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Phase Transformation and Chiasma Frequency Variation in Locusts

I. *Schistocerca gregaria*

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Abstract. Locusts exhibit two basic forms or phases, one characteristic of swarming populations, termed phase gregaria, and the other characteristic of low density populations, termed phase solitaria. It has been claimed by Nolte that locusts living at low density, both in the field and in the laboratory, have a reduced chiasma frequency compared with animals living at high density. A postulated gregarisation pheromone is held to be responsible for the stimulation of melanin biosynthesis in the swarming animals and an unknown metabolite in this pathway causes the increase in chiasma frequency, as well as other phenotypic changes associated with phase transformation. According to Nolte this represents a means of releasing stored genetic variation necessary for adaptation in the areas invaded by swarms. — This claim has been re-examined in laboratory stocks of *Schistocerca gregaria* using a methodology comparable to that of Nolte. No reduction in the chiasma frequency of isolated animals was observed in any of the experiments. The isolated animals did, however, develop a phenotype characteristic of phase solitaria in terms of their pigmentation and morphometrics.

1. Introduction

Locust populations are characterised by large fluctuations in population density. These changes are often accompanied by a radical alteration in the phenotype of the animals and this phenomenon has been the subject of extensive study in recent years (for reviews see Albrecht, 1967; Ellis, 1972; Kennedy, 1956, 1961; Uvarov, 1966). Although the phenotypic variation is continuous, it has been convenient to recognise two basic forms or phases, one characteristic of high density populations, termed phase gregaria, and the other characteristic of low density populations, termed phase solitaria (Uvarov, 1921). One can assign a list of typical characteristics to each phase and they appear to comprise two basic groups of phenotypes each uniquely adapted to the different mode of life engaged in by that particular phase (Albrecht, 1962).

The change from the solitaria to the gregaria phase is initiated by mutual contact between solitaria individuals. In the field the animals can be brought together by a wide range of environmental agencies such as converging air currents (Rainey, 1962) or patchy vegetation

(Uvarov, 1957). Gregarious behaviour is developed by the animals after a period of learning (Ellis, 1959a) and the stimuli associated with aggregation are thus reinforced. Certain aspects of this aggregation, involving both visual and tactile stimuli (Ellis, 1962), result in a change in the physiology of the animal.

This view of phase biology has recently been challenged by Nolte (Nolte, 1963, 1964a, b, 1966, 1968, 1969, 1972; Nolte *et al.*, 1969, 1970). He has proposed that the various phase characters are no more than mere by-products of a process concerned with the development of the only true phase character, which he claims is the amount of intrachromosomal genetic recombination occurring during meiosis. His hypothesis states that gregarisation is accompanied by an increase in chiasma frequency. This is held to be adaptive in that the presumed increase in phenotypic variation resulting from the increased chiasma frequency enables high density migrating populations to successfully colonise an alien invasion territory. He also claims that this increase in chiasma frequency, and hence the phase phenotype itself, is mediated by a single agent, a pheromone emanating from the faeces of all hoppers.

The present study was initiated because of the importance of Nolte's claims. For many years acridologists have been searching for the causation involved in phase transformation and, apart from the intrinsic academic interest of this phenomenon, it has obvious economic implications. The claims are of no less interest to cytologists concerned with the control of chiasma formation and to population geneticists examining the problems of genetic adaptation in natural populations.

Many aspects of the work carried out by Nolte are unsatisfactory. This includes both his experimental techniques and the analysis and interpretation of the data. It was felt necessary, therefore, to re-examine certain aspects of this work and in the present study a direct comparison will be made between the results obtained in a series of experiments involving the Desert Locust, *Schistocerca gregaria*, and those offered by Nolte from his work with the same organism. Results obtained with other material and a more general criticism of the Nolte hypothesis will be presented in subsequent papers in this series.

2. Methodology

a) General Features

The experimental analysis of phase presents particular problems which have not been wholly solved in the present studies. The basic difficulty arises from the fact that what we call phase characters are the result of the interaction of a wide spectrum of environmental factors with the genotype of the organism. Animals of the two phases will select different habitats if given a suitable choice. Thus the resultant phenotype is just as much a product of the physical environment in

question as it is of the degree of mutual stimulation experienced by the animals. The interaction of these various components have not yet been fully studied, but the evidence we do have indicates that the situation is exceedingly complex.

A rigorous experimental design would consist of two environmental regimes; one giving maximum expression of phase gregaria characteristics, and the other giving maximum expression of phase solitaria characteristics. An investigation of the effects of density on the expression of a particular character, for example chiasma frequency, would involve reciprocal experiments in the two environmental regimes. In practice, very few of these idealised requirements can be met. The practical problems of accurately controlling temperature and humidity at anything other than at a fairly crude level are enormous, and have certainly not been solved in Nolte's own experimental approach.

A simple, though generally accepted, method of isolation has been adopted in these experiments, namely placing animals singly in small glass jars screened on three sides and situated around a suitable heat and light source. Crowded controls were reared at high density in large rearing cages in a separate room. The fact that one is dealing, on the one hand, with a small glass jar heated externally, and on the other with a large cage with internal heating, imposes considerable problems with regard to the comparability of temperature and humidity gradients. Despite the arguments outlined above, one is forced to adopt the approach of trying, as far as possible, to equate the conditions experienced by the two groups of animals.

Because the only environmental component being examined is density, temperature and humidity being assumed equal, the experimental animals have been designated as either "crowded" or "isolated" in preference to the specific terms gregaria and solitaria.

b) The Animals

The culture of *S. gregaria* used in these experiments was derived from a strain supplied by the Centre for Overseas Pest Research known as the "Standard Strain". It has a mixed history which has been outlined by Shaw (1971).

Experiment 1 utilised egg pods obtained directly from the C.O.P.R. Subsequent work was carried out on a culture established at the Southampton laboratory.

c) The Rearing of Animals

The stock cultures and crowded experimental animals were maintained in standard 60 litre locust rearing cages in a room controlled at a constant 22° C. Each cage was heated and internally lit by a 60 W bulb operative for twelve hours each day. This supplied both the necessary photoperiod and a "day" temperature gradient of 32° C to 45° C. Each cage contained a high density of animals, initially in the order of 1000 hatchlings.

The isolated animals were separated within twenty four hours of hatching and reared individually in one litre glass jars screened on three sides with white paper to prevent visual contact between the animals. In an attempt to eliminate any possible pheromonal contamination, the isolated animals were maintained in a room that had never been previously exposed to locusts. The heat and light for the isolated animals was supplied by 150 W bulbs. The rearing jars were placed in circular groups of ten around the bulbs with the unscreened wall facing the light source and the centre of each jar ten inches from the bulb surface. The room itself was maintained at 22° C and during the twelve hour light period the temperature in the jars was 34–36° C.

Both the small jars and the large cages were cleaned and fresh grass supplied each day. The grass was obtained locally and was thus subject to seasonal variation in quality.

The addition of grass into the cages presented a serious problem in relation to humidity control. With a two-temperature cycle, artificial control of humidity was not feasible. Humidities in the large 60 litre cages ranged from 20% RH before feeding to 70% RH after the addition of grass. In the small one litre jars the humidity range was from 30% RH to 70% RH after feeding.

In the large cages, egg pods were laid in tubes filled with damp sand. Incubation was at a constant 30° C and under these conditions eggs took about 13 days to hatch. Excessive culling was not practiced, though wherever possible mature adults were kept for only a few weeks to reduce the incidence of the protozoan parasite *Malamoeba locustae*. Matings of isolated animals were carried out in special cages consisting of modified plant propagating chambers and utilising standard egg tubes.

d) Morphometrics and Pigmentation

The morphology of the adult animals was measured using Mauser dial calibrated callipers reading to 0.05 mm. The measurements used were those agreed to by the Fourth International Locust Conference held in 1936 at Cairo (see Dirsh, 1953 for details).

Fifth instar melanin distribution was scored using the quantitative scales devised by Stower (1959).

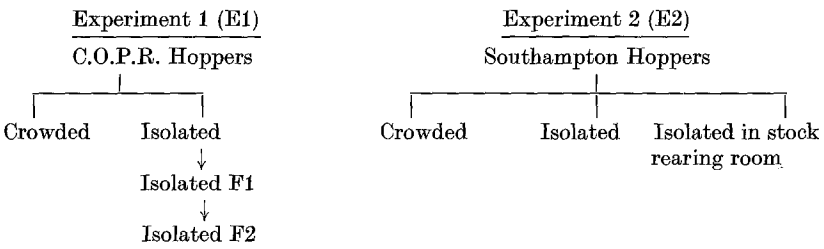
e) The Scoring of Chiasma Frequency

Testes were removed on the tenth day after the last moult since at this time the meiotic index is at its maximum for this species. To facilitate this, all experimental animals were labelled and coded individually just after fledging. The testes were removed *in situ* and fixed in freshly prepared 1:3 acetic alcohol after the fat body had been removed under insect saline.

Squash preparations were made in lacto-propionic orcein stain, and chiasmata scored at diplotene. In the first experiment twenty cells per individual were scored for the first generation of isolated and crowded animals. Thereafter, because of the large between-individual component of chiasma frequency variation, the mean individual chiasma frequencies were estimated from ten cells per individual.

f) The Experiments

Two experiments were carried out using the C.O.P.R. "Standard Strain" of *S. gregaria* and these are outlined below. The terminology F1 and F2 is used here to refer to successive generations of isolated hoppers obtained from isolated parents.



3. The Assessment of Phase Status

a) General Problems

Although this study was concerned primarily with chiasma frequency, it was felt essential to make some independent assessment of the phase

status of the experimental animals. To appreciate the rationale behind the actual experiments, it is necessary to recall three key aspects of phase biology:

i) The process of phase transformation in locusts is cumulative not only within but also between generations (Ellis, 1959b, 1962), the phase status of hatchlings being determined to some degree by the density of the maternal parent during her adult life (Albrecht *et al.*, 1959).

(ii) Although it is possible to simulate phase gregaria and phase solitaria in the laboratory by rearing the animals at particular densities it is not possible to produce the extreme forms seen in field material (Gunn and Hunter-Jones, 1952).

(iii) The characters showing phase variation are not necessarily correlated with each other, none are purely density dependent and all are differentially affected by environmental and social factors (Ellis, 1970). It is important therefore to examine these characters in relation to the environmental agencies which alter their development and appearance.

It was decided to measure both hopper pigmentation and adult morphometrics as a means of assessing phase status. Both of these characters can be scored directly without sacrificing the animal and, since they have been used extensively by locust workers, a direct comparison is possible with other studies using the same stock of insects reared under similar conditions.

b) Pigmentation

The pigmentation of locusts is made up of two principal components, a dark pattern due to melanin and insectorubin and a complex ground colour pattern whose main pigments include β-carotene and mesobiliverdin (Goodwin, 1952). Isolated locusts differ from crowded ones with respect to both these components (Goodwin, 1952). Because the ground colour pattern is highly variable and difficult to measure the melanin distribution of fifth instar hoppers, which incidently obscures the underlying insectorubin pattern, was chosen as the main phase indicator. Stower (1959) has devised quantitative scales covering the range of variation found in melanin patterns of *S. gregaria* hoppers and those which relate to the gena, the pronotum, the frons, the hind femur and the abdominal tergites have been used in the present study.

Fig. 1 shows the fifth instar melanin patterns of these five parts of the body for a group of experimental males isolated from hatching. Though there is considerable variation in the response to density between individuals within each group, the separation of the two groups is quite distinct and significantly different in each case ($P < 0.001$) using t test comparisons. Though the relevant data have not been included for each experiment, essentially identical pattern distributions were seen in each batch of isolated animals. Unlike morphometrics, pigmentation is quite sensitive as a phase indicator and the full range of patterns can be achieved after a comparatively short time of isolation.

Although humidity seems to have little effect on fifth instar melanin patterns (Dudley, 1964), temperature can have a marked effect. At 24° C isolated *S. gregaria*

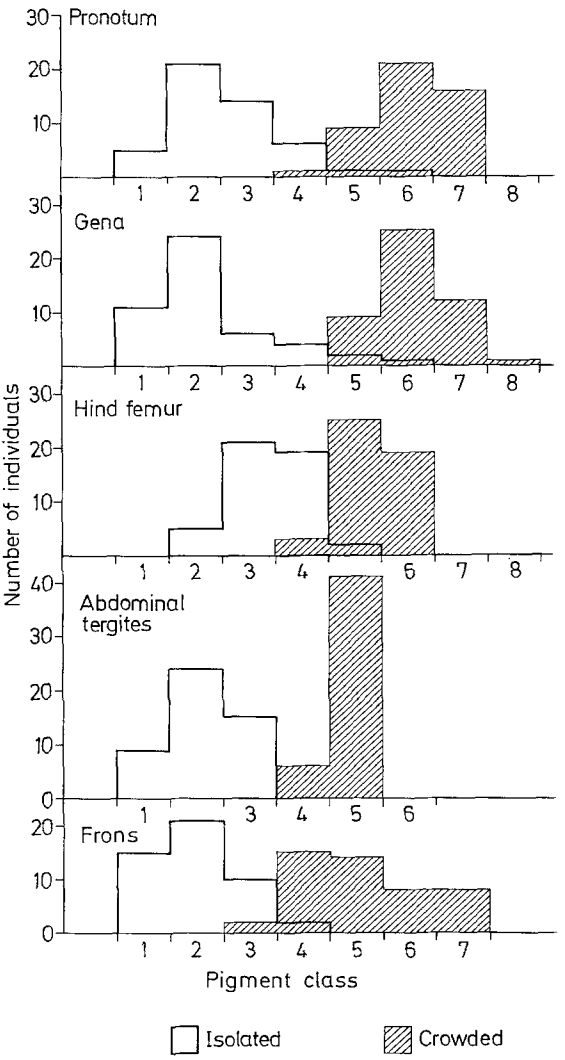


Fig. 1. Pigmentation scores for two groups of fifth instar male *Schistocerca gregaria* hoppers, one group kept isolated and the other crowded from hatching. The pigment classes are those devised by Stower (1959) and are shown for five parts of the body

hoppers show typical gregaria patterns while crowded animals maintained at a constant 42°C will have melanin patterns typical of phase solitaria (Husain and Ahmad, 1936). An equivalent temperature effect has also been observed in the field Stower (1959). In the present experiments these extreme temperatures were never experienced by the animals so that the solitaria patterns seen in the isolated animals

could not have resulted from high temperature. These animals, unlike the crowded controls, were not able to approach the light bulbs which formed the main heat source in each case.

The isolated animals did not develop the typical yellow or orange gregaria ground colour; instead they usually showed the bright green colour (formed by the conjugation of the yellow β-carotene and the solitaria specific blue tetrapyrrole pigment, mesobiliverdin), which is characteristic of solitaria animals. Some animals, however, developed a pale creamy white colour. This undoubtedly resulted from a combination of low humidity and a tendency for the animals to assume a pigmentation similar to the colour of their background (Rowell, 1971). Recall that all solitarised animals were reared on a completely white background formed by the paper screening the jars used for isolation.

We can thus be sure that the method of isolation employed in these experiments was sufficient to produce animals with at least the superficial appearance of phase solitaria. We can also rule out the possibility that the solitaria phenotype resulted from abnormal environmental temperatures.

c) Morphometrics

Locusts living at different densities can be distinguished by both their absolute morphological measurements and the relative length of various body parts. In the past the relative measurements, expressed by particular morphometric ratios, have been favoured for the purpose of phase discrimination, a practice initiated by Uvarov (1921). Dirsh (1953), in a detailed examination of field populations of *Schistocerca gregaria*, considered the ratios between the elytron (fore wing) and femur lengths (E/F) and the femur and caput (head width) (F/C) to give the best discrimination with regard to phase status in this species. These two measurements have dominated the morphological approach to phase discrimination of field populations. Such use of single morphometric ratios can be criticised on a number of grounds (Blackith, 1972). While it is agreed that the various multivariate techniques would offer a far more sensitive and biologically meaningful analysis of morphological data, it was decided to measure the E/F and F/C ratios in this study. There are two reasons for such a decision. First, the measures are used purely as a phase indicator in a system where density is the only experimental variable. Second, it enables a direct comparison to be made with the results obtained by other workers in the same field using the same stock of animals.

Dirsh (1953) showed clearly that in both sexes, solitaria animals of *S. gregaria* have a lower E/F and a higher F/C ratio than gregaria animals. A comparison between the sexes is complicated by the inherent sexual dimorphism which is always accentuated in the solitaria phase, the females being considerably larger in size. The degree of sexual dimorphism has in fact itself been used as a quantitative phase character by some workers.

Table 1 shows the E/F and F/C values obtained for both males and females in the experiments. Although the degree of discrimination does not at first sight appear particularly impressive, there has been a significant shift in both the E/F and F/C ratios towards the solitaria end of the distribution by the F2 generation. This applies to both the males and the females in the isolated lines (male E/F $t_{45} = 7.30$, male F/C $t_{45} = 7.29$, female E/F $t_{37} = 7.60$, female F/C $t_{38} = 6.37$, $P < 0.001$ in all cases). These results may be compared with those obtained by other workers using laboratory isolated animals from the same culture (Table 2).

The values after three generations of isolation (Table 1) still fall within the limits found for field material by Dirsh (1953). This applies equally well to the gregaria values and it is hard to see how crowding can be intensified in the labora-

Table 1. Morphometric ratios obtained with *Schistocerca gregaria*

Experiment		Morphometric ratio		
		Mean	S.D.	No.
E/F	Males crowded	2.18	0.06	22
	E2 isolated	2.20	0.06	19
	E1 isolated F1	2.13	0.06	27
	E1 isolated F2	2.07	0.04	25
F/C	Males crowded	3.34	0.07	22
	E2 isolated	3.39	0.10	19
	E1 isolated F1	3.47	0.10	27
	E1 isolated F2	3.54	0.11	25
E/F	Females crowded	2.24	0.05	20
	E1 isolated F1	2.16	0.05	23
	E1 isolated F2	2.09	0.08	19
F/C	Females crowded	3.37	0.09	20
	E1 isolated F1	3.50	0.09	23
	E1 isolated F2	3.62	0.15	20

Table 2. Morphometric data obtained by other workers isolating in the laboratory the same strain of *Schistocerca gregaria* as used in the present series of experiments

	Class	Morphometric ratio	Source
E/F	Isolated males	2.05	Norris (unpublished data)
	Crowded males	2.09	in Gunn and Hunter Jones (1952)
	Isolated females	2.07	
	Crowded females	2.12	
	Isolated males	2.06	Gunn and Hunter Jones (1952)
	Crowded males	2.10	
	Isolated females	2.07	
	Crowded females	2.13	
	Isolated males	2.07	Hunter-Jones (1958)
	Crowded males	2.12	
	Isolated females	2.10	
	Crowded females	2.16	
F/C	Isolated males	3.62	
	Crowded males	3.52	
	Isolated females	3.72	
	Crowded females	3.56	

tory above the levels employed. This indicates an overriding problem concerning laboratory experiments on phase transformation for clearly density levels in themselves do not appear sufficient to produce full phase phenotype.

The interpretation of morphological data, as for all other phase indicators, is extremely complex and must take into account density independent as well as density dependent factors. Husain and Mathur (1944) showed that rearing *Schistocerca gregaria* hoppers at a low temperature resulted in a low E/F ratio. The interactions between humidity and temperature in determining the morphometric ratios, however, appear to be extremely complex (Dudley, 1964).

d) Summary

The purpose of the pigmentation and morphometric studies was to monitor the experimental animals with respect to phase and to show that the isolated animals did in fact conform in some degree to accepted phase solitaria criteria. We can be confident that they have done so within the limits of laboratory rearing.

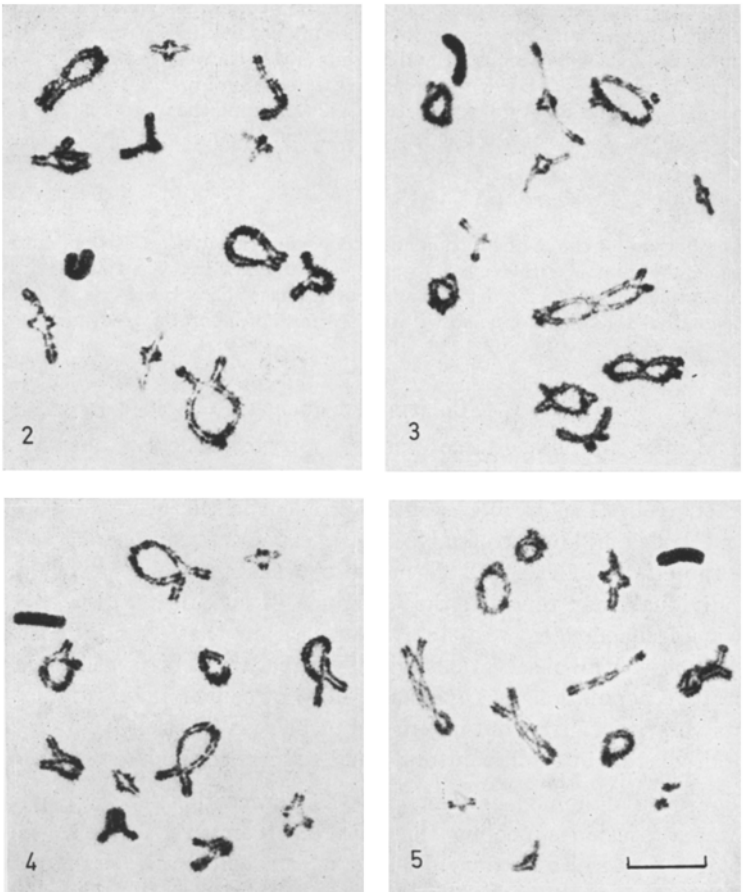
4. Chiasma Frequency

The main purpose of this study was to examine the chiasma frequencies of isolated locusts. In all cases data were obtained from male diplotene cells (Figs. 2–5). A central problem in this work has been in the methods employed to analyse the chiasma frequencies and to compare the effects of various treatments. An examination of every group of individuals has revealed the existance of significant differences in chiasma frequency between individuals within that group. This is in agreement with all observations made by other workers on both field and laboratory populations of *Orthoptera*. This, of course, makes the experiments very insensitive and it remains to be seen whether rigid environmental controls in the laboratory could reduce this source of variation.

The lack of homogeneity within the observations for a particular treatment precludes pooling the data and comparing groups with a simple c test as Nolte has done. Instead, a hierarchical analysis of variance must be employed (see Sokal and Rohlf, 1969, chapter 10). In addition, wherever possible, the number of individuals should be maximised at the expense of the number of cells per individual.

The data obtained in the present experiments are given in Tables 3 and 4. In each case an analysis of variance within each treatment showed that there were significant differences in chiasma frequency between individuals. The analyses of variance between groups in each experiment is shown in Tables 5 and 6. In no case has there been a significant drop in the mean cell chiasma frequency of the isolated animals.

Despite the large between-individual component of variation, the overall population mean has been stable throughout these experiments and is identical to that obtained by Shaw (1971) using the same culture of *S. gregaria*.



Figs. 2—5. Four diplotene cells in *Schistocerca gregaria*. Figs. 2 and 3. Cells from two crowded individuals showing 17 and 19 chiasmata respectively. Figs. 4 and 5. Cells from two animals kept isolated from hatching showing 19 and 21 chiasmata respectively. The scale bar represents 10 μm

5. Discussion

The initial aim of this study was to verify the basic claim that locusts isolated in the laboratory have markedly reduced chiasma frequencies compared with crowded controls. The detailed analysis of the causal determination of the chiasma phenotype, the ultimate purpose of this study, depended on the ability to consistently reproduce this drop in chiasma frequency under defined conditions. This would have subsequently served as the basic experimental system to be used in testing the effects of various treatments.

Table 3. Mean individual chiasma frequencies and standard deviations for animals in Experiment 1. In the crowded and isolated groups 20 cells per individual were scored. For the F1 and F2 groups only 10 cells per individual were scored (see text)

Crowded		Isolated		Isolated F1		Isolated F2	
Mean	S.D	Mean	S.D	Mean	S.D.	Mean	S.D.
19.90	1.17	21.45	1.15	22.20	1.03	20.00	1.05
21.30	1.56	20.70	1.18	22.30	1.34	19.40	1.35
20.50	1.05	19.50	0.83	21.40	0.84	21.80	0.92
21.60	0.60	20.10	1.33	17.70	0.95	19.50	0.97
20.35	1.43	21.65	1.09	19.90	0.99	21.20	0.79
18.90	1.21	21.00	0.98	20.00	1.63	20.20	1.03
19.65	1.35	20.70	1.84	21.00	1.05	20.20	1.14
18.90	1.12	19.55	1.19	20.10	1.52	19.00	1.05
21.60	1.190	21.45	1.05	20.90	1.29	19.80	1.32
20.50	1.54	21.10	1.55	20.30	1.16	19.80	0.63
18.80	1.07	20.80	1.54	21.10	1.29	20.80	1.32
19.50	1.10	20.05	1.67	22.40	0.84	19.30	1.42
20.75	1.37	19.20	1.51	20.10	0.99	20.30	0.82
20.25	1.29	20.25	0.91	19.50	1.08	21.70	1.49
21.35	1.23	19.80	1.06	21.40	1.51	22.00	1.49
20.70	0.98	19.10	0.97	20.10	1.45	18.20	1.03
19.35	1.14	20.60	0.94	21.10	1.20		
21.35	1.14	21.85	1.23	22.30	1.06		
20.05	1.23	21.50	1.28				
$\bar{X}_{19} = 20.28$		$\bar{X}_{19} = 20.55$		$\bar{X}_{18} = 20.77$		$\bar{X}_{16} = 20.20$	

In no case, however, did the isolated animals show a reduced chiasma frequency in comparison with crowded controls. This is in direct contradiction to the results obtained by Nolte. The degree of response he obtained is shown in Table 7. It is now pertinent to consider the possible reasons for this discrepancy in the two sets of data.

One could first argue that the isolated animals in these experiments did not conform to phase solitaria, that is the conditions of isolation were not rigid enough or alternatively the animals were not isolated for a sufficient period of time. This necessitates a comparison between the techniques employed here and those used by Nolte. In all his work Nolte isolated hoppers at mid-third instar and in no case did he continue any laboratory lines into further generations of isolation. By comparison the animals used in these experiments were either isolated at hatching or were the progeny of isolated mothers. Thus, on these grounds, the method of isolation used was a great deal more rigorous than that used by Nolte. There remains the possibility that it is the late isolation itself that is responsible for the results obtained by Nolte, though this,

Table 4. Mean individual chiasma frequencies and standard deviations for animals in Experiment 2. Ten cells per individual were scored

Crowded		Isolated		Isolated in Locust room	
Mean	S.D.	Mean	S.D.	Mean	S.D.
19.4	0.84	20.3	1.16	20.7	0.95
22.4	1.51	20.7	1.06	21.0	1.33
18.4	0.84	16.8	1.81	20.9	0.88
21.7	1.57	19.0	1.15	21.6	1.51
19.6	0.97	20.8	1.23	20.7	1.57
19.8	0.92	20.6	1.26	20.3	1.16
20.0	1.05	16.8	0.92	21.6	1.08
23.1	1.37	18.6	1.08	22.9	1.20
20.0	1.33	22.3	1.06	20.4	1.58
20.5	1.43	18.7	1.49	19.9	0.99
20.5	1.08	21.1	1.60	20.0	1.42
19.8	1.06	18.6	1.58	20.6	0.97
19.6	1.35	21.0	1.56	19.8	1.40
20.7	0.95	19.7	1.64	20.2	0.92
21.6	0.84	20.6	1.17	21.1	1.52
20.9	0.88	21.6	1.35	20.7	1.25
19.0	1.33	21.2	0.92	21.3	1.34
19.4	1.08	21.1	1.73	21.5	1.51
19.6	0.84	23.4	1.35	19.8	1.03
18.0	1.41				
$\bar{X}_{20} = 20.20$		$\bar{X}_{19} = 20.15$		$\bar{X}_{19} = 20.81$	

if true, would nullify the basic tenets of his hypothesis. To test this, a batch of hoppers were isolated at mid-third instar and ten adults were scored for chiasma frequency (Table 8). This gave a mean value of 20.1, not different from the crowded controls.

Another measure of the effect of isolation is provided by an examination of the other phase indicators, in this case pigmentation and morphometric ratios. The data, already presented and discussed in section 3, demonstrate clearly that the isolated animals in this study did in fact develop a phenotype corresponding to phase solitaria.

An accurate appraisal of the phase status of Nolte's animals is difficult for the information he gives is very vague. Morphometric ratio data are given but no mention is made of the number of animals measured or the degree of variation encountered. Similarly, with respect to the pigmentation of his hoppers, he merely states that they go from "black" to "green" after isolation. No quantitative scale is used for measurement and again no mention is made of the number of animals scored or the variation for this metric.

Table 5. Analysis of variance of chiasma frequency in Experiment 1

Comparison	Source of variation	df	SS	MS	VR	*P*
1. Crowded *vs.* isolated animals	Between treatments	1	12.90	12.90	0.82	N.S.
	Between individuals within treatments	36	565.30	15.70	10.28	<0.001
	Between cells within individuals	722	1102.25	1.53		
	Total	759	1680.44			
2. Crowded *vs.* isolated F1 animals	Between treatments	1	28.43	28.43	1.82	N.S.
	Between individuals within treatments	35	546.11	15.60	10.61	<0.001
	Between cells within individuals	523	767.40	1.47		
	Total	559	1341.94			
3. Crowded *vs.* isolated F2 animals	Between treatments	1	0.80	0.80	0.06	N.S.
	Between individuals within treatments	33	473.50	14.35	10.04	<0.001
	Between cells within individuals	505	721.40	1.43		
	Total	539	1195.70			

It is true that morphometric ratios and pigmentation are only superficial indicators of phase status. Nevertheless, the experimental animals were kept in complete isolation as regards visual and mechanical contact and, while it is clearly impossible to eliminate pheromonal contact, the isolated animals were reared in a room uncontaminated by previous exposure to locusts. This is as far as one can go without supplying each jar with its own purified air source.

Table 6. Analysis of variance of chiasma frequency in Experiment 2

Comparison	Source of variation	df	SS	MS	VR	*P*
1. Crowded *vs.* isolated animals	Between treatments	1	0.22	0.22	0.01	N.S.
	Between individuals within treatments	37	836.07	22.60	14.30	<0.001
	Between cells within individual	351	554.50	1.58		
	Total	389	1390.79			
2. Isolated animals *vs.* animals isolated in the locust breeding room	Between treatments	1	40.46	40.46	2.30	N.S.
	Between individuals within treatments	36	634.17	17.62	10.31	<0.001
	Between cells within individuals	342	584.20	1.71		
	Total	379	1258.83			

If the chiasma phenotype depends on the hopper density, which is the hypothesis under test, then the isolated animals should show the Nolte effect for they have been subjected to a far more stringent isolation regime than Nolte himself used. Nolte has consistently argued that chiasma frequency is the most sensitive phase character and has used this to accommodate the fact that although the isolated animals showed a marked reduction in chiasma frequency they did not show any change in their morphometric ratios over the short period of isolation.

Turning to chiasma frequency, it has proved impossible to re-analyse the data of Nolte since in no case has the number of cells or the number of individuals scored for a particular experiment been stated. The statistical analysis employed by Nolte has been one of the most disturbing aspects of his work. He has consistently pooled data for a given treatment regardless of any heterogeneity within the group. Indeed he has made no attempt to carry out an analysis of variance of his chiasma frequency data in order to justify his use of a simple *c* test.

Table 7. Results obtained by Nolte after isolating strains of *Schistocerca gregaria* in the laboratory

Strain	Year	Chiasma frequency	
		Crowded	Isolated
Sudan	1967	19.98	18.53
	1967	19.97	17.32
	1968	19.07	17.58
Sudan × S. Africa	1967	19.30	18.25
	1968	19.78	17.75

Table 8. Mean chiasma frequencies and standard deviations of ten males isolated at the third instar

Mean	S.D.	Mean	S.D.
21.3	0.95	19.4	1.35
20.6	0.70	20.2	1.03
21.8	1.03	20.5	1.27
20.3	1.06	17.8	1.14
20.6	0.97	18.6	1.08
		$\bar{X}_{10} = 20.1$	

It must also be pointed out that the material used by Nolte for scoring chiasma frequency, as judged by his only published photographs (Nolte, 1964 pp. 376–377), is of a poor quality. Of the six cells illustrated by him none can be scored unambiguously with any accuracy.

According to Nolte, the development of a high chiasma frequency, and subsequently the full gregaria phenotype, depends solely on the action of a postulated pheromone. The development of solitaria phase characteristics is thus prevented if the pheromone is present. It is surprising, therefore, that the isolated animals reared in the main breeding room not only developed a solitaria phenotype, but showed it to the same degree as animals reared in isolation in a separate building. One could explain these results on the basis of the Nolte hypothesis if one were to postulate that the threshold level of the pheromone was very high and that even being a few feet away from crowded locust cages was insufficient to trigger the development of the gregaria phase phenotype. Nolte, however, has always claimed that the threshold level of the pheromone is very low and significant differences in pigmentation and chiasma frequency were obtained even between animals reared at different positions in the same locust room (Nolte, 1963, 1968).

The involvement of a possible pheromone in the aggregation response of *S. gregaria* adults has been proposed by Gillet (1968). Her evidence for the pheromone is, however, indirect and there is no reason to suppose its relation to the other phase characters is causal, these developing in response to the induced aggregation and not to the pheromone *per se*.

There is, however, one further possibility worth considering. It is conceivable that the Southampton culture of *S. gregaria* did not show a reduced chiasma frequency when isolated because it had lost the genetic basis to do so. This must be seriously considered since the stock used has been laboratory bred for some 25 years and unconscious selection coupled with inbreeding will inevitably result in some modification of the gene pool. The animals clearly do not lack the capacity to respond to changes in density and have, therefore, the genetic basis for the expression of solitary phase characters. It is possible, however, that the animals lack the genetic basis for the expression of a low chiasma frequency phenotype. It can be demonstrated that this is not true. An albino stock, derived from mutants occurring in the *S. gregaria* culture and consisting of individuals lacking the melanin and ommochrome pigments, showed a marked reduction in chiasma frequency. This has been noted by Nolte for the same species (Nolte, 1968) and he considers the albino in effect to represent an extreme solitaria phase. His interpretation of this situation is also open to question and a detailed analysis of the albino mutant, especially in relationship to Nolte's claims, will be presented in the third paper in this series. The point of interest here is that the Southampton stock of *S. gregaria* clearly does not lack the genetic basis for developing a low chiasma frequency phenotype.

One is left then, with the observation that the animals reared in isolation at Southampton failed to show a reduction in chiasma frequency. Whatever the relationship between chiasma frequency and phase, and at this stage such a relationship cannot be dismissed, it is clear that it cannot be causal. Nolte has claimed that the phase phenotype is a by-product of the epigenetic mechanism determining the chiasma phenotype. The fact that it is possible to obtain animals that show phase solitaria and yet do not show a reduced chiasma frequency negates this hypothesis as it stands.

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