STUDIES ON *PERIPLANETA AMERICANA*

I. EXPERIMENTAL ANALYSIS OF MALE MEIOSIS

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I. INTRODUCTION

THE main cytological features of the american cockroach (*Periplaneta americana*) have been described by Morse (1909) and more particularly by Suomalainen (1946). The diploid number of the species is 33 ♂ 34 ♀ with an XO/XX type sex chromosome mechanism. The male meiotic sequence is of particular interest in relation to the problems of spindle development, bivalent association and chromosome movement. It is anomalous in the following respects :

- (i) The development of a polarised pachytene.
- (ii) The absence of visible chiasmata at all stages of meiosis.
- (iii) The complete absence of diplotene-diakinesis.
- (iv) The occurrence of a pre-metaphase stretch following spindle development at the end of pachytene.

A similar division sequence has been recorded for some mantids (White, 1941 ; Hughes-Schrader, 1943*a*, 1950), but all previous work has been concerned solely with the descriptive aspects of the sequence, no attempt having been made to analyse these anomalies experimentally. The present paper is concerned with such an analysis by the induction of ϵ -meiosis. Additional information has also been obtained from the study of spontaneous diploid interchange heterozygotes.

2. MATERIAL AND TECHNIQUE

Material for the present study was obtained from wild populations established in the following localities.

- (i) Deep Navigation Colliery, Treharris, Glamorgan (T).
- (ii) Wyndham Colliery, Ogmore Vale, Glamorgan (W).

For cytological analysis the testes of male imagines were dissected under saline (Baker, 1950), fixed in 1 : 3 acetic-alcohol and permanent squash preparations made in either acetic-orcein or aceto-carmin. Certain of the individuals were pre-treated for varying time periods by injecting solutions of ϵ -mitotic inductors into their hæmocœls between abdominal tergites four and five (table).

Each of the paired testes consists of numerous follicles which are small and globular. Each follicle is divided into a series of zones in which the sex cells are at distinct, though locally synchronised, stages of development. As there is only localised synchronisation of division, the treatment times outlined in the table limit only the maximum, and do not define the effective duration of treatment.

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3. OBSERVATIONS

(i) *Normal meiosis*

Previous workers have established the salient features of the meiotic sequence but their accounts require extending and correcting in certain respects. It is necessary, therefore, to summarise the course of male meiosis up until the organisation of the first metaphase plate :

- (i) Leptotene and zygotene are normal and there is no indication that zygotene pairing is in any way localised. At early pachytene the paired chromosomes are distributed at random throughout the nucleus.
- (ii) The polar organisers commence their activity during mid-pachytene and all the chromosome ends, though euchromatic, respond to this polarity resulting in a double bouquet-chromosome arrangement (plate, fig. 1).

TABLE
Schedule of treatments

G-mitotic inductor	Saline solution form injected	Treatment time
Alpha-bromo-naphthalene . . .	Saturated	2 hours
Coumarin	"	2 hours
Para-dichlorobenzene	"	4½ hours
Colchicine	0.05 per cent.	1 ; 2 ; 4½ ; 10½ hours 3 days

- (iii) Paired chromosomes retain a close parallel association until the end of pachytene but contract appreciably (plate, fig. 10). At this stage separation of homologous chromosomes is localised at the centromere and the nuclear membrane is still intact.
- (iv) On the dissolution of the nuclear membrane at the end of pachytene a spindle is organised in the nuclear area. The bivalents orientate on this spindle but do not congress to a metaphase plate. Instead there is a rapid and asynchronous polar movement of the centromeres with a consequent separation of homologous chromosomes (plate, fig. 2). This separation proceeds smoothly and progressively from the centromere but terminally the bivalents remain in association so that they become attenuated, often to extreme degrees, in a pre-metaphase stretch (plate, fig. 3).
- (v) With the exception of the single X chromosome, which is positively heteropycnotic and behaves as a univalent, the chromosomes stain feebly during the initial stage of the pre-metaphase stretch presenting a diffuse or "lampbrush" appearance (plate, fig. 2). This condition is similar to that in other species of Orthoptera (*cf.* Hsu, 1948, and Srivastava, 1954), but has not been previously recorded for *P. americana*.

- (vi) The pre-metaphase stretch is followed by an asynchronous re-approach of homologous centromeres, the congression of bivalents and the consequent organisation of a metaphase plate (plate, figs. 4 and 5). On recontraction the bivalents assume either a rod or ring type of configuration.

(ii) *C-meiosis*

The course of meiosis is considerably altered following the treatments summarised in the table. All the treatments produce the same general effects on the division sequence :

- (i) Late pachytene bivalents are morphologically similar to those observed in untreated material and the homologues are closely associated except for localised centromeric repulsion. There is, however, no indication of polar activity.
- (ii) On the dissolution of the nuclear membrane no spindle is organised, but the continued operation of repulsion in the centromeric loop results in the opening out of the bivalents until they remain associated terminally, rarely subterminally, either as rings or rods. This is accompanied by their progressive contraction (plate, figs. 6, 7 and 8).
- (iii) In the absence of a spindle the bivalents remain unorientated and dispersed so that this stage resembles diplotene-diakinesis except for the absence of the nuclear membrane.
- (iv) The highly condensed bivalents at "late diakinesis" have a pronounced tendency to clump, but even after three days' treatment with colchicine no indication of either restitution or pseudo-anaphase is observed, neither does any failure of pairing occur.

4. DISCUSSION

The problems of bivalent structure and chromosome movement implicit in the male meiosis of *P. americana*, apart from their own special evolutionary significance, are aspects of importance in relation to general chromosome mechanics. Although these have been discussed elsewhere (Cooper, 1949 ; Hughes-Schrader, 1950 ; Östergren, 1951 ; and Suomalainen, 1946), the present experiment makes a reconsideration of them desirable.

(i) *Bivalent structure*

Suomalainen (1946) argues that concealed chiasmata form the basis of the meiotic association in *P. americana*, supporting his argument by three principal observations :

- (i) The occurrence of visible chiasmata in certain other species of roaches.
- (ii) The occurrence of terminalisation resulting in the production of either ring or rod bivalents.
- (iii) The regular formation of a ring multiple in an individual heterozygous for a single interchange.

Cooper (1949), however, maintains that these observations are all open to alternative interpretation. To the observations suggesting chiasma formation can be added :

- (iv) The occasional observation of subterminal associations.
- (v) The development of open cross bivalents (plate, fig. 6).
- (vi) The persistence of terminal connections at the pre-metaphase stretch.
- (vii) The regular formation of multiple associations in spontaneous interchange heterozygotes.
- (viii) The relative constancy in the number of associated arms per nucleus at "induced diakinesis". In interchange heterozygotes the proportion of ring to rod bivalents decreases with increasing multiple formation, the total number of associated arms remaining relatively constant.

The association frequency is thus similar to that which one might expect were the chromosomes held together by chiasmata.

Individually these conditions can occur in the absence of chiasmata, but collectively the indirect evidence favours chiasma formation at least on occasion. The rapidity of terminalisation, however, would indicate that where chiasma occur they are formed near the ends of the chromosomes. It seems best to conclude that while the indirect evidence suggests the occurrence of chiasmata in some bivalents, the absence of non-centromeric loops indicates that an additional mechanism has been evolved to supplement or replace the mechanical association afforded by chiasmata. Hughes-Schrader (1950) suggests that this mechanism consists of a prolongation of parallel pairing by lateral attraction. While lateral attraction may operate in *P. americana*, it is more probable that the association of homologues, initiated by specific zygotene pairing, is maintained by a general and non-specific stickiness of the matrix. Three lines of evidence support this suggestion:

- (i) The centromeres are localised but, in addition to their association at the centromere, the pro-metaphase II chromatids are also held together at other regions (plate, fig. 9).
- (ii) Occasional bridges are observed connecting non-homologous bivalents (plate, fig. 10).
- (iii) In interchange heterozygotes, consequent upon failure of multiple formation, it follows that some of the "bivalents" must be incompletely homologous and therefore one would not expect a close parallel association of partner chromosomes along their entire length. Such a condition does, however, obtain and, since the interchanges generally involve long non-interstitial segments, torsion pairing can hardly account for such an association (plate, fig. 10).

These observations indicate the operation of a non-specific sticky

factor in maintaining chromosome association. During the stretch this matrical stickiness terminates: at maximum stretch and subsequent metaphase the meiotic association of bivalents is maintained solely by terminal affinity.

Summarising, it is suggested that, whilst chiasmata may be formed in *P. americana*, matrical stickiness and terminal affinity are the main factors which maintain the association of homologues.

(ii) Chromosome movement

Four independent forces have been suggested as contributing to the pre-metaphase stretch (Hughes-Schrader, 1943*a*, 1947):

- (i) Centromeric repulsion.
- (ii) Centrosome-centromere attraction.
- (iii) Spindle elongation.
- (iv) Half-spindle activity.

The first of these forces is operative during typical diplotene-diakinesis whereas the remainder are normally associated with the later meiotic stages. Obviously if the three latter forces could be suppressed experimentally then the pre-metaphase stretch would be largely inhibited and the continued action of repulsion in the centromeric loop might then be expected to result in the formation of diplotene-diakinesis type configurations. This expectation has been largely fulfilled. The main effect of *c*-inductors at meiosis in *P. americana* is comparable with that recorded by Levan in *Allium* (1939), namely an inactivation of the spindle apparatus. Spindle inhibition at *c*-meiosis has also been obtained in male *Chorthippus longicornis* (Coleman, 1947).

Östergren (1951), in discussing the subject of centromeric repulsion, is of the opinion that "there does not exist an action of this kind at any stage of mitosis and meiosis" and this despite evidence to the contrary (Hughes-Schrader, 1943*a*). There is no doubt from the present analysis that localised separation of homologues in the meiotic bivalent results from centromeric repulsion and is in no way connected with any centromere-centrosome attraction. Two observations support this contention:

- (i) Separation of the centromeres takes place at a time when the nuclear membrane is still intact. At this stage while the telomeres respond to polar activity, the centromeres show no such response and the plane of separation of the bivalent bears no relation to the polarity of the nucleus (*cf.* Le Calvez, 1947).
- (ii) Localised separation of the bivalents at the centromere takes place after *c*-induction treatment at a time when polar activity has been completely suppressed.

The movement of the centromeres during the pre-metaphase

stretch of *P. americana* is essentially similar to that which takes place at first anaphase. It differs from the latter, however, in two respects :

- (i) It occurs immediately following pachytene.
- (ii) It does not result in complete separation of the homologues.

From the mantid evidence, Hughes-Schrader (1950) has concluded that the absence of diplotene-diakinesis is in no way correlated with the pre-metaphase stretch. The present investigation shows that this is an over-generalisation.

Hughes-Schrader's own investigations support this contention. During the stretch phase in *Stagomantis carolina* (Virginia) the centromere is not the only locus of separation. Non-centromeric loops are also present which must result from generalised repulsion, or to use Hughes-Schrader's terminology, from a lapse of lateral attraction. This generalised repulsion is operative during typical diplotene-diakinesis and, in conjunction with centromeric repulsion, results in the opening out of the pachytene bivalents (Darlington, 1937). There is no reason to doubt that both these forces would continue to operate in the absence of a spindle as indeed they must do in most organisms, and that inhibition of the spindle in *Stagomantis* would result in the production of diplotene-diakinesis as it does in *P. americana*. Non-centromeric loops during the pre-metaphase stretch have also been described for *Tauromantis*, *Phyllovates* and *Stagomantis heterogamia* (Hughes-Schrader, 1950). It would appear, therefore, that all these mantids are comparable with *P. americana* : the absence of diplotene-diakinesis is due solely to the intervention of the pre-metaphase stretch.

Östergren (1951) has put forward two conditions on which the presence or the absence of the stretch depends :

- (i) The maintenance of parallel pairing.
- (ii) The degree of chromosome contraction prior to spindle formation.

The observations on *P. americana* support these suggestions. That there is a lapse of matrical stickiness is indicated by the fact that diplotene-diakinesis can be induced. Further the chromosomes, although contracted at late pachytene, become diffuse prior to their orientation. The pachytene stages of *Periplaneta* and *Callimantis* are comparable, but the difference in the subsequent behaviour of *Callimantis* can be attributed to the continued parallel pairing of the contracted chromosomes.

(iii) *Non-chiasmate meiosis*

It is now accepted that chiasmata are not an indispensable factor in maintaining the association of homologous chromosomes. This was first established by the classical work of Darlington (1934) on the autosomal bivalents of male *Drosophila pseudo-obscura* and has since

		MEIOTIC TYPE.				
		TYPE 1	NORMAL	TYPE 2		
CHIASMATA.		—	+	±		
PRE-MET. STRETCH.		—	—	±		
PROLONGED PAIRING.		+	—	—		
TERMINAL AFFINITY.		+	+	+		
PACHYTENE		MYCETOPHILID TYPE	L.P.P.	C.R.		
POST-PACHYTENE	PHRYNE TYPE	DROSOPHILA TYPE	C.R. DIPTOTENE	L.P.P.	S.F.
			DIKINESIS	DIFFUSE STAGE
		PRO-METAPHASE		PRE-METAPHASE STRETCH
METAPHASE		S.F.	S.F.
ANAPHASE		L.T.A.	L.T.A.
DIPTERA (REFS. 6,15,31,33,34)		MYCETOPHILIDAE BIBIONIDAE SCATOPSIDAE THAUMALEIDAE BLEPHAROCERIDAE PHRYNE	DROSOPHILIDAE MYCETOBIA	LIRIOPIDAE LIMONIIDAE PSYCHODIDAE SIMULIIDAE CULICIDAE CHIRONOMIDAE	...	
ORTHOPTERA	BLATTIDAE (REF. 28)	BLABERA	RAUSTRALASIAE	BLATTELLA	LOBOPTERA	P.AMERICANA BLATTA
	MANTIDAE (REFS. 11,13)	CALLIMANTIS PSEUDOMIOPTERYX HUMBERTIELLA	LITURGOUSA CHOERADODIS	PHYLLOVATES TAUROMANTIS STAGOMANTIS	STAGOMANTIS PARATENODERA ACANTHOTHESPIA MELLIERA ANGELA	

FIG. 1.—Schematic comparison of normal and non-chiasmate types of male meiosis.

C.R. = Centromeric repulsion : S.F. = Spindle formation.
L.P.P. = Lapse of parallel pairing : L.T.A. = Lapse of terminal attraction.

been confirmed by a number of workers on other species (Hughes-Schrader, 1943*b* ; Le Calvez, 1947 ; Piza, 1943 ; Wolf, 1941, 1950).

Meioses, where an alternative force either replaces or supplements chiasmata, are of two main types (fig. 1) ; types intermediate between normal meiosis and both of these variants are also known.

Type (1). In which parallel pairing is maintained until first anaphase. Here, homologues show no visible connection and chiasmata are clearly absent. The early repulsion of the centromeres may be suppressed as in *Drosophila*, *Blabera*, *Penthetria*, *Scatopse* and the mantids ; it may be evident during pachytene as in the mycetophilids, or during post-pachytene as in *Phryne* and *Thaumalea*. Finally, bivalents of the first and third categories may both occur in the same species as in *Periplaneta australasiae*. The observations on *Mycetobia* (Wolf, 1950), where bivalents with chiasmata occasionally show a persistence of parallel pairing, together with those on *Liturgousa* and *Choeradodis rhombicollis* (Hughes-Schrader, 1943*a*), where some bivalents are chiasmate whilst others are associated by lateral attraction, show stages intermediate between type (1) and the normal sequence.

Type (2). In which pachytene is followed by a pre-metaphase stretch which results in the opening out of the bivalents. Here there is no clear separation between homologous pairs except that localised at the centromere. The presence or absence of chiasmata in these cases cannot be established by observation. However, a number of mantid species (*Phyllovates impunctata*, *Tauromantis championi*, *Stagomantis heterogamia* and *Stagomantis carolina* (Virginia)), are intermediate between this type and normal meiosis : non-centromeric loops indicative of chiasma formation are apparent during the pre-metaphase stretch.

Normal meiosis has not been recorded in mantids, nor has the pre-metaphase stretch been found in dipterans. The mantids are, however, interesting in that they contain types intermediate between both extreme variants. The whole range of meiotic types from the normal to both extremes occurs only in the blattids. These considerations generally apply to only the autosomal bivalents.

The development of forces other than chiasmata capable of maintaining the meiotic association has permitted the independent evolution of a non-chiasmate meiotic sequence in several animal groups. Such a meiosis would tend to maintain gene sequences and would confer greater immediate fitness once favourable gene combinations had been built up. This may account for the evolution of this type of meiosis in outbreeding animals.

5. SUMMARY

1. In the normal meiotic sequence of male *P. americana* the spindle forms at the end of pachytene following polarisation of the nucleus.
2. Bivalent orientation is followed by a pre-metaphase stretch. Recontraction of the chromosomes with subsequent asynchronous congression leads to the formation of a metaphase plate.

Plate

Normal and *c*-meiosis in male *P. americana*. $\times 1800$.

(Figs. 6, 7, 8 and 10 are taken from interchange heterozygotes which have the following chromosome formula— $2.IV + 12.II + X$).

- FIG. 1.—Mid pachytene, double polarisation.
FIG. 2.—Early pre-metaphase stretch, chromosomes diffuse, orientated but not congressed.
FIG. 3.—Pre-metaphase stretch, chromosomes highly attenuated.
FIG. 4.—Pro-metaphase, chromosomes in congression.
FIG. 5.—Metaphase, chromosomes condensed, oriented and congressed.
FIG. 6.—Early induced "Diakinesis", chromosomes diffuse and non-oriented (α -bromonaphthalene 2 hr., individual T. 9).
FIG. 7.—Mid induced "diakinesis" chromosomes less diffuse (α -bromonaphthalene 2 hr., individual T. 1).
FIG. 8.—Late induced "diakinesis", chromosomes deeply stained and highly contracted (colchicine 1 hr., individual T. 23).
FIG. 9.—Pro-metaphase II, chromosomes with non-localised regions of association.
FIG. 10.—Late pachytene, with beginning of centromeric repulsion and non-homologous bridges present. Although this individual (T. 15) is heterozygous for two interchanges only one multiple has been formed in this nucleus. Two of the "bivalents" are therefore incompletely homologous yet all bivalents are closely associated along their entire length.

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3. The extreme pre-metaphase stretching of the bivalents is facilitated by a lapse of matrical stickiness and the diffuse nature of the chromosomes on their orientation.

4. Diplotene and diakinesis are suppressed by the pre-metaphase stretch. Injection of *c*-inductors results in the inhibition of the spindle and the absence of stretch. The continued operation of repulsion in the centromeric loop leads to the production of diplotene-diakinesis type nuclei.

5. The mechanical function normally performed by chiasmata is supplemented or replaced by matrical stickiness and terminal affinity. These forces are capable of maintaining the meiotic association in the absence of chiasmata.

6. The restriction of recombination, in the absence of chiasmata, may account for the establishment of non-chiasmate meiosis in outbreeding animals.