

INVESTIGATION
IN THE
GRAMINEAE

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A Karyosystematic Investigation in the Gramineae.

by

A. W. S. Hunter

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A Karyosystematic Investigation in the Gramineae

The history and present state of the classification of the Gramineae is briefly reviewed. Some of the different characteristics on which phylogenetic systems have been based are considered. The subject of chromosome morphology is discussed in detail, and the application of idiograms and karyotypes to taxonomic studies is explained. The main purpose of the present study was to scrutinize and extend the findings of Avdulov who has recently published a monograph in which extensive changes, based on karyological studies, have been made in the classification of the Gramineae. Taking the grasses in order by tribes, Avdulov's results are summarized, to them are added the results of an original investigation of thirty-three species belonging to twenty-nine genera from ten tribes, and also new results from other workers. The evolution of different forms within the family is touched upon.

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A KARYOSYSTEMATIC INVESTIGATION IN THE GRAMINEAE

Introduction:

The classification of the family Gramineae has long presented many difficulties to the systematist. Numerous workers have attempted to divide the family in a natural manner, but so far they have failed to reach agreement even on the division into the main groups---subfamilies and tribes.

Robert Brown, in his "Prodromus Florae Novae Hollandiae" published in 1810, was the first to recognize the two main subfamilies (which he called tribes), the Panicoideae and Pooideae. Following him Kunth, Endlicher and Steudel proposed classifications differing from one another only in details and in the number of genera included. They, however, did not recognize the division of the family into subfamilies as proposed by Brown, and it was not until the work of Bentham and Hooker, published in 1883, that this distinction was revived. They called the subfamilies Paniceae and Poaceae respectively. The system of Hackel (1887) differed very little from that of Bentham and Hooker. He replaced the names of the subfamilies by the terms "Series A" and "Series B" and changed the order of some of the tribes. Since, however, the arrangement of the tribes in any linear phylogenetic series is impossible, this difference is not very important. The system is still followed more or less closely by most modern

workers. It has been added to and modified mostly by Stapf (quoted by Bews, 1929) who has elevated many subtribes to the rank of tribes, and has also split up some of Hackel's larger genera, such as Andropogon, restoring many names of earlier authors to generic rank, and creating many new ones. The same has been done for other genera, such as Panicum, by Hitchcock and Chase (1910). Apart from this, Hitchcock and other American botanists generally follow Hackel in their delimitation of the tribes, though not in their order of arrangement. Bews (1929) whose system is followed in this study, draws upon the work of all these authors. Due value is given to viewpoints which differ by the simple device of including many genera in more than one position, often in more than one tribe.

As was quite natural, the earliest classifications were based on external morphology alone. Avdulov (1931) states, however, that in 1858 Trecul noted the organization of the starch grains in plant cells and classified them as simple and compound and that in 1881-1882 Harz used these data for the building up of a phylogenetic scheme for the development of the family. Hackel mentions the characteristics of the starch grains in his tribes but he does not regard them as of any great importance. The circumstance that very often the systematic placing and the structure of the starch grains did not agree with one another did not cause him to alter his system.

Avdulov goes on to say that in 1925 Hayek revived the question of the importance of the structure of the starch grains for the classifying of grasses. Hayek based his conclusions, however, not on material of his own but only

on Hackel's brief notes, overlooking entirely the work of Schimper and of Meyer on the structure and development of the starch grains. Meyer differentiated between solitary starch grains in a chromatophor and several such grains arising in the same chromatophor. On the basis of such a classification, the type of starch grain in any given plant cannot be determined simply by an examination of its structure in mature seeds, but only by a study of its ontogeny. The starch grains in a whole series of plants which had been considered simple were found to develop after the manner of the "adelphischen Stärkekörner" which is essentially characteristic of the clumped grains. This naturally threw the old classification into disorder. Meyer had gathered many valuable data on this question which were in sharp opposition to Hayek's conclusions, but the whole question is so involved that much work must be done before it becomes possible to use this character with accuracy in systematics.

Many workers who studied the classification of the Gramineae mentioned the peculiarities of the epidermis which, in this family, is a more highly specialized structure than in any other. Studies of the development and structure of the epidermal cells were made at an early date, but Duval-Jouve seems to have been the first to study this tissue from the point of view of its taxonomic applications. In 1870 (Duval-Jouve, 1875) he published a treatise on the identification of the species of Agropyrum, using as a basis the anatomy of the rhizome, stem and leaves. In 1875 he expanded this work to include the anatomy of the leaves of grasses in general, analyzing the different types of epidermis and the distribution of certain of the cell forms.

Grob (1896) extended the work of Duval-Jouve and assigned names to the various types of epidermal cells. He noted the presence or absence and arrangement of these types in representatives of most of the genera of the Gramineae and grouped them on this basis.

Pée-Laby (1898) worked along similar lines and established five groups on the basis of comparative number of stomata in upper and lower epidermis, the organization of the epidermal cells, the distribution of the chlorenchyma tissue, the organization of the vascular bundles, and the form of supporting tissue. He says that the arrangement of the chlorenchyma takes two different forms: one in which the green cells are nearly uniformly distributed and the other in which they form two separate concentric envelopes around the vascular bundles. The outer envelope consists of very long and narrow palisade cells and the inner, on the other hand, of very large cells. Those of the outer envelope contain ordinary chlorophyll, while those of the inner are characterized by enormous chloro-leucocytes in the shape of "massue", and have a marked bluish colour in contrast with the green of the outer layer. Pee-Laby says that "the presence of this sheath with all its special characters, constitutes, to my mind, a histo-taxonomic character sufficient to divide the family Gramineae into two categories containing plants very different in their properties and in their structure". It is this character that Avdulov (1931) uses in classifying species as "type 1" and "type 2". Type 1 is the type with the chlorenchyma localized around the vascular bundles and type 2 that having these cells uniformly distributed. By comparing them in this way

Avdulov shows that most of the species of the subfamily Panicoideae have type 1 chlorenchyma, and that type 2 is characteristic of most of the Pooideae. The exceptions in the latter group are more numerous than in the Panicoideae. Avdulov considers these to be due, not to variations in the character, but to errors in the classification.

Prat (1932) improved the field of study by analyzing epidermis from all parts of the plant; he found the numerous forms of epidermal cells to be grouped in large units or zones. Using this improved method of analysis he was able to show more clearly the relationship between the different groups and to mark more definitely the boundaries of each group.

The application of cytology to systematics has become possible chiefly through the work of Nawaschin on the morphology of the chromosomes. Previously, Häcker and Rückert (according to Lewitsky, 1931a) had discovered what they called

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"rod-shaped tetrads" in Cyclops and other related Copepoda. These occurred as groups of two parallel rods, each of them appearing as if ruptured in two at the middle. They explained them as two longitudinally split "univalent" (somatic) chromosomes joined by their ends and forming one "bivalent chromosome" or "geminus". In the reduction division, according to Weismann's theory, such a bivalent chromosome divides transversely, in consequence of which the univalents, having temporarily conjugated, become free and reach the different poles of the spindle. This gave rise to the theory of telosynapsis which, however, was soon questioned, and has now been completely replaced by the parasynaptic theory.

It is clear that what Häcker and Rückert were dealing with was not the beginning of a transverse division but what is now known as the "attachment constriction" or the point of articulation of the chromosome. Lewitsky (1931a) quotes Nawaschin as saying in 1910 that according to his observations "the chromosomes of Fritillaria tenella are distinctly double and distinctly divided in the place where the bundles of the spindle fibers are attached". A year later he distinguished between three types of bivalent chromosomes according to the position of the attachment constriction: (1) U-shaped, almost equal-armed: (2) U-shaped, distinctly unequal armed and (3) hook-shaped, one arm being very small. In 1912 he reported "on the dimorphism of the nuclei in somatic cells of Galtonia candicans, showing the presence of special, minute but perfectly constant appendages attached by means of a thread to two (out of four) medium sized chromosomes characteristic of this plant. He called these appendages "satellites", or in German "trabanten"

(the latter term is now more widely used), and noted that during the division of the nucleus they split along with the remaining body of the chromosome. Here, for the first time, was the possibility of distinguishing between chromosomes by the peculiarities of their structure. Nawaschin, according to Lewitsky, (1931a), states definitely that there is no such thing as terminal attachment of the spindle fibers. In all such apparent cases a closer examination reveals that "a minute part of the body of the chromosome is separated from it by a transverse slit, and the spindle threads are attached beyond it," i.e., at the attachment constriction. Nawaschin also distinguished between heads and trabants, stating that trabants are not modified heads but are appendages of them. Lewitsky (1931a) says that in 1915 Delaunay definitely proved the existence of tri-articulate chromosomes in representatives of the genus Muscari. Delaunay claimed that the number of segments and degree of differentiation were characteristic of definite chromosome types of definite species. This character represents the second type of secondary differentiation of the chromosomes, trabants being the first type.

During this period important advances were made in the field of cytological technique. Nawaschin introduced the use of "formalin Flemming", (chrom-acetic-formalin). This fixative gives a somewhat swollen appearance to the chromosomes but still allows such details as heads and trabants to be seen quite clearly. "Medium Flemming," a mixture of chromic, acetic and osmic acids introduced by Strasburger was also used successfully by Nawaschin. It gives a finer appearance to the chromosomes, shows up the split condition of the arms and renders the attachment constrictions clearly visible.

The work of Nawaschin here referred to and that of most of his colleagues was published in Russian. This, coupled with

the special character of the publications and the political unrest in Russia at the time, made it practically unavailable to most outside workers. Tschernoyarow, a student of Nawaschin had published a paper in 1914 in German which contained only the older data concerning the trabants of Galtonia and Muscari, and it was not until 1924 when Nawaschin himself published a paper in German that his findings became generally known. It is not surprising, therefore that other workers had by that time come to very similar conclusions. As a matter of fact, in the same year in which Nawaschin's German paper appeared we find two others, one by Newton (1924) and the other by Taylor (1924) from which it is apparent that they had discovered the heads of the chromosomes, the attachment of the spindle fibers at the constrictions and the presence of trabants. The latter, however, they regarded as modified heads somewhat more separated from the body of the chromosome, and not as appendages of the heads as Nawaschin considered them.

According to Lewitsky (1931a), Sakamura, working chiefly with Vicia Faba, made extensive use of artificial methods influencing the chromosomes in order to manifest or to intensify their morphological differentiation. He used narcotics such as chloral hydrate, chloroform and ether and other substances such as benzene, cocaine and carbon dioxide as well as high temperatures, electrical discharges and x-rays. All these influences produced a similar effect -- the chromosomes became conspicuously shorter and thicker and the constrictions more marked. This enabled him to demonstrate the presence of constrictions in plants where they had not before been observed. On comparing his data he came to the conclusion that the presence of constant constrictions in the chromosomes is a general phenomenon in both

plants and animals, and that at the point of attachment of the spindle fibers the chromosomes always show a constriction or a rudiment of one.

After the Great War there came a period of transition during which the "law of the constancy of chromosome number" came to be considered as a more general "law of the constancy of the morphological composition of the nucleus." This law became expressed in the form of the "idiogram." Changes of the idiogram in a series of successive species afforded material for drawing up particular phylogenetic lines as well as for establishing some facts in connection with the evolution of the species.

Lewitsky (1931b) discusses fully this question of the use of karyotypic characters in systematics. He defines an idiogram as the graphic representation of a chromosome set with all the diversity of its structure. Separate species of a genus are characterised by quite definite and constant idiograms. These idiograms, although varying somewhat from one species to another usually maintain the same general type throughout the genus. On the contrary, when passing from one genus to another, the type of idiogram undergoes a complete transformation and appears as a new "karyotype." It would seem, therefore, that in classifying plants all that would have to be done would be to group those species having similar idiograms into the same genus, and into different genera when the karyotype changed. The question, however, is not quite as simple as this for we are immediately faced with the difficulty of deciding when a difference in idiogram is great enough to justify the establishment of a new karyotype. This is a very difficult point, especially so when

it is known that there exists a uniformity in the number and main characteristics of chromosomes through whole groups of genera which, though allied, are yet unquestionably different. Instances of a real coincidence of idiograms for whole large families have been known on one hand and on the other of a clear divergence of idiograms in adjacent species, which are then characterized by different karyotypes.

Lewitsky (1931.b), in his analysis of the differences to be found in idiograms, notes that in general, chromosomes with equal arms indicate a more primitive stage of development, both morphologically and cytologically, than those with sharply unequal arms. Exceptions to this rule occur sometimes in the case of the formation of heads on chromosome fragments in X-rayed plants, (Lewitsky and Araratian, 1931). It should be noticed, however, that the extent to which these constrictions are functional has not yet been finally settled.

Distinction must be made, in the comparison of idiograms, between chromosome sets which have become differentiated through a process of gradual shortening and those which have undergone more complicated processes such as translocation, fragmentation, constriction-shifting, etc. Lewitsky and Araratian conclude that the sharp differences sometimes observed in the idiograms of closely related species may be due to fragmentation and subsequent loss of portions of the chromosomes, while close similarity of idiograms in morphologically dissimilar genera indicates that the differences between the genera are chiefly genic. Another explanation for the wide difference in idiograms sometimes noticed in related species is afforded by the trans-

location of parts of chromosomes following fragmentation. Lewitsky and Araratian cite an instance of such a change in a triploid Crepis. Similar transformations have been produced by the action of X-rays.

Thus, though the taxonomic significance of one or another of the karyotypic peculiarities is, generally speaking, variable, nevertheless some of them characterize large systematic units and others small ones. In this respect the first place is taken by the basic number and the size of the chromosomes. A basic number for a polyploid series is usually of great systematic importance, characterizing whole families and tribes. The size of the chromosomes is used to separate, for example, subfamilies in the Gramineae and tribes in the Leguminosae, as well as many genera, species and varieties.

Next in order of importance come idiogram types, characterized by changes of greater or lesser degree in the length and structure of the arms of the individual chromosomes. Of least importance is the polyploid multiplication of whole chromosome sets.

In conclusion, Lewitsky and Araratian state that "the idea of the karyotype as a karyological characteristic especially and exclusively applied to generic definitions or to any other definite systematic unit does not hold. One cannot state what extent of similarity of idiograms involves their subordination to the same karyotype. On the ground of larger or lesser community of characters, one may distinguish in one and the same complex of forms karyotypes of families, genera, species and races. Therefore, the karyotype is a karyological characteristic of a group of allied forms which may be applied to any systematic unit."

Smith (1932) has furnished a striking example of the significance of chromosome morphology in relation to systematics. His arrangement of the Anchuseae on this basis alone accords perfectly with the revision of the group made by Johnston (1924; Smith, 1932) from a general taxonomic study.

Schiemann and Sorokin (cited by Avdulov, 1931) in 1928 attempted a classification of the genus Aegilops but took into consideration the chromosome number only, and were barely able to establish that the various sections of the genus contained species with different chromosome numbers. In contrast, Avdulov mentions the work of Senjaninowa- Kortschagina who studied the same genus from the standpoint of chromosome morphology as well as number. She was able to make great advances in the construction of a system of classification and also to clear up, to a certain degree, the history of its evolution and the common connection between the individual species.

The essential fault in the work of many investigators in this field is the lack of complete research material, with the result that their conclusions are not based on a sufficiently wide foundation and their findings therefore inconclusive. Another important drawback has been that most workers preferred to study the karyotype in the course of reduction division. At this stage the chromosomes rarely retain their individuality and their study for the purpose of karyo-systematics becomes very difficult.

Avdulov was the first to apply a realization of these facts to the study of grasses. His material is derived from the botanical gardens of the world, it covers practically all

sections of each tribe and in many cases includes two or more species of the same genus, and his results are based on the study of somatic divisions only.

Materials and Methods.

The material used in this study was collected by Dr. C.L. Huskins while at the John Innes Horticultural Institution, Merton, England. The figures are from root-tip preparations fixed in La Cour's (1931) 2BE or 2BD fixatives. The sections are 14 μ thick and staining is by La Cour's chromic acid modification of Newton's gentian violet method, using crystal violet as suggested. The drawings are all made with the aid of a camera lucida, a 30x ocular and a Zeiss 1.5mm. objective, N. A., 1.3. This gave a magnification of approximately 6900x at table level, which was reduced in photographing to approximately 6200x.

Classification of the Gramineae.

As already mentioned, Bews classification is being used in this study. Its advantages over other standard works are that it covers the whole world, it is written in English, it is concise and it is more generally available than any other complete study.

Bews divides the family Gramineae into two subfamilies, the Pooideae and the Panicoideae. The Pooideae are in turn divided into eleven tribes and the Panicoideae into four. A summary of the classification of the species investigated in this study together with their chromosome numbers is given in Table 1. Hackel's monograph in Engler and Prantl's "Die N  rlichen Pflanzenfamilien", as well as the many works of Hitchcock and of Chase were also consulted from time to time.

TABLE 1: List of Species Examined and their Somatic Chromosome Number

 Subfamily Pooideae
Bambuseae

<u>Arundinaria Fortunei</u> Fenzi.	48
<u>Arundinaria pygmaea</u> Kurz.	54
<u>Phyllostachys flexuosa</u>	54

Festuceae

<u>Arundo donax</u> L.	110
<u>Phragmites communis</u> Trin.	48
<u>Gynerium argentea</u> (Nees) Stapf.	76
<u>Melica altissima</u> L.	18

Chlorideae

<u>Eleusine coracana</u> Gaertn.	36
<u>Chloris gayana</u> Kunth	20
<u>Eustachys gayana</u>	40
<u>Cynodon dactylon</u> Pers.	30

Hordeae

<u>Lepturus cylindricus</u> Trin.	52
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Agrostideae

<u>Sporobolus tenuissimus</u> Kuntze.	40
<u>Phleum phleoides</u> L.	14

Phalarideae

<u>Anthoxanthum odoratum</u> L.	20
<u>Phalaris arundinacea</u> L.	28

Subfamily PanicoideaeMelinideae

Melinis minutiflora Beauv. 36

Paniceae

Digitaria exilis Stapf. 54

Echinochloa frumentacea (Roxb.) Link. 36

Oplismenus burmanii (Retz.) Beauv. 72

Brachiaria mutica Stapf. 36

Pennisetum clandestinum Hochst. 36

Cenchrus tribuloides L. 34

Andropogoneae

Miscanthus saccharifera Benth. 64

Miscanthus sinensis Anderss. 42

Andropogon eliottii Chapm. 20

Andropogon scoparius Michx. 40

Apluda mutica L. 20

Maydeae

Tripsacum dactyloides L. 36

Coix lachryma-jobi L. 20

Euchlaena mexicana Schrad. 20

Euchlaena perennis Hitch. 40

Zea mays L. subsp. indentata 20

Purpose of Study

The purpose of this study is to check the findings of Avdulov (1931) and to augment his work at certain points. With this in view most of the species were chosen to cover some more or less contentious point. Other species which have not heretofore been investigated are included to give a broader foundation on which to base conclusions. Some of the anomalous situations appear to be due to wrong chromosome counts. In other cases the count in this material corresponds with that of earlier investigators and other explanations for the anomaly must be sought.

Presentation of Results

Subfamily Pooideae

Following Bew's classification we will deal first with the subfamily Pooideae which he describes as follows: "Spikelets two to many flowered or reduced from above downward to a one flowered condition, with or without one or more empty lemmas, or a prolongation of the rachilla above the single fertile floret. Sometimes there may be empty lemmas below the fertile floret, usually two in number. The mature spikelets break up, leaving the glumes behind, or if they fall off entire with the glumes, then they do not consist of two heteromorphous florets as in the Panicoideae. The spikelets are usually more or less laterally compressed. The basic chromosome number in a great many is seven."

Tribe Bambuseae

This tribe is characterized by an extraordinary variety in the reproductive organs, yet despite this diversity it forms throughout a natural group which is more readily separated from its neighbours than any of the other tribes. The connecting character is chiefly a vegetative one, the culm being lignified either throughout its entire length or only at the base. The sessile leaves are also a general characteristic.

The internal anatomy has not been much investigated. Prat (1932) maintains that the epidermis of such members of the Bambuseae as he has analyzed resemble more closely the epidermis of the Panicoideae than they do the epidermis of the Pooideae. Certain anatomical and morphological peculiarities, however, separate them from the former, and he considers it better to create a new subfamily, the Bambusoideae, for the tribe Bambuseae.

Cytological data are scarce. Avdulov examined an undetermined species from each of the genera Bambusa, Arundinaria and Phyllostachys. In each case he found the chromosome number to be somewhere between 68 and 74. More recently, Yamaura (1933) reported 48 as the diploid number for Sasa kozasa Nakai, Sasa kurilensis var. cernua Nakai, and var. Uchidai Makino, Sasa paniculata var. paniculata Nakai, Sasamorpha borealis Nakai and Pleioblastus Maximowiczii Nakai, and 72 as the diploid number for Bambusa nana Roxb. The present investigation included three species. Their chromosome numbers were as follows:

Arundinaria Fortunei Fenzi $2n = 48$. (Fig. 1)

Arundinaria pygmaea Kurz. $2n = 54$. (Fig. 2)

Phyllostachys flexuosa $2n = 54$. (Fig. 3)

Yamaura considers the basic number of the Bambuseae to be 12, but this is rendered doubtful by the results

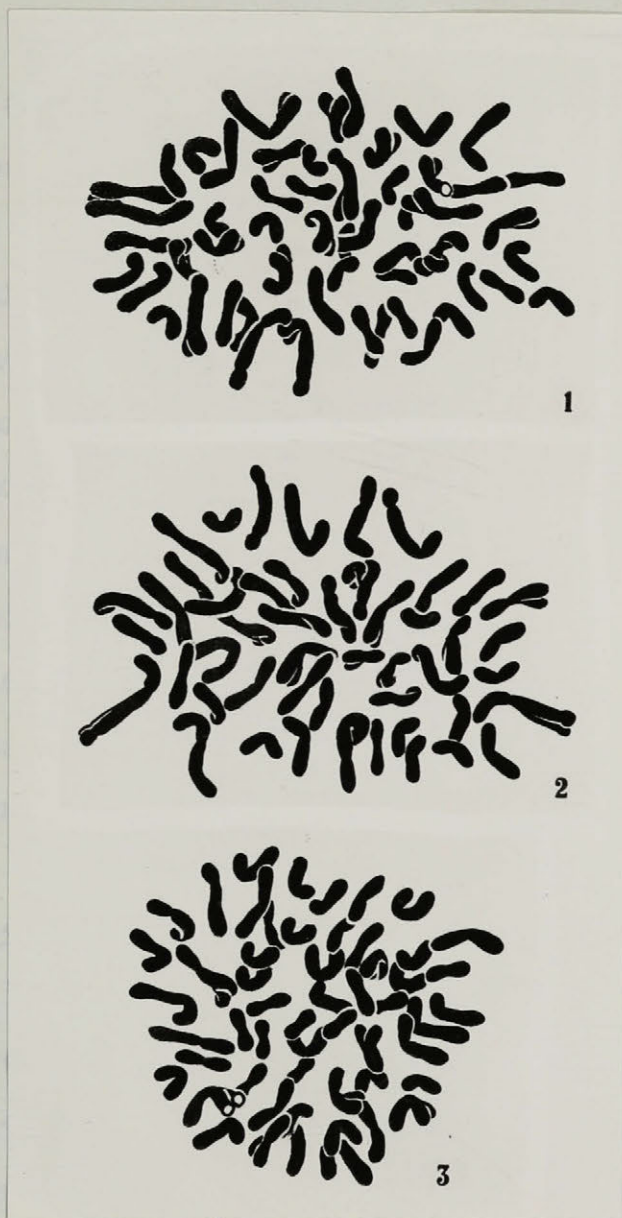


Fig. 1 Arundinaria Fortunei Fenzi ($2n = 48$)

Fig. 2 Arundinaria pygmaea Kurz. ($2n = 54$)

Fig. 3 Phyllostachys flexuosa ($2n = 54$)

here published unless great differentiation of chromosome number has occurred within the tribe. This question of

basic number is associated with that of the primitiveness of the tribe. Certain features of the flower of the Bamboos have led systematists to believe that they are not far removed from the original ancestor of the grasses. Lamb (1912) placed the tribe at the tip of the phylogenetic tree of the family but Schellenberg and also Hayek (Avdulov, 1931) considered it as a side branch of development, that is, as derived from the same ancestor as the rest of the grasses. Prat (1932) notes that the flowers of the Bambuseae approach closer to the typical monocotyledon flower than any of the other grasses, and that certain peculiar characteristics of the epidermis are found also in the Cyperaceae. Evolution in the epidermis of the Gramineae seems to have been in the direction of a progressive simplification, and it is interesting to note that the epidermal elements of the Bambuseae are the most variable and the most complex of all the grasses. Avdulov believes that the basic chromosome number in the Gramineae has undergone a progressive diminution, probably from some such number as 12 to the 7, 6 and even 5 found in the Pooideae, and that this reduction in the basic number was preceded by an increase in the size of the chromosomes. Lewitsky (1931a) maintains that, in general, small chromosomes with equal arms indicate a more primitive stage of development, both morphologically and cytologically, than those with sharply unequal arms. This primitive morphological condition is indicated in the species here illustrated and also in those reproduced by Avdulov and Yamaura.

Tribe Festuceae

Just as the majority of systematists agree on the naturalness of the Paniceae, Andropogoneae and Maydeae so they are agreed on the unnaturalness of the Festuceae. Avdulov says that Kunth originally set up three tribes instead of the one tribe Festuceae, out of which the Bambuseae were later divided off. Bentham and Hooker combined the three main tribes into one and allowed the fourth, Bambuseae, to stand. Hackel adhered to this classification and later, along with Bentham and Hooker, divided the tribe into a whole series of subtribes. Much confusion followed as the individual constituents of the tribe were interpolated here and there throughout the system. Finally the view was taken that the whole multiplicity of several-flowered Gramineae, with the exception of certain specially differentiated tribes, the Aveneae and Hordeae, should be classed together as the Festuceae.

Bews considers the Festuceae to be the most primitive of the common grasses. This claim to primitiveness is based chiefly on their many-flowered spikelets and short glumes. The tribe is divided by him into seven subtribes of which the first two, Centothecinae and Arundinae are the most primitive. The main line of evolutionary differentiation commences in the Festucinae and is followed through in the Dactylidinae-Seslerinae group, the Melicinae, the Eragrostinae and the Pappophorinae. This last subtribe is in some respects the most advanced of all the Festuceae though on the whole it is still rather specialized.

Cytologically the tribe may be divided into three groups which by no means agree with the established subtribes. The majority of genera which Avdulov investigated belong to the

temperate zones and are marked by large chromosomes having the basic number 7, type 2 leaf anatomy and clumped starch grains. In this first group belong the genera Glyceria, Puccinellia, Poa, Festuca, Sclerochloa, Scleropoa, Briza, Dactylis, Desmazeria, Lamarckia, Sesleria, Koeleria, Echinaria, Melica, Catabrosa, Bromus, Boissiera and Schismus. Polyploidy is observed in the genera Poa, Glyceria and Puccinellia, and a probable instance of the loss of a chromosome pair from a tetraploid form of a series with the basic number 7 is afforded by Koeleria phleoides which has 26 chromosomes in comparison with the multiples of seven of the other species investigated.

In the genera Echinaria, Melica and Catabrosa the basic number deviates from the 7 common to most of this group, being 9 in the first two and 10 in the last. Inasmuch as this difference in chromosome number is not bound up with any sharp difference in their phenotype or karyotype, Avdulov does not object to their inclusion in this group, considering it probable that they spring from the basic number 7. I examined Melica altissima (Fig.7) in this class and found it to resemble Avdulov's material in size and number of chromosomes.

Avdulov noticed that in Schismus calicinus the chromosomes were considerably smaller than ordinary and that the basic number was 6. However, other species of the group also have small chromosomes, and since it is morphologically closely connected with other members it is included in this position.

The second karyo-systematic group contains, so far, only the genera Diplachne and Eragrostis. The basic number is 10 and the chromosomes are small. These characters of the karyotypic

structure are connected with a series of morphological characters. In its cytology, the group is reminiscent of the Panicoideae and the Chlorideae. Both the genera belong to Bew's subtribe Eragrostinae which, he says, Stapf elevated to the rank of a tribe and separated from the Festuceae by placing the tribes Chlorideae, Pappophoreae (of Stapf), Oryzeae and Phalarideae between the two. Stapf removed the genus Diplachne from the Festuceae and placed it in the Chlorideae. The doubt of the systematists concerning their placing is therefore upheld by the cytologist.

The third group has small chromosomes, a basic number 12 and type 2 leaf anatomy. The genera Centotheca, Uniola, Phragmites and Arundo fall into this class. The characteristic of small chromosomes in multiples of 12 is common to the groups to which Oryza and Erharta belong, which they also resemble in structure of starch grains, leaf anatomy and geographical distribution.

Two races of Phragmites communis were reported by Tischler (1918) to have 18 chromosomes in the haploid condition. In 1929 he revised his statement for P.communis var. Pseudodona, saying that he was not sure whether the basic number, in common with the rest of the Festuceae was 7 or not. He had a few preparations which gave him 36 chromosomes but once or twice he had to admit the presence of 42 chromosomes. Avdulov was not able to be any more certain. He found different Phragmites races with different numbers of chromosomes, but outside of that he was able to say only that the number was probably 48 in some and 96 in others. This, along with Tischler's first count of 36, would make a polyploid series with 12 as its basic number. In my material,

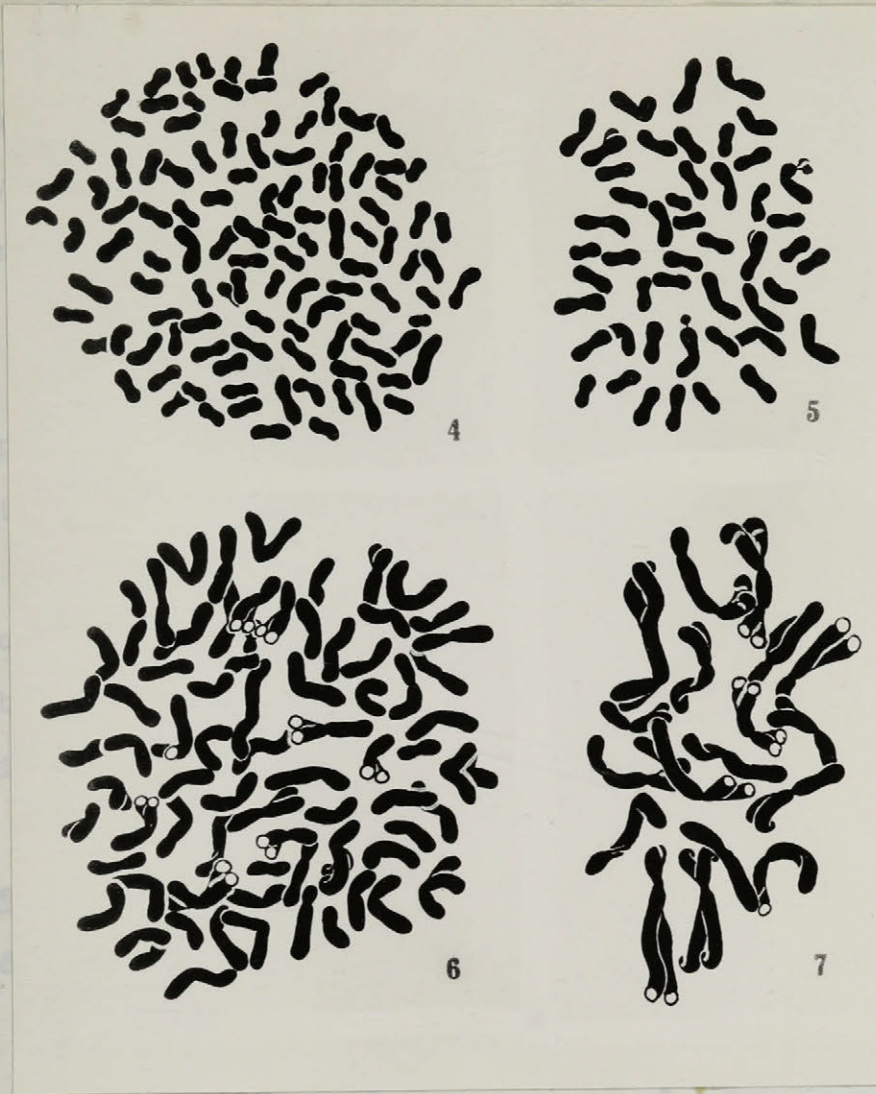


Fig.4 -- Arundo donax L. ($2n=110$)

Fig.5 -- Phragmites communis Trin. ($2n=48$)

Fig.6 -- Gynerium argentea (Nees) Stapf. ($2n=76$)

Fig.7 -- Melica altissima L. ($2n=18$)

a Californian specimen, I was able to determine definitely that the chromosome number is 48 (Fig.5), thus confirming Avdulov's supposition. The accurate establishment of a basic number for this genus is of considerable theoretical importance. The Festuceae of 12. The apparent lack of uniformity noted by the systematists is therefore confirmed cytologically and directions in which the classification could be improved are indicated.

are regarded as the point of origin of the grasses and Phragmites, with its nearness to the hexameric flowered form having many-flowered spikelets and a tropical distribution, along with a very long palaeontological record, becomes probably the most primitive genus.

Arundo is a closely allied form. Avdulov examined A. donax but was not able accurately to determine its chromosome number. He placed it as somewhat over 100. I was able to say definitely that the material which I examined had 110 chromosomes (Fig. 4). Its karyotype agrees so closely with that of Phragmites and in cytology, anatomy and morphology it is so different from the members of the first and second groups that Avdulov had no hesitation in placing it in this group.

I also investigated a member of one other genus, viz., Gynerium argentea (Fig. 6) and include it in this group. Morphologically it is closely related to Centotheca, Arundo and Phragmites and in its cytology is very similar to the species of Centotheca and Uniola described by Avdulov. They have all rather small chromosomes more or less equal in size and a few unequally armed. G. argentea has 76 chromosomes and is, therefore, possibly a modified hexaploid of a form having 12 as its basic number.

In the Festuceae, therefore, we have three cytological groups. The first, and by far the largest, has large chromosomes and the basic number 7 and is more or less independent of the other tribes. The second is characterized by small chromosomes in multiples of 10 and in this respect is similar to the Chlorideae. The third group joins the genera with small chromosomes in multiples of 12. The apparent lack of uniformity noted by the systematists is therefore confirmed cytologically and directions in which the classification could be improved are indicated.

Tribe Chlorideae

The Chlorideae are distinguished from other tribes by the presence of two series of closely touching spikelets which form a one sided spike. The differences within the tribe, however, are large. As stated by Avdulov, the standing of the tribe has undergone but little change since the time of Kunth. In 1917 Bessey advocated its complete abolishment and the division of the genera between the Agrostideae, Aveneae and Festuceae. Schellenberg (1922, from Avdulov 1931) favoured this change. In a later work on the phylogeny of grasses Hayek gave the Chlorideae the standing of a tribe leading out from the Festuceae.

Bews subdivides the tribe into three groups based on the reduction of the number of flowers in the spikelet. The first group contains those with two to several perfect florets in each spikelet, among which are Eleusine, Dactyloctenium, Beckmannia and Leptochloa. Some of the genera in this group are hardly distinguishable from the Festuceae.

The members of the second group have only one perfect floret and one or more imperfect florets in each spikelet. Bouteloua, Gymnopogon, Chloris, Eustachys and Munroa are some of the genera which fall in this class.

The most advanced group contains those genera with the spikelets having definitely one floret, with or without a pro/longation of the rachilla. The most important representatives are Cynodon and Spartina.

The anatomy of the leaves of the Chlorideae differs from that of most of the Pooideae in being type 1. Guntz (1886; Avdulov, 1931) placed Spartina in type 1, while Duval-Jouve (1875) put it in type 2. Avdulov unhesitatingly classifies it as type 1 after inspection of a colleague's material. The single exception to the rule, then, seems to be the genus Beckmannia which definitely has type 2 of chlorophyll arrangement.

In the structure of the starch grains all the genera, with the exception of Gymnopogon, have clumped grains. A difference is found in another character, however, investigated, according to Avdulov, by Grob (1898). He demonstrated the presence of two-celled hairs, "Winkelhaaren", on the epidermis of the leaves of the members of the subfamily Panicoideae and in a few cases throughout the Pooideae. It is interesting to note that all the Chlorideae with the exception of Beckmannia possess these two-celled hairs. Again, in geographical distribution, while the rest of the tribe is more or less tropical or subtropical in range, Beckmannia grows in temperate regions and is never found in the tropics.

Avdulov divides the tribe into two karyo-systematic groups. The first is characterized by small chromosomes and the basic numbers 9, 10 and rarely 12. Into this group fall the majority of genera investigated including Spartina, Cynodon, Trichloris, Dinebra, Eleusine, Dactyloctenium, Leptochloa, Buchloe and Bouteloua. I examined Eusteghys gayana (Fig. 10) and found it to have 40 chromosomes. This genus is given as synonymous with Chloris.

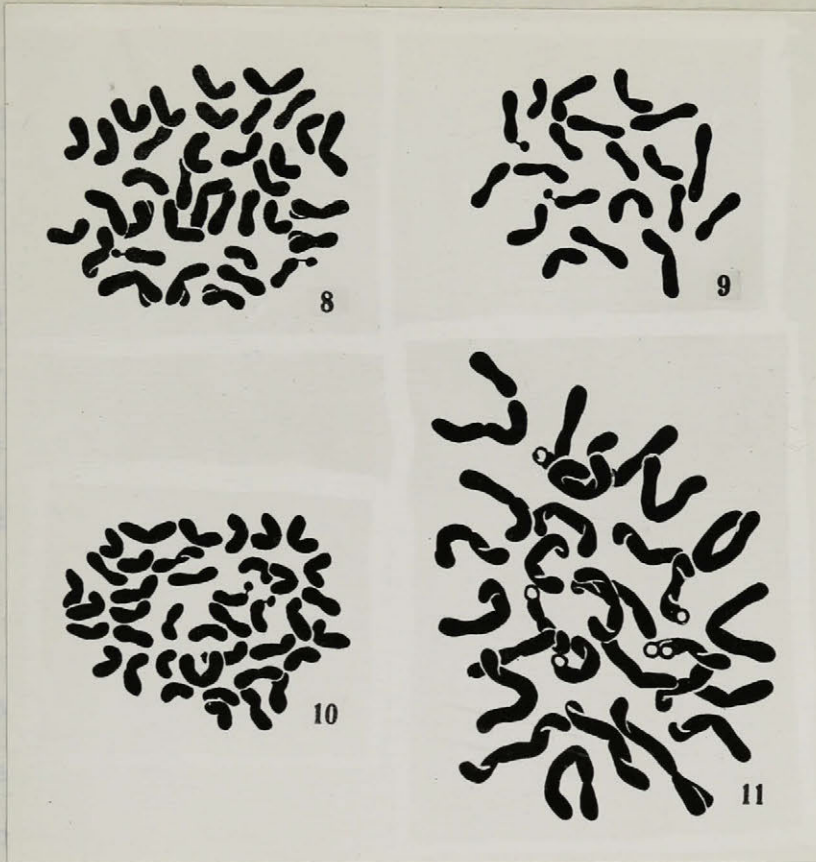


Fig.8. *Eleusine coracana* Gaertn. ($2n=36$)

Fig.9. *Chloris gayana* Kunth. ($2n=20$)

Fig.10. *Eustachys gayana* ($2n=40$)

Fig.11. *Cynodon dactylon* Pers. ($2n=30$)

In the material which I examined, *Chloris gayana* (Fig.9) had 20 chromosomes of which two pairs were long, four were medium sized and four pairs short, ~~and~~ one pair of which carried trabants. *E.gayana* on the other hand has two pairs of long chromosomes, ten pairs of medium and eight pairs of short ones. One short pair has trabants. On the basis of this disagreement in their karyotype, showing that *E. gayana* is not merely a tetraploid form of *C. gayana*, the placing of the two species in different genera seems justified.

A splendid example of the way in which cytology may strengthen more or less circumstantial evidence is afforded by an investigation of Spartina Townsendii. From its characteristics and the circumstances of its origin, practically all competent observers were convinced that S. Townsendii arose from hybridization between S. alterniflora and S. stricta. Huskins (1931) realized that if the chromosome number of S. Townsendii should prove to be the sum or a multiple of the sum of the chromosome numbers of its supposed parents then such a mode of origin would be practically assured. Upon examination he found S. Townsendii to have 126 chromosomes, S. alterniflora to have 70 and S. stricta to have 56. Thus S. Townsendii is apparently the result of chromosome doubling following on interspecific hybridization. If such is actually the case it is an outstanding example of the significance of allopolyploidy in plant evolution.

My observations on Eleusine coracana (Fig. 8) agreed with those of Avdulov, but in Cynodon dactylon (Fig. 11) he reported 36 chromosomes and I distinctly counted 30. This removes one of the exceptional cases with 12 as its basic number. The other exception is Dactyloctenium aegyptiacum Willd. Unfortunately there was no material of this species available for investigation.

The second group contains as yet only the genus Beckmannia. Avdulov examined B. erucaeformis Host var. ramosa Paczosky and B. erucaeformis Host var. baicalensis W. Kusnezow, and counted 14 chromosomes in each. The chromosomes are large and altogether resemble those of the northern grasses of the Phalarideae, Aveneae, Festuceae and Agrostideae. Therefore, in addition to leaf anatomy,

epidermal hairs and geographical distribution, the genus Beckmannia differs from the rest of the tribe in its cytology. These characteristics plus a whole series of morphological characters indicate that the genus would be more properly placed with the tribe Festuceae.

The correspondence of the remaining genera with the Panicoideae will become obvious after the discussion of that tribe. They will be seen to be similar in leaf anatomy, geographical distribution and in possessing two-celled epidermal hairs as well as in other epidermal characteristics (Prat, 1932). Their cytological similarity is also striking, both groups being characterized by more or less small chromosomes in multiples of 9 or 10.

Thus a complete revision of the systematic placing of the tribe is indicated. The bulk of it should be shifted, en masse, to the sub-family Panicoideae, and the genus Beckmannia would seem to be more at home with the Festuceae.

Tribe Hordeae.

The Hordeae are still more advanced than the Chlorideae in having the spikelets always definitely sessile and usually more or less sunk in the notches on opposite sides of a spike. With the exception of one isolated genus, Pariana, they are more or less confined to the temperate regions. The tribe as a whole is extremely uniform, both from the morphological and from the anatomical point of view. Prat (1932, 1933a and 1933b) made an exhaustive study of the epidermal characters of a number of the genera and came to the conclusion that with the exception of Nardus and Jouvea, and two other small genera, the tribe was remarkably homogeneous.

There are several differences within the tribe, however, which serve to break it up into sub-tribes. Harz, 1880-1882, and Hayek, 1925, (quoted by Avdulov) divided the tribe into the Triticeae or Hordeae with simple starch grains, and the Lolieae with compound starch grains. Hackel (1887) divided the tribe into six sub-tribes, the Nardeae, Lolieae, Leptureae, Triticeae, Elymeae, and Parianeae. Bews (1929) combined some of these and proposed the following classification:

1. Parianinae (= Parianeae Hackel)---contains the isolated genus Pariana.

2. Elyminae (= Elymeae Hackel)---contains the genera Elymus, Sitanion, Hystrix, Hordeum and Asprella.

3. Triticinae. (= Lolieae, Leptureae and Triticeae Hackel)---contains the genera Agropyron, Haynaldia, Heteranthelium, Triticum, Secale, Lolium, Kerinozoma, Jouvea, Scribneria, Psilurus, Ischnurus, Lepturus and Pholiurus.

4. Nardinae (= Nardeae Hackel)---contains only the genus Nardus.

Prat (1933) notes that the edge of the spikelet may be either directed towards the rachis or away from it, and also that the glumes may be one or two and that the spikelets may be solitary at the node or in clusters. These relationships are bound up with certain characteristic epidermal patterns, and he therefore considers the following subdivision of the tribe to be the more natural:

1. Triticeae---(2 glumes; spikelets transverse and solitary): Agropyron, Aegilops, Triticum, Secale, and Haynaldia.

2. Hordinae---(2 glumes; spikelets transverse and 2-6 at each node): Hordeum, Elymus and probably Hystrix and Sitanion.

3. Loliae---(1 glume; spikelets longitudinal): Lolium, Lepturus, Monerma, and Psilurus.

4. Pholiurae---(2 glumes; transverse spikelets but with deeply excavated rachis and epidermis of paleas very little differentiated): Pholiurus and Scribneria.

Cytological evidence will be sought to decide between these two classifications.

There are two genera now generally included in the Hordeae which have given taxonomists considerable trouble; these are Nardus and Jouvea. Hackel and Bews both construct a special sub-tribe for Nardus. Koch (1873 and 1907), Husnot (1896) and Ascherson and Grabner (1898-1902) set it apart as a separate tribe, while Krause first (1908) placed it, along with Psilurus, in the Chlorideae and later (1909) put them near the genera Arundo and Sieglingia in the tribe Festuceae. The retention of the genus within the Hordeae is morphologically dependent on the fact that the inflorescence is a simple spike. Numerous anatomical features clearly separate it from that tribe, however, and to these Prat (1932) has added a series of epidermal observations. It thus becomes evident that the retention of the genus within the Hordeae destroys the admirable unity of that tribe and the only alternative seems to be to create for it a special tribe. This tribe has been called the Nardoideae. Further investigation reveals that this new tribe does not properly belong in the sub-family Pooideae at all, but in the Panicoideae.

The genus Jouvea suffers a like fate. Bentham and Hooker seem to have been aware of its relationship for they placed it with the Chlorideae. Prat (1933a) suggests that it may belong to either the Andropogoneae or the Maydeae.

He refuses to commit himself on this point, however, until a more detailed study of the Panicoideae has been made.

Two other small genera, Kralikia Coss. and Kerinozoma Stend. also seem to require reclassification. Indications are that they are similar in nature to Nardus and Jouvea.

With the exception of Zea mays, the common cereals, of which three occur in this tribe, are probably the best known, both cytologically and genetically, of all the grasses. The genus Triticum forms a definite polyploid series with 7 as its basic number, and is an important example of the use of chromosome numbers alone in classification. Thus the members of the Einkorn group (T. monococcum etc.) have 14 chromosomes, the members of the Emmer group (T. dicoccum etc.) have 28 chromosomes and those of the Spelt group ~~Triticum vulgare~~ (T. vulgare etc.) have 42 chromosomes. This cytological grouping agrees exactly with the classification based on morphological and serological grounds, as well as on the basis of size of pollen grain, rust resistance and mutual fertility or sterility. Experimental crosses between Triticum and Secale or Aegilops giving new genera with 28 chromosomes have also been made.

Aegilops, Agropyron and Hordeum likewise form polyploid series within the genus, the first with 7 and 14 and the other two with 7, 14 and 21 chromosomes.

Prat (1933b) drew attention to the similarity which exists between the epidermis of the genera Triticum, Aegilops and part of Agropyron (sub-genus Eremopyrum and section Intermedia (Prat, 1932) of the sub-genus Euagropyrum). It is interesting to note that intergeneric crosses between

certain species of Triticum and species of Aegilops are quite possible. There is, however, no proof at present available that such crosses are only possible between species of Triticum and the species of Agropyron falling within the above sections, although the only successful ones reported to date have been with species in that category (Verushkine and Shechurdine, 1933).

Avdulov (1931) studied the cytology of a number of species of Agropyron. He found three types of chromosome sets within the genus. The first type of set is composed exclusively of V-shaped chromosomes (A. caninum, A. cristatum, etc.); the second type has every chromosome with one arm reduced to a head (A. prostratum), and the third type is an equal mixture of the first two types (A. orientale). All the members of the sub-genus Euagropyrum fall in the first class, while the sub-genus Eremopyrum contains representatives of all three classes. There is no evidence of any close morphological relationship between the sub-genus Euagropyrum and those species of the sub-genus Eremopyrum with V-shaped chromosomes, nor is there any clear distinction between the morphology of their chromosomes. The only correspondence between a karyological division and a systematic division in the sub-genus Eremopyrum lies in the fact that all the species (two of them) with only V-shaped chromosomes belong to the section Perennia Boiss., and the rest belong to the section Annua Boiss.

In Agropyron orientale Avdulov noticed one chromosome which, instead of being split only in two at the distal end of its arm, was divided into four parts. This is similar to the case which was observed in the present investigation in Anthoxanthum odoratum, and which will be considered in the discussion of the tribe Phalarideae.

Avdulov studied three species of Lepturus and one of Monerma and observed the following chromosome numbers:

Lepturus incurvatus (L.) Trin. $2n = 36$.

Lepturus filiformis (Roth.) Trin. $2n = 14$.

Lepturus pannonicus Kunth $2n = 14$.

Monerma cylindrica Coss and Dur. $2n = 26$.

The specimen corresponding to M. cylindrica which was examined in the present investigation (Fig. 12) had 52 chromosomes and is a tetraploid.

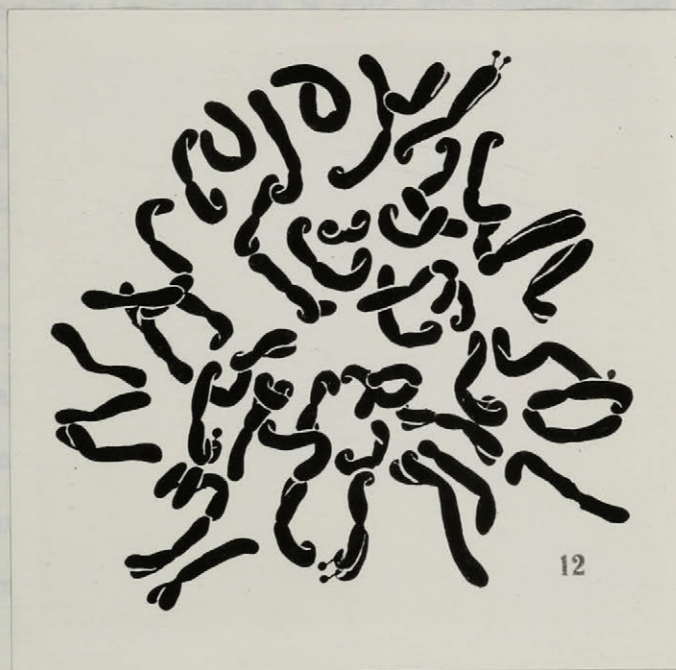


Fig. 12 Lepturus cylindricus Trin. $2n = 52$.

Camus (1922) notes that the genus Lepturus was created by Robert Brown in 1810 to separate the species of Rottboellia having a single glume from those having two glumes. In 1812 Palisot de Beauvois proposed the name Monerma to cover the same species. Lepturus has priority, however, and so Monerma cylindrica Coss and Dur. should be called Lepturus cylindricus Trin. Trinius, in 1820, proposed the name Pholiurus for

those members of the genus Rottboellia having two glumes. Since Lepturus incurvatus (L.) Trin., L. filiformis (Roth.) Trin., and L. pannonicus Kunth fall in this class, Camus rightly proposes that they should be called by the names Pholiurus incurvus A. Camus, P. filiformis A. Camus and P. pannonicus Trin., respectively. It is interesting to note that P. incurvus and P. filiformis are distinguished from one another only by the length of the glumes and the shape and size of the anthers, and yet Avdulov reports one as having the basic number 9 and the other the basic number 7. It is evident from his illustrations that all three species of Pholiurus and Lepturus cylindricus have chromosomes of similar types but that they have them in varying proportions. The close morphological relationship of the two genera is therefore reflected in their cytological similarity and it appears as if we have here an instance of redistribution of chromatin, such loss as has occurred not having any marked effect on the phenotype. Indeed, such is the uniqueness of the chromosome types here represented that, were it not for the very distinctive difference of the one and the two glumes, one would feel quite justified in grouping all the species into one genus.

Avdulov removes these four species to his tribe Festuceae which is a member of his series Festuciformes and contains most of the Festuceae and Aveneae. He also examined Nardus stricta (26 chromosomes), Lolium italicum (28 chromosomes) and Psilurus nardoides (28 chromosomes) and included them in his Festuceae. Prat (1932) found considerable similarity

between the epidermis of the Hordeae, Festuceae and Aveneae, but the inclusion of Nardus in ~~this~~ group is absolutely contrary to practically all existing morphological, anatomical and epidermal evidence. As has already been pointed out, this genus is very different from the rest of the tribe and approaches the Chlorideae in its resemblance to the Panicoideae. In spite of the almost entire lack of Panicoid characters (more or less small chromosomes in multiples of 9 or 10) in its karyotype, it nevertheless seems to belong naturally to that subfamily. This is a case where more or less definite karyological evidence must be subordinated to an overwhelming weight of evidence of another nature. In other words, the evidence to be derived from cytology is no more a foolproof basis of classification than is any other evidence. On the contrary, all available evidence must be used in order to obtain a favourable balance one way or the other.

The genera Jouvea, Kralikia and Kerinozoma have, unfortunately, so far not been cytologically examined. The remaining genera fall in Bews' subtribes Elyminae and Triticinae or into Prat's subtribes Triticeae and Hordinae. Avdulov finds this to be a very uniform group with chromosomes all more or less the same size and characterized by the basic number 7. He leaves these genera in their original position in what he calls the tribe Fruментaceae which contains also the genera Bromus and Boissiera of the Festuceae. Thus, with the exception of the genus Nardus, his classification agrees exactly with that of Prat and shows the unnaturalness of grouping the subtribes Lolieae-Leptureae and Triticeae. Such a correspondence between classifications based on two so entirely different characters is good evidence of its correctness.

Tribe Agrostideae-

According to Bews the tribe Agrostideae embraces 59 genera and is divided into three subtribes. "The first subtribe, Stipinae is separated from the rest of the tribe by having the lemma indurated, or, at least, much harder than the glumes at maturity, and closely enveloping the fruit. The others have a hyaline or membranaceous lemma, more delicate than the glumes. These are further subdivided into two other subtribes, the Phleinae with the stigmas having short branches all round and projecting from the apex of the lemmas, and the Agrostinae with the stigmas plumose, projecting from the side of the spikelet."

The Agrostinae are the most primitive of the subtribes and probably the most primitive genus among them is Calamagrostis to which is allied the genus Agrostis. These genera are hygrophilous or mesophytic and, therefore, primitive ecologically as well as floristically. The subtribe is morphologically connected with the Festuceae—Aveneae series at more than one point, but the separation of such forms as Calamagrostis and Agrostis probably goes a long way back.

The Phleinae are a distinct, but not important, subtribe. Phleum, Heleochloa and Alopecurus are the only genera of importance.

The genus Sporobolus is rather distinct from either the Agrostinae or Phleinae, but is included in the former by Hackel. Stapf (Bews, 1929) separates it as a distinct tribe, the Sporoboleae and places it next the Eragrosteae which he removed from the Festuceae.

The subtribe Stipinae is the most advanced of the three as is evident from their more efficient means of seed dispersal and adaptation to a more or less xerophytic habitat.

Cytologically the tribe may be divided into three fundamental groups. The first is represented by the genera Muhlenbergia and Lycurus on the one hand and Heleochloa and Sporobolus on the other. It is characterized by small chromosomes and a basic number of 10 for the first part and 9 for the second. Avdulov bases his conclusion as to the basic number of the second part on Heleochloa schoenoides which has 36 chromosomes and on two forms of Sporobolus indicus, one of which has 18 and the other 36 chromosomes. I examined S. tenuissimus (Fig. 13) and found it to have 40 chromosomes. Assuming it to be a tetraploid, which is indicated by the presence of two pairs of similar chromosomes with trabants, this gives it the basic number 10 and makes it conform with the first part of the group in this respect. The group as a whole resembles the Sacchariferae, i.e., the subfamily Panicoideae of Bews, in regard to its leaf anatomy, geographical distribution and structure of the ligula as well as its cytology. Krause, (according to Avdulov, (1931), demonstrated the morphological connection of Heleochloa with Sporobolus and of both with the Chlorideae and the genus Tragus of the Zoysieae. Both these tribes are similar to this group in cytology, structure of starch grains and leaf anatomy and, in fact, Avdulov mentions that Bessey suggests the division of the genera of the tribe Chlorideae among the Agrostideae, Aveneae and Festuceae.



Fig. 13 Sporobolus tenuissimus Kuntz ($2n = 40$)

Fig. 14 Phleum phleoides L. ($2n = 14$)

The second cytological group contains the genera Agrostis, Calamagrostis, Mibora, Cornucopiae, Phleum, Alopecurus and Milium. It is characterized by large chromosomes and the basic number 7, with the exception of Milium vernale which has 18 chromosomes. Of this group Phleum phleoides (Fig. 14) was examined in the present investigation and found to have 14 chromosomes.

This falls in line with the other species of the genus examined by Avdulov. He found in this genus a polyploid series of 14, 28 and 42 chromosomes.

Three genera of this group, viz., Phleum, Mibora and Alopecurus are connected morphologically with the genera Hierochloa, Anthoxanthum and Phalaris in the tribe Phalarideae through the fact that the beard springs from the tip of the glume. In Apera, however, it arises from the back of the glume as in the Aveneae. The group agrees with the above members of the Phalarideae and with the Aveneae in having clumped starch grains, type 2 leaf anatomy and in being distributed mostly in the northern hemisphere. The similarity extends also to their cytology. All have large chromosomes, and the basic number 7 is characteristic of the Aveneae and two of the three genera of Phalarideae. Avdulov considers the basic number 5 of the other genus, Anthoxanthum, to have been derived from the original number 7 which is characteristic of most of the Pooideae. It seems certain, therefore, that a connection exists between this group of the Agrostideae and the Phalarideae and Aveneae.

The third group, represented by the genera Stipa and Oryzopsis, is characterized by small chromosomes with a basic number 12. Of the eight species of Stipa investigated by Avdulov, however, one, S. sibirica has 24 chromosomes and the rest have 44. These species are presumably tetraploids derived from a diploid form with basic number 12, which had lost one pair of chromosomes. The two species of Oryzopsis reported by Avdulov, O. miliacea and O. virescens, both have 24 chromosomes.

Cytologically this group approaches Oryza (Oryzeae), Ehrharta (Phalarideae) and Phaenosperma (Melinideae) where the basic number 12 is also met with and where the chromosomes are also small. These genera are likewise similar floristically, all approaching a more or less primitive, hexameric type of flower structure. There thus seems to be a linkage between small chromosomes with the basic number 12 and the above mentioned flower type.

Each of these main cytological groups is doubtless of independent origin and forms an independent branch of development and in each case the divergence of the characters of the karyotype is linked up with a series of morphological or anatomical characters. The cytological data disclose the basis for the unnaturalness of the tribe and suggest the possibility of breaking up the tribe and giving it a new grouping.

Tribe Phalarideae

This is a small tribe containing only six genera.. It is divided naturally into two groups. The first contains Ehrharta, Microlaena and Tetrarrhena and is characterized by the third and fourth outer glumes being larger than the first and second. The second group, comprising the genera Phalaris, Anthoxanthum and Hierochloa has the third and fourth glumes smaller than the first and second. The two groups are also different in geographical distribution. The first is confined to the southern

hemisphere while the second, with the exception of one species of Anthoxanthum grows entirely in the northern hemisphere.

Both groups have compound starch grains and type 2 leaf anatomy.

Avdulov (1931) gives the following chromosome numbers:

<u>Ehrharta panicea</u> Smith	2n = 24.
<u>Phalaris paradoxa</u> L.	2n = 14.
<u>Phalaris minor</u> Retz.	2n = 28.
<u>Phalaris arundinacea</u> L.	2n = 28.
<u>Phalaris canariensis</u> L.	2n = 12.
<u>Anthoxanthum odoratum</u> L.	2n = 20.
<u>Anthoxanthum aristatum</u> Boiss.	2n = 10.
<u>Hierochloa odorata</u> (L.) Whlbg.	2n = 42.

Cytologically the tribe is divided into two groups corresponding, as far as present investigations indicate, to the above mentioned taxonomic groups. Avdulov finds that the chromosomes of the first group, represented in his work by Ehrharta panicea, are small and in multiples of 12. They therefore resemble the Oryzeae, and particularly the genus Oryza. The structure of the starch grains, anatomy of the leaves and geographical distribution are also the same as that found in the Oryzeae.

The second group is characterized by large chromosomes in multiples of 7 or less. In the genus Phalaris the chromosomes are at least three times as large as those of Ehrharta panicea and the basic number is 7. Phalaris canariensis formed an apparent exception since both Avdulov (1931) and Church (1929a) reported 12 as its diploid number. Recently, however, Nakajima (1933) has established its haploid number as 14 and its diploid number as 28.

The only species of this genus examined in the present

investigation was P. arundinacea (Fig. 16). The diploid number of 28 coincides with that of Avdulov's material.

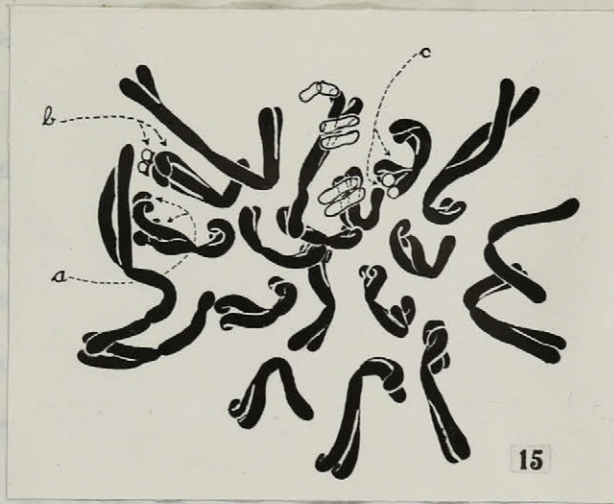


Fig. 15. Anthoxanthum odoratum L. ($2n=20$).

Fig. 16. Phalaris arundinacea L. ($2n=28$).

Anthoxanthum also has large chromosomes. Marchal (1920; Avdulov, 1931) counted 8 chromosomes in meiotic metaphase of A. odoratum, but Avdulov reported no deviation from 20 chromosomes in somatic tissue; the material examined in the present study also had 20 chromosomes (Fig. 15). A. aristatum has 10 chromosomes and therefore the basic number for the

genus is probably 5. Two interesting phenomena were observed in the material examined. In one cell it could very clearly be seen that the ends of three of the chromosomes (a, b, and c, Fig. 15) were four-partite instead of bi-partite as is commonly the case. This is similar to an instance reported by Avdulov in Agropyron orientale and already referred to. The significance of this "secondary" split was apparently not realized, however. Such a condition is not new, for Digby (1919), McClung (1927), Robertson (1931) and others have all noticed a double structure in the mitotic chromosomes in the anaphase preceding division, and Sharp (1929) showed that large somatic chromosomes first split in the prophase of the division preceding that in which the halves separate. Darlington (1932) has argued that the apparent doubleness is an optical illusion since "the fixed chromatid is probably a hollow cylinder which in optical section will appear double". His arguments have been shown by Huskins (1933) to be invalid. Avdulov assumes that it might be possible for this secondary split to become completed and result in the addition of an extra pair of chromosomes to the set. While this would be a very handy explanation for the presence of such extra chromosome pairs, it seems much more likely that the function of this split is that which has been assigned to it by the above investigators and that the secondary split will not become operative until the nuclear division following that in which it was conceived.

The other phenomenon noticed was the presence, in all the cells examined, of two pairs of large "fragments" and one pair of small, almost spherical, ones_x (shown in outline in Fig. 15) These "fragments" do not stain as deeply as the

rest of the chromosomes, they always lie in definite pairs as shown, and no attachment constriction is visible. They appear to be a constant feature of the plant, however, since they were found in more than the one root-tip. It seems, therefore, that they may be analagous either to the "chromosomal bodies" reported by Avdulov (1933) in Zea mays or the "additional chromosomes" reported by Avdulov and Titova (1933) in Paspalum stoloniferum.

Thus we have two basic numbers in this second group; 7 for the genera Phalaris and Hierochloa and 5 for the genus Anthoxanthum. Avdulov (1931) thinks that this difference between the basic numbers may be due to an evolutionary deviation of Anthoxanthum away from the more or less primitive number 7 characteristic of the other members of the group and, indeed, of the majority of the Poideae.

There seems to be some relationship between the second group and the Aveneae. In Anthoxanthum the lower lemmas sometimes bear male flowers and are awned from the back as in the Aveneae. In Hierochloa the lower lemmas, or at least one of them, always bears male flowers. The chromosomes of the two groups are also very similar, both in basic number and in morphology. There is also a marked correspondence in their geographical distribution.

On the other hand, the first group of the Phalarideae, of which Ehrharta panicea is so far the only representative to be examined cytologically, bears a close similarity to the Oryzeae.

Prat (1932) examined the epidermis of Phalaris paradoxa, Phalaris minor, Phalaris arundinacea and Anthoxanthum

odoratum and considers them to have well marked affinities with the Oryzeae. In the face of this evident disagreement between the two methods of classification it is advisable that further and more detailed study be made, both of their karyology and epidermology, before any definite placing can be given to either of the groups of genera. At present, however, the cytological data seem more conclusive, at least in so far as the second group is concerned. It therefore appears to be fairly certain that this second group and the Aveneae are quite closely related. Avdulov considers that this resemblance may be taken to represent a side-line development of the genera Phalaris, Anthoxanthum and Hierochloa that has not become differentiated to any great extent.

Subfamily Panicoideae

Bews' description of this subfamily is as follows:

"Except in unisexual and neuter spikelets, the spikelets have one terminal perfect floret, with a male floret or empty lemma below it. One glume, or rarely both glumes, may be wanting. The rachilla is not continued above the upper floret. The spikelets fall entire from their pedicels, singly or in groups, or together with the joints of the rachis. The spikelets are usually more or less dorsally compressed.

The Panicoid series seems to have had a distinct origin from the Pooideae but may connect somewhat remotely with ancestral forms which resemble the Bamboos. It is obvious that the whole of the Panicoideae are rather highly developed in so far as reduction in the number of flowers in the spikelet is concerned. But as we have seen, this reduction occurs in primitive tribes of the Pooideae as well. Though a very important evolutionary trend, it is, after all, only one among many. It has taken place within all the separate circles of affinity." Bews considers that

there are really only two main tribes of the Panicoideae, for the Maydeae are little more than a subtribe of the Andropogoneae and the Melinideae an unimportant transitional tribe which might be included in the Paniceae.

Tribe Melinideae

This tribe is of little importance. It is rather a heterogeneous collection of genera in which the spikelets have the two heteromorphous florets, the upper perfect, the lower male or barren, characteristic of the whole Panicoid series. The glumes and lemmas are both membranaceous, the latter not harder than the glumes, as in the Paniceae and the former not much harder either, or at least not closing round the spikelet as in the Andropogoneae. Bews says that Stapf includes the genera Rhynchelytrum, Tricholaena and Melinis in the Paniceae, and Beckera in the Oryzeae. Unfortunately only one of these genera has been examined cytologically. Bews is doubtful as to whether or not this tribe should be maintained, since the genera are not very closely connected.

Avdulov examined the genera Arundinella, Melinis and Phaenosperma. Hackel maintained that Arundinella was the genus from which the tribe Andropogoneae originated. Avdulov believes their morphological similarity to be confirmed cytologically through A. anomala and Miscanthus japonicus. Both species have the basic number 9. In two other species of Miscanthus which I examined, however, I found the basic numbers to be 7 and 8 respectively. Avdulov doubts that all the Andropogoneae were derived from the genus Arundinella but rather considers it more probable.

that Arundinella and Miscanthus form a connecting link between the Andropogoneae and the Melinideae.

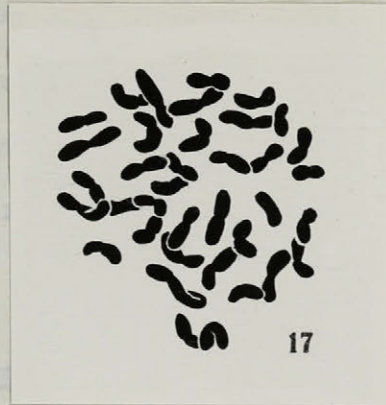


Fig.17. Melinis minutiflora Beauv. ($2n=36$)

Hackel believed the genus Melinis to have sprung from the Paniceae and, as was already mentioned, Stapf includes it in that tribe. Avdulov examined M. minutiflora and found its chromosome number to be 36. I confirmed this observation (Fig.17). Since the basic number of most of the Paniceae is 9 and since most of them have small chromosomes as has M. minutiflora, Avdulov considers it quite logical to regard the genus as a member of the Paniceae.

The third genus which Avdulov investigated was the monotypic Phaenosperma, represented by P. globosa. It is cytologically very different from the other members of the tribe in that it has the basic number 12. This change in basic number is accompanied by parallel morphological differences which, along with the size of its chromosomes place it in close relationship with Oryza, Ehrharta and the group within the Agrostideae represented by Stipa and Oryzopsis. Hackel, Bews and other systematists seem

to have erred, therefore, in including this genus in the Panicoideae.

On the evidence of cytological investigations already completed a thorough examination of the whole tribe is called for. Such an examination cannot fail to bring to light many important data for a more correct classification.

Tribe Paniceae

The Paniceae are less advanced than the Andropogoneae floristically and this is reflected very clearly in their distribution and ecological behavior. According to Bews, "they are grasses of the great tropical and subtropical regions of the world, where they are abundant. They do not enter very much into the composition of subtropical mesophytic grassland, a type of vegetation which, on the other hand, is completely dominated by the Andropogoneae!" It is true that the Paniceae have produced some rather highly evolved ecological types but the large majority belong to the primitive hygrophilous situations of the tropics. The more advanced types occupy drier situations in the warm regions and a few, e.g. a species of Paspalum in South America, occur in grasslands mixed with the Andropogoneae.

The Paniceae form a very large tribe having, according to Bews, about 60 genera and nearly 1400 species. Yet, as is to be expected from the uniformity of their habitat, many of the genera are very closely related and the tribe as a whole

forms one of the most natural groups within the family. Bews follows Stapf in dividing up some of Hackel's genera, chief among which is Panicum. Bew's nomenclature is followed in the present discussion of the tribe.

Hackel's section Digitaria of the genus Panicum becomes Bew's genus Digitaria. Avdulov examined two forms of the species Digitaria sanguinale Scop. viz. f. australis Mez. and subsp. horizontalis Mez. In both he found 36 chromosomes. In D. exilis (Fig. 18) I found 54 chromosomes. These are the only species so far reported, so that the basic number at present is definitely 9.

The section Echinochloa is also raised to the rank of a genus. According to Kihara^{et.al.} (1931) Panicum crusgalli L. var. frumentaceum Hook. = Echinochloa crusgalli Beauv. = Echinochloa frumentacea (Roxb.) Link. Hitchcock (1920b) gives Echinochloa frumentacea Link, as synonymous with Echinochloa crusgalli edulis Hitchc. Avdulov reports 54 chromosomes in Panicum crusgalli L. var. frumentaceum (Roxb.) Frimen. Church (1929b) gave 21 as the haploid number for Panicum crusgalli L. var. frumentaceum Hook. In Echinochloa frumentacea (Roxb.) Link I counted 36 chromosomes (Fig. 19). The basic number is the same as in Avdulov's material and it appears as if Church was mistaken in his observations.

The section Brachiaria becomes Bew's genus of the same name. The genus is based on Panicum eruciformis Sibth. (Hitchcock and Chase, 1910). Hitchcock (1920a) gives this species as synonymous with B. erucaeformis (J.E. Smith) Griseb. Avdulov reported 18 chromosomes for this species. I examined B. mutica and found 36 chromosomes (Fig. 21). According to Hubbard (1929)

B. mutica Stapf = Panicum barbinode Trin. and Bews says it is the same as P.muticum Forsk. In so far as it has been examined, then, the genus Bracharia forms a polyploid series with the basic number 9.

Hitchcock (1920b) makes the section Ptychophyllum of the genus Panicum a section of the genus Chaetochloa Scribn. This latter genus is synonymous with Setaria Beauv. Hackel's P.plicatum Lam. falls in this group and Avdulov counted 36 chromosomes. Of the other species of Setaria which Avdulov examined, two had 18 chromosomes and two 36, making a polyploid series with 9 as the basic number.

The remaining section which Avdulov examined, Hackel's Eupanicum, Bews retains in his genus Panicum. All species investigated have multiples of 9 chromosomes.

Throughout the whole of Hackel's genus Panicum, then, we find no divergence from the basic number 9, with one very doubtful exception. All the species are so similar in their chromosome morphology that it is impossible, as yet, to subdivide them on that score. A very careful comparison of their karyotypes may bring out definite generic differences, but until then their classification must be based on other than cytological grounds.

Avdulov found a characteristic case of polyploidy in the genus Pennisetum. Of the species so far investigated one is a diploid ($2n=18$), one is a triploid ($2n=27$), two are tetraploids ($2n=36$), two are pentaploids ($2n=45$) and two are hexaploids ($2n=54$). To this list I added the species P.clandestinum (Fig.22) with 36 chromosomes, a tetraploid.

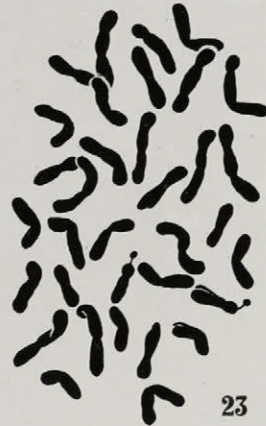
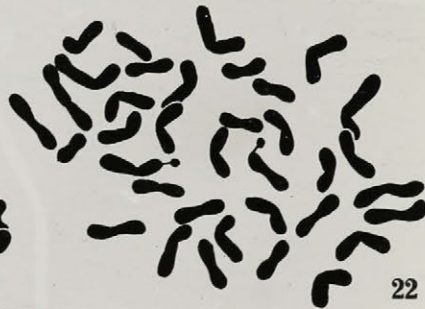
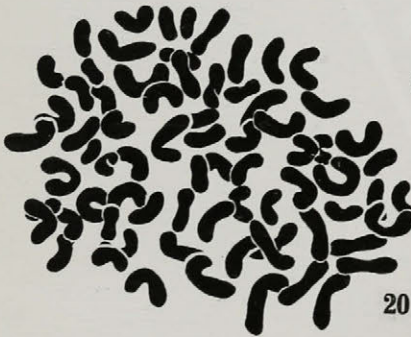
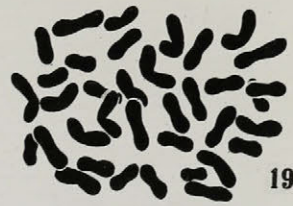
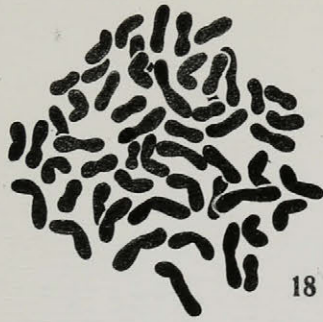


Fig.18 Digitaria exilis Stapf ($2n=54$)

Fig.19 Echinochloa frumentacea (Roxb.) Link. ($2n=36$)

Fig.20 Oplismenus burmanii (Retz.) Beauv. ($2n=72$)

Fig.21 Brachiaria mutica Stapf ($2n=36$)

Fig.22 Pennisetum clandestinum Hochst. ($2n=36$)

Fig.23 Cenchrus tribuloides L. ($2n=34$)

The genus Pennicillaria Willd. is included by Bews and Hackel in the genus Pennisetum Pers. Avdulov examined Pennicillaria spicata Willd. and found that it had 14 large chromosomes, larger than those of any other member of the tribe. In view of its peculiar karyotype it seems better to retain it in a separate genus as does Willdenow (quoted by Chase, 1921) rather than to group it with Pennisetum.

Of the three species of Oplismenus which Avdulov examined one had 54 chromosomes and the other two had 72. I found O. burmanii (Fig.20) to have 72 chromosomes.

In the genus Cenchrus, Avdulov finds three species with the basic number 17 and one with 35. C. tribuloides (Fig.23), which I examined, has 34 chromosomes. Avdulov believes that the number 34 must have originated from a tetraploid form of a series with the basic number 9, through the loss of one pair. The 70-chromosome form, in his opinion, might have arisen through the backcross of a 34 chromosome form with a 36 chromosome form. The 17 and 18 gametes formed, respectively, would give 70 chromosomes on doubling.

An interesting case is disclosed in the genus Paspalum. All the species so far examined have their chromosomes in multiples of 10. This basic number is characteristic of most of the Andropogoneae. In point of chromosome size there is also a decided similarity between the two groups. This evidence, along with the fact already noted that the genus is one of a few that desert the typical habitat of the Paniceae and are found growing mixed with the Andropogoneae, points to the possibility

that it should be included with the latter tribe rather than with the Paniceae.

Cytological investigations suggest very little change within the Paniceae. A redistribution of Hackel's Panicum may or may not be justified. The present state of knowledge in this respect does not contribute any information in favour of breaking it up. The only change so far indicated is the transfer of Paspalum to the Andropogoneae.

Tribe Andropogoneae

The Andropogoneae are marked by an extreme specialization in the inflorescence. This specialization marks an advance and applies, with variations, to the whole tribe. "Within the tribe, however, are further degrees of advance and specialization, and the genera fall into two series, the one with the joints and pedicels relatively slender and not approximate or fused together — relatively primitive in this respect — and the other more advanced, with the joints and pedicels more or less stout and approximate or fused together, forming a receptacle of the sessile spikelet." The first series consists of the subtribes Saccharinae and Andropogoninae, and the second of the subtribes Ischaeminae and Rottboellinae.

The Saccharinae are probably the most primitive and its members include Saccharum and its allies Imperata, Miscanthus and Erianthus as well as Eulalia, Polytrias and several others. They are all relatively primitive ecologically as well as floristically.

The Andropogoninae is a much larger and more progressive

subtribe. It includes only seven genera in Hackel's arrangement, for he grouped a large number into the single genus Andropogon. Other authorities, however, gave the numerous subgenera of Andropogon generic rank. Stapf, whom Bews follows, has retained most of them and also established several more so that the subtribe now contains about thirty genera. Some of these are Andropogon, Cymbopogon, Sorghum and Amphilophis, subdivisions of Hackel's Andropogon, as well as Themeda, Arthraxon and so on.

Under the subtribe Ischaeminae come Apluda, Jardinea and Elyonurus and under the Rottboellinae the most important is Rottboellia.

Hackel (1889), according to Avdulov (1931), monographed the tribe and considered it a very natural one, a view which very few subsequent workers have found any reason to question.

Avdulov examined representatives of a number of genera and found that in most cases the basic number was 10, but that in Miscanthus, Arthraxon and Rottboellia it was 9. I was able to examine two species of Miscanthus, M. sinensis (Fig.24) and M.saccharifera (Fig.25), and found them to have 42 and 64 chromosomes respectively. Avdulov examined only M.japonicus and found 36 chromosomes. Church (1929b) gave the haploid number for M.sinensis as 10, but Avdulov doubted his accuracy. Taking Avdulov's result for M.japonicus and mine for M.sinensis and M.saccharifera we have another case similar to that found in Lepturus of a change in the basic number within the genus. It should be noted that, as was mentioned in the introduction

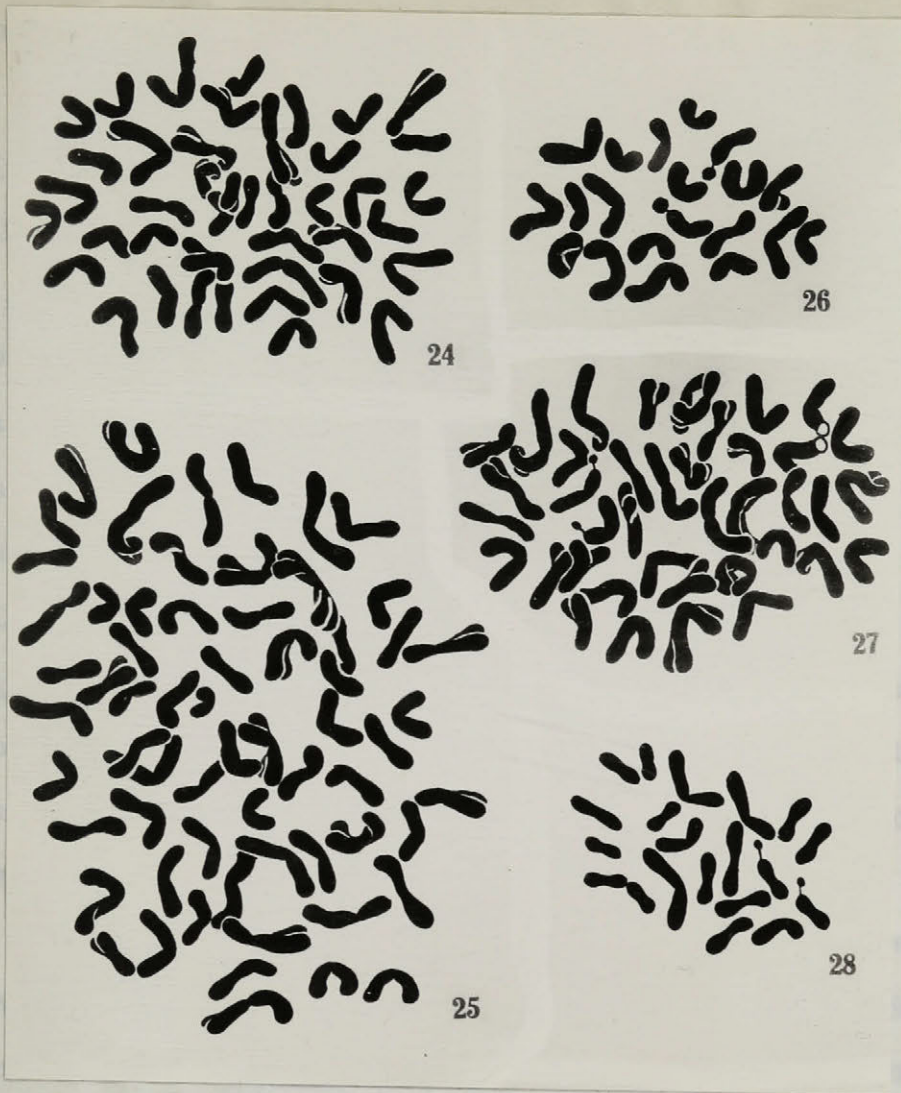


Fig.24 Miscanthus sinensis Anderss. ($2n=42$)

Fig.25 Miscanthus saccharifera Benth. ($2n=64$)

Fig.26 Andropogon eliottii Chapm. ($2n=20$)

Fig.27 Andropogon scoparius Michx. ($2n=40$)

Fig.28 Apluda mutica L. ($2n=20$)

under the discussion on the use of the karyotype in systematics, it is possible for such a change to occur without producing any great effect on the expression of the phenotype and since the three species are closely related they

are most likely derivatives of one another.

Avdulov follows Hackel's system of classifying the genus Andropogon and finds all the species investigated to have the basic number 10. I examined two species, A.eliottii (Fig.26) and A.scoparius (Fig.27) and found 20 and 40 chromosomes respectively. Huskins and Smith (1932) reported 40 as the somatic chromosome number of Sorghum halepense and 20 for ^{other} twenty-one species and varieties of Sorghum. They found also a number of tetraploid segments and one octoploid segment in root-tips of diploid plants. As is the case with the genus Panicum in the preceeding tribe, the similarity between the chromosomes in all the species so far investigated is so great that it is impossible to subdivide this large genus on purely cytological grounds.

In the subtribe Ischaeminae the only genera which have been examined are Ischaemum and Apluda. Avdulov doubts Kuwada's count of 68 in I.anthephoroides and gives 20 as the count in I.timorense. For Apluda mutica he reports 40 chromosomes. My material (Fig.28) had only 20 chromosomes, so that it is probably a diploid and Avdulov's a tetraploid.

The whole tribe, then, is characterized by a remarkable regularity in basic number and size of chromosomes. It thus becomes interesting to speculate on the course taken by evolution in the development of the tribe. Avdulov considers that the genus Miscanthus which he postulates as a link between the Andropogoneae and Melinideae, constitutes the point of origin for the whole tribe. From this point development has proceeded

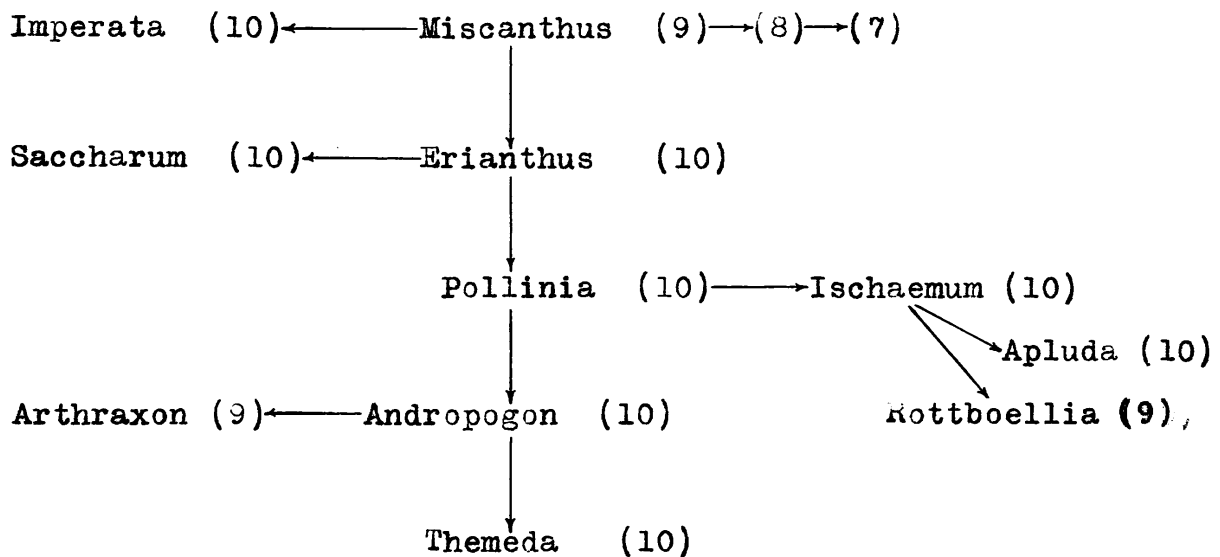
in two directions, towards Imperata and towards Erianthus, in both cases with an increase in basic number from 9 to 10.

Further variation ^{in Erianthus} follows a course through Pollinia to Ischaemum and Andropogon without a change in basic number.

From Ischaemum it goes in one direction to Apluda and in the other direction, with a decrease in the basic number to 9,

to Rottboellia. Andropogon likewise proceeds to Themeda without change and to Arthraxon with a drop to 9. In

addition there is a decrease in basic number from 9 to 8 to 7 within the genus Miscanthus. This may be more clearly demonstrated diagrammatically as follows (after Avdulov, 1931):



Tribe Maydeae

There are only seven genera included in this small tribe. Bews considers it as little more than a subtribe of the Andropogoneae. All the members are monoecious, the male and female flowers being in the same inflorescence in some, such as Tripsacum and Coix, while in Euchlaena and Zea the male spikes are terminal in a panicle and the female in the leaf axils. The whole tribe is obviously advanced and specialized to a supreme degree, in many ways the most specialized of all the grasses. Within the tribe the genera Euchlaena and Zea are the most advanced.

The basic chromosome number for most of the genera has been established as 10. Longley (1924) reported 35 as the basic number for Tripsacum. Avdulov examined T.dactyloides and maintained that it had not less than 72 and probably as many as 80 chromosomes. My material (Fig.29) definitely had 36 chromosomes. This rather destroys Avdulov's contention that the basic number is probably 10 like the rest of the tribe. This fact, along with the extreme difference in chromosome size, however, fits in well with the acknowledged morphological isolation of the genus.

Avdulov reported 40 as the chromosome number of Polytoca macrophylla. This gave it also the basic number 10.

Kuwada (from Avdulov 1931) gave 10 as the haploid number for Coix lachryma-jobi. Taylor (1925) agreed with him. Avdulov counted 20 chromosomes in the somatic cells and I found the same in my material (Fig.30). This genus, as well

as Tripsacum, occupies an isolated position in the tribe, and here again, morphological dissimilarity is reflected in the karyotype. Its chromosomes, though larger than those of Tripsacum are on the whole considerably smaller than those of the other investigated genera of Maydeae.

The genus Euchlaena is Zea's closest relative. Kuwada in 1915 (Avdulov 1931) reported 20 as the chromosome number of E.mexicana, and Longley (1924) counted 40 in E.perennis. My investigations agree with both of these (Figs.31 and 32). An interesting phenomenon presented itself in E.mexicana in the form of a heteromorphic pair of chromosomes. These are indicated in the figure by an X. As will be seen the chromosomes are three armed. One member of the pair is perfectly normal. It has a median primary constriction and a subterminal secondary one which divides off a head-like portion. The other chromosome has the secondary constriction greatly accentuated until it gives the distal portion the appearance of a slightly elongated trabant. This condition was constant in all the material examined.

In his survey of chromosome numbers in Zea mays, Randolph (1928) notes that Kuwada (1911, 1915 and 1919) reported the haploid number as varying from 9 to 12, sugary varieties usually having higher numbers than starchy varieties. Longley (1924) examined four varieties and found no deviation from 10, but later (1925) he found more than 10 in two starchy and two sugary strains. Risk in 1925 (Randolph 1928) investigated different strains and "found no clear evidence of numbers other than 20

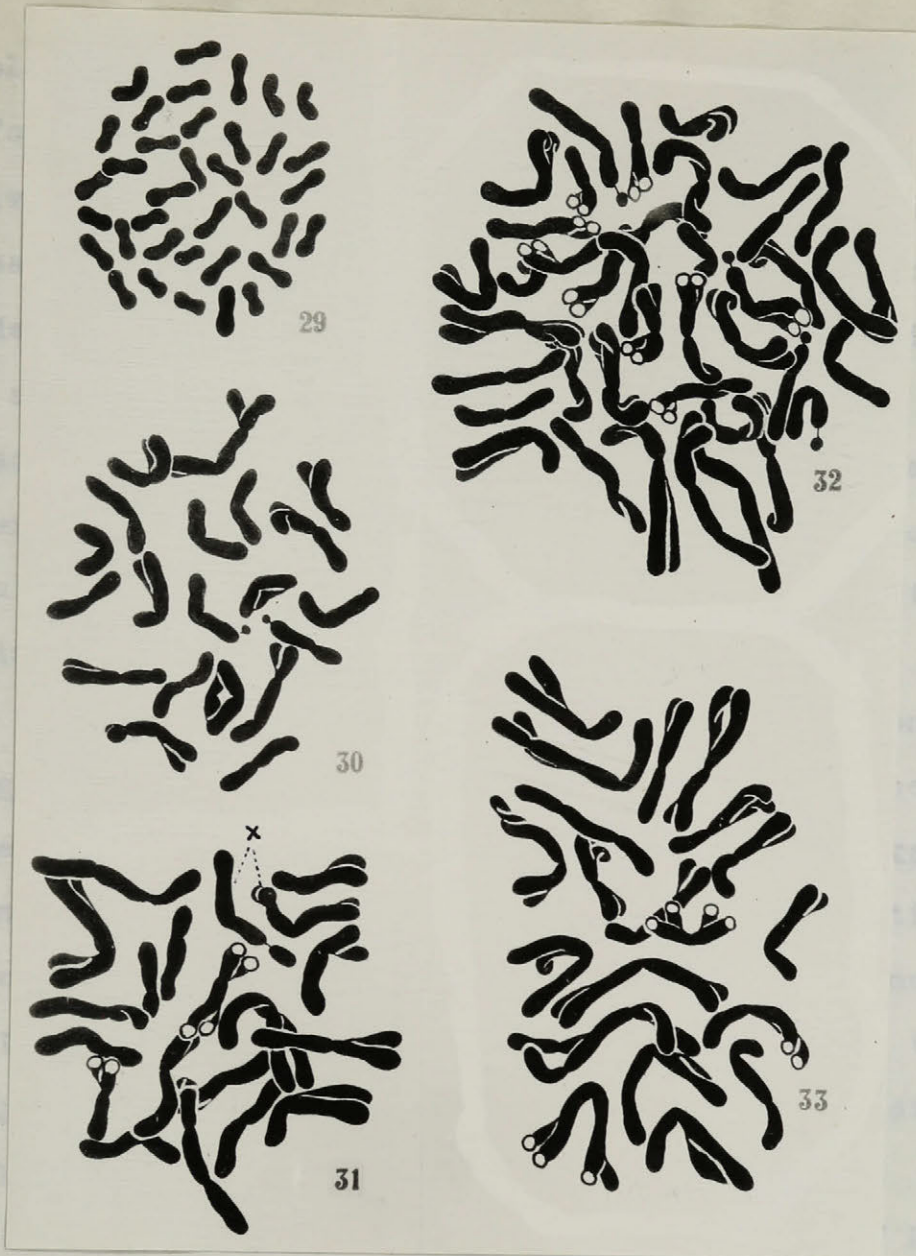


Fig.29 Tripsacum dactyloides L. ($2n=36$)

Fig.30 Coix lachryma-jobi L. ($2n=20$)

Fig.31 Euchlaena mexicana Schrad. ($2n=20$)

Fig.32 Euchlaena perennis Hitch. ($2n=40$)

Fig.33 Zea mays L. subsp. indentata. ($2n=20$)

(diploid) except in the sugary variety Black Mexican." Here he found, as did Kuwada, inconstancy of number both in different individuals and in different cells of the same individual. Kiesselbach and Petersen (1925) made a large number of counts. No deviations from 10 as the haploid number were discovered. The suggestion was made that the extreme variation reported by Kuwada was perhaps due to errors of counting. Kuwada (1925) re-examined the preparations on which his former accounts had been based and found additional evidence in support of his earlier observations.

This condition is analogous with the situation in Secale cereale L. which normally has 7 chromosomes but occasionally 8. Other examples have been found in Oenothera and Crepis, to mention only the most familiar. A number of explanations have been given to explain this divergence from the 20 chromosomes normal to the species, among which Randolph (1928) cites those of non-conjunction, non-disjunction and the crossing of diploid and triploid strains.

In my investigations I examined a variety of Dent corn, Zea mays indentata (Fig.33), and found no deviation from 20 chromosomes.

A comparison of the Andropogoneae and Maydeae with regard to basic number and chromosome size shows that while the basic number 10 is typical of both, the chromosomes of the latter are very much larger. Tripsacum and Coix are an exception and may be said to form a connecting link, cytologically, between the two tribes. Because of this almost general extreme

difference in chromosome size it seems more logical to consider the Maydeae as a distinct tribe rather than merely as a subtribe of the Andropogoneae.

Evolution in the Gramineae

Having completed a survey of chromosomes in the Gramineae it would be of interest to speculate on the evolution of the various tribes. Such a discussion, however, would occupy a paper by itself and I am not prepared to go into the required details at the present time. Suffice it to say that there is every reason to suppose that the family is monophyletic and so all the different types must have evolved one from the other. The groups regarded as primitive are characteristic, according to Avdulov, in the possession of small chromosomes in multiples of 12, and some of flowers with 6 stamens. The most advanced genera with dimerous flowers are characterized, on the other hand, by large chromosomes in multiples of 7, 6 or even 5. Evolution has therefore taken the form of an increase in size of chromosomes together with a reduction in their basic number. Avdulov considers that the first of these two processes took place first.

Polyploidy has only occurred within the genus or section of the genus. Whenever numbers higher than the basic number are observed in a genus the lower numbers are also observed, and whenever a genus is characterized by a constant chromosome number this is always equal to the basic number. In the

evolution of the karyotype of the genera, then, polyploidy has played little or no part.

In the earlier stages of evolution of the karyotype there was an undoubted gain in total quantity of chromatin. In the later stages, however, there was a fall in basic number not accompanied, or not compensated for, by an increase in the size of the chromosomes, resulting in a loss of chromatin material.

Another process by which the final stages of evolution have occurred in some genera is the change, not of the number, but of the idiogram, i.e., a changed disposition of the chromatin between the chromosomes.

Conclusions and Discussion.

On the basis of his investigations, Avdulov divided the grasses into three groups. The first group is characterized by (1) type 1 leaf anatomy, (2) chromosomes in multiples of 9 or 10, rarely more or less, (3) elliptical or lanceolate first leaf, growing horizontally, (4) usually a row of small hairs in place of the ligule, (5) tendency of the main shoots to tiller, (6) peculiar shape of the silicated cells, "Kieselkurzellen", and the presence of two-celled hairs, "Winkelhaaren", on the epidermis of the leaf, (7) alternation in the leaf of one primary with several or many secondary vascular bundles and (8) limitation almost entirely to tropical or subtropical regions. The group of genera possessing this complex of characters, which includes the Panicoideae, Chlorideae and a number of genera from other tribes, constitutes, therefore,

a very natural group. Avdulov thus defines the new subfamily Sacchariferae (Harz) Avdulov.

The second group differs from the first in having (1) its chromosomes in multiples of 7 or less, rarely 9, together with (2) a linear first leaf growing vertically, (3) type 2 of chlorophyll arrangement, (4) the primary vascular bundles of the leaf alternating with 1, 2 or, at the most, 4 secondary ones, (5) two-celled hairs entirely absent, (6) starch grains compound, except in a small group in the Hordeae, together with Bromus and Boissiera, where the starch grains are still different from those of the Sacchariferae, and (7) limitation to the temperate or cold zones.

The third group, Oryza, etc., lacks uniformity in many respects. Avdulov has examined only isolated genera of this group but believes it to constitute a tropical or subtropical group with small chromosomes in multiples of 12, but with a different type of leaf structure from that of the Sacchariferae.

The second and third groups are placed together in the second subfamily Pooateae (Hitchcock) Avdulov, the second as the Festuciformes Avdulov and the third as the Phragmitiformes (Harz) Avdulov. The group Festuciformes is subdivided into two tribes, the Fruentaceae (Harz) Avdulov and the Festuceaceae (Kunth) Avdulov, the first with simple and the second with clumped starch grains.

The present investigation, with a few minor exceptions, supports Avdulov's conclusions. It is evident that parts of the family have not yet received sufficient attention and that more work must be done on them before any really valid conclusions can be reached. This applies in particular to

Avdulov's third group which at present comprises a more or less heterogeneous collection of genera bound together only by rather scant resemblances. The tribe Bambuseae is also deserving of more attention. Many investigators claim that it forms a subfamily of its own which is probably intermediate in position between the other two subfamilies, or from which they have developed. Some smaller groups seem incapable of further development along cytological lines. Such are the genera Panicum and Andropogon of Hackel. In such a case all that cytology can do is indicate the existence of a more or less close relationship between its members. An examination of such groups confirms the important fact that was brought out in the introduction, viz., a karyotype cannot be exclusively applied to any particular systematic unit. This brings us to a recognition of the fact that cytology can no more be used as the sole basis of classification than can any other single form of anatomical or morphological study. It can, however, when properly used and correctly interpreted, form a very powerful tool in the hands of the systematist who will correlate its facts with those derived from other branches of study. As has already been stated, a classification based on one field of investigation alone is not very conclusive, but when two or more unrelated schemes of classification point to the same conclusion, then the correctness of that conclusion is almost a certainty.

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