BASE COMPOSITION OF NUCLEAR DNA WITHIN THE GENUS *ALLIUM*

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Received 3.xii.69

1. Introduction

In living organisms genetic information is stored in the form of DNA. Differences in genetic information result from changes in the sequence of bases in the polynucleotide chains. Gross changes in the base sequence are usually, although not always, accompanied by changes in the overall base composition. Organisms which are genetically very closely related will have much the same DNA base composition. Organisms which are distantly related or quite unrelated may, or on the other hand may not, have widely different base compositions.

Between species of micro-organisms the base composition varies widely. In bacteria, for example, the GC§ content ranges from 25 to 75 per cent. (Belozersky and Spirin, 1960; Sueoka, 1961). A correspondence between genetic relationship and base composition has proved useful in the taxonomy of this group. Between species of higher plants the base composition, in sharp contrast, shows a much narrower range, with GC values of from 35 to 49 per cent. (Belozersky, 1961). There is no indication as yet that base composition will prove as useful in higher plant taxonomy as in bacteria. The range in base composition is, perhaps, too narrow. However, information on base composition in higher plants is limited. Further investigations may reveal a wider range in base ratios than the 14 per cent. established to date.

The following is an account of measurements of nuclear DNA base composition in twenty *Allium* species and of a few species in other genera of the same family, the *Liliaceae*. The measurements are based on a method which gives substantially greater accuracy than was previously possible (Kirk, 1967). The purpose of the survey was, first, to determine to what extent species within the genus *Allium* vary in respect of their nuclear DNA base composition. Second, to ascertain whether differences in base composition are correlated with differences in nuclear DNA amount. The latter is of special interest because in *Allium*, as in other Angiosperm genera, the nuclear DNA amount varies considerably between species (Rees, Cameron Hazarika and Jones, 1966; Jones and Rees, 1969). The question arises, therefore, as to whether the gain or loss of nuclear DNA associated with the evolution of these *Allium* species is random with respect to base ratio or, conversely, biased and restricted to DNA of particular base composition.

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§ *Abbreviations:* GC content is the proportion of guanine + cytosine (+ 5-methylcytosine, where present) in DNA, expressed as a percentage of the total number of moles of purine and pyrimidine present.

2. Materials and methods

Plant material. Nuclei were isolated from commercially obtained bulbs of *Allium cepa*, *A. sativum*, *A. azureum*, *A. owstrowskianum*, *A. ascalonicum*, *Hyacinthus orientalis* (horticultural variety, " White "), *Narcissus* sp. (daffodil, cultivar " Carlton "), *Tulipa* sp. (cultivar " Clara Butt "), *Leucojum aestivum*, and *Puschkinia* sp., and from locally bought plants of *Allium porrum*. Nuclei were isolated from glasshouse-grown plants of *Allium schoenoprasum*, *A. flatunense*, *A. dicipiense*, *A. angulosum*, *A. cyrilli*, *A. galanthum*, *A. darwasicum*, *A. mutans*, *A. albopilosum*, *A. fistulosum*, *A. senescens*, *A. neapolitanum*, *A. cepa var. aggregatum* and *Triteleia* sp.; and also from plants of *A. ursinum* growing wild locally.

Isolation of nuclei. In all this work DNA has been isolated from nuclei rather than from whole cells. This is made necessary by the fact that chloroplasts (Kirk, 1963; Sager and Ishida, 1963; Edelman, Cowan, Epstein and Schiff, 1964) and mitochondria (Luck and Reich, 1964) are now known to contain their own DNA; DNA isolated from whole cells is likely to contain a certain amount of this extranuclear DNA, and so may not have exactly the same base composition as nuclear DNA.

The plant tissue was thoroughly washed and then surplus water removed. The tissue was homogenised with a pestle and mortar, or an MSE Atomix blender, in a medium containing 0·5 M-sucrose, 0·05 M-tris (hydroxymethylaminomethane), 0·1 M-disodium ethylenediaminetetraacetic acid, *p*H 7·23, at a ratio of 1·0-2·0 ml. medium to every 2·0 gm. fresh weight of tissue. The homogenate was strained through a double layer of muslin and then centrifuged at 1000 g_{av} for 20 minutes. The pellet (nuclei, starch grains; and chloroplasts if green tissue was used) was resuspended in sucrose/Tris/EDTA homogenisation medium containing 3·3 per cent. (v/v) of the non-ionic detergent, Triton X-100. Triton X-100 is known to bring chloroplasts, but not nuclei into solution (Spencer and Wildman, 1964). The suspension was centrifuged for 10 minutes at 1000 g_{av} and then the pellet (nuclei; starch grains) was washed twice more in the same medium by resuspension and centrifugation. All steps of the nuclear isolation procedure were carried out at 0°-4° C.

Preparation of DNA. DNA was isolated from the nuclear preparation by a simplified version of the Marmur procedure (Marmur, 1961) as described previously (Kirk, 1963). Two deproteinisations were carried out. RNA was removed by treatment with 0·5 N-NaOH as in previous work (Kirk, 1963).

Determination of base composition of the DNA. The base compositions of the DNA samples were determined by measuring the molar ratio of adenine to guanine, using the method recently described (Kirk, 1967). The GC content of each DNA was then calculated on the assumption that the number of moles of adnine in the DNA equalled the number of moles of thymine, and that the number of moles of guanine equalled the number of moles of cytosine plus 5-methyl-cytosine. Whenever possible, two or more analyses (each on about 0·7 mg. of DNA) were carried out, but in many cases the amount of nuclear DNA recovered was too small to permit more than one analysis. In the case of six of the *Allium* species (*A. flatunense*, *A. azureum*, *A. fistulosum*, *A. senescens*, *A. mutans*, *A. cyrilli*) and two of the other species (*Narcissus* sp., *Puschkinia* sp.), only half to two-thirds the normal amount of DNA was available for analysis.

When this method of DNA base analysis was first devised a standard deviation of 0·2 per cent. GC content was found on the basis of several analyses of a single batch of calf thymus DNA (Kirk, 1967). When a number of analyses is carried out each on a different sample of DNA from a given organism, an additional cause of variation may be present due to the possibility that a variable degree of fractionation of the DNA may occur during isolation. In recent work on *Phaseolus vulgaris* analyses were carried out on a series of separately isolated DNA samples (Baxter and Kirk, 1969): the standard deviation was 0·3 per cent. GC. Thus, in the present work it might be advisable to assume a standard deviation of 0·3 per cent. GC as an estimate of the total variability resulting from experimental manipulations. Those analyses, mentioned above, which were carried out on sub-optimal quantities of DNA, will have a somewhat higher intrinsic variability.

Measurement of DNA content of nuclei. DNA content of nuclei was measured by Feulgen photometry; most of the values have been taken from Jones and Rees (1969), the remainder have been determined in the present work. To ensure comparability of determinations carried out at different times, the value for *Allium cepa* was re-determined and used as a common standard on each occasion.

3. Results and discussion

Variation in base composition. The values for base compositions in the *Allium* and other species are given in table 1. It will be seen that the GC values range from 34·6 per cent. in *A. cepa* and *A. ascalonicum* to 39 per cent. in *A. cyrilli*. This range of 4 per cent., although on the face of it small, amounts to nearly a third of that applying to higher plants as a whole. Taking the other species into account, the range within the one family, the *Liliaceae*, is at least 10·8 per cent., from 34·6 in *A. cepa* to 45·4 per cent. in *Hyacinthus orientalis*.

From these results it is clear that base composition is unlikely to prove useful in distinguishing between families, because in this one family investigated the base composition covers three-quarters of the known range for all higher plants. At the same time the survey shows distinctive and clear-cut differences between many of the species within the family.

If it turns out that a GC range of the order of 14 to 15 per cent. is indeed the limit for higher plants it is pertinent to inquire why this should be so—especially when a range of more than 10 per cent. is found within a single family. What kinds of evolutionary constraints could operate to restrict the GC range to this degree? Put in another way, is there in higher plants some selective disadvantage in having a DNA base composition outside these comparatively narrow limits? If so, why in higher plants and not, for example, in bacteria and other micro-organisms?

Base composition and DNA amount. As mentioned earlier there is, between *Allium* species, a considerable variation in the nuclear DNA content (Jones and Rees, 1969). This variation may be quite independent of polyploidy and is of the order of two-fold between some diploid species. There is good evidence that the variation in DNA amount is the consequence of lengthwise loss or gain of chrosmosome segments (Jones and Rees, 1969). To find out if such quantitative DNA variation is correlated with change in base composition within the family we have plotted the GC percentage against DNA contents in the eleven species for which the data are available

(fig. 1; see also table 1). There is no indication from the graph of a correlation between DNA base composition and amount. An analysis of variance confirms that the regression of GC percentage against DNA amount is not significant ($P = 0{\cdot}10$).

It therefore appears that species with low nuclear DNA amount are as likely to have high GC as low GC values. That is to say, there does not seem to be any inherent tendency for loss of high GC DNA, or loss of low GC DNA; whether or not particular DNAs are gained or lost is determined by factors other than their base composition.

TABLE 1

Base compositions of nuclear DNA's

Plant species	No. of analyses	Mean adenine/guanine ratio	Percentage (guanine+cytosine/5-methylcytosine) content	2C (diploid) DNA amounts (arbitrary units)
Allium cepa	5	1·889	34·6	33·5
Allium ascalonicum	2	1·885	34·6	—
Allium ursinum	2	1·829	35·3	—
Allium galanthum	1	1·813	35·5	24·4
Allium cepa var. aggregatum	2	1·812	35·6	—
Allium flatunense	1	1·751	36·3	—
Allium dicipiense	2	1·730	36·6	21·5
Allium porrum	2	1·717	36·8	—
Allium sativum	3	1·708	36·9	—
Allium schoenoprasum	2	1·678	37·3	16·9
Allium neapolitanum	1	1·670	37·4	31·2
Allium albopilosum	2	1·660	37·6	—
Allium darwasicum	1	1·654	37·7	17·7
Allium azureum	1	1·652	37·7	17·8
Allium owstrowskianum	1	1·650	37·7	39·8
Allium fistulosum	1	1·634	37·9	26·3
Allium angulosum	1	1·631	38·0	20·6
Allium senescens	1	1·596	38·5	21·6
Allium mutans	1	1·593	38·6	—
Allium cyrilli	1	1·561	39·0	—
Triteleia sp.	2	1·638	37·9	—
**Leucojum aestivum*	2	1·487	40·2	—
Tulipa sp.	2	1·462	40·6	—
**Narcissus* sp.	1	1·403	41·6	—
Puschkinia sp.	1	1·205	45·3	—
Hyacinthus orientalis	2	1·203	45·4	—

* *Amaryllidaceae* (*Liliales*)

DNA loss or gain in conjunction with the evolution of *individual species* is however not random. On the contrary, the fact that the DNA base ratio, as well as DNA amount, varies at all between certain of the *Allium* species is, in itself, testimony to a qualitatively non-random change. The most feasible explanation is that the quantitative DNA changes are, in such cases, localised within chromosome segments whose DNA base composition is not characteristic of the complement as a whole. There is, indeed, cytological evidence which supports this view (Jones and Rees, 1969). That, localised, lengthwise replication of chromosome segments leading to increased DNA does not result in similar changes in base composition in different species,

as is apparent from fig. 1, is not surprising. The loci replicated, or lost, may be quite different, both in respect of their information and their base composition. Indeed, it is to be expected that selection would favour changes in different loci in different species, especially when we take into account the probability that the species would thrive in very different environments.

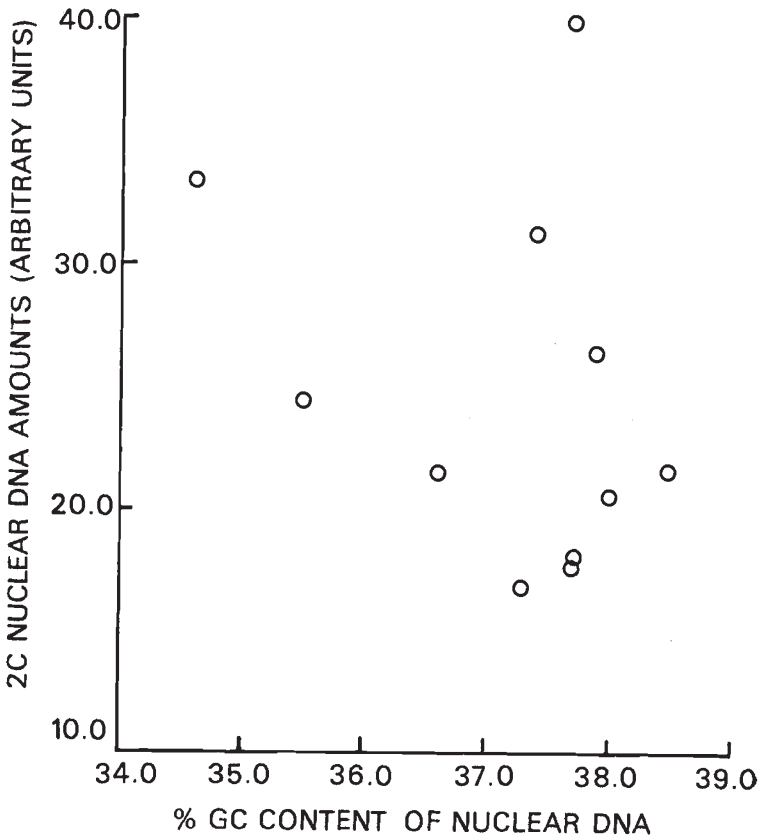

FIG. 1.—Base composition of nuclear DNA and amount of DNA per 2C (diploid) nucleus, in *Allium cepa*, *A. galanthum*, *A. dicipiense*, *A. schoenoprasum*, *A. neapolitanum*, *A. darwasicum*, *A. azureum*, *A. owstrowskianum*, *A. fistulosum*, *A. angulosum*, and *A. senescens*.

4. SUMMARY

1. A survey among species of *Allium* and of a few species in other genera in the *Liliaceae* reveals a significant variation in the base composition of nuclear DNA.

2. A range of at least 10 per cent. in GC content is found within the family.

3. Within the *Allium* genus there is no correlation between DNA base composition and nuclear DNA amount.

4. This shows that loss or gain of particular DNAs, associated with evolution within the genus as a whole, is determined by factors other than the base composition of these DNAs.

5. That certain *Allium* species differ from one another in base composition indicates, at the same time, that DNA fractions gained or lost do not always comprise a qualitatively representative sample of the chromosomal DNA.

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