STUDIES ON *PERIPLANETA AMERICANA*

IV. PAKISTANI POPULATIONS

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1. INTRODUCTION

INTERCHANGES occur spontaneously in animal populations from time to time (Staiger, 1955; Lewis and John, 1963a; John and Hewitt, 1963) and have undoubtedly played a role both in isolating the sexes within a species (evolution of sex-chromosome heterozygotes) and in isolating species from one another (evolution of structural homozygotes). But only two instances are known in animals where they have been preserved as permanent autosomal heterozygotes. The first of these to be described was Piza's demonstration of extensive multiple formation in Brazilian scorpions (summarised in White, 1954). Floating interchanges have also subsequently been described in the Indian scorpions *Buthus doriae* and *B. tamulus* (Sharma, Parshad and Joneja, 1959).

The second instance of persistent interchange hybridity in animals was discovered in 1957 by Lewis and John in British coal mine and culture populations of the American cockroach *Periplaneta americana*. In accounting for the presence of interchanges in these populations it was proposed that, by analogy with the situation in plants (Darlington, 1956), fixation of the interchange system was determined by a prior change in the breeding system since a measure of inbreeding must have been involved in the origin of both kinds of population studied.

If this explanation is correct one might expect to find heterozygotes either less frequently or else simply floating in areas where cockroaches are more widespread in occurrence and where inbreeding would be less rigorous. To this end we have sampled populations from Pakistan where cockroaches are still abundant in the more humid areas. Here then conditions are clearly quite different from those obtaining in coal mines or in culture.

2. TECHNIQUE

Specimens were collected from four populations; two of these were from Karachi, in the extreme south of West Pakistan, and two were from Lahore, which is about 900 miles north of Karachi (table 1). The material was sent from Pakistan by air. Unfortunately many specimens died *en route* so that in all only 46 males were available for study. In *Periplaneta* the detection of interchanges is complicated by the existence of an extensive pre-stretch phase following the dissolution of the nuclear membrane at the end of pachytene (John and Lewis, 1957, 1960). Specimens were therefore pre-treated with 0.05 per cent. colchicine before fixation.

TABLE 1
The sources of material

Population	Prefix	Origin	No. of ♂♂ examined
Karachi . . .	K ₁	House Sewer manhole	12
	K ₂		14
Lahore . . .	La ₁	House Covered fruit market	9
	La ₂		11
Total males examined			46

3. THE INCIDENCE OF HETEROZYGOSITY

All four populations contain structural heterozygotes (table 2, plate figs. 2-6). On account of the small number of individuals analysed it was not possible to compare the distribution of the different karyotypes within the individual populations. But a χ^2 test shows

TABLE 2
The incidence of interchange heterozygosity in 4 populations of Pakistani cockroaches

Locality	Classes						Total individuals
	16II	11V	21V	1VI	1VI+11V	1VI11	
K ₁	4	2	3	1	2	...	12
K ₂	7	4	2	1	...	...	14
La ₁	3	1	...	2	3	...	9
La ₂	7	1	...	2	...	1	11
Totals	21	8	5	6	5	1	46

no significant difference between the populations in the ratio of homo- to heterozygotes (table 3). Despite this the household populations from both Karachi (K₁) and Lahore (La₁) have proportionately more heterozygotes than the non-household ones. The homo- and heterozygotes from K₁ and La₁ were therefore pooled and tested against the equivalent pooled totals for K₂ and La₂. Although the comparison is still not significant (table 4) it does indicate that over 80 per cent. of the total χ^2 value of 2.82 can be attributed to the difference between household and non-household populations. And this in turn suggests that larger samples might well reveal a significant difference between the two categories.

TABLE 3

The contingency table for the distribution of homo- and heterozygotes in Pakistani populations

Locality	Classes		Total individuals
	Homo	Hetero	
K ₁	4	8	12
K ₂	7	7	14
La ₁	3	6	9
La ₂	7	4	11
Totals	21	25	46

$$\chi^2_{[3]} = 2.82 \quad P = 0.30-0.20.$$

TABLE 4

A comparison of household and non-household populations of Pakistani roaches

Locality	Classes		Total individuals
	Homo	Hetero	
K ₁ + La ₁	7	14	21
K ₂ + La ₂	14	11	25
Totals	21	25	46

$$\chi^2_{[1]} = 2.36 \quad P = 0.20-0.10.$$

TABLE 5

The degree of maximum multiple formation in Pakistani populations of P. americana

Karyotype	Percentage maximum multiple formation					Total cells	Total individuals
	11-20	21-70	71-80	81-90	91-100		
1·1V	...	...	...	1	7	181	8
2·1V	...	...	...	...	5	105	5
1·V1	1	...	...	2	3	138	6
1·V1 + 1·1V	...	...	1	1	3	122	5
1·V111	...	...	...	1	...	26	1
No. of individuals observed per class							

The degree of maximum multiple formation is high in all four populations; indeed with the exception of one ring-of-six heterozygote in K2 it never fell below 70 per cent. (table 5). No British population has ever been found to contain a multiple higher than a ring-of-six. Of particular interest, therefore, was the finding of a ring-of-eight in La2, more especially in view of its very regular pairing.

No univalents or numerically uneven multiples were encountered, though two X chromosomes were found in 4 cells each from 4 distinct individuals. Pre-meiotic non-disjunction of the X was also reported in British material (John and Lewis, 1958). Unfortunately there was insufficient material to study the disjunctional behaviour of the multiples but one of the factors leading to high fertility is present (high degree of maximum multiple formation) and since several of the conditions necessary for regular disjunction are present in all individuals (pre-stretch and terminal chiasma localisation, see Lewis and John 1957, 1963*b*) one would predict that the heterozygotes should enjoy high fertility.

4. THE LEVEL OF HETEROZYGOSITY

Since the distribution of homo- and heterozygotes in the Pakistani populations was not significantly different the data from the 4

TABLE 6

A comparison of the distribution of homo- and heterozygotes in British and Pakistani populations

Populations	Observed number		Total Individuals	P
	Homo	Hetero		
Pakistani . . .	21	25	46	$\chi^2_{[1]} = 4.84$ $P = 0.05-0.02$
British wild* . . .	33	86	119	
Totals	54	111	165	
Pakistani . . .	21	25	46	$\chi^2_{[1]} = 14.78$ $P < 0.001$
British culture* . . .	20	99	119	
Totals	41	124	165	

* Data of John and Lewis (1958).

populations was pooled and compared with that already available for British wild and culture populations (table 6), which two latter are known to show significant differences in their levels of heterozygosity (John and Lewis, 1958). Both comparisons show a significant difference. These differences can be demonstrated more forcefully by

a contingency comparison of the distribution of karyotypes (table 7). Once again both comparisons are significantly different. One further difference is revealed in table 7. Not only do the Pakistani populations

TABLE 7
*A comparison of the distribution of karyotypes in
British and Pakistani populations*

Populations	Classes					Total individuals	P
	16·11	1·1V	2·1V	1·V1	1·V1+1·1V		
Pakistani .	21	8	5	6	5	45	$\chi^2_{[4]} = 13.80$ $P = 0.01-0.001$
British wild* .	33	50	22	9	5	119	
Totals .	54	58	27	15	10	164	
Pakistani .	21	8	5	6	5	45	$\chi^2_{[4]} = 32.21$ $P < 0.001$
British culture* .	20	52	28	6	13	119	
Totals .	41	60	33	12	18	164	

* Data of John and Lewis (1958).

have a relatively larger number of homozygotes, they also have proportionately more of the higher heterozygotes. The increased frequency of heterozygotes in British populations is thus due to the larger numbers of individuals with one or two ring-of-four multiples.

5. THE MEANING OF HETEROZYGOSITY

(i) *Cockroaches*

The original explanation advanced by Lewis and John for the extensive interchange hybridity of *Periplaneta* populations was based on analogy with the experimental comparison of the survival of homo- and heterozygous types following cross- and self-fertilisation of interchanges of the same kind in the genus *Campanula* (Darlington and La Cour, 1950). In these experiments heterozygotes survived at the expense of homozygotes following both selfing and crossing, but the effect was most marked in selfed progeny. In terms of survival, therefore, the heterozygotes showed a considerable advantage. This depended on the fact that the chromosomes involved in interchange are both structurally and genically heterozygous so that heterozygotes are expected to show superior genetic balance. This would be particularly true in *Periplaneta* where, as in rye (Rees, 1961), chiasma formation is confined in the main to distal segments.

In these terms the level of hybridity would be expected to show a

clear relationship to the extent of inbreeding. Thus in culture the level of heterozygosity is significantly higher than that in coal mines (John and Lewis, 1958) and as we have seen there are indications that the same may be true of household as compared to non-household populations. Of course, cockroaches, being dicecious, cannot self-fertilise so that the complete elimination of homozygotes is not expected. There can be no doubt that cockroaches are much more common in Pakistan as compared to British coal mines and they unquestionably enjoy greater mobility so that the extent of inbreeding must be expected to be less intense. Significantly the level of heterozygosity is also less marked.

Three other studies have dealt with natural populations of *Periplaneta* from outside Britain. In two of these the authors did not report any measure of interchange hybridity (Suomalainen, 1946; Sharma, Parshad and Sehgal, 1959). This we are now inclined to attribute to the fact that, in the absence of colchicine pre-treatment, the detection of interchanges is difficult and tedious. More recently Rajasekaresetty and Ramanmurthy (1963) have figured ring and chain of four multiples in untreated specimens from Mysore but they give no indication of the frequency of their occurrence in the population. We would predict that probably all populations of *Periplaneta* will show interchange polymorphism but that the precise level of hybridity will depend on the extent of inbreeding. Our reason for making such a prediction is based on the fact that all *Periplaneta* populations must be expected to show a measure of inbreeding simply because the species is so closely associated with human habitation; indeed so far as the cockroach is concerned this association is a necessary one, for as a scavenger it has become virtually self-domesticated.

The presence of a high level of interchange heterozygosity in a single population of another roach, *Blaberus discoidalis* (John and Lewis, 1959), suggests that this situation may prove to be more widespread in cockroaches. Samples from a second population of *B. discoidalis* maintained in culture at the Zoology department at Birmingham confirm our earlier findings (table 8). The precise origin of this population is uncertain since it has been in culture for at least ten years, but we can be certain that it is quite remote from the Oxford population sampled previously. The nature and pattern of hybridity in the Birmingham population is similar to that in the Oxford population though it shows considerably more stability; the degree of maximum multiple formation was never below 75 per cent. It also contains one individual heterozygous for a ring-of-six and two independent rings-of-four. This means that the two populations of *B. discoidalis* so far sampled have a minimum of 7 distinct interchanges while the minimum of 7 interchanges necessary to account for the number and morphology of multiples in the British populations of *P. americana* must now be increased still further to accommodate the discovery of the ring-of-eight in the La2 population.

Plate

C-induced diplotene-diakinesis in Pakistani cockroaches. Acetic orcein squash preparations, all ca. $\times 2250$.

FIG. 1.—16II+X(La2).

FIG. 2.—R·1V+14II+X(K2).

FIG. 3.—R·V1+13II+X(K1).

FIG. 4.—R·V1+13II+X(La1).

FIGS. 5 & 6.—R·V111+12II+X(La2).

Whether the interchanges present in the British populations are the same as those in Pakistan cannot be answered with confidence, though this is a conclusion we would tend to favour at the moment. Thus some of the pachytene crosses we have seen in individuals from Pakistan agree in morphology with those described earlier (John and Lewis, 1958). On the other hand there does seem to be a greater range of interchanges in Pakistan, for higher multiples are unquestionably more prevalent in our samples (see table 7). The reduction in

TABLE 8

*The incidence of interchange heterozygotes in
two populations of Blaberus discoidalis (2n = 37♂)*

Population	Classes						Total	
	18·11	1·1V	2·1V	3·1V	4·1V	1·V1 + 2·1V	Cells	Individuals
Birmingham.	2	11	6	...	...	1	400	20
Oxford*	2	10	9	3	1	...	850	25

* Data of John and Lewis (1959).

multiple range might then be correlated with the increased inbreeding involved in the production of the British populations for as Thompson and Rees (1956) and Rees (1961) have shown selection for interchange heterozygosity in the face of inbreeding is not indiscriminate. Selection clearly favours particular heterozygotic combinations.

In summary, there is no reason to doubt that the interchange system of *P. americana* is a means of conserving genic heterozygosity (Lewis and John, 1963*b*)

(ii) *Scorpions*

Throughout his numerous studies Piza made no attempt to account for the prolific interchange pattern which he uncovered in scorpions. White (1954) therefore proposed that, since meiosis in male scorpions is achiasmate, interchanges might serve to replace chiasmata as a means of generating new combinations of genes. To be effective this highly improbable suggestion requires recurrent chromosome breakage and exchange and requires them at an unprecedented frequency. White claimed support for his suggestion both from the occurrence of occasional fragment-fusion heterozygotes in some populations and from the wide range of chromosome numbers reported for different "races" of *Tityus bahiensis* (see for example da Cunha and Pavan 1954).

Now, where meiosis is achiasmate traditional diplotene-diakinesis stages do not occur; pachytene is followed simply by a contraction phase during which homologues maintain parallel alignment (Ullerich,

1961). This means that interchange multiples will maintain the cross configurations produced at zygotene pairing and will not open out into rings and chains. The centromere system of scorpions has been variously interpreted but the balance of evidence unquestionably favours the view that it is non-localised (Hughes-Schrader and Schrader, 1961). Even so the first division is reductional. The net result of this combination of circumstances must mean that almost all the meiotic products of cells containing interchange multiples will be unbalanced since the twisting of an interchange cross, which is necessary to produce alternate orientation, would be difficult, if not impossible, to effect in practice. Indeed all the interchange crosses figured by Piza at first metaphase must give non-disjunctional separation. It is difficult to see how a system of this kind can function in the manner suggested by White.

Matthieson (1962) has drawn attention to the interesting fact that females are much more common than males in both *T. bahiensis* and *T. trivittatus*. He explains this disparity of males as a trend to the complete female parthenogenesis which he claims to have found in the related species *T. serrulatus*. And, in keeping with White's idea, he suggests that, where interchanges occur, thelytoky serves as a mechanism to fix any useful genetic combinations which they generate. The fact that males are also less common in the Indian scorpions studied by Sharma, Parshad and Joneja (1959), which lack regular interchange hybridity, shows that a propensity for parthenogenesis appears to be present in the group.

We do not know what kind of parthenogenetic system occurs in scorpions but the commonest type found in animals seems to be the ameiotic type where meiosis is replaced by an essentially mitotic mechanism (White, 1954). If this is so in scorpions too then the interchange system would work perfectly well in the female since the necessity for meiotic regularity would then have been circumvented without forfeiting the advantages obtained by combining heterozygous combinations of genes in new linkage relationships. And to the extent that parthenogenesis is not obligatory any balanced sperm produced by normal or interchanged males would result in the production of both males and females. Under such a system, however, one would expect the proportion of males to fall rapidly in many if not most populations. This may well occur for as Brieger and Graner (1943) comment, in several of the localities which they sampled for *T. bahiensis* no males at all were encountered among the several hundred females found.

If this explanation has any validity then the presence of interchanges in scorpions is understandable. We are witnessing, in part at least, a process similar in principle to that which operates in many plants in which fertility-affecting chromosome mutations accumulate in species which reproduce predominantly or exclusively by asexual means (Koosho and Sharma, 1959; Lewis and John, 1961).

6. SUMMARY

1. Four populations of *P. americana* from Pakistan have been found to contain interchange multiples of four, six and eight.

2. The frequency of heterozygotes in the Pakistani populations is relatively uniform but it differs significantly from that found in coal mine and culture populations from Britain. They also differ in the distribution of the various karyotypes represented.

3. This difference in the level and pattern of heterozygosity can be correlated with the more common occurrence and mobility of *Periplaneta* in Pakistan, factors which both suggest less intensive inbreeding than that which obtains in British populations.

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