

## SUPERGENES IN POLYMORPHIC LAND SNAILS II. *PARTULA SUTURALIS*

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### SUMMARY

The colour and banding of the shell of *Partula suturalis* are controlled by a single locus ( $M$ ) with a series of at least six alleles.  $M^X$ , giving apex as a homozygote, is dominant to  $M^{F1}$ , giving *frenata*, which is dominant to the other alleles.  $M^{F2}$  is similar to  $M^{F1}$  except in its relation with  $M^A$ .  $M^{F2}M^A$  produces *bisecta* and provides a striking example of a heterozygote that is qualitatively different from both homozygotes for the alleles producing it.  $M^A$  gives *atra* as a homozygote and is dominant to  $M^C$  and  $M^S$ .  $M^C$ , giving *cestata* as a homozygote, is recessive to all except  $M^S$ .  $M^S$ , giving *strigata*, is the universal recessive. It is suggested that the locus may be complex.

The direction of coiling of the shell is determined by the  $H$  locus with  $H^S$  (sinistrality) dominant to  $H^D$  (dextrality). The expression of coiling is delayed by one generation, the maternal genotype determining the phenotype of the offspring.  $M$  and  $H$  are not linked.

Self-fertilisation occurs infrequently and non-randomly.

### 1. INTRODUCTION

THE island of Moorea in French Polynesia is inhabited by a number of species of polymorphic land snails of the genus *Partula*. *P. taeniata* Mörch and *P. suturalis* Pfeiffer have the widest distributions, living in most of the forested parts of the island. The other species are found in more limited areas, usually sympatric with both *taeniata* and *suturalis*. In our work on the population genetics and speciation of *Partula* (Clarke, 1968; Murray and Clarke, 1968a; Clarke and Murray, 1969, 1971) we have bred both *taeniata* and *suturalis* in the laboratory in order to establish the mode of transmission of the principal colour and banding morphs. Some of our results have already been reported (Murray and Clarke, 1966, 1968b). The first paper in the current series (Murray and Clarke, 1976) describes the matings of *P. taeniata*, in which the colour and banding morphs are determined by at least six loci, in some cases with multiple alleles. All the loci for which linkage data are available are strongly linked to form a single functional supergene.

In this paper we describe the *Partula suturalis* matings and offer an interpretation of the polymorphism also suggesting the presence of supergenes. In addition to the data on colour and banding, the matings provide information on the inheritance of shell-coiling (dextrality and sinistrality).

2. METHODS

The breeding biology of *P. suturalis* and methods of rearing it have been described in Murray and Clarke (1966). The snails were kept in plastic boxes lined at the bottom with moistened toilet-paper and were fed on a diet of oatmeal, lettuce and powdered natural chalk.

The shells were scored according to Crampton's (1932) classification. The different morphs are patterned as follows: Apex is uniformly whitish or straw-coloured with a dot of dark pigment on the tip of the spire. Atra is uniformly dark brown or black. Strigata is also unbanded but shows alternating cross-striations of brownish-yellow and white. Bisecta is dark with a broad central longitudinal light band. Cestata is just the reverse, light with a dark central band. Finally, frenata is light with two narrow longitudinal dark lines. Any form may be either dextral or sinistral, although in nature the occurrence of both types in the same population is restricted to a few transitional areas (Clarke and Murray, 1969). Illustrations of the morphs may be seen in Crampton (1932) or Murray and Clarke (1966).

3. RESULTS

The progeny of the experimental matings are shown in table 1. Some of the matings reported in Murray and Clarke (1966) are included here, but only if they are essential to the genetic interpretation, or if significant numbers of additional offspring have been produced. Our interpretation of the genetics of the various colour and banding morphs is shown in table 2. The inheritance of the direction of shell-coiling is illustrated in fig. 1.

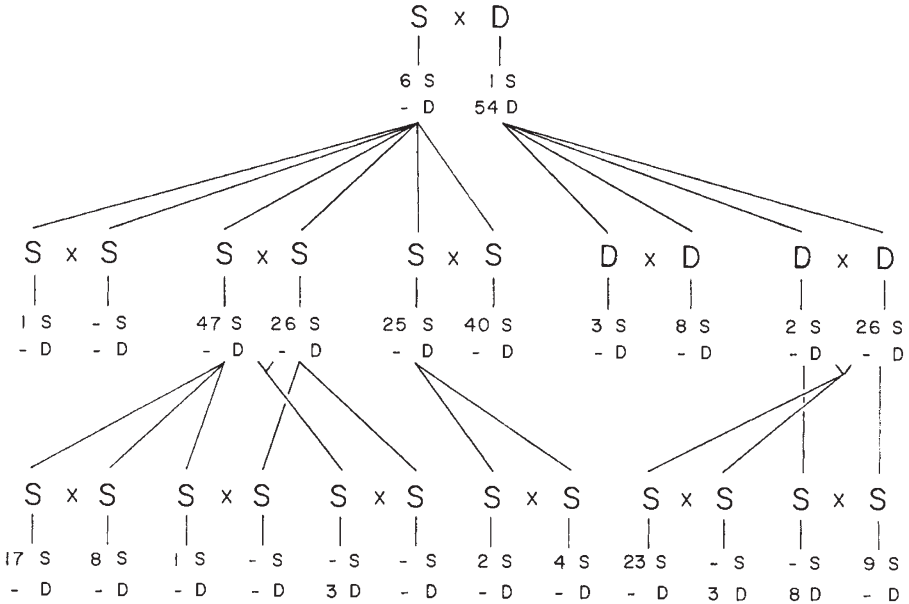


FIG. 1.—The inheritance of shell-coiling in the lineage of mating 28 of *Partula suturalis*. S and D show the phenotypes of sinistral and dextral individuals. Lines indicate the parentage of individuals used for breeding. In three cases, individuals used as parents were born prior to the separation of their parents and therefore can only be assigned to the pair. A fork at the origin of the line of parentage indicates this uncertainty.

All six colour patterns show clear segregation with one another. *Frenata* segregates with *bisecta* and *atra* in matings 154, 157, 174, 175 and 202; with *cestata* in 216 and 253; with *strigata* in 216; and with *apex* in the lineage of mating 27. *Bisecta* segregates with *apex* in 89. *Cestata* segregates with *strigata* in 215, 216 and 253 and with *atra* in the lineage of 28. *Strigata* segregates with *atra* in 29 and 247. *Atra* segregates with *apex* in 27 and 161. There are special problems with the segregation of *apex* and *strigata* (see below).

In the inheritance of the direction of coiling, the offspring of any individual are usually of only one type, although in exceptional cases one or two may differ from their sibs (see matings 25, 28, 83, 84, 86, 160 and 228). The coil of the offspring may be different from that of the parental shell.

#### 4. DISCUSSION

##### (i) *The genetics of shell colour and pattern*

(a) *The model.* In our preliminary account of the genetics of *P. suturalis* (Murray and Clarke, 1966) we interpreted our results in terms of at least four loci with two alleles at each locus. This model was suggested by the behaviour of the *frenata* phenotype. Several apparent backcrosses of *frenata* and *bisecta* (matings 20, 22 and 86) yielded different segregation ratios, and in the lineage of mating 27 *frenata* and *apex* showed rather complex interactions with each other. Further matings were set up to clarify these relationships. Since there was a suggestion that some of the curious results might be due to the hybridisation of animals from different localities, many of the new matings were set up with animals from a single population in Maaraarii Valley where most of the phenotypes are found together.

The new matings give no support to the hypothesis that there are differences from place to place in the genetic control of phenotypes in *P. suturalis*. On the contrary, the data show a satisfying consistency, and they lead us to propose the model set out in table 2. We find that there are never more than four of the six morphs expressed in any one mating. If several interacting loci are proposed, then it is necessary to postulate virtually absolute linkage between them. This is particularly well illustrated in the lineage from mating 27. Whatever the underlying genetic architecture, it therefore appears that the principal patterns of the shell are determined by a single functional locus ( $M$ ) with multiple alleles.

According to our model, the allele  $M^S$  (*strigata*) is the universal recessive. Matings of *strigata* × *strigata* have invariably produced nothing but *strigata* (matings 127, 204, 205, 223, 224 and 226).

The next allele,  $M^C$  (*cestata*), is dominant only to  $M^S$ . Matings that are almost certainly crosses between *cestata* homozygotes do not segregate (149, 151 and 172); those between *cestata/strigata* heterozygotes ( $M^C M^S$ ) yield 3:1 segregations (206 and 215). Matings between *cestata* and *strigata* either do not segregate (222) or do so as backcrosses (253).

The  $M^A$  (*atra*) allele is dominant to both  $M^C$  and  $M^S$ . The lineage from mating 28 establishes the relationship with  $M^C$ , matings 114, 115, 117 and 118 segregating 3:1 *atra* and *cestata*. Matings 29 and 247 show the same for  $M^S$ . The most interesting attribute of the  $M^A$  allele is that as a heterozygote

TABLE 1

The matings of *Partula saturalis*. Names of localities and phenotypes are from Crampton (1932). "Prog. 20" means that the parents of that mating are the progeny of mating 20. Genotypes are indicated by the superscript of the allele only, the M locus first and the H locus second. Young that cannot be scored for pattern are reported as S (sinistral) or D (dextral) under "remarks". The asterisk (\*) indicates that the offspring may have come from either of the two parents

Mating No.	Provenance	Parents		Mating No.	Provenance	Parents		Mating No.	Provenance	Parents		Mating No.	Remarks		
		Phenotype	Inferred genotype			Phenotype	Inferred genotype			Phenotype	Inferred genotype			Phenotype	Inferred genotype
20	Opunohu	Frenata S	F1/F2; S/D	92	Prog. 22	Frenata S	F1/A; S/?	12	Prog. 22	Frenata S	F1/A; S/?	12	& 33S		
		Bisecta S	F2/A; D/D			Frenata S	F1/A; S/?			Atra S				9	& 27S
		Bisecta D				Frenata S				Atra S				3	
21	Moorea	Frenata S	F1/F1; S/S	108	Prog. 27	Frenata S	F2/S; S/D	26	Prog. 27	Frenata S	F2/S; S/D	1	& 4S		
		Bisecta S				Frenata S				Frenata S				6	& 14S
		Bisecta S				Frenata S				Frenata S				6	
22	Moorea	Frenata D	F1/S; S/D	114	Prog. 28	Frenata S	A/C; S/D	3	Prog. 28	Atra S	A/C; S/D	19	& 21S		
		Bisecta D				Atra S				Atra S				2	& 17S
		Bisecta D				Atra S				Atra S				7	
23	Roroie	Frenata D	F2/F2; D/D	115	Prog. 28	Frenata S	A/C; S/D	43	Prog. 28	Atra S	A/C; S/D	13	& 9S		
		Cestata S				Atra S				Atra S				18	& 17S
		Cestata S				Atra S				Atra S				1	
24	Faamaariri	Frenata S	F2/F2; S/?	117	Prog. 28	Frenata S	A/C; S/D	7	Prog. 28	Atra D	A/C; S/D	3	& 2S		
		Frenata D				Atra D				Atra S				6	& 2S
		Frenata D				Atra D				Atra S				1	
25	Faatoai	Frenata S	F1/F1; S/S	118	Prog. 28	Frenata S	A/C; S/D	15	Prog. 28	Atra D	A/C; S/D	1	& 8S		
		Bisecta D				Atra D				Atra S				12	& 8S
		Bisecta D				Atra D				Atra S				18	
26	Faamaariri	Bisecta D	F2/A; D/D	119	Prog. 25	Frenata D	F1/F2; S/D	15	Prog. 25	Frenata D	F1/A; S/D	13	& 18S		
		Atra S	A/A; S/?			Frenata D				Bisecta S				7	& 32S
		Atra S				Frenata D				Bisecta S				1	
27	Faamaariri	Apex S	X/S; S/?	120	Prog. 25	Frenata D	F1/F2; S/D	7	Prog. 25	Frenata D	F1/A; S/D	2	& 4S		
		Bisecta D				Frenata D				Frenata D				38	& 8S
		Bisecta D				Frenata D				Frenata D				1	
28	Faatoai	Atra D	A/A; D/D	120	Prog. 25	Frenata D	F1/F2; S/D	38	Prog. 25	Frenata D	F1/F2; S/D	2	& 4S		
		Atra S	C/C; S/S			Frenata D				Frenata D				1	& 8S
		Cestata S				Frenata D				Frenata D				6	

(1) Parent derived by selfing.

29	Opunohu	Atra D	A/S; D/D	Atra D Strigata D	12	& 13D	122	Prog. 22 Prog. 22	Frenata S Atra S	F2/S; D/D A/S; D/D	— Frenata D Bisecta D Atra D Strigata D	7 4 5 1	& 4D & 59D
83	Prog. 20	Frenata S	F2/F2; S/D	Frenata S Bisecta S	11	& 35S	127	Prog. 29 Prog. 29	Strigata D Strigata D	S/S; D/D S/S; D/D	Strigata D Strigata D	13 12	& 8D & 10D
84	Prog. 20	Frenata D	F1/F2; S/D	Frenata S Bisecta S Frenata S Bisecta S	10 6 11 1	& 7D & 1S, 1D	148	Prog. 118	Atra S	A/C; S/?	Atra S Cestata S	4 3	& 16S
85	Prog. 25	Frenata S	F1/F2; S/D	Frenata S Bisecta S Frenata S Bisecta S	10 6 6 1	& 31S & 6S	149	Prog. 118 Prog. 118	Cestata S Cestata S Cestata S	C/C; S/? C/C; S/? C/C; D/D	Cestata S Cestata S Cestata D	3 2	& 6S & 6D
86	Prog. 20	Frenata D	F2/F2; S/D	Frenata S Bisecta S Frenata D Bisecta S	6 0 1 1	& 2S	150	Prog. 114 Prog. 114	Atra S Cestata S	A/A; D/D C/C; ?/?	—	2	& 3D
87	Prog. 24 Prog. 24	Frenata S Frenata S	F1/F2; S/D F2/S; S/D	Frenata S Bisecta S Frenata S Bisecta S	6 0 1 1	& 12S & 16S	151	Prog. 115 Prog. 115	Cestata S Cestata S*	C/C; S/? C/C; S/?	Cestata S Cestata S	2 4	& 2S
88	Prog. 26 Prog. 26	Bisecta D Atra S	F2/F2; S/D F2/A; D/D	Frenata S Bisecta S Frenata D Bisecta S	7 5 1 7	& 1S & 17S & 19D	152	Prog. 92 Prog. 26	Frenata S Atra S	F1/F1; S/? A/A; S/?	Frenata S Frenata S	12 3	& 19S & 3S
89	Prog. 27	Apex S	X/F2; S/D	Frenata S Atra S Atra S	14 7 8	& 1S & 26S	153	Prog. 89 Prog. 89	Bisecta S Apex D Apex*	F2/A; S/D F2/A; S/D	Frenata S Bisecta S Atra S	6 1 8	& 13S & 3S & 1S
90	Prog. 28 Prog. 28	Atra S Atra S	A/C; S/D A/C; S/D	Apex S Bisecta S Apex D Apex S Bisecta S	18 10 11 1 5	& 1S & 18S & 26S	154	Prog. 26	Bisecta S	F2/A; S/D	Frenata S Bisecta S Atra S	5 9 6	& 17S
91	Prog. 29 Prog. 28	Atra D Atra D	A/A; D/D A/A; S/D	Atra S Atra S	31 2	& 17D (1)	155	Prog. 89 Prog. 89	Bisecta D	F2/A; S/D	Bisecta S	1	& 1S
							156	Prog. 89 Prog. 89	Apex S Apex*	X/X; S/? X/?; ?/?	Apex S Apex S	1 2	& 4S
							157	Prog. 89	Bisecta S	F2/A; S/D	Apex S Apex S	8 3	& 9S & 1S
							158	Prog. 89	Bisecta S	F2/A; S/D	Frenata S Bisecta S Atra S	1 3 2	& 11S
								Prog. 89	Bisecta S	F2/A; S/D	Frenata D	1	& 3D
								Prog. 89	Bisecta S	F2/A; S/D	Frenata S Bisecta S Atra S	1 1 1	& 6S
								Prog. 89	Bisecta S	F2/A; S/D	—	1	& 2S

Mating No.	Provenance	Parents		Mating No.	Provenance	Parents		Mating No.	Remarks	Progeny		Remarks
		Phenotype	Inferred genotype			Phenotype	Inferred genotype			Phenotype	No.	
159	Prog. 108 Prog. 108	Frenata S Frenata S	F2/F2; S/? F2/?; ?/?	216	Maaraarii	Cestata S	C/S; S/S	17	& 4S	Frenata S Cestata S Strigata S	1 0 0	& 9S & 1S
160	Prog. 108 Prog. 108	"Apex" S "Apex" S	S/S; S/? S/S; S/?	217	Maaraarii Maaraarii	* Frenata S Frenata S	F2/?; S/S F2/?; S/S	12 5 1 1	& 6S & 11S	Frenata S Frenata S Frenata S	4	& 5S
161	Prog. 27 Prog. 27	* Apex S Atra S	XI/A; S/D A/S; S/D	218	Maaraarii Maaraarii	Frenata S Frenata S	F1/A; S/S F1/A; S/S	3 3 14 13	& 1S & 22S & 8S	Frenata S Atra S Atra S Frenata S	0 1 3 0 1	
162	Prog. 85 Prog. 85	Frenata S Frenata S	F1/F1; S/? F1/?; ?/?	222	Prog. 206 Maaraarii	Cestata S Strigata S	C/C; S/S S/S; S/S	5	& 10S	Cestata S	8	& 1S & 6S & 1S
163	Prog. 85 Prog. 85	Frenata S Bisecta S	F1/F2; S/? F2/A; S/?	223	Maaraarii Maaraarii	Strigata S Strigata S Strigata S	S/S; S/S S/S; S/S	4 2 4 0	& 14S & 9S	Strigata S Strigata S Strigata S	4	& 1S
169	Prog. 83 Prog. 28	Frenata S Atra S	F1/F2; S/D A/C; S/D	224	Maaraarii Maaraarii	Cestata S Cestata S	C/S; S/S C/S; S/S	6 3 7 2	& 8S & 5S	Cestata S Cestata S	5 1	& 14S & 3S
170	Prog. 108 Prog. 108	Frenata S Frenata S	F2/F2; S/? F2/?; S/?	225	Prog. 205 Prog. 205	Strigata S Strigata S	S/S; S/S S/S; S/S	13 6	& 1S & 7S & 7S	Strigata S Strigata S	14	& 7S & 1S & 1S
171	Prog. 108 Prog. 89	"Apex" S Apex S	S/S; S/? X/?; ?/?	226	Prog. 205	Strigata S Strigata S	S/S; S/S S/S; S/S	& 1S		Strigata S	6	& 3S
172	Prog. 114 Prog. 114	Cestata S Cestata S	C/C; S/? C/C; S/?	228	Parent 26 Prog. 154	Atra S Frenata S	A/A; S/? F2/F2; S/?	12 3	& 5S & 5S	Bisecta S Bisecta S Bisecta S	2 18 2	& 13S & 8S
173	Prog. 114 Prog. 114	Atra S Atra S	A/?; S/? A/?; ?/?	235	Prog. 200 Prog. 205	Atra S Strigata S	A/A; S/S S/S; S/S	& 1S		Atra S Atra S	1 1	& 1S & 16S
174	Prog. 88 Prog. 88	Bisecta S Bisecta S	F2/A; S/? F2/A; S/?	236	Prog. 200 Prog. 206	Atra S Cestata S	A/S; S/S C/C; S/S	4 5 4 2 6 1	& 17S & 8S	Cestata S	2	& 7S & 6S & 1S
175	Prog. 88 Prog. 88	Bisecta S Bisecta S	F2/A; S/? F2/A; S/?	244	Parent 89 Parent 170	Apex S Frenata S	X/?; S/D F2/F2; S/?	3 10	& 32S	Apex S Atra S	1 5 3	& 6S & 3S
	Prog. 88	Bisecta S	F2/A; S/?	247	Maaraarii	Atra S	A/S; S/S	3	& 1S	Strigata S	3	
	Prog. 88	Bisecta S	F2/A; S/?		Maaraarii	Atra S	A/S; S/S	1	& 1S	Atra S	1	

77	Prog. 88 Prog. 88	Atra S Atra S*	A/A; S/? A/A; ?/?	Atra S —	5 & 105 & 15	249	Prog. 202 Prog. 202	Frenata S Atra S*	F2/F2; S/S A/A; S/S	Bisecta S Bisecta S Bisecta S	3 1 & 19S & 16S
192	Prog. 154 Prog. 154	Frenata S Frenata S	F2/F2; D/D F2/F2; D/D	Frenata D Frenata D	27 6 & 14D & 10D	252	Prog. 247 Prog. 223	Atra S Strigata S*	A/A; S/S S/S; S/S	Atra S Atra S Atra S	6 3 & 17S & 21S
193	Prog. 92 Prog. 92	Atra S Atra S*	A/A; S/? A/A; S/?	Atra S Atra S	11 1 & 13S & 8S	253	Prog. 224 Prog. 223	Cestata S Strigata S	C/S; S/S S/S; S/S	Cestata S Strigata S Cestata S Strigata S	0 7 & 28S & 5S & 1S
194	Prog. 175	Bisecta S	F2/A; S/?	Frenata S Bisecta S Atra S	1 2 1 & 8S			*			
200	Prog. 175	Bisecta S	F2/A; S/?	—	1 & 6S	256	Prog. 202	Bisecta S	F2/A; S/S	Frenata S Atra S Frenata S Atra S	8 7 & 4S & 10S
201	Maaraarii Maaraarii	Atra S Atra S*	A/A; S/S A/A; S/S	Atra S Atra S	6 1 & 8S & 14S	257	Prog. 202 Prog. 222	Bisecta S Cestata S	F2/A; S/S C/S; S/S	Frenata S Atra S —	2 5 & 15S & 9S
202	Maaraarii	Bisecta S	F2/A; S/S	Frenata S Bisecta S Atra S	1 5 & 8S	258	Prog. 216 Prog. 222	Frenata S Cestata S*	F2/S; S/S C/S; S/S	Strigata S Strigata S	2 1 & 4S (2) & 8S & 1S
204	Maaraarii Maaraarii	Strigata S Strigata S	S/S; S/S S/S; S/S	Strigata S Strigata S	5 3 & 23S & 11S	261	Prog. 192 Prog. 122	Frenata D Atra D	F2/F2; D/D A/S; D/D	Frenata D Bisecta D Frenata D Bisecta D	5 7 4 2 & 15D
205	Maaraarii	Strigata S	S/S; S/S	Strigata S	9 & 10S	263	Prog. 249	Bisecta S	F2/A; S/S	Frenata S Atra S	5 8 & 3S
206	Maaraarii	Strigata S Cestata S	S/S; S/S C/S; S/S	Cestata S Strigata S	16 5 & 6S		Prog. 225	Strigata S	S/S; S/S	Frenata S Atra S	3 1 & 3S
	Maaraarii	Cestata S	C/S; S/S	Cestata S Strigata S Strigata S	6 1 1 & 6S			*		Frenata S	1
211	Prog. 154 Prog. 154	Frenata S Frenata S	F2/F2; S/? F2/F2; ?/?	— —	1 2 & 15	267	Prog. 228 Prog. 215	Bisecta S Cestata S	F2/A; ?/? C/S; S/S	— Frenata S	1 1 & 4S
214	Prog. 175 Prog. 175	Frenata S Frenata S*	F2/F2; S/? F2/F2; ?/?	Frenata S —	3 & 3S & 15	268	Parent 202 Prog. 253	Bisecta S Strigata S	F2/A; S/S S/S; S/S	Frenata S Atra S Frenata S Atra S	1 2 2 1 1 & 5S & 6S
215	Maaraarii	Cestata S	C/S; S/S	Cestata S Strigata S Cestata S Strigata S	4 2 4 0 & 17S	269	Prog. 261	Bisecta D	F2/A; D/D	Frenata D Atra D	2 0 & 7D
216	Maaraarii	Frenata S	F2/S; S/S	Frenata S Cestata S Strigata S	3 1 1 & 5S		Prog. 225	Strigata S	S/S; S/S	Frenata S Atra S Frenata D	1 2 1 & 5S

(2) Some young not strigata.



TABLE 2

The alleles of the M locus of *Partula suturalis*, indicating the phenotypes of the various combinations. The two types enclosed in brackets have not been produced in the matings

$M^X$	Apex					
$M^{F1}$	(Apex)	Frenata				
$M^{F2}$	Apex	Frenata	Frenata			
$M^A$	Apex	Frenata	Bisecta	Atra		
$M^C$	(Apex)	Frenata	Frenata	Atra	Cestata	
$M^S$	Apex	Frenata	Frenata	Atra	Cestata	Strigata
Alleles	$M^X$	$M^{F1}$	$M^{F2}$	$M^A$	$M^C$	$M^S$

with  $M^{F2}$  (frenata) it produces bisecta. This behaviour is shown in a number of matings (*e.g.* 154, 157, 174, 175 and 202) that segregate

$$1 : 2 : 1 \text{ frenata} : \text{bisecta} : \text{atra.}$$

In our matings the  $M^{F2}M^A$  heterozygote appears to be the only way in which the bisecta pattern can be produced, and provides an example of a heterozygote that is strikingly different from either of the homozygotes for the alleles producing it. In its other relationships  $M^{F2}$  is dominant to  $M^C$  and  $M^S$  (see below under *Testing the model*).

That there is another allele ( $M^{F1}$ ) also producing frenata is shown by crosses in which frenata behaves as a dominant to bisecta, but with unusual segregation ratios. Thus in mating 20 an expected backcross of frenata  $\times$  bisecta yields 3:1 frenata:bisecta instead of 1:1. Matings among the offspring also show unusual results. Mating 83, between two frenatas, is closer to a 1:1 than a 3:1 frenata:bisecta segregation; while mating 86, apparently similar to mating 20, nevertheless produces a reasonable 1:1 ratio. These results are to be expected if the original frenata parent of mating 20 is an  $M^{F1}M^{F2}$  heterozygote. Other genotypes are as given in table 1. Support for this interpretation is found in mating 25 in which frenata and bisecta produce all frenata. If the bisecta is  $M^{F2}M^A$ , then the frenata must be  $M^{F1}M^{F1}$ . Bisecta is recovered in matings of the offspring (85, 119 and 163).

These matings show that  $M^{F1}$ , unlike  $M^{F2}$ , is dominant to  $M^A$ . In other respects  $M^{F1}$  appears to be equivalent to  $M^{F2}$ , being dominant to  $M^C$  (169) and  $M^S$  (122), although here the evidence is not so strong.

Finally the  $M^X$  allele, producing apex, appears to be dominant to all the others, although two of the heterozygotes ( $M^X M^{F1}$  and  $M^X M^C$ ) have not been produced in any of these matings. The behaviour of  $M^X$  is shown in the lineage derived from mating 27. In the initial mating it behaves as a dominant to  $M^{F2}$ ,  $M^A$  and  $M^S$ . Bisectas ( $M^{F2}M^A$ ) are recovered from the apex  $\times$  apex cross of 89.

There is a difficulty in the interpretation of some of the  $M^X$  crosses, illustrated in matings 108 and 160. Here frenata  $\times$  frenata yields 3:1 frenata and a phenotype that is not distinguishably different from the apex parent of mating 27. Since it is not easy to see how  $M^X$  could behave as a dominant to  $M^{F2}$  in one generation and a recessive to it in the next, we suggest that the recessive here is  $M^S M^S$ , the strigata phenotype in this case resembling apex very closely. Alternatively, but less economically, we could postulate another allele recessive to frenata and indistinguishable from



apex. In the event, we have scored the phenotype as apex but noted the inferred genotype as  $M^S M^S$ , thereby calling attention to the problem.

(b) *Testing the model.* After the genetic model had been worked out, we next looked for matings that would provide a critical test of its assumptions. There were two such types of matings unrepresented up to that time among our crosses. They give precise and peculiar predictions which, in view of the degree of dominance of *frenata*, seem almost anti-intuitive.

The predictions rest on the unique constitution of *bisecta* ( $M^{F^2} M^A$ ) and on the dominance of both its alleles to  $M^C$  and  $M^S$ . Hence *bisecta* × *cestata* and *bisecta* × *strigata* should each give 1:1 *frenata* and *atra*. A number of matings were set up to make this test, and five of them have produced offspring. Matings 256, 257, 263, 268 and 269 all segregate for *frenata* and *atra*. Although the numbers of young in each case are rather small, the results are entirely in accord with the hypothesis.

(c) *Is the M locus a supergene?* In the light of the evidence that polymorphism in *P. taeniata* is controlled by a supergene (Murray and Clarke, 1976), it is interesting to consider whether a similar mechanism may be present in *P. suturalis*. Although separate elements of the shell polymorphism are not as easily recognisable in *suturalis* as they are in *taeniata*, there is a regularity in the pattern of inheritance suggesting that there may be subunits within the *M* locus. The top dominant and the bottom recessive are the two most similar phenotypes, and there is even some indication that they may occasionally be confused with one another (see above). As one progresses up through the intermediate alleles, the patterns become first darker (*cestata*, *atra*) and then lighter again (*bisecta*, *frenata*). In the case of *cestata* and *bisecta* the addition and subtraction of pigment are reciprocal. We suggest that the patterns are controlled by a series of genetic elements, each of which may exist in three states: (1) no effect, recessive to (2) positive effect or dark pigmentation, recessive to (3) repressive effect or suppression of dark pigmentation. Three linked elements (loci) could then provide the "alleles" observed in the matings. In the absence of observed crossing over it is impossible to test this hypothesis with our data. However, it is possible to predict the properties of some new cross-over "alleles". Perhaps the most interesting is the one that is predicted to produce *bisecta* as a homozygote and *atra* as a heterozygote with  $M^C$ , to be dominant to  $M^{F^2}$  and  $M^S$  and to be recessive to  $M^X$  and  $M^{F^1}$ .

#### (ii) *The genetics of coiling*

Our preliminary data on the inheritance of shell-coiling in *P. suturalis* (Murray and Clarke, 1966) supported the suggestion by Crampton (1916, 1932) that the maternal genotype controls the direction of coil among the offspring. With the segregation by whole broods in the  $F_3$  generation, the demonstration is now complete. Coil is determined by a single locus (*H*). Sinistrality ( $H^S$ ) is dominant to dextrality ( $H^D$ ) and segregation is delayed by one generation.

Fig. 1 shows our best example of this pattern of inheritance in four generations. The parents of the initial cross (mating 28) come from populations that are wholly sinistral in one case and wholly dextral in the other. Each parent produces offspring of its own phenotype, although the  $F_2$  offspring, which are all sinistral, indicate that the  $F_1$  are heterozygotes with

sinistral dominant. The phenotypically uniform  $F_2$ , however, show themselves to have been genetically diverse. Their offspring show segregation by broods, ten individuals producing sinistral offspring and three producing dextrals.

Occasionally, individuals are produced with a coil opposite to that of the majority of the brood. Nine parents in eight matings (25, 28, 83, 84, 86 (2), 89, 160 and 228) have given rise to 11 reversed young out of a total of 286. There does not seem to be any pattern in the occurrence of these exceptional young. We have suggested an ontogenetic origin for them, since they are probably too common to be mutants (Murray and Clarke, 1966).

Thus, coiling in *P. suturalis* follows the general pattern of inheritance established for *Lymnaea peregra* by Diver and his colleagues (Boycott and Diver, 1923, Diver *et al.*, 1925; Boycott *et al.*, 1930; Diver and Andersson-Kottö, 1938) except that sinistrality is dominant to dextrality, and aberrant phenotypes are much less common.

Another question of interest is whether linkage can be detected between the locus for coiling ( $H$ ) and that for shell pattern ( $M$ ). Unfortunately even in those cases where recombination might be observed, each whole brood provides only a single datum. Tests for linkage are therefore necessarily inexact. We can however look for evidence in three lineages. In lineage 28 cestata ( $M^C M^C$ ) and atra ( $M^A M^C$ ) parents in the  $F_3$  generation should produce broods in a ratio of 3:1 sinistral and dextral if the two loci are unlinked. There should be no dextral broods if linkage is absolute. There are, in fact, six sinistral broods (148, 149, 151 (2) and 172 (2)) and two dextral broods (148 and 149). In lineage 27 frenata parents in the  $F_3$  generation provide no information since both sinistral and dextral broods are expected in any case. However, apex ( $M^X M^?$ ) parents should produce all sinistral broods and bisecta ( $M^{F^2} M^A$ ), all dextral broods with complete linkage. Independent assortment should give 3:1 sinistral and dextral in each class. All the broods from apex parents (155 (2), 156 (2), 160 (2) and 171) do indeed produce sinistrals, but of the bisectas three (157 and 158 (2)) produce sinistrals and one (157), dextrals. Finally in a single observation from the  $F_3$  parents of lineage 25, a bisecta (163), which should produce dextrals with complete linkage, produces sinistrals instead. Thus there is no evidence of linkage between the  $M$  and  $H$  loci in *P. suturalis*.

### (iii) *Self-fertilisation*

Like *Partula taeniata*, *P. suturalis* is capable of self-fertilisation (Murray and Clarke, 1966, 1976). Estimating the frequency with which young are produced by this method is difficult, however, because there is evidence of great individual variation.

The most direct way to observe self-fertilisation is to record the offspring of individuals that have been kept in isolation since shortly after birth. Of 15 such animals, maintained over periods ranging from 1 to  $8\frac{1}{2}$  years, only one has produced any young at all; but that one had 11 in five years. Moreover, five of the 15 isolated virgins are offspring of the one self-fertile individual, indicating that there is no direct transmission of this tendency from parent to offspring. In these circumstances an "average frequency of self-fertilisation" is of doubtful validity, but it is the best one can do. Three methods of estimation may be used.

First, the production of young by the isolated virgins (0.015 offspring per month) may be compared with that by parents in the regular crosses (0.604 per month) to yield an estimate of 2.5 per cent of the normal reproductive rate. Second, we can calculate the frequency of selfing by observing deviations from the Mendelian ratios in backcross matings (Murray and Clarke, 1976). If the total deviations are weighted according to the frequencies of phenotypes expected from self-fertilisation, we obtain an estimate of 4.2 per cent selfing in the regular experimental crosses. Finally, a direct estimate may be derived by observing the number of offspring with phenotypes that could only have been produced by selfing, and comparing them with the number of offspring produced in all the matings where such types might be observed. Only one such individual has been found, giving a very rough estimate of  $1/195.5$  or 0.51 per cent self-fertilisation. Since all these methods are subject to objections of several kinds, the only firm conclusions are that self-fertilisation occurs, that it is rather rare (probably 1 or 2 per cent), and that it is not randomly distributed among the matings. There is no evidence that self-fertilisation is more frequent during early reproductive life, as it is in *P. taeniata* (Murray and Clarke, 1976).

(iv) *Problems of interpretation*

In closing, mention should be made of questions which have been left unresolved by our analysis. The difficulty of interpretation resulting from the association of apex and strigata in the lineage of mating 27 has been discussed above. If all of the individuals classed as apex are indeed of that type, then some additional explanation will be necessary, such as the inclusion of another allele recessive to one of those producing frenata.

Another scoring problem has been posed by the appearance of some individuals intermediate in phenotype between frenata and cestata which might be described either as frenata with a tendency toward fusion of bands or a cestata with an incipient split of the band. In two lineages our preliminary results (Murray and Clarke, 1966) have had to be revised in the light of subsequent experience. The types scored as cestata in matings 23, 24 and 87 in 1966, we now believe to be genetically frenata. In one other group of five matings in the lineage of mating 207, the scoring has proved to be so intractable that the results have been omitted here.

There are a few anomalous results which require comment. According to its antecedents mating 91 should segregate 3:1 atra and cestata. Instead it has produced all atra. This outcome is reasonable if one of the parents of the cross is a product of self-fertilisation in the former generation. Less easily accounted for are the single bisecta young in 153 and the atra in 160. Given these particular parents neither self-fertilisation nor recombination according to our hypothesis will explain them. These are possibly technical errors, although mutation cannot be ruled out.

Lastly, it should be pointed out that the inferred genotypes of table 1 are not always the only combinations that might be allowed by the data. They are the most likely interpretations under the circumstances, but others are often possible (*e.g.* in matings 23, 201 and 218). These inferred genotypes make it easier to follow the explanations, without introducing any distortion into the model.

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