STRUCTURE AND SEQUENCE ORGANIZATION OF DNA IN EUKARYOTES WITH SPECIAL REFERENCE TO THREE GRAMINEAE PLANT SPECIES

A THESIS SUBMITTED TO THE

UNIVERSITY OF POONA

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

(IN CHEMISTRY)

BY

MRS. VIDYA S. GUPTA

M. Sc.

DIVISION OF BIOCHEMISTRY

NATIONAL CHEMICAL LABORATORY

POONA - 411008 (INDIA)

1981

ACKNOWLEDGEMENTS

"There are teachers who are able to sow in you the seeds of enquiry and to lead you to the threshold of your own mind so that infinite depths of hidden treasure be yours". I consider myself extremely fortunate to get one such invaluable teacher, Dr. P.K.Ranjekar as my guide. His able guidance, constructive criticism and encouraging advice have become visible in the form of this thesis. To him, is due my lasting and sincere gratitude.

I record my deep sense of gratitude to Dr. V.Jagannathan, Head of Division of Biochemistry and Dr. L.K.Doraiswamy, Director, National Chemical Laboratory, Pune for extending all the facilities and kind permission to submit this work as a thesis.

I gratefully acknowledge the help I received from Dr. S.R. Gadre, Professor of Physical Chemistry, Department of Chemistry, University of Poona, Pune, in computer analysis of the data.

A full-hearted cooperation of my friends, Dr. Malathi, Lakshmi, Mrinal, Meena, Shekhar and Shirish made this journey very lively. I owe a lot to them for the successful completion of the work.

My thanks go to Mr. S.T.Dhume and Mr. M.N.Kamthe, who have been very helpful in instructing me in the use of different instruments. I am indebted to all the members of the staff of Division of Biochemistry for their kind cooperation. I also wish to thank Miss Usha Deshpande and Miss Indira for their skilful typing of the thesis.

To my parents and my husband, Shrikant, I owe to the fullest extent for inspiration, constant encouragement and tremendous patience throughout the course of the present investigations. It would not have been possible for me to complete my research and dissertation without their love and affection.

Lastly, I gratefully acknowledge the financial assistance from CSIR, New Delhi in the form of a Research Fellowship.

Gupta

Poona

Vidya S. Gupta

C O N T E N T S

ABBREVIATIONS

Cot	DNA concentration x time expressed in terms of moles x second/litre.
A + T	Adenine + Thymine.
G + C	Guanine + Cytosine.
HA	Hydroxyapatite.
K	Rate constant.
np	Nucleotide pairs.
PB	Sodium phosphate buffer.
pg	Picogram
PMSF	Phenyl methyl sulphonyl fluoride.
SDS	Sodium dodecyl sulphate.
SSC-X	0.15 M sodium chloride + 0.015 M trisodium citrate, pH 7.0.
TEMED	N,N,N',N'-Tetramethyl ethylenediamine.
Tm	Melting temperature.

SYNOPSIS

SYNOPSIS

Knowledge of properties of repeated DNA sequences and of their chromosomal organization with respect to single copy DNA sequences is crucial for understanding their biological role in eukaryotic cells. Plants are rather unique in having large variations in their nuclear DNA content, high proportion of repeated DNA sequences and a diversity in the nuclear organization. In plants, only a few species belonging to the family Gramineae have been studied in detail with respect to characterization of repeated DNA sequences, DNA sequence organization and DNA sequence homologies. However, all these species have nuclear DNA content (1C) more than 6.7 pg. In the present work, we have selected four Gramineae species namely finger millet (Eleusine coracana) Gaertn., great millet. (Sorghum vulgare) L., pearl millet (Pennisetum americanum)(L.)K. Schum and rice (Oryza sativa) Linn. The nuclear DNA content (1C) of finger millet, pearl millet and rice is less than 2.5 pg. In these three species, we have studied the reassociation kinetics of the total, sonicated DNAs, characterized the repeated DNA fractions by determination of their thermal denaturation and reassociation behaviour and have investigated the arrangement of repeated DNA sequences on the chromosomes. In the case of great millet, we have carried out only reassociation kinetics of total, sonicated DNA and thermal denaturation studies of native DNA. A preliminary study of interphase nuclear organization and of identification of histones in finger millet, pearl millet and rice was also carried out. This work was carried out with the following

objectives

1. How are the variations in the nuclear DNA content reflected in the proportion and the properties of repeated DNA sequences and in the mode of DNA sequence organization?

2. What is the molecular basis for the variation in the nuclear organization in plants?

The results from our work are summarized as follows:

1. Reassociation kinetics of the sonicated DNAs (an average fragment length of 550 np) of finger millet, great millet, pearl millet and rice were studied in the Cot range of 10^{-2} to 10^{4} mol x sec/l. Computer analysis of DNA reassociation curves was carried out in the case of finger millet, pearl millet and rice. DNA sequences reannealing by Cot 25 mol x sec/l in finger millet, Cot 10 mol x sec/l in pearl millet, Cot 50 mol x sec/l in rice and great millet were considered to be repetitive. The proportion of repeated DNA sequences varied in the range of 49 - 54% in all the four species.

2. . The repetitive DNA fraction was arbitrarily divided into very rapidly reassociating DNA (reannealing before Cot 0.1 mol x sec/l), rapidly reassociating DNA (Cot 0.1 to 1.0 mol x sec/l) and intermediately reassociating DNA (Cot 1.0 to respective limiting Cot values). The proportion of the very rapidly reassociating DNA in all the four species was in the range of 8.5 - 20.0%. The kinetic complexity of this fraction was estimated only in the case of rice, and was 1.05×10^{3} np. The rapidly reassociating fraction represented 7.0 - 18.5% of the genome with frequency of repetition and kinetic complexity in the range of 1.5×10^{3} to 1.2×10^{4} and 1.7×10^{4} to

4.3×10^4 np respectively. The intermediately reassociating DNA fraction was the largest among the repetitive DNA fractions and represented 21 - 25% of the genome. The frequency of repetition of this fraction was in the range of 1.4×10^2 - 1.0×10^3 with kinetic complexity as 3.3×10^5 - 3.2×10^6 np.

3. From the respective Cot 1/2 of non-repetitive DNA the kinetic complexity of the genome of these species was estimated as 1.6×10^8 - 1.2×10^9 np or 0.17 to 1.31 pg.

4. Thermal denaturation profiles of all the four, native, unsonicated DNAs were smooth and the Tms were 85.0°C in the case of finger millet and rice, 85.6°C in the case of great millet and 88.6°C in the case of pearl millet. From the melting temperature values, the G + C contents were determined to be 38.5 to 47.0%. The latter values compared well with those (38.37 to 48.0%) obtained from spectrophotometric method.

5. Thermal denaturation studies of different repetitive Cot fractions of finger millet, pearl millet and rice were carried out. The thermal stability of different repetitive fractions in the case of finger millet was in the range of 70.8 - 73.6°C and that in rice was in the range of 79.6 - 82.6°C. The melting profiles of these repetitive fractions were smooth. Pearl millet Cot 0.1, 1.0 and 10.0 fractions showed a presence of high melting component in them. The average proportion of this high melting component was 9.3% of the total genome. The melting temperatures of pearl millet repetitive fractions were in the range of 78.0 - 95.0°C.

6. When the thermal stabilities of the repetitive DNA

fractions of finger millet, pearl millet and rice were compared with those of respective native, sonicated DNAs, a lowering in the Tm values was observed. The lowering in the thermal stability of repeated DNA fractions indicated the presence of base mismatching in the range of 10.2 to 13.0% in the case of finger millet, 3.8 to 9.0% in the case of pearl millet and 0.8 to 3.8% in the case of rice.

7. Optical reassociation studies of Cot 1.0 DNAs of finger millet, pearl millet and rice revealed the presence of two kinetic components in the case of pearl millet and rice. The minor fast reassociating fraction which represented 28 to 35% of the total Cot 1.0 DNA, had a kinetic complexity of 330 to 390 np. Reassociation curve of finger millet Cot 1.0 DNA fraction was different from the above two species in having only one DNA component with an average kinetic complexity of 7.7×10^4 np. Close fit between the ideal curve for second order reaction and the experimental curve indicated the presence of only one distinct class of repetitive DNA in Cot 1.0 fraction of finger millet.

8. Arrangement of repetitive and nonrepetitive DNA sequences in finger millet, pearl millet and rice was studied. These studies revealed the presence of interspersion of repetitive and nonrepetitive DNA sequences in the case of finger millet and pearl millet and lack of interspersion at the fragment length of 6500 np in the case of rice. In the case of finger millet and pearl millet, 58% and 77.5% of the genomes, respectively, were interspersed. S_1 nuclease resistant repetitive duplexes of finger millet, pearl millet and rice DNAs

were sized on agarose (A50) column and by agarose gel electrophoresis. In finger millet, 20% of repetitive duplexes of length 4000 - 4200 np, 60% of 150 - 200 np and 20% of intermediate size were interspersed with 1900 np long unique DNA. Only 18% of the unique DNA was involved in interspersion. In pearl millet, 50% of single copy DNA of length 1900 np was interspersed with repetitive duplexes of size 4300 - 4500 np. The DNA sequence organization was unique in the case of rice. No interspersion was observed at a fragment length of 6500 np. The proportion of high molecular weight (6000 - 6400 np) S_1 nuclease resistant duplexes was 35% - 40% and that of very low molecular weight (less than 150 np) was 60 - 65%. The DNA sequence organization patterns in these species were thus different from each other as well as from other plants studied so far.

9. The information about the morphology of metaphase chromosomes and interphase chromatin was obtained by carrying out cytological studies. The number of chromosomes in finger millet, pearl millet and rice were 36, 14 and 24 respectively. They were metacentric and small. The interphase nuclear organization was of reticulate type and showed thin chromonemata and a few chromocenters.

10. The histone patterns of finger millet, pearl millet and rice were similar to some other Gramineae species such as wheat, rye, & barley. They showed presence of specific plant histones which were important in determining the reticulate organization of interphase nuclei. The molecular weights of these histones were in the range of 11,000 to 25,000 daltons

11. The low nuclear DNA content in finger millet, pearl millet and rice was thus reflected in the small chromosome size, low repetitive DNA content and diversed DNA sequence organization.

12. If we compare the molecular data in finger millet, great millet, pearl millet and rice with the available data on wheat rye, oat and barley, it appears that in Gramineae there is a decrease in nuclear DNA content as well as in the repetitive DNA content during evolution.

CHAPTER I

REVIEW OF LITERATURE

REVIEW OF LITERATURE

INTRODUCTION

Satellite and repeated DNA sequences have been identified approximately fifteen years before. The presence of these sequences in eukaryotes created a great interest in studying their properties and in speculating their biological functions. Determination of thermal denaturation and reassociation properties, buoyant density in CsCl gradients as well as in Cs_2SO_4 gradients containing Ag^+ and Hg^{++}, specific localization on chromosomes by *in situ* hybridization and behaviour towards the action of sequence specific restriction endonucleases are some of the most commonly used approaches to characterize satellite and repeated DNA sequences. These studies have yielded valuable information about their structural heterogeneity and kinetic complexity. The distribution of repeated DNA sequences with respect to single copy DNA sequences has been investigated and as a result, two predominantly occurring patterns of DNA sequence organization have emerged.

This Laboratory has been engaged in carrying out cytological and molecular biological studies of plant genomes since last five years. Our main interest in this study is to look for a correlation among several factors such as nuclear DNA content, repetitive DNA content, structural organization of interphase chromatin and DNA sequence organization. In this thesis, data are presented on the analysis of DNAs of mainly three Gramineae species, namely finger millet, pearl

in this area with special reference to that in plants.

Concept of repetitive DNA and satellite DNA in eukaryotes

The eukaryotic cell is characterized by the presence of a true nucleus, consisting of chromatin which is a complex of deoxyribonucleic acids (DNA) and proteins in a condensed (heterochromatin) or uncondensed (euchromatin) form. The occurrence of DNA in all the organisms imposed some basic and universal role on DNA in all the living cells. In the early 1940s, the first evidence of DNA being the genetic material was obtained by Avery, MacLeod and McCarthy (1944). Now, perhaps, it is the most widely accepted fact of genetics that DNA is the chemical basis of heredity; it stores genetic information and RNA helps cells in transmitting and expressin the genetic information in DNA. As better knowledge of function necessarily follows from better knowledge of structure, biochemists have been very much interested in understanding more about the structure of DNA and its organization in a genome.

According to classical genetics, the eukaryotic chromosomes were considered to be a linear sequence of linked genes. However, recent biochemical work has clearly indicated the presence of much more DNA than what appears to be required in terms of this simple model and this DNA is several times more complex than that of prokaryotes. The heterogeneity of this complex eukaryotic DNA was first extensively studied by Britten and Kohne (1966). In their studies on denaturation and reassociation of eukaryotic DNA they expected longer time for this DNA to reassociate as compared to that for prokaryotic

DNA. In reality, however, they observed a large fraction of eukaryotic DNA reassociating many times faster than bacterial DNA at the same concentration, indicating the presence of certain nucleotide pieces in many fold concentrations at the initial stage. This DNA was named as "Repeated DNA". Presence of repeated DNA is the characteristic of all the eukaryotic DNAs with the exception of some fungi (Timberlake, 1978).

Britten and Kohne (1966, 1968) and Kohne (1970) in their pioneering work on reassociation kinetics of many eukaryotic DNAs, classified repetitive DNA into two categories; highly repetitive and intermediately repetitive. This classification is dependent on their complexity and number of copies present (frequency of repetition) per genome. In general, highly repetitive DNA is assumed to include all the DNA sequences reassociating by Cot 1 mol x sec/l and is repeated atleast 1,000 to 100,000 times or more. Intermediately repetitive DNA is the fraction of the genome which forms duplexes in the Cot range of 1 to 100 mol x sec/l and is repeated 100 to 1,000 time All the DNA sequences reassociating beyond Cot 100 mol x sec/l are considered to be mostly unique or single copy sequences which include the sites of classical genetic activity, that is the structural gene loci (fibroin: Suzuki et al., 1972; globin Bishop and Freeman, 1973; Bishop and Rosbash, 1973; Harrison et al., 1974; ovalbumin: Harris et al., 1973), though not all unique DNA appears to function in this way (Davidson et al., 1977). However, this classification of repetitive DNA is very broad and superficial. Later on, depending on the nature of the reassociation curve, different values of Cot are also used

as the limiting values for repetitive DNA, such as Cot 10^3 mol x sec/l (Narayan and Rees, 1976), Cot 50 mol x sec/l (Smith and Flavell, 1974; Walbot and Dure, 1976), Cot 10 mol x sec/l (Seshadri and Ranjekar, 1979; Wimpee and Rawson, 1979) and Cot 5 mol x sec/l (Wu et al., 1977). Similarly, the highly repetitive DNA itself is also subdivided into different classes (Mitra and Bhatia, 1973; Smith and Flavell, 1975; Willey and Yunis, 1975).

In many cases, the frequency of repetition of a certain nucleotide sequence is very high (Salser et al., 1976) and its base composition is different from the bulk DNA. This repetitive DNA fraction bands in CsCl gradient at a buoyant density different than that of bulk DNA (main band DNA) and is referred to as a "Satellite" DNA. This phenomenon was actually observed by Kit (1961) and Sueoka (1961) before the concept of repetitive DNA was put forth. In some cases satellite band(s) can be observed after binding of DNA with antibiotics (such as actinomycin D, netropsin: Kersten et al., 1966; Peacock et al., 1974; Votavova and Sponar, 1975; Dennis et al., 1979), heavy metals like Hg^{++} and Ag^{+} (Nandi et al., 1965; Jensen and Davidson, 1966; Corneo et al., 1970a; Guille and Grisvard, 1971; Huguet and Jouanin, 1972; Filipski et al., 1973; Liberman, 1973; Skinner and Beattie, 1973; Timmis et al., 1975; Ranjekar et al., 1976; Zardi et al., 1977) or Guanidinium (Enea and Zinder, 1975); followed by centrifugation in a CsCl or Cs_2SO_4 gradient. Such satellite DNAs are called as "cryptic" satellites. However, the absence of satellite DNA in a density gradient does not necessarily mean the lack

of highly repeated DNA sequences in a genome.

In addition to these, some other techniques such as methylated albumin kieselguhr column chromatography (Cheng and Sueoka, 1963; Corneo et al., 1970c; 1972; Votavova and Sponar, 1974) and alkaline CsCl or Cs_2SO_4 gradient centrifugation (Corneo et al., 1968; Flamm et al., 1969; Hatch and Mazrimas, 1974; Fillipski and Rzeszowskalvolny, 1974) are also used to isolate the satellite DNAs and their individual strands.

The satellites isolated by these different techniques can be lighter or heavier than the main band DNA. Commonly occuring satellites are heavier or G + C rich and are reported in animals and plants (Beattie and Skinner, 1972; Curtain et al., 1973; Beridze et al., 1973; Ayres, 1978; Macaya et al., 1978 Beridze, 1980a; 1980b), whereas less reports are available about lighter or A + T satellites in animals (Corneo et al. 1972; Papaconstantinou et al., 1972; Markham et al., 1973, and plants (Ingle et al., 1973; Capesius et al., 1975).

Occurrence of repetitive and satellite DNA

In spite of the fact that the work on repetitive DNA and satellite DNA of animals and plants started almost simultaneously, the data available for plants are comparatively less. The most probable reasons are - (1) the various components such as cellulose, hemicellulose, lignin and pectin make the plant cell-wall resistant to mechanical breakage and (2) possible contamination of cell organelles and other reserve food materials in plant cells hamper the pure nuclear pellet formation.

The presence of different classes of repetitive nucleotide

sequences was demonstrated in a large number of evolutionarily diverged animal species such as bovine (Britten and Kohne, 1968; Votavova et al., 1972; 1973), human (Saunders et al., 1972; Mitchell, 1974), rodent (Santigo and Rake, 1973; Holmes and Bonner, 1974), Xenopus (Davidson et al., 1973), Chicken (De Jimenez et al., 1974), sea urchin (Graham et al., 1974), amphibian (Baldari and Amaldi, 1976), Chironomus tentans (Sachs and Clever, 1972), silkworm (Gage, 1974), Drosophila (Manning et al., 1975; Wensink, 1978), fish (Vladychenskaya et al., 1975), Microtus agrestis (Yasmineh and Yunis, 1975), Algerian hedgehog (Willey, 1975), oyster (Kamalay et al., 1976), mouse (Cech and Hearst, 1976; Ginelli et al., 1977), loach and trout (Kupriyanova and Timofeeva, 1974), mouse, syrian hamster and Chinese hamster (Rake, 1974), marine invertebrates (Weinblum et al., 1973) and buffalo, sheep and goat (Mehra and Ranjekar, 1979; Mehra et al., 1980). Necturus contains the highest known value (90%) of repetitive DNA (Straus, 1971).

Likewise, extensive studies were also carried out on satellite DNA in animal genomes using isopycnic centrifugation in CsCl or Cs_2SO_4 (reviewed by Walker, 1971; Bostock, 1971; Rae, 1972; Appels and Peacock, 1978; John and Miklos, 1979). One of the first satellite DNA studied in detail was that of mouse (Waring and Britten, 1966; Bond et al., 1967; Flamm et al 1967; Corneo et al., 1968; Schildkraut and Maio, 1968; Walker et al., 1969; Yasmineh and Yunis, 1969; Salomon et al., 1969; Henning and Walker, 1970; Mattocia and Comings, 1971; Rae and Franke, 1972; Sutton and McCallum, 1972; Sutton and Walker, 1972; Chilton, 1973; Markham et al., 1973 and

Wintzerith et al., 1973). This was followed by analysis of satellites in several other animal species, namely calf (Polli et al., 1965; Yasminch and Yunis, 1971; Filipski et al., 1973; Votavova and Sponar, 1974; Votavova et al., 1975; Thiery et al., 1976; Roizes, 1976; Cortadas et al., 1977; Macaya et al., 1978), Drosophila (Laird and McCarthy, 1969; Gall et al., 1971; Henning, 1972a; 1972b; Travaglini et al., 1972; Blumenfeld et al., 1973; Peacock et al., 1974; Gall and Atherton, 1974; Schweber, 1974; Cordeiro et al., 1975; Endow et al., 1975; Birnboim and Sederoff, 1975; Brutlag et al., 1977), human (Giacomoni and Corneo, 1968; Corneo et al., 1970b; 1971; 1972; Ginelli and Corneo, 1972; Chuang and Saunders, 1974), sheep and goat (Curtain et al., 1973; Forstova et al., 1979), guinea pig (Corneo et al., 1968; 1970a; Flamm et al., 1968), Crustaceans (Brodzicki, 1969; Skinner et al., 1970; Skinner and Kerr, 1971; Gray and Skinner, 1974; Skinner and Beattie, 1974), kangaroo rat (Hatch and Mazrimas, 1970; 1974; Mazrimas and Hatch, 1970; Bostock et al., 1972; Prescott et al., 1973; Fry et al., 1973; Dunsmuir, 1976), chicken (Ayres, 1978; Cortadas et al., 1979), sea urchin (Stafford and Guild, 1969; Sponar et al., 1970; Mistra, 1978), whale (Arnason et al., 1976), fish (Hudson et al., 1980) and some other mammals (Arrighi et al., 1970; Prosser et al., 1973).

In contrast to animals, the information about the plant genomes is much more limited. From the data available so far, it can be generalized that plant genomes contain a higher proportion (40 - 85%) of repetitive DNA (Flavell et al., 1974)

as compared to that in animal genomes. A few plant species such as wheat, oat, rye and barley belonging to the family Gramineae show very high percentage of repetitive DNA which is approximately 80% (Bendich and McCarthy, 1970a; Mitra and Bhatia, 1973; Smith and Flavell, 1975; Ranjekar et al., 1976). On the contrary, a few dicot species such as Vigna radiata (Murray et al., 1979), Phascolus mungo (Seshadri and Ranjekar, 1979) show a low repetitive DNA content of 35%. The repetitive DNA contents in different plants species are summarized in Table 1.1. As observed from this Table, the fungal genomes show comparatively low repetitive DNA. Neurospora crassa, for example, has only 10.0 - 15.0% repetitive DNA (Chaudhuri and Dutta, 1976; Krmlauf and Marzluf, 1980) and Aspergilus nidulans has only 2.0 - 3.0% repetitive DNA (Timberlake, 1978) which is very unusual in eukaryotic genomes.

The presence of nuclear satellite DNA has also been observed in a number of plant species (reviewed by Coudray et al.,1970 and Nagl, 1976). The primitive plant species such as yeast cytoplasmic petite mutant (Bernardi et al., 1968), Saccharomyces cerevisiae (Stevens and Moustacchi, 1971), some unicellular algae (Gense, 1971) and Chlorella pyrenoidosa (Bayen and Rode, 1970) were first studied to show the presence of satellite DNA. The credit of first analysis of higher plant DNA by isopycnic centrifugation in neutral CsCl gradients goes to Beridze (1972). Later on Beridze (1975) and Beridze et al. (1973) carried out extensive work on six Phaseolus and seven Brassica species, where the buoyant density of satellite DNA in the species belonging to the same genus showed

Table 1.1 Repetitive DNA content of different plant species

Plant species	Repetitive DNA content %	Reference
Fungi		
Dictyostelium discoidium	30.0	Firtel and Bonner, 1972.
Physarum polycephalum	45.0	Fouquet et al., 1974
Achlya	18.0	Hudspeth et al., 1974
Aspergilus nidulans	2.0 - 3.0	Timberlake, 1978
Neurospora crassa	10.0 - 15.0	Chaudhuri and Dutta, 1976; Krmlauf and Marzluf, 1980.
Phycomyces sp.	30.0	Dusenbery, 1975.
Saccharomyces cerevisiae	13.1	Lauer et al., 1977.
Schizophyllum commune	<5.0	Dons and Vessels, 1980.
Algae		
Blue green algae	5.0	Robertis, 1977.
Euglena gracilis	78.5	Rawson et al., 1979.
Pteridophytes		
Deer fern	50.0	Bendich and Anderson, 1977.
Osmunda (3 species)	50.0 - 60.0	Stein and Thompson, 1975.
Parsley fern	50.0	Bendich and Anderson, 1977.
Gymnosperms		
Conifers	40.0 - 50.0	Miksche and Hotta, 1973.
Angiosperms (Monocotyledons)		
Allium (7 species)	44.0 - 60.0	Ranjekar et al., 1978b.
Dafodils	50.0	Bendich and Anderson.

Plant species	Repetitive DNA content %	Reference
Lilium henryi	60.0	Smyth and Stern, 1973.
Orchids such as Brassia Cattleya, Cymbidium and Phalenopsis	45.0	Nagl and Capesius, 1977.
Pearl millet	69.0	Wimpee and Rawson, 1979.
Poa annua	87.0	Flavell and Smith, 1975.
Poa trivialis	82.0	Flavell and Smith, 1975.
Scilla sibrica	40.0	Stanziano and Maggini, 1975.
Wheat, oat, rye and barley	70.0 - 83.0	Bendich and McCarthy, 1970a; Ranjekar et al., 1974; 1976; Smith and Flavell, 1975.
Zea mays	78.0	Flavell and Smith, 1975.
Angiosperms (Dicotyledons)		
Anemone sp.	53.0 - 67.0	Cullis and Schweizer, 1974.
Artichoke	45.0	Nze-Ekekang et al., 1974.
Beans	40.0- 70.0	Chooi, 1971; Straus, 1972; Gnucheva et al., 1977.
Cucumber	55.0	Ranjekar et al., 1978c.
Cichoreae (11 species)	42.0 - 74.0	Bachmann and Price, 1977.
Gossypium hirsutum	40.0 - 50.0	Walbot and Dure, 1976; Wilson et al., 1976.
Gossypium thurberi and Gossypium arboreum	50.0 - 60.0	Wilson et al., 1976.
Lathyrus (9 species)	56.0 - 72.0	Narayan and Rees, 1976.
Pea	65.0 - 75.0	Sivolap and Bonner, 1971; Murray et al., 1978; Pearson et al., 1978a;b.

Plant species	Repetitive DNA content %	Reference
Phaseolus (4 species)	35.0 - 47.0	Seshadri and Ranjekar, 1979; 1980a.
Radish	45.0	Ranjekar et al., 1978c.
Soybean	60.0	Goldberg, 1978; Gurley et al., 1979.
Tobacco	70.0	Zimmerman and Goldberg, 1977.
Tropaeolum majus	70.0	Deumling and Nagl, 1978.
Umbelliferae (Parsley)	50.0 - 70.0	Kiper and Herzfeld, 1978.
Vigna radiata	35.0	Murray et al., 1979.

a similarity. Furthermore, the proportion of satellite DNA was shown upto 37% in Brassica and Phaseolus plant species. In 1973, Ingle et al., scanned a large number of plant species from a wide spectrum of families for the presence of satellite DNA. It was shown that the satellite DNA is scattered throughout the angiosperm families in an irregular fashion. Among Magnolidae, it is present in Drimys but absent from Magnolia and Ranunculus. In cucurbitaceae, prominent satellites were found in Cucumis, Cururbita, Citrullus, Bryonia, Lagenaria and Luffa but not in Momordica. In Cruciferae, satellite DNA was found in Lobularia and Raphanus. In Rutaceae, conspicuous satellite peaks appeared in the DNA of Citrus and Fortunella but not in Choisya or Skimma. Other families containing some genera having satellite DNA and some without it are Leguminosae, Solanaceae and Compositae. In the genus Linum, it was found in L. usitatissimum but not in L. grandiflorum. Some of the other plant species which showed the presence of satellite DNA in neutral CsCl gradients are Ammi and Carrot (Ohyama et al., 1972), cotton (Aripdzhanov et al., 1976), Citrus lemoni (Beridze, 1980b), cucumber and flax (Timmis and Ingle, 1977), cucumber and radish (Kadouri et al., 1975; Ranjekar et al., 1978c), Cymbidium (Capesius et al., 1975), pea seedling (Broekaert and Van Parijs, 1975), Phaseolus vulgaris (Bragvadze, 1974) and Tropaeolum majus (Deumling and Nagl, 1978).

From the above data, it can be easily stated that monocotyledons as against dicotyledons in general, do not show satellite bands in a CsCl density gradient. The orchid Cymbidium is the only monocot which shows a satellite in CsCl

gradient (Capesius et al., 1975). However, cryptic satellites have been observed in number of monocots (Deumling et al., 1976). Satellite DNA in Ag^{+}/Cs_2SO_4 density gradient was first observed in wheat (Huguet and Jouanin, 1972). Later on, the cryptic satellite DNA in wheat was detected by many research workers in either Hg^{++} or Ag^{+}/Cs_2SO_4 gradients (Deumling et al., 1976; Ranjekar et al., 1976; Peacock et al., 1977a; 1977b). In barley, two satellites were observed in Ag^{+}/Cs_2SO_4 density gradients and one satellite was noticed in Hg^{++}/Cs_2SO_4 gradients (Deumling et al., 1976; Ranjekar et al., 1976, Peacock et al., 1977b). The cryptic satellites were also noticed in rye (Appels et al., 1978) and Scilla sibrica (Timmis et al., 1975).

Analysis of satellite DNAs in species such as muskmelon (Bendich and Anderson, 1974; Bendich and Taylor, 1977), melon (Sinclair et al., 1975), Scilla sibrica (Timmis et al., 1975), tomato (Chilton, 1975), french bean (Beridze and Bragvadze, 1976), Cymbidium (Capesius, 1976), flax and cucumber (Timmis and Ingle, 1977), barley and wheat (Ranjekar et al., 1978a) and cucumber and radish (Ranjekar et al., 1978c) by thermal denaturation and optical reassociation kinetics measurements have revealed the presence of atleast two DNA components varying in Tm values and kinetic complexity. The satellite DNAs of nuclear and chloroplast origin have also been compared in higher plants and the differences in the characteristics of melting temperatures are shown (Pascoe and Ingle, 1978).

Recent approaches used in developing the subject of repetitive DNA and satellite DNA

(a) Thermal denaturation and buoyant density

Thermal denaturation and isopycnic centrifugation in CsCl or Cs_2SO_4 gradients are two important basic techniques used for the characterization of DNA. Thermal denaturation studies give a valuable information concerning the G + C content and the base composition heterogeneity of DNA (Mandel et al., 1968). Likewise, CsCl or Cs_2SO_4 centrifugation yields the buoyant density of a given DNA preparation from which G + C content can be estimated (Mandel and Marmur, 1968). These two parameters do not seem to differ much among different species within the same genus (Beridze et al., 1973; Beridze, 1975; 1980b; Dutta et al., 1976; Levings et al., 1976; Subramanyam and Azad, 1978; Ranjekar et al. 1978b). Similarities between buoyant densities and melting temperatures within a genus reflect the similarity of the base composition of their DNA. The difference in the G + C content from the melting temperature and buoyant density implies the presence of 5-methyl cytosine in the DNA molecule. Presence of 1% methylated cytosine is known to lower the buoyant density value of DNA by about 0.001 g/cm^3 (Kirk, 1967). Unequal distribution of 5-methyl cytosine has been shown in case of runner bean and lemon satellite DNAs (Beridze, 1980b). Lemon satellite shows a presence of as high as 24.1% of 5-methyl cytosine in its DNA molecule (Beridze, 1980b). The repetitive DNA of many animal species is analysed using these two techniques (Human: Britten, 1968; Saunders et al., 1972; Calf: Britten and Smith, 1969;

otavova et al., 1970; 1972; 1973; few Microtus species: asmineh and Yunis, 1971; 1973; Xenopus: Davidson and Hough, 1971; mphibians: Straus, 1971; Drosophila: Laird and McCarthy, 969; Wu et al., 1972; Nassaria: Davidson et al., 1971; hynchosciara: Balsamo et al., 1973). Relatively less nformation is available on the characterization of plant epetitive DNAs. Thornburg and Siegel (1973) for example, have hown the presence of two or more classes of repetitive omponents in four plant species by isopycnic centrifugation of heir repetitive DNAs in neutral CsCl gradients. Nze-Ekekang t al. (1974) have revealed the occurrence of two rather omogeneous fractions with an average complexity of 2 x 10^8 nd 10^9 daltons, respectively, in Jerusalem artichoke repetitive NA. Smith and Flavell (1975) isolated a very rapidly eassociating fraction (Cot 0.02 mol x sec/l) and a heterogenous ntermediately reannealing fraction from wheat genome and haracterized them by studying their denaturation-reassociation roperties. Ranjekar et al.(1974; 1976) isolated three epetitive fractions as Cot 0.01 mol x sec/l, Cot 0.01 to 1.0 ol x sec/l and Cot 1.0 to 100 mol x sec/l from rye, barley nd wheat and studied isopycnic centrifugation pattern in eutral CsCl gradients and thermal denaturation properties. Bachmann and Price (1977) have reported the analysis of epetitive DNA in Cichorieae and have demonstrated the presence f two distinct fractions of repetitive DNA in eleven plant pecies. In tobacco, (Zimmerman and Goldberg, 1977) three iscrete repetitive DNA fractions have been described. roperties of repetitive DNA fractions of seven Allium species

'ere studied by isopycnic centrifugation in neutral CsCl ;radients and by thermal denaturation (Ranjekar et al., 1978b).

b) In situ localisation

Accumulation of more and more information on repetitive)NA and satellite DNA components necessiated its localisation, .n situ on chromosomes, in order to assess its role in :hromosome organization. The first successful attempt which .ed to a workable procedure was made by Gall and Pardue Gall and Pardue, 1969; 1971; Pardue and Gall, 1969; 1970 ınd Pardue et al., 1970). In this technique, DNA/DNA or DNA/RNA ıybridization is carried out. DNA molecule embedded in the ıucleus or the chromosome is first fixed on the slide, and is ınown as 'receptor DNA'. The other component, either DNA or :NA which has to be localised is in the solution form and is 'adioactive. Then the hybridization between these two :omponents is carried out at proper conditions and the hybrid ıolecules are localised by autoradiography.

The first satellite DNA localised was that of mouse and .t was found to be exclusively in the centromeric heterochromatin f all the chromosomes except Y chromosomes (Pardue and Gall, 970; Jones, 1970; Jones and Corneo, 1971). Similar studies .n other animal species namely guinea pig (Yunis and Yasmineh, 970), Drosophila (Jones and Robertson, 1970; Gall et al., 1971), 'ly (Eckhardt, 1970), Calf (Yasmineh and Yunis, 1971; Kurnit t al., 1973), salamander (MacGregor and Kezer, 1971), hynchosciara hollaenderi (Papaconstantinou et al., 1972; :ckhardt and Gall, 1971), human (Arrighi and Hsu, 1971; Chen ınd Ruddle, 1971; Yunis et al., 1971; Jones et al., 1973; 1974;

Marx et al., 1976), birds (Brown and Jones, 1973) and Triturus cristatus (McCregor, 1979) have shown the localisation of satellite DNA either in centromeric heterochromatin or to wholly heterochromatic arms. The satellite DNA components when masked by main band DNA are difficult to resolve. However, all the eukaryotes contain repetitious sequences which can be isolated by reassociation kinetics. These sequences can also be localised by using in situ hybridization technique. This type of work is carried out in Drosophila (Rae, 1970; Botchan et al., 1971) and mammals (Arrighi et al., 1970; 1971; Saunders et al., 1972; Ahnstrom and Natarajan, 1974) and demonstrates the location of repetitive DNA, especially the highly repetitive DNA in constitutive heterochromatin. A review article by Eckhardt (1975) summarizes the use of in situ localisation technique in number of organisms and represents the respective locations of repetitive DNA.

The relatively small size of chromosomes in plants containing satellite DNA and the inherent impermeability of plant cell walls to various chemicals and radioactive materials have frustrated the earlier attempts to localise satellite DNA in plant chromosomes (Timmis et al., 1975). The first plant species studied for in situ localisation was that of Scilla sibrica where it was shown that the satellite DNA was distributed on the heterochromatic regions of the chromosomes (Timmis et al., 1975). In Vicia faba it is shown that satellite DNA is more generally spread over the nuclei and the chromosomes (Timmis et al., 1975). Similar studies in Cymbidium (Nagl and Capesius, 1977) and Tropaeolum majus

(Deumling and Nagl, 1978) have revealed the location of satellite DNA preferably in the regions of the heterochromatic chromocentres and at the nucleolar organizer regions respectively. In rye, the highly repetitive DNA (Cot 0.02 mol x sec/l) was isolated and shown to be localised mainly within C-bands near the distal ends of most chromosome arms (Appels et al., 1978). From the above data it can be concluded that satellite DNA and highly repetitive DNA in both plants and animals are generally localised at the heterochromatin blocks, in the regions of centromere, nucleolar organizer and telomeres of the chromosomes (Yunis and Yasmineh, 1971). The elucidation of the relation between satellite DNA and heterochromatin permits some conjecture concerning the origin and function of this significant portion of the genome of higher organisms (Yunis and Yasmineh, 1971; Sharma, 1978). Satellite DNAs possibly have evolved in eukaryotes in response to increase in genome size and the complexity of chromosomal and nuclear organization of cell. The specific location of satellite DNAs indicates their possible functions such as genetic regulators, spacers and mutation accumulating loci (Sharma, 1978). Thus, in situ hybridization serves as one important link between molecular biology and cytology.

(c) DNA-DNA hybridization

Traditional classification of plants is based mainly on morphological characters. Nowadays, besides morphology, evidence from protein analysis, secondary products (reviewed by Turner, 1969) and cytogenetic analysis are also considered when establishing relatedness and divergence among different

relationships is the analysis of DNA sequences themselves. DNA-DNA hybridization has proved useful for studying the taxonomic relationships and evolutionary patterns. The DNA sequences common between two organisms are detected by this method. In this techniques, labelled DNA of one species is mixed with an excess of DNA from another species and they are denatured by raising the temperature (thermal denaturation). The separated strands are then allowed to reassociate, at the appropriate temperature and salt concentration, to form a duplex. During such reassociation the separated strands of one species can react with those of another, provided there is homology in base sequences. The thermal stability of the hybrid DNA is taken as a measure of phylogenetic relatedness of two organisms in question (Schildkraut et al., 1961) or as an indication of conservation of sequences related to essential biological functions (Shearer and McCarthy, 1970; Whiteley et al., 1970).

This approach has already been applied to several species of related and unrelated plants (Bendich and Bolton, 1967), four species of Gramineae (Bendich and McCarthy, 1970a; Smith and Flavell, 1974), four biotypes of wheat (Bendich and McCarthy, 1970b), six species of Vicia (Chooi, 1971), three species of Osmunda (Stein and Thompson, 1975), four species of Atriplex (Strong and Thompson, 1975) and a few species of Lathyrus (Narayan and Rees, 1977) and Cucurbita (Goldberg et al., 1972). DNA-DNA hybridization in four Gramineae species was used to recognise different groups of repeated sequences and to distinguish different chromosomal regions : atleast 10,000 base

pairs long, which did not possess repeated sequences in common. Crude maps of sequence organization for almost all the DNA in the barley and oat genomes are produced (Rimpau et al., 1980). This study reveals that each species has its own type of repeated sequences which appear to have arisen after the species diverged from a common ancestor (Flavell et al., 1977). Ultimately, in Gramineae, the phylogenetic relationship that has been derived from morphological analyses, was confirmed and it was concluded that the amount of repeated fractions held in common diminishes with taxonomic separation of the species (Flavell et al., 1977).

The hypothesis that repetitive DNA arose during a short period of evolutionary time (Walker, 1971) and that single copy DNA (unique sequences) held a better palaeontological record (Kohne, 1970) than repetitive DNA, has led several investigators to prefer the use of single copy DNA for phylogenetic analysis (Angerer et al., 1976; Rice and Paul, 1971; Belford and Thompson, 1976). Thus contrary to the evidence from DNA-DNA hybridization using repetitive DNA, an equivalent study involving single copy DNA among the four species of Atriplex gave support to the traditional concept of the phylogenetic relationships between them (Belford and Thompson, 1976).

(d) Use of restriction endonuclease

Use of restriction endonucleases is an important tool in gene isolation, DNA sequencing, genetic engineering and characterization of satellites. Botchan et al.(1974) has shown that one of the mouse satellite DNAs has repeat length between 500 and 2000 base pairs. Further work on mouse was

done by Hoerz et al. (1974), Hoerz and Zachau (1977), Bernstine (1978) and Heller and Arnheim (1980). Some other species such as bovine (Mowbray et al., 1975; Phillippsen et al., 1975; Lipchitz and Axel, 1976; Kopecka et al., 1978), guinea pig (Altenburger et al., 1977), rat (Fuke et al., 1979), Apodemus (Cooke, 1975), monkey (Fittler, 1977), Drosophila (Shen et al., 1976; Endow, 1977; Kirsch and Cohen, 1980), sheep and goat (Forstova et al., 1979) and human (Manuelidis, 1976; 1978) are also analysed in detail by using a number of restriction endonucleases.

Restriction endonucleases Bsp I and Hpa II which recognize G + C rich cleavage sites attack runner bean and black mustard satellites in more or less similar manner as compared to lemon satellite (Beridze, 1980b). Bsp I cleaves lemon satellite DNA extensively, yielding bands and high periodicity; whereas the same DNA preparation is completely resistant to Hpa II indicating the occurrence of m^5C in lemon satellites (Beridze, 1980b). Long range periodicities after endonuclease action are observed in bean and black mustard satellites (Fodor and Beridze, 1980), whereas lemon shows short range periodicity (Beridze, 1980b). Recently restriction endonuclease analysis of a monocot lily DNA (Sugiura and Hotta, 1980) is also reported.

Fold back sequences

One important fraction of repeated sequences, organized in inverted register in the genome, is called as palindromic, fold back, hairpin or zero-time binding DNA (Wilson and Thomas, 1974). This DNA is easily separated from the remaining fraction of eukaryotic DNA due to the special property of

denatured fold back sequences to reassociate instantaneously. Intra-molecular base pairing between the complementary sequences of the inverted repeat takes place (Wilson and Thomas, 1974) and because of this highly characteristic appearance, they are identified very easily in electron microscopy (Cech and Hearst, 1975; Deininger and Schmid, 1976; Bell and Hardman, 1977). A novel structure of fold back sequences with a more complex appearance is also detected. These 'bubbled' hairpins are formed by reannealing of many distinctly apart homologous segments of inverted repeat sequences, separated by interspersed non-fold back sequences of various sizes (Hardman et al., 1979).

The presence of inverted repeats has been shown in lower eukaryotes (Engberg and Klenow, 1977; Hardman and Jack, 1977; Borchsenius et al., 1978; Klein and Welch, 1980; Krmlauf et al., 1980), animals (Davidson et al., 1973; Cech and Hearst, 1975; Szala et al., 1980) as well as in plant genomes such as wheat (Huguet et al., 1975; Smith and Flavell, 1975; Bazetoux et al., 1978), rye (Smith and Flavell, 1977), Cotton (Walbot and Dure, 1976), tobacco (Zimmerman and Goldberg, 1977), pea (Murray et al., 1978) and soybean (Gurley et al., 1979). The amount of palindromic sequences varies in the range of 1.5% (Bazetoux et al., 1978) to 6% (Dott et al., 1976) in animals and plants. The distribution of inverted repeats on the genome can be of four types: randomly distributed over all of the genome; distributed over all of the genome with uniform spacing; clustered in some fraction of the genome and clustered and spaced uniformly or randomly on the genome. In the case of Xenopus (Davidson et al., 1973), sea urchin (Graham et al.,

1974), Triturus (Wilson and Thomas, 1974), Drosophila (Schmid et al., 1975), Mollusc (Angerer et al., 1975), they are interspersed with either repetitive DNA or single copy DNA and are scattered throughout the genome. The examples of clustered inverted repeats are mouse (Cech and Hearst, 1975) and man (Wilson and Thomas, 1974; Deininger and Schmid, 1976). Among plants, the distribution of fold back sequences is somewhat similar to that in animal genomes. In wheat (Smith and Glavell, 1975) for example, the proportion of zero time binding fraction increases with increase in fragment length due to the presence of single strand tails on the fold back duplexes. These sequences are shown to be spaced throughout atleast 20% of the genomes and are probably clustered in groups. In cotton (Walbot and Dure, 1976), fold back sequences occur in a quasi-clustered arrangement.

The role of palindromic sequences in the eukaryotic genomes has become clear now. They serve as binding sites for regulatory proteins (Dickson et al., 1975; Darlix and Horaist, 1975), cleavage sites for the restriction enzymes (Kelly and Smith, 1970) or for the processing of nuclear RNA (Dunn and Studier, 1973). These sequences in DNA are transcribed into RNA. Ribosomal RNA as well as heterogeneous nuclear RNAs from mammals have been shown to contain a few percent of fold back sequences (Wellauer and Dawid, 1974; Jelinek et al., 1973; Ryskov et al., 1973). Inverted repeat sequences appear to be important in biological events which involve transposition of genetic material (Cohen, 1976; Ohtsubo and Ohtsubo, 1976; Deonier and Hadley, 1976; Wuilmart et al., 1977).

Functions of repeated and satellite DNA

Since a substantial proportion of repetitive DNA has been shown to be present in all the eukaryotes, it is naturally assumed that it has some fundamental role in nuclear activities. The exact function of repeated DNA is unclear and many suggestions have been made.

First, it was thought that repeated DNA is made locally for the process of differentiation. But this theory has been ruled out as all cells of the same organism have the same DNA composition and repetition (Willey and Yunis, 1975; De Jimenez et al., 1974). Britten and Davidson (1971) and Bishop and Freeman (1973) suggested the role of repetitive DNA in the expression of the rest of the genetic DNA by some switching 'on' and 'off' controlling mechanism. These reports supported the idea that they are redundant and inert as far as the genetic flow of information is concerned (Ohno et al., 1957; Jones, 1970; Pardue and Gall, 1970; Yasmineh and Yunis, 1970). However, mRNA for histone proteins has been shown to be transcribed from repetitive DNA (Weinberg et al., 1972). Furthermore some other mRNAs have been isolated from various tissues, which are transcribed from repetitive DNA (Greenberg and Perry, 1971; Goldberg et al., 1973; Firtel and Lodish, 1973; Bishop et al., 1974; Campo and Bishop, 1974; Galau et al., 1974; Klein et al., 1974; Spradling et al., 1974; Davidson et al., 1975). The repetitive DNAs have also been found to code for non-translatable RNA (Lima-de-Faria et al., 1975) and rRNA (Price, 1976; Lauer et al., 1977; Timberlake, 1978). Mazrimas and Hatch (1972) revealed that a heavy

satellite of Geocarcinus lateralis is transcribed during certain stages of the moult cycle. Walker (1971) has suggested the possible role of repetitive and satellite DNA in chromosome replication, chromosome folding and chromosome pairing. Chromosomal specificity of satellite DNA is important in homologous chromosomal recognition in the cell (Brutlag and Peacock, 1975). The amount and kind of heterochromatin can alter the firmness of attachment of centromere to spindle proteins and reduce chances of chromosome loss. Such sequences also perform some mechanical role of "house keeping" and are involved in arranging functional sequences in relation to higher order chromatin structure (Walker, 1978). Nagl (1978) has proposed that repeated sequences are important in evolution and speciation. According to him, non-coding, simple, highly repeated sequences may be involved in ontogenetic differentiation and phylogenetic diversification. It is thus concluded that the genetically inert, highly repetitive "chromosome engineering DNA" controls both the gross morphology of karyotypes and chromosomes and their evolution.

DNA sequence organization in eukaryotic genomes

The occurrence of repetitive and non-repetitive DNA fractions in all the higher organisms without exception raised an interesting question of organisation of these sequences in the eukaryotic genomes. Discussions of gene regulation and chromosome structure resulted in specific predictions regarding the arrangement of repetitive and non-repetitive sequences (Callan, 1967; Thomas et al., 1970; Crick, 1971; Britten and Davidson, 1969; 1971). Evidences to show the interspersion

of repetitive and unique sequences were collected.

(1) By the increasing proportion of DNA binding to hydroxyapatite at low Cot values with DNA fragments of increasing length (Britten and Smith, 1970; Davidson et al., 1973; Graham et al., 1974).

(2) By electron microscopy of reassociated DNA (Wu et al., 1972; Bonner et al., 1973; Manning et al., 1975).

(3) By inference from the presence of both repetitive and non-repetitive sequences transcribed into the same RNA molecule (Firtel and Lodish, 1973).

(4) By CsCl equilibrium sedimentation (Kram et al., 1972).

First two are the most widely used methodologies for studying DNA sequence organization. To this date, many animal and plant species have been studied in this respect. In all these species most of the repetitive DNA sequences are organized with an alternating arrangement of repetitive DNA and non-repetitive DNA sequences. However, the lengths of the interspersed repetitive and non-repetitive DNA sequences are observed to be different. The most commonly observed pattern of genome organization among eukaryotes, known as short period interspersion was first observed in Xenopus (Davidson et al., 1973). This is characterized as repeated DNA sequences 300 - 400 np in length interspersed with single copy DNA sequences 800 - 1500 np long (Sea urchin: Graham et al., 1974; Davidson et al., 1974; Vorobev and Kosjuk, 1974; Galau et al., 1976; Lee et al., 1977; Rat: Bonner et al., 1973; Pays and Ronsse, 1975; Wu et al., 1977; Pearson et al., 1978b; Marine invertebrates: Goldberg et al., 1975; Human: Deininger and Schmid, 1976;

Ginelli and Corneo, 1976; Houck et al., 1978; 1979; Amphibians: Bozzoni and Beccari, 1978). A striking exception to the 'Xenopus pattern' was found in Drosophila (Manning et al., 1975; Schmid et al., 1975; Crain et al., 1976b), honeybee (Crain et al., 1976a) and Chironomus tentanus (Wells et al., 1976). In the 'Drosophila pattern' or 'long period interspersion pattern', single copy DNA sequences extend for as long as 10,000 np without interruption by repeated sequences. The average repeated DNA sequence length in Drosophila is 5600 np with a small fraction less than 500 np. Animal genomes showing short period interspersion pattern also exhibit a small proportion of long period interspersion pattern (Xenopus: Davidson et al., 1973; Sea urchin: Graham et al., 1974 and Mollusc: Angerer et al., 1975; Angerer and Hough-Evans, 1977). An intermediate organization pattern has been reported recently in the case of chicken (Arthur and Straus, 1978; Eden and Hendrick, 1978), where the length of interspersed repetitive DNA is 2000 - 3400 np and that of unique DNA is 4500 np. Some other animals such as mouse (Cech et al., 1973; Cech and Hearst, 1975; 1976; Ginelli et al., 1977), nematodes (Schachat et al., 1978; Beauchamp et al., 1979), birds (Epplen et al.,1978), housefly (Efstratiadis et al., 1976) and syrian hamster (Moyzis et al., 1977) are also studied in detail for DNA sequence organization.

As compared to animals, less information is available about the plant genome organization. However, the information regarding the organization in plant genomes is very important. Higher plants offer an unique approach to the study of eukaryotic

gene expression due to the fact that single cells can be programmed to differentiate into mature plants (Street, 1973). Different plants studied for their DNA sequence organization pattern are fungi (Firtel and Kindle, 1975; Hudspeth, 1977; Dons and Wessels, 1980; Hardman et al., 1980), cotton (Walbot and Dure, 1976), Phaseolus species (Seshadri and Ranjekar, 1980b), parsley (Kiper and Herzfeld, 1978), pea (Thompson, 1976 Murray et al., 1977; 1978), pearl millet (Wimpee and Rawson, 1979), rye (Smith and Flavell, 1977), soybean (Goldberg, 1978; Gurley et al., 1979), tobacco (Zimmerman and Goldberg, 1977), Vigna radiata (Murray et al., 1979) and wheat (Flavell and Smith, 1976). Since plants often contain relatively large amounts of repetitive DNA, many repetitive regions are not continuous with single copy DNA sequences. The organization of these repetitive sequences is either a tandem arrangement (Ingle et al., 1973; Bendich and Anderson, 1977; Timmis et al., 1975) or a complex interspersion of unrelated families of repetitive sequences as is the case in the genomes of wheat (Flavell and Smith, 1976) and rye (Smith and Flavell, 1977). Fig. 1.1 and 1.2 represent the comparative picture of lengths of interspersed repetitive DNA and single copy DNA in the case of some animals and plants.

The high degree of order seen in the alternating interspersion of relatively brief and longer non-repetitive sequence elements in eukaryotic genomes must be having some basic biological meaning. Britten and Davidson (1969) constructed a model of gene regulation using Xenopus pattern as the basic structure. In this model, structural genes in different sections of the genome can be simultaneously controlled due to their

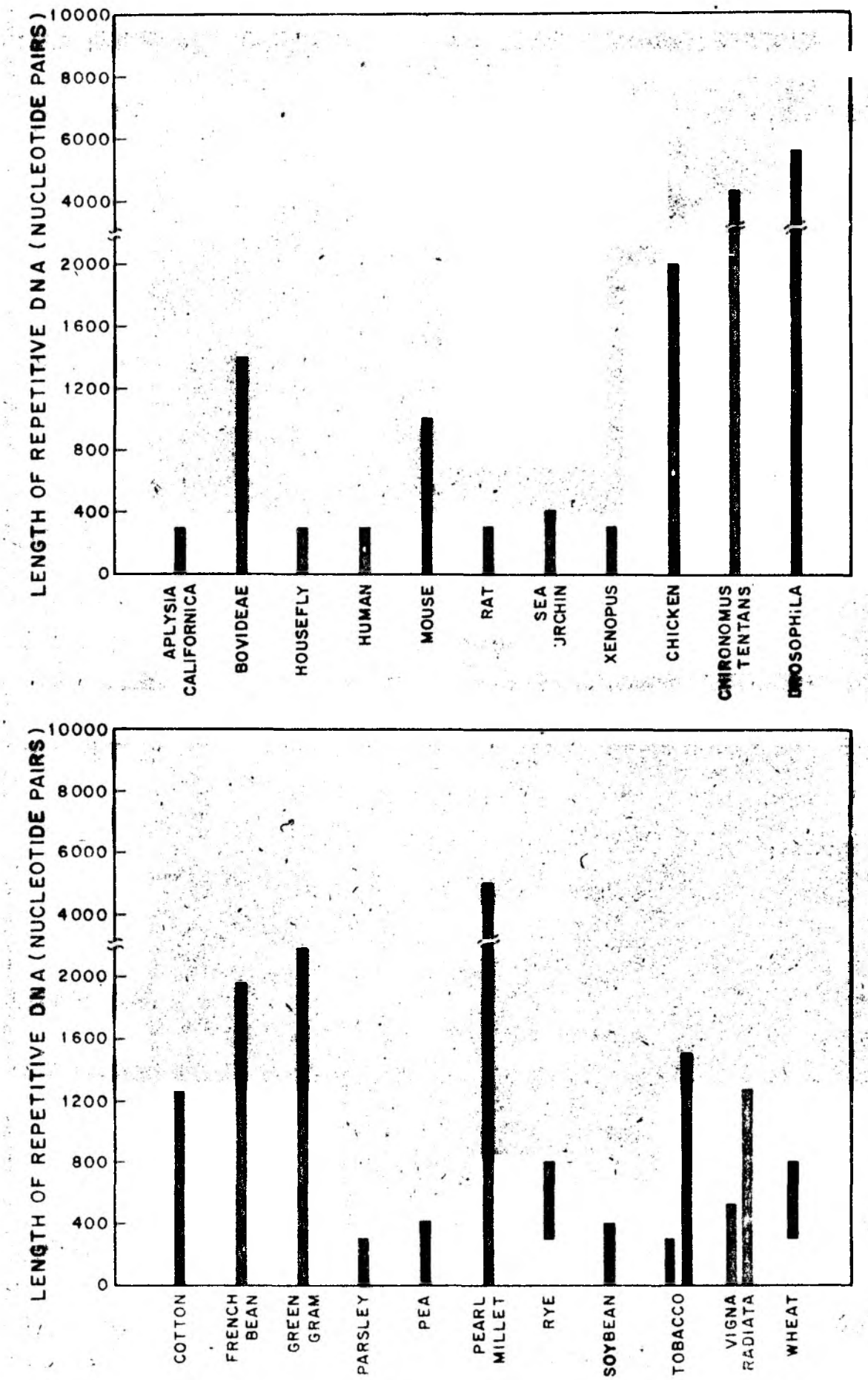
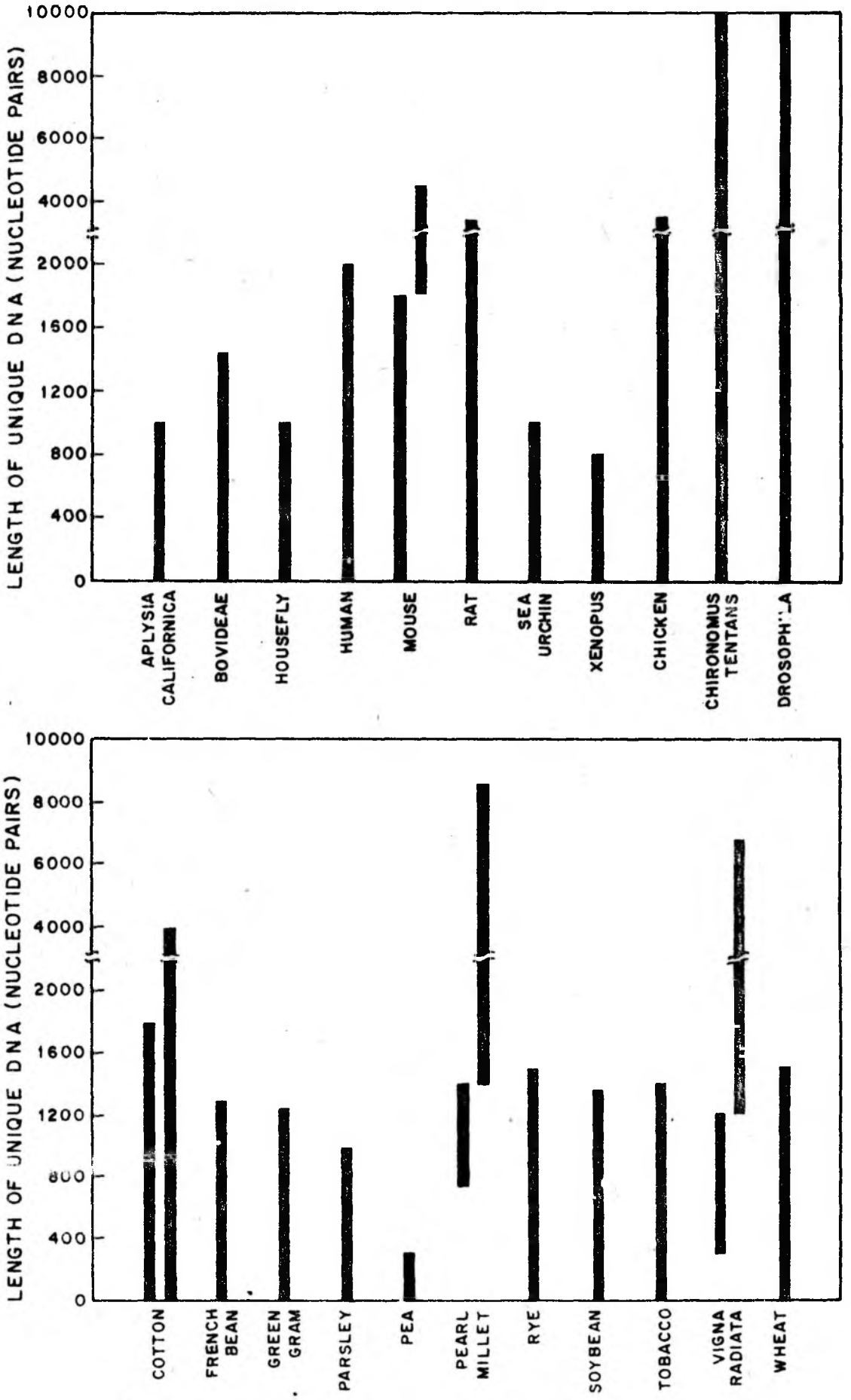


Fig. 1.1: Comparative picture of lengths of interspersed repetitive DNA in some animals and plants.



2: Comparative picture of lengths of interspersed unique DNA in some animals and plants

proximity to interspersed repeated regulatory elements. This model is strongly supported by the observation that 80 - 100% of mRNA sequences in sea urchin embryos are transcribed from repeat contiguous to unique DNA sequences. In their recent work, Davidson and Britten (1979) have proposed that nuclear RNA (nRNA) includes continuously synthesized RNA copies of the structural gene regions of the genome and that regulatory interactions occur between these copies and complementary repetitive sequence transcripts by the formation of RNA-RNA duplexes, Thus in this model nRNA also contains transcripts of repetitive DNA sequences which are represented at high levels in certain tissues and in low levels in others. The formation of repetitive RNA-RNA duplexes controls the production of mRNA.

Organization of chromatin

(a) Morphology and composition

Chromatin, the interphase forms of hereditary material of the cells is a complex of DNA, RNA, histones and non-histone chromosomal proteins (Johns, 1954). Majority of the mechanisms for controlling cellular proliferation, cell enlargement and the differential action of genes during development reside in the various histone and non-histone proteins that are complexed with DNA to form the chromatin (Arbuzova et al., 1968; Stein et al., 1975). Although chromatin is in diffused state during interphase, certain parts remain condensed as compared to the remaining chromatin. This condensed chromatin is known as 'heterochromatin', the term first introduced by Heitz (1928) and the diffuse portion is known as 'euchromatin'.

Two types of heterochromatin are recognised (1) facultative which is present in only one of the homologous chromosomes and (2) constitutive which is present in all the cells at identical positions on both the homologous chromosomes (Brown, 1966). This constitutive heterochromatin is present in chromosomes as centromeric, interstitial and telomeric heterochromatin (Arrighi and Hsu, 1971; Chen and Ruddle, 1971; Hsu and Arrighi, 1971) and represents a special kind of DNA "repetitive and satellite DNA" (Lee and Yunis, 1970; Yasmineh and Yunis, 1970).

Different cytological techniques to visualize the chromatin are based on the DNA level in the chromatin. In the morphological studies, staining the chromosomes with different dyes, in spite of its limitations, has a decided advantage over phase contrast and interference microscopy (Sharma and Sharma, 1980). Of all the different staining methods, the Feulgen reaction is considered to be the most effective one (Feulgen and Rossenbeck, 1924). Some other dyes are such as Carmine (Gatenby and Beams, 1950), Orcein (La Cour, 1941), Chlorazol black (Nebel, 1940), Crystal violet (Newton, 1926), Azure A (Himes and Moriber, 1956), Orange G-aniline blue (La Cour and Chayen, 1958), Toluidine blue (Robinson and Bacsich, 1958) and Giemsa (Robinow, 1941).

To locate the heterochromatic regions consisting of repetitive DNA sequences in the chromatin, different banding techniques have been employed. The denaturation of the chromatin at the cytological level, followed by renaturation and staining with different dyes, particularly Giemsa, shows intensively positive reaction at certain regions revealing the visualization of repetitive DNA because of its high reassociation

rates. This banding following denaturation-renaturation and Giemsa staining is termed as C-banding (Hsu, 1973; Arrighi, 1974). Different banding techniques such as Q, C, G, N, R are developed by different groups of scientists (Casperson and Zech, 1973; Dobel et al., 1973; Vosa, 1973; 1976; 1977; Marks and Schweizer, 1974; Yunis, 1974) in various animals and plants. These banding techniques permit the visualization of molecular sequences at the cellular and microscopic level. The microspectrophotometric analysis of bands at different phases of cell development and differentiation may lead to an understanding of the basic mechanism of genetic regulation in animals and plants.

(b) Histones

Histones are acid soluble, basic, low molecular weight (11,000 - 22,000 daltons) proteins without any enzymatic activity. Histones possess very low absorbance at 280 nm due to their low content of aromatic amino acids, with the exception of Arginine rich histone in higher organisms (Panyim et al., 1971; Brandt and Von Holt, 1972). They do not contain cystine or cysteine. There are five main types of histones. Two of them contain an excess of arginine over lysine and are classified as the arginine rich histone fractions. The remaining three histones contain more lysine than arginine and are known as the lysine rich histones.

In recent years, histones are considered to be involved in the structural organization of chromatin (Felsenfeld, 1978; Kornberg, 1977; Klug, 1978; Worcel, 1978). The four histones H_2A, H_2B, H_3 and H_4 form the first level of structural

organization into the core particle. The histones, H_3 and H_4 have the same primary structure in animal and plant kingdom (De Lange and Smith, 1971), whereas the histones, H_2A and H_2B are different in plants as well as animals (Nadeau et al., 1974; Spiker et al., 1976). The histone H_1 is important in the folding of the core particles into the next higher level of organization and is much variable in its sequence and seems to be species specific (De Lange and Smith, 1971).

(c) Non histone chromosomal proteins

The non-histone chromosomal proteins (NHC proteins) are defined to be those proteins, other than the histones, which are isolated in association with nuclear DNA in the purification of chromatin. Very little is known about NHC proteins since their isolation and fractionation is severely hampered by the tendency of these proteins to aggregate with DNA, histones and one another. However, it has been suggested that the NHC proteins play some role in the regulation of template activity. Marushige and Dixon (1969) indicated that the more template-active chromatin of a given organism contain more NHC protein than do less template-active chromatins. The NHC protein fraction includes DNA and RNA polymerases, nucleases and other enzymes involved in the metabolism of chromatin, structural proteins perhaps analogous to, but more acidic than histones, nuclear membrane components and possibly specific repressor or activator proteins.

(d) Present status of chromatin organization

Recent studies in the field of chromatin organization have indicated a presence of some basic repeating unit known

as 'ν-bodies' or 'nucleosomes' in the chromatin (Hewish and Burgoyne, 1973; Woodcock, 1973; Kornberg, 1974; 1977; Noll, 1974a; Olins and Olins, 1974). The nucleosome consists of DNA about 140 - 240 np (Compton et al., 1976; Morris, 1976; Noll, 1976; Lipps and Morris, 1977; Lohr et al., 1977; Thomas and Thompson, 1977) complexed with an octamer of histones (Kornberg, 1974). The octamer contains two molecules of lysine-rich histones H_2A and H_2B and arginine-rich histones, H_3 and H_4. H_1 is not the part of nucleosome, but is associated with linker DNA (Allan et al., 1980). Detail study of neutron and X-ray scattering (Richards et al., 1977) as well as X-ray diffraction and electron microscopy (Finch et al., 1977) have revealed cylindrical structure about 100 Å in diameter and 50 Å in height, with the DNA wrapped around it to form a pair of rings at the top and bottom.

Further characterization of nucleosomes is made by digesting the chromatin with micrococcal nuclease (Axel et al., 1974) as well as pancreatic DNAase (Noll, 1974b). Though the linker DNA is the preferred target, the nucleosome core is also attacked at a slower rate. Pancreatic DNAase attacks at 30, 80 and 110 nucleotides in the nucleosome from 5' terminus (Simpson and Whitlock, 1976). This symmetrical distribution of cleavage sites indicate that the nucleosome contains a dyad axis and is capable of unfolding into two symmetrical half-nucleosomes, as a part of some mechanism of replication or transcription (Weintraub et al., 1976).

The arginine-rich histone pair H_3-H_4 plays a central part in nucleosome formation (Kornberg, 1974). X-ray diffraction

patterns of fibres made from reconstituted complexes of DNA with H_3-H_4 are almost identical to those obtained from intact chromatin fibres (Boseley et al., 1976; Moss et al., 1977). H_1 histone also has a direct effect on the chromatin structure. Studies of the dependence of H_1 binding on the size of nucleosome oligomers showed that there is steady increase in affinity upto the octanucleosome suggesting that octanucleosomes are capable of forming a stable unit of higher order structure (Renz et al., 1977).

With this model of chromatin organization, certain conformational changes that take place during the cell cycle are not understandable. These changes may arise from modifications in composition of chromatin. Some other proteins, which are not histones, are found in large quantities associated with chromatin (Levy et al., 1977; Goldknopf and Busch, 1977) which may react and modify the chromatin structure further.

Scope of Thesis

Among all the groups of plants, cereal grains are of most importance to mankind. They are used as staple food all over the world. Some of the important Gramineae species such as rice, wheat, great millet, pearl millet, finger millet and minor millets are cultivated very commonly as field crops throughout India. Extensive cytogenetic studies have been carried with this group of plants from which their taxonomic and evolutionary relationships may be deduced (Bell, 1965; Riley, 1965).

Among the family Gramineae, the temperate plant species like wheat, oat, rye and barley were characterized thoroughly, when the present work was undertaken. However, no DNA

characterization data were available on the tropical plant species. In addition, the temperate plants were known as high DNA containing species. We, therefore, selected three tropical Gramineae species with low DNA content namely Eleusine coracana Gaertn. (finger millet), Pennisetum americanum (L)K. Schum (pearl millet) and Oryza sativa Linn. (rice) and one Gramineae species namely Sorghum vulgare L. with an intermediate DNA content. Extensive genome characterization of finger millet, pearl millet and rice was carried out. In the case of great millet, only a limited studies were done.

The present thesis is divided into six chapters, Chapter I being that of introduction in which the relevant literature is reviewed. Chapter II describes the isolation of DNAs from finger millet, great millet, pearl millet and rice and characterization of these DNAs by studying their reassociation kinetics using hydroxyapatite column chromatography. In the case of finger millet, pearl millet and rice, the Cot curves are analyzed using nonlinear least square analysis to reveal the components reassociating with second order kinetics. Haploid genome size is estimated from the Cot 1/2 of unique DNA in each of the four species. The reassociation data are also used to estimate the frequency of repetition and kinetic complexity of different Cot fractions which included mostly repeated DNA sequences.

In Chapter III, melting profiles and base composition of native, unsonicated DNAs are given. Different repeated DNA fractions (Cot fractions) are also characterized by studying their thermal denaturation behaviour and optical reassociation

kinetics. The kinetic heterogeneity and the kinetic complexity of the DNA sequences reannealing by Cot 1.0 mol x sec/l in finger millet, pearl millet and rice are reported.

Arrangement of repeated and single copy DNA sequences in finger millet, pearl millet and rice is described in Chapter IV.

Chapter V gives the data about the structure of interphase nucleus and identification of histones in finger millet, pearl millet and rice.

The important conclusions from the work described in Chapters II to V are discussed in Chapter VI. Bibliography is detailed in the end.

CHAPTER II

DNA REASSOCIATION KINETICS

DNA REASSOCIATION KINETICS

INTRODUCTION

It is well established that a fraction of an eukaryotic genome consists of repetitive DNA sequences. This conclusion is principally based on DNA reassociation kinetics measurements. Various techniques such as hydroxyapatite column chromatography and optical and fluorometric measurements are employed to collect the reassociation kinetics data. However, hydroxyapatite chromatography is the most convenient and widely used methodology for the fractionation of DNA on the basis of the reassociation rates of its components. A curve of percentage reassociation of denatured DNA corresponding to different Cot values which are dependent on the concentration of DNA and period of incubation can be obtained by using this technique. Furthermore, from this Cot curve the percentage of repetitive and non-repetitive DNA as well as the Cot 1/2 of non-repetitive DNA, fraction can be calculated. With the help of the latter, it is ultimately possible to arrive at the haploid genome size estimate. With nonlinear least square analysis of the Cot curve, it is also possible to resolve the repetitive DNA fraction into different components and to estimate their proportions, frequencies of repetition and kinetic complexities. We have, therefore, used this technique to measure the reassociation kinetics of the 550 np long DNAs of the four Gramineae species, namely finger millet, great millet, pearl millet and rice.

Bendich and McCarthy (1970a) first studied the homology of DNA sequences among wheat, oat, rye and barley by

reassociating one of these labelled DNAs at a time with the remaining species. Later, in 1974, Smith and Flavell revealed the presence of about 80% of repetitive DNA in these four Gramineae species. Further work on rye (Ranjekar et al., 1974), wheat (Smith and Flavell, 1975; Ranjekar et al.,1976) and barley (Ranjekar et al., 1976) has also confirmed the presence of very high percentage of repetitive DNA fraction in these species. A few more Gramineae species such as Avena sativa, Poa trivialis, Poa annua and Zea maya have also been shown to have the repetitive DNA content in the range of 78 - 87% (Flavell and Smith, 1975).

Except the above Gramineae species, nothing is known about the genomes of several other cereals belonging to the same family. In order to verify whether the high repetitive DNA is a general feature of Gramineae plant species, irrespective of their nuclear DNA content, we undertook the genome analysis of four more cereal species namely finger millet, great millet, pearl millet and rice. Recently, Wimpee and Rawson (1979) have reported the characterization of pearl millet genome and have demonstrated the presence of repetitive DNA representing 69% of the total DNA. In this chapter, we have described the isolation of DNAs from finger millet, great millet, pearl millet and rice and their characterization by determination of their reassociation kinetics.

MATERIALS AND METHODS

All the chemicals used throughout our work were of analytical reagent (AR) grade or guaranteed reagent (GR) grade obtained from BDH, Sarabhai Chemicals or E. Merck. Certain

chemicals such as RNAase, PIPES, Tris were obtained from Sigma Chemical Corporation, USA. λ DNA, T_4 DNA, S_1 nuclease, EcoRI and DNA molecular weight markers were secured from Boehringer Mannehiem, West Germany.

Seeds of finger millet (variety: Madwa VL 101), great millet (variety: Maldandi), pearl millet (variety: Awasari) and rice (variety: Sona) were obtained locally.

Hydroxyapatite preparation

Hydroxyapatite was prepared essentially according to the procedure described by Tiselius et al. (1956). Two litres of 0.5 M solution of $CaCl_2$ and two litres of 0.5 M solution of Na_2HPO_4 were mixed at a flow rate of 120 drops per minute from two separating funnels into a glass beaker under stirring to obtain a brushite ($CaHPO_4.2H_2O$) precipitate. The precipitate was washed four to five times with distilled water. Then distilled water was added to the precipitate to a final volume of four litres and to this was added 100 ml of freshly prepared 40% (w/v) NaOH solution under constant slow stirring. The mixture was boiled under stirring for one hour and the precipitate was washed with distilled water till the pH of the mixture was 7.0. Sodium phosphate buffer (0.012 M pH 6.8) was added to make the total volume of four litres and the solution was heated until it just started to boil. After decantation, 0.012 M PB (pH 6.8) was added to the precipitate and it was allowed to boil for 5 min. Another boiling with 0.0012 M PB was carried out for 15 min. HA was stored at 4°C with a few drops of chloroform.

Treatment and germination of seeds

Recent studies have revealed the association of micro-organisms with a variety of plants including those of Gramineae (Lange, 1966; Dunleavy and Urs, 1973; Mundt and Hinkle, 1976; Lewis and Crotty, 1977; Chakrabarti et al., 1978). It is not known whether these microorganisms should always be regarded as contaminants or whether associations may have a deeper biological significance. To effectively reduce the level of bacterial contamination without altering the germination of seeds, the latter were treated as follows: Seeds were thoroughly washed first with soap waterand then with distilled water and were allowed to remain in alcohol (50%) for 10 min. After this treatment, they were washed and soaked in 1% chlorine water for 5 min and washed again to remove all traces of chlorine (Mascarenhas et al., 1976). These seeds were soaked in distilled water for 5 - 6 h. The swollen seeds were then kept for germination on wet cotton in the dark. After 8 days, shoots (5 - 6 inches in length) were harvested about one inch above the surface to minimize bacterial contamination. These shoots were immediately frozen in liquid nitrogen and stored at -20°C till further use.

Extraction of unlabelled DNA

The DNA extraction procedure is a combination of that of Marmur (1961) and Ranjekar et al. (1976). The frozen shoots were homogenized in a Remi make blender at high speed in buffer I (0.5 M sucrose, 0.05 M tris, 0.05 M maleic acid and 0.003 M $CaCl_2$, pH 6.0) containing 0.1% Triton (X-100) for 90 seconds in the case of finger millet and pearl millet and 2 min in the

case of great millet and rice. The homogenate was filtered through four layers of cheese cloth and the filtrate centrifuged at 1000 x g for 10 min. The crude nuclear pellet was washed twice with buffer I, once with buffer II (1.0 M sucrose, 0.05 M tris, 0.05 M maleic acid and 0.003 M $CaCl_2$, pH 6.0) containing 0.1% triton (X-100) and then again washed once with buffer I, each washing involving homogenization of the pellet in the buffer and centrifugation at 1000 x g for 10 min. The washed pellet was suspended in saline - EDTA (0.15 M NaCl + 0.1 M sodium salt of EDTA, pH 8.0) solution and the DNA was extracted according to Marmur's procedure with slight modifications. The crude DNA dissolved in SSC (0.15 M NaCl and 0.015 M trisodium citrate, pH 7.0 abbreviated as SSC-X) was incubated with pretreated RNAase (RNAase was preheated at 80°C for 10 min to denature any DNAase present) at 37°C for one h. After the RNAase treatment, the DNA solution was deproteinized with chloroform-isoamyl alcohol (24:1 v/v) mixture and was precipitat from the aqueous layer by addition of 1.5 volumes of chilled alcohol. At least two deproteinizations were necessary for obtaining pure DNA preparations. From 1.0 kg of shoots, the yields of finger millet, great millet, pearl millet and rice DNAs were approximately 3 - 4 mg, 1 - 2 mg, 15 mg and 2.0 mg, respectively.

All the DNA preparations were routinely analyzed for their content in DNA, RNA and protein by diphenylamine assay (Burton, 1968), orcinol assay (Volkin and Cohn, 1954) and Lowry's assay (Lowry et al., 1951), respectively. Only those DNA preparations with RNA content of less than 1%

and protein content of less than 1 - 2% and 230 nm/260 nm optical density ratio of less than 0.45 were used for further experiments.

Extraction of labelled DNA

To obtain labelled DNA, a slightly modified procedure which was a combination of that of Bendich (1972), Mitra and Bhatia (1973) and Smith and Flavell (1974) was followed. The seeds of finger millet and pearl millet were sterilized as described in "Treatment and germination of seeds". The sterilized and soaked seeds were placed in sterile petri dishes each lined with Whatman No. 1 filter paper soaked in water. Approximately 50 seeds were placed in each petri dish. Thus 16 petri dishes of each species were prepared and were incubated in the dark at room temperature for various days. For example, after one day incubation, 5 ml of distilled water containing 50 μC and 100 μC ^{3}H - Thymidine (labelled in the 5 - CH_3 group, specific activity 23,000 cpm/umole) was added in two different petri dishes of the same species. These four petri dishes were further incubated for 7 days under the same conditions and then the shoots were removed carefully, washed repeatedly in running water and stored at -20°C. The second set was treated with ^{3}H-Thymidine after 3 days of incubation and was allowed to grow further for 5 days. Similarly, the doses were given on 4th, 5th, 6th and 7th day of incubation as well as 12 h and 6 h, before removing the shoots. Eight days old seedlings were used for DNA extraction from all the petri dishes. The isolation of labelled DNA was carried out by the same procedure as described for unlabelled DNA. The radioactivity was measured

in a Beckman Liquid Scintillation Counter (LS 100) using 5 ml of Dioxane containing PPO (0.5%) and Naphthalene (10%).

DNA shearing and sizing

The size of the DNA fragment, an important parameter in the study of reassociation kinetics, is controlled by shearing the DNA to small fragments. These small fragments give reproducible rates of reassociation and do not form large aggregates or network under the usual conditions for reassociation (Marmur et al., 1963).

DNA was fragmented using a sonic oscillator (Bronwill mode Biosonik III 250 W with 20KbZ) fitted with half an inch probe. Prior to sonication, nitrogen gas was bubbled through the DNA solution for 5 - 10 min (Walker and McLaren, 1965). Then, approximately, 30 ml of DNA solution was kept immersed in an icebath and sonication was carried out at maximum intensity for 3 min with 10 min interval for cooling between each successive minute of sonication. Care was taken to see that the temperature of the solution did not rise more than 4°C during sonication.

The average size of the DNA fragments after shearing was determined by sedimentation through neutral sucrose density gradients in a Beckman Preparatory Ultracentrifuge. Approximat 0.5 ml of DNA solution (100 - 200 µg/ml) was layered on sucrose gradients (5 - 25% sucrose in SSC-X) and was centrifuged at a speed of 34,000 rpm for 17 - 18 h (McConckey, 1967). From the sedimentation patterns, average sedimentation coefficient 'S' values were determined using McEwen's tables (McEwen, 1967). The molecular weight was then calculated using the formula:

$$S^{Sucrose}_{20,w} = KM^{a}$$

where K = a constant 0.047
a = a constant 0.38
for SSC-X

(Van der Schans et al., 1969). The estimated molecular weight of DNA fragments was in the range of 500 - 600 np (Fig. 2.1).

DNA reassociation studies

The slurry of HA was poured into a double jacketed, glass column (15 x 2.5 cm) to a bed volume of 15 ml. The column was equilibrated with 0.12 M PB (pH 6.8) and brought to 62°C by connecting it to a constant temperature water bath equipped with a circulating pump.

Reassociation of four cereal DNAs was carried out according to the method described by Britten et al. (1974) using HA column chromatography (Kohne and Britten, 1971). Sonicated DNA was dialyzed against 0.12 M PB (pH 6.8) and the final DNA concentration determined by absorbance at 260 nm. A small aliquot of each DNA in 0.12 M PB (pH 6.8) was run through HA column operating at 62°C to determine what percent (if any) was incapable of binding at this temperature and this value was corrected for subsequent calculations. The DNA solution was denatured by heating in a water bath at 100°C for 10 min and then incubated at 62°C for a specific time to achieve desired Cot values. Cot value was defined as the half product of absorbance of dissociated DNA x h(s) of incubation (Cot = A/2 x t (h)) (Britten and Kohne, 1968; Kohne and Britten, 1971). Thus the Cot value of one is equivalent to incubation of a DNA solution

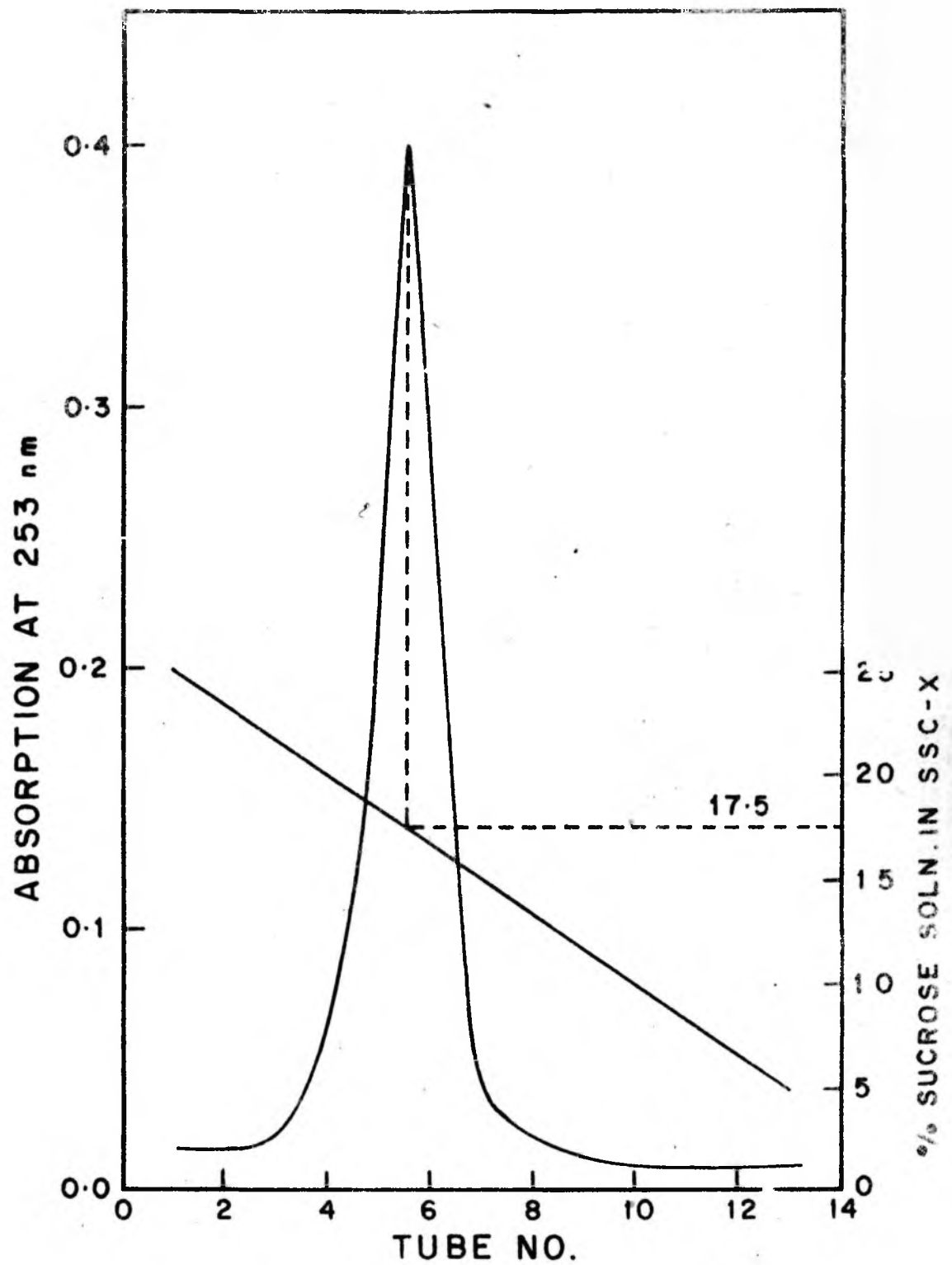


Fig. 2.1: Graph representing the concentration of sucrose corresponding to maximum absorption of DNA sheared as described in Materials and Methods and centrifuged on neutral sucrose density gradient.

of concentration 83 µg/ml (approximately 2 O.D.) for one h (Britten and Kohne, 1968). The incubated DNA was transferred to the HA column maintained at 62°C. After adsorption of the DNA solution at the top of the column, 0.12 M PB (pH 6.8, 62°C) was passed through in order to elute the single stranded or denatured DNA. The double stranded (reassociated) DNA was then removed with 0.4 M PB (pH 6.8). The optical density of the fractions was measured in a Beckman spectrophotometer (model 25). The percentage of reassociation was calculated using the formula:

$$\% \text{ reassociation} = \frac{B}{A + B} \times 100$$

where A = micrograms of DNA eluted with 0.12 M PB

B = micrograms of DNA eluted with 0.4 M PB

(Ranjekar and Murthy, 1973). In experiments at high Cot values (more than 1 x 10^2 mol x sec/l) the DNA solution was incubated in 0.36 M PB (pH 6.8) in order to decrease the incubation time. At this salt concentration, the reassociation rate increases by a factor 4.7778; hence the corresponding correction was applied to the reassociation rate (Britten et al., 1974). The percent DNA reassociated was then plotted against Cot on a semilogarithmic paper. The reassociation studies of E.coli and calf thymus DNA were carried out under our experimental conditions and wereused as standards throughout this work.

RESULTS

The wavelength scanning of the four cereal DNAs was carried out in the range of 225 nm to 300 nm. The absorbance of these

DNA preparations at 300 nm was always less than 0.1 and 230/260 and 280/260 O.D. ratios were found to be 0.45 and 0.55, respectively. Figure 2.2 represents the typical absorption spectrum for pearl millet DNA.

The results from the experiments to obtain labelled DNA of finger millet and pearl millet were not satisfactory. The specific activities for finger millet and pearl millet DNAs were in the range of 8 to 46 cpm/µg and 1.6 to 8.23 cpm/µg DNA, respectively. The maximum specific activity (46 cpm/µg) was obtained in the case of finger millet on 2 days of incubation after feeding tritiated Thymidine, which itself was very low to carry out further experiments. Hence no labelled DNA was used in DNA reassociation kinetics studies.

Reassociation kinetics of four cereal DNAs

The reassociation kinetics of the sonicated DNAs (550 **np**) of finger millet, great millet, pearl millet and rice were studied over a wide Cot range of 10^{-1} to 10^{4} mol x sec/l. The reassociation of sonicated **E.** coli DNA as well as of calf thymus DNA were also carried out under identical experimental conditions. E. coli DNA reassociated in a rather narrow Cot range of 10^{-1} to 10^{2} mol x sec/l. On the other hand, the reassociation of calf thymus DNA spread over a wide Cot range of 10^{-1} to 10^{4} mol x sec/l (Fig. 2.3). Since no labelled DNAs were used in these studies, the lowest Cot value was of the order of 10^{-1} mol x sec/l.

(a) Finger millet

In the case of finger millet Cot curve (Fig. 2.4), the solid curve represents the complete experimental reassociation

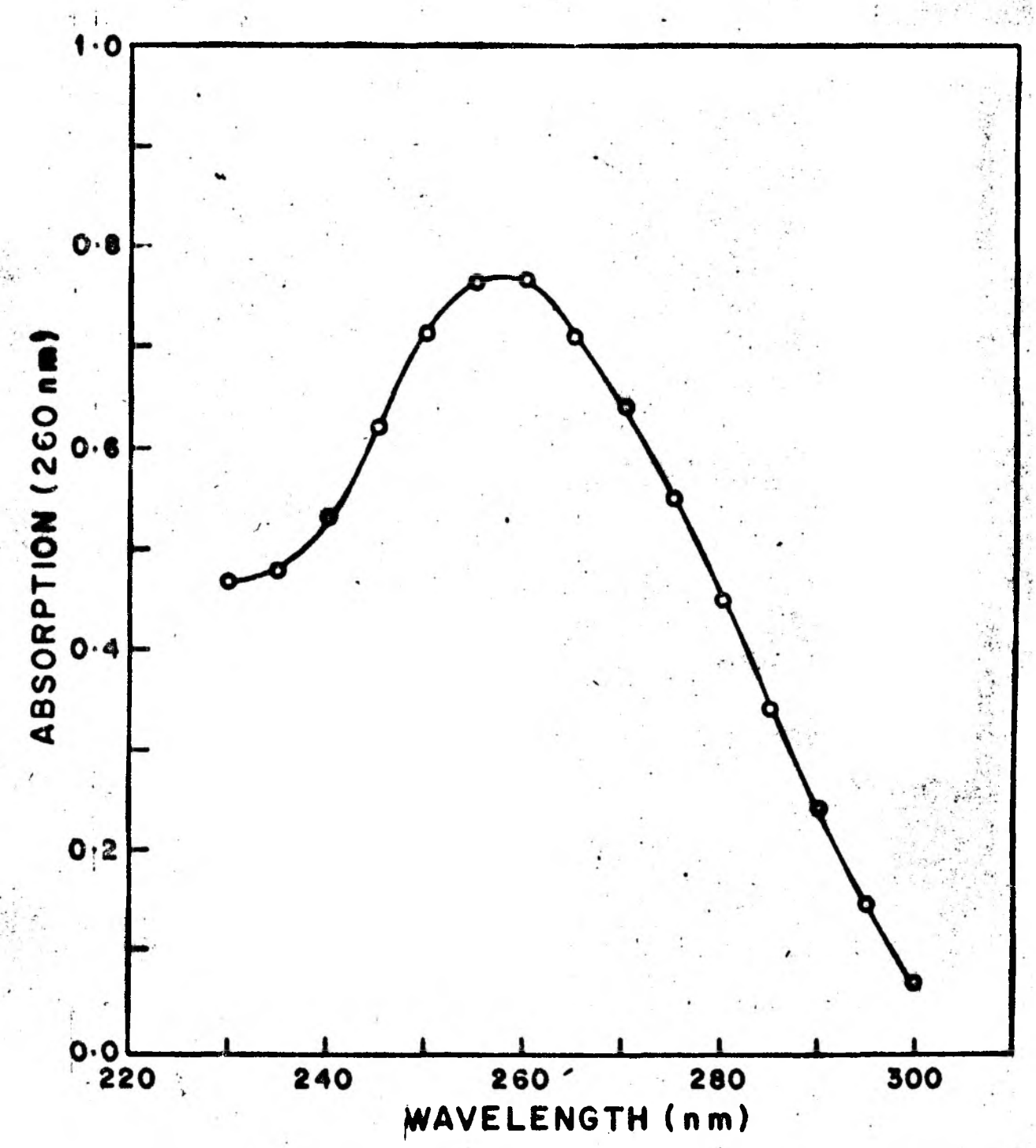


Fig. 2.2: Absorption spectrum of DNA prepared from pearl millet. DNA was dissolved in 0.12 M PB (pH 6.8).

curve of 550 np long DNA. The least square analysis of the reassociation curve was carried out by employing a standard optimization subroutine STEPIT (subroutine STEPIT written by J.P.Chandler and distributed by QCPE, Indiana University, Bloomington, Indiana, USA), and is shown by dashed curve through the data points. The error is given by the following equation:

$$\text{Error} = \sum \left\{ \left(\frac{C}{CO}\right)_{\text{expt}} - \left(\frac{C}{CO}\right)_{\text{fit}} \right\} \qquad \ldots 1$$

and was minimized by a direct search procedure. The form for $\frac{C}{CO}$ was assumed to be

$$\frac{C}{CO} = T + \sum \frac{F_i}{1 + K_i \, Cot} \qquad \ldots 2$$

where $\frac{C}{CO}$ is the fraction denatured, T is the fraction of the genome failed to reassociate, F_i is the fraction of the genome reassociating in the ith component and K_i is the reassociation rate for the ith component. These parameters T, F_i and K_i are all freely floated so as to minimize the error in equation 1. It is observed that the method converages quite well with wide starting choices for the parameters (root mean square 0.0261). This analysis of the data reveals two kinetic components and the lower dashed curves in Fig. 2.4 represent the predicted reassociation kinetics of the individual components if they existed alone. The proportion of the fast fraction, which included DNA sequence forming duplexes upto an approximate Cot

value of 25 mol x sec/l was 49%. The reassociation pattern of 17% of the genome reannealing before Cot 0.1 mol x sec/l is unknown. When the average Cot 1/2 values of the fast and slow reassociating DNA fractions were compared, it was observed that the former DNA fraction consisted of nucleotide sequences reassociating 160 times more rapidly than that of the latter DNA fraction. This observation reveals the presence of repetitive DNA sequences in the fast reassociating DNA fraction and mostly the non-repetitive DNA sequences in the latter DNA fraction. About 8.5% of the genome fails to reassociate at Cot 10,000 mol x sec/l and may be due to degradation or failure of some sequences to form stable duplexes at the criterion used for these experiments.

(b) Great millet

The reassociation curve of great millet DNA (Fig. 2.5) also reveals two kinetic components: fast reassociating DNA fraction including DNA sequences forming duplexes by Cot 50.0 mol x sec/l with Cot 1/2 as 6.5×10^{-1} mol x sec/l and slow reassociating DNA fraction reannealing after Cot 50.0 mol x sec/l with Cot 1/2 as 1000 mol x sec/l. The DNA sequences in the fast reassociating fraction, therefore, renature approximately 1530 times faster than those in the slow reassociating DNA fraction and are considered to be repetitive. Due to the poor yield of DNA in the case of great millet, a limited number of experiments were carried out in the Cot range of 10^{-1} to 10^{3} mol x sec/l. Only 70% of the DNA reassociated by Cot 10^{3} mol x sec/l and hence computer curve fitting by nonlinear least square regression analysis of the reassociation curve was not carried out.

(c) Pearl millet

Figure 2.6 represents the reassociation curve of pearl millet DNA. As described in finger millet, the solid line is the experimental curve and the dashed line through the data points is the computer fit with root mean square 0.036. The predicted reassociation of the individual components is shown by lower dashed curves. There are two distinct kinetic components in pearl millet DNA. Fast reassociating component (upto Cot 10 mol x sec/l) comprising 54% of the total genome reassociates 1180 times faster as compared to the slow reassociating fraction with an observed second order rate constant of 0.00045 mol^{-1}. sec^{-1}l. In the fast reassociating fraction of pearl millet genome, 20% fraction reassociates too rapidly to resolve (Cot< 0.1 mol x sec/l) while approximately 10% of the genome fails to reassociate.

(d) Rice

The Cot curve of rice DNA (Fig. 2.7), unlike those of finger millet, great millet and pearl millet, reveals the presence of three kinetic fractions. The rapidly reassociating DNA fraction, which included DNA sequences reassociating upto a Cot value of 1.0 mol x sec/l represents 27% of the total DNA. The nucleotide sequences forming duplexes in the Cot range of 1 to 50 mol x sec/l are referred to as intermediately reassociating and these account for an additional 25% of the total DNA. The DNA sequences present in these two fractions are considered to be repetitive, since the Cot 1/2 values of the rapidly and intermediately reassociating DNAs are 26,000 times and 150 times, respectively, less as compared to that of slow reassociating

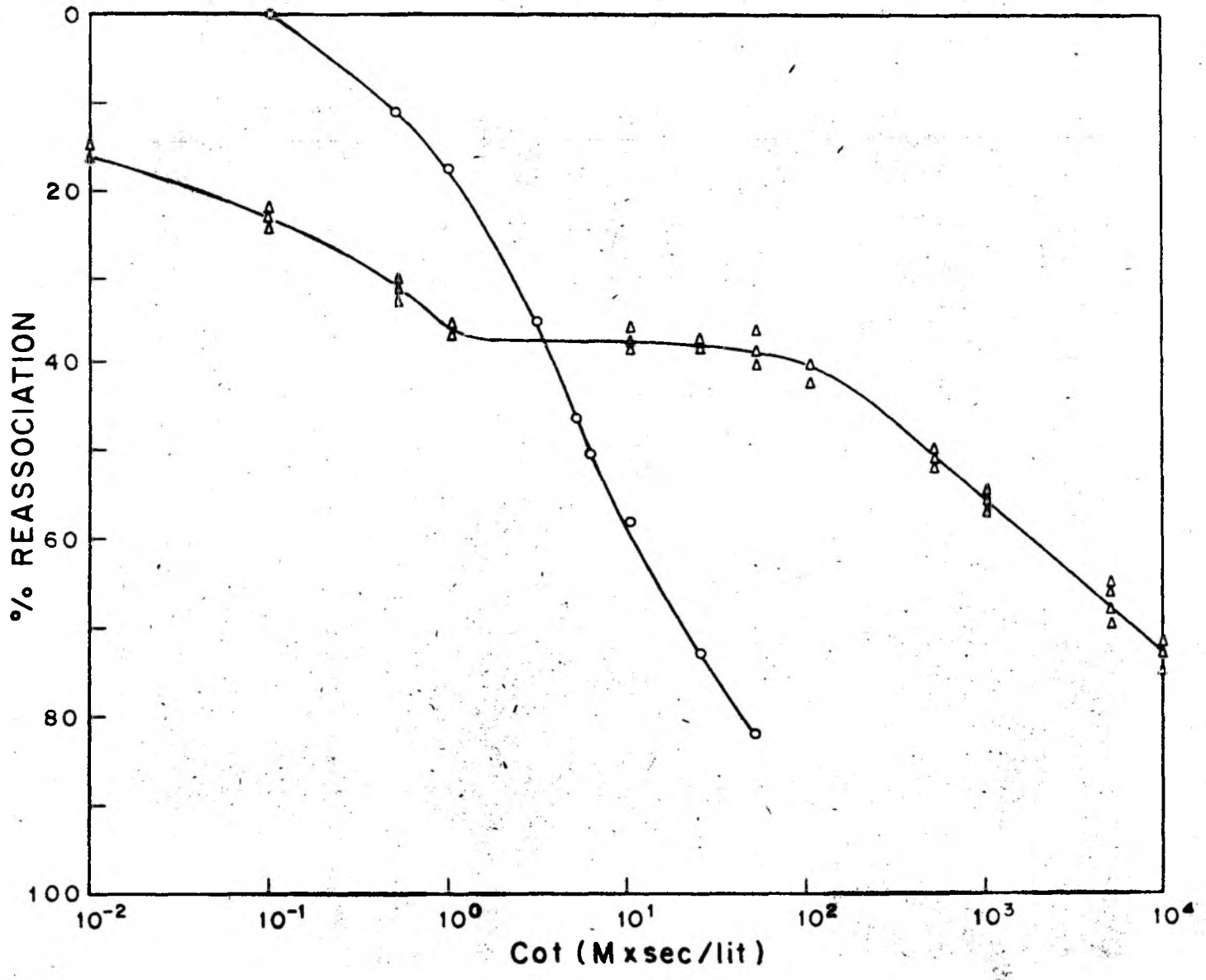


Fig. 2.3: Reassociation curve of E. coli and calf thymus DNA of length 550 np. (-o-o-) E. coli and (-Δ-Δ-), calf thymus.

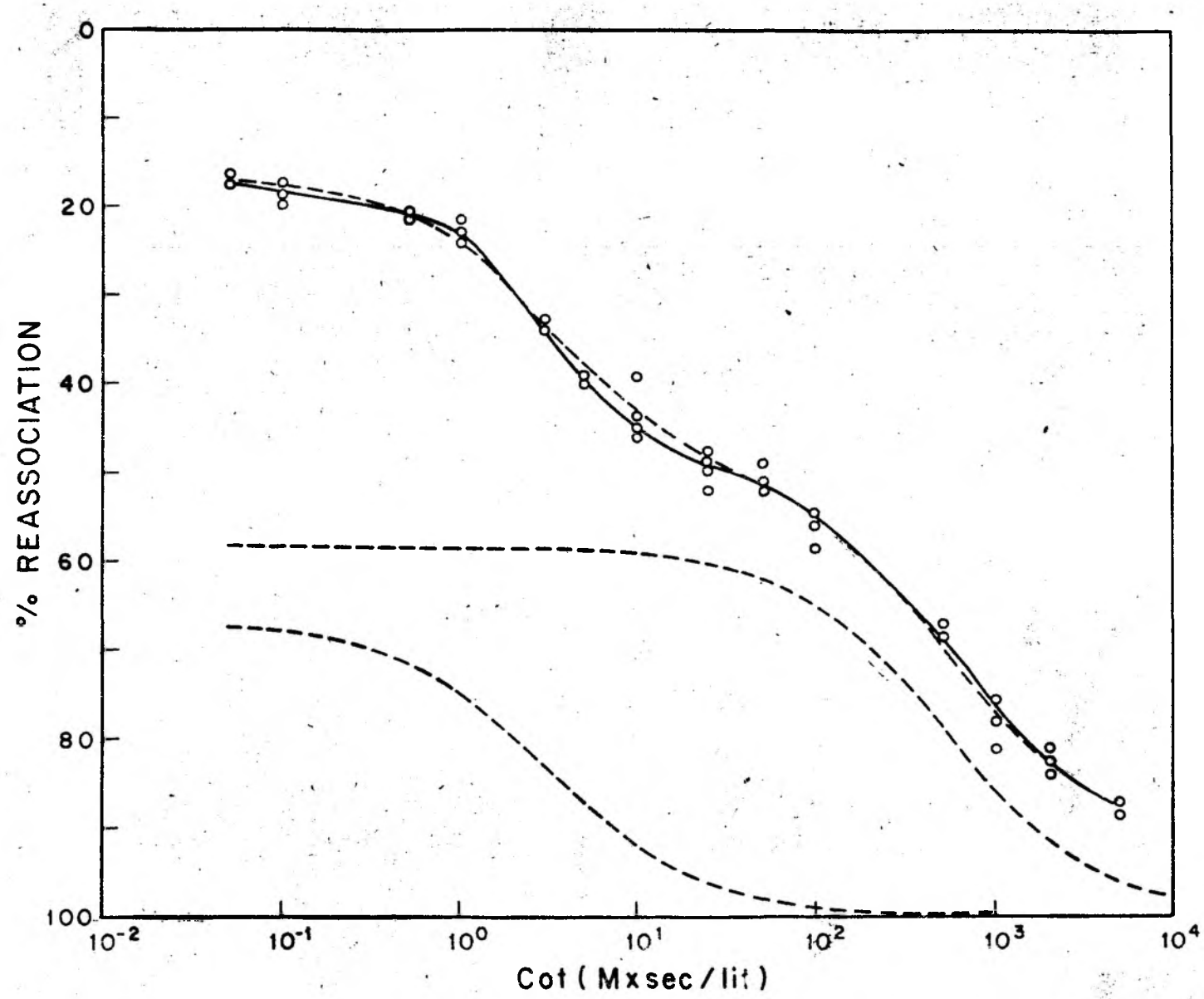


Fig. 2.4: Reassociation curve of finger millet DNA of length 550 np. The solid line represents the experimental curve and the dashed line through data points presents the least square fit for two component system allowin all the parameters to free float (rms 0. 0261). The lower dashed curves show reassociation kinetics of pi components.

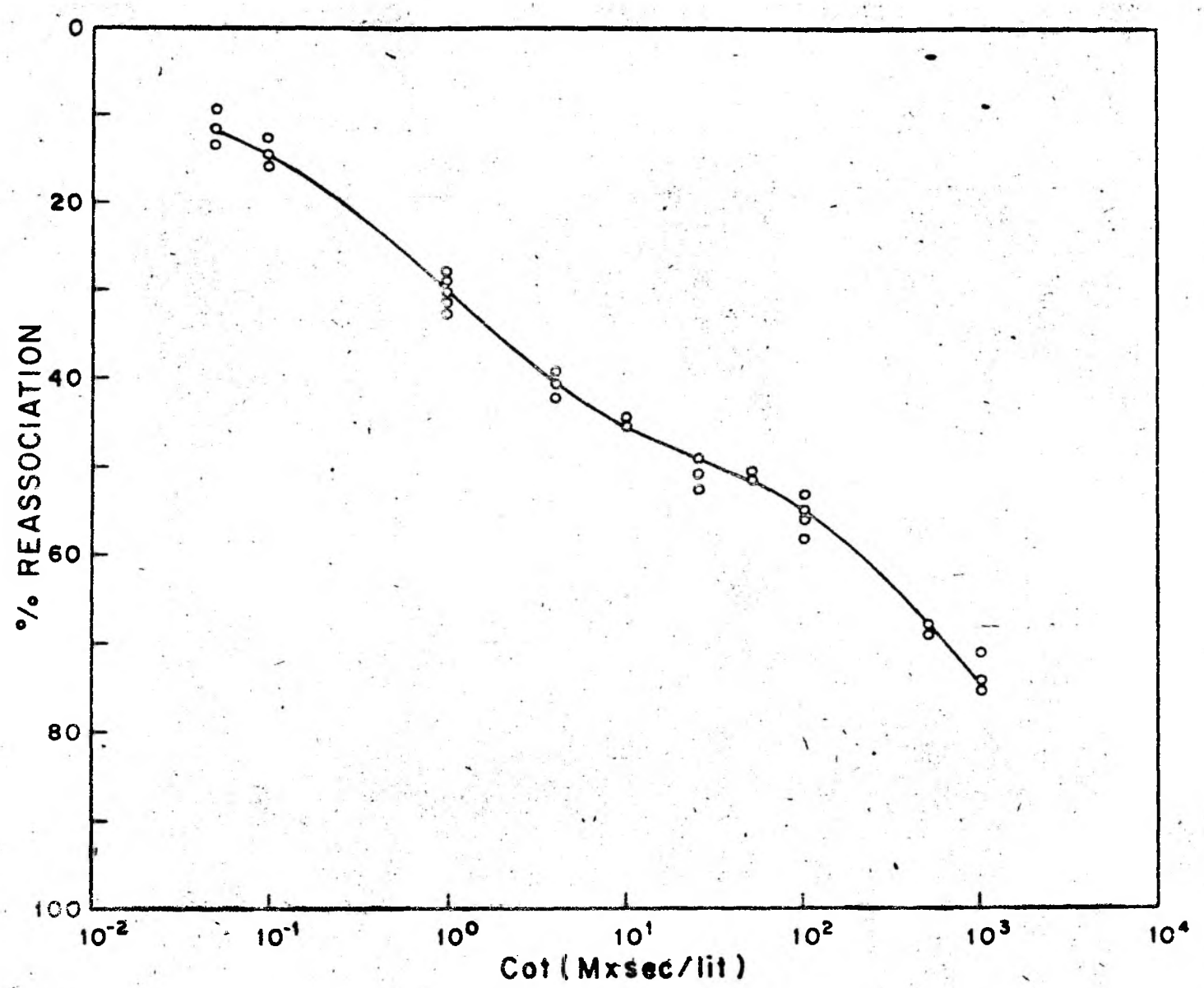


Fig. 2.5: Reassociation curve of great millet DNA of length 550 np.

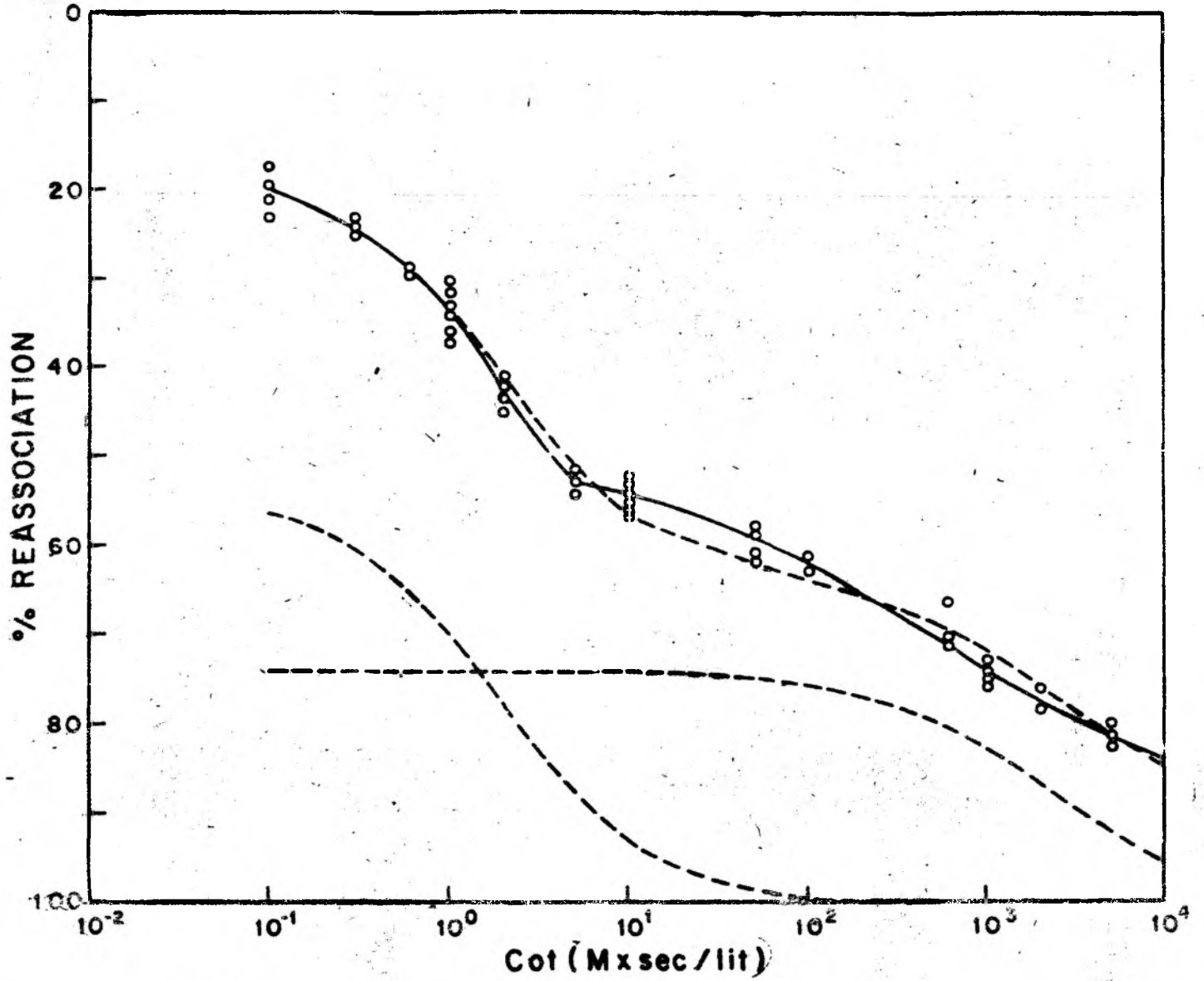


Fig. 2.6: Reassociation curve of pearl millet DNA of length 550 np. Solid line is the experimental curve, dashed line through the data points is computer fit with rms 0.036 and the lower dashed curves are the predicted reassociation curv of individual components.

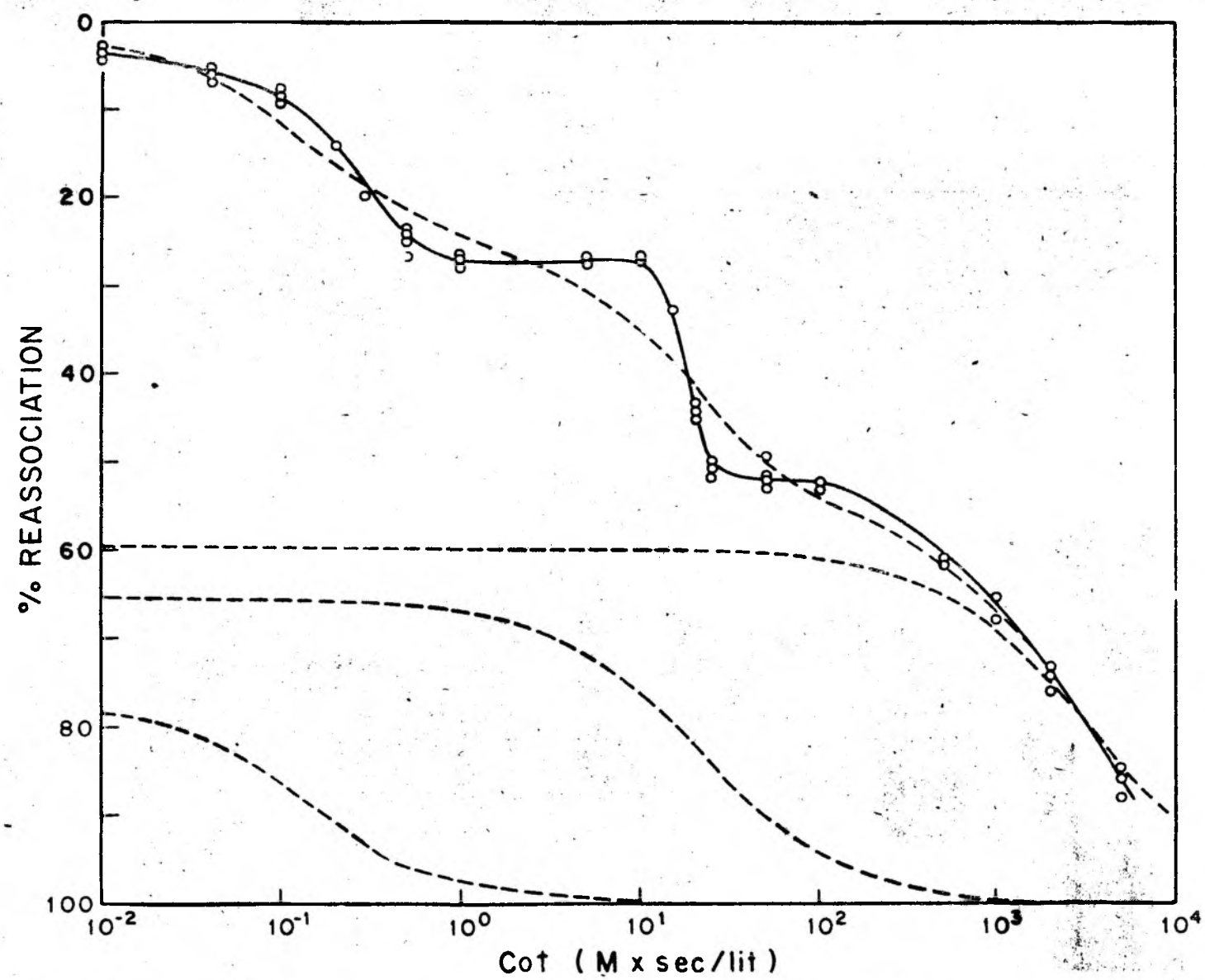


Fig. 2.7: Reassociation curve of rice DNA of length 550 np. The dashed line through the data points is the computer fit with rms 0.0454. The solid curve is the experimental curve. The lower dashed lines represent the predicted reassociation curves of pure components.

DNA fraction. Thus the proportion of the repetitive DNA in rice, is 52% of the total DNA. In the case of rice, as observed from Fig. 2.7, no computer fit is obtained for the DNA portion reassociating in the Cot range of 1.0 to 50.0 mol x sec/l, indicating thereby that this DNA is not reannealing with second order kinetics. Smith and Flavell (1977) have observed in rye that some repeated sequences do not reassociate with second order kinetics due to differences in their reiteration frequencies.

The fast reassociating DNA fractions in finger millet, great millet and pearl millet genomes which mainly consist of repetitive DNA sequences are further classified into three subfractions, namely very rapidly reassociating (upto Cot 0.1 mol x sec/l), rapidly reassociating (Cot 0.1 to 1.0 mol x sec/l) and intermediately reassociating (Cot 1.0 to 25.0 mol x sec/l in the case of finger millet, Cot 1.0 to 50 mol x sec/l in the case of great millet and Cot 1.0 to 10.0 mol x sec/l in the case of pearl millet) fractions. The fast reassociating fraction of rice DNA is also arbitrarily subdivided into very rapidly reassociating (upto Cot 0.1 mol x sec/l) and rapidly reassociating (between Cot 0.1 to 1.0 mol x sec/l) fractions. The proportion, the Cot 1/2 value, the frequency of repetition and the kinetic complexity of these repetitive DNA fractions are given in Table 2.1.

Haploid genome size in four Gramineae species

The Cot 1/2 of a DNA is directly proportional to genome size assuming that all the DNA sequences are nonrepetitive in character (Britten and Kohne, 1966). In eukaryotes, the haploid genome size is determined by comparing the Cot 1/2 of

Table 2.1: Kinetic complexity of repetitive DNA in four Gramineae species

Species	Fraction of DNA[a]	Cot 1/2 values observed[a] (mol x sec/l)	Frequency of repetition[b]	Cot 1/2 values pure[c] (mol x sec/l)	Kinetic complexity[d]	
					Daltons	nucleotide pairs
1	2	3	4	5	6	7
Finger millet						
Very rapidly reassociating DNA (Cot < 0.1 mol x sec/l)	0.17	-	-	-	-	-
Rapidly reassociating DNA (Cot 0.1 to 1.0 mol x sec/l)	0.07	3.3×10^{-1}	1.5×10^{3}	2.3×10^{-2}	1.1×10^{7}	1.7×10^{4}
Intermediately reassociating DNA (Cot 1.0 to 25.0 mol x sec/l)	0.25	3.7×10^{0}	1.4×10^{2}	9.3×10^{-1}	4.6×10^{8}	7.0×10^{5}
Great millet						
Very rapidly reassociating DNA (Cot < 0.1 mol x sec/l)	0.15	-	-	-	-	-
Rapidly reassociating DNA (Cot 0.1 to 1.0 mol x sec/l)	0.14	3.7×10^{-1}	2.7×10^{3}	5.2×10^{-2}	2.6×10^{7}	3.9×10^{4}

1	2	3	4	5	6	7
ıtermediately eassociating DNA]ot 1.0 to 50.0 ›l x sec/l)	0.23	4.0×10^{0}	2.5×10^{2}	9.2×10^{-1}	4.5×10^{8}	6.9×10^{5}
earl millet						
ry rapidly associating DNA ot < 0.1 mol x sec/l)	0.20	-	-	-	-	-
pidly reassociating A (Cot 0.1 to 1.0 l x sec/l)	0.13	4.5×10^{-1}	4.9×10^{3}	5.8×10^{-2}	2.8×10^{7}	4.3×10^{4}
termediately associating DNA ot 1.0 to 10.0 l x sec/l)	0.21	2.1×10^{0}	1.0×10^{3}	4.4×10^{-1}	2.2×10^{8}	3.3×10^{5}
ce						
ry rapidly associating DNA ot < 0.1 mol x c/l)	0.085	1.6×10^{-2}	2.0×10^{5}	1.4×10^{-3}	6.9×10^{5}	1.05×10^{3}
pidly reassociating A (Cot 0.1 to 1.0 l x sec/l)	0.185	2.8×10^{-1}	1.2×10^{4}	5.2×10^{-2}	2.5×10^{7}	3.9×10^{4}

1	2	3	4	5	6	7
termediately associating DNA ot 1.0 to 50.0 l x sec/l)	0.25	1.7×10^{1}	1.9×10^{2}	4.2×10^{0}	2.1×10^{9}	3.2×10^{6}

alues obtained from the respective Cot curves.

ot 1/2 values of unique DNA as described in Table 2.2 divided by Cot 1/2 values of given raction.

ot 1/2 values observed x fraction of the genome.

alues obtained from the standard relationship for E. coli DNA where Cot 1/2 is 6 mol x seo/l nd the number of nucleotide pairs is 4.5×10^{6} under our experimental conditions.

the unique DNA with that of E. coli DNA which is known to consist of nonrepeated DNA sequences (Britten and Kohne, 1966). In the latter, the Cot 1/2 value is 6.0 mol x sec/l under our experimental conditions and the number of nucleotide pairs present is 4.5×10^6 (Cairns, 1963). The Cot 1/2 of the slow reassociating DNA in all the four cereal species is determined and corrected for repeated DNA sequences. The kinetic complexities of these four species are then calculated by comparing these corrected Cot 1/2 values with that of E. coli DNA as given in Table 2.2. Thus the kinetic complexities for finger millet, great millet, pearl millet and rice DNA are 1.6×10^8, 3.6×10^8, 5.9×10^8 and 1.2×10^9 np, respectively. The haploid DNA content as summarized in Table 2.2 is obtained in the range of 0.17 to 1.31 pg from the kinetic complexity using the relationship of 1 pg = 9.13×10^8 nucleotide pairs (Laird, 1971).

DISCUSSION

Yield of DNAs

Among the four cereal species, pearl millet was the best species to obtain DNA in sufficient yields. Getting pure DNA i sufficient amounts from finger millet, great millet and rice was an extremely difficult task. We tried different extraction procedures including those of Bendich and Bolton (1967) and Flavell et al. (1974). However, all the methods gave low DNA yields in case of finger millet, great millet and rice. In plants, the two important difficulties in DNA extraction are resistance of cell wall towards its breakage and very high DNAase activity (Stern, 1968). In addition to this, the aerial

Table 2.2: Kinetic complexity and DNA content in four Gramineae species

Species	Fraction of DNA[a]	Cot 1/2 values observed[a] mol x sec/l	Cot 1/2 values pure[b] mol x sec/l	Kinetic complexity[c] Daltons	Kinetic complexity[c] Nucleotide pairs	DNA content[d] (1C pg)	DNA content[e] (1C pg)
Finger millet							
Slow reassociating DNA (Cot > 25 mol x sec/l)	0.42	500	210	1.0×10^{11}	1.6×10^{8}	0.17	1.6
Great millet							
Slow reassociating DNA (Cot > 50 mol x sec/l)	0.48	1000	480	2.3×10^{11}	3.6×10^{8}	0.39	3.0 - 7.0
Pearl millet							
Slow reassociating DNA (Cot > 10 mol x sec/l)	0.36	2200	792	3.9×10^{11}	5.9×10^{8}	0.64	2.5
Rice							
Slow reassociating DNA (Cot > 50 mol x sec/l)	0.48	3300	1584	7.9×10^{11}	1.2×10^{9}	1.31	0.6

[a] Values obtained from the respective Cot curves. [b] Cot 1/2 values observed x fraction of the genome.

[c] Values obtained from standard relationship of *E. coli* DNA where Cot 1/2 is 6 mol x sec/l and the number of nucleotide pairs present is 4.5×10^{6}.

[d] Values obtained from kinetic complexity using the relationship of 1 pg = 9.12×10^{8} np.

[e] Cytophotometric estimations of DNA content (Bennett and Smith, 1976).

tissue does not become crisp even after freezing it in liquid nitrogen, hence crushing the tissue in sucrose buffer I to make a paste was very difficult. These factors may be responsible for getting low DNA yields in these Gramineae species. Another important reason can be the low DNA content of these species. According to Bennett and Smith (1976), the cytophotometric genome size estimates (1C) in finger millet, pearl millet and rice are 1.6 pg, 2.5 pg and 0.6 pg, respectively. Though the DNA content in great millet is not very low (1C content 3.0 - 7.0 pg), the yield of DNA in great millet was also very low. Due to this problem, numerous extractions were required in the case of finger millet, great millet and rice. In fact, in great millet the problem of DNA yield was so serious that we were able to carry out only reassociation kinetics experiments.

The DNAs of these Gramineae species were isolated from nuclear pellets instead of total tissue homogenates, thus minimizing the possibility of contamination by chloroplast or mitochondrial DNAs. Further, precautions were taken against bacterial contamination by carrying out surface sterilization of seeds prior to germination and by using only the shoots for DNA extraction. It can, therefore, be concluded that different repetitive fractions observed in finger millet, great millet, pearl millet and rice are of mainly plant nuclear origin.

DNA labelling

Various experiments were tried to get labelled DNA from finger millet and pearl millet as explained in Materials and Methods. But there was no incorporation of ^{3}H - Thymidine in the cereal DNAs. Bendich (1972), Mitra and Bhatia (1973) and

Smith and Favell (1974) were successful in getting labelled DNA from a few Gramineae species such as wheat, oat, rye and barley with specific activities in the range of 8,000 - 13,000 cpm/µg DNA. Compared to their results, the specific activities obtained by us (maximum 46 cpm/µg) in finger millet and pearl millet were negligible. Due to the poor yields of DNA, no experiments were attempted to lable great millet and rice DNAs. The possible reason of less incorporation of label in plant DNAs can be the effective competition of bacteria on the roots with plant seedlings for the limited supply of labelled precursor (Barber, 1966). Contamination of labelled bacterial DNA was also observed by Lonberg-Holm (1967) in lettuce and radish DNA and in watermelon by Hock (1967). However, in our case, the chances of this possibility were very less for two reasons. First, we took utmost care to avoid bacterial contamination and secondly, we used ^{3}H - Thymidine as a labelled precursor on account of the fact that most bacteria do not readily take up nucleosides whereas phosphate uptake is efficient (Bodmer and Grether, 1965). Though there are no possible convincing reasons for such a low specific activity observed in finger millet and pearl millet, similar difficulties are reporte elsewhere also (Wimpee and Rawson, 1979). Besides this method, there are some other methods to label DNA, such as iodination of DNA *in vitro* (Houck *et al.*, 1978) and nick translation (Maniatis *et al.*, 1975; McGregor and Mizuno, 1976). However, due to some technical difficulties, we were unable to carry out these experiments.

Conditions for DNA reassociation kinetics

Throughout our work on DNA reassociation, maximum care was taken to maintain the proper conditions of reassociation. In order to achieve reproducible reassociation reactions, the cation concentration, temperature of incubation, DNA concentration and DNA fragment size must be controlled. Effects of different parameters are discussed by Marmur et al. (1963), Britten and Kohne (1966), Subirana and Doty (1966), Thrower and Peacock (1966) and Wetmur and Davidson (1968). For efficient reassociation, we used salt concentration of DNA as 0.18 M Na^+ and the temperature of incubation as 62°C. Same temperature was maintained throughout the HA column chromatography also. Sonicated DNAs of fragment size 550 np were used in these studies to avoid overestimations. Using these parameters for optimum reassociation, we studied the reassociation kinetics of E. coli and calf thymus DNA as standards. The Cot 1/2 of E. coli DNA obtained by us (6 mol x sec/l) is in reasonable agreement to the values reported in literature (Britten and Kohne, 1966; Miksche and Hotta, 1973; Rawson, 1975). Further, the reassociation data of bovine DNA are comparable with that of Britten and Kohne (1968). The DNA reassociation values at various Cots in our work compare well with those of Britten and Kohne (1968). There is slight lowering in the extent of DNA reassociation after Cot 1000. In addition, our estimate of the proportion of repetitive DNA (40%) in bovine compares well with that of Britten and Kohne (1966; 1968) and Votavova et al.(1972; 1973). Due to the close similarity of the reassociation patterns of bovine DNA with that of others,

bovine DNA was considered as a second standard in our work.

Repetitive DNA content in Gramineae

The DNA reassociation kinetics of the four Gramineae species have provided an estimate of repetitive DNA which is in the range of 49 - 54% of the total genome. The limiting Cot values used to determine percent of repetitive DNA are Cot 25 mol x sec/l and Cot 10 mol x sec/l in the case of fing millet and pearl millet, respectively and Cot 50 mol x sec/l in the case of great millet and rice. These values were confirmed by obtaining the closefit between the computer fit for second order kinetics and experimental curve in the case finger millet, pearl millet and rice, as described in Results In other Gramineae species studied so far such as wheat, oat, and barley, the Cot values used to determine the proportion o repetitive DNA were either Cot 50 or Cot 100 mol x sec/l (Mit and Bhatia, 1973; Smith and Flavell, 1974 and Ranjekar et al 1974; 1976). In the case of great millet, though there is no computer analysis of closefit, the sudden change in the reassociation rate at Cot 50 mol x sec/l made us to assume Cot 50 mol x sec/l as the limiting value.

In the case of pearl millet, we took Cot 10 mol x sec/l as the limiting value to determine the repetitive DNA content Wimpee and Rawson (1979) have also used the same Cot value to calculate repetitive DNA percentage. However, the proportion repetitive DNA sequences (54%) reannealing by Cot 10 mol x sec/l obtained by us is somewhat lower than that (69%) reported by Wimpee and Rawson (1979) under identical Cot conditions. There can be a number of reasons for such a discrepancy. First, we

have isolated the pearl millet DNA from the nuclear preparations as described in Materials and Methods. The advantage of isolating the DNA from the nuclei is that the contamination by the rapidly reassociating DNAs from the other organelles such as chloroplast and mitochondria is eliminated. Secondly, there can be some seed variety differences. Smith and Flavell (1974), for example, have obtained the values of 83% and 92% as against 76% and 75% of Ranjekar et al. (1974; 1976) for the proportion of repetitive DNA in wheat and rye, respectively. We conclude, therefore, that the repetitive DNA proportion may vary in the range of 54 to 69% in pearl millet.

The proportion of repetitive DNA in finger millet, great millet, pearl millet and rice, when compared to the other Gramineae species studied so far (Smith and Flavell, 1974; 1975; Ranjekar et al., 1974; 1976; Flavell and Smith, 1975) appears to be rather low. In general the proportion of repetitive DNA is more than 75% in those Gramineae species and in a few cases, such as Poa annua (Flavell and Smith, 1975) and rye (Smith and Flavell, 1974), it has reached to even 90%. In this respect, the four Gramineae species under our considerations differ significantly from the other Gramineae species.

Classification of repeated DNA sequences

We have used the terms such as very rapidly reassociating for DNA sequences reannealing before Cot 10^{-1} mol x sec/l, rapidly reassociating for sequences reannealing in the Cot range of 10^{-1} - 1.0 mol x sec/l and intermediately reassociating which includes DNA sequences forming duplexes in the Cot range of 1.0 to their respective limiting Cot values (Table 2.1).

As explained above each of these three classes of repeated DNA sequences does not necessarily represent a single homogeneous family and the estimated values of frequency of repetition and kinetic complexity of each class must be considered as average one.

The proportion of very rapidly reassociating DNA is high (15 - 20%) in the case of finger millet, great millet and pearl millet and low (8.5%) in the case of rice. The proportion of DNA sequences forming duplexes in the range of Cot 0.1 to 1.0 mol x sec/l is 13 - 18.5% in the case of great millet, pearl millet and rice and comparatively low (7%) in the case of the finger millet. Table 2.1 describes the proportion of DNA sequences reassociating at various Cot values. From this table it is possible to comment upon the interspecies differences in the content of different types of repetitive DNA sequences. For example, finger millet and rice have the maximum proportion (25%) of intermediately reassociating DNA and finger millet has minimum proportion (7%) of rapidly reassociating DNA. Similarly, pearl millet shows maximum proportion (20%) of very rapidly reassociating DNA. In general, the proportions of the various DNA fractions among the three Gramineae species are different from each other and appear to be species specific.

The frequency of repetition of different classes of repetitive DNA varies in these four Gramineae species. For example, the frequency of repetition of rapidly reassociating and intermediately reassociating DNA sequences is in the range of 1.5×10^3 to 1.2×10^4 and 1.4×10^2 to 1.0×10^3, respectively. The kinetic complexities of these fractions are

in the range of 1.7×10^4 to 4.3×10^4 np and 3.3×10^5 to 3.2×10^6 np, respectively. The frequency of repetition of the rapidly reassociating DNA fractions is thus 5 to 100 fold more than that of the intermediately reassociating fraction; while the kinetic complexity of the intermediately reassociating fraction is 10 to 100 fold more as compared to that of rapidly reassociating DNA fraction. These values compare with those reported for some other plant species such as wheat (Smith and Flavell, 1973), soybean (Goldberg, 1978; Gurley et al., 1979) and pearl millet (Wimpee and Rawson, 1979).

Comparison between kinetic and cytophotometric genome size estimates

Apart from furnishing information about the repetitive DNA content, the DNA reassociation kinetics of these four Gramineae species have also enabled us to estimate the respective haploid genome sizes from the Cot 1/2 value of unique or nonrepetitive DNA fraction. The haploid genome size is found to vary in the range of 1.6×10^8 to 1.2×10^9 np or 0.17 pg to 1.31 pg (Table 2.2). The cytophotometric genome size estimates (1C) in finger millet, pearl millet and rice are 1.6 pg, 2.5 pg and 0.6 pg, respectively and for great millet it is in the range of 3.0 to 7.0 pg (Bennett and Smith, 1976). The kinetic genome size estimates in the case of finger millet, great millet and pearl millet are lower than those obtained by cytophotometry. In the case of rice the kinetic genome size estimate is higher than the cytophotometric estimate. In plants, relationship between analytical DNA content and genome size estimate is not very straightforward. In tobacco

(Zimmerman and Goldberg, 1977), for example, the DNA content value (1.65 pg) obtained from reassociation analysis differs significantly from that of analytical DNA content (10 pg). Similar discrepancies have also been observed in other plant species such as cotton (Walbot and Dure, 1976), broad bean (Gnucheva et al., 1977), pea (Pearson et al.,1978b) and pearl millet (Wimpee and Rawson, 1979). There can be a number of explanations for such discrepancies in the genome size estimat One explanation is that truly unique sequences are not present in these species. For example, the genome of Vicia faba (Gnucheva et al., 1977) contains 60 identical copies of the so-called unique DNA sequences whereas Vicia sativa contains four identical copies (Gnucheva et al., 1977). Certain inaccuracies in the Cot 1/2 determination of unique DNA may also lead to erroneous estimation of genome size. According to Kemp and Merlo (1975) and Merlo and Kemp (1976), factors capable of accelerating the rate of reassociation may be prese in plant DNA preparations. The kinetic genome size estimates of the four Gramineae species can, therefore, be considered as approximate ones.

CONCLUDING REMARKS

The repetitive DNA contents estimated by nonlinear least square regression analysis are in the range of 49 - 54% in the case of finger millet, pearl millet and rice. From the reassociation curve of great millet (Fig. 2.5), Cot 50 mol x sec/l is taken as the limiting value for the determination of repetitive DNA. At this Cot, 52% of the toal DNA form duplexes. The proportion of repetitive DNA in these four

species is very low as compared to the other Gramineae species studied so far.

The kinetic complexities of unique DNA fractions of these four species are in the range of 1.6×10^8 to 1.2×10^9 np or 0.17 pg to 1.31 pg. The Gramineae species under our consideration thus differ from the other Gramineae species in having low nuclear DNA content and less proportion of repetitive DNA.

CHAPTER III

THERMAL DENATURATION AND OPTICAL REASSOCIATION OF NATIVE DNAs AND REPETITIVE DNA

THERMAL DENATURATION AND OPTICAL REASSOCIATION OF NATIVE DNAS AND REPETITIVE DNA

INTRODUCTION

The physicochemical characterization of individual DNAs is carried out using different methodologies such as CsCl centrifugation, thermal denaturation, optical rotarary dispersion, circular dichroism and X-ray crystallography. Among all these methods, CsCl centrifugation and thermal denaturation are most widely used. Equilibrium centrifugation of DNA in neutral CsCl yields the information about its buoyant density, base composition heterogeneity and about the presence of a satellite DNA. The G + C content can be estimated from the buoyant density value (Mandel and Marmur, 1968).

The thermal denaturation studies of native DNAs are very important due to the striking changes observed in some properti like viscosity, extinction coefficient and light scattering in a relatively narrow range of temperature. The temperature at which 50% of a DNA molecule is dissociated is called the mean thermal dissociation temperature or melting temperature (Tm) which is characteristic of each DNA. Thermal stability of a DNA molecule is a function of the complementarity in base sequences of its two component strands (Bautz and Bautz, 1964; Kotaka and Baldwin, 1964; Laird and McCarthy, 1968; Moore and McCarthy, 1968). A lowering in the thermal stability indicates less complementarity in base sequences of two component strands or more base mismatch (Britten et al., 1974). In addition to this, thermal denaturation is dependent and proportional to the G + C content of the DNA (Mandel et al.,

1968). Higher the G + C content, more will be the Tm. This linear variation of the Tm of DNAs with the G + C content is attributed to the formation of three hydrogen bonds in the G - C pair and only two in the A - T pair. The thermal denaturat studies thus provide a valuable information about the base composition heterogeneity of a DNA molecule. It reveals the presence of different components depending on their A + T and G + C composition. These A + T rich and G + C rich components can be isolated and studied further for their sequence analysis.

Although base composition can be determined from buoyant density and melting temperature, there are some direct methods to determine the base composition. One such method is based on the spectral changes accompanying the helix $\longrightarrow$ coil transition in DNA (Fredericq *et al.*, 1961; Felsenfeld and Sandeen, 1962; Marmur and Doty, 1962; Fresco *et al.*, 1963; Mahler *et al.*,1964; Felsenfeld and Hirschman, 1965; Hirschman and Felsenfeld, 1966; Russel *et al.*, 1969; Ulitzur, 1972). The method described by Ulitzur (1972) is comparatively simple and rapid.

In this Chapter, we have described the thermal denaturation characteristics of DNAs of finger millet, great millet, pearl millet and rice. The G + C content is determined from Tm values as well as using Ulitzur's method (1972). We have also isolated repetitive DNA fractions in finger millet, pearl millet and rice and studied their thermal denaturation and optical reassociation properties.

MATERIALS AND METHODS

Extraction of DNA

DNAs of all the four Gramineae species were isolated and purified as described in Materials and Methods of Chapter II.

Isolation of repetitive DNA fractions

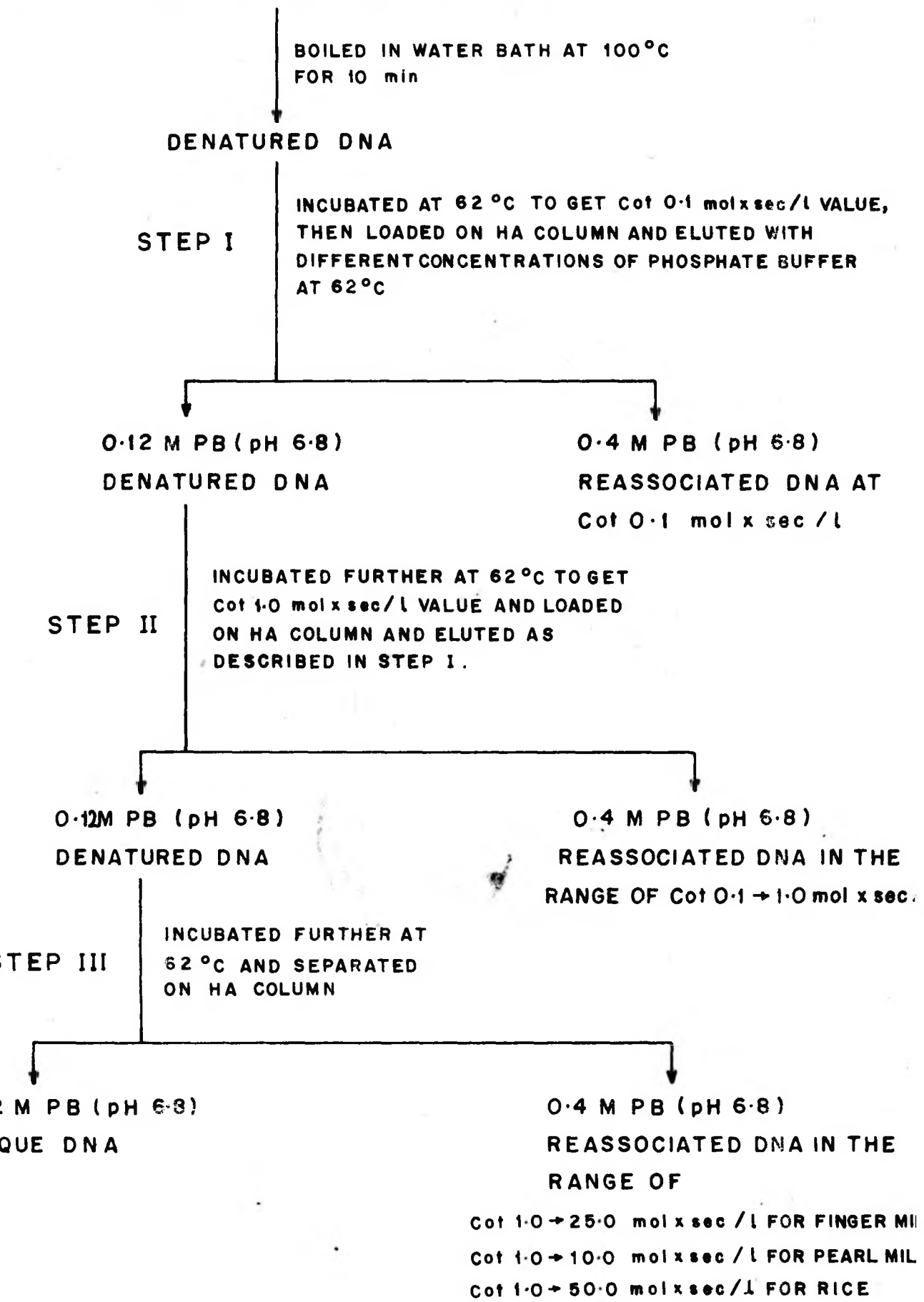
In Chapter II, we have described the arbitrary classification of repetitive DNA of these Gramineae species into three subclasses: (1) very rapidly reassociating DNA containing duplexes formed by Cot 0.1 mol x sec/l, (2) rapidly reassociating DNA forming duplexes in the Cot range of Cot 0.1 to Cot 1.0 mol x sec/l and (3) intermediately reassociating DNA forming duplexes in the Cot range of 1.0 to 25.0 mol x sec/l in the case of finger millet, 1.0 to 10.0 mol x sec/l in the case of pearl millet and 1.0 to 50.0 mol x sec/l in the case of rice. To isolate these fractions, total DNAs were sonicated to an average length of 550 np as described in Chapter II. The flow diagram of fractionation of DNAs is as shown. The reassociated 0.4 M PB (pH 6.8) DNA fractions were isolated and dialyzed against 0.12 M PB (pH 6.8) prior to their characterization.

In addition, Cot 1.0 DNA fraction of all the three DNAs and Cot 25.0, Cot 10.0 and Cot 50.0 DNA fractions of finger millet, pearl millet and rice DNA, respectively, were isolated using the same methodology.

Thermal denaturation

Thermal denaturation of various DNAs were carried out in Gilford 250 spectrophotometer equipped with thermoprogrammer (Model 2527), analog multiplexer (Model 6046) and automatic

FLOW DIAGRAM FOR FRACTIONATION OF DNAs



reference compensator (Ranjekar et al., 1976). Various DNA samples dialyzed with 0.12 M PB (pH 6.8) were used for studying their melting properties. Approximately, 0.2 ml of the DNA solution (25 to 50 µg/ml) was added to the thermal cuvettes and the temperature of the solution was raised at a rate of 1°C/min upto 98°C. The absorbance changes at 260 nm were continuously recorded during the heating process. From the absorbance change the hyperchromicity of the DNA sample was calculated using the formula:

$$H = \frac{A_{260}(98°C) - A_{260}(60°C)}{A_{260}(98°C)}$$

where H is the hyperchromicity and A_{260} is the absorbance at 260 nm (Zimmerman and Goldberg, 1977; Wimpee and Rawson, 1979). The total hyperchromicity was normalized to 100% and the graph of percent hyperchromicity versus temperature was plotted. E. coli, T_4 and calf thymus DNAs were used as standards for all the thermal denaturation studies.

Optical reassociation

Optical reassociation of sonicated DNAs and of Cot 1 fractions was studied to determine kinetic heterogeneity and kinetic complexity. Sonicated, 550 np long native DNAs as well as Cot 1.0 fractions of finger millet, pearl millet and rice in 0.12 M PB (pH 6.8) were added to thermal cuvettes and the absorbance of 260 nm at 25°C was recorded. DNA concentration (10 - 40 µg/ml) was such that after melting, the absorbance still remained within the linear regions of the spectrophotometer. The temperature of the DNA was raised to 98°C at a rate of

1°C/min. The DNA samples were maintained at this temperature for 4 - 5 min to ensure complete denaturation. The temperature of the cuvette was next quickly lowered to 62°C by circulation of water. This lowering process took about 60 - 90 seconds. The change in the absorbance of the DNA solutions was continuously recorded with time. For the determination of zero time or the start of reassociation, the midpoint (80°C) between 98°C and 62°C was taken (Britten et al., 1974).

The total change in absorbance from 25°C to 98°C was taken as 100% hyperchromicity. From the decrease in absorbance (hypochromicity), percentage reassociation was calculated by using the following formula at various Cot values:

$$\% \text{ reassociation} = 100 \times \left[\frac{A_{260} \text{ of denatured DNA} - A_{260} \text{ at a given time (t)}}{A_{260} \text{ of denatured DNA} - A_{260} \text{ of native DNA at 25°C}} \right]$$

Collapse hypochromicity was also calculated from the instantaneous loss in absorbance (260 nm) which was observed when totally denatured DNA was cooled from 98°C to 62°C (incubation temperature). Bacteriophage T_4 DNA, the genome complexity of which is already known (Laird, 1971) was taken as a standard DNA in these experiments and its optical reassociation was measured.

Ultraviolet spectroscopy for base composition

All native, unsonicated DNA samples in SSC-X giving absorbance in the range of 0.4 to 0.9 were used for UV spectroscopy. DNA samples having G + C content in the range

of 30 to 70% were used to plot a standard graph. DNAs from onion, calf thymus, salmon sperm, E. coli and M. lysodeikticus in SSC-X were used as standards. Their G + C contents were determined under our experimental conditions and the ratios at ultraviolet absorbance at the wavelengths (nm) 245/270, 240/280 and 240/275 were determined. We plotted the absorbance ratios found in this step against the base percent, using these points as loci for construction of standard lines. Then the absorbance ratios of our four unsonicated DNA samples were determined as explained above and their positions were located on each of the appropriate standard lines. The G + C% was thus calculated from each ratio and the average of three values was determined (Ulitzur, 1972).

RESULTS

Thermal denaturation of native DNAs

Native, high molecular weight DNAs of finger millet, great millet, pearl millet and rice are used for thermal denaturation studies and their melting properties are summarized in Table 3.1. The Tm values (the temperature at which 50% of the DNA melts) of the DNAs of E. coli, T_4 bacteriophage and calf thymus under our experimental conditions, are 90°C, 84°C and 85.5°C, respectively (Fig. 3.1) and compare well with the values reported in the literature. The melting curves of finger millet, great millet, pearl millet and rice are smooth and do not indicate any biphasic shape (Fig. 3.2 - 3.5). The melting temperatures are observed to be 85°C in the case of finger millet and rice, 85.6°C in the case of great millet and 88.6°C in the case of pearl millet. The hyperchromicities

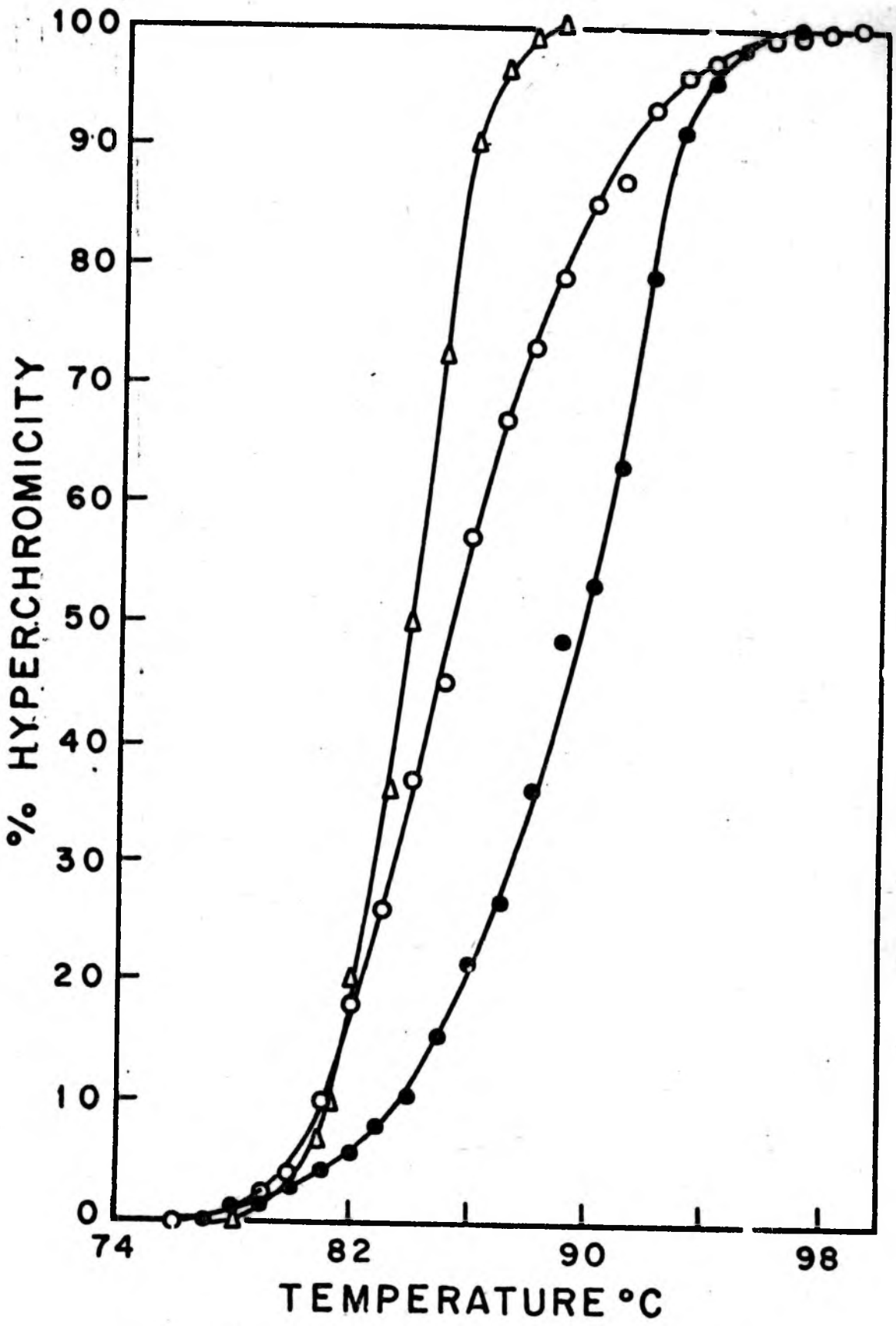


Fig. 3.1: Thermal denaturation profiles of native, unsonicated *E. coli* (—●—●—), T_4 bacteriophage (—Δ—Δ—) and calf thymus (—o—o—) DNAs in 0.12 M PB (pH 6.8).

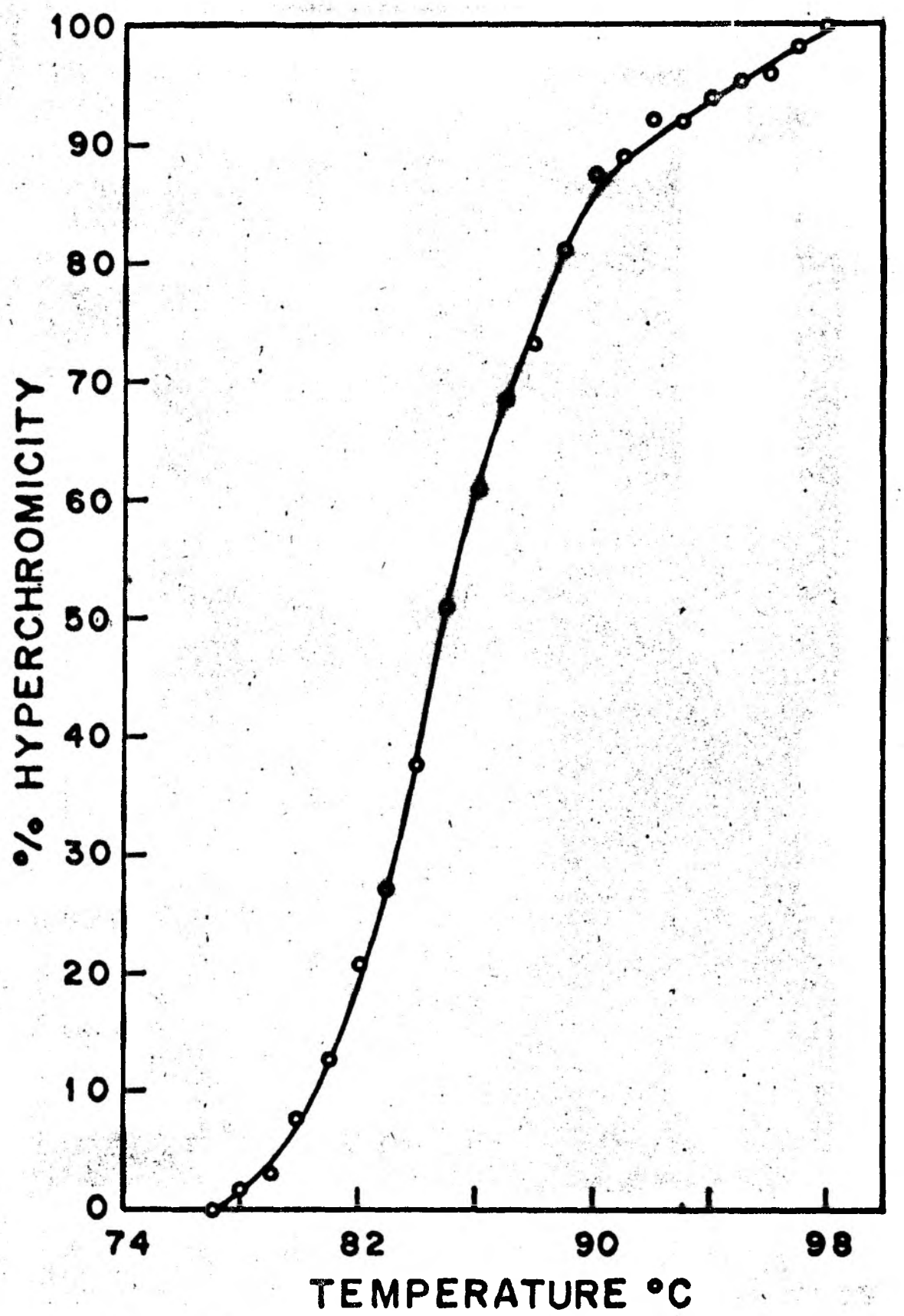


Fig. 3.2: Thermal denaturation profile of native, unsonicated finger millet DNA in 0.12 M PB (pH 6.8).

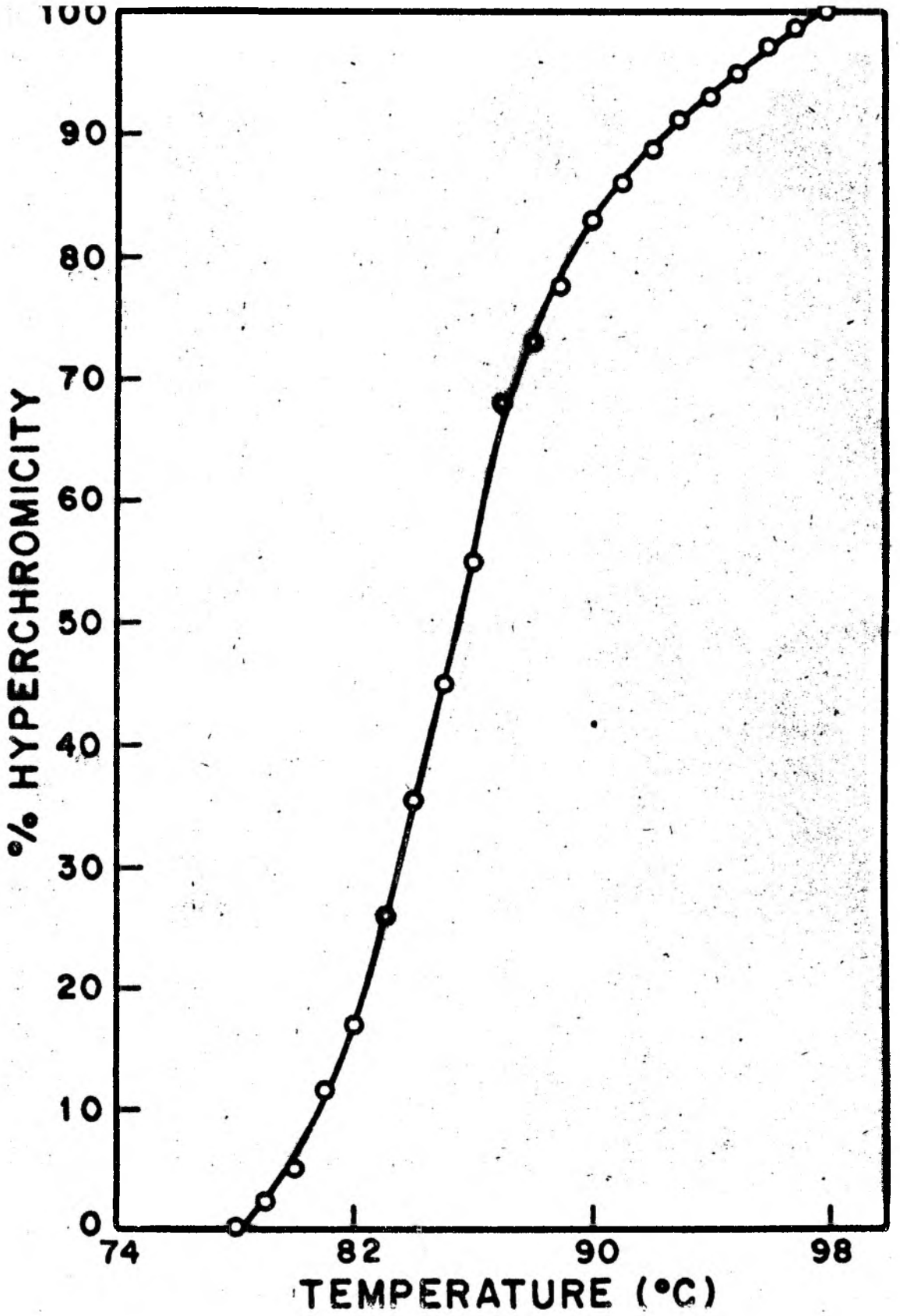


Fig. 3.3: Thermal denaturation profile of native, unsonicated great millet DNA in 0.12 M PB (pH 6.8).

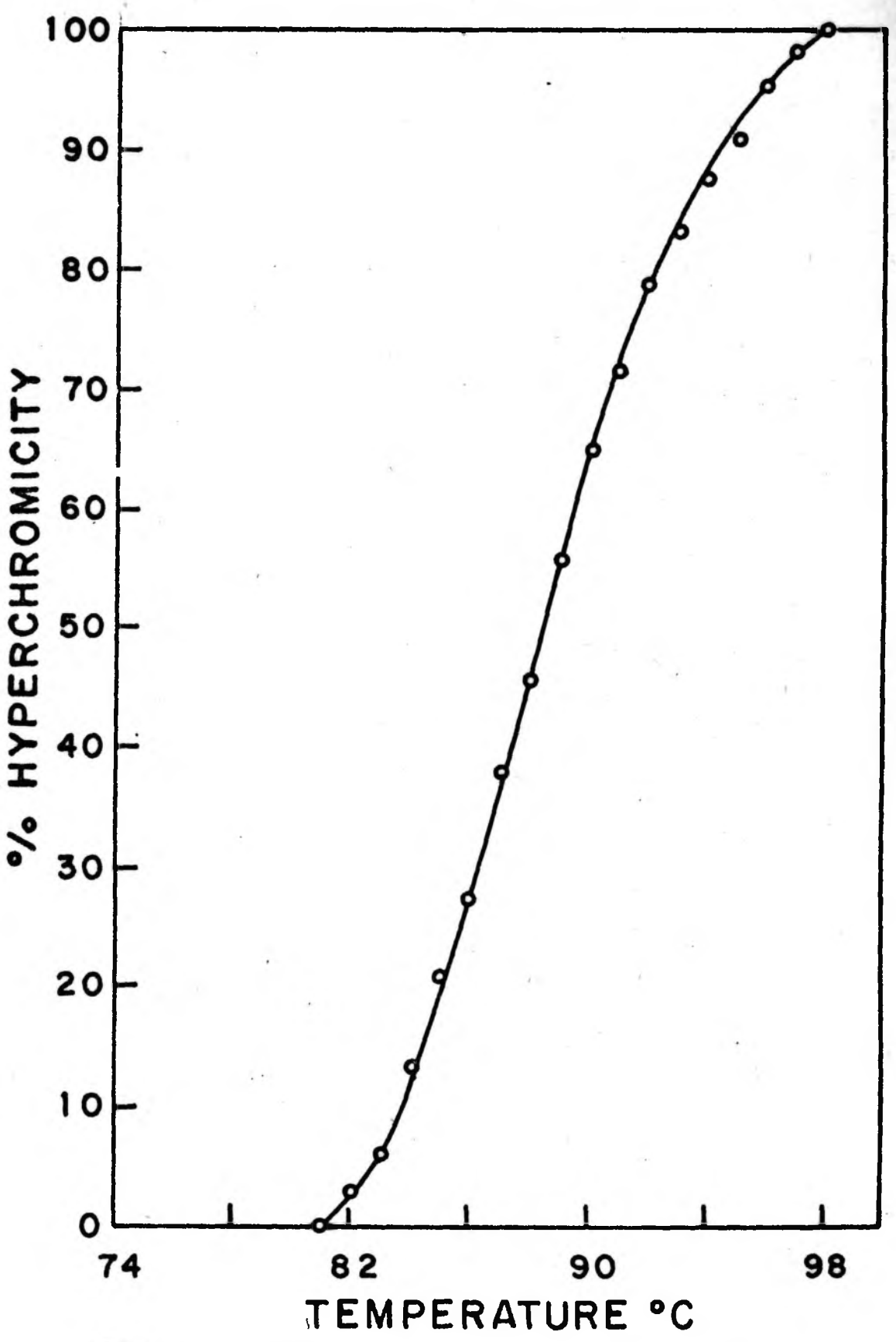


Fig. 3.4: Thermal denaturation profile of native, unsonicated pearl millet DNA in 0.12 M PB (pH 6.8).

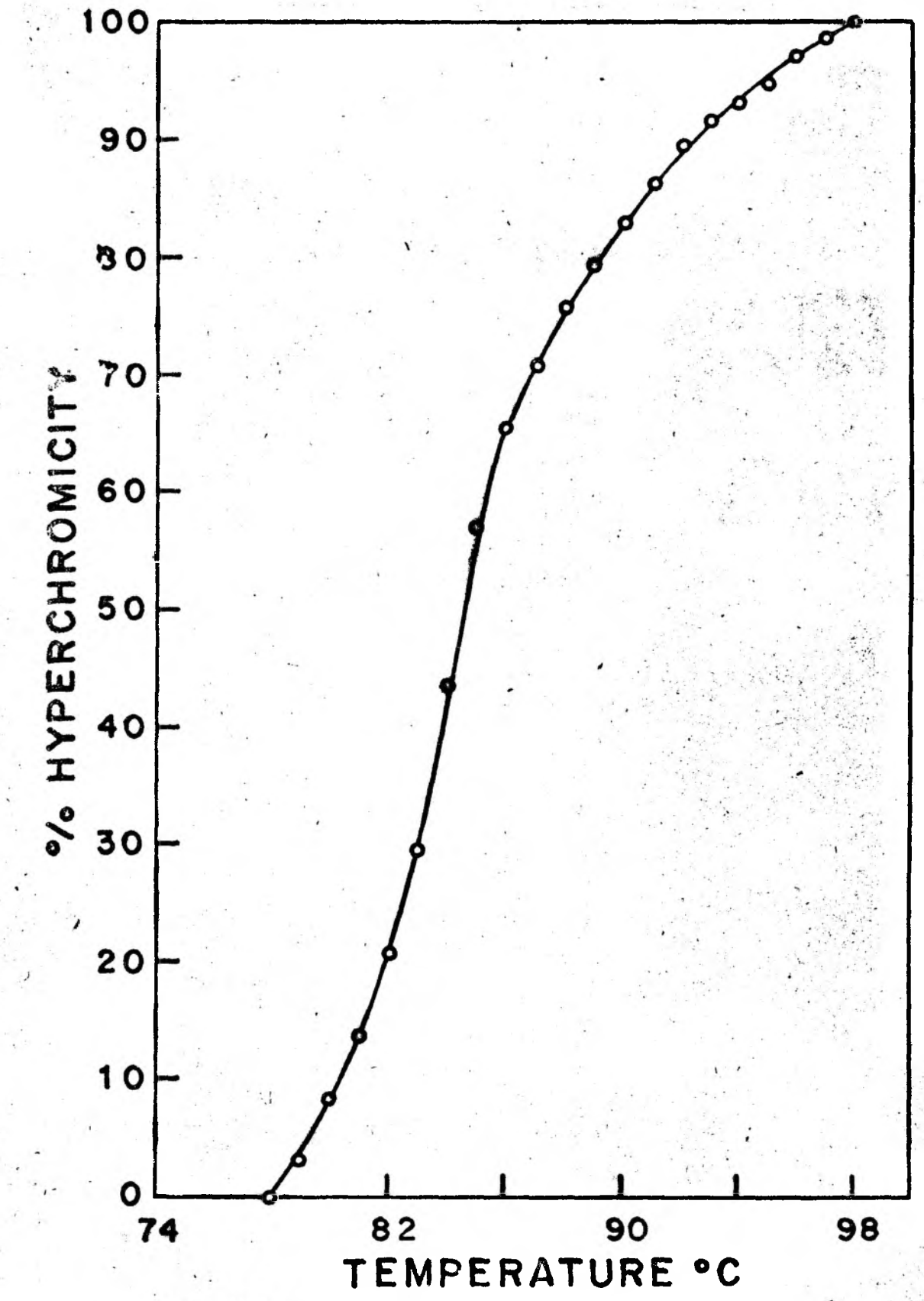


Fig. 3.5: Thermal denaturation profile of native, unsonicated rice DNA in 0.12 M PB

of finger millet, great millet and pearl millet are 20.0%, 22.0% and 25.0%, respectively. As compared to these, the hyperchromicity of rice DNA (12%) is very low. The base composition of these DNAs is then determined from the Tm values using the formula:

$$G + C \text{ content} = (Tm - 69.3)\ 2.44,$$

where 69.3 represents the Tm of a pure A + T polymer and every degree rise in the Tm above 69.3°C represents an increase of 2.44% in the G + C content (Marmur and Doty, 1959; Mandel et al., 1968). The percent G + C content is 38.5 in finger millet and rice, 39.8 in great millet and 47.0 in pearl millet (Table 3.1).

Base composition by absorbance ratios

Base composition of all the four Gramineae species was also determined by the method of Ulitzur (1972). Onion, salmon sperm, calf thymus, E. coli and M. lysodeikticus DNAs in SSC-X were used to plot a standard graph. Absorbance ratios at 240/280, 240/275 and 245/270 for these DNAs are calculated and are plotted against G + C contents (Table 3.2 and Fig. 3.6). This is used to estimate the G + C content of native, unsonicated finger millet, great millet, pearl millet and rice DNAs in SSC-X. Absorptions of these DNAs at 240, 245, 270, 275 and 280 nm are measured on Shimadzu spectrophotometer (Model No. UV 210A) and ratios 240/275, 240/280 and 245/270 are calculated. Using the standard curves, the G + C contents of each species at each absorption ratios are calculated. The average G + C contents of finger millet, great millet, pearl millet and rice are found

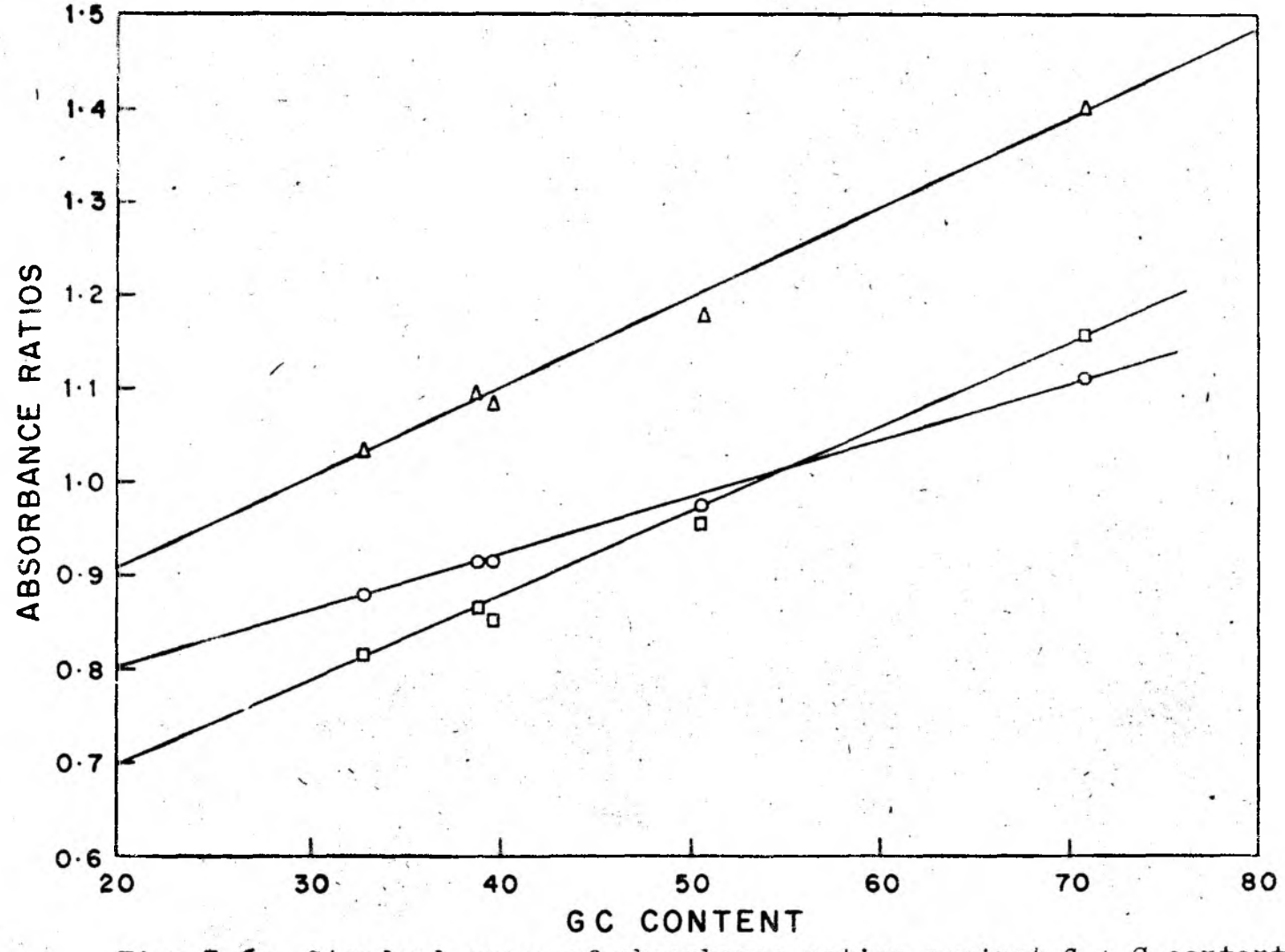


Fig. 3.6: Standard curve of absorbance ratios against G + C content for base composition. (-o-o-), 245/270; (-Δ-Δ-), 240/280 and (-□-□-), 240/275.

Table 3.1: Melting data of native DNAs

DNA	Tm °C	Hyperchromicity[a] %	G + C[b] content %
Finger millet (8)	85.0 ± 0.12	20.0 ± 0.31	38.5
Great millet (6)	85.6 ± 0.18	22.0 ± 0.53	39.8
Pearl millet (7)	88.6 ± 0.04	25.0 ± 0.48	47.0
Rice (11)	85.0 ± 0.22	12.0 ± 0.87	38.5
E. coli (3)	90.0 ± 0.02	24.48± 0.2	50.50
T_4 bacteriophage	84.0	27.0	35.85
Bovine (3)	85.5 + 0.03	25.03+ 0.16	39.53

[a] Hyperchromicity is calculated using the formula

$$H = \frac{A_{260}\ (98°C) - A_{260}\ (25°C)}{A_{260}\ (98°C)}$$

[b] % G + C is calculated according to the formula

$$\% \text{ G + C content} = (Tm - 69.3)\ 2.44$$

(Marmur and Doty, 1959; Mandel *et al.*, 1968).

Figures in the paranthesis indicate the number of DNA samples used to carry out these experiments.

Table 3.2: The absorption ratios and G + C content of standard DNA samples

DNA	Absorption ratios			G + C content[a]	G + C content[b]	Reference
	$\frac{240}{280}$	$\frac{240}{275}$	$\frac{245}{270}$	%	%	
Onion	1.0347	0.815	.0.881	32.69	27.3	Ranjekar et.al.,1978b
Salmon sperm	1.0944	0.865	0.913	38.79	44.4	Marmur and Doty, 1962.
Calf thymus	1.0866	0.857	0.9123	39.53	43.1	Marmur and Doty, 1962.
E. coli	1.178	0.961	0.975	50.5	51.3	Marmur and Doty, 1962.
M. lyso-deikticus	1.402	1.162	1.111	70.76	72.0	Schildkraut et al.,1962.

[a] G + C content calculated from Tm values under our experimental conditions.

[b] Literature values of G + C content as reported in the references mentioned in the neighbouring column.

to be 40.95%, 42.5%, 48.03% and 38.37%, respectively (Table 3.3), These values are in close agreement with those obtained from Tm values.

Thermal denaturation of repetitive DNA fractions

From Chapter II, it is clear that the repetitive DNA accounts for 49 to 54% of the total DNA in finger millet, pearl millet and rice. Since the repetitive DNA fractions of all these three species were isolated from sonicated DNA samples, the melting patterns of the latter are also studied.

In the case of finger millet, the comparative melting patterns of sonicated, native DNA and of Cot fractions are presented in Figs. 3.7 and 3.8. The Tm of 550 np long, native DNA is 83.8°C and the Tms of the Cot fractions are in the range of 70.8°C - 73.6°C (Table 3.4). All the melting profiles in the case of finger millet are monophasic in character.

Native, sonicated 550 np long pearl millet DNA exhibits a Tm of 87.0°C (Fig. 3.9) and the melting curve is monophasic. Melting profiles of Cot 0.1, 1.0 and 10.0 mol x sec/l are, however, biphasic (Figs. 3.9 and 3.10). The high melting fraction of Cot 0.1 pearl millet DNA accounts for 50% of the total Cot 0.1 fraction with a Tm of 95.0°C and the low melting fraction has a Tm of 83.2°C. In Cot 1.0 DNA, the proportion of low melting (Tm = 81.6°C) components is 65% and that of high melting component (Tm = 94.8°C) is 35%. In Cot 10.0 DNA, the proportion of high melting fraction is 12% with a Tm of 92°C and that of low melting component is 88% with a Tm of 78.0°C. However, the Cot fractions 0.1 to 1.0 and 1.0 to 10.0 do not

Table 3.3: Base composition of native DNAs by absorption ratio method

DNA	G + C content from ratios $\frac{245}{270}$	$\frac{240}{280}$	$\frac{240}{275}$	G + C content mean %	G + C content from Tm %
Finger millet (3)	40.60	40.75	41.50	40.95	38.5
Great millet (2)	42.0	42.50	43.0	42.5	39.8
Pearl millet (6)	48.45	46.75	48.90	48.03	47.0
Rice(4)	37.38	38.68	39.06	38.37	38.5

Figures in the parenthesis indicate the number of DNA samples used to calculate absorbance ratios.

show any biphasic nature and melt with Tms of 80.2°C and 80.0°C, respectively (Table 3.4).

Melting profiles of native, 550 np long rice DNA as well as of the Cot fractions are smooth and monophasic in nature (Fig. 3.11 and 3.12). The Tm of native, 550 np long rice DNA is 83.4°C and the Tms of the Cot fractions are in the range of 79.6°C to 82.6°C. Cot 50.0 fraction shows a Tm of 79.6°C and Cot 0.1 fraction has a Tm of 82.6°C (Table 3.4).

Hyperchromicity and base mismatch of repetitive DNA fractions

The hyperchromicities of finger millet and pearl millet 550 np long, native DNAs are 27.0 and 21.0%, respectively. The hyperchromicity of rice, native 550 np DNA is, however very low (7.0%). We also tried to estimate the hyperchromicities of native, rice DNAs with different fragment lengths as 4900 np and 1500 np and they were found to be 11.0% and 9.1%, respectively. In addition to these, the Cot fractions of rice also show very low hyperchromicities (5.5 - 12%). The hyperchromicities of finger millet Cot fractions are in the range of 10.0 - 18.0% and those of pearl millet Cot fractions are in the range of 13.1% - 19.0% (Table 3.4).

The Tm values of the repetitive DNA fractions are compared to that of the corresponding native, sonicated, 550 np long DNA preparations in each species. From the lowering in the Tm of the reassociated DNAs, the percentage base mismatch in these fractions can be estimated from the relationship explained by Britten et al. (1974) where lowering in Tm by 1°C is assumed to correspond to 1% base mismatch. In the case of finger millet, the extent of base mismatch of repetitive duplexes is

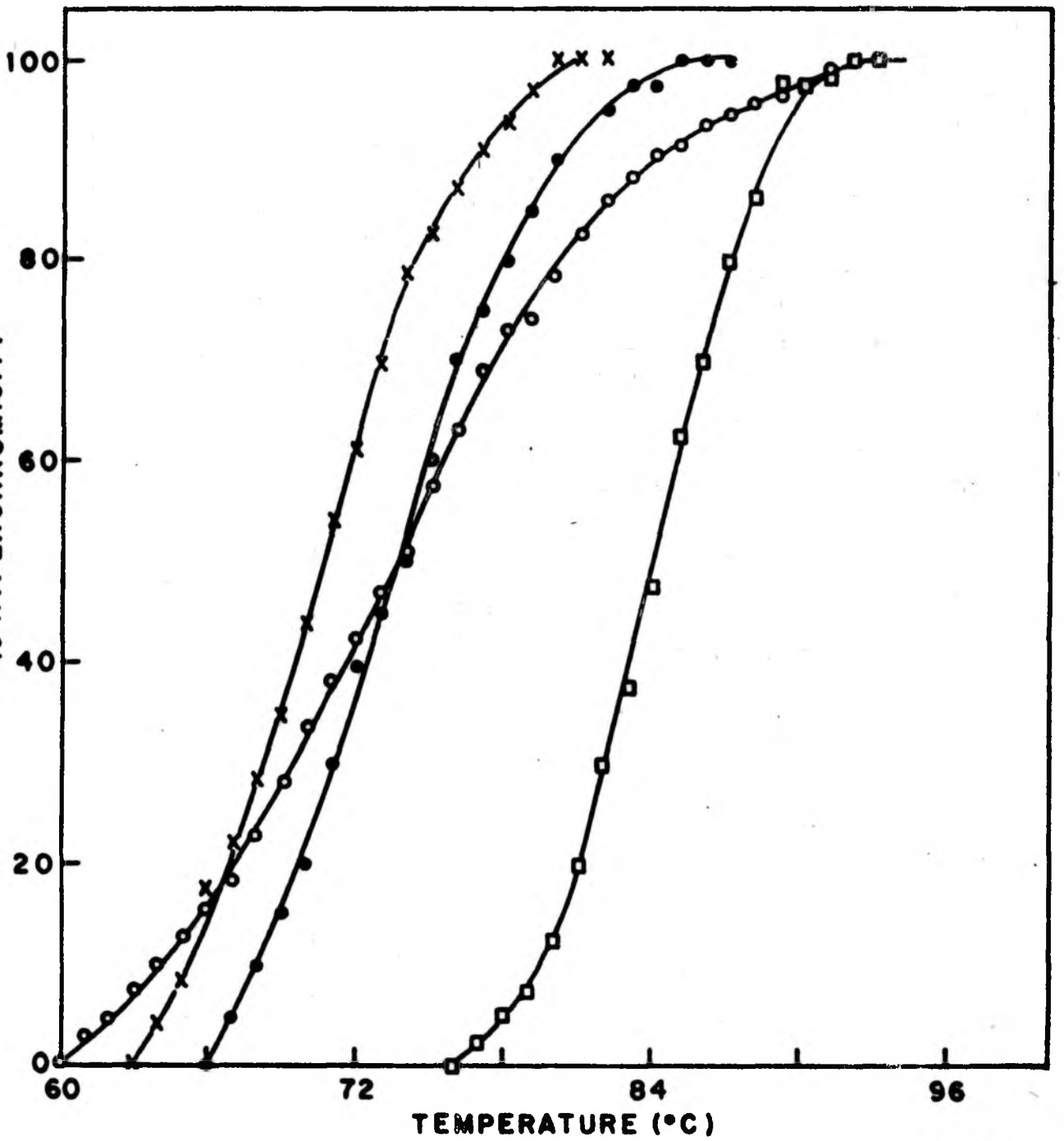


Fig. 3.7: Thermal denaturation profiles of Cot 0.1 (-o-o-), Cot 0.1 to 1.0 (-•-•-), Cot 1.0 to 25.0 (-×-×-) and total 550 np (-□-□-) DNAs of finger millet.

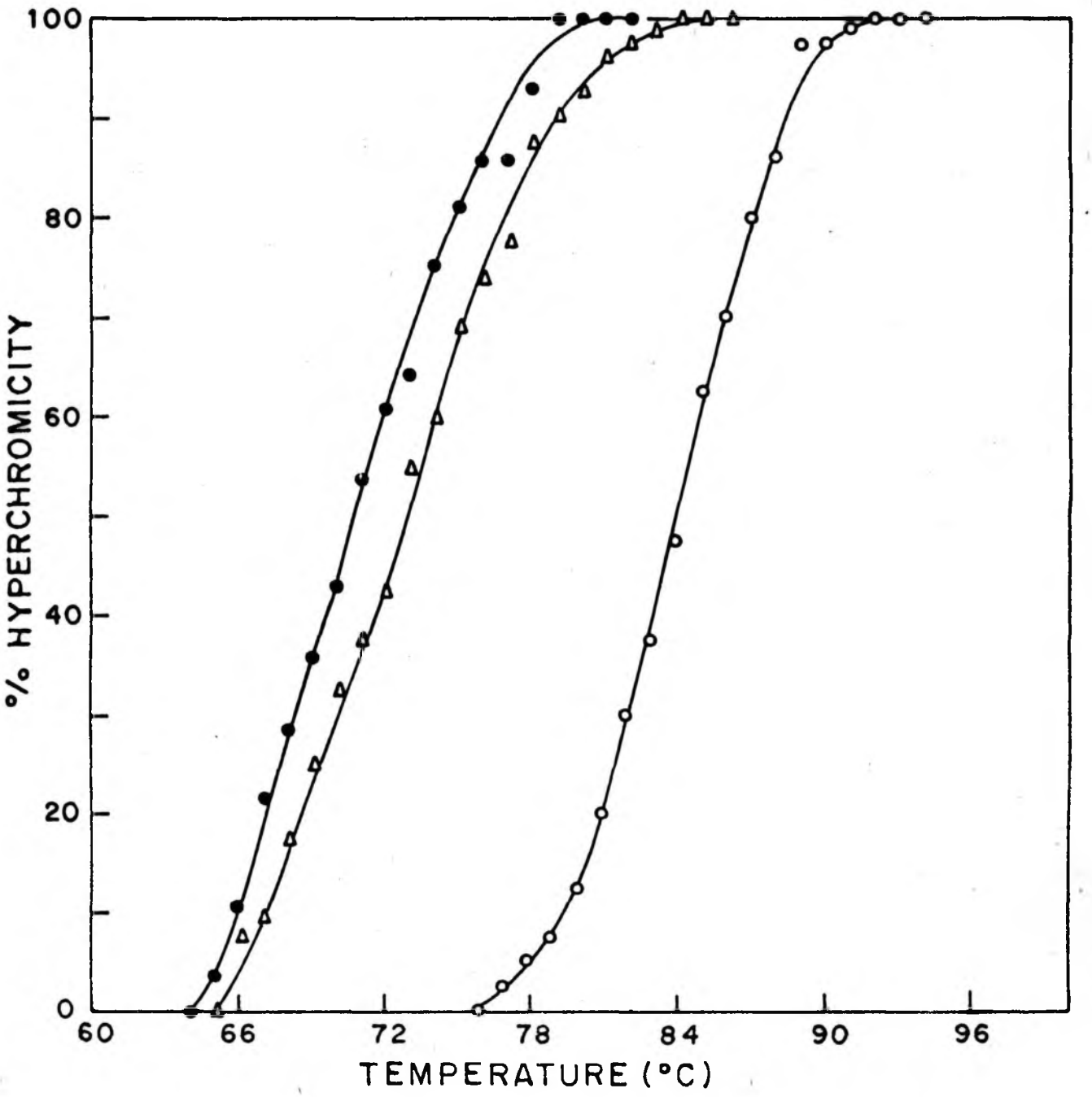


Fig. 3.8: Thermal denaturation profiles of Cot 1.0 (—●—●—), Cot 25.0 (—△—△—) and total 550 np (—o—o—) DNAs of finger millet.

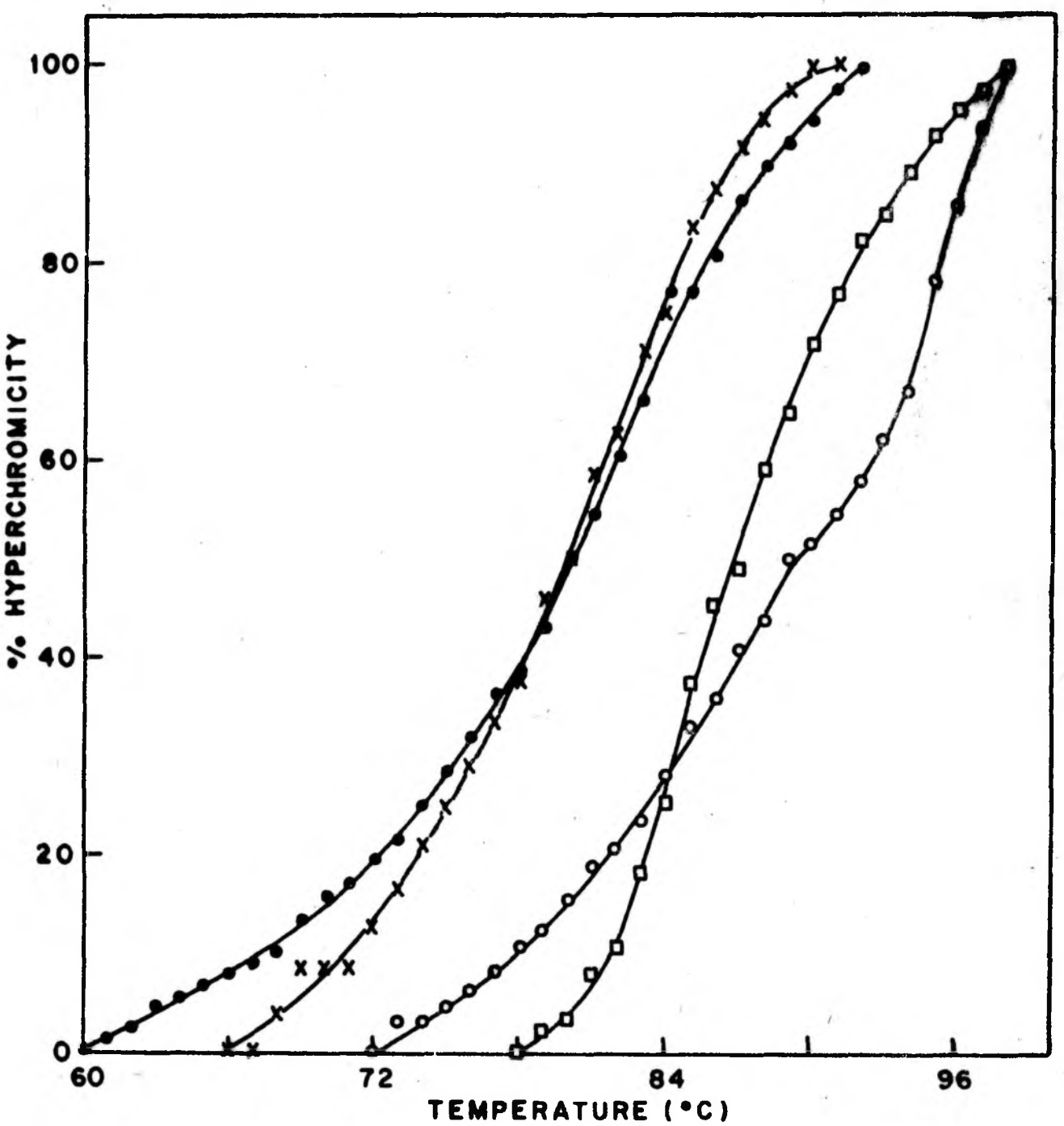


Fig. 3.9: Thermal denaturation profiles of Cot 0.1 (-o-o-), Cot 0.1 to 1.0 (-●-●-), Cot 1.0 to 10.0 (-×-×-) and native, 550 np long (-□-□-) DNAs of pearl millet.

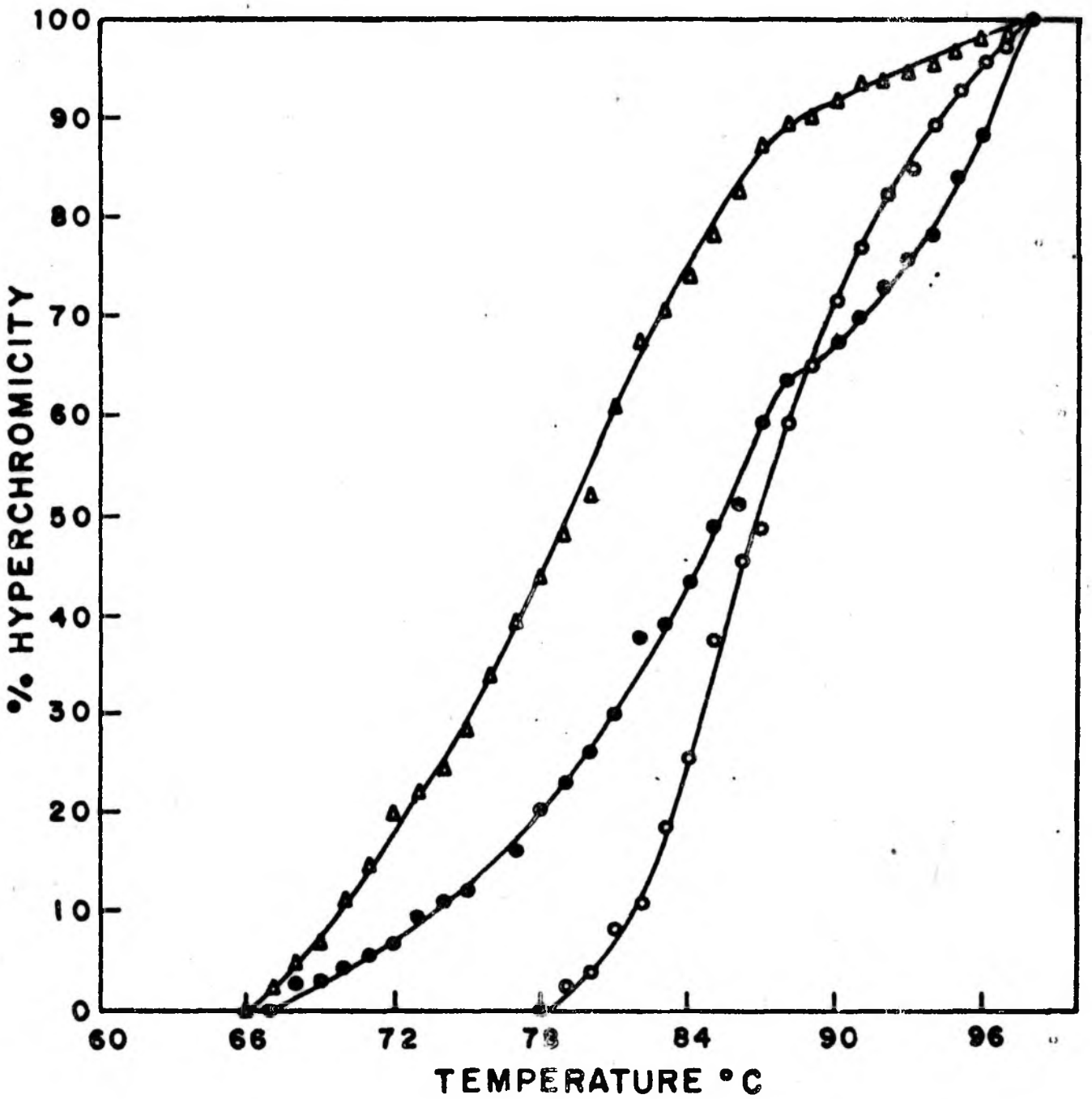


Fig. 3.10: Thermal denaturation profiles of Cot 1.0 (—●—●—), Cot 10.0 (—△—△—) and native, 550 np long (—o—o—) DNAs of pearl millet.

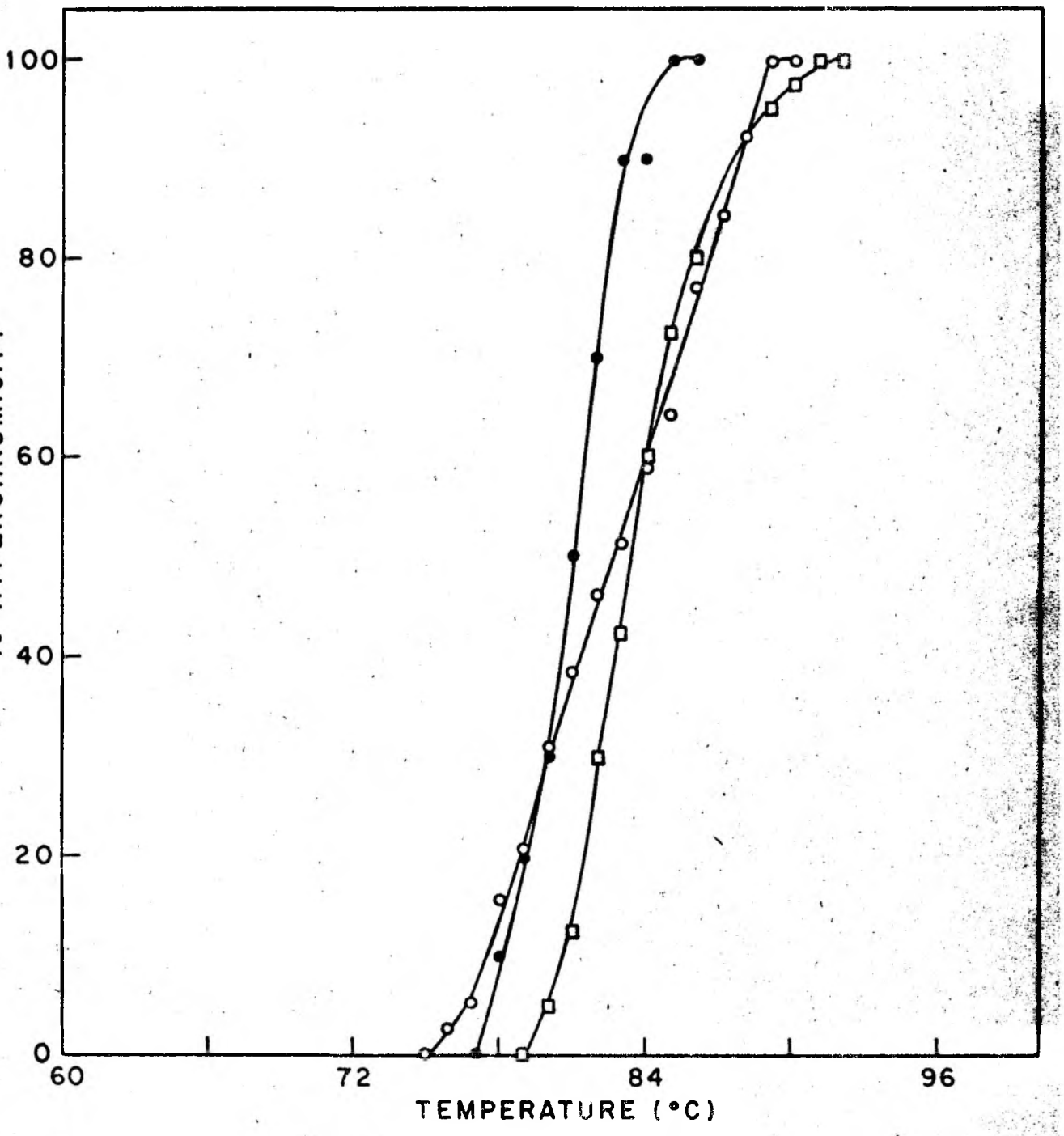


Fig. 3.11: Thermal denaturation profiles of Cot 0.1 (-o-o-), Cot 0.1 to 1.0 (-●-●-) and native 550 np long (-□-□-) DNAs of rice.

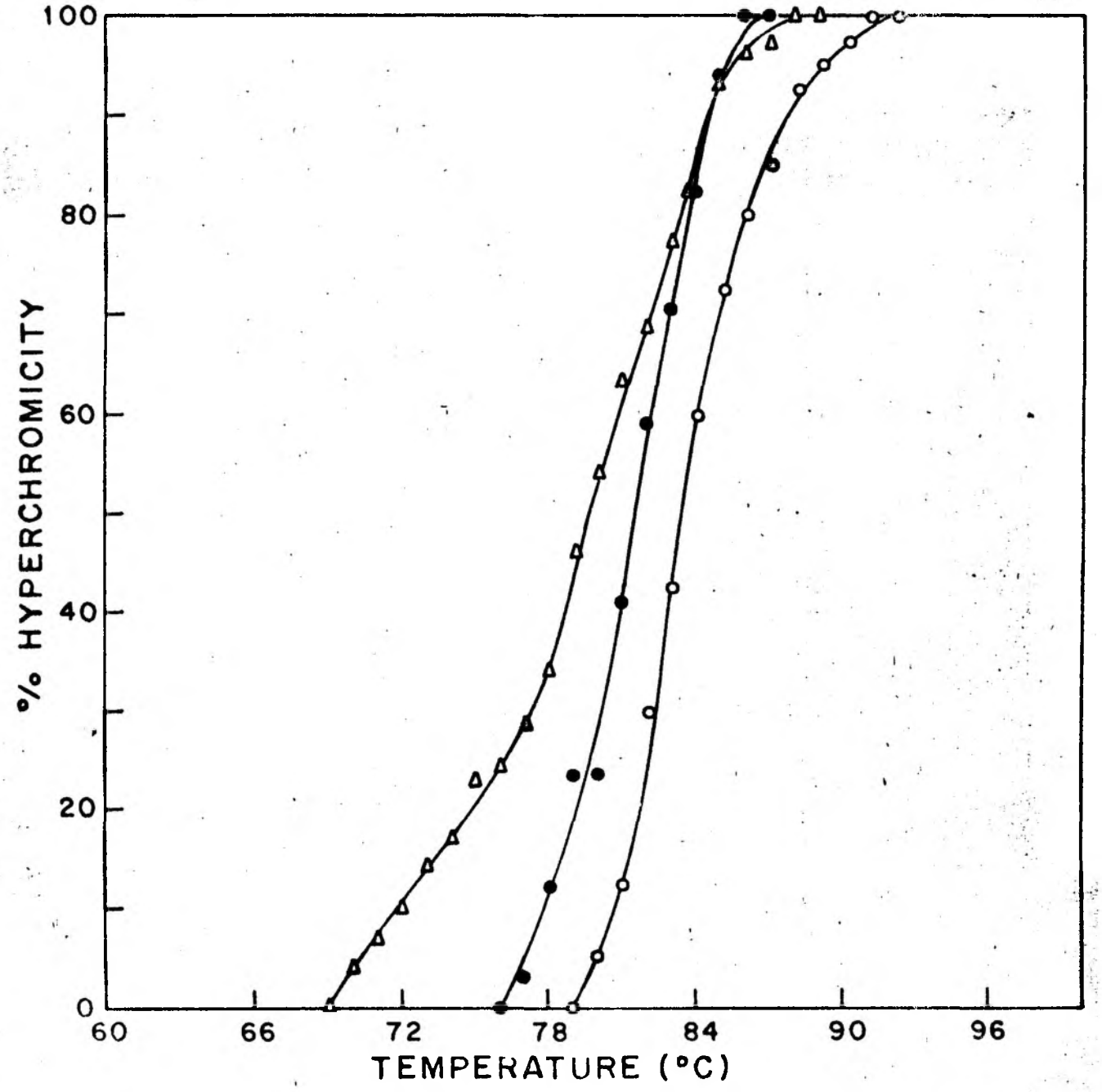


Fig. 3.12: Thermal denaturation profiles of Cot 1.0 (●—●), Cot 50.0 (△—△) and native 550 np long (○—○) DNAs of rice.

Table 3.4: Thermal denaturation properties of repetitive DNA fractions

Cot fraction	Reassociation %	Tm °C	Hyperchromicity[a] %	Δ Tm[b] °C	Base mismatching[c] %
1	2	3	4	5	6
Finger millet					
Native, 550 np DNA (9)	-	83.8 ± 0.022	27.0	-	-
Cot 0.1 (7)	18.0	73.6 ± 0.11	18.0	10.2	10.2
Cot 0.1 - 1.0 (3)	5.0	73.6 ± 0.20	13.0	10.2	10.2
Cot 1.0 (7)	23.0	71.0 ± 0.08	12.0	12.8	12.8
Cot 1.0 - 25.0 (3)	26.0	70.8 ± 0.61	10.0	13.0	13.0
Cot 25.0 (8)	49.0	73.0 ± 0.13	14.2	10.8	10.8
Pearl millet					
Native 550 np DNA (7)	-	87.0 ± 0.03	21.0	-	-
Cot 0.1 (8)	20.0	(50%) 83.2 ± 0.16	19.0	3.8	3.8
		(50%) 95.0 ± 0.13	-	-	-
Cot 0.1 - 1.0 (3)	13.0	80.2 ± 0.21	15.3	6.8	6.8
Cot 1.0 (9)	33.0	(65%) 81.6 ± 0.06	16.9	5.4	5.4
		(35%) 94.8 ± 0.072	-	-	-
Cot 1.0 - 10.0 (3)	21.0	80.0 ± 0.16	13.1	7.0	7.0
Cot 10.0 (8)	54.0	(88%) 78.0 ± 0.09	19.4	9.0	9.0
		(12%) 92.0 ± 0.063	-	-	-

1	2	3	4	5	6
Rice					
Native, 550 np DNA (12)	-	83.4 ± 0.21	7.0	-	-
Cot 0.1 (10)	8.5	82.6 ± 0.10	12.0	0.8	0.8
Cot 0.1 - 1.0 (3)	18.5	81.0 ± 0.26	5.5	2.4	2.4
Cot 1.0 (10)	27.0	81.6 ± 0.096	10.0	1.8	1.8
Cot 1.0 - 50.0 (3)	25.0	-	-	-	-
Cot 50.0 (7)	52.0	79.6 ± 0.128	7.8	3.8	3.8

[a] % Hyperchromicity was calculated using the formula described in Materials and Methods.

[b] Δ Tm was calculated by taking the difference in the Tm values of native, sonicated, 550 np DNA and the Cot fraction.

[c] % base mismatching was estimated by using the relationship 1°C lowering in Tm corresponds to 1% base mismatching (Britten et al., 1974).

Figures in the parenthesis indicate the number of experiments carried out.

in the range of 10.2% to 13.0%. The range of base mismatching in the case of pearl millet repetitive duplexes is 3.8% to 9.0% and that in the case of rice is very low (0.8% - 3.8%). In the case of finger millet, the intermediately reassociating fraction (Cot 1.0 - 25.0 mol x sec/l) has a maximum base mismatch as 13.0%. Pearl millet Cot 0.1, 1.0 and 10.0 fractions show biphasic nature, In these cases the low melting fraction shows base mismatching of 3.8%, 5.4% and 9.0%, respectively. Cot 0.1 fraction of rice shows a base mismatching of 0.8% only.

Optical reassociation of sonicated DNAs and Cot 1 DNA fractions

During the reassociation kinetics experiments of the total, sonicated DNAs by hydroxyapatite column chromatography, the lowest Cot value, at which the extent of reassociation was measured, was in the range of 10^{-1} to 10^{-2} mol x sec/l. We were interested in knowing the mode of reassociation at still lower Cot values. Hence the optical reassociation of sonicated DNAs as well as those of Cot 1.0 DNA fractions were measured using T_4 DNA as a standard (Laird, 1971).

The optical reassociation curves of native, 550 np long DNAs of finger millet, pearl millet and rice are shown in Figs. 3.13, 3.14 and 3.15, respectively. The optical reassociation value at Cot 1.0 of the total DNA compares well with that of hydroxyapatite in each Gramineae species (Table 3.5).

In the case of finger millet, approximately 92% of the Cot 1.0 DNA is found to reassociate in a rather narrow Cot range of 3×10^{-3} to 1.0 mol x sec/l (Fig. 3.13). An average Cot 1/2 value of this DNA fraction is estimated and compared with that of slow reassociating DNA fraction to calculate its average

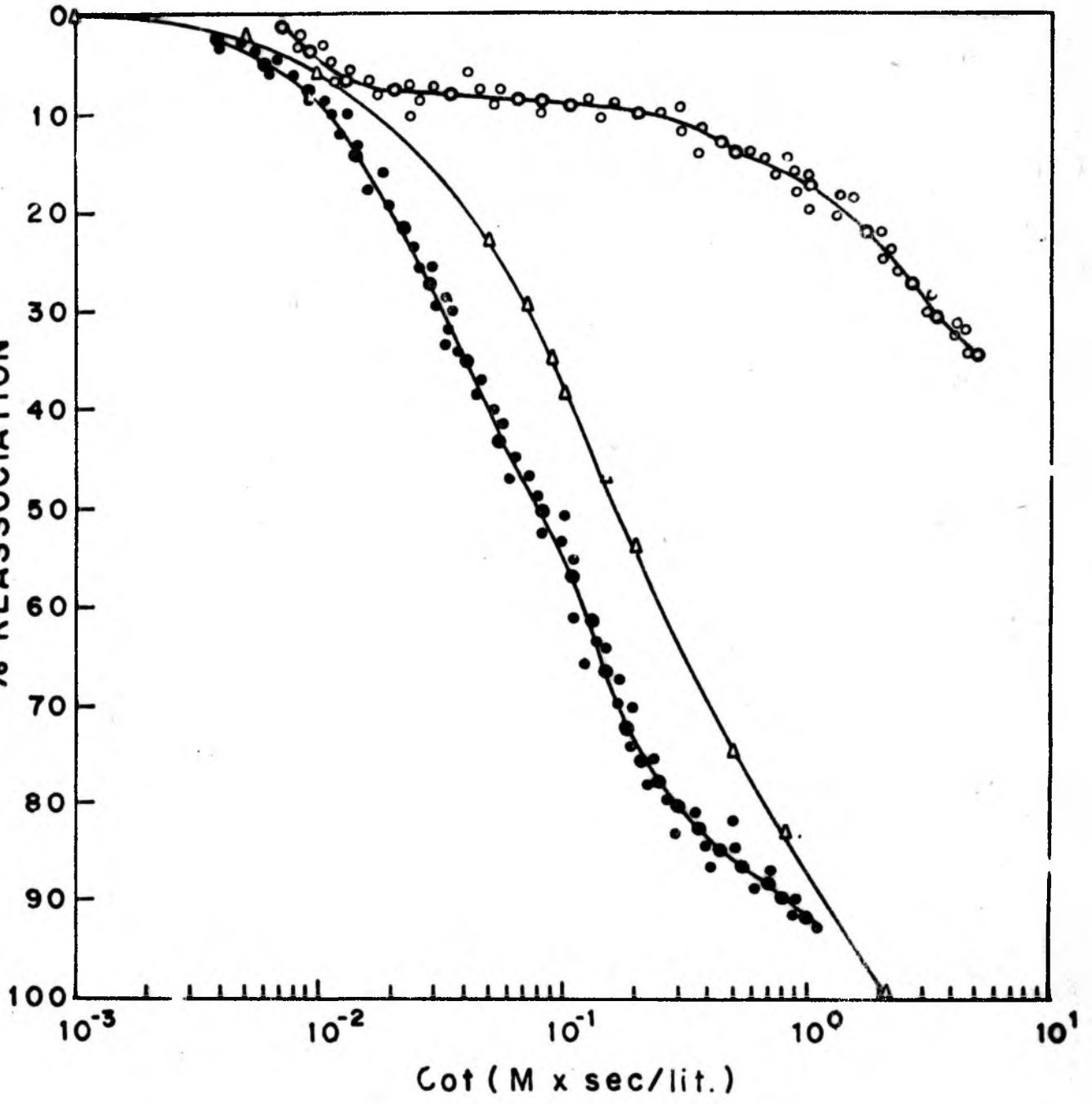


Fig. 3.13: Optical reassociation curves of total sonicated DNA (—o—o—) and Cot 1 fraction (—●—●—) of finger millet along with that of sonicated T_4 DNA (—Δ—Δ—).

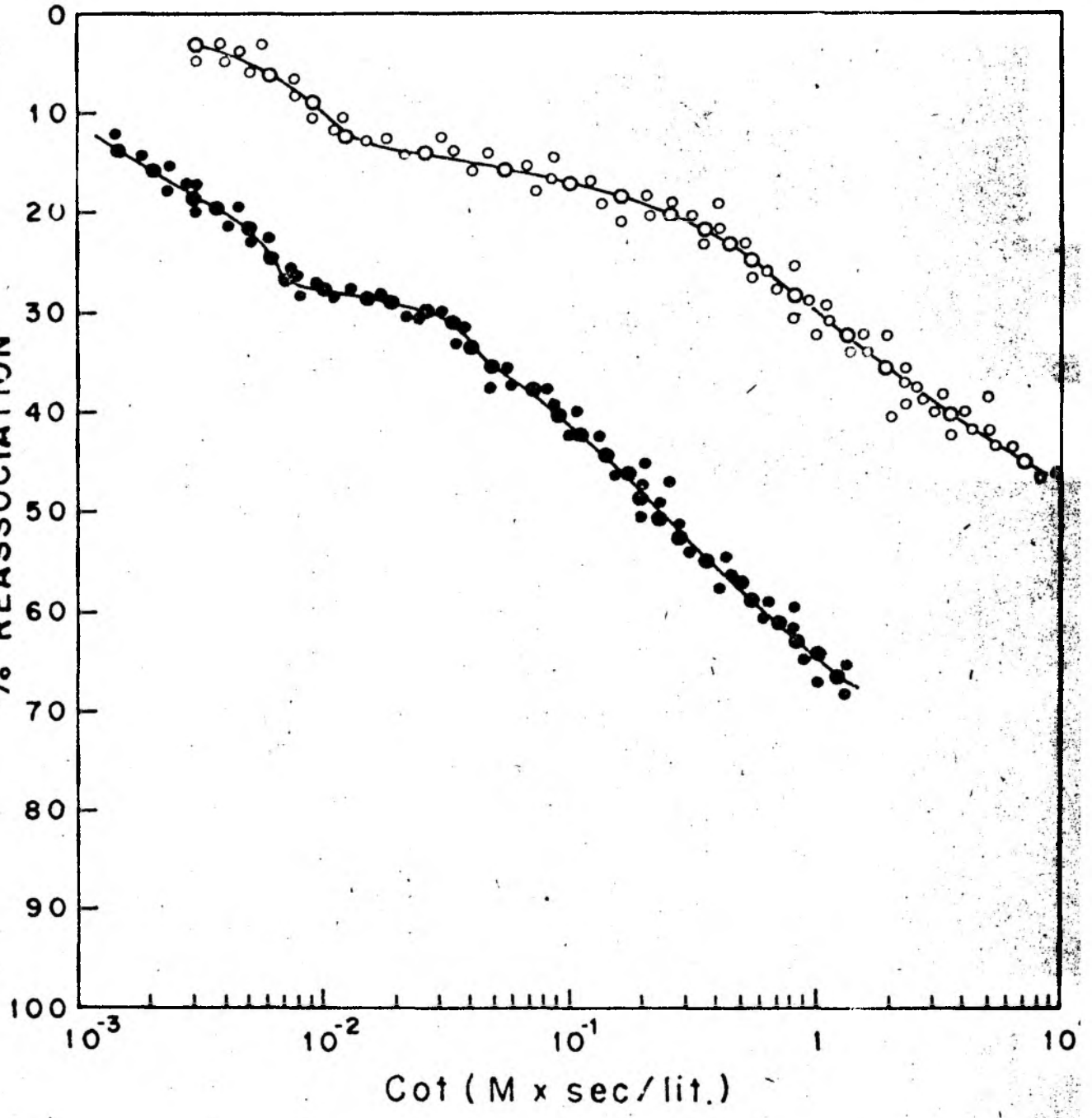


Fig. 3.14: Optical reassociation curves of total sonicated DNA (—o—o—) and Cot 1 fraction (—●—●—) of pearl millet.

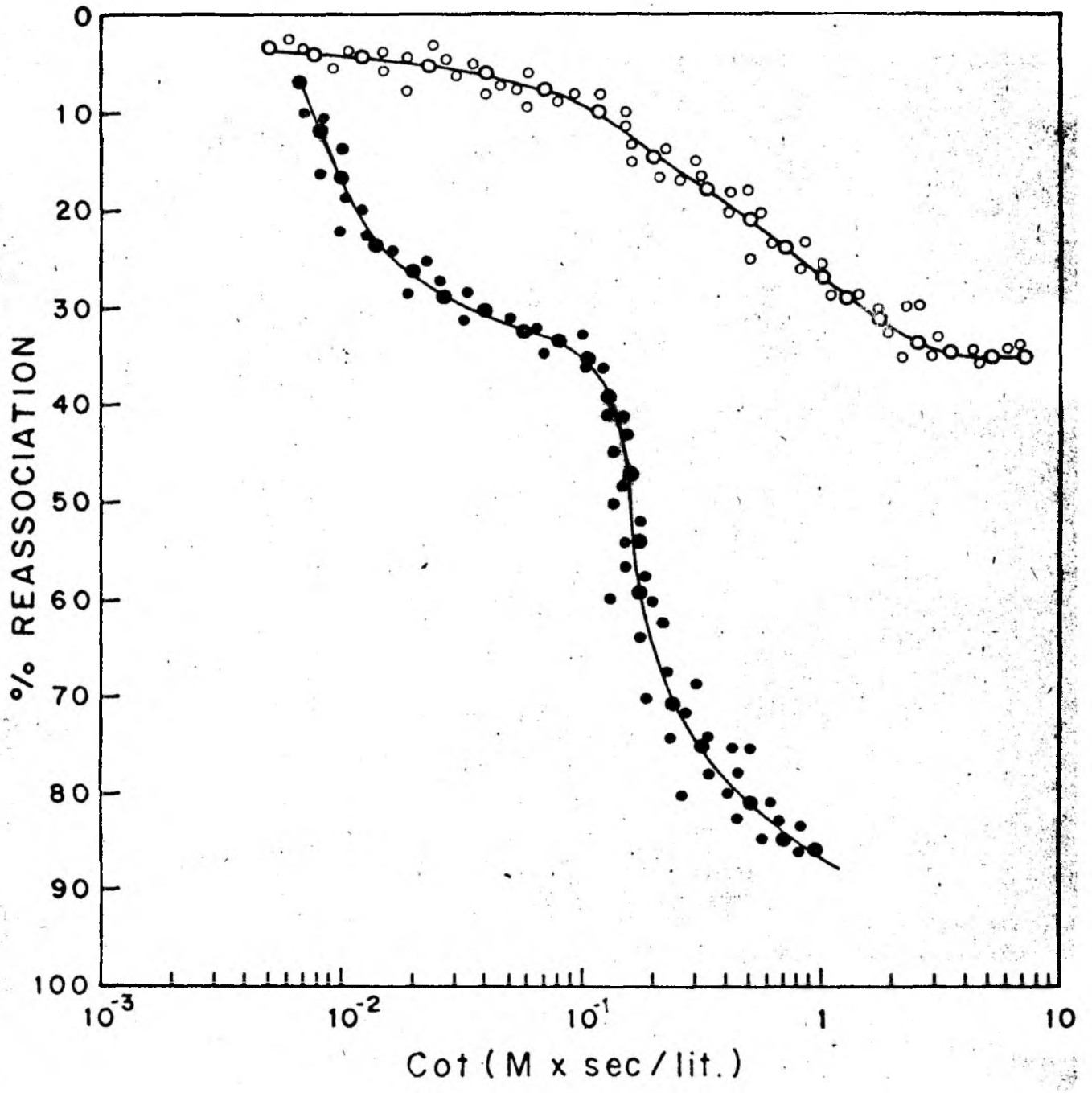


Fig. 3.15: Optical reassociation curves of total sonicated DNA (-O-O-) and Cot 1 fraction (-●-●-) of rice.

Table 3.5: Comparison of the extent of DNA reassociation by optical methods and by hydroxyapatite measurements

Total sonicated DNA	Reassociation at Cot 1.0 by hydroxyapatite measurements %	Reassociation at Cot 1.0 by optical reassociation measurements %
Finger millet	23.0	18.0
Pearl millet	33.0	32.0
Rice	27.0	27.0

frequency of repetition (Table 3.6). The optical reassociation curve in the case of pearl millet Cot 1.0 DNA fraction (Fig. 3.14) reveals the presence of at least two subfractions; rapidly reassociating upto Cot 0.01 mol x sec/l and slow reassociating in the Cot range of 0.01 to 1.0 mol x sec/l. The proportion of the rapidly reassociating fraction is 28% of the total Cot 1.0 DNA fraction. The average Cot 1/2 values of each rapidly and slow reassociating fractions are 1.5×10^{-3} and 0.9 mol x sec/l, respectively. The optical reassociation curve of rice Cot 1.0 DNA (Fig. 3.15) shows the presence of two DNA fractions separated by a clearcut break at Cot 0.1 mol x sec/l. The proportion of rapidly reassociating DNA is 35% of the total Cot 1.0 DNA and the DNA sequences in this fraction reassociate upto a Cot value of 0.1 mol x sec/l with a Cot 1/2 value of 1.0×10^{-2} mol x sec/l. The DNA sequences in the second fraction reanneal in a very narrow Cot range of 10^{-1} to 10^{0} mol x sec/l and have a Cot 1/2 of 2.1×10^{-1} mol x sec/l. The frequency of repetition and the kinetic complexities of various fractions are summarized in Table 3.6.

In order to determine the number of components reannealing with second order kinetics, the reassociation curve of the Cot 1 DNA in each species was analyzed by the curve fitting procedure of Laird and McCarthy (1969) using the formula:

$$\frac{C}{CO} = \frac{1}{1 + K_2\ Cot}$$

where $\frac{C}{CO}$ is the fraction of denatured DNA and K_2 is the reciprocal of Cot 1/2 (Britten and Kohne, 1966). The close fit of the ideal curve with the experimental reassociation

curve of finger millet Cot 1 DNA indicates that the complete reassociation curve of the Cot 1 finger millet DNA actually consists of nucleotide sequences reassociating with second order kinetics (Fig. 3.16). No curve fitting, however, was possible for the different components of pearl millet and rice Cot 1.0 DNAs by our procedure. Hence these fractions actually consist of different classes of repetitive DNA.

DISCUSSION

Tm of native DNAs

In Gramineae, the Tm values of only a few species like wheat, oat, rye and barley have been determined and are in the range of 85 - 87°C (Bendich and McCarthy, 1970a; Ranjekar *et al.* 1974; 1976). The melting curves of all these species have been found to be smooth. Among the DNAs of finger millet, great millet, pearl millet and rice the Tm value of pearl millet DNA is 88.6°C and is thus found to be the highest value among the Gramineae species studied so far. Wimpee and Rawson (1979) have earlier reported the Tm value of pearl millet DNA (89.2°C) and it compares well with that of ours. A question naturally arises, why the Tm of pearl millet DNA should be so high? Is there any ecological significance which is of value to the plant? Or alternatively is this only a species specific property? No data are available on a possible coorelation between the Tm of a plant DNA and its ecology. Hence species specificity may be the most likely explanation.

G + C content

We have used two methods to arrive at an estimate of G + C content in the DNAs of the present four species. From

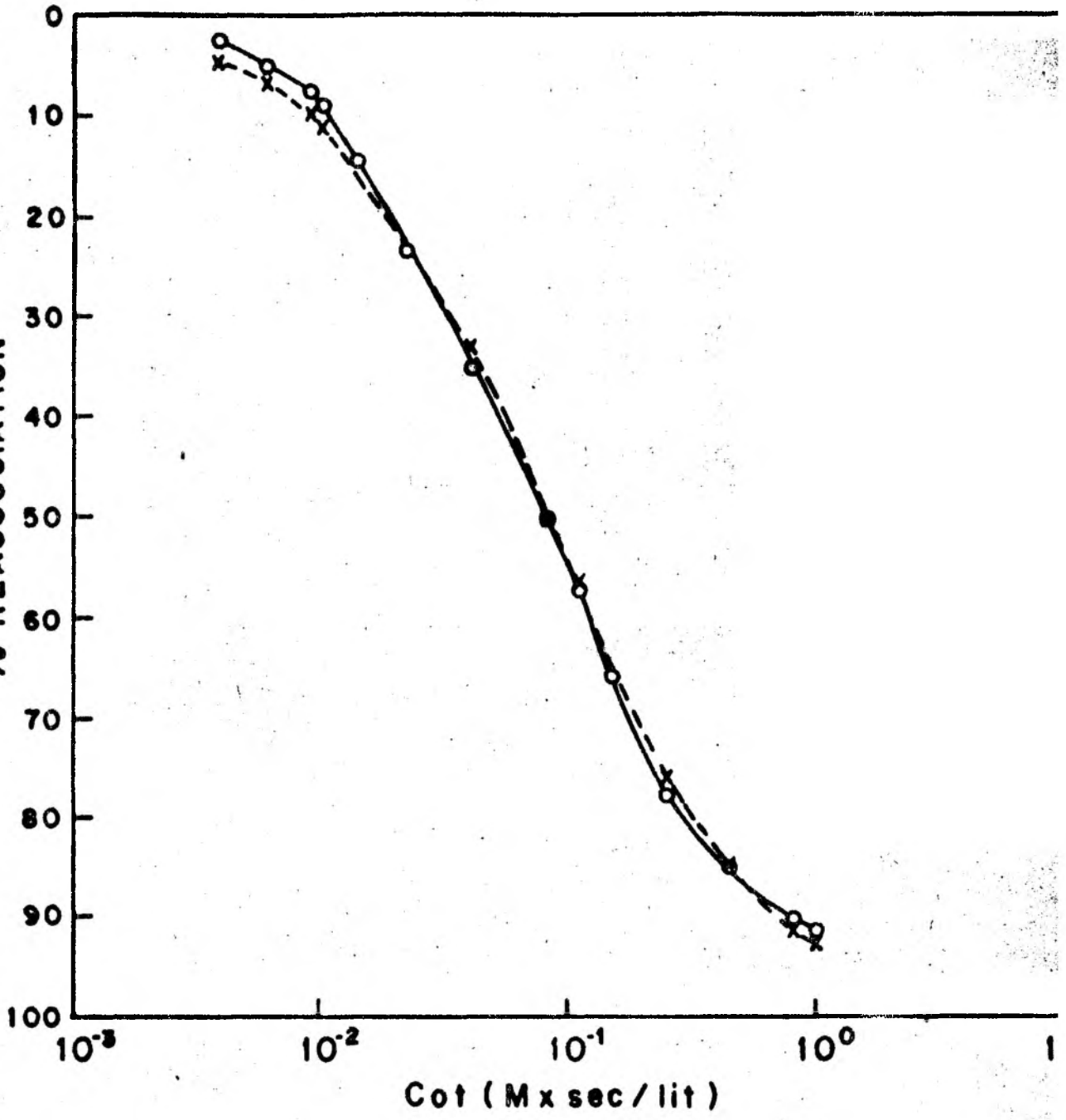


Fig. 3.16: Ideal Cot curve (-x--x-) and the experimental Cot curve (-o—o-) of the Cot 1.0 DNA of finger millet. The ideal curve is obtained by using the equation:

$$\frac{C}{CO} = \frac{1}{1 + K_2\ Cot}$$

where $\frac{C}{CO}$ = fraction of denatured DNA,

K_2 = reciprocal of Cot 1/2 of the Cot 1 DNA fraction.

Table 3.6: Kinetic complexity of Cot 1.0 DNA

	Fraction of Cot 1.0 DNA[a]	Cot 1/2 values observed[a] mol x sec/l	Frequency of repetition[b]	Cot 1/2 values pure[c] mol x sec/l	Kinetic complexity[d] Daltons	Kinetic complexity[d] Nucleotide pairs
Finger millet	1.0	0.082	9.7×10^3	0.082	5.1×10^7	7.7×10^4
Pearl millet Minor rapidly reassociating (Cot < 0.01 mol x sec/l	0.28	0.0015	1.5×10^6	0.00042	2.5×10^5	3.9×10^2
Major slowly reassociating (Cot 0.01 to Cot 1.0 mol x sec/l)	0.72	0.9	2.5×10^3	0.648	4.0×10^8	6.09×10^5
Rice Minor rapidly reassociating(Cot < 0.1 mol x sec/l)	0.35	0.01	2.5×10^6	0.0035	2.2×10^5	3.3×10^2
Major slowly Reassociating (Cot 0.1 to 1.0 mol x sec/l)	0.65	0.21	1.2×10^4	0.1365	8.4×10^7	1.28×10^5

[a] Values obtained from the optical Cot curves.

[b] Cot 1/2 values of unique DNA divided by Cot 1/2 values of given fraction.

[c] Cot 1/2 values observed x fraction of the genome.

[d] Values obtained from the standard relationship for Bacteriophage T_4 DNA where Cot 1/2 is 0.17 mol x sec/l and the number of np present is 1.6×10^5.

Table 3.3, it is clear that comparable values of G + C content are obtained using these two methods in the case of all the four plant species. Determination of G + C content from a Tm of a given DNA is a very standard method. Since the G + C content values estimated from the respective Tms are in close agreement with those derived from the absorption ratio method, the latter method can also be considered as an accurate method. In fact, the absorption ratio method appears to be a rapid and simple method and can be applied to DNA samples with high G + C content. This method is based on the aberration that there are relatively minor differences in the ultraviolet spectra in the 265 to 280 nm region, whereas the region from 240 to 265 nm including the λ maximum is progressively shifted towards shorter wavelengths with increasing values of the G + C mole % (Ulitzur, 1972). The standard deviation by this method is less than $\pm$ 0.85% as the possible error in the calculation of a ratio is decreased due to the use of a combination of five different wavelengths. In addition to these, one more important advantage of this method is that, even at protein concentrations three times (w/v) greater than the DNA concentration, a reliable determinatic of G + C % can be obtained using the absorbancy ratio of 245/270.

Interspecies comparison of thermal stability of Cot fractions

We have studied the thermal denaturation behaviour of different Cot fractions in finger millet, pearl millet and rice. These Cot fractions presumably consist of DNA sequences of varying degrees of frequency of repetition and kinetic complexity. By studying the melting profile of total, sonicated

DNA or of total repetitive DNA fraction, we only get an idea about the average Tm value and gross heterogeneity in base composition. Analysis of individual Cot fractions, which include DNA sequences reannealing in a narrow Cot range, enables us to pinpoint any specific differences which otherwise cannot be detected. This is very well illustrated in the case of pearl millet. In this species, the total DNA has a smooth melting curve, whereas Cot 0.1, 1.0 and 10.0 DNA fractions show a clear biphasic melting profile. The high melting component seen in the above DNA fractions is not observed in Cot 0.1 to 1.0 and Cot 1.0 to 10.0 DNA fractions (Table 3,4). This suggests that the high melting fraction is a component of very rapidly reassociating DNA (Cot $<$ 0.1 mol x sec/l) and is restricted to that fraction only. This is confirmed by the lowering in the proportion of high melting fraction in Cot 1.0 and Cot 10.0 DNA fractions. The proportion of high melting DNA component in Cot 0.1 DNA is 50% of the total Cot 0.1 DNA. Since Cot 0.1 DNA itself represents 20% of the total pearl millet DNA, the high melting component would account for 10% of the total DNA. Similar calculations carried out for Cot 1.0 and Cot 10.0 fractions reveal the proportion of high melting component as approximately 11% and 7% of the total DNA, respectively. The latter proportion is somewhat a low value than expected.

If we compare the Tm values of the different Cot fractions in each species, the variations are quite small suggesting a similarity in their G + C content. We have not been able to characterize these Cot fractions by using other methods such

as isopycnic centrifugation in neutral CsCl gradients. No conclusions can, therefore, be arrived at about the general similarity in the Tm values of different Cot fractions.

Nucleotide sequence divergence in repetitive DNA fractions

The thermal denaturation data of the repetitive DNA fractions in all the three Gramineae species (Table 3,4) have revealed varying percentages of base mismatch. In general, it is known that the degree of base mismatch in a given DNA fraction gives an indication of the extent of sequence divergence. Thus the DNA sequence divergence will be more if there is a substantial base mismatch in the DNA duplexes. In plants, only a limited data are reported on the thermal stability of repetitive DNA duplexes. In rye, wheat, barley and tobacco (Ranjekar et al., 1974; 1976; Smith and Flavell, 1975; Zimmerman and Goldberg, 1977) for example, upto 15% base mismatch is observed in repetitive duplexes indicating a considerable degree of sequenc divergence. The repetitive DNA fraction in finger millet shows a base mismatching of 10 - 13% and thus resembles the repetitiv DNAs in the above species. In the case of pearl millet Cot 0.1 and Cot 1.0 DNA fractions, there is comparatively less base mismatching (approximately 5%) and therefore less sequence divergence (Table 3.4). The repetitive DNA of rice is rather unique in consisting of nucleotide pairs with very low percent base mismatch and hence, negligible sequence divergence.

Kinetic complexity of Cot 1 DNAs

The optical reassociation data have enabled us to get some information about kinetic heterogeneity of DNA sequences reannealing by Cot 1.0. The lowest Cot values at which the

percentage reassociation is measured during optical reassociation studies is of the order of 10^{-3} mol x sec/l. At this Cot, approximately 5 - 11% of the Cot 1 DNA forms duplexes. We have determined the collapse hypochromicity as 2.5 - 3% caused by increased secondary structure when denatured DNA is cooled from 98°C to 62°C. The collapse hypochromicity is also calculated using the formula of Bendich and Anderson (1977).

$$\% \text{ Collapse} = a + (t_{25} - t_i)\ (0.0078\ GC + b)$$

where a = 12.6 for 0.12 M PB

t_{25} = Tm -25°C

t_i = incubation temperature

GC = percentage G + C value obtained from Tm

b = 0.31 for 0.12 M PB

From above calculation, we get % collapse hypochromicity as 11.37% for finger millet. The hyperchromicity of total finger millet DNA is 20%. So the collapse hypochromicity is (20% x 11.37%)/100 = 2.27%. This value of 2.27 is similar to the experimental value of 2.5%. Similarly the calculated value of 3.4% for pearl millet is very close to the experimental value (3.0%). Only in the case of rice, the calculated value 1.36% is rather low as compared to the experimental value (2.5%). If we substract the collapse hypochromicity value from the percent reassociation at Cot 10^{-3} mol x sec/l, the remaining 2 - 8% would most probably represent mainly the highly repetitive DNA sequences.

During the study of optical reassociation of Cot 1.0 DNA fraction in all three species (Figs. 3.13, 3.14 and 3.15), only

one kinetic component was observed in the case of finger mille with frequency of repetition as 9.7×10^3 and complexity as 7.7×10^4 np. The curve fitting procedure revealed that this fraction followed a second order kinetics indicating the presence of one discrete frequency class of DNA sequences (Fig. 3.16). Relatively discrete frequency components which can be physically isolated and shown to reassociate essentiall by second order kinetics, do exist in several (but not all) an genomes (Britten and Davidson, 1971; Hough and Davidsnn, 1972 Of the higher plant genomes so far analyzed in this way, only tobacco has been shown to contain a large fraction of repetiti DNA clearly belonging to three discrete frequency classes (Zimmerman and Goldberg, 1977).

Two distinct kinetic fractions are observed in the case of pearl millet and rice Cot 1.0 fractions. The minor fast reassociating fraction, which represents 28 to 35% of the tota Cot 1.0 DNA in these two species, has a kinetic complexity in the range of 330 to 390 np. The major slow-reassociating fraction is more complex and consists of 1.3 to 6.0×10^5 np. The Cot 1.0 DNA fraction in pearl millet and rice, therefore, resembles some of the plant satellite DNAs which have been shown to consist of at least two kinetic components (Bendich and Anderson, 1974; Chilton 1975; Sinclair et al., 1975; Timmis et al., 1975; Beridze and Bragvadze, 1976; Capesius, 1976; Bendich and Taylor, 1977; Timmis and Ingle, 1977; Ranjekar et al., 1978a; 1978c; Beridze, 1980a; 1980b).

These fractions in pearl millet and rice do not show the close fit between the experimental curve and the ideal curve for second order reaction. This clearly indicates the

heterogeneity of these fractions. One reason for lack of close fiting can be the reassociation of completely complementary base pairs between essential homologous sequences does not take place in the case of pearl millet and rice Cot 1 DNA fractions. This can be explained by comparatively low thermal stability in the case of pearl millet Cot 1.0 DNA. However, in the case of rice, the Cot 1.0 duplexes are as stable as native, sonicated DNA. Hence the possible reason of lack of close fit between experimental and ideal curve may be the existence of many unrelated families of repetitive DNA sequences with different kinetic complexities in rice Cot 1.0 DNA.

From the thermal denaturation data in Table 3.4, it is clear that in finger millet the nucleotide sequences in Cot 1.0 DNA exhibit a base mismatching of the order of 12.8%, whereas, those in the low melting component of the Cot 1 DNA fraction in pearl millet reveal a base mismatching of 5.4%. Since base mismatching has been shown to cause an increase in the Cot 1/2 value of a DNA fraction (Sutton and McCallum, 1971) the complexities of the Cot 1 DNA fractions in finger millet and pearl millet are likely to be still simpler than those calculated in Table 3.6. In the case of pearl millet Cot 1.0 DNA, the correlation between the two thermal fractions and the two kinetic fractions has not yet been studied. Even at lower Cot values such as 0.1 mol x sec/l, the two thermal and the two kinetic fractions are evident. In view of a high Tm value of 94.8°C of one of the two thermal fractions of Cot 1.0 DNA, it is very likely that one of the kinetic components of Cot 1.0 DNA consists of very similar nucleotide sequences.

CONCLUDING REMARKS

Thermal stability of native, unsonicated DNAs of four Gramineae species is in the range of 85°C to 88.6°C. The Tm of pearl millet DNA (88.6°C) is the highest in all the four species as well as in other plant species studied so far. The G + C contents estimated from thermal stability compare well with those from spectrophotometric method and are in the range of 38.0 to 48.0%.

Thermal denaturation properties of different Cot fractions of finger millet, pearl millet and rice are studied to know more about their base composition and heterogeneity. The melting temperatures of different Cot fractions of finger millet and rice are in the range of 70.8 - 73.6°C and 79.6 - 82.6°C, respectively.

In the case of pearl millet, Cot 0.1, 1.0 and 10.0 DNA fractions show biphasic melting profiles. The melting curves of Cot 0.1 to 1.0 and 1.0 to 10.0 DNA fractions are monophasic. This clearly indicates that the DNA sequences of high melting fractions reanneal by Cot 0.1 mol x sec/l.

The base mismatching is observed to be maximum (10 - 13%) in the case of finger millet, intermediate (3.8 - 9%) in the case of pearl millet and negligible (0.8 - 3.8%) in the case of rice.

Optical reassociation of Cot 1.0 DNAs of these three species reveals the presence of two components in the case of pearl millet and rice and only one component in the case of finger millet. Finger millet Cot 1.0 fraction reassociates with second order kinetics indicating the presence of one discrete component

On the other hand, pearl millet and rice Cot 1.0 fractions do not reassociate with second order kinetics and are heterogeneous.

CHAPTER IV

DNA SEQUENCE ORGANIZATION IN THREE GRAMINEAE SPECIES

DNA SEQUENCE ORGANIZATION IN THREE GRAMINEAE SPECIES

INTRODUCTION

DNA sequence organization refers to the arrangement of repeated and nonrepeated DNA sequences in a genome. After having known that the fraction of an eukaryotic genome consists of repeated sequences, it is of interest to know how these sequences are arranged or distributed with respect to single copy sequences and what are their approximate lengths. Such information is expected to throw light on several important questions such as gene regulation.

In earlier studies on repetitive DNA in bovine and mouse, it was indicated that some repetitive sequences were scattered throughout the genome (Bolton et al., 1966; Waring and Britten, 1966; Britten, 1969; Britten and Smith, 1970). However, during the period of last ten years, DNA sequence organization has been studied in detail in a large number of animal and plant species. Based on these data, it is now known that there is an interspersion of repeated and single copy DNA sequences and that there are two main patterns of DNA sequence interspersion namely short period and long period.

Plant genomes have been observed to differ from those of animals in two important respects. Firstly, they have relatively more proportion of repetitive DNA sequences. Secondly, the lengths of the interspersed repeated sequences are highly variable in different species. In Chapter II and III, we have described the genome characterization of four Gramineae species. We have also carried out the DNA sequence organization studies in finger millet, pearl millet and rice

to determine whether they differ from the other well characterized Gramineae species, namely wheat (Flavell and Smith, 1976) and rye (Smith and Flavell, 1977) in the mode of arrangement of repeated and single copy DNA sequences. For these studies, the reassociation kinetics of DNAs of different fragment lengths were first determined in the Cot range of 10^{-4} to 5×10^{1} mol x sec/l. The reassociated duplexes were characterized by hyperchromicity measurements. Sizing of the S_1 nuclease resistant repetitive duplexes was carried out by agarose column chromatography as well as agarose gel electrophoresis.

The present work on DNA sequence organization in finger millet, pearl millet and rice was undertaken in early 1979. However, by the time the work on pearl millet genome was compl a report on DNA sequence organization in pearl millet genome appeared by Wimpee and Rawson (1979). Our data on pearl millet genome are found to be comparable to that of Wimpee and Rawson (1979) and are given in full details in this thesis

MATERIALS AND METHODS

Extraction of DNA

DNAs of finger millet, pearl millet and rice were isolate from eight days old plants as described in Chapter II.

DNA shearing and sizing

DNA fragments of an average length of 550 np were obtaine by sonication for three minutes using Biosonic III fitted with half an inch probe as described in Chapter II. Sonication wit 1/4 inch probe for one minute at 20 setting yielded DNA fragments of 1500 np. Larger DNA fragments (4900 np) were

obtained by homogenizing the DNA solution in Sorvall Omnimixer (Model No. 17106) for 6 mins at 25,000 rpm. DNAs of fragment size in the range of 1000 - 3000 np were obtained using Virtis 60 K homogenizer by varying the conditions of speed and time.

The average fragment lengths of the above sheared preparations and of native DNA samples were estimated by ultracentrifugation in neutral sucrose density gradients as described in Chapter II as well as by agarose gel electrophoresis as described later.

DNA reassociation kinetics

Reassociation kinetics of finger millet, pearl millet and rice DNAs of all sizes were measured as described in Chapter II. Separation and estimation of denatured and reassociated DNA fractions were carried out as described in Chapter II.

Thermal denaturation of repetitive DNA fractions

Thermal denaturation studies of repetitive DNA fractions from DNAs of all sizes were carried out in a Gilford spectrophotometer 250 equipped with thermoprogrammer (Model No. 2527), analog multiplexer (Model No. 6046) and reference compensator as described in Chapter III, In these studies, the data were used without any correction.

Single strand collapse was determined according to the procedure of Davidson et al. (1973). Unreassociated, unique DNA which included the DNA sequences reassociating after Cot 25 mol x sec/l in finger millet, Cot 10 mol x sec/l in pearl millet and Cot 50 mol x sec/l in rice was isolated by HA column chromatography. It was melted in 0.12 M PB, pH 6.8 and the hyperchromicity value was observed to be in the range of

1% to 1.5% in all the species.

S_1 nuclease digestion

These experiments were carried out according to the procedure of Murray et al., (1978) with some modifications. Native DNAs of fragment size in the range of 5700 - 8200 np were dialyzed against 0.18 M NaCl plus 0.006 M PIPES buffer, pH 6.8. The DNA samples were denatured in boiling water bath for 10 min and allowed to reassociate at 62°C to a specific Cot value. After reassociation to specific Cots, the DNA samples were adjusted to 25 mM sodium acetate (pH 4.5), 0.1 mM $ZnSO_4$, 25 mM 2-mercaptoethanol (Goldberg et al., 1975; Britten at al., 1976). At least 1.5 to 2 mg of DNA was used for each experiment. 10 units of S_1 nuclease (Boehringer) per µg of DNA were added and the samples incubated for one hour at 37°C so as to remove all single stranded regions. The reaction was terminated by adjusting the samples to 0.12 M PB (pH 6.8) and cooling the samples to 4°C. S_1 nuclease resistant duplexes were then separated by HA column chromatography by their specific elution at 0.4 M PB (pH 6.8). These samples were dialyzed against 0.12 M PB (pH 6.8) for melting analysis as well as to determine the size of the duplex DNA.

Sizing of S_1 nuclease resistant reassociated duplexes

Agarose column chromatography

The size distribution of S_1 resistant duplexes was determined by gel filtration on agarose A_{50} (100 - 200 mesh size, Biorad Laboratories) column, previously calibrated with calf thymus DNA of known duplex lengths.

(a) Isolation of calf thymus DNA of known fragment length

Calf thymus DNA of size more than 1500 np was obtained commercially. It was then sonicated for three minutes using Biosonik III as described in Materials and Methods of Chapter to get 550 np long DNA. Calf thymus DNA of 250 np fragment size was obtained by homogenizing it in Virtis 60K homogenizer for 50 min with 55,000 rpm. The sizes were determined by sucrose density gradient centrifugation and agarose gel electrophoresis.

(b) Preparation of agarose column

The agarose gel matrix (fully hydrated in 0.001 M Tris-EDTA buffer, Biorad Laboratories) was poured around 4 mm glass beads in a 92 cm x 1.5 cm column. The column was equlibriated with 0.12 M PB (pH 6.8). The column was reused a number of times, by washing it with 0.12 M PB (pH 6.8) each time after eluting the sample completely.

(c) Elution of DNA fragments

Approximately 70 - 100 μg of S_1 nuclease resistant duplexes, separated by HA column chromatography, were loaded the column and the elution was carried out with 0.12 M PB (pH 6.8). The effluent was continuously monitored with the help of LKB Uvicord at 253 nm (Sachs and Painter, 1972; Wimp and Rawson, 1979). The exclusion limit of this column was 1500 nucleotide pairs.

Agarose gel electrophoresis

The DNA fractions eluted from the agarose columns were analyzed by agarose gel electrophoresis for exact sizing. These experiments were carried out as described by Thomas

and Davis (1975) with slight modifications.

Agarose solution was prepared by dissolving agarose (1.4% w/v) in Tris/Borate buffer (0.089 M Tris, 0.089 M boric acid, pH 8.5 and 2.5 mM Na_2 EDTA) and heated in a boiling water bath. The hot agarose solution (around 60 - 80°C) was poured in glass tubes (15 cm x 0.6 cm inner diameter) which were sealed at the bottom with dialysis tubing. The gels were allowed to set for about one hour. Then the tubes were filled with Tris/borate buffer and the gels stored in cold (4°C) till further use. Abo 2 - 3 µg of DNA sample in Tris/borate buffer adjusted to 10% sucrose was layered under the buffer on the top of each gel and electrophoresis was carried out at 50 volts for 5 h at room temperature. After electrophoresis, the gels were stained overnight with 0.004% solution of toluidine blue and were then destained in distilled water (Phillippsen and Zachau, 1972; Phillippsen et al., 1974). As a standard, λ DNA (Boehringer) hydrolysate was used. About 5 µg of λ DNA in 0.2 ml of Tris/ borate buffer was taken and 0.049 ml of 0.5 M Tris-HCl, 0.25 M NaCl and 0.05 M $MgCl_2$ at pH 7.5 was added. To this, 1 µl of restriction endo nuclease EcoRI (Boehringer) was added and the mixture was incubated at 37°C for 1 h. To stop the reaction, 0.05ml of 7 mole/l urea solution, 50% (w/v) sucrose, 0.1 M mole/ l Na_2 EDTA, pH 7.0 was added. This λ DNA hydrolysate was applied to the gel and electrophoresis was carried out as described above. The gels were stained with toluidine blue and destained in water. These gels were scanned at 546 nm in Gilford 250 spectrophotometer equipped with gel scanner. Six bands of different molecular weights were observed. As the separation

and resolution of nucleic acids in agarose gel electrophoresis is depending on the basis of molecular size (Johnson and Grossman, 1977), within a limited range, the electrophoretic mobility of DNA can be an approximately linear function of the logarithum of molecular weight (Helling et al., 1974; Moore et al., 1977; Fangman, 1978). Hence, a graph of log molecular weight against electrophoretic mobility for known molecular weights of fragments of λ DNA cleaved by EcoRI was plotted. From this graph, the molecular weights of S_1 nuclease resistant repetitive duplexes of finger millet, pearl millet and rice DNA as well as native DNAs of different fragment sizes were determined.

RESULTS

Reassociation kinetics of DNAs of different fragment lengths

The most reliable method used in understanding the DNA sequence organization in a genome is to study the reassociation kinetics of DNAs of different fragment sizes under identical Cot conditions (Davidson et al., 1973; Graham et al., 1974; Goldberg et al., 1975). The reassociation kinetics of DNAs of finger millet (Fig. 4.1), pearl millet (Fig. 4.2) and rice (Fig. 4.3) of four different fragment lengths in the range of 550 - 8200 np were studied. As no labelled DNAs were used, the reassociation experiments were carried out in the Cot range of 10^{-2} to 5.0×10^{1} mol x sec/l only. By Cot 5.0×10^{1} mol x sec/l, almost all repetitive DNA sequences and very few single copy sequences were assumed to reassociate (Deshpande and Ranjekar, 1980).

In the case of finger millet (Fig. 4.1), the extent of

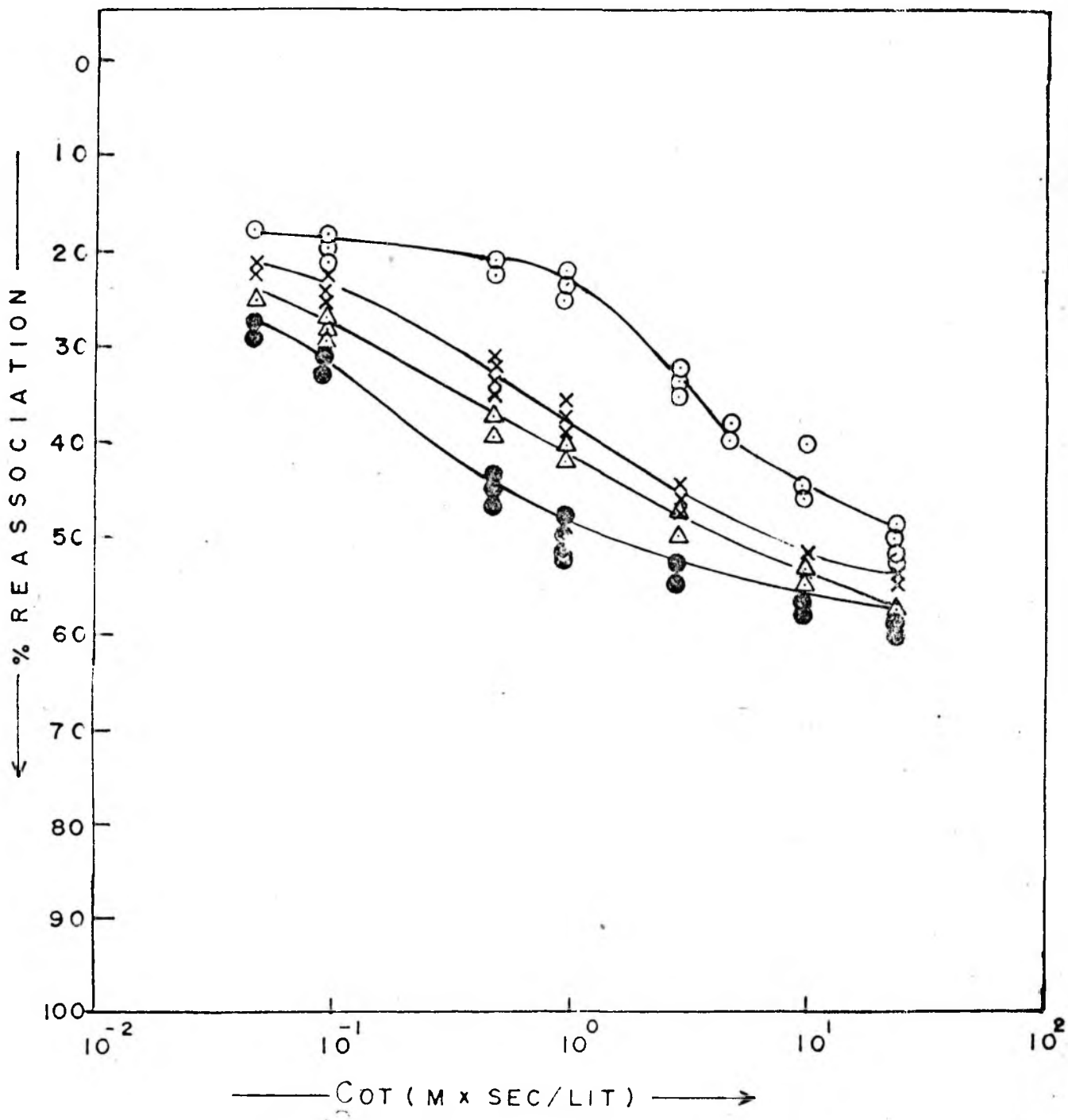


Fig..4.1: Reassociation kinetics of DNAs of long fragment lengths. The DNAs of four fragment lengths of finger millet were obtained as described in Materials and Methods and their reassociation was studied in the Cot range of 10^{-1} to 2.5×10^{1} mol x sec/l. Reassociation curves of finger millet DNA (-O-O-) 550 np; (-*-*-) 1500 np; (-Δ-Δ-) 4900 np and (-●-●-) 5700 np.

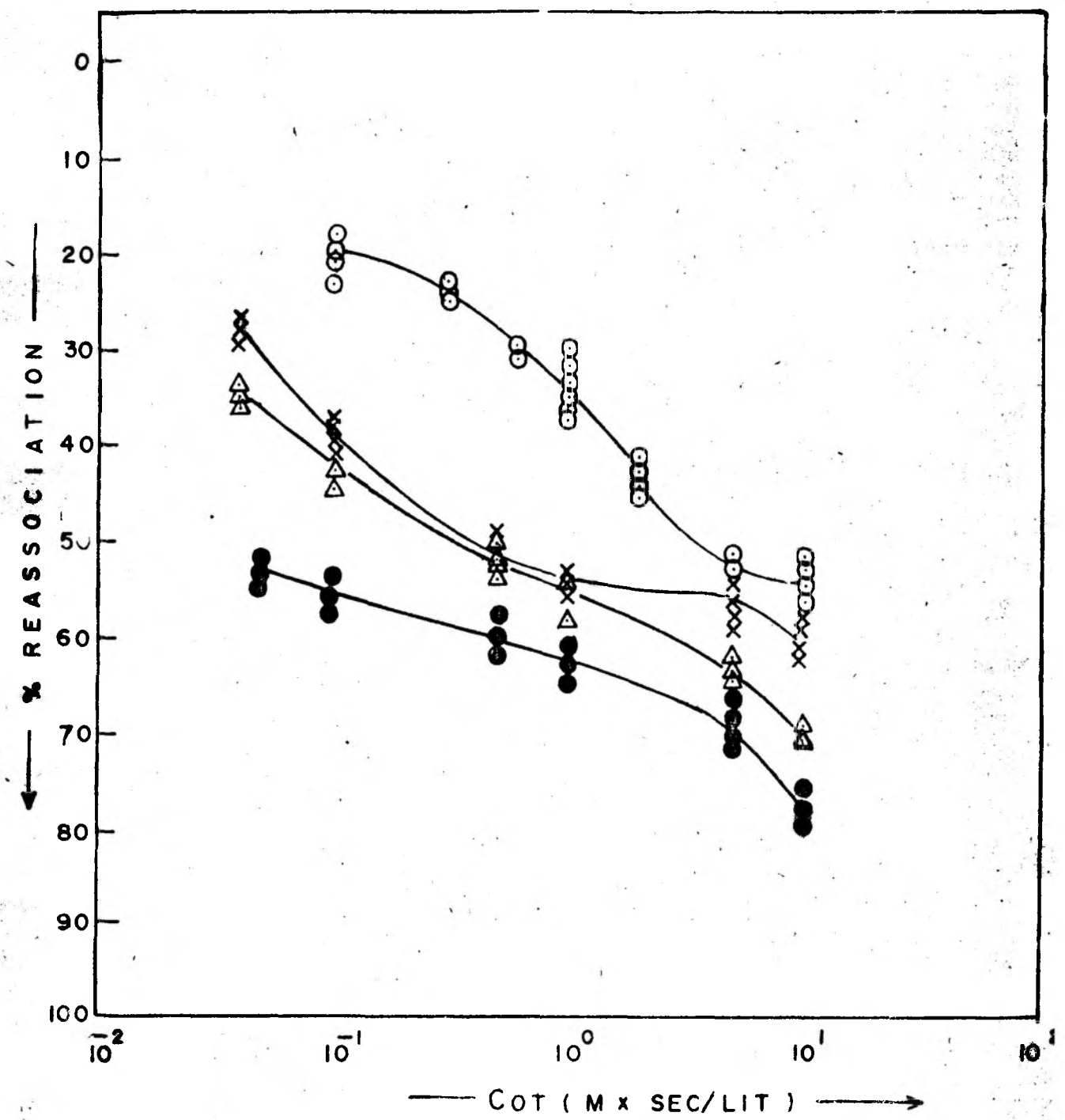


Fig. 4.2: Reassociation kinetics of DNAs of long fragment lengths. The DNAs of four fragment lengths of pearl millet were obtained as described in Materials and Methods and their reassociation was studied in the Cot range of 10^{-1} to 10^{1} mol x sec/l. Reassociation curves of pearl millet DNA (-o-o-) 550 np; (-x-x-) 1500 np; (-Δ-Δ-) 4900 np and (-●-●-) 8200 np.

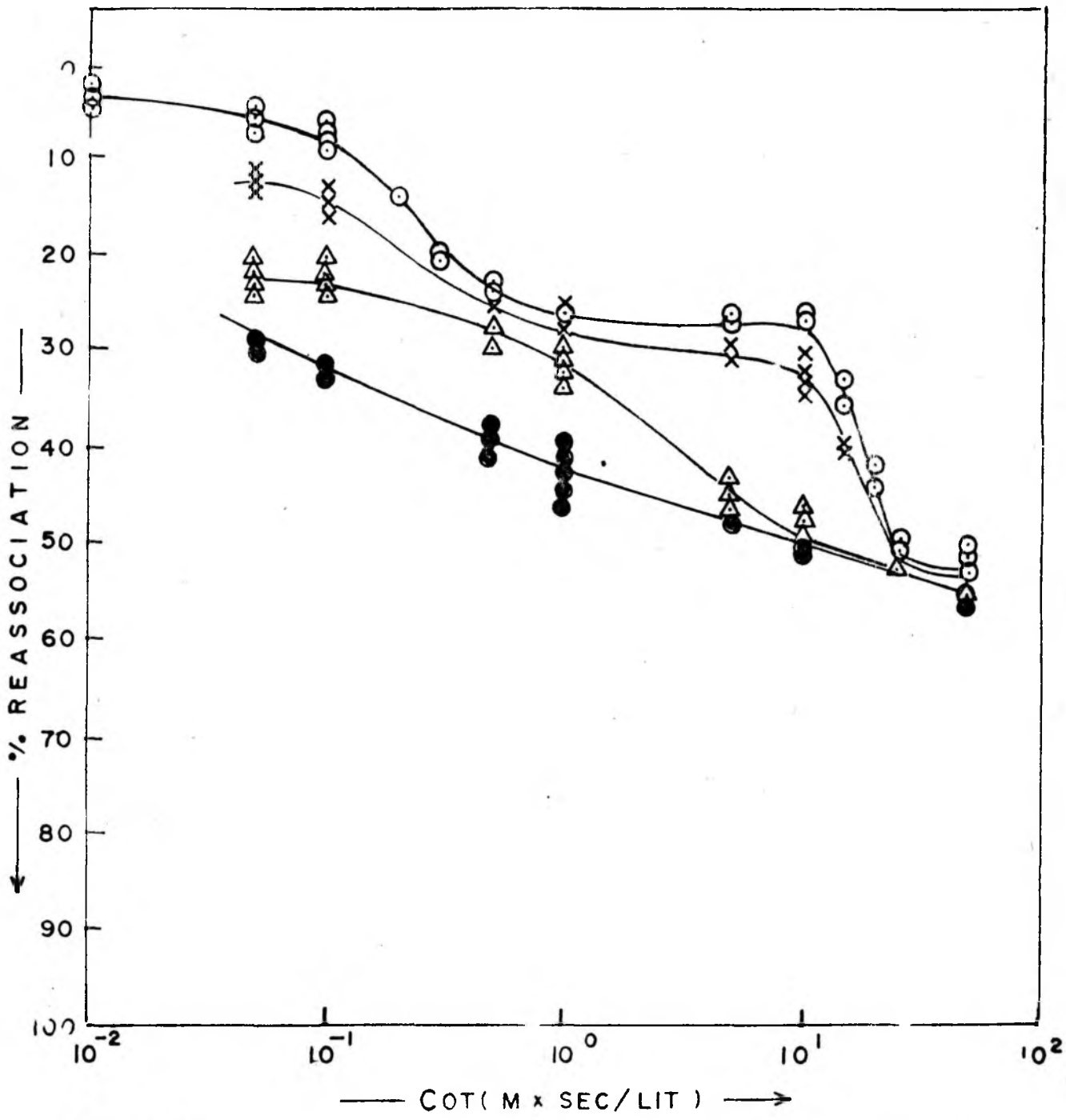


Fig. 4.3: Reassociation kinetics of DNAs of long fragment lengths. The DNAs of four fragment lengths of rice were obtained as described in Materials and Methods and their reassociation was studied in the Cot range of 10^{-2} to 5×10^{1} mol x sec/l. Reassociation curves of rice DNA (-o-o-) 550 np; (-x-x-) 1500 np; (-Δ-Δ-) 4900 np; (-●-●-) 6500 np.

HA binding increases from 18% to 28% and from 49% to 58% at Cot 0.5 and 25.0 mol x sec/l, respectively, as the fragment length increases from 550 np to 5700 np. This is quite in contrast to the situation in pearl millet (Fig. 4.2). In the latter, 19% and 54% of 550 np long DNA reassociates by Cot 0.1 and Cot 10.0 mol x sec/l, respectively, while 56% and 77.5% of 8200 np long DNA is retained on the HAP column at Cot 0.1 and Cot 10.0 mol x sec/l, respectively. Thus the increase in the reassociation (37% at Cot 0.1 and 23.5% at Cot 10.0 mol x sec/l) as the fragment length increases, is very significant in the cas of pearl millet.

The reassociation kinetics of rice DNAs of different fragment lengths are strikingly different from those of finger millet and pearl millet (Fig. 4.3). The Cot curves of 550 np and 1500 np rice DNA reveal the presence of two different kineti fractions separable at Cot 1.0 mol x sec/l. This type of distinction becomes unclear in the reassociation curve of 4900 n long DNA and is completely absent in the Cot curve of 6500 np long DNA. There is a slight increase (52% to 55%) in the reassociation value at Cot 50 mol x sec/l with increasing DNA fragment lengths from 550 np to 6500 np. However, since the experimental scatter of 5% to 8% is observed in the percentage reassociation values at each Cot point, this increase in the reassociation value is negligible. The reassociation at lower Cot values shows some differences with the DNAs of different fragment lengths. For example, 6% of the DNA 550 np long and 29% of the DNA 6500 np long are retained on HA at Cot 0.05 mol x sec/l.

An increase in the binding to HA as a function of DNA fragment length is considered to be an indication of the occurrence of interspersion of repetitive and nonrepetitive DNA sequences (Wetmur and Davidson, 1968; Davidson et al., 1973). The reassociation data on finger millet and pearl millet indicate that the repeated sequences may be interspersed with nonrepeated DNA sequences. The results in the case of rice, however, reveal that there may not be an interspersion of repeated and single copy DNA sequences.

Before arriving at the conclusion that there is DNA sequence interspersion, it is necessary to study the effect of DNA fragment length on the extent of binding of DNA fragments to HA. DNA fragments containing both single copy and repetitive sequence elements on the same strands will have rate constants greater than those predicted by the effect of fragment length alone (Wetmur and Davidson, 1968; Davidson et al., 1973). If there is no interspersion of different kinetic components then the reassociation rate will solely be affected by the fragment length and the observed rate constants of repetitive DNA fractions will only vary as a function of the square root of the ratio of the fragment lengths of the two fractions in comparison; this being represented by the following formula:

$$K1/K2 = (L1/L2)^{0.5}$$

where L1 = short fragment size in np

L2 = long fragment size in np

K1 = rate constant for reassociation of short (L1) fragments

K2 = rate constant for reassociation of long (L2) fragments

(Wetmur and Davidson, 1968). Table 4.1 summarizes the observed and the predicted rate constants for different fragment lengths of finger millet, pearl millet and rice. If there is no interspersion of repetitive and nonrepetitive DNA, then the observed rate constant (K_{obs}) will be equal to the predicted rate constant (K_{pred}) and the ratio of K_{obs}/K_{pred} will become 1. In the case of finger millet and pearl millet the observed rate constants are in the range of 0.7 to 13.0 and 2.0 to 25.0 $l.mol^{-1}.sec^{-1}$, respectively (Table 4.1) and are greater than the rate constant predicted by considering the effect of fragment length alone. Thus the ratios K_{obs}/K_{pred} vary in the range of 3.0 to 6.0 in the case of finger millet and pearl millet, suggesting the presence of single copy DNA sequences in an unreassociated form contiguous to repetitive duplexes. Hence it appeares that about 58% of the total genome of finger millet (5700 np) and 77.5% of that of pearl millet (8200 np) consist of interspersed sequences.

In the case of rice, the observed rate constants of the repeated DNA sequences at different fragment lengths are not greater than the predicted rate constants which are calculated by taking into consideration the fragment lengths alone, except for the maximum size of 6500 np. Here the increase in the observed rate constant is due to the interspersion of different repetitive components. Similar studies carried out for the first kinetic component namely, highly repetitive DNA reassociating upto Cot 1.0 mol x sec/l, shows that the observed rate constants are greater than the predicted values for all the different sizes (Table 4.1). The most probable explanation

Table 4.1: Comparison of the experimental and the predicted rate constants (K) of the repeated DNA fractions of different fragment lengths

DNA	Fragment length np	Cot 1/2 observed[a] mol x sec/l	Observed K[b] $l.mol^{-1} sec^{-1}$	Predicted K[c] $l. mol^{-1} sec^{-1}$	$K_{observed}/K_{predicted}$
1	2	3	4	5	6
Finger	550	1.4×10^{0}	0.714	-	-
millet	1500	2.0×10^{-1}	5.000	1.179	4.24
Cot 25.0	4900	1.25×10^{-1}	8.000	2.1319	3.7525
mol x sec/l	5700	7.5×10^{-2}	13.300	2.2994	5.784
Pearl	550	4.8×10^{-1}	2.083	-	-
millet	1500	4.7×10^{-2}	21.27	3.44	6.183
Cot 10.0	4900	4.0×10^{-2}	25.00	6.217	4.0206
mol x sec/l	8200	$<4.0 \times 10^{-2}>$	25.00	8.045	>3.000
Rice	550	7.0×10^{-1}	1.428	-	-
Cot 50.0	1500	6.0×10^{-1}	1.666	2.358	0.7065
mol x sec/l	4900	3.8×10^{-1}	2.631	4.262	0.6173
	6500	4.0×10^{-2}	25.000	4.910	5.0916
Rice	550	1.9×10^{-1}	5.26	-	-
Cot 1.0	1500	8.2×10^{-2}	12.196	8.687	1.40
mol x sec/l	4900	$<5.0 \times 10^{-2}>$	20.00	15.701	> 1.0
	6500	$<5.0 \times 10^{-2}>$	20.00	18.088	> 1.0

[a]Calculated from the reassociation curves upto Cot 25.0, Cot 10.0

and Cot 50.0 mol x sec/l in case of finger millet, pearl millet and rice respectively (Fig. 4.1, 4.2 and 4.3).

$$b_K = \frac{1}{\text{Cot } 1/2}$$

[c]Calculated using $K_1/K_2 = (L1/L2)^{0.5}$ where K_1 and K_2 are the rate constants for the reassociation of short (L1) and long (L2) fragments, respectively.

of such phenomenon could be the absence of interspersion of repetitive and nonrepetitive DNA sequences and the presence of interspersion within repetitive sequence elements.

Hyperchromicity studies of reassociated repetitive DNA fragments

Since hyperchromicity of a given DNA fraction is nearly proportional to its duplex content (Davidson et al., 1973), the melting behaviour of repetitive DNA fractions of different fragment lengths was studied.

As shown in Fig. 4.4 and 4.5 and Table 4.2; there is a slight increase in the melting temperature of the reassociated duplexes (1.5 - 4.0°C) while the hyperchromicity decreases with increasing fragment length. The decrease in the hyperchromicity of finger millet repetitive duplexes is small (14.2% - 11.4%) whereas in pearl millet the decrease is significant (19.4% - 11.6%). Such decrease in the hyperchromicity of a repetitive DNA fraction with an increase in DNA fragment size is attributed to the increasing presence of single strand regions in the reassociated fragments. These single strand regions are most likely unique sequences which remain unreassociated at Cot 25.0 and Cot 10.0 mol x sec/l in the case of finger millet and pearl millet respectively and contribute very little (1% - 1.5%) to DNA hyperchromicity. These data, thus provide an additional evidence for the existence of repetitive and nonrepetitive DNA sequences on the same strand in finger millet and pearl millet genomes.

Hyperchromicity measurements of reassociated long fragments of DNA can be used to determine the average fraction of reassociated fragments which are in duplex structures (Davidson

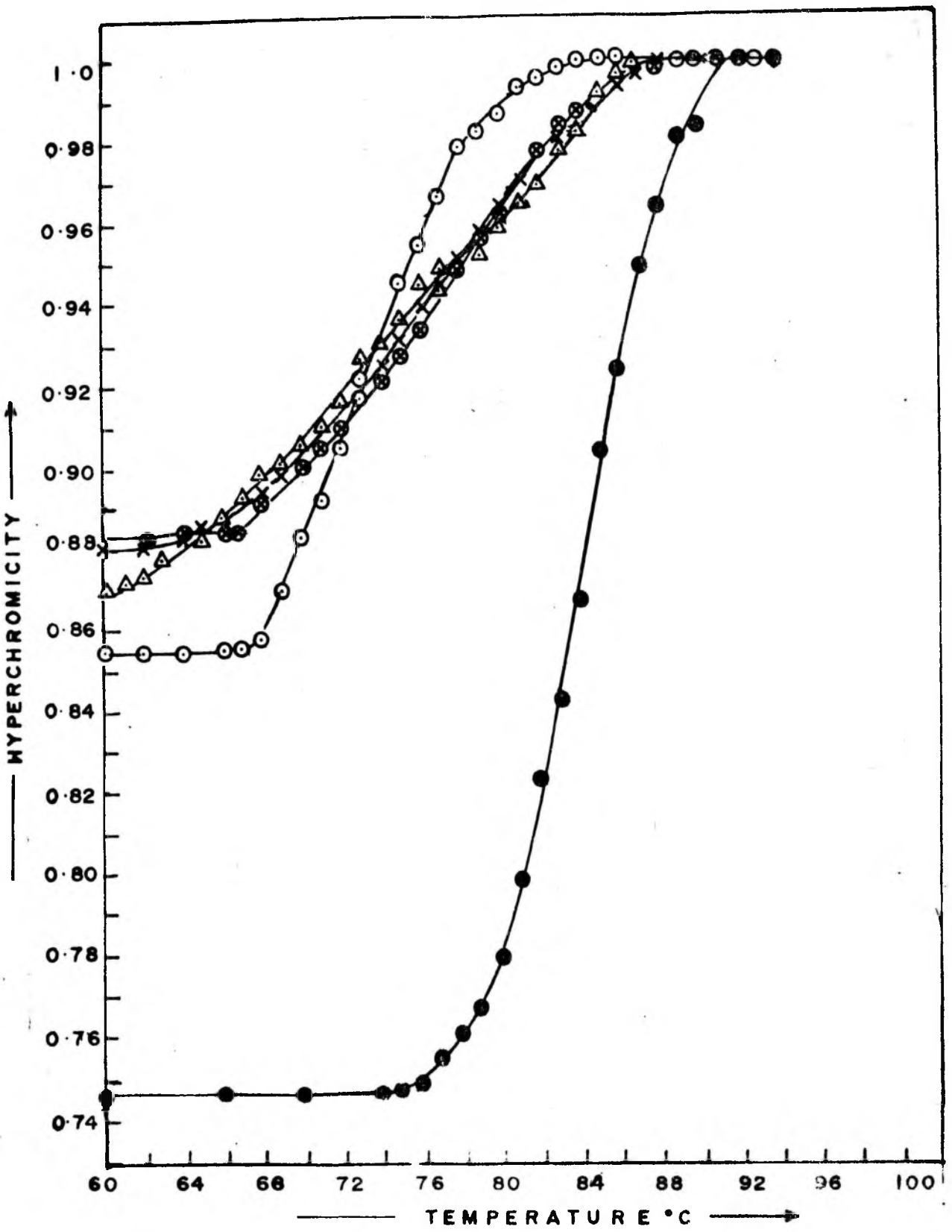


Fig. 4.4: Thermal denaturation profiles of finger millet Cot 25 DNAs of various fragment lengths, compared to that of native; 550 np long native DNA; (-o-o-) 550 np; (-Δ-Δ-) 1500 np; (-✕-✕-) 4900 np; (-⊗-⊗-) 5700 np and (-●-●-), native 550 np long DNA. Each point in these graphs represented an average of at least five experiments.

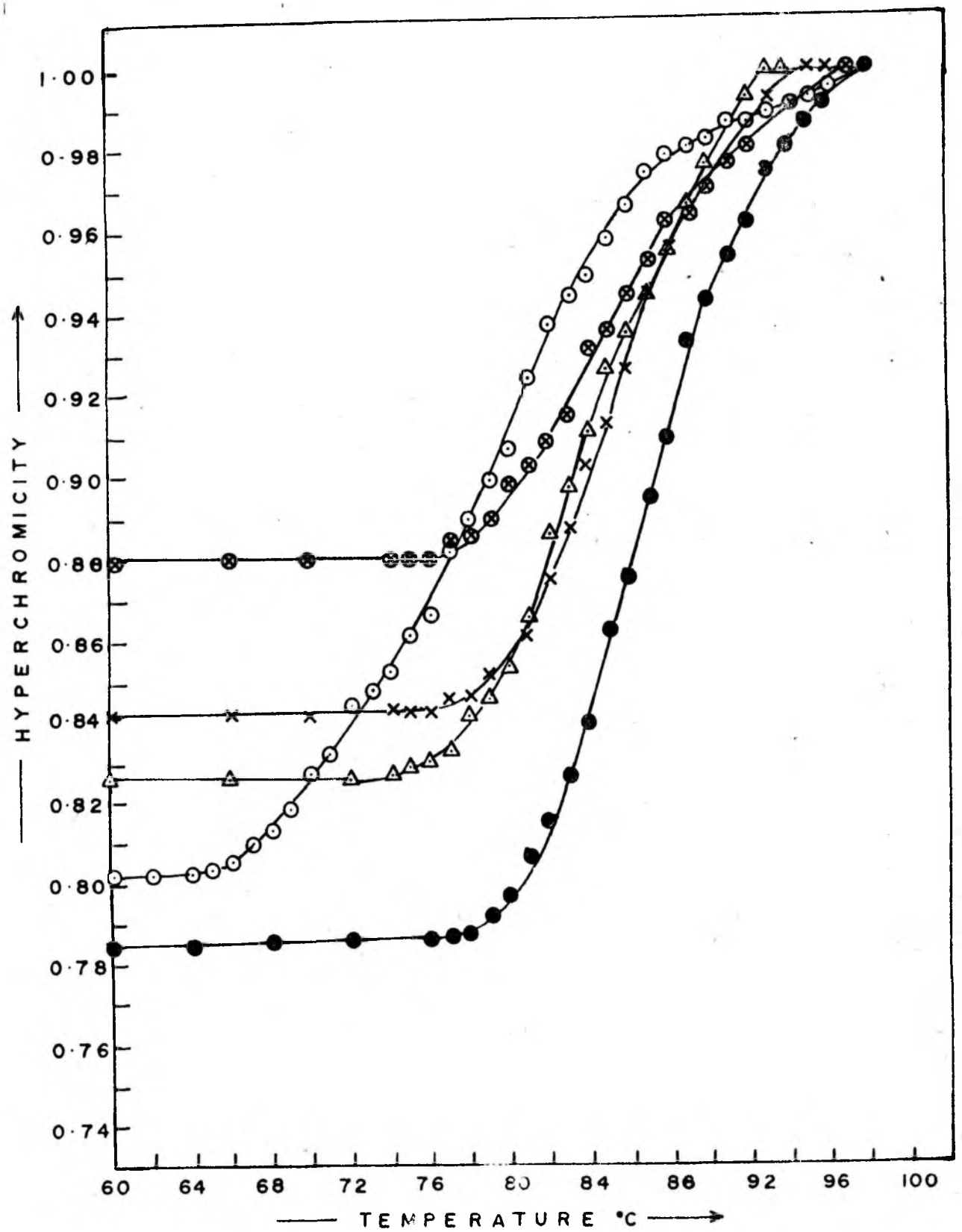


Fig. 4.5: Thermal denaturation profiles of pearl millet Cot 10 DNAs of various fragment lengths, compared to that of native 550 np long DNA; (-O-O-) 550 np; (-Δ-Δ-) 1500 np; (-✕-✕-) 4900 np; (-⊗-⊗-) 8200 np and (-●-●-) native, 550 np. long DNA. Each point in these graphs represented an average of atleast five experiments.

Table 4.2: Hyperchromicity and fragment lengths

					Native sonicated DNA
Finger millet					
1. Fragment size (np)	550	1500	4900	5700	550
2. Fraction bound to HA at Cot 25.0[a]	0.49	0.535	0.575	0.58	-
3. Hyperchromicity[b]	0.142	0.13	0.118	0.114	0.27
4. Tm °C[c]	73.0	75.0	76.5	77.0	83.8
5. Duplex content (D)[d]	0.498	0.459	0.404	0.39	-
6. Average duplex length[e]	273	676	1979	2223	-
7. Duplex content from S_1 nuclease[f]	-	-	-	0.41	-
Pearl millet					
1. Fragment size (np)	550	1500	4900	8200	550
2. Fraction bound to HA at Cot 10.0[a]	0.54	0.605	0.70	0.775	-
3. Hyperchromicity[b]	0.194	0.172	0.157	0.116	0.21
4. Tm °C[c]	(88%) 78.0	84.0	85.0	85.4	87.0
	(12%) 92.0				
5. Duplex content (D)[d]	0.918	0.805	0.728	0.517	-
6. Average duplex length[e]	504	1207	3568	4239	-
7. Duplex content from S_1 nuclease[f]	-	-	-	0.4955	-

[a]Obtained from Fig. 4.1 and 4.2.

[b]Obtained from Fig. 4.4 and 4.5. Hyperchromicity (H) was

calculated using the formula:

$$H = \frac{A_{260}\ (98°C) - A_{260}\ (60°C)}{A_{260}\ (98°C)}$$

(Zimmerman and Goldberg, 1977; Wimpee and Rawson, 1979).

[c]Obtained from Fig. 4.4 and 4.5.

[d]The average duplex content (D) of bound fragment was estimated using the formula:

$$D = \frac{H - \text{single strand collapse}}{H\ (\text{native sonicated DNA}) - \text{single strand collapse}}$$

The hyperchromicity of the denatured single copy was determined in order to obtain a value for single strand collapse and was of the order of 1.5% (Graham et al., 1974).

[e]The average length of duplex region is the product of the duplex content (D) and the fragment length (np) of DNA.

[f]Duplex content of S_1 nuclease treated repetitive DNA fragments was obtained as

$$\frac{\text{Micrograms of } S_1 \text{ nuclease resistant repetitive duplexes bound to HA column}}{\text{Micrograms of } S_1 \text{ nuclease treated total DNA loaded on HA column.}}$$

et al., 1973; Graham et al., 1974; Goldberg et al., 1975). Using hyperchromicity values, we calculate that about 50% of the 550 np long DNA is base paired, while only 39% of the 5700 np long fragments is base paired in finger millet. The latter is a slightly lower value as compared to the repetitive DNA content revealed from the reassociation curve of the 550 np long DNA. Similarly in the case of pearl millet, 91.8% and 51.7% of the duplexes are base paired of the 550 and 8200 np long fragments, respectively. The estimated duplex content (51.7%) of 8200 np long DNA is in good agreement with the repetitive DNA content of 54% obtained from the reassociation curve of 550 np long DNA. The average size of the interspersed repetitive DNA sequences which reassociate within 5700 np fragment in finger millet and 8200 np fragment in pearl millet is computed to be 2223 np and 4239 np, respectively (Table 4.2).

S_1 nuclease studies of reassociated repetitive DNA fragments

An independent approach to determine the presence of single strand regions in the reassociated DNA fractions is to use S_1 nuclease which is known to digest the single strand regions specifically on the reassociated DNA fragments without digesting the duplex regions. Native DNAs of finger millet and pearl millet (fragment size in the range of 5700 - 8200 np) were reassociated to Cot 25.0 and Cot 10.0 mol x sec/l, respectively. S_1 nuclease was then added and the S_1 nuclease resistant duplexes were isolated by hydroxyapatite column chromatography. The hyperchromicities of the DNA duplexes before and after S_1 nuclease treatment were determined. An increase in the hyperchromicity of the S_1 nuclease resistant duplexes is assumed

to be due to degradation of single stranded tails which are generally present ~~contiguous~~ contiguous to repeated DNA sequences.

In the case of finger millet, 58% of 5700 np DNA reassociates by Cot 25.0 mol x sec/l and this DNA fraction has an hyperchromicity of 11.4%. After S_1 nuclease treatment, the hyperchromicity of the S_1 nuclease resistant DNA is 21.5% (Table 4.2 and 4.3). Similarly in pearl millet, 77.5% of 8200 np DNA reassociates at Cot 10.0 mol x sec/l and has an hyperchromicity of 11.6%. Approximately 49.5% of the Cot 10.0 DNA is S_1 nuclease resistant and has a hyperchromicity of 18% (Table 4.2 and 4.3). There is thus a substantial increase in the hyperchromicity of the S_1 nuclease resistant DNA duplexes in finger millet as well as pearl millet. This can be a direct proof for the occurrence of interspersion of repetitive and single copy DNA sequences in these two species.

No significant changes are observed in the Tm values of Cot 25.0 and Cot 10.0 DNA fractions of finger millet and pearl millet, before and after S_1 nuclease treatment (Fig. 4.6 and 4.7). Since the Tms of the reassociated fragments are lower than those of native, sonicated DNAs, the hyperchromicities of these DNAs are also expected to be lower than those of native DNAs. Taking into account the nucleotide base mismatching, the expected hyperchromicities of the DNA fractions are also estimated. For example, native, sonicated finger millet DNA has a Tm of 83.8°C and hyperchromicity of 27.0%. The S_1 nuclease resistant duplexes have a Tm of 78.0°C and thus have a base mismatching of 5.8% (Britten et al.,1974). Due to the base mismatch, the hyperchromicity of this DNA

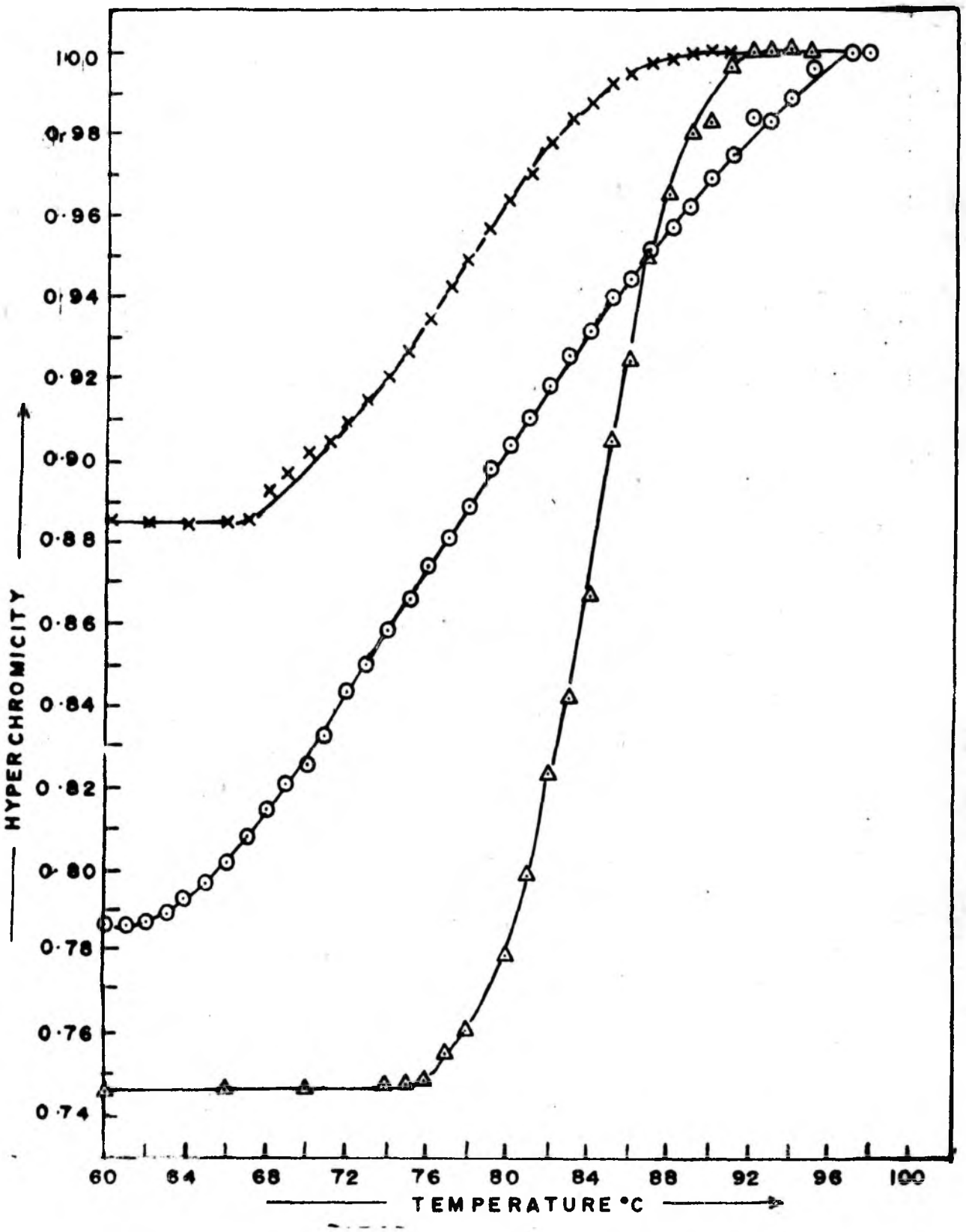


Fig. 4.6: Thermal denaturation profiles of Cot 25 finger millet DNA before and after S_1 nuclease digestion along with native 550 np long DNA. (-x-x-) before S_1 nuclease treatment; (-o-o-), after S_1 nuclease treatment and (-Δ-Δ-) native, 550 np long DNA. In these experiments, each point represented an average of at least three different experiments using three different DNA

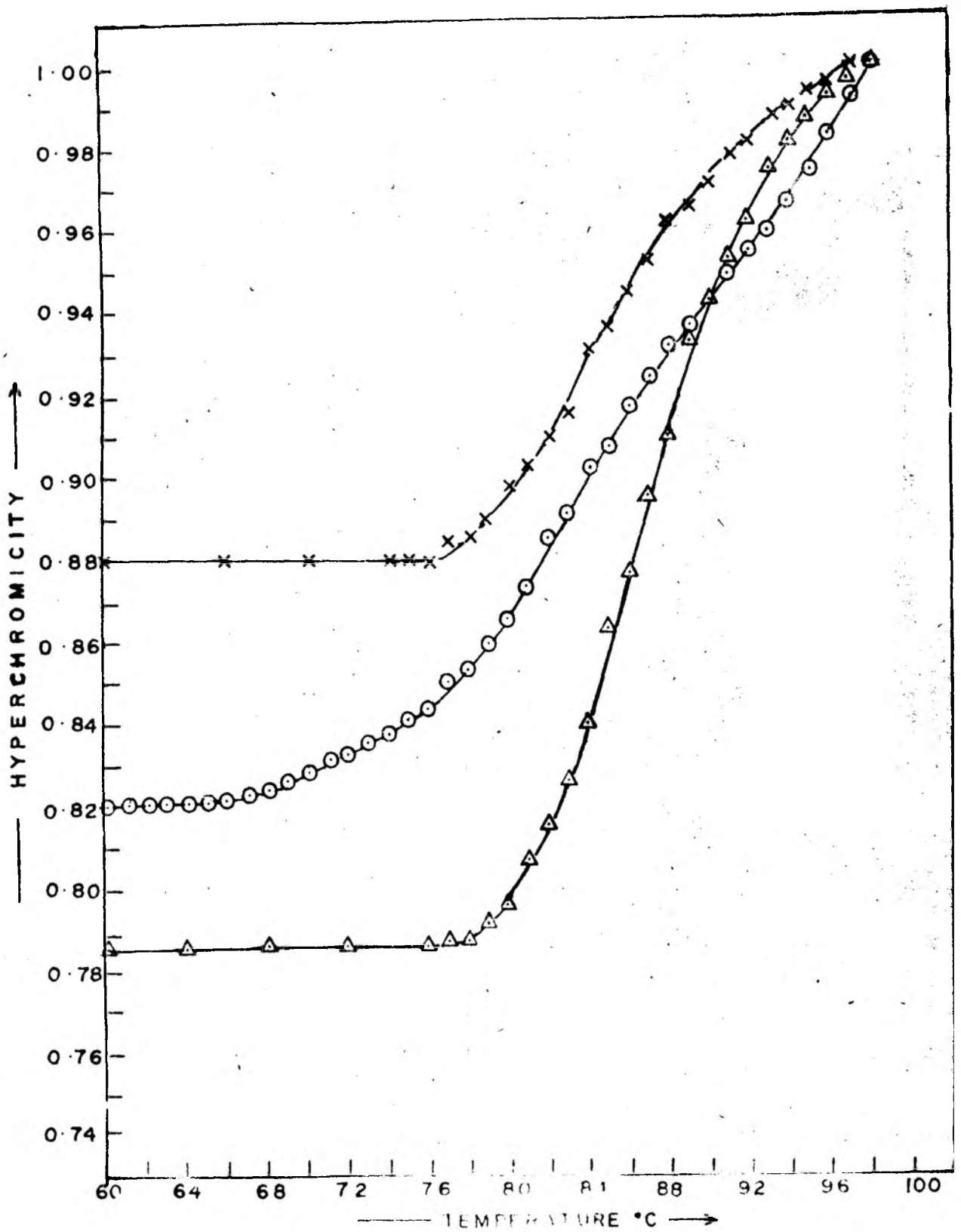


Fig. 4.7: Thermal denaturation profiles of Cot 10 pearl millet I before and after S_1 nuclease digestion along with nati 550 np long DNA. (-X-X-), before S_1 nuclease treatmen (-O-O-) after S_1 nuclease treatment and (-Δ-Δ-) native 550 np long DNA. In these experiments each point represented an average of at least three different experiments using three different DNA preparations.

Table 4.3: Melting properties of S_1 nuclease treated repetitive duplexes

DNA	Hyperchromicity		Tm
	Experimental	Expected[a]	°C
Finger millet	21.5	25.4	78.0
Pearl millet	18.0	20.6	85.4
Rice	8.9	6.5	75.8

[a]Calculated using the formula (Graham et al., 1974):

Fractions base paired = sonicated, native DNA hyperchromicity.

For example, native, sonicated finger millet DNA has a Tm of 83.8°C and a hyperchromicity of 27.0%. The S_1 nuclease resistant Cot 25.0 fraction has a Tm of 78.0°C and thus a base mismatch of (83.8 - 78.0) = 5.8%. Due to base mismatch, the hyperchromicity of this DNA fraction is expected to be (27.0 x (1 - 0.058)) = 25.4%,

fraction is expected to be (27.0 x (1.0 - 0.58)) = 25.4% (Graham et al., 1974). The latter estimated value compares with the experimental value of 21.5% (Table 4.3). Similarly in the case of pearl millet, there is a good agreement between the expected and experimental hyperchromicity values (Table 4.3). This further indicates that the single strand regions in DNA fractions are mostly digested by S_1 nuclease.

Since we assumed that all the repetitive DNA sequences reassociated by Cot 25.0 and Cot 10.0 mol x sec/l in finger millet and pearl millet,respectively (Deshpande and Ranjekar, 1980), the fractions of hydroxyapatite bindable 5700 np DNA in finger millet and 8200 np DNA in pearl millet fragments which are in duplex form, most probably represent the percent of repetitive sequences. For example, approximately 41% of 5700 np long hydroxyapatite bindable fraction at Cot 25.0 mol x sec/l in the case of finger millet is S_1 nuclease resistan and hence in duplex form. This estimate compares well with the estimated value of 39% obtained from hyperchromicity measurements. Likewise in pearl millet, 49.5% of 8200 np long hydroxyapatite bindable fraction at Cot 10.0 mol x sec/l is in duplex structures. This is in agreement with the hyperchromicity measurements (51.7%) at this length.

In the case of rice, 52 - 55% of 6500 np DNA reassociates at Cot 50.0 mol x sec/l. After S_1 nuclease treatment, the proportion of S_1 nuclease resistant DNA is only slightly reduced to 48%. This is in contrast to the situation observed in finger millet and pearl millet, where the percent reassociation values are significantly lower after S_1 nuclease

treatment. This clearly indicates the near absence of single strand regions in Cot 50.0 DNA fraction isolated from 6500 np DNA and thus supports the view that there is no DNA sequence interspersion in rice. The proportion of S_1 nuclease resistant DNA (48%) in rice can also represent the estimate of repetitive DNA content. The value compares with the estimate of 52% as obtained from the reassociation kinetics experiments of 550 np rice DNA.

Size distribution of repetitive duplexes

Agarose gel column chromatography and agarose gel electrophoresis of S_1 nuclease resistant repetitive DNA

The duplex length values as obtained from hyperchromicity measurements represent average size estimates of the interspersed repeated segments. A direct estimate of the size distribution of repetitive DNA sequences is obtained by analysing S_1 nuclease resistant repetitive duplexes using agarose column chromatography. In these gel filtration measurements, calf thymus DNA of known molecular weights was used as a standard (Fig. 4.8). From Fig. 4.9 and 4.11, it is quite clear that there are different sizes of repetitive duplexes in case of finger millet and rice. In the case of finger millet, 20% of the repetitive duplexes has a size more than 1500 np, 60% has a size of 150 - 200 np and 20% are of intermediate size. Similarly, in the case of rice, 35 - 40% is of size more than 1500 np and 60 - 65% is around 50 - 100 np. Contrary to this, pearl millet duplexes (Fig. 4.10) show only one peak of size more than 1500 np.

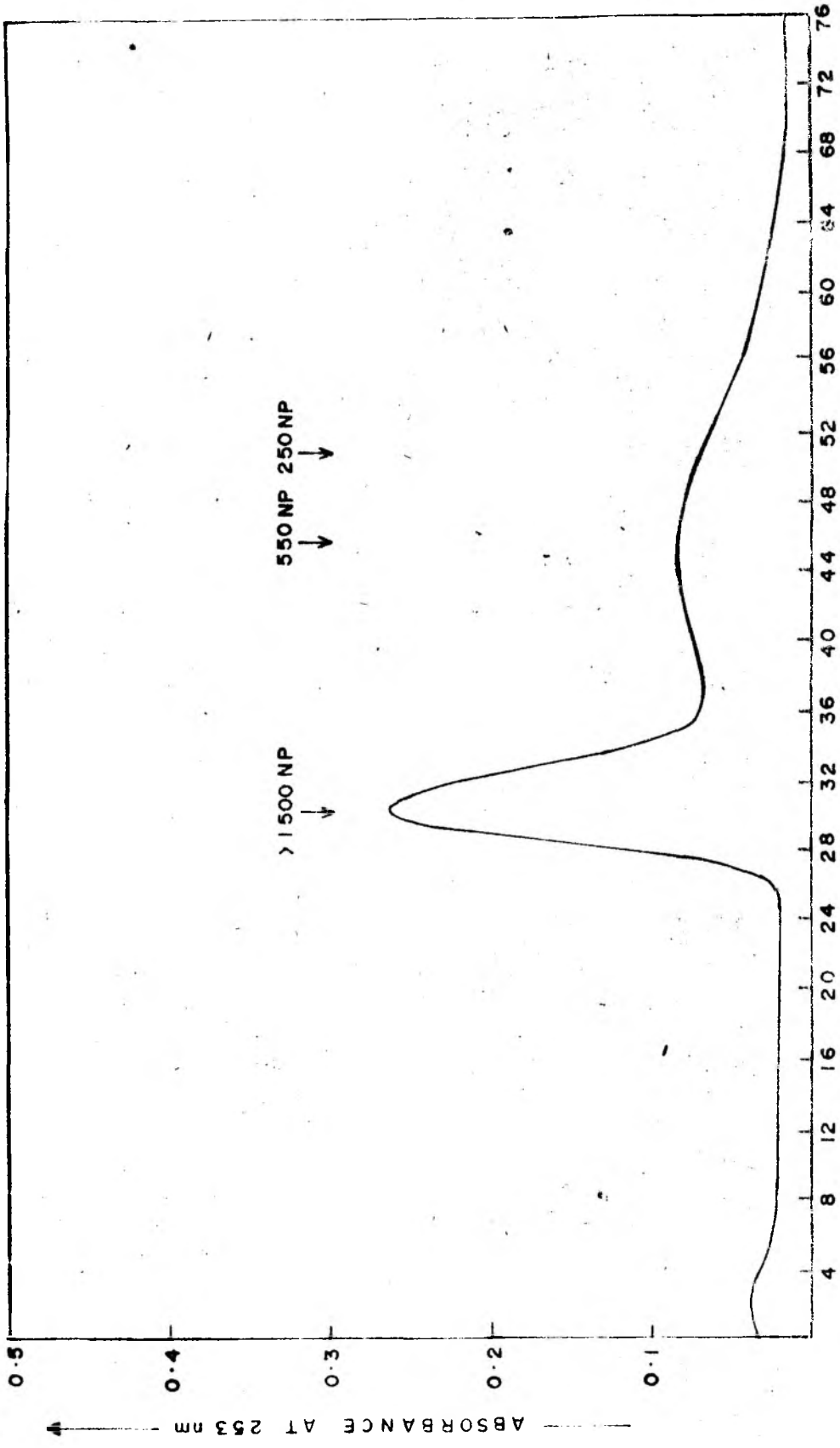


Fig. 4.8: Agarose A_{50} column chromatography profile of calf thymus DNA of known duplex lengths.

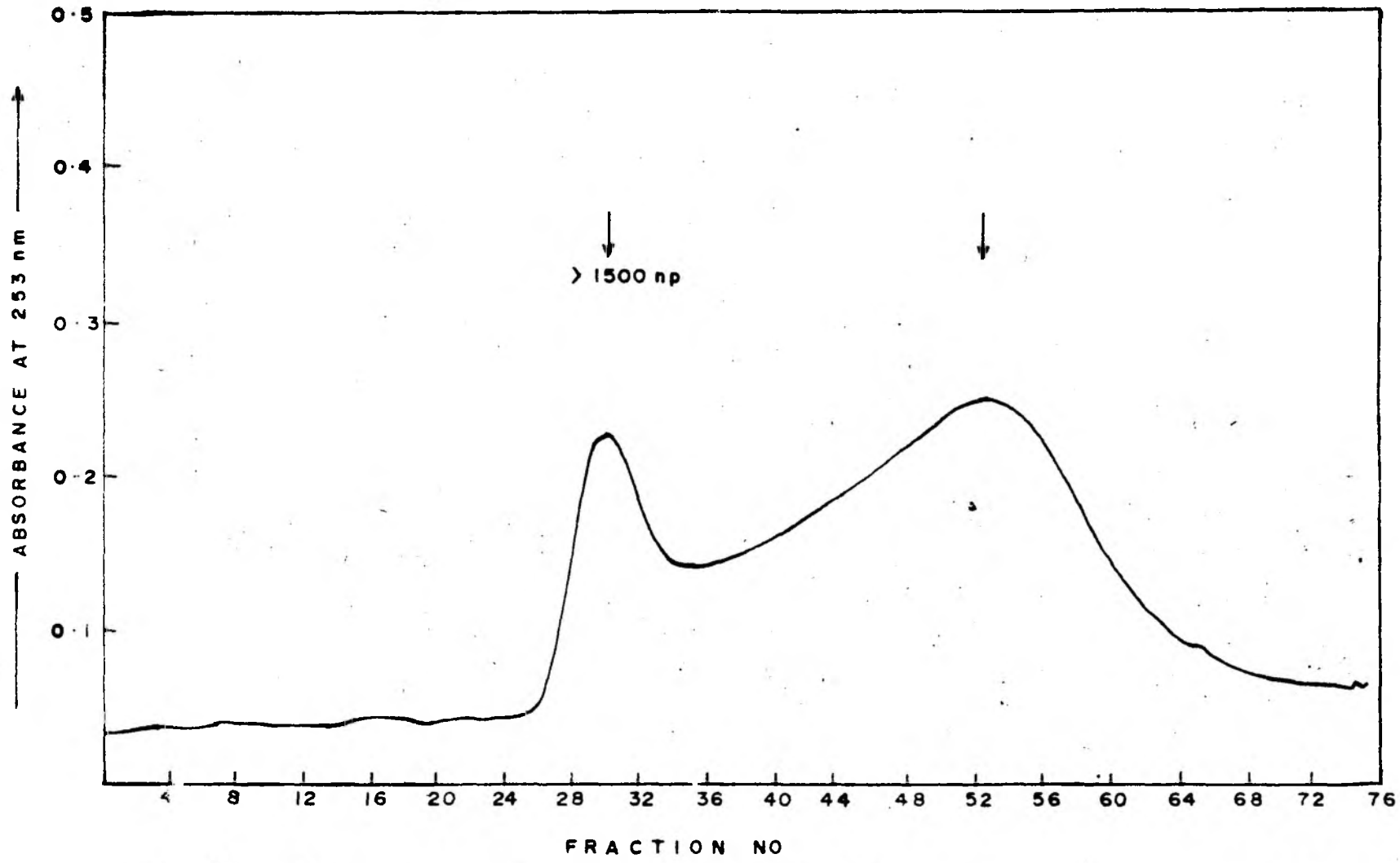


Fig. 4.9: Agarose A_{50} column chromatography profile of S_1 nuclease resistant Cot 25 DNA fraction of finger millet.

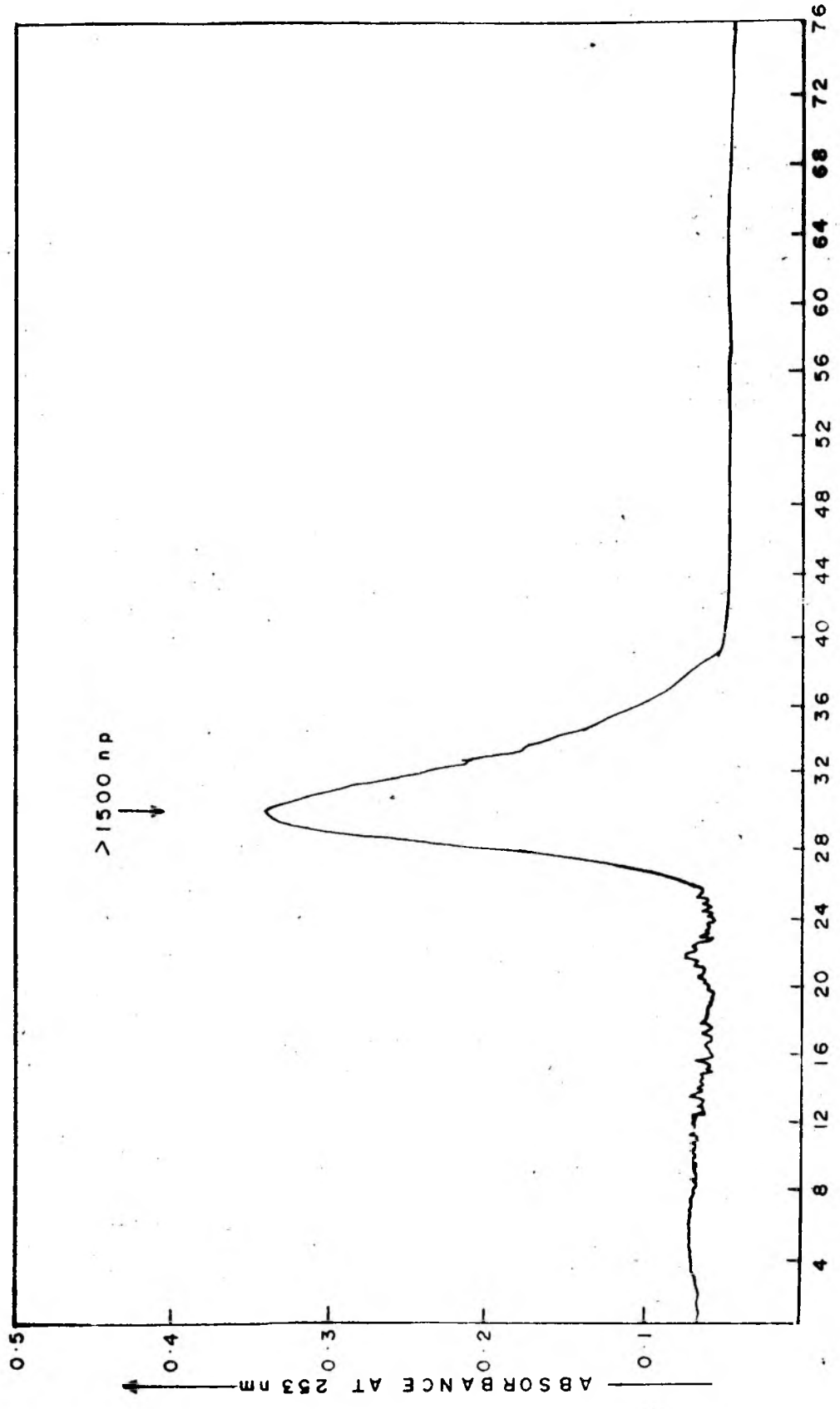


Fig. 4.10: Agarose A_{50} column chromatography profile of S_1 nuclease resistant Cot 10 DNA fraction of pearl millet.

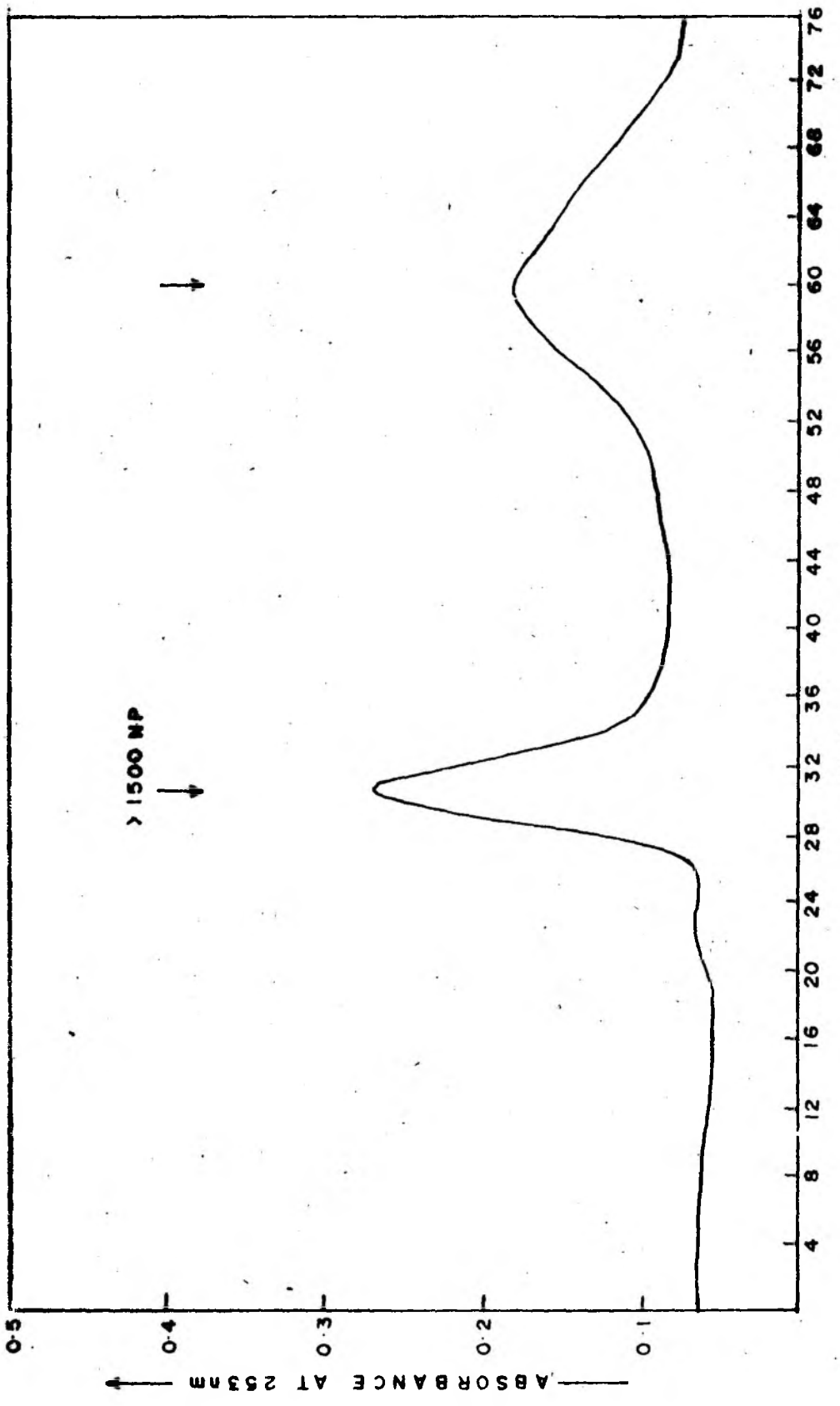


Fig. 4.11: Agarose A_{50} column chromatography profile of S_1 nuclease resistant Cot 50 DNA fraction of rice

Precise lengths of these DNA fractions are then determined by agarose gel electrophoresis. Here, λ DNA digest with EcoRI is used as a molecular weight marker (Fig. 4.12). The banding patterns of high and low molecular weight fractions from agarose gel columns are as shown in Fig. 4.13 for finger millet, Fig. 4.14 for pearl millet and Fig. 4.15 for rice. From the mobilities of the excluded fractions, the molecular weights are estimated to be 4000 - 4200 np, 4300 - 4500 np and 6000 - 6400 np in the case of finger millet, pearl millet and rice, respectively. Mobilities of the low molecular weight fractions of finger millet and rice go beyond the range of the molecular weight marker suggesting its length to be less than 500 np. Considering agarose column chromatography and agarose electrophoresis results together, the lengths of the repetitive duplexes are estimated to be 4000 - 4200 np and 150 - 200 np in the case of finger millet, 4300 - 4500 np in the case of pearl millet and 6000 - 6400 np and 50 - 100 np in the case of rice.

Spacing of repetitive sequence elements

The HA chromatography data of the total DNA of different fragment sizes as well as the hyperchromicity measurements and the S_1 nuclease resistance of the reassociated repetitive duplexes of different fragment size have shown that repetitive and nonrepetitive sequences are interspersed in finger millet and pearl millet only. Further more, these studies have revealed the proportion and the sequence length of the repetitive DNA segments. The next question which arises is about the length of single copy sequences which are present

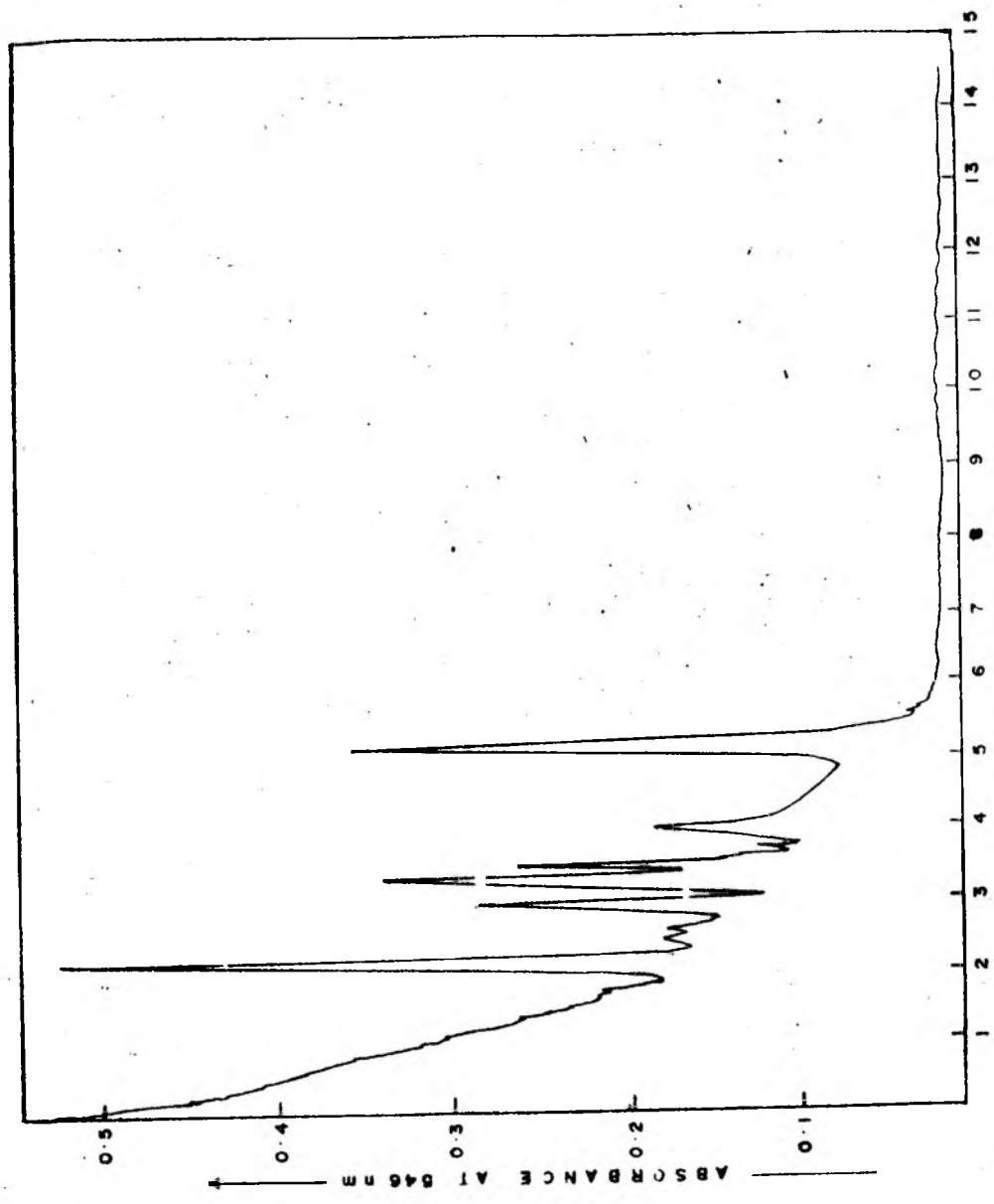


Fig. 4.12: Agarose gel electrophoresis pattern of λ DNA digest with EcoRI used as a molecular weight marker in these experiments. The six peaks observed were corresponding to the molecular weights 20757 np, 7196 np, 5303 np, 4545 np and 3257 np taken from Thomas and Davis (1975)

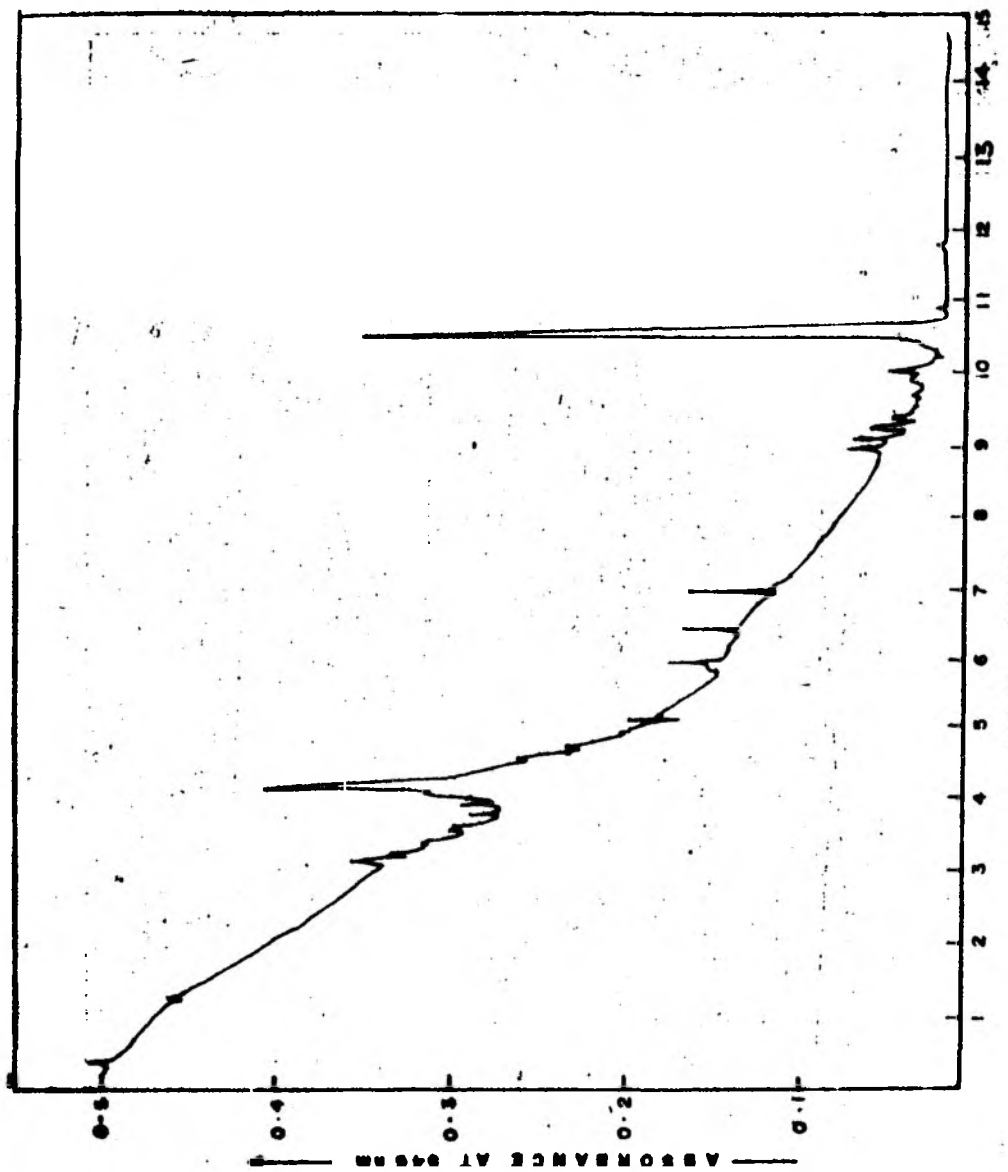


Fig. 4.13: Agarose gel electrophoresis pattern of S_1 nuclea
resistant finger millet repetitive duplexes.

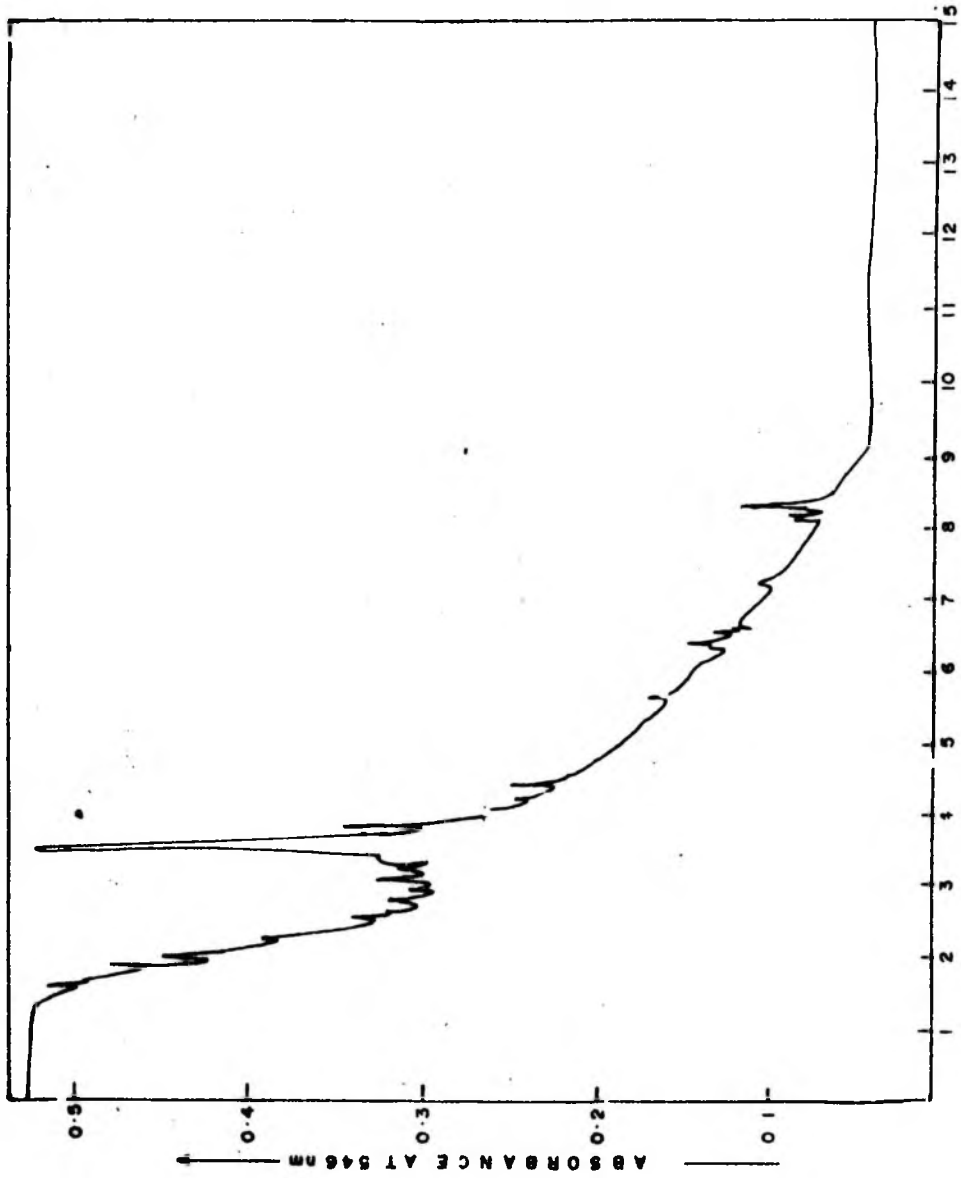


Fig. 4.14: Agarose gel electrophoresis pattern of S_1 nuclease resistant pearl millet repetitive duplexes.

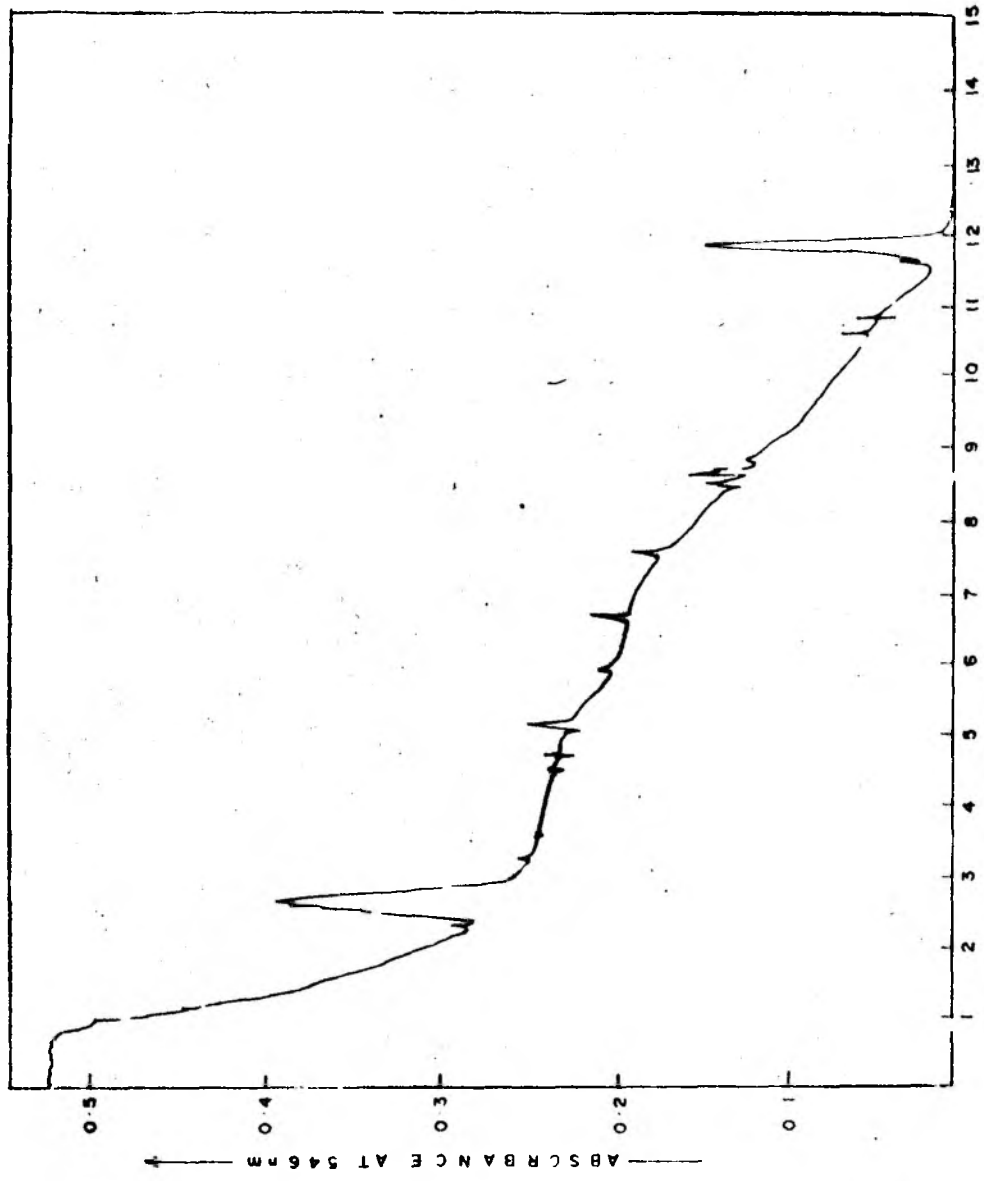


Fig. 4.15: Agarose gel electrophoresis pattern of S_1 nuclease resistant rice repetitive duplexes.

adjacent to the repetitive DNA sequences. A direct estimate of the length of the interspersed single copy sequences can be obtained from a curve relating the fraction of DNA fragments binding to HA and the DNA fragment length (Wetmur and Davidson, 1968). As mentioned earlier Cot 25.0, Cot 10.0 and Cot 50.0 mo: x sec/l were selected to measure the percent of DNA binding to I in finger millet, pearl millet and rice, respectively. Whenever there is an interspersion of repeated and single copy DNA sequences, a certain amount of single copy DNA will also bind to HA at these Cot values. The amount of single copy DNA prese as single strand tails on reassociated repetitive duplexes will increase with fragment length. This will be true to a fragment length which is equal to the average distance between repeated sequences (Wetmur and Davidson, 1968; Davidson et al., 1973). From Fig. 4.16 and 4.17, it is observed that two slopes are present in case of finger millet and pearl millet and the change in the slope occurs at a fragment length of approximatel 1800 - 2000 np. Upto 1900 np, there is a linear relationship between fragment length and percent binding to HA and thereafte the increase in binding is more gradual. The position of the change in slope is interpreted as the approximate length of single copy sequences. Therefore, the length of the single copy DNA of finger millet and pearl millet is in the range of 1800 - 2000 np. In contrast to these observations, the binding curve of Cot 50.0 rice DNA (Fig. 4.18) is a straight line with one slope only and is a clear indication of the fact that there is no sequence interspersion of repeated and single copy DNA

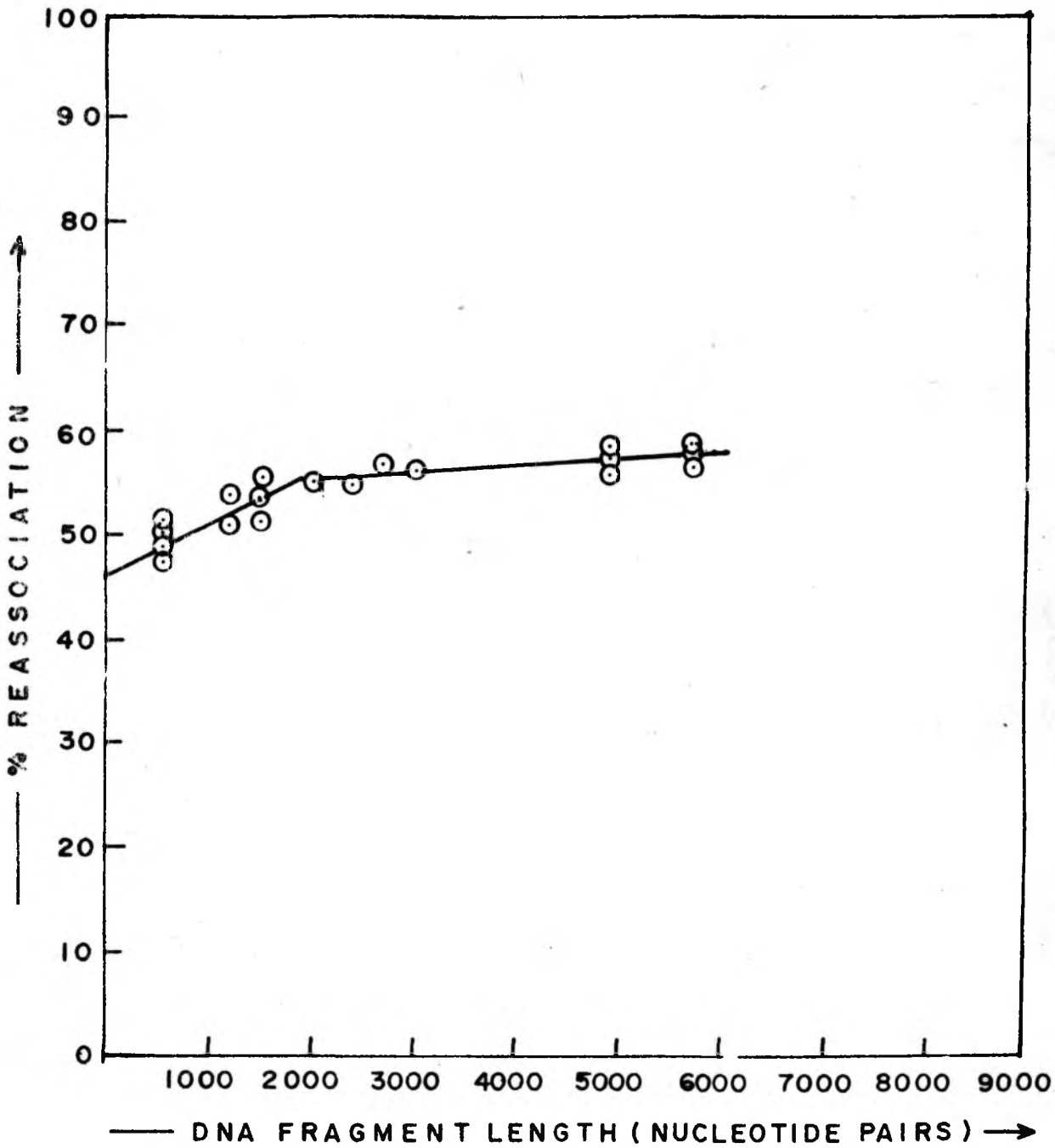


Fig. 4.16: The percentage binding of finger millet Cot 25 DNA to hydroxyapatite as a function of fragment length.

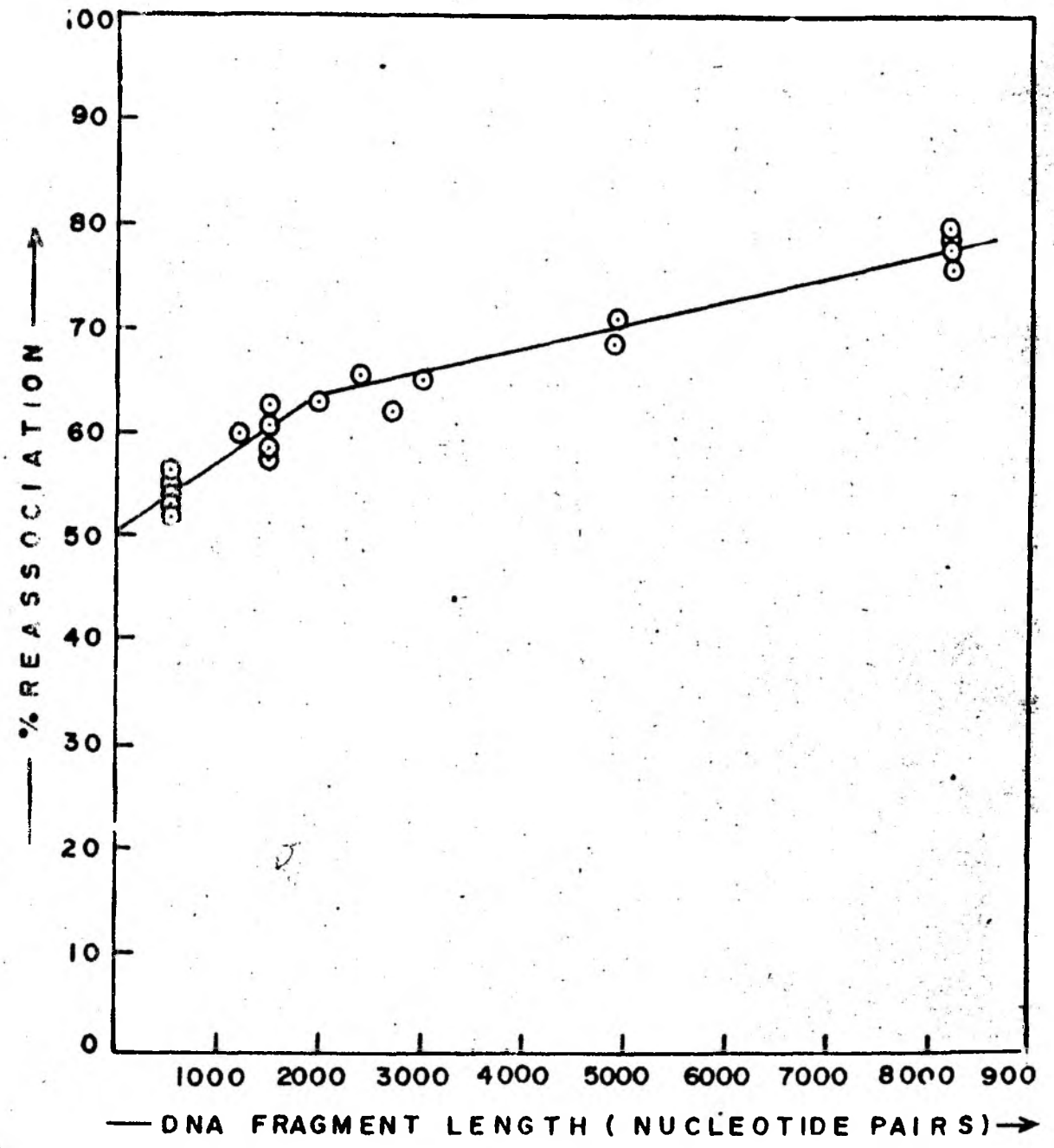


Fig. 4.17: The percentage binding of pearl millet Cot 10 DNA to hydroxyapatite as a function of fragment length.

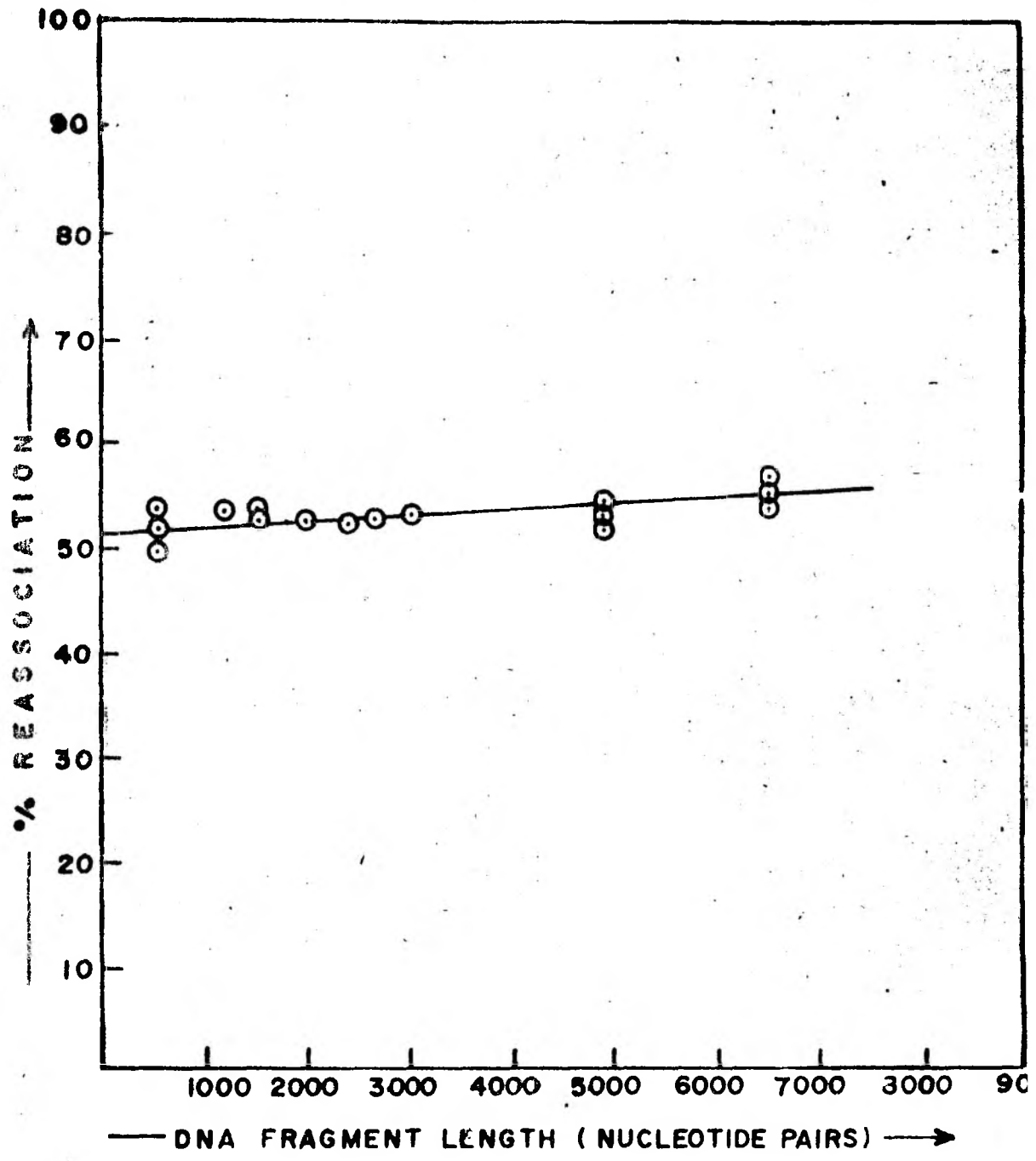


Fig. 4.18: The percentage binding of rice Cot 50 DNA to hydroxyapatite as a function of fragment length.

An estimate of the percentage of repetitive DNA content in the present three plant species can be obtained by extrapolating the curves of greater slopes in each case to zero fragment length. The intercept revealed the percentage of repetitive DNA as 46%, 50% and 51.5% in case of finger millet, pearl millet and rice, respectively. These values are in clos agreement with the values obtained from reassociation kinetics hyperchromicity and S_1 nuclease measurements (Table 4.4).

DISCUSSION

DNA sequence arrangement

We have employed the most commonly used approaches for studying the DNA sequence organization in eukaryotic genomes. Several authors have adopted the approach of using labelled tracer DNAs to study DNA reassociation with different fragmen lengths. We have not been able to obtain labelled DNA of sufficiently high specific activity and hence have not used labelled tracer DNAs. Vorobev and Kosjuk (1974) and Baldri Amaldi (1976) have clearly shown that their results obtained from sea urchin and Xenopus, using unlabelled DNAs are in gooc agreement with those of Graham et al. (1974) and Davidson et a (1973) where labelled DNA was used. In plants, Wimpee and Rawson (1979) have used unlabelled DNA for studying DNA reassociation kinetics. Since only cold DNA was used in our experiments, we have been unable to estimate the zero time binding fraction and to apply the corresponding correction to the reassociation data.

We have isolated Cot 25.0 in finger millet, Cot 10.0 in

Table 4.4: Duplex contents (D) of repetitive DNA fractions of three Gramineae species from various independent methods

	Finger millet	Pearl millet	Rice
1. From reassociation curve of 550 np DNA (Fig. 4.1, 4.2 amd 4.3).	0.49	0.54	0.52
2. Hyperchromicity measurements (Table 4.2)	0.39	0.51	-
3. S_1 nuclease resistance measurements (Table 4.2)	0.41	0.495	0.48
4. From graph (% reassociation Vs fragment length) (Fig. 4.15, 4.17 and 4.18).	0.46	0.50	0.515

fragment sizes and characterized them by studying their hyperchromicity and S_1 nuclease resistance. Here we have assumed that all the repeated DNA sequences and very few single copy sequences reanneal by these Cot values. In fact several authors have used Cot 50.0 and Cot 100.0 (Xenopus: Davidson et al., 1973; Mollusc: Angerer et al.,1975; Cotton: Walbot and Dure, 1976; Tobacco: Zimmerman and Goldberg, 1977) as limiting Cot values for the reassociation of repetitive DNA sequences.

Using three main approaches, namely reassociation kinetics of DNAs of different fragment lengths, hyperchromicity measurements of repetitive DNA fractions of varying fragment size and S_1 nuclease resistance of repetitive duplexes isolated from the highest fragment length DNA, we have been able to show the occurrence of interspersion of repeated and single copy DNA sequences in finger millet and pearl millet genomes and absence of DNA sequence interspersion in the case of rice genome.

Repetitive duplex content and its sizing

From Table 4.4, it can be seen that estimates of repetitive duplex contents are arrived at by four methods independent of each other. Comparable values of duplex content were obtained in the case of pearl millet (49.5% to 54%) and rice (48% to 52%). In finger millet, the duplex contents estimated from hyperchromicity measurements and S_1 nuclease resistance (39% to 41%) were in close agreement with each other. The estimate obtained from the reassociation kinetics of 550 np long DNA fragments (49%) was,however higher (Table 4.4).

For the length estimation of the repeated DNA duplexes,

repetitive DNA fraction is determined. From the hyperchromicity, the average duplex length is computed. For precise sizing, the repetitive DNA fraction is freed from the single strand tails by single strand specific S_1 nuclease and then analysed by agarose column chromatography followed by agarose gel electrophoresis.

In pearl millet, there is a good comparision of the size of repetitive sequence elements as determined by hyperchromicity measurements (4239 np) and by agarose gel electrophoresis of S_1 nuclease resistant duplexes (4300 - 4500 np). In the case of finger millet, since 20% of the S_1 resistant repetitive duplexes are of high molecular weight (4000 - 4200 np), 60% are of low molecular weight (150 - 200 np) and 20% are of intermediate size, there is no direct comparision between these data and those obtained from hyperchromicity measurements. In the case of rice, two different molecular weights of S_1 resistant repetitive duplexes (35 - 40% of 6000 - 6400 np and 60 - 65% of 50 - 100 np) are observed.

Tm of the repetitive DNA fractions of increasing fragment length

In both the millet species, the Tm values of repetitive DNA fractions were observed to increase with an increase in the DNA fragment length. However, such increase in the thermal stability of the repetitive duplexes of finger millet and pearl millet with increase in the fragment lengths was not only due to the effect of fragment length alone. This was confirmed by calculating the melting temperatures of DNAs of various lengths effected by fragment lengths only (Crothers et al.,

pearl millet repetitive duplexes predicted using the formula:

$$T_n - Tm = B/L$$

where T_n = melting temperature of native DNA.

L = fragment length

B = a constant, 650 (Britten et al., 1974)

were in the ranges of 82.6 - 83.7°C and 85.8 - 86.9°C, respectively. These ranges were very narrow as compared to the observed ranges of 73.0 - 77.0°C and 79.2 - 85.4°C in finger millet and pearl millet, respectively. A likely explanation for this observation is that much of the repeated duplexes in these two millet species are present in long arrays consisting of smaller elements varying slightly in their nucleotide sequences. When the DNAs are sheared, due to the greater probability of mismatch in the reassociated duplexes, the Tm values are lower as compared to those of longer fragments which reanneal with a minimum of mismatch in the repetitive duplexes (Lee et al., 1970).

Comparative analysis of DNA sequence organization in finger millet, pearl millet and rice

Among the three species under present investigation, the DNA sequence interspersion is observed only in finger millet and pearl millet. There are a number of differences observed in these two species with respect to the proportion of interspersed repeated and single copy DNA sequences and the modes of DNA sequence interspersion. These differences can be summarized as follows:

fragment lengths, it is quite clear that in finger millet approximately 58% of the total DNA consists of interspersed DNA sequences. Since the content of repetitive DNA is 49% of the total genome, it can be concluded that about 18% of the unique DNA is involved in DNA sequence interspersion. Unlike in finger millet, in pearl millet nearly 78% of the total genome consists of interspersed DNA sequences. Here repeated DNA sequences represent 54% of the total genome and are interspersed with 50% of the single copy DNA sequences.

(2) In finger millet, the sizes of the interspersed repeated DNA sequences are of three types. Approximately, 60% of the repeated DNA sequences are 150 - 200 np long, 20% are 4000 - 4200 np long and remaining 20% are of intermediate size. In pearl millet, the interspersed repeated DNA sequences belong to only one class with a fragment length of 4300 - 4500 np.

Thus the mode of DNA sequence interspersion in finger millet is of mixed type that is of short period as well as of long period while that of pearl millet is typically of long period nature.

The data obtained in the case of rice genome by reassociation kinetics of various fragment lengths are in contrast to those observed in the case of finger millet and pearl millet. The increase in the DNA binding to HA as the fragment length increases from 550 np to 6500 np is very little (52% to 55%). Approximately 94% of the unique DNA sequences are present in the form of long stretches of minimum length of 6500 np. Thus the repeated sequences of rice DNA do not show interspersion with

tried to isolate the DNAs with higher molecular weights to determine their reassociation kinetics. However, we were unable to isolate such DNAs. The possibility, therefore, remains that at the DNAs of fragment lengths higher than 6500 np, interspersion of repeated and single copy DNA may occur. A number of interesting questions can be raised from our data on rice genome: Is there interspersion at all of repeated and nonrepeated DNA sequences? What is the function of very short repeats of less than 150 np? How are rapidly reassociating DNA sequences and intermediately reassociating DNA sequences organized with respect to one another?

Interspersed single copy DNA length in finger millet and pearl millet

Though the differences are observed in the sizes of interspersed repeated DNA sequences, in proportions of single copy DNA involved in interspersion and in the modes of DNA sequence organization in these two species, the length of the interspersed single copy DNA is observed to be similar and ranges from 1800 to 2000 np. Since most of the single copy sequences apparently code for structural genes (Greenberg and Perry, 1971; Firtel et al., 1972; Goldberg et al., 1973), the average length of the single copy DNA stretch must not be smaller than that of mRNA. We have not isolated and characterized mRNA in either of these species. However, from the literature, the average length of mRNA containing poly (A) is shown to be minimum of 1500 np (Key and Silflow, 1975;

pearl millet are 1800 - 2000 np and can compare with the expecte length of mRNA. This is consistent with the idea that mRNA may be transcribed from the former. This concept is supported by the results of Davidson et al. (1975) showing that 30 - 100% of embryonic mRNA from sea urchin is transcribed from single copy DNA objacent to interspersed repetitive sequences.

Pearl millet genome: Comparison of our data with that of Wimpee and Rawson (1979)

Most of our data on DNA sequence organization in pearl millet genome are comparable to that of Wimpee and Rawson (1979). The proportion of the total genome consisting of interspersed repeated and single copy DNA sequences is the same in both the cases (78% - 80%). Approximately 50% of the single copy DNA is interspersed with the repeated DNA sequences in both the cases. The length of the interspersed repetitive DNA determined by Wimpee and Rawson (1979) is 4500 - 5000 np and compares with our estimate of 4300 to 4500 np.

In their report, the hyperchromicity of Cot 10.0 DNA fraction is in the range of 19.2% to 21.6% for the fragment size range of 430 to 7200 np. In our data, the hyperchromicity of Cot 10.0 DNA fraction decreases from 19.6% to 11.4% with the increase in the fragment size from 550 np to 8200 np. The length estimate (1900 np) of the interspersed single copy DNA in our work is slightly higher than that given by Wimpee and Rawson (750 - 1400 np). The size distribution of repeated sequences by agarose A_{50} column in the report of Wimpee and

column having molecular weight greater than 1500 np and the other insignificant fraction has a broad size distribution averaging about 400 np. No such minor component is observed in our gel filtration profiles. Only one peak with a molecular weight greater than 1500 np is apparent.

CONCLUDING REMARKS

The information on DNA sequence organization in finger millet has clearly shown that the plant genomes exhibit a great diversity in the length of the interspersed repeated DNA sequences. In fact, finger millet offers an unique example, where the lengths of the interspersed repeated DNA sequences are as large as that seen in pearl millet and are smaller than those exhibiting typical short period interspersion pattern as in the case of wheat (Flavell and Smith, 1976), rye (Smith and Flavell 1977), soybean (Goldberg, 1978; Gurley et al., 1979) & tobacco (Zimmerman and Goldberg, 1978). In the case of pearl millet, the overall DNA sequence organization pattern compares well with that reported by Wimpee and Rawson (1979) as discussed earlier. From the comparison of the reassociation kinetics data of rice DNAs of different fragment lengths with the available data on plant genomes, rice is the only genome where no DNA sequence interspersion is observed at a DNA length of 6500 np. Similarly, the lengths of repeated DNA sequences in rice are of two types: 35% of the DNA is very long (6000 - 6400 np) and 65% of the DNA is very short (less than 150 np considering the agarose gel chromatography and electrophoresis data together). Both the sizes are extremes as compared to the data available on plant genome organization.

Thus the DNA sequence interspersion patterns in finger millet, pearl millet and rice are different from each other as well as from those in other plants studied so far.

CHAPTER V

CHROMATIN: VISUALIZATION, ISOLATION AND CHARACTERIZATION

CHROMATIN: VISUALIZATION, ISOLATION AND CHARACTERIZATION

INTRODUCTION

Structural organization of chromatin in interphase nucleus has been a subject of interest for cytologists since long time (Reviewed by Lafontaine and Lord, 1969; Lafontaine, 1974). The various techniques in light, phase contrast, fluorescence and electron microscopy were utilised for elucidation of this puzzle. The advancement of knowledge about interphase nucleus added many more dimensions to the picture, once thought to be non-dividing or resting nucleus. Nonrandom arrangement of chromosomes, indication of somatic association and specific orientation of chromosomes maintaining their telophasic configuration are some of the known interesting features of interphase nucleus. In many laboratories, Giemsa banding technique has been utilized for visualization of interphase nucleus structure in plants (Fussell, 1977; Ghosh and Roy, 1977; Stack et al., 1977; Joshi and Ranjekar, 1980). Lafontaine (1974) has proposed a relationship between the nuclear DNA content and the interphase chromatin organization in plants. According to him, plant species with low DNA content exhibit chromocentric nuclear organization, while those with high DNA content have reticulate interphase nuclei. Most of the Gramineae species such as wheat, oat, rye and barley which have been characterized for their high repetitive DNA content have nuclear DNA content (1C) more than 5 pg and have reticulate nuclei. The three plant species under present consideration have nuclear DNA content less than 3 pg. We were, therefore, interested in studying the interphase chromatin organization in these species.

Biochemically, the organization of chromatin involves participation of DNA, RNA, histone proteins and non-histone proteins. Histones stabilize the DNA duplex, induce a tertiary structure and block the accessibility of the DNA for enzymes (Nagl, 1978). In recent years, histones have been isolated and characterized from a few plant species (Fambrough and Bonner, 1969; Pipkin and Larson, 1972; 1973; Strokov et al., 1973; Spiker et al., 1976; Hayashi et al., 1977; Rodrigues et al., 1979; Iqbal Ahmed and Padayatty, 1980). Detail comparison of chromatographic and electrophoretic behaviour of pea histones and calf thymus histones has been carried out to show the striking similarities in the plant and animal histones (Fambrough and Bonner, 1966; De Lange et al., 1969; Elgin and Weintraub, 1975) Some other plants also possess histones of same electrophoretic mobilities as calf and pea H_3 and H_4 histones (Spiker, 1975). However, some dissimilarities have also been shown in animal and plant histones (Mandal et al., 1971; Hsiang and Cole, 1973; Spiker and Krishnaswamy, 1973; Nadeau et al., 1974). DNA sequence organization in finger millet, pearl millet and rice is observed to be significantly different from other plant species as well as animal species. Since histones are also suggested to play an important role in gene regulation, we were curious to know the histone patterns in these three species.

This Chapter describes the cytological organization of interphase nucleus and comparison of histones from finger millet, pearl millet and rice with those from calf thymus.

MATERIALS AND METHODS

Giemsa staining method

Root tips of finger millet, pearl millet and rice were excised and fixed in cold acetic alcohol (1:3) for 12 - 16 h followed by storage in 70% alcohol at 4 °C. Root tips were squashed in a drop of 45% acetic acid after 10 min maceration in 1 N HCl at room temperature. Coverslip separation was achieved by slide inversion in a dish of absolute alcohol or alternatively with liquid nitrogen. Both slides and coverslips were flame dried. Selective disruption of euchromatin was carried out with 8 N HCl for 1 - 35 min at room temperature. Prior to HCl treatment, slides were immersed in distilled water for 10 - 15 min. Slides were rinsed several times with running cold water which abruptly discontinues hydrolysis and brings the pH of the material to neutrality. Staining was done in 5% Giemsa (BDH) diluted with M/15 Sorensen's phosphate buffer (pH 6.8). Excess of stain was washed off with distilled water. Preparations were air dried and mounted in DPX mounting medium.

Isolation of nuclei

Plants of finger millet, pearl millet and rice were grown on wet cotton and the aerial tissue was cut as described in Materials and Methods of Chapter II.

Frozen aerial tissue was crushed in a Waring blender for 90 sec in the case of finger millet and pearl millet and 2 min in the case of rice in sucrose buffer (0.5 M sucrose, 0.05 M Tris, 0.05 M maleic acid, 0.003 M $CaCl_2$ and 0.05 mM phenyl methyl sulphonyl fluoride, pH 6.0) containing 0.1% Triton. The homogenate was filtered through four layers of cheese cloth

and centrifuged at 1000 g for 15 min to obtain a crude nuclear pellet . The latter was suspended in the crushing medium and centrifuged at 1000 g for 15 min. The nuclear pellet was washed once again with sucrose buffer and centrifuged as above. The pellet was checked under the microscope to determine the quality of nuclear preparation. Generally, a clean nuclear pellet was obtained after 2 - 3 washings with sucrose buffer. All the operations were carried out at 4 °C.

Isolation of chromatin

Chromatin was isolated from the nuclear pellet using the procedure of Hossainy et al., (1973) with some modifications. The nuclei were suspended in saline - EDTA (80 mM NaCl, 20 mM EDTA, 0.05 mM PMSF, pH 6.2) for one hour under stirring at 4 °C. The suspension was then centrifuged at 1000 g for 10 min. The pellet was resuspended in 0.5 mM EDTA/Tris, pH 7.5 containing 1% Triton X100 and 0.05 mM PMSF, without stirring and was centrifuged at 2000 g for 10 min. The pellet was then homogenized in 0.5 mM EDTA/Tris, pH 7.5 containing 0.05 mM PMSF using a Waring blender at 100 v for 1 min. The homogenate was centrifuged at 2000 g for 10 min. The supernatant which contained solubilized chromatin was used for precipitation of chromatin by slowly adding 1 M NaCl with stirring to make the final concentration of NaCl as 0.14 M. The precipitated chromatin was collected by centrifugation at 2000 g for 30 min. and dissolved in 0.2 mM EDTA/Tris at pH 7.0. Throughout the extraction procedure, care was taken to avoid the temperature increase above 4 °C.

Isolation of histones

Histones were isolated either from chromatin or directly from nuclear pellet using Panyim and Chalkley's method (1969). The nuclear pellet was homogenized in SSC-X containing 0.05 mM PMSF and was centrifuged at 1000 g for 15 min (Rubio et al., 1980). The resulting pellet was washed twice with 0.1 M Tris-HCl buffer, pH 7.4 and centrifuged at 1000 g for 15 min. The pellet was suspended in 0.1 M Tris-HCl buffer, pH 7.4. An equal volume of cold 0.8 N H_2SO_4 was added to this solution or to chromatin solution and the mixture was stirred for one hour at 4 °C. The preparation was next centrifuged at 18,000 g for 10 min. The resulting pellet was reextracted with cold 0.4 N H_2SO_4 and centrifuged with the same speed. Both the supernatants were pooled together and five volumes of absolute ethanol was added. The histones were allowed to precipitate for 3 days at -20 °C. The proteins were recovered by centrifugation and were dissolved in 0.9 N acetic acid. The quantitative estimation of proteins was carried out by the method of Lowry et al. (1951).

Polyacrylamide urea gel electrophoresis of histones

(a) Preparation of gels

Preparation of gels was carried out as described by Panyim and Chalkley (1969). The final form of the solution was expected to be 15% polyacrylamide in 6.25 M urea at a final pH 3.2.

Solution A - 60% acrylamide (w/v) and 0.4% N,N'-bisacrylamide (w/v) in water.

Solution B - 43.2% glacial acetic acid (v/v) and 4% TEMED

(w/v) in water.

Solution C - 0.2% ammonium persulphate (w/v) in 10 M urea freshly prepared.

Gels of size 10 x 0.6 cm were prepared by mixing 1 part of solution B, 2 parts of solution A and 5 parts of solution C. In the presence of 6.25 M urea, the polymerization occurred in 40 - 50 min. Electrophoresis was performed at room temperature in a standard vertical gel system using tray buffer as 0.9 N acetic acid.

(b) Electrophoresis of histones

Histones of finger millet, pearl millet, rice and calf thymus (commercial preparation) were dissolved in 0.9 N acetic acid. To this, sucrose was added to a final concentration of 15%. Basic fuschin was used as a marker dye. Before applying the histone solutions to the gel, preelectrophoresis was carried out for about half an hour. About 0.05 ml of each histone solution containing 50 - 70 µg of protein was applied to the gel and then electrophoresis was carried out with 1 mA/tube current for one hour and then 2 mA/tube till the marker dye moved nearly to the bottom of the tube. Before removing the gels from the tubes, they were immersed in cold water for 5 - 10 min, which made the removing of the gels from the tubes particularly easy.

(c) Staining and destaining

The gels were stained with 1% Coomassie Blue in 7% acetic acid overnight and destained in a solution containing 50 ml acetic acid + 150 ml absolute alcohol + 300 ml distilled water.

The gels were photographed and scanned in a Gilford 250

spectrophotometer attached with gel scanner at 600 nm to calculate relative mobilities.

SDS polyacrylamide gel electrophoresis of histones

SDS polyacrylamide gel electrophoresis was carried out by the procedure described by Weber and Osborn (1969). Various solutions used in this electrophoresis were prepared as follows:

1. Phosphate buffer stock solution (0.2 M, pH 7.1) - 44.0 g, $NaH_2PO_4.2H_2O$ + 258.0 g, $Na_2HPO_4.12H_2O$ + 10.0 g, SDS. Volume made upto 5000 ml with distilled water.

2. Electrode buffer - 1 part buffer stock solution + 1 part distilled water.

3. Phosphate sample buffer (0.01 M, pH 7.1)- 5.0 ml phosphate buffer stock solution + 1.0 ml 2-mercaptoethanol + 1.0 g, SDS. Volume made upto 100 ml with distilled water.

4. Acrylamide solution - 22.2 g, acrylamide + 0.6 g, Bis dissolved in distilled water to make the volume 100 ml. The solution was filtered through a Whatman No. 1 filter paper.

5. Ammonium persulphate solution - 150 mg of ammonium persulphate dissolved in distilled water to make volume of 10 ml.

To prepare 7.5% polyacrylamide gels, 22.2 ml acrylamide solution, 3.2 ml ammonium persulphate, 0.1 ml TEMED, 33.0 ml phosphate buffer stock solution and 7.5 ml distilled water were mixed and poured into vertical glass tubes of 0.6 cm diameter to make the height of the gels as 15 cm. Polymerization took about 40 to 50 min. The histone samples were dissolved in sample buffer and then incubated in a boiling water bath for 5 min. The samples were chilled and then 0.01 ml Bromophenol

Blue marker dye and 0.01 ml 2-mercaptoethanol were added to 0.25 ml of the sample. Before loading the samples on the gels, preelectrophoresis was carried out for 20 - 30 min with 150 mA current. Samples were then loaded on the respective gels and then electrophoresis was carried out with 6 mA/tube current for 1 h and further 8 mA/tube current till the marker dye moved 13 cm. Gels were then removed and stained with Coomassie Blue and destained with ethanol, acetic acid, water as described in polyacrylamide urea gel electrophoresis. The gels were scanned in Gilford 250 spectrophotometer equipped with gel scanner at wavelength 600 nm. Commercial preparations of calf thymus histones of known molecular weights were used as standard in this system. As the mobility of the dye was kept constant (13 cm) the graph of log molecular weight against mobility was plotted in case of calf thymus histones. From this standard graph, the molecular weights of finger millet, pearl millet and rice histones were determined.

RESULTS AND DISCUSSION

Size and number of chromosomes

Karyomorphological studies of temperate Gramineae species such as wheat, oat, rye and barley have indicated the presence of long chromosomes with reticulate interphase organization.

In finger millet, chromosome size was observed to be in the range of 2 to 3 µm and 36 chromosomes (Fig. 5.1a) were observed in mitotic metaphase. Kempanna et al. (1976) have reported similar results.

In pearl millet, 14 chromosomes were observed in mitotic

metaphase which range in size from 3.0 to 5.0 µm (Fig. 5.1b). This compares well with the reports of Sree Ramlu and Rangaswamy (1971).

In rice, 24 chromosomes were observed which were of 1 - 2.8 µm in size with median or submedian primary constriction (Fig. 5.1c). Similar observations are reported by Mukherjee and Mukherjee (1979).

Structural organization of interphase nucleus

Monocotyledons are often characterized by presence of reticulate nuclei. Lafontaine (1974) has associated presence of reticulate nuclei with high nuclear DNA content. In finger millet, pearl millet and rice, nuclear DNA content (1C) ranges from 0.6 to 2.5 pg and these are considered to be low DNA containing species. In order to assess the role of DNA content in nuclear structure, we have studied interphase nuclei in the above mentioned species. In finger millet, deeply stained chromocentres are present with very thin chromonemata. In certain cases, matrix of the nucleus appears to be structureless (Fig. 5.2a). In pearl millet a similar situation is apparent (Fig. 5.2b). However, rice shows diffuse interphase nuclei with less demarked chromocentres (Fig. 5.2c). Even though general structure of interphase nuclei in these three species is similar to reticulate nuclei, no distinct reticulum has been observed in all these cases. Small size of the chromosomes, low nuclear DNA content and reticulate nuclear organization suggest that genomes of these species are remarkably distinct from the other temperate, monocotyledonous Gramineae species.

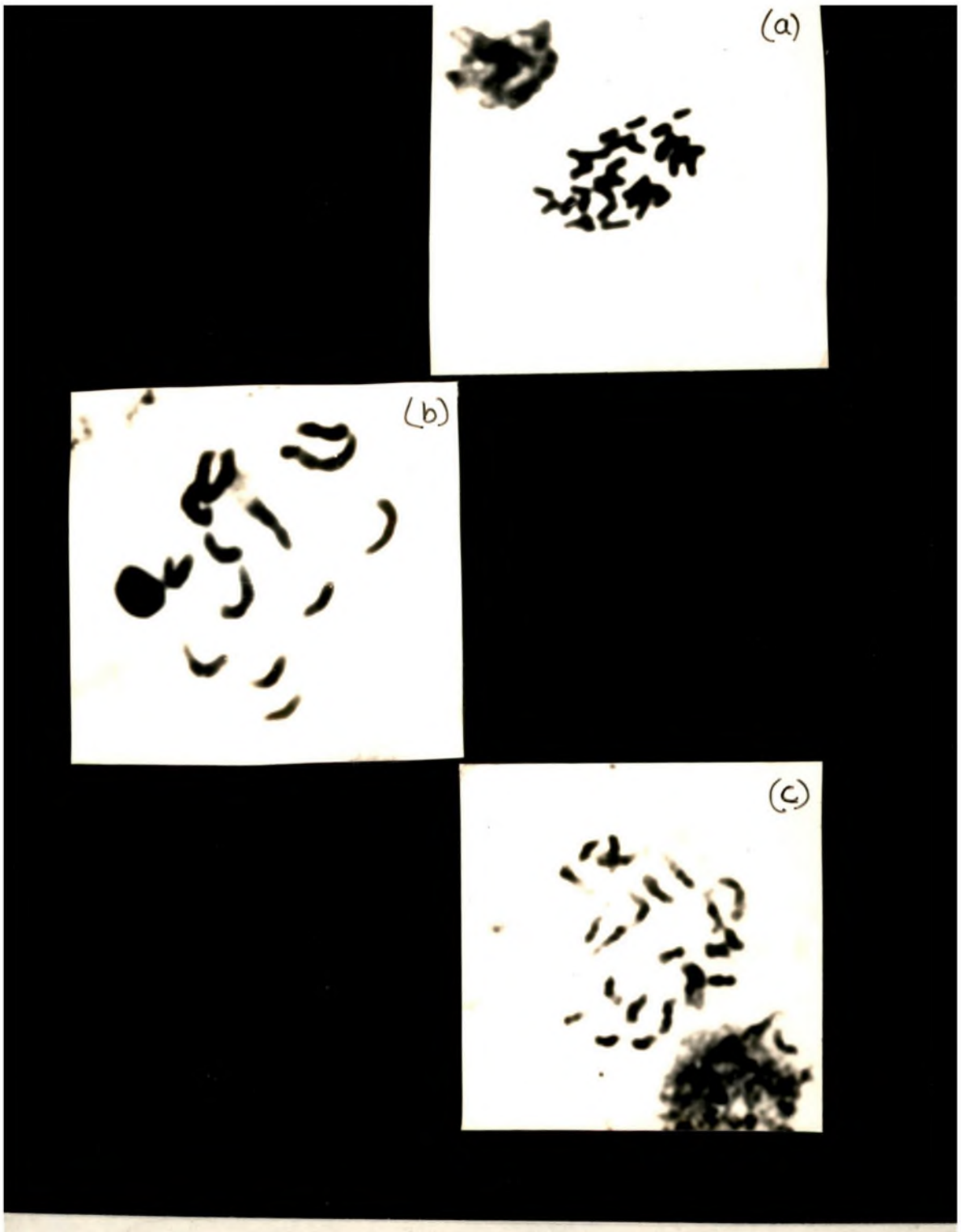


Fig. 5.1: Metaphase chromosomes of
(a) finger millet (b) pearl millet and (c) rice
(magnification 1600 X)

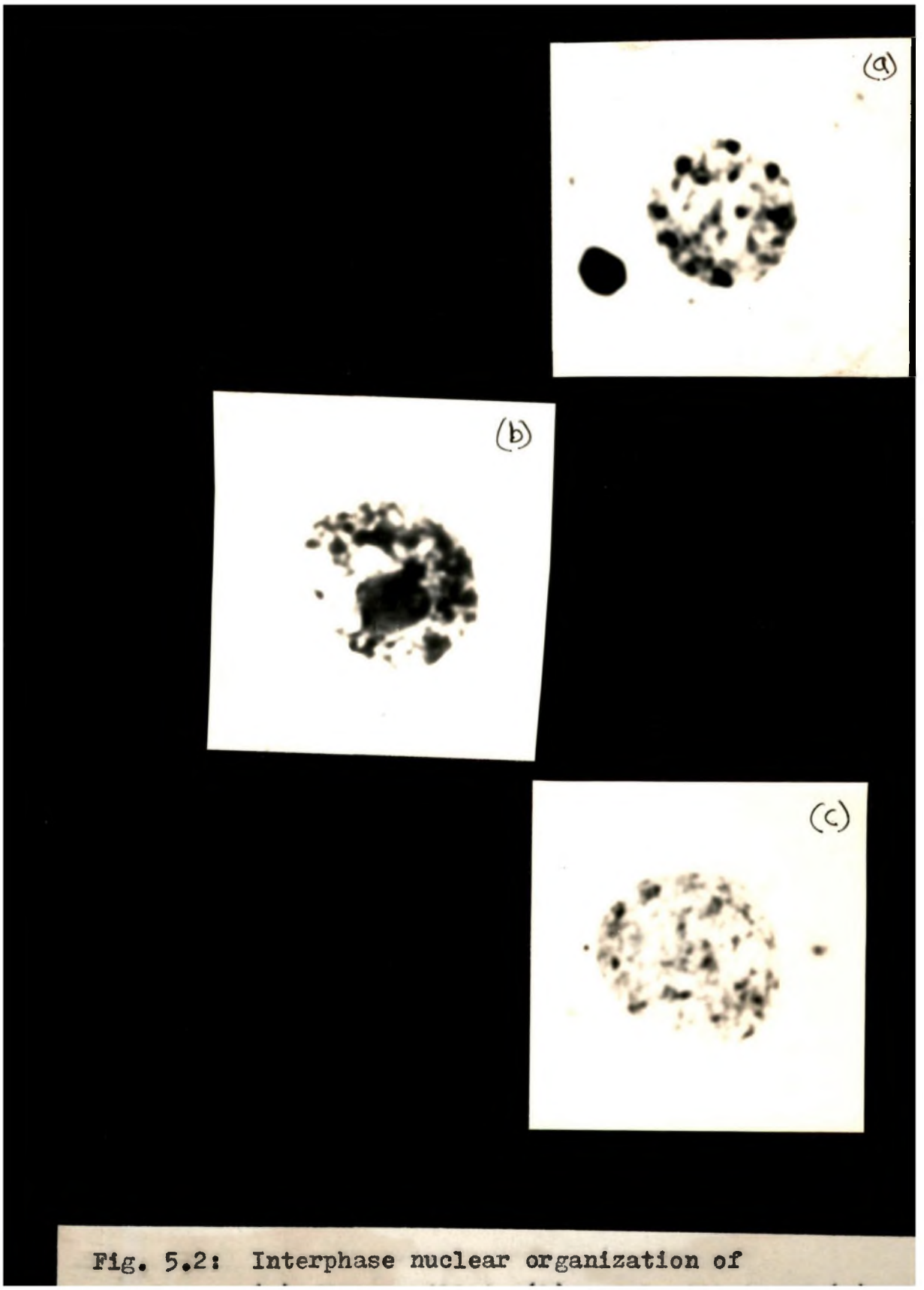


Fig. 5.2: Interphase nuclear organization of

Chromatin isolation

The ultraviolet absorption spectra (320 - 220 nm) of finger millet (Fig. 5.3), pearl millet (Fig. 5.4) and rice (Fig. 5.5) chromatin are similar to those of calf thymus and pea bud (Bonner et al., 1968). They show maximum absorption in the range of 255 to 260 nm and a minima at 240 nm which is a characteristic feature of chromatin. The A_{280}/A_{260} and A_{240}/A_{260} ratios for finger millet, pearl millet and rice vary in the range of 0.61 - 0.7 and 0.7 - 0.9, respectively, which are comparable with the reported values of purified chromatin (Bonner et al., 1968; Bhorjee and Pederson, 1973). In the case of rice (Fig. 5.5), the A_{240}/A_{260} ratio is high (0.92) as compared to the one (0.7) reported by Iqbal Ahmed and Padayatty (1980). The observed A_{320}/A_{260} ratios of 0.09 - 0.2 suggest the absence of any contaminating aggregates of nonchromosomal protei

Histone characterization

For the identification of histones, polyacrylamide urea gel electrophoresis experiments were carried out. Histones were identified by comparing the electrophoretic mobilities of finger millet, pearl millet and rice histones with those of calf thymus histones (Fig. 5.6). The relative mobilities of lysine rich histones H_1 and arginine rich histones H_3 and H_4 of finger millet, pearl millet and rice are quite similar to those of calf thymus histones. In the case of pearl millet, H_3 and H_4 bands consist of several minor bands. The histones H_2B and H_2A of finger millet, pearl millet and rice show different relative mobilities from those of calf thymus H_2B and H_2A histones (Fig. 5.6). These histones which show some

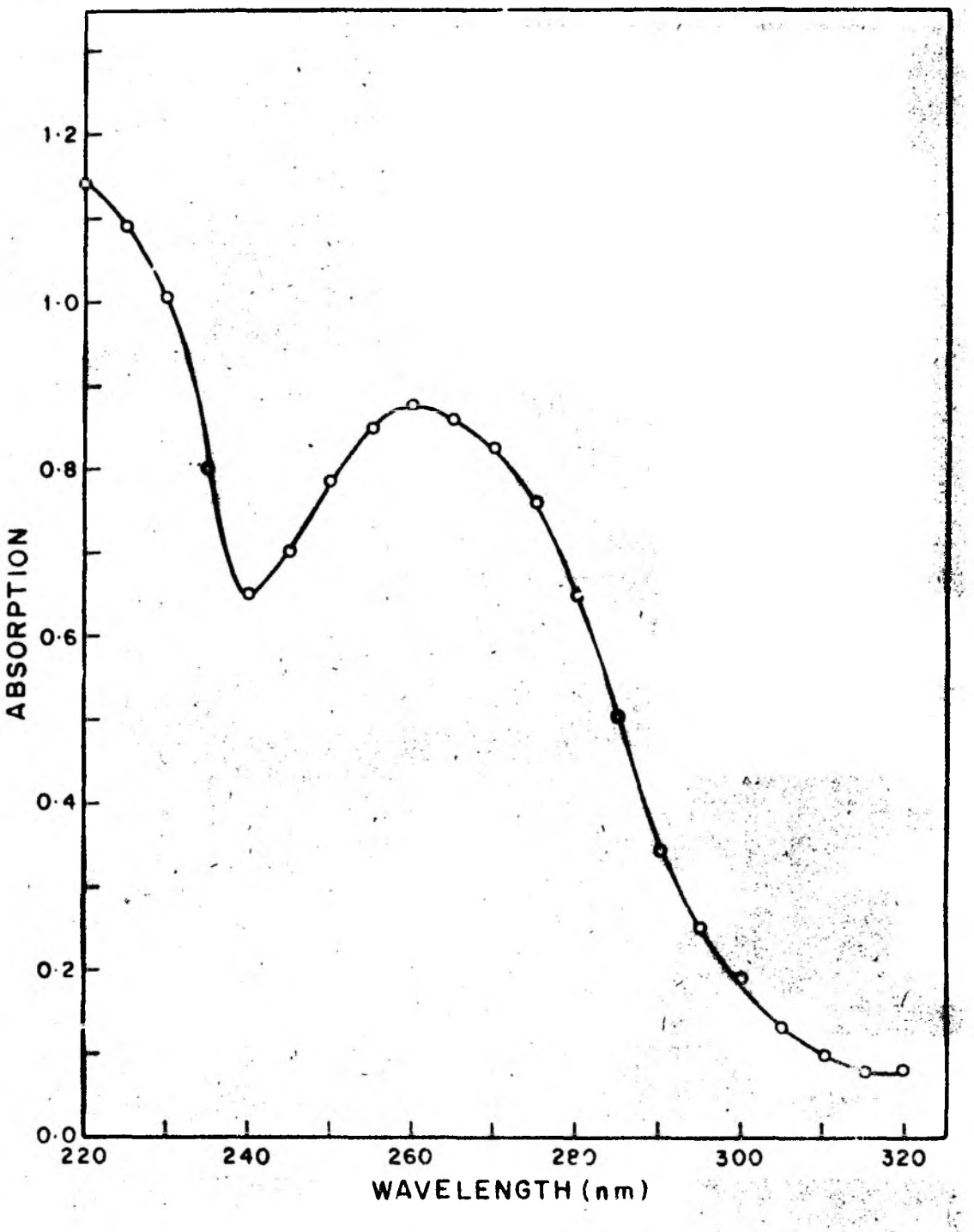


Fig. 5.3: Ultraviolet absorption spectrum of finger millet chromatin in 0.2 mM EDTA/Tris pH 8.0.

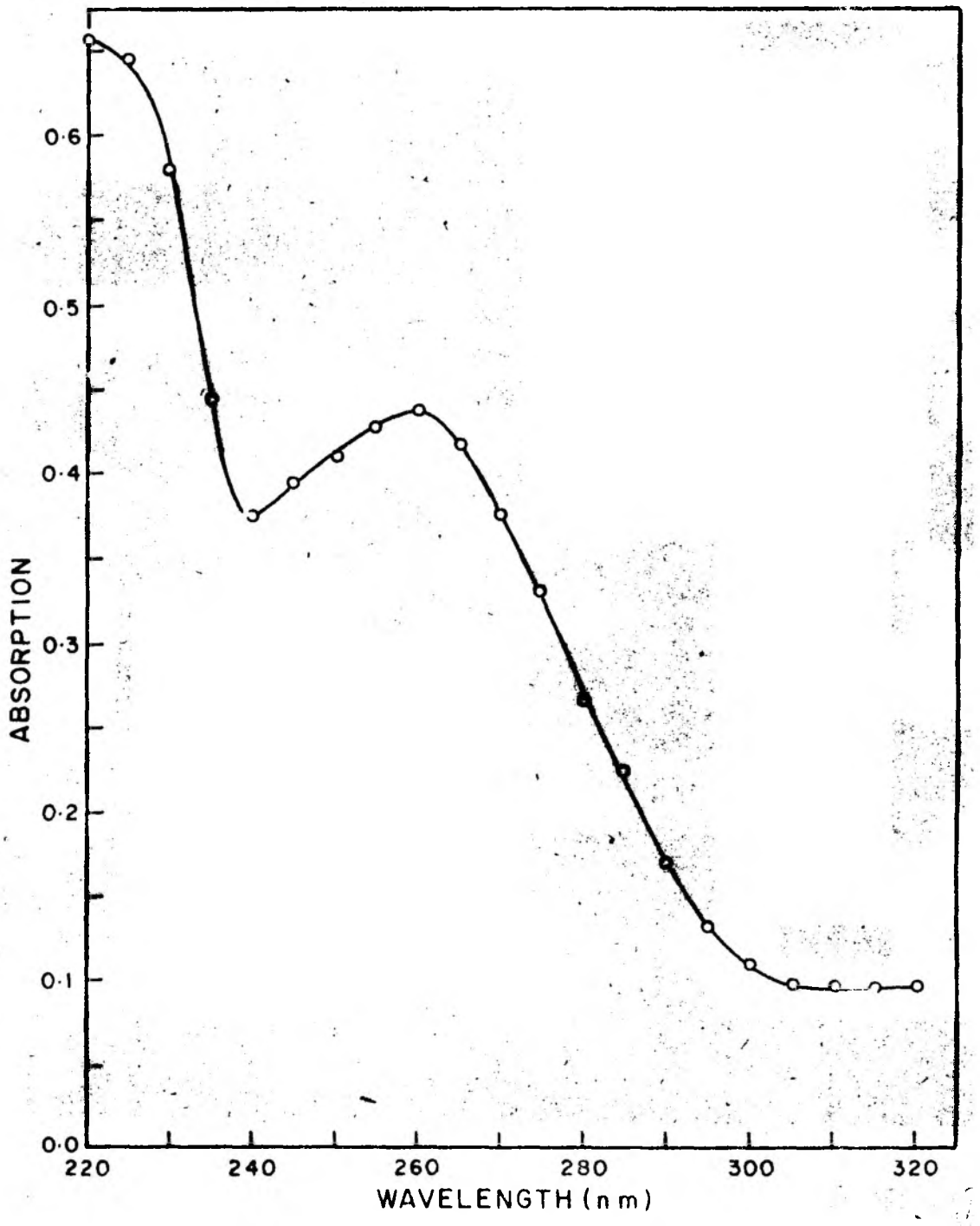


Fig. 5.4: Ultraviolet absorption spectrum of pearl millet chromatin in 0.2 mM EDTA/Tris, pH 8.

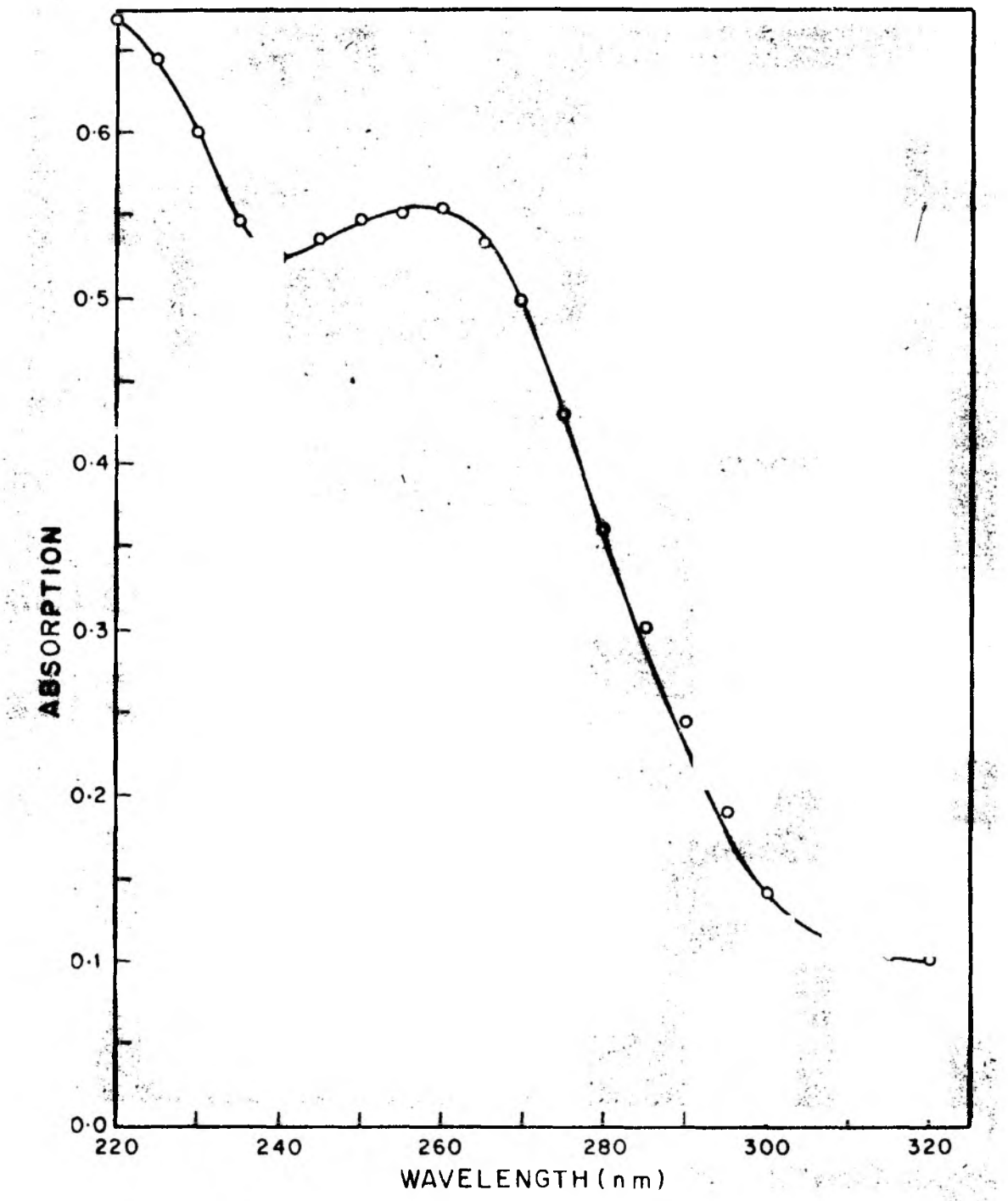


Fig. 5.5: Ultraviolet absorption spectrum of rice chromatin in 0.2 mM EDTA/Tris pH 8.0.

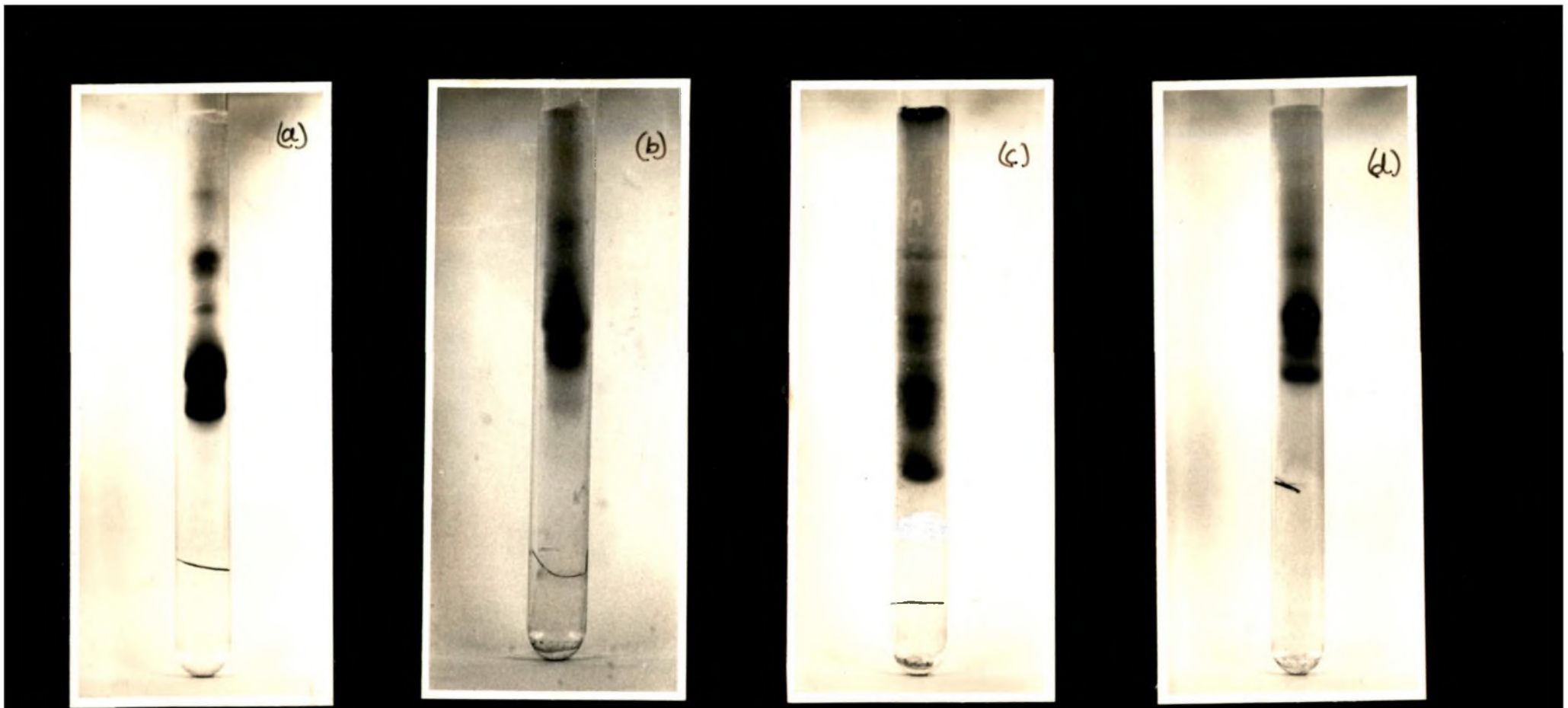


Fig. 5.6: Polyacrylamide urea gel electrophoresis pattern of
(a) calf thymus (b) finger millet (c) pearl millet and (d) rice histones

differences from the vertebrate histones were also studied by Nadeau et al. (1974) and were named as 'Plant histones (PH)' specifically. It was assumed that these peculiar 'plant histone play a key role in determining the characteristic reticulate organization of the interphase nuclei in many plants (Lafontain 1968). Later on Nadeau et al. (1974) reported the histone pattern of radish which was quite similar to the other plant species containing specific 'plant histones'. The interphase nuclear organization of radish, however, resembles the typical animal cell nuclear structure, thus ruling out the hypothesis put forward by Lafontaine (1968). This hypothesis can be reviv in the case of finger millet, pearl millet and rice. Putting cytological and biochemical observations together, it can be said that finger millet, pearl millet and rice contain specific plant histones and their interphase nuclei show reticulate organization, thus indicating the key role of plant histones i determining the interphase nuclear organization.

SDS polyacrylamide gel electrophoresis

This is the most commonly used methodology in protein chemistry to determine the molecular weights of proteins. We have employed the same technique to determine the molecular weights of finger millet, pearl millet and rice histones using calf thymus histones as molecular weight markers. The electrophoresis was carried out as described in Materials and Methods. Fig. 5.7, 5.8, 5.9 and 5.10 represent the scans of SDS polyacrylamide gels of histones of calf thymus, finger millet, pearl millet and rice, respectively. Mobilities of cal thymus histones corresponding to the peaks in the scan were

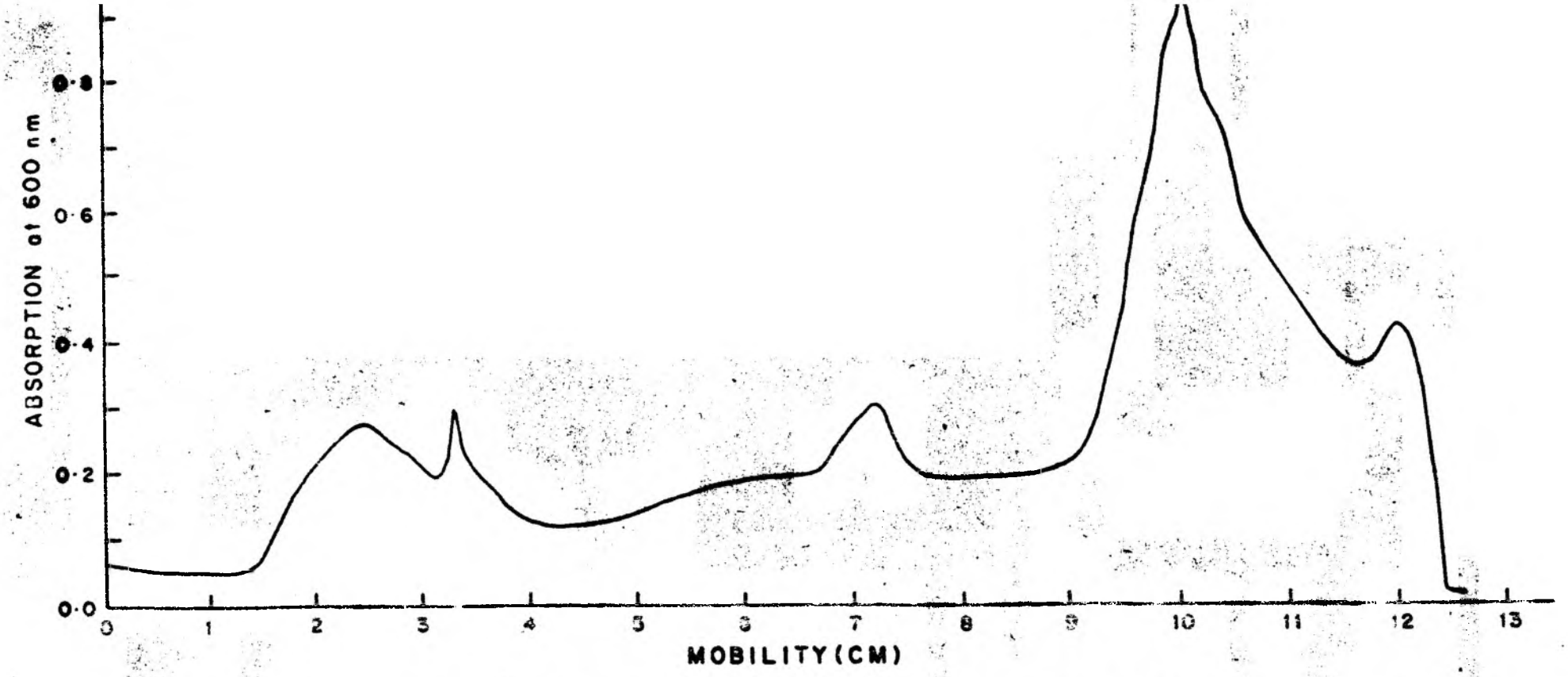


Fig. 5.7: The spectrophotometric scan of SDS-polyacrylamide gel of calf thymus histone at 600 nm.

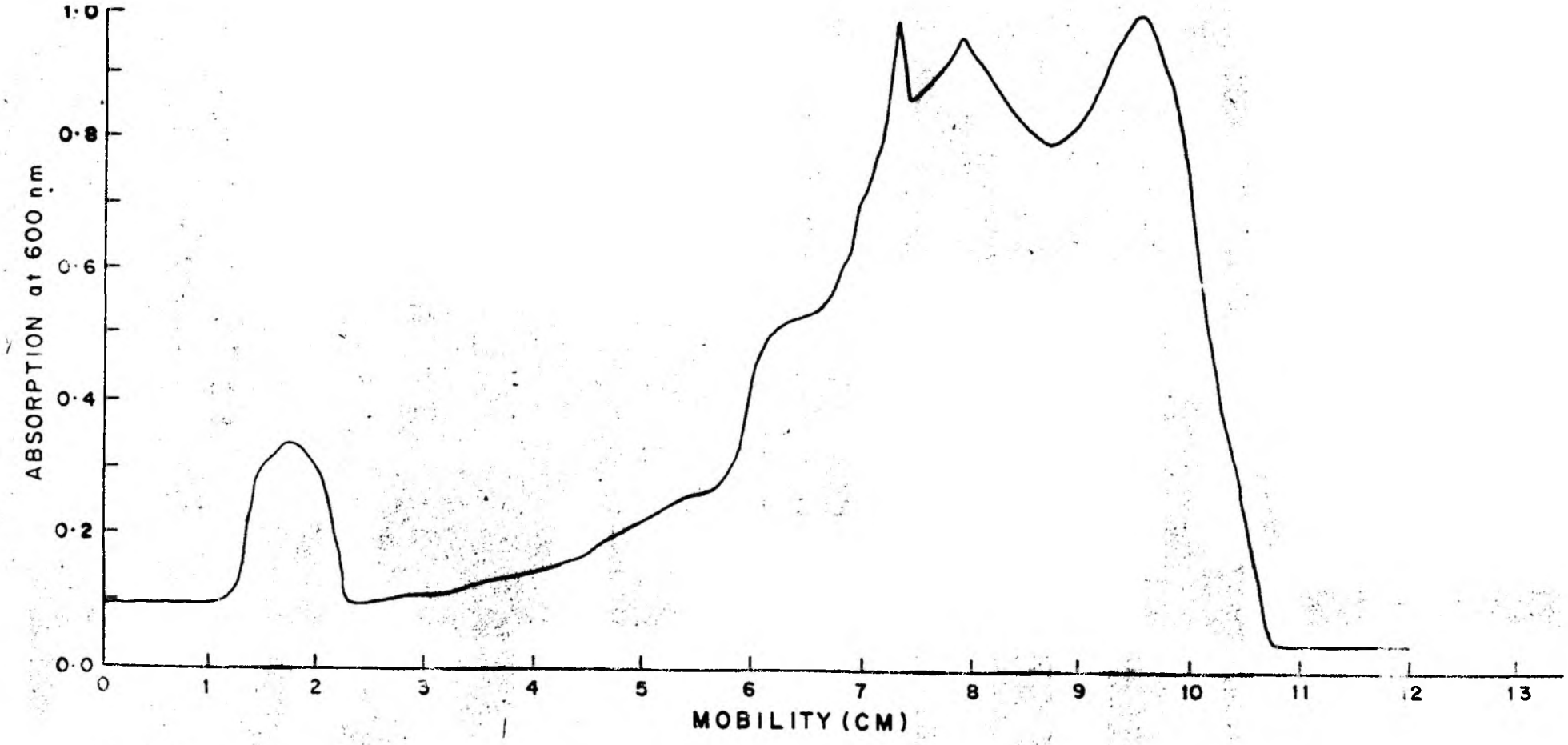


Fig. 5.8: The spectrophotometric scan of SDS-polyacrylamide gel of finger millet histones at 600 nm.

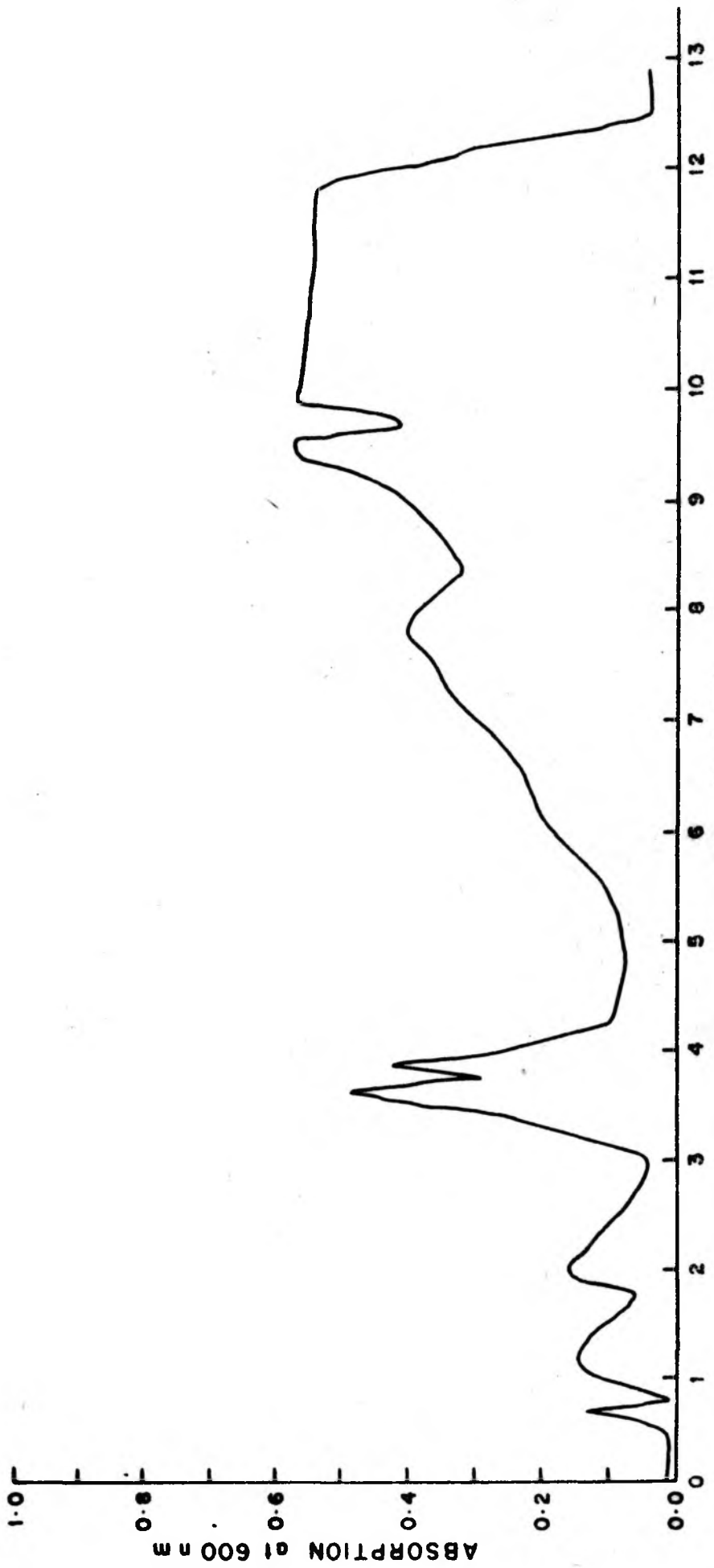


Fig. 5.9: The speotrophotometric soan of SDS-polyacrylamide gel of pearl millet histones at 600 nm.

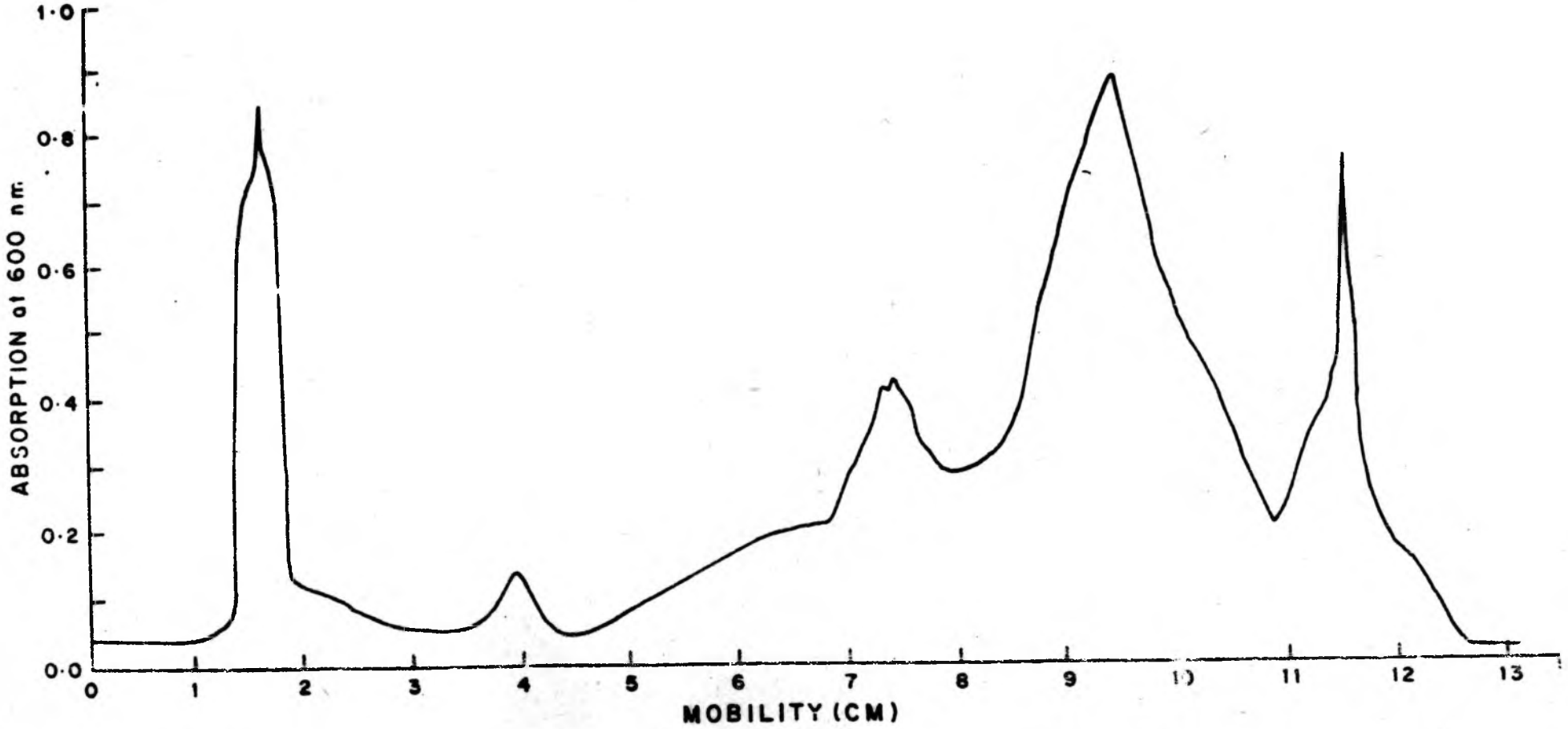


Fig. 5.10: The spectrophotometric scan of SDS-polyacrylamide gel of rice histones at 600 nm.

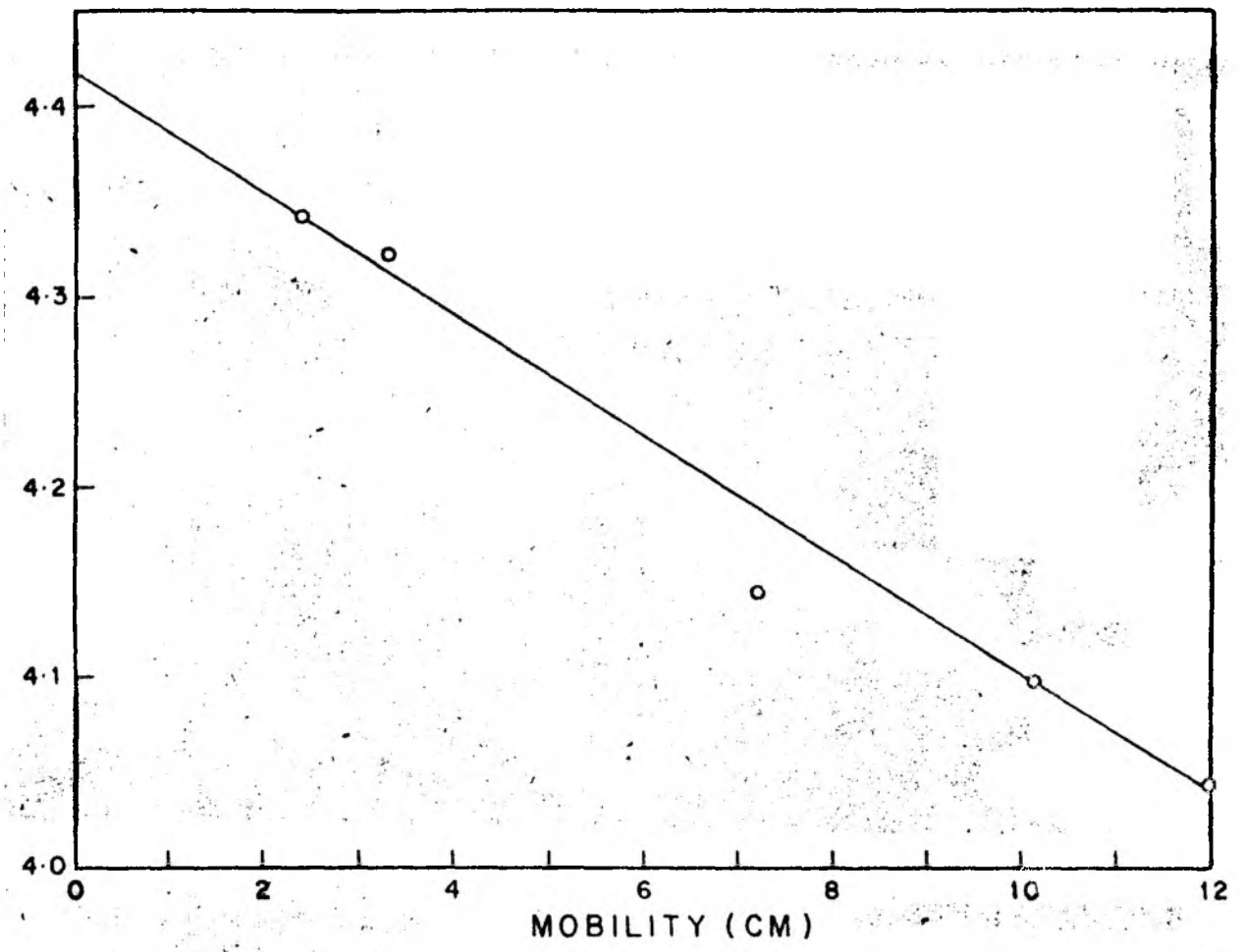


Fig. 5.11: The graph of log of known molecular weights of calf thymus histones as against their mobilities in SDS-polyacrylamide gel electrophoresis

Table 5.1: Comparison of molecular weights of histones of three Gramineae species with that of calf thymus histones

Histones	Calf thymus	Finger millet	Pearl millet	Rice
			24,900	
			24,000	
		23,000		23,000
			22,600	
H_1	22,000			
H_3	21,000			
			20,000	
			19,600	19,600
		16,000		
		15,400		15,200
		14,800	14,800	
H_2B	14,000			
PH		13,000	13,100	13,200
H_2A	12,500		12,600	
				11,300
H_4	11,000		11,100	

then plotted against the logarithum of molecular weights of calf thymus histones (Weber and Osborn, 1969), which is a linear function (Fig. 5.11). From the mobilities of finger millet, pearl millet and rice histones on SDS polyacrylamide gels, their molecular weights were determined using Fig. 5.11. These are as summarized in Table 5.1. Molecular weights of finger millet, pearl millet and rice histones are in the range of 11,000 to 25,000 as revealed from Table 5.1, which is a common range for histone molecular weights.

CONCLUDING REMARKS

When the mobilities and the molecular weights of histones of finger millet, pearl millet and rice are compared among themselves and with calf thymus, certain variations are observed. Finger millet and rice histones are similar to each other in revealing only five histones bands. Pearl millet differs from finger millet and rice in having two or three small bands grouped together to give one band corresponding to the major histones. However, the overall pattern of five histones remains the same in all the three species suggesting that a limited number of genes code for histone proteins. They must be originated early in evolution and must have maintained all of their major features since then. This further indicates that the histones carry out similar functions in an identical manner in all the species which contain histones. This supports the fact that unlike enzymes, histones possess no active sites and are especially resistant to evolutionary change.

CHAPTER VI

GENERAL DISCUSSION

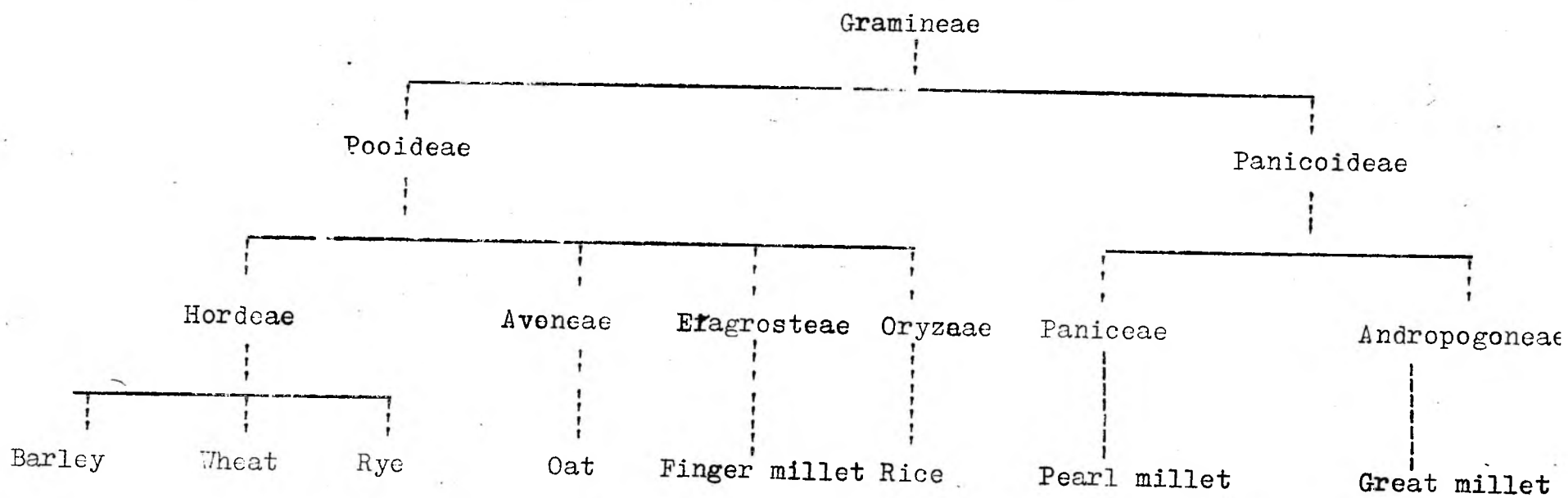
GENERAL DISCUSSION

Classification and origin of Gramineae species

Most of the cereals are poorly understood from taxonomic point of view, Many controversial points exist about their placement in the family Gramineae. According to the recent classification (Hutchinson, 1973), family Gramineae is divide into two subfamilies namely Pooideae and Panicoideae, each consisting of a number of tribes. Finger millet and rice com under Pooideae belonging to the tribes Eragrosteae and Oryzeae, respectively; while great millet and pearl millet fall under Panicoideae and belong to tribes Andropogonae and Paniceae, respectively. Comparative classification of common known, Gramineae species is shown in Fig. 6.1. Finger millet great millet and pearl millet are of African origin and may have reached India as domesticated cereals during the second millennium B.C. These are typically Kharif crops and are use as staple food in African and Asian countries. Rice is of Indian origin and was later cultivated all over the world. It is one of the world's most important food plants.

From Hutchinson's classification scheme (1973), it appea that the present four species are more evolved as compared to wheat, oat, rye and barley. Nuclear DNA contents in our four species are also very low when compared to the other Graminea species. We have, therefore, selected finger millet, great millet, pearl millet and rice for analysis of their DNAs. Our main interest in characterizing the DNAs of these species is to compare the data with that in other well charactrized Gramineae species and to assess the changes that may have tak

Fig. 6.1: Classification of some of the Gramineae species according to Hutchinson (1973)



place during evolution. We also wish to understand the speciation events at the molecular level in these four species.

Computer analysis of reassociation kinetics data

Reassociation kinetics by HA column chromatography is the most commonly used methodology for estimation of different DNA components reannealing with different rate constants. Recent use of computer to analyse these data has made this technique precise and perfect. Computer analysis is applied to a number of functions used to interpret kinetic measurements. Least square analysis is used to resolve repetitive and single copy components in DNA reassociation experiments. In most of the eukaryotes, the repetitive DNA component is heterogeneous in nature and it is very difficult to predict the number and reassociation rates of pure, individual component With the aid of computer analysis, an excellent solution can be derived for such a problem. Moreover, if the repetitive DNA fraction is homogeneous, least square analysis provides accurate rate constant to calculate its kinetic complexity. Hence we have carried out computer analysis of finger millet, pearl millet and rice reassociation data. No such analysis is carried out in the case of great millet. Use of versatile STEPIT programm with direct search method gives equal weight to all the individual measurements and resolves the reassociatic curves of finger millet and pearl millet into two components and that of rice into three components with root mean square value in the range of 0.026 to 0.045. The latter value is very low and comparable to that found in the literature (Wimpee and Rawson, 1979; Gurley et al., 1979). The solutions of

non-linear least square regression analysis are discussed in Chapter II. The repetitive DNA content estimated by this method is in the range of 49% - 54%. The reassociation kinetics of great millet reveals the proportion of repetitive DNA as 53% which is also in the same range.

As observed from Fig. 2.7 in Chapter II, the computer fit for second order reaction obtained for the intermediately reassociating DNA, in the case of rice is not very close to the experimental curve, thus clearly indicating that the intermediately repetitive rice DNA does not reassociate with second order kinetics. The apparent order of this reaction is not known in the case of rice. We are studying the heterogeneity of the reacting components in further details using the form of the reaction as $C/CO = (1 + K\ Cot)^{-n}$ with the help of computer analysis (Pearson et al., 1977).

Highly repetitive DNA and its correlation with satellite DNA

The proportion of very rapidly reassociating DNA is very high (15 - 20%) in the case of finger millet, great millet and pearl millet and is comparatively low (8.5%) in the case of rice (Table 2.1). Though there is only one report of monocotyledons showing the presence of satellite DNA on neutral CsCl density gradient centrifugation (Capesius et al., 1975; Capesius, 1976), the existence of cryptic satellites in some monocotyledons on Ag^{+}/Cs_2SO_4 and Hg^{++}/Cs_2SO_4 density gradient centrifugation (Huguet and Jouanin, 1972; Timmis et al., 1975; Deumling et al., 1976; Ranjekar et al., 1976; 1978a; Wimpee and Rawson, 1979) has been shown. From the study of these satellite DNAs, it is now known that very

highly repetitive DNA sequences form duplexes by Cot 10^{-2} mol x sec/l. It is, therefore, likely that a substantial proportion of Cot 10^{-1} DNA may represent the cryptic satellite DNA. It can also be possible that a small portion of the Cot 10^{-1} DNA is due to the presence of fold back or palindromic DNA sequences. Our experimental data, however, cannot throw any light on the presence and proportion of palindromic sequences in the four cereal species. As no labelled DNA was used in these studies, determination of reassociation at very low Cot values was not feasible and hence no data are available about the frequency of repetition and the kinetic complexity of the nucleotide sequences present in these fractions except in the case of rice. The Cot 1/2 value of very rapidly reassociating DNA fraction of rice is 1.6 x 10^{-2} mol x sec/l and the kinetic complexity is of the order of 1.05 x 10^{3} np.

In the case of pearl millet repetitive DNA, Cot 0.1, 1.0 and 10.0 fractions show presence of high melting component. The proportions of this component in Cot 0.1, 1.0 and 10.0 DNA fractions are 10%, 11% and 7% of the total genome, respectively. Interestingly enough, in spite of the presence of such a proportion (average 9.3%) of high melting component, which is supposedly also very G + C rich, pearl millet DNA does not form a satellite DNA band upon isopycnic centrifugation in CsCl gradients (Wimpee and Rawson, 1979). However, cryptic satellites have been reported to be present in this plant species (Wimpee and Rawson, 1979). It is, therefore, very likely that the nucleotide sequences present in the high melting DNA component of pearl millet may contribute, at least in

part, to the formation of a satellite DNA peak in either Ag^{+}/Cs_2SO_4 or Hg^{++}/Cs_2SO_4 gradients. In fact, similar high melting DNA components have been shown to be present in quite a few plant satellites (Bendich and Anderson, 1974; Sinclair et al.,1975; Timmis et al., 1975; Chilton, 1975; Ranjekar et al., 1978c).

DNA content and proportion of repetitive DNA

With our data on the repetitive DNA content determinations of four more Gramineae species, it is now possible to comment on the relationships between the amount of repetitive DNA and the nuclear DNA content in Gramineae. Flavell et al. (1974), in their extensive survey of 23 angiosperms have shown that plant species with DNA content above 5 pg have more repetitive DNA than those with DNA content less than 4 pg. Studies on beans (Straus, 1972), conifers (Miksche and Hotta, 1973), Lathyrus (Narayan and Rees, 1977) and Phaseolus species (Seshadri and Ranjekar, 1979; 1980a) tend to support the conclusions of Flavell et al., (1974); while contradictory results are obtained in the case of Anemone (Cullis and Schweizer, 1974) and Allium (Ranjekar et al., 1978b). As described in Chapter II, the haploid nuclear DNA content in finger millet, pearl millet and rice are in the range of 0.6 to 2.5 pg and the repetitive DNA content varies from 49% to 54%. These data, therefore, appear to support the results of Flavell et al. (1974). The haploid DNA content of great millet is in the range of 3.0 - 7.0 pg which is on the border of high and low DNA containing species and repetitive DNA content is 52%. Hence, it is difficult to apply any of these

Relationship between DNA content and DNA sequence organization

From the available data on plant species, a relationship can be postulated between the DNA content and the DNA sequence organization pattern. Plant species with an haploid genome size more than 2.5 pg exhibit mostly short period interspersion pattern which is typical of animal genome. Examples are wheat (18.1 pg), rye (7.9 pg), tobacco (3.9 pg), soybean (6.5 pg) per cell) and pea (4.9 - 5.2 pg). On the other hand, plant species with the haploid DNA content less than 2.5 pg show diversed nature of genome organization. This is evident in cotton (0.795 pg per cell), pearl millet (2.5 pg), mung bean (0.5 pg) and french bean (1.8 pg). In these species, the length of the interspersed repeated sequences varies in the range of 1200 - 5000 np. Finger millet, pearl millet and rice fall under this category. In view of its wide occurrence, short period interspersion pattern in eukaryotic genomes is assumed to be preferred to long period interspersion pattern during the course of evolution. Further, Britten and Davidson (1969) and Davidson and Britten (1979) have proposed a model which is based on the significance of short period interspersion pattern in the coordinate regulation of transcription. However, considering the recent data where interspersion patterns are not typically short period, it appears that the above model may have to be either altered or modified.

Role of DNA content in interphase nuclear organization

According to Lafontaine (1974) low DNA content in plants plays an important role in the chromocentric organization of

interphase nucleus. The plant species with high DNA content usually show reticulate interphase nuclear organization. The same observation is further confirmed by Nagl (1978). Howeve the three Gramineae species, namely finger millet, pearl mill and rice contain low DNA and show reticulate type of interpha nuclear organization. On further detail analysis of his observation, Lafontaine (1974) suggested that the average amo of DNA per chromosome plays role in determining the nuclear organization. In this respect, certain species (for example, Crepis cappillaris and Haplopappus gracilus) with relatively low DNA content exhibit reticulate interphase nuclei. In suc species, as the number of chromosomes is less, the amount of DNA per chromosome increases and reaches to a sufficient leve of DNA so as to take on a rather compact organization at interphase. This observation is also not true in the case of three Gramineae species under present discussion. Relatively more number of chromosomes and less amount of DNA bring down the level of DNA per chromosome. The unclear visualization o chromonemata in these three species confirms the reticulate interphase nuclear organization which is an exception to the hypothesis stated by Lafontaine (1974).

Molecular approach to phylogenetic study

As mentioned in the begining of this Chapter, our main interest in studying the genomes of present four species was to assess the changes in their DNAs as a result of their low nuclear DNA content and their advanced evolutionary status. Information is available showing that the amount of nuclear D content can influence various morphological, physiological an

biochemical characteristics of plants and can play an important role in evolution (Stebbins, 1976). Many phenotypic characters of plants such as structure of the involucre, pappus parts, achene, inflorescence and mature fruits are correlated with the DNA content in the genus Microseris (Price and Bachmann, 1975; Bachmann and Price, 1979; Bachmann et al., 1979). At the genotype level, DNA content affects size of the chromosomes, nuclear organization and the repetitive DNA content. In Microseris, correlation of mitotic cell time, the proportion of intermediately reassociating DNA and number of ribosomal cistrons with that of nuclear DNA content is discussed in detail by Price and Bachmann (1976), Bachmann and Price (1977) and Hemleben et al. (1978). According to Nagl (1978), low nuclear DNA content corresponds to less proportion of repetitive DNA and varied types of DNA sequence arrangements. He has also proposed chromocentric type of interphase nuclear organization with low nuclear DNA content and reticulate type with high nuclear DNA content.

If we compare the chromosome size, chromatin organization, repetitive DNA content and arrangement of repetitive and nonrepetitive DNA sequences on the chromosomes of finger millet, pearl millet and rice with those of other Gramineae species with high DNA content, we find the following observations:

1. The amount of repetitive DNA content in our species (49 - 54%) is low.
2. The DNA sequence organization patterns in the case of finger millet, pearl millet and rice are different from each other as well as from the other Gramineae plant species such

as wheat and rye.

3. The size of the chromosomes is very small.

4. The interphase chromatin organization is of reticulate type.

Except the last observation, all the other data in our Gramineae species are in agreement with the Nagl's hypothesis.

The second important question is about the effect of evolution on the DNA content in plant species. During evolution, many variations are observed in the DNA content of plants (Sparrow et al., 1972; Price, 1976). Increase as well as decrease in the DNA contents are noticed as the species in a series evolve from primitive to advanced. Many theories have been put forth to explain these variations in eukaryotes. One reason of this variation can be due to the repetitive DNA sequences present in eukaryotes. According to Nagl (1978), increase in the DNA content is due to the saltatory replication of repetitive DNA sequences as well as due to duplication and mutation of coding genes. Stebbins (1966) and Rees and Jones (1972) have proposed the decrease in the DNA content during evolution and according to them, this decrease is due to the loss of repetitive DNA sequences as the cells can not afford to loose some important structural genes during evolution. This can be the basis of the observation of Flavell et al. (1974) that repetitive DNA content is proportional to the DNA content. In the comparative study of molecular properties of DNAs of seven Allium species (Ranjekar et al., 1978b), two possible explanations have been given to account for variations in the nuclear DNA content. First, there are duplications or deletions of large chromosomal segments containing repetitive

as well as nonrepetitive segments. This explanation is similar to that of Nagl (1978) as described above. Secondly, superimposed on these variations are changes in the amount of repetitive DNA contents. Such changes are responsible for producing the variations in the percentages of repetitive DNA sequences.

From the available data on phylogenetic evolution in the family Gramineae (Hutchinson, 1973), there appears to be a decrease in the nuclear DNA content as well as repetitive DNA content during evolution. Wheat, oat, rye and barley are less evolved compared to the present four species and have haploid nuclear DNA content of more than 6.7 pg and repetitive DNA content more than 75% (Table 6.1). In finger millet, pearl millet and rice, the haploid nuclear DNA content is less than 2.5 pg and in great millet it is in the range of 3.0 - 7.0 pg and the repetitive DNA content is in the range of 49 - 54%. There is thus a loss of nuclear DNA as well as of repeated DNA sequences in Gramineae during evolution.

The repetitive DNA fractions of all the four species reveal remarkable differences in the extent of nucleotide sequence divergence and kinetic heterogeneity. Finger millet Cot 1.0 DNA fraction consists of only one component of discrete frequency class reassociating with second order kinetics. Pearl millet and rice Cot 1.0 DNAs show two kinetic components which are heterogeneous in nature. Rice genome is conspicous in having a discrete DNA fraction reassociating in the Cot range of 10^{-2} to 5×10^{-1} mol x sec/l. Pearl millet genome differs from those of rice and finger millet, in having the presence of a high melting component in its repetitive DNA

Table 6.1: Relationship between nuclear DNA content and repetitive DNA content in Gramineae

Species	Haploid DNA content pg	Repetitive DNA cont %
Poa trivalis	3.45	82
Zea mays	5.5	78
Barley	6.7	76
Poa annua	6.9	87
Rye	9.45	92
Wheat	18.1	83
Oat	21.5	83
Finger millet	1.6	49
Great millet	3.0 - 7.0	52
Pearl millet	2.5	54
Rice	0.6	52

fraction. The sequence organization patterns are of different types in finger millet, pearl millet and rice. Thus these differences can be considered as the events responsible for speciation.

BIBLIOGRAPHY

Ahnstrom, G. and Natarajan, A.T. (1974) Hereditas, 76, 316-320.

Allan, J., Hartman, P.G., Crane-Robinson, C. and Aviles, F.X. (1980) Nature, 288, 675-680.

Altenburger, W., Hoerz, W. and Zachau, H.G. (1977) Eur. J. Biochem., 73, 393-400.

Angerer, R.C., Davidson, E.H. and Britten, R.J. (1975) Cell, 6, 29-40.

Angerer, R.C., Davidson, E.H. and Britten, R.J. (1976) Carnegie Inst. Wash. Yearbook, 75, 944-948.

Angerer, R.C. and Hough-Evans, B.R. (1977) Receptors and Hormone Action, 1, 1-30.

Appels, R., Driscoll, C. and Peacock, W.J. (1978) Chromosoma (Berl.), 70, 67-89.

Appels, R. and Peacock, W.J. (1978) Int. Rev. Cytol., Supplement 8, 69-126.

Arbuzova, G.S., Gryaznova, I.M., Morozova, T.M. and Salganik, R.I. (1968) Mol. Biol., 2, 249-253.

Aripdzhano., Sh. A., Bespal'ko, N.V. and Ibraginsov, A.P. (1976) Uzb. Biol. Zh., 1, 9-10.

Arnason, U., Pero, R. and Lima-de-Faria, A. (1976) Hereditas, 84, 221-223.

Arrighi, F.E. (1974). In the Cell Nucleus (H. Busch, Ed.) Vol. 2, pp. 1, Academic Press, New York.

Arrighi, F.E., Mandel, M., Bergendahl, J. and Hsu, T.C. (1970) Biochem. Genet., 4, 367-376.

Arrighi, F.E. and Hsu, T.C. (1971) Cytogenetics, 10, 81-86.

Arrighi, F.E., Saunders, P.D., Saunders, G.F. and Hsu, T.C. (1971) Experientia, 27, 964-966.

Arthur, R.R. and Straus, N.A. (1978) Can. J. Biochem., 56, 257-263.

Avery, O.T., MacLeod, C.M. and McCarthy, M. (1944) J. Exp. Med., 79, 137-158.

Axel, R., Melchior, W., Sollner-Webb, B. and Felsenfeld, G. (1974) Proc. Natl. Acad. Sci. (USA), 71, 4101-4105.

Ayres, K.N. (1973) J. of Cellular Physiology, 96, 105-113.

Bachmann, K., Chambers, K.L. and Price, H.J. (1979) Plant Syst. Evol. Supplement 2, 41-46.

Bachmann, K. and Price, H.J. (1977) Chromosoma (Berl.),61, 267-275.

Bachmann, K. and Price, H.J. (1979) Plant Syst. Evol., 131, 17-34.

Baldari, C.T. and Amaldi, F. (1976) Chromosoma (Berl.), 59, 13-22.

Balsamo, J., Hierro, J.M. and Lara, F.J.S. (1973) Cell Differentiation, 2, 131-141.

Barber, D.A. (1966) Nature, 212, 638-640.

Bautz, E.K.F. and Bautz, F.A. (1964) Proc. Natl. Acad. Sci. (USA), 52, 1476-1481.

Bayen, M. and Rode, A. (1970) C.R. Acad. Sci., Ser. D., 271, 2046-2049.

Bazetoux, S., Jouanin, L. and Huguet, T. (1978), Nucl. Acids Res., 5, 751-769.

Beattie, W.G. and Skinner, D.M. (1972) Biochim. Biophys. Acta, 281, 168-178.

Beauchamp, R.S., Pasternak, T. and Straus, N.A. (1979) Biochemistry, 18, 245-251.

Belford, H.S. and Thompson, W.F. (1976) Carnegie Inst. Wash. Yearbook, 75, 362-365.

Bell, G.D.H. (1965), In Essays on Crop Plant Evolution (J. Hutchinson Ed.), pp. 70-102, Cambridge Univ. Press,London.

Bell, A.J. and Hardman, N. (1977) Nucl. Acids Res., 4, 247-268.

Bendich, A.J. (1972) Biochim. Biophys. Acta, 272, 494-503.

Bendich, A.J. and Anderson, R.S. (1974) Proc. Natl. Acad. Sci. (USA), 71, 1511-1515.

Bendich, A.J. and Anderson, R.S. (1977) Biochemistry, 16, 4655-4663.

Bendich, A.J. and Bolton, E.T. (1967) Plant Physiol., 42 , 959-967.

Bendich, A.J. and McCarthy, B.J. (1970a) Genetics, 65, 545-565.

Bendich, A.J. and McCarthy, B.J. (1970b) Genetics, 65, 567-573.

Bendich, A.J. and Taylor, W.C. (1977) Plant Physiol. 59, 604-609.

Bennett, M.D. and Smith, J.B. (1976) Philos. Trans. R. Soc. London, Ser. B., 274 (B933), 227-274.

Beridze, T.G. (1972) Molekul. Biol., 6, 908-914.

Beridze, T.G. (1975) Biochim. Biophys. Acta., 395, 274-279.

Beridze, T.G. (1980a) Biochemistry (USSR), 44, 1546-1553.

Beridze, T.G. (1980b) Plant Sci. Letters, 19,325-338.

Beridze, T.G. and Bragvadze, G.P. (1976) Molekul. Biol., 10, 1279-1289.

Beridze, T.G., Durmishidze, S.V. and Khachidze, O.T. (1973) Dokl. Acad. Nauk. (USSR), 211, 468-469.

Bernardi, G., Carnevali, F., Giorgio, N., Antonio, P. and Gianni, T. (1968) J. Mol. Biol., 37, 493-505.

Bernstine, E.G. (1978) Exp. Cell. Res., 113, 205-208.

Bhorjee, J.S. and Pederson, T. (1973) Biochemistry, 12, 2766-2773.

Birnboim, H.C. and Sederoff, R. (1975) Cell, 5, 173-181.

Bishop, J.O. and Freeman, K.B. (1973) Cold Spring Harbor Symp. Quant. Biol., 38, 707-716.

Bishop, J.O., Morton, J.C., Rosbash, M. and Richardson, M. (1974) Nature, 250, 199-204.

Bishop, J.O. and Rosbash, M. (1973) Nature New Biol., 241, 204-207.

Blumenfeld, M., Fox, A.S. and Forrest, H.S., (1973) Proc. Natl. Acad. Sci. (USA), 70, 2772-2775.

Bodmer, W.F. and Grether, S. (1965) J. Bacteriol. 89, 1011-1014.

Bolton, E.T., Britten, R.J., Cowie, D.B., Roberts, R.B., Szafranski, P. and Waring, M.J. (1966) Carnegie Inst. Wash. Yearbook, 64, 316-332.

Bond, H.E., Flamm, W.G., Burr. H.E. and Bond, S.B. (1967) J. Mol. Biol., 27, 289-302.

Bonner, J., Chalkley, G.R., Dhamus, M., Fambrough, D., Fujimura, F., Huang, R.C., Huberman, J., Jensen, R., Marushige, K., Ohlenbusch, H., Olivera, B. and Wildhom, J. (1968). In Methods in Enzymology (L. Grossman and K. Moldave Eds.), Vol. 12B, pp. 3-65, Academic Press, New York.

Bonner, J., Gerrard, W.T., Gottesfeld, J., Holmes, D.S., Sevall, J.S. and Wilks, M. (1973) Cold Spring Harbor Symp. Quant. Biol., 38, 303-310.

Borchsenius, S., Belozerskaya, N.A., Merkulova, N.A. and Vorobev, V.I. (1978) Molekul. Biol., 12, 676-688.

Bostock, C.J. (1971) Advan. Cell. Biol. 2, 153-223.

Bostock, C.J., Prescott, D.M. and Hatch, F.T. (1972) Exp. Cell. Res., 74, 487-495.

Boseley, P.G., Bradbury, E.M., Butler-Browne, G.S., Carpenter, B.G. and Stephens, R.M. (1976) Eur. J. Biochem., 62, 21-31.

Botchan, M., Kram. R., Schmid, C.W. and Hearst, J.E. (1971) Proc. Natl. Acad. Sci. (USA), 68, 1125-1129.

Botchan, M., McKenna, G. and Sharp, P.A. (1974) Cold Spring Harbor Symp. Quant. Biol., 38, 383-395.

Bozzoni, I. and Beccari, E. (1978) Biochim. Biophys. Acta, 520, 245-252.

Bragvadze, G.P. (1974) Tezisy. Dokl. Simp. "Strukt. Funkts. Nukleinovykh Kishot Rst.", 11.

Brandt, W.F. and Von Holt, C. (1972) FEBS Letters, 23, 357-360.

Britten, R.J. (1968) Carnegie Inst. Wash. Yearbook, 67, 327-330

Britten, R.J. (1969). In Problems in Biology: RNA in Development (E.W.Hanley Ed.), pp. 187-216, Univ. of Utah Press, Salt Lake City.

Britten, R.J. and Davidson, E.H. (1969) Science, 165, 349-357.

Britten, R.J. and Davidson, E.H. (1971) Quart. Rev. Biol., 46, 111-119.

Britten, R.J., Graham, D.E., Eden, F.C., Painchand, D.M. and Davidson, E.H. (1976) J. Mol. Evol., 9, 1-23.

Britten, R.J., Graham, D.E. and Neufeld, B.R. (1974). In Methods in Enzymology (G.L. Grossman and K. Moldave Eds.), Vol. 29E, pp. 363-418, Academic Press, New York.

Britten, R.J. and Kohne, D.E. (1966) Carnegie Inst. Wash. Yearbook, 65, 78-106.

Britten, R.J. and Kohne, D.E. (1968) Science, 161, 529-540.

Britten, R.J. and Smith, J. (1969) Carnegie Inst. Wash. Yearbook, 68, 378-386.

Britten, R.J. and Smith, J.E. (1970) Carnegie Inst. Wash. Yearbook, 69, 506-507.

Brodzicki, S. (1969) Ser. A., 18, 393-401.

Broekaert, D. and Van Parijs, R. (1975) Cell Differentiation, 4, 139-145.

Brown, S.W. (1966) Science, 151, 417-425.

Brown, J.E. and Jones, K.W. (1973) Chromosoma (Berl.), 38, 313-318.

Brutlag, D., Appels, R., Dennis, E.S. and Peacock, W.T. (1977) J. Mol. Biol. 112, 31-47.

Brutlag, D.L. and Peacock, W.J. (1975). In The Eukaryotic Chromosomes (W.J. Peacock and R.D.Brock Ed.) pp. 35-45, ANU Press, Canberra.

Burton, K. (1968). In Methods in Enzymology (L. Grossman and K. Moldave Ed.), Vol. 12A, pp. 163-166, Academic Press, New York.

Cairns, J. (1963) Cold Spring Harbor Symp. Quant. Biol., 28, 43-46.

Callan, H.G. (1967) J. Cell Science, 2, 1-4.

Campo, M.S. and Bishop, J.O. (1974) J. Mol. Biol., 90, 649-664

Capesius, I. (1976) FEBS Letters, 68, 255-258.

Capesius, I., Bierweiler, B., Bachmann, K., Rucker, W. and Nagl., W. (1975) Biochim. Biophys. Acta, 395, 67-73.

Casperson, T. and Zech, L. (1973). In Chromosome Identificati - Techniques and Applications in Biology and Medicine

(T. Casperson and L. Zech Eds.) Academic Press, New York.

Cech, T.R. and Hearst, J.E. (1975) Cell, 5, 429-446.

Cech, T.R. and Hearst, J.E. (1976) J. Mol. Biol., 100, 227-256.

Cech, T.R., Rosenfeld, A. and Hearst, J.E. (1973) J. Mol. Biol. 81, 299-325.

Chakrabarti, T., Doy, C.H. and Subrahmanyam, N. (1978) Barley Genetics News Letters, 8, 25-29.

Chaudhuri, R.K. and Dutta, S.K. (1976) Indian J. Exp. Biol., 14, 677-679.

Chen, T.R. and Ruddle, F.H. (1971) Chromosoma (Berl.), 34, 51-

Cheng, T.Y. and Sueoka, N. (1963) Science, 141, 1194-1196.

Chilton, M.D. (1973) Nature New Biol., 246, 16-17.

Chilton, M.D. (1975) Genetics (USSR), 81, 469-483.

Chooi, W.Y. (1971) Genetics, 68, 213-230.

Chuang, C.R. and Saunders, G.F. (1974) Biochem. Biophys. Res. Commun., 57, 1221-1230.

Cohen, S.N. (1976) Nature, 263, 731-738.

Compton, J.L., Bellard, M. and Chambon, P. (1976) Proc. Natl. Acad. Sci. (USA), 73, 4382-4386.

Cooke, H.J. (1975) J. Mol. Biol., 94, 87-89.

Cordeiro, M., Wheeler, L., Lee, C.S., Kastritsis, C.D. and Richardson, R.H. (1975) Chromosome (Berl.) 51, 65-73.

Corneo, G., Ginelli, E. and Polli, E. (1970a) Biochemistry, 9, 1565-1571.

Corneo, G., Ginelli, E. and Polli, E. (1970b) J. Mol. Biol., 48, 319-327.

Corneo, G., Zardi, L. and Polli, E. (1970c) Biochim. Biophys. Acta, 217, 249-258.

Corneo, G., Ginelli, E. and Polli, E. (1971) Biochim. Biophys. Acta, 247, 528-534.

Corneo, G., Ginelli, E., Soave, C. and Bernardi, G. (1968) Biochemistry, 7, 4373-4379.

Corneo, G., Zardi, L. and Polli, E. (1972) Biochim. Biophys. Acta, 269, 201-204.

Cortadas, J., Macaya, G. and Bernardi, G. (1977) Eur. J. Biochem., 76, 13-19.

Cortadas, J., Olofsson, B., Meunier-rotival, M., Macaya, G. and Bernardi, G. (1979) Eur. J. Biochem., 99, 179-186.

Coudray, Y., Quetier, F. and Guille, E. (1970) Biochim. Biophys. Acta, 217, 259-267.

Crain, W.R., Davidson, E.H. and Britten, R.J. (1976a) Chromosoma (Berl.), 59, 1-12.

Crain, W.R., Eden, F.C., Pearson, W., Davidson, E.H. and Britten, R.J. (1976b) Chromosoma (Berl.) 59, 309-326.

Crick, F.H.C. (1971) Nature, 234, 25-27.

Crothers, D.M., Kallenbach, N.R. and Zimm. B.H. (1965) J. Mol. Biol., 11, 802-820.

Cullis, C.A. and Schweizer, D. (1974) Chromosoma (Berl.) 44, 417-421.

Curtain, C.C., Pascoe, G. and Hayman, R. (1973) Biochem. Genet., 10, 253-262.

Darlix, J.L. and Horaist, M. (1975) Nature 256, 288-292.

Davidson, E.H. and Britten, R.J. (1979) Science, 204, 1052-1059.

Davidson, E.H., Hough, B.R., Klein, W.H. and Britten, R.J. (1975) Cell, 4, 217-238.

Davidson, E.H., Graham, D.E., Neufeld, B.R., Chamberlin, M.E., Amenson, C.S., Hough, B.R. and Britten, R.J. (1974) Cold Spring Harbor Symp. Quant. Biol., 38, 295-301.

Davidson, E.H. and Hough, B.R. (1971) J. Mol. Biol., 56, 491-506.

Davidson, E.H., Hough, B.R., Amenson, C.S. and Britten, R.J. (1973) J. Mol. Biol., 77, 1-23.

Davidson, E.H., Hough, B.R., Chamberlin, M.R. and Britten, R.J. (1971) Develop. Biol., 25, 445-463.

Davidson, E.H., Klein, W.H. and Britten, R.J. (1977) Develop. Biol., 55, 69-84.

De Jimenez, E.S., Gonzalez, J.L., Dominquez, J.L. and Saloma, E.S. (1974) Eur. J. Biochem., 45, 25-29.

De Lange, R.J. and Fambrough, D.M., Smith, E.L., and Bonner, J. (1969) J. Biol. Chem., 244, 5669-5679.

De Lange, R.J. and Smith, C.L. (1971) Annu. Rev. Biochem., 40, 279-314.

Deininger, P.L., and Schmid, C.W. (1976) J. Mol. Biol., 106, 773-790.

Dennis, E.S., Pryor, A.J., Peacock, W.J. and Rhoades, M.M. (197 Proceed. Australian Biochem. Soc., 12, 70.

Deonier, R.C. and Hadley, R.G. (1976) Nature, 264, 191-193.

Deshpande, V.G. and Ranjekar, P.K. (1980) Hoppe-Seyler's Z. Physiol. Chem., 361, 1223-1233.

Deumling, B. and Nagl., W. (1978) Cytobiologie, 16, 412-420.

Deumling, B., Sinclair, J., Timmis, J.N. and Ingle, J. (1976) Cytobiologie, 13, 224-232.

Dickson, R.C., Abelson, J., Barnes, W.M. and Reznikoff, W.S.

(1975) Science, 187, 27-35.

Dobel, P., Rieger, R. and Michaelis, A. (1973) Chromosoma (Berl.), 43, 409-422.

Dons, J.J.M. and Wessels, J.G.H. (1980) Biochim. Biophys. Acta, 607, 385-396.

Dott, P.J., Chuang, C.R. and Saunders, G.F. (1976) Biochemistry, 15, 4120-4125.

Dunleavy, J.M. and Urs, N.V.R. (1973) J. Virology, 12(1), 188-192.

Dunn, J.J. and Studier, F.W. (1973) Proc. Natl. Acad. Sci. (USA), 70, 3296-3300.

Dunsmuir, P. (1976) Chromosoma (Berl.), 56, 111-125.

Dusenbery, R. (1975) Biochim. Biophys. Acta, 378, 363-377.

Dutta, S.K., Sheikh, I., Choppala, J., Aulakh, G.S. and Nelson, W.H. (1976) Mol. Gen. Genetics, 147, 325.

Eckhardt, R.A. (1970) J. Cell. Biol., 47, 55a.

Eckhardt, R.A. (1976), In Handbook of Genetics (R. C. King Ed.) Vol. 5, pp. 31-53, Plenum Press, New York.

Eckhardt, R.A. and Gall, J.G. (1971) Chromosoma (Berl.),32, 407-427.

Eden, F.C. and Hendrick, J.P. (1978) Biochemistry, 17, 5838-5844.

Efstratiadis, A., Crain, W.R., Britten, R.J., Davidson, E.H. and Kafatos, F.C. (1976) Proc. Natl. Acad. Sci. (USA), 73, 2289-2293.

Elgin, S.C.R. and Weintraub, H. (1975) Annu. Rev. Biochem., 44, 725-774.

Endow, S.A. (1977) J. Mol. Biol., 114, 441-449.

Endow, S.A., Polan, M.L. and Gall, J.G. (1975) J. Mol. Biol., 96, 665-692.

Enea, V. and Zinder, N.D. (1975) Science, 190, 584-586.

Engberg, J. and Klenow, H. (1977) Trends Biochem. Sci., 2, 183-185.

Epplen, J.T., Leipoldt, M., Engel, W. and Schmidtke, J. (1978) Chromosoma (Berl.), 69, 307-321.

Fambrough, D.M. and Bonner, J. (1966) Biochemistry, 5, 2563-2570.

Fambrough, D.M. and Bonner, J. (1969) Biochim. Biophys. Acta, 175, 113-122.

Fangman, W.L. (1978) Nucl. Acids. Res., 5, 653-665.

Felsenfeld, G. (1978), Nature, 271, 115-122.

Felsenfeld, C. and Hirschman, S.Z. (1965) J. Mol. Biol., 13, 407-427.

Felsenfeld, C. and Sandeen, G. (1962) J. Mol. Biol., 5, 587-610.

Feulgen, R. and Rossenbeck, H. (1924) Hoppe-Seyler's Z. Physiol. Chem., 135, 203-248.

Filipski, J. and Rzeszowskalvolny, J. (1974) Acta Biochim. (Pol.), 21, 323-329.

Filipski, J., Thiery, J. and Bernardi, G. (1973) J. Mol. Biol., 80, 177-197.

Finch, J.T., Lutter, L.C., Rhodes, D., Brown, R.S. Rushton, B., Levitt, M. and Klug, A. (1977) Nature, 269, 29-36.

Firtel, R.A. and Bonner, J. (1972) J. Mol. Biol., 66, 339-361.

Firtel, R.A., Jacobson, A. and Lodish, H.F. (1972) Nature New Biol., 239, 225-228.

Firtel, R.A. and Kindle, K. (1975) Cell, 5, 401-411.

Firtel, R.A. and Lodish, H.F. (1973) J. Mol. Biol., 49, 295-314.

Fittler, F. (1977) Eur. J. Biochem., 74, 343-352.

Flamm, W.G., McCallum, M. and Walker, P.M.B. (1967) Proc. Natl. Acad. Sci. (USA), 57, 1729-1734.

Flamm, W.G., McCallum, M. and Walker, P.M.B. (1968) Biochem. J., 108, 42p.

Flamm, W.G., Walker, P.M.B. and McCallum, M. (1969) J. Mol. Biol. 40, 423-443.

Flavell, R.B., Bennett, M.D., Smith, J.B. and Smith, D.B. (1974) Biochem. Genet., 12, 257-269.

Flavell, R.B., Rimpau, J. and Smith, D.B. (1977) Chromosoma (berl.),63, 205-222.

Flavell, R.B., and Smith, D.B. (1975) Stadler Symp., 7, 47-70.

Flavell, R.B. and Smith D.B. (1976) Heredity, 37, 231-252.

Fodor, I. and Beridze, T. (1980) Mol. Biol. Rep., 6, 17-20.

Forstova, J., Votavova, H., Guttmann, T., Pivec, L. and Doskocil, J. (1979) Nucl. Acids. Res., 6, 57-70.

Fouquet, H., Bierweiler, B. and Sauer, H.W. (1974) Eur. J. Biochem., 44, 407-410.

Fredericq, E., Oth, A. and Fontaine, F. (1961) J. Mol. Biol., 3, 11-17.

Fresco, J.R., Klotz, L.C. and Richards, E.G. (1963) Cold Spring Harbor Symp. Quant. Biol., 28, 83-90.

Fry, K., Poon, R., Whitcome, P., Idriss, J., Salser, W., Mazrimas, J. and Hatch, F. (1973) Proc. Natl. Acad. Sci. (USA), 70, 2642-2646.

Fuke, M., Davis, T.M. and Busch, H. (1979) FEBS Letters, 102, 46-51.

Fussell, C.P. (1977) Exp. Cell. Res., 110, 111-117.

Gage, L.F. (1974) Chromosoma, 45, 27-42.

Galau, G.A., Britten, R.J. and Davidson, E.H. (1974) Cell, 2, 9-22,

Galau, G.A., Chamberlin, M.E,, Hough, B.R., Britten, R.J. and Davidson, E.H. (1976). In Molecular Evolution, pp. 200-204, Sinauer Assoc., Sunderland, Mass.

Gall, J.G. and Antherton, D.D. (1974) J. Mol. Biol., 85, 633-664.

Gall, J.G., Cohen, E.H. and Polan, M.L. (1971) Chromosoma (Berl.), 33, 319-344.

Gall, J.G. and Pardue, M.L. (1969) Proc. Natl. Acad. Sci. (USA) 63, 378-383.

Gall, J.G. and Pardue, M.L. (1971). In Methods in Enzymology (L. Grossman and K. Moldave, Eds.) Vol. 21D, pp. 470, Academic Press, New York.

Gatenby, J.B. and Beams, H.W. (1950). In the Microtomists' Vade-mecum (B. Lee Ed.) London.

Gense, M.T. (1971) Biochem. Biophys. Res. Commun., 42, 347-352.

Ghosh, S. and Roy, S.C. (1977) Chromosoma (Berl.), 61, 49-55.

Giacomoni, D. and Corneo, G. (1968) Biochim. Biophys. Acta, 166, 586-588.

Ginelli, E. and Corneo, G. (1972) Atti Accad. Naz. Lincei, Cl. Sci. Fis., Mat. Natur., Rend. (Ital.), 53, 621-629.

Ginelli, E. and Corneo, G. (1976) Chromosoma (Berl.), 56, 55-58

Ginelli, E., Lerna, R.D. and Corneo, G. (1977) Chromosoma (Berl 61, 215-226.

Gnucheva, F. Yu., Kir'yanov, G.I., Polyakov, V. Yu. and

Chentsov, Yu. S. (1977) Biokhimya, 42, 2186-2191.

Goldberg, R.B. (1978) Biochem. Genet., 16, 45-68.

Goldberg, R.B., Bemis, W.P. and Siegel, A. (1972) Genetics, 72, 253-266.

Goldberg, R.B., Crain, W.R., Ruderman, J.V., Moore, G.P., Barnett, T.R., Higgins, R.C., Gelfand, R.A., Galau, G.A., Britten, R.J. and Davidson, E.H. (1975) Chromosoma (Berl.), 51, 225-251.

Goldberg, R.B., Galau, G.A., Britten, R.J. and Davidson, E.H. (1973) Proc. Natl. Acad. Sci. (USA), 70, 3516-3520.

Goldknopf, I.L. and Busch, H. (1977) Proc. Natl. Acad. Sci. (USA), 74, 864-868.

Gurley, W.B., Hepburn, A.G. and Key, J.L. (1979) Biochim. Biophys. Acta, 561, 167-183.

Graham, D.E., Neufeld, B.R., Davidson, E.H. and Britten, R.J. (1974) Cell, 1, 127-137.

Gray, D.M. and Skinner, D.M. (1974) Biopolymers, 13, 843-852.

Greenberg, J.R. and Perry, R.P. (1971) J. Cell Biol., 50, 774-786.

Guille, E. and Grisvard, J. (1971) Biochem. Biophys. Res. Commun., 44, 1402-1409.

Hardman, N. and Jack, P.L. (1977) Eur. J. Biochem., 74, 275-283.

Hardman, N., Jack, P.L., Brown, A.J.P. and McLachlan, A. (1979) Eur. J. Biochem., 94, 179-187.

Hardman, N., Jack, P.L., Fergie, R.C. and Gerrie, L.M. (1980) Eur. J. Biochem., 103, 247-257.

Harris, S.E., Means, A.R., Mitchell, W.M. and O'Malley, B.W. (1973) Proc. Natl. Acad. Sci. (USA), 40, 3776-3780.

Harrison, P.R., Birnie, G.D., Hell, A., Humphries, S., Young, B.D. and Paul, J. (1974) J. Mol. Biol., 84, 539-554.

Hatch, F.T. and Mazrimas, J.A. (1970) Biochim. Biophys. Acta, 224, 291-294.

Hatch, F.T. and Mazrimas, J.A. (1974) Nucl. Acids Res., 1, 559-575.

Hayashi, H., Iwai, K., Johnson, I.D. and Bonner, J. (1977) J. Biochem., 82, 503-510.

Hayes, F.N., Lilly, E.H., Ratliff, R.L., Smith, D.A. and Williams, D.L. (1970) Biopolymers, 9, 1105-1117.

Heitz, E. (1928) Jb. Wiss, Bot., 69, 762-818.

Heller, R. and Arnheim, N. (1980) Nucl. Acids Res., 8, 5031-5042.

Helling, R.B., Goodman, H.M. and Boyer, H.W. (1974) J. Virol., 14, 1235-1244.

Hemleben, V., Bachmann, K. and Price, H.J. (1978) Experientia, 34, 1452-1453.

Henning, W. (1972a) J, Mol. Biol., 71, 407-417.

Henning, W. (1972b) J. Mol. Biol., 71, 419-431.

Henning, W. and Walker, P.M.B. (1970) Nature, 225, 915-919.

Hewish, D.R. and Burgoyne, L.A. (1973) Biochem. Biophys. Res. Commun., 52, 504-510.

Himes, M. and Moriber, L. (1956) Stain Tech., 31, 67-70.

Hirschman, S.Z. and Felsenfeld, G. (1966) J. Mol. Biol., 16, 347-358.

Hock, B. (1967) Plant Physiol., 42, 1149-1152.

Hoerz, W., Hess, I. and Zachau, H.G. (1974) Eur. J. Biochem., 45, 501-512.

Hoerz, W. and Zachau, H.G. (1977) Eur. J. Biochem., 73, 383-392.

Holmes, D.S. and Bonner, J. (1974) Biochemistry, 13, 841-848.

Hossainy, E., Zweidler, A. and Bloch, D.P. (1973) J. Mol. Biol., 74, 283-289.

Hough, B.R. and Davidson, E.H. (1972) J. Mol. Biol., 70, 491-509.

Houck, C.M., Rinehart, F.P. and Schmid, C.W. (1978) Biochim. Biophys. Acta, 518, 37-52.

Houck, C.M., Rinehart, F.P. and Schmid, C.W. (1979) J. Mol. Biol., 132, 239-306.

Hsiang, M.W. and Cole, R.D. (1973) J. Biol. Chem., 248, 2007-2013.

Hsu, T.C. (1973) In Chromosome Identification, Nobel Symp. (T. Casperson and L. Zech Eds). pp 32, Academic Press, New York.

Hsu, T.C. and Arrighi, F.E. (1971) Chromosoma (Berl.), 34, 243-253.

Hudson, A.P., Guny, G., Cortadas, J., Haschemeyer, A.E.V. and Bernardi, G. (1980) Eur. J. Biochem., 112, 203-210.

Hudspeth, M.E.S., Timberlake, W.E. and Goldberg, R.B. (1977) Proc. Natl. Acad. Sci. (USA), 74, 4322-4336.

Huguet, T. and Jouanin, L. (1972) Biochem. Biophys. Res. Commun., 46, 1169-1174.

Huguet, T., Jouanin, L. and Bazetoux, S. (1975) Plant Sci. Letters, 5, 379-383.

Hutchinson, J. (1973). In The Families of Flowering Plants, Third Edition, Oxford University Press, Oxford.

Ingle, J., Pearson, G.G. and Sinclair, J. (1973) Nature New Biol., 242, 193-197.

Iqbal Ahmed, C.M. and Padayatty, J.D. (1980) Indian J. Biochem. Biophys., 17, 104-108.

Jelinek, W., Molloy, G., Solditt, M., Wall, R., Sheiness, D. and Darnell, J.E. Jr. (1973) Cold Spring Harbor Symp. Quant. Biol., 38, 891-898.

Jensen, H. and Davidson, N. (1966) Biopolymers, 4, 17-32.

John, B. and Miklos, G.L.G. (1979) Int. Rev. Cytol., 58, 1-114.

Johns, E.W., (1954) Biochem. J., 92, 55-59.

Johnson, P.H. and Grossman, L.I.(1977) Biochemistry, 16, 4217-4225.

Jones, K.W. (1970) Nature, 225, 912-915.

Jones, K.W. and Corneo, G. (1971) Nature New Biol., 233, 268-271.

Jones, K.W., Prosser, J., Corneo, G. and Ginelli, E. (1973) Chromosoma (Berl.), 42, 445-454.

Jones, K.W., Purdom, I.F., Prosser, J. and Corneo, G. (1974) Chromosoma (Berl.), 49, 161-171.

Jones, K.W. and Robertson, F.W. (1970) Chromosoma (Berl.), 31, 331-345.

Joshi, C.P. and Ranjekar, P.K. (1980) Cytologia, In Press.

Kadouri, A., Atsmon, D. and Edelman, M. (1975) Proc. Natl. Acad. Sci. (USA), 72, 2260-2264.

Kamalay, J.C., Ruderman, J.V. and Goldberg, R.B. (1976) Biochim. Biophys. Acta, 432, 121-128.

Kelly, T.J. and Smith, H.O. (1970) J. Mol. Biol., 51, 393-409.

Kemp, J.D. and Merlo, D.J. (1975) Biochem. Biophys. Res. Commun. 67, 1522-1526.

Kempanna, G., Laxmi, P.V. and Nasrath, R. (1976) Nucleus, 19, 200-203.

Kersten, W., Kersten, H. and Szybalski, W. (1966) Biochemistry, 5, 236-244.

Key, J.L. and Silflow, C. (1975) Plant Physiol., 56, 364-369.

Kiper, M. and Herzfeld, F. (1978) Chromosoma (Berl.), 65, 335-351.

Kirk, J.T.O. (1967) J. Mol. Biol., 28, 171-172.

Kirsch, D.B. and Cohen, E.H. (1980) Biochem. Biophys. Res. Commun., 96, 1603-1610.

Kit, S. (1961) J. Mol. Biol., 3, 711-716.

Klein, W.H., Murphy, W., Attardi, G., Britten, R.J. and Davidson, E.H. (1974) Proc. Natl. Acad. Sci. (USA), 71, 1785-1789.

Klein, H.L. and Welch, S.K. (1980) Nucl. Acids Res., 8, 4651-4670.

Klug, A. (1978) Phil. Trans. Royal Soc. (London) 283, 233-239.

Kohne, D.E. (1970) Quart. Rev. Biophys., 3, 327-375.

Kohne, D.E. and Britten, R.J. (1971). In Procedures in Nucleic Acids Research (G.L. Cantoni and D.R.Davies, Eds.) Vol. 2, pp. 500.

Kopecka, H., Macaya, G., Cortadas, J., Thiery, J.P. and Bernardi, G. (1978) Eur. J. Biochem., 84, 189-195.

Kornberg, R.D. (1974) Science, 184, 868-871.

Kornberg, R.D. (1977) Annu. Rev. Biochem., 46, 931-954.

Kotaka, T. and Baldwin, R.K. (1964) J. Mol. Biol., 9, 323-339.

Kram, R., Botcham, M. and Hearst, J.E. (1972) J. Mol. Biol., 70, 103-117.

Krmlauf, R. and Marzluf, G.A. (1980) J. Biol. Chem., 255, 1138-1145.

Kupriyanova, N.S. and Timofeeva, M. Ya. (1974) Eur. J. Biochem., 44, 59-65.

Kurnit, D.M., Shafit, B.R. and Maio, J.J. (1973) J. Mol. Biol., 81, 272-284.

La Cour, L.F. (1941) Stain Tech., 16, 169-174.

La Cour, L.F. and Chayen, S. (1958) Exp. Cell Res., 14, 462-468.

Lafontaine, J.G. (1968). In The Nucleus (A.J. Dalton and F. Haguenau Eds.) Vol. 3, pp. 151-196, Academic Press, New York.

Lafontaine, J.G. (1974). In The Cell Nucleus (H. Busch Ed.) Vol. 1, pp. 149-181, Academic Press, New York.

Lafontaine, J.G. and Lord, A. (1969). In Handbook of Molecular Cytology (Lima-De-Faria Ed.) pp. 381-411, North Holland Publishing Co., Amsterdam.

Laird, C.D. (1971) Chromosoma (Berl.) 32, 378-406.

Laird, C.D. and McCarthy, B.J. (1968) Genetics, 60, 303-332.

Laird, C.D. and McCarthy, B.J. (1969) Genetics, 63, 865-882.

Lange, R.T. (1966). In Symbiosis (S.M. Henry Ed.) Vol. 1, pp. 99-170, Academic Press, New York.

Lauer, G.D., Roberts, T.M. and Klotz, L.C. (1977) J. Mol. Biol., 114, 507-526.

Lee, A.S., Britten, R.J. and Davidson, E.H. (1977) Science, 196, 189-192.

Lee, C.S., Davis, R.W. and Davidson, N. (1970) J. Mol. Biol., 48, 1-22.

Lee, J.C. and Yunis, J.J. (1970) Exp. Cell Res., 59, 339-341.

Levings III, C.S., Timothy, D.H. and Hu, W.W. (1976) Crop Science, 16, 63-66.

Levy, W.B., Wong, N.C.W. and Dixon, G.H. (1977) Proc. Natl. Acad. Sci. (USA), 74, 2810-2814.

Lewis, R.F. and Crotty, W.J. (1977) Am. J. Bot., 64, 190-198.

Lieberman, M.W. (1973) Biochim. Biophys. Acta, 324, 309-319.

Lima-de-Faria, A., Pero, A., Avanzi, S., Durante, M., Stahle, U. D'Amato, F. and Granstrom, H. (1975) Hereditas, 79, 5-19.

Lipchitz, L. and Axel, R. (1976) Cell, 9, 355-364.

Lipps, H.J. and Morris, N.R. (1977) Biochem. Biophys. Res. Commun., 74, 230-234.

Lohr, D., Corden, J., Tatchell, K., Kovacic, R.T. and Van Holde, K.E. (1977) Proc. Natl. Acad. Sci. (USA) 74, 79-83.

Lonberg-Holm, K.K. (1967) Nature, 213, 454-457.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.L. (1951) J. Biol. Chem., 193, 265-275.

Macaya, G., Cortadas, J. and Bernardi, G. (1978) Eur. J. Biochem., 84, 179-188.

Mahler, H.R., Kling, B. and Mehrotra, B.D. (1964) J. Mol. Biol., 9, 801-811.

Mandal, R.K., Mondal, H., Som, S. and Biswas, B.B. (1971) Indian J. Biochem. Biophys., 8, 50-51.

Mandel, M. and Marmur, J. (1968). In Methods in Enzymology (L. Grossman and K. Moldave Eds.) Vol. 12B, pp. 195-206, Academic Press, New York.

Mandel, M., Schildkraut, C.L. and Marmur, J. (1968). In Methods in Enzymology (L. Grossman and K. Moldave Eds.) Vol. 12B, pp. 184-195, Academic Press, New York.

Maniatis, T., Jeffrey, A. and Kleid, D.G. (1975) Proc. Natl. Acad. Sci. (USA), 72, 1184-1188.

Manning, J.E., Schmid, C.W. and Davidson, N. (1975) Cell, 4, 141-155.

Manuelidis, L. (1976) Nucl. Acids Res., 3, 3063-3076.

Manuelidis, L. (1978) Chromosoma (Berl.), 66, 1-21.

Markham, R., Blackwell, P.M. and Reid, B.L. (1973) Aust. J. Med. Technol., 4, 164-167.

Marks, G.E. and Schweizer, D. (1974) Chromosoma (Berl.), 44, 405-416.

Marmur, J. (1961) J. Mol. Biol., 3, 208-218.

Marmur, J. and Doty, P. (1959) Nature, 183, 1427-1429,

Marmur, J. and Doty, P. (1962) J. Mol. Biol., 5, 109-118.

Marmur, J., Rownd, R. and Schildkraut, C.L. (1963) Progr. Nucl. Acid Res., 1, 231-300.

Marushige, K. and Dixon, G.H. (1969) Develop. Biol., 19, 397-414.

Marx, K.A., Allen, J.R. and Hearst, J.E. (1976) Chromosoma (Berl.), 59, 23-42.

Mascarenhas, A.F., Hendre, R.R., Nadgir, A.L., Ghugale, D.D., Godbole, D.A., Prabhu, R.A. and Jagannathan, V. (1976) Indian J. Expt. Biol., 14, 604-606.

Mazrimas, J.A. and Hatch, F.T. (1970) Exp. Cell Res., 63, 462-466.

Mazrimas, J.A. and Hatch, F.T. (1972) Nature New Biol., 240, 102-105.

McConkey, E.H.(1967). In Methods in Enzymology (L. Grossman and K. Moldave Eds.) Vol. 12A, pp. 620-634, Academic Press, New York.

McEwen, C.R. (1967) Anal. Biochem., 20, 114-148.

McGregor, H.C. (1979) Chromosoma (Berl.), 71, 57-65.

McGregor, H.C. and Kezer, J. (1971) Chromosoma (Berl.), 33, 167-182.

McGregor, H.C. and Mizuno, S. (1976) Chromosoma (Berl.), 54,

15-25.

Mehra, U., Patil, B.G. and Ranjekar, P.K. (1980) Indian J. Biochem. Biophys., 17, 8-12.

Mehra, U. and Ranjekar, P.K. (1979) Indian J. Biochem. Biophys., 16, 56-60.

Merlo, D.J. and Kemp, J.D. (1976) Plant Physiol., 58, 100-106.

Miksche, J.P. and Hotta, Y. (1973) Chromosoma (Berl.), 41, 29-36.

Mistra, N.K. (1978) Experientia., 34, 846-848

Mitchell, A.R. (1974) Biochim. Biophys. Acta, 374, 12-22.

Mitra, R. and Bhatia, C.R. (1973) Heredity, 31, 251-262.

Moore, D.H., Johnson, P.H., Chandler, S.E.V. and Grossman, L.I. (1977) Nucl. Acids Res., 4, 1273-1289.

Moore, R.L. and McCarthy, B.J. (1968) Biochem. Genet., 2, 75-86.

Morris, N.R. (1976) Cell, 8, 357-364.

Moss, T., Stephens, R.M., Crane-Robinson, L. and Bradbury, E.M. (1977) Nucl. Acids Res., 4, 2477-2485.

Mowbray, S.L., Gerbi, S.A. and Landy, A. (1975) Nature, 253, 367-370.

Moyzis, R., Bonnet, J. and Ts'O, P.O.P., (1977) J. Cell Biol., 75, 130a.

Mukharjee, P. and Mukharjee, D.K. (1979) Curr. Sci., 48, 496-497.

Mundt, J.O. and Hinkle, N.F. (1976) Appl. Env. Microbiol., 32, 694.

Murrey, M.G., Cuellar, R.E. and Thompson, W.F. (1978) Biochemistry, 17, 5781-5790.

Murray, M.G., Palmer, J.D., Cuellar, R.E. and Thompson, W.F. (1979) Biochemistry, 18, 5259-5266.

Murray, M.G., Preisler, R.S. and Thompson, W.F. (1977) Carnegie Inst. Wash. Yearbook, 76, 240-246.

Nadeau, P., Pallotta, D. and Lafontaine, J.G. (1974) Arch. Biochem. Biophys., 161, 171-177.

Nagl, W. (1976) Ann. Rev. Plant Physiol., 27, 39-69.

Nagl, W. (1978). In Endopolyploidy and Polyteny in Differentiation and Evolution. North Holland Publishing Co., Amsterdam.

Nagl, W. and Capesius, L. (1977) Chromosomes Today, 6, 141-152.

Nandi, U.S., Wang, J.C. and Davidson, N. (1965) Biochemistry, 4, 1687-1696.

Narayan, R.K.J. and Rees, H. (1976) Chromosoma (Berl.), 54, 141-154.

Narayan, R.K.J. and Rees, H. (1977) Chromosoma (Berl.), 63, 101-107.

Nebel, B.R. (1940) Stain Tech., 15, 69-72.

Newton, W.F.C. (1926) J. Linn. Soc. (Bot.), 47, 339-354.

Noll, M. (1974a) Nature, 251, 249-251.

Noll, M. (1974b) Nucl. Acids Res., 1, 1573-1578.

Noll, M. (1976) Cell, 8, 349-355.

Nze-Ekekang, L., Patillon, M., Schafer, A. and Kovoor, A. (1974) J. Expt. Bot., 25, 320-329.

Ohno, S., Kaplan, W.D. and Kinosita, R. (1957) Exp. Cell Res., 13, 358-364.

Ohtsubo, H. and Ohtsubo, E. (1976) Proc. Natl. Acad. Sci. (USA) 73, 2316-2320.

Ohyama, K., Ganshorg. O.L. and Miller, R.A. (1972) Plant Physiol., 50, 319-321.

Olins, A.L. and Olins, D.E. (1974) Science, 183, 330-332.

Panyim, S. and Chalkley, R. (1969) Arch. Biochem. Biophys., 130, 337-346.

Panyim, S., Sommer, K.R. and Chalkley, R. (1971) Biochemistry, 10, 3911-3917.

Papconstantinou, J., Bradshaw, W.S., Chin, E.T. and Julku, E.M. (1972) Develop. Biol., 28, 649-661.

Pardue, M.L. and Gall, J.G. (1969) Proc. Natl. Acad. Sci. (USA), 64, 600-604.

Pardue, M.L. and Gall, J.G. (1970) Science, 168, 1356-1358.

Pardue, M.L., Gerbi, S.A., Eckhardt, R.A. and Gall, J.G. (1970) Chromosoma (Berl.), 29, 268-290.

Pascoe, M.J. and Ingle, J. (1978) Plant Physiol., 62, 975-977.

Pays, E. and Ronsse, A. (1975) Biochem. Biophys. Res. Commun., 62, 862-867.

Peacock, W.J., Appels, R., Dunsmuir, P., Lohe, A.R. and Gerlach, W.L. (1977a). In Int. Cong. Cell. Biol. (B.R.Brinkely and K. R. Porter Eds.) 1976-77, pp. 494-506, Rockefeller Univ. Press, New York.

Peacock, W.J., Brutlag, D., Goldring, E., Appels, R., Hinton, C.W. and Lindsley, D.L. (1974) Cold Spring Harbor Symp. Quant. Biol., 38, 405-416.

Peacock, W.J., Lohe, A.R., Gerlach, W.L., Dunsmuir, P., Dennis, E.S. and Appels, R. (1977b) Cold Spring Harbor Symp. Quant. Biol. 42, 1121-1135.

Pearson, W.R., Davidson, E.H. and Britten, B.J. (1977) Nucl. Acids Res., 4, 1727-1737.

Pearson, W.R., Smith, S.L., Wu, J. and Bonner, J. (1978a) Plant Physiol.. 62. 112-115.

Pearson, W.R., Wu, J.R. and Bonner, J. (1978b) Biochemistry, 17, 51-59.

Phillippsen, P., Streeck, R.E. and Zachau, H.G. (1974) Eur. J. Biochem., 45, 479-488.

Phillippsen, P., Streeck, R.E., Zachau, H.G. and Mueller, W. (1975) Eur. J. Biochem., 57, 55-68.

Phillippsen, P. and Zachau, H.G. (1972) Biochim. Biophys. Acta, 277, 523-538.

Pipkin, J.L. and Larson, D.A. (1972) Exp. Cell Res., 71, 249-260.

Pipkin, J.L. and Larson, D.A. (1973) Exp. Cell. Res., 79, 28-42.

Polli, E., Corneo, G., Ginelli, E. and Bianchi, P. (1965) Biochim. Biophys. Acta, 103, 672-677.

Prescott, D.M., Bostock, C.J., Hatch, F.T. and Mazrimas, J.A. (1973) Chromosoma (Berl.), 42, 205-213.

Price, H.J. (1976) Bot. Reviews, 42, 27-52.

Price, H.J. and Bachmann, K. (1975) Am. J. Bot., 62, 262-267.

Price, H.J. and Bachmann, K. (1976) Plant Syst. Evolut. 126, 323-330.

Prosser, J., Moar, M., Bobrow, N. and Jones, K.W. (1973) Biochim. Biophys. Acta, 319, 122-134.

Rae, P.M.M. (1970) Proc. Natl. Acad. Sci. (USA), 67, 1018-1025.

Rae, P.M.M. (1972) Adv. in Cell and Mol. Biol., 2, 109-149.

Rae, P.M.M. and Franke, W.W. (1972) Chromosoma (Berl.), 39, 443-456.

Rake, A.V. (1974) Biochem. Genet., 11, 261-277.

Ranjekar, P.K., Lafontaine, J.G. and Pallotta, D. (1974) Chromosoma (Berl.), 48, 427-440.

Ranjekar, P.K. and Murthy, M.R.V. (1973) J. Neurochem., 20 1257-1264.

Ranjekar, P.K., Pallotta, D. and Lafontaine, J.G. (1976) Biochim. Biophys. Acta, 425, 30-40.

Ranjekar, P.K., Pallotta, D. and Lafontaine, J.G. (1978a) Biochim. Biophys. Acta, 520, 103-110.

Ranjekar, P.K., Pallotta, D. and Lafontaine, J.G. (1978b) Biochem. Genet., 16, 957-970.

Ranjekar, P.K., Pallotta, D. and Lafontaine, J.G. (1978c) Can. J. Biochem., 402, 171-178.

Rawson, J.R.Y., Kckenrode, V.K., Boerma, C.L. and Curtis, S. (1979) Biochim. Biophys. Acta, 563, 1-16.

Rees, H. and Jones, R.N. (1972) Int. Rev. Cytol., 32, 53-92,

Renz, M., Nehls, P. and Hozier, J. (1977) Proc. Natl. Acad. Sci. (USA), 74, 1879-1883.

Rice, N. and Paul, P. (1971) Carnegie Inst. Wash. Yearbook, 71, 262-264.

Richards, B., Pardon, J., Lilley, D., Cotter, R. and Wooley, J. (1977) Cell Biol. Int. Rep., 1, 107-116.

Riley, R. (1965). In Essays on Crops Plant Evolution (J. Hutchinson Ed.) pp. 103-122, Cambridge Univ. Press, London.

Rimpau, J., Smith, D.B. and Flavell, R.B. (1980) Heredity, 44, 131-149.

Robertis, T.M. (1977) J. Mol. Biol., 110, 341-362.

Robinow, C. (1941) Proc. Roy. Soc. B., 130, 299-324.

Robinson, R.L. and Bacsich, P. (1958) Stain Tech., 33, 171-173.

Rodrigues, J. De A., Brandt, W.F. and Von Holt, C. (1979) Biochim. Biophys. Acta, 578, 196-206.

Roizes, G. (1976) Nucl. Acids Res., 3, 2677-2696.

Rubio, J., Rosado, Y. and Castaneda, M. (1980) Can. J. Biochem. 58, 1247-1251.

Russel, A.P., Herrmann, R.L. and Dowling, L.E. (1969) Biophys. J., 9, 473-488.

Ryskov, A.P., Saunders, G.F., Farashyan, V.R. and Gorgiev, G.P. (1973) Biochim. Biophys. Acta, 312, 152-164.

Sachs, R.E., and Clever, U. (1972) Exp. Cell Res., 74, 587-591.

Sachs, D.H. and Painter, E. (1972) Science, 175, 781-782.

Salomon, R., Kaye, A.M. and Herzberg, M. (1969) J. Mol. Biol., 43, 581-592.

Salser, W., Bowen, S., Browne, D., Adli, F.E., Fedoroff, N., Fry, K., Heindell, H., Paddock, G., Poon, R., Wallace, B. and Whitcome, P. (1976) Fed. Proc., 35, 23-35.

Santiago, L. and Rake, A.V. (1973) Biochem. Genet., 9, 275-282.

Saunders, G.F., Shirakawa, S., Saunders, P.P., Arrighi, F.E. and Hsu, T.C. (1972) J. Mol. Biol., 63, 323-334.

Schachat, F., O'Cornor, D.J. and Epstein, H.F. (1978) Biochim. Biophys. Acta, 520, 638-692.

Schildkraut, C.L. and Maio, J.J. (1968) Biochim. Biophys. Acta, 161, 76-93.

Schildkraut, C.I., Marmur, J. and Doty, P. (1961) J. Mol. Biol., 3, 595-617.

Schildkraut, C.I., Marmur, J, and Doty, P. (1962) J. Mol. Biol., 4, 430-443.

Schmid, C.W., Manning, J.E. and Davidson, N. (1975) Cell, 5, 159-172.

Schweber, M.S. (1974) Chromosoma (Berl.), 44, 371-382.

Seshadri, M. and Ranjekar, P.K. (1979) Indian J. Biochem. Biophys., 16, 1-5.

Seshadri, M. and Ranjekar, P.K. (1980a) Hoppe-Seyler's Z. Physiol. Chem., 361, 1041-1048.

Seshadri, M. and Ranjekar, P.K. (1980b) Biochim. Biophys. Acta, 610, 211-221.

Sharma, A.K. (1978) Nucleus, 21, 113-116.

Sharma, A.K. and Sharma, A. (1980). In Chromosome Techniques: Theory and Practice, Third Edition, Butterworth and Company (Publishers) Limited.

Shearer, R.W. and McCarthy, B.J. (1970) Biochem. Genet., 4, 395-408.

Shen, Che-Kun, J., Wiesehahn, G. and Hearst, J.E. (1976) Nucl. Acids Res., 3, 931-951.

Simpson, R.T. and Whitlock, J.P. Jr. (1976) Cell, 9, 347-357.

Sinclair, J., Wells, R., Deumling, B. and Ingle, J. (1975) Biochem. J., 149, 31-38.

Sivolap, Y.M. and Bonner, J. (1971) Proc. Natl. Acad. Sci. (USA), 68, 387-389.

Skinner, D.M. and Beattie, W.G. (1973) Proc. Natl. Acad. Sci. (USA), 70, 3108-3110.

Skinner, D.M. and Beattie, W.G. (1974) Biochemistry, 13, 3922-3929.

Skinner, D.M., Beattie, W.G., Kerr, M.S. and Graham, D.E. (1970) Nature, 227, 837-839.

Skinner, D.M. and Kerr, M.S. (1971) Biochemistry, 10, 1864-1872.

Smith, D.B. and Flavell, R.B. (1974) Biochem. Genet., 12, 243-256.

Smith, D.B. and Flavell, R.B. (1975) Chromosoma (Berl.), 50, 223-242.

Smith, D.B. and Flavell, R.B. (1977) Biochim. Biophys. Acta, 474, 82-97.

Smyth, D.R. and Stern, H. (1973) Nature New Biol., 245, 94-96.

Sparrow, A.H., Price, H.J. and Underbrink, A.G. (1972) Brookhaven Symp. Biol., 23, 451-494.

Spiker, S. (1975) Biochim. Biophys. Acta, 400, 461-467.

Spiker, S., Key, J.L. and Wakim, B. (1976) Arch. Biochem. Biophys., 176, 510-518.

Spiker, S. and Krishnaswamy, L. (1973) Planta, 110, 71-76.

Sponar, J., Sormova, Z. and Glisin, V. (1970) FEBS Letters, 11, 254-256.

Spradling, A., Penman, S., Campo, M.S. and Bishop, J.O. (1974) Cell, 3, 23-30.

Sree Ramlu, K. and Rangaswami, S.R. (1971) Cytologia, 36, 480-486.

Stack, S.M., Brown, D.B. and Dewey, W.C. (1977) J. Cell Sci., 26, 281-299.

Stafford, D.W. and Guild, W.R. (1969) Exp. Cell Res., 55, 347-350.

Stanziano, P. and Maggini, F. (1976) ATTI Associazione Genetica Italiana, 21, 66-69.

Stebbins, G.L. (1966) Science, 152, 1463-1469.

Stebbins, G.L. (1974). In Evolutionary Biology (H.K. Hecht, W.C. Steere and E. Wallace Eds.) Vol. 9, pp. 1-34, Plenum Press, New York.

Stein, G.S., Stein, J.S. and Kleinsmith, L.J. (1975) Sci. Amm., 232, 46-57.

Stein, D.B. and Thompson, W.F. (1975) Science, 185, 888-890.

Stern, H. (1968). In Methods in Enzymology (L. Grossman and K. Moldave Eds.) Vol. 12B, pp. 100-112, Academic Press, New York.

Stevens, B.J. and Moustacchi, E. (1971) Exp. Cell Res., 64, 259-266.

Straus, N.A. (1971) Proc. Natl. Acad. Sci. (USA), 68, 799-802.

Straus, N.A. (1972) Carnegie Inst. Wash. Yearbook, 71, 257-259.

Street, H.E. (1973). "Plant Cell and Tissue Culture", University of California Press, Los Angeles.

Strokov, A.A., Bogdanov, Yu. F. and Reznickova, S.A. (1973) Chromosoma (Berl.), 43, 247-260.

Strong, H.G. and Thompson, W.F. (1975) Carnegie Inst. Wash. Yearbook, 74, 789-792.

Subirana, J.A. and Doty, P. (1966) Biopolymers, 4, 171-187.

Subramanyam. N.C. and Azad, A.A. (1978) Chromosoma (Berl.), 69, 265-273.

Sueoka, N. (1961) J. Mol. Biol., 3, 31-40.

Sugiura, M. and Hotta, Y. (1980) Plant and Cell Physiol., 21, 1129.

Sutton, W.D. and McCallum, M. (1971) Nature New Biol., 232, 82-85.

Sutton, W.D. and McCallum, M. (1972) J. Mol. Biol., 71, 633-656.

Sutton, W.D. and Walker, P.M.B. (1972) Biochem. J., 128, 193-198.

Suzuki, Y., Gage, L.P. and Brown, D.D. (1972) J. Mol. Biol., 70, 637-656.

Szala, S., Paterak, H. and Chorazy, M. (1980) FEBS Letters, 117, 349-353.

Thiery, J.O., Macaya, G. and Bernardi, G. (1976) J. Mol. Biol., 108, 219-235.

Thomas, M. and Davis, R.W. (1975) J. Mol. Biol., 91, 315-328.

Thomas, Jr. C.A., Hamkalo, B.A., Misra, D.N. and Lee, C.S. (1970) J. Mol. Biol., 51, 621-632.

Thomas, J.O. and Thompson, R.J. (1977) Cell, 10, 633-640.

Thompson, W.F. (1976) Plant Physiol., 57, 617-622.

Thornburg, W. and Siegel, A. (1973) Biochim. Biophys. Acta, 312, 211-214.

Thrower, K.J. and Peacock, A.R. (1966) Biochim. Biophys. Acta, 119, 652-654.

Timberlake, W.E. (1978) Science, 202, 973-975.

Timmis, J.N., Deumling, B. and Ingle, J. (1975) Nature, 257, 152-155.

Timmis, J.N. and Ingle, J. (1977) Biochem. Genet. 15, 1159-1173.

Tiselius, A., Hjerten, S. and Levin, O. (1956) Arch. Biochem. Biophys., 65, 132-155.

Travaglini, E.C., Petrovic, T. and Schultz, J. (1972) Genetics, 72, 431-439.

Turner, B.L. (1969) Taxon, 18, 134-151.

Ulitzur, S. (1972) Biochim. Biophys. Acta, 272, 1-11.

Vander Schans, G.P., Aten, J.B.T. and Blok, J. (1969) Anal. Biochem., 32, 14-30.

Vladychenskaya, N.S., Kedrova, O.S. and Antonov, A.S. (1975) Biol. Nauki (Moscow), 18, 105-110.

Volkin, E. and Cohn, W.E. (1954) Methods Biochem. Anal., 1, 287-303.

Vorobev, V.I. and Kosjuk, G.N. (1974) FEBS Letters, 47, 43-46.

Vosa, C.G. (1973) Chromosoma (Berl.), 43, 269-278.

Vosa, C.G. (1976) Heredity, 37, 395-403.

Vosa, C.G. (1977). In Current Chromosome Research (K. James and P.E. Brandham Eds.) pp. 105, Amterdam, Elseuer Press.

Votavova, H. and Sponar, J. (1974) Coll. Czech. Chem. Commun., 39, 2312-2334.

Votavova, H. and Sponar, J. (1975) Nucl. Acids Res., 2, 431-446.

Votavova, H., Sponar, J. and Sormova, Z. (1970) Eur. J. Biochem., 12, 208-216.

Votavova, H., Sponar, J. and Sormova, Z. (1972) Coll. Czech. Chem. Commun., 37, 1412-1419.

Votavova, H., Sponar, J. and Sormova, Z. (1973) Coll. Czech. Chem. Commun., 38, 3004-3013.

Votavova, H., Strokrova, J. and Sponar, J. (1975) Biochim. Biophys. Acta, 383, 370-378.

Walbot, V. and Dure, L.S. (1976) J. Mol. Biol., 101, 503-536.

Walker, P.M.B. (1971) Prog. Biophys. Mol. Biol., 23, 145-190.

Walker, P.M.B. (1978) Phil. Trans. R. Soc. Lon., Ser. B., 282, 305-307.

Walker, P.M.B., Flamm, W.G. and McLaren, A. (1969). In handbook of Molecular Cytology (Lima-de Faria Ed.) pp. 52-66, North Holland Publishing Company, Amsterdam.

Walker, P.M.B. and McLaren, A. (1965) J. Mol. Biol., 12, 394-409.

Waring, M. and Britten, R. (1966) Science, 154, 791-794.

Weber, K. and Osborn, M. (1969) J. Mol. Biol., 244, 4406-4412.

Weinberg, E.S., Birnstiel, M.L., Purdom, I.F. and Williamson, R. (1972) Nature, 240, 225-228.

Weinblum, D., Guengerich, U., Geisert, M. and Zahr, R.K. (1973)

Biochim. Biophys. Acta, 299, 231-240.

Weintraub, H., Worcel, A. and Alberts, B. (1976) Cell, 9, 409-417.

Wellauer, P.K. and Dawid, I.B. (1974) J. Mol. Biol., 89, 379-395.

Wells, R., Royer, H.D. and Hollenberg, C.P. (1976) Mol. Gen. Genet., 147, 45-51.

Wensink, P.C. (1978) Cold Spring Harbor Symp. Quant. Biol., 42, 1033-1039.

Wetmur, J.G. and Davidson, N. (1968) J. Mol. Biol., 31, 349-370.

Whiteley, H.R., McCarthy, B.H., Whiteley, A.N. (1970) Develop. Biol., 21, 216-242.

Willey, A.M. (1975). "The Characterization and Localization of the repetitive DNA of the Algerian Hedgehogn" Ph.D. Thesis. Abstr. Diss. Int. B., 35, 5942.

Willey, A.M. and Yunis, J.J. (1975) Exp. Cell Res., 91, 223-232.

Wilson, J.T., Katterman, F.R.H. and Endrizzi, J.E. (1976) Biochem. Genet., 14, 1071-1075.

Wilson, D.A. and Thomas, C.A. Jr. (1974) J. Mol. Biol. 84, 115-138.

Wimpee, C.F. and Rawson, J.R.Y. (1979) Biochim. Biophys. Acta, 562, 192-206.

Wintzerith, M., Mori, K. and Mandel, P. (1973) J. Neurochem., 21, 1341-1343.

Woodcock, C.J. (1973) Cell Biol., 59, 368a.

Worcel, A. (1978) Cold Spring Harbor Symp. Quant. Biol., 42, 313-324.

Wu, J., Hurn, J. and Bonner, J. (1972) J. Mol. Biol., 64, 211-219.

Wu, J., Pearson, W.R., Posakony, J.W. and Bonner, J. (1977) Proc. Natl. Acad. Sci. (USA), 74, 4382-4386.

Wuilmart, C., Urbain, J. and Givol., D. (1977) Proc. Natl. Acad. Sci. (USA), 74, 2526-2530.

Yasmineh, W.G. and Yunis, J.J. (1969) Biochem. Biophys. Res. Commun., 35, 779-782.

Yasmineh, W.G. and Yunis, J.J. (1970) Exp. Cell Res., 59, 69-75.

Yasmineh, W.G. and Yunis, J.J. (1971) Biochem. Biophys. Res. Commun., 43, 580-587.

Yasmineh, W.G. and Yunis, J.J. (1973) Exp. Cell Res., 81, 432-446.

Yasmineh, W.G. and Yunis, J.J. (1975) Exp. Cell Res., 90, 191-200.

Yunis, J.J. (1974). In Human Chromosome Methodology, Second Edition, Academic Press, New York.

Yunis, J.J., Roldan, L., Yasmineh, W.G. and Lee, J,C. (1971) Nature, 231, 532-533.

Yunis, J.J. and Yasmineh, W.G. (1970) Science, 168, 263-265.

Yunis, J.J. and Yasmineh, W.G. (1971) Science, 174, 1200-1209.

Zardi, L., Siri Annalisa, Santi, L. and Rovera, G. (1977) FEBS Letters, 79, 188-190.

Zimmerman, J.L. and Goldberg, R.B. (1977) Chromosoma (Berl.), 59, 227-252.

LIST OF PUBLICATIONS

1. Vidya G. Deshpande and P.K.Ranjekar
   "Repetitive DNA in three Gramineae species with low DNA cc
   Hoppe-Seyler's Z. Physiol. Chem. (1980), 361, 1223-1233.
2. Vidya S. Gupta (nee Vidya G. Deshpande) and P.K.Ranjekar
   "Novel DNA sequence organization in rice genome"
   Biochim. Biophys. Acta (Communicated).
3. Vidya S. Gupta (nee Vidya G. Deshpande) and P.K.Ranjekar
   "DNA sequence organization in finger millet genome"
   Biochem. J. (Communicated).
4. Vidya S. Gupta and P.K.Ranjekar
   "The histone pattern in finger millet and pearl millet"
   Indian J. Biochem. Biophys. (Communicated).
5. C.P.Joshi, Vidya S. Gupta and P.K.Ranjekar
   "The molecular basis of interphase nuclear organization ir
   plants"
   J. Cell Sci. (Communicated).

LIST OF PAPERS PRESENTED

1. Vidya G. Deshpande and P.K.Ranjekar
   "DNA sequence organization in rice (Oryza sativa)"
   Presented at the 48th Annual General Meeting of the
   Society of Biological Chemists, New Delhi, India (1978).
2. Vidya G. Deshpande, Lakshmi SivaRaman and P.K.Ranjekar
   "Nuclear DNA characterization in three millet species".
   Presented at the Golden Jubilee Meeting of the Society
   of Biological Chemists and Second Congress of FAOB, Banga
   India (1980).