PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Genetics of Some Morphs and Varieties of Cepaea nemoralis (L.)

A. J. Cain, P. M. Sheppard and J. M. B. King

Phil. Trans. R. Soc. Lond. B 1968 **253**, 383-396
doi: 10.1098/rstb.1968.0005

STUDIES ON *CEPAEA*

I. THE GENETICS OF SOME MORPHS AND VARIETIES OF *CEPAEA NEMORALIS* (L.)

By A. J. CAIN*, P. M. SHEPPARD, F.R.S.† and J. M. B. KING‡

* *Department of Zoology, University of Manchester*
† *Department of Genetics, University of Liverpool*
‡ *Formerly Department of Zoology and Comparative Anatomy, University Museum, Oxford*

(*Received* 16 *February* 1967)

CONTENTS

	PAGE
Introduction	384
Shell colour	384
Faint pink	384
Pale brown and faint brown	385
Yellow-white	385
Banding and lip-colour	390
Suppression of the band in mid-bandeds	390
Spread-banded	390
White lip	391
Hyalozonate	393
Complementarity between orange-banded and hyalozonate	393
Punctata	394
Body colour	395
Discussion	395
References	396

The following segregants of shell colour are described: pale brown, faint brown, faint pink, yellow-white. Faint pink is dominant to dark and pale yellow and recessive to pale pink. Murray's finding that the hyalozonate gene when homozygous pales shell colour as well as removing the lip and banding pigment is confirmed; faint pink or yellow-white shells homozygous for hyalozonate may be genetically faint pink or pale pink. Deep pink hyalozonates exist and transmit their phenotype to their offspring; in these the paling effect is not noticeable.

Shells with no bands but a strip of fascialbate opaque material in the position for bordering the middle band are genetically mid-banded. The condition is due to a multifactorial suppression of the pigmented band.

Spread-banded is described in the five-banded form as well as the mid-banded; the strong linkage between it and shell colour previously suggested is confirmed, but crossovers are recorded.

White lip (with normally pigmented bands) is recessive to normal lip, linked to the locus for shell-colour, and allelic to hyalozonate. The normal lip/white lip heterozygote appears to be pale-lipped, but the degree of pallor is modified by the presence of yellow or pink shell colour. White lip, when homozygous, may reduce the intensity of shell colour in some cases, as does hyalozonate.

Hyalozonate is shown to be linked to the banding locus, which itself is strongly linked to that for shell colour.

Orange-banded is complementary to hyalozonate, not allelic to it as is the very similar form *lurida* described by Murray in *Cepaea hortensis*.

A form of the var. *punctata* producing only traces of bands and occasionally a definite punctate band is described, which is linked to the shell colour locus and dominant to normal bands. This and the form figured by Lang and by Taylor with well-developed punctate bands (the segregation of which from normal bands is confirmed) may well be alleles.

A segregation of medium grey to very pale body is described, with medium grey dominant to pale. It appears to be unlinked to shell colour, banding and mid-banded.

Introduction

This paper continues the contribution from our breeding programme to the genetics of the polymorphism in *Cepaea nemoralis* (Cain & Sheppard 1957; Cain, King & Sheppard 1960).

Shell colour

Faint pink

Faint pinks (and browns) have been noted by several authors, some of whom have called them 'creams'. There are two different ways in which faint pinks are produced: (i) by the action of an allelomorph at the shell colour locus which does not affect the lip and banding pigmentation, and (ii) by the action of the hyalozonate gene which abolishes lip and banding pigmentation and in addition greatly reduces the shell colour in some shells, as Murray (1963) has shown.

Faint pinks (FP) may have a nearly white shell with only a very slight flush of colour near the apex, or there may be a more general slight tinge of pink, best seen when the shell is put beside that of a pale yellow. Faint pinks heterozygous for yellow may have a noticeable yellow colour at the apex, as may pale pinks (Cain *et al.* 1960). In these very pale shells, the slightest trace of pink, whether yellow can be seen or not, is evidence that an allele for pink is present. The periostracum is itself a pale slightly brownish yellow, and may hinder the recognition of the presence of pink; but even if it is completely removed and the calcareous layers of the shell appear to show no pink whatever, the animal may still be genetically pink if the shell is also hyalozonate.

(i) In mating LaPoE 1 (table 1) a faint pink, with normally pigmented bands, progeny of mating PoE 1 reported by Cain *et al.* (1960) in which all the pale pinks should be recorded as faint pinks, gave on mating with a yellow five-banded a clear segregation between dark yellows and faint pinks. A similar segregation was given by PoE 1 (table 1, this paper), from which faint pink is, as previously shown, linked with banding (as the shell colour locus is known to be), and is dominant to dark yellow, since unbanded is known to be dominant to five-banded. From LLt 1, faint pink is recessive to pale pink, and this is supported by PoER 1, and by Ry 1 in which it is dominant to pale yellow. The data suggest strongly that it is another allelomorph in the colour series, fitting into the dominance hierarchy below pale pink and above the yellows.

(ii) Murray (1963) has shown that in the hyalozonate form not only is the pigment of the lip and bands suppressed but the general shell colour may be greatly reduced. Mating ZLn 1 confirms Murray's findings. It is of a pale yellow with normal bands and white lip mated with a phenotypically yellow-white hyalozonate, i.e. with yellow periostracum and plain white calcareous layers, and with no pink at all. All the offspring are pink, which is impossible unless the hyalozonate was a pink with suppressed shell colour. Similarly, in ELn 1, PoEBn 1 and EXLn 1 at least one parent must be homozygous for pale pink, and in the latter it can only be the yellow-white hyalozonate. However, the abolition of pigment in the lip and bands does not always involve reduction of the shell colour to this extent; Taylor (1914) figures a roseozonate shell which is deep pink and hyalozonate, and in mating ChMP 1 such a shell is seen in one parent and in several offspring which vary from deep pink to pink, not to pale or faint pink. In heavily coloured shells, therefore, the paling may be inconspicuous or absent.

Pale brown and faint brown

Deep or medium-deep brown shells have often been recorded from British populations and are figured well by Taylor (1914) under various varietal names including *castanea* and *olivacea*. Such shells are very seldom banded; for example, Cain & Sheppard (1954) recorded only 169 bandeds out of 1607 browns (10·5%), but 5820 bandeds in 6978 yellows (83·4%) and 6865 in 8949 pinks (76·7%); similarly, Cain & Currey (1963*a*) recorded 363 bandeds in 2605 browns (13·9%) but 4949 in 6319 yellows (78·3%) and 2610 in 3865 pinks (67·5%). Clarke & Murray (1962), however, noticed in a random sample from the sand-dunes at Berrow, Somerset, pale browns which were not less banded than the yellows and pinks in the same samples, and which segregated clearly from both them and dark browns. We have seen such shells in several sand-dune collections (Cain, this volume, p. 507) and verified the segregation; in table 2 is shown the score of a large random sample from sand-dunes at Streedagh, western Ireland, kindly given to us by Mr D. P. T. Burke. In this, pale browns, many with bands, segregate from various pinks and yellows, but also from a class of very pale shells with only a faint flush of the bluish pigment which characterizes the calcareous layers of brown shells. (The brown effect is due to a bluish pigment overlaid by the yellowish periostracum.) These shells clearly correspond in the brown class to the faint pinks described above as the palest in the pink class. The fresh shell with periostracum looks a rather brownish or straw yellow, with a slight darker brown flush on the spire, whereas pale browns are well tinged with sepia and deep browns are deep chestnut or olive brown, sometimes almost black-brown. The faint browns are banded as are the pale browns in this sample, and as most of them are white-lipped, it might be thought that the greater paleness of the shell colour comes from the paling action of the white lip gene on a pale brown shell (see below, p. 393). However, one is only pale-lipped; this condition, as can be seen from table 2, is not regularly associated with a paling of shell colour, and indeed seems to have no effect on it at all. Faint browns without white lips have been seen also in other samples and appear to segregate clearly from pale brown and other shell colours. In this sample, then, we have both faint browns which may result from paling by white lip, the action of which is similar to that of hyalozonate described above, and a faint brown which is probably (on the analogy of pinks) due to an allele at the colour locus.

Yellow-white

In table 2 are also shown seven yellow-white shells with normal or pale lip, three of them banded with normal bands, as well as ten which are hyalozonate. A yellow-white shell has pure white calcareous layers with no trace of pigment, and the usual straw-yellow periostracum. Such shells with no, or virtually no, shell pigment can be produced from genetically pale pink or faint pink (and no doubt pale or faint brown) shells by the action of the hyalozonate gene. They also exist with normal bands and dark lips, segregate from yellows, and have been seen by us in several random samples from the Marlborough Downs where pale and faint browns and white lips are absent, and hyalozonate is extremely rare.

TABLE 1. GENERAL LIST OF BROODS

Key. Column 1: brood number consists of letters giving provenance of stock followed by a serial number. Bn = Bundoran, W. Ireland; C = Cantabrian Mountains, Spain; E = Eynsham road near Oxford; Ff = Faringdon, Berkshire; La = Les Alpilles, S. France; Ln = Lambourn, Berkshire; Lt = near Liercourt, Somme Valley, France; M = Marley Wood, Wytham, Berkshire; Mv = Mevagissey, Cornwall; P = Pentridge, Dorset; Po = Portnoo, W. Ireland; R = Rockley, Wiltshire; Ry = Rycote, Oxford; Sa = Steeple Aston, Oxford; St = Stroud, Gloucester; Ta = Tan Hill, Wiltshire; X = unknown origin; Y = Pyrenees, Spain; Z = abbreviation for BnMRTa.

Columns 2 and 3: B = brown; P = pink; Y = yellow; (with prefix D (dark), M (medium), P (pale), F (faint). If the depth of colour is uncertain, no prefix is given); YW = white shell with yellow periostracum. 0 = unbanded; 3 = mid-banded; 5 = five-banded, minor variations being ignored. i = indication, a faint uniform line marking the position of a band instead of a normal darkly pigmented band. : = interrupted or punctate band. 00fa00 = a shell with only a strip of opaque material (fascialbate) in the usual position for bordering band 3 but with no actual band. S = spread of banding pigment.

al = white lip (albolabiate); pl = pale lip; nl = normally pigmented lip; all are unscorable in juveniles. nb = normal bands; pb = punctate bands; hz = hyalozonate (transparent bands and white lip); rz = roseozonate (hz on a deep pink shell); ob = orange bands.

ad = adult when obtained and could have mated previously in the wild—in all matings involving adults the progeny shown are from the virgin parent; juv = juvenile (without lip); prog. = progeny—followed by a brood number means progeny of the mating given.

Column 4: notes, and scores of body colour; med = medium grey body; pl = pale (no or very little melanin).

brood number	parents	progeny	
	linkage of faint pink to banded and its dominance to dark yellow		
PoE 1	FP 0 Portnoo Ireland	FP 5 nb	30
	Y 5 Oxford (Eynsham)	Y 0 nb	28
LaPoE 1	Y 5 *punctata* ad	FP 5 pb	14
	Les Alpilles	FP 5 nb	10
	FP 5 normal prog.	DY 5 pb	13
	PoE 1	DY 5 nb	15
	recessiveness of faint pink to pale pink		
LLt 1	PP 3 near Liercourt	PP 3	16 pl
	PP 5 prog. L 13	PP 3	5 med
		PP 5	7 pl
		PP 5	9 med
		FP 3	5 pl
		FP 3	3 med
		FP 5	1 pl
		FP 5	0 med
PoER 1	FP 5 prog. PoE 1	PP 3	7
	PP 3 prog. RE 3	PP 5	4
		FP 3	0
		FP 5	5
		Y 3	1
		Y 5	2
Ry 1	PP 5 both Rycote	PP 5	11
	PY 5 near Oxford	FP 5	9
	effect of hyalozonate on pale pink		
ZLn 1	PY 5 al prog ad al	PP 5 pl? nb	4
	Tan Hill	FP 5 al nb	4
	Y 5 hz ad Lambourn		
ELn 1	YW 5 ob prog. E 2	PP 5 nb	27
	YW 5 hz ad Lambourn		
EXLn 1	DY 3 prog. EX 1	PP 3 nb	69
	heterozygous for ob	PP 5 nb	49
	YW 5 hz ad Lambourn		
PoEBn 1	FP 5 prog. PoE 1	FP 5 hz	19
	YW 5 hz prog. of Portnoo and Bundoran hz	FP 5 nb	13

Table 1 (*cont.*)

effect of hyalozonate on dark pink			
ChMP 1	DP 3 rz ad Chester DP 0 nl prog. MP 1	DP 0 nl	8
		DP to P 3 rz	9
suppression of the band in mid-banded snails			
LX 1	P 5 prog. L 13 P 00fa00 ad Berkshire Downs	P 0	1
		P 3	19
		P 5	17
StLX 1	PP 00fa00 ad Stroud Gloucestershire DP 5 prog. LX 1	P 0	3 juv
		P 3	11
		P 5	18
LX 3	DP 3 sibs, prog. DP 3 LX 1	DP 3	16 pl
		DP 3	10 med
		DP 5	2 pl
		DP 5	3 med
effect of spread-banded in five-banded individuals and the linkage between spread-banded and shell colour			
REL 4	DY 3 S prog. RE 1 DP 5 prog. L 12	DP 3	5
		DP 3 S	4
		DP 5	6
		DP 5 S	3
FfSa 1	PP (12345) Faringdon DY 3 Steeple Aston (and DY 5 prog. ME 4?)	PP 5 S	15
		PP 5	0
		DY 5 S	3
		DY '0'	1
		DY 3	5
		DY 5	5
RESa 1	MB 0 prog. RE 1 (genetically banded) DY 5 juv Steeple Aston near Oxford	B 0	1
		B 3	19
		B 5	9
		Y 3	12
		Y 5	11
distinction between broad band and spread band			
BrEX 1	DY 3 very broad band, Brittany DY 3 prog. EX 1	DY 3	26 med
		DY '0'	1 med
		DY 3	6 pl
		DY '0'	2 pl
genetic control of white lip and pale lip			
Z 1	PY 5 pl ad virg. prog. BnMR 1 DY 5 al ad Tan Hill, Wilts.	DY 5 pl	3
		PY 5 al	10
BnMR 1	PP 0 nl prog. MR 4 YW 5 nb al Bundoran	PP '00i:0' juv	13 very variable
		PP '00i:0' pl	1 light pb
		PP small juv	4 too small to score details of light bands
		PY 5 pl	3 normal dark
		PY 5 juv	11 bands
ZY 2	PY 5 al prog. Z 1 Y 12305 pl ad Pyrenees	Y 5 pl	3
		Y 5 al	5

TABLE 1 (*cont.*)

brood number	parents	progeny	
CMv 2	DY 0 al prog. Cantabrian stock DY 3 al ad Mevagissey, Cornwall	DY 0 al	18 med
		DY 0 al	14 pl
		DY 0 juv	2 med
		DY 0 juv	2 pl
CZ 1	PY 5 al prog. Z 1 DY 0 al ad Cantabrian Mts.	DY 0 al	18 med
		DY 0 al	14 pl
		DY 0 juv	8 med
		DY 0 juv	9 pl
complementation test between white lip and hyalozonate			
ZLn 1	PY 5 al prog. ad al Tan Hill Y 5 hz ad Lambourn	PP 5 pl? nb	4
		FP 5 al nb	4
linkage between hyalozonate and banding			
ChMP 1	DP 3 rz ad Chester DP 0 nl prog. MP 1	DP 0 nl	8
		DP to P 3 rz	9
complementation test between orange banded and hyalozonate			
ELn 1	YW 5 ob prog. E 2 YW 5 hz ad Lambourn	PP 5 nb	27
EXLn 1	DY 3 prog. EX 1 heterozygous for ob YW 5 hz ad Lambourn	PP 3 nb	69
		PP 5 nb	49
EXPoBn 1	PP 5 prog. EX 1 heterozygous for ob YW 5 hz prog. PoBn 1	PP 5 nb	5
dominance of *punctata*			
PoE 1	FP 0 Portnoo, Ireland Y 5 Oxford (Eynsham)	FP 5 nb	30
		Y 0 nb	28
PoEM 1	FP 5 prog. PoE 1 must be mated with a sib FP 5 nb	FP 5 nb	5
		FP 5 hz	2
		Y 5 nb	2
		Y 5 hz	0
LaPoE 1	Y 5 *punctata* ad Les Alpilles FP 5 normal prog. PoE 1	FP 5 pb	14
		FP 5 nb	10
		DY 5 pb	13
		DY 5 nb	15
linkage between *punctata* and shell colour			
BnMR 1	PP 0 nl prog. MR 4 YW 5 nb al Bundoran	PP ‘00i:0’ juv	13 very variable
		PP ‘00i:0’ pl	1 light pb
		PP small juv	4 too small to score details of light bands
		PY 5 pl	3 normal dark bands
		PY 5 juv	11
recessiveness of pale body colour and absence of linkage with shell colour			
BrEX 1	DY 3 very broad band, Brittany DY 3 prog. EX 1	DY 3	26 med
		DY ‘0’	1 med
		DY 3	6 pl
		DY ‘0’	2 pl

TABLE 1 (*cont.*)

CZ 1	PY 5 al prog. Z 1 DY 0 al ad Cantabrian Mts.	DY 0 al	18 med
		DY 0 al	14 pl
		DY 0 juv	8 med
		DY 0 juv	9 pl
CMv 2	DY 0 al prog. Cantabrian stock DY 3 al ad Mevagissey, Cornwall	DY 0 al	18 med
		DY 0 al	14 pl
		DY 0 juv	2 med
		DY 0 juv	2 pl
LLt 1	PP 3 near Liercourt PP 5 ad prog. L 13	PP 3	16 pl
		PP 3	5 med
		PP 5	7 pl
		PP 5	9 med
		FP 3	5 pl
		FP 3	3 med
		FP 5	1 pl
		FP 5	0 med
LX 3	DP 3 sibs, prog. DP 3 LX 1	DP 3	16 pl
		DP 3	10 med
		DP 5	2 pl
		DP 5	3 med

TABLE 2. SCORE OF SHELL CHARACTERS IN A RANDOM SAMPLE FROM STREEDAGH, W. IRELAND

shell colour and banding	normal lip	pale lip	white lip	hyalozonate
browns				
pale brown 0	3	—	—	—
pale brown 3	—	6	—	—
pale brown 5	6	17	—	—
faint brown 0	—	—	—	—
faint brown 3	—	1	10	—
faint brown 5	—	—	5	—
pinks				
dark pink 0	1	—	—	—
dark pink 3	5	—	—	—
dark pink 5	3	2	—	—
pale pink 0	3	—	—	—
pale pink 3	2	2	—	—
pale pink 5	6	6	—	—
faint pink 0	—	—	—	—
faint pink 3	—	3	2	—
faint pink 5	1	—	1	—
yellows				
dark yellow 0	3	3	—	—
dark yellow 3	3	9	—	—
dark yellow 5	7	7	—	—
pale yellow 0	—	—	—	—
pale yellow 3	1	—	4	—
pale yellow 5	—	4	3	—
yellow-white 0	3	1	—	—
yellow-white 3	—	—	—	4
yellow-white 5	2	1	—	6
totals	49	62	25	10

All the yellows we have bred have some trace of yellow pigment in the shell, and usually a noticeable amount at the apex. Yellow-whites have none and so look straw-yellow. They probably indicate yet another allele at the shell colour locus.

Banding and lip-colour

Suppression of the band in mid-bandeds

Those parts of the shell that lie between the pigmented bands in a five-banded shell are a rather opaque white, contrasting with the glassy transparency of the shell-structure in the bands themselves which is well seen in hyalozonates. The contrast is visible with transmitted light even in shells with pigmented bands. In the unbanded shell there is no contrast, the structure being intermediate in opacity throughout. In mid-bandeds, there are narrow strips of more opaque material bordering the band on either side and very variable in width; when well developed they can be seen clearly on the outside of the shell especially if it is deeply coloured, and are var. *fascialba* Picard (Taylor 1914). The pigmented band itself is very variable in width, and a few shells occur in which it is absent but a strip of fascialbation is present.

Mating LX1 is between one of these and a normal five-banded, and gave 36 normal bandeds and one apparently unbanded. One of the normals mated to a second snail with only fascialbation (StLX1) gave 29 normals and three apparently unbandeds, all small juveniles. The condition is obviously not dominant to normal banding, and if it were recessive it should appear in LX3, the progeny of which are an F2 from LX1; none were seen in 31 offspring. It seems therefore to have no simple genetic basis and, occurring as it does as an extreme of variation of width of the middle band, is probably controlled multifactorially. The presence of the fascialbation distinguishes it from unbandeds; if this also were to disappear there would be no distinction, but from the rarity of the shells with only fascialbation in random samples, errors are likely to be made very seldom.

Spread-banded

This form was described (Cain *et al.* 1960) as a mid-banded with a flush of banding pigment spreading on both sides of the band but not beyond the position of the upper edge of band 1 or the lower edge of band 5. A heavy spread may obscure the middle band completely and be indistinguishable from a five-banded with complete fusion of bands. The form came from the Marlborough Downs where it occurs in a restricted area (Cain & Currey 1963*a*) in which five-bandeds are absent. It has since been found in both mid-bandeds and five-bandeds on Salisbury Plain (Cain & Currey 1963*b*). Mating REL4 shows the spread in the five-banded form. It produces a shell with rather badly defined but well pigmented bands, obscured to some extent by the spread. In the bred material, the separate bands can be seen on the higher whorls or close to a varix; elsewhere on the shell there is often a complete fusion of spread pigment and bands. In mating FfSa1 of a pale pink heavily spread five-banded to a normal yellow mid-banded the linkage between spread and shell colour suggested by Cain *et al.* (1960) is again obvious but there appear to be three crossovers (yellow five-banded spread) in 29 progeny. One large juvenile yellow appears unbanded although from the mating it must be genetically banded; there must be either a failure of penetrance or a mistake.

In mating RE1 (Cain *et al.* 1960) linkage between shell colour and spread was indicated, with the allele for spread on the chromosome carrying yellow shell colour. Consequently none of the brown progeny should be carrying spread, whereas if the association is due to differences in penetrance of spread in yellows and browns, all should have spread. A test mating RESa 1 (this paper) using one of the two exceptional shells from RE 1 in which banding was apparently absent (presumably because of incomplete penetrance) gave no spreads in 29 brown and 23 yellow offspring, confirming strong linkage. In the browns all but one is banded, showing that the apparently unbanded brown was, as expected, genetically banded.

The brown parent of RE 1 had only a broad mid-band but must have been spread-banded; that such a broad band is not necessarily a spread-band is shown by BrEX 1 in which no spread were produced in 34 progeny from a yellow broad-banded.

White lip

A large sample from the Pyrenees collected by Dr L. R. B. Emberton is scorable (table 3) for normal dark lip (nl), albolabiate or white lip (al) and intermediate or pale lip (pl). On division into white lip and others, there is a significant association ($P < 1 \times 10^{-8}$) of lip colour and shell colour strongly suggestive of linkage. Since the genetics of shell colour is known, one can obtain the maximum likelihood estimate (Smith 1956) of frequencies of the four types of chromosome carrying respectively yellow and white lip, yellow and non-white lip, pink and white lip, and pink and non-white lip, assuming either dominance or recessiveness of white lip (table 3). The assumption of dominance gives a highly significant χ^2_2 (17·70, $P < 0·001$), while assumption of recessiveness gives a very good agreement ($\chi^2_2 < 0·0001$) between expected and observed phenotype frequencies. Cook (1966) also has evidence that al is recessive, in material from Malham Tarn.

Table 3. Scores of lip pigmentation in a random sample from the Pyrenees

shell colour and bands	al	pl	nl
Y 0	46	3	1
Y 3	14	...	...
Y 5	153	25	1
P 0	1	4	1
P 3	...	...	...
P 5	...	8	...

estimated frequencies of chromosomes

	al assumed recessive	al assumed dominant
Y al	0·9104	0·6025
Y non-al	0·0620	0·3694
P al	0·0021	0·0000
P non-al	0·0255	0·0028

Since the pale lips are frequent, it seems possible that they may be heterozygous for white and normal lip. The expected frequencies of the three types on this hypothesis agree well with the observed ($\chi^2_1 = 0·556$, $P > 0·3$). This might be coincidence, but is supported by similar agreements in other samples, from the Pyrenees and one from Britain, collected by Dr R. W. Arnold (data in table 4, comparison in table 5). Further, it is consistent with the breeding data obtained from British material (table 1). In Z 1 a cross between a white lip and a pale lip segregates al and pl in a ratio not significantly different from 1:1. In

BnMR 1 and ZY 2, four known heterozygotes are pale-lipped; and crosses of two al give only al, even when involving crossing Spanish and British stock (CMv 2, CZ 1).

Since al appears to be linked to the colour and banding loci in the samples discussed above, and hyalozonate (in which both lip and bands have no pigment) is also linked (below, p. 393), these two forms have been tested for allelomorphism by a complementation test in mating ZLn 1. The progeny consist of undoubted white lips and others with a suggestion of lip pigment, in one case quite noticeable but far less than in other pale lips we have seen. Since neither an al mated to a non-albolabiate homozygote, nor a hyalozonate mated to a non-hyalozonate homozygote should give any white-lipped individuals, the cross gives strong evidence of non-complementarity and suggests that the forms hz and al are allelomorphic. Cook's data (1967) also support non-complementarity. The possibility that the hz individual was heterozygous or homozygous for al is remote since al has not been found in the locality from which it came. Although the faint pink (FP) progeny were all al, traces of lip pigment were present in the pale pinks (PP), still indicating the absence of complete complementarity but suggesting that the pink allele at the closely linked colour locus may modify the phenotype slightly. In the Pyrenean samples it is noticeable that the pale lips are markedly darker in the pinks than in the yellows; this cannot be due to an infusion of pink shell-colour pigment in the lip, both since it is a much

Table 4. Scores of lip pigmentation in random samples collected by Dr R. W. Arnold

sample no. and data	shell score	lip score		
		al	pl	nl
1098. Valle de Aran; Valarties Hill	Y 0	6	7	0
	Y 3	0	0	0
	Y 5	2	5	2
	YW 0	0	1	1
	YW 3	3	0	0
	YW 5	27	23	3
	P 0	0	0	0
	P 3	0	1	0
	P 5	0	5	4
1127. Valle de Aran; plateau, 5–6000 ft.	Y 0	40	14	0
	P 0	3	0	0
1375. St Béat, Lower Garona	Y 0	0	11	10
	Y 3	0	2	5
	Y 5	1	5	2
	P 0	0	2	3
	P 3	0	2	2
	P 5	0	6	5
1377. St Béat-Gastrade, Lower Garona	Y 0	8	17	2
	Y 3	0	0	1
	Y 5	3	8	4
	P 0	0	3	1
	P 3	0	0	1
	P 5	0	6	5
1599. Minera, N. Wales	Y 0	9	1	0
	Y S (3 and 5)	0	14	6
	P 0	1	2	0
	P S (3 and 5)	0	7	5

paler pigment than lip and banding pigment and of a different hue, and because the pinks in the Pyrenean samples are well flushed with the pink pigment only high up on the shell and not close to the lip.

Further evidence for allelomorphism comes from the effect of al on shell colour. Mating Z1 suggests that the white-lipped individuals tend to be paler yellow than the heterozygotes, which cannot be explained in this mating by linkage. However, in the Pyrenean sample there is no indication of paling in these rather brilliant yellows, nor in the pinks. Such a paling of shell colour occurring in some forms, but not in others, is reported above for hz; the actions of the two alleles on shell colour are therefore similar. The random sample from Streedagh reported in table 2 contains both hz and al, as is not infrequent in samples from western Ireland. While all the hz in this sample are so pale as to be indeterminate in colour, virtually white, those with al are assignable to the usual colour-morph classes but are always found in the paler colour classes.

TABLE 5. OBSERVED AND EXPECTED VALUES FOR THE THREE PHENOTYPES WHITE LIP (al), PALE LIP (pl) AND NORMAL LIP (nl)

	al		pl		nl	
locality	exp.	obs.	exp.	obs.	exp.	obs.
Streedagh (table 2, excluding hz)	23·07	25	65·88	62	47·05	49
Pyrenees (table 3)	213·06	214	41·88	40	2·06	3
1098 (table 4)	38·68	38	40·64	42	10·68	10
1127 (table 4)	43·86	43	12·28	14	0·86	0
1375 (table 4)	4·02	1	21·97	28	30·01	27
1377 (table 4)	13·29	11	29·42	34	16·29	14
1599 (table 4)	10·76	10	22·49	24	11·76	11

Hyalozonate

White shells with normal banding but a very pale tinge of pink shell pigment are faint pinks (FP), shells with this appearance or paler, even white, may be genetically faint pinks or pale pinks, as mating ELn1 shows. The earlier statement by Cain & Sheppard (1954) that hyalozonate shows an association with the shade of colour which must be due to linkage must be treated with reserve since pale shells were involved; but in mating ChMP1 there is clear evidence of linkage between hyalozonate and banding which itself is known to be linked to shell colour. The dark pink unbanded is heterozygous for banded and unbanded and for normal pigmentation and hyalozonate (progeny of mating MP1, Cain *et al.* 1960) with the alleles in coupling, and in ChMP1 only double recessive and double dominant phenotypes appear, giving good evidence for linkage ($P < 0{\cdot}001$). Cook (1967) has shown recombination between hyalozonate and colour and banding, with fairly strong linkage.

Complementarity between orange-banded and hyalozonate

The form orange-banded ob, with dilute banding and lip pigmentation, described by Cain *et al.* (1960) is recessive to normally pigmented bands and lip as is hyalozonate in which the banding and lip pigment is wholly absent. Mating ELn1 is between an orange-banded and a hyalozonate and has given only normally pigmented progeny. The forms ob and hz behave as complementary, which suggests that they are determined at different loci. This is confirmed by EXLn 1 and EXPoBn 1 both being a cross between an hz and a normal-banded heterozygous for ob (progeny of EX 1, Cain *et al.* 1960), which has given 118 progeny, all normal-banded. If ob and hz were alleles at the same locus, a 1:1 segregation would be expected, of normal bands and whatever were the result of heterozygosity for ob and hz. All the ob shells we have seen from the Oxford District, Salisbury Plain (Cain & Currey 1963*b*), and the Channel Isles (kindly shown to us by Dr R. W. Arnold) have very pale ground colour; the only exceptions are a few deep pink orange-bandeds from Cornwall. It seems highly likely that ob, like hz, often but not always pales the ground colour of the shell.

Punctata

The condition *punctata* or pale bands or *bandes pâles* has been investigated by Lang (1904), and Lamotte (1951) who concludes it to be a dominant. In it, as well illustrated by Lang, the bands, instead of being uniformly darkly pigmented, are pale with occasional dark or black spots of pigment, which usually occur simultaneously in shell production in all the bands present. It is this punctation of pale bands by dark spots which is characteristic; pale bands, as Lang originally called it, is not a good name since it could apply better to more uniformly pale bands such as those in orange-banded or the form *lurida* in *Cepaea hortensis* (Murray 1963) or in *nemoralis* (Cook & Murray 1966).

Mating LaPoE 1, a cross between a yellow punctate from Les Alpilles, France, collected by H. B. Ginn, and a very pale pink normal banded progeny of PoE 1 (Cain *et al.* 1960) segregated normals and punctates, confirming Lang's original finding. In BnMR 1, very feeble punctate bands are confined to the pink progeny, while the yellow progeny have strong and dark normal bands, thus giving evidence of linkage and of the recessiveness of normal bands ($P < 0.001$). The allelomorph for *punctata* originally came from a pale pink individual from Marley Wood, Wytham Woods, Berkshire, which appeared to be unbanded except for a 'trace' of band 4—a single dark spot in that position, almost at the lip. It produced pink individuals (MR 4, Cain *et al.* 1960), the majority of which appeared unbanded but one had a trace, again in the position of band 4. A sib-mating (MR 28, Cain *et al.* 1960) produced three juvenile pale pink individuals apparently unbanded, but one of them on re-inspection has indications (narrow lines, faintly pigmented) of bands 3 and 4. A third sib, a parent in the present mating BnMR 1, appears unbanded but has given no certainly unbanded progeny; in those pinks showing bands, band 4 is best represented in two individuals by a very narrow but typically punctate band, while 3 is represented usually only by an indication. Thus *punctata* is controlled as a dominant (or semi-dominant) closely linked to colour. The expression of this gene in the progeny of MR 4 was very slight, greater in MR 28, and still more so in BnMR 1, but even the best-marked shells are far less marked than in LaPoE 1 and in most (but not all) examples of *punctata* from the wild. While the increase in expression in broods subsequent to MR 4 suggests some action of modifiers, the most extreme shells so far produced are so much less marked than most *punctata* that we may well have two alleles at this locus. The heavily punctate form described by Lang and Lamotte is shown to be dominant to normal bands and linked to the colour locus, by Cook (1967), examining the data of Stelfox.

BODY COLOUR

In five matings, BrEX 1, CMv 2, CZ 1, LLt 1 and LX 3, there is segregation for colour, of the body. In these broods, the animals can be separated into the same two classes whether extended, when the head and body can be seen, or retracted inside the shell, when only the mantle can be seen through the body-whorl of the shell. It seems, therefore, that pigmentation of the head and protrusible parts generally and of that part of the mantle covering the 'lung' is controlled as a single unit. In samples from the wild, an apparently continuous variation of body colour can be seen from nearly black to a pale yellowish in which there seems to be no melanin at all (Cain & Sheppard 1952). In these broods the segregation is for a medium grey to pale yellowish.

In BrEX 1 there is a 3:1 segregation showing that pale is recessive. In CZ 1 and CMv 2 there is a 1:1; in each, one parent is from Cantabrian stock which has been bred through several generations, in which every individual has been pale, without exception. As all the live sibs of the medium grey parent of CZ 1 are more or less medium grey like it, this suggests that again pale is recessive. In LLt 1, the segregation (medium 17, pale 29) is not significantly different from a 1:1 ratio; there is no association of body colour with mid-banded (00300) and five-banded, but since neither parent can have been a double heterozygote for body colour and banding, this is no evidence against linkage. There is equally no association of body colour with shell colour, but since the segregation for shell colour is 3:1, one parent must have been a double heterozygote for body and shell colour, and the lack of association is evidence against strong linkage. The same is true for body colour and mid-banded against five-banded in LX 3; in both matings LX 3 and LLt 1, with strong linkage, the recessive class of the 3:1 segregation can show only one class of the 1:1 segregation in body colour, and the figures available, although small, suggest no linkage at all.

DISCUSSION

The data reported here extend the number of loci contributing to the polymorphism which are known to be strongly or very strongly linked. These are now (extending the nomenclature of Cain & Currey (1963*a*) and altering it to conform with the agreements reached at the Second International Conference on Snail Genetics (York 1967)) C ground colour of shell; alleles (in dominance order with the top dominant first) C^B dark and medium brown, C^{DP} dark pink, C^{PP} pale pink, C^{FP} faint pink, C^{DY} dark yellow, C^{PY} pale yellow. (The segregants PB pale brown, FP faint pink, and YW yellow-white almost certainly belong here also.)

B presence or absence of banding: alleles B^0 unbanded, B^B bands present.

S spread bands: S^S spread of banding pigment, S^- unmodified.

I punctate bands (interrupta); I^I punctate, I^- unmodified.

P pigmentation of lip and bands or lip alone; P^N normally pigmented bands and lip, P^A white lip or albolabiate (no pigment in lip, bands normal), P^T transparent bands or hyalozonate (no pigment in lip or bands).

Those known not to be linked to the above or each other are:

U mid-banded (unifasciata); U^3 bands 1, 2, 4 and 5 suppressed, U^- unmodified.

T suppression of two upper bands (trifasciata) (Lamotte 1954); T^{345} bands 1 and 2 suppressed, T^- unmodified.

Body colour segregation for grey to reddish brown has been described by Murray (1963) as D, with D^R reddish dermal pigment, D^G grey dermal pigment.

That given here for quantity of pigment, Q, has Q^M medium grey, Q^P very pale, and appears to be unlinked to C, B or U. Darkening bands R (Cain *et al.* 1960) has R^- unmodified, R^D bands gradually darkening down the shell, and is not or not closely linked to C. The linkage of orange bands O (Cain *et al.* 1960) which has O^- unmodified, O^O orange bands and lip, is undetermined as yet, but it is not at the P locus.

B^O is epistatic to all modifications of banding, and U^3 is epistatic to T^{345}.

We are grateful to all those who have given us live material for breeding or allowed us to examine random samples, especially Dr R. W. Arnold, Dr A. Comfort, Dr L. R. B. Emberton, Mr D. P. T. Burke and Mr H. B. Ginn. This breeding programme was initially supported by the Nuffield Foundation, and is now supported by a grant from the Department of Scientific and Industrial Research (now the Science Research Council) to Professor A. J. Cain as part of a programme of work in functional systematics; grateful acknowledgement is made accordingly.

References

Cain, A. J. 1968 Sand-dune populations of *Cepaea nemoralis* (L.). *Phil. Trans.* B **253**, 499–517, (Part V).

Cain, A. J. & Currey, J. D. 1963*a* Area effects in *Cepaea*. *Phil. Trans.* B **246**, 1–81.

Cain, A. J. & Currey, J. D. 1963*b* Area effects in *Cepaea* on the Larkhill Artillery Ranges, Salisbury Plain. *J. Linn. Soc.* (*Zool.*) **45**, 1–15.

Cain, A. J., King, J. M. B. & Sheppard, P. M. 1960 New data on the genetics of polymorphism in the snail *Cepaea nemoralis* L. *Genetics* **45**, 393–411.

Cain, A. J. & Sheppard, P. M. 1952 The effects of natural selection on body colour in the land snail *Cepaea nemoralis*. *Heredity* **6**, 217–231.

Cain, A. J. & Sheppard, P. M. 1954 Natural selection in *Cepaea*. *Genetics* **39**, 89–116.

Cain, A. J. & Sheppard, P. M. 1957 Some breeding experiments with *Cepaea nemoralis* (L.). *J. Genetics* **55**, 195–199.

Clarke, B. C. & Murray, J. J. 1962 Changes of gene frequency in *Cepaea nemoralis* (L.). *Heredity* **17**, 445–465.

Cook, L. M. 1966 Notes on two colonies of *Cepaea nemoralis* (L.) polymorphic for white lip. *J. Conch.* **26**, 125–130.

Cook, L. M. 1967 The genetics of *Cepaea nemoralis*. *Heredity* **22**, 397–410.

Cook, L. M. & Murray, J. J. 1966 New information on the inheritance of polymorphic characters in *Cepaea hortensis*. *J. Hered.* **57**, 245–247.

Lamotte, M. 1951 Recherches sur la structure génétique des populations naturelles de *Cepaea nemoralis* (L.). *Bull. biol. Fr.* (Suppl.) **35**, 1–239.

Lamotte, M. 1954 Sur le déterminisme génétique du polymorphisme chez *Cepaea nemoralis* (L.). *C. r. Acad. sci. Paris* **239**, 365–367.

Lang, A. 1904 Ueber Vorversuche zu Untersuchungen über die Varietätenbildung von *Helix hortensis* Müll. und *Helix nemoralis* L. *Denkschr. med.-naturw. Ges. Jena* **11**, 439–506.

Lang, A. 1908 Ueber die Bastarde von *Helix hortensis* Müller und *Helix nemoralis* L. *Festschr. Univ. Jena* pp. 1–120.

Murray, J. J. 1963 The inheritance of some characters in *Cepaea hortensis* and *Cepaea nemoralis* (Gastropoda). *Genetics* **48**, 605–615.

Smith, C. A. B. 1956 Counting methods in genetical statistics. *Ann. hum. Genet.* **21**, 254–276.

Taylor, J. W. 1914 *Monograph of the land and freshwater mollusca of the British Isles*. **3**. Leeds: Taylor.