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Chromosomal Variation and Evolution

Polyploidy and chromosome size and number shed light on evolutionary processes in higher plants.

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The comprehensive, many-sided monograph of the genus *Crepis* which Ernest Babcock completed almost 20 years ago (*1*) gave us a model of the kind of exploratory study which can bring to our attention major problems concerning the processes of evolution in higher plants. In this article I shall reconsider three problems which Babcock called to our attention. These are phylogenetic reduction in chromosome size, phylogenetic reduction in chromosome number, and polyploidy. The wealth of information about biological and evolutionary problems which has accumulated since Babcock's book was published has provided new insight into the nature of these problems and has given rise to hypotheses which may lead to reasonable solutions to them. Furthermore, if these solutions are correct, they constitute important evidence from cytogenetics concerning that most mystifying and elusive of botanical problems, the origin and early evolution of the angiosperms.

Chromosome Size

Babcock (*1*, pp. 11–12) pointed out that with respect to the total length of the chromosomal complement at metaphase, the species of *Crepis* which on morphological grounds appear to be the most primitive have the largest chromosomes, and the smallest chromosomes are found in certain specialized annual species with reduced life cycles. The ratio between total chromosomal lengths is about 5 to 1. Although Babcock did not calculate the total volume of the chromosomes, his illustrations show that the shorter chromosomes of the annual species are also narrower than those of the perennials. Consequently, with respect to volume the difference between the species is probably even greater than that in length.

This phylogenetic reduction in chromosomal volume has always been difficult to reconcile with the fact that deletions of even a small chromosomal segment are lethal or severely detrimental to a homozygous organism. It becomes even harder to understand in the light of the now recognized fact that the stainable component of the chromosomes is largely DNA. Furthermore, Sparrow and Evans (*2*) have demonstrated a high degree of correlation in angiosperms between nuclear volume and DNA content. Hence the fact that among species of *Crepis* there is a good correlation between total chromosome length at metaphase and interphase-nuclear volume indicates strongly that the specialized, short-lived annuals of this genus have undergone a reduction in their amounts of nuclear DNA. Does this mean that genes have been lost during the evolution of the more advanced species of *Crepis*? If so, how have these species managed to survive such a loss?

The first clue to understanding this problem can be obtained from a comparison of DNA content in the nuclei in various organisms. Sparrow and Evans have made such a comparison, using their own data on higher plants along with those of Sinsheimer (*3*) and others on animals and microorganisms. These data, somewhat rearranged, are presented in Table 1. They show that the DNA content of viruses and bacteria is less than 1 percent of that found in cells of higher animals and plants and that unicellular algae, as well as primitive Metazoa such as sponges and coelenterates, contain considerably less than do vascular plants and vertebrate animals. Although these data need to be supplemented by a large number of additional observations, they point toward an important generalization: this is, that in unicellular organisms and in multicellular organisms having a relatively simple organization and a low degree of integration, there is a reasonably good correlation between increasing DNA content and degree of evolutionary advancement, but that among the most complex forms, such as vascular plants and vertebrates, such a correlation does not exist.

This lack of correlation among vascular plants will become clearer as more data on DNA content are obtained, particularly from the spore-bearing archegoniates, but the data of Sparrow and Evans on angiosperms appear to be representative of that group. For instance, the DNA content of the bean *Vicia faba* is more than five times that of the soybean *Glycine max*. Both *Vicia* and *Glycine* are relatively advanced members of the family Leguminosae, being the end points of very dif-

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ferent lines of evolution. The species of *Lilium*, which along with *Trillium* have the highest DNA content in the order Liliales, are neither the most advanced nor the most primitive members of their order. Smaller nuclei, which probably contain less DNA, are found both in more primitive genera such as *Tofieldia* and in more specialized forms such as *Asparagus* and *Sansevieria* (*4*). In the Gramineae, as Avdulov (*5*) pointed out, variation in chromosome size is correlated not with phylogenetic primitiveness or advancement but with climatic adaptation. The genera having large chromosomes are found exclusively in temperate climates, whereas those having medium-sized or small chromosomes are predominantly tropical or subtropical.

In spite of the absence of actual data on DNA content, the excellent illustrations presented by Manton (*6*) of chromosomes in ferns and other spore-bearing archegoniates indicate that the lack of correlation between DNA content and phylogenetic position which is found in the angiosperms prevails also in these more primitive vascular plants. For instance, the genus *Selaginella*, which is characterized by the advanced condition of heterospory, and which with respect to vegetative structure has specialized xylem vessels in some of its species, has the smallest nuclei found in any of these groups. In the more generalized species of *Lycopodium*, and particularly in *Psilotum* and *Tmesipteris*, which are usually regarded as the most primitive living vascular plants, the nuclei are many times larger and their DNA content may be as much as 10 to 20 times greater. This suggests that loss of DNA in association with advancing specialization may have taken place during even the earliest evolution of the vascular plants.

In vertebrates, the same lack of correlation between phylogenetic position and DNA content can be seen from the data obtained by Mirsky and Ris (*7*), as well as from those compiled by Sinsheimer. For instance, the relatively primitive lungfish (*Protopterus*) has 30 to 100 times as much DNA in its nuclei as that found in nuclei of the more advanced teleost fishes. The salamander (*Amphiuma*) has more than ten times the nuclear DNA content of the frog, and more than 30 times that of reptiles such as the turtle. In the evolution of birds from reptiles, a 50-percent reduction in DNA content appears to have taken place.

How can we explain this apparent anomaly? There are three clues, all of which point toward the same explanation. One is that in several groups of higher plants a definite correlation exists between DNA content or nuclear volume and ecological adaptation. In the grass family, Avdulov (*5*) first pointed out that the tribes and genera which are centered in tropical regions, and which in temperate climates grow only during the warm season, have uniformly small to medium-sized chromosomes and nuclei, whereas most of the grasses that grow chiefly in cool temperate regions have large chromosomes and nuclei. The same situation exists to some extent in two other groups of angiosperms, the Leguminosae and the order Liliales. All of the tropical members of the Leguminosae have small to medium-sized chromosomes, as do most of the temperate-climate genera in this family. In one strictly temperate tribe, the Vicieae, however, the chromosomes and nuclei are large. In the Liliales, the genera *Lilium* and *Trillium*, both strictly temperate in distribution, have the highest nuclear DNA content yet recorded in plants. Genera of Liliales which are primarily tropical or subtropical, such as *Sansevieria, Asparagus*, and *Smilax* (*4*), have much smaller chromosomes and presumably a much lower DNA content.

The high content of DNA in certain temperate grasses, vetches, and lilies is probably not directly adaptive to low temperatures, as Avdulov (*5*) suggested, for a few strictly tropical groups have relatively large chromosomes, and probably a high DNA content. Among these groups are *Rhoeo* in the Commelinaceae, members of the mistletoe family (Loranthaceae) (*8*), and members of the Proteaceae (*9*).

Although these data, too, are still scanty, they suggest that variation in DNA content in higher plants and animals, though not correlated with evolutionary advancement, is nevertheless not random and probably has an adaptive significance. A clue to this significance is the correlation between DNA content and length of the mitotic cycle reported by Van't Hoff and Sparrow (*10*). These authors, comparing six genera of angiosperms having medium-sized to large chromosomes (*Trillium, Tulipa, Tradescantia, Vicia, Pisum, Helianthus*), found that nuclear volume is positively correlated with the length of the mitotic cycle. The higher the volume of the nuclei, and probably also their DNA content, the slower is their mitotic rhythm. As yet, no information is available as to whether this correlation affects all stages of the mitotic cycle equally or is confined to certain particular stages.

The significance of this correlation must be considered in relation to the elegant experiments of Stern and Hotta (*11*), which have revealed two important facts. First, the onset and completion of mitosis in the microspores of *Lilium* are associated with very specific inductions and inhibitions of enzymatic activities. These, in turn, result from the stage-specific action of genes, that is, from their production of messenger RNA. Second, the very slow course of the prophase of meiosis in *Trillium* is associated not with correspondingly low rates of metabolic processes, but with great prolongation of certain phases of gene action. In this way, the research of Stern's group has definitely established that the lengths of the mitotic and the meiotic cycles are related to the duration of specific phases in carefully regulated sequences of gene action. The correlation found by Van't Hoff and Sparrow extends this association to differences in chromosome size.

On the basis of these deductions, we can regard both the correlation between nuclear volume and length of the mitotic cycle and the fact that differences in chromosome size are often associated with adaptation to different environments as clues pointing in the

Table 1. DNA content of nuclei in various animals and plants. Content is recorded as percent of that found in *Lilium henryi*. Data from *2*, *3*, and *6*.

Organism	Content
Microorganisms	
T2 Bacteriophage	0.0004
Escherichia coli	.02
Animals	
Sponge	0.12
Coelenterate	.6
Echinoderm	1.8
Lungfish	100.0
Teleost fishes	1.0–3.0
Salamander (*Amphiuma*)	168.0
Frog	15.0
Turtle	5.0
Mammals (man, rat, cow)	5.8–6.4
Plants	
Chlorella sp.	0.25
Scenedesmus sp.	0.9
Tmesipteris tannensis	~25–30*
Selaginella spinulosa	~ 1.5*
Arabidopsis thaliana	4.0
Glycine max	6.5
Vicia faba	38.4
Zea mays	34.1
Secale cereale	~45
Allium cepa	54.3
Tradescantia paludosa	59.4

* Rough estimates from drawings.

same direction. They suggest that variations in nuclear DNA content in higher organisms have an adaptive significance as one method of regulating sequences of gene action.

A third clue points toward a possible mechanism by which this regulation might be brought about. This is the fact that differences in chromosome size and nuclear volume are often associated with a different distribution of heterochromatin in the metabolic or interphase nucleus. Avdulov (*5*) observed that in those species of grass that have large chromosomes heterochromatic regions are scattered throughout the nuclei, whereas in those with small chromosomes these regions are aggregated into "chromocenters" in the regions of the centromeres. The same situation exists also in *Crepis*. For instance, in the four-paired *Crepis neglecta* the total chromosome length at metaphase is considerably greater than it is in the closely related but three-paired *C. fuliginosa*. In *C. neglecta* the metabolic nuclei are filled with large numbers of heterochromatic regions, whereas the metabolic nuclei of *C. fuliginosa* contain a much smaller number of clearly defined heterochromatic chromocenters (*12*). As a rule, large chromosomes and a high nuclear DNA content appear to be associated with a large amount of heterochromatin, distributed irregularly throughout the nucleus, whereas plants with smaller chromosomes generally have smaller ratios of heterochromatin to euchromatin and their heterochromatin is mostly concentrated into chromocenters which surround the centromeres.

Although the genetic significance of heterochromatin is by no means fully clarified, connections between heterochromatin and gene action have long been recognized. The older view, that heterochromatic regions of chromosomes are devoid of normal Mendelian genes, has now been shown to be invalid in animals as well as in plants (*13*). Nevertheless, the presence of heterochromatin, or the heterochromatization of chromosomes, definitely influences the action of those genes associated with it. In *Drosophila*, it has long been known that when genes are transferred by means of translocations to heterochromatic regions, their action during development becomes irregularly inhibited, so that the adult tissue is a mosaic of normal cells and cells exhibiting the recessive phenotype (*13*). In mice the heterochromatization of the X chromosome is associated with temporary inactivation of its genes (*14*). The same is true of whole genomes in certain coccids (*15*).

This genetic evidence concerning the influence of heterochromatin on gene action has recently been supplemented by biochemical evidence. A number of workers have shown in a variety of organisms that the DNA in heterochromatic regions of the nucleus replicates later than it does in euchromatic regions of the same nucleus (*16*). Late replication is apparently a constant property of heterochromatic regions (*17*).

In the nuclei of calf lymphocytes, up to 80 percent of the DNA is visible as heterochromatin and is in the "repressed" condition, so that it does not participate in the synthesis of messenger RNA (*18*).

Furthermore, the repression of gene action which is characteristic of heterochromatin appears to be associated with the binding of DNA with basic protein or histone into a supramolecular complex. The presence of basic protein in heterochromatic regions and of acidic, globulin-like proteins in euchromatic regions of the giant salivary chromosomes of insects has long been recognized (*13*). Bonner and Huang (*19*), using material from pea nuclei, and Allfrey, Littau, and Mirsky (*20*), using calf thymus tissue, have demonstrated that histone suppresses DNA-dependent synthesis of RNA in vitro. Although, as Sonnenberg and Zubay (*21*) have pointed out, these demonstrations can by no means be taken as direct evidence that histone acts similarly in vivo, the association of histone with heterochromatin, which clearly inhibits gene action, suggests that basic proteins at least form part of an inhibitory complex which has the function of regulating gene action. Allfrey *et al.* (*22*) have obtained evidence that acetylation or methylation, or both, of the ends of histone molecules may play a role in this regulation.

All this evidence has led me to the following hypothesis. The large chromosomes and high nuclear DNA content which prevail in such plants as *Lilium* and *Trillium* and in such animals as the lungfishes and salamanders indicate an increase in the number of genes but not in the number of different gene-controlled metabolic processes. Instead, each of several different gene-controlled processes is controlled by a system consisting of many gene loci responsible for the same process. Heterochromatinization of the DNA in these loci, which probably involves association of DNA with histones, a special kind of RNA (*23*), and perhaps other proteins, represses the action of each gene unit except at certain specific times in development. The action of these similar gene units is coordinated by a system of macro-operons, like that postulated by Stern (*11*), in which the product of one gene in a series activates the next unit of that series. In such a system, the length of time over which a particular cell or tissue carries on a particular process would depend on the number of sequentially activated gene units responsible for that process. Hence, adding similar gene units would slow down development by prolonging particular stages, and losses of such units would correspondingly speed up development. At the same time, loss of heterochromatin, of euchromatization of certain genic regions, would prolong the time span of activity for individual genes and so would speed up development by permitting a large number of gene-controlled metabolic reactions to take place at the same time.

According to this hypothesis, the phylogenetic reduction in chromosome size observed by Babcock in *Crepis* involved the loss of gene units in particular metabolic series, plus the euchromatization of other units, and these changes led to an increase in time of action for each gene unit. The DNA which was lost by this process did not provide a unique code for particular enzymatic functions, but merely a modified duplication of portions of the code carried by other genes.

The presence of numerous genes with limited action times could be an adaptation to metabolic activity at low temperatures, when photosynthesis is taking place at minimal rates and when the active transport system is also operating very slowly so that the supply of raw materials to each growing cell at any one time is very limited. Natural selection has adjusted cellular metabolism to this limited supply by reducing the number of gene-controlled synthetic processes occurring at any one time. In the annual species, growth takes place under highly favorable temperature and moisture conditions, so that a synthetic system in which many gene-controlled processes are taking place simultaneously can be supplied with an abundance of raw materials. The evolution of such a system enables the species to complete its developmental cycle during the short period of time available to it.

Reduction in Chromosome Number

The second problem raised by Babcock's cytotaxonomic survey of *Crepis* is the reduction in basic chromosome numbers which has taken place in the genus. The most primitive species have the basic monoploid number $x=6$, while in various separate lines of evolution the number has been reduced to 5, 4, and 3. Similar trends of reduction in basic number have taken place in other genera of the tribe Cichorieae (*24*), as well as in other tribes of the family Compositae and in many other families of flowering plants. An example in gymnosperms is the family Podocarpaceae (*25*).

The longest aneuploid reduction series known to me is in the genus *Haplopappus* (*26*). Primitive species such as *H. eximius* Hall and *H. linearifolius* DC, as well as advanced shrubby species in the section *Ericameria* [*H. Palmeri* Gray and *H. arborescens* (Gray) Hall] all have $x=9$. The basic numbers $x=8$ and $x=7$ are not yet known in the genus, but may well exist in some of the numerous species which have not yet been counted. The basic number $x=6$ is found in the sections *Isocoma* [*H. venetus* (HBK) Blake and *H. acradenius* (Greene) Blake] and *Pyrrocoma* [*H. racemosus* (Nutt.) Torr.], and $x=5$ is characteristic of the section *Hazardia* [*H. canus* (Gray) Blake and *H. squarrosus* H. & A.]. Finally the section *Blepharodon*, which has been studied extensively by Jackson (*27*), contains forms with $x=4$, $x=3$, and $x=2$, the latter being the now famous *H. gracilis* (Nutt.) Gray.

The association of phylogenetic reduction with the occurrence of translocations was first pointed out by Navashin (*28*) and was carefully documented through studies of meiotic pairing in species hybrids of *Crepis* by Tobgy (*12*) and Sherman (*29*). The same association has been demonstrated in *Astranthium* by DeJong (*30*), in *Chaenactis* by Kyhos (*31*), and in *Haplopappus* section *Blepharodon* by Jackson (*27*). It is also implicit in the concept of "centric fusion," elaborated by Robertson for insects of the order Orthoptera and found in many different groups of animals (*32*). We can conclude, therefore, that phylogenetic reduction in chromosome number is usually associated with the occurrence of unequal translocations between nonhomologous chromosomes, followed by the loss of heterochromatic, genetically inert, or nonessential centromeric regions.

As Grant and I (*33*) have pointed out, this association between translocation and aneuploid reduction is the key to understanding the selective forces which have guided the latter phenomenon. Aneuploid reduction has not occurred at random throughout the plant kingdom, but has been characteristic of species groups in which outcrossing prevails, so that the plants are heterozygons at a large proportion of their gene loci, and which occupy pioneer habitats. Species which occupy such habitats must repeatedly colonize new areas and their success depends on the speed with which they can do this in competition with other colonizers. As I pointed out many years ago (*34*), this maximum rate of colonization is achieved when a small number of initially successful individuals produce offspring which are genetically like themselves. Genetic constancy is favored. The variability which results from excessive segregation and recombination of genes is disadvantageous for colonization, since it produces too many segregates with inferior adaptive properties.

In a sexually reproducing, outcrossing species, the most efficient method of reducing genetic variability is the tightening of genetic linkages. This can be done in two ways. One is by reduction in frequency and localization of crossovers, so that large segments of chromosomes are virtually devoid of genetic recombination; and the other is by reduction of the number of chromosomes or linkage groups. Both of these processes have been characteristic of the annual species of *Crepis* as well as the other pioneer species mentioned above, insofar as their cytological behavior is known.

This explanation of the adaptive significance of reduction in chromosome number has been greatly strengthened by the computer simulation experiment of Fraser and Burnell (*35*). These workers have shown that given two postulates, (i) that a modal condition which is determined by cooperation between many genes has a maximum adaptive value and (ii) that outcrossing is the rule, the selection will inevitably guide the population into a condition in which adaptive complexes of genes are linked together on the same chromosome arm. As long as the organism-environment interaction remains constant, these linkages will be maintained.

We can extrapolate from this demonstrated adaptive advantage of specific linked gene combinations in a constant environment to the effects of adaptation to a radically new environment in the following manner. The linked combinations already present can in many instances form the basis for the necessary new adaptation, but they must be modified in various ways. Some of this modification can take place by mutation, and some by introgression of genes from related but differently adapted subspecies or species. In any case, the newly acquired genes can exist anywhere on the chromosomal complement and are not likely to be linked to the preexisting combination. Consequently, translocations which provide this linkage will have high adaptive value. In most instances such translocations will not alter the chromosome number, and subspeciation or speciation at the homoploid level will result. Occasionally, however, translocations will occur which will transfer to a single chromosome pair the genes which previously were distributed over two pairs, and aneuploid reduction will be the result. The species with the reduced basic number may sometimes have limited success; *Crepis fuliginosa* ($n=3$) has a narrower geographic distribution than the related *C. neglecta* ($n=4$). In other instances, as in *C. capillaris*, *C. zacintha*, and *Haplopappus gracilis*, the species with a reduced chromosome number may be much more successful than its relatives with higher numbers.

Significance of Polyploidy

The third problem which was raised in Babcock's monograph of *Crepis* was that of the evolutionary significance of polyploidy. In *Crepis*, polyploidy is relatively uncommon and is most highly developed in the North American species. These species, as worked out by Babcock and me, formed the prototype of the polyploid complex (*36*). Such complexes consist of a number of diploid species which represent the extremes of morphological and ecological variation and which are connected to each other by means of a vast superstructure of polyploids. The polyploid complexes exhibit a complete series of intergradations and recombinations of the characteristics found in the diploids. In the North American complex of *Crepis*, these polyploids are largely apomictic, and the fact that their collective variation pattern does not trans-

gress the limits set by the diploids could be ascribed to their lack of sexual reproduction. Nevertheless, there are many polyploid complexes in which the polyploids are both sexually reproducing and largely outcrossed, and still do not transgress the limits of variation set by the diploids. In previous publications (*34, 37*) I have discussed *Paeonia, Zauschneria, Eriogonum fasciculatum, Vaccinium* subgenus *Cyanococcus*, and *Dactylis*. In these examples, the failure of the polyploids to evolve new characteristics can best be ascribed to the retardation of evolutionary progress which results from the presence of many duplicated gene loci.

Two other evolutionary aspects of polyploidy are illustrated by the American species of *Crepis*. One of these points toward the ecological conditions which are most favorable for the origin of polyploidy. This is the difference between two species complexes among the American species. The complex of *C. runcinata*, which is centered in the Rocky Mountain region, consists entirely of sexual diploids. It is just as widespread as the complex containing the polyploid apomicts, in which the seven diploid species are centered in northeastern California and the adjacent part of Oregon. The two complexes, however, differ greatly in their ecological habitats. *Crepis runcinata* and its subspecies inhabit stream margins, moist meadows, and other habitats which are largely associated with the drainage systems of the major rivers, and which consequently tend to be continuous over large areas or, if discontinuous, differ little from each other. The diploids which gave rise to the polyploid complex, such as *C. acuminata, C. modocensis*, and *C. occidentalis* are, on the other hand, marked xerophytes, and occupy pioneer habitats on open mountain slopes. Over long periods of time, such habitats are likely to change their location frequently, so that plant species adapted to them must often become adapted to environments with entirely new conditions. It seems likely that this difference in ecological adaptation had much to do with the great development of polyploidy in the xeric montane species and its absence in the species of moist meadows. A parallel situation can be seen in other groups. For instance, the genus *Betula*, which in northern climates often invades dry areas and mountain slopes, contains many polyploids, whereas the related genus *Alnus*, the species of which are largely confined to stream banks and lowlands, does not. A similar difference exists to a lesser degree between *Salix*, which has developed an elaborate polyploid complex, and *Populus*, which is strictly homoploid. There is good reason, therefore, for believing that existence in pioneer habitats, particularly in mountain regions, favors the development of polyploid complexes.

When we consider a series of polyploid complexes such as those mentioned above, we gain a better understanding of why the polyploids belonging to them have been so successful. In *Paeonia,* the diploid hybrids from which the polyploids arose are highly sterile and have irregular meiosis. In this and in many other examples we are dealing with amphiploids derived from two or more different species. The polyploid state serves to restore fertility, and preferential pairing between chromosomes belonging to the same parental species prevents genetic segregation of the character differences between the two species. In this way a fertile and stable intermediate form is evolved. In *Dactylis*, on the other hand, the diploid hybrids are fully fertile and vigorous in the F_1 and later generations, and polyploidy does not have the function of restoring fertility. It does, however, tend to stabilize a condition intermediate between the parental types, largely because of the great complexities involved in tetrasomic segregation and the larger number of intermediate conditions which this type of segregation makes possible (*34*). The other polyploid complexes mentioned represent intermediate situations between these two extremes. The two effects which polyploidy has in all of them are, first, an increase in vigor, and, second, a stabilization of certain intermediate genotypes. In general, therefore, polyploidy most often has the adaptive function of stabilizing genotypes which are intermediate between or represent recombinations of the characteristics found in the ancestral diploids. It will be successful only when such intermediate types possess an adaptive advantage in some new environment which is available to them.

The second aspect of polyploidy which is illustrated by the American species of *Crepis* is the occurrence of separate cycles of polyploidy. The basic number upon which the American complex is built, $x=11$, is itself almost certainly derived by amphiploidy (*1, 36*). The ancestral species forming the complex were in all probability amphiploid derivatives from one group of Old World species with $x=4$ and another with $x=7$. Such cycles of polyploidy have probably occurred many times during the evolution of angiosperms. In the tribe Pomoideae of the family Rosaceae the basic number $x=17$ was probably formed by amphiploidy from hybridization between ancestral forms having $x=9$ and $x=8$ as their basic numbers (*34*, p. 361). This event must have occurred a very long time ago, perhaps at the beginning of the Tertiary or even during the Cretaceous Period. Much later, probably during the Pleistocene epoch, individual genera of this tribe, such as *Amelanchier, Crataegus, Cotoneaster, Malus*, and *Sorbus*, formed separate polyploid complexes on the basic number $x=17$ (*38*).

The occurrence of successive cycles of polyploidy is the most likely explanation for the high chromosome numbers in the spore-bearing vascular plants, or "Pteridophytes" (*6*). In the Lycopsida, for instance, the low basic numbers of $x=9$, $x=10$, and $x=11$ occur respectively in the heterosporous genera *Selaginella* and *Isoetes* (*39*), whereas in the homosporous and presumably more primitive genus *Lycopodium* the lowest numbers definitely known are $x=23$ and $x=34$ (*40*). This situation is best explained by assuming that the original common ancestor of the three genera, which is now extinct, had a low basic number like that of *Selaginella*. From it, *Lycopodium* evolved through polyploidy, which tended to conserve primitive characteristics, while *Selaginella* and *Isoetes* evolved more specialized characteristics at the diploid level. On this basis, the high number found in *Lycopodium selago*, $2n$ = about 260, was probably derived through two or more separate cycles of polyploidy. The same kind of reasoning leads to the conclusion that the high numbers found in the only living representatives of the Psilotales, $2n$ = about 106 and $2n$ = about 200 for *Psilotum* and $2n$ = about 400 for *Tmesipteris*, represent the culmination of several cycles of polyploidy.

The most extensive development of successive cycles of polyploidy has apparently occurred in the family Ophioglossaceae, which is the most primitive living family of the order Filicales, or true ferns. The lowest basic number known, $x=45$ in *Botrychium* (*41*) may be a hexaploid derivative of $x=15$, present in extinct ancestors or primi-

tive members of the family. The monotypic genus *Helminthostachys* has the haploid number $x=94$, which Ninan (*41*) regards as a modified polyploid derivative of the primitive basic number $x=15$. Finally, the genus *Ophioglossum* consists of a polyploid complex of which the basic number is $x=120$, and contains diploid, tetraploid, hexaploid, octoploid, and approximately decaploid species. One of its species, *O. reticulatum*, contains races with $2n =$ about 1260, probably the highest chromosome number in the plant kingdom. At meiosis, these chromosomes pair regularly to form about 630 bivalents, a feat which to cytologists is as remarkable a wonder of nature as are the fantastic elaborations of form exhibited by orchids, insectivorous plants, and many animals.

The evolution of the Ophioglossaceae provides a definite answer to a question which cytologists have sometimes asked: What kind of a ceiling exists to the increase of chromosome number? Studies of newly produced artificial polyploids (*42*) have shown that levels above tetraploidy, such as the octoploid state, are rarely tolerated when they arise directly from diploid forms. Apparently, however, successful polyploids can become adjusted or "diploidized" over long periods of time so that further elevation of the chromosome number by successive cycles of polyploidy then becomes possible. The series of chromosome numbers found in the Ophioglossaceae suggests that, given a sufficiently long span of time, accompanied by diploidization at successively higher chromosomal levels, almost no limit exists to the upward trend of polyploid chromosome numbers.

Cytology and Ecology of the Earliest Angiosperms

Now that the chromosome numbers of the woody Ranales, which are generally regarded as the most primitive angiosperms, are becoming reasonably well known, speculations as to the cytological nature of the earliest flowering plants are in order. Raven and Kyhos (*43*) have recently summarized the available evidence, which is presented in Table 2. This table includes numbers for herbaceous Ranales and for the more primitive families of the orders Dilleniales and Rosales which, in my opinion, also deserve consideration as very primitive angiosperms.

Two facts emerge from this table.

Table 2. Chromosome numbers in apocarpous dicotyledons.

Family	Basic monoploid number
Annonaceae* (6 genera)	7,8,9
Calycanthaceae*	11
Cercidiphyllaceae*	19
Crossosomataceae*	12
Degeneriaceae*	12
Dilleniaceae* (3 genera)	8,13
Eupomatiaceae*	10
Illiciaceae*	14
Lauraceae* (6 genera)	12
Leguminosae* (many genera)	6,7,8,9,10, 11,12,13,14
Magnoliaceae* (6 genera)	19
Nymphaeaceae	8,10,12,14,17
Paeoniaceae	5
Ranunculaceae	7,8
Rosaceae	7,8,9
Saxifragaceae (s. str.)	7,8,9,10, 11,12,13,15
Schisandraceae*	14
Trochodendraceae*	19
Winteraceae*	13,43

* Woody.

In the first place, many different basic numbers are represented, and no particular number can be immediately singled out as the most primitive one. Second, the families which are strictly woody have higher basic numbers than do those which are either predominantly herbaceous or have many herbaceous representatives. Moreover, the numbers most commonly found in the woody families, such as $x=12$, 13, 14, and 19, seem to be secondary polyploid derivatives.

These numbers in themselves indicate that both aneuploid alterations of basic number and polyploidy took place during the early evolution of the angiosperms. Raven and Kyhos believe that the numbers are best explained by assuming an initial polyploidy followed by aneuploid changes, but alternative explanations are by no means excluded. This is particularly true in view of the fact that, as Bailey and Nast (*44*) have stated, the modern representatives of the woody Ranales are the few survivors of what must have once been a much larger and more diverse group, and the connecting forms between them have become extinct. If we recognize such families as the Dilleniaceae and Paeoniaceae as nearly or quite as primitive as the Ranales proper, then the existing numbers strongly suggest that aneuploid changes in chromosome number first produced basic numbers of $x=9$, 8, 7, 6, and 5, after which polyploidy gave rise to the higher numbers now found in the Magnoliaceae, Winteraceae, and Degeneriaceae. The three highest of these numbers, $x=9$, 8, 7, occur in the Annonaceae, and $x=8$ is characteristic of *Hibbertia*, of the Dilleniaceae. The basic number $x=5$ in *Paeonia* must be very old, because of the numerous primitive and anomalous morphological characteristics found in this genus. That *Paeonia* could have been derived from herbaceous Ranales is very unlikely on the basis of morphological evidence, and cytological considerations make its origin from woody types with $x=14$, $x=13$, or $x=12$ equally unlikely. The most probable connection between *Paeonia* and other primitive angiosperms is, therefore, via extinct forms which had $x=7$ and $x=6$. If such forms existed, the derivation by direct polyploidy of the numbers $x=14$, 13, and 12 is just as probable as Raven and Kyhos's hypothesis that the numbers 13 and 12 were derived by reduction from $x=14$.

Even more striking than this extensive variation in basic number between different primitive families is the constancy exhibited within many woody genera and families, both primitive and more advanced (*38*). Examples are the number $x=19$, found throughout the Magnoliaceae; $x=12$, characteristic of the Lauraceae, as well as the Fagaceae; $x=13$, found in most of the large genera of the Leguminosae, subfamily Mimosoideae; $x=14$, found in many genera of the subfamily Caesalpinoideae of the Leguminosae; and $x=36$, which apparently occurs uniformly throughout the family Bombacaceae (*45*). All these basic numbers appear to be of ancient polyploid derivation, but since they came into being there has been extensive evolution in the groups concerned without further changes in chromosome number.

These series of chromosome numbers suggest that the early history of the angiosperms was divided into three stages, characterized by different kinds of chromosomal changes. During the first of these stages, the earliest woody forms, now largely extinct, underwent aneuploid changes of basic number at the relatively low levels of $x=9$ to $x=5$, perhaps through both upward and downward progression from an original number of $x=7$ or $x=6$. Then ensued a period when the woody forms built up higher basic numbers by means of polyploidy. At the same time some of them probably gave rise to the earliest herbaceous angiosperms, such as the Ranunculaceae, Saxifragaceae, and Berberidaceae, without alteration of the basic number. During the third period of evolution the predominant process was speciation and generic differentia-

tion of woody forms at the higher chromosomal levels which they had reached by polyploidy. During this time, the primitive herbs were probably few in number and restricted in distribution, so that they did not evolve extensively. A fourth period of angiosperm evolution, which probably took place during the middle and latter part of the Tertiary Period, saw the decline of the woody groups except in the moist tropics and the rise to prominence of the previously subordinate herbaceous vegetation. This last phase was accompanied by extensive polyploidy in the herbaceous groups. These four periods almost certainly overlapped extensively at various times and places.

On the basis of our knowledge of the ecological conditions which favor the different kinds of chromosomal changes in modern forms, we can tentatively reconstruct the angiosperm flora of these three earliest periods. During the period of aneuploid change, the angiosperms were insect-pollinated shrubs occupying relatively dry pioneer habitats (*46*). They were less common than other groups of vascular plants, and their populations moved from place to place as the environment changed. Such conditions would have been highly unfavorable for the preservation of either macrofossils or pollen, so this earliest period is believed to have occurred before the known fossil record of angiosperms began in the Cretaceous Period. The second stage, that of polyploidization among the woody forms, probably coincided with the rapid spread of angiosperms into new habitats, and so most likely accompanied their "explosive" appearance and expansion in the fossil record during the Cretaceous Period. The period of speciation and generic differentiation of woody forms at secondary homoploid levels of polyploid derivation probably took place during the latter part of the Cretaceous and the beginning of the Tertiary Period, when woody angiosperm floras were widespread and dominant over most of the earth.

This final speculation has led us a long way from the evolutionary generalizations which Babcock made upon the basis of his investigations of *Crepis*. It is presented in the hope that it will stimulate further explorations of evolutionary processes at the level of the species as well as of higher categories. The opportunities for profitable investigations of this sort are by no means at an end, and new techniques may extend them to degrees of clarity and certainty which at present can hardly be imagined.

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