NOTES AND COMMENTS

EFFECT OF HETEROCHROMATIN ON THE RELATIONSHIP BETWEEN NUCLEAR *DNA* CONTENT AND CHROMOSOME VOLUME

F. A. LINTS* and R. N. JONES†
Department of Agricultural Botany, University College of Wales, Aberystwyth, Wales

16.vii.68

1. Introduction

Studies on quantitative variation in the nuclear *DNA* content of species from several genera of higher plants and animals, show that in general, there exists a correlation between the amount of nuclear *DNA* at interphase, and chromosome volume measured at metaphase of *c*-mitosis (McLeish and Sunderland, 1961; Keyl, 1964; Keyl, 1965; Rees *et al.*, 1966; Rothfels *et al.*, 1966; Martin, 1966; John and Hewitt, 1966; Lima de Faria, 1959). This is not to say of course, that detailed studies on certain chromosomes within complements, or between whole complements of certain species, would not reveal some differences in the *DNA* amount per unit volume of chromosome, or chromosome complement. Indeed several such instances have been reported. Lima de Faria (1959) has shown that heterochromatin at interphase may contain two or three times more *DNA* per unit area than euchromatin. Jones and Rees (in press) have indicated that heterochromatic supernumerary *B*-chromosomes in rye contain 1·5 times as much *DNA* per unit volume as *A*-chromosomes. Nirula, Bhaskaran and Swaminathan (1961) studied the effect of linear differentiation of chromosomes on the proportionality between chromosome length and *DNA* content in three species of *Sorghum*. They found that the *DNA* content per micron of chromosome varied between the species and ascribed this variation to differences in the amount of heterochromatin relative to euchromatin. They concluded that no regular relationship between *DNA* content and chromosome length may thus occur in species with varying amounts of heterochromatin. It does not follow of course that measurements involving the volume of chromosome complements, rather than length of chromosomes, would give the same result.

The opportunity to investigate this relationship between *DNA* content and chromosome volume (with reference to heterochromatin) exists in two *Allium* species, *cepa* and *pulchellum*. They do not differ much with regard to their nuclear *DNA* content and chromosome size, but differ considerably in respect of heterochromatin, *A. cepa* having relatively little heterochromatin in comparison with *A. pulchellum*.

* Present Address: F. A. Janssens Memorial Laboratory for Genetics, Agricultural Institute of the University , Heverlee-Louvain, Belgium.

† Present Address: Sub-Department of Genetics, The Queen's University, Belfast 7, Northern Ireland.

2. Methods

(i) *Estimation of DNA by Feulgen Microspectrophotometry*

The fixation and staining technique used was that described 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 2C telophase and early interphase nuclei (of root tips).

(ii) *Measurement of Chromosome Volume*

For calculating chromosome volumes, the total chromatid length was measured at metaphase (*c*-mitosis in root tips) 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 following colchicine treatment and Feulgen staining.

Root tips of both species were taken from mature plants at similar stages of development, which were growing in John Innes potting compost in an unheated glasshouse.

3. Observations

The results of the *DNA* estimations for the two species are given in table 1. Analysis of the data confirms that the difference in nuclear *DNA* is significant

Table 1

Mean DNA *values of* 2c *nuclei in the two* Allium *species* (*arbitrary units*)

	Allium cepa	Allium pulchellum
Rep. 1	13·75 ± 0·23	11·75 ± 0·38
2	13·35 ± 0·32	11·45 ± 0·24
3	12·40 ± 0·27	11·50 ± 0·27
4	13·00 ± 0·37	10·48 ± 0·24
5	12·78 ± 0·50	11·40 ± 0·31
Mean	13·07 ± 0·15	11·31 ± 0·14

($P < 0{\cdot}01$). The data for chromosome dimensions and calculated chromosome volumes (table 2) indicate that the two species are identical in respect to their chromosome volumes (although the relative proportions of length to width of chromatids can be seen to differ). The chromosome complements are represented in fig. 1. These results suggest that *A. pulchellum*, characterised by the presence of heterochromatin at interphase, has less *DNA* per unit volume of metaphase chromosome than *A. cepa*, which lacks heterochromatic chromocentres.

4. Discussion

There are two possible explanations for these results, both of which merit further investigation.

(*a*) That the *DNA* results are not true estimates and merely reflect differences in the state of organisation of the chromatin in the nuclei of the

TABLE 2

Chromosome dimensions and calculated chromosome volumes in the two Allium species (in microns)

	Allium cepa			Allium pulchellum		
	Total chromatid length (μ)	Average chromatid width (μ)	Total chromosome volume (μ^3)	Total chromatid length (μ)	Average chromatid width (μ)	Total chromosome volume (μ^3)
Cell— 1	301·22	0·964	219·7	326·23	0·714	130·6
2	251·54	1·086	238·4	309·27	0·866	182·1
3	277·31	0·946	194·8	328·55	0·933	224·5
4	259·62	0·842	144·5	382·96	0·866	225·5
5	263·64	1·000	207·0	374·17	0·878	226·4
6	227·53	0·921	151·5	345·63	0·824	184·2
7	198·37	1·177	215·7	377·71	0·842	210·2
8	298·17	0·872	178·0	289·25	0·927	174·9
9	330·01	0·830	178·5	312·32	0·921	208·0
10	285·24	0·939	197·4	311·71	0·842	173·5
11	288·41	0·927	194·6	340·75	0·836	186·9
12	322·45	0·830	174·4	341·36	0·805	173·6
13	296·70	0·964	216·4	306·46	0·964	223·6
14	257·18	0·946	180·7	284·63	0·903	182·2
15	282·67	0·964	206·2	295·36	0·939	204·4
Mean	276·40 ± 8·84	0·947 ± 0·024	193·2 ± 6·6	326·42 ± 9·06	0·871 ± 0·016	194·0 ± 6·9

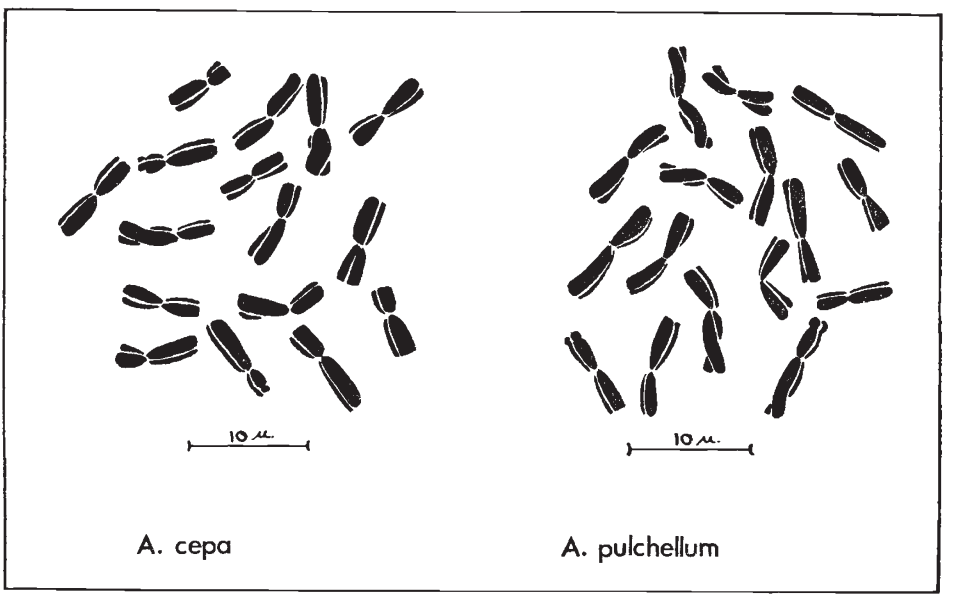

FIG. 1.—Chromosome complements of *Allium cepa* and *A. pulchellum*.

two species, *i.e.* that the *DNA* is underestimated in nuclei with deeply staining heteropycnotic chromocentres (*pulchellum*) as opposed to those with the more diffuse and evenly distributed nuclear *DNA* (*cepa*). Mittwoch (1968) has provided evidence which supports this kind of explanation. She finds that in human fibroblast-like cultured cells the estimated *DNA* values are slightly lower in cells with multiple chromocentres as compared with cells lacking chromocentres, and has attributed this difference in *DNA* to an underestimation in the case of cells with chromocentres, rather than to any real difference in *DNA* amount.

(*b*) That there is a real difference in nuclear *DNA* amount between the two species, and that *A. pulchellum* has therefore less *DNA* per unit volume of metaphase chromosome than *A. cepa*.

It is known of course that there are differences in the coiling behaviour of heterochromatin relative to euchromatin at certain phases of the cell cycle.

Dyer (1964) working with certain *Trillium* species, finds that heterochromatin is present as densely staining chromocentres in interphase nuclei, and easily distinguishable from euchromatin. At metaphase of mitosis on the other hand the heterochromatin and euchromatin were exactly synchronised in their behaviour and thus indistinguishable under the light microscope, as far as staining intensity is concerned. At low temperatures however heterochromatin is visibly understained and probably undercoiled relative to the euchromatin. Such a condition (negative heterochromatin) gives rise to an increase in the length of metaphase chromosomes at these low temperatures. It is not impossible therefore that in the present experiment, the lower amount of *DNA*/unit volume in *A. pulchellum* chromosomes, as compared with the largely euchromatic *cepa* ones, reflects a certain degree of " negative heterochromaticity ". (Such an effect would presumably be exaggerated at low temperatures).

If this finding can be established, it is of interest in that it contrasts with the situation pertaining in rye *B*-chromosomes (Jones and Rees, in press) (and probably those of maize), which show " positive heterochromaticity " at metaphase of mitosis, and as such it merits further investigation.

5. Summary

1. The relationship between *DNA* content and chromosome volume was investigated in two species of *Allium*, *A. cepa* and *A. pulchellum*, which differ considerably in respect of the amount of heterochromatin they reveal at interphase of mitosis but do not differ much with regard to their nuclear *DNA* content and chromosome size.

2. The results suggest that *A. pulchellum*, characterised by the presence of heterochromatin at interphase of mitosis, has less *DNA* per unit volume of metaphase chromosome.

3. Two hypotheses are advanced to explain those results, one of " negative heterochromaticity ", the other of underestimation of *DNA* content in nuclei with heteropycnotic chromocentres.

Acknowledgments.—One of us (F. A. L.) express his sincere thanks to Dr H. Rees for his warm hospitality and friendly advice during a stay at the Department of Agricultural Botany of the University College of Wales in Aberystwyth which was made possible through a grant of the British Council.

6. References

DYER, A. F. 1964. Heterochromatin in American and Japanese species of *Trillium*. *Cytologia*, *29*, 171-190.

JOHN, B., AND HEWITT, G. M. 1966. Karyotype stability and DNA variability in the Acrididae. *Chromosoma*, *20*, 165-172.

JONES, R. NEIL, AND REES, H. 1968. Influence of *B*-chromosomes in rye upon the nuclear phenotype. In press.

KEYL, H. G. 1964. Verdopplung des DNA Gehalte kleiner Chromosomen Abschnitte als Faktor der Evolution. *Naturwissenschaften*, *51*, 46-47.

KEYL, H. G. 1965. A demonstrable local and geometric increase in the chromosomal DNA of *Chironomus*. *Experientia*, *21*, 191.

LIMA DE FARIA, A. 1959. Differential uptake of initiated thymidine into hetero- and euchromatin in *Melanopus* and *Secale*. *J. Biophys. Biochem. Cytology*, *6*, 457-466.

MARTIN, P. G. 1966. Variations in the amounts of nucleic acids in the cells of different species of higher plants. *Exp. Cell Res.*, *44*, 84-94.

MCLEISH, J., AND SUNDERLAND, N. 1961. Measurements of deoxyribonucleic acid (DNA) in higher plants by Feulgen photometry and chemical methods. *Exp. Cell Res.*, *24*, 527-540.

MITTWOCH, U. 1968. Scope and limitations of feulgen microdensitometry. Chromosomes today. (In press.)

NIRULA, S., BHASKARAN, S., AND SWAMINATHAN, M. S. 1961. Effect of linear differentiation of chromosomes on the proportionality between chromosome length and DNA content. *Exp. Cell Res.*, *24*, 160-162.

REES, H., CAMERON, M. F., HAZARIKA, M. H., AND JONES, G. H. 1966. Nuclear variation between diploid angiosperms. *Nature*, *211*, 828-830.

ROTHFELS, K., SEXSMITH, E., HEIMBURGER, M., AND KRAUSE, M. O. 1966. Chromosome size and DNA content of species of *Anemone* L. and related genera (*Ranum culaceae*). *Chromosoma*, *20*, 54-74.

SUNDERLAND, N., AND MCLEISH, J. 1961. Nucleic acid content and concentration in root cells of higher plants. *Exp. Cell Res.*, *24*, 541-554.

THE EFFECT OF POPULATION STRUCTURE ON THE SUCCESS OF INSECT INTRODUCTIONS

A. M. LUCAS

School of Biological Sciences, The Flinders University of South Australia, Bedford Park, South Australia 5042, Australia

Received 7.viii.68

Introduction

It would be an advantage to be able to predict the conditions that will give the best chance of success when an insect species is introduced into an area to control a pest. Remington (1968) has produced a model which, he suggests, enables such predictions to be made. However, some inconsistencies appear in his paper.

Remington's model and predictions

Remington summarises his model of the structure of an insect population as follows:

"... there are two opposite genetic structures and their intermediates.

1. The ecologically marginal portions of the population are small inbred units with high homozygosity and a very low frequency of deleterious