Occurrence of Multiploid Sporocytes in the Genus *Sorghum**

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Summary. The breakdown of the meiotic cycle in the plasmodial microsporocytes is described in detail for the interspecific hybrid, *S. almum* (2n = 40) × *S. halepense* (2n = 40). The plasmodial masses probably result from a suppression of wall formation during premeiotic mitosis. The possible role played by some of the mitotic organelles in the formation of atypical as well as multipolar spindles in the plasmodial cells is also considered.

A fairly large number of genetically determined meiotic irregularities have been reported (see DARLINGTON and THOMAS, 1937; SMITH, 1942; REES, 1961 and MAGOON and KHANNA, 1963). These affect almost every conceivable aspect of meiotic behaviour capable of being disturbed and for the most part, the effects of these genes have been extensively investigated in the microsporocytes of plants (SWANSON, 1957). The present paper describes the breakdown of meiotic division cycle and the probable role of some mitotic organelles in the formation of atypical spindles in the plasmodial pollen mother cells. The *Sorghum* material utilized in the present investigation provided an opportunity to study meiosis under condition of incomplete separation or fusion of pollen mother cells.

Material and methods

Material used in the present investigation are listed below.

1. *Sorghum almum* parodi. P.I. 240996 (2 n = 40)
2. *Sorghum halepense* (Linn.) pers. I.S. 3304 (2 n = 40)
3. *Sorghum almum* × *Sorghum halepense*

Seeds of the parental species were obtained through the courtesy of the Rockefeller Foundation, Division of Botany, I.A.R.I., New Delhi, and were sown in pots in the green house as well as under field conditions. About 50 spikelets were emasculated in *S. almum* and pollinated with the pollen of *S. halepense* under controlled conditions. Ten crossed seeds thus obtained were sown in pots in the green house along with the parental species, but only five seeds germinated. It was established from morphological studies that all these five plants were real hybrids as they had purple stigma colour which is a characteristic feature only of the male parent. However, one out of these five F_1 plants showed stunted growth, small leaves and a few tillers etc. as compared to the other four F_1 plants. The detailed cytomorphology of this plant alone which exhibited distinct morphological differences from the other four F_1 plants is dealt with in the present investigation. For the study of microsporogenesis, the simple propiono-carmine smear method (see SWAMINATHAN et al., 1954; MAGOON et al., 1962; KITA et al., 1959) was followed.

Table 1. *Showing the external morphological features of the parents and their F_1 hybrid.*

Characters	*S. almum*	*S. almum* × *S. halepense*	*S. halepense*
Plant height (cm.)	221.5	154.9	217.4
Number of leaves	16	12	14
Length of the 5th leaf (cm.)	64.5	48.3	61.3
Breadth of the 5th leaf (cm.)	3.7	2.1	3.2
Number of tillers	12	5	18
Stigma colour	Yellow	Purple	Purple

Observations

a) External morphology: The comparative morphological data of both the parental species as well as the F_1 hybrid between them is presented in Table I.

The F_1 plant exhibited a remarkably stunted growth with very thin culms and small leaves. The panicle was also small and loose. The purple stigma colour found in the male parent (*S. halepense*) showed dominance.

b) Cytology of the F_1 plant: Critical observations on the early prophase stages revealed the presence of masses of cytoplasm with no cell wall and containing many nuclei (Fig. 1). This condition may

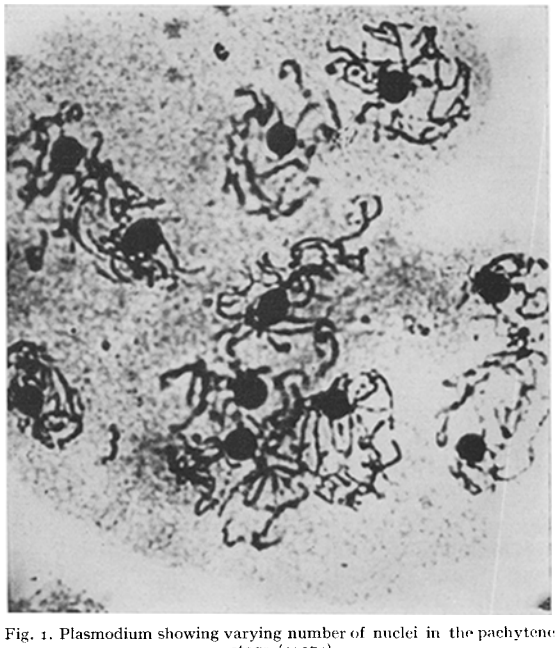

Fig. 1. Plasmodium showing varying number of nuclei in the pachytene stage (×374).

* These studies were largely undertaken at the Division of Botany, I.A.R.I., New Delhi where the authors were previously located. They are, therefore, grateful to the Director, I.A.R.I., Dean of P. G. School and Head of the Division of Botany for their keen interest and facilities. One of us (R.S.S.) is also thankful to I.C.A.R. as well as to C.S.I.R. for the award of Senior fellowship. Cooperation of Rockefeller Foundation, I.A.R.I., and C.T.C.R.I., is also hereby acknowledged.

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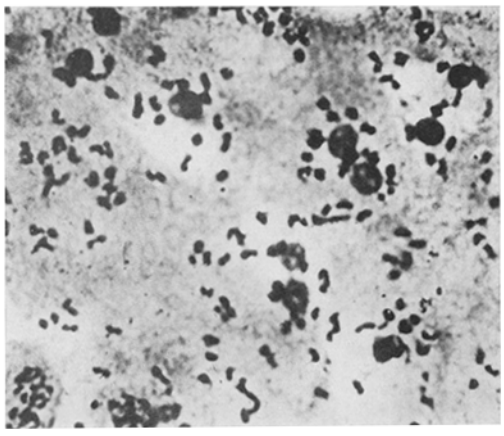
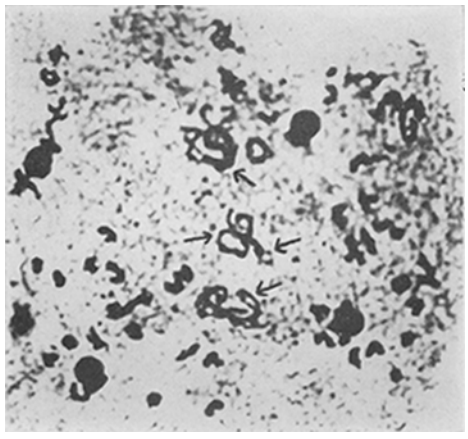


Fig. 2.



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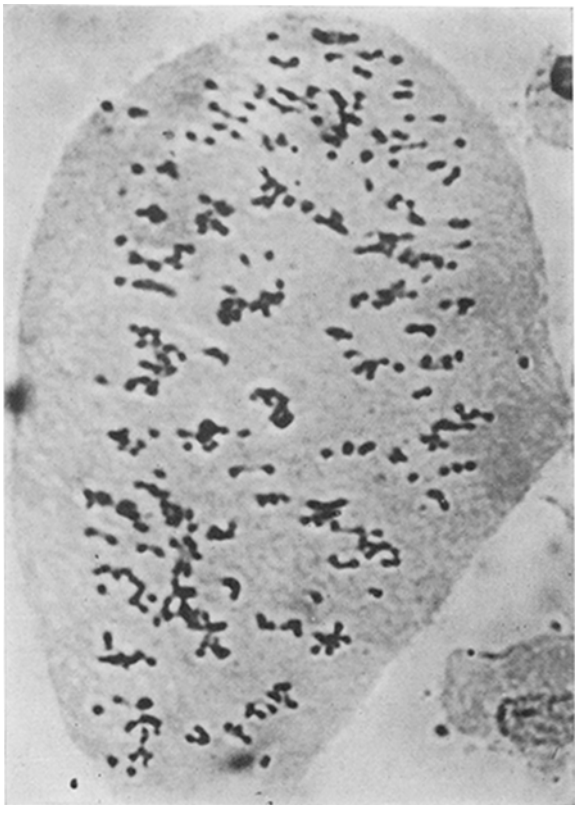

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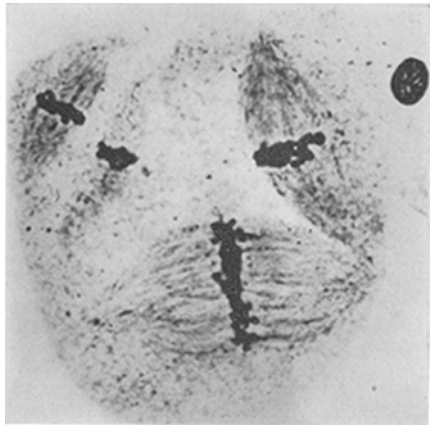

Fig. 5.

Fig. 2. Plasmodium showing varying number of nuclei in diakinesis stage (× 374).

Fig. 3. Diakinesis showing the differential condensation of bivalents (↑) (× 393).

Fig. 4. Oversized metaphase plate with bivalents showing normal orientation (× 468).

Fig. 5. Plasmodium showing polypolar spindle in metaphase I (× 374).

be the result of the fusion of variable number of pollen mother cells into plasmodial masses. The plasmodia vary greatly in size and number and occur regularly in all the anthers. A clear boundary of individual cells could not be made out either at this stage or at diakinesis. In a large number of plasmodia examined, all the nuclei are apparently capable of beginning meiosis.

At diakinesis, a number of plasmodial masses were seen with varying number of nuclei lying in them (Fig. 2). In majority of the plasmodia, the prophase development of chromosomes in various nuclei was found to be perfectly synchronised. However, in a few plasmodial cells the chromosomes also appeared to undergo differential condensation at diakinesis (Fig. 3). The chromosome pairing at diakinesis was higher than might be expected in the abnormal tissues such as these (Fig. 2). The mechanism which keeps the various nuclei in the common cytoplasm separate from one another disappears at metaphase I and consequently the bivalents from different nuclei arrange themselves on a common equatorial plate in many of the plasmodia. This indicates that a single giant spindle is organised in these (Fig. 4). However, occasionally, several spindles may be organised in each plasmodium. In that case, the spindles vary in size having different chromosome number on the equatorial region. Besides these abnormalities, the occurrence of polypolar spindle was also occasionally noted in these plasmodial cells (Fig. 5).

The chromosome pairing at MI was almost normal though sometimes a few univalents were also present. No clear-cut multivalents were noted at this stage. Normally, at MI, it was found that the chromosome numbers in different plasmodia were not limited to a doubling series such as 20, 40, 60, 80, 100 etc.; odd numbers of nuclei often occurred in complete masses of cytoplasm. Typically, the plasmodia at MI exhibited a smooth and regular outline (Fig. 4) and deviation occurred rarely particularly when the contour

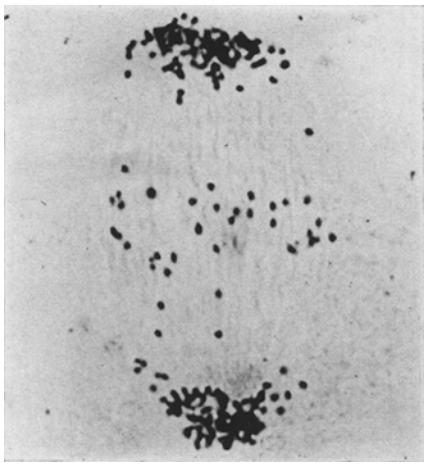

Fig. 6. Anaphase I showing varying number of lagging chromosomes (× 411).

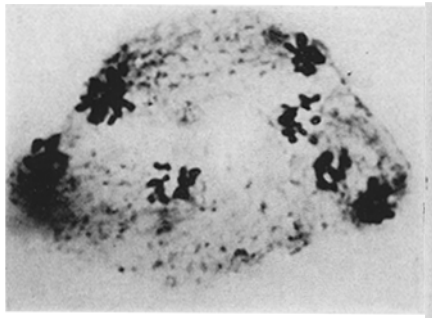

Fig. 7. Plasmodium showing several groups of chromosomes at telophase (× 431).

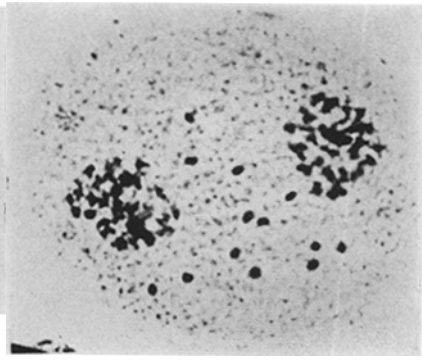

Fig. 8. Telophase I showing lagging chromosomes (× 449).

was broken by the presence of a nucleus greatly out of phase with the remaining mass. This suggests the presence of a partially separating membrane between the cytoplasm containing the nucleus with the divergent behaviour and the main body of the plasmodium. In many cases, a thin peripheral membrane around the individual plasmodium was also suspected (Fig. 4).

Anaphase I was highly abnormal with different types of irregularities such as lagging of varying number of chromosomes (Fig. 6), division of univalents, delayed separation of bivalents, sticky chromosomes etc. In many groups the spindle is obviously much shorter in proportion to its width than the normal. In anaphase the chromosomes move apart about the same distance as in normal plants, though often the movement is not limited by the bounds of the PMC material (plasmodium). The movement therefore appears to be stopped by some other mechanism, such as possibly the inability of the repelling force (electro-magnetic or protoplasmic streaming) to push them further apart. At late anaphase, in many larger groups, the chromosomes do not converge on a point. Therefore, from the shape and size of the spindle in these groups, it is doubtful if there is any point which can function as a 'pole' in the normal cytological sense of the term. The widely scattered anaphase chromosomes failed to be included into a single telophase nucleus. Instead, large and several smaller nuclei of different sizes were formed (Fig. 7). Sometimes the cytoplasm between the two groups of chromosomes divides, but this is not followed by the formation of cell walls.

At telophase I, the chromosomes usually formed a spherical nucleus as in the normal plants. However, when the chromosome number is large, an elongated nucleus is formed. Varying number of lagging chromosomes were also noted at this stage (Fig. 8). In one out of 30 plasmodial cells analysed at telophase I, non-synchronisation of nuclear division was also noted. In other words, one nucleus was at late telophase whereas the other was still at pachytene stage (Fig. 9).

Usually, the plasmodial cells do not seem to proceed further with the second meiotic cycle although there is an indication that in some cases the cytoplasm may continue to develop physiologically. It becomes more granular and coarse and resembled the condition observed in the formation of normal pollen grain (Fig. 10). In a few cases where the nuclei entered second division they showed a regular mitotic metaphase but the chromosomes undergo disorganization of the chromatin material soon after anaphase movement.

The anthers failed to dehisce and as a result no seeds were set when selfed. The pollen fertility was tested by teasing out the anthers. It was found that all the pollen grains were empty (Fig. 11). However, 1 to 8 seeds were obtained when pollinated with the normal pollen of its parental species.

Discussion

The fusion of several pollen mother cells into plasmodial masses of varying size was noted as early as

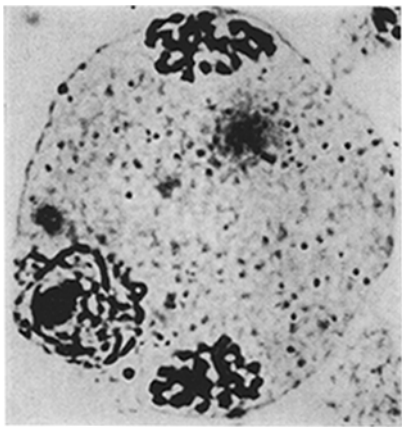

Fig. 9. Non-synchronization of nuclear division (× 524).

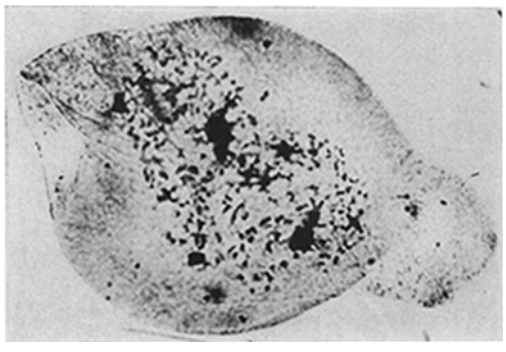

Fig. 10. Deterioration of plasmodium cell (× 224).

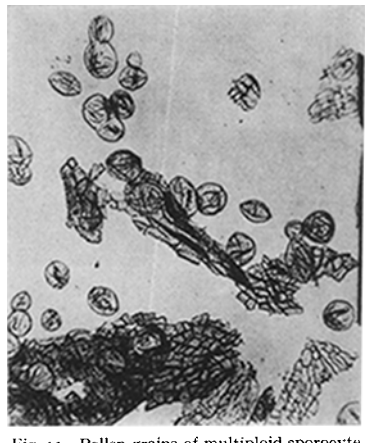

Fig. 11. Pollen grains of multiploid sporocyte plant (× 224).

pachytene stage (Fig. 1). These plasmodial masses probably result from a suppression of wall formation during several premeiotic mitosis. Similar observations have also been made by some workers in the different plant species (BEADLE, 1932 in *Zea*; DARLINGTON and THOMAS, 1937 in a trisomic derivative of a *Lolium-Festuca* hybrid; LEBEDEFF, 1940 in *Zea*; SMITH, 1942 in *Hordeum*; SNOAD, 1954 in *Helianthemum*; MORGAN, 1956 in *Zea* and JAIN, 1962 in *Lolium*). In a majority of these cases the reported abnormality has been shown to be genetically determined. However, in *Calamagrostis*, it is inferred that the occurrence of plasmodia may have been the result of extreme environmental condition, since several plants growing in the alpine regions of Swedish mountains were all abnormal and produce no pollen (NYGREN, 1946). In the material under investigation, however, fluctuations in climatic conditions can be ruled out as several sister plants from the same cross growing side by side with the abnormal one did not show such plasmodial masses. It may be possible that the phenomenon noticed in the present material has a genetical basis as has been observed in certain other plant material by some authors. The present case, however, differs in that the abnormal plant has been noted only in hybrid progenies and therefore, in postulating genetical determination of this condition, this fact has to be kept in mind. Because the fairly large number of selfed progenies from the parents which were analyzed did not exhibit such abnormalities, it does not appear likely that this phenomenon is due to a simple recessive gene as has been postulated in other crops (BEADLE, 1932 in *Zea*; SMITH, 1942 in *Hordeum*; SNOAD, 1954 in *Helianthemum*). It may be that this condition in the present case is probably due to a dominant mutation occurring in one of the gametes or later or it may be that a more complicated genetical basis is involved. Nothing definite can, however, be said at present as to the exact cause for this abnormality. Further studies particularly on the few progenies which may be obtained from this abnormal plant will be needed.

Usually, the nuclei in these plasmodial cells were at the same stage of division. However, occasionally it was also found that the nuclei were in different stages of division (Fig. 9). At MI, the bivalents are not widely spaced in the plasmodial mass, but on the contrary bivalents from many cells tend to congregate into a single metaphase plate. Such aggregation appears to suggest more the effect of protoplasmic streaming than the effects of electro-magnetic forces (SMITH, 1942).

The course which meiotic division takes in a plasmodium is variable. It is usually influenced by several factors including the size and mode of origin of the plasmodium. In the present material, the course taken by meiotic division in the plasmodial cells is similar in some respects with the observations made by SMITH (1942) in barley and JAIN (1962) in *Lolium*. They noted, as in the present study, large size metaphase plates and diffuse nature of spindle poles which results in divergent type of anaphase separation. These observations appear to have a good bearing in understanding the mode of organization of the spindle and the mechanism determining metakinesis and separation and movement of chromosomes during anaphase.

Several authors have given plausible explanations for the formation of typical spindle shape (see SWANN, 1957; RIS, 1957 and REES, 1961). Though the contribution of centrosomes in the organization of spindle is well recognized, the role played by centromere in this regard is still not clearly known. It may be of interest to note that atypical spindles occur frequently in the plasmodial masses in the present material. The lack of well defined poles in them is probably due to the inactivity of centrosomes. The organization of such atypical spindles can, therefore, be best explained if an active contribution of the centromeres in organizing these bodies is visualized as well. The formation of such giant spindles lacking well defined poles and regular form of metaphase plates provide therefore support both for the active role of centromeres in organization and of centrosomes in determining the form of these bodies (see also DARLINGTON and THOMAS, 1937 and JAIN, 1962). Again, several workers (see review by SWANN, 1957) have emphasised the significant role which the polar forces play in the establishment of the metakinesis and in the anaphase separation of chromosomes. However, it may be noted that in the present material both metaphase configuration as well as the anaphase separation of chromosomes in atypical spindles appear to occur independently of the polar or centrosome forces. Hence, in so far as the organization of these atypical spindles as well as the determination of metakinesis and anaphase separation of chromosomes in them are concerned, the role played by centrosomes does not appear to be well marked. However, centrosomes appear to take an active part in the organization of multipolar spindles which also occur, though in a very low frequency, in the present material. They also seem to determine metakinesis as well as the anaphase separation in these multipolar spindles. The probable nature of the more importance of these mechanisms has been discussed in recent years among others by SWANN (1957), HUGHES (1952), RIS (1957) and REES (1961). It is likely that gene(s) or factor(s) involved may directly affect one or more of the mechanisms which are responsible for carrying through of the division cycle.

It has also been observed in the present study that the spindle may be greatly increased in width without materially affecting its length. It was also evident that the length of the spindle was largely independent of the size and shape of the plasmodial mass.

Zusammenfassung

In plasmodialen, wahrscheinlich durch Ausbleiben der Zellwandbildung während der prämeiotischen Mitose entstehenden Pollenmutterzellen des Bastards *Sorghum almum* (2 n = 40) × *S. halepense* (2 n = 40) traten Meiosestörungen auf, deren Einzelheiten beschrieben werden. Die möglichen Einflüsse der Zentromere und Zentrosomen auf die Bildung von atypischen und multipolaren Spindeln werden diskutiert.

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Buchbesprechungen / Book Reviews

DESSAUER, F.: Quantenbiologie. Einführung in einen neuen Wissenszweig. 2. Auflage, herausgegeben und ergänzt von K. SOMMERMEYER. Berlin-Göttingen-Heidelberg: Springer-Verlag 1964. XIX, 286 S., 68 Abb., 1 Porträt, zahlr. Tabellen. Geb. DM 39,60.

In der 1954 erschienenen „Quantenbiologie" hat FR. DESSAUER Ursprung und Entwicklung der von ihm inaugurierten Treffertheorie in einer so ausgefeilten Form dargestellt, daß sie auch heute noch in ihren wesentlichen Punkten gültig ist; es ist daher nur zu begrüßen, daß in der von K. SOMMERMEYER herausgegebenen Neuauflage und Bearbeitung der Text dieses als klassisch zu bezeichnenden Buches unverändert wiedergegeben ist und daß die seither gewonnenen Erkenntnisse in einem besonderen zweiten Teil angefügt werden. B. RAJEWSKI hat als langjähriger Mitarbeiter und Freund dem Buch eine Würdigung der Person und des Lebenswerkes des 1963 verstorbenen Verfassers vorangestellt, in der er auch zeigt, wie — ausgehend von der Vorstellung über die quantenhafte Natur der Strahlenwirkung, die ursprünglich nur eine formalistische statistische Beschreibung der quantitativen Beziehungen zwischen energiereicher Strahlung und biologischem Effekt sein konnte — durch Präzisierung der biologischen Bedeutung der Begriffe Treffer und Treffbereich der Weg zu einem Verständnis der daran beteiligten Primärvorgänge erschlossen werden kann. Wie weit das bis heute gelungen ist, geht nun aus dem von K. SOMMERMEYER bearbeiteten zweiten Teil des Buches hervor, in dem die Literatur bis 1963 verarbeitet worden ist. Hier wird zunächst allgemein im Anschluß an die Formulierung von BLAU und ALTENBURGER die Form und Deutung der verschiedenen Dosis-Wirkungskurven und die Theorie der Treffwahrscheinlichkeit bei direkter und indirekter Strahlenwirkung diskutiert. Einen breiten Raum nehmen dann Erörterungen über die Energieleitung in festen Substanzen und innerhalb von Makromolekülen ein, wobei besonders auf die Energieübertragungsmöglichkeit durch Dipol-Resonanz hingewiesen wird. Für die Beurteilung indirekter Strahlenwirkung spielen die bei der Bestrahlung von Wasser entstehenden chemisch aktiven Radikale eine entscheidende Rolle. Die Ergebnisse erlauben die Anwendung der zunächst für energiereiche Strahlung entwickelten Treffertheorie auch in der allgemeinen Strahlenbiologie: Bestrahlungseffekte mit energiereicher Strahlung und ultraviolettem Licht an Enzymen, Viren, Phagen, Bakterien und Hefen werden besprochen. Besonderem Interesse wird die treffertheoretische Deutung der strahlenbedingten Mutationsauslösung begegnen. So wird z. B. berichtet, daß die Genmutationen grundsätzlich durch einen einzigen Treffer erzeugt werden, und es wird der Versuch einer Deutung des Mutationsvorganges unternommen. In diesem Zusammenhang wird die Möglichkeit diskutiert, daß eventuell ein engbegrenzter Abschnitt eines DNS-Fadens das „strahlenempfindliche Volumen" darstellen kann. Es gibt aber auch Beispiele für Inaktivierung und Mutation durch mutagene Stoffe, die bei der Bestrahlung im Plasma gebildet werden, also indirekte Strahlenwirkung anzeigen. Von Strahlungseffekten im sichtbaren Licht werden die Elementarreaktionen im Auge beim Sehen und die Physik der Photosynthese genauer besprochen. Speziell die Ergebnisse der Beobachtungen über die durch Fluoreszenzänderungen nachweisbaren Energiewanderungen im Chloroplasten werden an Hand der neuesten Arbeiten ausgewertet. Der Rahmen der Strahlungswirkungen ist also sehr weit gespannt, und es zeigt sich, daß trotz der Einheitlichkeit der physikalischen Primärwirkungen ein vielgestaltiges Bild der biologischen Wirkung entsteht, das aber auf dem Weg der statistischen Bearbeitung auch der physiologischen Deutung zugänglich ist. Dem vorbildlich ausgestatteten Buch ist ein Autornamenverzeichnis angefügt, das gute Dienste leistet, die kapitelweise aufgeteilten Literaturangaben aufzufinden; angesichts der Tatsache, daß viele Probleme (wie z. B. Mutation, Sehvorgang oder Photosynthese) an mehreren Stellen des Buches behandelt werden, wäre bei einer neuen Ausgabe zur Orientierung noch ein Sachverzeichnis wünschenswert. Für den an mikrophysiologischen Fragen interessierten Biologen ist das Buch unentbehrlich. *P. Metzner, Gatersleben*

Geerts, S. J. (Edit.): **Genetics Today.** Proceedings of the XI International Congress of Genetics, The Hague, The Netherlands, September 1963. Oxford–London–New York–Paris: Pergamon Press 1963/65.

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Volume 3 (1965): Symposia 14–25, Lists of Members, Index of Authors. 582 Seiten, zahlr. Tabellen u. Abbildungen. Geb. je Bd. £ 5.–.

Vom 2. bis 10. September 1963 fand in Den Haag der XI. Internationale Genetikerkongreß statt. Er übertraf mit 2290 registrierten Teilnehmern an Größe alle vorherigen Genetikerkongresse.

Die wissenschaftlichen Veranstaltungen dieses Kongresses gliederten sich auf Grund der Unterscheidung in „eingeladene" (invited) Vorträge und in „beigetragene" (contributed) Vorträge, Demonstrationen und Filme.

Von allen durch die Teilnehmer angemeldeten „beigetragenen" Vorträgen, Demonstrationen und Filmen wurden Zusammenfassungen (abstracts) angefordert, die im Band 1 der Verhandlungen des Kongresses enthalten sind. Dieser Band wurde den Teilnehmern am Kongreßbeginn übergeben. Die Zusammenfassungen sind thematisch geordnet und in 18 Sektionen eingegliedert worden: 1. Complex loci, 2. Recombination, 3. Molecular and microbial genetics, 4. Gene action, 5. Mutagenesis, 6. Cytology, 7. Cytogenetics, 8. Cytotaxonomy and experimental taxonomy, 9. Population genetics, 10. Developmental genetics, 11. Immunogenetics, 12. Plasmatic inheritance, 13. Plant genetics and breeding, 14. Animal genetics and breeding, 15. Human genetics, 16. Human cytogenetics, 17. Dermatoglyphics, 18. Citation indexing. Die 885 Zusammenfassungen sind auf 321 Seiten durch Kleindruck und zweispaltigen Satzspiegel übersichtlich angeordnet. Jede Zusammenfassung ist außer durch die Seitenzahl