CHAPTER XII

Evolutionary Trends I:
The Karyotype

THERE HAS BEEN a tendency among many systematists to regard differences between species and genera in the number, form, and size of their chromosomes as merely additional taxonomic characters. For those who are interested in "pigeon hole" classification and nothing else, this attitude may be justified. But systematists should realize that, considering the amount of time and labor needed to prepare and to observe them, chromosomes often provide inefficient and uncertain diagnostic characters. On the other hand, they often provide to the student of evolution and phylogeny valuable signposts indicating the nature of the evolutionary processes at work and the trends which evolution has taken.

The important fact to remember here is that chromosomes are not only structures which result as end products of a series of gene-controlled developmental processes; they are themselves the bearers of the genes or hereditary factors. This at once puts them into a different category from all other structures of the body. Furthermore, as White (1945, p. 152) has observed in this connection, chromosomes are not merely aggregates of discrete genic units. To a certain extent they are units in themselves. We should expect changes in the chromosomes to bear a more direct relationship to genetic-evolutionary processes than do any other types of changes.

Before studying visible chromosomal differences, however, we must remind ourselves of the fact that chromosomes which resemble each other in outward appearance are not necessarily alike in genic content or in hereditary potentiality. The changes in the chromosomes produced by gene mutations are by definition invisible even under the most powerful microscope. In some genera, like *Pinus* and *Quercus* (see Chapter II), differentiation

of species appears to have been entirely by this process, since in their gross structure the chromosomes of all the species are so similar as to be indistinguishable. This is shown both by comparative karyology of somatic chromosomes and by the regular pairing of the chromosomes at meiosis in species hybrids. Furthermore, the chromosomes of different species may look exactly alike as to size and form, but may nevertheless possess many differences in gross structure, such as translocations and inversions, which become evident only when they pair with each other in species hybrids. This is notably true of the genus *Paeonia* (Stebbins 1938a).

On the other hand, the superficial appearance of the chromosomes may be completely altered in two entirely different ways, without comparable changes in the genotype. One way is by means of large unequal reciprocal translocations of chromosomal segments. Such changes have little or no effect on the external morphology or the physiological reactions of the plant. They have been induced artificially in *Crepis tectorum* (Gerassimova 1939) and in several other examples. Another is by the accumulation of chromosomes with little or no genetic activity, such as the "B-type" chromosomes found in certain strains of *Zea mays* (Longley 1927, Randolph 1941b, Darlington and Upcott 1941), and the fragment-type chromosomes sometimes found in species of *Tradescantia* (Darlington 1929, Whitaker 1936). We cannot, therefore, estimate either the amount or the directional trend of evolution by studying only the external, visible characteristics of the chromosomes. Nevertheless, comparison of the chromosomes of related species has in many groups disclosed certain regular differences, which are often correlated with trends of specialization in the external morphology of the plant. The full explanation of these correlations has not yet been obtained for any example, but they nevertheless deserve careful consideration, since they very likely will provide valuable guides toward the solution of fundamental problems concerning the relation between chromosomes, genes, and visible characters or character complexes.

THE CONCEPT OF THE KARYOTYPE

The pioneering work of the Russian School of cytologists,

headed by S. Navashin, established firmly the fact that most species of plants and animals possess a definite individuality in their somatic chromosomes, which is evident in their size, shape, position of primary constrictions or centromeres, and in such additional features as secondary constrictions and satellites. Furthermore, closely related species are usually similar in these respects, and distantly related ones are often recognizably different, at least in those species with large, easily studied chromosomes. These facts led to the early formulation of the concept of the *karyotype*. Delaunay (1926) formulated the concept of the karyotype as a group of species resembling each other in the number, size, and form of their chromosomes. In the liliaceous genera with which he worked *(Muscari, Bellevalia, Ornithogalum)*, the karyotype seemed to correspond to the genus, but Levitzky (1924, 1931b) soon showed that this situation is not at all general, and that in many genera the evolution of the karyotype can be traced through a series of gradual changes in the external appearance of the chromosomes. He therefore redefined the term karyotype as the phenotypic appearance of the somatic chromosomes, in contrast to their genic contents. An earlier term, the idiogram, was used in a somewhat similar sense by S. Navashin, but the term karyotype is the one which has been established by general usage, whereas the term idiogram is now applied to the diagrammatic representation of the karyotype.

The principal ways in which karyotypes differ from each other are well described in the classic work of Levitzky (1931a,b), and additional information has been supplied by Heitz (1926, 1928, 1932) and others (cf. Darlington 1937). These distinguishing characteristics are as follows: first, basic chromosome number; second, form and relative size of different chromosomes of the same set; third, number and size of the satellites and secondary constrictions; fourth, absolute size of the chromosomes; and fifth, distribution of material with different staining properties, that is, "euchromatin" and "heterochromatin." These five types of difference will be discussed in turn.

In this discussion, frequent reference will be made to the primitive or advanced condition of the various genera and species in respect to various characteristics of external morphology. In most instances, the reasons for assuming that the characteristics in

question are primitive or advanced are discussed by the authors whose papers are cited, and the present writer believes that their conclusions are based on satisfactory evidence. Other examples are judged on the basis of criteria familiar to and accepted by most systematists and morphologists. Some of the principal ones, of these are that the annual habit is usually derived from the perennial one; that genera or species groups which form connecting links between other groups are likely to be primitive, unless they are of allopolyploid origin; and that such conditions as sympetaly, epigyny, zygomorphy, and dioecism are derived conditions. Some of these points are discussed in Chapter V, and particularly in Chapter XIII.

CHANGES IN BASIC NUMBER

The commonest type of change in chromosome number found in the higher plants is polyploidy. This type of change is an irreversible one, so that in any polyploid series the oldest, most primitive members are those with the lowest chromosome numbers. But in many genera and families of the higher plants we find an entirely different type of change in chromosome number, occurring most often on the diploid level, without multiplication of chromosome sets or even of whole chromosomes. In the best-known examples these changes in basic haploid number may be seen to involve an increase or decrease by one chromosome at a time. Furthermore, the trend may be in either direction, depending on the group concerned.

The principles governing this stepwise, aneuploid alteration of the basic chromosome number are now well established in cytological literature. They are summarized by Darlington (1937, Chap. XII) and by White (1945, Chaps. IV, VIII). The postulate of M. Navashin (1932), that centromeres (that is, "kinetochores" or "primary constrictions") cannot arise de novo, is now well supported by a body of observational and experimental evidence, as is also that of the permanence of chromosomal ends. Furthermore, chromosomes with two centromeres cannot function efficiently and are unknown in natural populations of plant species. Once these principles are understood, the impossibility of changes in basic chromosome number through simple transverse fragmentation and end-to-end fusion of single chromosomes becomes

evident. Reduction of the basic number must involve loss of a centromere plus at least a small amount of adjacent chromosomal material, while increase must involve at first a duplicated chromosome, or centromere-bearing fragment.

¶ Darlington (1937, pp. 559–560) has shown how conditions favoring the loss or gain of a chromosome can be produced by
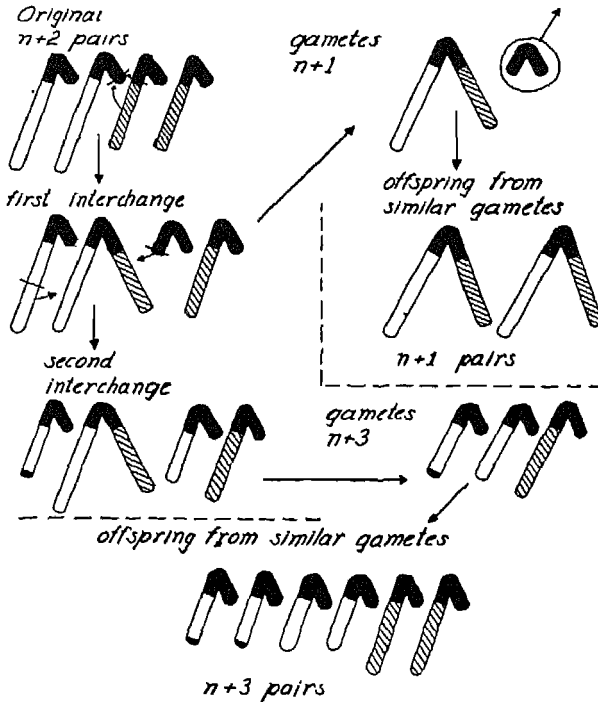

FIG. 41. Diagram showing how, by means of reciprocal translocation of unequal chromosomal segments, the basic chromosome number can be decreased or increased. The parts of the chromosomes colored black are assumed to be inert. Modified from Darlington 1937.

means of unequal translocations. If two different nonhomologous chromosomes both have subterminal or nearly terminal centromeres (the "acrocentric" chromosomes of White, 1945), then a segmental interchange involving the long arm of one chromosome and the short arm of the other will produce one long chromosome with a median centromere ("metacentric," in White's terminology) and one very short, fragment type of chromosome

(see Fig. 41). If such an interchange type becomes homozygous, it will form at meiotic metaphase one exceptionally large bivalent and one very small one. In the latter bivalent, both chromosomes are likely to pass to the same pole of the spindle, as Gerassimova (1939) observed in a chromosomal race of this nature produced artificially in *Crepis tectorum*. This will yield, in addition to normal gametes, others with either two or none of the small chromosomes. The behavior of such gametes will depend on whether these small chromosomes are genetically active or consist entirely of inert material. The latter is likely to be the case in many organisms, since the portions of the chromosomes on either side of the centromere are often inert. If this is true, gametes lacking such small chromosomes will function as well as normal ones, and the fusion of two such gametes will give rise to a true-breeding strain with the basic number reduced by one (see Fig. 41). If, on the other hand, the small chromosomes are genetically active, reduction of the basic number is impossible by this method, but individuals with an increased number, possessing three or four of the small chromosomes, are likely to occur frequently (see Fig. 41). These will at first be impermanent tri- or tetrasomics, but they could be converted into races or species with a permanently increased basic number in two ways, either by divergent gene mutations in the duplicated small chromosomes or by reciprocal translocation between one of these and one of the large chromosomes of the set (see Fig. 41). Therefore, as Darlington has pointed out, the question of whether the basic number will be increased or decreased as a consequence of unequal translocations depends largely on whether the regions about the centromeres are active or inert.

This aneuploid alteration of the basic number has been carefully studied in *Crepis* and its relatives (Babcock and Cameron 1934, Babcock, Stebbins, and Jenkins 1937, 1942, Babcock and Jenkins 1943, Babcock 1942, 1947). Here the evidence is conclusive that the most primitive species have the highest basic numbers, and that the trend has been toward reduction. In the genus *Dubyaea,* which in both vegetative and floral characteristics is more primitive and generalized than *Crepis,* and which forms a connecting link between the large genera *Crepis, Lactuca, Prenanthes,* and *Hieracium* (Stebbins 1940), all the species

counted have the haploid number $x = 8$, while the basic numbers of most of the genera related to *Crepis*, namely, *Youngia, Prenanthes, Hieracium, Lactuca, Sonchus, Launaea,* and *Taraxacum,* are either $x = 8$ or $x = 9$. In *Crepis* itself, the species which in one way or another approach one of these other genera have

IDIOGRAMS SHOWING KARYOTYPE EVOLUTION IN CREPIS
REDUCTION IN NUMBER, TOTAL LENGTH AND SYMMETRY OF THE CHROMOSOMES

LARGEST CHROMOSOMES   SMALLEST CHROMOSOMES

KASHMIRICA   100   46   MUNGIERI

SIBIRICA   81   38   LEONTODON-
                                                              TOIDES

CONYZAEFOLIA   71   21   SUFFRENIANA

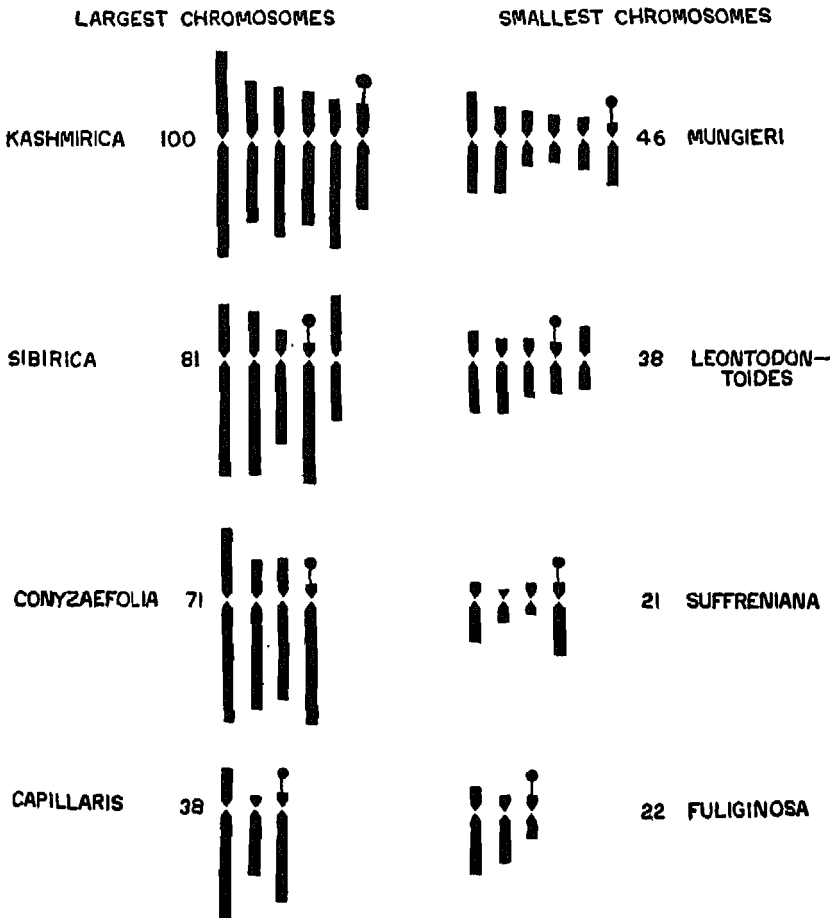CAPILLARIS   38   22   FULIGINOSA

Fig. 42. Ideograms showing the basic haploid chromosome complements of various species of *Crepis* and illustrating the phylogenetic progression in the reduction of chromosome number and size. From Babcock 1947, by permission of the University of California Press.

$x = 7$, $x = 6$, or $x = 5$, while those with $x = 4$ and $x = 3$ are the most typical of the genus and the farthest removed from the other genera (see Fig. 42). In addition, the reduction in basic number has been accompanied by certain definite trends of specialization in external morphology. The most primitive species of *Crepis* are those with $x = 6$ (the seven-paired species are a specialized off-shoot, perhaps forming a transition toward the related genus *Ixeris*). Their primitiveness consists in the perennial habit, the presence of shallow rooting rhizomes, entire or shallowly dissected leaves, relatively large involucres in a few-headed inflorescence, more or less imbricated, unspecialized involucral bracts, and un-specialized, unbeaked fruits or achenes. The species with $x = 5$, $x = 4$, and $x = 3$ possess to an increasing degree some of the following specializations: annual habit; deep taproots; deeply pinnatifid leaves; smaller and more numerous involucres; special-ized involucral bracts in two series, the outer reduced and the inner variously thickened and otherwise modified; and beaked, sometimes strongly dimorphic, achenes. This correlation between reduction in basic number and increasing morphological special-ization is not complete; some species with $x = 5$ (*C. foetida* et aff.) are highly specialized in nearly all of their characteristics, while at least one three-paired species, *C. capillaris,* is less special-ized in most respects than are some species with $x = 4$. But when all the facts are considered, the series can be read in only one way. The three-paired species are related only to four-paired species of *Crepis;* all the latter are typical of the genus and show clear con-nections with the former and with the five-paired species, while it is only in those species with six and five pairs that we can see the evolutionary connection between *Crepis* and other genera. Both morphological and genetic evidence show that the lower numbers have arisen independently several times. The three species with $x = 3$, *C. capillaris*, *C. fuliginosa,* and *C. zacintha,* are all more closely related to various unconnected four-paired species than they are to each other. Similar independent connec-tions can be traced between various four-paired species and related ones with $x = 5$.

Darlington's postulates concerning the mechanism by which basic chromosome numbers are reduced have now been fully con-firmed for this material. Tobgy (1943) has shown that the three-

paired *C. fuliginosa* was derived from the four-paired *C. neglecta* or its ancestor through a system of reciprocal translocations (Fig. 43). One of the four chromosomes of *C. neglecta* (designated as C) is heterochromatic, and probably inert genetically except for the distal part of one arm. In *C. fuliginosa*, the active material of
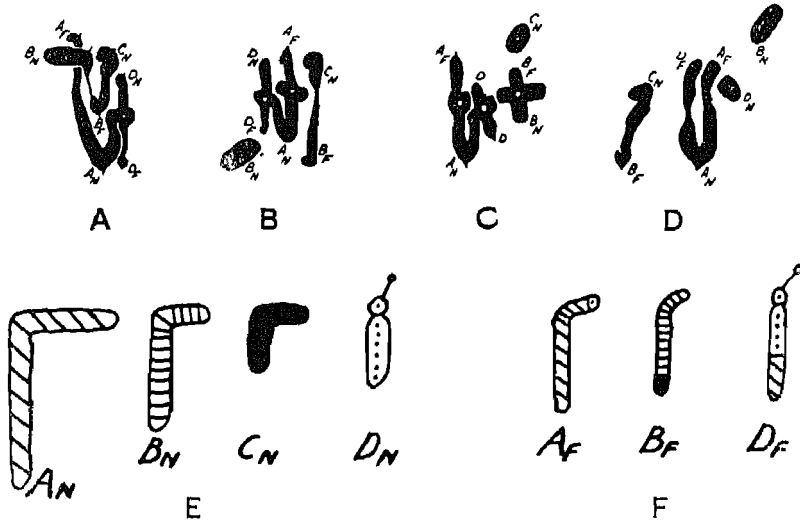

FIG. 43. A to D, four different types of metaphase configurations found at metaphase of meiosis in the F_1 hybrid between *Crepis neglecta* and *C. fuliginosa*. E, the haploid complement of *C. neglecta*, with distinctive shading for each chromosome. F, the haploid complement of *C. fuliginosa*, with the chromosome segments shaded according to their homology with the various chromosomes of *C. neglecta*. A to D, from Tobgy 1943; E and F, redrawn from the figures and data of Tobgy.

the *neglecta* C has been translocated onto the short arm of another chromosome, designated as A. This is clearly demonstrated by the pairing found in the F_1 hybrid between the two species. Tobgy believes, on the basis of indirect evidence, that the translocation was reciprocal, but that the segment of the ancestral A translocated onto the ancestral C of *fuliginosa* was so small that the latter chromosome consisted entirely of inert material, and so could be lost with impunity. Sherman (1946) has shown that the four-paired *C. kotschyana* was derived in a similar manner from a five-paired form related to *C. foetida*. From these examples we may make the general statement that if the region of a chromosome near the centromere is genetically inert, its active distal

portions may occasionally be translocated to another, nonhomologous chromosome, its centromere may be lost, and reduction of the basic number will result. This type of reduction has taken place several times in the evolution of the genera of the tribe Cichorieae, family Compositae. In *Youngia*, a close relative of *Crepis*, most of the species have the basic haploid number $x = 8$, but in *Y. tenuifolia* the number has been reduced to $x = 5$ (Babcock and Stebbins 1937). In *Ixeris*, the progression has been from $x = 8$ (*I. chinensis, I. japonica, I. stolonifera*) to $x = 7$ (*I. dentata*) and $x = 5$ (*I. denticulata, I. lanceolata*, etc., Babcock, Stebbins, and Jenkins 1937).

In *Godetia whitneyi*, the combined efforts of Hiorth and Håkansson (1946a) have demonstrated the production of a fertile type with six pairs of chromosomes out of a species which normally has seven. Hiorth crossed two structurally heterozygous monosomic plants of this species, and among the progeny obtained a relatively fertile F_1 plant with six pairs and essentially regular meiosis. The fact that this result was obtained only when structural heterozygotes were used as parents supports strongly the other evidence indicating that reduction of the basic chromosome number usually, if not always, involves rearrangement of chromosomal segments.

Progressive increase in basic chromosome number has been suggested in a much smaller number of examples among plants, and no natural example of such increase has as yet been supported by experimental evidence. Darlington (1936c) has suggested that *Fritillaria pudica*, with $x = 13$, has arisen from species with $x = 12$, since the latter number is the most common one in the genus and the only one found in the related genera *Lilium* and *Tulipa*. Levan (1932, 1935) has concluded that the most primitive species of *Allium* are the North American ones with $x = 7$, while the Eurasian species with $x = 8$ are derived from them. Krause (1931) has shown that in the genus *Dorstenia* (Moraceae) the primitive number is $x = 14$, corresponding with that for the Urticales as a whole. From this, the basic number of 12 and probably 10 have evolved in the Old World species, while in the New World the more specialized species have progressively increasing basic numbers of $x = 15$ and $x = 16$.

The simplest process by which the basic number could be pro-

gressively increased would be by the duplication of one whole pair of chromosomes. Plants containing such extra chromosomes, known as tetrasomics, have been found as occasional aberrants in a number of species grown experimentally, such as *Datura* (Blakeslee and Belling 1924), *Nicotiana* (Goodspeed and Avery 1939), and Maize (McClintock 1929). They are usually unstable, and since they are not isolated genetically from their normal relatives, they would under natural conditions lose their identity through crossing with plants having the normal chromosome number, followed by selection of the more viable, genetically balanced normal disomic types. Somewhat similar types of plants with increased chromosome numbers are the alien addition races produced by Gerstel (1945a,b) in *Nicotiana.* He crossed tetraploid *N. tabacum* $(2n = 96)$ with diploid *N. glutinosa* $(2n = 24)$ and pollinated F_1 plants of this cross with diploid *N. tabacum.* A great variety of segregant types resulted from these pollinations, but among them were constant, true-breeding lines with 25 and 26 pairs of chromosomes. These contained the complete diploid set of *N. tabacum* plus one or two pairs from *N. glutinosa.* Crossing between these alien addition races and normal *N. tabacum* produced trisomic or tetrasomic types in the F_1 generation, as expected, and only a small proportion of 25- or 26-paired types was recovered in the F_2 generation. Gerstel therefore concluded that alien addition races could exist in nature only in plants with regular self-fertilization. Although similar types have been produced as a result of hybridization by Florell (1931) and by O'Mara (1940) in wheat, with the addition of a chromosome pair from rye, and by Beasley and Brown (1943) in cotton, no example is know to the writer of such types existing in nature.

Such evidence as is available suggests that in nature progressive increase of the basic chromosome number is usually brought about by a process involving duplication of a centromere plus a system of translocations. This may occur most easily through the medium of extra centric fragment chromosomes, as described above (p. 446, and Fig. 41). Unfortunately, no clear examples of this type of alteration are yet available.

Another type of aneuploidy which simulates a progressive increase in chromosome number can be derived by a combination of progressive decrease or increase in basic number followed or

accompanied by amphidiploidy. If, for instance, a genus with an initial basic number of $x = 7$ produces by progressive reduction according to the *Crepis* scheme derivatives with $x = 6$ and $x = 5$, then various polyploid and amphidiploid combinations from these species can produce every haploid number from $n = 10$ upward. Polyploids of five-paired species will produce those with $n = 10$, while $n = 11$ can arise from amphidiploids of species with $x = 6$ and $x = 5$. In a similar manner may be derived $n = 12$ (6 + 6 or 7 + 5), $n = 13$ (7 + 6), $n = 14$ (7 + 7), $n = 15$ (5 + 10), $n = 16$ (6 + 10 or 5 + 11), $n = 17$ (7 + 10, 6 + 11, or 5 + 12), and so on. It will be seen that the higher the number, the more the different ways in which it can be derived; hence, in such series the higher numbers should be more common and found in more different unrelated species than the lower ones.

Part of such a series has been produced experimentally in *Brassica* by Nagahuru U (1935) and by Frandsen (1943). The basic diploid numbers in this genus are $x = 8$, 9, and 10, which probably represent a phylogenetically ascending series (Manton 1932). The amphidiploid combinations possible are those with $n = 16$, 17, 18, 19, 20, 24, 25, 26, 27, 28, 29, 30, and so on, and of these the numbers $n = 17$, 18, 19, 27, and 29 are known either in natural or in artificially produced amphidiploids. In *Erophila* (or *Draba* subg. *Erophila*) Winge (1933, 1940) has synthesized by amphidiploidy higher numbers in an aneuploid series. In the genus *Stipa*, which has an extensive aneuploid series extending from $x = 12$ to $n = $ c.42, Love (1946 and unpublished) has shown that among the four species, *S. leucotricha* ($n = 14$), *S. lepida* ($n = 17$), *S. pulchra* ($n = 32$), and *S. cernua* ($n = 35$), the chromosome behavior in hybrids indicates that the two latter species are allopolyploids containing genomes derived from one or both of the two former or from their relatives.

The most extensive aneuploid series in the plant kingdom is that in the genus *Carex,* in which haploid numbers ranging from $n = 6$ to $n = 56$ have been reported, and every number from 12 to 43 is represented by one or more species (Heilborn 1924, 1928, 1932, 1939, Tanaka 1937, 1939, Wahl 1940). Heilborn (1939) has considered that the most important processes in the origin of this series were structural changes of the chromosomes and autopolyploidy. The evidence produced by him in favor of structural

changes consists of the demonstration that within the comple-
ments of several species there exist chromosomes of very different
sizes, and that in some instances there are pairs of closely related
species with haploid numbers differing by one, of which the
species with the smaller number of chromosomes possesses a large
pair, while that with the larger number lacks it. Wahl (1940) has
produced even more convincing evidence of structural differences
between species. In several interspecific hybrids, both artificial
and natural (*C. pennsylvanica* $\times$ *umbellata, C. platyphylla* $\times$
plantaginea, C. swanii $\times$ *gracillima*), chains of three to six or
eight chromosomes occur regularly at meiosis, although bivalents
are the rule in the parental species. These interspecific hybrids,
therefore, are structural heterozygotes for translocations. Since
the chromosomes not forming these higher associations usually
pair as bivalents, so that meiosis in the hybrids is more or less
regular, this evidence also indicates a high degree of homology
between the chromosomes of different species belonging to the
same section.

This evidence weakens the assumption by Heilborn of auto-
polyploidy, which is based chiefly on the presence of associations
of three to eight chromosomes in *C. glauca*, which he therefore
interprets as an autopolyploid. For the evidence of Wahl sug-
gests that *C. glauca* could just as well be a segmental amphidi-
ploid derived from a hybrid between two species belonging to
the same section. At present, therefore, the only safe conclusion
is that in *Carex* structural changes and polyploidy or amphiploidy
have been the major processes responsible for its aneuploid series,
but that the type of polyploidy or amphiploidy involved is uncer-
tain and will remain so until a systematic series of hybridizations
is undertaken to unravel species relationships in at least one
portion of this most interesting and highly complex genus. Wahl
has provided indirect evidence that at least four original basic
numbers existed in *Carex*, namely, $x = 5$, 6, 7, and 8, with 7
probably the most common. The similar extensive aneuploid
series found in other Cyperaceae, as well as in the Juncaceae,
have probably originated in a manner similar to that in *Carex*.

We can thus classify naturally occurring aneuploid series of
chromosome numbers in sexually reproducing plants into three
types, as follows: 1) descending basic (*Crepis* and other Cicho-

TABLE 8

SUMMARY OF THE TYPES OF ANEUPLOID SERIES IN HIGHER PLANTS

Type and genus	Growth habit (P = perennial) (A = annual) (W = woody)	Range of numbers involved	Authority
DESCENDING BASIC			
Cycadaceae	P	11 (13)–8	Sax and Beal 1934
Dorstenia	P	14–12	Krause 1931
Polygonaceae	P	11–7	Jaretzky 1928
Delphinium-Nigella	P–A	8–6	Levitzky 1931b
Arabis	P	8–6	Rollins 1941
Lesquerella-Physaria	P–A	8–4	Rollins 1939
Cruciferae, several groups	P, P–A, A	8–7 and 7–6	Manton 1932, Jaretzky 1932
Primula	P	12–9	Bruun 1932
Polemoniaceae	P	9–7	Flory 1937, Wherry 1939
Phacelia (3 series)	P–A (3)	11–7, 11–9, 9–5	Cave and Constance 1942, 1944, 1947
Verbena-Glandularia	P–A	7–5	Dermen 1936a, Schnack and Covas 1944, Covas and Schnack 1944
Nicotiana (?)	P–A	12–9	Goodspeed 1934, 1945
Plantago	P–A	6–4	McCullagh 1934
Hemizonia	A	7(?)–4	Johansen 1933
Calycadenia	A	7–4	Clausen, Keck, and Heusi 1934
Crepis	P–A	6–3	Babcock 1947; see text

TABLE 8 (*Continued*)

SUMMARY OF THE TYPES OF ANEUPLOID SERIES
IN HIGHER PLANTS

Type and genus	Growth habit (P = perennial) (A = annual) (W = woody)	Range of numbers involved	Authority
DESCENDING BASIC			
Youngia	P	8–5	Babcock and Stebbins 1937
Leontodon	P	7–4	Bergman 1935b
Briza	P–A	7–5	Avdulov 1931
Hierochloe-Anthoxanthum	P–A	7–5	Avdulov 1931
Pennisetum	P–A	9–7	Avdulov 1931, Krishnaswamy 1940
Scilla-Bellevalia	P	8–4	Delaunay 1926
Ornithogalum	P	8–5	Delaunay 1926, Darlington and Janaki-Ammal 1945
Triteleia	P	7–5	Burbanck, 1941, 1944
Fritillaria	P	12–9	Darlington 1936c
ASCENDING BASIC			
Dorstenia	P	14–16	Krause 1931
Biscutella	P	8–9	Manton 1932
Phacelia	A	11–13	Cave and Constance 1942, 1944, 1947
Allium	P	7–9	Levan 1932, 1935
Triteleia-Dichelostemma	P	7–9	Burbanck 1941, 1944
Fritillaria	P	12–13	Darlington 1936c
INTERCHANGE-AMPHIDIPLOID			
Draba, subg. Erophila	A	7, 15–32, 47	Winge 1933, 1940
Brassica	A	8–11, 17–19	Manton 1932, Nagahuru U 1935

TABLE 8 (*Continued*)

SUMMARY OF THE TYPES OF ANEUPLOID SERIES
IN HIGHER PLANTS

Type and genus	Growth habit (P = perennial) (A = annual) (W = woody)	Range of numbers involved	Authority
INTERCHANGE-AMPHIDIPLOID			
Sedum	P + A	4–34, 56, 64	Baldwin 1935, 1937, 1939, 1940, R. T. Clausen 1942, Hollingshead 1942
Euphorbia	P + A	6–15, 20, 28, 30, c.50, c.100	Perry 1943
Viola	P + A	6–13, 17–24, 27, 28, 36, c.40	Miyaji 1929, Clausen1929,1931b, Gershoy 1934
Salvia	P + A	7–19, 22, 27, 32	Scheel 1931, Yakovleva 1933, Benoist 1938
Nicotiana	P–A	9, 10, 12, 16, 18–22, 24, 32	Kostoff 1943, Goodspeed 1947
Veronica	P + A	7–9, 14–21, 24, 26, 28, 32, 34	Hofelich 1935, Darlington and Janaki-Ammal 1945
Stipa	P	12, 14, 16–24, 32–35, 41	Avdulov 1931, Stebbins and Love 1941, Love (unpubl.)
Carex	P	6, 9, 12–56	Heilborn 1924, etc.; see text
Scirpus	P	13, 18, 20–34, 38, 39, 52, 55	Tischler 1931
Eleocharis	P	5, 8–10, 15–19, 23, 28	Tischler 1931
Iris	P	8–24, 27, 28, 30, 35, 36, 41–44, 54	Simonet 1934, Anderson 1936b, Foster 1937
Crocus	P	3–16, 20, 23	Mather 1932, Darlington and Janaki-Ammal 1945

rieae), 2) ascending basic (*Fritillaria pudica* et aff., *Allium,* New World *Dorstenia*), and 3) interchange polyploid-amphiploid (*Brassica, Erophila, Carex, Stipa*). A fourth type of aneuploid series, containing unbalanced numbers which have resulted from polyploidy and apomixis, was mentioned in Chapter X.

Table 8 gives a summary of all of the examples of these three types of series known to the writer. This summary shows that the number of descending basic series (25) is far greater than that of either ascending basic (6) or interchange-amphidiploid (13). More than half (12) of the descending series culminate in annual species or occur in strictly annual groups, while only one of the ascending series is of this nature. This supports the hypothesis, suggested in Chapter V, that a low chromosome number has a selective advantage in a cross-fertilized annual species, since it increases the amount of linkage and therefore the degree of constancy of a population over short periods of time. In the interchange-amphidiploid series, most of the higher numbers are derived, but the lowest basic number is not necessarily the original one. In fact, indirect evidence in five of the genera listed (*Euphorbia, Salvia, Veronica, Eleocharis,* and *Crocus*) suggests that the lowest numbers found in them have been derived by stepwise reduction.

Although more complete evidence may change somewhat the relative frequency of the known examples of these three types of aneuploid series, nevertheless we can safely say that changes in basic diploid chromosome number proceed more frequently in the direction of decrease than of increase. In plants higher chromosome numbers are usually produced by means of either euploid polyploidy or aneuploid amphidiploidy, and therefore through duplication of entire chromosome sets. It must be noted again, however, that in a number of families (Pinaceae, Fagaceae, Asclepiadaceae, Caprifoliaceae, Rubiaceae), a high degree of morphological divergence and differentiation of species and genera has been reached without any changes in chromosome number.

CHANGES IN FORM AND RELATIVE SIZE OF THE CHROMOSOMES

In many genera and families of flowering plants, conspicuous differences in the appearance of the karyotype have been found

in species having the same chromosome number. In some instances these differences follow definite trends, associated with trends of morphological specialization. The most significant of these correlations, in addition to the ones in *Crepis* and its relatives, is that recorded by Levitzky (1931b) in the family Ranunculaceae, tribe Helleboreae. In this tribe the genus most primitive in floral structures, *Helleborus*, has a karyotype in which the chromosomes differ little from each other in size and most of them are V-shaped or isobrachial, with median or submedian centromeres. Karyotypes of this nature are the most common ones in the higher plants as a whole and are the only ones found in most of the families and genera which are relatively homogeneous karyologically. Therefore, such karyotypes, as Levitzky has pointed out, can be considered as generalized types from which various specialized ones have been derived. Two types of specialization were traced out in the tribe Helleboreae. The first is the reduction in length of one of the chromosome arms, altering the V-shaped chromosomes first to J-shaped and then to headed types, with subterminal centromeres. Rod chromosomes with terminal or apparently terminal centromeres, the acrocentric chromosomes of White (1945), are the ultimate stage in this increasing asymmetry, but these are rare in plants, although they are rather common in animals. The second type of specialization consists of the reduction in size of some chromosomes in relation to others of the same set, so that the specialized karyotype contains chromosomes of very unequal sizes. Specialization along both of these lines in the Helleboreae reaches its climax in *Aconitum* and *Delphinium*, the two genera which have highly specialized zygomorphic flowers. In the latter genus the more primitive species, like *D. staphysagria*, have the largest number of V- or J-shaped chromosomes, while the reduced annual species of the subgenus Consolida (*D. ajacis*, *D. consolida*) have the most specialized karyotypes in both the form and the relative size of the component chromosomes. A karyotype consisting of chromosomes all essentially similar to each other in size and with median or submedian centromeres may be termed a symmetrical one. Asymmetrical karyotypes possess many chromosomes with subterminal centromeres, or great differences in size between the largest and the smallest chromosome, or both.

It·should be noted here that these trends of specialization· in the karyotype do not occur in any tribes of the Ranunculaceae other than the Helleboreae. In the Clematideae and the Anemoneae, both of which are highly specialized in their achenes, the karyotypes are essentially symmetrical (Gregory 1941, Meurman and Therman 1939). The apparently sporadic distribution of this and other trends of divergence in the karyotype is characteristic of the higher plants.

In the tribe Cichorieae, family Compositae, karyotype specialization follows essentially the same lines as in the Ranunculaceae (Babcock, Stebbins, and Jenkins 1937, Babcock and Jenkins 1943, Babcock 1947). Species of *Dubyaea* which are morphologically unspecialized in both vegetative and floral characteristics have symmetrical karyotypes. In *Crepis*, most of the chromosomes have submedian or subterminal centromeres, but the species with $n = 6$ and relatively primitive external morphology have on the whole more symmetrical karyotypes than do most of the species with $n = 4$ or $n = 3$.

In *Lactuca* the same tendency accompanies the increasing specialization of the species in external morphology, though to a lesser extent. But in *Hieracium, Taraxacum,* and *Youngia,* considerable specialization in external morphology has been associated with the retention of a karyotype of the symmetrical, unspecialized type similar to that of *Dubyaea.*

Examples of such trends in chromosome morphology are scattered through the higher plants, but are easily studied only if the chromosomes are reasonably large. In *Vicia* and in *Lathyrus,* the ·perennial species with $x = 7$ have mostly symmetrical karyotypes. But in the annual species, *Vicia sativa, V. angustifolia, V. faba,* and others, the reduction of the number to $n = 6$ and $n = 5$ is accompanied by the appearance of chromosomes with subterminal centromeres and considerable differences in size between the largest and the smallest chromosomes (Sveshnikova 1927, 1936).

In the Gramineae, tribe Hordeae, most of the species have symmetrical karyotypes, but the genus *Aegilops* is a notable exception. In this genus of reduced annual grasses increasing specialization of the fertile scales or lemmas, associated with a fragile rachis of the inflorescence and the development of a system of awns which is a great aid to the transportation of seeds, is asso-

ciated with increasing specialization of the karyotype. Elsewhere in the tribe, the karyotypes of species ordinarily placed in different genera are indistinguishable from each other, but in *Aegilops* each section has a distinctive karyotype (Senjaninova-Korczagina 1932, Kihara 1940). On the other hand, the genus *Hordeum* has developed a number of annual species with a fragile rachis on the inflorescence and a system of awns which aid in seed dispersal, and are therefore functionally similar, though not homologous, to the awn systems of *Aegilops*. But these annual *Hordeum* species (*H. murinum, H. gussoneanum, H. marinum*) have symmetrical karyotypes essentially similar to those of their perennial relatives.

The greatest diversity of karyotypes to be found in any single family of plants is probably that in the Liliaceae. Here, the most asymmetric examples are found in plants which are definitely specialized morphologically. The most striking of these is in *Yucca* and in *Agave*, which have five pairs of relatively large chromosomes with mostly subterminal centromeres and 25 much smaller ones (McKelvey and Sax 1933, Whitaker 1934a). A similar karyotype is found in the eastern Asiatic genus *Hosta*, but Whitaker's suggestion that this genus and *Yucca* are descended from a common ancestor is unlikely on both morphological and distributional grounds. Another type of highly asymmetrical karyotype is common to three morphologically specialized genera of the tribe Aloineae — *Aloe, Gasteria*, and *Haworthia* (Sato 1937, Resende 1937). This consists of seven pairs of chromosomes, four long and three very short, all with subterminal centromeres. It is noteworthy that *Kniphofia*, a genus of the Aloineae with much less specialization in its vegetative habit, has a much more symmetrical karyotype. Its six pairs of chromosomes are about equal in size, and three have median to submedian centromeres (Moffett 1932). Two other genera of Liliaceae, *Nothoscordum* (Levan 1935) and *Miersia* (Cave and Bradley 1943), are among the few plant genera with chromosomes having apparently terminal centromeres. Both of them are rather specialized as to flowers, as well as vegetative parts.

Speaking generally, the statement can be made that plants with asymmetrical karyotypes are usually specialized morphologically, sometimes in vegetative parts, sometimes in flowers or fruits, and

sometimes in both. On the other hand, while symmetrical karyo-
types are most often found in plants which are morphologically
more or less generalized, they also occur not uncommonly in rela-
tively specialized species or genera. In other words, the evidence
indicates that the karyotypes of the original, unspecialized pro-
genitors of most families of plants were essentially symmetrical.
Increased asymmetry of the karyotype, consisting in the evolution
both of chromosomes with subterminal centromeres and of in-
equality in size between the different chromosomes of the same
karyotypes, has been a frequent, but far from universal, type of
change accompanying increased specialization in external
morphology.

That this kind of change has taken place at various times
throughout the history of the seed plants is suggested by the
presence of a definitely asymmetrical karotype in the genera *Cycas*
(Sax and Beal 1934) and *Ginkgo* (Sax and Sax 1933). Both of
these genera, although in many characteristics among the most
primitive of seed plants, are in other respects highly specialized.
Fossil evidence, reviewed in Chapter XIV, indicates that both rep-
resent the end products of evolutionary trends which took place
during the Mesozoic era or even in the latter part of the Paleozoic
era, hundreds of millions of years ago. On the other hand, equally
specialized genera of conifers, such as *Pinus* and *Taxus*, have es-
sentially symmetrical karyotypes (Sax and Sax 1933).

As compared with plants, the karyotypes of most animals are
far more asymmetrical. Rod chromosomes with apparently
terminal centromeres are much more common and in many
groups, like the reptiles and the birds, the difference in size be-
tween the largest and the smallest chromosome of the set is
enormous (White 1945). The statement of White, that spon-
taneous breakage and translocation of chromosomes usually
involves whole arms that break near the centromere, does not hold
for plants. In them translocations usually involve breaks near
the middle or distal parts of the chromosomes, as is evident from
the presence of "interstitial segments" in the translocation
heterozygotes of *Oenothera* (see Chapter XI), of figure-of-eight,
"necktie," and similar configurations in *Pisum* (Sansome 1932)
in *Datura* (Bergner, Satina, and Blakeslee 1933), in *Paeonia*
(Stebbins and Ellerton 1939), and in other plants, and from chro-

mosome pairing in interspecific hybrids involving translocations (Tobgy 1943, Sherman 1946). It is tempting to speculate on the reason why animals and plants, which are in many other respects so very similar in their hereditary mechanism, should differ in the morphology of their karyotypes. The fact that animals possess a vastly more differentiated and specialized body than that of plants may be significant in this respect.

The reason for these trends in karyotype evolution — both the progressive reduction of the basic chromosome number and the increasing asymmetry — is not easy to find, although a tentative hypothesis in respect to the former has already been advanced. Both types of changes result directly from gross structural alterations of the chromosomes, either translocations or inversions. As explained above, progressive reduction is produced by unequal translocations, but it cannot take place unless some of the chromosomes possess genetically inert material near their centromeres. Also, it occurs more easily if they have at least one arm that is relatively short and completely inert. Increasing asymmetry may result either from unequal translocations or from inversions involving the centromere. If such an inversion occurs as a result of a break in one arm near the centromere, and in the other near its distal end, the chromosome can be converted at one step from a V-shaped one with a median centromere to a "headed" type with a subterminal centromere.

The difficulty of explaining these regular trends in karyotype differentiation as a result of structural alterations alone is that, in spite of a large body of knowledge about such alterations produced artificially by X radiation, no mechanism has been discovered by which they could lead to the progressive alteration of the karyotype which has been observed. To be sure, viable translocation and inversion types induced by X rays do not result from breaks occurring at random. The most frequent regions of breakage are the heterochromatic ones (Bauer, Demerec, and Kaufmann 1938, Heitz 1940). Furthermore, short arms have a greater chance of receiving chromosomal material from translocations, while long ones tend to give it up (Levitzky and Sizova 1934). Hence, if all viable translocation types had an equally high survival value, the tendency would be for translocations to alter an asymmetrical karyotype in the direction of greater symmetry rather than in that

of progressively increasing asymmetry. The occurrence of this latter tendency, therefore, would be most easily explained if in certain groups of organisms translocations which lead toward a more asymmetrical karyotype have a relatively high selective value. At present, however, evidence is not available for the formulation of such a hypothesis.

In regard to the third series of differences between karyotypes, those affecting the nucleoli and the satellites, a vast literature has accumulated, which is reviewed in detail by Gates (1942). The work of Heitz (1931), Navashin (1934), and McClintock (1934) established firmly the fact that the small beadlike appendages known as satellites or trabants, which in many species occur on the ends of one or a few pairs of chromosomes, are directly related to those portions of the chromosomes which form the nucleoli. The satellite is separated from the rest of the chromosome either by a slender thread or by a "secondary constriction," which corresponds with the nucleolar organizing region of the chromosomes. The size of the satellite depends on the distance of this constriction from the end of the chromosome. Usually it is nearly terminal and the satellite is small; but in some plants the presence of an interstitial nucleolar organizing region and secondary constriction produces a chromosome with a large "satellite" which may include as much as one fourth of the chromosome (Fig. 44). Usually the satellite is borne on the end of the short arm of a chromosome with a subterminal centromere, but V-type chromosomes bearing satellites are not uncommon. In most diploid species, only one pair of satellites is found, but species with two, three, or more satellited chromosome pairs are known. As mentioned earlier (page 362), this weakens greatly the value of the number of satellites and nucleoli as evidence for the existence of polyploidy. Furthermore, the genetic experiments of Navashin (1934) and of McClintock (1934) have shown that nucleolar organizers vary greatly in the strength of their activity. If by hybridization a chromosome with a strong nucleolar organizer is placed in the same cell with a weak one, the activity of the latter may not be expressed, and the plant may have one less than the expected number of nucleoli and satellites.

Very little is known about evolutionary changes in the satellites and nucleoli. The wide distribution of these structures through-
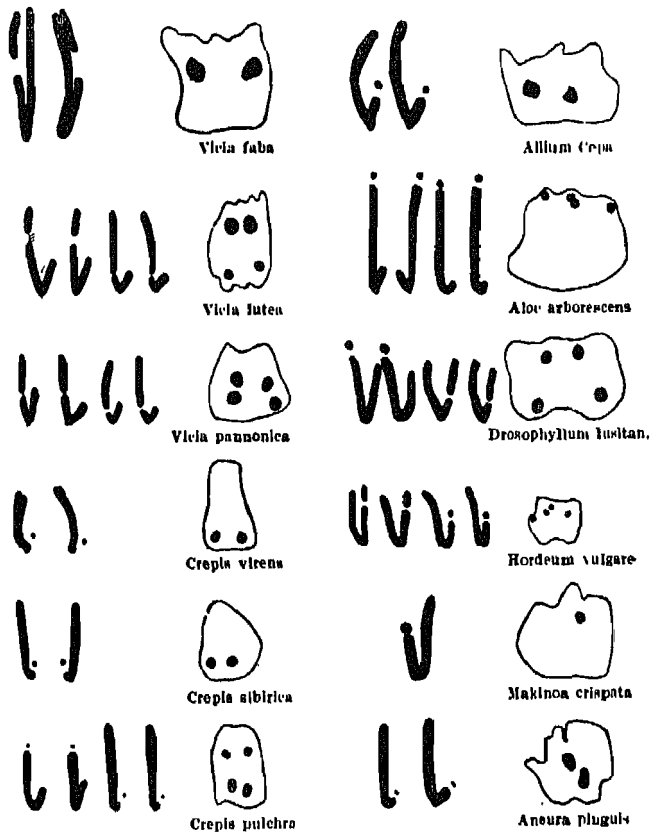
Fig. 44. Drawings showing various types of satellited chromosomes and their relationship to nucleoli. From Heitz 1931.

out the plant kingdom shows that they are a valuable, if not essential, part of the chromosomal complement. Furthermore, the distribution and relationships of species having different numbers and sizes of nucleoli and satellites suggests that a nucleolar organizer with its accompanying satellite may be either lost or gained during evolution, and that its size may either increase or decrease.

EVOLUTIONARY CHANGES IN CHROMOSOME SIZE

Differences in chromosome size are of two types. The first consists of differences in *relative* size between different chromosomes of the same set, as was discussed in a preceding section of this

chapter. Such changes are produced by unequal translocations, which increase the size of some chromosomes at the expense of others, without changing the total amount of chromosomal material present. In contrast to these are differences in *absolute* size of all the chromosomes of a set. The fact that some organisms have very small chromosomes and others very large ones is well known to cytologists. Darlington (1937, p. 82) has estimated that in the protozoan *Aulacantha* the total bulk of the somatic chromosomes at metaphase is more than 10,000 times that in the fungus *Saprolegnia,* which has very small chromosomes and a low basic number. Even in comparisons between different genera of the same family, as *Drosera: Drosophyllum,* the ratio of bulk is 1:1,000.

These differences in absolute chromosome size are to some extent controlled by factors outside of the chromosomes themselves. Pierce (1937) found that lack of phosphorus in the nutrition of the plant causes considerable reduction in the size of the chromosomes of *Viola.* Navashin (1934) found that in certain hybrids between species of *Crepis* having different chromosome sizes, the differences in the sizes of the chromosomes in the hybrid nuclei were much less than would be expected from comparison between the cells of the parental species. On the other hand, Tobgy (1943) found that the size differences between the chromosomes of *Crepis neglecta* and *C. fuliginosa* were just as evident in the hybrid nuclei as in those of the parental species, and therefore were apparently controlled by the chromosomes themselves. The factors controlling absolute chromosome size need much further exploration before the full significance of the trends to be described below will be evident.

In contrast to alterations of the basic number and the symmetry of the karyotype, changes in absolute size of the chromosomes appear to have involved both phylogenetic increase and decrease with about equal frequency, and are probably reversible. Phylogenetic reduction in chromosome size was first described by Delaunay (1926), in the genus *Muscari* of the Liliaceae. In this genus the species which show greater morphological specialization, like *M. tenuiflorum, M. caucasicum,* and *M. monstrosum,* have a smaller absolute chromosome size than the relatively primitive species, like *M. longipes.* This is associated with a reduction

in the amount of meristematic tissue in the root tips, and probably also in the shoots, since in M. *tenuiflorum* the rachis of the inflorescence is shorter and more slender, the flower pedicels are shorter, and the flowers are smaller than in M. *longipes*. In *Crepis*, a similar reduction in chromosome size, correlated with the appearance of the annual habit of growth and the reduction in size of all the parts of the involucre and the flower, was observed by Babcock and Cameron (1934). Other genera of the tribe Cichorieae in which phylogenetic reduction in chromosome size has taken place are *Youngia, Ixeris, Taraxacum*, and *Sonchus*. In these genera this reduction has not always been accompanied by appearance of the annual habit or decrease in size of the floral parts. The species of *Taraxacum* are nearly all long-lived perennials with large involucres and flowers, while some of the species of *Sonchus*, which nevertheless have relatively small chromosomes, are shrubby in habit and have large floral parts.

Other examples of phylogenetic reduction in chromosome size are scattered throughout the plant kingdom. In the leptosporangiate ferns the more primitive families Osmundaceae and Hymenophyllaceae have relatively large chromosomes; those in the Cyatheaceae and the Polypodiaceae are of intermediate size, while the smallest ones are in the vegetatively reduced and reproductively specialized heterosporous family Salviniaceae (de Litardière 1921). Among angiosperms, in addition to *Muscari* and the Cichorieae, phylogenetic reduction in chromosome size apparently occurs in the genus *Dianthus* of the Caryophyllaceae. Rohweder (1934) has reported that the reduced and specialized annual species, D. *armeria*, has smaller chromosomes than the perennials, while within the perennials themselves considerable differences in chromosome size exist. These are associated with resistance to cold, since the species with the largest chromosomes are found in high alpine areas. It is possible that the original species of *Dianthus* had chromosomes of an intermediate size, and that phylogenetic progression has been toward decrease in some lines and toward increase in others. The two families Juncaceae and Cyperaceae, both of which are almost certainly reduced, specialized derivatives of ancestors similar to the Liliaceae, have chromosomes considerably smaller in size than most species of the latter family.

The best example of phylogenetic increase in chromosome size is in the family Gramineae. Avdulov (1931) has pointed out that the most primitive grasses, of the tribes Bambuseae and his series Phragmitiformes, have relatively small chromosomes, as do also the more specialized grasses of tropical regions. But the grasses predominant in temperate regions, contained in his series Festucaeformes, usually have the basic number $x = 7$ and much larger chromosomes. Avdulov considers that the phylogenetic increase in size has occurred as an adaptation to the cool climate in which these grasses live, and he has suggested that it took place during the period of Pleistocene glaciation. The latter hypothesis is very unlikely in view of the wide distribution and extensive differentiation into tribes and genera found in the Festucaeformes, and in fact certain distribution patterns of species groups in this series suggest that many of its subgenera date back to the Tertiary period. But the correlation found in the Gramineae between large chromosome size and occupation of regions with a temperate climate may have some significance. Within certain groups of the Festucaeformes, on the other hand, the reverse tendency toward reduction in chromosome size may be observed. In the genus *Agropyron*, for instance, the reduced annual species *A. prostratum* has considerably smaller chromosomes than those of more typical species of the genus (Avdulov 1931, p. 260), while in another tribe the genus *Phalaris* has two specialized annual species, *P. canariensis* and *P. paradoxa*, which have smaller chromosomes than the perennial, more generalized species of *Phalaris*. The two genera *Poa* and *Puccinellia*, which have lemmas reduced in size and often with specialized, elongate cobwebby hairs, have chromosomes considerably smaller than those typical for the series Festucaeformes. This series of grass genera, therefore, provides the best evidence among plants for the reversibility of trends in absolute chromosome size.

Other examples of phylogenetic increase in chromosome size are scattered through the class of angiosperms. In the Polygonaceae, the group of *Rumex acetosa* and its relatives, which are specialized particularly in their dioecious condition, have larger chromosomes than any other species of the genus or family to which they belong (Jaretzky 1928). In the Cruciferae, of which most of the species have small chromosomes, two specialized

groups of annual species in *Hesperis* and *Matthiola* have large chromosomes for the family (Manton 1932). In the Leguminosae, the genera *Vicia*, *Pisum*, and *Lathyrus*, all closely related to each other and specialized, tendril-bearing vines, have the largest chromosomes found in the family, and therefore certainly represent end points of a trend of increase in chromosome size (Senn 1938a). In the Onagraceae considerable differences in chromosome size are found within the genus *Godetia* (Håkansson 1941, 1943b). Most remarkable is the fact that two closely related species, *Godetia bottae* and *G. deflexa*, were found to be strikingly different in chromosome size, the chromosomes of *G. deflexa* being more than four times as large as those of *G. bottae*. In addition, two related species, *G. amoena* and *G. whitneyi*, have larger chromosomes than most of the other species of the genus. These species, although their phylogenetic position in *Godetia* is at present uncertain, are significant in being among the most northerly in distribution of the genus.

Another probable example of phylogenetic increase in chromosome size is in the Rubiaceae. The extensive survey of Fagerlind (1937) has revealed one genus, *Galium*, in which many of the species have larger chromosomes than those typical of the family. This is a specialized genus of herbaceous perennials and annuals, and it is significant in being one of the few genera of this predominantly tropical family which are distributed in the North Temperate Zone.

Turning to the Monocotyledons, two further groups deserve mention. In the family Commelinaceae the temperate species of *Tradescantia* are conspicuous in having larger chromosomes than any other members of the family, which are mostly tropical in distribution (Darlington 1929). The phylogenetic position of these species is uncertain, so that one cannot say whether this family is an example of phylogenetic reduction or of increase in chromosome size. But in this, as in several previous examples, the significant fact is that the species inhabiting the temperate zone have conspicuously larger chromosomes than their tropical relatives. Finally, in the tribe Paridae of the family Liliaceae, the genera *Trillium* and *Paris* have the largest chromosomes known in the plant kingdom (Huskins and Wilson 1938, Geitler 1937, 1938). These genera are certainly specialized in their vege-

tative characteristics, and the tribe as a whole is related to and perhaps descended from the tribe Uvularieae (Hutchinson 1934), the members of which have large chromosomes, but nevertheless smaller ones than those in *Trillium* and *Paris*.

The facts which stand out from this survey are, first, that phylogenetic reduction and phylogenetic increase in chromosome size are about equally common in the higher plants and that both of these processes are reversible. Second, in about one half of the groups reviewed there is no difference in geographic distribution between the species with large chromosomes and those with small chromosomes. But in the other half such a difference exists, and the species with larger chromosomes invariably occupy cooler climates than those with small ones.

Another correlation between chromosome size and a characteristic of external morphology was pointed out earlier by the writer (Stebbins 1938b). Woody angiosperms nearly all have small chromosomes, while in herbaceous members of this class all sizes of chromosomes exist. The explanation of this correlation may lie in two characteristics of these woody types. First, the wood of angiosperms contains fiber cells which are very small in their transverse dimensions and must originate from small-sized cambial initials. Second, in woody plants the slow rate of replacement of individuals in a population puts a high selective premium on a genetic system permitting the maximum amount of recombination, as explained in Chapter V. This would favor a relatively high chromosome number and a symmetrical karyotype, both of which are characteristic of most woody plants (Stebbins 1938b). If the absolute size of the chromosomes were large, such a karyotype would have a relatively large total volume, and because of the relative constancy of the nuclear-cytoplasmic ratio this would require that all of the meristematic cells of the plant be relatively large. This would be incompatible with the necessary small cambial initials mentioned above, so that in woody angiosperms we should expect genetic changes tending toward small absolute chromosome size to have a high selective value.

In support of this hypothesis is the fact that the principal woody gymnosperms, the Coniferales, which lack wood fibers and have cambial initials all about equal in size, have symmetrical karyotypes and a relatively high basic number (mostly $x = 11$,

12, or 13), but also have rather large chromosomes. Among the few woody dicotyledonous angiosperms with medium-sized rather than small chromosomes are members of the most primitive order, Ranales, namely, *Illicium* (Whitaker 1933) and *Michelia* (Sugiura 1936, p. 572) of the Magnoliaceae, *Schizandra* and *Kadsura* of the Schizandraceae (Whitaker 1933), and various genera of the Anonaceae (Asana and Adatia 1945, Bowden 1945). It is likely, therefore, that phylogenetic reduction of chromosome size took place early in the evolution of woody dicotyledons.

Throughout this discussion the question naturally arises as to whether these differences in chromosome size involve corresponding differences in the number or size of the genes themselves. To this question no direct answer can be given as yet. The only plant species thoroughly known genetically is *Zea mays*, which has medium-sized to small metaphase chromosomes. The next best-known species are *Lycopersicum esculentum*, *Antirrhinum majus*, and *Hordeum vulgare*, of which the two former species have small chromosomes and the latter, large chromosomes. There is no evidence for the presence of more gene loci in *Hordeum* than in the other species mentioned, although this might possibly become evident when the genetics of *H. vulgare* are better known. Four other familiar cytogenetic objects with large chromosomes which should be watched in this connection are *Secale cereale*, *Vicia faba*, *Lathyrus odoratus*, and *Pisum sativum*.

Indirect evidence on the nature of differences in absolute chromosome size is provided by Tobgy's analysis (1943) of the difference between *Crepis neglecta* and *C. fuliginosa*. The metaphase chromosomes of *C. fuliginosa* are all smaller than the corresponding ones of *C. neglecta*, and this difference is evident in the F_1 hybrid nuclei at both somatic and meiotic metaphase. On the other hand, observations of chromosome pairing in the prophase of meiosis (pachytene) revealed that the pairing threads at that stage are of equal length. The difference in size between the metaphase chromosomes of the two species results from the greater contraction and presumably the tighter coiling of the chromonema in *C. fuliginosa*, and from the smaller amount of heavily staining substance, presumably desoxyribose nucleic acid, which forms around this chromonema. Examination of chromosomes in segregating individuals of the F_2 generation showed that in spite of the

fact that these chromosomes had acquired through crossing over in the bivalents of the F₁ plants chromosomal segments belonging to both parental species, each chromosome had dimensions characterizing it as typical of either *C. neglecta* or *C. fuliginosa*. Apparently, the dimensions of each chromosome are determined autonomously according to the genetic constitution of the region around its centromere.

A further difference between the nuclei of *C. neglecta* and *C. fuliginosa* is associated with the differences between them in chromosome size. The resting nuclei of *C. neglecta* contain many moderate-sized regions of heterochromatic material, that is, of substance which stains deeply after Feulgen treatment and is presumably desoxyribose nucleic acid. On the other hand, the nuclei of *C. fuliginosa* contain at the same stage a much smaller number of relatively large bodies of this substance. It is possible, therefore, that differences in absolute size of the chromosomes have nothing to do with the size of the chromonema or gene string, but are related to the coiling properties of this structure and to the amount and distribution of certain chemical substances in the chromosomes.

This possibility deserves further exploration in view of the fact that phylogenetic trends in absolute chromosome size, both toward decrease and toward increase, are often associated with a corresponding decrease or increase of the plant as a whole or of certain of its parts. Furthermore, if the relationship observed by Delaunay between reduction in chromosome size and decrease of the amount of meristematic tissue holds for other groups of plants, then there probably exists a definite influence of the amount of chromatin in the nucleus on the extent of meristematic activity of the tissue. In other words, it may become possible here to establish a definite relationship between the chemical constitution of the nucleus and certain highly significant morphological and physiological characteristics of the plant.

DIFFERENCES IN THE RESTING AND PROPHASE NUCLEUS

Until now we have been considering only differences and trends in the karyotype itself, that is, in the chromosomes as they appear at somatic metaphase. But in the preceding section it was shown that in one of these characteristics, absolute chromosome size, the

differences observed in the metaphase karyotype are closely connected with and perhaps dependent upon differences in the physicochemical nature of the nucleus which are observable only in the resting and prophase stages. The subject of evolutionary trends in the karyotype is, therefore, not complete without a survey of the comparative karyology of the resting and prophase nucleus.

The background for such a survey is the pioneering and fundamental work of Heitz (1932), on which the following account is based. He recognized a number of different types of resting nucleus, depending on the distribution of the so-called heterochromatic substance, that is, the substance which stains darkly when nuclei are prepared according to the Feulgen method, and is presumably desoxyribose nucleic acid. In one type, exemplified by *Paeonia,* this substance is found almost throughout the nucleus, so that the chromosome threads can be stained at any stage of the resting or mitotic cycle. In addition, small centers which stain more strongly are found near the centromere. These are known as chromocenters. This type of nucleus is found in many groups of plants with large chromosomes (Ranunculaceae, Liliaceae, many Gramineae) and is probably the commonest type in such groups. A modification of it is found in *Vicia faba,* in which small chromocenters occur at definite positions on the distal parts of the chromosome arms, as well as at the centromere.

The second type of nucleus is termed by Heitz the "cap nucleus," and it is exemplified by *Hordeum vulgare.* In this, the chromosome substance at one side of the nucleus stains much more heavily than that at the other. The staining region can be identified at mitotic telophase as the one away from the equator and toward the pole of the spindle, so that the parts of the chromosomes which stain are large chromocenters situated about the centromere and the proximal portions of the arms. Cap nuclei are not common and are found usually in plants with relatively large chromosomes (*Hordeum, Collinsia, Scorzonera*).

The two other types of nucleus are those in which all the heterochromatic substance is aggregated in a more or less definite and relatively small number of chromocenters, the remainder of the resting nucleus being clear when stained by the Feulgen technique. These chromocenters may be located at various positions

along the chromosome arms in addition to the region of the centromeres, or they may be confined to the latter regions, so that the number of chromocenters corresponds to the diploid number of chromosomes. Such large, localized chromocenters have been termed prochromosomes by many authors. Most resting nuclei, therefore, belong to one of four principal types, as follows.

Diffuse staining type. — Associated with large chromosomes; no conspicuous aggregation of heterochromatic material. Examples: *Paeonia, Bromus,* some species of *Allium.*

Cap nucleus. — Associated with large or medium-sized chromosomes. Heterochromatic material concentrated in the proximal regions of the chromosome arms. Examples: *Hordeum, Collinsia, Scorzonera.*

Multiple chromocenter nucleus. — Associated with medium-sized or small chromosomes. Heterochromatic substance contained in dark-staining chromocenters, which, at least in early prophase, are of a higher number than the diploid number of chromosomes and are found in the distal parts of the chromosome arms as well as near the centromeres. Examples: *Pellia* (Heitz 1928), *Crepis neglecta, C. fuliginosa.*

Prochromosome nucleus. — Associated chiefly with small chromosomes. Heterochromatic substance confined to a number of dark-staining bodies or chromocenters which, at least at early prophase, often equal the diploid chromosome number, each chromocenter consisting of the proximal portions of the two chromosome arms. Examples: *Thalictrum, Impatiens, Oenothera, Sorghum vulgare.*

Nuclei intermediate between some of these types are occasionally found. There is little doubt that the evolutionary trend from the diffuse to the multiple chromocenter type of nucleus and vice versa can proceed in either direction, since the differences between these two types are usually associated with differences in absolute chromosome size. It is likely, also, that the evolution from a cap nucleus to a prochromosome nucleus, as well as the reverse trend, can occasionally take place. On the other hand, the trend from the diffuse to the cap nucleus or from the multiple chromocenter to the prochromosome type may be irreversible. The few definitely established examples of cap nuclei are in relatively specialized species or genera, of which the more primitive

relatives have the diffuse type. *Hordeum vulgare,* for instance, is one of the most specialized members of its genus. In most of the more primitive perennial species of *Hordeum,* as well as in most species of the more generalized and perhaps ancestral genus *Elymus,* the nuclei are of the diffuse type. The same may very well be true of prochromosome nuclei, which also seem to be found in relatively specialized groups. *Sorghum vulgare,* the best-known example in the Gramineae, is certainly a specialized end line in its tribe. The evidence suggests that in certain unrelated lines the tendency develops for the chromosomal regions with a high content of desoxyribose nucleic acid to become aggregated near the centromeres, but that the reverse tendency rarely, if ever, takes place. The significance of this tendency is at present obscure.

None of the examples presented in this chapter of evolutionary trends in the karyotype are consistent for all groups of plants. But every one of these trends has occurred repeatedly in several different unrelated groups of plants, and in many cases has persisted over the time needed to differentiate whole genera. Such long-continued trends cannot be explained by chance, and there appears to be nothing in the structure of the chromosomes themselves which would force them to continue evolving in the same direction. They can be explained best on the assumption that in certain types of organisms under some particular environmental conditions alteration of the chromosomes in the direction of one of these trends has a definite selective value. At present, the study of the comparative karyology is in its infancy, so that the nature of these selective values can only be suggested in the form of tentative hypotheses. But they may eventually provide valuable clues as to the relation between changes in the chromosomes and the evolution of the individual and the population in visible, external characteristics.