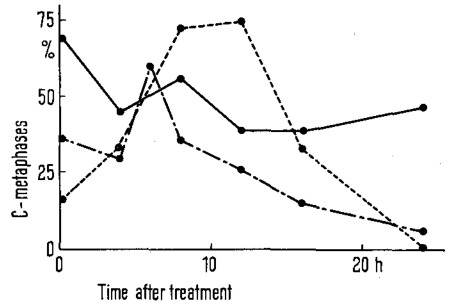

Fig. 1. C-metaphases induced by vinblastine ($1.10^{-4}M$/3 h) in *Hordeum* (—), *Vicia* (– – –) and *Nigella* (...).

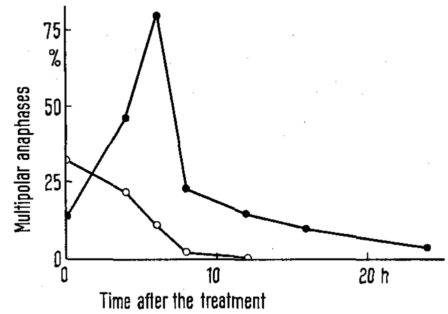

Fig. 2. Multipolar anaphases induced in *Nigella* by vinblastine $1.10^{-4}M$ (●) and $1.10^{-5}M$ (○).

Table II. Percentages of metaphase aberrations in *Nigella damascena*

Concentration (M)	Time after treatment (h)				
	0	4	8	12	24
1.10^{-4}	1.7	0.3	0.7	3.0	0.0
1.10^{-5}	0.3	1.0	–	–	–
Control	0.3	not investigated			2.0

300 cells analyzed. –, Absence of C-metaphases.

$1.10^{-5}M$ immediately after treatment, but decreased quickly with the recovery period. The lowest concentration only yielded aberrations immediately after treatment.

Besides the radiomimetic aberrations, we also observed stathmokinetic effects which confirm for plant materials the data obtained in animals so far. We noticed colchicometaphases which were sometimes so numerous that anaphase analysis was prevented. This was the case for barley and broad bean at the highest concentration but it should be pointed out that this effect occurred somewhat later in *Vicia* (Figure 1).

The modification of C-mitotic effects runs parallel to the radiomimetic ones in the 3 species. Merostathmokinetic effects (incomplete C-mitosis) were observed all the time which consisted in multipolar anaphases (Figure 2), metaphases-anaphases suggesting a partial disorganization of spindle fibers.

The sensitivity of *Nigella damascena* incited us to analyse further metaphase aberrations. In root tips the C-mitotic induction is roughly proportional to the concentration, no C-metaphases remaining at the lower concentration for duration longer than 4 h after treatment. In Table II, the chromosomal aberrations observed mainly consisted in breaks, giving rise to acentric fragments. The amount of such aberrations is very low as compared with that obtained for anaphases. These results suggest that the anaphase aberrations were induced subsequently to the metaphase, probably at early anaphase. They can be related to disturbances of the spindle apparatus. Their origin is the same as that of multipolar anaphases.

Discussion and conclusions. *Nigella damascena* has always been reported to be a very sensitive plant material suitable for investigations in mutagenesis. Thus it is not surprising that the highest amount of chromosomal aberrations was observed with that species, on the one hand. On the other hand, the radiomimetic effect is all the time low, if existing at all, in the two other species. Recent investigations using dominant lethal mutations in mouse[7] failed to detect mutagenic effects. It can thus be concluded that in all biological systems so far investigated the mutagenic action of the vinblastine is slight. However, the C-mitotic effect could result indirectly in mutations by giving rise to polyploid cells[8].

Résumé. Des racines de *Hordeum sativum*, *Vicia faba* et *Nigella damascena* ont été traitées par des solutions ($1.10^{-4}M$ à $1.10^{-6}M$) de vinblastine. Chez les trois espèces, nous avons pu observer des effets stathmocinétiques: C-métaphases et anaphases multipolaires. Chez *Vicia* et *Hordeum*, les taux d'aberrations sont très faibles; par contre, *Nigella* s'est montrée plus sensible à l'action radiomimétique de l'alcaloïde.

N. DEGRAEVE[9] and J. GILOT-DELHALLE[10]

Université de Liège, Laboratoire de Génétique, 15, rue Forgeur, B-4000 Liège (Belgium), 22 October 1971.

[7] N. DEGRAEVE, to be published.

[8] The authors are greatful to Prof. J. MOUTSCHEN for his valuable advice and helpful criticism during the preparation of the manuscript. They are indepted to Christiaens S.A. (Bruxelles) for the gift of the VELBE.

[9] Assistant au Fonds de la Recherche Fondamentale Collective.

[10] Aspirant au Fonds National de la Recherche Scientifique.

Characterization of DNAs from *Coprinus lagopus* and *Mucor azygospora*

Very little information exists on the characteristics of fungal DNAs. Since DNA serves as the basis for the functional and genetical differentiation of an organism, its characterization becomes necessary for any study of differentiation at molecular level. BRITTEN and KOHNE[1] have shown that the studies of DNA:DNA dissociation and reassociation reactions give very reliable information regarding the nature of DNA and genome size (total DNA/

haploid nucleus) of an organism. Earlier[2–4] we have reported our preliminary observations on the characteristics of *Neurospora* and *C. lagopus* DNAs. This paper describes our studies on yield, purity and thermal profile of *C. lagopus* and *M. azygospora* DNAs and kinetics of reassociation and occurrence of repeated sequences in *C. lagopus* DNA.

The wild type strains, $H_2A_6B_5$ and No. 99.1 of *C. lagopus* were obtained from Dr. PETER DAY of Connecticut Agricultural Experiment Station, New Haven, Conn. *M. azygospora* (strain ACTC 1105) was obtained from the American Type Culture Center, Bethesda, Maryland. ^{14}C-labelled DNA of *Escherichia coli* was kindly supplied by Dr. ROY BRITTEN of the Carnegie Institution of Washington, D.C.

C. lagopus, both in stock culture or in experiments, was grown in FRIES[5] minimal medium. Mycelial suspension was used as inoculum for either mycelial growth or oidial production. Mycelia were grown in liquid culture whereas oidia were produced on solid medium. After 72 h growth the mycelia were harvested, washed with deionized water and frozen. After the incubation period, necessary for their development (7–10 days), the oidia were harvested from the agar surface by brushing with a glass rod in presence of deionized water and passing through a 4-fold cheese cloth. Oidial cells were then pelleted from the suspension by centrifugation at 5000 *g*.

Mucor azygospora stock culture was maintained on potato dextrose agar plants at 24 °C. The sporangiospores were used as inoculum and mass culture was grown in complete liquid medium under aeration at 24 °C in VOGEL's[6] medium containing soluble starch.

Mycelia grown in phosphate deficient medium were used as inoculum for ^{32}P labelling of the DNA following the procedure described by DUTTA[7]. DNA was isolated either from the freeze-dried (for unlabelled DNA) or wet mycelia (for ^{32}P labelled DNA) using a newly developed DNA isolation technique[8]. The use of 1 *M* sodium perchlorate was found necessary for the isolation of *Coprinus* DNA.

In order to get rid of cross-linked DNA, ^{14}C *E. coli* DNA and ^{32}P *C. lagopus* DNA in 0.14 *M* phosphate buffer (PB) were denatured separately by boiling for 2–3 min in a water bath. The denatured DNAs were then quickly cooled to 60 °C and passed rapidly over a hydroxyapatite column equilibrated at 60 °C with 0.14 *M* PB. Under these conditions cross-linked DNA are adsorbed to the hydroxyapatite while single strand DNA pass through the column. Both in *C. lagopus* and *E. coli* the values for cross-linked DNA and zero time hybridization were found to be 2 to 4% and less than 0.1%, respectively. The DNA of *M. azygospora* was not labelled.

The purity of DNAs was tested by hyperchromic shift of the isolated DNA samples, by melting the DNA in 0.12 *M* PB and recording the increase in absorbance at 260 nm with a Gilford 2400 spectrophotometer. Tests done for the purity of ^{32}P labelled DNA were: acid solubility (0.2–3%), alkaline lability (0.2–0.3%), RNase lability (none), and DNase lability (95–98%).

After the above steps of purification, *E. coli* and *C. lagopus* DNAs were mixed and sheared together at 50,000 ψ for DNA:DNA reassociation kinetics studies. Reassociation was performed according to the method of BRITTEN and KOHNE[1].

Oidial cells were used for the estimation of total DNA per haploid nucleus using trichloroacetic acid (TCA) procedure as described by SCHNEIDER[9]. An oidial cell, containing apparently a single nucleus, was estimated to contain approximately 0.7×10^{-13} g DNA which correspond to about 4.2×10^{10} daltons approximately. This estimate was found to be greater than that obtained by DNA:DNA reassociation kinetics studies. The values obtained by the reassociation kinetics reflect the genome size with respect to unique sequences only. Fractionation of ^{32}P labelled whole-DNA on hydroxyapatite reveals that approximately 10% of the *C. lagopus* DNA is composed of repeated DNA sequences. This might partly account for the discriepancy as stated. These repeated DNA sequences are probably of non-nuclear origin[2].

Approximately 300 to 400 µg of highly purified DNA was obtained on an average from 1 g of (equivalent to approximately 10 g wet wt.) lyophylized mycelial powder of *Coprinus lagopus* using urea-phosphate-Na-perchlorate method[8]. From 1 g of wet wt. of oidia an average of approximately 3 O.D.s (150 µg) of DNA was obtained.

Figure 1 shows the thermal profile curve for *C. lagopus* mycelial DNA. The hyperchromicity of DNA isolated was approximately 25–27%. The greater part (more than 90%) of the DNA seems to contain 1 major fraction having a temperature of 91.5 °C (G+C = 52%). A very small fraction (less than 10%) of the DNA having a low temperature of 82.5 (G+C content 32%) is also evident from the Figure 1. This minor component of *Coprinus* DNA could not be seen in preparations by previous isolation procedures[10]. The *Coprinus* DNA is markedly different in this respect from the *Neurospora*[12] DNA which has at least 25% of low GC (32%) and approximately 75% high GC (52%) content.

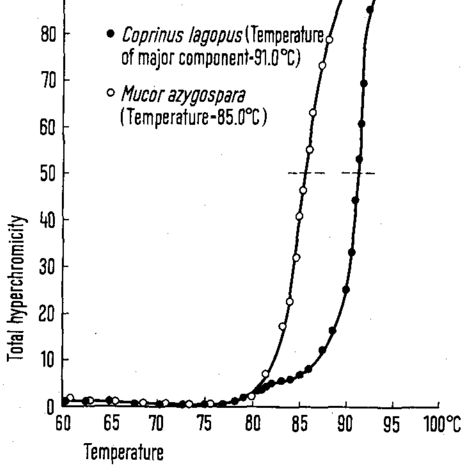

Fig. 1. Optical melting curves of DNAs in 0.12 *M* PB from mycelia of *C. lagopus* and *M. azygospora* recorded in Gilford (260 nm) spectrophotometer. The mycelia cells were freeze-dried and the DNA was isolated by Urea-Phosphate-sodium perchlorate method[7] on hydroxyapatite column.

[1] R. J. BRITTEN and D. E. KOHNE, Science *161*, 529 (1968).
[2] S. K. DUTTA and D. E. KOHNE, Proc. XII Intl. Botanical Congress (1969).
[3] S. PENN and S. K. DUTTA, Genetics *64* (1970).
[4] S. K. DUTTA, Neurospora Newslett. *15*, 25 (1969).
[5] L. FRIES, Physiologia Plant. *6*, 551 (1953).
[6] H. J. VOGEL, Naturalist *98*, 435 (1964).
[7] S. K. DUTTA, Microb. Genet. Bull. *26*, 6 (1967).
[8] R. J. BRITTEN, M. PAVICH and J. SMITH, Rep. Carnegie Inst. *68*, 400 (1970).
[9] W. C. SCHNEIDER, Meth. Enzym. 680 (1956).
[10] S. K. DUTTA, N. RICHMAN, V. W. WOODWARD and M. MANDEL, Genetics *57*, 719 (1967).

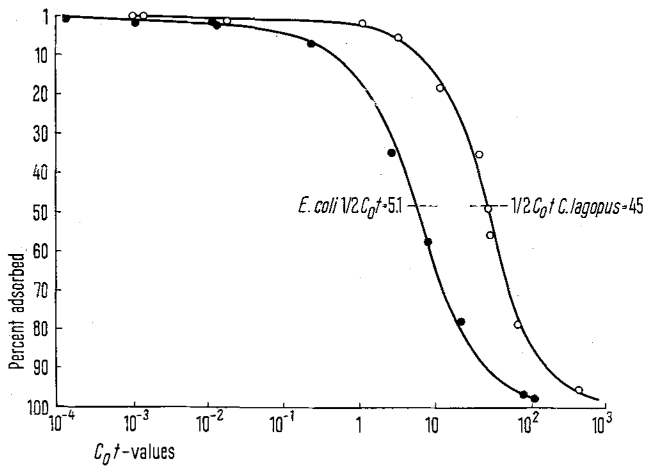

Fig. 2. The kinetics of reassociation of ^{32}P *C. lagopus* and ^{14}C *E. coli* DNAs, measured by hydroxyapatite chromatography. The DNAs were mixed, sheared together at 50,000 Ψ and incubated in the proportion of 10:1 by O.D. (*C. lagopus : E. coli*). For low C_0t values, the reaction mixture was adjusted to $0.14\,M$ phosphate buffer and incubated at 60 °C. For higher C_0t values the phosphate concentration was adjusted to $0.48\,M$ and incubated at 65 °C. For uniformity in expression all C_0t values were converted in equivalent to $0.12\,M$ PB.

Thermal profile curve (Figure 1) of *M. azygospora* DNA suggest distinctly one component having 38 G+C mole percent. One g freeze-dried mycelial cells yielded approximately 500–600 µg DNA.

Figure 2 summarizes the results of experiments of reassociation kinetics which gives a good estimate of the genome size of *C. lagopus*. Since *C. lagopus* DNA contains (less than 12%) repeated DNA (fast reassociating) this fraction was removed by giving a C_0t of 1.0 approximately. These results show that *E. coli* reassociates 8.8 times faster than *C. lagopus* DNA. It is well kmown[11] that an *E. coli* genome contains about 2.8×10^9 daltons of DNA, thus a *Coprinus* haploid nucleus should contain about 2.5×10^{10} daltons of DNA. The experimental details of this study are explained in the legend of Figure 2. Thus apparently the *Coprinus* genome is slightly larger than *N. crassa*[12] genome size, 2.2×10^{10}. We have not made such studies for *M. azygospora* but a recent study made in *M. bacilliformis* by R. SEIDLER (taken from reference[13]) suggest a genome size of 2×10^{10} daltons.

These data fit in very well when one considers the evolution from simplicity towards complexity. The order of evolution in these 3 organisms could be *Mucor – Neurospora – Coprinus*; which in turn agrees excellently with the experimental evidence of genome evolution. An elaboration of these points can be found elsewhere[14,15].

Occurrence of repeated DNA sequences in *C. lagopus*

Whole DNA		Non-repeated DNA	
C_0t	Hybridization (%)	C_0t	Hybridization (%)
0.47	7.5	0.03	0.047
1.26	9.0	0.64	1.05
2.52	17.6	2.00	3.80
8.8	42.9	6.30	18.00
25.25	64.4	22.10	34.00
416.00	89.9	632.30	95.05

Summary of ^{32}P labelled and unlabelled DNA:DNA reassociation within the whole and non-repeated DNAs. Cross-linked DNAs were removed from ^{32}p-labelled DNA before the reaction was run. Non-repeated DNA was obtained by eluting single strand DNA from hydroxyapatite column by giving a C_0t of approximately 1.0. $C_0t = M \times$ sec/l.

Fractionation of ^{32}P labelled whole DNA on hydroxyapatite reveals that perhaps less than 12% of the DNA is composed of repeated DNA sequences. It is not known yet what percent of these repeated DNA sequences are nuclear. In *N. crassa* it is observed that ribosomal RNA[15] cistrons are repeated which are nuclear DNA. The Table summarizes results of the hybridization reactions of whole and non-repeated DNAs. Reassociation of dissociated (native) DNA by optical method, in Gilford 2400 (by quickly changing 100 °C to 60 °C), done by Dr. D. E. KOHNE of Carnegie Institution of Washington, D. C., also suggest that *Coprinus* has very low percentage of repeated DNA sequences. We have not studied the occurrence of repeated DNA sequences in *M. azygospora* yet.

Resumé. Les rapports G+C des ADN de *Coprinus lagopus* et *Mucor azygospora* ont été étudiés. Le profil de fusion indique que l'ADN du *C. lagopus* est composé de deux fractions, une principale (90%) de rapport G+C 52 moles pourcent, une mineure (10%) de G+C 32 moles pourcent. Par contre l'ADN de *M. azygospora* contient une fraction unique de G+C 38 moles pourcent. L'étude de la cinétique de réassociation DNA:DNA montre que la dimension génomique («genome size») de *C. lagopus* est de 2×10^{12} et qu'il y a moin de 10% de DNA à séquences répétées de nucléotides.

S. K. DUTTA, S. R. PENN, A. R. KNIGHT and M. OJHA[16]

Department of Botany, Howard University, Washington D.C. 20001, USA), 16 November 1971.

[11] J. CAIRNS, Cold Spring Harb. Symp. Quant. Biol. *28*, 43 (1963).
[12] S. K. DUTTA, S. K. CHATTOPADHYAY and D. E. KOHNE, in preparation (1971).
[13] R. STORCK, Brookhaven Symp. *23*, in press (1971).
[14] S. K. CHATTOPADHYAY, D. E. KOHNE and S. K. DUTTA, in preparation (1971).
[15] D. E. KOHNE, Q. Rev. Biophys. *33*, 327 (1970).
[16] M. OJHA was visiting investigator from the Laboratory of Microbiology, Institute of Botany, University of Geneva (Switzerland). This research was supported in part by the U.S. Atomic Energy Commission Contract No. AT (40-1) 4182 and the Research Corporation, New York, to S.K.D. We are grateful to Professor G. TURIAN, University of Geneva, for making possible M. OJHA's participation in this research.