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# SEGREGATION AND RECOMBINATION OF NON-MENDELIAN GENES IN CHLAMYDOMONAS[1]

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ABSTRACT

Non-Mendelian genes in *Chamydomonas reinhardtii* are inherited in a uniparental (UP) fashion. Most zygotes and their progeny receive UP genes only from the $mt^+$ or maternal parent. However, a few exceptional zygotes are also found in which the $mt^-$ or paternal UP genome is transmitted. Most of the exceptional zygotes are biparental in that their progeny segregate UP genes transmitted by both parents. As a result, biparental zygotes have been extensively used to study the rules governing UP inheritance.

The frequency of biparental zygotes can be greatly increased if the maternal parent is irradiated with ultraviolet light prior to mating. Based principally on studies with ultraviolet-induced biparental zygotes, SAGER has argued that a vegetative cell contains two copies of the UP genome and that the progeny of a biparental zygote receive a copy derived from each parent. Results reported in this paper with spontaneous and ultraviolet-induced biparental zygotes do not support the two copy model, but argue for a mulitple copy model with most of the copies normally being transmitted by the maternal parent. A multiple copy model which accounts for both SAGER's results and ours is presented.

NON-MENDELIAN mutations exhibiting uniparental (UP) inheritance in *Chlamydomonas reinhardtii* were first reported by SAGER in 1954. Since that time the UP genetic system has been subject to extensive investigation at both the molecular and genetical levels (see reviews by GILLHAM 1969; SAGER 1972). There is strongly suggestive evidence that UP genes are localized in chloroplast DNA (SAGER and LANE 1972; LEE and JONES 1973) but this is not rigorously proven as demonstrated by the conflicting results of CHIANG (1968, 1971) and the fact that mitochondrial DNA has not been eliminated as a candidate.

In crosses the mating type plus ($mt^+$) or *maternal* parent transmits the UP marker(s) it carries to all four meiotic products in 90% or more of all zygotes (*maternal zygotes*). UP genes from the mating type minus ($mt^-$) or *paternal* parent are transmitted in 10% or less of the zygotes (*exceptional zygotes*). Two kinds of exceptional zygotes occur. *Biparental exceptional zygotes* transmit UP genes from both parents to the meiotic products. In the progeny of such zygotes UP genes, unlike Mendelian genes, continue to segregate during the postmeiotic,

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mitotic divisions as well as during the initial meiotic divisions as well as during the initial meiotic divisions (GILLHAM 1969; SAGER 1972). *Paternal exceptional zygotes*, the rarest class of all, transmit only UP genes from the $mt^-$ parent. The terms maternal and paternal are used advisedly since, in zygote formation in Chlamydomonas, gametes of opposite mating type fuse completely with the chloroplasts as well as the nuclei fusing (BASTIA, CHIANG and SWIFT 1969; CAVALIER-SMITH 1970).

Studies on the segregation and recombination of UP genes in biparental zygotes and their progeny (SAGER and RAMANIS 1963, 1965, 1968, 1970) together with experiments on the inheritance of chloroplast DNA in maternal zygotes (SAGER and LANE 1972) have led to an explicit model of UP inheritance in *C. reinhardtii* (SAGER 1972). The salient features of SAGER's model appear to be: (1) UP genes are localized in chloroplast DNA and form a single linkage group genetically. (2) Each parent contributes one genetically competent copy of this chloroplast "chromosome" to the zygote. (3) Chloroplast DNA from the $mt^-$ parent is normally destroyed in the zygote whereas that from the $mt^+$ parent is conserved. Selective destruction is assumed to occur by a mechanism akin to host-induced restriction and modification in bacteria. The $mt^-$ chloroplast DNA is presumed to be restricted by an enzyme synthesized by the $mt^-$ parent whereas the $mt^+$ chloroplast DNA is not restricted because it is modified by an enzyme synthesized by the $mt^+$ parent. Both enzymes are activated at the time of gamete fusion (SAGER and RAMANIS 1973). Biparental zygotes result when the restriction system fails. (4) Vegetative cells are genetically diploid for the UP linkage group and the two linkage groups are inherited in an oriented fashion such that each daughter cell receives two copies, one derived from each of the two original copies (Figure 1). (5) Among the progeny of biparental zygotes three types of segregation occur (Figure 1). In type I segregation no recombination occurs and two hybrid daughter cells are produced. In type II segregation nonreciprocal recombination (gene conversion) converts an allele (e.g., $b^+$) carried by one copy to that (e.g., $b$) carried by the other copy. Type II segregations produce a daughter cell hybrid for the converted marker (e.g., $b^+/b$) and one homozygous for that marker (e.g., $b/b$ or $b^+/b^+$ which occur with equal frequency). In type III segregations, the rarest class, reciprocal recombination yields cells homozygous for a pair of alleles (e.g., $b/b$ and $b^+/b^+$) by a mechanism resembling mitotic recombination. Type III segregations show a polarity in that pairs of alleles at different loci segregate at different rates and thus can be mapped with respect to a hypothetical attachment point which behaves like a mitotic centromere. Markers proximal to the attachment point segregate by the type III mechanism less frequently than those more distal. Polarity of type III segregations is the principal method used for mapping UP genes by SAGER and RAMANIS (1971) and SAGER (1972).

Our objective is to examine critically SAGER's model in terms of our own published (GILLHAM 1963, 1965, 1969; GILLHAM and FIFER 1968) and our heretofore unpublished studies of UP gene segregation and recombination in *C. reinhardtii*. These studies provide evidence *both* for significant deviations from a 1:1

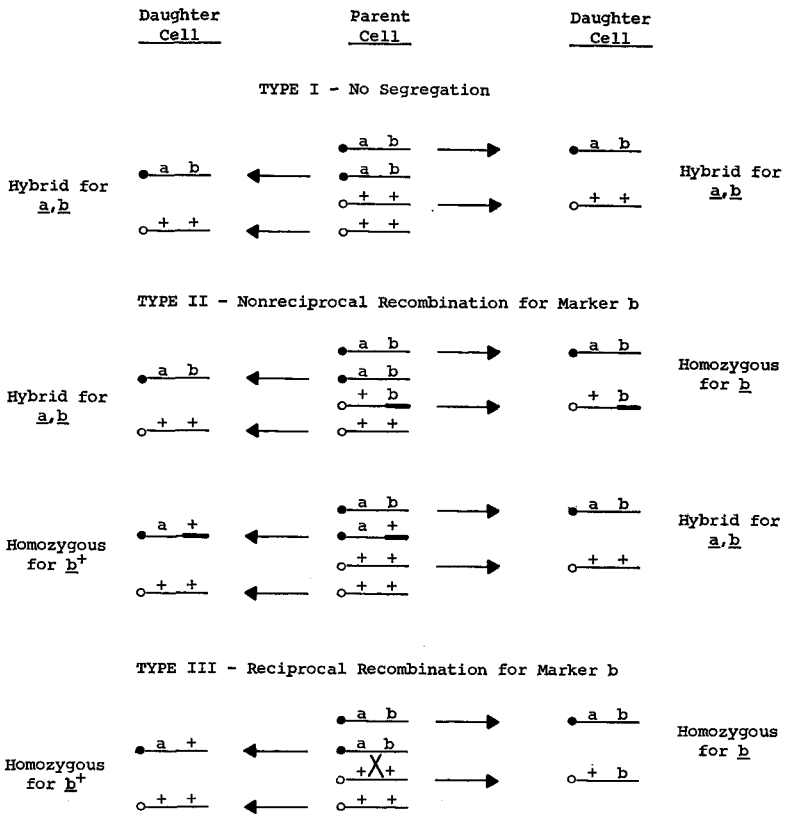


FIGURE 1.—UP gene segregation patterns in SAGER's two copy model. Recombination is depicted as taking place after the two copies have replicated. The UP genomes are shown as linear for the sake of clarity although they may really be circular (SAGER 1972).

ratio of pairs of UP alleles and from a 1:1 ratio of reciprocal recombinants among the progeny of biparental zygotes. Neither observation is predicted by SAGER's model . The possible influence of methods used to recover biparental zygotes on copy number is discussed and a multiple copy modification of SAGER's model is proposed.

## RECOVORY OF BIPARENTAL ZYGOTES

Early studies of UP gene recombination and segregation involved either direct selection of rare, spontaneous biparental zygotes by selection of progeny, immediately following meiosis, which carry specific UP genes from the paternal parent (SAGER and RAMANIS 1963, 1965) or indirect selection of exceptional zygote colonies at later times (GILLHAM 1963, 1965a, 1969; GILLHAM and FIFER 1968).

In a major breakthrough, SAGER and RAMANIS (1967) showed that the frequency of biparental zygotes could be greatly increased if the maternal parent was irradiated with ultraviolet light (UV) prior to mating. SAGER's model is based on genetic studies with directly selected and UV-induced biparental zygotes.

SAGER and RAMANIS (1963, 1965, 1968) report that hybrid cells derived either from directly-selected spontaneous biparental zygotes or from UV-induced biparental zygotes produce on the average, whether by type II or III segregation, daughters homozygous for each member of an allelic pair in a 1:1 ratio. Furthermore, in the case of hybrid vegetative cells derived mitotically from hybrid meiotic products of UV-induced biparental zygotes, about 90% of the cells tested segregate pure types in equal frequency (SAGER and RAMANIS 1968, SAGER 1972). This observed 1:1 allelic ratio is central to the two copy model as pointed out by SAGER and RAMANIS (1968). If the copy number were greater than two, hybrid cells would produce clones in which most of the progeny were homozygous for one or the other member of an allelic pair and the allelic ratio in the clone would depend on the number of UP genomes which the hybrid cell received from each parent.

In analyzing the progeny of spontaneous, unselected biparental zygote colonies we (GILLHAM 1965, 1969; GILLHAM and FIFER 1968) have consistently reported deviations from a 1:1 allelic ratio. A similar deviation was seen among the progeny of hybrid meiotic products obtained by tetrad analysis of spontaneous biparental zygotes (GILLHAM 1963) and among hybrid cells that had undergone several postmeiotic mitotic divisions (GILLHAM 1969). In all cases the UP alleles contributed by the $mt^+$ parent predominated. The deviation for a 1:1 allelic ratio observed in these experiments is most easily explained by a multiple copy model in which the majority of copies is donated to the zygote by the $mt^+$ parent.

How can these results in support of a multiple copy model be reconciled with those of SAGER which argue for a two copy model? Two interpretations are possible. The unselected spontaneous biparental zygotes could differ from the vast majority of maternal zygotes by containing more than two copies of the UP genome, with the excess copies usually provided by the maternal parent. Alternatively, spontaneous, unselected biparental zygotes could approximate the norm and biparental zygotes obtained either by selection of a specific UP marker or through the use of UV could contain less than the normal number of UP genomes from the maternal parent so that allelic pairs are present in approximately a 1:1 ratio.

Selection of biparental zygotes through the use of a specific UP marker involves selection *for* a specific allele carried by the $mt^-$ parent and *against* an allele carried by the $mt^+$ parent (SAGER and RAMANIS 1963, 1965). For example, in a cross of an acetate-requiring ($ac_1$) streptomycin-dependent (*sd*) $mt^+$ strain with an $mt^-$ streptomycin-resistant (*sr-2*) strain carrying a different acetate mutant ($ac_2$), biparental zygotes were recovered by plating the zygotes on an acetate-containing medium lacking streptomycin (SAGER and RAMANIS 1965). This simultaneously selects against the *sd* allele from the $mt^+$ parent and for the *sr-2* allele from the $mt^-$ parent. Allelic ratios of the unselected $ac_1$ and $ac_2$ markers among these progeny were found to approximate 1:1. One may suppose that progeny of biparental zygotes carrying an excess of *sd* alleles from the $mt^+$ parent would be killed during selection on streptomycin-free medium because they have a streptomycin-dependent phenotype. Those biparental zygotes surviving with-

out streptomycin would have an allelic ratio of *sd*:*sr* of approximately 1:1 to achieve a streptomycin-independent phenotype. Since $ac_1$ and $ac_2$ appear to be linked to *sr-2* (SAGER 1972), establishment of a 1:1 ratio for the *sr-2* and *sd* markers will also yield a 1:1 ratio for the acetate markers. This argument is supported by the fact that spontaneous biparental zygotes are 100-fold less frequent in the selection experiments of SAGER and RAMANIS (1963, 1965) than originally reported by SAGER (1954) for unselected biparental zygotes or routinely found in all of our experiments with unselected biparental zygotes (GILLHAM 1963, 1965, 1969; GILLHAM and FIFER 1968).

Thus it seems to us that the difference in allelic ratios seen between selected and unselected spontaneous, biparental zygotes may be related to their method of selection.

The foregoing hypothesis does not explain why SAGER and RAMANIS (1968) report a 1:1 allelic ratio among the progeny of biparental zygotes produced by irradiation of the maternal parent with UV. We felt that the best way of reconciling the discrepancies between our earlier results and those of SAGER and RAMANIS (1968) was to repeat the UV experiment of SAGER and RAMANIS (1967) over a range of UV doses and compare the allelic ratios of individual biparental zygotes thus obtained with those of an unirradiated control. If spontaneous, unselected biparental zygotes have abnormally high numbers of UP genomes contributed by the maternal parent and UV-induced biparental zygotes have one copy of the UP genome from each parent, one would expect the unirradiated control to give a skewed allelic ratio and the UV-induced biparental zygotes a 1:1 allelic ratio. If, on the other hand, the skewed allelic ratio of spontaneous unselected, biparental zygotes is normal and UV effectively reduces the number of UP genomes contributed by the maternal parent, one would expect the skew in allelic ratio to disappear gradually with increasing UV dose until a 1:1 allelic ratio is approximated.

## THE EFFECT OF ULTRAVIOLET LIGHT ON ALLELIC RATIOS

Reciprocal crosses between stocks carrying UP markers conferring resistance to spectinomycin (*spr-u-1-6-2*), erythromycin (*ery-u-37*), and streptomycin (*sr-u-2-60*) were made. Gametes were differentiated by suspending vegetative cells grown for six days on yeast extract acetate (YA) plates in 30 ml medium lacking nitrogen ($N^-$) and shaking them under ~15,000 lux cool white fluorescent light for five hours.[3] In each cross, four ml aliquots containing equal numbers of $mt^+$ gametes were irradiated with 0, 15, 30, 45 and 60 seconds of UV light. The aliquots were irradiated in a 5.5 cm petri dish bottom with continuous stirring at an intensity of 11,000 ergs $cm^{-2}$ $sec^{-1}$ with a G8T5 Sylvania Lamp mounted on a Chromato Vue CC20 cabinet. As described by SAGER and RAMANIS (1970), the

[3] The media used in these experiments were solidified with 15 g of Bacto Agar (Difco) per l unless otherwise noted and are as follows: HSA = high salt medium (HS) of Sueoka (1960) plus 2 g sodium acetate per l; YA = HSA plus 4 g yeast extract per l; ZM = minimal medium of EBERSOLD and LEVINE (1958) plus 40 g agar per l $N^-$ = liquid HSA meidum lacking $NH_4Cl$. Antibiotic media were prepared by adding filter-sterilized antibiotics equivalent to 100 mg streptomycin or spectinomycin and 200 mg erythromycin base per liter of HSA medium. The antibiotics and their sources were as follows: streptomycin sulfate, Lilly; erythromycin lactobionate, Abbott Laboratories; spectinomycin sulfate, Upjohn Co.

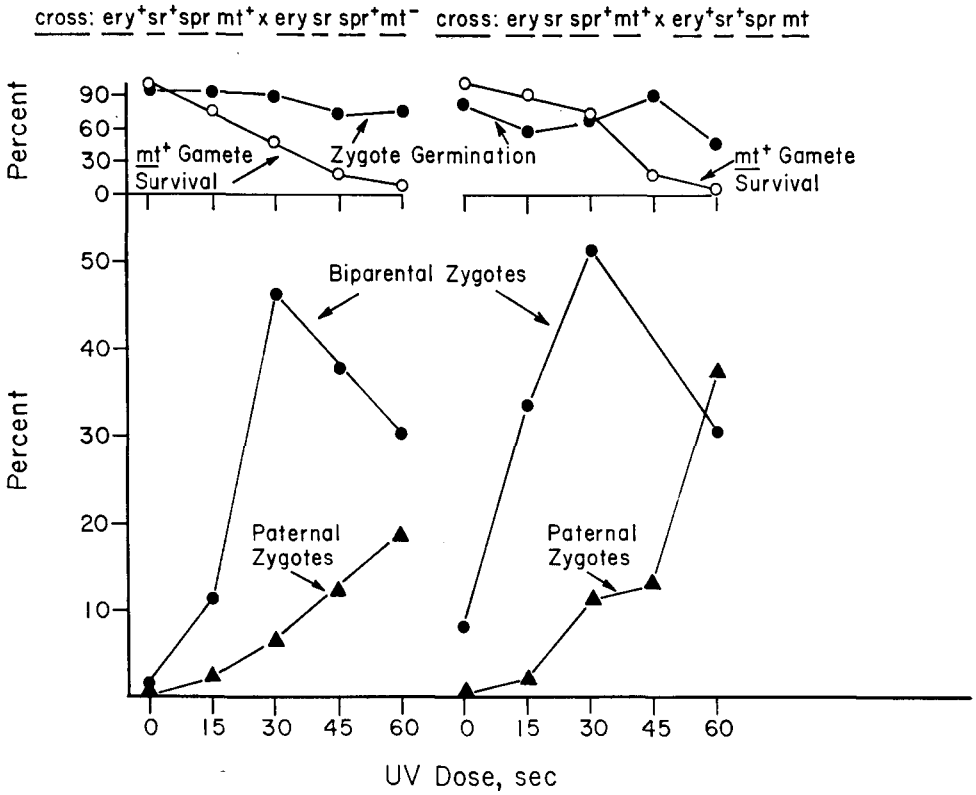


FIGURE 2.—Increase in the frequencies of biparental and paternal zygotes with increasing UV dose. The inset curves at the top of the figure show the effects of increasing UV dose on *mt*+ gamete survival and zygote germination.

irradiated *mt*+ gametes were mated in the dark for two hours with equal numbers of unirradiated *mt*− gametes, the mating mixture plated on zygote maturation (ZM) medium, the plates left in the light for 24 hours (~6,000 lux cool white fluorescent bulbs) and then matured in dark for a week or more. The *mt*+ gametes from the different UV treatments were plated on high salt acetate medium (HSA) in the light after a similar two hour dark incubation to determine killing. Under these conditions there is considerable photoreactivation of both lethality of *mt*+ gametes and the ability to form exceptional zygotes (SAGER and RAMANIS 1967).

Following maturation, zygotes were germinated on nonselective medium (HSA), allowed to form colonies, and these were replica-plated to medium containing each of the three antibiotics. Maternal zygote colonies grew only on media to which the maternal parent was resistant; paternal zygote colonies grew only on antibiotic-containing media to which the paternal parent was resistant; the biparental zygote colonies grew on all three antibiotic-containing media. Biparental zygote colonies identified by this method were picked from the original nonselective (HSA) plate, resuspended, and equal aliquots were replated on non-

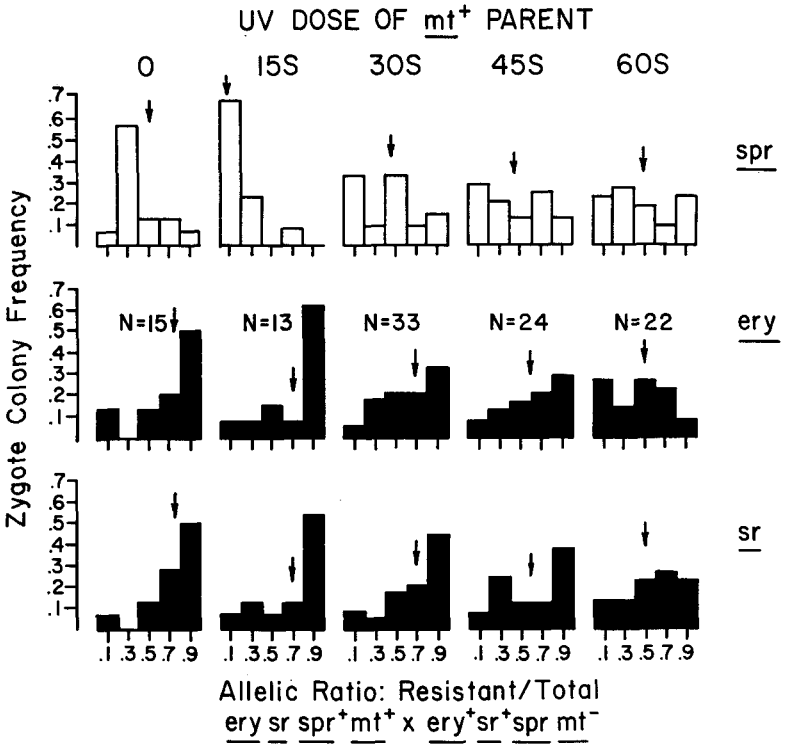


FIGURE 3.—Allelic ratios among biparental zygotes from the cross *ery sr spr*+ *mt*+ × *ery*+ *sr*+ *spr mt*−. The *mt*+ parent was treated with varying doses of UV prior to mating (see text for details). Zygote clones have been grouped into arbitrary classes based on the fraction of resistant cells ($R$) per clone. The number of colonies counted per zygote colony varied between 150 and 1000 on each of the diagnostic media.

selective medium and media containing individually each of the three antibiotics. The ratio ($R$) of resistant colonies growing on a particular antibiotic to the number of colonies growing on nonselective medium was then computed for each of hte UP markers studied. When the allelic ratio for a given zygote is 1:1, $R$ has a value of 0.5 since $R$ = resistant colonies/resistant + sensitive colonies. The *paternal allelic ratio* ($P$), defined as the ratio of colonies carrying a specific paternal allele over the total (i.e., $P$ = paternal colonies/paternal + maternal colonies), was also computed. When the paternal allele is the resistant allele $P=R$, but when the paternal allele is the sensitive allele $P=1-R$.

We found, as reported by SAGER and RAMANIS (1967), that the yield of biparental zygotes increased dramatically at low UV doses but that at higher UV doses biparental zygotes began to decrease in frequency and be replaced by paternal zygotes (Figure 2). Maximal numbers of biparental zygotes were induced by a UV dose which permitted survival of between 40 and 70 percent of the treated, unmated *mt*+ gametes (Figure 2). Zygote germination was not markedly affected by UV dose. In both crosses the three markers from the maternal parent were in excess in the majority of unirradiated, biparental zygotes (Fig-

ures 3 and 4). This excess persisted following 15 seconds irradiation in the cross *ery*$^+$ *sr*$^+$ *spr mt*$^+$ × *ery sr spr*$^+$ *mt*$^-$ and up to 45 seconds irradiation in the reciprocal cross. In the first cross the frequency of biparental zygotes had increased 8-fold at 15 seconds and the increase was over 4-fold at this time in the second cross. Clearly, maternal markers predominate among the progeny of most UV-induced biparental zygotes at low UV doses. At higher doses the distributions flatten out dramatically, but never approach normal. This change in the shape of the distribution away from the skew in favor of biparental zygotes with a high frequency of maternal alleles is accompanied by a shift in the mean value of *R* towards 0.5 (arrows, Figures 3 and 4). The UV experiment strongly supports the hypothesis that spontaneous biparental zygotes approximate the norm and that a deviation from this norm toward an average 1:1 allelic ratio is approached with increasing UV dose because the number of alleles contributed by the maternal parent is reduced as the UV dose increases to a point where *R* averages 0.5. Despite the fact that an average *R* value of 0.5 can be reached we do not believe this average has biological significance in terms of a two copy model since the frequency distribution around this mean is exceedingly broad and the values not normally distributed.

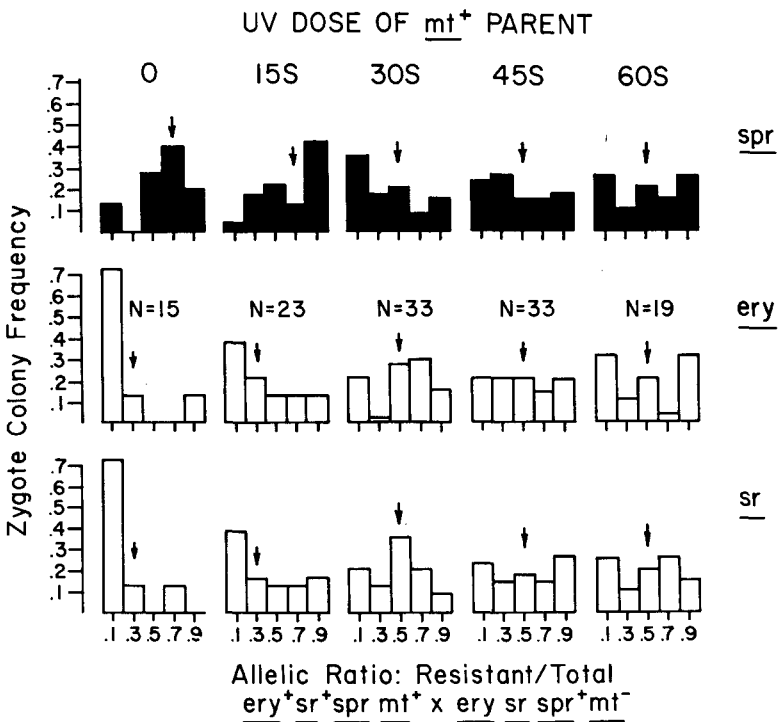


FIGURE 4.—Allelic ratios among biparental zygotes from the cross *ery*+ *sr*+ *spr mt*+ × *ery sr spr*+ *mt*−. The *mt*+ parent was treated with varying doses of UV prior to mating (see text for details). Zygote clones have been grouped into arbitrary classes based on the fraction of resistant cells (*R*) per clone. The number of colonies counted per zygote colony varied between 150 and 1000 on each of the diagnostic media.

TABLE 1

*Use of allelic ratios to establish predominant genotypes in an exceptional zygote colony*

| Zygote colony | Allelic ratio (paternal/total) *ery* | *sr* | *spr* | Majority genotypes in zygote colony |
|---|---|---|---|---|
| 1 | 0.89 | 0.79 | 0.65 | *ery sr spr*+, *ery*+ *sr*+ *spr* |
| 2 | <0.01 | 0.09 | 0.61 | *ery sr spr*+, *ery sr spr* |
| 3 | 0.29 | 0.30 | 0.18 | *ery sr spr*+, *ery*+ *sr*+ *spr* |
| 4 | 0.08 | <0.004 | 0.22 | *ery sr spr*+, *ery sr spr* |
| 5 | 0.07 | 0.04 | 0.26 | *ery sr spr*+, *ery sr spr* |
| 6 | 0.21 | 0.16 | 0.21 | *ery sr spr*+, *ery*+ *sr*+ *spr* |
| 7 | 0.07 | 0.04 | 0.21 | *ery sr spr*+, *ery sr spr* |
| 8 | 0.82 | 0.36 | 0.92 | *ery*+ *sr*+ *spr*, *ery*+ *sr spr* |
| 9 | 0.10 | 0.08 | 0.22 | *ery sr spr*+, *ery sr spr*? |
| 10 | 0.43 | 0.41 | 0.45 | *ery sr spr*+, *ery*+ *sr*+ *spr* |

The data are taken from the cross *ery sr spr*+ *mt*+ × *ery*+ *sr*+ *spr mt*− without UV treatment.

THE EFFECT OF ULTRAVIOLET LIGHT ON RECOMBINANT PRODUCTION

Examination of paternal allelic ratios ($P$) for each of the markers in the crosses analyzed in the UV experiment revealed that the predominant genotypes present in the biparental zygote colony could frequently be deduced. The method is exemplified by some sample data in Table 1. For example, in the case of zygote colony two, $P$ has values of 0.61 for *spr*, <0.01 for *ery*+ and 0.09 for *sr*+. This indicates that most cells carrying the *spr* marker from the parental parent are recombinant and carry the *ery* and *sr* alleles from the maternal parent. Similar sorts of deductions could be made for many but by no means all zygote colonies. Where doubt existed the paternal allelic ratios for each marker were tested by

TABLE 2

*Classification of recombinants isolated in the UV experiment*

| Cross | Recombinant class | UV dose (seconds) 0 | 15 | 30 | 45 | 60 |
|---|---|---|---|---|---|---|
| *ery sr spr*+ *mt*+ × | *ery sr spr* | 6 | 1 | 4 | 1 | 0 |
| *ery*+ *sr*+ *spr mt*− | *ery*+ *sr*+ *spr*+ | 0 | 7 | 1 | 1 | 0 |
| | *ery*+ *sr spr*+ | 0 | 1 | 1 | 0 | 4 |
| | *ery sr*+ *spr* | 0 | 0 | 2 | 1 | 4 |
| | *ery sr*+ *spr*+ | 0 | 3 | 0 | 0 | 0 |
| | *ery*+ *sr spr* | 1 | 0 | 3 | 2 | 3 |
| *ery*+ *sr*+ *spr mt*+ × | *ery sr spr* | 0 | 6 | 1 | 2 | 2 |
| *ery sr spr*+ *mt*− | *ery*+ *sr*+ *spr*+ | 6 | 3 | 5 | 3 | 1 |
| | *ery*+ *sr spr*+ | 1 | 1 | 1 | 1 | 0 |
| | *ery sr*+ *spr* | 0 | 0 | 1 | 1 | 0 |
| | *ery sr*+ *spr*+ | 1 | 0 | 3 | 0 | 2 |
| | *ery*+ *sr spr* | 0 | 0 | 0 | 2 | 0 |

The data tabulated represent the number of recombinants of each kind identified at each UV dose.

Chi-square to determine whether significant differences existed between them.

The data on recombinant frequencies compiled by this deductive method are summarized in Table 2. Without UV, the predominant recombinants are the reciprocals *ery sr spr* and *ery*$^+$ *sr*$^+$ *spr*$^+$. *However, only one of the two reciprocals is found in each cross and it is the one carrying two maternal alleles and one paternal allele.* This result is expected since the allelic ratio in the absence of UV is strongly skewed in favor of maternal markers.

UV greatly reduces the bias towards maternal markers among reciprocal recombinants (Table 2). Computation of the average ratio of recombinants carrying two maternal and one paternal marker to recombinants carrying one maternal and two paternal markers shows a drop from 4 in the absence of UV to between 0.5 and 1 with UV (Figure 5). That is, UV causes reciprocal recombinants to appear in more nearly equal frequencies among the progeny of a given cross.

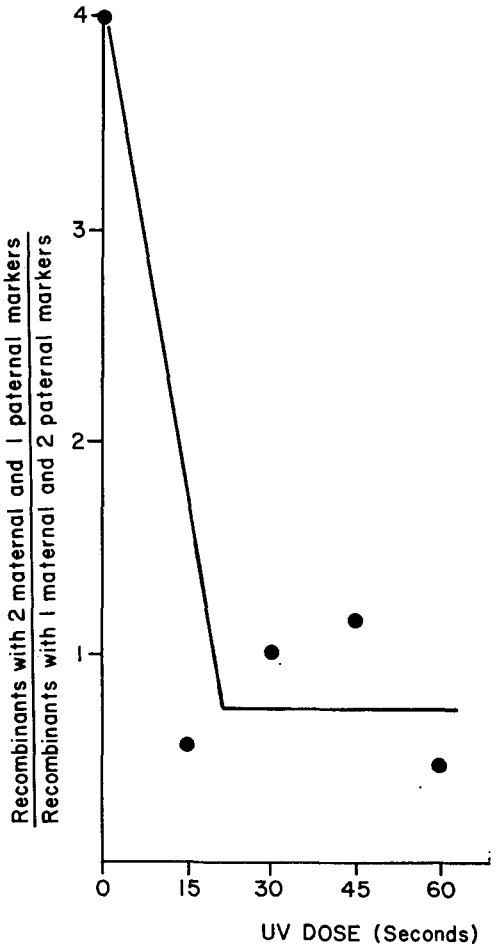


FIGURE 5.—Ratio of recombinants carrying two maternal and one paternal markers to recombinants carrying one maternal and two paternal markers plotted versus UV dose. Results of the two crosses have been pooled.

## A VARIABLE COPY MODEL OF UNIPARENTAL INHERITANCE

Results presented in this paper suggest that irradiation with increasing doses of UV of the maternal parent prior to mating gradually shifts the allelic ratio among the progeny of biparental zygotes from a distribution skewed in favor of maternal alleles towards one in which alleles from both parents are present in more nearly similar frequencies. This distribution is still not normal around a mean allelic ratio of 0.5. Similarly UV alters the ratio of reciprocal recombinants towards 1:1 from a ratio which favors recombinants carrying a majority of maternal and a minority of paternal alleles.

These observations have led us to question SAGER's two copy model of UP inheritance and to explore multiple copy models in which the ratio of maternal to paternal genomes varies, with maternal genomes usually predominating. Although fully aware of the limitations inherent in model making, we wish to elaborate one possible multiple copy model here to show how it might account both for our results and SAGER's and to serve as a departure point for future experimentation.

Our model is based upon the behavior of incompatible plasmids in bacteria (see CLOWES 1972 for a review). We assume that in a maternal zygote, paternal UP genomes are excluded by maternal UP genomes, much as certain bacterial plasmids exclude others. The mechanism of exclusion may be similar in both cases. The zygote of *C. reinhardtii* would contain a fixed number of membrane attachment sites required for replication and segregation of UP genomes by the oriented mechanism proposed by SAGER (1972). These sites would be occupied preferentially by maternal genomes. In a biparental zygote, one or more attachment sites would become occupied by a paternal UP genome. In a paternal zygote, attachment sites would be occupied by paternal UP genomes because of the spontaneous loss or destruction of all maternal UP genomes. Alternatively a change in the specificity of the paternal UP genomes in the $mt^-$ parent for attachment sites could occur leading to a reversal in the direction of exclusion. This may explain not only the occurrence of paternal zygotes but also the low frequency of spontaneous biparental zygotes which show reversal of the expected allelic ratio in favor of paternal genomes. (Figures 3, 4). Immediate destruction of paternal UP DNA in maternal zygotes is not a necessary prerequisite of the plasmid model as it is in the restriction-modification model of SAGER. Paternal UP DNA could simply be diluted out during the postmeiotic mitotic divisions. However, destruction of such "unattached" DNA would not be inconsistent with our model either.

A feature shared by both the restriction-modification and plasmid models is that their specificity is in some way conferred by the nuclear *mt* locus of *C. reinhardtii*. When maternal UP genomes from the $mt^+$ parent are introduced into the $mt^-$ meiotic products of a maternal zygote, they become functionally converted, at some point into paternal UP genomes (i.e., they are no longer transmitted at high frequencies in subsequent crosses). Similarly, when paternal UP genomes from the $mt^-$ parent are transmitted to $mt^+$ meiotic products in an

exceptional zygote, they become functionally converted to maternal UP genomes (i.e., they are transmitted at a high frequency in subsequent crosses). Thus the ability of the UP genome to be transmitted in a cross is clearly dependent upon the nuclear mating type gene of the cell in which it resides.

The two copy restriction-modification model of SAGER and the multiple copy plasmid model proposed here differ in the way they interpret the effects of UV on maternal gametes. The restriction-modification model assumes two UV effects both of which are photoreactivable since visible light converts paternal zygotes to biparental zygotes at a faster rate than biparental zygotes are converted to maternal zygotes (SAGER and RAMANIS 1967; SAGER 1972). Sager assumes that conversion of paternal to biparental zygotes results from restoration of one or both of the two UV-inactivated maternal UP genomes to a functional state by visible light. Biparental zygotes are not rapidly converted to maternal zygotes because the second UV effect eliminates the capability of the $mt^+$ parent to synthesize the regulator substance which activates the restriction enzyme present in the $mt^-$ parent (SAGER and RAMANIS 1973) and this process proceeds at a slower rate. In contrast, the plasmid model assumes a single site of action for the UV effect. UV inactivates maternal UP genomes. Paternal zygotes are converted to biparental zygotes and biparental zygotes to maternal zygotes at different rates by photoreactivation because the former involves only the conversion of a single nonfunctional maternal UP genome to a functional state whereas the latter requires conversion of all nonfunctional UP genomes present to a functional state. In short the plasmid model predicts that the two processes should show different photoreactivation kinetics because the number of maternal UP genomes which must be photoreactivated is different for the two processes and not because two different UV effects are occurring.

We have tried to approach a quantitative formulation of our variable copy plasmid model in the following way. Suppose that in a population of zygotes, paternal UP genomes are able to occupy vacant attachment sites at random, but that the number of vacant attachment sites will normally be very low because almost all are occupied by maternal UP genomes. Gametes contributing a full complement of maternal UP genomes produce maternal zygotes; those contributing less than normal, biparental zygotes; and those contributing none, paternal zygotes. The average number of paternal genomes transmitted per zygote ($m_p$) can be estimated from the number of zygotes in which no paternal genomes were transmitted from the $P_0$ term of the Poisson distribution

$$\text{maternal zygotes/total zygotes} = e^{-m_p} \tag{1}$$

Similarly the mean number of maternal genomes transmitted per zygote ($m_m$) can be calculated from the fraction of zygotes which received no maternal genomes at all

$$\text{paternal zygotes/total zygotes} = e^{-m_m} \tag{2}$$

Using these relationships, values of $m_p$ and $m_m$ are easily computed for the UV experiment (Table 3). The value of $m_m$ for the unirradiated control is difficult to determine since spontaneous paternal zygotes are very rare and a rare chance

TABLE 3

*Mean number of paternal ($m_p$) and maternal ($m_m$) UP genomes per zygote following different doses of UV irradiation*

| Cross | UV dose (sec) | Fraction of zygotes not receiving paternal genome ($MZ/TZ = e^{-m_p}$) | $m_p$ | Fraction of zygotes not receiving maternal genome ($PZ/TZ = e^{-m_m}$) | $m_m$ |
|---|---|---|---|---|---|
| | 0 | .985 | .015 | 0 | >10 |
| *ery+ sr+ spr mt+* | 15 | .870 | .140 | .02 | 3.90 |
| × | 30 | .475 | .740 | .065 | 2.73 |
| *ery sr spr+ mt−* | 45 | .500 | .690 | .125 | 2.08 |
| | 60 | .540 | .620 | .185 | 1.68 |
| | 0 | .920 | .080 | .005 | 5.30 |
| *ery sr spr+ mt+* | 15 | .664 | .410 | .020 | 3.90 |
| × | 30 | .390 | .940 | .110 | 2.20 |
| *ery+ sr+ spr mt−* | 45 | .740* | .750 | .130 | 2.04 |
| | 60 | .326 | 1.120 | .370 | 0.99 |

Abbreviations: MZ = maternal zygotes; PZ = paternal zygotes; TZ = total zygotes.
* Due to an apparent error in scoring which yielded too many maternal zygotes and too few biparental zygotes, this value is extrapolated from the curve shown in Figure 3.

event will have a big effect on the subsequent computations. The data suggest $m_m$ could be 5, 10 or more. Table 3 also shows that the decrease in the number of maternal UP genomes per zygote with increasing UV dose is accompanied by an increase in the number of paternal UP genomes. However, the two do not balance out, so that a net reduction in the number of UP genomes per zygote occurs with increasing dose as a 1:1 allelic ratio is approached.

Using the values of $m_m$ and $m_p$ in Table 3, the theoretical distributions of allelic frequencies among the different biparental zygote populations were calculated from the Poisson distribution (Figure 6). For example, in the cross *ery sr spr+ mt+* × *ery+ sr+ spr mt−* at 0 UV dose the probabilities that a zygote will receive 0, 1 or 2 copies of the paternal UP genome are 0.92, 0.074 and 0.0066 respectively. Therefore, among biparental zygotes 0.92 (0.074/0.074 + 0.0066) will receive one copy of the paternal UP genome and 0.075 two. Similar calculations for maternal UP genomes show that only 0.027 will receive a single maternal UP genome but 0.175 will receive five. Therefore, the proportion of biparental zygotes receiving one allele from each parent ($P = 0.5$) equals (0.92) (0.027) = 0.025 while the proportion receiving one allele from the paternal parent and five from the maternal paraent ($P = 0.17$) equals (0.92) (0.175) = 0.161. If class sizes for $P$ similar to those of the observed data (0.01–0.20, 0.21–0.40 etc.) are set up for the theoretical distributions, the fraction of zygotes in which $P$ is 0.2 or less is 0.719.

The theoretical and observed paternal allelic ratios ($P$) are compared in Figure 6. The observed ratios for each biparental zygote are computed as the mean ratio for all three markers. This is justified because computation of correlation coefficients revealed a strong correlation in the frequencies with which paternal

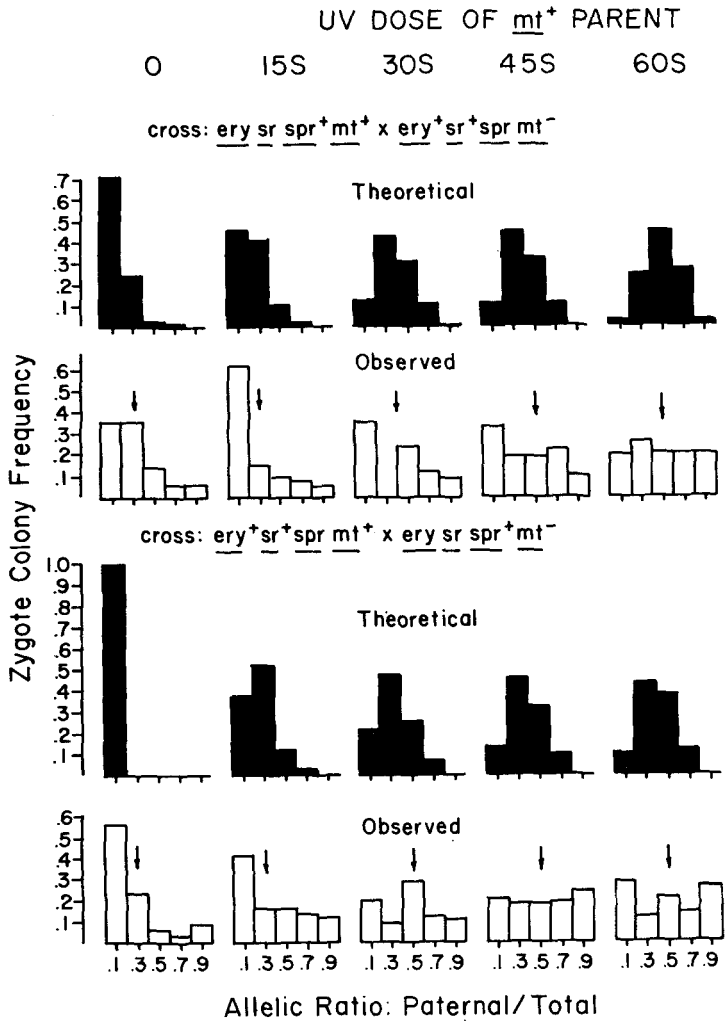


FIGURE 6.—Theoretical and observed paternal allelic ratios ($P$) from the UV experiment. The observed paternal allelic ratios were calculated from the mean paternal allelic ratio for all three markers in each cross (see text for explanation). Zygote clones have been grouped into arbitrary classes based on the mean fraction of cells carrying paternal markers per clone.

alleles are transmitted in biparental zygotes ($p < .001$ for each marker pair) despite the fact that recombination occasionally distorts this ratio in specific clones.

Both the theoretical and observed distributions are skewed towards a high proportion of biparental zygotes with low values of $P$ in the absence of UV and at low UV doses. This skew becomes less pronounced at higher UV doses and the theoretical distribution begins to approach a normal distribution as the values of $m_p$ and $m_m$ become more similar. In contrast, the observed allelic ratios tend to be skewed towards the extremes. This departure from a normal distribution may

result from a random "drift" of paternal and maternal genomes into a reduced number of functional attachment sites. Such a reduction in the number of functional attachment sites is suggested by the fact that the sum of $m_m$ plus $m_p$ (Table 3) drops to a little over three after 30 seconds of UV irradiation. It might occur either because of a direct effect of UV on the attachment sites or because a fraction of total attachment sites becomes occupied by UV-inactivated maternal UP genomes. Whether or not this interpretation is correct, our results show that the input number of genomes as calculated from the Poisson means in Table 3 does not mirror the output as calculated from the final allelic ratio as UV dose is increased.

Our preliminary results on production of recombinants also deserve comment. Without UV we find a highly asymmetric distribution of the two predominant reciprocal recombinant types among the population of biparental zygotes such that in each cross the predominant recombinant carries two maternal and one paternal markers. This is precisely the result one would predict if the paternal genome is in the minority in these biparental zygotes and if it can undergo successive rounds of recombination with the majority maternal genome. Such successive rounds of recombination will produce a pool of recombinants most of whom contain a single paternal marker and two maternal markers. More nearly symmetric frequencies of reciprocal recombinants will be found among the population of biparental zygotes produced by UV since irradiation reduces the input number of functional maternal genomes, on the average, to a level not greatly different from the input number of paternal genomes. We feel that it should be possible to analyze UP gene recombination in Chlamydomonas in terms of the theory developed by VISCONTI and DELBRUCK (1953) for phage. Assuming no intracellular selection for specific genotypes, allelic ratios can be used as a measure of input of genomes from both parents in a biparental zygote, in a manner analagous to the multiplicity of infection in a phage cross. Recombination frequency can then be measured as a function of input. We would predict a decrease in the asymmetric frequency of reciprocal recombinant types and an increase in recombination frequency in those zygotes having more nearly equal allelic ratios. Results presented in this paper together with an analysis of data of GILLHAM (1965) suggest that both of these expectations are, in fact, fulfilled.

The variable copy model can also generate the three types of oriented segregation patterns described by SAGER (1972). Type I segregations (hybrid daughters) are produced by hybrid cells whose copy number is two or more (Figure 7). Type II segregations (hybrid daughter + daughter homozygous for alleles at one or more loci) are produced by hybrid cells with a copy number greater than two in which reciprocal recombination has occurred (Figure 7). In contrast, SAGER's two copy model requires that such recombinants arise by nonreciprocal recombination (gene conversion) (Figure 1). Type III segregations (each daughter cell homozygous for an allele of a pair) arise as they do in SAGER's model from hybrid cells which contain only two copies and in which reciprocal recombination has occurred (Figure 1). Polarity of type III segregations occur for the reasons described by SAGER (1972). Homozygosity for a given allele is more often achieved

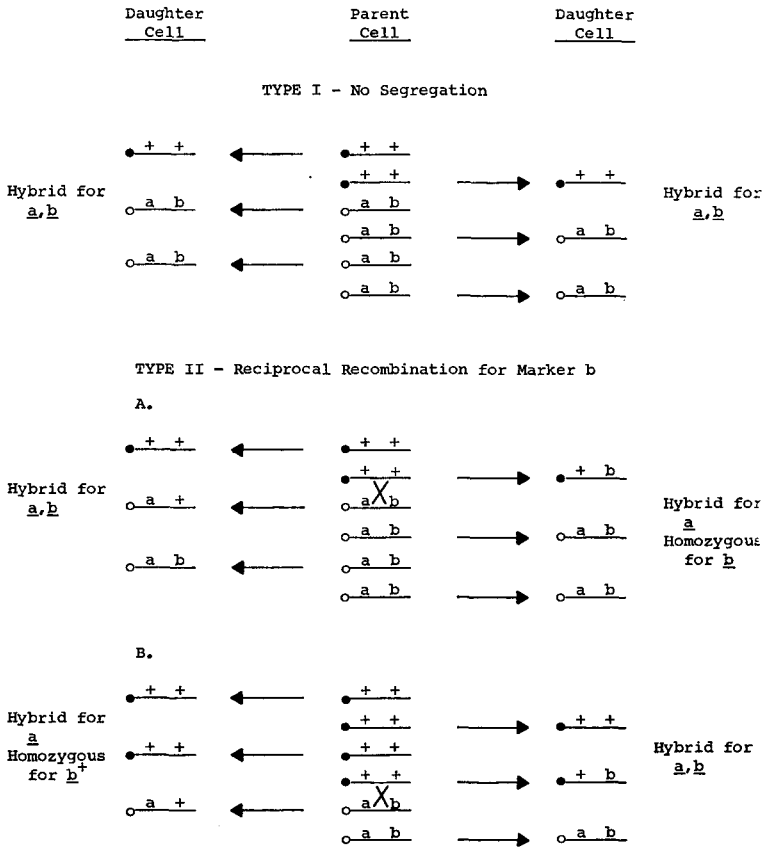


FIGURE 7.—Origin of type I and II segregants from hybrid cells containing four copies of one genome and two of the other.

by Type II segregation than by type III segregation (SAGER and RAMANIS 1968; GILLHAM 1969). This is in keeping with the hypothesis that among biparental zygotes homozygosity for an allele is most often achieved by recombination in cells having more than two copies of the UP genome. We would predict that with increasing UV dose the ratio of type III to type II segregations will increase due to the decline in functional maternal genomes.

Since type I segregations yield only hybrid daughter cells and type III segregations yield allelic ratios of 1:1, explanation of the clear deviations from these ratios reported here and elsewhere (GILLHAM 1963, 1965, 1969; GILLHAM and FIFER 1968) must be sought among the type II segregations. That is, if maternal UP alleles are in excess, type II segregants for these alleles should exceed type II segregants for paternal UP alleles. Evidence that this is the case for spontaneous unselected biparental zygotes has been published elsewhere (GILLHAM 1969). One way in which type II segregants could give rise to a skewed allelic ratio by oriented segregation and reciprocal recombination is shown in Figure 8. The orig-

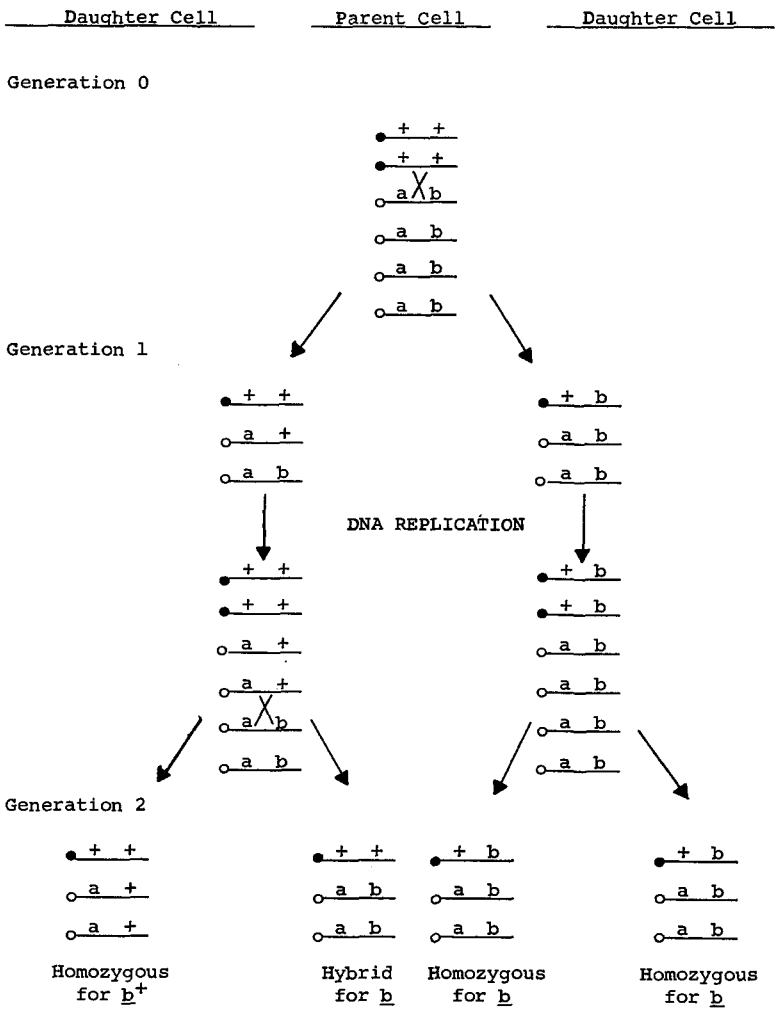


FIGURE 8.—Skewing of marker ratio by type II segregation among the progeny of a hybrid cell which originally contained four copies of one genome and two of the other. Notice that type II segregation for marker *b* by reciprocal recombination at the first division changes the allelic ratio for *b*+:*b* from 1:2 in generation 0 to 2:1 in the resulting hybrid cell of generation 1. By generation 2 a cell homozygous for *b*+ can be segregated by type II segregation. However, if this event occurs, the allelic ratio of *b*/total will only be 0.33 since two of the four cells are homozygous for *b*, one homozygous for *b*+ and the other hybrid for *b* and *b*+.

inal hybrid cell in this example is assumed, following DNA replication, to contain four maternal UP genomes and two paternal UP genomes. This general pattern is easily expanded to other genomic ratios.

In conclusion, we wish to emphasize that our model is only one of several possible multiple copy models. For example, one can construct a model in which the paternal genome is "rescued" in a biparental zygote by recombination with a maternal UP genome. Nevertheless, we believe the results discussed in this paper

are not easily explained by a two copy model and that future investigations on the uniparental genetic system should, at the very least, take into account the fact that the maternal parent appears to contribute multiple copies of its UP genome to the zygote. Such a result is not unexpected in view of the fact that individual chloroplasts and mitochondria appear to contain a minimum of several copies of their respective, unique DNA's (see SAGER 1972 for a review).

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