A CYTOLOGICAL STUDY OF THE GENUS *SORGHUM* PERS.

II. THE MEIOTIC CHROMOSOMES.

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(With Twenty-seven Text-figures.)

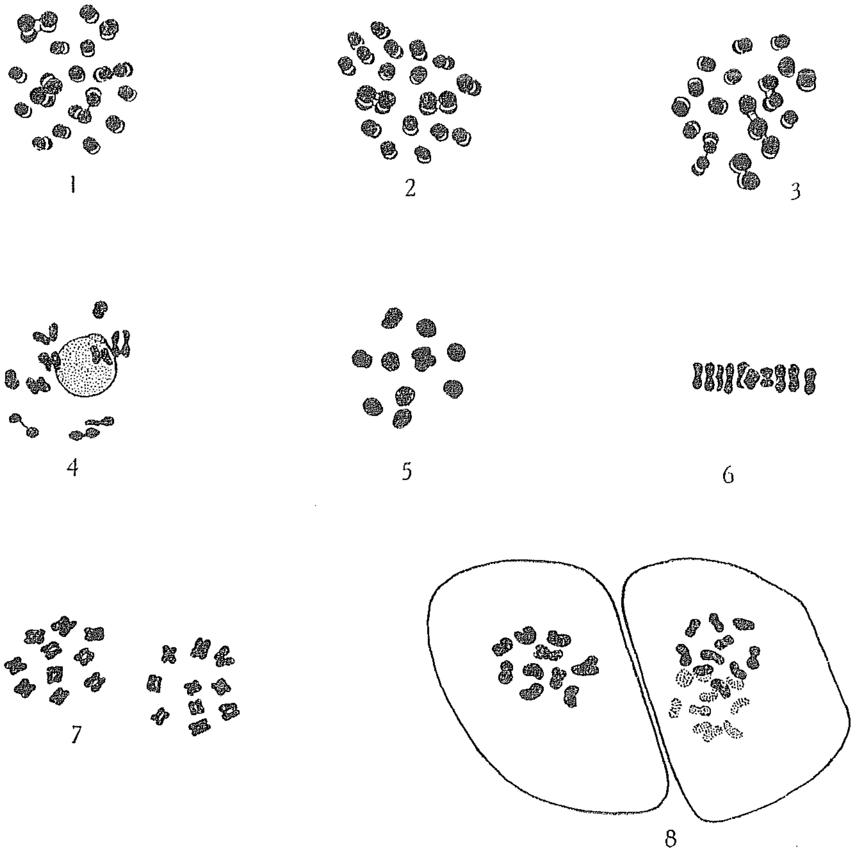
In the first section of this paper (1932) an account of the somatic chromosomes of the section *Eu-sorghum* of the genus *Sorghum* Pers. was presented. In this section meiosis in pollen mother cells of some of the species will be described. The material is the same. Spikelets from the plants grown at the John Innes Horticultural Institution, Merton, in 1930, under the conditions described in the preceding section, were fixed for 1 min. in Carnoy's 6 : 3 : 1 fluid and then for 24 hours in La Cour's 2 Bd, the end of the glumes having previously been clipped off to facilitate penetration. After embedding in paraffin, sections were cut at 16μ, and stained by Newton's iodine gentian-violet method. Drawings were made at the same magnification as those in the preceding section but are reduced to 3000× in reproduction.

Observations.

Meiosis has been studied in practically all of the species and varieties from which observations of mitosis have been recorded in the preceding section, but since many of them exhibit no distinctive features, only a few are here illustrated.

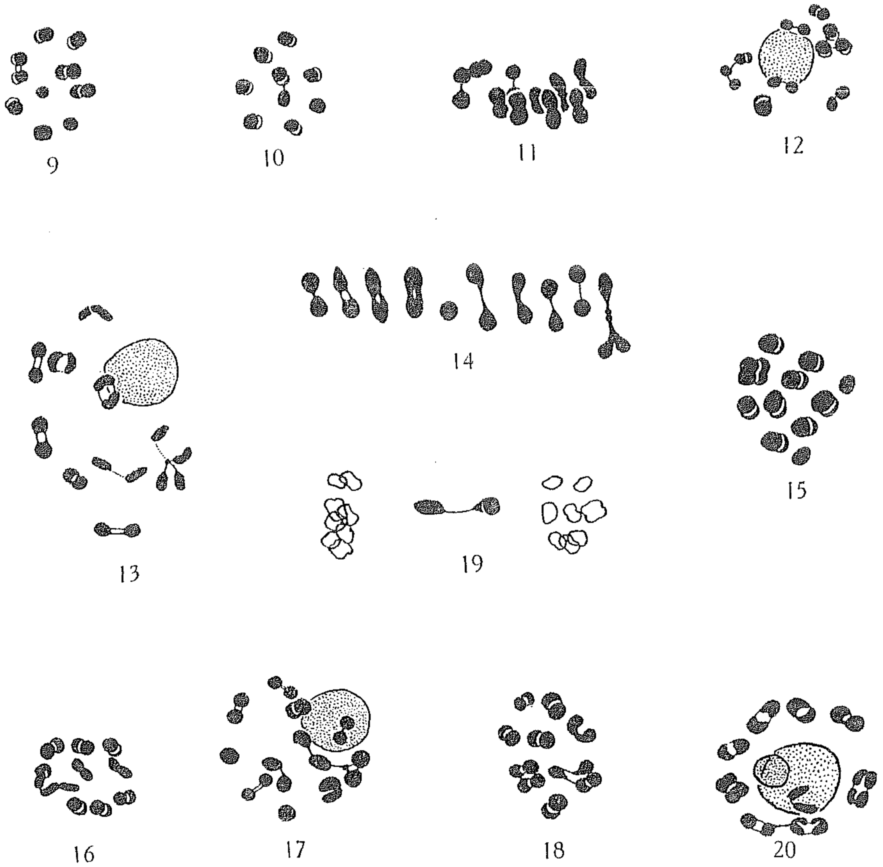
In *S. halepense* there are most commonly from 10 to 14 bivalents, and the remainder of the 40 chromosomes are in quadrivalent or higher associations. In Fig. 1 there are $12_{II} + 4_{IV}$; in Fig. 2 $16_{II} + 2_{IV}$; in Fig. 3 $11_{II} + 3_{IV} + 1_{VI}$.

In all the diploid forms of *Sorghum* examined, 10 bivalents are most commonly formed, but quadrivalent associations are also common and sexivalents are found occasionally. Fig. 4 represents a diaphase in *S. sudanense*, in which there are 10 distinct bivalents: and Fig. 5, a metaphase of *S. cernuum*, also having 10 distinct bivalents. Fig. 6 is a 10-bivalent metaphase of *S. cernuum* seen in side view. Fig. 7 is a polar view of a heterotypic anaphase of *S. virgatum*. (The slide was moved after the upper plate had been drawn so that the lower plate should not be superimposed upon it.) A homoeotypic division in *S. sudanense* is shown in Fig. 8, one of the daughter cells being at the metaphase and the other at late anaphase.



Figs. 1–8.
Figs. 1–3. *S. halepense*. Figs. 4 and 8. *S. sudanense*. Figs. 5 and 6. *S. cernuum*. Fig. 7. *S. virgatum*.

Figs. 9–20 illustrate multiple configurations in pollen mother cells of diploid species. In Figs. 9 and 10, polar metaphase views of *S. margaritiferum*, the 20 chromosomes are seen to be associated as $8_{II} + 1_{IV}$ and as $7_{II} + 1_{VI}$ respectively. Fig. 11 represents a side metaphase view of the same species, in which there are $8_{II} + 1_{IV}$. The associations found at late diaphase in one cell of *S. cernuum*, shown in Fig. 12, are $5_{II} + 1_{IV} + 1_{VI}$. Figs. 13, 14 and 15 are from *S. subglabrescens*. In the middle diaphase cell shown in Fig. 13 there are $8_{II} + 1_{IV}$; in the side view anaphase of Fig. 14 (the chromosomes being spaced out for the sake of clearness) there are $8_{II} + 1_{III} + 1_{I}$; and in the polar view metaphase of Fig. 15 there are $9_{II} + 2_{I}$. Fig. 16 shows $8_{II} + 1_{IV}$ in a polar metaphase view of *S. melaleucum*. In Fig. 17, a very late diaphase of *S. durra*, there are $7_{II} + 1_{IV} + 2_{I}$. In Fig. 18, a polar metaphase of another strain of *S. durra*, there are $6_{II} + 2_{IV}$, and in Fig. 19, a heterotypic anaphase of a third strain of *S. durra*, eight chromosomes have reached each pole, and a quadrivalent is just dividing at the equator. In Fig. 20 a middle diaphase of *S. sudanense*, there are $6_{II} + 2_{IV}$, the constituent chromosomes of one

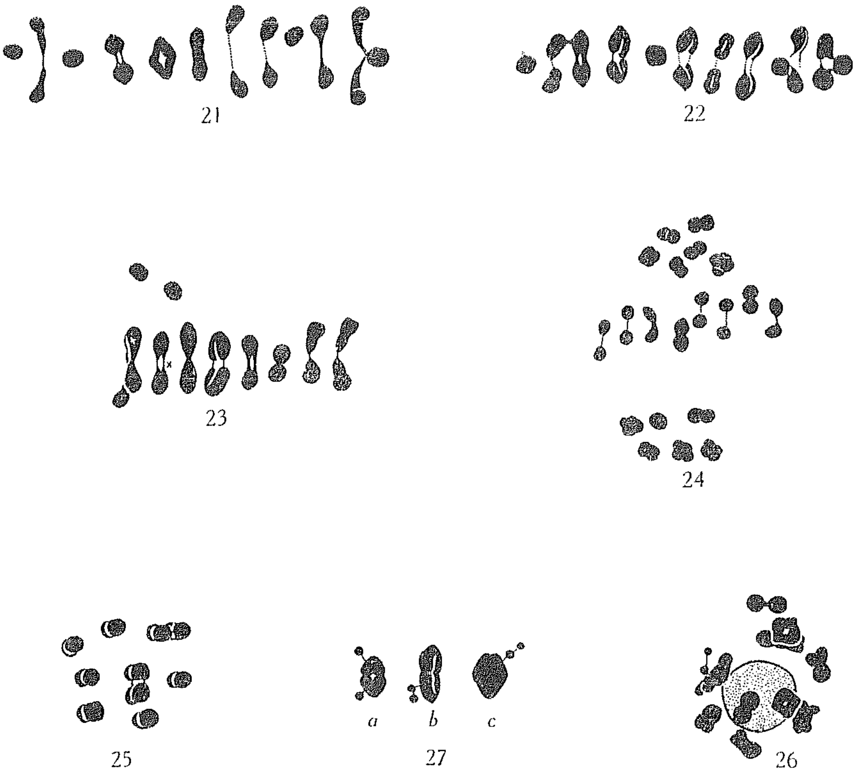


Figs. 9–20.

Figs. 9–11. *S. margaritiferum*. Fig. 12. *S. cernuum*. Figs. 13–15. *S. subglabrescens*. Fig. 16. *S. melaleucum*. Figs. 17–19. *S. durra*. Fig. 20. *S. sudanense*.

of the two quadrivalents being loosely, and those of the other closely associated.

Figs. 21–4 are all from Dakota Amber Sorgo, Figs. 21–3 being heterotypic metaphases and Fig. 24 a heterotypic anaphase. In all of these four figures, the chromosomes have been spaced in drawing. In Fig. 21 there are $7_{II} + 1_{III} + 3_{I}$. In Fig. 22 there are definitely $4_{II} + 2_{III} + 2_{I}$. In addition there is possibly a quadrivalent, since there appears to be a connection between the two pairs of chromosomes on the left. But we do not think this is a real union and consider that the complement of this cell is almost certainly $6_{II} + 2_{III} + 2_{I}$, not $4_{II} + 1_{IV} + 2_{III} + 2_{I}$. In Fig. 23 there are $7_{II} + 1_{IV} + 2_{I}$, and in Fig. 24 there are 6 chromosomes in each anaphase plate and 8 univalents dividing at the equator.



Figs. 21–27.

Figs. 21–24. Dakota Amber Sorgo. Figs. 25–27. *S. verticilliflorum.*

In this plant of Dakota Amber Sorgo there are univalents in at least 20 per cent. of the cells, their numbers ranging from 1–16, the mode being about 4, and the multivalents are much more common than in any other variety. It is difficult to state even an approximate percentage of occurrence, since in many cells there are apparent multivalents which cannot positively be identified as such, but perhaps 10 per cent. of all cells clearly seen would be a reasonable estimate of the proportion showing multivalents.

Figs. 25–7 are from a plant of *S. verticilliflorum* having an additional pair of fragments. Fig. 25 is a polar view of the heterotypic metaphase showing $6_{II} + 2_{IV}$. The fragments could not be seen in this cell. Fig. 26 is a late diaphase in which there are $10_{II} + 1_{II}$ ff. The paired fragments, though lying against a bivalent in the drawing, are actually well away from it in a different focal plane. Figs. 27 *a*, *b* and *c* are bivalents from three different heterotypic metaphase plates, each having the pair of fragments attached. In Fig. 27*a* one fragment is attached to each member of the bivalent. In Figs. 27*b* and 27*c* the fragments are paired and only one of them attached directly to the bivalent.

Secondary associations are very common in all the species, but since it is always difficult to distinguish real secondary pairing from accidental juxtapositions, and definite multivalents are relatively so common in *Sorghum*, only the latter have here received serious consideration.

DISCUSSION.

The occurrence of multivalent chromosome associations in all the "diploid" species of *Sorghum* studied, and of associations higher than quadrivalent in the "tetraploid" *S. halepense*, obviously raises many points of genetic interest. These have significance for other genera also, since ten has commonly been considered the basic chromosome number of the Andropogoneae and Maydeae. They must especially be considered in relation to *Zea Mays*, since it has been so extensively investigated cytogenetically and is in so many respects similar to *Sorghum* (cf. Karper 1931 and Karper and Conner, 1931).

In maize, multivalents have been reported only in cases where cytogenetic evidence showed them to result from either reciprocal segmental translocation or simple translocation followed by duplication. But Beadle (1931) shows occasional pairing in the haploid pollen grain divisions of polymitotic maize.

The multivalents of *Sorghum* can scarcely be due to translocation, unless, as is extremely improbable, all the translocations necessary to explain the observations have been advantageous ones, for *Sorghum* is about 94 per cent. self-fertilised (Karper and Conner, 1931) and they would therefore continually be eliminated from the population (cf. Brink and Burnham, 1929).

The observation of additional paired fragments in one plant of *S. verticilliflorum* also bears on the general problem, since tetrasomy commonly has a greater effect in diploids than polyploids. There were no obvious differences in either the morphology or fertility of this plant. Variations in chromosome number and supernumerary fragments occur likewise in maize.

The occurrence of duplicate or polymeric factors, as defined by Bateson (cf. Tjebbes, 1931) may, with certain reservations, be taken as an indication of polyploidy. Sprague (1932) cites ten cases of duplicate genes and three of triplicate in maize. Though relatively few studies have yet been made on *Sorghum*, duplicate genes for peduncle shape are known (Hayes and Garber, 1927), and Karper and Conner (1931) have found indications of polymeric genes governing chlorophyll development in *S. sudanense*. Though, as shown by Sprague, the "residual genetic mass" may determine whether a pair of factors will produce 9 : 7 or 15 : 1 ratios, the relative frequency of duplicate factors in maize and *Sorghum* agrees with the cytological evidence that 10 is not their basic chromosome number. In *Sorghum* there is more direct evidence in the discovery by Karper (1930) of a 5-chromosome species, *S. versicolor*, though he states that its chromosomes appear like "tetrasomes rather than disomes."

Fewer than 7 units of association were not found in our *Sorghum* material. This, together with the frequency with which the chromosome number 7 and its multiples occurs in the Gramineae, raises the possibility of it, rather than 5, being the basic number. It may be noted that in *Oryza sativa*, having 12 pairs of chromosomes, 5 independent groups of polymeric factors are known (Chao, 1928), and that in it Kuwada (1910) has recorded "secondary pairing" at metaphase II, and we have observed multivalents at metaphase I. Again, though multiples of 10 are characteristic of *Saccharum*, as of the Andropogoneae in general, Bremer (1932) has found a Java clone of *Saccharum spontaneum* and also *S. biflorum* from North Africa to have 56 chromosomes.

The multivalent formation in *Sorghum*, though not frequent enough seriously to disturb most genetic studies, would be expected to produce chromosome mutations. Where these involve only small segments of chromosomes they will often, in the absence of detailed analyses by linkage tests, pass for gene mutations. The mutation rate for a certain "gene" in *Sorghum* is considerably higher than in any in maize (Karper, 1932), and there is evidence that this applies to a number of "genes"

(Karper and Conner, 1931). This would be expected from our observations, if they are really duplications or deficiencies, or reverse changes from such conditions. The differences in linkage values found in different strains of maize by Stadler (1926) and Collins and Kempton (1927), and especially the latter's observation of lower crossing-over in plants heterozygous for R, are, together with observations of pairing within the haploid chromosome set of maize, in accord with the suggestion that in it also many mutations may be quantitative rather than qualitative changes. As a corollary, the evidence of polyploidy supplies an alternative explanation to that of Brink (1932) for the fact which he cites of translocations (*x*-normals) being the same as *o*-normals in maize but non-viable in *Drosophila*. In the former they may result from crossing-over between "homoeologous" chromosomes and therefore not involve any abnormal process. Further, any duplication or deficiency that might be involved would have less effect in a polyploid.

The possibilities relative to hybrid vigour which are inherent particularly in Goldschmidt's (1927) quantitative theory of the gene, and in Fisher's (1930) genetical theory of natural selection, have special significance for maize and *Sorghum* if they are either primary or secondarily-balanced polyploids. On these theories, in any population in which heterozygotes predominate, the heterozygous state should be the optimum one for many pairs of genes. Obligatory allogamous organisms should therefore exhibit a generalised type of hybrid vigour in addition to a more specific type associated with highly differentiated allelomorphs or interacting non-allelomorphs. Hybrid polyploids have in their immediate diploid ancestor hybrid vigour which is presumably due chiefly to the interaction of a number of such highly differentiated allelomorphs. By chromosome doubling these become polymeric genes (using this term here in a wide sense). An autogamous allopolyploid should retain the specific type of hybrid vigour of its diploid ancestor. An allogamous polyploid should come to have in addition a generalised type of hybrid vigour, depending upon small differences between many allelomorphs. Maize and *Sorghum*, which are respectively largely cross-fertilised and largely self-fertilised, appear to fulfil expectation on this hypothesis, so far as comparative evidence is available. The argument is, of course, distinct from the heterosis and dominant gene theories of hybrid vigour, though it embraces elements of both. If valid, it indicates difficulties, apart from those usually considered, cf. Richey and Sprague (1931), in the way of obtaining fully vigorous homozygous lines of maize and has obvious applications to methods of maize breeding.

Since the cytological work here described was completed, several more plants of Dakota Amber Sorgo have flowered and been found to be partially asynaptic. We may conclude, since the seed was from a hand-selected plant, that it is a mutant type accidentally picked out, which is analogous to Beadle's (1930) asynaptic maize. From the present point of view, the most interesting feature in the asynaptic form is the frequent formation of multivalents. It has been shown (cf. Darlington, 1932) that there is competition in chromosome pairing. If the asynapsis is due to irregularities in splitting and contraction of the leptotene threads, as it has been shown to be in asynaptic oat and wheat dwarfs (Huskins and Hearne, 1933), then release from competition in pairing with certain segments of their complete homologues would leave parts of chromosomes free to pair with homologous segments of other chromosomes which are only "homoeologous," or incompletely homologous, when considered as a whole. Pairing in normal 10-chromosome *Sorghum* species is analogous to pairing in pure line hexaploid wheat and oats, in which the 42 chromosomes ordinarily form 21 bivalents and multivalents occur only rarely. The asynaptic *Sorghum*, on the other hand, resembles *Zea Mays* × *Euchlaena perennis* hybrids (Longley, 1924), triploid and pentaploid wheat—*Aegilops* hybrids (Kihara and Nishiyama, 1930), and euploid wheat and oat hybrids (Huskins, 1928, strain 26–54, and unpublished data), in all of which multivalents occur much more commonly than in the parent species.

SUMMARY.

In "diploid" *Sorghum* species, $2n = 20$, 10 bivalents are usually found, but quadrivalents and sexivalents occur occasionally.

In the "tetraploid" *S. halepense*, $2n = 40$, quadrivalents, sexivalents and octavalents sometimes occur.

A fragmentally tetrasomic plant of *S. verticilliflorum* was found which was phenotypically normal.

A strain of Dakota Amber Sorgo was found to be partially asynaptic. Multivalents, however, occur in it with unusual frequency.

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