NUCLEAR DNA VARIATION IN *ALLIUM*

R. NEIL JONES* and H. REES

Department of Agricultural Botany, University College of Wales, Aberystwyth

Received 18.ii.68

1. Introduction

Now that DNA is firmly established to be the carrier of genetic information much can be learned about the nature and origin of heritable variation by directly investigating qualitative and quantitative changes in the DNA itself. Qualitative differences in the base sequences and the base ratios of nucleic acids are reported for a number of species (*e.g.* Chargaff, 1955; Reddi, 1959). A growing body of evidence also testifies to widespread variation in the quantity of nuclear DNA between species (*e.g.* McLeish and Sunderland, 1961; Keyl, 1964, 1965; Rees *et al.*, 1966; Rothfels *et al.*, 1966; Martin and Shanks, 1966; Martin, 1966; John and Hewitt, 1966). Such variation is especially common among Angiosperms and may be quite independent of change in chromosome number. The DNA differences are often large—even between species closely related. For example, *Vicia faba* has seven times more DNA than *V. sativa* (Rees *et al.*, *loc. cit.*). Both are diploids, yet the variation in DNA content is equivalent to that between $2x$ and $14x$.

The following is an investigation of nuclear DNA variation in *Allium*. It reveals that widespread changes in nuclear DNA content accompanied the divergence and evolution of species within the genus. In addition it provides information about the nature and the distribution of the chromosome structural changes which account for the variation in nuclear DNA.

2. Material and methods

(a) *Material*

The genus *Allium*, characterised by its bulbous plants smelling of onion or garlic, is world wide in distribution and comprises over 500 species. Their collective range covers almost the whole northern hemisphere, from the Atlantic coasts of Europe and North Africa to the Pacific, and from the western to the eastern coast of North America. Most of them are to be found in temperate mountain regions with well-marked seasons, notably in California, north-west Persia and Central Asia. Several species are grown as vegetables and others for their flowers (Stearn, 1944; Clapham, Tutin, Warburg, 1962). There is considerable variation in morphology and breeding system; some reproduce sexually as outbreeders, inbreeders, or versatile breeders, and others are asexual with vivipary and apomixis (Ved Brat, 1965). Successful interspecific hybridisation has been reported in some species, notably *Allium cepa* × *A. fistulosum* (Emsweller and Jones, 1935; Levan, 1936, 1941; Maeda, 1937), and *Allium ascalonicium* × *A. fistulosum* (Cochran, 1942).

* Present address: Sub-Department of Genetics, The Queen's University of Belfast.

Most species are diploid with basic chromosome numbers of $x = 7$, $x = 8$, or $x = 9$; $x = 8$ being the most common. The "New World" North American species (which form a group of their own) nearly all have a basic number of $x = 7$. In addition to these differences in basic numbers many species are polyploid, and some have polyploid series. *B*-chromosomes, are also found in several *Allium* species (Ved Brat, 1964). As far as chromosome size is concerned, Levan (1935) has noted considerable differences in the size of the chromosomes of various species. Taking the length of the longest chromosome as his measure of size, he gives a range of 7 μ (*Allium yunnanense*, $x = 8$) to 22 μ (*Allium fragrans*, $x = 9$). He also reports that, in general, there is little variation in chromosome size within *Allium* complements (see also Sharma, 1964). Arm-length asymmetry is more pronounced in the "16" and "18"-chromosomes types. Levan (1932, 1935) took this to mean that the "14"-chromosome types were the most primitive and that the "16" and "18"-chromosome types were derived from them.

The variation in chromosome size established by these earlier studies strongly suggests a variation in nuclear DNA in association with the evolution of *Allium* species. In the present work nuclear DNA measurements were made in the 25 species listed in table 1.

TABLE 1

The Allium *species*

Species	2*n*	Earlier references*	Source
A. karataviense	18	Levan, 1935	Holland (de Jaeger)
A. zebdanense	18	Levan, 1935	Holland (de Jaeger)
A. zebdanense	45	Jones, 1967	Latvia
A. triquetrum	18	Levan, 1935	Kew (Royal Bot. Gd.)
A. pulchellum	16	Levan, 1935	Kew (Royal Bot. Gd.)
A. cepa	16	D'Amato, 1948	Aberystwyth
A. fistulosum	16	Levan, 1935	Holland (Wageningen)
A. stellatum	16	Jones, 1967	Leeds (Univ. Bot. Gd.)
A. galanthum	16	Ved Brat, 1964	Latvia
A. jesolianum	16	Jones, 1967	Bruxelles (Univ. Gds.)
A. dicipience	16	Mensinkai, 1940	Bruxelles (Univ. Gds.)
A. roseum	16	Levan, 1935	Bruxelles (Univ. Gds.)
A. azureum	16	Jones, 1967	Holland (de Jaeger)
A. darwasicum	16	Ved Brat, 1964	Bruxelles (Univ. Gds.)
A. schoenoprasum	16	Levan, 1936	Aberystwyth
A. odoratissimum	16	Jones, 1967	Bruxelles (Univ. Gds.)
A. sibiricum	16	Levan, 1936	Latvia
A. globosum	32	LaCour, 1945	Paris (Nat. Hist. Mus.)
A. margaritaceum	32	Mensinkai, 1940	Chelsea (Physic Gds.)
A. senescens	32	Ono, 1935	Hungary
A. angulosum	32	Levan, 1935	Chelsea (Physic Gds.)
A. hirsutum	14	Feinbrun, 1950	Oxford (Univ. Bot. Gds.)
A. subhirsutum	14	Kurita, 1960	Oxford (Univ. Bot. Gds.)
A. cernum	14	Levan, 1935	Kew (Royal Bot. Gds.)
A. neapolitanum	14	Levan, 1935	Oxford (Univ. Bot. Gds.)
A. fuscum	14	Ved Brat, 1964	Oxford (Univ. Bot. Gds.)
A. moly	14	Levan, 1932	Holland (de Jaeger)
A. ursinum	14	Levan, 1932	Oxford (Univ. Bot. Gds.)

* From Darlington and Wylie (1955) and Vad Brat (1964).

(b) *Methods*

(i) *Cultivation of* Allium *species.* Plants used in the present study were either raised from seed, and subsequently transferred to large pots, or grown directly from bulbs, again in large pots. The pots were filled with John Innes potting compost, and plants kept under observation in an unheated glasshouse.

(ii) *Estimation of nuclear DNA by Feulgen photometry.* The fixation and staining technique used was that described in detail by McLeish and Sunderland (1961) and Sunderland and McLeish (1961). Photometric measurements were made on a Barr and Stroud integrating microdensitometer. All measurements were made on 2*C* telophase and early interphase nuclei. Estimates of DNA values can fluctuate between experiments due to many factors, *e.g.* different preparations of fixative, different preparations of stain, fluctuations in *p*H of stain, temperature variations, etc. Because it was not practical to handle more than four or five species in any one experiment, the precaution was taken of measuring a standard species (root-tip nuclei form the same bulb of *Allium cepa*—which could be used over more than one season) with each experiment. Results could then be weighted according to whether the values for the standard species were higher or lower than in the initial experiment. For each experiment actively growing roots were taken directly from pots in the glasshouse and thoroughly washed in water before fixation.

(iii) *Estimation of chromosome volume.* To calculate chromosome volumes the total chromatid length was measured at metaphase in each cell by means of a micrometer eyepiece and a mean estimate of chromatid width obtained from a random sample of five chromatids per cell. The calculation of the total chromosome volume assumes each chromatid to be cylindrical in form. Metaphases were scored in root tips following colchicine treatment and Feulgen staining. There were five replicates (cells) per species, taken from five different plants where possible.

3. Results

(a) *Nuclear DNA variation*

DNA values for the 25 *Allium* species are presented in table 2. (These are the weighted values, as described in Material and Methods.) The values are based on measurements of ten nuclei in each of two (sometimes four) plants in each species. The main conclusions to be drawn from these results are as follows:

(i) There are significant differences in nuclear DNA content between diploid *Allium* species within each basic chromosome number group ($P < 0{\cdot}001$). There are differences of the order of twofold between diploids in all three groups, equivalent to that between $2x$ and $4x$ in terms of ploidy. Constancy of chromosome number within this genus clearly does not reflect a constancy in the amount of nuclear genetic material.

(ii) Basic chromosome number in itself is not a reliable guide to DNA amount. For example, several species in the 14-chromosome group have higher DNA values than some species in both the 16 and 18 groups. There are also significant differences between the nuclear DNA contents of the three groups ($P < 0{\cdot}01$), the 18 group having the highest value, the 16 group the lowest.

(iii) Some polyploids have less DNA than diploids in the same genus, cf. *A. senescens* ($x = 8$, $4x$) and *A. karataviense* ($x = 9$, $2x$).

TABLE 2

Mean DNA amounts (in arbitrary units) in 2C nuclei of Allium *species (weighted values, each based on 10 cells in each replicate)*

Basic chr. no.	Species	Replicate 1	2	3	4	Mean DNA value
9	*A. karataviense*	45·4	—	—	—	45·4
	A. triquetrum	35·0	35·5	37·4	37·2	36·3
	A. zebdanense	25·3	25·3	—	—	25·3
	A. zebdanense (5×)	63·7	61·0	61·4	60·2	61·6
8	*A. cepa*	33·5	33·5	—	—	33·5
	A. fistulosum	27·2	25·6	25·5	26·9	26·3
	A. stellatum	26·0	25·0	—	—	25·5
	A. galanthum	24·8	24·0	—	—	24·4
	A. jesolianum	24·0	24·1	—	—	24·1
	A. dicipience	20·3	22·6	—	—	21·5
	A. roseum	18·1	20·6	21·3	21·6	20·4
	A. azureum	18·1	17·4	—	—	17·8
	A. darwasicum	18·1	17·2	—	—	17·7
	A. schoenoprasum	15·4	18·4	16·7	17·2	16·9
	A. odoratissimum	14·9	15·4	15·6	16·3	15·6
	A. sibiricum	14·8	15·8	—	—	15·2
	A. globosum (4×)	76·4	75·2	—	—	75·8
	A. margaritaceum (4×)	44·2	43·4	—	—	43·8
	A. senescens (4×)	43·8	42·5	—	—	43·2
	A. angulosum (4×)	43·1	39·3	—	—	41·2
7	*A. hirsutum*	35·5	36·3	—	—	35·9
	A. subhirsutum	35·1	34·4	—	—	35·7
	A. cernum	34·1	34·3	—	—	34·2
	A. neapolitanum	32·5	31·8	—	—	31·2
	A. fuscum	18·0	18·8	—	—	18·4

(b) *Chromosome volume and nuclear DNA*

From figs. 1, 2 and 3 it is clear that there is considerable variation in chromosome size between species. In fig. 4 the chromosome volumes are plotted against nuclear DNA content. They are clearly correlated and a regression analysis of variance of chromosome volume on to nuclear DNA confirms a positive regression ($P < 0{\cdot}01$). The correlation is in keeping with the results of other investigations (*e.g.* Rees, Cameron, Hazarika and G. H. Jones, 1966; Rothfels, Sexsmith, Heimburger and Krause, 1966).

(c) *Chromosome change within complements*

Since DNA amount is, in general, correlated with chromosome size, it is possible to ascertain whether the DNA differences between species are restricted to particular chromosomes or distributed more or less symmetrically between all chromosomes in the complement. This may be done in two ways, as discussed below.

(i) *A general comparison.* If it is assumed that the changes in chromosomal DNA are spread regularly throughout all chromosomes in the complement

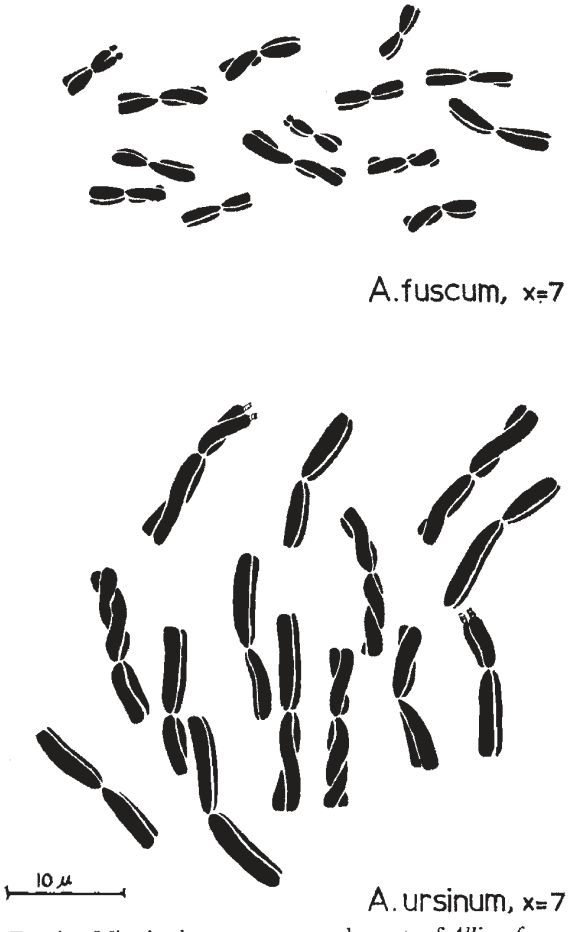


FIG. 1.—Mitotic chromosome complements of *Allium fuscum* and *Allium ursinum*, both $x = 7$.

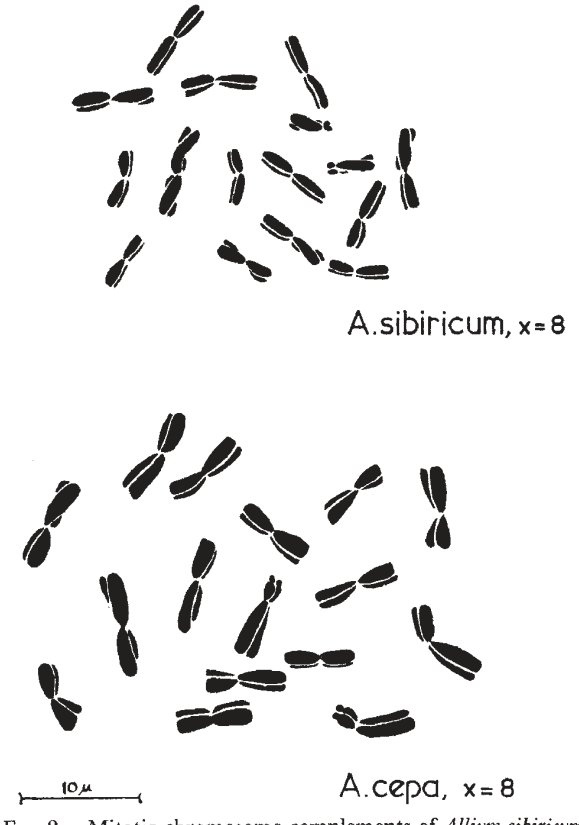


FIG. 2.—Mitotic chromosome complements of *Allium sibiricum* and *Allium cepa*, both $x = 8$.

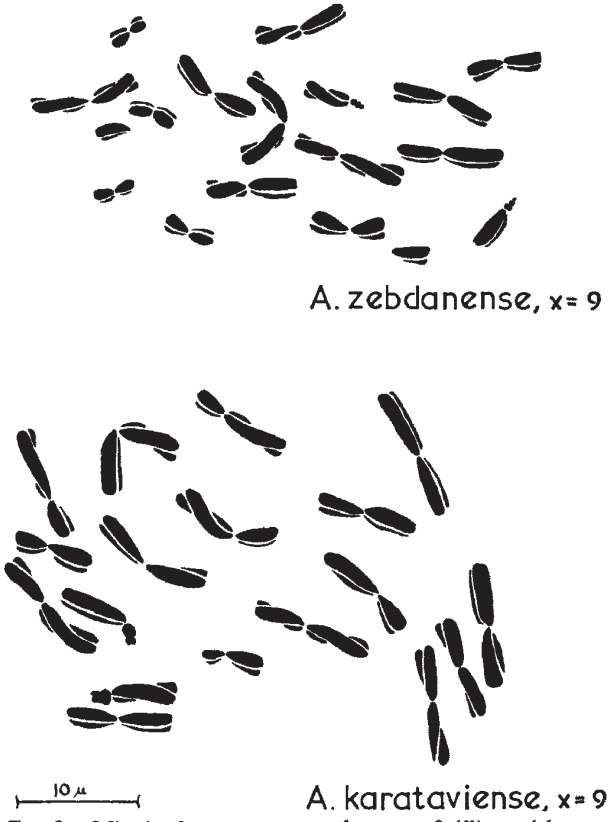


FIG. 3.—Mitotic chromosome complements of *Allium zebdanense* and *Allium karataviense*, both $x = 9$.

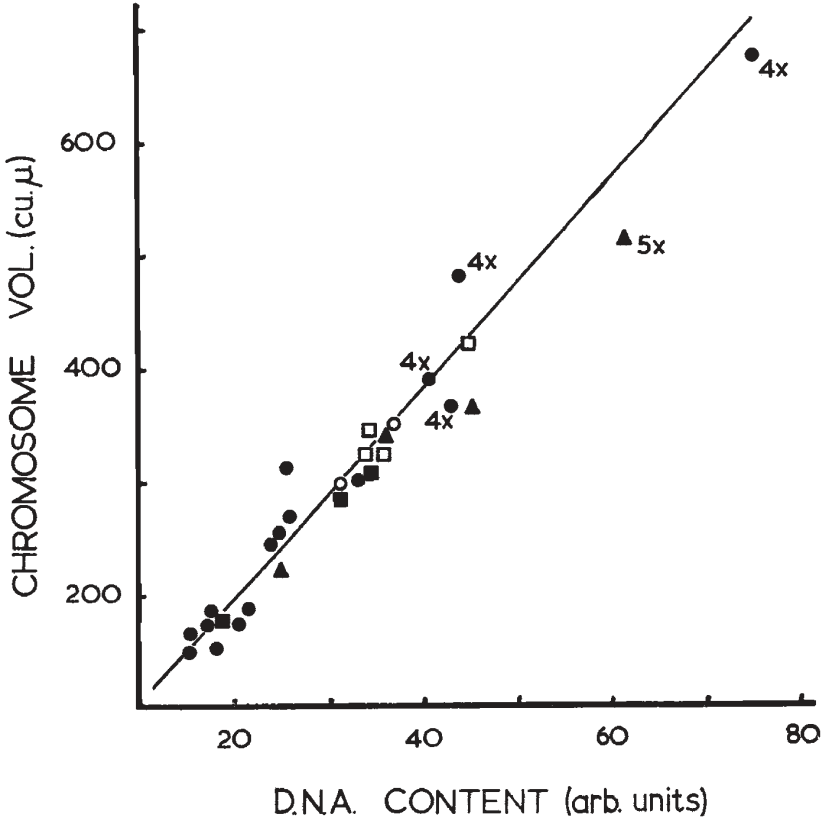


FIG. 4.—Mean chromosome volumes (cu. u) of *Allium* species, plotted against their DNA values (arb, units). Diploids unless indicated otherwise on the graph. ■ $x = 7$; ● $x = 8$; ▲ $x = 9$; □, ○ are species for which only the chromosome volume, or only the DNA value, is known.

then the relative sizes of chromosomes will be consistent throughout all species complements. For example, if the nuclear DNA content of one species is twice that of another we should expect all its chromosomes to have increased their DNA by a factor of two and all chromosomes therefore to

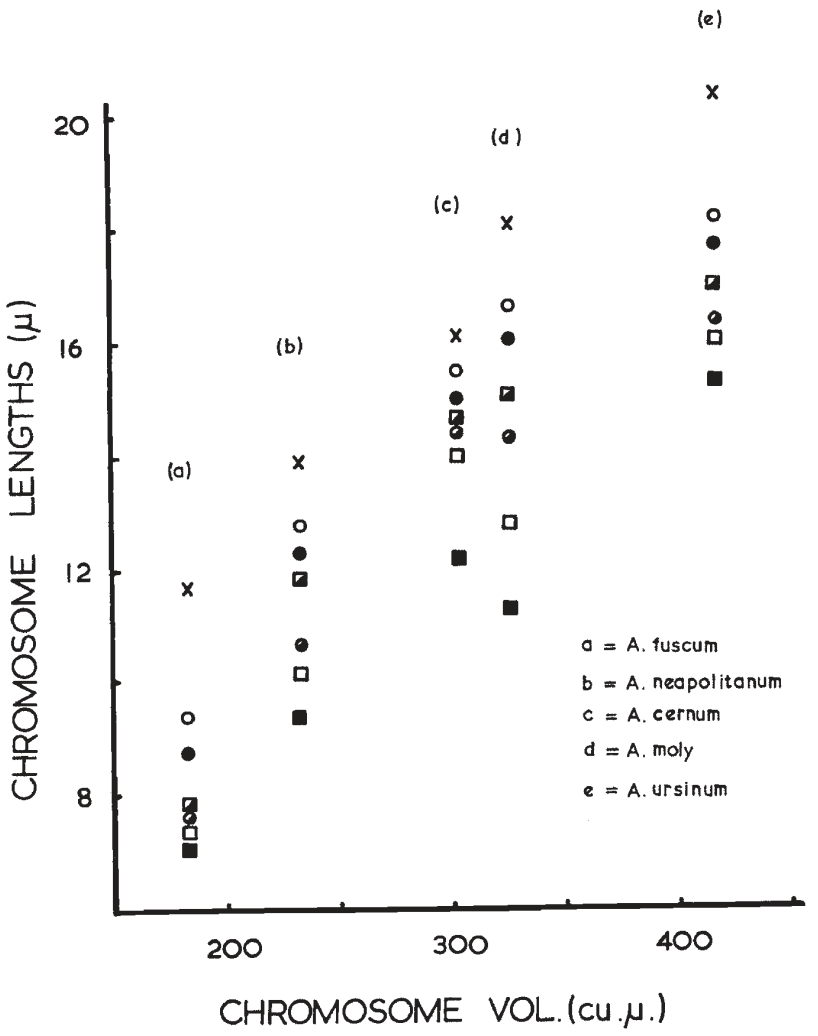


FIG. 5.—Chromosome lengths plotted against mean total chromosome volume for species with $x = 7$.

× chromosome I; ○ chr. II; ● chr. III; ◪ chr. IV; ◑ chr. V; □ chr. VI; ■ chr. VII, in order of length.

have increased their size to the same degree. There is one qualification. Interchanges or transpositions would confuse and confound this consistency within complements.

In the graphs (figs. 5, 6 and 7) the lengths of individual chromosomes are plotted against the total chromosome volume for each species. From the graphs it is possible to establish whether the increase in length is consistent

for all chromosomes in the complement as the total chromosome size (and nuclear DNA content) increases. There is good indication from the graphs for each of the three chromosome groups that the increase in size is indeed consistent throughout all chromosomes of the complement and, therefore, that the DNA changes are distributed fairly uniformly throughout all the chromosomes. This conclusion is supported by regression analyses of variance. The slopes of the regression of the increase in length of the longest

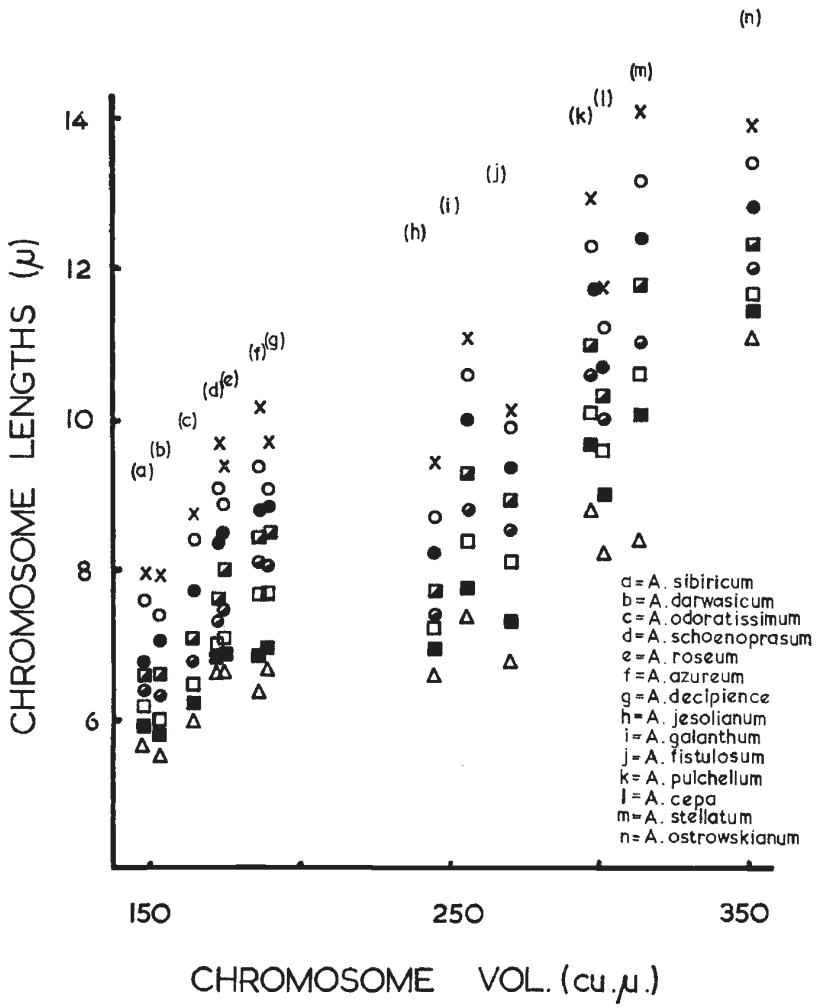


FIG. 6.—Chromosome lengths plotted against mean total chromosome volume for species with $x = 8$.

× chromosome I; ○ chr. II; ● chr. III; ◪ chr. IV; ◑ chr. V; □ chr. VI; ■ chr. VII; △ chr. VIII, in order of length.

chromosome and of the second and third longest, etc., on the total chromosome volume in each group are not significantly heterogeneous. In short, there appears to be a notable regularity in the distribution of DNA changes between chromosomes within complements.

The comparisons assume, of course, that the chromosomes in order of size in any one complement correspond and are homologous with the chromosomes similarly arranged in the complements of other species. As mentioned earlier, structural changes may have taken place which to some degree would invalidate this assumption. In very general terms, however, it appears reasonable to conclude that the DNA changes are widely distributed between chromosomes.

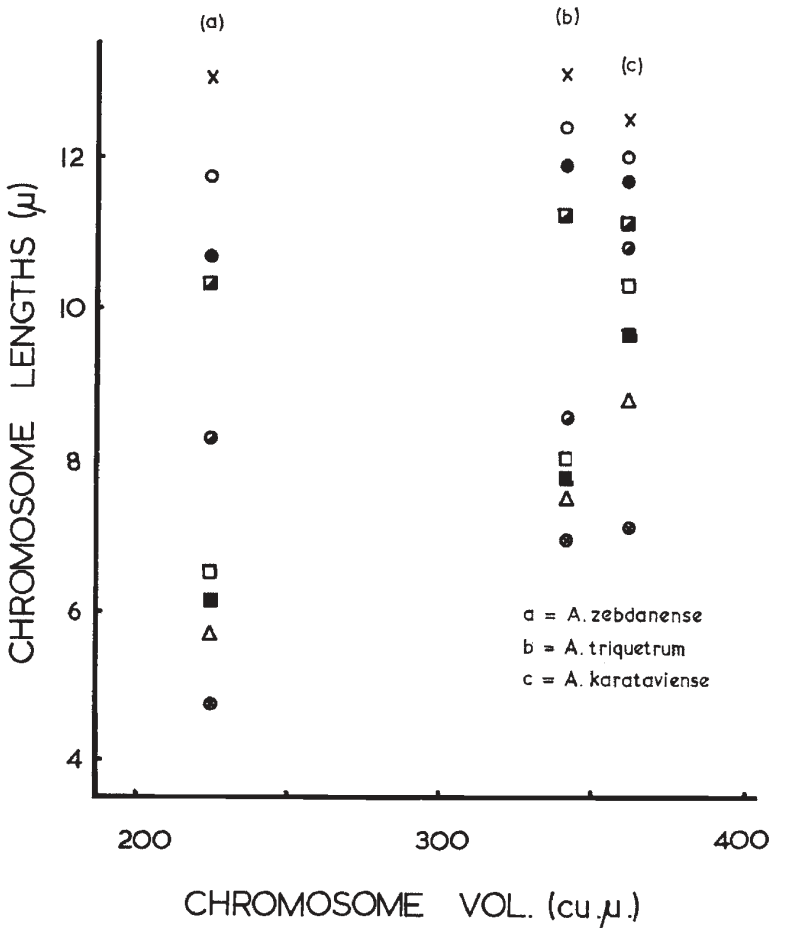


FIG. 7.—Chromosome lengths plotted against mean total chromosome volume for species with $x = 9$.

× chromosome I; ○ chr. II; ● chr. III; ◪ chr. IV; ◑ chr. V; □ chr. VI; ■ chr. VII; △ chr. VIII; ⊗ chr. IX, in order of size.

(ii) *The hybrid* A. cepa × A. fistulosum. *Allium cepa* has about 27 per cent. more nuclear DNA than *A. fistulosum* and the total volume of its chromosomes at metaphase of mitosis is about 30 per cent. greater. The chromosomes of both species, in order of size, are shown in fig. 8. Superficially it would appear that every *A. cepa* chromosome is about 25-30 per cent. larger than its *A. fistulosum* " homologue ". If this were the case we

should expect, first, all bivalents at first metaphase of meiosis in the *A. cepa* × *A. fistulosum* hybrid to be asymmetrical and, secondly, that the bivalents would show a similar degree of asymmetry. Plate fig. 1*a* confirms that all bivalents are asymmetrical. At the same time it is clear from this figure that the degree of asymmetry varies very considerably between the different bivalents. Measurements in first metaphases show that in some bivalents the differences in length between " homologues " is more than 60 per cent. In others it is less than 20 per cent. Evidently the ranking in order of size in fig. 8 does not correspond to the order of homology. These observations at meiosis nevertheless confirm the earlier conclusion that the variation in chromosome size and hence in DNA content involves all chromosomes in the *Allium* complement. They also indicate, however, that the extent of structural changes which account for the DNA change is not consistent for all chromosomes of the complement. It could, of course, be argued that extensive interchanges between non-homologous chromosomes subsequent to the DNA changes could account for the disproportionate variation in size

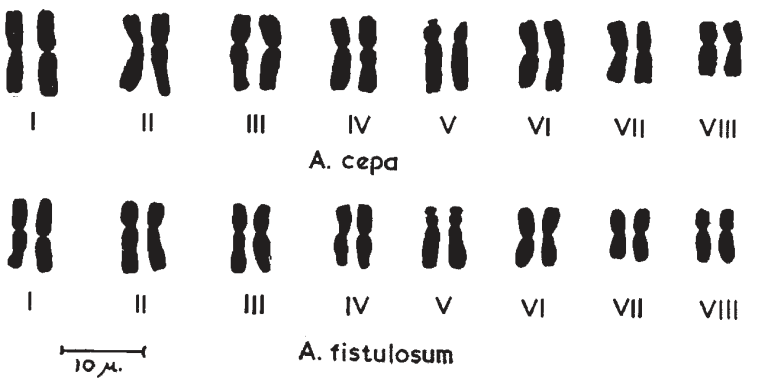


FIG. 8.—The chromosome complements of *Allium cepa* and *Allium fistulosum*.

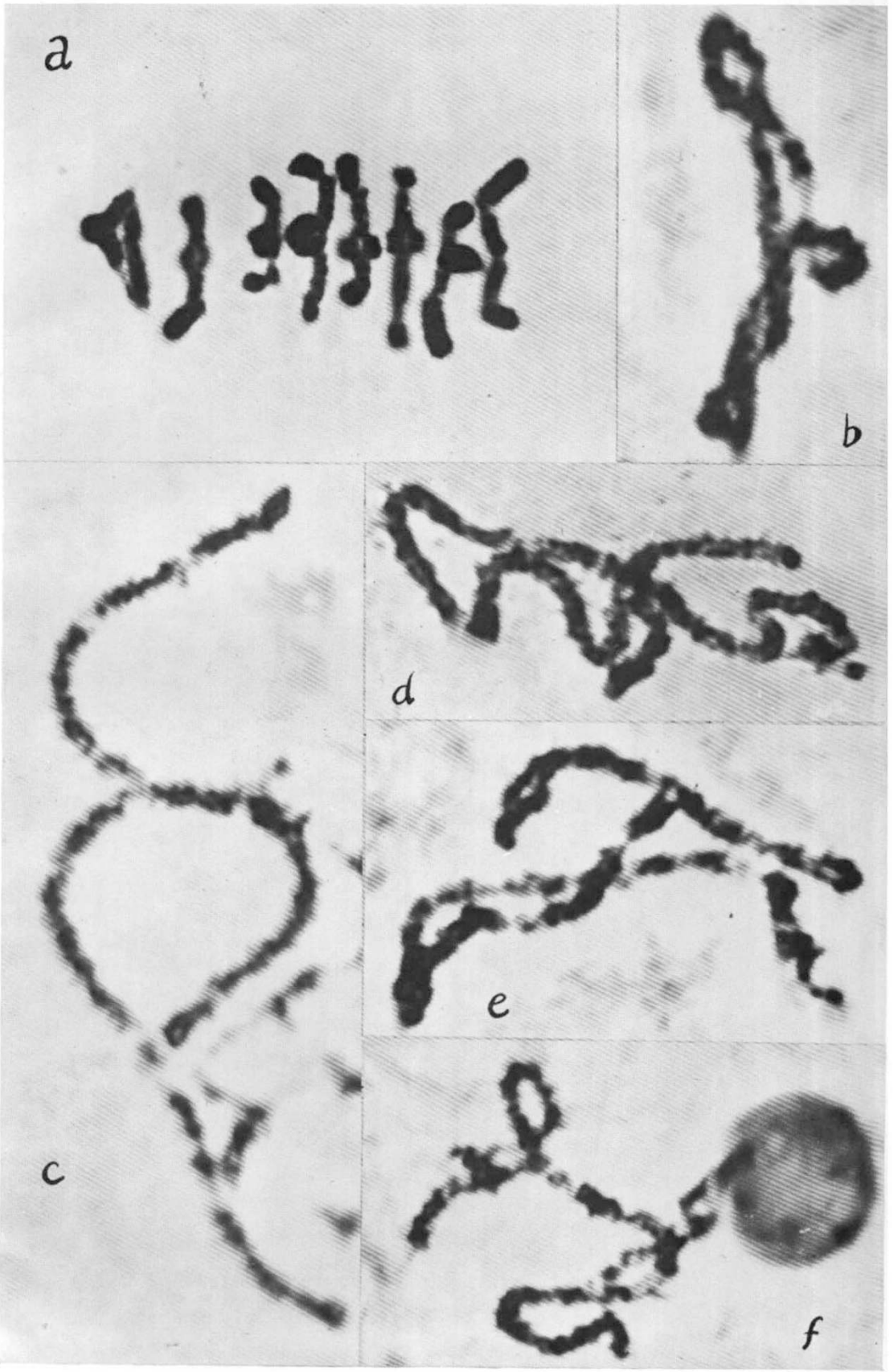
between homologous chromosomes and hence the degree of asymmetry between bivalents. This is unlikely, because in more than 400 metaphases scored at meiosis in the hybrid only four cells contained a multivalent association such as would be expected as a consequence of segmental interchange. Further reference to the distribution of DNA changes is made in the following section.

(d) *The structural basis*

If the difference in chromosome size and in chromosomal DNA between *A. cepa* and *A. fistulosum* is due entirely to a lengthwise incorporation (or loss) of chromosome segments (see Rees and Jones, 1967*b*) we should expect, in the hybrid, unpaired segments in pachytene " bivalents " corresponding in length to the DNA difference between the " homologous " chromosomes. It will be recalled that, on the basis of measurements at first metaphase, the DNA differences between " homologous " chromosomes range from about 10 per cent. to 60 per cent. In fig. 9 and plate fig. 1 are drawings and photographs of isolated pachytene chromosomes. These, like all pachytene chromosomes observed, show " duplicated " segments in the form of loops or

Plate fig. 1

Meiosis in *Allium cepa* × *A. fistulosum*. *a*, metaphase 1 showing asymmetry of bivalents. *b*, Early diplotene with relic of pachytene loop, *c*, *d*, *e*, *f*, Pachytene associations with " duplication " loops and overlaps.


a
b
c
d
e
f

overlaps. The organisation and distribution of loops are also clearly manifested by relic loops in early diplotene (plate fig. 1*b*). The loops and overlaps range from about 10 per cent. of the total chromosome length (fig. 9*b*, *e*) to more than 60 per cent. (fig. 9*a*)—precisely what would have been expected on the grounds that the nuclear DNA variation between *A. cepa* and *A. fistulosum* is due to a lengthwise incorporation or duplication of chromosome segments.

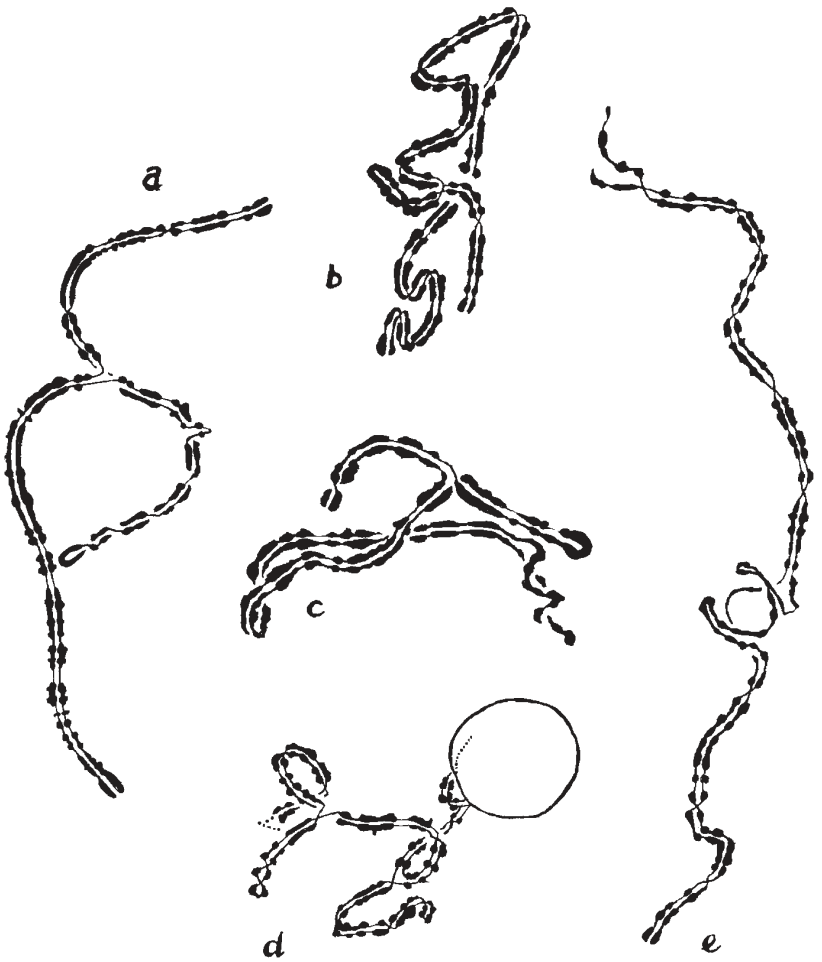


FIG. 9.—Isolated pachytene bivalents of *A. cepa* × *A. fistulosum*. Note large " duplication " loops and terminal overlaps.

It needs to be emphasised that only segmental " duplication " or loss will satisfactorily account for the loops and overlaps at pachytene. The common occurrence of one loop per chromosome rules out the possibility of their origin by segmental exchange or transposition *within* chromosomes. The possibility of widespread transposition or of exchange *between* chromosomes (of such large segments as are involved in loops) is ruled out by the virtual absence of multivalents at first metaphase. Neither did we find multivalents at pachytene. Levan (1936, 1941), it is true, describes " tri-

valents " and other multivalents both at pachytene and at metaphase in this hybrid. Emsweller and Jones (1935) and Maeda (1937), like ourselves, found very few or none. We believe that some pachytene " trivalents " illustrated by Levan may well have been bivalents with long loops as in fig. 9*a*. We think it probable that bivalents in which the duplication loops were severed in preparation (a common feature of many of the pachytene nuclei) may also have been mistaken for multivalents (*e.g.* Levan, 1941, fig. 2*g*, p. 258).

The nucleolar chromosomes. From fig. 8 it will be seen that the *A. cepa* nucleolar chromosome is about 25 per cent. bigger than that of *A. fistulosum*. In fig. 1*d* and plate fig. 1*f* is their pachytene association in the hybrid, showing two large loops. These loops involve separate chromosomes, *i.e.* there is one in the *A. cepa* chromosome and one in the *A. fistulosum* chromosome.* This is of particular interest in showing that a lengthwise replication of segments has not been confined to the species with the large chromosomes (*i.e. A. cepa*). Taking into account the loops in both chromosomes there is a difference of the order of 20-30 per cent. in the total chromosome length. This is what we should have expected if the DNA variation between the species is *completely* attributable to lengthwise structural changes involving the " duplication " or " loss " of chromosome segments.

With few exceptions the loops at pachytene are large and usually number one, sometimes two, per association. Assuming that elsewhere in the pachytene associations the pairing is between completely, or at least mainly, homologous parts (which appears to be the case in so far as one may judge visually, see plate) it follows that the DNA differences result from the duplication, or loss, of a few large highly localised chromosome segments rather than from many small ones, widely dispersed. In this connection it is not, of course, clear whether the extra segments are to be interpreted literally as " duplications " of existing segments. They may result from the incorporation of " new " DNA. Or again, a large extra segment could conceivably arise by the repeated replication of one or a few loci. In this connection the work of Gerstel and Burns (1967) and Burns and Gerstel (1967) in *Nicotiana* is especially relevant. They showed that a relatively small, restricted, heterochromatic, segment of a chromosome may vary enormously in size. Keyl's work on *Chironomus* (*e.g.* 1965) also shows how one " locus " may be enlarged by a factor of eightfold or more by repeated duplication. While the precise mechanism is not known it is clear either that particular regions of *Allium* chromosomes are especially prone to lengthwise replication or else that the replication in such regions, whether it be of single loci or of large segments, confers special advantage or, at the very least, is tolerated to an extraordinary degree in comparison with replication in other regions (*cf.* Beermann, 1966). It is worth noting that in *Chironomus* as in *Allium* the structural changes within chromosomes are also localised; the loci showing DNA gains in Chromosome III of *Chironomus thummi thummi* are clustered in the middle section (see Keyl and Pelling, 1963).

4. Discussion

The results described in the present work show that considerable differences in the mean DNA content per nucleus have arisen during the diverg-

* That the loops are located in separate chromosomes was confirmed by observations in early diplotene bivalents.

ence and evolution of species within the genus *Allium*. Further, the differences which occur amongst diploid species may be as great or greater than those resulting from polyploidy.

There is good evidence to show that the variation in overall nuclear DNA content is the result of changes affecting all chromosomes of the *Allium* complements. This finding is in agreement with the work of Rees and Jones (1967*a*) and Rees and Hazarika (in press) on *Lolium* and *Lathyrus* respectively. It may be, as Rees and Jones (*loc. cit.*) have suggested, that a large-scale quantitative variation in nuclear DNA could only be tolerated when distributed between chromosomes throughout the whole complement. They cite as a familiar analogy the contrasting consequences of polysomy as opposed to polyploidy in higher plants.

These observations in *Allium*, considered in conjunction with those in *Lolium* (Rees and Jones, *loc. cit.*) and *Chironomus* (Keyl, 1964, 1965), lead to the firm conclusion that, in structural terms, lengthwise " duplication " or loss accounts entirely for the widespread nuclear DNA variation reported for many genera in higher plants and, to a lesser degree, in animals. There are no grounds to sustain the argument that a differential polynemy, a variation in the lateral multiplicity of DNA strands, accounts for the DNA changes.

It is regrettable that one cannot, at present, correlate with confidence the direction of the cytochemical changes (*i.e.* DNA loss or gain) with the phylogeny of the *Allium* species. The reason for this is that the taxonomy of the genus *Allium* is poorly understood. Stearn (1944) states that the *Alliums* remain " . . . a vast almost unwieldy genus ". It is, however, of interest that taxonomists in general place the 14-chromosome group as the most primitive of the *Allium* species (see Stebbins, 1963). It will be recalled that the 16-chromosome group had the lowest nuclear DNA value, the 18-chromosome group the highest. From this it would seem, therefore, that DNA change during the evolution of these species involves both increase and decrease in the DNA content and the size of chromosomes.

The recent investigations of quantitative nuclear DNA variation between predominantly diploid species of plant and animal genera, although limited to comparatively few cases (*e.g.* Sunderland and McLeish, 1961; Keyl, 1963, 1964, 1965; Rees, Cameron, Hazarika and G. H. Jones, 1966; John and Hewitt, 1966; Martin, 1966; Rothfels, Sexsmith, Heimburger and Krause, 1966; Rees and Jones, 1967*a*; Rees and Hazarika, in press; Southern, 1967; Becak, Becak, Lavalle and Schreiber, 1967; Atkin and Ohno, 1967), give, nevertheless, a clear indication of the enormous extent of variation in nuclear DNA amounts which can exist between closely related diploid species. It is appreciated, as Stebbins (1966) emphasises, that there is much we do not understand about these nuclear changes and their consequences. Rather than speculate in general terms it seems more appropriate at this stage to pin-point a few aspects of the problem that are of particular and immediate interest.

(i) *Direction of change.* Stebbins (1966) has pointed out that within the most complex groups, such as flowering plants and vertebrates, there is no general correlation between nuclear DNA content and the degree of evolutionary " advancement ". Although one can find definite trends of this kind within certain individual genera (see Rees and Hazarika, in press), Stebbins would prefer to accept that in several groups of higher plants a

more meaningful correlation is that found between nuclear DNA content and ecological distribution. For example, there is some indication that tropical plants in general have less nuclear DNA and smaller chromosomes than temperate plants. This kind of pattern would clearly be worth investigating in more detail.

(ii) *The problem of loss.* On the basis of chromosome size comparisons there is, in *Crepis*, evidence which indicates strongly that the specialised short-lived annuals of this genus have undergone a reduction in nuclear DNA amount (Babcock, 1947; see also Darlington, 1965). There is also evidence for a diminution of DNA in *Lathyrus* (Rees and Hazarika, in press). On the assumption that DNA changes involve deletion of chromosome segments (see, Rees and Hazarika, *loc. cit.*), this phylogenetic reduction is difficult to reconcile with the fact that even small deletions are often lethal or severely detrimental to a homozygous organism (Stebbins, 1966; Lewis and John, 1963). In this context it is, however, worth emphasising that deletions which are *not* lethal or deleterious are less likely to be detectable, just as are major genes which do not mutate. The fact that deletions are *often* deleterious does not mean they always are.

(iii) *Metabolic significance of DNA variation.* Stebbins (1966) suggests that the variation in nuclear DNA amount influences the general *rate* of nuclear activity as distinct from contributing information relating to particular, specific metabolic steps or pathways (see also Commoner, 1964). The relation between mitotic cycle times and nuclear DNA content (Van't Hoff and Sparrow, 1963) fits well with this notion. There is to date, however, too little information to be positive on the matter.

5. Summary

1. The evolution of species within the genus *Allium* is associated with variation in nuclear DNA amounts. This variation is, to a large extent, independent of change in basic chromosome number and polyploidy.

2. Nuclear DNA content is proportional to chromosome volume.

3. The DNA changes are widely distributed *between* chromosomes within complements, but there is evidence which indicates that such changes are highly localised *within* chromosomes.

4. The DNA variation can be accounted for by lengthwise " duplications ", or loss, of chromosome segments.

5. It is probable that the evolution of the " 18 "- and " 16 "-chromosome *Alliums* from the primitive " 14 "-chromosome species was accompanied by gain and loss, respectively, of nuclear DNA.

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