

# INVERSE MEIOSIS IN TRIPLOID FEMALES OF THE MEALY BUG, *PLANOCOCCUS CITRI*<sup>1,2</sup>

H. SHARAT CHANDRA<sup>3</sup>

*Department of Genetics, University of California, Berkeley, California*

Received June 5, 1962

**H**OLOKINETIC chromosomes, that is, chromosomes with diffuse kinetic activity, were first described by SCHRADER (1935). Since then they have been experimentally demonstrated in several hemipteran insects (HUGHES-SCHRADER and RIS 1941; BROWN 1960; BROWN and NELSON-REES 1961; HUGHES-SCHRADER and SCHRADER 1961) a louse (BAYREUTHER 1955) and in one genus of plants, *Luzula* (CASTRO, CAMARA and MALHEIROS 1949). This major variant in chromosome organization is probably characteristic of all the hemiptera and the Juncaeeae. In addition, diffuse or nonlocalized kinetochores have been suggested for chromosomes of several other groups of organisms, including crustaceans, certain algae and the Cyperaceae.

Concurrent with holokinetic organization the chromosomes of these organisms exhibit several departures from conventional behavior. Since there are no localized kinetochores, the four chromatids cannot be segregated two-by-two at the first meiotic anaphase by means of undivided centromeres. Apparently as an adaptation to the holokinetic condition (HUGHES-SCHRADER and SCHRADER 1961), modified meiotic sequences have arisen to assure an accurate segregation. The inversion of the normal meiotic sequence to give equational first and reductional second divisions is the chief, and, perhaps the only important modification. During cytogenetic studies with the mealy bug, *Planococcus* (= *Pseudococcus*) *citri* (Risso), it became apparent that triploid females afforded unusual possibilities for studying the inverted meiotic sequence.

## MATERIAL AND METHODS

Cultures of mealy bugs were grown on potatoes in glass jars according to methods described by NELSON-REES (1960). For the present work, the standard dose for routine production of triploid females was 90,000rep, delivered to adult males from a cobalt<sup>60</sup> source belonging to the Bio-organic Chemistry Group of

<sup>1</sup> This work was supported in part by a grant from the National Science Foundation (G9772) to PROFESSOR SPENCER W. BROWN.

<sup>2</sup> Part of a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Genetics.

<sup>3</sup> Predoctoral trainee in Genetics (1961-62), N.I.H. research training grant (#26-367, U.S. Public Health Service). Present address: The Institute for Cancer Research, Philadelphia 11, Pennsylvania.

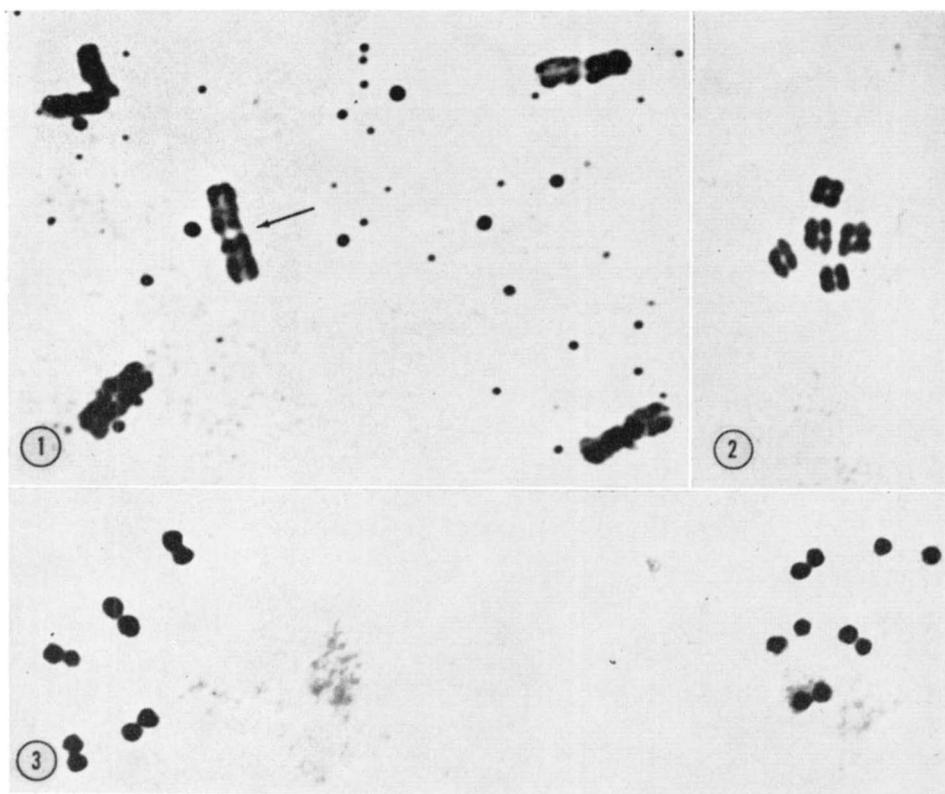
the University's Lawrence Radiation Laboratory. For further study the females that developed after high doses of paternal irradiation were isolated in glass vials when adult and mated to stock males. They were fixed about 70 hours after mating in Bradley-Carnoy (four parts chloroform : three parts absolute alcohol: one part glacial acetic acid) and kept in a refrigerator until used. A few drops of mordant, a saturated solution of ferric acetate in propionic acid, which were added to the fixed material about 24 hours before squashing in acetocarmine, greatly improved the stainability of chromosomes with carmine. All negatives were exposed at  $1140\times$  and enlarged subsequently.

### Observations

*Origin of triploids:* After high dosage paternal irradiation of 60,000–120,000rep, nearly all the offspring are female and these appear at about 40 percent of the control value. The large majority of these females are triploid; the remainder are diploid and  $3N/2N$  and  $2N/N$  mosaics. After such heavy doses, one set of chromosomes is grossly damaged in all the biparental embryos. No such broken chromosomes are seen in the daughters developing after the high doses and these are known to be gynogenetic (CHANDRA, Unpublished).

- The origin of the gynogenetic females is quite complex, and a separate report is being prepared on the embryonic cytology following the high dosage treatments. For the present purposes suffice it to say that the triploid complement stems from the polar bodies; these normally fuse to give rise to a *polar nucleus* (SCHRADER 1923) which then participates in the formation of a special structure, the mycetome, containing the intracellular symbionts (SCHRADER 1923). The triploid adults arise from embryos in which the zygotic derivatives are moribund and the triploid sector has successfully undertaken the task of embryogeny. All data on triploid cytology in the present report were obtained from gynogenetic females in which there was no evidence for chromosomal mosaicism.

*Meiosis in the diploid:* Chromosome behavior in our diploid material is essentially like that described by SCHRADER (1923). As in other coccids investigated so far it is not possible to analyze early meiotic events in the female. The earliest analyzable stage is early diakinesis, and the cytology becomes very clear from late diakinesis onwards. During diakinesis the oocyte usually has a large number of stained droplets which disappear just prior to metaphase. The five bivalents at diakinesis are mostly X- or cross-shaped and have one chiasma each. Extensive observations by several investigators in this laboratory have not revealed a single case of more than one chiasma per bivalent. Completion of terminalization of the chiasma leads to a bivalent with the two homologous chromosomes lying end to end (Figure 1). The four chromatids continue condensing until the long and short axes become indistinguishable (Figure 2). At metaphase, the five bivalents come together and form a compact entity in which individual chromosomes are difficult to identify. At late anaphase I five dyads are countable at either side (Figure 3). One of the groups of ten chromatids becomes the first polar body. The dyads in the secondary oocyte fall apart and, after condensing somewhat,



FIGURES 1-3.—First meiotic division in diploid females. Figure 1. Late diakinesis showing five bivalents, each with a terminalized chiasma (indicated by arrow for one bivalent). Figure 2. Polar view of prometaphase. Figure 3. Post anaphase I showing ten chromatids on either side (all 1500 $\times$ ).

reassociate for segregation at metaphase II. After second division is completed, the first and second polar bodies remain static until the first few cleavage divisions in the embryo; they then fuse to form a polar nucleus with 15 chromosomes (Figure 18). These 15-chromosome nuclei proceed to form a sector of even higher ploidy in the embryo by fusing *inter se* and with cleavage nuclei.

*Meiosis in the triploid:* A varying number of tri-, bi-, and univalents were seen at diakinesis in triploid oocytes. Trivalents always had two and only two chiasmata during diakinesis; if neither were terminalized, the configuration was a "double cross" structure. Complete terminalization of chiasmata resulted in a typical chain of three chromosomes (Figure 4). In four oocytes configurations resembling nonhomologous associations were noticed; because of difficulties in interpreting these, they have not been included in Table 1. Bivalents always had one chiasma each as in the diploid material. The frequency of the two kinds of associations and of univalents in 60 ovarioles is given in Table 1. A total of 447 chiasmata were observed, in remarkably close agreement to one and a half times that found in diploids; (in 60 diploid ovarioles, 300 chiasmata would be found;

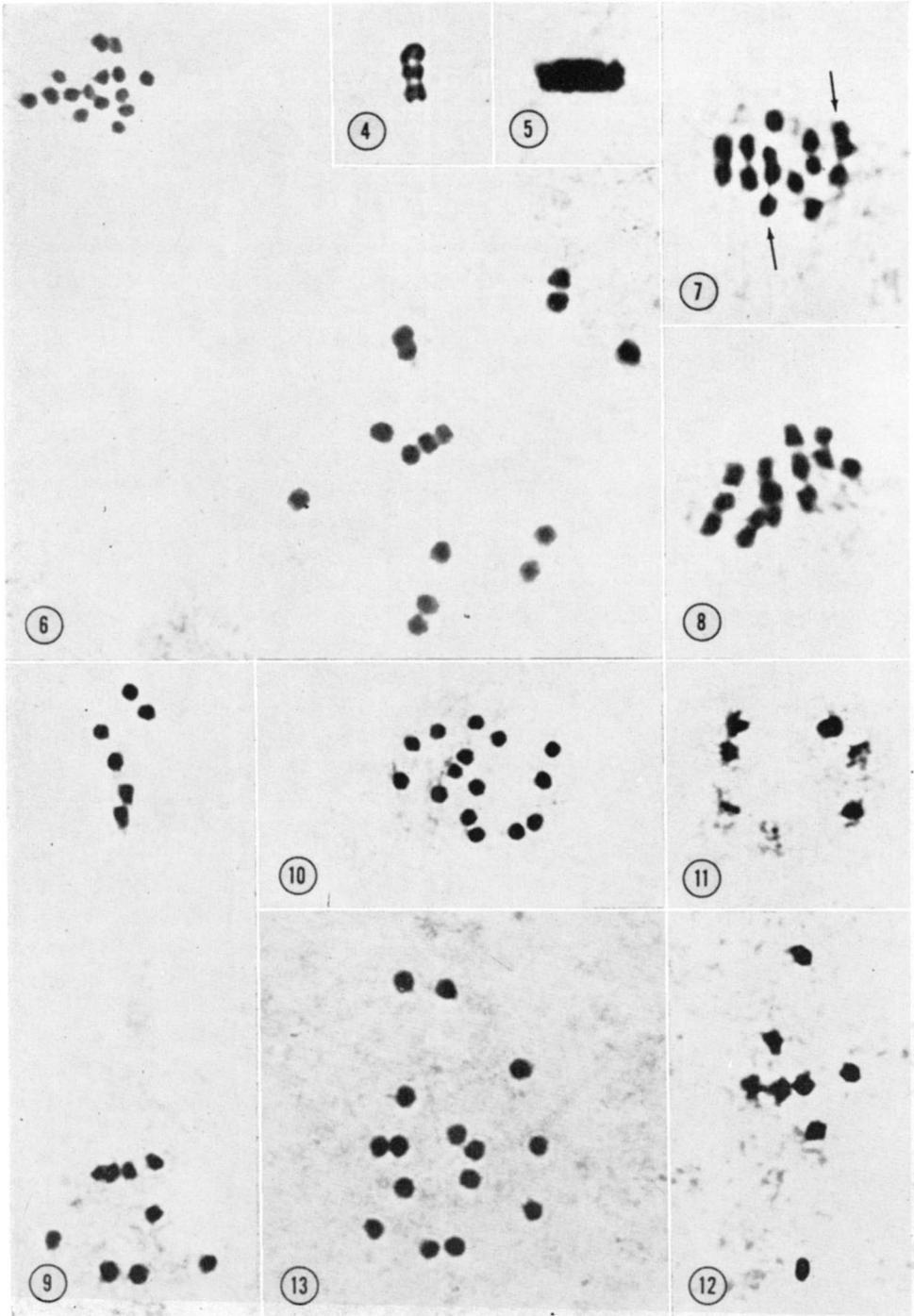


TABLE 1

*Frequency of tri-, bi-, and univalents and number of chiasmata in 60 triploid oocytes*

Number of oocytes	Trivalents	Bivalents	Univalents	Number of chiasmata per oocyte	Total number of chiasmata
2	5	0	0	10	20
10	4	1	1	9	90
3	4	0	3	8	24
16	3	2	2	8	128
4	3	1	4	7	28
11	2	3	3	7	77
4	2	2	5	6	24
7	1	4	4	6	42
2	1	3	6	5	10
1	0	4	7	4	4
Total 60					447

one and a half times this is 450). Such a relationship in number of chiasmata between diploid and triploid material has been found in several autotriploid plants (DARLINGTON 1937; WILSON 1958).

At metaphase I, the chromosomes form a compact rectangular group (Figure 5). Over 50 such first metaphases were observed and all of them had a uniformly rectangular appearance; such a rectangular structure can be consistently formed only when all chromosomes, especially the trivalents, always auto-orient (see legend, Figure 20). At anaphase I 15 chromatids moved to each of the two poles. Since the segregation was *always* 15:15 (Figure 6), it was obviously not influenced by the nature or frequency of prior associations.

*Reassociation during second division:* In the secondary oocyte, the chromatids fell apart and subsequently came together for reassociation at prophase II. All chromatids appeared as closely packed dots of uniform size which made it difficult to distinguish true associations from those that were not, especially since squashing pressure during preparation of the slide may have altered the real relationships. Consequently, evidence for reassociation can be obtained only by the examination of figures at metaphase II or very early anaphase II, just prior to separation. The second meiotic division is apparently completed very rapidly

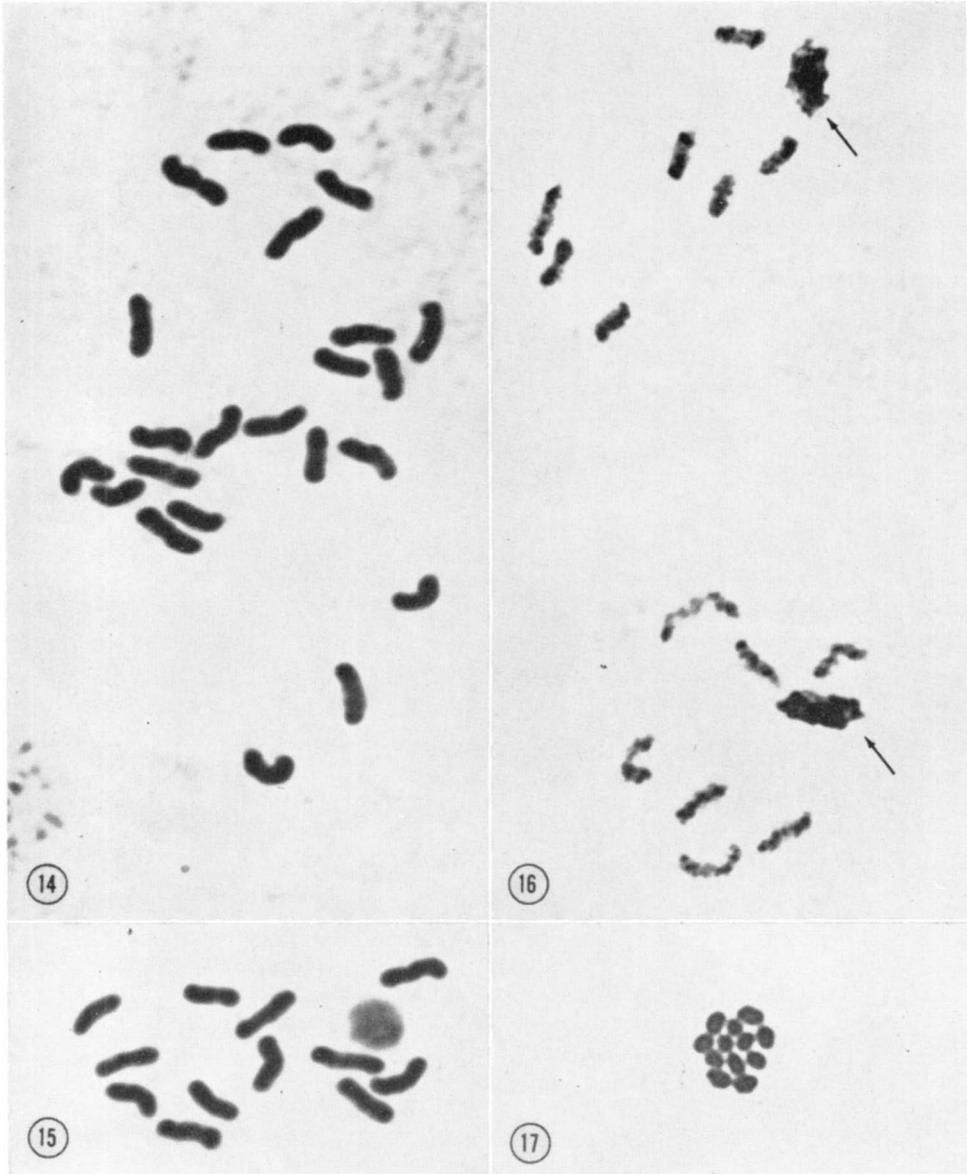
FIGURES 4-13.—Meiosis in the triploid. Figure 4. A trivalent with two terminalized chiasmata. Figure 5. Metaphase I; note lack of any protruding chromosomes. Figure 6. Post anaphase I showing 15:15 segregation of chromatids. The group to the left is the secondary oocyte preparatory to second division; "half-chiasmata" are still evident in the first polar body to the right. Figures 7 and 8. Secondary oocyte and first polar body from the same ovariole. Figure 7. Meta-anaphase II; two triads indicated by arrows. Figure 8. Polar body I; note complete reassociation into five triads. Figures 9 and 10. Division of the secondary oocyte and polar body I from the same ovariole. Figure 9. 9:6 segregation. Figure 10. Polar body I with 15 chromosomes. Figures 11-13. Egg, polar body II and polar body I from the same ovariole. Figure 11. Egg nucleus with seven chromosomes. Figure 12. Eight chromosomes in polar body II. Figure 13. Polar body I with 15 chromosomes. (Figures 4, 7 and 8, 2000 $\times$ ; all others 1500 $\times$ .)

since an extensive search revealed plenty of earlier and later stages but only two early anaphases. One of these, illustrated as Figure 7, shows two definite triads while the remaining chromatids can be interpreted as forming three dyads and three apparently unassociated chromatids. The remaining early second anaphase figure showed three triads, two dyads and two unassociated chromatids. In both these oocytes there was *complete* reassociation of the homologous chromatids in the first polar body to form five triads even though the latter does not divide prior to fusion with the second polar body (Figure 8). Because second division stages were quite infrequently observed, it was necessary to determine the products of this division by studying the resultant embryos.

*Tetrad analysis:* In young embryos, a cytological kind of tetrad analysis (BROWN 1960) is possible. As previously mentioned, the two polar bodies participate in the formation of the symbiont-bearing polyploid cells. In the diploid material, the diploid first polar body and the haploid second polar body fuse to form a triploid polar nucleus (Figure 18); the triploid nucleus then divides several times and these division products fuse with cleavage nuclei to give pentaploid nuclei. The pentaploid nuclei thus contain all the four chromosome complements resulting from meiosis in the oocyte, three of the four from the polar bodies and one from the egg via the zygote and cleavage nuclei, plus an additional complement from the sperm, also via the zygote and cleavage nuclei. In the triploid, the chromosomes which had been partitioned unequally between the egg and polar body II are thus added together again in the formation of the polyploid nuclei and, barring chromosome loss, are expected to total uniformly to 35 (Figures 14, 15). In other words,  $15(\text{Polar body I}) + m(\text{Polar body II}) + n(\text{egg}) + 5(\text{sperm})$  will always equal 35 since  $m + n$  always equals 15.

*Frequency distribution of chromosome numbers in embryos following  $3N\text{♀} \times 2N\text{♂}$  matings:* Over 300 embryos from 12 triploid mothers were studied for chromosome number. All embryos inside a gravid mother were dissected out and squashed; almost all unanalyzable embryos were also recorded. Embryogenesis in the triploid showed the typical lecanoid heterochromatization as previously reported for the diploids: in embryos developing as males, the paternal chromosome set becomes heterochromatic at blastula and remains so throughout development (HUGHES-SCHRADER 1948; BROWN and NELSON-REES 1961). Most of the eggs produced by triploid mothers were aneuploid; only those embryos with five heterochromatic plus five euchromatic chromosomes (males), and ten or 15 euchromatic chromosomes (females) survived; all others were lethal before gastrulation. Among the embryos with aneuploid numbers, the female embryos simply degenerated while the males showed repeated endomitosis of the euchromatic set prior to the onset of degeneration. There was no indication of any kind of differential viability among the different embryo classes with aneuploid numbers. The cytological aspects of lethality due to chromosome imbalance is being described in greater detail in a separate report.

The frequency distribution of the maternal contribution to the zygote in 272 embryos in which chromosome number could be determined is given in Figure 19. The maternal contribution was determined for each embryo by deleting the



FIGURES 14-15.—Cytological tetrad analysis; division products of polar and cleavage nuclei from a female embryo. Figure 14. Polar nucleus derivative with 23 chromosomes (15 from polar body I + eight from polar body II). Figure 15. A cleavage nucleus with 12 chromosomes (seven from the egg + five from the sperm) and a nucleolus. Figures 16 and 17. Cleavage nuclei from the same male embryo. Figure 16. Two adjacent nuclei each with seven euchromatic chromosomes from the mother and the five paternal chromosomes in the heterochromatic clump (arrows). Figure 17. Mitotic metaphase with 12 chromosomes; note lack of distinction between euchromatic and heterochromatic sets at this stage (all  $2000\times$ ).

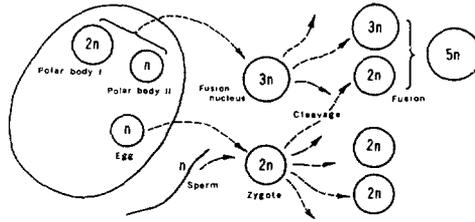


FIGURE 18.—Origin of pentaploid nuclei during early embryogeny in diploid embryos. Some of the cleavage nuclei of the triploid fusion nucleus (= polar nucleus) fuse with cleavage nuclei from the zygote to form pentaploid nuclei.

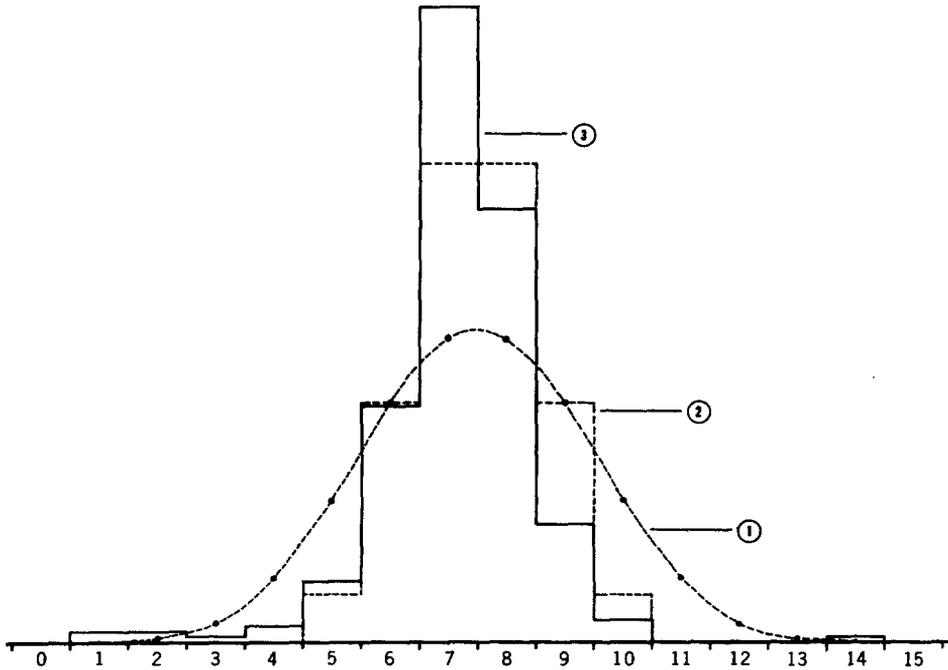


FIGURE 19.—Expected and observed frequency distribution of chromosome numbers among 272 embryos from triploid mothers. The numbers cited are five less than those in the embryos because paternal contribution of five has been subtracted in each instance. 1. Expected curve if segregation during the second meiotic division is random; it is given as a continuous distribution to avoid overlapping with the other histograms. 2. Expected distribution if segregation is based on pairing in the secondary oocyte. 3. Observed distribution. The actual numbers observed were: 1 = 2; 2 = 2; 3 = 1; 4 = 3; 5 = 11; 6 = 42; 7 = 108; 8 = 77; 9 = 21; 10 = 4; 14 = 1.

paternal contribution of five chromosomes from the number observed in the embryonic nuclei.

On the assumption that the three homologous chromatids reassociate at prophase II to give a triad, then this triad will probably disjoin in a 1-2 fashion, with perhaps equal probability of "1" or "2" going to either pole. The formation of balanced gametes will then conform to the binomial  $(\frac{1}{2} "1" + \frac{1}{2} "2")^5$ . Even

if prophase II association should happen to be a dyad and a monad instead of a triad, with the dyad disjoining regularly and the monad moving at random, the expectation would remain the same. In Figure 19, histograms of numbers expected if there is pairing and that of the observed numbers are given along with the one expected if the chromatids segregated at random. The observed frequencies compare well with those expected if segregation is mainly through pairing during second prophase. The direct cytological evidence mentioned before coupled with this indirect evidence indicates that second division segregation takes place mainly through reassociation of the homologous chromatids in most of the ovarioles. However, embryos with less than ten chromosomes and also with more than 15 (that is, less than five or more than ten chromosomes from the mother, respectively) do occur. One embryo with 14 chromosomes from the mother (14 euchromatic + five heterochromatic) was observed. This embryo, a "male," was in an advanced state of degeneration but two clear figures with 5H + 14E chromosomes could be counted. The other classes of embryos with less than ten chromosomes (that is, less than five from the mother) were clear-cut examples with many division figures. It is not known what sort of pairing, if any, preceded the production of eggs with these low and high numbers; it is possible that they arose through random segregation following failure of pairing. If they did arise from such a failure to reassociate at prophase II, then some embryos in the 5-10 classes of Figure 19 probably also arose from ovarioles in which second division pairing was either absent or extremely low.

It is also apparent from Figure 19 that there were more embryos in the classes with lower numbers; the difference between embryo classes 6-12 and 13-19 (which are expected in equal frequency) is highly significant ( $\chi^2 = 8$ ,  $P < .01$ ). If embryos with 6, 7, 8, 9 and 14 chromosomes are eliminated, since they might have come about through some unusual events during second division, the difference between classes 10-12 and 13-15 is still significant ( $\chi^2 = 6.7$ ,  $P < .01$ ). Even among embryos which are out of the range expected after pairing, there is again bias in favor of lower numbers (Figure 19, classes 1-4 and 11-14). Four possible reasons for this bias in favor of lower numbers have been considered. Firstly, meiotic loss of chromosomes, if it occurred, would lower the total number of chromosomes available for distribution and thus be responsible for the increase in embryo classes with fewer chromosomes. In this material loss of chromosomes during meiosis has yet to be encountered. During these and other studies, a large number of first polar bodies have been observed since they remain cytologically favorable for counting for a considerable period following I anaphase; all of them had 15 chromosomes. Among the 272 embryos analyzed, it was possible to do "tetrad analysis" in 18 young embryos; in all of them, the number of chromosomes totaled 35, with no evidence of loss. Although it is possible that loss of chromosomes occurs at a low frequency, it could not have contributed in any significant measure to the observed shift toward lower numbers among embryos listed in Figure 19.

Secondly, bias in scoring, that is, greater difficulty in scoring higher chromosome numbers, might bring about a shift to the lower numbers. In moribund

embryos in this species many mitoses apparently stop at mid to late prophase. Since most of the embryos in the triploid mothers died prior to gastrulation, a large number of clearly separated division figures with well-spread chromosomes were usually present. Furthermore, of the 272 embryos scored, 149 were male, that is, showed heterochromatization of the paternal set; scoring the chromosome constitution of male embryos is done simply by counting the number of euchromatic chromosomes since the heteropycnotic paternal complement is always five. In addition to the 272 embryos in which chromosome number could be determined there were about 35 embryos which could not be analyzed either because they were in an advanced stage of degeneration or lacked division figures. It is believed that omission of these 35 would not lead to bias since there was otherwise no indication of differential viability among the aneuploid embryos. For these reasons, it does not seem likely that bias in scoring or differential viability has contributed in any appreciable manner to the bias. Consequently, it seems highly likely that the shift toward embryo classes with lower numbers is a real one, based on a tendency on the part of the triploid mothers to deliver fewer chromosomes to the egg and more to the second polar body.

#### DISCUSSION

The terms equational and reductional have different meanings depending upon their usage in a cytological or genetical sense. This has led to considerable confusion in the past in spite of repeated clarification (HUGHES-SCHRADER 1955; see RHOADES 1961 for a recent appraisal of these terms).

In the holokinetic chromosome terminalization of chiasmata results in a bivalent in which the two homologous chromosomes come to lie end to end. If these two chromosomes orient *independently*, with one chromatid of each chromosome on either side of the equator, then the bivalent, or more precisely, the two chromosomes comprising the bivalent, are said to have auto-oriented (Figure 20); the division that follows is then said to be equational in a cytological sense without reference to the genetic makeup of the chromatids on either side of the metaphase plate. A first meiotic division which is always equational in this cytological sense will be genetically equational for some chromosomes or chromosome segments depending upon prior meiotic events, particularly, points of crossing-over.

In the early part of this century the detection of sex chromosomes in several species (see DARLINGTON 1937, p. 373) which auto-oriented and divided equationally during the first meiotic division opened the possibility that the entire chromosome complement of a species may do so. That the whole complement may divide equationally at anaphase I, with reduction accomplished in the second division, is a conclusion based on a considerable body of observational evidence from several species with holokinetic chromosomes. It was first suggested on the bases of THOMSEN'S (1927) observations on a soft scale, *Lecanium hemisphaericum*. During oogenesis in this species metaphase I cytology was favorable enough for the observation of auto-orientation. On the other hand, in

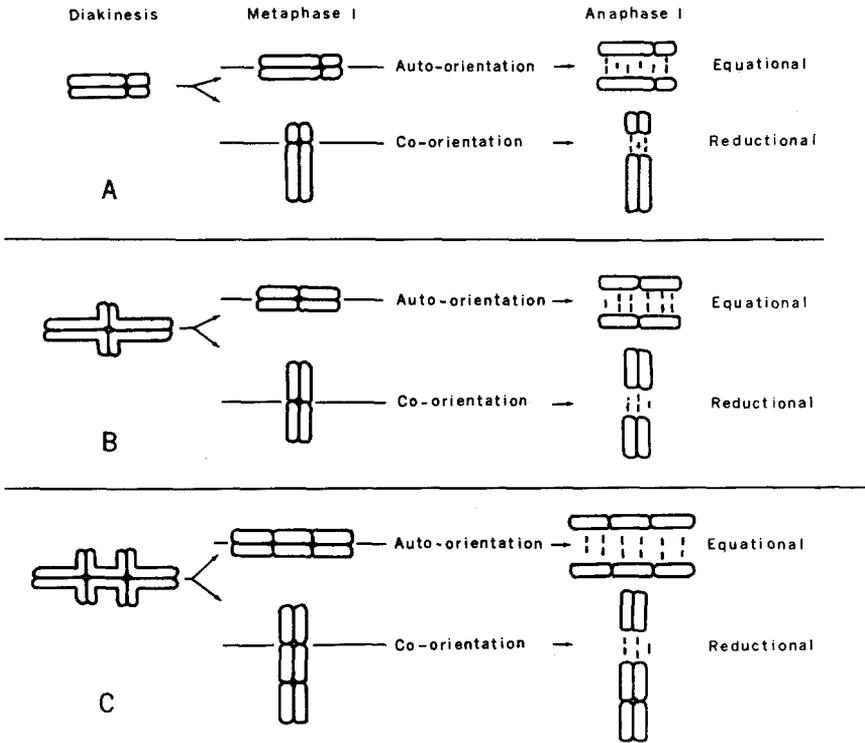


FIGURE 20.—Meiotic behavior of holokinetic chromosomes from diakinesis to anaphase I to illustrate orientation and division. For each association the two possible methods of orientation are given with the consequent division. A. Heteromorphic bivalent. B. Bivalent with one chiasma. C. Trivalent with two chiasmata. Note that in a material like *P. citri*, if all chromosomes, including the trivalents, auto-orient, metaphase I will be a compact rectangular entity (Figure 5). If they co-orient, the polar surfaces of the metaphase I group will be uneven because of co-orientation of the trivalents.

most other coccids investigated so far, the chromosomes of a bivalent condense to such a degree that the long and short axes become indistinguishable. Such extreme condensation makes a study of the manner of orientation impossible. However, additional observations on orientation of chromosomes at metaphase I in favorable material by HUGHES-SCHRADER (1944, 1948, 1955) and others on the other coccids (THOMSEN 1927; SUOMALAINEN 1940) and by RIS (1942) on the aphid, *Tamalia coweni*, have made an equational I division, with reduction in the second, one of the main features of the "aphid-coccid" type of meiosis (HUGHES-SCHRADER 1948).

Experimental evidence in favor of an equational first division comes from LACOUR's (1953) investigations of irradiated pollen mother cells of *Luzula purpurea*. He provided an example of a translocation ring-of-four dividing "mitotically" at anaphase I. In addition he observed, in tetraploid material, a half-ring-of-four, two half-trivalents and a half-bivalent—all retaining their respective half-chiasmata—in each of the two daughter nuclei following the first

meiotic anaphase. Such a distribution is positive evidence for an equational separation at anaphase I in this species.

In triploid females of the mealy bug, if all chromosomes irrespective of their valency and prior participation in pairing and exchanges auto-orient at metaphase I, there should *always* be a 15:15 segregation of chromatids at anaphase I (Figure 20C). This is indeed observed. Thus, a numerically equational division demonstrates its cytologically equational nature and must stem from auto-orientation of chromosomes at metaphase I; a numerically reductional division would have demonstrated reduction in the cytological sense as the result of co-orientation. The reverse relationship is true in the second division.

As the limited amount of direct cytological evidence and the frequency distribution of chromosome numbers in  $F_1$  embryos indicated, the homologous chromatids co-orient at metaphase II as triads or dyads, at least in the great majority of ovarioles, for segregation; some may have segregated as monads.

*Distribution of chromosome numbers in embryos:* Studies on the frequency distribution of chromosome counts in embryos following  $3N\text{♀} \times 2N\text{♂}$  matings have been made for a number of plant species. These include petunia, *Lolium*, sugar beet, maize, tomato, *Datura* and *Tulipa*. Among animals such analysis has been carried out in detail only for the axolotl by FANKHAUSER and HUMPHREY (1950); their Figure 7 gives the distribution of chromosome counts for axolotl and in the plants mentioned above, with the necessary references. The table also shows that there is a shift toward embryo classes with lower numbers in all these seven species of plants as well as in the axolotl. In all these species the chromosomes have localized centromeres. Chromosome lagging and other abnormalities leading to loss were frequently observed in those cases where chromosome behavior during female meiosis was studied. Hence, the investigators rightly ascribed the shift in almost all cases as due mainly to chromosome loss during meiosis. In *Datura*, SATINA and BLAKESLEE (1937) made a detailed study of the frequency of laggards, restitution nuclei and other sources of loss during megasporogenesis in the same stock of triploid plants from which the frequency distribution of chromosome counts in the progeny was obtained. The frequency of chromosome loss was adequate to explain the shift toward lower chromosome numbers.

Recently, NUR (1962) has found in another mealy bug a case of preferential recovery of a supernumerary chromosome similar to that observed in triploid females of the present study. The heterochromatic supernumerary chromosomes, in the females of the species, divided equationally during the first meiotic division, reassociated mostly in pairs and segregated during the second. They were never observed to lag or be eliminated during either of the meiotic divisions. When an even number of supernumeraries (two or four) was present, they reassociated in pairs and segregated normally. If a single supernumerary or an odd number, three or five, was present in the female, the odd chromosome was twice as likely to end up in polar body II as in the egg, thus resulting in a higher number of embryos with fewer supernumerary chromosomes than expected. The probability of an odd chromosome being included in the egg *vs.* the second polar

body was very similar in females with one (0.34), three (0.39) and five (0.33) supernumeraries.

Among the eggs with five to ten chromosomes formed by triploid *P. citri* (see Figure 19), each of which received at least one haploid set of chromosomes from the mother, the probability of a chromosome from the third set going to the egg was 0.39. This value, very close to those observed for the supernumerary chromosomes by NUR, is probably therefore the consequence of a general phenomenon of meiotic behavior in the secondary oocyte when an odd number of chromatids is present.

In the mealy bug, as emphasized earlier, loss of chromosomes has never been observed. Holokinetic chromosomes show more stability than chromosomes with a localized kinetochore under conditions such as those imposed by triploidy. The regular orientation and division of univalents during the first meiotic division and segregation rather than loss of single, free chromatids during the second are evidences for this conclusion.

#### SUMMARY

The sequence of meiotic divisions, the first usually being reductional and the second, equational, has been demonstrated to be inverted in a mealy bug, using triploid females. At first anaphase in the triploid there was always a 15:15 separation of chromatids; the diffuse nature of the kinetochore permits such a numerically equational separation. Reduction is accomplished in the second division in which there is evidence for reassociation or "secondary pairing" of the homologous chromatids into triads or dyads in most of the oocytes. The maternal contribution to the zygote following  $3N\text{♀} \times 2N\text{♂}$  matings was studied in 272 embryos. There was a significant bias in distribution in favor of lower chromosome numbers. It is suggested that the bias is probably real, and based on a definite tendency of triploid mothers to deliver fewer chromosomes to the egg than to the second polar body.

#### ACKNOWLEDGMENTS

I am very grateful to PROFESSOR SPENCER W. BROWN for many suggestions during the work and help during the preparation of the manuscript. DR. UZI NUR kindly provided the negative for Figure 1 and contributed much in the way of discussion. MRS. LORA WEIGMANN assisted with the cultures.

#### LITERATURE CITED

- BAYREUTHER, K., 1955 Holokinetic chromosomes bei *Haematopinus suis* (Anoplura, Haematopiniidae). *Chromosoma* **7**: 260-270.
- BROWN, S. W., 1960 Chromosome aberration in two aspidiotine species of the armored scale insects (Coccoidea-Diaspididae). *Nucleus* **3**: 135-160.
- BROWN, S. W., and W. A. NELSON-REES, 1961 Radiation analysis of a lecanoid genetic system. *Genetics* **46**: 983-1007.
- CASTRO, D., A. CÂMARA, and N. MALHEIROS, 1949 X-rays in the centromere problem of *Luzula purpurea* Link. *Genet. Iberica* **1**: 48-54.

- DARLINGTON, C. D., 1937 *Recent Advances in Cytology*. Second edition. Churchill. London.
- FANKHAUSER, G., and R. R. HUMPHREY, 1950 Chromosome number and development of progeny of triploid axolotl females mated with diploid males. *J. Exptl. Zool.* **115**: 207-249.
- HUGHES-SCHRADER, S., 1944 A primitive coccid chromosome cycle in *Puto* sp. *Biol. Bull.* **87**: 167-176.
- 1948 Cytology of coccids (Coccoidea-Homoptera). *Advan. Genet.* **2**: 127-203.
- 1955 The chromosomes of the giant scale *Aspidoproctus maximus* (Coccoidea-Margarodidae) with special reference to asynapsis and sperm formation. *Chromosoma* **7**: 420-438.
- HUGHES-SCHRADER, S., and H. RIS, 1941 The diffuse spindle attachment of coccids, verified by the mitotic behavior of induced chromosome fragments. *J. Exptl. Zool.* **87**: 429-456.
- HUGHES-SCHRADER, S., and F. SCHRADER, 1961 The kinetochore of the Hemiptera. *Chromosoma* **12**: 327-350.
- LACOUR, L. F., 1953 The *Luzula* system analyzed by X-rays. *Heredity (Suppl.)* **6**: 77-81.
- NELSON-REES, W. A., 1960 A study of sex predetermination in the mealy bug, *Planococcus citri* (Risso). *J. Exptl. Zool.* **144**: 111-137.
- NUR, U., 1962 Cytogenetics and population studies of supernumerary chromosomes in a mealy bug. Ph.D. dissertation. University of California, Berkeley.
- RHOADES, M. M., 1961 Meiosis. Pp. 1-75. *The Cell*. Edited by J. BRACHET and E. MIRSKY, Vol. 3. Academic Press. New York and London.
- RIS, H., 1942 A cytological and experimental analysis of the meiotic behavior of the univalent X-chromosome in the bearberry aphid *Tamalia (=Phyllaphis) Coweni* (Ckll.). *J. Exptl. Zool.* **90**: 267-326.
- SATINA, S., and A. F. BLAKESLEE, 1937 Chromosome behavior in triploid *Datura*. II. The female gametophyte. *Am. J. Botany* **24**: 621-627.
- SCHRADER, F., 1923 The sex ratio and oogenesis of *Pseudococcus citri*. *Z. Ind. Abst. Vererb.* **30**: 163-182.
- 1935 Notes on the mitotic behavior of long chromosomes. *Cytologia* **6**: 422-430.
- SUOMALAINEN, E., 1940 Beiträge zur zytologie der parthenogenetischen insekten. II. *Lecanium hemisphaericum* (Coccidae). *Ann. Acad. Sci. Fennicae (Ser. A)* **57**: 1-30.
- THOMSEN, M., 1927 Studien über die Parthenogenese bei einigen Cocciden und Aleurodiden. *Z. Zellforsch.* **5**: 1-116.
- WILSON, J. Y., 1958 Cytogenetics of triploid bluebells *Endymion nonscriptus* (L.) Garcke and *E. hispanicus* (Mill.) Chourad. *Cytologia* **23**: 435-446.