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CYTOGENETIC STUDIES FOLLOWING HIGH DOSAGE PATERNAL IRRADIATION IN THE MEALY BUG, PLANOCOCCUS CITRI

I. CYTOLOGY OF X_1 EMBRYOS * **

By

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With 41 Figures in the Text

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The mealy bug, *Planococcus citri* (RISSO) *(Coccoidea-Homoptera)* has a "lecanoid" system of chromosome behavior (HUGHES-SCHRADER 1948). The salient features of this system, as exemplified by *P. citri*, may be briefly summarized as follows: both males and females start development as diploids with 10 chromosomes each. The adult female goes through an aphid-coccid type of meiosis (HUGHES-SCHRADER loc. cit.). In the male, one set of chromosomes becomes heterochromatic during early embryogeny and remains so until it is eliminated in the adult following spermatogenesis. In common with those of other coccids (HUGHES-SCHRADER and RIS 1941), the chromosomes of the mealy bug are endowed with diffuse kinetic activity so that chromosome fragments persist through successive mitotic cycles.

By using radiation-induced chromosome fragmentation and dominant lethality, BROWN and NELSON-Rees (1961) have recently reported their analysis of the lecanoid system in *P. citri*. Since the present report is an outgrowth of their experiments, their results will be briefly summarized.

BROWN and NELSON-REES tested the two hypotheses of the SCHRADERS (SCHRADER and HUGHES-SCHRADER 1931, HUGHES-SCHRADER 1948) concerning the lecanoid male: that the heterochromatic set is paternal in origin and that it is genetically inert. After paternal irradiation (P. I.) chromosome fragmentation in the male embryos was limited to the heterochromatic set, thereby demonstrating its paternal origin. The inertness of the heterochromatic set was shown by the response of the X_1 (F_1) progeny following P. I. with low doses. Thus, following 2,000 to 16,000 r of P. I. the number of daughters declined drastically with increasing dose while the number of sons remained unaffected; at 16,000 r, for example, the number of daughters that survived was less than 5% of the control value while a preponderance of sons — about 120% of the control value — survived. The inability of damage to the heterochromatic set to induce lethality in males even after 30,000 rep (i. e., roentgens equivalent physical) of P. I. showed its considerable inertness.

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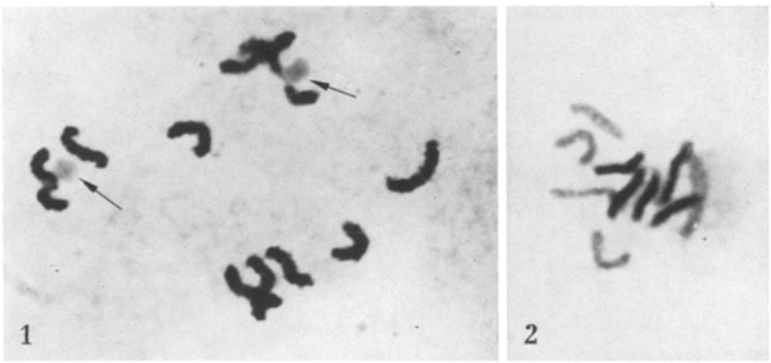
When BROWN and NELSON-REES tested higher doses (30, 60, 90, 120 × 10^3 rep) of P. I., however, the number of daughters *increased* to about 40% of the unirradiated control value at 60,000 rep and stayed at about that level until 120,000 rep—the highest dose used by them after which a male was capable of mating. After 60,000 rep of P. I., the dose at which the increase in surviving daughters was first noticed, the number of surviving sons abruptly dropped to less than 10% of the control value and further decreased with higher doses (see Fig. 4 of BROWN and NELSON-REES 1961).

Cytological studies of adult females surviving these heavy doses of P. I. (CHANDRA 1963) showed that they all had unbroken chromosomes



Figs. 1 and 2. Normal chromosome complements from young embryos (1500 ×). Fig. 1. Ten chromosomes from a female embryo; arrows indicate nucleoli organized at submedian organizers. Fig. 2. Five euchromatic and 5 heterochromatic chromosomes in early prophase, from a male embryo

and were therefore gynogenetic in origin. Triploids, diploids, 3N/2N mosaics, and 2N/N mosaics were found among these X_1 gynogenetic females. In the following paper (CHANDRA 1963), the cytology of X_1 females, their fertility and related problems will be described. Meiosis in triploid females is of special interest because of the holokinetic nature of the chromosomes and has been reported separately (CHANDRA 1962). In addition, NELSON-REES (1962) has recently reported on his studies of sons that survived heavy doses of P. I., with particular reference to spermatogenesis and fertility.

It is the purpose of this paper to describe the cytology of X_1 embryos following 90,000 rep P. I. in order to clarify the chromosomal mechanisms responsible for the production of gynogenetic females and the lethality of most of the X_1 male progeny.

Materials and Methods

Cultures of mealy bugs were grown on potatoes in glass jars according to the methods described by NELSON-REES (1961). The dose chosen as standard for studying X_1 embryos cytologically was 90,000 rep. The gamma radiation was delivered from a cobalt^{60} source belonging to the Bio-organic Group of the University's Lawrence Radiation Laboratory. Irradiated adult males were immediately mated to virgin females from stock cultures; appropriate controls were kept.

For cytology, females were fixed in Bradley-Carnoy (BRADLEY 1948) at varying intervals after mating, usually about 70 hours later. The material was stored in a refrigerator until used. A few drops of a saturated solution of ferric acetate in propionic acid were added as a mordant and this substantially increased the stainability of chromosomes upon squashing in acetocarmine. Most of the photographs were made from temporary preparations.

Observations

For the sake of comparison, normal chromosome behavior during early embryogeny will be described first, followed by cytological observations on X_1 embryos resulting from (a) low dosage paternal irradiation and (b) high dosage (90,000 rep) P. I.

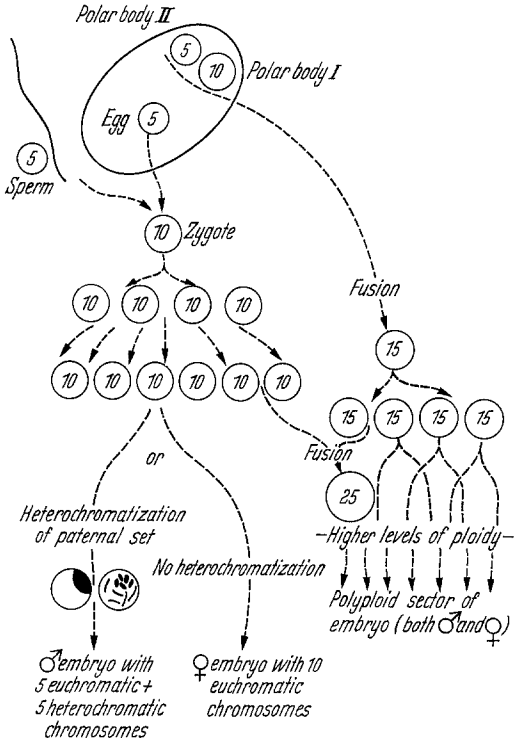


Fig. 3. Diagram of chromosome behavior during early embryogeny of *P. citri*. The numbers refer to the chromosome numbers of the respective nuclei

Normal chromosome behavior during early embryogeny The diploid complement consists of ten chromosomes of approximately equal size (Figs. 1, 2). A nucleolus organizer is present submedially in one of the chromosomes of the haploid set.

Following meiosis in the female, the two polar bodies (polar body I with 10 chromosomes and polar body II with 5) do not degenerate but persist and subsequently fuse with each other. The fusion does not occur until the zygote nucleus has undergone at least two or three cleavage divisions. This triploid fusion nucleus, which has been called a *polar nucleus* by SCHRADER (1923), divides at a slower rate than the cleavage nuclei of zygotic origin, thus there are always more nuclei of the latter kind. The triploid nuclei originating from the polar nucleus increase their 'ploidy level mainly through fusion with cleavage nuclei and fusion with each other. These polyploid cells finally come to house the intracellular symbionts and form a particular organ in the adult mealy bug, called the *mycetome*.

In embryos that develop as males, heterochromatization of the paternal set of chromosomes occurs usually when there are 32 to 64 nuclei in the zygotic sector (Fig. 2). No such visible change in the condensation of chromosomes occurs in female embryos. These major aspects of chromosome behavior during early embryogeny have been illustrated in Fig. 3.

Cytology of X_1 embryos following low doses of P. I. These cytological observations of X_1 embryos are taken from the report of BROWN and NELSON-REES (1961). After 8,000 r, the highest dose after which cytology of X_1 embryos was studied by them, an average of 95% of the embryos showed detectable structural changes in one set of the chromosomes. In male embryos fragmentation and other structural changes appeared in the heterochromatic set only. Excluding very rare exceptions, no chromosome bridging or restitution was observed during mitoses of the cleavage nuclei. Small fragments were occasionally lost but larger entities resulting from breakage were maintained in successive divisions.

Cytology of X_1 embryos following 90,000 rep P. I. For purposes of clarity, the observations will be presented in the following order: (1) the early cleavage divisions of the zygote nucleus and restitutions; (2) rates of division of zygote derivatives and of the triploid polar nuclei; (3) chromosome bridging and fragment loss. Sectors of the embryo derived from the zygote will be referred to as such to distinguish them from those stemming from the polar bodies.

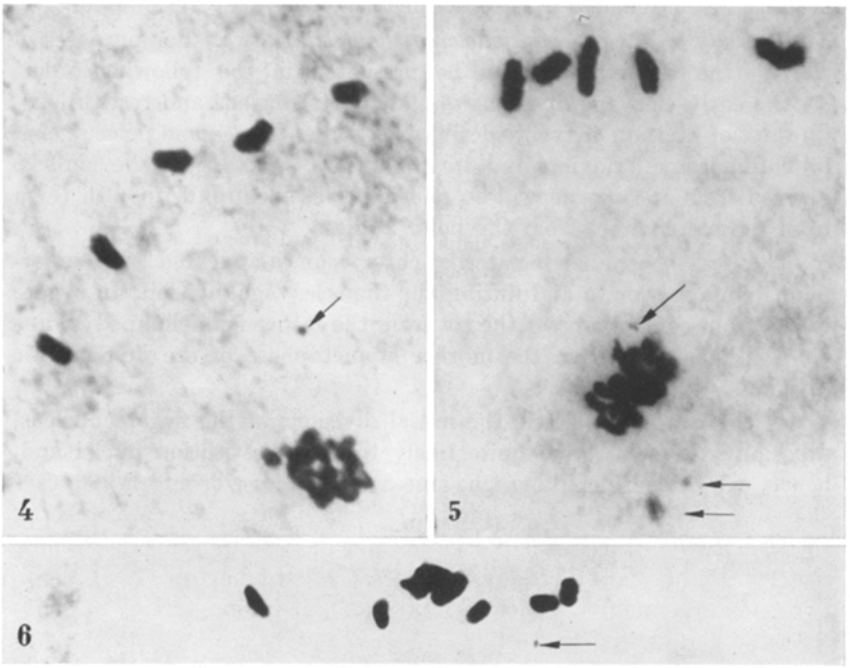
The heavily irradiated paternal chromosomes appeared as an amorphous clump prior to and during the first cleavage division. In zygote nuclei from control crosses the set from the father is unclumped and as condensed as that from the mother at metaphase of the first mitotic division.

In the irradiated series, the initial divisions of the zygote nucleus, especially the first, were quite likely to yield restitution nuclei and, hence, will be considered here in some detail. It may be added here that the term 'restitution' is used in this report in a broad sense to cover the attempted or successful divisions of the nuclei of zygotic origin which result in the formation of only one instead of two daughter nuclei or in two or more daughter nuclei with unequal partitioning of the chromosomes of the mother cell.

At late prophase of the first division of the zygote the entire male complement may be clumped together, or one or more fragments may lie away from the main bulk (Figs. 4—6). Because of their holokinetic nature, the fragments are not easily lost, and most of them apparently manage to orient themselves on the first mitotic spindle, since embryos in which cytology was exceptionally clear did not show any excluded fragments. The irradiated set appeared rather sticky during the first division as may be seen from a typical anaphase of the first cleavage division (Fig. 7). Here the two maternal complements moved ahead towards the poles. The damaged set, including a large number of fragments, occupied most of the space between the two maternal complements, and the small fragments formed delicate trails of chromatin during their movement towards the poles.

During the first few divisions, in both the controls and in the treated series, the cleavage nuclei did not always go through a noticeable inter-

phase. The chromosomes started condensing for the next division almost immediately after anaphase of the previous division. After anaphase of the first division in the treated series, and probably in part because of the rapid condensation for the next division, the damaged paternal set (P) was usually left between the division products of the maternal (M) set (Figs. 8—10). As judged by the constitution of nuclei

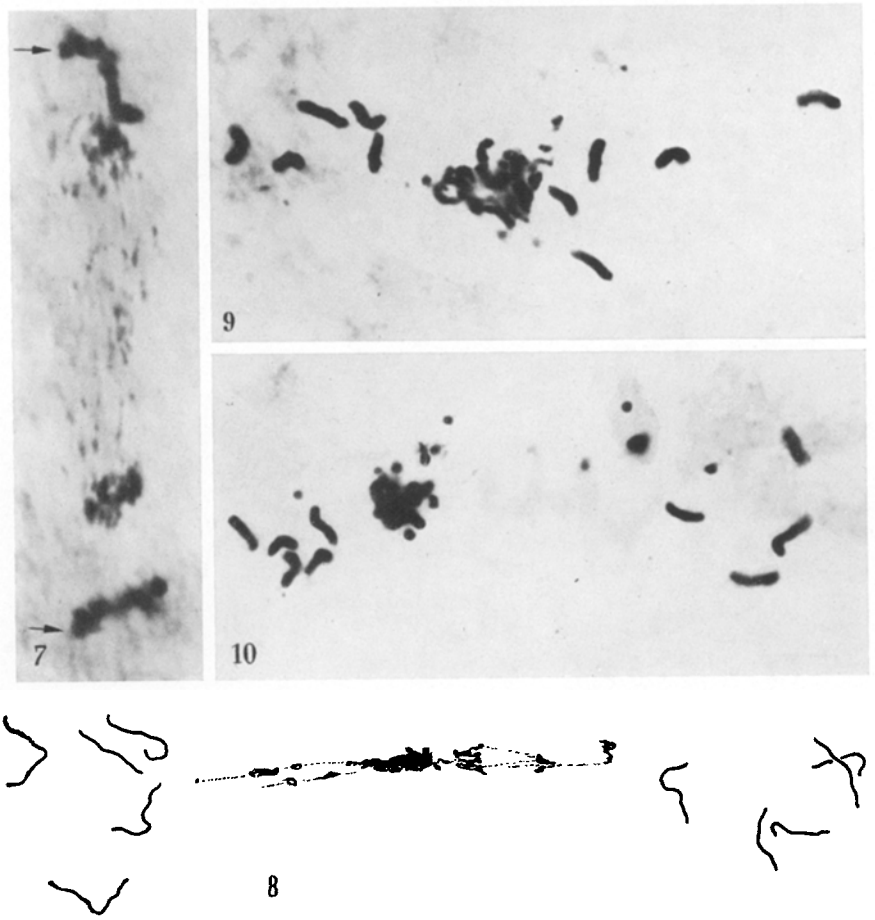


Figs. 4—6. Zygote nuclei in division (all 1500 ×). Arrows indicate fragments of paternal origin lying away from the main clump of paternal chromatin. Fig. 4. Mid prophase; 5 normal maternal chromosomes above and irradiated paternal set in the form of a clump below. Fig. 5. Same as Fig. 4. Fig. 6. Prometaphase; damaged complement above

at later stages, several types of restitution must have occurred at the first cleavage. (A) a single restitution nucleus of 10 maternal chromosomes (2 M sets) and 2 damaged P sets was formed from the entire division figure (Figs. 11, 12). Rarely (B) a restitution of the two P sets plus one maternal set occurred at one pole, the maternal set at the other pole forming a haploid nucleus. More frequently, (C) there were restitution nuclei with a haploid maternal complement and a varying number of fragments of paternal origin (Figs. 16—19).

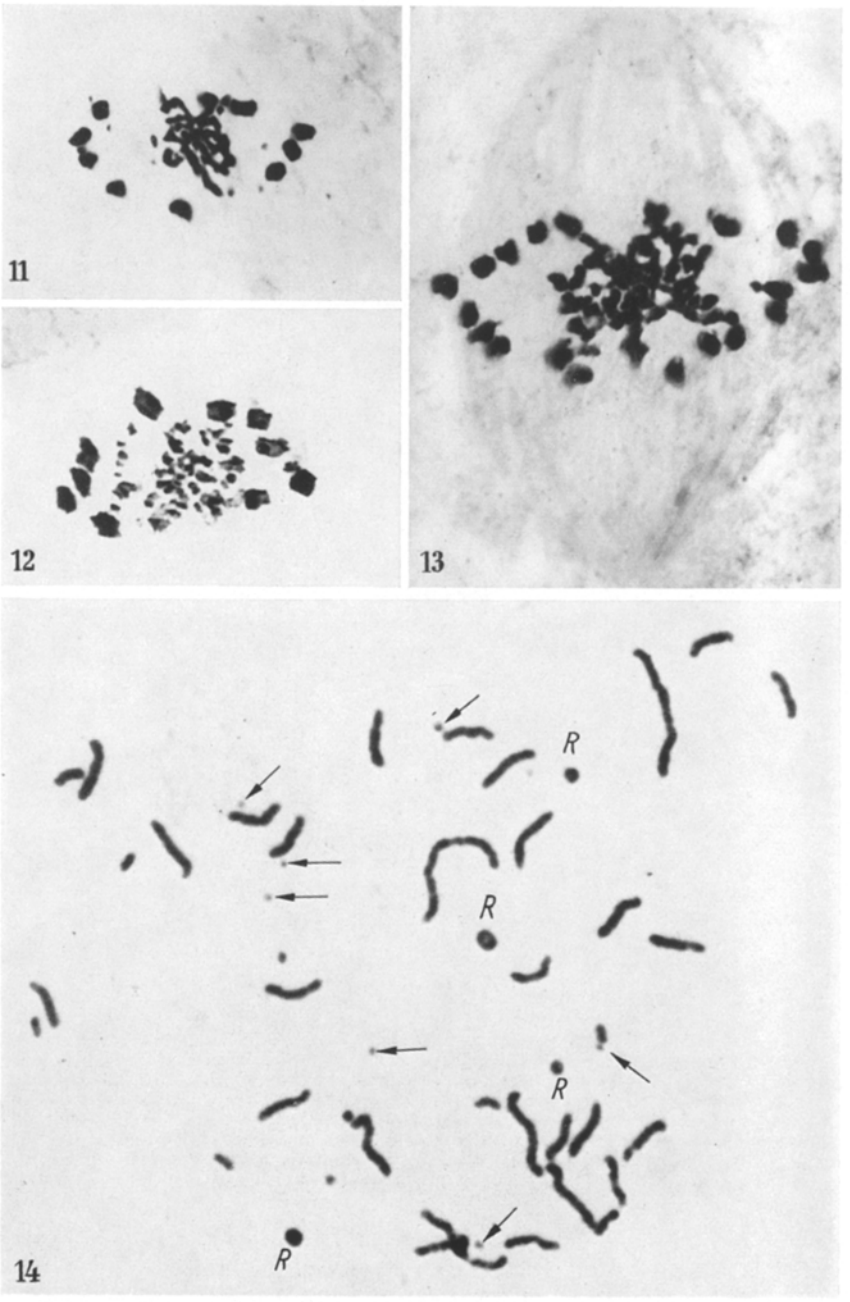
When type A restitution occurred, the single cleavage nucleus with 2 M + 2 P sets often went through another restitution division forming an octoploid cleavage nucleus (4 M + 4 P sets, Fig. 13). At later stages,

embryos with large zygotic sectors composed of nuclei with 2 M + 2 P sets or 4 M + 4 P sets (Fig. 14) were found. Zygotic sectors of high 'ploidy were not observed to differentiate. That restitution from a

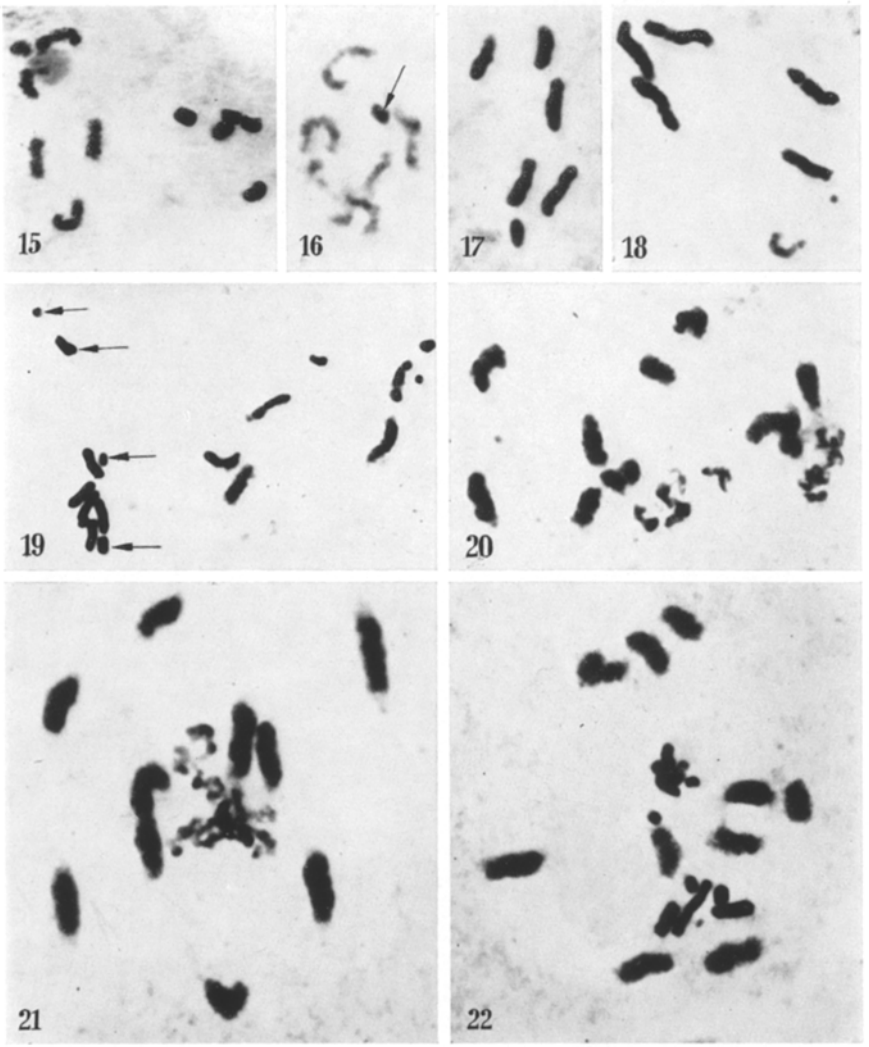


Figs. 7—10. First division of the zygote nucleus (all 1500 ×). Fig. 7. Anaphase. The normal maternal chromosomes (arrows) have moved ahead while the damaged paternal set is in between. Notice fine shower of fragments showing sticky-type bridges. Fig. 8. Drawing of early prophase, preparatory to second mitotic division. The damaged set is lying in between the two normal haploid complements of maternal origin. Figs. 9 and 10. Mid prophase of second mitotic division, with five maternal chromosomes on either side with the main bulk of the paternal set in between. Note some fragments of paternal origin lying along with the maternal chromosomes

2 M + 2 P to 4 M + 4 P state may occur later than the first cleavage stage is indicated by the observation of an embryo in which the zygotic sector was mosaic for 2 M + 2 P and 4 M + 4 P tissues. Since nuclear fusion among cleavage nuclei is extremely rare, the 4 M + 4 P sector

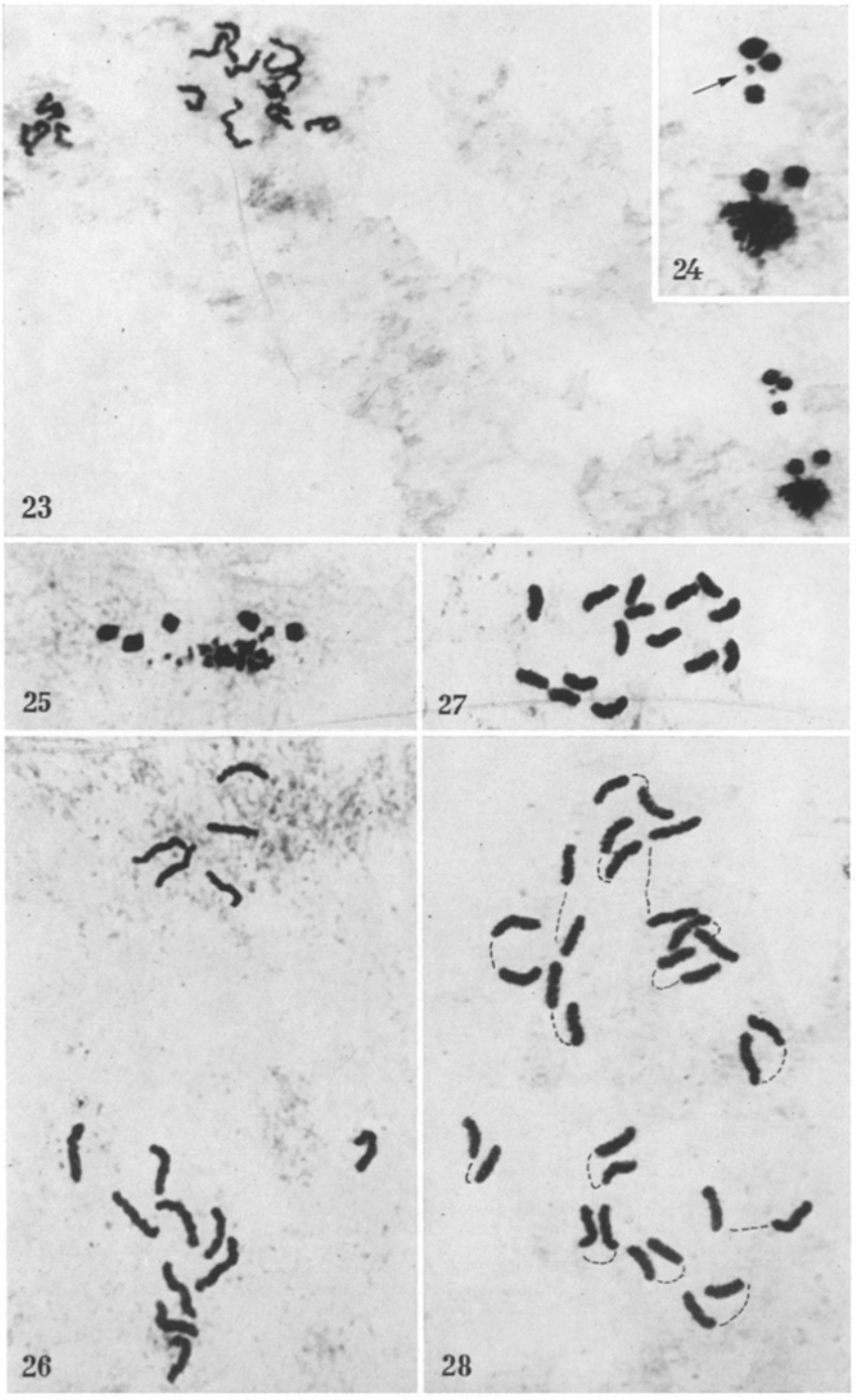


Figs. 11—14. Polyploid restitution nuclei (all 1500 ×). Fig. 11. A tetraploid (2M + 2P sets) zygote nucleus in metaphase; damaged paternal sets in the center, surrounded by the ten maternal chromosomes. Fig. 12. Same as Fig. 11. Fig. 13. Octoploid (4M + 4P sets) zygote nucleus in metaphase; as in Figs. 11 and 12, the damaged sets are in the center,



Figs. 15—22. Chromosome complements in cleavage nuclei in the zygotic sector in different embryos (all 1500 ×). Fig. 15. Two adjacent nuclei with 5 maternal chromosomes each. Fig. 16. Five euchromatic chromosomes + 1 heterochromatic fragment (arrow) from a male embryo. Fig. 17. Five chromosomes + 1 fragment; from a female embryo. Fig. 18. Five normal chromosomes + 2 paternal fragments (1 long + 1 small); note the thin, apparently less condensed state of the two paternal chromosomes. Fig. 19. Two adjacent division figures from a female embryo with 5 normal chromosomes + 4 fragments, indicated by arrows for the left division figure. Figs. 20—22. Division figures from a male embryo with 10 euchromatic chromosomes + 2 damaged sets. Fig. 20. Ten euchromatic chromosomes + damaged set in a diffuse state. Fig. 21. Damaged sets more condensed than in Fig. 20. Fig. 22. Damaged sets fully condensed

surrounded by the normal maternal chromosomes. Fig. 14. An octoploid nucleus (±) in midprophase from an embryo which had about 20 such zygotic nuclei. Rings are indicated by *R's* and smal fragments, by arrows



Figs. 23—24

probably arose through a restitution division of one of the 2 M + 2 P nuclei.

Octoploidy (4 M + 4 P sets), presumably resulting from repeated failures in division, was the highest level of polyploidy observed for a single zygote nucleus. In a few older embryos, however, zygotic sectors of very high polyploidy — definitely over the octoploid level — were seen.

A characteristic metaphase arrangement was found in division figures of polyploid nuclei with damaged paternal complements. The normal (and therefore maternal) chromosomes always surrounded the damaged complements which occupied the center of the arrangement (Figs. 11—13).

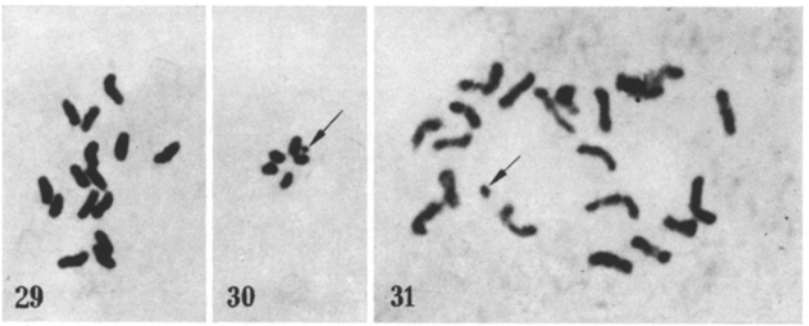
In addition to the complete restitutions described above, partial restitutions were also found (types B and C). At late blastula, in most embryos, a particular nuclear type was usually predominant. Embryos with zygotic sectors which were almost exclusively haploid (5 normal chromosomes), hyperhaploid (5 normal chromosomes + one or more small fragments), diploid (10 normal chromosomes), or hyperdiploid (10 normal chromosomes + one or two fragments) were observed. At late blastula, the zygotic sector most frequently encountered had a haploid maternal complement with or without a varying number of small fragments from the father.

In embryos which would have developed as males, the damaged paternal set or fragments of paternal origin showed heterochromatization irrespective of their number and size (Fig. 16) or the polyploid state of the nucleus (Figs. 20—22).

Fusion of polar bodies to form a triploid polar nucleus proceeded normally in most embryos (Figs. 23—27). This triploid nucleus divides and some of these division products fuse with each other and with cleavage nuclei to form a polyploid sector in the embryo. Rarely some of the triploid polar nuclei increased their 'ploidy level through endomitosis. Such an endomitotic doubling of chromosomes in an embryo whose father had been irradiated with 90,000 rep is illustrated as Fig. 28.

Figs. 23—28. Polar bodies and zygote nuclei. (Fig. 23, 1200 ×; Fig. 24, 2000 ×; all others, 1500 ×.) Fig. 23. An ovariole showing polar bodies and the zygote nucleus which is approaching metaphase. Above left, the first and second polar bodies in early prophase; polar body I to the right. Fig. 24. Zygote nucleus from Fig. 23 enlarged. The damaged paternal set in the form of a clump below the five normal chromosomes from the mother; arrow indicates fragment of paternal origin condensing away from the main bulk of the sperm contribution. Figs. 25 and 26. Zygote nucleus in metaphase and polar bodies, respectively, from the same ovariole; in Fig. 25 note the irradiated, amorphous-appearing paternal set orienting normally with the five maternal chromosomes. In Fig. 26 polar body II (above) and polar body I, both in mid prophase, are about to fuse. Fig. 27. A triploid polar nucleus with 15 chromosomes formed by the fusion of the two polar bodies. Fig. 28. A 30-chromosome nucleus formed as the result of an endomitotic division of a triploid polar nucleus derivative; probable sister chromosomes are connected by dotted lines

In control material, diagrammed in Fig. 3, the triploid polar nuclei usually fuse with diploid cleavage nuclei to form pentaploid nuclei. Fusion of triploid nuclei with cleavage nuclei also took place in X_1 embryos following heavy P. I. even when restitution divisions in the latter resulted in haploid or hyperhaploid nuclei. When haploid nuclei, each with 5 normal chromosomes, were formed as a result of restitution of the zygote and its division products, triploid polar nuclei fused with them to form cells with 20 chromosomes. In an embryo with a zygotic sector of 5 normal chromosomes + 1 fragment chromosome, fusion with



Figs. 29—31. A triploid nucleus, a zygote nucleus derivative and a fusion nucleus respectively, from the same embryo (all 1500 ×). Arrow in Figs. 30 and 31 indicates the fragment of paternal origin which acts as a convenient marker. Fig. 29. Triploid polar nucleus derivative with 15 chromosomes. Fig. 30. Zygote nucleus derivative with 5 chromosomes + 1 fragment. Fig. 31. Fusion nucleus with 20 + 1 fragment

triploid nuclei produced nuclei with 20 normal chromosomes + 1 fragment chromosome, the fragment serving as a convenient cytological marker (Figs. 29—31).

Origin of diploid nuclei. About five to ten per cent of the gynogenetic females recovered following high dosage P. I. are diploid (CHANDRA 1963). Since diploid zygotic sectors had been observed in X_1 embryos, the origin of these diploid females was at first considered to be exclusively the result of a doubling of the maternal complement of the zygote during an early cleavage division with prior or concomitant loss of the damaged paternal set. But, as will be discussed below, at least a few of these gynogenetic diploid females may possibly originate from polar bodies.

Among the nearly sixty very young X_1 embryos that were studied following P. I. with 90,000 rep, in all but one case there was normal fusion of the two polar bodies to form triploid nuclei (Fig. 27). In the one exception, however, both the polar bodies had divided once without prior fusion while the zygote nucleus remained in metaphase. The division of polar bodies without fusion would be an easy source of diploid and haploid nuclei. Such haploid and diploid polar body derivatives,

if successful in competing against the damaged zygotic sector, could account for the 2N/N mosaics that have been observed among X_1 gynogenetic adults (CHANDRA 1963).

The division of polar bodies prior to fusion, observed in one embryo, may not be the result of paternal irradiation. SCHRADER (1923) observed, in normal material, a probable case of division of the first polar body by itself and commented that "it is barely possible that the first polar body may actually divide on occasion, but this must be an exceptional condition for only one very doubtful case was encountered". In normal embryos such diploid nuclei originating from polar body I probably would not have much competitive advantage since normal diploid zygotic nuclei would also be present in the same embryo.

All X_1 embryos in mid-blastula showed normal triploid polar nuclei, indicating that fusion of the polar bodies had taken place normally. But the possibility must be considered that if the first and second polar bodies have started dividing, one or more of their products may fuse to give rise to triploid nuclei in addition to diploid and haploid nuclei. In this study, however, no young embryos were encountered which showed the predicted 3N/2N/N mosaic tissues in the non-zygotic sector.

Although the available data suggest that most of the diploid females develop from the maternal complement to the zygote after loss of the irradiated paternal complement, the actual mechanism(s) of origin of the diploid nuclei via the zygote is (are) uncertain. That diploid nuclei may be formed by unsuccessful division is shown by the fact that products of prior restitution with 10 normal chromosomes + 1 or 2 small fragments have been observed in young embryos; the fragments attest to their origin from zygotic nuclei. Diploid nuclei with only maternal chromosomes result if the elimination of the damaged set is complete. Haploid nuclei of maternal origin, which were frequently observed in zygotic sectors of young embryos, could possibly give rise to diploid nuclei through fusion or other processes but there was no clear evidence for the occurrence of such events. Further cytology and experiments with genetic and cytological markers are necessary to fully clarify these problems of origin(s) of diploids and mosaics and experiments in this direction are planned.

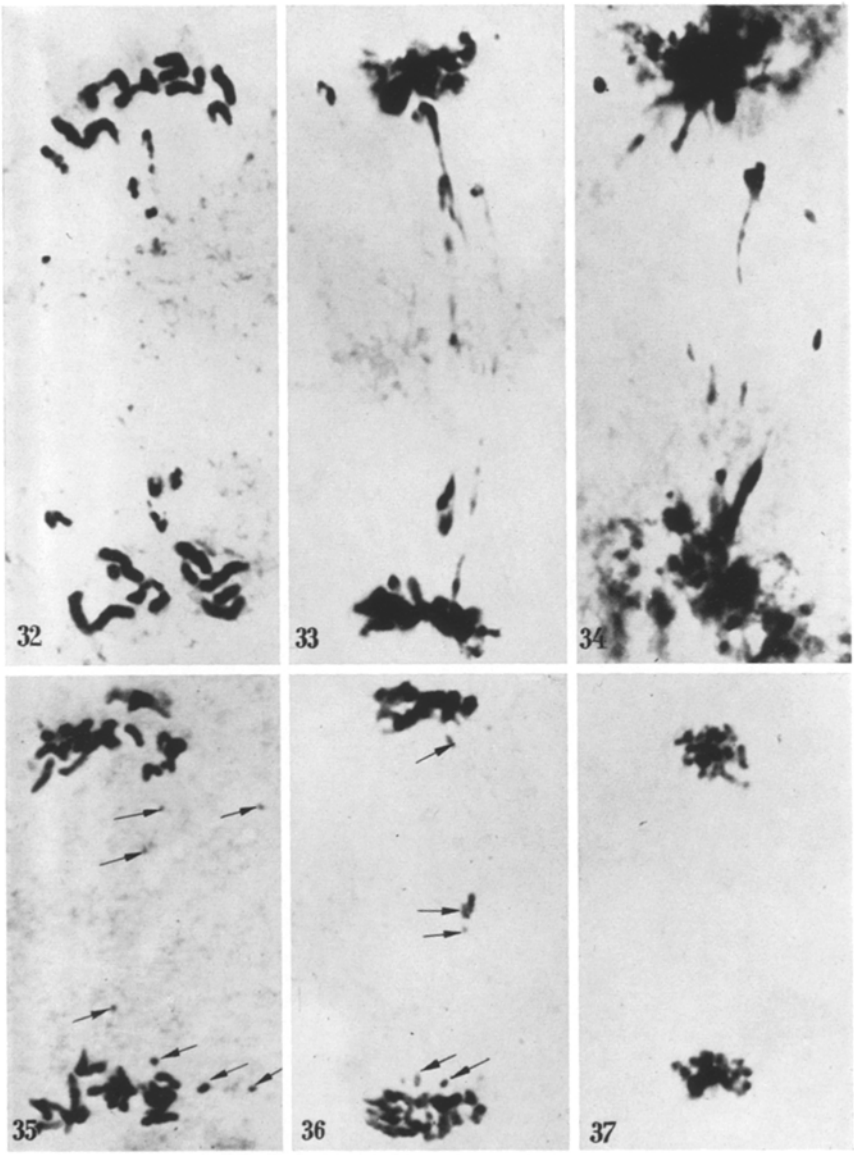
Chromosome bridging and the behavior of fragments. Except for the infrequent loss of small fragments and unsuccessful segregation, the damaged set showed remarkable stability as evidenced by its normal orientation on metaphase plate and movement on the spindle (Figs. 11 to 13 and 25). Considering the enormous amount of damage suffered by the P set, irregularities in division were not as frequent as one would expect. In fact it appeared that if the zygote nucleus succeeded in

dividing normally during the first few cleavage divisions, it would give rise to older embryos consisting of zygotic nuclei with similar and stable chromosome constitution. The following is a description of bridging and fragment loss that were observed. It was mentioned earlier that during the first cleavage division, a large number of fragments from the paternal set appeared as fine trails of chromatin as they moved towards the poles. Such trailing of small fragments was often noticed in the next two divisions but to a much lesser extent. Such trails were rarely seen in later divisions. Since this definitely was not due to loss of all such fragments, it probably reflects a change in the state of the fragments or in their relationship to their environment. During anaphases of subsequent divisions, no definite breakage-fusion-bridge cycles (McCLINTOCK 1938) were observed. However, three bridges, which appeared to be the result of misdividing rings (Figs. 32, 33), were observed among a total of over sixty young embryos. Most of the bridging in older embryos was attributable to stickiness (Figs. 34, 38, 40, 41). In some older embryos very sticky bridges were seen in zygotic sectors and were probably a sign of the onset of degeneration of such embryos (Fig. 41).

Small fragments showed some instability, especially during the first few cleavage divisions. Figs. 35—37 illustrate 3 of the 4 post-anaphases from a young embryo. Late anaphase (Fig. 35) and early telophase (Fig. 36) showed lagging fragments. Since the remaining two clear late telophases (one of them is Fig. 37) did not show any excluded fragments, it appears that many of the small fragments which lag at anaphases eventually manage to reach the poles and get incorporated into telophase nuclei. Apparent instability of small fragments gave rise to a few embryos in which the zygotic sectors were mosaic for small chromosome fragments. For example, an embryo was observed which had a zygotic sector with the following types of nuclei: 5 chromosomes + 1 fragment, 5 + 2 fragments and 5 + 3 fragments; the fragments involved were extremely small in size.

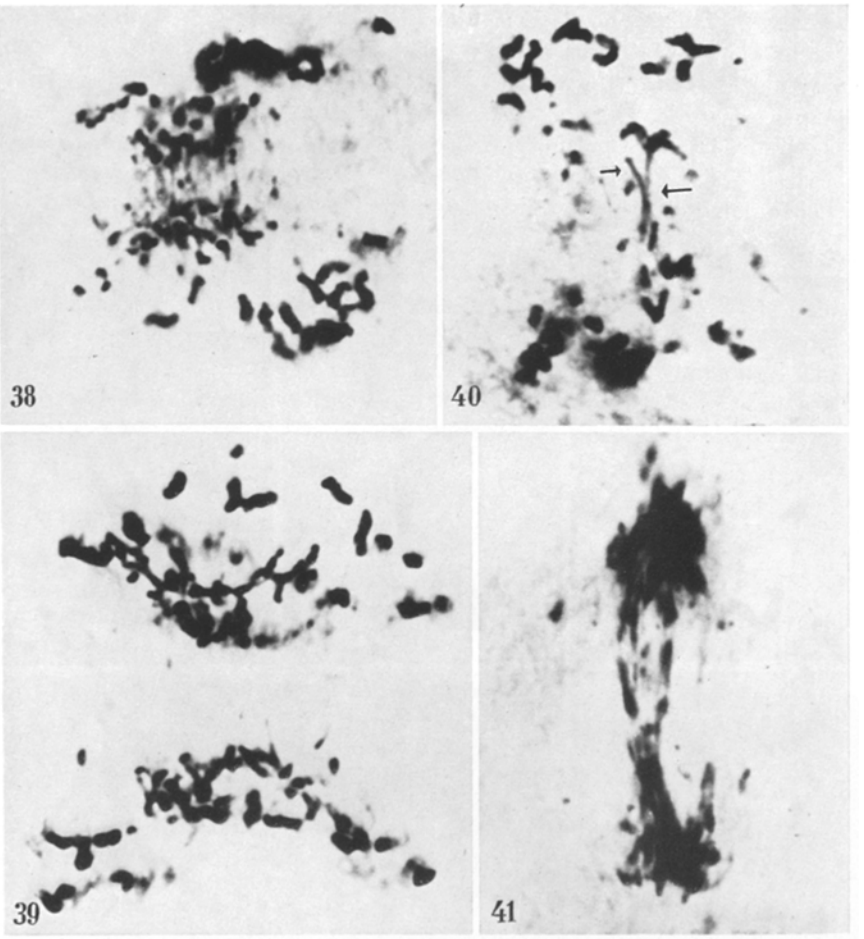
That the bulk of a fragment had something to do with its performance during anaphase movement is indicated by the following observation. During most early anaphases of nuclei with 1 M + 1 P or 2 M + 2 P or 4 M + 4 P, the normal chromosomes from the mother along with the bigger entities of paternal origin moved ahead while smaller entities moved at a slower rate (Figs. 38, 39). These observations on the behavior of radiation-induced fragments parallel those of BROWN (1960) on small fragments occurring spontaneously in an armored scale insect.

Rates of division of zygote derivatives and of triploid polar nuclei. In normal embryogeny, the fusion of polar bodies to form the polar nucleus occurs after 3 to 5 cleavage (zygotic) divisions. From there on, there are



Figs. 32—37. Mitotic anaphases showing bridges and behavior of fragments (all 2000 ×). Fig. 32. Lagging of small chromosomes at anaphase. Arrows indicate what appears to be a misdividing ring. Fig. 33. Chromosome bridge in late anaphase. Fig. 34. Sticky bridge in telophase. Figs. 35—37. Three of the 4 post-anahpases of cleavage nuclei in a young embryo. Arrows indicate fragments. Fig. 37. Telophase showing no excluded fragments

always more cleavage nuclei than polar nucleus derivatives. In addition to their initial advantage of starting with higher numbers, the cleavage



Figs. 38—41. Mitotic anaphases (2000 ×). Fig. 38. Anaphase separation of an early cleavage nucleus with 10 normal chromosomes + damaged sets. The undamaged chromosomes and the bigger fragments from the damaged set have moved ahead of the smaller fragments of paternal origin which, in addition to slower separation, are showing heavy bridging. Fig. 39. Cleavage nucleus with 20 normal chromosomes + damaged sets. The two halves in the form of bouquets show almost "clean" separation except for a few lagging fragments (arrows). As observed under the microscope, the 20 big chromosomes were situated at the periphery of the bouquet. Fig. 40. Cleavage nucleus with 10 normal chromosomes + damaged set in anaphase, from an embryo in mid blastula. Note faster movement of bigger chromosomes; arrows indicate bridges. Fig. 41. Anaphase showing extremely heavy bridging, with a sticky appearance: from an embryo with over 300 nuclei

nuclei actually divide at a faster rate than polar nuclei (SCHRADER 1923), giving them an advantage over the polar nuclei in embryogenesis. The rate of division of polar nuclei vs. cleavage nuclei appeared to a critical factor in the production of gynogenetic 3N females after high doses of P. I. It was therefore necessary to check the division rates of the two

sectors during early embryogeny following low doses of P. I. since there was no evidence for gynogenesis at lower doses (2,000—16,000 r) (BROWN and NELSON-REES l. c.). Several slides of young embryos in the 8,000 r P. I. series, which had been prepared by Dr. UZI NUR, were examined for this purpose. In no case was the number of polar nuclei greater than the number of cleavage nuclei. Data from the first 6 clearly analyzable young embryos are given in the Table, along with data from embryos following 90,000 rep P. I. Only data from very young embryos with clear cytology were included because it was difficult

Table. *Number of nuclei in zygotic and non-zygotic sectors in young embryos after paternal irradiation of 8,000 r and 90,000 rep*

	Embryo no.	No. of nuclei in zygotic secto. and their 'ploidy	No. of nuclei in non-zygotic sector		No. of unanalyzable nuclei
			No. of triploid polar nuclei	No. of nuclei with other 'ploidy	
P.I. 8,000 r	1	12 (2N)	3	1 (5N)[1]	—
	2	18 (2N)	2	—	2
	3	14 (2N)	3	—	—
	4	6 (2N)	1	—	—
	5	2 (2N)	—	—	4
	6	22 (2N)	2	1 (6N)[2]	3
P.I. 90.000 rep	1	2 (2N)	6	—	—
	2	4 (2N?)	1	—	2
	3	2 (4N)	4	—	—
	4	3 (4N)	3	5 (6N)[2]	—
	5	1 (4N)	2	—	—
	6	2 (4N)	9	—	2
	7	1 (2N)	1	—	—
	8	1 (2N)	1	—	—
	9	1 (4N)	1	—	—
	10	1 (4N)	6	—	—
	11	2 (8N) + 2 (4N)	6	—	—
	12	1 (4N)	7	—	—
	13	Zygote in first anaphase	Polar bodies about to fuse	—	—
	14	1 (8N)	10	—	—
	15	1 (4N)	2	—	—
	16	1 (2N)	Polar bodies about to fuse	—	—
	17	1 (over 4N)	12	—	1
	18	3 (N)	4	—	2**
	19	> 80 (N)*	3	—	ca 10
	20	> 34 (N)	6	—	ca 10

[1] Pentaploid nucleus formed by the fusion of a cleavage nucleus with a triploid polar nucleus.

[2] Formed by doubling of a triploid polar nucleus through fusion or endomitosis.

* Embryo mosaic for nuclei with 5 normal chromosomes, 5 + 1 fragment, 5 + 2 fragments.

** Nuclei of zygotic origin since they contained fragmented chromosomes.

to determine accurately the number and chromosome constitution of nuclei in older embryos. The division rates as expressed by number of nuclei in the 8,000 r P. I. series were comparable to those observed for controls but, as will be shown, differed markedly from those observed in embryos following 90,000 rep P. I.

Following 90,000 rep P. I. there was a marked suppression of the activity of the zygote nucleus in almost all embryos. The polar nuclei fused and often divided once before the zygote nucleus had gone beyond metaphase of the first mitosis. The Table gives data from 17 embryos after 90,000 rep P. I. Note that in 9 of the 11 embryos where the number of polar nuclei was greater than the number of cleavage nuclei, the cleavage nuclei were at 4N or 8N 'ploidy levels.

The fact that the zygote nucleus following heavy P. I. usually did not divide successfully but often underwent restitution to form tetraploid and octoploid nuclei gave the polar bodies a further advantage over the zygote nucleus. The latter, as a result of unsuccessful divisions, would only increase in 'ploidy level whereas every division of the polar nucleus at this stage would result in a doubling of the number of nuclei. This selective proliferation of triploid nuclei during early embryogeny is believed to be the important cause in the production of triploid gynogenetic females following heavy doses of P. I.

During the study of older embryos, another observation of interest was made. Haploid or hyperhaploid (haploid + 1 or 2 small fragments) or diploid nuclei, when found, occupied a considerable portion of the embryo, indicating that normal haploid or diploid nuclei might have had considerable competitive advantage over triploid polar nuclei, especially if the former arose during early cleavage. Some evidence for this conclusion comes from the last three embryos, 18, 19 and 20, of the Table in which haploid sectors were the predominant.

Discussion

Since O. HERTWIG's (1911) demenstration, in the frog, that radium treatment of sperm with low doses leads to abnormal embryos whereas heavy dosesl give normal development, this so-called "Hertwig effect" has been noticed, among others, in other amphibians, in rabbit eggs and in the mouse (see BEATTY 1957 for a review and references on amphibian and mammalian examples). Besides X rays, ultra-violet radiation and several chemicals — especially the basic fluorescent dyes — have been successfully used to induce gynogenesis. In most of the experiments with amphibian material, the gynogenetic embryos that hatched were haploid and their subsequent difficulties in development have been attributed as being primarily due to their haploid state. The origin of triploids in the mealy bug through a fusion nucleus of the polar bodies, on

the other hand, is a unique kind of gynogenesis; no other established case of this particular kind of gynogenesis has been reported.

No comparison will be made with other examples of gynogenesis, such as the extensive studies with frog, for two reasons: firstly, the arganization of the kinetochore is radically different in the two systems ond this has an important bearing on the mitotic behavior of chromosomes following treatment with radiation or chemicals. The localized kinetochore in organisms such as the frog makes the entire complement susceptible to elimination *in toto* following treatment with heavy doses of radiation or chemicals. With the holokinetic chromosomes found in the mealy bug, each chromosome fragment of any considerable size is potentially capable of perpetuating indefinitely. This restricts the number of gynogenetic embryos (of zygotic origin) that can develop following heavy doses of P. I. In fact, in several of the frog experiments, beyond a certain dose of radiation or concentration of a chemical, the number of gynogenetic haploids recovered approached 100% (RUGH 1939, BRIGGS 1952).

Secondly, there seem to be two qualitatively different kinds of gynogenesis occurring in the mealy bug. The triploid females develop from a fusion nucleus of the polar bodies whereas most of the diploid females appear to come from the maternal contribution to the zygote.

An interesting question, which is intimately linked with the general problem of sex determination in this species, deserves mentioning here. It has to do with the source of the gynogenetic females with respect to the sex potentiality of the ovarioles from which they originate; that is, do these gynogenetic females develop from ovarioles which would have normally given rise to male embryos or female embryos or either? This question cannot be answered at the present time.

Lethality of males following heavy doses of P. I. BROWN and NELSON-REES (1961), in their study of survival following paternal irradiation, noticed that the number of males surviving 2,000 r — 30,000 rep was not less than that in the control crosses. After 60,000 rep P. I., however, the number of surviving sons dropped suddenly and further decreased, up to 120,000 rep. On the basis of cytology of the X_1 embryos following 90,000 rep P. I. reported here and the cytology of the few adult males surviving heavy doses of P. I. reported by NELSON-REES (1962), the probable major reasons for lethality of X_1 males will be considered.

NELSON-REES (1962) observed that the few males surviving heavy doses of P. I. had badly damaged heterochromatic chromosomes but the total bulk of the heterochromatic (H) set in these survivors closely approximated the bulk of the H set in normal males, indicating that perhaps a near-haploid bulk is necessary for survival. If this is so, then obviously embryos which, due to restitution divisions, have zygotic

sectors such as 5 normal maternal chromosomes + 1, 2 or a few heterochromatic fragments would not survive. In addition, restitution divisions also produce polyploid zygotic nuclei (2 M + 2 heterochromatic P, 4 M + 4 heterochromatic P, etc.). Embryos with such polyploid zygotic nuclei are most likely lethal since NELSON-REES did not observe any polyploid adults among the male survivors studied by him.

Summary

In the mealy bug, *Planococcus citri*, following high dosage paternal irradiation (60,000—120,000 rep), the survivors are mostly female (about 30—40% of the unirradiated control value) whereas very few males survive (about 5% of control value). After lower doses of paternal irradiation (P. I.), however, few or no females survive while the normal number of males (never less than the control value) survive.

The females developing after high dosage P. I. are gynogenetic and are triploid or diploid or 3N/2N or 2N/N mosaics (CHANDRA 1963).

The cytology of X_1 embryos following 90,000 rep is described in this report, in comparison with data from embryos following lower doses (8,000 r) of P. I. and unirradiated controls, to illustrate the chromosomal mechanisms leading to the production of gynogenetic females and the probable reasons for lethality of X_1 males after heavy P. I.

It has been shown that triploid females stem from a fusion nucleus of the first and second polar bodies. This triploid *polar nucleus*, which normally participates in the formation of a polyploid sector in the young embryo, undertakes a successful embryogeny in many embryos when the zygote nucleus is unable to do so because of the heavily damaged paternal complement of chromosomes. Since the chromosomes are characterized by holokinetic activity, the irradiated paternal set manages to divide with the maternal complement but did not always segregate as successfully. Restitution divisions of the zygotic nuclei result in haploid, hyperhaploid, diploid and polyploid nuclei. Most of the diploid gynogenetic females probably originate from diploid nuclei of zygotic origin although it is possible that a few diploid females and the 2N/N mosaic females develop from polar bodies.

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