POLYMORPHISM IN THE AUSTRALIAN ACRIDIDÆ

II. CHANGES IN COLOUR PATTERN GENE FREQUENCIES IN THE PLAGUE LOCUST, *CHORTOICETES TERMINIFERA*

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1. INTRODUCTION

MATHEMATICAL theory in population genetics formulated by Fisher, Haldane and Wright during the 1930's paved the way for intensive studies of evolutionary processes in both laboratory and natural populations of plants and animals. It is particularly true in many polymorphic situations that selective pressures of a much higher order than at first postulated are operative, bringing about remarkable genetic changes in a relatively short time, as in industrial melanism (Kettlewell, 1956).

In most species where polymorphism has been investigated (for example colour patterns in adult butterflies, beetles or gene arrangements in larvæ of *Drosophila*) it is possible to estimate gene frequencies only for a particular stage in the life-cycle. For such organisms information on the net effect of selection operating throughout their life-cycle can be obtained. It is often desirable to know if a particular selective value is relatively constant throughout the life-cycle or if it is operative only during a certain stage of development, *e.g.* adult or pupal stage. In grasshoppers, colour patterns which appear during the first nymphal instar and remain obvious through the life of the insect provide an excellent opportunity to study the immediate effect of natural selection.

No detailed study of the population genetics of colour patterns in the Orthoptera has been made although inheritance studies in some species have been carried out. Fisher's (1930, 1939) analysis of Nabour's extensive breeding data indicated that in *Apotettix eurycephalus*, heterozygotes had a selective advantage of 7 per cent. over the dominant homozygotes and about 5 per cent. over the common recessive form. Also from the data for natural populations of *Paratettix texanus* Fisher showed that the frequency of individuals heterozygous for two or more dominant genes was less than expected and he suggested that selective forces of the order of 40 per cent. were operating against these forms in the field. Rubtsov (1935) reported relative frequencies of four different colour patterns in *Chorthippus albomarginatus* from habitats which varied from dry to humid. His data suggest that in moist habitats, green individuals have a selective advantage over brown forms whereas in dry habitats the situation is reversed. There were also marked differences in size and behaviour between the two forms.

The Australian plague locust, *Chortoicetes terminifera*, is polymorphic

for at least nine colour patterns (Byrne, 1967). These colour patterns are inherited as though determined by four multiple alleles, F^a , F^n , F^r and F^t . Heterozygotes can be recognised except in the case of F^aF^r , which is indistinguishable from F^aF^a .

2. MATERIAL AND METHODS

Six separate populations located in the central western district of New South Wales were sampled between October 1960 and March 1961. Each population was sampled weekly during the nymphal stage and fortnightly during the adult stage of the first generation. Only two of these (Pop. A and Pop. B) produced a second generation with adequate numbers of individuals for study. Difficulties in publication of extensive tables of data do not permit a detailed treatment of all the data

TABLE 1

Test for heterogeneity of colour patterns among replicate samples using three sampling methods

Method	No. of reps.	D.F.	Chi-square	P
Suction	4	13	21.4	0.1 P 0.05
Net	2	6	5.3	0.5 P 0.3
Cage	2	6	3.1	0.7 P 0.8

collected from the six populations. This paper will consider only changes in colour pattern frequency in populations A and B for which more extensive data are available. However, except for some minor irregularities, the same general trend in changes in colour pattern frequency occurred in all populations.

Samples were obtained using three methods. First instar nymphs are relatively inactive for the first few days and they rarely hop high enough from the ground to be caught by sweeping with a hand net. It was found that adequate samples of these individuals could be obtained by sucking them into a flask. After most individuals in a population had completed their first instar, a hand net was used for sampling. However, it was difficult to collect adequate numbers of adults in this way unless they were in a fairly dense population. The third sampling method involved using a cage attached to the front of a car. This was used to capture adults wherever possible.

Naturally, only one sampling method was used on any one date. Replicate samples obtained by each method were homogeneous with respect to colour pattern frequency. This indicates that on each occasion random samples of the populations were obtained. Values of chi-square for heterogeneity are given in table 1.

3. LIFE HISTORY UNDER NATURAL CONDITIONS

A generation of *Chortoicetes* may be completed within 7 weeks under laboratory conditions (Byrne, 1967). Swan (1956) has recorded a generation time of 11 weeks under favourable conditions in the field. During the studies reported here, considerable data on generation time and developmental rates for nymphal instars were obtained. In population A, development in which was typical of the populations studied,

hatching commenced on 30th September, after a fall of rain on 23rd September. The relative frequencies of the six different instars for males and females collected from population A at the different dates are given in table 2. First instar nymphs were captured up to 26 days after the commencement of hatching. Therefore, hatching must have continued for about three weeks even though no further rainfall was

TABLE 2

Relative frequencies of the six instars among males and females from population A

Instar	No. of days after start of hatching						
	4	11	19	26	33	47	60
1 ♀	0.91	0.87	0.34	0.05	0.01	...	...
1 ♂	0.95	0.87	0.32	0.03	0.00	...	...
2 ♀	0.09	0.12	0.33	0.42	0.04	...	...
2 ♂	0.05	0.12	0.40	0.17	0.01	...	...
3 ♀	...	0.01	0.28	0.28	0.20	...	...
3 ♂	...	0.01	0.17	0.19	0.02	...	...
4 ♀	...	...	0.05	0.23	0.43	0.01	...
4 ♂	...	...	0.11	0.59	0.38	0.00	...
5 ♀	...	...	...	0.02	0.31	0.15	...
5 ♂	...	...	...	0.02	0.58	0.03	...
6 ♀	...	...	...	...	0.01	0.84	1.0
6 ♂	...	...	...	...	0.01	0.97	1.0
P'tion ♀	0.508	0.494	0.476	0.451	0.409	0.329	0.576
S.E.	0.0262	0.0249	0.0231	0.0279	0.0273	0.0233	0.0177

Total number of females = 1436.
Total number of males = 1558.

recorded. More than half the males were adult by the forty-fourth day and after 8 weeks the population consisted almost entirely of adults. The second generation emerged on 18th December, approximately 11 weeks after the first generation.

For the first two weeks after hatching both sexes developed at approximately the same rate. However, after the third week most of the males were fourth and fifth instar and the females were second and third instar. This increase in rate of development in males was maintained and they were adult a week earlier on the average than females.

Active stages of *C. terminifera* rarely survive the winter period, which is normally passed in the egg stage. Eggs which are laid during autumn commence hatching in spring after the onset of warm weather and rainfall. In the areas most favourable for locusts, three generations may be completed in the one season.

4. DESCRIPTION OF THE HABITATS

Populations A and B were located approximately 20 miles apart in central western New South Wales. In describing the habitats, particular reference is made to those temporal changes in the environment which appear to be most relevant to the changes in frequency of colour patterns.

Population A (10 miles south-south-west of Coonamble). The soil is sandy loam with rather small isolated areas of compact soil. A shallow, expansive watercourse runs along the southern end of the site.

When hatching commenced on 1st October 1960, the vegetation was mostly *Medicago denticulata* and dormant tussocks of *Stipa aristaglumis*. The annual medic provided a green cover for the nymphs until mid-November, by which time it had died. The background colour of the habitat changed from green at hatching time to brown within six weeks. After December 1960, the perennial grass *Stipa aristaglumis* grew vigorously. Collecting was done over an area of about 12 acres.

Population B (8 miles north-west of Coonamble). This site has well-defined oviposition and food-shelter habitats. The soil on the oviposition habitat is sandy and compact. Plant species included *Eucalyptus populnea*, *Cynodon dactylon* and *Argemone mexicana*.

The food-shelter habitat is on lower ground than the oviposition site. It has black, self-mulching soil. The only plants were *Bassia quinquecuspis* and a few plants of *Astrebla lappacea*.

When hatching started on 1st October 1960, the only food on the oviposition site was couch grass which covered a portion of the ground. Medic, which was abundant at the site for population A, was absent. Notwithstanding the arid appearance of this habitat, the population survived to the adult stage. However, even after summer rains, there was inadequate food on both oviposition and food-shelter habitats and the majority of adults migrated from the site.

5. CHANGE IN COLOUR PATTERN FREQUENCY

Table 3 shows the relative frequencies of colour pattern genotypes for populations A and B which were sampled at intervals during the first generation. The dates on which samples were collected are expressed in number of days after the date on which hatching was observed to commence. The size of the samples are given in the extreme right-hand column.

Out of 5252 locusts, 2392 were females. Sex was not recorded for first instars because external genitalia are not obvious at this stage. To maintain uniformity in presentation of the data, it was assumed that the sex ratio among first instars is 1:1 and that colour patterns are distributed equally among first instar males and females. However, among each of the later instars it was found that males and females had different colour pattern frequencies and therefore the data for each sex are analysed separately.

The data indicate that in each population:

- (a) genotypes with the *albomedia* allele (F^a) were rare. At hatching, their total proportion did not exceed 15 per cent. of the population and after about 8 weeks it declined to less than 3 per cent.;
- (b) the proportion of *rubiginosa* (F^rF^r) increased gradually;
- (c) the relative frequency of F^nF^n decreased whereas F^nF^r and F^nF^t either remained constant or decreased in frequency;
- (d) the relative frequency of F^rF^t and F^tF^t either increased or remained stationary.

Colour pattern gene frequencies estimated from the data in table 3 are given in table 4. F^aF^a and F^aF^r are not distinguishable phenotypically and therefore gene frequencies of F^a and F^r cannot be accurately determined from the data unless relative selective values and the type of mating system are known. However, the *albomedia* phenotype was fairly rare in all populations, and if mating were random the proportion of homozygotes would be negligible in comparison with the heterozygotes. For example, if $F^r = 0.5$ and $F^a = 0.01$ the proportion of $F^aF^r:F^aF^a$ would be 100:1, assuming random mating and no differential selection. In calculating the gene frequencies, all individuals with this phenotype were assumed to have the F^aF^r genotype.

The relative frequency of F^a was less than 10 per cent. in each population and in fact it rarely exceeded 5 per cent. In both populations the frequency of this allele gradually decreased to 1.2 per cent. during the first 8 weeks. Although the data indicate that selection was acting against this gene during the active stages of the first generation, there must be counter-selection favouring it either during the egg stage or, perhaps, towards the latter part of the season, otherwise a rather high mutation rate would be required to maintain the gene frequency anywhere near the observed level. The proportion of F^r increased at the expense of F^n in both populations; F^t was relatively constant among females but showed some increase among males during the fourth and fifth instars.

The changes in colour pattern frequency which have been reported are considerable. Are these changes due to natural selection and, if so, are the different instars subject to different selective forces? If the data already presented in table 3 are arranged according to instars, it is found that similar changes in genotypic frequencies occur. This is because the successive samples reflected to a large extent the progression in development of first instars through to adults.

In order to investigate the nature of changes in genotypic frequencies, an analysis was carried out treating the colour pattern frequencies among the instars for each sample separately, *i.e.* each sample was partitioned with respect to sex, colour pattern and instar.

In table 5 are given the numbers of F^nF^r , F^nF^t , F^rF^r and F^rF^t for both sexes observed in those samples where at least two different instars occurred. The analysis of the four colour pattern genotypes for both

TABLE 4(a)
Frequency of colour pattern genes in the samples from population A

Days after hatching	F^a	F^n	F^r	F^t	$2N$
4 ♀	0.056	0.379	0.325	0.240	372
♂	0.064	0.360	0.349	0.226	358
11 ♀	0.043	0.407	0.332	0.198	398
♂	0.064	0.369	0.347	0.220	404
19 ♀	0.038	0.317	0.382	0.263	442
♂	0.049	0.294	0.394	0.263	494
26 ♀	0.033	0.333	0.374	0.260	270
♂	0.052	0.241	0.363	0.344	344
33 ♀	0.036	0.300	0.350	0.314	220
♂	0.025	0.253	0.375	0.347	320
47 ♀	0.008	0.203	0.489	0.300	266
♂	0.017	0.210	0.513	0.260	542
60 ♀	0.007	0.172	0.532	0.289	888
♂	0.015	0.235	0.491	0.258	654

TABLE 4(b)
Frequency of colour pattern genes in the samples from population B

Days after hatching	F^a	F^n	F^r	F^t	$2N$
15 ♀	0.071	0.409	0.291	0.229	350
♂	0.049	0.355	0.370	0.226	408
22 ♀	0.014	0.290	0.417	0.279	279
♂	0.013	0.257	0.465	0.265	370
29 ♀	0.027	0.282	0.391	0.300	376
♂	0.026	0.246	0.460	0.268	430
36 ♀	0.009	0.365	0.355	0.271	458
♂	0.012	0.282	0.422	0.284	792
44 ♀	0.021	0.321	0.369	0.289	290
♂	0.008	0.267	0.495	0.230	386
56 ♀	0.000	0.180	0.596	0.224	178
♂	0.005	0.243	0.495	0.257	218

populations is summarised in table 6. The chi-square test of significance used in the analysis was derived by Fisher (see Holt, 1947). The chi-square tests the significance of the regression of colour pattern frequency on instar and takes into account the actual numbers in each sample.

TABLE 5(a)
Sample data from population A showing genotype frequencies of FⁿF^r, FⁿF^t, F^rF^r and F^rF^t in the different instars

Instar	4 DAYS		11 DAYS		19 DAYS												
	1	2	1	2	1	2	3	4									
$F^n F^r$ $F^n F^t$ $F^r F^r$ $F^r F^t$	♂+♀ 80 52 52 51	♂ 1 2	♀ ... 14	♂ 6 1 1 9	♀ 11 3 ... 2	♂+♀ 32 21 9 45	♂ 29 8 11 32	♀ 27 9 4 17	♂ 14 5 4 12	♀ 20 3 8 22	♂ 9 3 3 5	♀ 5 2 1 2					
Sample total	338	7	17	24	24	348	97	73	43	63	26	11					
Instar	26 DAYS					33 DAYS			47 DAYS								
	1	2	3	4	5	3	4	5	5	6							
$F^n F^r$ $F^n F^t$ $F^r F^r$ $F^r F^t$	♂+♀ ... 4 3 2	♂ 5 4 1 11	♀ 16 8 5 15	♂ 25 13 17 24	♀ 8 6 3 4	♂ 25 13 17 24	♀ 9 7 7 10	♂ 5 7 1 7	♀ 10 8 5 15	♂ 19 8 15 26	♀ 5 7 8 5	♂ 3 3 2 ...	♀ 9 16 30 42				
Sample total	11	29	60	33	40	103	33	4	23	60	47	93	34	8	20	262	112

TABLE 5(b)
Sample data from population B showing frequencies of F^uF^r , F^uF^t , F^rF^r and F^rF^t in the different instars

Instar	13 DAYS						20 DAYS						27 DAYS					
	2		3		4		1		2		3		4		5		6	
	δ	ϕ	δ	ϕ	δ	ϕ	$\delta + \phi$	δ	ϕ	δ	ϕ	δ	ϕ	δ	ϕ	δ	ϕ	δ
F^uF^r	33	24	6	9	11	9	8	5	6	4	18	6	17	16	20	4	...	...
F^uF^t	12	10	15	6	4	1	10	3	3	1	6	3	6	23	13	4	1	1
F^rF^r	3	6	8	6	4	2	2	4	2	1	7	1	12	9	30	9	...	...
F^rF^t	20	23	6	14	7	...	8	6	10	8	22	14	26	21	33	15	...	...
Sample total	104	83	38	52	33	5	35	21	27	18	64	28	80	78	111	37	1	1
Instar	34 DAYS						42 DAYS						54 DAYS					
	2		3		4		5		6		3		4		5		6	
	δ	ϕ	δ	ϕ	δ	ϕ	δ	ϕ	δ	ϕ	δ	ϕ	δ	ϕ	δ	ϕ	δ	ϕ
F^uF^r	1	1	1	4	16	33	15	8	2	2	2	2	2	5	2	5	...	...
F^uF^t	...	2	1	5	16	28	25	8	...	1	1	1	2	4	1	...	11	10
F^rF^r	...	1	1	3	6	12	17	27	...	...	...	...	...	1	...	4	25	33
F^rF^t	2	2	...	1	7	10	18	27	4	1	1	1	2	3	2	5	30	11
Sample total	3	13	3	17	66	99	90	84	6	5	18	91	117	31	8	17	101	72

TABLE 6(a)

Statistical analysis of the genotypic frequencies of FⁿF^r, FⁿF^t, F^rF^r and F^rF^t in population A

Sample (days after hatching)	MALES				FEMALES			
	($qA-pA^1$)	$pq\Sigma(a-\bar{a})^2$	Chi- square	D.F.	($qA-pA^1$)	$pq\Sigma(a-\bar{a})^2$	Chi- square	D.F.
Genotype: F^nF^r								
11	— 0.5026	4.2592	0.06	1	+ 4.1613	4.5756	3.78	1
19	+ 15.4875	56.1672	4.27	1	+ 17.2558	49.3308	6.04	1
26	+ 6.0473	18.5046	1.98	1	— 1.3007	16.3310	0.10	1
33	+ 0.7647	5.7480	0.10	1	+ 0.0962	7.9902	0.001	1
47	— 1.4296	1.2250	1.67	1	— 1.1818	1.4017	0.99	1
	+ 20.3673	85.9040	8.08	5	+ 19.0308	79.6293	10.91	5
	Total effect within samples		4.83	1	Total effect within samples		4.55	1
	Heterogeneity between samples		3.25	4	Heterogeneity between samples		6.36	4
Genotype: F^nF^t								
4	+ 0.9044	0.9240	0.89	1	+ 0.3662	2.1187	0.63	1
11	— 2.5484	2.7720	2.34	1	+ 0.9355	2.9458	0.30	1
19	— 3.1781	29.6670	0.34	1	— 5.9767	9.0464	3.95	1
26	— 7.2727	21.5418	2.46	1	— 1.4390	12.6280	0.18	1
33	— 1.7255	3.3696	0.88	1	— 2.2269	9.3408	0.53	1
47	...	...	...	...	— 0.1212	2.0944	0.01	1
	— 13.8203	58.2744	6.91	5	— 8.3409	38.1741	5.60	6
	Total effect within samples		3.28	1	Total effect within samples		1.82	1
	Heterogeneity between samples		3.63	4	Heterogeneity between samples		3.78	5
Genotype: F^rF^r								
11	— 1.5161	2.1060	1.09	1	...	...	...	...
19	+ 5.9782	22.4264	1.59	1	+ 5.1338	17.0800	1.54	1
26	+ 4.3333	19.1780	0.98	1	— 2.6667	14.5236	0.49	1
33	+ 3.4509	3.9680	3.00	1	+ 4.4711	13.7700	1.45	1
47	+ 0.0444	1.4765	0.001	1	+ 0.2385	3.4797	0.16	1
	+ 12.2907	49.1549	6.66	5	+ 7.1767	48.8533	3.64	4
	Total effect within samples		3.07	1	Total effect within samples		1.05	1
	Heterogeneity between samples		3.59	4	Heterogeneity between samples		2.59	3
Genotype: F^rF^t								
11	+ 4.6774	3.2494	6.73	1	— 1.8700	3.6531	0.96	1
19	— 5.6688	60.1750	0.53	1	+ 0.7143	50.0045	0.001	1
26	— 4.0000	32.2500	0.50	1	— 4.4514	20.2680	0.98	1
33	— 0.1373	7.1350	0.003	1	— 2.2211	9.0272	0.55	1
47	...	...	...	...	+ 2.1212	3.8981	1.15	1
	— 5.1287	102.7479	7.76	4	— 5.7070	86.8509	3.64	5
	Total effect within samples		0.26	1	Total effect within samples		0.38	1
	Heterogeneity between samples		7.50	3	Heterogeneity between samples		3.26	4

TABLE 6(b)

Statistical analysis of the genotypic frequencies of F^aF^r, F^aF^t, F^rF^r and F^rF^t in population B

Sample (days after hatching)	MALES				FEMALES			
	(<i>qA-pA</i> ¹)	<i>pqΣ(a-ā)</i> ²	Chi- square	D.F.	(<i>qA-pA</i> ¹)	<i>pqΣ(a-ā)</i> ²	Chi- square	D.F.
Genotype: <i>F^aF^r</i>								
13	— 5·0000	56·0604	0·45	1	— 12·0000	30·4752	4·70	1
20	+ 1·6552	56·0286	0·05	1	+ 1·0260	30·4876	0·04	1
27	— 0·2066	20·8236	0·002	1	+ 0·0327	20·0166	0·00	1
34	— 6·6499	32·0733	1·38	1	+ 3·0045	33·1968	0·27	1
42	— 6·1330	11·6424	3·23	1	— 6·6828	12·1302	3·68	1
54	— 0·1652	1·2376	0·02	1	— 1·9439	2·0650	1·83	1
	— 16·4995	177·8659	5·13	6	— 16·5635	124·6534	10·52	6
	Total effect within samples		1·53	1	Total effect within samples		2·20	1
	Heterogeneity between samples		3·60	5	Heterogeneity between samples		8·32	5
Genotype: <i>F^aF^t</i>								
13	— 1·5029	29·7564	0·08	1	+ 4·5454	22·1088	0·93	1
20	— 16·5877	30·0268	9·16	1	— 10·1428	20·0736	5·12	1
27	+ 2·2944	13·0424	0·40	1	— 0·2896	24·9368	0·03	1
34	— 12·1625	32·7894	4·51	1	+ 3·1233	29·6361	0·33	1
42	— 5·7515	6·9216	4·78	1	+ 1·6207	11·3229	0·23	1
54	— 0·1193	1·0339	0·01	1	...	...	...	...
	— 33·8295	113·5705	18·94	6	— 4·3844	108·0782	6·64	5
	Total effect within samples		10·08	1	Total effects within samples		0·18	1
	Heterogeneity between samples		8·86	5	Heterogeneity between samples		6·46	4
Genotype: <i>F^rF^r</i>								
13	+ 11·0000	18·6868	6·47	1	+ 10·4545	12·6840	8·62	1
20	+ 12·1281	42·6564	3·45	1	+ 6·9162	13·8900	3·44	1
27	+ 6·5419	12·7350	3·36	1	+ 1·5683	21·0678	0·12	1
34	+ 16·7158	24·9480	11·20	1	+ 4·9179	19·0720	1·27	1
42	+ 8·2182	6·4538	10·47	1	+ 7·0500	6·1200	8·12	1
54	...	...	...	...	+ 3·0674	3·4006	2·77	1
	+ 54·6040	105·4794	34·95	5	+ 33·9743	76·2344	24·34	6
	Total effect within samples		28·27	1	Total effect within samples		15·14	1
	Heterogeneity between samples		6·68	4	Heterogeneity between samples		9·20	5
Genotype: <i>F^rF^t</i>								
13	+ 10·2472	13·6727	7·68	1	— 5·4070	17·9568	1·63	1
20	+ 9·6454	64·9026	1·43	1	+ 12·1948	37·0148	4·02	1
27	— 3·9273	36·5085	0·42	1	+ 8·1579	38·9648	1·71	1
34	+ 11·8299	29·6460	4·72	1	+ 11·8223	22·0752	6·33	1
42	+ 5·9480	9·0384	3·91	1	+ 4·5793	11·3229	1·85	1
54	+ 0·3486	1·4518	0·08	1	— 1·9439	2·0636	1·83	1
	+ 34·0918	155·2199	18·24	6	+ 29·4034	129·3981	17·37	6
	Total effect within samples		7·49	1	Total effect within samples		6·68	1
	Heterogeneity between samples		10·75	5	Heterogeneity between samples		10·69	5

Interpretation of analysis

For population A the total maximum likelihood score for F^nF^r (males) was positive, indicating that within samples the relative frequency of this genotype increased during successive instars. The total effect of this increase within the samples was significant ($\chi^2 = 4.83$, I.D.F.) and there was no significant heterogeneity among the samples. Similar changes were recorded for F^nF^t females. Also for population A, the frequency of F^rF^r increased with the age of individuals but it decreased in the case of F^rF^t and F^nF^t respectively. These changes were not significant at the 5 per cent. level.

TABLE 7

Colour pattern genotype frequencies in two samples each from populations A and B during the second generation

Sample	Colour pattern genotype									Total
	F^aF^a , F^aF^r	F^aF^n	F^aF^t	F^nF^n	F^nF^r	F^nF^t	F^rF^r	F^rF^t	F^tF^t	
<i>Population A</i> 23 days ♀	1	1	0	11	31	20	37	32	3	136
	4	1	1	4	23	6	25	36	3	103
	4	0	2	17	54	17	98	168	24	384
	2	2	6	21	53	20	76	113	16	309
<i>Population B</i> 23 days ♀	1	5	2	88	138	96	79	108	9	526
	4	2	6	41	115	72	137	188	16	581
	0	1	0	6	13	5	22	19	3	69
	1	0	2	2	14	7	32	31	4	93

In population B, the frequencies of both F^nF^r and F^nF^t decreased with increasing age of individuals, the change not being significant in the case of F^nF^t females. On the other hand, F^rF^r and F^rF^t showed significant increases.

Natural selection is the most likely explanation for the regression which has been shown to occur for some of the colour pattern genotypes. The homogeneity among samples indicates that there were no real differences in the rate of change in genotype frequency over all samples. One could conclude from this that the relative selective values were fairly constant and were the same for the different stages of development.

Second-generation hatching in these two populations commenced on or just before 18th December 1960. Samples of nymphs were obtained from both populations on 10th January 1961—these two samples will be referred to as the 23-day samples. Adults were collected from both populations on 9th February 1961 and they comprise the 53-day samples. The colour pattern frequencies for the two populations are given in table 7.

For population A the colour pattern frequencies among males and females in the 23-day sample were homogeneous. When they are pooled, the total frequencies agree with the expected frequencies generated by the adult gene frequencies in the previous generation assuming random mating ($\chi^2 = 7.16$ with 5 D.F.).

The proportions of the different instars in this sample were almost equivalent to those in the 26-day sample from the first generation. However, the colour pattern frequencies in the two samples were significantly different both for males and females ($\chi^2 = 15.49$ with 5 D.F. for females and $\chi^2 = 19.47$ with 6 D.F. for males). The colour pattern frequencies among adults in first and second generations also differed significantly.

Table 8 gives the colour pattern gene frequencies for the two samples from the second generation, and also the gene frequencies for

TABLE 8

Gene frequencies for the second generation of populations A and B. Gene frequencies at the corresponding stages in the first generation are given in brackets

Sample	F^a	F^n	F^r	F^l
<i>Population A</i>				
23 days ♀	0.008 (0.033)	0.272 (0.333)	0.507 (0.374)	0.213 (0.260)
♂	0.029 (0.052)	0.185 (0.241)	0.548 (0.363)	0.238 (0.344)
53 days ♀	0.008 (0.007)	0.137 (0.172)	0.549 (0.532)	0.306 (0.289)
♂	0.016 (0.015)	0.189 (0.235)	0.518 (0.491)	0.277 (0.258)
<i>Population B</i>				
23 days ♀	0.008 (0.027)	0.394 (0.282)	0.385 (0.391)	0.213 (0.300)
♂	0.010 (0.026)	0.233 (0.246)	0.500 (0.460)	0.257 (0.268)
53 days ♀	0.007 (0.000)	0.225 (0.180)	0.551 (0.596)	0.217 (0.224)
♂	0.016 (0.005)	0.134 (0.243)	0.591 (0.495)	0.259 (0.257)

the comparable samples from the first generation. The relative frequency of F^r , which increased from 0.3 to 0.5 during the first generation, did not differ significantly from 0.5 in the two samples from the second generation. The frequency of F^n in each sample was less than that in the corresponding samples from the first generation although the difference was not significant. However, as in the first generation, there was a significant decrease in the frequency of this gene between nymphal and adult females. The frequency of F^l did not change significantly from its value for adults in the previous generation.

In population B, genotypic frequencies among males and females in the 23-day sample were different and neither agreed with the expected frequencies as derived from the adult gene frequencies for the previous generation. The 29-day and 56-day samples from the first generation had an age distribution comparable to the 23-day and 53-day samples respectively from the second generation. The colour pattern frequencies in both generations were homogeneous

except among nymphal females, where the heterogeneity was caused by a rather high frequency of F^nF^n and F^nF^r genotypes in the second generation. The gene frequencies in each of the second generation samples agree reasonably well with the gene frequencies in the corresponding first generation samples. However, among females there is a marked discrepancy in the gene frequencies of F^n and F^r between first generation adults and second generation nymphs. This discrepancy was due largely to the high frequency of the F^nF^n and F^nF^r genotypes collected in the 23-day sample.

6. PROPORTION OF GREEN "FORMS" AMONG THE COLOUR PATTERN GENOTYPES

The colour patterns already described (Byrne, 1967) may include green pigment. The green is located in the integument and may vary from a trace amount usually located on the posterior portion of the disk

TABLE 9

Proportion of green individuals in colour pattern genotype frequencies among females in populations A to F

Population	Colour pattern genotype									Proportion green in Population
	F^aF^a , F^aF^r	F^aF^n	F^aF^t	F^nF^n	F^nF^r	F^nF^t	F^rF^r	F^rF^t	F^tF^t	
A	0.11	0.29	0.18	0.29	0.17	0.44	0.10	0.26	0.13	0.23
B	0.08	0.20	0.14	0.27	0.08	0.35	0.02	0.24	0.09	0.18
C	0.00	0.14	0.00	0.26	0.21	0.27	0.00	0.09	0.00	0.17
D	0.20	0.44	0.67	0.67	0.47	0.61	0.39	0.51	0.55	0.50
E	0.00	0.14	0.00	0.12	0.07	0.11	0.02	0.19	0.05	0.10
F	0.00	1.00	0.33	0.39	0.13	0.61	0.12	0.51	0.00	0.31

to an extreme where individuals are completely green except for a few small regions of the body surface. Specimens were classified according to whether or not green pigment was present. The pigment is restricted almost entirely to females, although green males are occasionally found. The proportion of green females among the different colour pattern genotypes for the six populations are given in table 9.

The percentage of green females in the different populations varied from 10 per cent. in E to almost 50 per cent. in D. F^rF^r females consistently had the lowest proportion of green forms. About 10 per cent. or less of F^rF^r individuals were green except in D, where green forms comprised 40 per cent. of F^rF^r females and 50 per cent. of all females in the population. F^nF^n and F^nF^t genotypes had the highest proportion of green phenotypes followed by F^rF^t and F^nF^r respectively. The incidence of green forms among the colour pattern genotypes is clearly non-random. A 2×2 contingency table comparing the incidence of green forms between the F^rF^r genotype and non- F^rF^r genotypes is given in table 10. Chi-square for independence equals 81.1 with

1 degree of freedom indicating a negative association between the incidence of green forms and the F^rF^r genotype. When environmental conditions are apparently most favourable for the production of the green pigment, green forms comprise a greater portion of the frequency of F^rF^r individuals, as in population D.

TABLE 10

2×2 contingency table showing a negative association between the incidence of the F^rF^r genotype and the green form among females

	F^rF^r	non- F^rF^r	Total
Green	68	853	921
Non-green	625	2463	3088
Total	693	3316	4009

$$\chi^2 = 81.1 \text{ with 1 D.F.}$$

7. EFFECT OF DISPERSION AND MIGRATION ON COLOUR PATTERN FREQUENCIES

During its life-cycle, *C. terminifera* is often exposed to ecologically diverse environments as afforded by oviposition and food-shelter habitats. Some of the nymphs may disperse from the oviposition site and complete their development in the food-shelter habitat. By studying colour pattern frequencies simultaneously in both environments it may be possible to determine whether or not dispersion of nymphs is random with respect to colour pattern. Kettlewell (1958) showed that in *Biston betularia* there is a marked preference by melanic forms for dark backgrounds when landing, whereas the non-melanics prefer light-coloured backgrounds. Such a study may also indicate if the relative selective values were the same in both environments.

The area occupied by population B consisted of an oviposition habitat with sandy soil and a food-shelter habitat with black, self-mulching soil. Nymphs started dispersing from the oviposition site to the food-shelter during the third and fourth instar. Samples were collected from each habitat 27 and 34 days after hatching. The colour pattern frequencies for these samples are given in table 11. The colour pattern frequencies in the two samples collected on the 27th day were homogeneous ($\chi^2 = 3.57$ with 5 D.F. for females and $\chi^2 = 7.7$ with 5 D.F. for males). It would therefore appear that nymphs disperse at random with respect to colour pattern. However, from the samples collected on the 34th day, there was a significant difference between the two environments in colour pattern frequencies ($\chi^2 = 15.39$ for females, $\chi^2 = 11.5$ for males, each with 5 D.F.). When the samples were obtained on the 27th day, there were relatively few nymphs in

TABLE 11
Colour pattern frequencies among nymphs in two consecutive samples from both the oviposition and food-shelter habitats of population B

Sample	Total									
	F^aF^a , F^aF^r	F^aF^n	F^aF^r	F^nF^n	F^nF^r	F^nF^r	F^rF^r	F^rF^r	F^rF^r	
<i>Oviposition Habitat</i>										
27 days ♂ ♀	4 1	3 5	4 1	15 12	31 19	13 30	37 19	37 32	9 7	153 126
34 days ♂ ♀	3 ...		5 1	10 15	43 24	37 32	42 16	60 12	15 1	215 101
<i>Food-shelter habitat</i>										
27 days ♂ ♀	... 1		... 2	4 3	10 11	10 12	7 13	8 20	2 2	41 64
34 days ♂ ♀	1 1		2 2	23 8	43 37	34 28	33 17	35 23	11 12	182 128

the food-shelter habitat but there was a dense band of them moving across the transitional zone. By the 34th day, the nymphs had become distributed over a large area of the food-shelter habitat. It seems likely that the difference in colour pattern frequencies between the two samples collected on this occasion was the result of different selective forces operating in the two environments. In fact, in the food-shelter habitat, conditions for survival must have been extremely adverse. By the 45th day very few grasshoppers had survived and it was not possible to obtain enough individuals for a reliable sample.

In population F, a large portion of the adults migrated from the habitat as a "loose swarm". Samples which were obtained on 2nd and 14th November 1960 were taken both from migrating and non-migrating locusts. For each occasion the two samples had similar colour pattern frequencies. (For 2/11/60, $\chi^2 = 6.5$ with 5 D.F. and for 14/11/60 $\chi^2 = 6.28$ with 6 D.F.). This suggests that there is no differential migration with respect to colour pattern.

8. DISCUSSION

For most of the populations studied, colour pattern frequencies differed between males and females. This discrepancy could result if males and females had different genotypic selective values. If this were so, then greater differences in colour pattern frequencies between the sexes would be expected among adults than among nymphs. This was not observed. Of course, it is possible that opposing selective forces may have been operating at different stages of the life-cycle and this could lead to a similar situation to that reported here.

For nymphs, the relative genotypic frequencies of $F^n F^n$ and $F^n F^t$ were generally greater in females than in males from the same sample. The incidence of green forms is higher among females of either of these genotypes than of any other colour pattern genotype. If the presence of green pigment in females improves their adaptation, which may be the case particularly on a green background, then the genotypic selective value would be expected to be higher for females than for males. Presumably, the green pigment would have to be somewhat deleterious in adult females to account for the difference in frequency of these genotypes among fifth instar nymphs and adults. It is likely that green adults are less active as fliers as reported by Rubtsov in *Chorthippus albomarginatus* and hence more prone to predation, or perhaps green forms are more susceptible to disease and parasitism both of which appear to be more prevalent among adults than nymphs—at least from laboratory studies (Byrne, 1967).

Differences in the relative frequencies of colour patterns between the sexes were greater within instars than within samples. For population A, although the frequencies in the samples were homogeneous, they were not homogeneous within each instar. Either males and females do have different selective values or, if they are the same, the

difference may be due to the fact that females develop at a slower rate than males.

The changes in relative frequency of the different colour patterns during the first generation were rather similar in each of the populations. Colour patterns which were either heterozygous or homozygous for the F^a allele were selected against. However, this gene must be favoured by selection at some stage otherwise its frequency could not be maintained except by a very high mutation rate. The F^a allele may have survival value either during the later generations or in the egg stage. It could also be favoured by gametic selection.

The increase in frequency of the homozygote F^rF^r indicates that this colour pattern has a selective advantage during the first generation. To explain this increase in frequency one must consider not only the environment for this particular generation but also that in which the previous generation was reared. These two generations were separated in time by at least five months during which the eggs over-wintered. The ecological characteristics of the habitat at these two different times of the year would be different, particularly with regard to temperature, humidity and vegetation. It is also possible that the ancestors of some of the populations were immigrants from geographically different habitats. The gene frequency of F^r among the adult ancestors must have been considerably lower than among adults of the first generation. After hatching of the first generation commenced in October 1960, there was a period of 10 weeks during which no rainfall occurred. In this period soil moisture was gradually depleted and daily temperatures increased. The pasture plants which were present at the time of hatching were mainly annual species and these had completed their growth. The subsequent death of these plants resulted in a shortage of green food and a change in the background colour of the habitats. Also, the area of bare patches of soil increased considerably. In other words, during the first generation each habitat became increasingly drier and its background colour became relatively more uniform. The *rubiginosa* (F^rF^r) colour pattern is apparently better adapted than other colour patterns to this kind of environment. It is uniformly brown with black mottling and it certainly appears to be less conspicuous than other colour patterns when on backgrounds such as dead plants and exposed areas of soil. The F^rF^r genotype would most likely have a selective advantage in such an environment against the action of predators. It is also likely that individuals with this colour pattern are physiologically better adapted to withstand dry conditions than are most other genotypes. They may utilise the available moisture more efficiently or perhaps they are better equipped to survive in the absence of an adequate supply of green food. Rubtzov (1935) recorded that the *rubiginosa* colour pattern in *Chorthippus albomarginatus* was relatively more abundant in dry than in humid habitats. Descriptions of this colour pattern suggest that its appearance is similar to the F^rF^r pattern in *C. terminifera*; however, it may well have a different genetical basis.

During the second generation rainfall was adequate and summer growing plants were abundant in most of the habitats. In this generation the frequency of F^rF^r in population A did not change appreciably from its value for adults in the first generation.

No data were obtained on the effect of predation on colour pattern frequencies. However, the capture of specimens by other invertebrate animals was observed on several occasions. These predators were mainly spiders, Psammocharid wasps and Asilid flies. The work of Carrick (1959) on the food and feeding habitats of two species of Ibis suggest that these birds may be important predators of *C. terminifera*. Although visual selection by predators may play a part in maintaining the polymorphism, it is likely that physiological selection is more important.

From inadequate data it would appear that migration does not affect the genetic structure of populations, at least with respect to colour patterns. Migration is an important feature of behaviour in gregarious phases of this locust, and for those populations which colonise habitats similar to Key's outbreak centres its occurrence must be common. Little is known either of the factors which trigger off migration in *C. terminifera* or of the fate of the migrating swarm. Individuals which leave a population certainly become mixed with those from other populations and they may traverse several hundred miles. The migratory phase is generally short-lived and perhaps the majority of adults die without reproducing. It is unlikely that migrants found new populations which can be maintained for a long period of time. However, it is reasonable to expect new populations to be formed in fairly unfavourable habitats and they may persist for a small number of generations if climatic conditions permit. From the evolutionary viewpoint migration is perhaps an adaptive mechanism operating for the survival of those individuals which remain in their habitat. It is a way of eliminating a high proportion of the numbers in a population when competition for space and food become important for survival. It can be regarded as an environmentally determined density-regulating mechanism acting to regulate population density before all resources are depleted and doing so quite independently of the genetic structure of the population. If this large-scale migration resulted in important changes in the population gene pool, it would impose an unnecessary evolutionary load on the population. Several generations of selection would then be required to equilibrate the gene frequencies to levels approaching those determined by the habitat for the population in a non-migratory phase.

It cannot be inferred from the data whether colour pattern polymorphism is transient or balanced. However, it seems unlikely that multiple alleles would be in a transient polymorphism unless the polymorphism was previously balanced. Marked changes in the selective values could upset the equilibrium and the polymorphism could become transient for one or more of the alleles. Data from a

number of successive generations are necessary to decide this point. For multiple alleles in a selectively balanced polymorphism, the necessary conditions for a stable equilibrium require that no heterozygote be less fit than both the associated homozygotes (Owen, 1953; Kimura, 1956; Mandel, 1959). It is therefore possible to have a balanced polymorphism without all heterozygotes showing overdominance. The equilibrium conditions assume random mating and constant selective values. Further studies in progress should indicate if the average values for the different genotypes satisfy the conditions for a stable equilibrium.

The studies up to date have revealed a number of problems which require further investigation before the evolutionary significance of colour patterns in this species becomes clear. Field studies throughout a number of different seasons should indicate whether or not those colour patterns which were favoured by selection during the first generation maintained their advantage for the remainder of the season. The importance of predators, particularly the ibis, in maintaining the polymorphism is also being investigated. A study of the effect of different environments on genotypic frequencies in separate populations reared in laboratory cages may indicate if some colour patterns are better adapted physiologically than others, and if so, the basis for the adaption.

9. SUMMARY

1. The Australian plague locust, *Chortoicetes terminifera*, occurs in a wide range of habitats throughout Australia. It is strongly migratory and subject to phase variation.

2. Under field conditions, the generation time is about 11 weeks, and three generations may be completed in a year if environmental conditions are favourable.

3. Data on colour pattern frequencies in natural populations were obtained for part of the 1960-61 season. There is evidence that the polymorphism is maintained by selection and that selective values vary from population to population.

4. The effect of natural selection on colour pattern gene frequencies can be studied during all stages of the life-cycle except the egg stage. Data from the first generation in the 1960-61 season suggest that the genotypic selective values were the same for the different instars.

5. At different times in a season, and in different seasons, selective values are likely to be different.

6. Migration appears to be independent of colour pattern genotype.

7. The polymorphism may be maintained primarily through physiological selection, although visual selection by predators could also be important.

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