POLYMORPHISM IN WEST AFRICAN SNAILS

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1. INTRODUCTION

SHELL colour polymorphism occurs in many terrestrial snails and has been studied intensively in the genus *Cepaea*. The present paper deals with the genus *Limicolaria* from the tropical rainforest zone of West Africa (Nigeria). Two species have been studied—*L. flammulata* (Pfeiffer) and *L. aurora* (Jay). These snails, like *Cepaea*, are polymorphic for two shell colour characters—banding and background colouration. The genetical basis of these polymorphisms and the genetical structure of natural populations have been studied. The hypothesis is advanced that these polymorphisms are controlled by a super-gene and that selection is acting against the coupling arrangements of two of the genes.

2. MATERIAL AND METHODS

All the populations studied were in the Western Region of Nigeria. Three or more samples were taken from each population. An attempt was made to collect every snail at each sample site. The breeding techniques will be described elsewhere.

3. DESCRIPTION OF THE POLYMORPHISMS AND NOTATION

The banding polymorphism

In both species some snails have bands of brownish pigment parallel to the long axis of the shell, *i.e.* across the whorls rather than along them; other snails lack such bands. The bands may be either dark (brownish-black) or light (pale brown) and almost all banded snails can be unequivocally classified in this respect. Hence there are three phenotypes: dark banded, light banded and unbanded. Dark banded snails have normally and perhaps always a grey background colouration.

The background colouration polymorphism

In *L. aurora* the two phenotypes are pink and grey, which is usually a clear-cut difference. Variation in the darkness of pink is ignored. Banded pink snails are normally *light* banded. In *L. flammulata* there seem to be two main colour types—grey and brown, but there are intermediate shades of brown which often make classification difficult.

Notation for genes

The banding, darkness of banding, and background colouration genes will be referred to as *U*, *u*; *D*, *d*; *G*, *g*, respectively, the corresponding recessive phenotypes being unbanded, dark and grey. The letters *p* and *b* are used as superscripts to distinguish between pink and brown thus: G^p , G^b .

4. BREEDING RESULTS

L. aurora

The results (table 1) are largely consistent with the hypothesis that (i) the background polymorphism is controlled by one gene with two alleles G^b , g , with pink dominant over grey; (ii) the banding polymorphism is basically controlled by one gene, U , u , with banded dominant to unbanded. Thus most of the crosses give a good agreement with the ratios expected on this hypothesis (statistical tests, table 2). The F_1 crosses 2, 3 and 4 should

TABLE 1
L. aurora: breeding results

Parents	Cross code No.	Offspring			
		Banded Pink	Unbanded Pink	Banded Grey	Unbanded Grey
Banded Pink $\times$ Banded Pink	56	5	3	2	—
Banded Grey $\times$ Banded Grey	47	—	—	9	2
Banded Grey $\times$ Banded Grey	69	—	—	54	16
Unbanded Pink $\times$ Unbanded Pink	78	—	38	—	15
Unbanded Pink $\times$ Unbanded Grey	2T	—	28	—	—
Unbanded Pink $\times$ Unbanded Grey	2U	—	17	—	—
Banded Pink $\times$ Unbanded Pink	48	10	10	16	—
Banded Pink $\times$ Unbanded Pink	60	—	4	—	—
Banded Pink $\times$ Unbanded Pink	72	18	36	21	1
Banded Grey $\times$ Unbanded Grey	2L	—	—	9	—
Banded Pink $\times$ Banded Grey	57	1	1	7	—
Banded Pink $\times$ Banded Grey	68	5	1	4	—
Banded Pink $\times$ Unbanded Grey	59	—	32	25	—
Banded Pink $\times$ Unbanded Grey	64	—	25	11	5
Banded Pink $\times$ Unbanded Grey	73	—	19	29	—
Banded Grey $\times$ Unbanded Pink	2R	4	—	3	2
Banded Grey $\times$ Unbanded Pink	45	16	17	—	—
F_1 CROSSES					
Unbanded Pink* $\times$ Unbanded Grey†	2	—	53	—	49
Unbanded Pink* $\times$ Unbanded Grey†	3	—	6	—	10
Unbanded Pink* $\times$ Unbanded Grey†	4	—	9	—	9

* Unbanded pink parents of these crosses came from the P_1 cross 2U.

† Unbanded grey parents were from field samples.

especially be noted as providing clear evidence about the background colouration gene. However, two crosses (48 and 64) gave significant departures from expected. Such departures could be caused by faulty experimental technique or by linked recessive lethals. The deviation chi-square for the 3:1 ratio of the background colouration gene is significant at the 0.05 level, there being a deficiency of pink. This raises the possibility that homozygous pink may be less viable than the other genotypes, at least under the culture conditions used.

It is necessary to postulate tight linkage to account for the results of crosses such as 59 and 72; so the unbanded grey snails in crosses 64 and 72 should be recombinants. The recombination fraction was estimated as

3 per cent. with 95 per cent. confidence limits of 1 per cent. and 23 per cent. In making these estimates the data of crosses 59, 64 and 73 were combined, since these crosses are of identical type; for the same reason the results of crosses 48 and 72 were combined.

However, *Limicolaria* is hermaphrodite, and the possibility cannot be excluded that the unbanded grey offspring of crosses 64 and 72 are the products of self-fertilisation rather than recombinants. The egg-laying parent of all the cross 64 offspring was unbanded grey and the egg-laying parent of the unbanded grey offspring of cross 72 was unbanded and heterozygous for pink. So if self-fertilisation was taking place, it could lead to the production of unbanded grey snails in these crosses. Clearly, if these unbanded grey snails were produced in this way, the intensity of linkage between the two genes would be greater than estimated above. It does, however, seem unlikely that self-fertilisation is taking place since the few snails of both *L. aurora* and *L. flammulata* that were reared without partners did not produce young. Also, the writer knows of no published evidence that self-fertilisation occurs in *Limicolaria* and related genera.

It is possible that a third gene is involved in the control of the polymorphisms, a gene for darkness of bands. For banded pink snails are normally and perhaps always light banded, while banded grey snails are dark banded. This difference could be caused either by interaction between G^b and U or by a closely-linked third gene such as the gene D , d , postulated in the next section for *L. flammulata*. The latter hypothesis will be adopted here in order to produce a unified hypothesis to explain the polymorphisms of both species.

L. flammulata

The banding polymorphism will be dealt with first (results, table 3; statistical tests, table 4). Most of the results of the first generation (P_1) crosses can be explained by the simple hypothesis of one gene, two alleles and no dominance, so that dark banded and unbanded are homozygotes, and light banded is the heterozygote. For example, light banded $\times$ light banded crosses give a good agreement with the expected 1:2:1 ratio in the F_1 (chi-square test 1), and a good agreement with expected in the F_2 .

But the results of P_1 crosses 35 and R cannot be explained in this way. These crosses were dark banded $\times$ unbanded and should therefore have given all light banded offspring, as cross 3C did. But they gave a 1:1 ratio of dark banded:light banded. So some other factor is present in addition to the two alleles postulated. This factor could be either a third allele at the banding locus or a different but closely linked gene, these two possibilities being indistinguishable at present. But the hypothesis of another gene allows the development of a unified hypothesis to explain the polymorphism of both species, so will be adopted here (see next section).

The present hypothesis, then, is that two tightly linked genes are involved in the banding polymorphism: U , u , concerned with the production of bands, and D , d , concerned with the darkness of the bands. U is needed to produce bands, dd makes the bands dark. Crosses 35 and R should therefore be $Ud/Ud \times uD/ud \rightarrow Ud/uD$ (light banded) + Ud/ud (dark banded). The dark banded offspring should be heterozygous for U but homozygous dd and therefore when mated together should give a 3:1 ratio of dark banded:unbanded, which they did (crosses MB, MC (chi-square test 3)). The rest of

TABLE 2

L. aurora: statistical tests of the hypothesis that banding and background colouration are each controlled by 1 gene with pink background dominant to grey, banded dominant to unbanded. Data from table 1

(a) Background colouration gene—3:1 ratio

Cross	Results		χ^2	P	Source	χ^2	d.f.	P
	Pink	Grey						
56	8	2	—	1.0*				
48	20	16	7.259		Deviation	4.463	1	0.05-0.02
72	54	22	0.632		Heterogeneity	3.736	2	0.2-0.1
78	38	15	0.308		Total	8.199	3	
			8.199					
	112	53	4.463					

* By binomial expansion.

1:1 ratio

Cross	Results		χ^2	Source	χ^2	d.f.	P
	Pink	Grey					
57	2	7	2.778				
59	32	25	0.860				
64	25	16	1.976				
68	5	4	0.112	Deviation	0.004	1	0.95
73	19	29	2.084	Heterogeneity	9.074	8	0.5-0.3
2R	4	5	0.112	Total	9.078	9	
2	53	49	0.156				
3	6	10	1.000				
4	9	9	0.000				
			9.078				
	155	154	0.004				

(b) Banding gene—3:1 ratio

Cross	Results		χ^2	P*
	Banded	Unbanded		
47	9	2		0.74
56	7	3		1.00
57	8	1		0.47
68	9	1		0.47
69	54	16	0.172	0.7-0.5

* Using the binomial expansion except for cross 69.

1:1 ratio

Cross	Results		χ^2	Source	χ^2	d.f.	P
	Banded	Unbanded					
45	16	17	0.030				
48	26	10	7.112				
59	25	32	0.860	Deviation	0.120	1	0.8-0.7
64	11	30	8.804	Heterogeneity	21.600	6	<0.01
72	39	37	0.052	Total	21.720	7	
73	29	19	2.084				
2R	7	2	2.778				
			21.720				
	153	147	0.120				

TABLE 3

L. flammulata: breeding results for the banding polymorphism. Snails which appear to be intermediate between two phenotypes scored with a number printed small between the phenotypes concerned

		P ₁ CROSSES	Offspring		
Parents		Cross code No.	Dark Banded	Light Banded	Unbanded
Dark Banded×Dark Banded		<i>MB13</i>	24	0	0
		<i>SPP4</i>	19	0	0
		<i>SPP5</i>	59	0	0
		<i>MB17</i>	26	0	0
Light Banded×Light Banded		<i>α52</i>	2	1	1
		<i>I</i>	16	34	21
		<i>2V</i>	17	36	14
		<i>2W</i>	10	37	12
		<i>2Y</i>	27	43	21
		<i>2Z</i>	13	30	16
		<i>10</i>	0	0	29
Unbanded×Unbanded		<i>34</i>	0	0	13
		<i>Q</i>	0	0	33
		<i>SPP8</i>	0	0	46
		<i>19</i>	43	24	0
Dark Banded×Light Banded		<i>T</i>	17	10	0
		<i>V</i>	10	1	11
		<i>B2</i>	13	24	0
		<i>B7</i>	7	1	10
		<i>35</i>	24	1	18
Dark Banded×Unbanded		<i>R</i>	12	14	0
		<i>α38</i>	0	5	0
		<i>3C</i>	0	63	0
		<i>α49</i>	0	20	19
Light Banded×Unbanded		<i>3H</i>	1	37	33
		<i>OTP</i>	2	4	4
Doubtfully Banded×Doubtfully Banded (see text)					

F₁ CROSSES. (A) From P₁ cross *I*

Parents		Cross code No.	Offspring		
			Dark Banded	Light Banded	Unbanded
Dark Banded×Dark Banded		<i>M8</i>	44	0	0
Dark Banded×Unbanded		<i>M9</i>	0	37	0
Unbanded×Unbanded		<i>M11</i>	0	0	76
Light Banded×Unbanded		<i>M37</i>	0	13	20

F₁ CROSSES.* (B) From P₁ crosses 35 and *R*

Parents		Cross code No.	Offspring		
			Dark Banded	Light Banded	Unbanded
Dark Banded×Dark Banded		(35)MB	49	0	10
		(35)MC	46	0	17
Dark Banded×Light Banded		(35)MD	6	6	6
		†(R)MV	35	2	37
Light Banded×Light Banded		(R)M41	44	1	35
		(35)M3K	13		27
		(35)M3P	10	1	27
Dark Banded×Unbanded		†(35)M35L	0	3	5
		†(R)MRU	0	37	33

(continued overleaf)

TABLE 3—(continued)

F₂ CROSSES.* (A) From F₁ crosses MB, MC, MD

Parents	Cross code No.	Offspring		
		Dark Banded	Light Banded	Unbanded
Dark Banded × Dark Banded	(MB)N25	52	0	15
	(MB)N26	37	0	0
	(MB)N28	6	3	0
Dark Banded × Unbanded	(MC)N34	13	0	0
Light Banded × Unbanded	(MD)N50	9	1	10
				24

F₂ CROSSES. (B) From F₁ cross MRU

Parents	Cross code No.	Offspring			
		Dark Banded	Light Banded	Very Light Banded	Unbanded
Light Banded × Unbanded	N16	5	4	0	2
	N17	25	9	7	5
	N18	8	2	2	0
	N19	8	4	4	2
	N20	2	1	1	0
	N21	3	1	4	0
					18

* Origin of parents of the F₁ and F₂ crosses indicated where necessary with a bracketed cross code number, e.g. (35). Such a number is always placed immediately before the cross code number of the cross concerned.

† Crosses MV, MRU and M35L are outcrosses. Their light banded and unbanded parents came from field samples.

the results which run to three generations are in good general agreement with hypothesis.

A discrepancy occurs with crosses MD, M41 and MV. These give a segregation of all three phenotypes as expected and also agree with the expected 3:1 ratio of banded:unbanded (chi-square test 4). But it is also expected that they should give a 2:1:1 ratio of dark banded:light banded:unbanded, and there is a significant departure from this ratio (test 5). This is taken simply to mean that some other factor(s) affects the darkness of banding as well as the gene *D*, *d*, while the hypothesis advanced remains basically valid.

Two other features of the results should be mentioned. One P₁ cross, number 19, gave a disturbed ratio, possibly caused by linked recessive lethals (test number 2). With the F₂ crosses N16-N21 considerable difficulty was experienced in classifying the offspring into phenotypes. This difficulty may be caused by deterioration of "developmental canalisation" following upon inbreeding (Waddington, 1957), since the progeny are the F₂ of an outcross. For the purposes of the chi-square test 6, those snails that were intermediate between dark and light banded were divided equally between these two phenotypes. There was also a fourth phenotype scored in these crosses—"very light banded"—in which *very* faint brown marks occupy the position where bands are normally found on the lower part of the biggest whorl, the rest of the shell being without marks or bands. Such snails were classified as light banded for the chi-square test. This seems justified since one P₁

TABLE 4

L. flammulata: statistical tests based on the hypothesis that banding is controlled by two closely linked genes—U, u (banded, unbanded) and D, d (light banded, dark banded). Data from table 3

Test 1. P_1 crosses I, 2V, 2W, 2Y, 2Z. Light banded $\times$ light banded

$Ud/ud \times Ud/ud \rightarrow 1:2:1$ ratio dark banded:light banded:unbanded.

Cross	Results			χ^2		χ^2	d.f.	P
I	16	34	21	0.831				
2V	17	36	14	0.643	Deviation	0.493	2	0.8-0.7
2W	10	37	12	3.950	Heterogeneity	6.319	8	0.7-0.5
2Y	27	43	21	1.066	Total	6.812	10	
2Z	13	30	16	0.322				
				6.812				
	83	180	84	0.493				

Test 2. P_1 crosses 19, T, V, B2, B7. Dark banded $\times$ light banded

Various possibilities $\rightarrow 1:1$ ratio dark banded:light banded.

Cross	Results		χ^2		χ^2	d.f.	P
19	43	24	5.388				
T	17	10	1.814	Deviation	0.716	1	0.5-0.3
V	10	11	0.048	Heterogeneity	10.334	4	0.05-0.02
B2	13	24	3.270	Total	11.050	5	
B7	7	10	0.530				
			11.050				
	90	79	0.716				

Test 3. F_1 crosses MB, MC; F_2 cross N25. Dark banded $\times$ dark banded

$Ud/ud \times Ud/ud \rightarrow 3:1$ ratio dark banded:unbanded.

Cross	Results		χ^2		χ^2	d.f.	P
MB	49	10	2.040	Deviation	0.777	1	0.5-0.3
MC	46	17	0.132	Heterogeneity	1.639	2	0.5-0.3
N25	52	15	0.244	Total	2.416	3	
			2.416				
	147	42	0.777				

Test 4. F_1 crosses MD, MV, M41. Dark banded $\times$ light banded

$Ud/ud \times Ud/ud \rightarrow 3:1$ ratio banded:unbanded.

Cross	Results		χ^2		χ^2	d.f.	P
MD	12	6	0.667	Deviation	1.519	1	0.3-0.2
MV	74	19	1.036	Heterogeneity	1.517	2	0.5-0.3
M41	80	20	1.333	Total	3.036	3	
			3.036				
	166	45	1.519				

(continued overleaf)

TABLE 4 (continued)

Test 5. F_1 crosses MD, MV, M41. Dark banded $\times$ light banded $Ud/ud \times Ud/ud \rightarrow 2:1:1$ ratio dark banded:light banded:unbanded.

Cross	Results			χ^2		χ^2	d.f.	P
MD	6	6	6	2.000	Deviation	17.413	2	<0.001
MV	35	37	19	11.967	Heterogeneity	2.322	4	0.7-0.5
M41	44	35	20	5.768	Total	19.735	6	
				19.735				
				85 78 45 17.413				

This test ignores three banded snails that were classified as intermediate between dark and light banded.

Test 6. F_2 crosses N16, N17, N18, N19, N21, N50. Light banded $\times$ unbanded $Ud/ud \times ud/ud \rightarrow 1:1:2$ ratio dark banded:light banded:unbanded.

Cross	Results			χ^2		χ^2	d.f.	P
N16	5	4	10	0.157				
N17	29.5	18.5	33	5.765	Deviation	2.259	2	0.5-0.3
N18	9	3	13	2.920	Heterogeneity	10.916	10	0.5-0.3
N19	8	8	16	0.000	Total	13.175	12	
N21	3.5	4.5	18	3.923				
N50	9.5	10.5	24	0.410				
				13.175				
				64.5 48.5 114 2.259				

cross (OTP) was between two snails with similar faint marks and this gave some dark and some light banded offspring, suggesting that the marks really are bands. However, this phenotype may be caused by the segregation of a different recessive gene and the marks may simply be structural features marking the position of absent bands. Such a phenotype does occur in *Cepaea* as the recessive phenotype hyalozonate (Lang, 1912; Cain, King and Sheppard, 1960).

It was only possible to classify some of the crosses with respect to background colouration. The results are summarised in table 5, and table 6 gives the statistical tests. It appears that brown is dominant to grey. Thus all grey $\times$ grey crosses gave only grey offspring (crosses 2Y, 10, 34, N18 and N21). The brown $\times$ brown cross Q gave only brown offspring, but other brown $\times$ brown crosses gave a 3:1 ratio of brown:grey (crosses 2V, 20 and probably 16). The brown $\times$ grey cross SPP8 gave all brown offspring, but several other crosses of this type gave a 1:1 ratio of brown:grey (crosses 2Z, 3C, 3H, N17 and N19). It is likely that the background colouration gene is linked with the banding gene, for it is difficult to account for the results of crosses 2V and 2Z without this.

Most or all of the very light banded and doubtfully banded snails of crosses N17 and N19 had a brownish background colouration, and it is difficult to account for this if these band-like marks really are bands. For letting G^b and g stand for the alleles of the background colouration gene, these crosses should be $Udg/udG^b \times uDg/udg$ (without implying gene order

on the chromosome). So banded brown offspring would be cross-overs and the reciprocal cross-over class unbanded grey should appear. Now unless the two unbanded snails of cross 19 that could not be classified for background colour are in fact grey, this reciprocal class did not occur.

Finally, it should be pointed out that *dark* banded *brown* snails did not occur in the breeding programme and the writer has never seen an unequivocal example of such a phenotype in natural populations. Perhaps the combination $U-ddG^b$ — is lethal.

Comparison between the species

On the hypothesis that there are three linked loci, u , d and g involved in the polymorphisms, each with two alleles, there are eight theoretically possible chromosomal arrangements of which five may be identified in the crosses already discussed (table 7). For example, the *L. flammulata* cross 3C must be $Udg/Udg \times uDG^b/uDg \rightarrow Udg/uDG^b + Udg/uDg$, so that the chromosomes Udg , uDG^b and uDg are identified.

TABLE 5

L. flammulata: breeding results for the background polymorphism. Offspring of each cross classified in two rows: upper, brown snails; lower, grey. Snails which appear to be intermediate between two phenotypes scored with a number printed small between the phenotypes concerned

		P ₁ CROSSES	Offspring		
Parents		Cross Code No.	Dark Banded	Light Banded	Un-banded
×	Light Banded Brown Light Banded Brown	2V	$\begin{bmatrix} - \\ 17 \end{bmatrix}$	$\begin{bmatrix} 36 \\ - \end{bmatrix}$	$\begin{bmatrix} 14 \\ - \end{bmatrix}$
×	Light Banded Grey Light Banded Grey	2Y	$\begin{bmatrix} - \\ 17 \end{bmatrix}$	$\begin{bmatrix} - \\ 29 \end{bmatrix}$	$\begin{bmatrix} - \\ 16 \end{bmatrix}$
×	Light Banded Brown Light Banded Grey	2Z	$\begin{bmatrix} - \\ 13 \end{bmatrix}$	$\begin{bmatrix} 14 \\ 1 \\ 15 \end{bmatrix}$	$\begin{bmatrix} 16 \\ - \end{bmatrix}$
×	Unbanded Grey Unbanded Grey	10	$\begin{bmatrix} - \\ - \end{bmatrix}$	$\begin{bmatrix} - \\ - \end{bmatrix}$	$\begin{bmatrix} - \\ 29 \end{bmatrix}$
×	Unbanded Grey Unbanded Grey	34	$\begin{bmatrix} - \\ - \end{bmatrix}$	$\begin{bmatrix} - \\ - \end{bmatrix}$	$\begin{bmatrix} - \\ 13 \end{bmatrix}$
×	Unbanded Brown Unbanded Brown	Q	$\begin{bmatrix} - \\ - \end{bmatrix}$	$\begin{bmatrix} - \\ - \end{bmatrix}$	$\begin{bmatrix} 33 \\ - \end{bmatrix}$
×	Unbanded Brown Unbanded Grey	SPP8	$\begin{bmatrix} - \\ - \end{bmatrix}$	$\begin{bmatrix} - \\ - \end{bmatrix}$	$\begin{bmatrix} 46 \\ - \end{bmatrix}$
×	Dark Banded Grey Unbanded Brown	3C	$\begin{bmatrix} - \\ - \end{bmatrix}$	$\begin{bmatrix} 33 \\ 1 \\ 29 \end{bmatrix}$	$\begin{bmatrix} - \\ - \end{bmatrix}$
×	Light Banded Grey Unbanded Brown	3H	$\begin{bmatrix} - \\ 1 \end{bmatrix}$	$\begin{bmatrix} 26 \\ 11 \end{bmatrix}$	$\begin{bmatrix} 13 \\ 20 \end{bmatrix}$

(continued overleaf)

TABLE 5 (continued)

		F ₂ CROSSES		Offspring			
Parents		Cross Code No.	Dark Banded	Light Banded	Very Light Banded	Un- banded	
×	Light Banded Brown Unbanded Brown	N20	$\begin{bmatrix} - \\ 2 \end{bmatrix}$	1 $\begin{bmatrix} 1 \\ - \end{bmatrix}$	$\begin{bmatrix} - \\ - \end{bmatrix}$	$\begin{bmatrix} 5 \\ - \end{bmatrix}$	
×	Light Banded, Doubtful Brown Unbanded Brown	N16	$\begin{bmatrix} - \\ 5 \end{bmatrix}$	$\begin{bmatrix} 4 \\ - \end{bmatrix}$	$\begin{bmatrix} - \\ - \end{bmatrix}$	2 $\begin{bmatrix} 10 \\ - \end{bmatrix}$	
×	Light Banded Brown Unbanded Grey	N17	$\begin{bmatrix} - \\ 25 \end{bmatrix}$	1 $\begin{bmatrix} - \\ 7 \end{bmatrix}$ 8	$\begin{bmatrix} 7 \\ - \end{bmatrix}$	5 $\begin{bmatrix} 33 \\ - \end{bmatrix}$	
×	Light Banded Brown Unbanded Grey	N19	$\begin{bmatrix} - \\ 8 \end{bmatrix}$	$\begin{bmatrix} - \\ 4 \end{bmatrix}$	$\begin{bmatrix} 1 \\ 3 \\ - \end{bmatrix}$	1 $\begin{bmatrix} 14 \\ 2 \\ - \end{bmatrix}$ 1	
×	Light Banded Grey Unbanded Grey	N18	$\begin{bmatrix} - \\ 8 \end{bmatrix}$	2 $\begin{bmatrix} - \\ 2 \end{bmatrix}$	$\begin{bmatrix} - \\ - \end{bmatrix}$	$\begin{bmatrix} - \\ 13 \end{bmatrix}$	
×	Light Banded Grey Unbanded Grey	N21	$\begin{bmatrix} - \\ 3 \end{bmatrix}$	1 $\begin{bmatrix} - \\ 4 \end{bmatrix}$	$\begin{bmatrix} - \\ - \end{bmatrix}$	$\begin{bmatrix} - \\ 18 \end{bmatrix}$	

* All the parents of the F₂ crosses were offspring of the F₁ cross MRU (see table 3).

Of these five arrangements, all are found in *L. flammulata* but only three in *L. aurora* (table 7). This difference is related to the presence of the phenotype light banded grey in *L. flammulata* and its apparent absence in *L. aurora*. For light banded grey will only occur in the presence of both *U* and *D* and the absence of *G*, and in *L. aurora*, *D* has only been found in the chromosome *uDG*, i.e. coupled with *G*. So all light banded snails in *L. aurora* will have the pink background colour. But the two chromosomes *UDg* and *uDg*, which are found in *L. flammulata* and not in *L. aurora*, have *D* coupled to *g*, allowing the production of the light banded grey phenotype.

Table 7 shows that the only chromosomes not yet definitely identified on the above hypothesis are *UDG*, *UdG* and *uDG*, although their reciprocal arrangements occur. Hence there is no clear evidence from the breeding results for the existence of the *U-G* coupling arrangements.

5. POPULATION GENETICS

L. aurora

The population data is summarised in table 8, where for each population all the samples have been pooled together. It can be seen that the least common phenotype is usually light banded pink. Considering background colouration only, the commonest phenotype is usually grey, which varies in frequency from 0.4 (Orile Odo) to 1.0 (Oluwo). Taking the banding polymorphism by itself, unbanded is usually the commonest phenotype, varying in frequency from 0.28 (Epe) to 0.94 (Iperu). The recessive phenotypes, then, tend to be the commonest, but they are not always so. It should be

pointed out that the samples from two populations, Odo Ona and Abu Uku 1, contained a high proportion of *L. flammulata* (see table 8). Although most snails can be readily classified as either *aurora* or *flammulata*, some prove difficult to classify, so the results from these two populations should be treated with caution.

Tests were performed to see if the polymorphisms are in Hardy-Weinberg equilibrium. For this purpose the gene *D*, *d* was ignored, since *D* is probably always coupled with *G^b* in *L. aurora* and since the breeding results for this

TABLE 6

L. flammulata: statistical tests of the hypothesis that background colouration is controlled by one gene with brown dominant to grey. Data from table 5

3:1 ratio							
Cross	Results		χ^2	Source	χ^2	d.f.	P
	Brown	Grey					
2V	50	17	0.005	Deviation	0.000	1	1.0-0.99
N16	16	5	0.016	Heterogeneity	0.021	1	0.9-0.8
				Total	0.021	2	
			0.021				
	66	22	0.000				
1:1 ratio							
Cross	Results		χ^2	Source	χ^2	d.f.	P
	Brown	Grey					
2Z	30	28	0.068				
3C	33	29	0.258	Deviation	0.474	1	0.5-0.3
3H	39	32	0.690	Heterogeneity	1.408	4	0.9-0.8
N17	45	40	0.294	Total	1.882	5	
N19	16	12	0.572				
			1.882				
	158	146	0.474				

TABLE 7

Chromosomes found among breeding programme snails assuming the three gene hypothesis is true

Possible chromosomes*	<i>L. aurora</i>	<i>L. flammulata</i>
UDG	May occur†	May occur
UDg	Does not occur	Found
UdG	Does not occur	Does not occur
uDG	Found	Found
Udg	Found	Found
udG	May occur	May occur
uDg	May occur	Found
udg	Found	Found

* No order of the genes along the chromosome is implied.

† "May occur" means that the breeding results do not provide critical evidence for presence or absence.

TABLE 8

Summary of samples from natural populations

<i>L. aurora</i>										
Locality	Numbers*						$f\ddagger$ Un- banded	$f\ddagger$ Grey	None§ <i>aurora</i> snails	
	Banded Pink	Banded Grey	Un- banded Pink	Un- banded Grey	Total					
Aba Ajao	6	—	14	30	—	11	61	0.67	0.41	8
Odo Ona	10	1	21	33	3	17	85	0.62	0.47	47
Orile Odo	72	—	84	140	5	53	354	0.56	0.39	—
Abanla 1	5	—	24	17	—	17	63	0.54	0.65	—
Abanla 2	7	—	16	15	—	12	50	0.54	0.56	—
Abu Uku 1	6	—	17	15	—	9	47	0.51	0.55	70
Abu Uku 2	4	—	7	25	2	28	66	0.83	0.55	8
Odetola	107	20	403	310	24	454	1318	0.60	0.67	26
Oluwo	—	—	47	—	—	35	82	0.43	1.00	—
Ipara	27	4	57	29	3	25	145	0.39	0.59	—
Iperu	2	—	4	42	—	52	100	0.94	0.56	—
Shagamu-Ijebu Rd.	8	—	10	36	2	25	81	0.78	0.44	—
Itele	4	—	84	5	4	86	183	0.52	0.95	5
Epe	2	—	156	—	—	60	218	0.28	0.99	9

L. flammulata

Locality	Numbers*					Total	$f\parallel$ Unbanded
	Banded		Banded		Unbanded		
	Dark		Light				
Balogun	176	—	183	2	58	419	0.14
Majeroku	578	—	111	1	8	698	0.01
Iroku	45	3	92	—	36	176	0.20
Ibadan 1	15	—	22	1	7	45	0.16
Ibadan 2	76	1	156	—	65	298	0.22
Ibadan 3	9	2	45	—	29	85	0.34
Ibadan 4	26	—	28	—	16	70	0.23
Ibadan 5	19	—	29	—	6	54	0.11
Odolagaye	70	—	17	—	3	90	0.03
Oshunmaiyegan 1	130	9	46	—	5	190	0.03
Oshunmaiyegan 2	362	5	58	—	5	430	0.01
Omo	199	3	3	—	—	205	0.00
Achilla	342	25	38	—	2	407	0.00
Onishere	1495	8	336	3	41	1883	0.02
Ashipa	134	2	31	2	4	173	0.02

* In the "Numbers" sections, unlabelled columns are for snails that could not be definitely assigned to one particular phenotype and are therefore placed between the two phenotypes concerned. The totals include such snails.

† The frequency (f) of unbanded makes use of the totals as given.

‡ The frequency of grey makes use of the totals minus snails that could not be definitely classed as either pink or grey.

§ The column headed "None *aurora* snails" is the number of specimens of *flammulata* in the corresponding *aurora* sample; such snails are not included in the "Total" column.

|| Snails that could not definitely be classed as either banded or unbanded were omitted from the total before the frequency (f) of unbanded was calculated.

species can be explained without it. Gene frequencies were estimated (Li, 1955) and then used to calculate expected phenotype frequencies. The chi-square test was then employed to test if the observed values are consistent with the Hardy-Weinberg expectations. There was a significant departure from the expected proportions in the following populations: Aba Ajao, Odo Ona, Orile Odo, Abanla 1, Abu Uku 1 and Odetola. In all these populations there were fewer banded pinks and unbanded greys than expected. In five other populations there were non-significant deviations in the same directions. Details are given in table 9 for Odetola, the population from which most data has been obtained.

TABLE 9

L. aurora: genetics of the Odetola population. The gene D, d is ignored

Gene frequencies

U , 0.2256 u , 0.7744 G^p , 0.1798 g , 0.8202

Hardy-Weinberg equilibrium chi-square test

	Banded Pink	Banded Grey	Unbanded Pink	Unbanded Grey
Observed	107	403	310	454
Expected	166.89	343.09	250.09	513.93

Chi-square = 53.29. d.f. = 4-2-1 = 1. $P = < 0.001$.

Frequencies of chromosomes

	UG^p	Ug	uG^p	ug
Expected on Hardy-Weinberg	0.0405	0.1850	0.1393	0.6351
Estimated by chromosome counting	0.0024	0.2232	0.1774	0.5970

Frequencies of genotypes

	Genotype				
	UG/UG	UG/Ug	UG/uG	UG/ug	Ug/uG
Hardy-Weinberg	0.0016	0.0150	0.0113	0.0515	0.0515
Estimated by counting	0.0000	0.0011	0.0009	0.0029	0.0792
	Ug/Ug	Ug/ug	uG/uG	uG/ug	ug/ug
Hardy-Weinberg	0.0342	0.2350	0.0194	0.1769	0.4034
Estimated by counting	0.0498	0.2665	0.0315	0.2118	0.3564

L. flammulata

The population data is summarised in table 8; only the banding polymorphism is recorded, since it was normally difficult to classify for background colouration. The least common phenotype is unbanded, its frequency only exceeding 0.3 in one population (Ibadan 3, $f = 0.34$). This is in marked contrast to *L. aurora*, where unbanded is frequently the commonest phenotype, with a frequency that only falls below 0.4 in two populations. Since the populations of both species are located in various parts of the same large area of Western Nigeria, this difference in the frequency of unbanded may well be a real difference between the species. And since the frequency of the gene u is the square root of the unbanded frequency, it looks as if the two species may differ in the frequencies of the U and u alleles.

Although it is normally difficult to classify *L. flammulata* for background colour, it was possible to do this with one population, Balogun. When the data for this population was analysed, the results were essentially the same as those obtained with *L. aurora*: *i.e.* there are similar departures from the Hardy-Weinberg equilibrium frequencies (see table 10).

TABLE 10

L. flammulata: genetics of the Balogun population. The gene D, d is ignored

Gene frequencies				
	U, 0.6295	u, 0.3705	G ^b , 0.1274	g, 0.8726
Hardy-Weinberg equilibrium chi-square test				
	Banded Brown	Banded Grey	Unbanded Brown	Unbanded Grey
Observed	66	292	33	24
Expected	85.41	272.61	13.61	43.37

Chi-square = 42.06. d.f. = 4 - 2 - 1 = 1. P = <0.001.

Frequencies of chromosomes				
	UG ^b	Ug	uG ^b	ug
Expected on Hardy-Weinberg	0.0802	0.5493	0.0472	0.3233
Estimated by chromosome counting	0.0000	0.6302	0.1276	0.2422

The action of selection

It seems highly probable that in both species the departures from Hardy-Weinberg equilibrium are caused by natural selection, and evidence will now be presented that they are associated with a reduction in the frequencies of the coupling arrangements *UG^b* and *ug* from the Hardy-Weinberg equilibrium frequencies.

(i) In *L. aurora*, the frequencies of the four chromosomal arrangements *UG^b*, *Ug*, *uG^b* and *ug* have been estimated for each population using the iterative process of "gene counting" which is applicable (as here) to chromosome counting (Ceppellini, Siniscalco and Smith, 1955; Smith 1956). The calculation is a very long one, so the Ibadan University IBM computer was programmed to carry it out. The calculations were performed to six decimal places of accuracy to avoid "round off" errors. The results are shown in table 11, and estimates of genotype frequencies for the Odetola population are shown in table 9; table 11 also gives for comparison the frequencies of the coupling arrangements as expected on the basis of Hardy-Weinberg equilibrium.

It can be seen that the frequency of the *UG^b* chromosome is very low or zero in all populations. Also the frequencies of both the coupling arrangements are usually lower than the Hardy-Weinberg equilibrium values. Table 9 shows that within the light banded pink phenotype, all genotypes containing the *UG^b* chromosome have very much lower values than expected, while the repulsion double heterozygote *Ug/uG^b* is slightly more frequent than expected, and is much commoner than the coupling double heterozygote *UG^b/ug*. Table 10 shows that the *UG* chromosome is also rare in the Balogun population of *L. flammulata*.

(ii) All ten light banded pink parents in the *L. aurora* breeding programme are double heterozygotes, since both unbanded and grey snails segregate out in their progenies. Further, eight of these parents must be in the repulsion phase, the phase of the remaining two being unknown (one parent of cross 56 and the light banded pink parent of cross 60). In the absence of selection one would expect a 1:1 ratio of coupling and repulsion phases, so the probability of getting eight out of ten may be calculated using the binomial expansion. Since there are already grounds (section (i)) for suspecting that the departure from the 1:1 ratio will be in favour of the repulsion phase, the appropriate test is the "one-tail" one. The probability is 0.0547—i.e. ≈ 0.05 , which is on the verge of formal significance. Since

TABLE 11

L. aurora: estimated frequencies of chromosomes in natural populations compared with Hardy-Weinberg equilibrium frequencies. The gene D, d is ignored

Population	Chromosome frequencies					
	Estimated by counting				Hardy-Weinberg equilibrium (coupling chromosomes only)	
	<i>UG^p</i>	<i>Ug</i>	<i>uG^p</i>	<i>ug</i>	<i>UG^p</i>	<i>ug</i>
Aba Ajao	0.0000	0.1833	0.3661	0.4505	0.0649	0.5248
Odo Ona	0.0000	0.2157	0.3170	0.4673	0.0676	0.5382
Orile Odo	0.0195	0.2368	0.3539	0.3897	0.0958	0.4659
Abanla 1	0.0000	0.2683	0.1954	0.5363	0.0513	0.5926
Abanla 2	0.0067	0.2584	0.2449	0.4899	0.0668	0.5499
Abu Uku 1	0.0000	0.2884	0.2589	0.4528	0.0732	0.5316
Abu Uku 2	0.0119	0.0781	0.2486	0.6614	0.0234	0.6731
Odetola	0.0024	0.2232	0.1774	0.5970	0.0405	0.6351
Oluwo	0.0000	0.3467	0.0000	0.6533	0.0000	0.6533
Ipara	0.0292	0.3452	0.1999	0.4256	0.0859	0.4822
Iperu	0.0032	0.0272	0.2484	0.7211	0.0077	0.7256
Shagamu-Ijebu Rd.	0.0182	0.1031	0.3162	0.5625	0.0405	0.5850
Itele	0.0056	0.2814	0.0199	0.6931	0.0074	0.6949
Epe	0.0046	0.4701	0.0000	0.5246	0.0022	0.5222

selection seems to be acting in a similar way in both *L. aurora* and *L. flammulata*, it seems legitimate to combine the data of the two species when considering the proportions of coupling and repulsion phases. In *L. flammulata*, all seven of the light banded brown parents must be doubly heterozygous and at least six of these must be in the repulsion phase. Summing over species gives a minimum of fourteen repulsions out of a total of seventeen double heterozygotes. This gives a probability of <0.01 .

(iii) In *L. aurora*, the rarity of the *UG^p* chromosome may also be shown by ignoring it and treating the other chromosomes as a system of multiple alleles. Representing the chromosomes *Ug*, *uG^p* and *ug* as alleles *p*, *q* and *r*, respectively, the composition of the phenotypes becomes: banded pink, $2pq$; banded grey, $p^2 + 2pr$; unbanded pink, $q^2 + 2qr$; unbanded grey, r^2 . The frequencies of *p*, *q* and *r* may be estimated and used to calculate phenotype frequencies expected on the basis of Hardy-Weinberg equilibrium. When this is done, there is normally a very good agreement between observed and expected frequencies. Thus with the Odetola population, when the gene frequencies are calculated by the method used by Taylor and Prior (1938),

the chi-square is 0.217, which with one degree of freedom gives a probability of 0.7-0.5; estimating gene frequencies by Fisher's method (described in Race and Sanger, 1962) leads to a chi-square of 0.234, again with a probability of 0.7-0.5.

6. DISCUSSION

There are a number of similarities between the shell colour polymorphisms of *Limicolaria* and *Cepaea*. In *Cepaea* the genes for banding, background colouration, and almost certainly for "spread bands" are tightly linked to form a super-gene (Fisher and Diver, 1934; Cain and Sheppard, 1957; Cain, King and Sheppard, 1960). In *Limicolaria* the genes for banding and background colouration are probably tightly linked and there may be a third linked gene which is concerned with the darkness of bands. The evidence therefore suggests that the polymorphisms in *Limicolaria* are under the control of a super-gene, as they are in *Cepaea*. It has, of course, long been recognised that the genes controlling a polymorphism are often linked (Haldane, 1930; Sheppard, 1953). There are other similarities between the genera. In the background polymorphism, pink and brown are dominant, in *Cepaea* over yellow (Lamotte, 1951; Cain, King and Sheppard, 1960), in *Limicolaria* over grey. Further, in *Cepaea* there are probably differences between species in the chromosomal arrangements that occur (Professor P. M. Sheppard, personal communication), and if the three gene hypothesis advanced in the present paper is true, similar differences occur in *Limicolaria*.

In the banding polymorphism, banded is dominant to unbanded in *Limicolaria* but the reverse is true of *Cepaea*. However, the bands of *Limicolaria* are probably not homologous with those of *Cepaea* because they have a different orientation on the shell, presupposing a different pattern of activity and distribution of the pigment-producing cells. Thus the bands in *Limicolaria* run across the whorls, those in *Cepaea* along the whorls. There is, however, a rare phenotype in *Cepaea*, the variety *undulata*, with bands running across the whorls (Lang, 1912; Lamotte, 1951). Lamotte thinks this is a variant of the phenotype "pale bands", and there is evidence that the gene for pale bands is closely linked to the background colouration and banding genes (Dr L. M. Cook, personal communication). The bands of *Limicolaria* may then be homologous to those of the variety *undulata*.

In the polymorphisms of *Limicolaria* selection seems to be acting against coupling phases, particularly of the dominant genes. In *L. aurora* it has been estimated by chromosome counting that the frequency of the *UG^b* chromosome is less than 0.02 in all populations except Ipara, where its frequency is about 0.03. Indeed, the frequency of this chromosome is so low that when it is ignored and the other chromosomes treated as a system of multiple alleles, a very good agreement between observed and expected phenotype frequencies is obtained. The situation seems to be similar to that in the colour pattern polymorphism of the Grouse Locust *Paratettix texanus*, where the coupling arrangements of the various dominant genes are so rare as to be virtually absent (Fisher, 1939).

The magnitude of the selection pressures against coupling phases in *Limicolaria* is uncertain, since it depends upon the tightness of linkage. Now, the recombination fraction for *U*, *u* and *G^b*, *g*, was estimated as 3 per cent., but it was pointed out that the linkage would be much tighter than this if the snails classed as recombinants were in fact the products of self-fertilisation.

In the absence of suitable genetical or cytological markers, it is not possible to decide whether self-fertilisation has taken place or not. But even with 3 per cent. recombination the selection pressures are probably small.

Where selection acts against the coupling phases of two genes and in favour of the repulsion phases, it is to be expected that both alleles of both genes will be maintained in equilibrium. It is therefore suggested that the polymorphisms of *Limicolaria* are balanced rather than transient in most populations. This conclusion is strengthened by the fact that the banding polymorphism in another species of *Limicolaria* (*L. martensiana*) is of ancient origin, since it is found in Pleistocene populations (Owen, 1963).

7. SUMMARY

1. *Limicolaria aurora* and *L. flammulata* are polymorphic for the shell colour characters banding and background colouration. Banded is dominant to unbanded (the gene *U, u*); pink and brown backgrounds are dominant to grey (the gene *G, g*). There may be a third gene which controls the darkness of bands. These genes are tightly linked.

2. In several *L. aurora* populations there are fewer banded pink and unbanded grey snails than expected on the basis of Hardy-Weinberg equilibrium.

3. Estimates of the frequencies of the different chromosomal arrangements of the genes *U, u* and *G, g* show that the coupling arrangement *UG* is rare in all populations and the frequencies of both coupling arrangements are lower than the Hardy-Weinberg equilibrium values.

4. The *UG* chromosome only occurs in the banded pink phenotype and the unbanded grey phenotype is *ug/ug*. It is therefore suggested that the departures of phenotype frequencies from the Hardy-Weinberg equilibrium values may be caused by selection acting against the coupling arrangements of the genes.

5. Selection is probably acting in a similar manner in *L. flammulata*.

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