MODE OF REPRODUCTION AND EVOLUTION IN THE COMPILOSPECIES *DICHANTHIUM INTERMEDIUM* (R. Br.) DE WET ET HARLAN

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PREFACE

Each chapter of this dissertation is written, with minor modifications, in the form and style of the biological journal to which it will be presented for publication. It is believed that this method of presentation will allow for more accurate and comprehensive interpretation of the material.

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CHAPTER I

INTRODUCTION

In order to interpret the evolutionary dynamics of a plant group, an understanding of its breeding system and reproductive physiology is of utmost importance. The overall reproductive potentiality of any group controls the spread of genetic variability, and will reflect its evolutionary trends. Many plant groups display a variable degree of versatility in their reproductive behavior which may be manifested by a side-by-side persistence of alternative pathways of seed production either via agamospermous apomixis, or through normal sexual reproduction. Such facultative apomicts provide excellent materials for the study of evolution as directed by mode of reproduction. Facultatively apomictic plants further present a unique opportunity for a study of mechanisms that control sexual and asexual systems of seed formation. In the ovule of some facultatively apomictic angiosperms two developmental pathways, the formation of a normal sexual embryo sac with a cytologically reduced egg and the formation of a cytologically unreduced aposporous embryo sac, function at the same time. Finally, one prevails over the other in forming the seed. In the sexual embryo sac, embryo development starts after

fertilization of the egg by a male gamete, whereas in the aposporous embryo sac meiosis and usually also fertilization are circumvented, and the cytologically unreduced egg develops parthenogenetically to form the embryo and finally the seed is produced. Occasionally, a cytologically reduced egg may develop parthenogenetically to form a haploid embryo, and the cytologically unreduced egg in an aposporous sac may be fertilized.

In facultative apomicts, factors determining the ultimate success of one system of reproduction over the other remain largely elusive. However, in many plants the method of reproduction has been found to change from place to place and time to time, and to explain this both genetical as well as environmental factors have been implicated (Fryxell, 1957). Likewise a study of the physiology of apomixis has revealed a certain degree of direct or indirect environmental control (Böcher, 1951; Nygren, 1951; Knox and Heslop-Harrison, 1963). The control of reproduction by environment has tremendous evolutionary implications, and a study of such a system by manipulating the external factors in a facultatively apomictic plant is likely to yield valuable information concerning the elusive problems of reproductive morphogenesis. The genes, undoubtedly, confer on the organism the ability to perform certain biochemical steps mediated by the enzymes toward the final fulfillment of a specific metabolic process. Steward (1963), however, has pointed out that the course of metabolism requires

something more than mere genetic control. The extent to which the genetically determined reactions actually occur is influenced by a great range of nutritional and environmental factors that intervene to control, or modulate, the genetically feasible events. Various factors that were found to modify sex expression including apomixis, in flowering plants, are mineral nutrition, light and temperature regimes, mutilation, grafting, age, and chemicals, such as auxins and gibberellins (Nygren, 1951; Heslop-Harrison, 1957a; Zatyko, 1962, 1963; Knox and Heslop-Harrison, 1963).

The genus Dichanthium is ideally suited for a study of variability related to mode of reproduction, reproductive physiology, and environmental control of the mechanisms of sexual and asexual reproduction. The Old World species of Dichanthium are characterized by sexually reproducing diploids and largely facultatively apomictic polyploids (de Wet, Borgaonkar and Richardson, 1963). The apomictic polyploids display two types of embryo sac formation (Celarier and Harlan, 1957; Brown and Emery, 1957; Harlan et al., 1964). The sexual embryo sac develops from the functional chalazal megaspore, and the final structure is comprised of an egg, two synergids, two polar nuclei and a large cluster of antipodal cells. The aposporous embryo sac, which originates directly from the nucellar tissue, is characterized by an unreduced egg, two synergids and only one polar nucleus with the antipodal cells completely lacking. Either the reduced egg in the sexual sac or the

unreduced egg in the aposporous sac may function directly to form the embryo parthenogenetically, or either of them may be fertilized and function sexually. Haploid, maternal tetraploid as well as hexaploid offspring of a self fertilized facultatively apomictic tetraploid individual have been reported (Harlan and de Wet, 1963a). In predominantly apomictic individuals, the development of aposporous sacs take a lead over development of sexual sacs, which may disintegrate prior to maturation and fertilization, with the result that the true sexual reproduction may be masked. Conversely, in a facultatively apomictic but predominantly sexual individual, it is the aposporous sac which degenerates in the early stages of its development. Parthenogenetic development in Dichanthium, however, is closely correlated with pollination, and pseudogamous apospory is the rule in apomictic development.

The overall apomictic mode of reproduction in Dichanthium is inherited as a dominant character over sexuality. Harlan et al. (1964) demonstrated that fully sexual plants, and facultative as well as essentially obligate apomicts can be obtained from a single cross between facultative apomicts. Completely sterile plants have also been reported as a result of such a cross (de Wet and Borgaonkar, 1963).

According to Harlan and de Wet (1963a), sexual reproduction and aposporous apomixis are neither genetical nor operational alternatives. The degree of sexuality in an apomictic individual depends on the interaction of various

mechanisms associated with reproduction. These mechanisms are strongly influenced by the environment. Knox and Heslop-Harrison (1963) demonstrated that the apomictic Dichanthium aristatum responds to day length and the length of application with regard to the percentage of aposporous sacs produced.

The genetics of apomixis is difficult to study. The various processes controlling seed formation in facultative apomicts are probably inherited independently from each other, although the overall apomictic mode of reproduction functions as a dominant character over sexuality in Dichanthium. Genetic studies in apomictic representatives of Dichanthium are further complicated by autosyndetic preferential pairing of chromosomes during microsporogenesis (de Wet, Mehra and Borgaonkar, 1961; Chheda and Harlan, 1962). The progeny of a single cross often differ greatly from each other in chromosomal behavior. Most hybrids between these apomictic segmental allopolyploids are characterized by more or less normal chromosome behavior during microsporogenesis, while in others meiosis is very irregular.

Plants that are comparatively regular in their chromosomal behavior during sporogenesis are the only ones that can produce cytologically balanced gametes, and thus function sexually. This may explain the essentially obligate apomictic behavior of some biotypes. In general, however, completely agamospermous apomicts probably do not occur, since some small degree of sexuality may be present but pass unnoticed

in breeding behavior (Davis and Heywood, 1965). In Dichanthium, the individuals that behave as essentially obligate apomicts are usually pentaploids or hexaploids, and are characterized by numerous univalents and multivalents during microsporogenesis. Some hybrid plants, although comparatively regular in chromosomal behavior during microsporogenesis, are completely sterile; neither sexual reproduction nor agamospermous apomixis can function. These always represent hybrids between distantly related species. Apparently not even apomixis can operate when two essentially alien genomes are brought together. Comparatively regular association of meiotic chromosomes in these hybrids is due to preferential autosyndetic pairing.

The present investigation was designed primarily to study:

1. Patterns of variation in reproductive behavior and morphological features in the complex compilospecies Dichanthium intermedium (R.Br.) de Wet et Harlan under experimental conditions, and to correlate these observations with known climatic variables as evidenced by the extent of geographical distribution of the species.
2. Mechanisms of reproduction in controlled environments, particularly of light regime, to ascertain the effect of day length on the physiology of apomixis.
3. Evolutionary trends in Dichanthium intermedium

based on modes of reproduction, cytological behavior and the selective action of local climates.

CHAPTER II

THE EFFECTS OF ENVIRONMENT ON REPRODUCTION IN FLOWERING PLANTS

Morphologically, a flower is a modified shoot with metamorphosized leaves adapted to the special function of sexual reproduction, and preservation of the species. In many plants, flowering is not merely a product of a series of autonomous processes determined solely by the genetic constitution, but is controlled, to a great extent, by the environmental factors in a specific manner. Onset of the reproductive phase is characterized by the phenomenon of an apical meristem being induced to initiate floral primordia instead of producing leaf primordia. In essence, therefore, reproductive development is a problem of differentiation. The manner in which environment controls the qualitative differentiation in the shoot apex is adequately known in a descriptive sense but the underlying biochemical phenomena are relatively less well understood. Broadly speaking, therefore, flowering may be looked upon as a genetically inherited response involving an interaction of genes and their products with the environment. The two environmental factors which play by far the most significant role in controlling reproductive development

in plants are temperature and day length.

History

Whyte (1960) has given a brief account of the early history of the study of flowering. In 1865, Sachs presented experimental evidence to show that leaves produce in light 'flower-forming' substances which may be accumulated in the storage organs. In 1918, Klebs made a critical analysis of the various external and internal factors concerned in flowering. According to Salisbury (1963), Klebs probably did more relating to the effects of environment upon plant growth and development than anyone else before Garner and Allard. His work with Sempervivum funkii led to the postulation of a balance of carbohydrate and soil nutrients as a causal factor in flowering. He also recognized three stages in flowering: (1) ripeness-to-flower, (2) flower initiation, and (3) bud development and ultimate opening. The Soviet physiologists have based their phasic development theories mainly on Klebs' formulation. Following Klebs' studies, three major aspects of modern research on the physiology of flowering were introduced: reaction to temperature or vernalization, reaction to light or photoperiodism and hormonal regulation of functions in plants. Workers credited with fundamental research in these aspects are Gassner (1918), who studied the effect of temperature during germination on subsequent flowering behavior in spring and winter cereals, Garner

and Allard (1920) who stressed the importance of duration of alternate light and dark periods in controlling flowering, and Went (1928) who isolated plant hormones involved in growth and meristematic activity. Heslop-Harrison (1957a) feels that Tournois, who published his work in 1911, 1912, and 1914 on Humulus japonicus (Japanese hop), has a priority over Garner and Allard in the discovery of photoperiodism.

Commenting on flowering and environment, Whyte (1960) has emphasized that external factors which govern the change from vegetative to a reproductive state are those of aerial environment, primarily temperature and light (presence or absence), always "necessarily supplemented or complemented by the secondary factors." Important interactions exist between several of the controlling factors, such as photosynthetic light and temperature, photoperiod and temperature, and soil moisture and relative humidity of air.

Temperature and Flowering

The temperature of the natural or artificial environment in which a plant is germinated and grown is, in many species, one of the dominant factors in controlling the growth and reproductive development of plants. Temperature may act alone or in association with other environmental factors especially light, and may be effective at low or higher degrees.

With regard to the effect of temperature on flower formation, Zeevaart (1963) distinguishes two aspects, direct or non-inductive effect and indirect or inductive effect (vernalization).

A. Direct Effect of Temperature. Most of the information in this field comes from the classic work of Blaauw and his coworkers with flower bulbs. Salisbury (1963) mentioned that Blaauw began his work in 1918 continuing until his death in 1942, and that he used carefully controlled conditions in his experiments long before environmental chambers and phytotrons were known. It was found that each species has its own optimum temperature for flower formation. In tulip, the optimum is between 17°C and 20°C, whereas in hyacinth it is 25°C. Under extremely low or high temperatures, flower formation never occurs, but shifting such bulbs to the optimum temperature leads to subsequent flowering. Little is known about the mechanism by which temperature induces flower formation in these bulbs. Rodrigues Pereira (1961) has shown that isolated buds of Wedgewood Iris cultivated on agar medium initiate flower buds at 13°C only if leaf primordia, scales or both are present. From

his experiments, he came to the important conclusion that young leaves and scales rather than the growing points may be the sites of temperature perception, and that the substance coming out of the scales is of hormonal rather than nutritional nature. This provides experimental evidence that flower initiation in bulbs may be controlled by a hormone which in its own turn is activated under optimum temperature.

B. Vernalization, the Indirect Effect of Temperature. Vernalization is the treatment, also referred to as the process, which accelerates the onset of ripeness-to-flower. The term was first used by Klebs in 1918 to describe the transition from the juvenile state (inability to form flowers) to the adult state (ability to form flowers) usually by a cold treatment. The main aspects of vernalization, as discussed by Zeevaart (1963) are:

a. The Juvenile Phase. In certain plants both germinating seeds and growing plants can be vernalized while in others seeds are not vernalizable. In such plants the seedlings must reach 'ripeness-to-flower' stage before they

can react to a cold treatment and flower subsequently. This is known as the juvenile phase, and its duration varies from plant-to-plant. The nature of the juvenile phase is not fully understood. Experiments have shown that sugar is necessary for vernalization in winter rye. It was therefore considered possible that the reserve material in the seeds may determine the vernalizability. No correlation, however, has been found between seed size and the possibility of seed vernalization; therefore, it has been suggested that it is the quality rather than the quantity of the reserve material that matters. In some plants it has been further shown that the juvenile phase is not a fixed character, and can be shortened by strong additional light increasing photosynthesis and reserve materials.

b. The Site of Perception. It is widely held that low temperature is perceived by the apical meristem, although cases of leaf vernalization have also been reported. Wellensiek (1961) has found that in Lunaria biennis leaves can be

vernalized without buds. Such leaves regenerate flowering plants, but non-vernalized leaves produce only vegetative plants. It has been further suggested that the meristematic tissue at the base of the petiole perceives low temperature and acts in regeneration in the same manner as an apex does in an intact plant. Some support has also been found for the view that vernalization proceeds only in dividing cells. But Grif (1958) has shown that in winter rye, vernalization proceeds normally at -4°C although mitotic activity stops at -2°C.

c. Maintenance of Vernalized State. The after effect of vernalization is transmitted through cell division. Thus, all tillers of vernalized winter rye will flower although they are formed after the cold treatment.

Research workers have made biochemical investigations looking for any new protein or ribonucleic acid produced during vernalization. Finch and Carr (1956) were unable to find any quantitative differences in nucleic

acids between vernalized and non-vernalized winter rye. However, Hess found that 2-thio-uracil, an inhibitor of RNA metabolism, inhibits flower formation in Streptocarpus. From his later work, Hess (1961) concluded that the inhibitor acts by selectively blocking the production of "reproductive" RNA. According to Zeevaart (1963), no rigorous proof of this conclusion has been provided.

d. A Transmissible Stimulus. The transmissible stimulus has been called "vernalin" so as to distinguish it from the photoperiodically controlled stimulus "florigen." It is considered as a product of low temperature. The relationship between vernalin and florigen is not known, but at least in a physiological sense vernalin must be a precursor of florigen (Zeevaart, 1963).

Thermoperiodism

According to Whyte (1960), in the temperature responses of plants, a distinction should be made between seasonal and daily thermo-periodicity. In many plants it has been found that each morphological or physiological stage has a different temperature requirement,

necessitating passing through a succession of higher and lower temperatures with a cycle of approximately one year's duration.

Sachs showed that growth in many plants takes place mainly at night. According to Went (1957) the temperature of night, the "nyctotemperature" is also of great importance. Daily thermoperiodicity is considered very important for flowering in plants like tomato, potato, chillies, and maize.

In order to find out the effect of different kinds of environment on growth and reproduction in Poa, Hiesey (1953) conducted a number of experiments under controlled environmental conditions. He studied 33 individuals of Poa including contrasting ecologic races of species like Poa ampla, P. arida, P. compressa, P. pratensis, P. scabrella, and interspecific hybrids between these species under different conditions of controlled day and night temperatures. He found that different races of Poa ampla and P. compressa failed to flower when subjected to a warm night temperature of 17°C but the same plants flowered freely at cold nights of 6°C, the day temperature, day-length, and other external factors remaining the same. The responses of the interspecific hybrids could be correlated to the characteristics of their parents.

Thus, it is now known that flowering is induced or promoted by low temperature in a great many species of plants, and as might be expected, there are many

manifestations of this response. According to Salisbury (1963), the main problem in the study of the effects of temperature on flowering is the one concerning a better understanding of the physiological and biochemical mechanisms which control these processes.

Photoperiodism and Photoreactions

The response of plants to the relative length of day and night has been called photoperiodism. Flowering, as controlled by day length is an inductive process, and exposure to a minimum number of photoperiods results in subsequent flowering even if the plant is returned to a day length which of itself does not cause flowering. Certain plants have a dual day length requirement for flowering. Such plants stay vegetative if grown continuously under long- or short-day conditions. They flower, however, if exposed to long days followed by short days. Such plants are called long-short-day plants. Their counterparts, short-long-day plants are also known. Photoperiodically sensitive plants do not respond primarily to the length of the daily light period, but respond rather to the length of the dark period. Long nights induce flowering in short-day plants, and inhibit flowering in long-day plants.

Natural Light Intensities and Flowering

According to Salisbury (1963), the response of living

organisms to different light intensities is a fascinating subject for study. The response in photoperiodism will occur under very low intensities. The increasing intensity causes an increased response up to a certain point, and then the response stays the same even though intensity increases. The level of intensity beyond which no change in response occurs is called the saturation intensity. "In flowering we are concerned with the light absorbing reaction which informs the plant by adjusting its metabolism as to whether it is day or night."

Twilight and the Flowering Response to Day-Length

In nature the change from light to dark or back to light is gradual, and there is a definite flowering response to twilight. It has been found that plants respond to light or its absence through a reversible pigment system called phytochrome. Salisbury (1963) described in detail the response of phytochrome to illumination. When the pigment system is illuminated with natural light, it is driven in one direction; at the same time metabolic processes are tending to convert it back to its first condition.

> In a given interval of time, illumination with a given intensity will produce a certain amount of F-phytochrome. In the same interval of time another amount of F-phytochrome will be reconverted to R-phytochrome by metabolic processes. Thus, the amount of F-phytochrome present at any time will reflect the rates of the conversions brought about in one direction by light and in the other direction by metabolism. When the amount of F-phytochrome is great the plant

> 'knows' it is in light; when the amount of F-phytochrome is small the plant 'knows' it is in the dark.

In the evening, then, as light intensity decreases, a point will be reached at which metabolic removal of F-phtyochrome begins to exceed its production by light, and dark phase of flowering begins. Two factors will determine the time of the beginning of the dark phase: first, the quantity of F-phytochrome as a function of light and metabolism, and second, the sensitivity of the flowering process to F-phytochrome. Plants might well differ in this sensitivity. In the morning, similarly, the situation is exactly reversed.

According to Salisbury (1963), a practical experimental approach has been to transfer plants to complete darkness at various times during evening twilight, and, thus, to learn how dim the light intensity must be before plants left in twilight flower as much as plants placed in total darkness. Takimoto and Ikeda (1960) performed a number of experiments of this type in Japan. They found that Japanese morning glory plants placed in the twilight with light intensity of 10 to 20 ft-c flowered as well as those placed in complete darkness. In the morning, however, plants were inhibited in their flowering if they were moved from darkness to twilight any time after the light intensity had reached 0.1 ft-c. They concluded from this that the biological night for the Japanese morning glory begins in the evening at about the start of astronomical

sunset and ends in the morning at the time of the beginning of civil twilight. Studies with other plants have indicated that sensitivity in the morning or the evening varies considerably from species-to-species.

The effect of clouds during twilight could be quite complicated. This problem has yet to be fully investigated, but it should be fairly apparent that clouds during sunrise or sunset could, indeed, influence flowering to a certain extent, and this might readily account for the observation that many plants sensitive to photoperiod do not always flower exactly at the same time each year (Salisbury, 1963).

Floral Stimulus

The leaves are the organs which perceive the day length, whereas the floral primordia are differentiated by the apical meristems. The initial perception and final expression of the photoperiodic stimulus are separated by the petiole and a small portion of stem. Therefore, a signal must move from leaf to bud. This signal has been called the floral stimulus, flower hormone or florigen.

Studies on changes in metabolism during floral induction have remained fruitless. According to Zeevaart (1962), numerous metabolic differences between flowering and vegetative plants have been found, but these seem to be "merely symptoms of flowering rather than its primary cause." Another approach has been the application of

antimetabolites and metabolic inhibitors, in the hope that one might find specific inhibitor of hormone synthesis. Such studies have been mainly carried out with the short-day plant, Xanthium.

Partial Reactions in Short-Day Plants

Xanthium requires one dark period longer than 8½ hours for floral induction. Low temperature during the dark period prevents flowering. Zeevaart (1962) has described the phytochrome reaction during floral induction in a different way. During the dark period the plant measures time. The first event during darkness is the conversion of phytochrome P_{730} to red absorbing form P_{660}, thus

$$P_{660} \underset{\text{Far red (730 m}\mu\text{)}}{\overset{\text{Red (660 m}\mu\text{)}}{\rightleftharpoons}} P_{730}$$

Results of various experiments indicate that this conversion probably does not take more than 2 to 3 hours, whereas the critical dark period for flowering is 8½ hours. Thus, there still remain 6 to 7 hours for measuring time. It has been suggested that some special preparatory process goes on during this period to permit the processes leading toward flowering to proceed. Brief irradiation with red light after 8 hours of darkness converting P_{660} to P_{730} still completely inhibits flowering. However, if the flash of red light is immediately followed

by one of far-red irradiation, switching P_{730} back to P_{660}, flowering proceeds normally. This strongly suggests that even after 8 hours in darkness no reaction beyond pigment conversion has taken place. Zeevaart (1962) found that application of cobaltous ion extended the critical period by 2 hours. The effect could be reversed by application of cysteine or glutathione.

As soon as the time-measuring mechanism has registered a period equal to the critical dark period, synthesis of the floral stimulus appears to start. The flowering response increases with increasing length of the dark period up to a length of about 15 hours. Interruption by red light during the second half of the dark period does not nullify the effect of the preceding dark period, but presumably, merely stops hormone synthesis by conversion of P_{660} to P_{730}.

Bonner et al., (1963) found that the compound called SKF 7997, Tris (2-diethylaminoethyl) phosphate, fully suppresses flowering in Xanthium and Pharbitis if applied to the leaves shortly before the inductive night. This compound is also known to suppress cholestrol biosynthesis in animals (Holmes and DiTullio, 1962). It was found that flowering was not suppressed when the compound was applied to the leaves after the dark period, or to the buds. This suggests that a reaction leading to hormone synthesis in the leaf is blocked by SKF 7997. In animal tissue, this compound blocks cholestrol biosynthesis after lanosterol.

This led to the working hypothesis that the hormone might be a steroid or an isoprenoid-like compound. Hendricks (1960) has also speculated that the hormone might be a sterol, solubilized for transport by the formation of a glycoside with a sugar. According to Zeevaart (1963), however, further experiments are required to elucidate by what mechanism SKF 7997 blocks the inductive processes in the leaf.

Analysis of the reactions of the inductive period indicates that the reactions of the earlier part of the night are qualitatively different from those of the later part. This conclusion is supported by results obtained by exposing short-day plants to a temperature of 35°C during different parts of the dark period. In all species high temperature is inhibitory only when applied towards the end of the dark period. Results of detailed experiments under controlled conditions in Xanthium have shown that most of the stimulus is produced between the 9th and 16th hours of darkness (Searle, 1961). Once produced, the stimulus is stable under both strong and weak light, and in darkness.

According to Hendricks and Borthwick (1963), phytochrome is a bright blue protein having two interconvertible forms with absorption maxima in the red part of the spectrum at 660 mμ and near the limit of vision at 730 mμ; control of growth probably arises from the action of P_{730} as an enzyme. While discussing the role of phytochrome in

the relation of plants to the environment, these workers have further mentioned that phytochrome has four distinct ways of linking a plant to the environment. First, it changes with light quality independently of the intensity above low values. Second, it reverts in darkness from P_{730} to P_{660}, and, thereby, determines photoperiodism. Third, the substrates upon which it acts, and the products it forms depend upon photosynthetic and reserve metabolic activity. Fourth, the rates of the crucial reactions in which P_{730} is involved, including its own dark transformation, are temperature dependent.

> Adaptation of a plant to an environment implies some type of interaction between them. The manner of adaptation through phytochrome action can follow the general adaptation for any protein action; either the level of the particular protein is changed or the actions of other pacemakers are altered.

Endogenous Rhythms

The theory of endogenous rhythm was evolved by Bünning (1956) to describe biological processes which alter periodically. He states that, as in pendular movements, biological systems require an impulse to set them in motion. A single impulse brought about by some external factor, such as a transition from darkness to light, or from low to high temperature, may evoke an endogenous rhythm which was previously unrecognizable. In discussing the relation between endogenous rhythms and photoperiodism, Bünning states that it has become clear

that two phases, each of 12 hours, alternate during the course of the endogenous diurnal rhythm. These phases differ in a quantitative manner with respect to certain partial processes, and in a qualitative manner to certain others. Thus, during these phases, plants respond to external stimuli quantitatively, or, even qualitatively with different reactions. Primarily, plants react differently to light and darkness. For normal development, a light/dark rhythm must be present, and this should be in the period of the endogenous rhythm, i.e., in a 24-hour cycle.

Bünning's endogenous rhythm theory has found support with a number of workers, including Salisbury (1963) who states - "one is almost compelled to assume that the several reactions in the photoperiodic responses are nothing but phases of the endogenous diurnal rhythm." According to Bünning (1956), the endogenous diurnal rhythm must also be regarded as a cause for the diurnal variation of sensitivity of plants to temperature characteristics of thermoperiodism.

Growth Substances, Gibberellins and Flowering

Auxin appears to control reproduction through a strong influence on developmental activities taking place in the flower. Evidences have been presented to demonstrate that major structural and functional alterations may be induced in intact flowers by externally applied

auxins. Heslop-Harrison (1957b) reported that auxin stimulates development of the orchid embryo sac. He found that, in nature, auxin is introduced to the orchid ovary by pollination, and this triggers the entire development of the embryo sac. Until the flower is pollinated or supplied with an external source of auxin, the orchid embryo sac does not develop beyond the single-cell stage. In a different experiment Heslop-Harrison (1959) described the effect of treatment with 2, 3, 5-triiodo-benzoic acid (TIBA) in Cannabis sativa. He found that in dioecious hemp, externally applied auxins induce the production of female or inter-sexual flowers.

From his experiments, Heslop-Harrison has concluded that since floral morphogenesis can be influenced by applied auxins, it would be expected that environmental factors which alter auxin metabolism should influence the structure of flowers. This will, in turn, also affect the mode of reproduction.

The effect of growth substances on flower induction and sex determination has been well documented in two symposia held in London (Journ. Linn. Soc. Lond. 56: 153-302 (1959) and Proc. Linn. Soc. Lond. 172: 90-127 (1961).

Gibberellins are the first compounds with which one can induce flowering in many long-day as well as cold-requiring plants grown under strictly non-inductive conditions. In some cases negative results have also been obtained. In certain short-day plants, application of

gibberellin promotes but does not induce flowering.

Anton Lang and his co-workers at California Institute of Technology made valuable contributions to the understanding of the role of gibberellins in flower formation. From their extensive investigations on gibberellins and flowering Lang (1957), and Lang and Reinhard (1961) made the following conclusions:

A. Flowering of many cold requiring plants with a rosette form is promoted in the absence of cold treatment by application of gibberellins, showing that gibberellins will substitute for a cold requirement. If the plant also has a long-day requirement, effects of gibberellin are best observed when plants are treated with the chemical under long-day conditions. In a few instances such plants have been made to flower with gibberellins in warm, short-day conditions, but often this results only in stem elongation, and no flowering.

B. Long-day plants without a cold requirement may often be made to flower under short days by treatment with gibberellins. In a long-short-day species of Bryophyllum, application of gibberellin under short-days promptly resulted in flowering, but application under long days did not. Thus, gibberellins can substitute for the long-day requirement.

C. Applied gibberellins do not cause flowering of short-day plants under long-day conditions. There are reports of promotion in flower development by gibberellins in short-day plants when the plants have been induced to flower by short-day treatment, but gibberellins by themselves do not seem capable of substituting for short days.

D. There are a few cases where applied gibberellins do not cause flowering of cold- or long-day requiring plants. This is especially true of such plants that have a caulescent growth habit with an elongate stem instead of only a rosette of leaves.

A more popular view on the effect of gibberellin on flower formation is that it may be indirect, acting primarily via stem elongation (Lang, 1957; Zeevaart, 1963).

The name "florigen" has been proposed for the flower hormone of photoperiodic plants, and "vernalin" for that of cold-requiring plants. According to Chailakhian (1961), the flowering hormone "florigen" consists of two substances: anthesin and gibberellin. Anthesin would limit flowering in short-day plants, and gibberellin in long-day plants. But this idea is not supported by conclusive evidence. For example, grafting of a vegetative short-day plant to a vegetative long-day plant should result in flowering, since each partner should supply the

complementary factor to the other. Such grafts have, however, consistently yielded negative results (Zeevaart, 1962).

Floral Initiation in Total Darkness

Tashima and Imamura (1953) have shown that plants grown on nutrient media form flowers even in total darkness. But, if the cultures are exposed to light each species shows its usual response. It has been found in green plants that have accumulated much reserve material are also able to initiate flower promordia when kept in darkness for a long period. This has led to the belief that floral induction is autonomous, but sensitive to light. Once the light inhibition is imposed, this can be removed only by certain ratios of light and darkness.

Floral Differentiation

The subject of floral differentiation in relation to gene action and environment has been discussed by Zeevaart (1962).

It is generally accepted that all cells of a plant have the same genetic make-up or genotype. All characteristics are potentially present in each cell, although not all of them are expressed in every cell at the same time. The possibility of a change in genotype directed by environment has been rigorously ruled out. Genetic information remains constant during development. The reaction

of an organism to its environment must then be determined by its genotype. From this it follows that the amount of and kind of genetic information used and expressed during ontogenesis must vary. The property to flower, therefore, becomes expressed only as a result of an interaction between the genes for flowering and environment. When an apical meristem starts floral differentiation, the floral genes must have become operative. Melchers (1961) has pointed out that external factors such as day-length, and low temperature may exert their influence on gene action via a change in metabolism; the hormone produced under these conditions might have the nature of a coenzyme. At the molecular level, each gene has the capacity to produce one kind of enzyme through RNA as an intermediate product. It is likely that the photoperiodic stimulus interacts with the product of gene in the form of a coenzyme, or possibly it activates the floral genes directly.

Gifford and Tepper (1961, 1962), studying floral differentiation in the short-day plant Chenopodium album by histochemical methods have observed a marked decrease in histone content, and an increase in RNA and protein as some of the earliest chemical changes in the apices after photoperiodic induction. Histones appear to regulate gene activity, and the lowering of histone content in induced apices would mean gene activity leading to a subsequent increase in RNA and protein synthesis. Huang and Bonner

(1962) have implicated histones as regulators of genetic activity.

Genetic Control of Environmental Responses

According to Cooper (1963) most climatic responses which have been investigated show continuous variation and prove to be polygenically controlled. This also includes such factors as winter induction and photoperiodic response to flowering. Cooper has further pointed out that polygenic control allows not only a continuous variation in response to climatic factors leading to close local adaptation, but also the production of a similar phenotype by many different combinations of genes. Selection in each environment is phenotypic only, and an adapted population usually consists of many different genotypes, all phenotypically similar. When the population is transferred to a different environment, considerable genetic variation between individuals is often revealed. In Lolium perenne, the Irish commercial variety flowers very uniformly after an autumn sowing in Britain, all plants having received the required winter induction followed by appropriate combinations of photoperiod and temperature for floral development. If the same variety is grown with threshold inductive exposure, or at threshold photoperiods, a wide range of heading and nonheading plants is revealed (Cooper, 1959). This genetic divergence under altered climatic conditions, provides

suitable variation for selection to work upon.

Similarly, Clausen and Hiesey (1958), in their transplant studies on Achillea and Potentilla, have demonstrated that different sets of genes operate under different environments.

Each population, although fairly uniform phenotypically in its natural environment, may be gentically quite diverse. Much genetic variation is carried between individuals, and can often be revealed by growing the population in other environments.

Environment and Reproduction in Grasses

Whyte (1960) has described the effect of environment on the reproductive development of grasses. One of the most important environmental factors controlling reproductive development in grasses is the photoperiod, the exact daylength requirement varying with species and strains. Most grasses from temperate regions of moderate and high latitudes are long-day plants, and they cannot form heads until the hours of day-light exceed a certain critical value. Even if the temperature is adequate for vegetative growth during winter months, no heads can be produced until the required photoperiod is reached in the spring. On the other hand, grasses from lower latitudes, where the optimal seasons for flowering and seed production may coincide with shorter daylengths, are short-day plants with an upper critical period, above which heads

cannot be formed. In South African bluestem, Hyparrhenia hirta, flowering is delayed by long days and by high night temperatures, while several species of Bouteloua from South Arizona show short-day flowering responses.

According to Cooper (1963), in the Mediterranean environment, flowering responses have usually been selected which result in seed production at the beginning of the dry season, soon after the water supply becomes exhausted. In the Mediterranean annual, Lolium rigidum, there is little or no obligatory winter requirement for floral induction, and initiation can occur in the comparatively short photoperiods of the Mediterranean winter followed by flowering in early spring (Cooper, 1959).

Cooper (1963) further pointed out that in Northern Europe summer drought is not usually limiting, and the long days of summer provide the optimum conditions for seed production. Floral initiation and elongation in north temperate varieties of such forage species as Dactylis glomerata and Lolium perenne require a fairly long photoperiod, and local populations often follow a latitudinal cline in this respect.

In cultivated grasses, the optimal season of flowering, and, hence, the response to photoperiod may be modified by agronomic requirements.

Environment and Apomixis in Plants

In a general sense the term apomixis is employed to

include all types of asexual reproduction which substitute or replace the sexual method. Apomictic phenomena have been divided into two main classes: (1) Vegetative reproduction and (2) Agamospermy. Vegetative reproduction includes all cases where structures such as bulbils, tubers, rhizomes, stolons, etc., which are normally accessory means of reproduction take over the whole reproductive function, whereas agamospermy includes all those types of reproduction which result in the formation of seeds and embryos by means of non-sexual processes (Davis and Heywood, 1965).

Some grasses are known to reproduce by means of vegetative proliferation. According to Whyte (1960), it is possible to induce spikelet proliferation by subjecting some of the grasses to photoperiods intermediate in effect between normal flowering and vegetative growth, or by removing the normal long-day plants from long day conditions. This effect has been produced in Cynosurus cristatus, S.48 timothy, and other grasses.

According to Harlan and de Wet (1963a), sexual reproduction and agamospermous apomixis are neither genetical nor operational alternatives. The degree of sexuality in an apomictic individual depends on the interaction of various mechanisms associated with reproduction. These mechanisms are strongly influenced by the environmental factors.

Environmental control of the incidence of apomixis

has been well demonstrated in some facultative apomicts. Environmental factors, such as temperature, and light regime have been implicated for such a control. Age and external application of chemicals have also been reported to induce apomixis in plants.

A control of incidence of apomictic embryo sacs by temperature has been reported by Hjelmqvist and Grazi (1964). They found that when Limonium transwallianum, an apomict, was cultivated under different temperatures, the unreduced embryo sacs were more common at higher temperatures in the glasshouse, whereas under cool conditions or outside, the reduced sacs were almost as frequent as the unreduced ones.

Knox and Heslop-Harrison (1963) have conducted controlled experiments in which an unequivocal effect of the light regime upon relative numbers of reduced (sexual) and unreduced (aposporous) embryo sacs has been demonstrated in a facultatively apomictic tetraploid race of Dichanthium aristatum. This species was found to be a sensitive short-day reactor. Plants were grown in greenhouses or growth chambers with the air temperature regulated above 22°C. Long-day treatment was given to the plants by exposing them to the available natural day-light supplemented with incandescent light at ca. 100 f.c. to give a day length exceeding 16 hours. During short-day treatment, plants were placed in growth chambers and given a 'night' duration of 16 hours. After having grown the

plants for 135 days from germination under long day conditions, they were divided into different groups and given varying short-day treatments for a study of the effect of light regime on reproductive behavior. It was found that there was a significant increase in the percentage of aposporous sacs in plants grown under continuous exposure to short-day (68.5 - 79.0%), as compared to the plants with 40 short days (27.0 - 46.5%) followed by long days. From these results, Knox and Heslop-Harrison concluded that the production of unreduced sacs may be affected in a systematic way in D. aristatum through the agency of light regime in which plants are grown.

Recently, Knox (1967) presented data to show that in experimental populations of Dichanthium aristatum, grown at six stations covering 27 degrees of south latitude, length of day during development of inflorescences correlates with the incidence of apomixis. He found that at the three southerly stations, where the day exceeded 14 hours throughout development of inflorescence, the incidence of apomictic sacs was low: 54.82, 60.69, and 63.08 percent. On the other hand, at the three northern stations, where photoperiods were less than 14 hours, it was high: 92.96, 87.45, and 91.40 percent. Other climatic factors, such as temperature, however, showed no clear association with the degree of apomixis in Dichanthium aristatum.

In the apomictic species of Calamagrostis, Nygren

(1949, 1951) found a correlation between age and incidence of apomixis. In 174 clones that he investigated from different parts of Sweden, pollen was present only in one. And this clone too varied in pollen formation from one year to another. There was also an evident difference in regard to the type of division in various panicles depending on their age. The panicles first developed during vegetative period had pollen mother cells with meiotic division, while later developed ones had mother cells with mitotic chromosomes. A similar behavior was found in megasporogenesis. Thus, the over-all reproductive behavior in this clone, a race of Calamagrostis purpurea showed that early panicles were sexual while later ones were apomictic.

Zatyko (1962, 1963) has reported induction of parthenocarpy and apomixis by externally applied gibberellic acid in the genus Ribes. Emasculated and isolated flowers of red, white, and black currant varieties were sprayed with gibberellic acid, B-indoleacetic acid, or a mixture of the two. In the black currants Amas Black, Seabrook's Black, and Goliath H, gibberellic + B-indoleacetic acid mixture induced, or by inhibiting fruit drop, allowed the development of apomictic seeds.

Conclusion

In recent years, significant advances have been made in elucidating the effects of environment on reproduction

in flowering plants. On surveying some of the data in this field, one is struck by the tremendous diversity of responses among the many species that have been so far studied. It emphasizes the complexity of the reproductive process, the number of genetically controlled steps which must be directly or indirectly involved, and also implies an intricate ecology of flowering. In the physiological field, advance has been rapid. With the use of gibberellins, the flowering response to cold is being increasingly understood. Elucidation on the control of plant processes by phytochrome offers a great insight into photo- and biochemical interaction of a plant with the low intensity light environment. Advances in biochemistry may take us even closer to an understanding of the response to photoperiod and of the metabolic pathways involved in the synthesis of flower hormone. Studies on environmental control of incidence of unreduced embryo sacs in facultative apomicts offer a challenging prospect for the understanding of the metabolic pathway selected by the plant for sexual or asexual development under specific response to the environment. It is reasonable to hope that further studies will provide new insights into the intricate problems of the environmental control of reproduction in flowering plants.

CHAPTER III

A RAPID SQUASH METHOD FOR GRASS EMBRYOLOGY

The aceto-carmine squash technique of ovules provides a rapid and efficient method to study embryo sac development in apomictic grasses. In facultative apomicts where sexual and asexual embryo sacs develop simultaneously, and ultimately one dominates over the other, it is essential to study each ovule in a spike individually for a full understanding of the mechanism involved in the embryo sac development and embryogeny. An aceto-carmine squash technique for mature embryo sacs which involves maceration and squashing of many ovules simultaneously was described by Bradley (1948). This technique, however, is good only for plants with many small ovules in a single ovary, more commonly found in dicot than in monocot families, and cannot be used with success where each floret has a single ovule and a serial scoring is required. A modified Bradley squash technique used by Brooks (1958) for embryological investigations in grasses has the same drawback. Maxwell and Valentine (1966), in their embryological studies in Primula, found that using Bradley's aceto-carmine squash technique, it was difficult to obtain, with consistency, a high yield of embryo sacs from a single ovary. When they treated the ovaries for 12 to 24 hours in

a mixture of one gram of Michrome pectinase ground up in 10 ml of water and acidified with 1 ml of NHCl, the proportion of successful embryo sac dissection was increased to 60-70 per cent.

For embryological studies in the present investigation a very simple aceto-carmine squash technique for ovules was developed, and employed with success in the Andropogoneae genus Dichanthium.

Materials and Methods

Spikes at different stages of development were fixed in freshly prepared Carnoy's fluid; six parts 95 per cent ethanol, three parts glacial acetic acid and one part chloroform. Fixatives with other combinations of these chemicals were also tried but they caused inferior staining of the embryo sac nuclei.

After approximately one hour of fixation 25 drops of a saturated aqueous solution of ferric chloride were added for every 50 ml of fixative. The ovules were ready for squashing after 48 hours of fixation in this mixture. Inflorescences could be stored in this fluid for several months without any evident deterioration.

The fixed inflorescence was placed in a petri dish and each floret studied beginning with the youngest raceme. With the help of a pair of fine needles the pistil was removed and placed on the slide in a drop of aceto-carmine. The styles were cut off at their bases and the ovary was tapped

gently with the needle until the ovule popped out. With a little practice it was easy to take out the ovule without the help of a dissecting microscope. While viewing the slide on the microscope under low power, the top of the cover glass was pressed carefully with the needle to spread the ovule. Gentle heating of the slide helped in further separating the cells. With the desired pressing and tapping of the cover glass, the intact embryo sac was separated from the ovule.

Temporary preparations could be preserved for four to five days by sealing the cover glass with a mixture of gum mastic and paraffin. Permanent preparations could also be made by any of the usual procedures.

Results and Discussion

In embryological studies sectioning of paraffin-embedded material is most frequently employed. The sections are stained and studied serially for the interpretation of embryological processes. This method is, undoubtedly, of great value in studying the ontogeny of integuments and nucellus, but presents several difficulties in the interpretation of embryo sac development (Bradley, 1948; Maxwell and Valentine, 1966). The advantages of the aceto-carmine squash technique over paraffin sectioning have been emphasized by Bradley (1948), the prominent ones being, great reduction in time and labor involved, and absence of the uncertainty of interpretation encountered in studies of

paraffin sections when the picture of the entire embryo sac has to be built up from examinations of several serial sections. The use of the aceto-carmine squash technique in the embryological investigations of facultative apomicts is especially suitable since it facilitates tracing the pathway of both sexual and asexual embryo sac development. Knox and Heslop-Harrison (1963) have shown in a tetraploid race of Dichanthium aristatum (Poir) C. E. Hubbard, a facultative apomict, that different day-length conditions have a significant effect on the incidence of sexuality and apomixis. For an investigation of this type, where it is necessary to ascertain the variation from ovule to ovule in the inflorescence serially, the aceto-carmine squash technique is much more convenient and reliable than paraffin sectioning, particularly when the plant has small ovules.

Bradley's aceto-carmine squash technique which involves maceration and squashing of several ovules at one time is not suited for this type of study. Since no difficulty was encountered in the separation of embryo sacs, the suggestion of Maxwell and Valentine (1966) for the use of pectinase was considered unnecessary and was not employed in the present investigations.

The technique outlined above makes the embryo sac squash as simple as anther squashing. The fact that ferric chloride is used, in this method, directly with the fixative makes it much more convenient as compared to other compounds of iron, such as iron alum or iron acetate. Ferric chloride,

as a mordant, provides excellent nuclear differentiation and helps to keep the embryo sac intact. Using this technique it has been possible to study and trace various stages in the development of embryo sacs, sexual as well as asexual, and their ultimate fate. Early stages of embryogeny have also been studied.

Although this technique has been employed only in the generic complex Dichanthium, which according to de Wet and Harlan (1966), includes the classically recognized genera Bothriochloa O. Kuntze, Capillipedium Stapf and Dichanthium Willemet, it is being extended to other members of Andropogoneae. It is expected that this technique will prove to be a rapid method for embryological studies in other plants also, especially grasses with small ovules.

CHAPTER IV

REPRODUCTION IN *DICHANTHIUM INTERMEDIUM*

The genus *Dichanthium* Willemet is recognized here in the sense of de Wet and Harlan (1966) to include *Bothriochloa* O. Kuntze and *Capillipedium* Stapf. Belonging to this genus is the morphologically extremely variable compilospecies *D. intermedium* (R. Br.) de Wet et Harlan. It has a wide geographical distribution extending all along the tropical and subtropical regions of the Old World, and is known to have absorbed germplasm from the related species *D. annulatum*, *D. parviflorum*, and *D. ischaemum* (Harlan and de Wet, 1963b; de Wet and Harlan, 1966). The generic complex *Dichanthium*, as a whole, consists of diploid sexual races and polyploids that vary from almost completely sexual to essentially obligate apomicts (Celarier and Harlan, 1957; Harlan et al., 1964). Such a diverse mode of reproduction must have played an important role in the origin of the compilospecies by allowing it a wide range of hybridizational limit, thus, a knowledge of the various mechanisms of reproduction involved is of utmost importance in fully interpreting its evolutionary dynamics.

This paper outlines megagametophyte development and early embryogeny in a facultatively apomictic biotype of

Dichanthium intermedium, and two of its progeny developed as a result of chance operation of sexuality.

Materials and Methods

Three plants were selected for this study. The plant designated as 5450 is a tetraploid ($2n = 40$) facultatively apomictic biotype of D. intermedium collected at Delhi, India; X750 is an artificial tetraploid hybrid obtained from a cross between two natural Indian biotypes of this species, and X750-X-5450, consequently, is a backcross offspring.

For embryological studies, the aceto-carmine squash technique (Saran and de Wet, 1966) was employed. The technique is quite suitable for other grasses as well, and is described here in some detail.

Inflorescences at different stages of development were fixed in a modified, freshly prepared, Carnoy's fluid (six parts 95 per cent ethyl alcohol, three parts glacial acetic acid and one part chloroform). Any time after an hour of fixation, 25 drops of a saturated aqueous solution of ferric chloride were added for every 50 ml of the fixative. The ovules were ready for squashing about 48 hours after the addition of ferric chloride. Inflorescences could be stored in this mixture in refrigerator for several months without any evident deterioration. The fixed inflorescence was placed in a petri dish containing the fixative, and, beginning with the youngest raceme, each floret was studied serially. With the help of a pair of fine needles, the pistil was

taken out and placed on the slide in a drop of aceto-carmine. The styles were cut off at their bases, and the ovary was tapped gently with the needle until the ovule popped out. With a little practice it was a simple operation to take the ovule out and time was saved since the dissection of the ovule under dissecting microscope was eliminated. While viewing the slide on the microscope under low power, the top of the cover glass was pressed carefully. Gentle heating of the slide further helped in better spreading apart of the cells. With the desired pressing and tapping of the cover glass, the intact embryo sac was separated and studied. For each plant 1000 ovules were examined serially.

Photomicrographs were obtained from temporary preparations, all at a magnification of X450. Slides were also made permanent using the tertiary butyl alcohol dehydration technique (Celarier, 1956).

Results

The overall embryological and reproductive behavior of the plants under study have been summarized in Table I.

Dichanthium intermedium (5450)

In this plant, each ovule had an average number of four embryo sacs with a maximum of seven in some cases. Two systems of reproduction, sexual and aposporous apomixis, were found to be functioning side-by-side in many ovules, thus, confirming the facultatively apomictic nature of the

TABLE I

EMBRYOLOGICAL AND REPRODUCTIVE BEHAVIOR OF DICHANTHIUM INTERMEDIUM

No.	No. of ovules examined	No. of embryo sacs in each ovule							Total No. of embryo sacs	4-nucleate		8-nucleate		Reproductive behavior
		1	2	3	4	5	6	7		No.	%	No.	%	
5450	1000	0	67	84	683	105	46	15	4024	3814	94.79%	210	5.21%	Fac. Apomict
X750	1000	784	216	0	0	0	0	0	1216	216	17.76%*	1000	82.24%	Essentially Sexual
X750 X 5450	1000	1000	0	0	0	0	0	0	1000	0	0	1000	100%	Sexual

*These aposporous sacs degenerated at or before the 2-nucleate stage

plant.

The sexual system was characterized by the development of a monosporic, 8-nucleate Polygonum type (Maheshwari, 1950) of embryo sac, directly below the micropyle from the functional chalazal megaspore. Following three mitotic divisions in the megaspore, the typical 8-nucleate structure was obtained with three micropylar, two polar and three chalazal nuclei (Plate I). The three nuclei at the micropylar end gave rise to the egg and two synergids. Several sacs were observed with two synergids together on one side of the micropylar end and the egg on the other, while in others the egg was in center flanked by two synergids. The 8-nucleate stage was rather ephemeral in nature, because the three chalazal nuclei were soon replaced by a massive cluster of antipodal cells as a result of secondary mitotic divisions followed by cytokinesis (Plate I). An interesting pore like opening was consistently noted towards the micropylar end at all stages of development in the sexual sac. This was probably a weak spot which looked like a distinct opening in squash preparations.

Except for the indication of a weak spot, and the massive cluster of antipodal cells, consisting sometimes of 60 cells, the development of the sexual embryo sac was found to be typical of that described for other grasses. Some of the antipodal cells had two to four nuclei of different sizes probably due to incomplete formation of the cell walls.

The asexual system of seed production was characterized

PLATE I

Stages Showing Development of the Sexual Embryo Sac in *Dichanthium intermedium*. All figures ca. X450.

Legend:

Figure 1. 1-Nucleate Embryo Sac of *D. intermedium* (5450)

Figure 2. 2-Nucleate Embryo Sac of *D. intermedium* (5450)

Figure 3. 4-Nucleate Embryo Sac of *D. intermedium* (5450)

Figure 4. 8-Nucleate Embryo Sac of *D. intermedium* (5450) Showing the Egg, Two Synergids, Two Polar Nuclei and Three Antipodsls

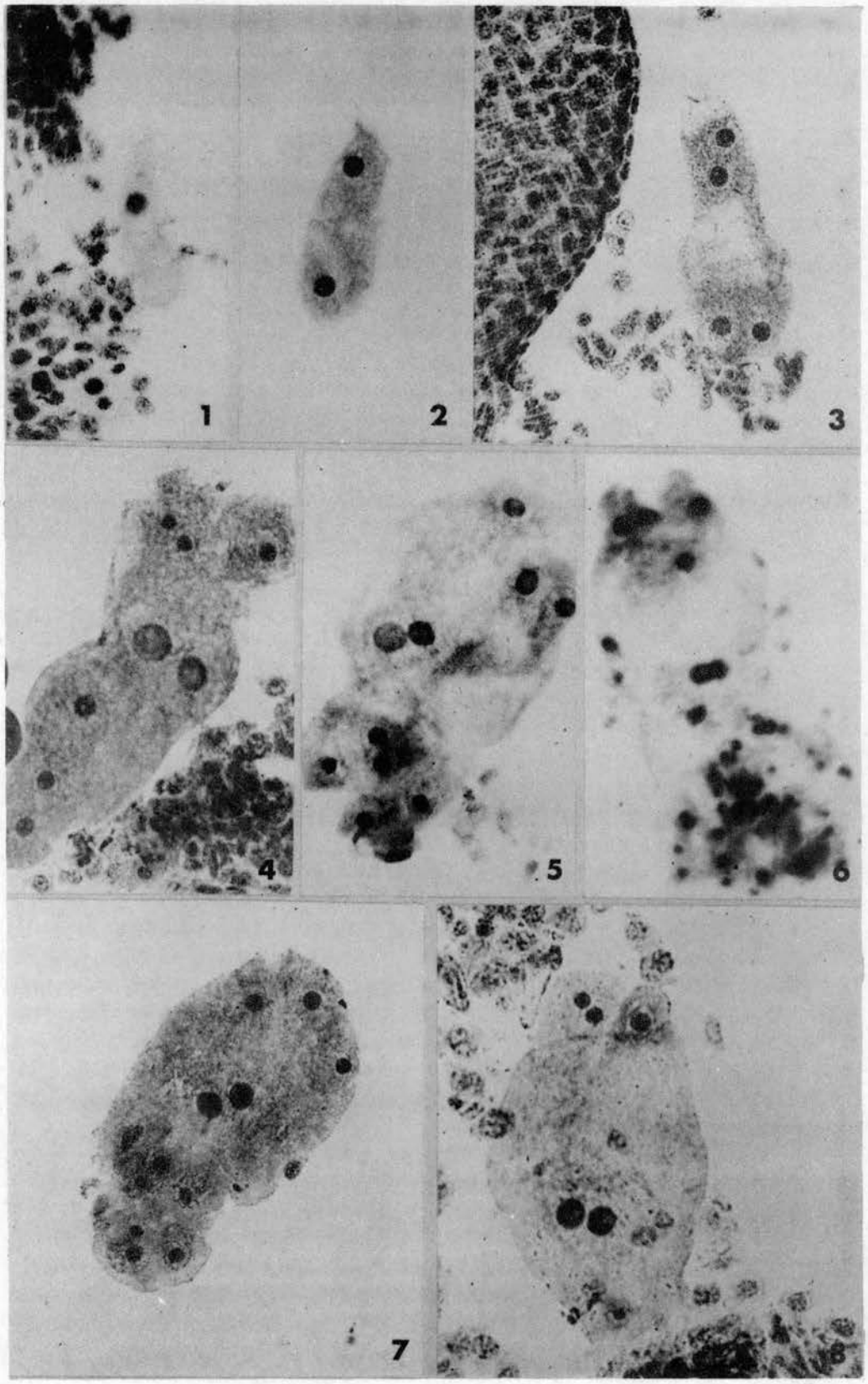
Figure 5. A Mature Embryo Sac of *D. intermedium* (5450) With Several Antipodals

Figure 6. A Mature Embryo Sac of *D. intermedium* (5450) With A Large Cluster of Antipodals

Figure 7. A Mature Embryo Sac of *D. intermedium* (X-750)

Figure 8. A Mature Embryo Sac of *D. intermedium* (X750 X 5450)

PLATE I



by the presence of usually more than one 4-nucleate aposporous sac developed from the nucellar tissue in each ovule (Plate II). The stages in the development of the aposporous sacs were similar to that reported in other apomictic genera, such as Pennisetum (Warmke, 1954; Snyder, Hernandez, and Warmke, 1955), and members of Panicoideae (Brown and Emery, 1957, 1958; Brooks, 1958; Knox and Heslop-Harrison, 1963). The uninucleate initials for the aposporous sacs developed directly from the individual cells in the nucellar tissue. The nucleus in the initial divided mitotically to form a 2-nucleate sac. Unlike the sexual embryo sac, the two nuclei in the developing aposporous sacs were located at the same pole, and this was probably caused by a prior vacuolation in the aposporous sac as was reported by Brown and Emery (1957) in Bothriochloa ischaemum. Following second mitosis, the aposporous sacs matured into 4-nucleate structures, each with two synergids flanking an egg, and a single polar nucleus (Plate II). No opening was noticed in the aposporous sacs, and this was used as a diagnostic feature along with the presence of only one polar nucleus and complete absence of antipodal cells to distinguish them from the sexual embryo sac.

In many ovules fully developed 4-nucleate aposporous sacs accompanied a sexual sac that was still in the 2-nucleate stage (Plate II) indicating that the development of aposporous sacs was initiated prior to or simultaneously with the megasporogenesis. In some ovules developing sexual sacs

PLATE II

Stages in the Development of the Aposporous Embryo Sacs in *Dichanthium intermedium* (5450).

Legend:

Figure 9. An Ovule Squash Showing A Mature Aposporous Embryo Sac, Several Aposporous Sac Initials and A 2-Nucleate Sexual Sac (ca. X450)

Figure 10. A Mature 4-Nucleate Aposporous Embryo Sac With an Egg, Two Synergids, and A Single Polar Nucleus (ca. X450)

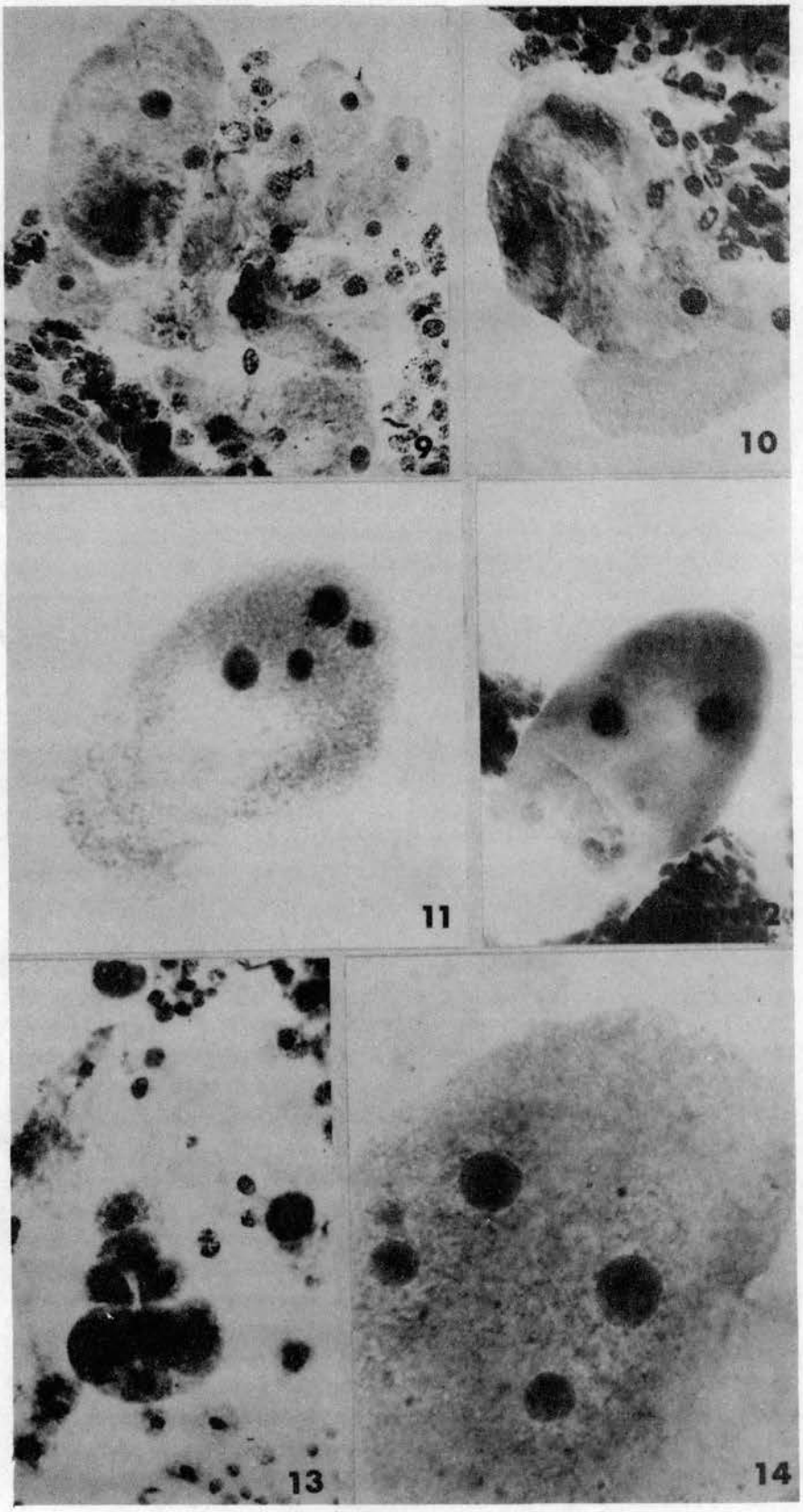
Figure 11. A Mature Aposporous Sac Showing A Large Vacuole (ca. X450)

Figure 12. A Mature Aposporous Sac With the Egg, Polar Nucleus, and Two Disintegrating Synergids (ca. X450)

Figure 13. 8-Nucleate Pro-Embryo With the Polar Nucleus (ca. X450)

Figure 14. A Mature 4-Nucleate Aposporous Sac Showing Fertilization (ca. X1350)

PLATE II



degenerated very soon after initiation, while in others, fully developed sexual, as well as aposporous, sacs were present in a single ovule. The enlargement of a nucellar cell to form an aposporous sac was found to be independent of the sporogenous cell degeneration, and even in ovules with more than two sacs no direct crowding out of the sexual sac by asexual sacs was ever observed. In the aposporous sac, after an early degeneration of synergids, the egg frequently divided precociously prior to anthesis, and embryo development preceded the development of endosperm (Plate II). No correlation was observed between the rate of development of embryo and that of endosperm. In sexual sacs the synergids, as well as the antipodals, disintegrated before the development of the embryo. Sometimes fertilization was observed in the aposporous sac (Plate II).

Out of a total number of 1000 ovules and 4024 embryo sacs examined in this plant, there were only 210 sexual sacs (8-nucleate) while the rest were aposporous including the sexual sacs that did not develop to maturity. Although, compared to the aposporous sacs the sexual sacs formed only about five per cent of the total number, the potentiality of the plant for sexuality was quite good since out of a total of 1000 ovules 210 had a well developed sexual sac.

Dichanthium intermedium (X750)

Pertaining to the breeding behavior, earlier studies had indicated this plant to be almost completely sexual as

well as self-sterile (Harlan, Chheda and Richardson, 1962). However, it was interesting to note in the present study that, in the early stages of development, many ovules had supernumerary embryo sacs indicating a potentiality for aposporous apomixis. The average number of embryo sacs was 1.2 per ovule. In the early stages of development, ovules usually contained two developing sacs. The sexual sac was located directly below the micropyle and was recognized by the orientation of the embryo sac mitoses and final disposition of the nuclei as described above for 5450. The aposporous sac was located away from the micropyle, and the usual criteria were applied to recognize it. The aposporous sac never developed beyond the 2-nucleate stage, and at the time of anthesis only the mature sexual sac was observed in each ovule.

The development of the sexual embryo sac was typical of the species described for 5450 (Plate I). No fertilization or development of embryo was observed. In this plant, out of a total of 1000 ovules and 1216 embryo sacs examined, each ovule had a normally developing sexual embryo sac. In 216 ovules early rudiments of aposporous sac development were found, but the aposporous sac never developed beyond the 2-nucleate stage.

Dichanthium intermedium (X750 X 5450)

This backcross offspring was found to be completely sexual with no trace of agamospermous apomixis. Each ovule

had a normally developing sexual sac (Plate I). Stages of fertilization were also observed and the development of endosperm preceded division in the fertilized egg.

Discussion

The majority of embryological studies in plants are based on sectioning of paraffin-embedded material. The sections are stained and studied serially for the interpretation of embryological features. This method is, undoubtedly, of great value in studying the ontogeny of integuments, nucellus and embryo, but several difficulties are encountered in the interpretation of embryo sac development (Bradley, 1948; Maxwell and Valentine, 1966). The main difficulty lies in the fact that the entire picture of embryo sac development has to be built from a serial examination of several sections, and many times the sections may not be complete enough to permit a reliable interpretation. In a grass inflorescence, especially, it is very difficult to obtain a full picture of embryo sac development in each and every ovule of the entire inflorescence. In apomictic grasses where two developmental pathways of embryo sac development, sexual and aposporous, function simultaneously, a sequential and complete study of every single ovule is of vital importance. For this purpose, an aceto-carmine squash technique for embryological studies has definite advantages over paraffin sectioning (Saran and de Wet, 1966). Using this technique it was possible to analyze and score each

ovule serially for the type and the final product of embryo sac development in the present investigation. An aceto-carmine squash technique for mature embryo sacs described by Bradley (1948), which involves maceration and squashing of several ovules at one time, is suitable for plants with several ovules in an ovary, but it cannot be employed with success in plants where each floret has a single ovule, and each ovule has to be studied serially. The modified Bradley squash technique used by Brooks (1958) for embryological studies in grasses has the same drawback. The technique outlined in this study (Saran and de Wet, 1966) provides a rapid method for embryological studies, and in investigations where thousands of ovules are involved for individual study, this method is highly recommended. The use of pectinase (Maxwell and Valentine, 1966) for easy dissection of embryo sac was found to be unnecessary in D. intermedium.

By using ferric chloride as a mordant with the fixative, it was possible to squash the ovules directly in aceto-carmine and get immediate staining. In the squash preparations the nucleoli were deeply stained and the outlines of nuclei were also delineated. There was a marked increase in the nucleolar volume of the polar nuclei as compared to the nucleoli of synergids and antipodals. The nucleolus of the egg nucleus also showed a slight increase. The presence of large nucleoli in the megagametophyte of corn has been implicated with storage of material to be used during endosperm and embryo development (Diboll and Larson, 1966), and a similar role of

nucleolus enlargement is plausible in D. intermedium.

The degeneration of the sexual sac in facultative apomicts has been attributed in a crowding out and crushing by aposporous sacs, or to a direct competition for nutritive material (Akerberg, 1942; Gustafsson, 1946; Brown and Emery, 1957). However, in D. intermedium (5450) mature sexual and aposporous sacs were often observed in the same ovule, and degeneration of the developing sexual sac always appeared to be independent of nucellar cell enlargement. This was in agreement with the observations by Kiellander (1937) in Poa palustris and Grazi, Unaerus and Akerberg (1961) in Poa pratensis, that the degeneration of megaspores or sexual sacs was not due to an encroachment of the aposporous sacs, and could be initiated at any time during development.

Although in the facultatively apomictic D. intermedium (5450), about 95 per cent of the ovules studied produced mature aposporous sacs, these rarely became fertilized, because the cytologically unreduced egg in the aposporous sac quite frequently started to develop a proembryo before anthesis. This was also reported in subsexual species of Agropyron (Hair, 1956). However, fertilization was observed in some aposporous sacs. As was found in Parthenium by Esau (1946), the embryo development in D. intermedium (5450) preceded endosperm development. However, in fully sexual offspring (X750 X 5450) of this species endosperm development always preceded division of the fertilized egg.

Presence of a weak spot towards the micropyle was a

unique but consistent feature of the sexual sacs which probably facilitated fertilization by providing a path of least resistance to the advancing pollen tube. Such a spot was totally lacking in the aposporous sacs.

The overall mode of reproduction in D. intermedium as revealed by the present study is characterized by a high degree of versatility. Primarily the facultatively apomictic biotypes have played a significant role in the origin and evolution of the compilospecies. The facultative apomict, in its reproductive mechanisms, harbors a dual system of seed production, sexual and aposporous and each system is supported by a set of independent factors. Agamospermous apomixis is favored by numerical superiority of the aposporous sacs, and their ability to form embryos before anthesis and endosperm development. On the other hand, the sexual sac gains advantage in its location directly below the micropyle, and the presence of a weak spot, both favoring fertilization. And, indeed, essentially sexual to fully sexual plants were obtained as a result of a cross involving two facultatively apomictic biotypes of D. intermedium. Moreover, as pointed out by Clausen (1961) for Poa, apomixis facilitated introgression in Dichanthium also because essentially sexual plants must occur commonly in natural populations of D. intermedium. These plants will provide an ideal opportunity for massive introgression and enormous variability. Harlan (1963) indicated such an active hybridization between Bothriochloa ischaemum, and the introgressants of D. annulatum

and B. intermedia (syn. D. intermedium) in areas disturbed by man along the foothills of northern West Pakistan. This area, referred to as a microcenter by Harlan, was characterized by extremely polymorphic populations with plants mostly apomictic but showing a low frequency of sexual reproduction. Hybrids like X750, self-sterile and essentially sexual with the incipient potentiality for apomictic development characterized by the presence of rudiments of aposporous sacs, provide an ideal material permitting distant hybridizations. In fact, D. intermedium (X750) has been used extensively as a female parent for making distant interspecific crosses, which shows how well it can function as a compilospecies (Celarier et al., 1961; de Wet, Borgaonkar and Chheda, 1961; Harlan, Chheda and Richardson, 1962; Harlan and de Wet, 1963b).

The hybrid, D. intermedium (X750) provides further, an ideal material for the study of the effect of environment on the mode of reproduction. Knox and Heslop-Harrison (1963) pointed out for Dichanthium aristatum that day-length had a marked effect on the incidence of apomixis in this facultative apomict. A study of this problem in D. intermedium will help to elucidate its wide distribution and enormous variability.

CHAPTER V

A STRUCTURAL PECULIARITY OBSERVED IN THE SEXUAL EMBRYO SACS OF *DICHANTHIUM INTERMEDIUM*

Facultatively apomictic members of the compilospecies *Dichanthium intermedium* (R. Br.) de Wet et Harlan (Gramineae) are characterized by the development of two types of embryo sacs, sexual and aposporous, in the same ovule. These sacs differ from each other characteristically both in mode of origin and final morphological structure. The sexual embryo sac develops from the cytologically reduced, functional chalazal megaspore, whereas the initial for aposporous sac development is cytologically unreduced and nucellar in origin. In development of the sexual embryo sac, the chalazal megaspore undergoes three successive mitotic divisions to form a normal, 8-nucleate, *Polygonum* type (Maheshwari, 1950) of sac. However, the 8-nucleate stage is short-lived, and the three antipodal nuclei, shortly after their formation, undergo repeated secondary mitotic divisions to form finally a large group of antipodal cells. The aposporous sac, on the other hand, develops from an initial differentiated in the nucellar tissue. The initial nucleus divides twice mitotically to form a 4-nucleate embryo sac which represents

a characteristic product of apospory in most Paniceae and Andropogoneae (Warmke, 1954; Brown and Emery, 1957; Harlan et al., 1958). In embryological studies of facultative apomicts in the Andropogoneae, the main diagnostic criteria utilized in distinguishing the two kinds of embryo sacs are the presence of antipodal cells and two polar nuclei in the sexual sacs, and absence of these features in the aposporous sacs. A comparative study of the orientation of the embryo sac mitoses, and the final arrangement of the nuclei at the 4-nucleate stage in the two types of sacs has also been very useful (Knox and Heslop-Harrison, 1963). This paper reports on an interesting pore-like opening consistently observed towards the micropylar end in the sexual embryo sacs of Dichanthium intermedium. Such an opening was never observed in the aposporous sacs.

Materials and Methods

Plants included in this study formed a part of several hundred collections of Dichanthium intermedium assembled from different parts of Africa, Asia and Australia representing the entire range of geographical distribution of this species. These plants were grown in a uniform nursery as described by Celarier and Harlan (1956). Ten plants were selected for a study of the overall mode of reproduction in this compilospecies. For embryological investigations a rapid method for aceto-carmine squash of ovules (Saran and de Wet, 1966) was used. Photomicrographs were obtained from

temporary preparations. Slides were made permanent using tertiary butyl alcohol dehydration technique (Celarier, 1956).

Results and Discussion

The sexual embryo sac developed directly below the micropyle from the functional chalazal megaspore. The pattern of development of the sexual sac was similar to that worked out for other grasses such as Zea. The only peculiarity observed in the sexual sac was the presence of an opening on the megagametophyte wall towards the micropylar end (Plate III). This was observed at all stages of development of the sexual embryo sac. Since such an opening was completely lacking in the aposporous sacs, this feature was also helpful in delineating the two kinds of sacs. Moreover, due to the development of a large vacuole in the aposporous sac initial, the four nuclei produced as a result of two mitotic divisions remained at one pole of the mature sac (Plate III). The 4-nucleate stage was, therefore, found to be the best for distinguishing the two kinds of sacs.

The opening on the wall of the sexual embryo sac probably represents a weak spot which appears like a distinct opening due to tapping and heating involved in squash preparation of ovule. It is likely that the weak spot develops due to unequal thickening of the megagametophyte wall. Diboll and Larson (1966), in an electron microscopic study, reported that the wall of mature megagametophyte of Zea mays

PLATE III

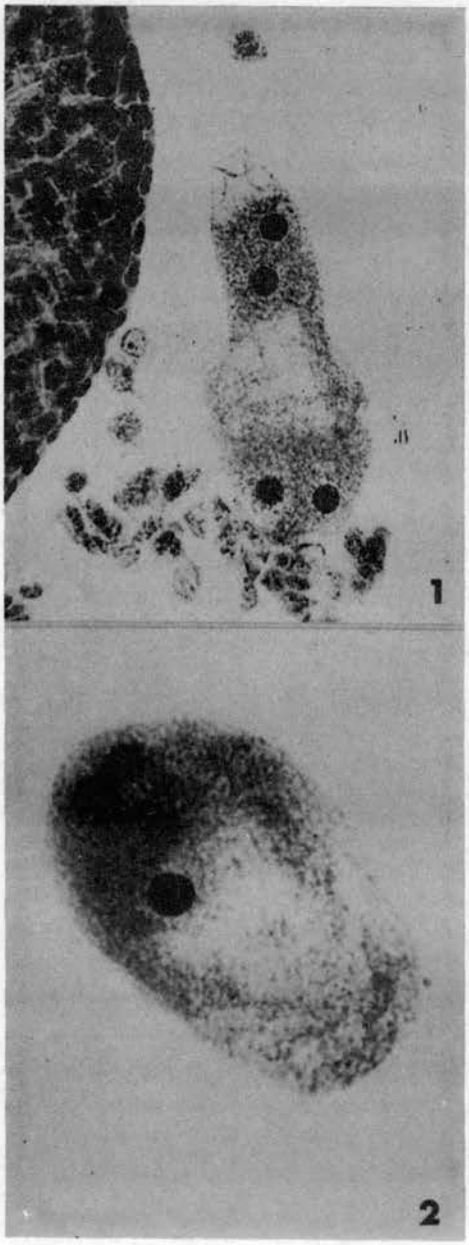
Four-Nucleate Sexual and Aposporous Embryo Sacs of *Dichanthium intermedium* Showing Structural Peculiarities (ca. X450).

Legend:

Figure 1. Sexual Embryo Sac

Figure 2. Aposporous Embryo Sac

PLATE III



was made up of layers of thin lamellae appressed to each other. They further pointed out that the innermost layer of the wall was the original megaspore wall while the outer layers were remnants of nucellus cell walls crushed and stretched by the enlarging megagametophyte. A similar situation can be visualized in the development of the megagametophyte wall in Dichanthium intermedium. However, in this species, a nucellar cap is formed directly over the micropyle, and fits over the nucellus like a hood. The cells of the nucellar cap, lying at the top of the embryo sac, are conspicuously thickened, and as compared to the nucellar tissue, they would impede the progress of stretching megagametophyte wall. It is likely, therefore, that due to the presence of the firm nucellar cap there is an unequal appression of lamellae of crushed cell walls towards the micropylar end leading to the development of a weak spot. On the other hand, such a weak spot will not be encountered in the aposporous sacs since they develop from the nucellar tissue not in immediate contact with the nucellar cap or what was termed as epistase by Van Tiegham (Maheshwari, 1950).

The apparent opening in the sexual embryo sac will facilitate fertilization by providing a path of least resistance to the growing pollen tube. Since it favors sexuality, such a structure will have a definite selective advantage in facultative apomicts. In facultatively apomictic members of the compilospecies Dichanthium intermedium where sexuality and agamospermous apomixis function

side-by-side, independent factors tend to favor the success of one system rather than the other. In the aposporous sac, the cytologically unreduced egg usually develops precociously before anthesis, therefore, chances of fertilization become less. On the other hand, in the sexual embryo sacs, due to their position directly below the micropyle and the presence of an opening or a weak spot on the wall, the chances of fertilization are very much enhanced.

CHAPTER VI

ENVIRONMENTAL CONTROL OF THE MODE OF REPRODUCTION IN *DICHANTHIUM INTERMEDIUM*

In a facultatively apomictic grass species, *Dichanthium aristatum*, the incidence of cytologically unreduced, aposporous and reduced, sexual embryo sacs was demonstrated to be controlled by the photoperiod (Knox and Heslop-Harrison, 1963). Other environmental factors implicated with the control of apomixis, directly or indirectly, in facultative apomicts are temperature in *Limonium transwallianum* (Hjelmqvist and Grazi, 1964), and age in *Calamagrostis purpurea* (Nygren, 1949, 1951). Zatyko (1962) was able to induce apomictic seed production in *Ribes* by spraying emasculated and isolated flowers with a mixture of gibberellic acid and B-indole acetic acid. Such a control of the mode of reproduction in facultative apomicts by environmental, or biochemical means could have a great evolutionary significance (Clausen, 1954; Brooks, 1958; Heslop-Harrison, 1961). The present paper deals with the effect of photoperiod on the mode of reproduction in the facultatively apomictic compilospecies, *Dichanthium intermedium* (R. Br.) de Wet et Harlan.

Materials and Methods

The progeny obtained as a result of artificial crosses involving two biotypes of D. intermedium from India were found to range from predominantly apomictic to essentially sexual individuals in reproductive behavior. One such hybrid, designated as D. intermedium X-750, was selected for this study. Earlier experiments had demonstrated this hybrid to be highly self-incompatible, and essentially sexual (Harlan, Chheda, and Richardson, 1962). A study of the megagametophyte development in this hybrid by aceto-carmine squash technique of ovules revealed rudiments of aposporous sacs, but only the sexual embryo sac ever matured (Saran and de Wet, 1967). This plant could be reproduced vegatatively by means of stem cuttings, ensuring thereby, essentially homogeneous populations to work with.

Plants from stem cuttings were placed in four pots, and grown in the greenhouse under natural photoperiod in September, 1964. In January of 1965, following the technique used by Olmsted (1944) for Bouteloua, plants were clipped to within one inch of the ground, and transferred to a walk-in growth chamber. At the time of clipping, the plants were in vegetative growth, and no flower initiation had taken place. The air temperature in the growth chamber was maintained above 22°C throughout the experiment. For the first treatment, a day-length of 14 hours was maintained. Illumination was provided from fluorescent tubes at 1000 f.c. The first inflorescences appeared approximately 104 days after

clipping the plants. Inflorescences were collected at different stages of development, and fixed in a freshly prepared mixture of six parts 95 per cent ethanol, three parts glacial acetic acid, and one part chloroform for studying embryo sac development. For collecting data on apomictic seed set, inflorescences were simply bagged, since D. intermedium X-750 had been earlier demonstrated to be highly self-incompatible.

For the second treatment, the plants were clipped back again to within one inch of the ground, and maintained at a reduced day-length of 12 hours. As soon as the plants started heading, inflorescences were collected for mega-gametophyte study. Inflorescences were also bagged to see if there was any seed set under the changed photoperiod.

In the present investigation, a serial study of each and every ovule was essential, therefore, a rapid aceto-carmine squash technique for ovules (Saran and de Wet, 1966) was adopted. Sectioning of paraffin-embedded material was considered unsuitable for this study. More than 1000 ovules were examined from six to eight inflorescences obtained under each treatment.

Diagnostic criteria employed for classifying apomictic and sexual sacs have been described earlier (Saran and de Wet, 1967). During early stages of megagametophyte development, when it was not possible to classify sexual and apomictic sacs on the basis of the morphology, presence of supernumerary embryo sacs in an ovule was taken as an

indication for aposporous sac development. In a fully sexual "embryological" race of D. intermedium, only one sexual embryo sac develops in each ovule.

Results

Embryo Sac Development

Under continuous day-length of 14 hours, the average number of embryo sacs per ovule was found to be 1.2. During the early stages of megagametogenesis, two developing embryo sacs were observed. One was the normal, sexual sac, developing from the functional chalazal megaspore, directly below the micropyle, and the other was an aposporous sac originating in the nucellar tissue somewhat away from the micropyle. The aposporous sac degenerated early, and was never observed to grow beyond the 2-nucleate stage. In 784 ovules out of 1000 studied, only the sexual sac was present. In some ovules, an aposporous sac initial was observed when the sexual sac had already fully matured (Plate IV). Finally, only the sexual sac ever matured and functioned.

Under continuous day-length of 12 hours, there was a dramatic change in the incidence of apomictic sacs. The percentage of apomictic sacs went up from 21.6 under 14 hours to 63.4 under 12 hours (Table II). Furthermore, out of 634 apomictic sacs observed in a total of 1000 ovules studied, 434 were in the mature, 4-nucleate stage (Plate V). A sexual sac was, however, observed in each ovule, and in 434 ovules mature sexual as well as aposporous embryo sacs

TABLE II

DATA ON APOMICTIC AND SEXUAL EMBRYO SAC DEVELOPMENT IN D. INTERMEDIUM X-750 UNDER DIFFERENT PHOTOPERIODS

Day-length	No. of ovules examined	No. of embryo sacs in each ovule					Total No. of embryo sacs	Apomictic*		Sexual*		Remarks
		1	2	3	4	5		No.	%	No.	%	
14 hours	1000	784	216	0	0	0	1216	216	21.6%	1000	100%	All apomictic sacs degenerated at the 2-nucleate stage
12 hours	1000	366	634	0	0	0	1634	634	63.4%	1000	100%	In 434 ovule mature, 4-nucleate apomictic sacs observed

*Data based on the number of ovules examined

PLATE IV

Sexual and Aposporous Embryo Sac Development in *Dichanthium intermedium* X-750 Under Different Photoperiods.

Legend:

Figure 1. Ovule Squash Showing A Mature Sexual Embryo Sac and an Aposporous Sac Initial Under A Continuous Day-Length of 14 Hours

Figure 2. Ovule Squash Showing A Mature Sexual and A Mature Aposporous Embryo Sac Under A Continuous Day-Length of 12 Hours

PLATE IV

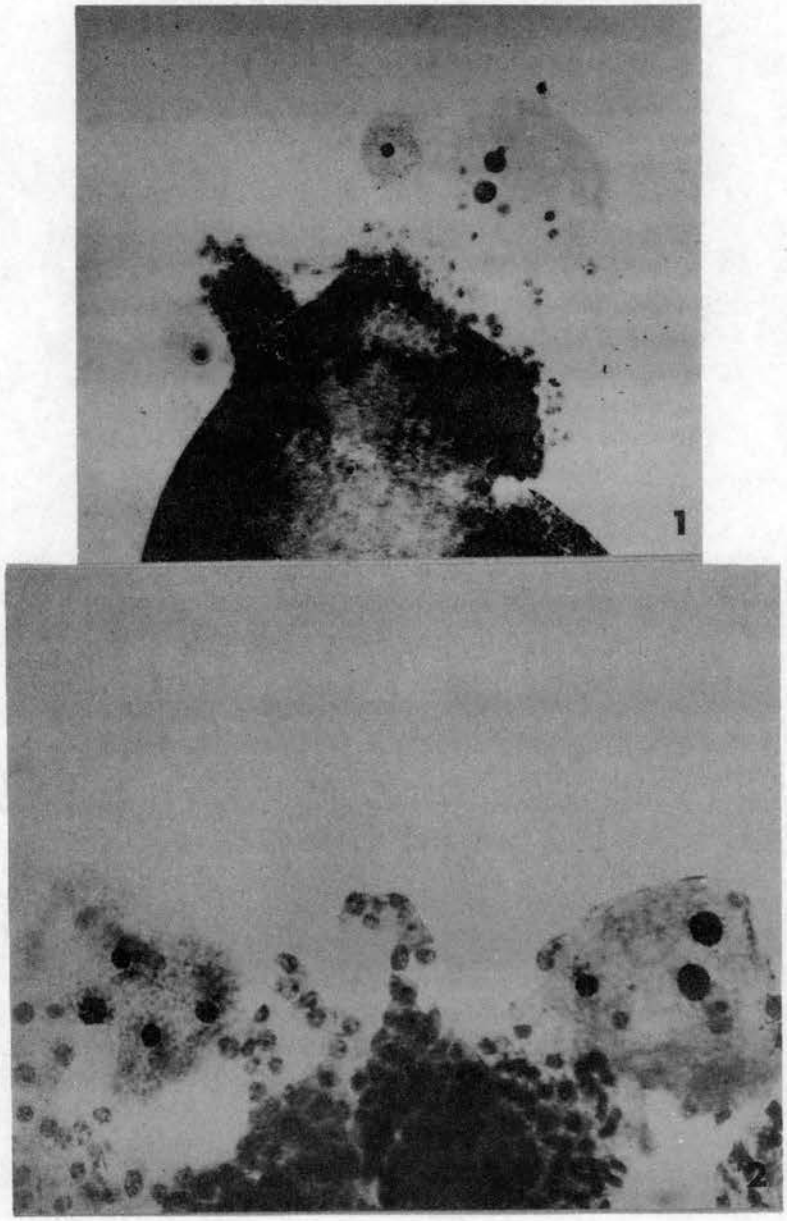
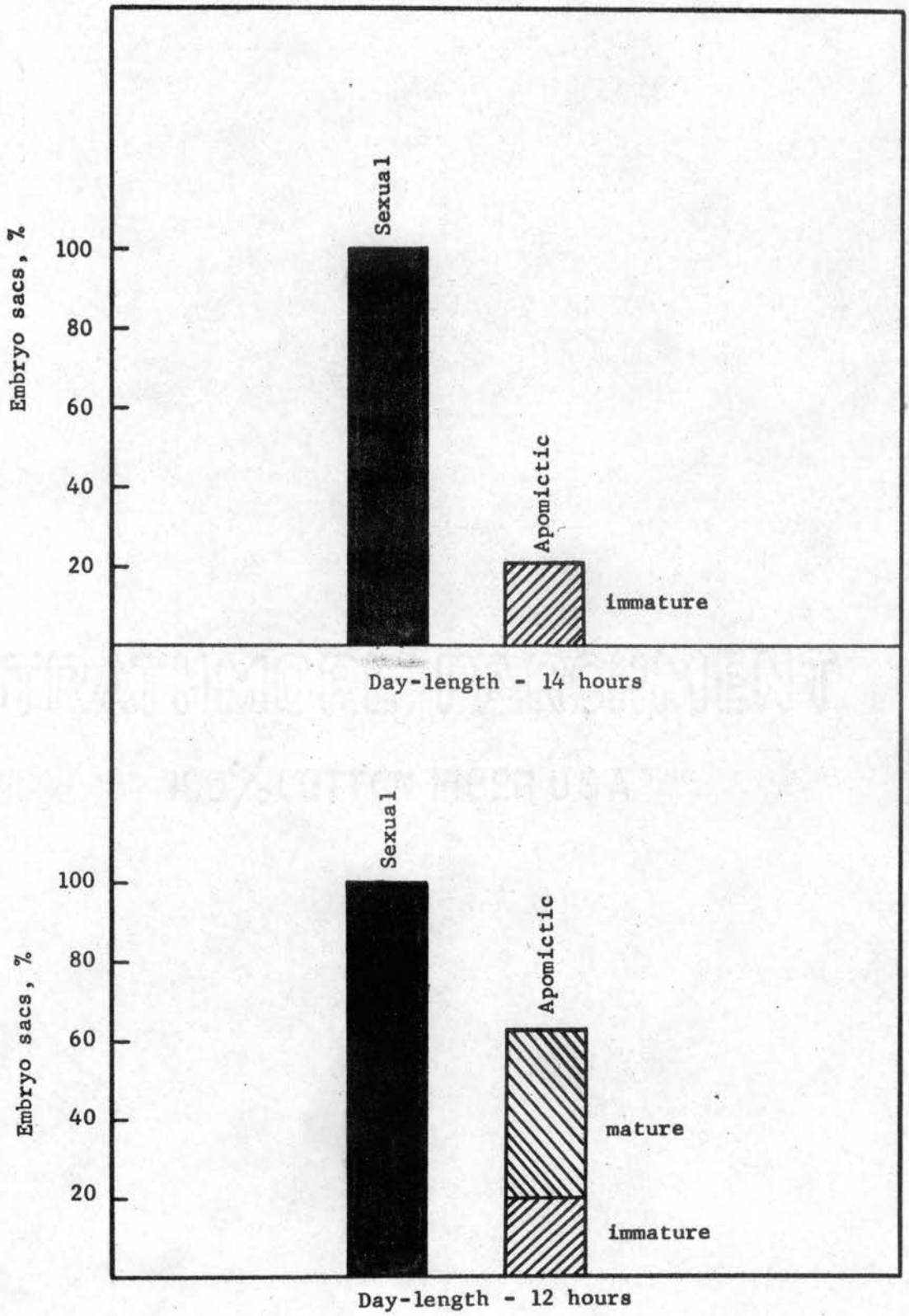


PLATE V

Graph Showing Percentage of Sexual and Aposporous Embryo Sacs Under Day-Lengths of 14 and 12 Hours.

PLATE V


Embryo sacs, %
100
80
60
40
20
Sexual
Apomictic
immature
Day-length - 14 hours
Embryo sacs, %
100
80
60
40
20
Sexual
Apomictic
mature
immature
Day-length - 12 hours

persisted side-by-side (Plate IV). Data on embryo sac development are summarized in Table II.

The data on apomictic embryo sac development with regard to the duration of photoperiod was analyzed statistically by approximating binomial distribution to normal distribution. The lower and upper limits of the distribution of apomictic sacs for both the treatments were calculated by taking two standard deviations. Under 14-hour photoperiod, the distribution of apomictic sacs was calculated to be 216 ± 26.64, whereas for 12-hour photoperiod it was 634 ± 39.4. The results of statistical analysis showed that distributions for the two treatments were separate, therefore, the incidence of apomictic sac formation under changed photoperiod was highly significant.

Data on apomictic seed set under the two different treatments of photoperiod are summarized in Table III. Out of five inflorescences bagged under a day-length of 14 hours, four set no seeds. Only two seeds were obtained from one inflorescence, out of 132 florets. These two seeds could have developed due to chance success of self-fertilization. Under a day-length of 12 hours, however, all the five inflorescences bagged set seeds. The number of seeds set was quite variable from one inflorescence to another. In one inflorescence, the seed set was as high as 26 out of 76 florets.

TABLE III

DATA ON APOMICTIC SEED SET IN D. INTERMEDIUM X-750 UNDER DIFFERENT PHOTOPERIODS

Day-length - 14 hours

Name	No. of florets	No. of seeds set
65-G-2	75	0
65-G-14	72	0
65-G-40	79	0
65-G-43	81	0
65-G-44	132	2

Day-length - 12 hours

Name	No. of florets	No. of seeds set
66-G-26	89	2
66-G-28	76	26
66-G-44	49	1
66-G-45	34	3
66-G-48	56	14

Discussion

A significant approach in elucidating the environmental control of apomictic embryo sac formation in a facultatively apomictic, tetraploid race of Dichanthium aristatum was made by Knox and Heslop-Harrison (1963). They found that under continuous short-day condition (eight hours illumination), the percentage of aposporous sacs went up to 79 per cent, while in plants receiving a minimum photoperiodic induction, and followed by long days (more than 16 hours illumination), a maximum of 47 per cent aposporous sacs were formed. They, however, raised their experimental materials from caryopses rather than vegetative cuttings. Recently, Knox (1967) reported in the same species that, under natural conditions, the mean percentage of apomictic embryo sacs for photoperiods longer than 14 hours was 59.58 ± 2.03, while for photoperiods shorter than 14 hours, 90.84 ± 0.94. No data on actual increase in apomictic seed set was given in either of the studies.

In the present investigation, three main experimental approaches were considered very important, therefore, they were emphasized. (1) Ensuring genetically homogeneous experimental material by using vegetative cuttings, (2) one by one serial, cytological study of each ovule in randomly selected inflorescences obtained under each treatment, and (3) obtaining data on apomictic seed set.

In an experiment where the environmental control on mode of reproduction is under investigation, it is essential

that the experimental materials be genetically homogeneous. As Hiesey and Milner (1965) pointed out,

> ... interpretation of much recent work is rendered difficult by not knowing what part of the observed effects is to be attributed to physiological response of races, and what part to unknown genetic differences among them.

The best solution of this problem is to investigate plant materials that can be propagated vegetatively for the study of physiological responses.

In cytological studies of ovules, sectioning of paraffin-embedded materials is commonly employed. The sections are stained, and studied serially for the interpretation of embryological processes. This method, however, presents several difficulties in the interpretation of embryo sac development (Bradley, 1948; Maxwell and Valentine, 1966). For an investigation where it is essential to ascertain the variation from ovule to ovule, the aceto-carmine squash technique is much more convenient and reliable than paraffin sectioning, especially in plants with small ovules.

Finally, a knowledge of actual change in the incidence of apomictic seed production as directed by a change in the photoperiod is very important. An increase in aposporous sac formation is, at best, only an indirect estimate.

In the present study conclusive evidence was found that in D. intermedium X-750, there was an increase in the incidence of apomictic sac formation under a continuous photoperiod of 12 hours. The data on apomictic seed set support that concomitant with a change in the photoperiod,

there was significant seed set in this plant which was demonstrated to be highly self-incompatible. The two seeds obtained under 14 hours illumination were evidently formed as a result of self-fertilization, because in the cytological study not a single mature aposporous sac was encountered under this treatment. The data on seed set under 12 hours illumination is quite variable. This may be due to the fact that the development of apomictic seed is dependent upon fertilization of the polar nucleus to form the endosperm in the apomictic members of Dichanthium (Brooks, 1958). The seed set in one inflorescence was quite high.

It is difficult to interpret the physiology of apomixis at the biochemical level. Heslop-Harrison (1957b) implicated auxin in the development of the sexual embryo sac in the orchid flower. He found that until the flower was pollinated or supplied with an external source of auxin, the orchid embryo sac did not develop beyond the single cell stage. In Ribes, Zatyko (1962) was able to induce apomictic seed set by spraying the flowers with a mixture of gibberellic acid and indole acetic acid. These experiments suggest that a certain degree of balance between gibberellic acid and auxins might be involved in the production of apomictic and sexual sacs in the facultative apomicts, but the mechanism that triggers the development of aposporous sacs and determines the success of one sac rather than the other continues to be elusive. Among the external environmental factors, at least the role of photoperiod, in determining

the incidence of apomictic and sexual sac formation in two facultatively apomictic members of the genus Dichanthium, appear to be reasonably established.

Versatility of reproductive behavior has undoubtedly contributed significantly to the origin and evolution of the compilospecies D. intermedium. The range of morphological variation, and extensive geographical distribution of this compilospecies further suggest that latitudinal morphological variations might be associated with the control of the mode of reproduction by the natural photoperiod.

CHAPTER VII

SOME NEW DATA ON INTERRELATIONSHIPS OF *DICHANTHIUM INTERMEDIUM* AND *DICHANTHIUM FECUNDUM*

On the basis of cytogenetical studies involving hybrids between *Dichanthium fecundum* S. T. Blake and *D. annulatum* (Forssk.) Stapf, Borgaonkar and de Wet (1964) reduced the former species to a varietal rank, and recognized a new combination *D. annulatum* var. *fecundum*. This variety is characterized by having, except for the lower 1-3 spikelet pairs that are male or neuter, bisexual pedicellate spikelets. Bisexuality of the pedicellate spikelet is often accompanied by a lemma ending in a well developed awn. In geographical distribution, this variety is restricted to the more tropical regions of Australia and New Guinea. In reproductive behavior it has been demonstrated to be essentially obligately apomictic (de Wet, Borgaonkar and Richardson, 1963). Evidences have also been presented to show that this variety is an apomictic derivative of *D. annulatum* (Borgaonkar and Singh, 1962; Mehra, 1962; Borgaonkar and de Wet, 1964). The cytological behavior of a tetraploid ($2n = 40$) race of this variety was reported by Chheda and Harlan (1962).

Following an extensive collection of Old World species of Dichanthium in 1963-64, additional data were assembled for a better understanding of species relationship in this generic complex. A cytological survey of the various collections revealed that D. annulatum var. fecundum had both tetraploid ($2n = 40$) and hexaploid ($2n = 60$) races in Australia. Furthermore, at several places, throughout the range of its geographical distribution, this variety is sympatric with the compilospecies D. intermedium (R. Br.) de Wet et Harlan, but there was little evidence of any introgression between them. The compilospecies D. intermedium is rather unique in its ability for distant hybridization. Studies were, therefore, undertaken to determine the relationship between the compilospecies and D. annulatum var. fecundum.

Materials and Methods

A preliminary cytological survey was made of five natural biotypes of Dichanthium annulatum var. fecundum collected from Australia. Two plants of this variety, a tetraploid ($2n = 40$) and a hexaploid ($2n = 60$), were then selected for hybridization with D. intermedium designated as X-750. This tetraploid D. intermedium X-750 ($2n = 40$) was obtained as a result of artificial hybridization between two naturally occurring, facultatively apomictic, biotypes of this species from India. It was highly self-incompatible, and essentially sexual in reproductive behavior. For all

crosses D. intermedium X-750 was used as the female parent. Reciprocal crossing was not attempted because D. annulatum var. fecundum had already been demonstrated to be essentially obligately apomictic. In all, five crosses were made, and on maturity the seeds were harvested. The hybrids obtained as a result of crosses between D. intermedium X-750 and the hexaploid D. annulatum var. fecundum 10791 were designated as 65-X-17, 65-X-19, and 65-X-37, and those from the tetraploid race of D. annulatum var. fecundum were designated as 65-X-38 and 65-X-39, respectively.

Data on the crossability of D. intermedium X-750 with D. annulatum var. fecundum were obtained. Among the hybrids, detailed meiotic studies were made in 15 plants by squashing microsporocytes in aceto-carmine. For all cytological studies 25 cells each at metaphase I, anaphase I and anaphase II were analyzed.

Results

Crossability

This term is used here in the sense of Khush and Stebbins (1961) to denote

> ... the relative ease with which hybrid seed can be obtained from a cross between two species, the germination ability of the hybrid seed, and the percentage of hybrid seedlings which reach the age of reproduction.

Lower crossability, usually, indicates a more distant relationship.

In the present study hybrid seeds were obtained in all

the crosses. Seed set in one cross was as high as 20.4 per cent. On the whole, the germination ability of the hybrid seeds was also good. Only one cross produced hybrid seeds that did not germinate at all. All F_1 plants looked vigorous and healthy. The study, therefore, showed that crossing barriers between D. intermedium and D. annulatum var. fecundum were not strongly developed (Table IV).

Meiosis in the Parents

Meiosis in D. intermedium X-750 was comparatively normal, with 20 bivalents usually formed at metaphase I. In D. annulatum var. fecundum meiotic irregularities were frequently observed in both tetraploid, as well as hexaploid biotypes. The tetraploid often had cells with two univalents and one tetravalent. Cells with two univalents were more frequent in the hexaploid, and some cells often, also, had a tetravalent. In both cases there were a number of cells showing perfectly normal meiosis with 20 bivalents in the tetraploid, and 30 bivalents in the hexaploid at metaphase I.

In the later stages of meiosis, the tetraploid D. annulatum var. fecundum showed a majority of cells with the normal 20:20 segregation of chromosomes at anaphase I. Some cells, however, had two laggards. Cells were also observed where the two laggards divided precociously at anaphase I. Some of the microspores contained a micronucleus, but on the whole, pollen fertility was quite high. Likewise, the hexaploid race of this variety showed a normal 30:30 distribution

TABLE IV

DATA ON THE CROSSABILITY OF *DICHANTHIUM INTERMEDIUM* WITH *D. FECUNDUM*

Cross	No. of florets pollinated	No. of seeds set	% seed set	No. of seeds sown	No. of seeds germinated	% germination	No. of plants grown	No. of false hybrids	No. of F_1's matured	% cross-ability
65-X-17	93	5	5.3	3	0	0	0	0	0	0
65-X-19	102	9	8.8	4	1	25.0	1	0	1	2.2
65-X-37	191	39	20.4	39	19	48.7	19	0	19	9.9
65-X-38	97	12	12.3	12	2	16.6	2	0	2	2.04
65-X-39	76	6	7.8	6	3	50.0	3	0	3	3.9

of chromosomes on the two poles at anaphase I in a majority of the cells. Some cells had two laggards, and 2-3 cells showed a 29:31 segregation of chromosomes at anaphase I. Micronuclei were also observed in some of the microspores of the hexaploid race, but the pollen fertility, on the whole, was quite high. The data on the cytology of the parents are summarized in Table V.

Meiosis in the Hybrids

The data on the meiotic behavior of the hybrids are summarized in Table V. Meiosis in all the hybrids was greatly disturbed.

Cytological study of the hybrid 65-X-19 showed that it had 2n = 50 chromosomes. At diakinesis and metaphase I of meiosis in this plant, 6-10 univalents were observed in every cell studied, and a trivalent was found in some cells (Plate VII). During subsequent stages of meiosis, also, irregularities were present. At anaphase I laggards were frequent. In a large number of cells there was an unequal segregation of chromosomes at anaphase I (Plate VII), but no micronuclei were observed in the microspores.

The hybrid 65-X-19 was further characterized by the presence of several multiploid microsporocytes. These cells were unusually large, each with a polyploid nucleus (Plate VI). In each anther 8-10 of these multiploid cells or syncytes were found. As opposed to 2n microsporocytes with 50 chromosomes, these syncytes were of two kinds, 4n with

TABLE V

CYTOLOGY OF PARENTS AND HYBRIDS

Plant	No. Plants Studied	Diploid Chromosome Number	Chromosome Association at Meiotic Metaphase I* I	II	III	IV	Remarks
Parents							
D. intermedium X-750	1	40	0	20	0	0	Meiosis normal
D. annulatum var. fecundum							
Accession Number 10792	1	40	0.5 0-2	18.5 18-20	0	0.62 0-1	Some cells with 2 dividing laggards at Anaphase I.
Accession Number 10791	1	60	1.75 0-2	28.6 27-30	0 0	0.25 0-1	Some cells showing 29:31 disjunction at Anaphase I. Others normal.
Hybrids							
65-X-19	1	50	6.6 6-10	21.4 20-22	0.2 0-1	0 0	Several multiploid microsporocytes were present with 2n=100 and 200
65-X-37	10	50	13.6 12-13	14.4 12-15	2.2 2-3	0 0	few cells with pentavalents
65-X-38	2	40	8.6 8-10	9.9 7-8	0 0	2.9 2-4	

TABLE V (CONTINUED)

Plant	No. Plants Studied	Diploid Chromosome Number	Chromosome Association at Meiotic Metapahase I* I	II	III	IV	Remarks
65-X-39	2	40	11.1 8-24	14.2 6-16	0 0	0 0	Dividing and nondividing laggards present at Anaphase I

*Average number and range of various configurations are listed.
I = univalents; II = bivalent; III = trivalents; IV = quadrivalent

100 chromosomes, and 8n with 200 chromosomes. In aceto-carmine squash studies of microsporocytes, the syncytes could be traced from the very early stages of meiosis, and no evidence was found to indicate that there was any migration of a nucleus from one pollen mother cell into another, or that any fusion of pollen mother cells had taken place. The chromosome associations were quite distinct in 4n syncytes at metaphase I with an average of 15 univalents, 41 bivalents, and one trivalent per cell. Metaphase I in the 8n syncytes was quite disturbed, showing a large number of univalents and multivalents (Plate VI). Furthermore, in the 8n syncytes, the chromosomes at metaphase I tended to drift away from the equatorial plate (Plate VI). No micronuclei were observed in the microspores developing from the syncytes; however, these microspores looked like giants as compared to the n microspores in size.

Ten plants were studied in the hybrid designated as 65-X-37. All of these plants had a 2n = 50 chromosomes. Meiosis in this hybrid also was quite disturbed. Univalents were present at metaphase I (Plate VII) in all the cells, sometimes as many as 14 in a single cell. At anaphase I laggards were quite frequent, and in one cell up to 22 laggards were observed (Plate VII). A large number of microspores had 2-3 micronuclei.

Two plants each in the other two hybrids 65-X-38 and 65-X-39, respectively, were studied. Both the hybrids had a 2n = 40 chromosomes. Meiosis was highly disturbed in

PLATE VI

Cytology of the Hybrids Between *Dichanthium intermedium* and *D. fecundum*.

Legend:

Figure 1. Metaphase I in A Syncyte in the Hybrid 65-X-19 With 2*n* = 200 (ca. X450)

Figure 2. Enlarged View of the Equatorial Plate of Figure 1 Showing Univalents, Bivalents and Multivalents (ca. X1350)

Figure 3. Anaphase I in A syncyte in the Hybrid 65-X-19 With 2*n* = 100 (ca. X1350)

PLATE VI

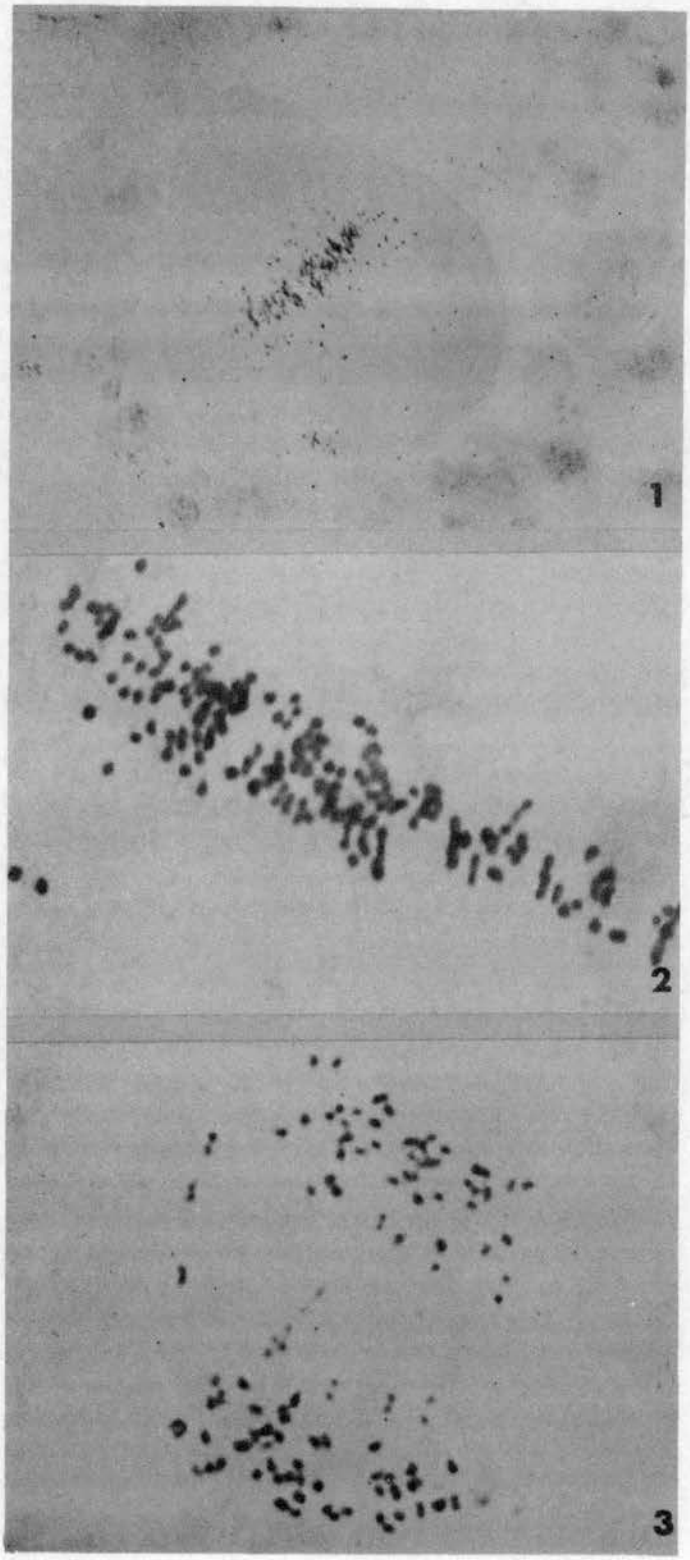


PLATE VII

Cytology of the Hybrids Between *D. intermedium* and *D. fecundum* (continued). All figures ca. X1350.

Legend:

Figure 4. Metaphase I in the Hybrid 65-X-19 Showing Two Univalents and 24 Bivalents ($2n = 50$)

Figure 5. Anaphase I in the Hybrid 65-X-38 ($2n = 40$)

Figure 6. Metaphase I in the Hybrid 65-X-37 Showing Six Univalents, Nine Bivalents, Two Trivalents and Five Tetravalents ($2n = 50$)

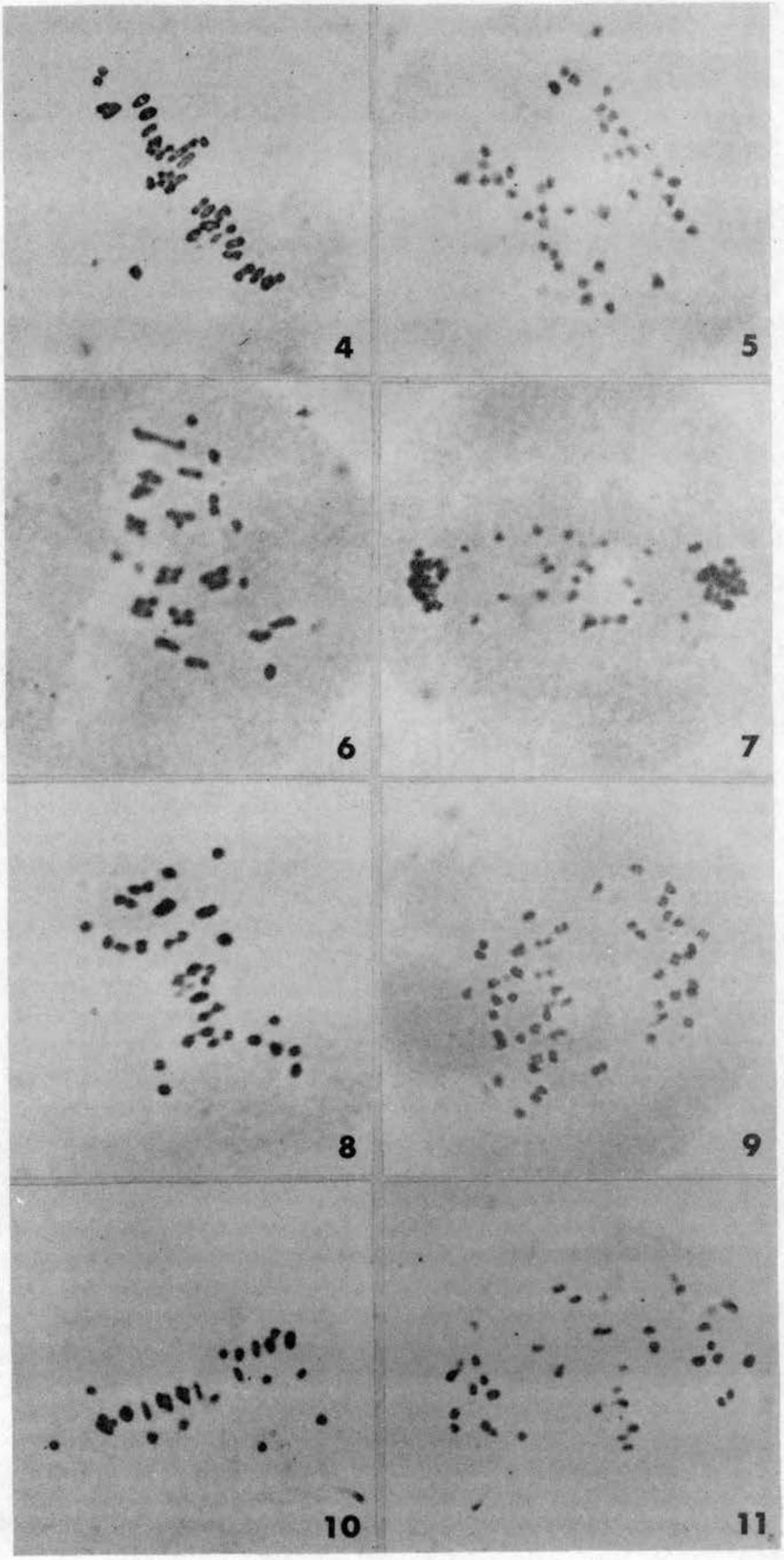
Figure 7. Anaphase I in the Hybrid 65-X-37 Showing 22 Laggards ($2n = 50$)

Figure 8. Metaphase I in the Hybrid 65-X-38 Showing 12 Univalents, 13 Bivalents and One Tetravalent ($2n = 40$)

Figure 9. Anaphase I in the Hybrid 65-X-19 Showing Unequal, 30:20 Segregation of Chromosomes ($2n = 50$)

Figure 10. Metaphase I in the Hybrid 65-X-39 Showing Ten Univalents, Seven Bivalents and Four Tetravalents ($2n = 40$)

Figure 11. Anaphase I in the Hybrid 65-X-39 Showing Several Laggards ($2n = 40$)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these hybrids (Plate VII). Univalents were present in all the cells, and in one cell as many as 24 univalents were found. At anaphase I laggards were quite frequent, and 2-3 micronuclei were observed in a large number of microspores.

In all the hybrids shrivelled pollen grains were frequent indicating pollen abortion. Furthermore, with all the irregularities in chromosome association, pollen inviability must be quite high among the hybrids.

Discussion

One of the interesting features in the study of meiotic behavior of the hybrids between D. intermedium and D. annulatum var. fecundum was the presence of syncytes in the anthers of one of the hybrids. The literature on syncyte formation in plants was reviewed by Price (1956). In this hybrid the syncytes appeared as unusually large pollen mother cells, each with a single nucleus, and multiple sets of chromosomes. Since the syncytes were of only two kinds, either with 4n or 8n chromosomes, their origin appeared to be archesporial. The 4n syncytes probably developed as a result of a multiplication in the archesporial cells one cell generation before microsporogenesis. Likewise, 8n syncytes were products of the original multiplication taking place two cell generations prior to the onset of meiosis. No indications for either migration or fusion syncyte formation were obtained. It was clear, therefore, that syncyte formation in D. intermedium X annulatum var. fecundum (65-X-19) was due to the failure of cytokinesis in archesporial divisions.

The general role of syncytes is not clear, although they have been implicated by various workers as a major source of polyploidy. Syncytes, where an increase in the number of sets of chromosomes is accompanied by an increase in the volume of cytoplasm, have a definite advantage over microspores with simple polyploidization of chromosomes. From the point of view of metabolism, among the syncytes a more favorable relationship between surface area of the nucleus and the volume of the cytoplasm is created (Lewis and John, 1963). Price (1956) observed syncytes in Saccharum and its relatives, and suggested that in this group syncytes might lead to the formation of diploid pollen grains, and, perhaps, male transmission of 2n chromosomes.

More studies will be needed to establish a definite role for syncytes as a major source of polyploidy in Dichanthium, but male transmission of 2n chromosomes is not unknown in this generic complex. Borgaonkar and de Wet (1963) reported an octaploid progeny (2n = 80) resulting from a cross between two tetraploid (2n = 40) individuals of the species Bothriochloa intermedia (syn. D. intermedium). Occurrence of unreduced female gamete with 2n chromosomes is quite common in Dichanthium, and the octaploid progeny demonstrated that a male transmission of 2n chromosomes is likely. Furthermore, in the present study, it was observed that the 4n syncytes had more regular meiosis as compared to the normal 2n microsporocytes. Therefore, the 4n syncytes appeared to have a better chance of giving rise to

functional 2n microspores. These 2n microspores would be capable of raising the level of polyploidy of the group in one step.

In nature hybrids involving D. intermedium and D. annulatum var. fecundum are rare. This can be explained on the basis of the cytogenetic behavior of the artificially obtained hybrids in this study, and also from the fact that in nature D. intermedium is predominantly facultatively apomictic. The data on crossability indicate that isolation barriers between D. intermedium and D. annulatum var. fecundum have not strongly developed. They cross readily to yield hybrid seeds. On the other hand, data on meiosis in the hybrids show that considerable cytological and genetic differentiation has taken place between these two members. All the hybrids appeared meiotically unable to reproduce sexually, although the production of apomictic seed might be possible. According to Baker (1959) such meiotically impossible hybrids can persist as long as apomictic reproduction can carry them. The rare hybrids that are encountered throughout the range of sympatric distribution of D. intermedium and D. annulatum var. fecundum are probably F_1 plants of a cross involving these two individuals, reproducing through apomictic seed production. No segregation was apparent in the hybrid population. In a situation like this only the compilospecies D. intermedium can benefit by enriching its germplasm through introgression. Due to essentially obligate apomixis in D. annulatum var.

fecundum there can be no flow of intermedium genes in this variety which appears to have entered an evolutionary blind alley. On the other hand, back-crosses involving chance fertile pollen of F_1 plants and female D. intermedium can be salvaged through facultative apomixis.

In a cytogenetic study of hybrids between B. intermedia (BBDD), and D. fecundum ($D_fD_fD'_fD'_f$), Chheda and Harlan (1962) assigned $BDD_fD'_f$ as the genomic constitution of the hybrids, but they did not discuss the interrelationships between the two species.

Taxonomic treatment of hybrids in a group where essentially obligate apomixis exists is very difficult. Morphologically it is difficult to distinguish between the hybrids involving D. intermedium and D. annulatum var. annulatum, quite common in the Gangetic Plains of India, and S. E. Asia, and those produced through hybridization between D. intermedium and D. annulatum var. fecundum in New Guinea and Australia. Taxonomically both of these hybrids have been included under D. intermedium var. grahamii (de Wet and Harlan, n.d.). In a separate cytological study of D. intermedium var. grahamii it was found that there was very little irregularity in neiosis in the plants collected from the Gangetic Plains of India. On the other hand, the hybrids in the present study demonstrated considerable meiotic irregularity. It was found, therefore, that in its relationship with the compilospecies D. intermedium, D. annulatum var. fecundum was cytogenetically distant as

compared to D. annulatum var. annulatum.

CHAPTER VIII

EVOLUTIONARY TRENDS IN *DICHANTHIUM INTERMEDIUM*

The compilospecies *Dichanthium intermedium* (R. Br.) de Wet et Harlan (Gramineae) has had quite a varied taxonomic history, as described in detail along with its origin by Harlan and de Wet (1963b) and de Wet and Harlan (1966). Intensive biosystematic studies in this laboratory, extending over a period of a decade, have led to a better understanding of the wide range of variability, and the consequent complexity of this species. It has been shown that this compilospecies is absorbing genes through hybridization from several other species generally recognized as belonging to the three genera of the Andropogoneae, *Bothriochloa* O. Ktze., *Capillipedium* Stapf and *Dichanthium* Willemet. Due to the aggressiveness of this species, a large assemblage of hybrid derivatives has resulted in which various members have been either recognized as distinct species, or have been placed with the parental species they resemble more closely. The ability of this species to reproduce through agamospermous apomixis along with the normal amphimictic seed production has further complicated the taxonomic treatment.

In the present paper meiotic behavior of species contributing to variability in *D. intermedium* is presented

along with chromosome associations in their assumed natural hybrids, and an attempt is made to recognize different varieties on the basis of cytogeographical considerations as well as morphological distinctness. It is hoped that these features will throw some light on the evolutionary trends in this compilospecies.

Following de Wet and Harlan (1966), all species contributing to the variability in D. intermedium will be treated as belonging to the genus Dichanthium, and their phylogenetic position identified by referring to the classically recognized genera as sections.

Species that have directly contributed towards variability in Dichanthium intermedium are D. ewartianum and D. ischaemum of section Bothriochloa, D. parviflorum of section Capillipedium, and D. annulatum of section Dichanthium.

Materials and Methods

Plants included in this paper formed a part of approximately 1000 collections representing all the Old World species belonging to the three sections of the genus Dichanthium. These plants were grown in a uniform nursery as described by Celarier and Harlan (1956). Chromosome numbers and chromosome associations were determined from developing microspore mother cells stained with aceto-carmine. For embryological studies a rapid method for aceto-carmine squash of ovules (Saran and de Wet, 1966) was used. Morphological data were obtained from a study of as many plants as were

available, both in the field and in the herbarium. Hybrids were produced by W. L. Richardson, using the technique described by Richardson (1958).

Results and Discussion

The cytology of the four species of Dichanthium is summarized together in Table VI, whereas the cytology of each assumed hybrid is discussed separately along with its geographical distribution and morphological features. The key characters utilized for distinguishing the different taxa, as recognized in this paper, are given in Table VII.

Dichanthium ewartianum

This species is widely distributed in tropical and subtropical Australia. It is facultatively apomictic with $2n$ = 40, 50 and 60. The plant with $2n$ = 40 chromosomes represents the basic species with the hexaploid being derived as a product of fertilization between an unreduced female gamete and a reduced male, whereas the pentaploid is a product of a cross between hexaploid and tetraploid biotypes. Cytologically this species behaves as a segmental allopolyploid with a large number of bivalents and a few univalents and multivalents during meiosis of microsporogenesis. An increase in the number of univalents appears to be directly correlated with increase in ploidy. The average number of univalents per cell for tetraploid, pentaploid and hexaploid is 4.8, 6.2, and 8.9, respectively.

Hybrids between D. ewartianum and D. intermedium are frequently encountered wherever these species are sympatric, and an attempted hybridization involving tetraploid biotypes of these two species yielded a hexaploid offspring which resembled the assumed natural hybrids in morphological and cytological details (de Wet and Harlan, 1966). The natural hybrids form a weed complex, widely distributed in northern Queensland.

Dichanthium ischaemum

In distribution, this species extends from southwestern Europe to the China coast. Celarier and Harlan (1957) recognized two varieties in this species, var. ischaemum and var. songarica. Celarier (1957) also described the cytology of the species. This species has biotypes ranging from facultatively apomictic to essentially obligate apomicts in reproductive behavior with 2n = 40, 50 and 60 chromosomes. Hybrid populations intermediate between D. ischaemum and D. intermedium in morphological features have been found in the foothills of the Himalayas. These two species are known to grow in the same areas in Burma, and this could be the site of hybridization between them. Comparative morphological studies further indicate that D. ischaemum var. songarica, which is an essentially obligate apomict, is a product of backcross with the D. intermedium and D. ischaemum var. ischaemum introgressants. Meiotic studies in the two varieties of this species indicate some

irregularities, but most developing microspore mother cells, at all levels of ploidy, showed normal meiosis. Due to the presence of univalents, trivalents and tetravalents, laggards were found at the anaphase of both divisions. Cytologically this species also behaved as a segmental allopolyploid.

Dichanthium parviflorum

This species extends from southern Africa to southern Japan and eastern Australia. It has a sexual diploid race with 2n = 20 chromosomes, and a tetraploid race that is facultatively apomictic and hybridizes readily with tetraploid D. intermedium in areas where they are sympatric. The diploids were found to be normal in cytological behavior. The tetraploids also showed almost normal meiotic behavior with an average number of 19 bivalents per cell. Some of the cells were characterized by a few univalents and a tetravalent, the maximum number of univalents being four.

Dichanthium annulatum

This species has a wide geographical distribution extending from Africa to India, and throughout South-East Asia to Australia. It is also characterized by a diploid race (2n = 20) that is sexual, and tetraploids that are facultative apomicts and hybridize with D. intermedium throughout the extent of their sympatric distribution. A greater prepondrence of hybridization between these two species is reported from India. Cytologically the diploids

TABLE VI

CYTOLOGY OF *DICHANTHIUM* SPECIES

Name	No. Collections Studied	Diploid Chromosome Number	Chromosome Association at Meiotic Metaphase* I	II	III	IV
Dichanthium ewartianum	1	40	4.8 4-6	15.57 15-17	1.14 0-2	0.14 0-1
	4	50	6.2 4-10	20.6 19-23	0.2 0-1	0.5 0-1
	22	60	8.97 6-12	25.3 23-27	0.03 0-1	0.01 0-1
D. ischaemum						
var. *ischaemum*	141	40	1.05 0-10	18.6 15-20	0.01 0-1	0.43 0-3
	3	50	5.14 0-10	22.0 16-25	0.02 0-1	0.2 0-1
	4	60	3.3 0-8	26.4 20-30	0.1 0-1	0.9 0-3
var. *songaricum*	6	50	3.6 2-10	21.3 15-25	0.2 0-1	0.8 0-3
	4	60	4.88 2-10	26.7 20-30	0.04 0-1	0.4 0-2

TABLE VI (CONTINUED)

Name	No. Collection Studied	Diploid Chromosome Number	Chromosome Association at Meiotic Metaphase* I	II	III	IV
D. parviflorum	13	20	----	10	----	----
	2	22	----	11	----	----
	29	40	1.2 0-4	19.0 17-20	---- ----	0.2 0-1
D. annulatum	10	20	----	10	----	----
	122	40	0.9 0-4	18.6 16-20	0.1 0-1	0.4 0-2

*Average number and range of various configurations are listed.
I = univalents; II = bivalent; III = trivalents; IV = quadrivalent

TABLE VII

KEY TO THE DIFFERENT TAXA RECOGNIZED IN THIS STUDY

1. All sessile spikelets in a raceme bisexual and usually awned; glumes lanceolate, acute or acuminate; inflorescence variable.
   2. Inflorescence of strongly divided branches arranged on an elongated primary axis; ultimate racemes consisting of 10 or less spikelet pairs .....section Capillipedium
      3. Plants with bamboo-like culms; leaves conspicuously narrowed at the base ..... D. assimile
      3. Culms not bamboo-like; leaves at most slightly narrowed at the base ..... D. parviflorum
   2. Inflorescence composed of simple or divided branches, subdigitate or arranged along an elongated axis; racemes with 15 or more spikelet pairs ..... section Bothriochloa
      4. Inflorescence composed of subdigitately arranged racemes.
         5. Leaves mostly cauline; Australian D. ewartianum
         5. Leaves mostly basal; Eurasian D. ischaemum
      4. Inflorescence composed of divided or simple branches arranged along an elongated primary axis
         6. Primary axis of inflorescence distinctly longer than the lower branches.
            7. Inflorescence branches simple or at the most the lowest few divided; racemes of 25 or more spikelet pairs D. intermedium var. intermedium
            7. Inflorescence branches strongly divided; racemes

TABLE VII (CONTINUED)

with 15 to 20 spikelet pairs — D. intermedium var. glabrum

6. Primary axis of inflorescence about as long as the lower branches.

8. Glumes acute or acuminate, variously hairy or glabrous, but without long hairs near the apex.

9. Leaves basal as well as cauline; Australian — D. intermedium var. australis

9. Leaves primarily basal; distributed along the Himalayan foothills — D. intermedium var. montanum

8. Glumes somewhat oblong and obtuse, usually with a few long hairs near the apex.

10. Pedicel of pedicellate spikelet grooved on one side; spikelets almost lanceolate and acute. — D. intermedium var. indicum

10. Pedicel of pedicellate spikelet without a translucent middle-line; spikelets oblong-lanceolate, somewhat obtuse. — D. intermedium var. grahamii

1. Lower 1-6 sessile spikelets in a raceme usually male or neuter and awnless; glumes oblong-lanceolate, obtuse; racemes subdigitately arranged on a short axis. — section Dichanthium

TABLE VII (CONTINUED)

11. Peduncle to the inflorescence pubescent	D. aristatum
11. Peduncle to the inflorescence glabrous	
12. Glumes with long hairs near the apex, often forming a transverse fringe.	D. annulatum
12. Glumes variously pubescent but without long hairs near the apex.	D. caricosum

were normal, but the tetraploids showed some irregularities during microsporogenesis due to the presence of univalents and multivalents as is typical of a segmental allopolyploid as defined by Stebbins (1947).

Based on the materials available and studies conducted to 1963, Faruqui published in 1964 cytogenetical data on the Bothriochloa intermedia complex (syn. Dichanthium intermedium). He made a broad survey of the complex which indicated a wide range of morphological and cytological variability. During 1963-64, extensive collections were made numbering around 400, from various parts of Africa, India, S. E. Asia and Australia where D. intermedium was growing sympatrically with other species and numerous hybrid populations were indicated. Since that time further information on reproductive mechanisms, physiology of reproduction, cytology and genetic behavior of the compilospecies D. intermedium have been obtained. On the basis of this information, and the morphological distinctness of the various populations within the compilospecies, de Wet and Harlan (n.d.) described in detail the taxonomy of D. intermedium, and divided it into six varieties. These varieties are believed to have resulted from interspecific crosses involving D. intermedium and four other species, as already discussed. The following account deals in detail with the meiotic behavior of the varieties and their geographical distribution along with the distribution of the putative parents; the morphological characters separating

these varieties have been summarized in Table VII.

Dichanthium intermedium var. intermedium

This is the basic variety of the compilospecies, and was recognized in the sense of Bor (1960) to include plants with at most the lower inflorescence branches moderately divided. The inflorescence is elongated, with the lower branches only about half as long as the primary axis. The racemes are composed of 25 or more apikelet pairs and the spikelets may be pitted or not pitted, often in the same raceme.

This variety is widely distributed from tropical Aftrica to India, S. E. Asia and Australia (Plate VIII). Its cytology is summarized in Table VIII. It has biotypes with 2n = 40, 50 and more rarely 60 chromosones. The variety, as a whole, could be classified cytologically as a segmental allopolyploid with the majority of the chromosomes associating into bivalents during meiosis with a few univalents and occasional multivalents. The tetraploid race from India shows more or less normal behavior cytologically (Plate XIV). The tetraploid race from Hongkong is characterized by the presence of a hexavalent and a tetravalent which are probably due to reciprocal translocations. From Australia, tetraploid, pentaploid and hexaploid races have been collected. The cytology of the tetraploid and the pentaploid is given in Table VIII, and the cytology of the hexaploid (Galton, Australia; A. No. 4596) was described by Faruqui (1964).

PLATE VIII

Distribution of *Dichanthium intermedium* var. *intermedium*

Each dot represents an area where collections were made.

PLATE VIII

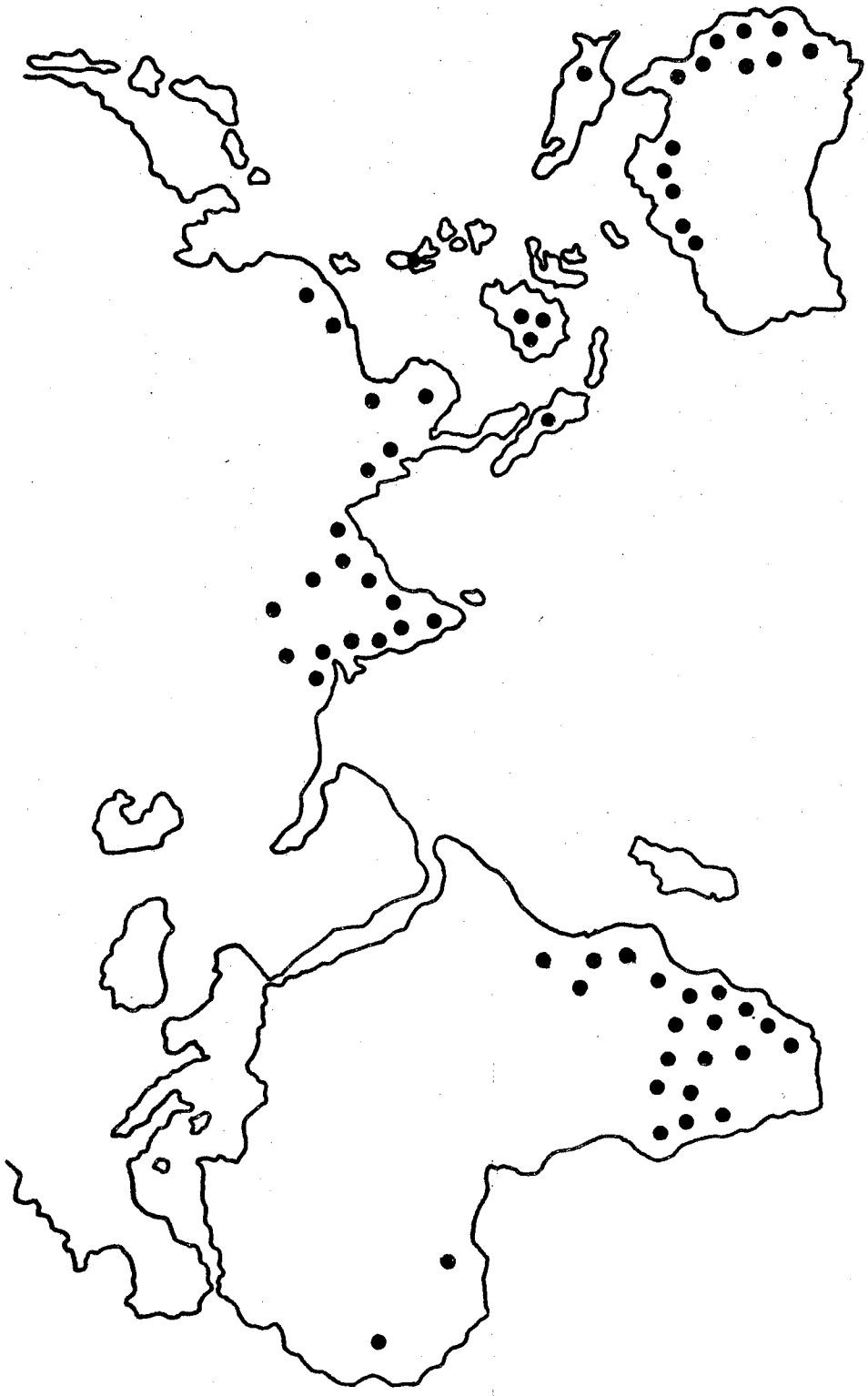


TABLE VIII

CYTOLOGY OF DICHANTHIUM INTERMEDIUM VAR. INTERMEDIUM

A.no.	Locality	Diploid Chromosome Number	Chromosome Association at Meiotic Metaphase*			
			I	II	III	IV
9070	Lohnavla, India	40	2.5 2-4	18.25 17-19	---	0.25 0-1
A9071	Lohnavla, India	40	0.5 0-2	19.75 19-20	---	----
9073	Lohnavla, India	40	0.6 0-2	19.7 19-20	---	----
9074	Lohnavla, India	40	1.0 0-2	19.5 19-20	---	----
9082	Igatpuri, India	40	---	20	---	----
10640	Hongkong	40	2 2	14 14	---	1 1
10899	Mackay, Australia	40	4 4	14 14	---	2 2
10900	Mackay, Australia	50	5.8 4-6	21.1 20-22	---	0.5 0-1
10785	Townsville, Australia	50	6.0 6	21.0 20-22	--- ---	0.5 0-1

TABLE VIII (CONTINUED)

A.no.	Locality	Diploid Chromosome Number	Chromosome Association at Meiotic Metaphase*			
			I	II	III	IV
10805	Townsville, Australia	50	6.2 6-8	17.7 16-20	---	2.1 2.3

*Average number and range of various configurations are listed.
I = univalents; II = bivalent; III = trivalents; IV = quadrivalent

The embryo sac squash studies of this variety revealed a range of reproduction from agamospermous apomixis to fully sexual (Saran and de Wet, 1967). This dual mode of reproduction must have played an important role in the origin of the different cytological races.

Dichanthium intermedium var. australis de Wet et Harlan

This variety was recognized by de Wet and Harlan (n.d.) to include plants with the inflorescence composed of undivided or moderately divided branches, arranged on an axis which they more or less equal in length. It is considered to have resulted from a cross between D. intermedium and the Australian D. ewartianum. Although this variety was collected only in coastal Queensland, it is likely to be found wherever the two parental species are sympatric. The distribution of the variety is given in Plate IX.

In reproductive behavior, this variety ranges from facultative to essentially obligate apomicts. It has three cytological races with 2n = 40, 50 and 60 chromosomes. The cytological behavior during meiosis of microsporogenesis was irregular in all the three races, with univalents and multivalents present in almost every developing spore mother cell (Plate XIV). However, in the tetraploid some cells always had only four univalents, and the rest were all bivalents. Some cytologically balanced and functional gametes were formed in the tetraploid, while in the pentaploid and the hexaploid the dyads and tetrads had micronuclei

PLATE IX

Distribution of *Dichanthium intermedium* var. *australis*

Each dot represents an area where collections were made.

PLATE IX

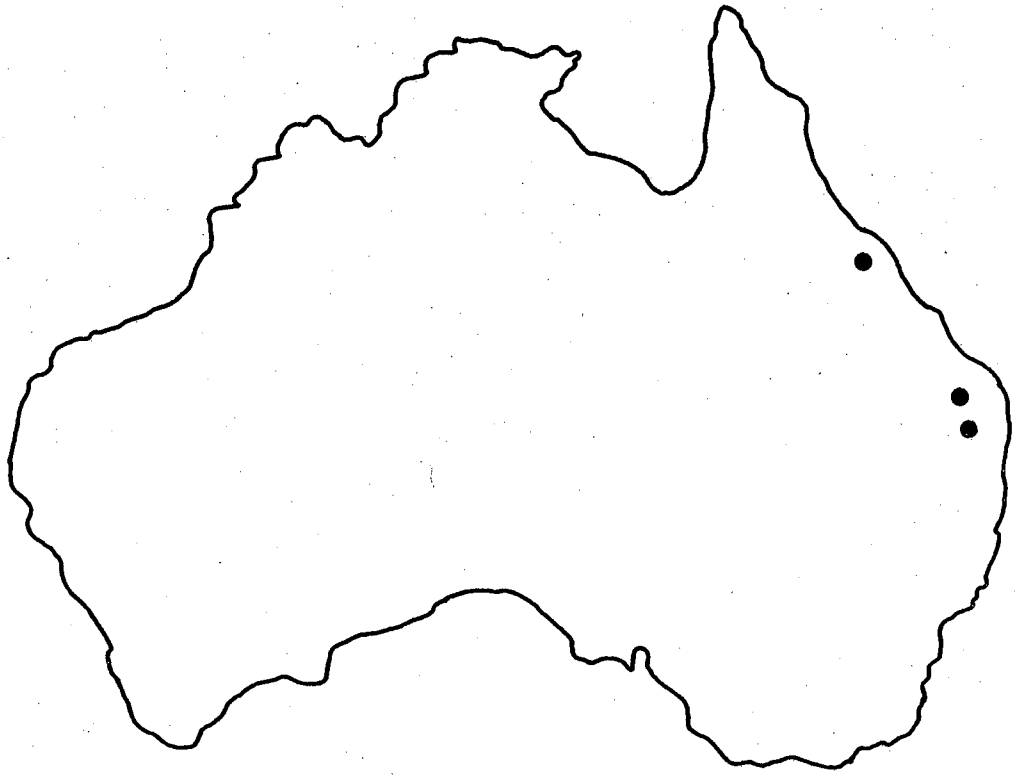


TABLE IX

CYTOLOGY OF *DICHANTHIUM INTERMEDIUM* VAR. *AUSTRALIS*

A.no.	Locality	Diploid Chromosome Number	Chromosome Association at Meiotic Metaphase*			
			I	II	III	IV
10784	Townsville, Australia	40	8.5 4-12	15.4 14-18	---	0.28 0-1
10876	Rockhampton, Australia	50	7.78 2-12	17.28 14-21	.07 0-1	1.85 0-2
10874	Rockhampton, Australia	60	6.8 6-8	16.7 12-24	5.4 2-8	0.9 0-3

*Average number and range of various configurations are listed.
I = univalents; II = bivalent; III = trivalents; IV = quadrivalent

and the pollen grains were generally empty. The cytology of this variety is summarized in Table IX.

Dichanthium intermedium var. glabrum (Roxb.) de Wet et Harlan

This variety is recognized in the sense of de Wet and Harlan (n.d.) to include plants characterized by strongly divided inflorescence branches, having individual racemes with 15-20 spikelet pairs. The primary branches are distinctly shorter than the primary axis of the inflorescence. This variety is considered to have resulted from a cross between D. intermedium and D. parviflorum. In morphological features this variety lies essentially intermediate between the two assumed parental species. Backcrossing with D. intermedium reduces the degree of secondary and tertiary branching, while introgression with D. parviflorum increases secondary and higher degree of branching.

This variety is widely distributed in the warm and somewhat humid parts of Africa, India, South-East Asia and Australia (Plate X). In reproductive behavior, plants in this variety, range from facultative to essentially obligate apomicts.

Cytologically, the variety follows an interesting pattern. All Asian individuals are tetraploids; African members are both tetraploid and hexaploid, and the maximum diversity is present in Australia where tetraploid, pentaploid as well as hexaploid individuals are found. The

PLATE X

Distribution of *Dichanthium intermedium* var. *glabrum*

Each dot represents an area where collections were made.

PLATE X

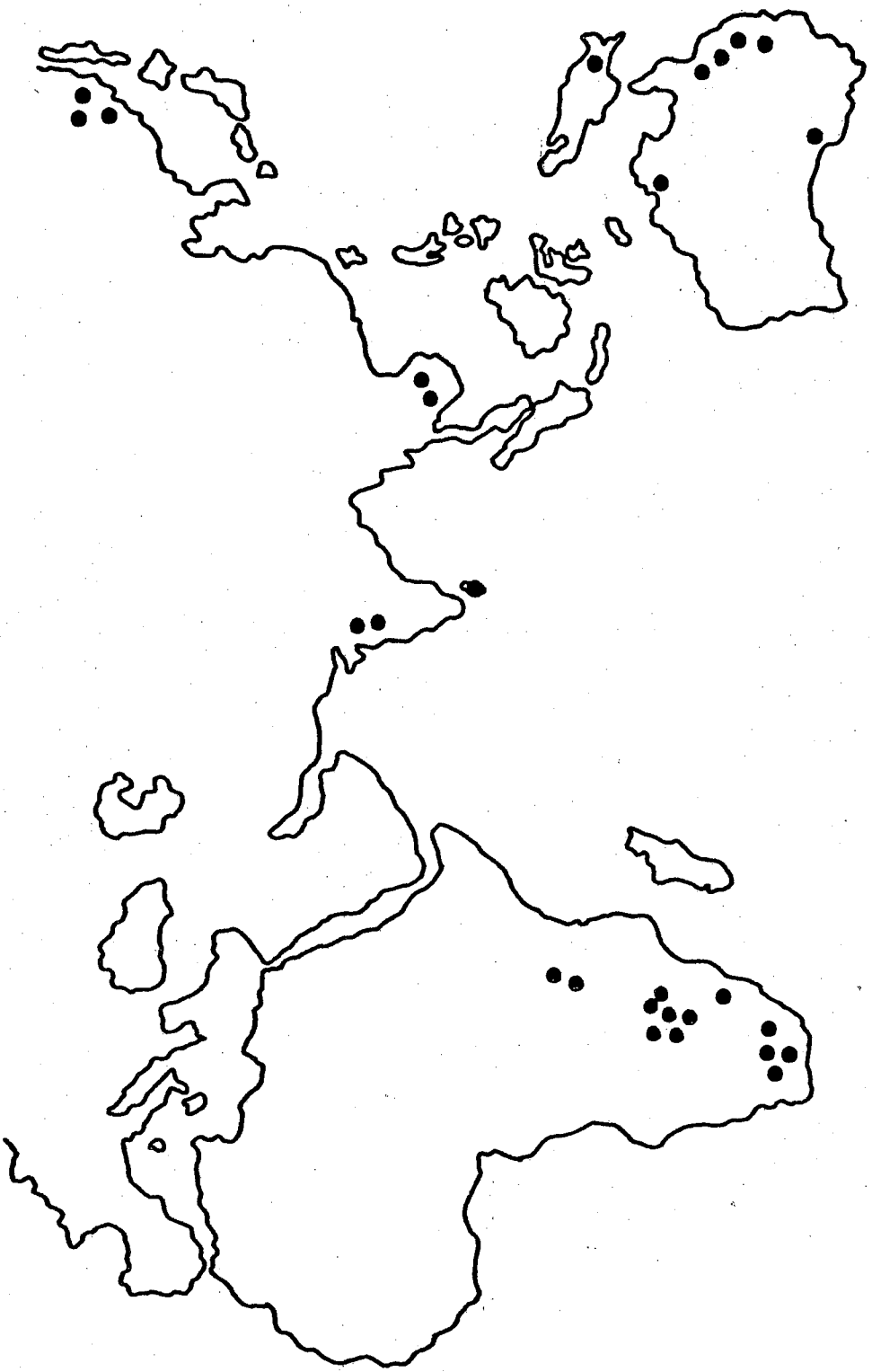


TABLE X

CYTOLOGY OF DICHANTHIUM INTERMEDIUM VAR. GLABRUM

A. No.	Locality	Diploid Chromosome Number	Chromosome Association at Meiotic Metaphase*						
			I	II	III	IV	V	VI	More
9450	Bankert-Sinoia Road, Rhodesia	40	----	20	----	----	---	---	
9451	Sinoia-Karoi Road, Rhodesia	40	1.14 0-4	18.85 17-20	----	0.28 0-1			
9454	Kafue, Rhodesia	40	0.50 0-2	18.0 15-20	0.09 0-1	0.81 0-2			
9455	Choma, Rhodesia	40	----	18.85 12-20	----	0.57 0-4			
9453	Kafue, Rhodesia	60	----	30.0	----	----			
9456	Zimba, Rhodesia	60	1.4 0-4	29.0 26-30	----	0.20 0-1			
9452	Sinoia, Rhodesia	60	0.60 0-4	27.10 23-30	----	0.28 0-3			
9457	Zimba, Rhodesia	60	0.5 0-2	27.20 24.30	0.10 0-1	1.20 0-3			
9458	Wankie, Rhodesia	60	1.7 0-8	29.0 27-30	0.10 0-1	----			

TABLE X (CONTINUED)

A. No.	Locality	Diploid Chromosome Number	Chromosome Association at Meiotic Metaphase*						
			I	II	III	IV	V	VI	More
9462	Beatrice, Rhodesia	60	0.57 0-2	28.28 24-30	----	0.85 0-3			
9551	Nylstroom, S. Africa	40	----	19.50 18-20	----	0.25 0-1			
9552	Nylstroom, S. Africa	40	0.5 0-2	17.5 15-20	0.5 0-2	0.75 0-2			
9553	Nylstroom, S. Africa	40	0.28 0-2	19.85 19-20	----	----			
9554	Nylstroom, S. Africa	40	0.28 0-2	18.71 16-20	----	0.57 0-2			
9555	Newcastle, S. Africa	40	0.2 0-2	19.3 16-20	----	0.3 0-2			
9556	Tongaat, S. Africa	40	1.1 0-4	18.9 18-20	0.1 0-1	0.2 0-1			
9558	Ixopo, S. Africa	40	0.4 0-2	17.2 15-20	0.4 0-2	1.0 0-2			
9559-A	Richmond, S. Africa	40	----	20.0	----	----			

TABLE X (CONTINUED)

A. No.	Locality	Diploid Chromosome Number	Chromosome Association at Meiotic Metaphase*						
			I	II	III	IV	V	VI	More
9560	Nelspruit, S. Africa	40	0.4 0-2	19.6 18-20	----	0.1 0-1			
9561	Nelspruit, S. Africa	40	0.5 0-2	17.9 15-20	0.1 0-1	0.9 0-3			
9562	Nelspruit, S. Africa	40	0.2 0-2	17.9 16-20	----	1.0 0-2			
9563	Piet Retief, S. Africa	40	----	19.42 18-20	----	0.28 0-2			
9460	Lupani-Kenmar, S. Africa	40	0.20 0-1	17.20 12-20	0.20 0-1	1.20 0-4			
9539	East Lynn, S. Africa	40	----	17.60 14-20	----	1.20 0-3			
9540	East Lynn, S. Africa	40	1.0 0-4	18.25 16-20	0.25 0-1	0.50 0-2			
9541	East Lynn, S. Africa	40	0.40 0-2	19.80 19-20	----	----			
9542	East Lynn, S. Africa	40	0.80 0-2	19.30 16-20	0.20 0-2	----			

TABLE X (CONTINUED)

A. No.	Locality	Diploid Chromosome Number	Chromosome Association at Meiotic Metaphase*						
			I	II	III	IV	V	VI	More
9544	Nelsprint, S. Africa	40	0.33 0-2	17.66 12-20	0.33 0-2	1.0 0-4			
9545	Barbarton, S. Africa	40	----	20.0	----	----			
9546	Bremersdorp, Swaziland	40	----	20.0	----	----			
9547	Bremersdorp, Swaziland	40	1.30 0-4	19.0 16-20	0.10 0-1	0.10 0-1			
9548	Groblersdal, S. Africa	40	1.30 0-3	18.40 16-20	0.50 0-1	0.10 0-1			
9549	Groblersdal, S. Africa	40	0.6 0-4	17.90 16-20	----	0.90 0-2			
9550	Groblersdal, S. Africa	40	----	20.0	----	----			
9564	Cape Town, S. Africa	40	----	19.5 18-20	----	0.34 0-1			
9565	Rooipoierspruit, S. Africa	40	0.5 0-2	17.9 16-20	0.3 0-2	0.7 0-2			
9677	Bremersdorp, Swaziland	40	0.7 0-2	18.7 18-20	0.5 0-3	0.1 0-1			

TABLE X (CONTINUED)

A. No.	Locality	Diploid Chromosome Number	Chromosome Association at Meiotic Metaphase*						
			I	II	III	IV	V	VI	More
9678	Bremersdorp, Swaziland	40	0.5 0-2	18.4 18-20	0.1 0-1	0.7 0-4			
9879	Pretoria, S. Africa	40	1.4 0-5	18.5 15-20	0.2 0-1	0.3 0-2			
9557	Ixopo, S. Africa	60	1.6 0-6	29.0 25-30	----	0.1 0-1			
9079	Lohnavla, India	40	0.42 0-1	18.28 18-20	0.42 0-1	0.42 0-1			
9083	Igatpuri, India	40	0.8 0-2	19.5 19-20	----	----			
10-115	Colombo, Ceylon	39-40	1.0 0-2	15.75 15-16	----	1.25 1-2			
10-605	Bangkok, Thailand	36	----	18.0	----	----			
10-624	Hongkong, China	38	0.6 0-2	13.3 12-15	----	2.7 2.3			
10625	Hongkong, China	40	0.5 0-1	9.0 8-10	----	0.5 0-1			chain. of 19.5 19-20
10630	Hongkong, China	40	2.0 0-4	16.0 11-19	----	1.5 0-4			

TABLE X (CONTINUED)

A. No.	Locality	Diploid Chromosome Number	Chromosome Association at Meiotic Metaphase*							
			I	II	III	IV	V	VI	More	
10631	Hongkong, China	40	3.0 0-4	15.1 14-19	----	1.7 0-3				
10635	Hongkong, China	40	0.4 0-4	17.4 16-20	----	1.2 0-2				
10647	Singapore, Malaya	40	----	4.0 4.0	----	2.0 2.0	---	3.2 0-4	1.6 0-3	VIII
8145	Port Moresby, New Guinea	40	0.85 0-2	19.57 19-20	----	----				
8147	Port Moresby, New Guinea	40	0.28 0-2	19.85 19-20	----	----				
10676	Darwin, Australia	40	2.0 2	17.2 15-19	----	0.9 0-3				
10678	Adelaide, Australia	40	2.0 2	14.0 14	----	1.0 1	---	1.0 1		
10688	Darwin, Australia	40	----	20	----	----				
10732	Ipswich, Australia	40	4.0 4	10.0 10	----	----	---	---	2.0 2	VIII
10733	Brisbane, Australia	40	5.3 4-8	8.0 6-10	----	4.1 2-6	---	0.33 0-1		

TABLE X (CONTINUED)

A. No.	Locality	Diploid Chromosome Number	Chromosome Association at Meiotic Metaphase*						
			I	II	III	IV	V	VI	More
10734	Brisbane, Australia	40	4.0 4	11.6 10-18	----	3.4 2-4			
10771	Townsville, Australia	40	1.0 0-2	12.0 9-20	----	2.2 0-4	---	0.75 0-1	
10821	Townsville, Australia	40	2.0 2	14.4 11-19	----	2.2 0-4			
10904	Mackay, Australia	40	5.0 4-8	17.5 16-18	----	----			
10905	Mackay, Australia	40	4.0 4	4.0 4	----	7.0 7			
10788	Townsville, Australia	50	10.0 10	16.0 16	----	2.0 2			
10817	Townsville, Australia	50	5.0 5	9.0 9	3.0 3	4.0 4			
10880	Mackay, Australia	50	4.5 4-6	18.7 18-19	----	2.0 2			
10806	Townsville, Australia	60	11.2 6-12	19.9 17-22	0.4 0-4	2.0 2			

TABLE X (CONTINUED)

A. No.	Locality	Diploid Chromosome Number	Chromosome Association at Meiotic Metaphase*						
			I	II	III	IV	V	VI	More

*Average number and range of various configurations are listed.
I = univalents; II = bivalent; III = trivalents; IV = quadrivalent; V = pentavalent; VI = hexavalent

tetraploids from India show, more or less, normal chromosome behavior during meiosis with occasionally 1-2 univalents and a multivalent. This is also true for tetraploids from Africa, but some of the tetraploids from S. E. Asia and Australia show extensive abnormality in the meiosis of microsporogenesis, characterized by frequent multivalents. Aneuploids with $2n$ = 36, 38 and 39 were also found among tetraploids from Hongkong, Bangkok and Ceylon (Plate XIV). A tetraploid from Hongkong (10625) had a chain of 19-20 chromosomes in several cells, whereas associations of eight chromosomes were quite frequent in Australian tetraploids. These abnormalities are often associated with almost 100 per cent pollen sterility, and in such plants the mode of reproduction is essentially obligate apomixis. Sexuality among the tetraploids is maintained where cytologically balanced gametes are formed, and this, in a facultatively apomictic population, readily leads to higher levels of ploidy as in Africa and, especially, in Australia. The hexaploids from Africa were, more or less, normal in cytological behavior with several cells in each plant studied showing 30 bivalents. A hexaploid from Rhodesia (9453) showed completely normal meiosis. On the other hand, both pentaploids and hexaploids from Australia were quite irregular in their chromosome associations characterized by a number of univalents and multivalents. The cytology of this variety is summarized in Table X.

Dichanthium intermedium var. grahamii (Haines) de Wet et Harlan

This variety is of hybrid origin involving D. intermedium and D. annulatum, and according to de Wet and Harlan (n.d.) can be easily recognized by its oblong-lanceolate spikelets with scattered long hairs along the margins and near the apex of the lower glume. The lower inflorescence branches are about as long as the primary axis, and are sometimes moderately divided.

In distribution, the variety is quite common in the Gangetic plain of India, but also sporadically present in Africa, Pakistan, central India, South-East Asia and Australia (Plate XI).

The mode of reproduction is by facultative apomixis. Races with 2n = 40, 50 and 60 chromosomes were found. Cytological studies indicate that in India and Pakistan tetraploid as well as hexaploid races are present, in Africa only tetraploids are found, and in Australia tetraploids, pentaploids and hexaploids, all three races, are present. The cytology of the pentaploids was reported by Faruqui (1964). These were listed as A. Nos. 50, 52 and 5803-b, all from Australia. Most of the tetraploids showed almost normal cytological behavior, however, a certain degree of irregularity always accompanied the pentaploids and hexaploids as well as some tetraploids (Plate XIV). The cytology is summarized in Table XI.

PLATE XI

Distribution of *Dichanthium intermedium* var. *grahamii*

Each dot represents an area where collections were made.

PLATE XI

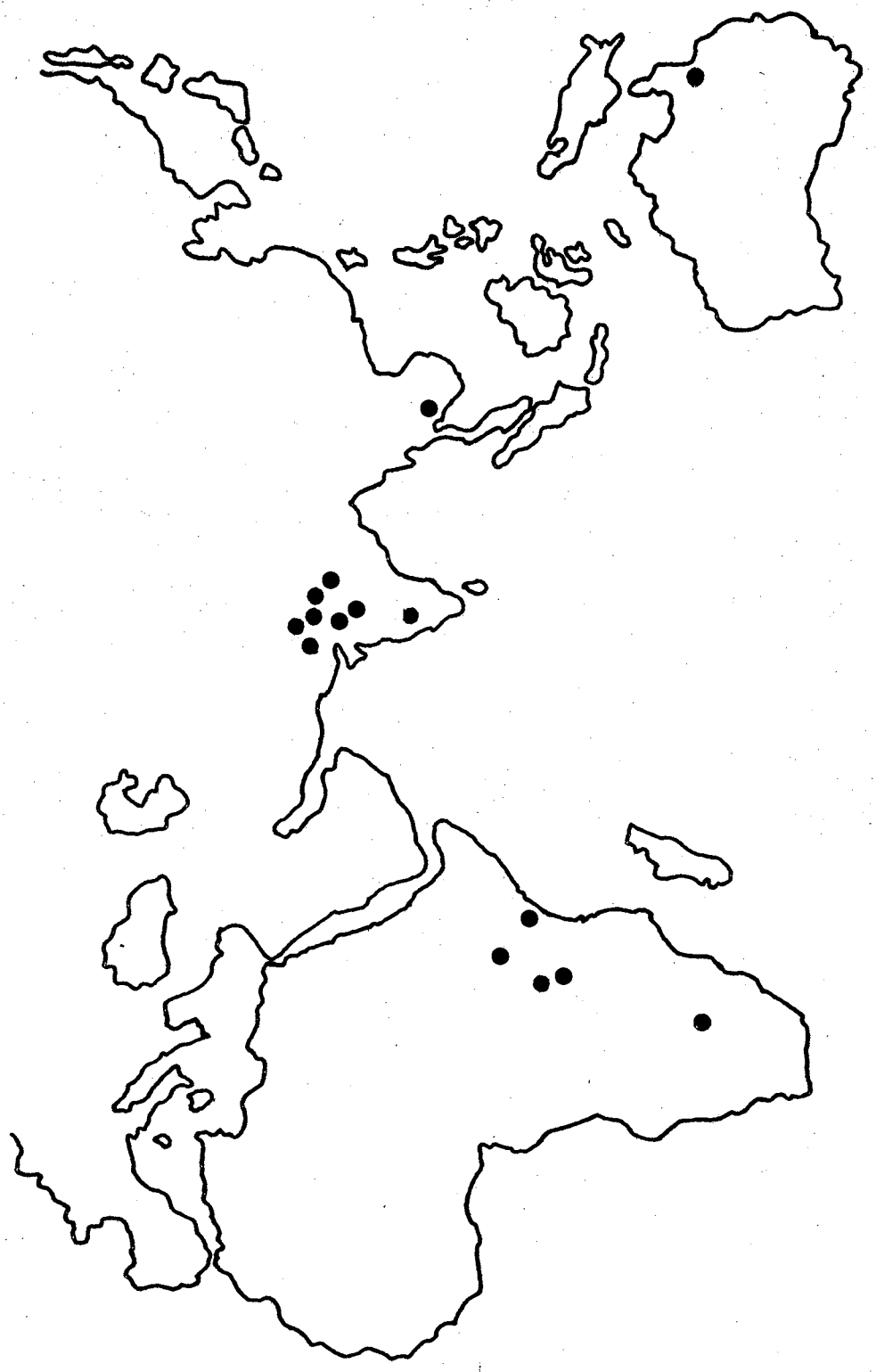


TABLE XI

CYTOLOGY OF DICHANTHIUM INTERMEDIUM VAR. GRAHAMII

A.no.	Locality	Diploid Chromosome Number	Chromosome Association at Meiotic Metaphase*			
			I	II	III	IV
9504	Lupani, Rhodesia	40	0.8 0-4	19.6 18-20		
9505	Lupani, Rhodesia	40	1.8 0-1	14.6 12-16	0.6 0-1	3.0 2-3
10568	Zanzibar, Tanzania	40	2.2 2-4	18.9 18-20		
8863	Rawalpindi, W. Pakistan	40	1.0 0-4	19.5 18-20		
8864	Rawalpindi, W. Pakistan	40	0.6 0-2	19.7 19-20		
8873-b	Parachinar, W. Pakistan	40	1.71 0-4	18.85 18-20		
8945	Rawalpindi, W. Pakistan	40	0.6 0-2	19.7 19-20		
8946	Rawalpindi, W. Pakistan	40	---	20		
8946-a	Rawalpindi, W. Pakistan	40	---	20		
8948	Jehum, W. Pakistan	40	---	20		

TABLE XI (CONTINUED)

A.no.	Locality	Diploid Chromosome Number	Chromosome Association at Meiotic Metaphase*			
			I	II	III	IV
8955	Parachinar, W. Pakistan	40	---	20		
8955-a	Parachinar, W. Pakistan	40	0.8 0-4	19.6 18-20		
8861	Nothal, W. Pakistan	60	1.7 0-4	27.4 22-30	---	1.7 0.3
8949	Gujarat, India	40	---	20		
8950	Gujarat, India	40	---	20		
8950-b	Gujarat, India	40	---	20		
8951	Gujarat, India	40	---	20		
8951-b	Gujarat, India	40	8.6 4-16	12.4 9-14	1.5 0-3	
8960-a	Dalhousie, India	40	1.6 0-4	19.2 18-20		
9026	New Delhi, India	40	---	20		
9064	Nasik, India	40	2.0 0-4	19.0 18-20		

TABLE XI (CONTINUED)

A.no.	Locality	Diploid Chromosome Number	Chromosome Association at Meiotic Metaphase*			
			I	II	III	IV
9064-b	Nasik, India	40	1.33 0-4	19.33 18-20		
9115	Mt. Abu, India	40	1.0 0-1	12.0 12-16	1.0 0-1	3.0 2-3
8976-b	Aligarh, India	60	4.0 2-6	28.0 27-29		
10603	Bangkok, Thailand	40	4.6 2-6	18.7 16-19	0 0	0 0
10789	Townsville, Australia	60	9.2 8-12	19.3 16-22	---	3.0 2.4

*Average number and range of various configurations are listed.
I = univalents; II = bivalent; III = trivalents; IV = quadrivalent

Dichanthium intermedium var. indicum de Wet et Harlan

This variety, in the sense of de Wet and Harlan (n.d.) is a hybrid between D. intermedium var. grahamii and D. ischaemum var. ischaemum, and differs from the latter parent by the somewhat elongated primary axis of the inflorescence, and from its other parent by more lanceolate spikelets which are very sparsely hairy above the middle.

This variety is mainly confined to disturbed areas of the mountains of West Pakistan, Kashmir and northwestern India (Plate XII).

The mode of reproduction is by facultative apomixis, and only two races are known with $2n = 40$ and 60 chromosomes. Chromosome association during microsporogenesis is comparatively regular (Table XII). Univalents and multivalents are present in some cells of both tetraploids and hexaploids (Plate XV), but with most of the plants producing cytologically balanced and functional gametes, the variety flourishes both sexually as well as through agamospermous apomixis. As reported by Harlan (1963), however, there is a low frequency of sexual reproduction in one such hybrid population growing in areas disturbed by man along the foothills of northern West Pakistan.

Dichanthium intermedium var. montanum de Wet et Harlan

According to de Wet and Harlan (n.d.) this variety is a product of hybridization between D. intermedium var. intermedium and D. ischaemum var. ischaemum. In morphological

PLATE XII

Distribution of *Dichanthium intermedium* var. *indicum*

Each dot represents an area where collections were made.

PLATE XII

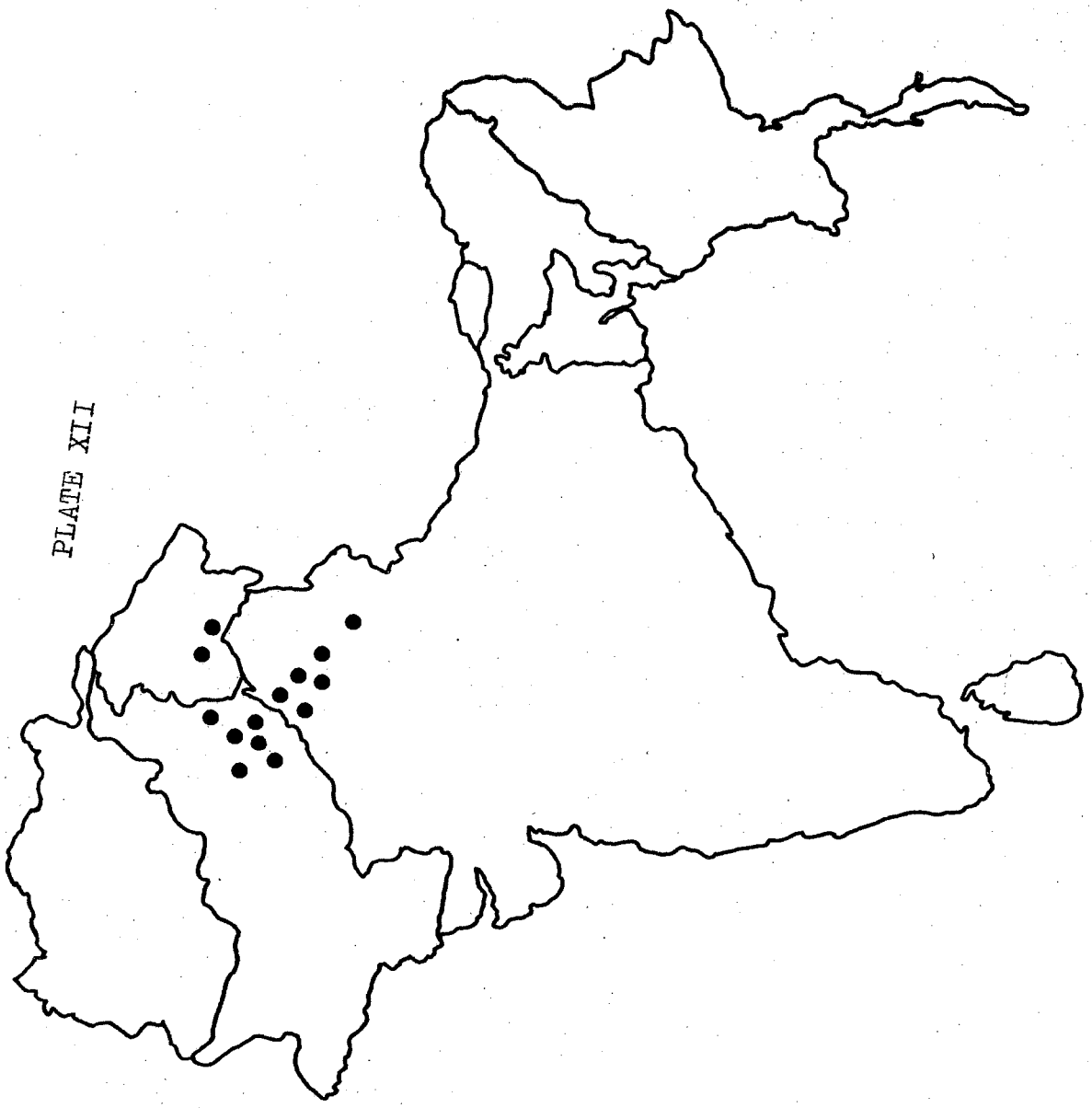


TABLE XII

CYTOLOGY OF DICHANTHIUM INTERMEDIUM VAR. INDICUM

A. No.	Locality	Diploid Chromosome Number	Chromosome Association at Meiotic Metaphase*					
			I	II	III	IV	V	VI
8957	Udampur, India	40	0.8 0-2	19.6 19-20				
8958	Kud, W. Pakistan	40	0.8 0-2	19.6 19-20				
4634	Sargodha, W. Pakistan	40	1.0 0-4	17.28 14-20	0.04 0-1	1.08 0-3		
8873-c	Parachinar, W. Pakistan	40	2.6 0-4	18.8 18-20				
8874	Parachinar, W. Pakistan	40	0.8 0-2	19.6 19-20				
8943	Thal, W. Pakistan	40	0.85 0-2	19.57 19-20				
9889-a	West Pakistan	40	5.5 0-10	16.9 15-20	0.1 0-1	0.07 0-1		
8944	Rawalpindi, W. Pakistan	60	2.2 0-6	27.14 25-30	----	0.85 0-2		
8947	Rawalpindi, W. Pakistan	60	1.7 0-4	27.87 24-30	----	0.62 0.2		

TABLE XII (CONTINUED)

A. No.	Locality	Diploid Chromosome Number	Chromosome Association at Meiotic Metaphase*					
			I	II	III	IV	V	VI
8952	Sargodha, W. Pakistan	60	3.0 2-4	27.5 25-29	----	0.5 0-2		
8953	Sargodha, W. Pakistan	60	4.0 2-6	26.5 24-29	----	0.75 0-2		
8955-b	Parachinar, W. Pakistan	60	1.25 0-4	28.37 26-30	----	0.25 0-1		
8955-c	Parachinar, W. Pakistan	60	0.8 0-2	28.0 26-30	----	0.8 0-2		
8959	Kud, W. Pakistan	60	3.0 2-4	28.5 28-29	----	----		
8875-b	Hargu, W. Pakistan	60	3.3 2-4	27.0 26-28	----	0.66 0-1		
8899	Rawalpindi, W. Pakistan	60	3.0 0-6	28.5 27-30	----	----		
8900	Rawalpindi, W. Pakistan	60	6.8 4-12	25.0 23-28	----	0.57 0-1		
8973	Kalka, India	40	1.33 0-4	19.33 18-20				

TABLE XII (CONTINUED)

A. No.	Locality	Diploid Chromosome Number	Chromosome Association at Meiotic Metaphase*					
			I	II	III	IV	V	VI
8974	Kalka, India	40	0.8 0-2	19.6 19-20				
8975	Kalka, India	40	----	20				
8975-b	Kalka, India	40	1.0 0-2	19.5 19-20				
8942	Hanzei, Kashmir	40	6.2 4-8	14.6 12-18	2.6 2-4			
8942	Hanzei, Kashmir	60	1.7 0-6	25.7 23-27	----	1.3 0-3	---	0.2 0-1

*Average number and range of various configurations are listed.
I = univalents; II = bivalent; III = trivalents; IV = quadrivalent; V = pentavalent; VI = hexavalent

features it resembles the latter species in having the pedicels and rachis strongly pilose, but differs from it in having somewhat elongated inflorescences with the primary axis at least as long as the lower branches. The available specimens have pitted sessile spikelets, and the leaf-blades as well as sheaths are somewhat hairy.

In reproductive behavior, plants belonging to this variety are facultatively apomictic. They are distributed in the foothills of northwestern India, and possibly eastward, at least, to Burma (Plate XIII). This variety was also collected from Taiwan, therefore, it may be present sporadically in S. E. Asia.

Three cytological races of this variety are known with $2n = 40$, 50 and 60. Almost all tetraploids have the majority of the cells showing regular chromosome association and normal meiosis during microsporogenesis. There were, however, a few cells showing irregular behavior of chromosomes with 2-4 univalents, and rarely a multivalent. The pentaploids and hexaploids were more irregular showing 2-7 and 2-13 univalents in each cell, respectively (Plate XV). In both pentaploids and hexaploids 0-2 multivalents were present (Table XIII).

With regard to the general mode of reproduction in the entire compilospecies D. intermedium and the species contributing to its variability, embryological studies showed that the diploids were fully sexual, tetraploids were facultatively apomictic, whereas pentaploids and hexaploids

PLATE XIII

Distribution of *Dichanthium intermedium* var. *montanum*

Each dot represents an area where collections were made.

PLATE XIII

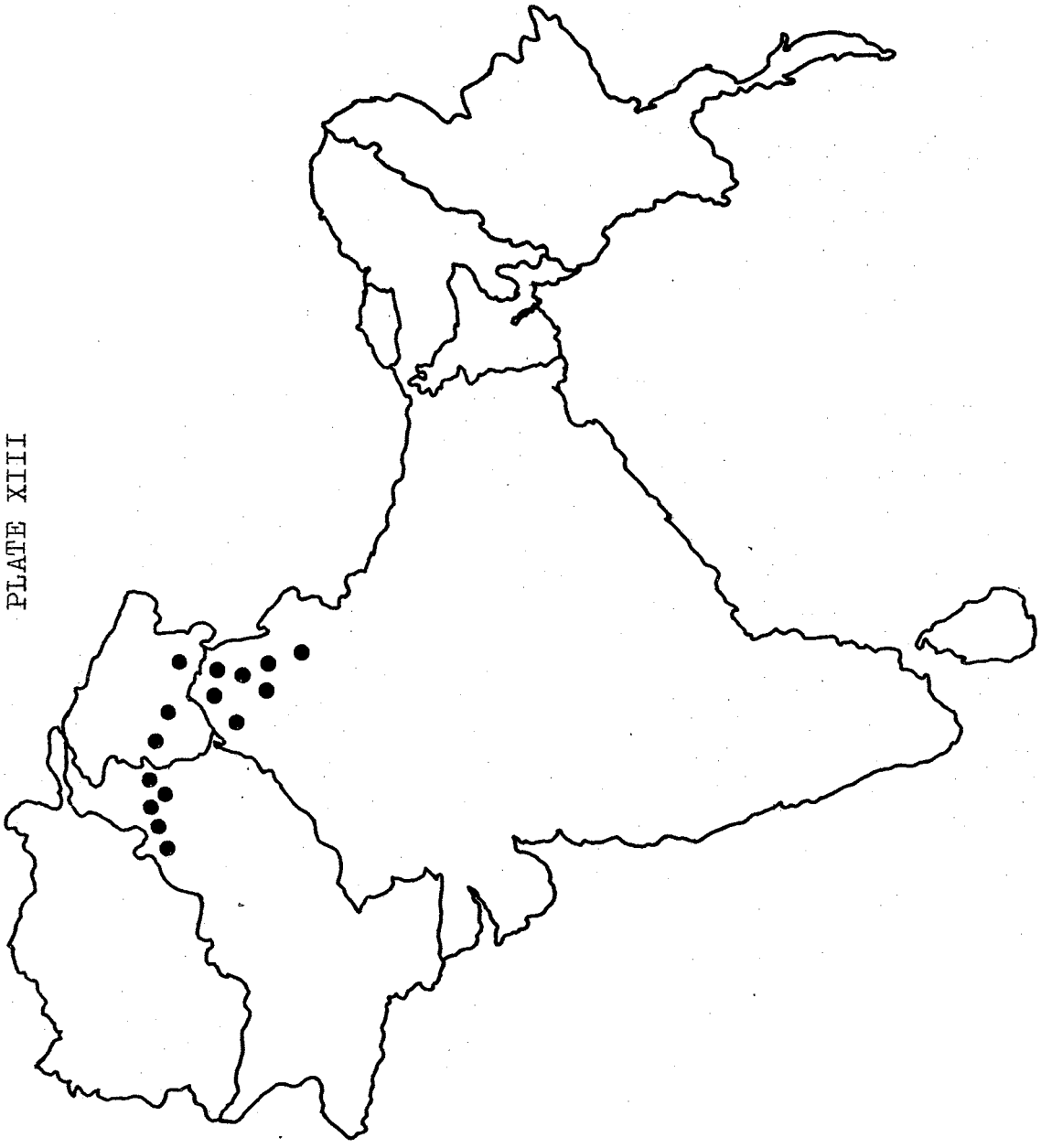


TABLE XIII

CYTOLOGY OF DICHANTHIUM INTERMEDIUM VAR. MONTANUM

A. No.	Locality	Diploid Chromosome Number	Chromosome Association at Meiotic Metaphase*			
			I	II	III	IV
8875	Hangu, W. Pakistan	40	0.5 0-2	19.7 19-20		
8902-b	Murree, W. Pakistan	40	1.11 0-2	19.44 19-20		
8904-c	Murree, W. Pakistan	40	0.8 0-2	19.6 19-20		
8873	Parachinar, W. Pakistan	50	4.1 2-7	20.3 16-22	----	0.8 0-2
8964	Joginder Nagar, India	40	0.84 0-4	18.56 16-20	2.24 0-4	
8964	Joginder Nagar, India	40	0.28 0-2	19.57 19-20		0.14 0-1
8965	Manali, India	40	----	20		
8965-a	Manali, India	40	----	20		
8969-b	Manali, India	40	1.78 0-6	19.0 17-20		

TABLE XIII (CONTINUED)

A. No.	Locality	Diploid Chromosome Number	Chromosome Association at Meiotic Metaphase*			
			I	II	III	IV
8971	Simla, India	40	----	20		
8977	Dehra Dun, India	40	1.25 0-4	19.37 18-20		
8983	Udampur, India	40	0.8 0-2	19.6 19-20		
8983-a	Udampur, India	40	0.8 0-2	19.6 19-20		
9108	Delhi, India	60	5.80 2-13	26.50 22-27	2.36 0-4	

*Average number and range of various configurations are listed.
I = univalents; II = bivalent; III = trivalents; IV = quadrivalent

PLATE XIV

Cytology of the Varieties of *Dichanthium intermedium*. All figures ca. X1350.

Legend:

Figure 1. Metaphase I Showing 20 Bivalents in *D. intermedium* var. *intermedium* ($2n = 40$)

Figure 2. Anaphase I in *D. intermedium* var. *intermedium* ($2n = 40$)

Figure 3. Metaphase I Showing Eight Univalents, 14 Bivalents and One Tetravalent in *D. intermedium* var. *australis* ($2n = 40$)

Figure 4. Anaphase I in *D. intermedium* var. *australis* ($2n = 40$)

Figure 5. Metaphase I in *D. intermedium* var. *glabrum* ($2n = 60$) Showing 30 Bivalents

Figure 6. Metaphase I Showing Two Univalents and 18 Bivalents in an Aneuploid *D. intermedium* var. *glabrum* ($2n = 38$)

Figure 7. Metaphase I in *D. intermedium* var. *grahamii* ($2n = 40$) Showing 18 Bivalents and A Tetravalent

Figure 8. Metaphase I Showing One Univalent, 12 Bivalents, One Trivalent and Three Tetravalents in *D. intermedium* var. *grahamii* ($2n = 40$)

Figure 9. Metaphase I Showing 16 Univalents, Nine Bivalents and Two Trivalents in *D. intermedium* var. *grahamii* ($2n = 40$)

Figure 10. Metaphase I Showing Six Univalents and 17 Bivalents in *D. intermedium* var. *grahamii* ($2n = 40$)

PLATE XIV

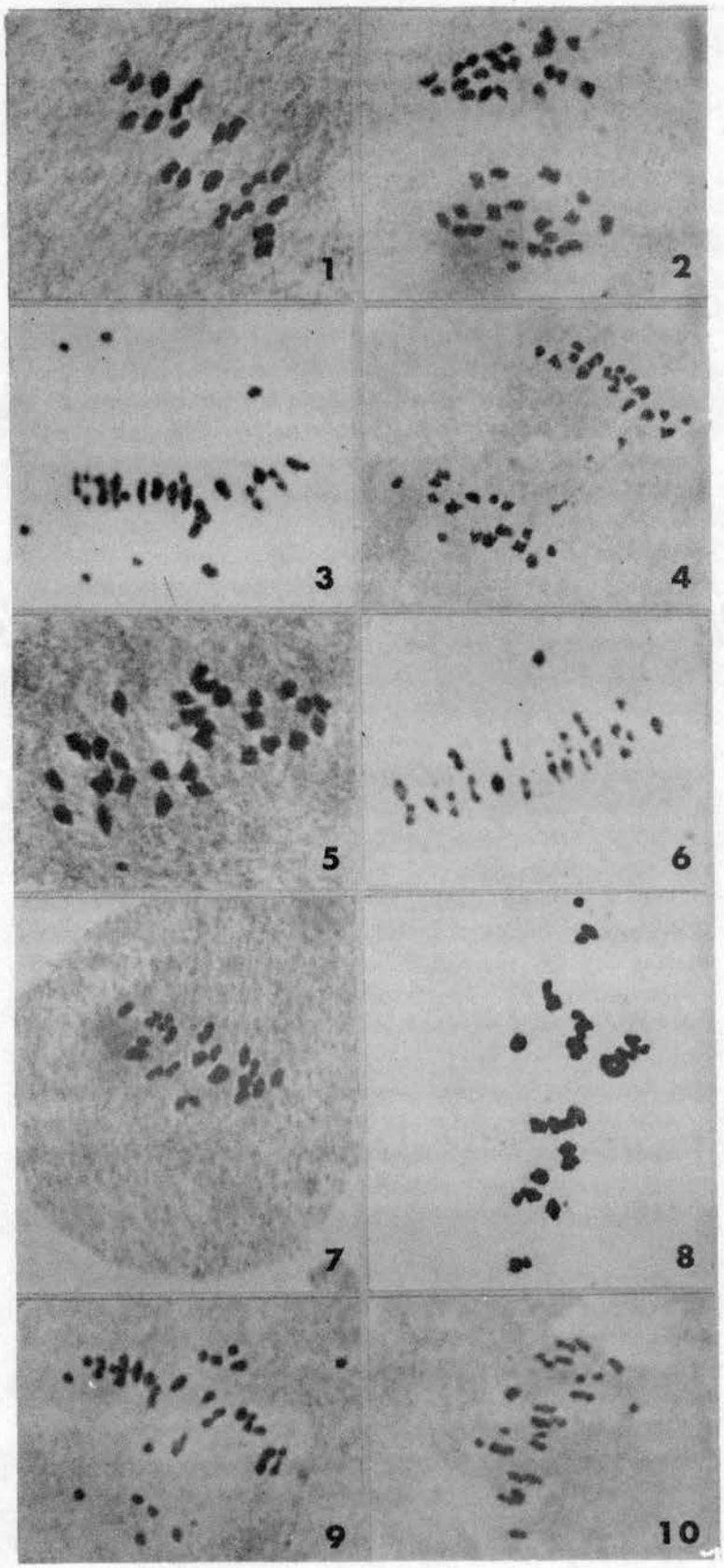


PLATE XV

Cytology of the Varieties of *Dichanthium intermedium* (continued). All figures ca. X1350.

Legend:

Figure 11. Metaphase I Showing Eight Univalents, Ten Bivalents and Four Trivalents in *D. intermedium* var. *indicum* ($2n = 40$)

Figure 12. Anaphase I Showing Laggards and Dividing Univalents in *D. intermedium* var. *indicum* ($2n = 40$)

Figure 13. Metaphase I Showing Two Univalents and 19 Bivalents in *D. intermedium* var. *indicum* ($2n = 40$)

Figure 14. Anaphase I in *D. intermedium* var. *indicum* ($2n = 40$)

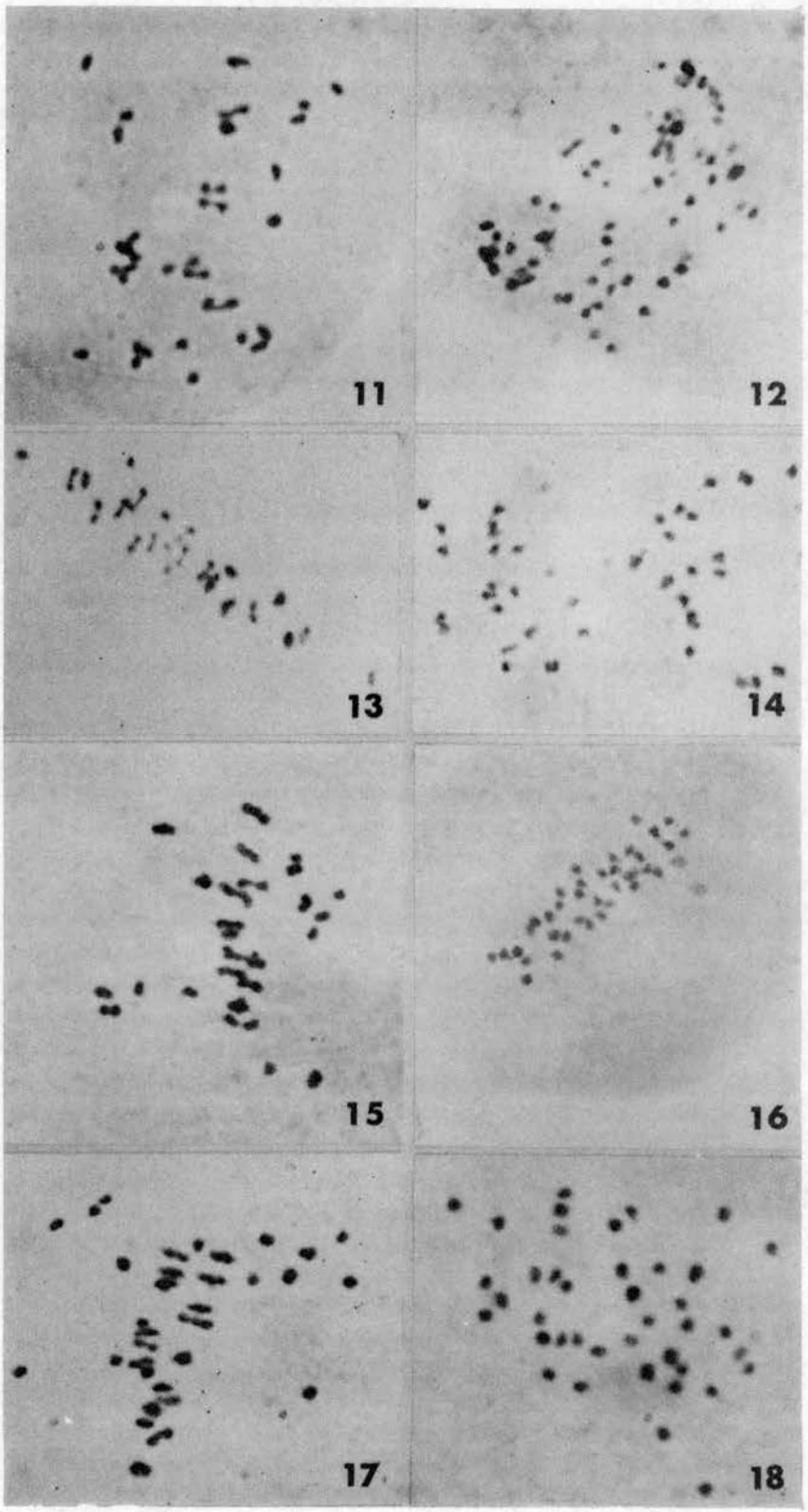
Figure 15. Metaphase I Showing Six Univalents, 16 Bivalents and Four Trivalents in *D. intermedium* var. *montanum* ($2n = 50$)

Figure 16. Anaphase I in *D. intermedium* var. *montanum* ($2n = 40$)

Figure 17. Metaphase I Showing Six Univalents, 21 Bivalents and Four Trivalents in *D. intermedium* var. *montanum* ($2n = 60$)

Figure 18. Anaphase I in *D. intermedium* var. *montanum* ($2n = 50$) With Ten Bivalents Showing Non-Disjunction

PLATE XV



were largely apomictic. The embryological studies revealed further that obligate apomicts, probably, do not occur at all. Some small degree of sexuality may be present at all levels of ploidy, and may pass unnoticed in breeding behavior leading to wrong conclusions.

Classification and the mode of origin of different varieties of Dichanthium intermedium have been summarized in Plate XVI.

Phylogenetic Survey of the Genus Dichanthium and the Status of the Compilosecies Dichanthium intermedium

Only those species will be discussed that have a direct bearing on the composition of the present day germplasm of Dichanthium intermedium.

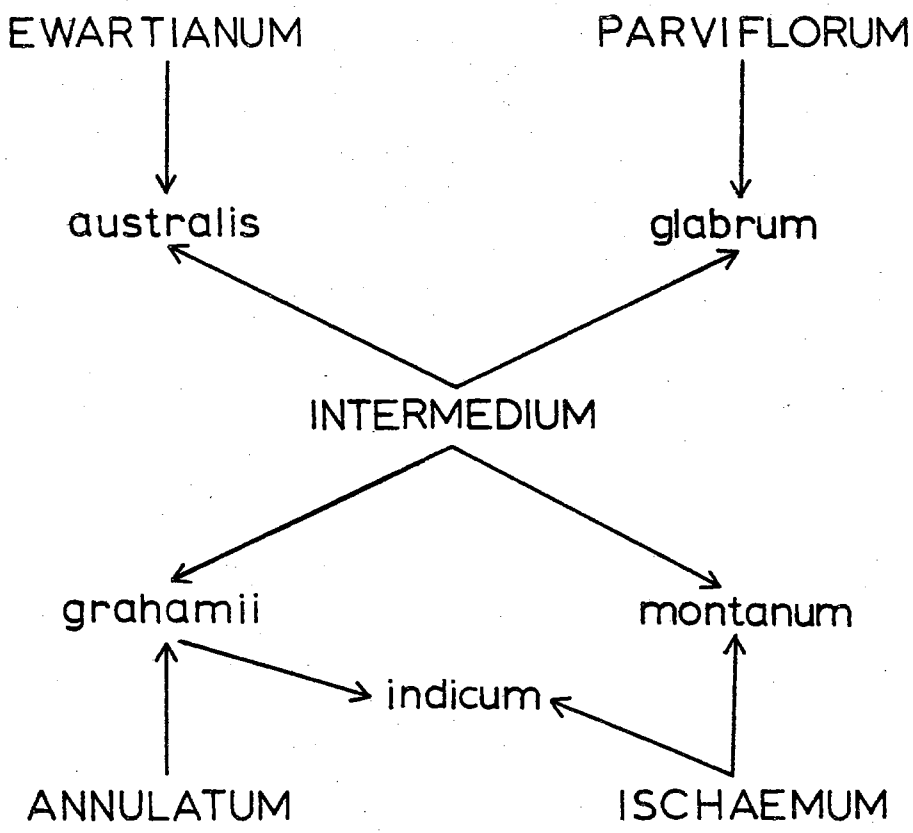
The majority of species included in the complex genus Dichanthium are polyploids, a few are diploids, and some are characterized by cytological races with different levels of ploidy (de Wet, Borgaonkar and Richardson, 1963).

The diploids are relatively rare, endemic and restricted to stable habitats. They are genetically isolated and hundreds of attempts made to cross the diploids in any combination, completely failed (de Wet and Singh, 1964). Of the twenty-three diploids known in the generic complex, only five bear any relationship with the compilospecies D. intermedium. These are; D. annulatum, D. caricosum, D. aristatum, D. assimle, and D. parviflorum, each with a diploid and a

PLATE XVI

Classification of the Contributing Species and Their Hybrids

PLATE XVI


EWARTIANUM
PARVIFLORUM
australis
glabrum
INTERMEDIUM
grahamii
montanum
indicum
ANNULATUM
ISCHAEMUM

tetraploid race. Tetraploid races, probably, arose as a result of fusion of two unreduced gametes. Therefore, in genome constitution, the tetraploids range from true autoploids to well defined segmental allopolyploids. Once the tetraploids had originated, further evolution was augmented by agamospermous apomixis providing an alternate pathway for seed production. Contact with sexuality was insured by a diploidizing gene complex leading to cytologically balanced and functional gametes. Moreover, polyploidy helped to buffer the genotype against the shock of alien germplasm accommodation. Thus, the genetic raw materials for the emergence of a compilospecies were assembled at the tetraploid level.

The two diploids of the section Capillipedium, D. assimile and D. parviflorum are genetically isolated. Hybrids involving tetraploid races of these two species are quite common in nature. The hybrids are largely apomictic, and resemble D. parviflorum morphologically except for retaining the bamboo-like habit of D. assimile.

The remaining three diploids belong to the section Dichanthium and are D. annulatum, D. caricosum, and D. aristatum. At the diploid level these species are genetically isolated from each other. However, at the tetraploid level, D. caricosum crosses in nature and under experimental conditions with D. annulatum and D. aristatum, while the last two mentioned species could not be crossed experimentally.

The species of the section Bothriochloa, directly associated with the compilospecies are D. ewartianum and D. ischaemum. These two species have no diploid races, and, as already mentioned, they are represented by tetraploid, pentaploid and hexaploid races. They are geographically isolated, D. ewartianum being confined to Australia and D. ischaenum to Eurasia.

The central species, D. intermedium of the section Bothriochloa has no diploid race, and exists today as tetraploids, pentaploids and hexaploids. Faruqui (1964) suggested a close ancestral affinity between the diploid B. longifolia and B. intermedia (syn. D. intermedium) on the basis of radially arranged epidermal cells, but this has not been substantiated by other morphological features and genetic evidence. Therefore, the mode of origin and relationship of the original tetraploid D. intermedium remain obscure. However, the original tetraploid may have represented relicts of an ancient species that combined genetic material of the ancestral complexes from which the present day sections of Dichanthium were derived.

The section Capillipedium is primarily Southern-Asian in distribution with only D. parviflorum extending to Africa and Australia. Over its complete range of distribution, D. parviflorum introgresses with D. intermedium. In India and South-East Asia, D. assimile and D. parviflorum are sympatric and some degree of introgressive hybridization between them takes place. The question as to whether these

last two mentioned species are phylogenetically distinct can only be solved when section Capillipedium has been studied in more detail. Hundreds of attempted crosses between the diploid sexual races of these two species failed to produce a single hybrid. The few hybrids obtained when tetraploids were crossed reproduced apomictically, and always combined the complete female genome of D. parviflorum with a haploid male of D. assimile.

The three species of the section Dichanthium, D. annulatum, D. caricosum, and D. aristatum occupy identical open habitats, and are sympatric in tropical and subtropical southern Asia with D. annulatum extending to Africa and Australia. Hybrids could not be produced between D. aristatum and D. annulatum. On the other hand, D. caricosum crossed with both D. annulatum and D. aristatum. When hybrids which combined genomes of D. annulatum and D. caricosum were crossed with ones genetically intermediate between D. caricosum and D. aristatum, the resulting offspring were completely sterile. Not even apomixis could function when genetic material of D. annulatum and D. aristatum were combined, suggesting that the genomes of these two species are only distantly related. Detailed cytological studies further revealed that in hybrids involving either D. annulatum or D. aristatum with D. caricosum as a common denominator, the chromosomes always paired preferentially within genomes derived from each parental species. This suggested that the genomes of the species which do cross are

only distantly related to each other, and functional gametes are produced only because of preferential pairing. The three species D. annulatum, D. aristatum and D. caricosum therefore appear to be phylogenetically distinct.

The two species from the section Bothriochloa, D. ewartianum and D. ischaemum are almost completely isolated genetically from species of the sections Dichanthium and Capillipedium, as well as from each other. The only hybrid produced, resulted from the fertilization of a cytologically unreduced 2n = 50 chromosome female gamete of D. ischaemum with a normal n = 20 male gamete of D. annulatum. This plant was completely sterile. The species D. intermedium of the section Bothriochloa is the only common denominator between these species, and it can cross with members from any section to form fertile offspring. However, hybrids that combine genomes of D. intermedium and of any other two contributing species are always completely sterile, suggesting that these are truly contributing species, and not species that have originated from the D. intermedium complex. If members of D. intermedium were completely removed from the phylogenetic scene, the three sections Dichanthium, Bothriochloa and Capillipedium would have represented valid groups of species of generic rank, morphologically distinct, and genetically isolated. But the actual picture is immensely complicated due to genetic aggressiveness and central position of D. intermedium which hybridizes, in nature and under experimental conditions, with members from

each section, and stands like a triradiate link joining Dichanthium, Bothriochloa and Capillipedium. The present day D. intermedium, as was demonstrated by Harlan and de Wet (1963a), is a taxonomic nightmare. It is difficult to decide what the original tetraploid that initiated the hybridization looked like. Comparative morphological studies suggested that it must have had an undivided raceme arranged on an elongated primary axis. Such plants are scattered in wet habitats throughout the tropical regions of the distribution range of D. intermedium, extending almost continuously from southern Africa to Australia. As was demonstrated for D. ischaemum, by Harlan (1963), agriculture probably played a major role in disturbing the habitat and bringing the other contributing species into contact with D. intermedium. The contributing species as well as the relicts of D. intermedium are mainly tetraploids, and range in genetic constitution from autoploids to well defined segmental allopolyploids, as demonstrated by extensive cytological studies. The original hybrids with D. intermedium approach alloploids in genetic constitution. Artificially produced hybrids as well as assumed natural hybrids are characterized by chromosome pairing within genomes derived from each parental species. Although univalents and sometimes multivalents were also observed in some developing microspore mother cells, functional and cytologically balanced gametes were produced in all hybrids. Being facultatively apomictic and adapted to disturbed habitats,

these hybrids are today widely distributed, and for the greater part characterized respectively by the unity of their morphological type. Introgression with either parent takes place, but usually not too extensively.

From a phylogenetic point of view, therefore, the assumed basic tetraploid within the D. intermedium complex is as good a biological species as the contributing species. The hybrids between D. intermedium and the contributing species are essentially allopolyploids, as shown by their chromosome behavior, and deserve distinct ranks of taxonomic species. However, as the relict B. intermedia (syn. D. intermedium) is being genetically swamped, and introgressive hybridization in all possible combinations slowly overcoming sterility barriers between the hybrids, the suggestion of Harlan and de Wet (1963a) to combine all hybrids into a compilospecies seems the most logical solution.

The compilospecies Dichanthium intermedium occupies today a central yet highly labile and dynamic status in the genus Dichanthium. Aided by polyploidy, gene controlled preferential pairing of chromosomes during meiosis, facultatively agamospermous apomixis and introgressive hybridization, the compilospecies has succeeded in absorbing germplasm from its relatives by breaking down species barriers. It has developed a system of evolution which is rapid, highly adaptable and capable of a high degree of adaptive polymorphism, but as pointed out by Harlan and de Wet (1963a) this system is better designed for the destruction of

species than production of new ones. The future of this compilospecies is difficult to predict, but, it spells probably a continued taxonomic catastrophe for Dichanthium.

CHAPTER IX

SUMMARY

To study megagametophyte development and early embryogeny in Dichanthium, the aceto-carmine squash technique for ovules was found much more convenient and reliable than sectioning of paraffin-embedded material. A saturated aqueous solution of ferric chloride, added to the fixative, acted as a mordant, and provided excellent staining of the embryo sac nuclei.

Studies on mode of reproduction by aceto-carmine squash of ovules showed that natural biotypes of tetraploid Dichanthium intermedium are facultatively apomictic. In many ovules, mature sexual as well as aposporous embryo sacs persisted side-by-side, and no evidence was found for any crushing or crowding out of the sexual sac by the aposporous sacs. One of the hybrids produced by crossing such facultative apomicts, and designated as X-750, appeared to be wholly sexual in breeding behavior. Detailed studies, however, demonstrated that X-750 had early rudiments of aposporous sacs, but they degenerated before reaching maturity. Therefore, only the sexual sac functioned.

The sexual embryo sacs were observed to have a weak spot towards the micropylar end. In squash preparations,

this spot appeared as an opening. It was suggested that the weak spot might have a selective advantage for facilitating sexual reproduction in facultative apomicts. Such a weak spot was totally lacking in aposporous sacs.

Experiments conducted under controlled conditions, demonstrated that light regime had a definite control over incidence of apomixis in D. intermedium. A continuous photoperiod of 14 hours caused an early degeneration of aposporous sacs in D. intermedium (X-750). At a continuous photoperiod of 12 hours there was a significant increase in the incidence of aposporous sacs, and a majority of these sacs reached maturity. Under 12 hours day-length apomictic seed set, as well as sexual seed production were obtained, indicating that the mode of reproduction in the compilospecies D. intermedium could be controlled by the environment.

Cytogenetic studies demonstrated that considerable cytological and genetic differentiation had taken place between D. intermedium and D. fecundum. Syncytes were found in one of the hybrids involving these two species. It was likely that the syncytes developed due to the failure of cytokinesis in archesporial divisions.

A survey of the trends of evolution in the compilospecies D. intermedium, based on morphology, cytogeography, and reproductive mechanisms, demonstrated that two main features influenced the course of evolution of this compilospecies. These are, buffering of the genotype by polyploidy which permitted a wide range for hybridization, and

environmental control of reproductive behavior leading to geographical variation due to increase or decrease of sexuality.

BIBLIOGRAPHY

Akerberg, E. 1942. "Cytogenetic Studies in Poa pratensis and its Hybrid with Poa alpina." Hereditas, 28: 1-126.

Baker, H. G. 1959. "Reproductive Methods as Factors in Speciation in Flowering Plants." Cold Spring Harb. Sym. Quant. Biol., 24: 177-191.

Blake, S. T. 1944. "Monographic Studies in Australian Andropogoneae." Univ. Queensl. Papers Biol. , 2: 1-62.

Böcher, T. W. 1951. "Cytological and Embryological Studies in the Amphi-Apomictic Arabis holboellii Complex." Danske Videnskab. Selskab. Biol. Skrifter, 6: 1-59.

Bonner, J., E. Heftmann, and J. A. D. Zeevaart. 1963. "Suppression of Floral Induction by Inhibitors of Steriod Biosynthesis." Plant Physiol., 38: 81-88.

Bor, N. L. 1960. The Grasses of Burma, Ceylon, India and Pakistan. Pergamon Press, Oxford.

Borgaonkar, D. S., J. R. Harlan, and J. M. J. de Wet. 1962. "Cytogenetical Study of Hybrids Between Dichanthium annulatum and D. fecundum. II." Proc. Okla. Acad. Sci., 42: 13-16.

Borgaonkar, D. S., and A. P. Singh. 1962. "Species Relationships in Dichanthium. IV. D. annulatum and D. papillosum." Phyton, 19: 101-107.

Borgaonkar, D. S., and J. M. J. de Wet. 1964. "Cytological Studies of Hybrids Between Dichanthium annulatum and D. fecundum. III." Phyton, 21: 50-60.

Bradley, M. V. 1948. "An Aceto-Carmine Squash Technique for Mature Embryo Sacs." Stain Tech., 23: 29-40.

Brooks, M. H. 1958. "A Study of the Reproductive Mechanisms in Certain Species of Bothriochloa and Dichanthium Complexes." (unpub. Ph. D. thesis, Oklahoma State University., Stillwater).

Brown, W. V., and W. H. P. Emery. 1957. "Apomixis in the Gramineae, Tribe Andropogoneae: Themeda triandra and Bothriochloa ischaemum." Bot. Gaz., 118: 246-253.

Brown, W. V., and W. H. P. Emery. 1958. "Apomixis in the Gramineae: Panicoideae." Amer. J. Bot., 45: 249-263.

Bünning, E. 1956. "Endogenous Rhythms." Ann. Rev. Plant Phys., 7: 71-90.

Celarier, R. P. 1956. "Tertiary Butyl Alcohol Dehydration of Chromosome Smears." Stain Tech., 31: 155-157.

Celarier, R. P. 1957. "The Cytogeography of the Bothriochloa ischaemum Complex. II. Chromosome Behavior." Amer. J. Bot., 44: 729-738.

Celarier, R. P., and J. R. Harlan. 1956. "An Andropogoneae Garden in Oklahoma." Taxon, 5: 183-186.

Celarier, R. P., and J. R. Harlan. 1957. "Apomixis in Bothriochloa, Dichanthium and Capillipedium." Phytomorph., 7: 93-102.

Chailakhian, M. Kh. 1961. "Principle of Ontogenesis and Physiology of Flowering in Higher Plants." Can. J. Botany, 39: 1817-1845.

Chheda, H. R., and J. R. Harlan. 1962. "A Cytogenetical Study of Introgeneric Hybrids Between Bothriochloa intermedia and Dichanthium fecundum." Cytologia, 27: 418-423.

Clausen, J. 1954. "Partial Apomixis as an Equilibrium System in Evolution." Caryologia, 1: 469-479.

Clausen, J., and W. M. Hiesey. 1958. Experimental Studies on Nature of Species. IV. Genetic Structure of Ecological Races. Carnegie Inst. Washington Publ. No. 615.

Cooper, J. P. 1959. "Selection and Population Structure in Lolium. I. The Initial Populations." Heredity, 13: 317-340.

Cooper, J. P. 1963. "Species and Population Differences in Climatic Response." In Environmental Control of Plant Growth. (ed. L. T. Evans) Academic Press, New York and London.

Davis, P. H., and V. H. Heywood. 1965. Principles of Angiosperm Taxonomy. D. Van. Nostrand Co., Inc., New York.

de Wet, J. M. J., D. S. Borgaonkar and H. R. Chheda. 1961. "Intergeneric Hybrids in the Bothriochloininae. II. Bothriochloa and Capillipedium." Cytologia, 26: 268-273.

de Wet, J. M. J., K. L. Mehra and D. S. Borgaonkar. 1961. "Chromosome Association in Dichanthium Hybrids." Cytologia, 26: 78-82.

de Wet, J. M. J., and D. S. Borgaonkar. 1963. "Aneuploidy and Apomixis in Bothriochloa and Dichanthium." Bot. Gaz., 124: 437-440.

de Wet, J. M. J., D. S. Borgaonkar and W. L. Richardson. 1963. "Chromosome Number and Mode of Reproduction in Bothriochloininae." Caryologia, 16: 47-55.

de Wet, J. M. J., and A. P. Singh. 1964. "Species Relationships in Dichanthium. V. The Diploid Species." Caryologia, 17: 153-160.

de Wet, J. M. J., and J. R. Harlan. 1966. "Morphology of the Compilospecies Bothriochloa intermedia." Amer. J. Bot., 53: 94-98.

de Wet, J. M. J., and J. R. Harlan. (n.d.). Taxonomy of Dichanthium intermedium (Gramineae).

Diboll, A. G., and D. A. Larson. 1966. "An Electron Microscopic Study of the Mature Megagametophyte in Zea mays." Amer. J. Bot., 53: 391-402.

Esau, K. 1946. "Morphology of Reproduction in Guayule and Certain Other Species of Parthenium." Hilgardia, 17: 61-120.

Faruqui, S. A. 1964. "Cytogenetical Studies of the Bothrichloa intermedia Complex." Cytologia, 29: 280-297.

Finch, L. R., and D. J. Carr. 1956. "Nucleic Acid Content of Petkus Rye Embryos in Relation to Vernalization and Devernalization." Australian J. Biol. Sci., 9: 355-363.

Fryxell, P. A. 1957. "Mode of Reproduction of Higher Plants." Bot. Rev., 23: 135-233.

Garner, W. W., and H. A. Allard. 1920. "Effect of Relative Length of Day and Night and Other Factors of Environment on Growth and Reproduction in Plants." J. Agric. Res., 18: 553-606.

Gassner, G. 1918. "Beiträge Zur Physiologischen Charakteristik Sommer-Und Winterannueller Gewächse, Insbesondere Der Getreidepflangen." Z. Bot., 10: 407-430.

Gifford, E. M., and H. B. Tepper. 1961. "Ontogeny of the Inflorescence in Chenopodium album." Amer. J. Bot., 48: 657.

Gifford, E. M., and H. B. Tepper. 1962. "Histochemical and Autoradiographic Studies of Floral Induction in Chenopodium album." Amer. J. Bot., 49: 706-714.

Grazi, F., M. Unaerus and E. Akerberg. 1961. "Observations in the Mode of Reproduction and the Embryology of Poa prantensis." Hereditas, 47: 489-541.

Grif, V. G. 1958. Plant Physiol. (U.S.S.R.) (English Translation, 5: 532).

Gustafsson, A. 1946. "Apomixis in Higher Plants. I. The Mechanism of Apomixis." Lund Univ. Arsskr., 42: 1-66.

Hair, J. B. 1956. "Subsexual Reproduction in Agropyron." Heredity, 10: 129-159.

Harlan, J. R. 1963. "Natural Introgression Between Bothriochloa ischaemum and B. intermedia in West Pakistan." Bot. Gaz., 124: 294-300.

Harlan, J. R., and J. M. J. de Wet. 1963a. "Role of Apomixis in the Evolution of the Bothriochloa-Dichanthium Complex." Crop Sci., 3: 314-316.

Harlan, J. R., and J. M. J. de Wet. 1963b. "The Compilospecies Concept." Evolution, 17: 497-501.

Harlan, J. R., H. R. Chheda, and W. L. Richardson. 1962. "Range of Hybridization with Bothriochloa intermedia." Crop Sci., 2: 480-483.

Harlan, J. R., R. P. Celarier, W. L. Richardson, M. H. Brooks and K. L. Mehra. 1958. "Studies on Old World Bluestems. II." Oklahoma Agric. Exp. Sta. Tech. Bull. T-72.

Harlan, J. R., J. M. J. de Wet, W. L. Richardson and H. R. Chheda. 1961. "Studies on Old World Bluestems. III." Okla. Agric. Exp. Sta. Tech. Bull. T-92.

Harlan, J. R., M. H. Brooks, D. S. Borgaonkar and J. M. J. de Wet. 1964. "Nature of Inheritance of Apomixis in Bothriochloa and Dichanthium." Bot. Gaz., 125: 41-46.

Hendricks, S. B. 1956. "Control of Growth and Reproduction by Light and Darkness." Amer. Scientist, 44: 229-247.

Hendricks, S. B. 1960. "Rates of Change of Phytochrome as an Essential Factor Determining Photoperiodism in Plants." Cold Spring Harb. Symp. Quant. Biol., 25: 245-256.

Hendricks, S. B., and Borthwick. 1963. "Control of Plant Growth by Light." In Environmental Control of Plant Growth. (ed. L. T. Evans) Academic Press, New York and London.

Heslop-Harrison, J. 1957a. "The Experimental Modification of Sex Expression in Flowering Plants." Biol. Rev., 32: 38-90.

Heslop-Harrison, J. 1957b. "The Physiology of Reproduction in Dactylorchis. I." Botan. Notiser, 110: 28-49.

Heslop-Harrison, J. 1959. "Growth Substances and Flower Morphogenesis." J. Linn. Soc. (Bot.), 65: 269-281.

Heslop-Harrison, J. 1961. "Apomixis, Environment and Adaptation." Rec. Adv. Bot., 891-895.

Hess, D. 1961. "Die Beteiligung Spezifischer Ribonucleinsäure an der Blükinduktion." Planta, 57: 13-28.

Hiesey, W. M. 1953. "Growth and Development of Species and Hybrids of Poa Under Controlled Temperature." Amer. J. Bot., 40: 205-216.

Hiesey, W. M., and H. W. Milner. 1965. "Physiology of Ecological Races and Species." Ann. Rev. Plant Phys., 16: 203-216.

Hjelmqvist, H., and F. Grazi. 1964. "Studies on Variation in Embryo Sac Development." Botan. Notiser, 117: 141-166.

Holmes. W. L., and N. W. DiTullio. 1962. "Inhibitors of Cholestrol Biosynthesis Which Act at or Beyond the mevalonic Acid Stage." Am. J. Clin. Nutrition, 10: 310-322.

Huang, R. C., and J. Bonner. 1962. "Histone, A Suppressor of Chromosomal RNA Synthesis." Proc. Natl. Acad. Sci. U. S., 48: 1216-1222.

Khush, G. S., and G. L. Stebbins. 1961. "Cytogenetic and Evolutionary Studies in Secale. I. Some New Data on the Ancestry of S. cereale." Amer. J. Bot., 48: 723-730.

Kiellander, C. L. 1937. "On the Embryological Basis of Apomixis in Poa palustris." Sv. Bot. Tidskr., 31: 425-429.

Klebs, G. 1918. "Über die Blütenbildung von Sempervivum." Flora, 128-151.

Knox, R. B. 1967. "Apomixis: Seasonal and Population Differences in a Grass." Science, 157: 325-326.

Knox, R. B., and J. Heslop-Harrison. 1963. "Experimental Control of Aposporous Apomixis in a Grass of the Andropogoneae." Botaniska Notiser, 116: 127-141.

Lang, A. 1957. "The Effects of Gibberellin Upon Flower Formation." Proc. Natl. Acad. Sci. (Wash.), 43: 285-300.

Lang, A., and E. Reinhard. 1961. "Gibberellins and Flower Formation." Advances in Chemistry, Series 28: 71-79.

Lewis, K. R., and B. John. 1963. Chromosome Marker. Little, Brown and Co., Boston.

Maheshwari, P. 1950. An Introduction to the Embryology of Angiosperms. McGraw-Hill Book Co., New York, Toronto and London.

Maxwell, C. D., and D. H. Valentine. 1966. "A Dissection Technique for Embryo Sacs." New Phytol., 65: 75-76.

Mehra, K. L. 1962. "The Dichanthium annulatum Complex. I. Morphology." Phyton, 18: 87-94.

Melchers, G. 1961. In Handbuch der Pflanzenphysiologie. (ed. W. Ruhland). Vol. XVI, Springer, Berlin.

Nygren, A. 1949. "Apomictic and Sexual Reproduction in Calamagrostis purpurea." Hereditas, 35: 285-300.

Nygren, A. 1951. "Embryology of Poa." Carnegie Inst. Washington Year Book, 50: 113-115.

Olmstead, C. E. 1944. "Photoperiodic Responses in Twelve Geographic Strains of Side-Oats Grama." Bot. Gaz., 106: 46-74.

Price, S. 1956. "Cytological Studies in Saccharum and Allied Genera. I. Syncytes in Certain Clones of Saccharum and Erianthus." Cytologia, 21: 21-37.

Richardson, W. L. 1958. "A Technique of Emasculating Small Grass Florets." Indian J. Genet. Plt. Breed., 18: 69-73.

Rodrigues, Pereira. 1961. "Flower Initiation in Excised Stem Disks of Wedgwood Iris." Science, 134: 2044-2045.

Salisbury, F. B. 1963. The Flowering Process. The Macmillan Company, New York.

Saran, Sunil, and J. M. J. de Wet. 1966. "A Rapid Squash Method for Grass Embryology." Proc. Okla. Acad. Sci., 46: 17-19.

Saran, Sunil, and J. M. J. de Wet. 1967. "Embryo Sac Squash Studies in Dichanthium." Amer. J. Bot., 54: 658 (abstr.).

Searle, N. E. 1961. "Persistence of Transport of Flowering Stimulus in Xanthium." Plant Physsiol., 36: 656-662.

Snyder, L. A., A. R. Hernandez and H. E. Warmke. 1955. "The Mechanism of Apomixis in Pennisetum ciliare." Bot. Gaz., 116: 209-221.

Stebbins, G. L. 1947. "Apomixis in the Angiosperms." Bot. Rev., 7: 507-542.

Stebbins, G. L. 1950. Variation and Evolution in Plants. Columbia Univ. Press.

Steward, F. C. 1963. "Effects of Environment on Metabolic Patterns." In Environmental Control of Plant Growth. (ed. L. T. Evans) Academic Press, New York and London.

Takimoto, A., and K. Ikeda. 1960. "Studies on Light Controlling Flower Initiation of Pharbitis nil. VI. Effect of Natural Twilight." Bot. Mag. Tokyo, 73: 175-181.

Tashima, Y., and S. Imamura. 1953. Proc. Japan Acad., 29: 581.

Vickery, J. W. 1961. "Flora of New South Wales. I. Gramineae." Contr. N. S. W. Nat. Herb., 19: 1-124.

Warmke, H. E. 1954. "Apomixis in Panicum maximum." Amer. J. Bot., 41: 5-11.

Wellensiek, S. J. 1961. "Leaf Vernalization." Nature, 192: 1097-1098.

Went, F. W. 1928. "Wuchsstoff und Wachstum." Rec. Trav. Bot. Neerl., 25: 1-116.

Went, F. W. 1957. The Experimental Control of Plant Growth. Chronica Botanica Co., Waltham, Mass.

Whyte, R. O. 1960. Crop Production and Environment. Faber and Faber, London.

Zatyko, J. M. 1962. "Parthenocarpy and Apomixis in the Ribes Genus Induced by Gibberellic Acid." Naturwissenschaften, 49: 212-213.

Zatyko, J. M. 1963. "Parthenocarpy Induced in Sterile Ribes Species by Gibberellic Acid." Naturwissenschaften, 50: 230-231.

Zeevaart, A. D. 1962. "Physiology of Flowering." Science, 137: 723.

Zeevaart, A. D. 1963. "Climatic Control of Reproductive Development." In Environmental Control of Plant Growth. (ed. L. T. Evans) Academic Press, New York and London.

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