Chromosome Cytology and Arboreta:  
A Marriage of Convenience

In this paper I would like to review the role cytology as a discipline, and cytologists, the practitioners of that discipline, play, or should play, in a botanical garden or arboretum. My thesis is that cytologists can play an important role in a botanical garden in fostering a better knowledge of the biology of the plants that are grown there, and in providing information of use for the breeding of new varieties, and that a botanical garden, all other things being equal, is a good place for a cytologist to be because he can benefit greatly from the use of a collection of living plants. Therefore my title: it is to the advantage of both parties to associate.

Let us start first by restricting the term cytology. Strictly speaking, cytology is the study of the cell. As a modern discipline cytology arose with the development of good quality compound microscopes in the second half of the 19th century. The great German botanist Edward Strassburger is the first outstanding cytologist, who by discovering chromosomes and karyokinesis, drew attention to the phenomena taking place in the nucleus of the cell. Soon after the rediscovery of the Mendelian laws of inheritance, W. S. Sutton drew attention to the similarity of the phenomena that occur in the cell nucleus during cell division and the predicted behavior of the genes. It was, as we all know, the American geneticist Thomas Morgan and his brilliant collaborators and students, Hermann Müller, C. B. Bridges, and Alfred H. Sturtevant, who demonstrated that indeed the chromosomes are the carriers of the genes. This led to a concentration of interest in the nucleus and the chromosomes on the side of cytologists, almost to the exclusion of every other aspect. In the classical textbook on cytology by A. J. Sharp, that dominated the scene in the United States for almost twenty years from 1932 on, two-thirds of its pages are dedicated to the nucleus and chromosomes, while C. D. Darlington's classical work, Recent Advances in Cytology, from about the same time is entirely concerned with chromosomes in spite of its title.
The equation of chromosome cytology with cytology comes from that time. Today the emphasis has shifted back to the cytoplasm with fantastic results as any of you who have followed the literature in cell biology knows. Nevertheless, when we speak of cytology in this paper, we are going to refer exclusively to chromosomal cytology.

Soon after chromosomes were discovered by Strassburger, it was noticed that their number was constant in the nucleus of all cells but for the nucleus of the gametes and cells of the gametophyte where it was exactly one-half. It also was soon noted that different species had different chromosome numbers in their nuclei. This fact was not let go unnoticed by taxonomists who saw the possibility of finding a constant, non-arbitrary character for use in classification. Alas, as we all know, chromosome number per se cannot provide a firm criterion of classification because related species often have the same chromosome number, and in a few cases, different populations of what on other criteria have to be considered the same species have different chromosome numbers. But the possibility that they might be the key to a non-arbitrary system of classification led to a great deal of work in the interface between cytology and taxonomy, what today is called cytotaxonomy.

I will not attempt to review the history of cytotaxonomy, but very briefly and very appropriately, point out the role that some members of the staff of the Arnold Arboretum played in the development of the field.

Although chromosomes do not provide absolutely reliable characters, as carriers of the genes, they can provide valuable information regarding the nature of the evolutionary process and the phylogeny of the species under study. Even when two species have the same number of chromosomes, they may differ in their shape and size. But even in those cases where the chromosomes are alike in their gross structure, they may differ in the internal content and arrangement of the genes. That species possess a unique individuality in their somatic chromosomes in respect to size, shape, position of centromeres and secondary constrictions and genic content was established in the 1920's by a number of workers in Russia, Europe and the United States, such as Navashin, Delaunay, Levitsky, Goodspeed, Darlington, Babcock and others. During this early period arose the concept of the karyotype, which can be defined as the phenotypic appearance of the somatic chromosomes, in contrast to their genic contents. Every species or group of closely related species has a unique karyotype, which is modified by natural
selection during the course of evolution. Consequently the more similar the karyotypes of two species, the more related they are. What are the ways a karyotype can evolve? It can evolve in a variety of ways, the most important of which are (1) change in basic number (aneuploidy); (2) duplication of all chromosomes (polyploidy); (3) change in size and (4) change in shape. During evolution one or more of these changes can and do occur, either simultaneously or consecutively, leading to the great variety of karyotypes.

One of the most unique karyotypes is that possessed by some members of the family Agavaceae, particularly the genera Agave and Yucca. These genera have five pairs of relatively large chromosomes with mostly subterminal centromeres and 25 smaller ones. This was pointed out first by J. O'Mara, Susan McKelvey and Karl Sax in 1933 (O'Mara, 1932; McKelvey and Sax, 1933) when they were on the staff of the Arnold Arboretum. At the time these genera were considered to belong to two different families: Yucca to the Liliaceae and Agave to the Amaryllidaceae. Their unique and identical karyotypes, as well as their morphological similarity, led to a reclassification of the group and the erection of the family Agavaceae by Hutchinson.

Polyploidy, that is the existence in related species of chromosome numbers that are multiples of each other, was one of the earliest cytological characteristics to be studied. An example is furnished by the genus Triticum, the cultivated wheat.

The earliest chromosome counts for any species of wheat are those of Overton who in 1893 reports $n = 8$ for Triticum vulgare. This is followed by a number of authors (Körnicke, 1896; Dudley, 1908; Nakao, 1911) all reporting the same erroneous number. The first accurate count is by Karl Sax (1918) who reports $2n = 28$ for Triticum durum. At about the same time, Sakamura (1918) reports the now well known polyploid series of $n = 7$ for T. monococcum, $n = 14$ for T. dicoccum, T. durum, T. polonicum and T. turgidum, and $n = 21$ for T. spelta, T. vulgare and T. compactum. Since Sakamura's paper did not contain illustrations, its results were not immediately accepted. It was Sax (1921, 1922) who in a series of very fine papers established definitely that (1) the cultivated species of wheat can be divided into three definite groups according to their sterility relationships in interspecific crosses, (2) that each of the three groups is characterized by a unique chromosome number, the three forming a polyploid series on the base of $x = 7$, and (3) classified all cultivated species into either the einkorn, emmer or vulgare group, according to their chromosome number and
crossing relationships. The discovery of the polyploid series of wheat is a very important step in the development of cytology, since it showed clearly how cytology could aid in unravelling the phylogenetic history of a group.

Finally, an example of aneuploidy with important taxonomic implications is that of Verbena investigated by Haig Dermen of the cytological laboratory of the Arnold Arboretum in the 1930's. On the basis of an analysis of the chromosome number of 25 taxa of this genus, Dermen was able to establish the existence of two basic chromosome numbers: \( n = 5 \) and \( n = 7 \). He also established the existence of polyploidy within each of the two groups. Species with \( n = 7 \) have in general smaller flowers, the flowers are borne in spikes and they never have a glandular appendage to the anthers. Species with \( n = 5 \) have larger flowers, borne in cymes and often have glandular appendages. The \( n = 7 \) species correspond to the section Verbenaca, while \( n = 5 \) species belong to section Glandularia. Hybridization studies by Dermen, as well as by later authors (Schnack, 1971; Solbrig, 1968) have shown that species of different sections cannot be hybridized while species of the same section can. This and other evidence has led Schnack and Covas (1944) to erect the section Glandularia into a separate genus.

Chromosomal cytology is an invaluable addition to the arsenal of techniques and approaches at the disposal of the botanist who is interested in unravelling the past history of plants, as this very brief review hopefully has shown. We may ask ourselves, however, whether this is a valid activity for a botanical garden or arboretum. My feeling is that indeed it is. Let us see how it fits into the framework of a garden or arboretum.

The three main activities of a botanical garden can be listed as being (1) the cultivation of a large number of species of plants, both foreign and domestic, for the education and enjoyment of the public; (2) the introduction of new species and varieties of plants; and (3) research on cultivated plants and their relatives. Although many gardens restrict their activities to certain groups of plants (for example, the Arnold Arboretum restricts its activities to woody plants), every major garden comprises all three of the mentioned activities. We may then ask what kind of research should be of first priority for a botanical garden. Here it is harder to obtain complete agreement, but if we accept as a valid criterion that first priority should go to activities that will increase our understanding of the relationships of plants, in order to be able to best further a program of
introduction and cultivation, then systematics in a broad sense, including cytotaxonomy, is a valid research activity for a botanical garden. That this is a valid assumption is attested by the fact that many of the great centers of systematic activity of the world (Paris, Kew, Edinburgh, Missouri, and my own institution, Harvard) are part of, or have a botanical garden associated with it, and that most botanical gardens large and small engage in some kind of systematic research. Using the same criterion, another activity that should be placed high in the list of research priorities is plant physiology and physiological ecology, but curiously enough, research in plant physiology and physiological ecology has not been pursued by botanical gardens with the same intensity as systematic research.

If it is accepted that research in systematics is a valid activity for a botanical garden, then there is no problem in justifying cytological research, as it is widely accepted that cytotaxonomy is an integral part of modern systematics. Many arboreta and botanical gardens maintain active laboratories in cytology. The Arnold Arboretum maintained such a laboratory for some 30 years (1928–1959) under the direction of Professor Karl Sax. Kew Gardens in England has the Jodrell Laboratories with a very active cytological group under the direction of Dr. Keith Jones; in Denmark, the botanical gardens there have an active group working on cytology and cytotaxonomy under the direction of Dr. Tyge Böcher. The Botanical Gardens of the University of California started cytological work shortly after their inception in 1908, when Dr. Thomas Goodspeed joined the staff; that tradition is being maintained today by Drs. Herbert Baker and Robert Ornduff and their collaborators. These are but a few examples that show that it is valid to say that cytology should have a high priority in the research activities of botanical gardens.

But not only do cytologists aid in systematic research, they play a very important role in plant breeding. Dr. Santamour is going to refer to this phase in more detail, so I only will mention the example of the Arnold Arboretum and the extensive work on hybridization and improvement done by Karl and Holly Sax with crab apples and other members of the family Rosaceae, and with the genus Syringa, the lilacs, work which is documented in a long list of papers that appeared in the Journal of the Arnold Arboretum, and in the present day living collections of the Arboretum.

Having established that cytology can be of great aid in systematic research and that systematic research is a valid ac-
tivity for a botanical garden, I would like now to address myself to some of the important unanswered questions within cytology that can be best researched in a laboratory associated with a botanical garden.

The most important general area still open within chromosomal cytology is no doubt the architecture and biochemical composition of the chromosome and the events of mitosis and meiosis. Although we know a great deal regarding the gross morphology of chromosomes at one end, and we know the atomic structure of DNA, we know very little regarding the fine structure of the chromosome. The ordinary electron microscope has been of no help in this respect, but the scanning electron microscope and the imaginative use of physico-chemical and biochemical techniques may still succeed in unravelling the secrets of the chromosome. Although the question of the nature of the chromosome and the events of mitosis and meiosis is the most important unanswered question in the field of chromosome cytology, it is not one that should have first priority in a laboratory associated with a botanical garden or arboretum. The reason that I feel so is that it is a kind of research that does not require nor takes advantage of the richness and diversity of the living collection, while on the other hand, making great demands of resources and expertise in electron microscopy, biochemistry and biophysics which are not likely to be found in a botanical garden. The kind of cytological research that can be best pursued in a botanical garden or arboretum is that which takes advantage of the diversity of plant collections that exist in a botanical garden. What are some of these activities?

First there is still a great deal of routine inventorying of chromosome numbers to be done. At present we have established the chromosome number of only approximately ten percent of all vascular plants. Furthermore, while our knowledge is fairly good for certain groups, such as ferns and Compositae, it is almost nonexistent in other groups. But routine surveys, although useful, are not the most efficient way of acquiring knowledge, nor the most imaginative way of spending one's time. Whenever possible, such surveys should be done in conjunction with systematic investigations, in order to be able to interpret the meaning of the results in an evolutionary framework. Furthermore, such investigations, whenever possible, should not be restricted to chromosome number alone but should include investigations of other aspects of the karyotype as well. A good
example of what can be done is furnished by the collaborative activity of my associate, Mrs. Lily Rüdenberg, and Mr. Peter Green, a former staff member of the Arnold Arboretum, in their work on the genus *Lonicera*.

The Arnold Arboretum possesses an unusually rich collection of species and varieties of *Lonicera*. The genus was monographed by Alfred Rehder, who was head curator of the herbarium of the Arnold Arboretum for many years. Rehder worked with the collection of *Lonicera* and was responsible for many of its identifications. The collection is therefore not only unusually rich in taxa, but they are in excellent taxonomic order. Mrs. Rüdenberg with the taxonomic assistance of Mr. Green systematically established the chromosome number for every growing shrub in the collection, a total of over 100 taxa. She established that the basic chromosome number in the genus is nine, and that the majority of species are diploid. They also established that many of the varieties and cultivars of diploid species are tetraploid. Mrs. Rüdenberg was also able to establish that the chromosomes of *Lonicera* have interesting heterochromatic areas, a phenomenon which she is still investigating.

Once enough information has been gathered from a routine survey, a number of interesting higher-level questions appear which I feel are one of the most challenging areas to be investigated. Let me illustrate this point with an example.

Almost fifteen years ago, Dr. Peter Raven and I decided to initiate a survey of the chromosome numbers of the family Compositae. In this enterprise we were joined by a number of colleagues, and in the intervening years we have published over one thousand counts, including first counts for many species and genera (Raven et al., 1960; Raven and Kyhos, 1961; Ornduff et al., 1963; Payne, Raven and Kyhos, 1964; Solbrig et al., 1964; Ornduff et al., 1967; Solbrig et al., 1969, 1972).

As a result of our work, and a similar parallel survey being undertaken by Dr. B. L. Turner and collaborators, as well as many other reports in the literature, approximately 25 percent of all species in this large family of flowering plants have been counted. Figure 1 shows a summary of the results obtained to date. Several aspects are revealed in the figure. First we see that the chromosome numbers in the Compositae are not randomly distributed but that they follow a very definite pattern, with a mode of nine and a more or less lognormal distribution. From this we concluded that nine is probably the ancestral chromosome number, although this of course is only a probability statement inferred from the evidence at hand. But there are
Figs. 1 and 2: Frequency of species with different chromosome numbers by habit in Compositae and Leguminosae, respectively.
other interesting observations that can be made. A very significant one is the correlation between habit and chromosome number. For the family as a whole as well as within each tribe, annual herbs have as a group a lower chromosome number than perennial herbs, which in turn have a lower chromosome number than shrubs or trees, although the sample number for this latter category is so low as to render this statement statistically suspect for the family Compositae. Finally the distribution of chromosome numbers is not uniform throughout the family, but certain tribes such as Cichorieae and Astereae have more species with lower chromosome numbers as well as lower numbers in an absolute sense (n = 2 for Astereae, n = 3 for Cichorieae) than Heliantheae (n = 5). From this information a number of interesting questions arise, such as the following: (1) Why do annual herbs have a lower chromosome number than shrubs and trees? (2) Why do different tribes have a different distribution of chromosome numbers? (3) What is the significance of a “basic” chromosome number? The answers to these and similar questions are among the still unresolved aspects of cytology. They involve the role of chromosomes as regulators of recombination, and the past history and geographical distribution of the various tribes (Solbrig, et al, 1964; Solbrig, 1972).

We can go a step further and compare the Compositae with other families. I have made such a comparison with the published chromosome numbers for the family Leguminosae (Solbrig, 1972). Figure 2 shows the distribution of chromosome numbers in that family. We can see that in this entirely unrelated family we again observe that annual plants as a group have lower chromosome numbers than perennial herbs, which in turn have lower numbers than shrubs and trees. This leads me to believe that we are dealing with a phenomenon that is general to the plant kingdom. The explanation of the reason of the correlation between habit and chromosome number is to be found in the functioning of the recombination system. Annual plants have of course shorter generation times and consequently a higher recombination index, all other things being equal, than populations of species with longer generations. A way to compensate for this higher rate of recombination is to increase genetic linkage by lowering the number of chromosomes. Although this explanation is highly plausible (Grant, 1958; Solbrig, 1972), more experimental and observational evidence is still needed.

Another observation that can be made from Figure 2 is that the modal chromosome number of Leguminosae and Com-
positae is different, as well as the distribution of numbers in the two families, although the general form of the curve is similar. So far this difference has been attributed always to a more or less mythical "phylogenetic component", but that non-explanation is to me very unsatisfying. I believe that as more information is accumulated for more families, more satisfying hypotheses based on firm foundations taken from genetical, ecological and evolutionary theory will be forthcoming. This is one of the still unresolved areas, and one which is very appropriate for a cytological laboratory connected with a botanical garden or arboretum to pursue. Only where a great variety of plants grow can this work be undertaken.

In summary, then, chromosome cytology and botanical gardens are a marriage of convenience. For the institution the existence of a cytological laboratory will ensure that active research will be done with the collection, research that is interesting in itself and significant and of general interest in terms of botanical science as a whole. Furthermore, it is research which has direct bearing on systematics and plant breeding, two aspects that are very central to the research activities of any botanical garden or arboretum. For the cytologist, the existence of living plants that can be monitored and studied throughout the year, as well as the presence of representatives of many genera and families, and the possibility of cultivating under expert care those organisms that are of particular interest to the researcher offer unique possibilities, with which most of us dream, but seldom see realized. The cytological laboratory of the Arnold Arboretum, which has counted over the years such outstanding researchers as Karl and Holly Sax, Edgar Anderson, Dermen and more recently, Joab Thomas, and their numerous students like Carl Swanson, Allan Conger, and Arnold Sparrow to name just a few, is a fine example of the role a cytological laboratory can play in a botanical garden. We wish it an equal or greater success in its second century.

Otto T. Solbrig
Department of Biology
and Gray Herbarium
Harvard University
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