POLYMORPHISM IN THE ISOPOD *SPHAEROMA RUGICAUDA*

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The spatial variation of gene frequencies has been recorded in several polymorphic species, including *Biston betularia* (Kettlewell, 1958), *Cepaea nemoralis* (Lamotte, 1951, 1959; Cain and Sheppard, 1954), *C. hortensis* (Clarke, 1962), *Littorina obtusata* (Sacchi, 1961), *Homo sapiens* (Allison, 1955), *Drosophila* spp. (Dobzhansky, 1951), and *Spaeroma serratum* (Bocquet, Lévi, and Teissier, 1951; Hoestlandt, various papers). In at least the first five species, there are indications of the reasons for the variation. In some species changes in gene frequencies have been recorded as well (see, for example, Sheppard, 1961, on *Panaxia dominula*; Dobzhansky, 1958; Clarke and Murray, 1962). The causes of spatial variation and changes in gene frequencies in natural populations are controversial, with more importance ascribed to selection (Cain and Sheppard, 1954; Fisher and Ford, 1947) or to random drift (Lamotte, 1959). As Wright (1948) has emphasized, however, these are not exclusive categories but may interact in certain circumstances.

The purpose of this paper is to describe the genetics of the polymorphic isopod, *Sphaeroma rugicauda* Leach, and to consider some aspects of gene-frequency data from 46 British populations of the species which suggest that natural selection is responsible for differences between populations.

ECOLOGY AND LIFE HISTORY

Sphaeroma rugicauda is common in most of the estuarine marshes of the British Isles, with its range extending from West Africa to northern Scotland and Sweden (Omer-Cooper and Rawson, 1934; Forsman, 1953). It is polymorphic for color and pattern of the chromatophores, and the frequencies of the various forms often differ from one population to the next, as Baig has noted (1959).

Fertilization is internal, and the young are held in the brood pouches of the mother for about 35 days and emerge with most of their adult color developed. Most of the young are released in early summer (June), but there are irregular bursts of breeding activity throughout the summer and early autumn. The animals mature the following spring, breed, and die before the end of the next winter. Thus the generations are annual and separate, at least in Britain. Farther south they may overlap.

S. rugicauda is most abundant near the upper level of high spring tides, so that when their habitat is submerged the isopods are exposed to water of rather uniform salinity (Day, 1951). Between high spring tides the isopods retreat into the pools and among the grass roots. Severe drought forces them into the remaining pools and ultimately into the mud where they remain until the next tide high enough to cover the habitat, or until the next heavy rain. Baig (1959) recorded salinities in the marsh pools at Oakenholt, Flints. between September, 1958 and May, 1959 and measured the density of *S. rugicauda* as a per cent of a standard catch. The salinity varied from 5‰ to 30‰, and the animals from about 1% to 30%. The isopods were densest in pools with low salinity (5–10‰). During severe winter weather the isopods hibernate in the mud, the time of resumed activity in the spring varying from year to year.

METHODS

Sampling and scoring.—Random samples were collected with a long-handled net in marsh pools or in the grass at high tide. The best time for collecting is on the incoming tide, when the animals are swimming

TABLE 1. *Results of matings: single phenotypes*

Type of mating	Number of matings	Progeny	Total	Probability of chance deviation
stripe × *grey*	8	164 *stripe* : 162 *grey*	326	> 0.90
stripe ♀ × *stripe* ♂	3	49 *stripe* : 17 non-*stripe*	66	> 0.80
pattern ♀ × *grey* ♂	4	36 *pattern* : 48 *grey*	84	> 0.20
grey ♀ × *pattern* ♂	5	48 *pattern* : 46 *grey*	94	> 0.80
pattern ♀ × *pattern* ♂	11	186 *pattern* : 78 *grey*	264	> 0.05
grey ♀ × *red* ♂	2	18 *red* : 19 non-*red*	37	> 0.80
red ♀ × *grey* ♂	4	49 *red* : 43 non-*red*	92	> 0.50
yellow ♀ × *grey* ♂	8	38 *yellow* : 42 *grey*	80	> 0.50
yellow ♀ × *red* ♂	6	44 *yellow* : 46 non-*yellow*	90	> 0.80
grey ♀ × *yellow* ♂	4	44 *yellow* : 33 *grey*	77	> 0.90
red ♀ × *yellow* ♂	3	51 *yellow* : 47 non-*yellow*	98	> 0.80
yellow ♀ × *yellow* ♂	12	124 *yellow* : 56 non-*yellow*	180	> 0.05
wine ♀ × *wine* ♂	2	18 *wine* : 9 *grey*	27	> 0.30
ruddy ♀ × *grey* ♂	2	13 *ruddy* : 11 *grey*	24	> 0.50
grey ♀ × *ruddy* ♂	2	5 *ruddy* : 7 *grey*	12	> 0.70
grey ♀ × *grey* ♂	3	75 *grey*	75	

actively about in the shallow water. As the tide turns, they settle down. The phenotypes were scored within an hour or two of collection. They can be recognized with the naked eye in good light (daylight is preferable to artificial light).

Breeding.—Virgin females were brought into the laboratory in the spring and paired with the desired males in 3 × 4-inch glass or plastic dishes with lids. They were kept in brackish water (5 parts seawater to 8 parts tap water), and fed on crumbled rat food and elm leaves which had been dried and soaked in tap water for a day or two. When the young were seen in the brood pouches, the females were isolated until emergence of the brood. Of the phenotypes *yellow* is recognizable at emergence, *stripe* and *pattern* at a few days of age, and *red*, *wine*, and *red–yellow* only after a few weeks, when the red pigments appear.

Calculation of gene frequencies.—Gene frequencies have been calculated using the Hardy–Weinberg principle: $p = 1 - \sqrt{q^2}$, where q^2 is the frequency of all individuals other than those of the phenotype under consideration. This is valid because there seems to be no epistasis between the genes in question, and each is dominant to the common morph, *grey*. Composite phenotypes have been listed separately under each constituent in table 5 except for *red–yellow*, for which there is evidence of close linkage between the two dominants (see below). Hence, the totals of all phenotypes will not always sum to the sample size. For *red–yellow* the gene frequencies (table 6) have been corrected by subtracting the expected number of repulsion-phase individuals. The standard deviations are calculated as $s = \sqrt{\frac{pq}{2n}}$.

RESULTS

Descriptions of Phenotypes and Genetic Results

Table 1 records the breeding results. The major phenotypes are shown diagrammatically in fig. 1.

1. *Grey* is the most common form in British populations. The melanophores and pale chromatophores are fairly evenly distributed on the body, with a faint dorsal band outlined in darker spots (fig. 1A). The dorsal band appears on all other forms as well. In some *greys* the melanophores are in a permanent state of expansion, rendering the body almost completely black. Similar "non-adaptable" individuals, which do not alter their color in response to intensity of illumination, have been described in *S. serratum* (Bocquet et

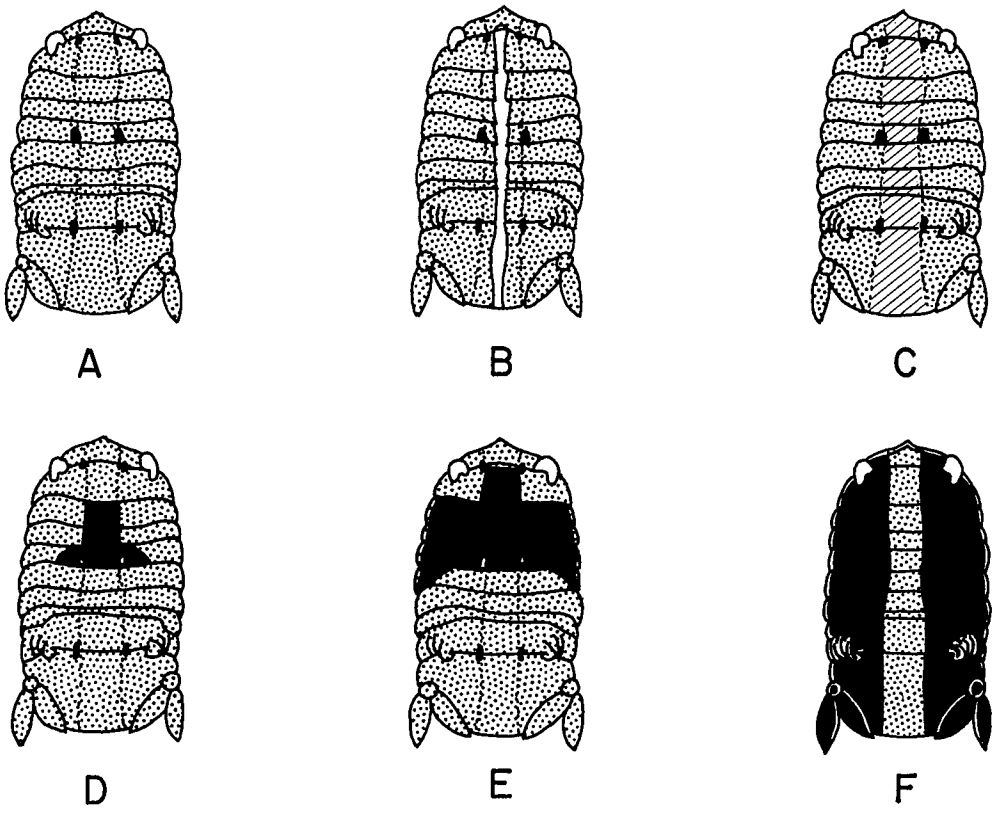


FIG. 1. Diagrammatic drawings of the major phenotypes of *Sphaeroma rugicauda*. A. *Grey*. B. *Yellow*. C. *Red*. D. and E. *Pattern* (two extremes). F. *Stripe* (unusually black sides). Stippling = scattered pale and dark chromatophores; white = pale yellow or pink; hatched = orange-red; black = black.

al., 1951). Most *S. rugicauda* become pale at night and in artificial darkness.

2. *Stripe* resembles *grey*, but the dorsal band is paler than the rest of the body (fig. 1F).

3. *Pattern* is variable but is characterized by a roughly "T"-shaped black patch on the dorsal forepart. Two extremes are shown in the figure (D and E). Otherwise, it is colored like *grey*. *Pattern* is probably the same form as that described by Lejuez (1961) as "plastron noir."

4. *Red* is apparently "bande mediodorsale" of Lejuez (1961). It has a dorsal orange-red band which runs the length of the body (fig. 1C).

5. *Yellow* has a narrow dense band of pale yellow or pink extending from the posterior edge of the head to the caudal end of the body, in the center of the dorsal band. Otherwise it resembles *grey* (fig. 1B).

6. *Wine* is similar to red, but the dorsal band is a dull wine-red.

7. *Ruddy* is probably homologous to one or two of the red forms of *S. serratum*, described by Bocquet et al. (1951) under the name of "rouges" (including forms *rubrum*, *aurantiacum*, and *hesperidium*), since *ruddy* occurs in an orange and a wine form. Because of its rarity, this form is not treated in the later section on gene frequencies.

8. Composite phenotypes. Most composites are simply double or triple heterozygotes, but one of them, *red–yellow*, ap-

TABLE 2. *Results of matings:* red–yellow *composite phenotypes*

Type of mating	Number of matings	Progeny	Total	Chi-square and probability of chance deviation
red–yellow × *grey*	5	32 *red–yellow* : 35 *grey*	67	$\chi^2 = 0.06$; $P > 0.80$
red–yellow × *grey*	21	(*red* not scorable in all progeny) 202 "*yellow*," including 12 *red–yellow* 171 "*grey*," including 1 probable *red*	373	$\chi^2 = 2.42$; $P > 0.10$
grey × *red–yellow*	1	11 *red–yellow* : 5 *grey*	16	$\chi^2 = 1.56$; $P > 0.20$
grey × *red–yellow*	11	(*red* not scorable in all progeny) 142 "*yellow*," including 19 *red–yellow* 177 "*grey*"; no *red*	319	$\chi^2 = 3.62$; $P > 0.05$

pears to be exceptional. *Red–yellow* looks like a composite of forms *red* and *yellow*, but in wild populations it occurs far in excess of expected numbers, and in addition the breeding results suggest that the genes controlling it are closely linked and are inherited as a single factor (supergene).

Combining exact probabilities for 27 localities (Fisher, 1946), the chi-square for 54 degrees of freedom is over 250 ($P < 0.0001$). In all but one of these samples *red–yellow* is in excess, and in 17 samples the excess is significant ($P < 0.05$). In six additional samples of especially large size, for which chi-squares could be calculated directly, the values ranged from 5.81 to 199.67 ($P < 0.02$ to $P < 0.001$) with *red–yellow* in excess in all. The excess could be explained by assortative mating or strong selection for this particular double heterozygote, but the breeding data suggest that such individuals have the genes for *red* and *yellow* closely linked in coupling. If this is so, it would indicate that there has been selection for the linked combination. The nature of the linkage is not known.

Among other composite phenotypes, *wine–yellow* and *stripe–red* are usually present in expected frequencies, while *stripe–pattern* appears in great deficiency in most samples, but the data for all are heterogeneous (see tables 3 and 4).

The results of matings are given in tables 1 and 2. They indicate that *grey* is recessive to the other morphs and there is no epistasis. Two aspects of the breeding data are notable: (1) the progeny from matings of like heterozygotes (e.g., *yellow* × *yellow*) contain too many recessive individuals, or, conversely, too few dominants, and (2) in matings of *red–yellow* × *grey* there is an excess of young like the female parent.

Assuming that the heterozygote × heterozygote matings should produce two phenotypes in the ratio 3 : 1, the χ^2 for the combined data (1 degree of freedom) is 6.72; $P < 0.01$. The data are homogeneous,

TABLE 3. *Observed numbers of* stripe–pattern *composites*

	Stripe	*Pattern*	*Stripe–Pattern*	Total	*P*
Christchurch	24	11	2	156	0.33
Test	79	112	7	493	< 0.001*
Medway	91	226	7	1,222	< 0.001*
Axe, 1961	59	80	14	261	< 0.001*
Axe, 1962	184	181	23	881	< 0.001*
Frome	144	255	20	1,163	< 0.001*

	degrees of freedom	χ^2	*P*
Total	6	77.25	< 0.001*
Deviation	1	55.28	< 0.001*
Heterogeneity	5	21.67	< 0.001*

TABLE 4. *Observed numbers of* stripe–red *composites: Pembrokeshire*

Locality	*Stripe*	*Red*	*Stripe–Red*	Total	*P*
Dale	161	26	4	1,120	1.000
Sprinkle	150	65	6	1,304	> 0.30
Garron	121	71	45	1,016	< 0.001*
Cresswell	121	76	6	1,414	> 0.80
Carew	82	16	3	856	0.424
Angle	40	10	2	257	0.499

$P > 0.99$. The abnormal ratio may be due to low viability of the dominant homozygotes, in which case the ratio should approach 2 : 1. The data are at least consistent with this possibility (χ^2 (1 degree of freedom) is 3.02; $P > 0.05$). The ratio lies nearer 2 : 1 than it does to 3 : 1, and the data thus suggest that there is heterozygote advantage to some of the dominant genes of *S. rugicauda*, at least relative to the dominant homozygotes.

The excess of progeny of the female parental type from matings of *red–yellow* × *grey* is more difficult to explain. Combining all data from table 2, the χ^2 (1 degree of freedom) is 4.19; $P < 0.05$. Heterogeneity χ^2 is 0.10; $P > 0.70$. If the phenomenon is real, it is restricted to matings involving *red–yellow*. The results of matings between *yellow* and *grey* are as expected.

Gene Frequencies

Between the autumn of 1960 and the summer of 1962, 46 British populations were sampled, some repeatedly. Table 5 records the composition of the samples. The gene frequencies for *stripe*, *pattern*, and *red* are shown graphically in fig. 3. I have indicated the localities on the map (fig. 2). Gene frequencies are given in table 6.

Discussion

In the following discussion I shall consider two aspects of the data on gene frequencies, (1) geographical trends and (2) changes within populations.

Pattern is common in the Bristol Channel and along the south coast, but it is present at low frequency in populations of the two peninsulas, Cornwall and Pembrokeshire, which jut out of southwestern Britain. In the gap of about 20 miles which separates the River Camel from the Gannel in north Cornwall, the frequency of the *pattern* gene drops from 7.7% to 0.4%, and in the 13 miles of unsuitable coast between the Rivers Otter and Axe of south Devon, the gene frequency rises from 0.2% to 14.2%. Both of these discontinuities are highly significant ($P < 0.001$). A similar discontinuity separates the populations of Milford Haven and the Cleddau from those of the Bristol Channel and Severn.

For *stripe* and *red* the situation is reversed, the peninsular populations having higher frequencies of these genes than do those of the Bristol and English channels. For *stripe* the information is incomplete but consistent as far as it goes. The obvious environmental correlation is with temperature. Coastal seawater temperatures are higher in the southwest (Lumb, 1961) in the winter, and the peninsulas enjoy a more temperate climate than do areas to the east. The season of the year at which temperature might exert a selective effect is important, however, since *S. rugicauda* hibernates in severe winter weather. For *S. serratum* Hoestlandt (1955a) suggested that the ambient temperature at the time of oocyte formation might be responsible for the northern limit of the range. It is difficult to see how temperature can be very important in determining the distribution of the gene for *pattern*, especially as the populations far up Milford Haven will be exposed to greater fluctuations of environmental temperature than the coastal water temperatures would indicate. Nevertheless, the average temperatures may be sufficiently different from those of the Bristol Channel and south coast to be of some importance, and there may be a relationship between the fitness of *pattern*, *red*, and *stripe* and temperature at some time in the life cycle.

Further support for the role of temperature comes from the population in the Sulby River, Isle of Man, which, although located at the latitude of northern Lancashire, has gene frequencies of *red*, *yellow*, and *stripe* which are significantly higher than those in neighboring coastal populations and which resemble those of populations to the south, in west Wales (between Sulby and Dee, $P < 0.001$ for all three gene frequencies).

TABLE 5. *Composition of samples of* Sphaeroma rugicauda *taken in Britain, 1960–1962. (* indicates unscored phenotype)*

Locality and date	*N*	*Grey*	*Stripe*	*Pattern*	*Red*	*Yellow*	*Wine*	*Red–Yellow*	*Ruddy* and misc.
1. Cree, Wights., June 16, 1961	305	284	13	–	2	5	–	1	–
2. Waver, Cumbs., May 31, 1961	684	624	46	–	4	5	–	5	–
3. Sulby, I. o. M., Nov. 20–21, 1960	4,371	3,549	419	–	140	174	–	82	7
4. Ribble, Lancs., Oct. 15, 18, 1960	674	663	*	–	3	7	–	1	–
5. Dee, Ches.–Flints., 1960–1962	5,730	2,015/2,122	60/2,122	1?	31/4,685	76	–	11/4,685	1
6. Clwyd, Denbs., Nov. 12, 1960	288	284	*	–	1	3	–	–	–
7. Malltraeth, Angl., Nov. 11, 1960	163	154	*	–	7	1	–	1	–
8. Traeth Bach, Mer., Nov. 10, 1960	175	166	*	–	1	2	–	3	3
9. Mawddach, Mer., Oct. 3, 1960									
" " Mar. 18, 1961	842	765	*	–	38	22	–	14	3
" " Oct. 26, 1961	1,182	1,045	76	–	26	20	–	6	9
10. Dovey, Cards., Oct. 3, 1960	386	363	*	–	15	4	–	4	–
11. Dale, Pembs., Sept. 23, 1961	293	228	40	8	9	7	–	1	–
" " May 21, 1962	1,120	870	165	32	30	19	–	2	2
12. Sprinkle, Pembs., Sept. 23, 1961	1,063	777	165	4	54	47	–	16	–
" " May 22, 1962	1,304	987	156	5	71	74	–	11	–
13. Black Hill, Pembs., Sept. 24, 1961	1,381	1,167	119	6	23	49	–	16	1
" " " May 21, 1962	538	457	36	3	13	22	–	7	–
14. Garron Pill, Pembs., Oct. 1, 1960	367	274	*	1	61	18	–	10	3
" " " May 15, 1961	1,050	744	106	6	118	41	–	28	6
" " " Sept. 22, 1961	857	608	125	4	66	20	–	26	8
" " " May 19, 1962	1,016	664	166	4	116	31	–	23	12
15. Cresswell, Pembs., Sept. 25, 1961	1,111	897	122	11	39	32	–	10	–
" " May 20, 1962	1,414	1,136	127	18	82	34	–	15	2
16. Carew, Pembs., May 13, 1961	832	721	50	14	33	9	–	5	–
" " Sept. 26, 1961	1,087	880	135	18	24	23	–	7	–
" " May 21, 1962	856	707	88	13	19	14	–	8	7
17. Radford, Pembs., Sept. 26, 1961	1,003	807	104	11	47	22		12	–
18. Angle, Pembs., Sept. 27, 1961	484	326	101	37	5	12	–	3	–
" " May 23, 1962	257	174	42	18	12	6	–	5	–

TABLE 5. *(Continued)*

Locality and date	*N*	*Grey*	*Stripe*	*Pattern*	*Red*	*Yellow*	*Wine*	*Red–Yellow*	*Ruddy* and misc.
19. Kidwelly, Carms., Sept. 27, 1961	1,266	827	98	316	6	18	–	–	1
20. Aust Ferry, Glos., Sept. 29, 1960, Oct. 27, 1960	1,549	1,373	*	103/743	10	62	*	1	–
21. Avonmouth, (Som.), Sept. 28, 1960, Oct. 25, 1960	1,386	1,133	*	172/660	9	70	*	2	–
22. Parrett, Som., Apr. 15, 1961	108	76	3	26	–	5	1	–	–
23. Taw, Devon, Apr. 16, 1961	757	489	71	128	6	17	12	–	–
24. Camel, Corn., Apr. 17, 1961	1,104	747	93	164	47	36	31	6	3
25. Gannel, Corn., Apr. 18, 1961	1,357	1,146	38	10	–	12	150	1	–
" " Apr. 4, 1962	481	392	19	7	2	3	63	–	–
26. Gweek, Corn., Apr. 19, 1961	141	120	6	1	7	4	–	2	1
27. Fal, Corn., Apr. 19, 1961	1,241	906	113	36	82	50	–	55	–
28. Teign, Devon, Apr. 22, 1961	770	541	140	40	11	36	–	2	–
29. Otter, Devon, Apr. 23, 1961	1,330	983	291	5	11	51	–	1	–
30. Axe, Devon, Apr. 23, 1961	261	78	73	80	5	6	20	3	–
" " Apr. 1, 1962	881	369	218	222	16	35	74	4	–
31. Frome, Dorset, Apr. 24, 1961	1,163	666	164	275	17	23	30	14	13
32. Christchurch, Hants., Apr. 24, 1961	156	108	24	13	6	4	3	–	–
33. Test, Hants., Apr. 25, 1961	601	153	79/493	112/493	9	45	106	6	8
34. Adur, Sussex, May 1, 1961	1,086	718	39	211	21	34	64	1	1
35. Rother, Sussex, May 2, 1961	89	60	–	17	–	3	9	–	–
36. Medway, Kent, May 3, 1961	1,222	787	91	226	20	32	64	8	18
37. Salcott, Essex, Mar. 31, 1961	348	274	10	43	3	4	13	1	–
38. Roman, Essex, Apr. 3, 1961	1,044	794	27	155	7	11	47	1	4
39. Stour, Suff., Apr. 2, 1961, Dec. 2, 1960, Mar. 30, 1961	2,363	1,136	186	676	55	70	220	12	27
40. Alde, Suff., Apr. 4, 1961	628	295	58	144	6	62	60	3	7
41. Kirton, Lincs., Apr. 5, 1961	356	222	36	76	2	6	12	4	–
42. Coquet, Northumb., May 29, 1961	501	373	56	2?	19	37	20	–	–
43. Aln, Northumb., May 29, 1961	1,485	1,256	99	4	20	69	34	3	–
44. Tyne, E. Lothian, May 30, 1961	1,335	819	279	118	1	94	24	–	–
45. Moray, Inverness, June 9, 1961	221	96	120	1	–	–	4	–	–
46. Munlochy, Ross., June 8, 1961	301	196	70	25	3	–	7	–	–

TABLE 6. *Frequencies of major genes in British populations of* S. rugicauda. *Numbers given are* p ± 1.96 s. *For* RY *the gene frequency has been reduced by that fraction of* red–yellows *expected to be in repulsion phase.*

Locality	*N*	*S*	*P*	*R*	*Y*	*W*	*RY*
1. Cree	305	0.022±0.012	–	0.003 ±0.004	0.008±0.007	–	0.002 ±0.004
2. Waver	684	0.034±0.003	–	0.003 ±0.003	0.004±0.003	–	0.004 ±0.004
3. Sulby	4,371	0.049±0.005	–	0.016 ±0.003	0.020±0.003	–	0.009 ±0.003
4. Ribble	674	*	–	0.002 ±0.003	0.005±0.004	–	0.008 ±0.002
5. Dee	5,730	0.014±0.004	–?	0.003 ±0.001	0.007±0.002	–	0.001 ±0.001
6. Clwyd	288	*	–	0.002 ±0.004	0.005±0.006	–	–
7. Malltraeth	163	*	–	0.022 ±0.016	0.003±0.006	–	0.003 ±0.008
8. Traeth B.	175	*	–	0.003 ±0.006	0.006±0.008	–	0.009 ±0.014
9. Mawddach (1)	842	*	–	0.023 ±0.007	0.013±0.005	–	0.008 ±0.006
" (2)	1,182	0.033±0.007	–	0.011 ±0.004	0.008±0.004	–	0.003 ±0.003
10. Dovey	386	*	–	0.020 ±0.010	0.005±0.005	–	0.005 ±0.007
11. Dale (1)	293	0.078±0.022	0.014±0.009	0.016 ±0.010	0.012±0.009	–	0.002 ±0.003
" (2)	1,120	0.077±0.011	0.014±0.005	0.014 ±0.005	0.008±0.004	–	0.006 ±0.003
12. Sprinkle (1)	1,063	0.092±0.012	0.002±0.002	0.026 ±0.007	0.022±0.006	–	0.007 ±0.004
" (2)	1,304	0.062±0.009	0.002±0.002	0.028 ±0.006	0.029±0.006	–	0.003 ±0.002
13. Black Hill (1)	1,381	0.047±0.008	0.001±0.001	0.008 ±0.003	0.018±0.005	–	0.006 ±0.003
" (2)	538	0.034±0.011	0.003±0.003	0.012 ±0.006	0.021±0.008	–	0.006 ±0.005
14. Garron (1)	367	*	0.001±0.003	0.087 ±0.020	0.025±0.011	–	0.012 ±0.008
" (2)	1,050	0.052±0.009	0.003±0.002	0.058 ±0.010	0.020±0.006	–	0.013 ±0.005
" (3)	857	0.076±0.013	0.002±0.002	0.039 ±0.009	0.012±0.005	–	0.015 ±0.006
" (4)	1,016	0.085±0.012	0.002±0.002	0.059 ±0.010	0.015±0.005	–	0.010 ±0.005
15. Cresswell (1)	1,111	0.062±0.010	0.005±0.003	0.018 ±0.005	0.014±0.005	–	0.004 ±0.003
" (2)	1,414	0.046±0.008	0.006±0.003	0.029 ±0.006	0.012±0.004	–	0.005 ±0.003
16. Carew (1)	832	0.030±0.008	0.008±0.004	0.020 ±0.007	0.005±0.004	–	0.003 ±0.002
" (2)	1,087	0.069±0.011	0.008±0.004	0.011 ±0.004	0.011±0.004	–	0.003 ±0.002
" (3)	856	0.054±0.011	0.008±0.004	0.011 ±0.005	0.008±0.004	–	0.005 ±0.003
17. Radford	1,003	0.053±0.010	0.006±0.003	0.024 ±0.007	0.011±0.006	–	0.006 ±0.003
18. Angle (1)	484	0.126±0.021	0.039±0.012	0.005 ±0.004	0.012±0.007	–	0.004 ±0.004
" (2)	257	0.085±0.024	0.036±0.016	0.024 ±0.013	0.012±0.009	–	0.010 ±0.008

Yellow is at low frequency in most populations on the west coast and reaches its highest frequencies in the east. There is a slight trend from lower frequency in the northwest to high in the southwest, but all frequencies are very close.

It is obvious that temperature alone is an insufficient explanation of gene frequencies on the west coast, especially when the populations of the east and northeast are considered. Only a few populations of *S. rugicauda* were found in East Anglia, where seawalls and draining have eliminated the isopods from many localities. For all forms except *red–yellow*, the two populations in Suffolk (Stour and Alde) have significantly higher gene frequencies than those from Essex (Salcott and Roman); $P < 0.001$, except $P < 0.002$ for *red*. There are no simple ecological differences between these pairs of localities, although organic pollution is greater in Salcott and Roman (Colchester area) than in the two rivers in Suffolk. In fact, the two samples from the Stour are from very different habitats. That from Flatford comes from a phragmites marsh where the salinity fluctuates from nearly fresh to almost that of seawater (Bingley, pers. comm.). The sample from Cattawade is from a typical East Anglican brackish marsh, about 2 miles below Flatford where the estuary widens. The two samples are homogeneous.

From an extensive series of collections of *S. serratum* on the eastern shores of the Atlantic, Hoestlandt has shown that the frequencies of some of the major genes are correlated with environment (Hoestlandt and Teissier, 1952; Hoestlandt, 1952, 1954, 1955b, 1956a, 1956b). The genetic characteristics of populations on exposed rocky coasts are similar, whether in En-

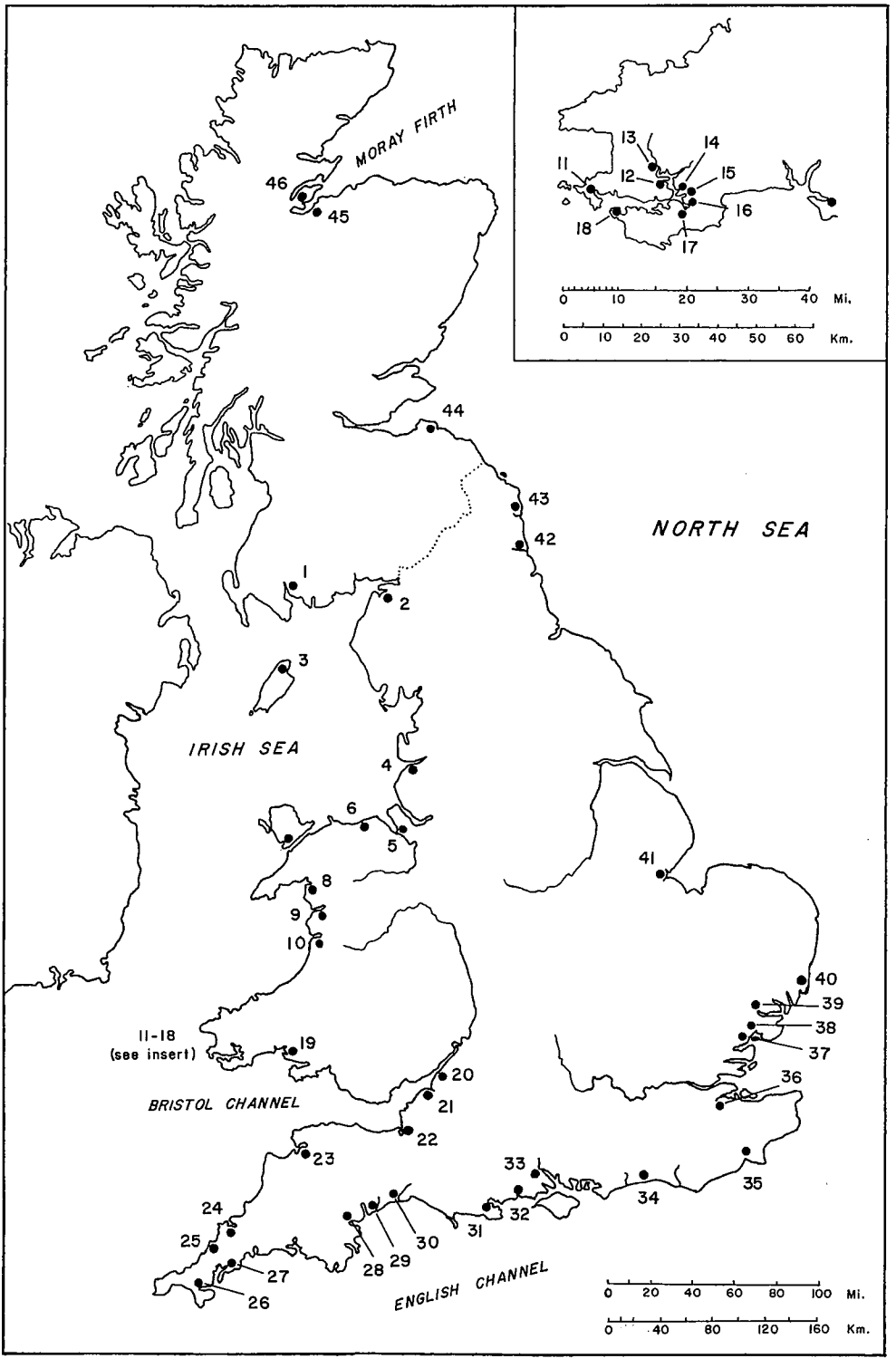
TABLE 6. *(Continued)*

Locality	*N*	*S*	*P*	*R*	*Y*	*W*	*RY*
19. Kidwelly	1,266	0.040±0.008	0.134±0.013	0.002 ±0.020	0.007±0.003	*	–
20. Aust	1,549	*	0.072±0.013	0.002 ±0.002	0.020±0.005	*	0.0003±0.0008
21. Avonmouth	1,386	*	0.140±0.019	0.002 ±0.002	0.026±0.006	*	0.001 ±0.001
22. Parrett	108	0.012±0.015	0.123±0.044	–	0.023±0.020	0.005±0.009	–
23. Taw	757	0.053±0.012	0.115±0.016	0.004 ±0.003	0.011±0.005	0.008±0.005	–
24. Camel	1,104	0.043±0.008	0.077±0.011	0.022 ±0.006	0.016±0.005	0.010±0.004	0.002 ±0.003
25. Gannel (1)	1,357	0.033±0.007	0.004±0.003	–	0.004±0.002	0.060±0.008	0.0004±0.001
" (2)	481	0.015±0.008	0.007±0.005	0.002 ±0.003			–
26. Gweek	141	0.022±0.017	0.004±0.007	0.025 ±0.018	0.014±0.014	–	0.007 ±0.014
27. Fal	1,241	0.050±0.009	0.015±0.005	0.034 ±0.007	0.020±0.005	–	0.022 ±0.008
28. Teign	770	0.098±0.015	0.026±0.008	0.007 ±0.004	0.004±0.003	–	0.001 ±0.003
29. Otter	1,330	0.116±0.012	0.002±0.002	0.004 ±0.003	0.019±0.005	–	0.0003±0.001
30. Axe (1)	261	0.137±0.014	0.142±0.014	0.009 ±0.004	0.018±0.005	0.042±0.008	0.0003±0.002
" (2)	881						0.002 ±0.003
31. Frome	1,163	0.073±0.011	0.126±0.014	0.007 ±0.004	0.010±0.004	0.013±0.005	0.006 ±0.004
32. Christchurch	156	0.080±0.030	0.043±0.022	0.019 ±0.015	0.013±0.013	0.010±0.011	–
33. Test	601	0.084±0.017	0.121±0.020	0.008 ±0.005	0.038±0.011	0.092±0.016	0.005 ±0.005
34. Adur	1,086	0.018±0.006	0.102±0.013	0.010 ±0.004	0.016±0.005	0.030±0.007	0.0002±0.001
35. Rother	89	–	0.101±0.044	–	0.017±0.019	0.052±0.033	–
36. Medway	1,222	0.038±0.010	0.097±0.012	0.008 ±0.004	0.013±0.005	0.026±0.006	0.003 ±0.003
37. Salcott	348	0.014±0.009	0.064±0.018	0.004 ±0.005	0.006±0.006	0.019±0.010	0.001 ±0.004
38. Roman	1,044	0.013±0.005	0.076±0.011	0.003 ±0.003	0.005±0.003	0.023±0.006	0.0005±0.001
39. Stour	2,363	0.039±0.006	0.117±0.015	0.012 ±0.003	0.017±0.004	0.049±0.006	0.002 ±0.001
40. Alde	628	0.047±0.012	0.122±0.018	0.005 ±0.004	0.051±0.012	0.049±0.012	0.002 ±0.004
41. Kirton	356	0.052±0.016	0.112±0.023	0.003 ±0.004	0.008±0.007	0.017±0.009	0.005 ±0.007
42. Coquet	501	0.058±0.014	0.002±0.003	0.019 ±0.008	0.038±0.012	0.021±0.009	–
43. Aln	1,485	0.034±0.006	0.001±0.001	0.007 ±0.003	0.024±0.005	0.012±0.004	0.001 ±0.002
44. Tyne	1,335	0.111±0.012	0.045±0.008	0.0004±0.0008	0.036±0.007	0.009±0.004	–
45. Moray	221	0.324±0.044	0.002±0.004	–	–	0.009±0.009	–
46. Munlochy	301	0.124±0.026	0.042±0.016	0.005 ±0.006	–	0.012±0.009	–

gland, Ireland, France, Spain, or the Azores, with certain morphs absent or very scarce. *S. rugicauda* shows similar geographical correlations, although the data are less complete.

Hoestlandt invokes rather tenuous explanations for these similarities (late Pliocene connection between Cornwall and Brittany; land bridge to the Azores). A more reasonable explanation is that environmental selection has brought about similar adjustments of the gene pools of the species and resulted in similar frequencies of the major genes.

Several populations of *S. rugicauda* were sampled more than once over a 2-year period, and the results were variable. In some populations there was no change in gene frequencies. In the Axe, for example, the samples of April 23, 1961 and April 1, 1962 are completely homogeneous. Likewise, the samples from Flatford Mill taken on December 2, 1960 and April 30, 1961 are homogeneous. The samples from the Dee, five in Flintshire (Oakenholt) and five in Cheshire (Burton) across the estuary, were taken in August, September, and October, 1960 and in February, July, August, and October, 1961, and all are homogeneous (for the *yellow* gene, $P > 0.90$). The population here is very large and occupies a wide range of habitat with apparent panmixia, and it might be expected to show broad adaptations to the various environments to which parts of the population are exposed. During high spring tides accompanied by a strong onshore wind, the entire estuary may be flooded, and waves will sweep across its entire width. It is not surprising that gene frequencies on both sides of this broad estuary are similar.



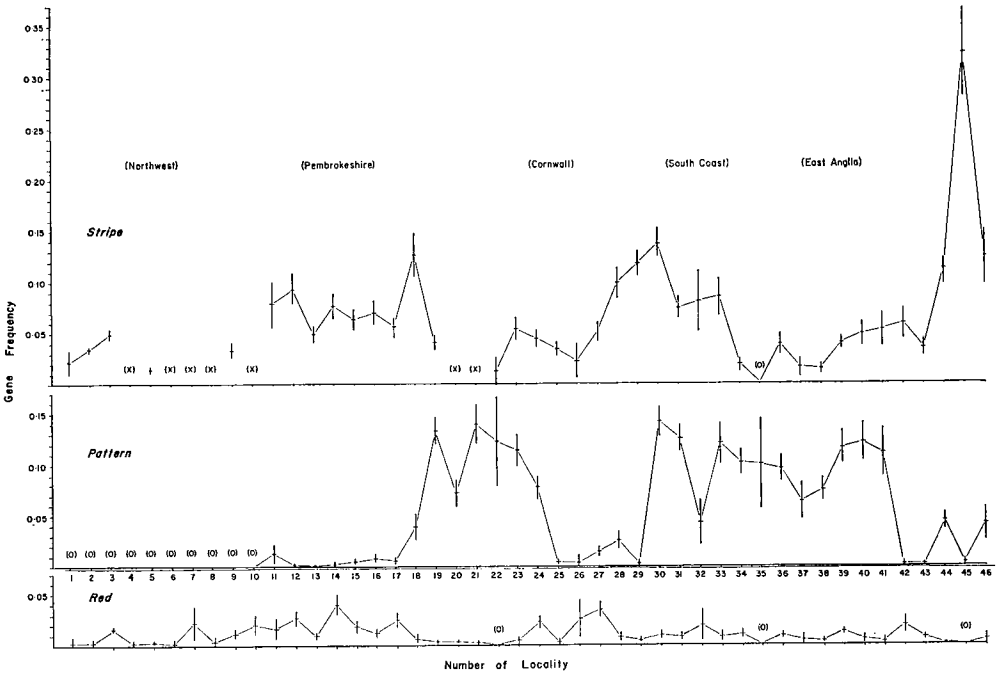


FIG. 3. Gene-frequency data for *stripe, pattern*, and *red*. Localities are numbered as in table 5, and the major geographical regions are labeled approximately. Vertical lines represent two standard deviations above and below the frequency.

The population at Penmaenpool, Mawddach River was sampled three times, in October, 1960, March, 1961, and October, 1961. The first two samples are homogeneous, but they differ from the third in the gene frequency of *red* ($P < 0.001$). The most striking changes in gene frequencies, however, were those recorded for the genes controlling *red, stripe*, and *yellow* in the populations of Milford Haven and the Cleddau River, Pembrokeshire. Table 6 records the changes in gene frequencies (localities 11–18).

Woolf's method (Race and Sanger, 1962: 398) was used to measure the reality and homogeneity of the changes in the several populations. Table 7 gives the chi-squares for the frequencies of *red* and *stripe*. The frequency of *yellow* increased an average of 14%, but although the data are homogeneous ($P > 0.10$), the change is not significant ($P > 0.20$).

There are several possible explanations for the homogeneous changes.

1. An unconscious change in scoring technique is unlikely to be the cause, since it would have affected all populations, and many showed no changes (see above).

2. Random changes are eliminated by the homogeneity of the changes as well as the almost uniformly large populations.

3. Immigration could not be responsible, since at Garron, for example, the frequency of *red* started and remained higher than in any neighboring populations. In addition, *S. rugicauda* is not capable of such massive dispersal as would be required.

4. The simplest explanation is natural selection. Over the period between samples similar environmental selection operated in

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FIG. 2. Sampling localities in the British Isles. Localities are numbered as in table 5. Pembrokeshire is enlarged in the insert.

TABLE 7. *Chi-square analysis of changes in frequency of* red *and* stripe *phenotypes of* S. rugicauda *in Pembrokeshire, September, 1961 to May, 1962. (Woolf's method; Race and Sanger, 1962)*

	Degrees of freedom	χ^2	P
Red			
V (weighted mean)	1	13.25	< 0.001*
Heterogeneity	6	11.22	> 0.05
Total	7	24.47	< 0.001*
Stripe			
V (weighted mean)	1	23.90	< 0.001*
Heterogeneity	6	10.24	> 0.10
Total	7	34.14	< 0.001*

all of these neighboring populations, and the result was a similar change in the gene pools of all of them. At Garron and Carew there were also homogeneous changes in gene frequencies during the previous period (May, 1961 to September, 1961); the other populations were not sampled before September, 1961.

Most of the samples taken in September, 1961 were of adults at the end of the breeding season, and in May, 1962 the samples consisted of the mature offspring of those adults. At Garron Pill, however, the autumn sample was of young of that year, and the following May the same young were resampled as adults. Thus, the changes in gene frequencies here reflect selection on one generation between youth and maturity. At Garron the frequency of the gene for *red* in May, 1962 showed a return to the frequency recorded in May, 1961, although the intervening sample had a far lower frequency.

At Sprinkle Pill the September, 1961 sample consisted of adults and their young, while in May, 1962 the same young were resampled as adults. Because of these inconsistencies I have not calculated selection coefficients. In addition, the polymorphism may not be maintained by the superiority of heterozygotes, and it would be premature to calculate coefficients on that assumption.

In *S. serratum* Prof. Bocquet and his colleagues have found great stability of gene frequencies over a 10-year period in the Breton populations (Bocquet and Teissier, 1960), while in some populations from the Cotentin there are changes in gene frequencies over shorter periods (a year or two; Bocquet, Lejuez, and Teissier, 1960). The authors suggest that the changes may not reflect accurately the true situation and that there may be greater stability than there appears to be. The presence of a cline in gene frequencies in populations of *S. serratum* in northeastern France (Hoestlandt and Teissier, 1952) suggests the action of natural selection, and the stability of gene frequencies within a population may result from stabilizing selection of the sort often demonstrated (see Haldane, 1960).

SUMMARY

1. The genetics of the major forms of the polymorphic isopod *Sphaeroma rugicauda* are described. The forms *pattern, stripe, red, yellow, wine, red–yellow*, and *ruddy* appear to be dominant to *grey*, which is the most common form in wild populations. There is no epistasis. Form *red–yellow* is controlled by a single segregating factor, perhaps the closely linked combination of the genes for *red* and *yellow*.

2. Samples of 46 British populations were analyzed for gene frequencies. The populations of southwest England and Pembrokeshire are similar in having lower frequencies of the gene for *pattern* than do those populations lying between in the Bristol Channel and on the south coast. Conversely, *stripe* and *red* are more common in the southwestern peninsulas.

3. Repeated sampling of seven populations of Pembrokeshire revealed significant changes in the frequencies of *red* and *stripe* over a 9-month period. The changes were homogeneous in all populations. The frequency of *yellow* also changed, but without significance.

4. The geographical trends and changes in gene frequencies in some populations

suggest that natural selection is responsible for the differences in gene frequencies between populations.

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