—	83	—	—	—	2	—	—	—	—	—	—	55	—	97	BC, JM	9.8.60
Carry forward		3	333	109	142	4487	12	124	101	69	1786	16	17	6	163	—	47			7493		

D 52	9	—	29	35	24	206	—	4	3	4	19	—	—	—	—	—	5	28	67	X	$\left\{ \begin{array}{l} 1-Y10045 \\ 1-Y12045 \\ 1-Po2345 \end{array} \right\}$	427	AEB, CD	29.9.26
	1	29	13	12	148	—	—	—	1	1	7	—	—	—	—	—	—	28	24	$\left\{ \begin{array}{l} 1-Y10045 \\ 2-Yo2345 \\ 1-Po2345 \end{array} \right\}$	244	BC, JM	8.7.59	
	—	8	4	7	45	—	—	—	—	—	1	—	—	—	—	—	—	3	5	7	$\left\{ \begin{array}{l} 1-Y10045 \\ 1-Po2345 \end{array} \right\}$	74	BC, JM	8.6.60
	—	3	1	2	24	—	1	—	—	—	2	—	—	—	—	—	—	1	4	6	$\left\{ \begin{array}{l} 1-Y12045 \\ 1-Po2345 \end{array} \right\}$	39	BC, JM	8.8.60
D 54	1	36	1	24	133	—	5	—	—	3	23	—	—	—	—	—	—	18	X	$\left\{ \begin{array}{l} 1-Y10045 \\ 3-Yo2345 \\ 1-Po2345 \end{array} \right\}$	249	AEB, CD	22.9.26	
	—	38	5	15	53	—	4	—	—	—	6	—	—	—	—	—	1	1	13	$\left\{ \begin{array}{l} 1-Yalbino \\ 12345 \\ 4-Yo2345 \end{array} \right\}$	128	BC, JM	8.7.59	
	2	80	6	43	196	—	14	1	9	18	—	—	—	—	—	—	—	5	3	26	—	377	BC, JM	8.8.60
B 26	—	20	—	5	40	—	3	—	1	4	—	—	—	—	—	—	—	2	8	8	—	75	BC, JM	14.9.60
	—	21	1	1	50	—	2	—	—	6	—	—	—	—	—	—	—	11	3	3	1-Yalbino	94	BC, JM	8.7.59
																					12345			
																					1-Y12045			
D 55	—	43	1	46	138	—	8	1	5	11	—	—	—	—	—	—	—	55	X	$\left\{ \begin{array}{l} 1-Yalbino \\ 10345 \\ 2-Yalbino \\ 12345 \end{array} \right\}$	311	AEB, CD	20.9.26	
B 59	—	7	—	4	62	—	—	—	—	—	1	—	—	—	—	—	8	5	9	—	87	BC, JM	16.9.60	
D 56	—	19	—	2	70	—	—	—	—	1	4	—	—	—	—	—	5	1	13	X	—	115	AEB, CD	20.9.26
	—	40	—	7	205	—	5	—	—	1	14	—	—	—	—	—	9	2	16	48	$\left\{ \begin{array}{l} 1-Yalbino \\ 12345 \\ 1-Y12045 \\ 1-P12045 \end{array} \right\}$	304	BC, JM	8.8.60
B 1	—	4	—	1	50	—	1	—	—	—	10	—	—	—	—	—	—	1	7	14	—	74	BC, JM	3.6.59
B 60	—	—	—	1	46	—	—	—	—	—	4	—	—	—	—	—	2	1	1	11	—	55	BC, JM	16.9.60
B 42	—	4	—	—	5	—	—	—	—	—	—	—	—	—	—	—	—	—	2	1-Po2345	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	449	118	118	12,658		

Figure 5 shows the distribution of single-banded forms (00300). Because the genes for dark brown shell colour and for the unbanded condition (00000) are both known to interact with the gene for 00300 (Lamotte, 1954; Cain and Sheppard, 1957; Cain, King and Sheppard,

FIG. 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 per cent., except when they are less than one per cent. Brackets indicate samples of less than 30 shells.

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 interacts with 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

FIG. 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. Brackets indicate samples of less than 30 shells.

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 browns 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.

FIG. 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. Brackets indicate samples of less than 30 shells.

If all the twenty collections taken in 1926 are added together, the overall percentage of 00300 is found to be 7.3 per cent. The overall percentage for 1959-60 is 14.7 per cent. 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 per cent. and for 1959-60 it is 15.1 per cent. 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 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

FIG. 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). Brackets indicate samples of less than 30 shells.

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 per cent. The overall percentage for 1959-60 is 3.2 per cent. The *average* percentage for 1926 is 6.2 per cent., and for 1959-60 is 3.9 per cent. 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. Their

FIG. 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). Brackets indicate samples of less than 30 shells.

development and expression are apparently unaffected by the environment, and phenocopies have not reliably been reported. 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

FIG. 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 fig. 5. Note scale at top left.

happened among our samples at locality B 45), 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.

FIG. 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. Note scale of percentages at top left.

(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, 1962).

(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).

These points also argue against any suggestion that the populations have changed as a direct result of the sampling by Boycott and Diver. We have detected no consistent differences, at the present day, between localities sampled by the earlier workers and those sampled only by us.

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 of 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 that occur within it. Lamotte (1951) has calculated that the panmictic unit in *Cepaea* is not more than fifty yards in diameter. Our own observations on movements of *C. nemoralis* at Berrow support Lamotte's conclusions (Clarke and Murray, in preparation). 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 as much as 50 per cent. 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 (1951*b*) 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 firstly, the overgrowth of sea buckthorn and secondly, the general stabilisation 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 whose selective values are most likely to have been improved by such a change of background are yellow and "effectively unbanded" shells (Cain and Sheppard, 1950, 1954). The latter category includes all shells that have the upper two bands missing (*i.e.* 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. The unbanded phenotype, on the other hand, is so rare throughout the area that a relatively large change in its frequency would not be detectable.

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 the average 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.

It should be possible to estimate the magnitude of the selective forces necessary to bring about the changes observed at Berrow. This problem will be considered in a forthcoming paper (Clarke and Murray, in preparation).

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 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 (00300) 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 visual and non-visual selection have been acting simultaneously.

6. The data presented in this paper will allow an estimate of the magnitude of natural selective forces acting on individual Mendelian populations (Clarke and Murray, in preparation).

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8. REFERENCES

- ALLISON, A. C. 1954. Notes on sickle-cell polymorphism. *Ann. Human Genet.*, 19, 39-51.
- BOETTGER, C. R. 1954. Zur Frage der Verteilung bestimmter Varianten bei der Landschneckengattung *Cepaea* Held. *Biol. Zbl.*, 73, 318-333.
- BOLEY, G. M. 1944. The vegetation at Berrow, North Somerset. 2. The sand dune succession. *Proc. Bristol Nat. Soc.*, 9, 510-520.
- CAIN, A. J., KING, J. M. B., AND SHEPPARD, P. M. 1960. New data on the genetics of polymorphism in the Snail *Cepaea nemoralis*, L. *Genetics*, 45, 393-411.
- CAIN, A. J., AND SHEPPARD, P. M. 1950. Selection in the polymorphic land snail *Cepaea nemoralis*. *Heredity*, 4, 275-294.
- CAIN, A. J., AND SHEPPARD, P. M. 1954. Natural selection in *Cepaea*. *Genetics*, 39, 89-116.
- CAIN, A. J., AND SHEPPARD, P. M. 1957. Some breeding experiments with *Cepaea nemoralis* (L.). *J. Genet.*, 55, 195-199.
- CARSON, H. L., AND STALKER, H. D. 1949. Seasonal variation in gene arrangement frequencies over a three-year period in *Drosophila robusta* Sturtevant. *Evolution*, 3, 322-329.
- CLARKE, B. 1960. Divergent effects of natural selection on two closely-related polymorphic snails. *Heredity*, 14, 423-443.
- CLARKE, B. 1962. Natural selection in mixed populations of two polymorphic snails. *Heredity*, 17, 319-345.
- DOBZHANSKY, T. 1943. Genetics of natural populations. IX. Temporal changes in the composition of populations of *Drosophila pseudoobscura*. *Genetics*, 28, 162-186.

- DOBZHANSKY, T. 1948. Genetics of natural populations. XVI. Altitudinal and seasonal changes produced by natural selection in certain populations of *Drosophila pseudoobscura* and *Drosophila persimilis*. *Genetics*, 33, 158-176.
- DOBZHANSKY, T. 1958. Genetics of natural populations. XXVII. The genetic changes in populations of *Drosophila pseudoobscura* in the American Southwest. *Evolution*, 12, 385-401.
- DUBININ, N. P. AND TINIAKOV, G. G. 1945. Seasonal cycles and the concentration of inversions in populations of *Drosophila funebris*. *Amer. Nat.*, 79, 570-572.
- DUBININ, N. P., AND TINIAKOV, G. G. 1946. Inversion gradients and natural selection in ecological races of *Drosophila funebris*. *Genetics*, 31, 537-545.
- FISHER, R. A., AND FORD, E. B. 1947. The spread of a gene in natural conditions in a colony of the moth *Panaxia dominula* L. *Heredity*, 1, 143-174.
- GERSHENSON, S. 1945. Evolutionary studies on the distribution and dynamics of melanism in the hamster (*Cricetus cricetus* L.). *Genetics*, 30, 207-251.
- GOODHART, C. B. 1956. Genetic stability in populations of the polymorphic snail, *Cepaea nemoralis* (L.). *Proc. Linn. Soc. Lond.*, 167, 50-67.
- GOODHART, C. B. 1958. Genetic stability in the snail *Cepaea nemoralis* (L.); a further example. *Proc. Linn. Soc. Lond.*, 169, 163-167.
- KETTLEWELL, H. B. D. 1958. A survey of the frequencies of *Biston betularia* (L.) Lep. and its melanic forms in Great Britain. *Heredity*, 12, 51-72.
- KOMAI, T. 1956. Genetics of ladybeetles. *Advanc. Genet.*, 8, 155-188.
- LAMOTTE, M. 1951. Recherches sur la structure génétique des populations naturelles de *Cepaea nemoralis* (L.). *Bull. biol. Suppl.*, 35, 1-239.
- LAMOTTE, M. 1954. Sur la déterminisme génétique du polymorphisme, chez *Cepaea nemoralis* L. *C. R. Acad. Sci. Paris*, 239, 365-367.
- LAMOTTE, M. 1959. Polymorphism of natural populations of *Cepaea nemoralis*. *Cold Spr. Harb. Sym. quant. Biol.*, 24, 65-86.
- SEDLMAIR, H. 1956. Verhaltens-, Resistenz- und Genäuseunterschiede bei den polymorphen Bänderschnecken *Cepaea hortensis* (Müll.), und *Cepaea nemoralis* (L.). *Biol. Zbl.*, 75, 281-313.
- SCHNETTER, M. 1950. Veränderungen der genetischen Konstitution in natürlichen Populationen der polymorphen Bänderschnecken. *Verh. dtsh. zool. Ges.*, 1950, 192-206.
- SHEPPARD, P. M. 1951a. A quantitative study of two populations of the moth *Panaxia dominula* (L.). *Heredity*, 5, 349-378.
- SHEPPARD, P. M. 1951b. Fluctuations in the selective value of certain phenotypes in the polymorphic land snail *Cepaea nemoralis* (L.). *Heredity*, 5, 125-134.
- SHEPPARD, P. M. 1956. Ecology and its bearing on population genetics. *Proc. roy. Soc. B.*, 145, 308-315.
- TIMOFEEF-RESSOVSKY, N. W. 1940. Zur Analyse des Polymorphismus bei *Adalia bipunctata* L. *Biol. Zbl.*, 60, 130-137.
- VAN VALEN, L. 1960. Nonadaptive aspects of evolution. *Amer. Nat.*, 94, 305-308.
- WRIGHT, S. 1940. The statistical consequences of Mendelian heredity in relation to speciation. In *The New Systematics*, ed. J. Huxley, Oxford, 161-183.
- WRIGHT, S. 1955. Classification of the factors of evolution. *Cold Spr. Harb. Sym. quant. Biol.*, 20, 16-24.